COCAINE

Spot Tests

1. 2% aqueous cobalt thiocyanate - fast blue precipitate.
   If negative, add conc. HCl to detect cocaine base.

2. Methyl benzoate (5% NaOH in MeOH) - odor of wintergreen.

3. Sanchez - positive with procaine or benzocaine.

4. Marquis - slow pink.

TLC

1. Clarke's T1 - (10 ml MeOH plus 7 drops conc. NH_4OH) - visualize with UV and acidified iodoplatinate.

2. Other appropriate systems can be found in the literature.

3. TOXI-LAB.

GLC

OV-101, OB-1, OV-17 and DB 17. Generally, small amount of powder plus MeOH is the only sample preparation required. Quantitate on any appropriate column using external or internal standard method.

Microcrystal

1. Ruybal's Gold Bromide - small x's.

2. Platinic Chloride - feathers.

3. Gold Chloride - fans.

IR

1. Cobalt thiocyanate derivative - paper included.

2. "Lumpology" - If your cocaine is lumpy or contains two kinds of material which you can separate under the stereoscope, run a IR direct on the lumps.

3. Dry extract with CH_2Cl_2, recrystallize with petroleum ether - make HCl salt with Methanolic HCl.

4. Acid-base shake out - use a weak base and make the base.

GC-MS - if available

I. Stimulant/analgesic

A. Cocaine

B. Phencyclidine

C. Procaine

II. Color test (refer to laboratory analytical manual--color tests)

A. Cobalt thiocyanate: blue ppt

B. Lieberman's: faint or light yellow

C. Mayer's: white ppt

D. Ruybal's: blue ppt

E. Compare to known reactions of known substances

Note: Free base cocaine may react slowly or not at all with cobalt thiocyanate or Crook's test. Free base may have to be converted to Cl - form - dissolve in hexane and bubble con. HCl fumes through hexane.

III. Microcrystals

A. Gold chloride (railroad tracks - cocaine)

Note: Usually have to use 0.02NHCl form to get good crystals.

IV. Ultraviolet spectrophotometry

A. Place portion of sample in 0.02NHCl (refer to UV section).

B. Run UV scan with solvent reference cell.

C. Examine charts - note peaks (cocaine: max 275-76 + 233).

D. Compare to known charts, reference materials, or known substance.

V. Infrared spectrophotometry

A. Prepare KBr window - neat sample.

B. Run IR.

C. Compare to known library reference spectrum or known substance.

Note: May have to cleanup sample with celite column and solvents acetone, chloroform, methanol, ether (acetone fraction seems the best for cocaine).

D. Other tests performed:

1. Thin layer chromatography

a. Refer to references.

2. Microcrystaline tests

a. Platinum chloride -- for cocaine and procaine.

b. 10% aqueous potassium iodide solution for PCP.

VI. Gas liquid chromatography

A. Extract sample with methanol.

B. Inject GC

1. Isothermal: OV-17 or OV-1 at 220°C and 250°C.

2. Programmed: OV-17 or OV-1 at 220°C and 250°C.

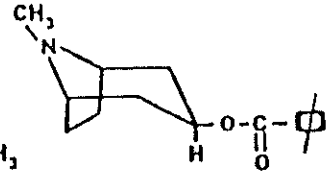
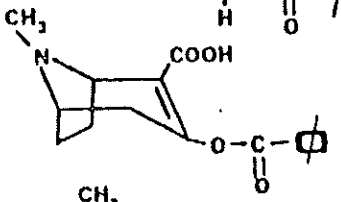
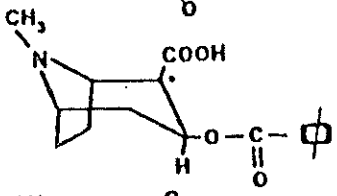
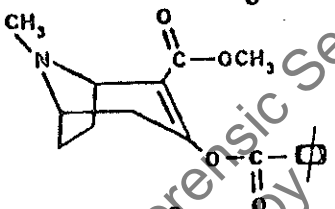
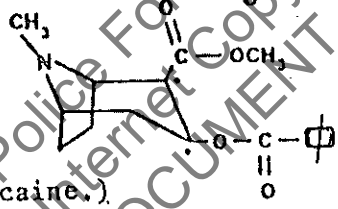
C. Examine charts - note peaks.

D. Compare to charts of known substances under same conditions. (Note the presence of peaks that may be dilutants.)

SYNTHETIC COCAINES

History

1862	Niemann and Wohler isolate l-cocaine from natural sources and begin comprehensive chemical analysis of a potentially important medicinal compound.
1903	Willstätter et. al., clarifies the two dimensional structure of cocaine and postulates the existence of three other diastereomers.
1923	Synthesis of pseudococaine by Willstätter. Willstätter also resolves d- and l-cocaine and publishes the melting point data in the open literature.
1935	Robinson-Schöpf reaction allows the synthesis of tropinones from dialdehyde, methylamine and β-keto-glutaric acid.
1954	Findlay synthesizes pseudococaine from cocaine.
1957	Findlay synthesizes 2-carbomethoxytropinone and demonstrates its significance as a precursor to the diastereomers of cocaine.
1959	Findlay synthesizes the allococaines via catalytic reduction. Although Findlay correctly assigns the stereochemical configurations, the nomenclature of the two compounds is reversed.
1963	Gabe and Barnes verify the absolute configuration of l-cocaine via X-Ray diffraction of cocaine crystals.
1964	Preobrazhenskii et. al., assign configurations of the four methyl esters of ecgonine via infrared spectroscopy. Synthesizes allococaines and publishes melting point data that has since been discredited.
1968	Sinnema et. al., verifies configuration and conformation of the four diastereomers of cocaine via NMR. Confirms prior suppositions that the tropane nucleus of cocaine exists in predominantly the chair form.
1978	Allen and Cooper synthesize racemic cocaine and outline forensic procedures to distinguish presence of d-cocaine.
1978	Aerospace Corporation partially resolve racemic cocaine. Presented at AAFS meeting.
1979	Allen and Cooper synthesize the four racemic cocaines and characterize via IR, NMR, and Mass Spectrometry.

Molecular Weight	Name	Structure	Reactions
245	3-Benzoxytropane (Tropacocaine)		Benzoylation of pseudotropine.
287	3-Benzoxytropidine-2-carboxylic acid		Alkaline/neutral hydrolysis of 2-carbomethoxy-3-benzoxytropidine.
289	3-Benzoxytropane-2-carboxylic acid (Benzoylecgonine)		Alkaline/neutral hydrolysis of cocaine.
301	2-Carbomethoxy-3-benzoxytropidine		Benzoylation of 2-carbometho tropinone is enhanced due to the ease of enol formation of the tropinone.
303	2-Carbomethoxy-3-benzoxytropane (Cocaine, pseudococaine, allococaine, pseudoallococaine.)		Desired end products.

Note: Asterisk (*) indicates that the substituent group at that carbon can exist in an "endo" or "exo" configuration.

(9)

Microgram Vol. VIII, No. 1
Jan. 1975

A SIMPLE FIELD TEST FOR COCAINE NOT RELYING ON COBALT THIOCYANATE

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OBJECTIVE

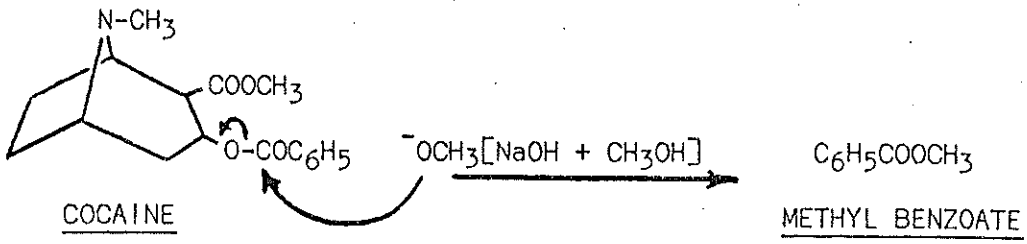
Our purpose was to improve the sensitivity and specificity of present methods of cocaine detection in a simple, easily interpreted field test.

BACKGROUND

Efforts to improve the specificity of cobalt thiocyanate in cocaine field testing (1,2,3,4,5) have tended to compromise field adaptability. Recently Lorch (4) had to add a fourth reagent to the already involved three reagent test of Scott (3,5) to further improve specificity. It is our impression that cobalt thiocyanate has been pushed to the limit in cocaine detection and that perhaps a fresh start should be made in another direction.

METHOD

Our approach relates to the fact that cocaine is quite unique among drugs in being a benzoate ester. We have screened several hundred drugs, including virtually all of the common drugs of abuse, by molecular formula and find only the relatively obscure "piperocaine" sharing this property with cocaine. While we knew of no available color test specific for benzoate esters we were aware of the distinctive odors of the lower alkyl benzoate esters. It is an easy matter to cleave off the benzoate function of cocaine in the form of methyl benzoate by treatment with sodium methoxide in methanol. Metallic sodium need not be used in the formation of sodium methoxide since sodium hydroxide in methanol will serve this purpose. The reaction is indicated below.



PROCEDURE

A few drops of a 5% solution of sodium hydroxide or potassium hydroxide in methyl alcohol are used to moisten the suspected cocaine specimen. A few minutes are allowed for the alcohol to evaporate and any odor is noted.

RESULTS AND DISCUSSION

The wintergreen-like odor of methyl benzoate is strong and distinctive. In presenting a test based on odor detection one faces a longstanding prejudice that rightfully maintains that subtle qualitative or quantitative distinctions based on odor cannot be made reproducibly by the human nose. However, in this connection it is of interest that a New York State Appeals Court recently ruled that the smell of marijuana smoke provided legal cause for Police to search an automobile and its occupants without a search warrant.

The saving grace of this test resides in the absence of any odor whatsoever when the test is applied to the vast majority of drugs likely to be encountered in the field. Occasionally, a very faint fishy odor arises when a low molecular weight amine, such as amphetamine, is released from its salt. Because of its specificity, the test is unaffected by the presence of excipients or other drugs. Methyl acetate is a product of the test when applied to heroin, aspirin, and other acetate esters but it is removed during the evaporation step. Benzoic acid itself fails to give a positive test. WATER WILL INTERFERE WITH THE TEST SO REAGENT AND SPECIMEN SHOULD BE KEPT DRY.

The test has been field tested by local County and State Police who now consider it the test of choice in the field identification of cocaine.

REFERENCES

1) R. Ruybal, Microgram, Vol. 5, No. 3 (1972)
2) C. N. Ruybal, Ibid., Vol. 6, No. 2 (1973)
3) L. J. Scott, Jr., Ibid., Vol. 6, No. 11 (1973)
4) S. K. Lorch, Ibid., Vol. 7, No. 8 (1974)
5) Drug Enforcement, Vol. , No. 3, pp. 26-27 (1974)

COCAINE CELITE COLUMN

Mix 4 grams of Celite 545 with 2 ml. of 1M potassium nitrate in 0.1N hydrochloric acid and transfer to the column in about two equal portions, pressing down each portion lightly with a glass packing rod. Place a pledget of glass wool on top of the mixture. Pipette a 1 ml. aliquot of solution containing 50 mg. of sample in 1M potassium nitrate in 0.1N hydrochloric acid onto the surface of the column.

Sniff Test for Cocaine:

Reagents:

5% KOH in MeOH

Procedure:

1. Place a small amount (a few crystals will do) suspected powder on glass slide.
2. Place a drop or two of the 5% methanolic KOH on this powder.
3. As evaporation progresses, waft towards or hold slide under nose and smell.
4. A wintergreen odor indicates the presence of cocaine.

Interfering substances:

The only other caine known to give the same odor is Pipericaine.

COCAINE
TARTARIC ACID

1. Dissolve sample in water in a small test tube.
2. Make basic with a drop of NH_4OH
3. Add one drop of sat'd NaCl solution
4. Extract with pet ether
5. Run pet ether through cotton plugged pipet
6. Reduce volume to acouple of drops
7. Estimate amount of cocaine present and add corresponding amount of Di-p-toluoyl-l-tartaric acid monohydrate (end of spatula)
8. Add approx. ½ ml acetone. Drip down side to rinse in tartaric acid.
9. Let stand until crystals form. Pipet off excess liquid & use blotter paper to remove all liquid.
10. Rinse crystals with acetone - dry
11. Run IR scan

FREE BASE COCAINE/COCAINE HYDROCHLORIDE

This laboratory has felt a need to determine if a suspect cocaine sample is the hydrochloride form or the free base form. This information is considered to be only for investigative purposes.

Testing of the free base cocaine form via cobalt thiocyanate reveals a slow light blue color reaction, as opposed to the rapid intense blue normally observed for cocaine hydrochloride.

The 5%KOH "smell" test has been found to be an excellent test for cocaine; however, it does not differentiate between the HCl or FB forms.

Microcrystal tests work well for both the HCl and FB forms.

This laboratory "usually" cleans up cocaine samples before instrumental analysis (UV, IR, GC); therefore, these techniques provide no information as to the "form" of the cocaine.

The following is a relatively quick procedure to determine the "form" of a cocaine sample:

A. Place a portion of the suspect white powder in a test tube and add water.

   1. The hydrochloride form will readily dissolve in the water.

      (a) Bubbling concentrated hydrochloric acid fumes through the water will not change the amount of substance dissolved.

   2. The free base form will not readily dissolve in the water.

      (a) Bubbling concentrated hydrochloric acid fumes through the water will cause the free base form to dissolve.

B. Place a portion of the suspect white powder in a test tube with hexane or ether used as a solvent:

   1. Filter solvent (#4 Whatman) into a beaker or test tube.

   2. Bubble concentrated hydrochloric acid fumes through the solvent.

      (a) The free base will be converted to the hydrochloric form and is precipitated out (this is a "good" clean up).

      (b) The hydrochloric form will not precipitate out of the solvent as it is not converted.

   3. Testing of the converted free base form and comparison to the unconverted form will reveal:

      (a) The unconverted free base form produces a slow light blue reaction to cobalt thiocyanate.

      (b) The converted free base form (HCl) gives a rapid intense blue reaction to cobalt thiocyante.

EPHEDRINE/DYCLONINE MIXTURE (WHITE POWDER)

1) Sample + Cobalt Thyocyanate + H_2O

2) Make basic with sat. NaOH.

3) Add Hexane - Extract.

4) Pull off Hexane - Dry with $HaSO_4$ - Bubble con. HCl fumes through - Ephedrine will precipitate out - Dry down - Run FTIR.

5) Make basic solution acidic with concentrated HCl, Extract with $CHCl_3$, Dry $CHCl_3$ through $NaSO_4$, evaporate in KBr, press and run FTIR for Dyclonine.

ABSTRACT OF A PAPER PRESENTED AT THE SPRING 1975 MEETING OF THE MIDWESTERN ASSOCIATION OF FORENSIC SCIENTISTS.

"A Simple Procedure for the Sepatation and Identification of Cocaine". by Jon D. Naylor, Carl R. Phillips, Robert J. McCurdy & Stephen A. Koers.

The selective extraction of the blue complex of cocaine with cobalt thiocyanate from aqueous acid into chloroform in the presence of common street excipients, e.g. lidocaine, tetracaine, procaine, etc., has suggested a very simple purification and identification procedure. The infrared spectrum of the dried blue extract has been found to be quite characteristic for cocaine. The spectrum consists of a simple addition of the absorption peaks for cocaine and cobalt thiocyanate with very few shifts in frequency or distortions of component peak shapes. The procedure is: a.) addition of about 2 ml of cobalt thiocyanate reagent (2g of cobalt thiocyanate in 100ml of water) to enough street sample to contain one to two mg. of cocaine, b.) addition of up to one-half ml. of concentrated hydrochloric acid (excess HCl results in displacement of cocaine by chloride ion in the complex to form a blue solution), c.) dropwise addition of enough water to dissolve all of the blue precipitate (vigorous shaking is necessary), d.) extraction of the blue cocaine-cobalt thiocyanate complex into chloroform, e.) drying the complex in an evaporating dish, and f.) running the infrared spectrum of the dried blue complex as a KBr mull. The resultant spectrum has been found to be totally free of other materials when mixtures of cocaine with procaine, lidocaine, and tetracaine were treated in the above manner.

If pure cocaine instead of the complex is desired, the chloroform extract in (d) above may be washed with aqueous ammonia to displace the cocaine from the complex. The tan-colored ammonia complex will be extracted into the aqueous layer, and the clear chloroform layer will contain cocaine free base which can be treated in the usual manner for further identification.

The greater speed, more complete separations, and ability to perform the entire operation in a test tube makes this procedure far superior to the traditional multiple extraction and chromatographic separation procedures.

STREET TESTS FOR COCAINE

As many Agents know, street lore describes three (3) tests to distinguish Cocaine from Turkey. It is important for an agent to be familiar with these tests if he is working undercover.

These tests were tried on various materials in the Western Regional Laboratory and the results are given below. The tests aren't great, but surprisingly they aren't bad either !!

DESCRIPTION OF TESTS

WATER TEST: Fill a large clear drinking glass with cold tap water; let trapped air bubbles rise and escape. Take a pinch of suspect cocaine about the size of a match head and throw it on the water. (NOTE: We found this test does not work well in a small diameter glass like an orange juice glass !! Do the test in a regular, large, clear, tumbler.)

BLEACH TEST: Pour liquid bleach (PUREX, CHLOROX, etc.) into a clear juice glass to a depth of at least two inches. Use a larger glass if you don't mind wasting the bleach. Take a pinch of suspect cocaine about the size of a match head and throw it on the bleach.

BURN TEST: Take a pinch of suspect cocaine about the size of a pea and place it on aluminum foil. Hold a lit paper match under the foil.

OBSERVED RESULTS OF TESTS

Material Tested	Water Test	Bleach Test	Burn Test
Cocaine Hydrochloride	Crystals dissolve at or just below the surface. Dissolve so fast they appear to "flash". Oily surface may appear on surface.	Crystals form scum and spin and jump around on surface. In a short time particles will sink, leaving white "contrails" in the bleach.	Produces smoke rapidly, total volume reduces quickly, ends up a black charred spot.
Procaine Hydrochloride	Crystals fall slowly to bottom, slow to dissolve.	Turns reddish brown and sinks to bottom rapidly.	Melts first, then produces smoke, turns yellow; doesn't reduce in volume.
Lactose	Sinks to bottom, no dissolving. May go to bottom in a lump.	Sinks to bottom, doesn't dissolve.	Bubbles up, doesn't smoke until it begins to turn black; then smokes and increases in volume. Odor of burning sugar.

Material Tested	Water Test	Bleach Test	Burn Test
PCP	Dissolves rapidly.	Floats on top, no "contrails" formed.	Smokes, but no residue is left expect a trace of ash.
Lidocaine	Very similar to cocaine.	Very similar to cocaine, but no "contrails" observed.	Smokes, then disappears to no residue expect a trace of ash.
Benzocaine	Floats on top as dry islands of powder.	Floats on top as dry islands of powder.	Melts immediately, runs around on foil, then disappears.
Tetracaine	Similar to cocaine	Similar to cocaine at first then forms yellow to dark brown oil spots on surface.	Smokes, turns yellow, then black, but does not reduce in volume.
Street Cocaine about 50% cocaine, 40% lactose and 10% procaine	Some crystals dissolve fast like cocaine, some particles go to the bottom.	Some crystals float on top, some material goes reddish brown and sinks to bottom, other material sinks to bottom with no color change.	Smokes, turns black, volume doesn't change much.

Although these tests work fairly well, don't get carried away. The sole purpose of this paper is to make sure you know as much as the street people. These tests are not specific for cocaine. You may obtain a false indication that cocaine is present or on the other hand you may not be able to detect cocaine which is actually present.

Robert K. Sager

ROBERT K. SAGER
Laboratory Director

RKS:fh

ANALYSIS OF PLANT MATERIAL FOR THE PRESENCE OF MARIHUANA

I. General Testing Procedure

A. Microscopic examination (7x to 40x)

Plant material must possess the following characteristics:

1. Cystolythic hairs - Small clawlike protrusions on upper surface of leaves.
2. Unicellular hairs - Long, tapered hairs on underside of leaves, giving a fuzzy appearance. Also found along stems.
3. Compound palmate leaves (if whole leaves are present) - Leaves are compound palmate with serrated edges. Three to five leaves are attached to the branches.

(NOTE: Presence of cystolythic hairs can be tested for by adding a drop of hydrochloric acid to a leave and looking for the foaming as $CaCO_3$ is formed.)

Seeds must possess the following characteristics:

1. Mosaic patterned shell - Veining gives the shell a mosaic appearance.
2. Coconut shell-like appearance - Shell resembles a small coconut with a ridge that runs along its circumference.

B. Modified Duquenois-Levine

1. Pet ether is added to plant material in a small test tube.
2. Decant a portion of pet ether extract into a second test tube.
3. Evaporate pet ether with a stream of air or heat.
4. Add Duquenois reagent to extract.
5. Add an equal amount of concentrated hydrochloric acid and shake test tube to mix. If marihuana is present, a deep purple-colored complex will result. The purple color will form if any phenolic compound with an alkyl side chain is present, eg. resorcinol. Unless a lot of resin is present, the purple color will develop gradually over a period of 1 to 2 minutes. Some materials such as patchouli oil develop a purple color immediately - other materials develop the purple color over a period of 10 minutes or more.
6. Add chloroform to test tube and mix. If purple complex enters chloroform (is soluble in), the test is positive for the presence of marihuana. If the purple color does not enter the chloroform layer, the test is considered to be negative.

C. Thin Layer Chromatography (TLC)

1. Pet ether extract of plant material is spotted on an appropriately labelled silica gel thin layer chromatography plates.
2. A marihuana standard is spotted on the plate.
3. The plate is then eluted in:
   (a) Petroleum ether - ether 4:1,
   (b) Chloroform,
   (c) Benzene, or
   (d) Hexane-diethylether 4:1.
4. The plate is visualized by spraying with Fast Blue BB salt solution. Separated cannabinoid components of extract will form a characteristic color.
   eg. Pet ether/ether 4:1 - cannabidiol (CBD) orange
   tetrahydrocannabinol (THC) scarlet
   cannabinol (CBN) purple

5. Visualized spots are compared to marihuana standard. If scarlet spot for THC is present, the test is considered positive. This three-part test sequence is utilized to confirm the presence of marihuana because each of the tests independently supports the other.

D. Germination of Seeds

1. Place a known number of seeds in a moist chamber at room temperature.
2. Check in two days. If negative, remoisten and check again next day.
3. Seeds are considered to be fertile if greater than 1% sprout.
4. To find germination, count total number of seeds and total number of sprouts.

II. Requirements for Specific Samples

A. Plant material.

1. Microscopic.
2. Modified Duquenois-Levine.
3. TLC.

B. Seeds

1. Microscopic.
2. Duquenois.
3. TLC.
4. Germination.

C. Hash

1. Microscopic (optional) - dissolve in $CHCl_3$ and look for crushed plant hairs.
2. Duquenois.
3. Two different TLC systems.

D. Pipes

1. Microscopic on ash (optional).
2. Duquenois.
3. Two different TLC systems - only one TLC is necessary if microscopic is positive.

III. Conclusions

The sample may be designated as "marihuana, non-narcotic schedule I" if microscopic, Duquenois and TLC are all positive.

The sample may be designated as "resins of marihuana, non-narcotic, schedule I" if microscopic was not done, but Duquenois and two TLC systems were positive.

ANALYSIS OF PLANT MATERIAL FOR THE PRESENCE OF MARIHUANA

I. GENERAL TESTING PROCEDURE

A. Microscopic Examination (1X-4X)

Plant material must possess the following characteristics:

1. CYSTOLYTHIC HAIRS

   Small clawlike protrusions along the topside and edges of leaf.

2. FINE HAIRS

   Long tapered hairs on underside of leaf. Give fuzzy appearance. Also appear along stems.

3. COMPOUND PALMATE LEAVES (If whole leaves are present)

   Leaves are compound-palmate. Edges are serrated.

Seeds must possess the following characteristics:

1. MOSAIC PATTERNED SHELL

   Veining gives the shell a mosaic appearance.

2. COCONUT SHELL-LIKE SHAPE

   Shell resembles a small coconut with a ridge that runs along its circumference.

B. Chemical Test-Modified Duquenois (Duquenois-Levine)

This test independently supports the microscopic examination.

1. Pet ether is added to material in small test tube.
2. Decant a portion of pet ether into additional test tube.
3. Evaporate pet ether with a stream of air.
4. Add Duquenois Reagent to extract.
5. Add an equal amount of concentrated hydrochloric acid and shake test tube to mix.

If MARIHUANA is present, a deep purple colored complex will result. The purple color will form if any phenolic compound with an alkyl side chain is present, e.g. resorcinol.

6. Add chloroform to test tube.

If purple complex enters chloroform (is soluble in), the ENTIRE test is positive for the presence of MARIHUANA. This is the crucial part of the test sequence. If the purple color does not enter the chloroform, the test is considered NEGATIVE.

C. Thin Layer Chromatography (TLC)

1. Pet ehter remaining in test tube with material from chemical test is spotted upon two appropriately labeled silica gel (5X10 or 5X20) thin layer chromatography plates.

2. A MARIHUANA standard is spotted on each plate.

3. Plates are then eluted in two of the following solvent systems:

   a. Hexane-diethylether 4:1
   b. Benzene
   c. Chloroform
   d. Petroleum ehter-ethanol 95:5 (use if suspect PCP)

4. Plates are visualized by spraying with a fast blue BB salt solution. Solution should be a pale yellow and prepared fresh.

   Separated cannabinoid components of extract will form a characteristic color.

   e.g. for Hexane/diethyl ether 4:1 Benzene

cannabidiol (CBD)	orange	CBD	orange
tetrahydrocannabinol (THC)	scarlet	CBN	purple
cannabinol (CBN)	purple	THC	scarlet
	lt. red		

5. Visualized spots are compared to MARIHUANA standard. If scarlet spot for THC is present, the test is considered POSITIVE.

This three part test sequence is utilized to confirm the presence of MARIHUANA because each of the tests independently supports each other.

e.g. Although other plant material may exhibit cystolytic hairs, none of these plants will provide a positive Duquenois-Levine test or a positive TLC plate.

D. Germination of Seeds

1. Place seeds in moist chamber.

2. Seeds should germinate in two to three days.

3. Seeds are considered fertile if greater than 10% sprout.

4. Estimate what percentage sprouted.

DUQUENOIS REAGENT

1. Dissolve 5 drops of Acetaldehyde and 0.4 grams vanillin in 20 ml. of 95% alcohol

or) 1.5 ml. of acetaldehyde and 12 grams of vanillin in 600 ml. of 95% alcohol

or)

2. Dissolve 12 drops acetaldehyde (fresh) and 1 g. vanillin in 50 ml. alcohol.

Ethanol

AOAC Methods of Analysis, 11th Ed. 1970, pg. 705

IRS Manual, pg. 136

FAST BLUE 2B

Visualizing Reagent for Marihuana TLC Plates

0.4% Solvent

Mix 0.1 g of FAST BLUE 2B salt in 25 ml. of distilled water.

NOTE: If kept refrigerated and covered, this reagent can be used for up to 1 week.

II. REQUIREMENTS FOR SPECIFIC SAMPLES

1. Plant material

   Steps A through C

2. Seeds

   Steps A through D

   When sample is predominantly or all seeds, after the initial microscopic examination, one of the following procedures must be covered:

   a. Perform Duquenois and TLC on extract of seeds themselves.

   b. Perform Duquenois and TLC on extract of germiniated seedlings.

3. Hash

   Steps A through C. Microscopic examination will involve looking for crushed plant hairs.

III. CONCLUSIONS

The sample will be scheduled as "MARIHUANA or the resin thereof" is the following conditions are met:

1. Plant Material

   Microscopic, modified Duquenois and TLC tests must all be positive for marihuana as previously outlined.

2. Seeds

   Seeds must germinate to be controlled. Additionally, microscopic examination, modified Duquenois (on seeds or seedlings) and TLC (on seeds or seedlings) must all be positive.

3. Hash

   Microscopic, modified Duquenois and TLC tests must all be positive for marihuana as previously outlined.

STANDARD METHODS OF ANALYSIS FOR HEROIN, OPIUM ALKALOIDS & SYNTHETIC OPIATES

I. Spot Tests

Marquis: Purple reaction indicates probable presence of an opium alkaloid.

II. TLC

Plates: Silica gel, may have fluorescent indicator.

System: a) MeOH and NH_4OH (conc.) 100:1.5.
b) H_2O sat. ethyl ether/acetone/diethylamine 85:8:7. (This system will separate heroin, acetylcodeine, codeine, morphine and monoacetylmorphine).

Visualization: Short wave UV: With fluorescent indicator plates, the plate will fluoresce green and spots will remain dark due to quenching.
Acid iodoplatinate: Opium alkaloids and opiates will react, turning various shades of brown through purple.

Results: Always run standard(s) of suspected substance(s) on same plate as samples. Samples should be consistent with standard(s) in both retention distance and color reaction to iodoplatinate.

Special TLC system: Toxi-Lab: Follow manufacturers' instructions for this procedure.

III. GC

Columns: Any column which provides adequate separation may be used. Those listed below have all been successfully used to identify opium alkaloids and synthetic opiates: OV-1, SE-30, OV-101, DB-1, OV-17, DB-17, SPB-5.

Results: Adjust column temperatures and/or carrier gas flow rate such that analyte of interest has a retention time of at least two minutes. Under identical conditions, sample and standard should have the same or very nearly the same retention time.

IV. IR

Sample cleanup: Use the appropriate method below.

a) Dry extract powdered sample with $CHCl_3$ or CH_2Cl_2. Sample may be recrystallized with solvents such as heptane or petroleum ether, or dried directly onto KBr. This method works with single-ingredient tablets and some powders.

b) Acid/base extraction. Heroin and some other members of this drug class may be extracted from aqueous solution either as bases or as ion pairs (from HCl solution). After extraction, either recrystallize with heptane or petroleum ether, or dry directly onto KBr.
Warning: Heroin and other drugs with ester linkages will often be hydrolyzed if extracted from strong basic solution. Use NH_4OH, Na_2CO_3, or $NaHCO_3$ to avoid this. This will also prevent amphoteric drugs such as heroin from going back into aqueous solution at high pH's.

c) "Panning" technique: See enclosure. This method works well with brown tar heroin. It may be done quite effectively on a smaller scale in test tubes instead of the separatory glassware described. Once the heroin base has been extracted into chloroform in step 4 (either Na_2CO_3 or $NaHCO_3$ may be used in this step), it may be dried down onto KBr, recrystallized with non-polar solvents, or converted to the HCl salt and recrystallized.

d) Celite column - this may be done in standard column chromatography apparatus or on a micro scale in a Pasteur pipet.

Standard procedure: Dissolve sample in 0.1N HCl. Mix with appropriate amount of Celite until fluffy, and pack into column. Elute with $CHCl_3$, preferably water-washed $CHCl_3$. (The column may also be eluted first with water-washed ethyl ether to obtain a cleaner product.) Dry $CHCl_3$ eluate through Sodium Sulfate column. Sample may now be dried onto KBr or recrystallized using heptane or petroleum ether.

Alternate procedure: (Preferred for brown tar heroin as it is faster.) Mix appropriate amount of Celite with 0.1N HCl until fluffy and pack into column. Dissolve sample in a few drops of $CHCl_3$ and place at top of column. Do not use so much $CHCl_3$ that sample begins to elute from column. Continue with standard procedure.

Results: Obtain IR scan of sample. Compare to a standard you have run by same procedure or to well accepted reference scan such as that from Sadtler, Aldrich, or Georgia State Crime Lab. Note: Some opium alkaloids will exhibit polymorphism, which will affect the ability to make a match to reference spectra.

V. GC-MS

Run both sample and standard of suspected material. Sample and standard should be consistent in GC retention time. Mass spectra of sample and standard should show major peaks at same mass-to-charge values with similar peak height ratios.

I. Narcotic class

A. Heroin

B. Codiene

C. Morphine

D. Dihydromorphine

II. Color tests (refer to laboratory analytical manual - spot tests)

A. Marquis: dark red-violet----vlue-violet.

B. Lieberman's: greenish-brown----dark brown.

C. Mecke's: green

D. Froehde's: purple----greenish.

E. Mayer's: ----white precipitate.

F. Concentrated Nitric Acid: yellow----green (Intensity of green color seems to be at least somewhat indicative of the quality of the heroin; the more intense the green, the better the heroin. Poor quality material will not turn green at all.)

G. Compare to known reactions of known substances.

III. Ultraviolet spectrophotometry

A. Place portion of sample in 0.02NHCl (refer to laboratory analytical manual - UV section).

B. Run UV with solvent reference cell.

C. Examine chart - note peak(s).

D. Compare to known chart, reference material or known substance.

Note: May have to do basic aqueous into solvent extracts ($CHCl_3$) for cleanup and then push into 0.02NHCl.

IV. Infrared spectrophotometry.

A. Prepare KBr window - neat sample.

B. Run IR.

C. Compare to known library reference chart or known substance.

Note: With Mexican "tar" heroin, may have to do solvent extracts through a celite column with chloroform, methanol, acetone, ether, etc. (acetone seems to work the best) for a cleanup before IR.

V. Gas liquid chromatography

A. Extract sample with methanol.

B. Inject GC

1. Isothermal OV-17 or OV-1 at 250°C; SE-30 at 250°C.

2. Programmed OV-17 or OV-1 at 180-250°C; SE-30 at 180-250°C.

C. Examine chart - note peak(s).

D. Compare to known chart, reference material or known substance under some conditions.

Note: May have to use celite column cleanup for some form.

VI. Microcrystaline

A. Gold chloride (refer to laboratory analytical manual).

1. Place suspect powder in wells on crystal slides.

2. Add reagent.

3. Examine crystals.

4. Compare to known crystals prepared in same manner.

Note: May have to use 0.02NHCl liquid form to enhance crystals.

B. Mycrocrystaline (additional)

1. Refer to main file on microcrystals

(A) Mercuric chloride

EXTRACTION OF HEROIN BY A "PANNING" TECHNIQUE

The procedure outlined below will afford reasonably pure white to off-white heroin base from "brown tar" materials. This extraction method offers several advantages over other methods in current use:

1. A minimum of materials are required (separation funnel, two test tubes, watch glass)
2. The extraction procedure is quick, taking less than 15 minutes to accomplish, and
3. It yields high quality heroin base free from colored impurities (meconin, meconic acid derivatives, etc.) and other adulterants, such as procaine.

The infrared spectra of extracted material are generally excellent.

Methodology

1. Dissolve 3 mg - 100 mg of "brown tar" in about 3-5 ml of water containing a little HCl (10% HCl works fine - Ed.). Filter through cotton.
2. Extract with about 5 ml of chloroform in a 30 ml separation funnel. Discard the aqueous layer, and rinse out the separation funnel with water.
3. Back extract the chloroform layer from step 2 twice with 3-5 ml of plain water. Discard the chloroform layer after the second wash, saving BOTH water washes. Rinse out the separation funnel (many of the highly colored impurities present in "tar"heroin are left behind in the choroform in this step.)
4. Make the aqueous extract basic with sodium bicarbonate, extract with about 5 ml of chloroform and evaporate the chloroform on a watch glass.
5. The residue on the watch glass is generally pure enough to yield a useable IR spectrum or definitive crystal test as is.

If pure crystalline material is desired, add a couple of milliliters of 35-60 degree petroleum ether containing 10-20% methylene chloride. The methylene chloride will dissolve the residue on the glass and evaporate first; from this heroin can be fractionally crystallized from any materials which may have "carried" along with the extraction.

HEROIN EXTRACTION FROM BLACK TAR

50 mg. of sample.

Dissolve in 1 - 2 ml. of .1N HCl.

Mix 4 grams of celite with 3 mls. of .1N HCl.

Pack column.

Run 50 ml. of $CHCl_3$ through column, collect in beaker.

Boil down.

Run through $NaSO_4$ column.

Recrystalize with Heptane.

HEROIN CELITE COLUMN

Mix 3 grams of Celite 545 and 2 ml. of 0.1N hydrochloric acid in a small beaker. Transfer to a chromatographic column having a pledget of glass wool at the base and tamp. Place enough glass wool on top of the packing to hold in place. Pass Chloroform through the column and collect the eluate.

HEROIN EXTRATION FROM "BLACK TAR HEROIN"

Reagents:

- 0.1N HCl Solution
- Hexane or Cyclohexane
- $CHCl_3$

Procedure -

Place 25-50 milligrams of the sample in a test tube or separatory funnel. Add 2 milliters of 0.1N HCl and shake to mix. The sample should begin to disintegrate and dissolve. Add 2 milliters of $CHCl_3$ and mix the two layers well. Let separate. Remove the lower organic layer and transfer it to an evaporation dish. Dry the extract down to a spot about the size of a quarter. Add hexane or cyclohexane dropwise to the sides of the dish above the dried extract in a circular fashion. Approximately 6-8 drops should suffice. Scratch the dried extract in the center of the dish to break it up in the (cyclo)hexane. Light brown crystals will begin to form in the center as the solvent evaporates. Use these crystals for IR analysis, either grinding with KBr powder for use in a pellet or applying a solution to a KBr crystal and evaporating.

SEPARATION OF HEROIN FROM ACETYLCODEINE

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OBJECTIVE: To provide a method to isolate small quantities of heroin from large amounts of acetylcodeine and other drugs and contaminants in order to obtain an infrared identification.

BACKGROUND: This Laboratory received a sample of brown powder suspected to contain heroin. Routine screening, including TLC in two solvent systems (T_1 and Davidow), indicated the presence of heroin, morphine, monoacetylmorphine, codeine, aspirin and salicylic acid. Extraction for heroin, however, did not produce the expected IR. GC analysis [3% OV-1, 120° (2 min) - 300° C, 10° C/min] of this extract revealed the presence of two compounds which had not been resolved by either TLC system used. These compounds appeared to be heroin and acetylcodeine. The following method was then developed to separate these two compounds for IR analysis.

MATERIALS:

1. TLC Plates - Merck Pre-coated Glass Silica Gel 60 F-254
   Layer thickness 0.25 mm

2. Solvent Systems - (A) T_1 - MeOH:NH_4OH (100:1.5)
   (B) Davidow - Ethyl Acetate:MeOH:NH_4OH (85:15:5)
   (C) Diethylether:Diethylamine (9:1) */

3. Chemicals and Solvents -

0.4N H_2SO_4	$CHCl_3$ (Fisher Scientific HPLC Grade)
4N NaOH or NH_4OH	Hexane (Fisher Scientific HPLC Grade)
2.8N HCl	Diethylether (Mallinkrodt AR)
$NaHCO_3$ (Saturated solution)	Diethylamine Free Base (Sigma)

PROCEDURE:

A. EXTRACTION - An initial cleanup of the brown powder is performed using the following procedure:

1. Dissolve a 50 mg sample in 3-5 ml of 0.4N H_2SO_4.
2. Extract with 6 - (3-5 ml) portions of $CHCl_3$. Discard the $CHCl_3$ (contains acidic drugs - ex: Salicylic acid, and neutral drugs - ex: caffeine).
3. Make the aqueous layer basic (pH 10-11) with 3-4 drops of 4N NaOH (or NH_4OH).

---

*/ Mix solvent freshly; can use immediately. Solvent system was adapted from Ref. 1.

4. Extract with 3 - (2 ml) portions of diethylether.

5. Remove the ether layer to a clean test tube, back extract into 2-3 ml of 2.8N HCl (ether layer turns cloudy initially). Vortex well to mix.

6. Discard the ether layer.
Extract the aqueous layer with 3 - (1-2 ml) portions of $CHCl_3$ (pass the $CHCl_3$ thru a pipette with a cotton plug and sodium sulfate).

7. Evaporate the $CHCl_3$ in a small mortar. (Do not apply heat!)

8. Dry in a dessicator (normally, if the sample is clean or the acetylcodeine concentration is very low, the IR can now be run as the HCl salt).

B. TLC PREP - The extracted sample is further treated using solvent system (C) in order to separate acetylcodeine from heroin.

1. Streak the extracted sample across a 5 or 10 cm wide plate, approximately 2 cm from the bottom of the plate.

2. Develop for 10 cm in solvent system (C). Remove the plate, air dry and visualize under shortwave UV.

	Approximate Rfs
Acetylcodeine	.7
Heroin	.6
Monoacetylmorphine	.5

3. Scrape the silica gel-heroin streak from the plate and place in a small test tube.

4. Add 2 ml of a saturated $NaHCO_3$ solution to the test tube and vortex well.

5. Extract 3X with a $CHCl_3$/hexane mixed solvent**/.
Centrifuge after each extraction.

6. Evaporate the $CHCl_3$/hexane layer in a small mortar.

7. Add KBr to the residue in the mortar, grind well and prepare an IR pellet using a 3 or 5 mm die.

8. The acetylcodeine streak may also be scraped from the TLC plate and extracted repeating steps 4 thru 7, above.

**/ $CHCl_3$/hexane mixed solvent (Ref. 2.), approximately a 1:2 ratio. In a 25 ml graduated cylinder with ground glass stopper, add 5 ml distilled water and approximately 6 ml $CHCl_3$. Add hexane until the organic mixture floats on the water layer (approximately 10-12 ml). The solvent is stable and does not need to be freshly prepared.

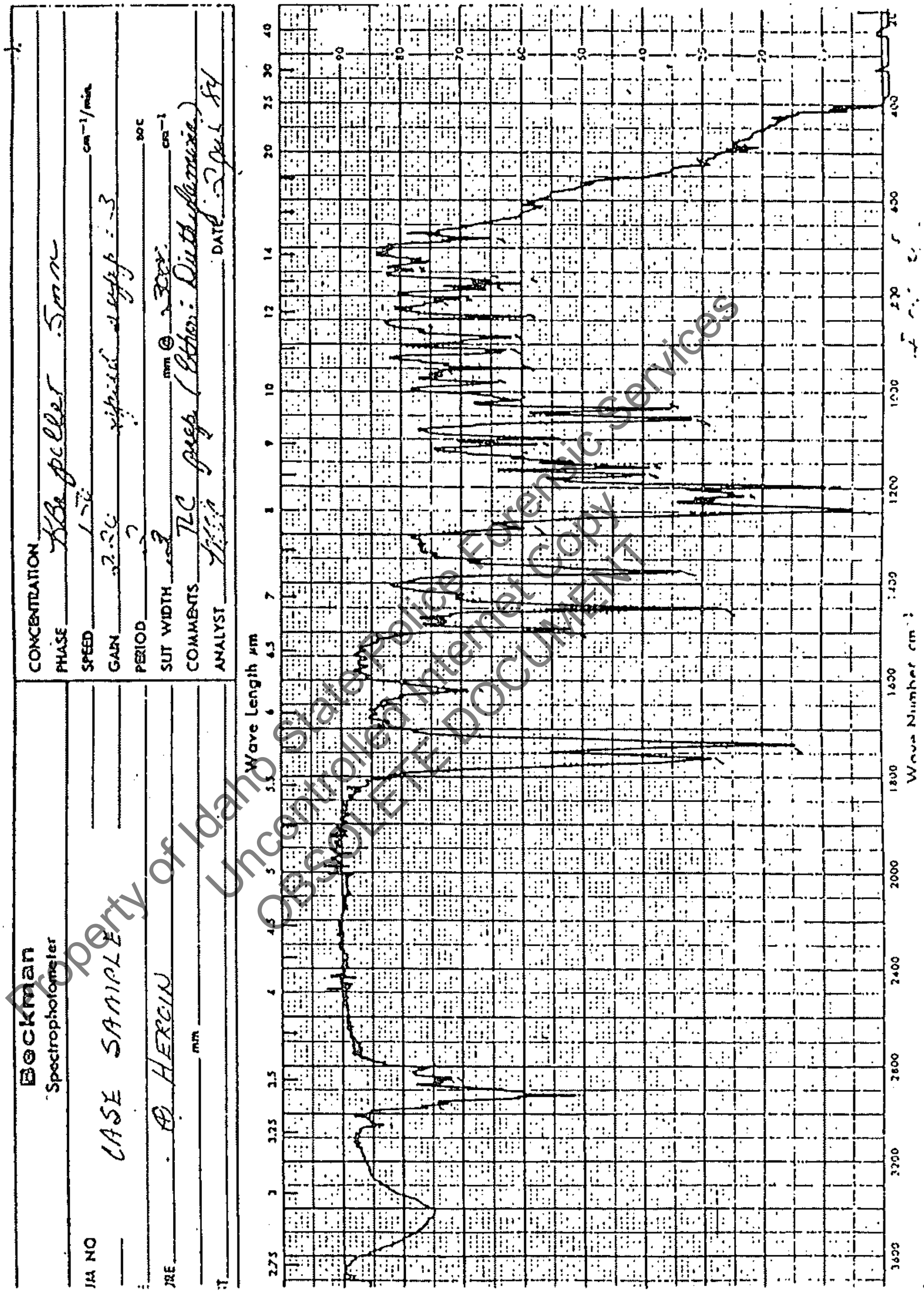
DISCUSSION: In a sample powder containing many other compounds, the initial extraction procedure is necessary prior to TLC prep. This eliminates unwanted streaking of the sample on the prep plate and ensures much better separation of heroin and acetylcodeine. This is especially important when the heroin concentration is extremely low in comparison to the other compounds present.

The extraction volumes are kept to a minimum in order to minimize introducing IR interfering impurities from the solvents. This becomes important when working with IR pellets of 3 mm and 5 mm sizes.

REFERENCES:

1. Delmar, Peter, MICROGRAM, Vol. IV, No. 11, pp. 147-148, Dec. 1971
2. Jacobs, James L., NISRFL-SD, personal communication.

Maureen E. O'Connor
Aug 1984
Revised: Mar 1985



HEROIN
IR PROCEDURES

Especially for small amounts

Dissolve in water

↓

make basic
Use NH_4OH/Na_2CO_3 if procaine is present.

↓

Extract w/ pet ether

↓

Na_2SO_4
(or just a cotton filled pipet)

↓

Don't let sit or it will super-sat. and ppt out in column- push liquid out with bulb

↓

Boil down
Let sit & slowly recrystallize

Or:

Dissolve in CH_2Cl_2

↓

Run through Alumina column*
Wet w/CH_2Cl_2 first

↓

Rinse column w/CH_2Cl_2

↓

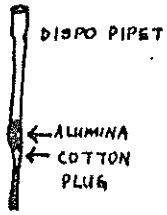
Evaporate-reduce volume

↓

Recrystallize w/pet ether

↓

Yields the base



*The alumina will absorb part of the heroin that passes through it which is why this technique is less effective for small amounts.

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ANALYSIS OF ILLICIT HEROIN

I. AN EFFECTIVE THIN-LAYER CHROMATOGRAPHIC SYSTEM FOR SEPARATING EIGHT OPIATES AND FIVE ADULTERANTS

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(First received June 4th, 1985; revised manuscript received May 12th, 1986)

SUMMARY

A thin-layer chromatographic (TLC) system with chloroform–*n*-hexane–triethylamine (9:9:4) as eluent that is capable of separating eight opiates and five potential adulterants, has been developed for the analysis of illicit heroin. The system was tested using illicit heroin samples and the results were confirmed by gas chromatography. The limit of detection is 0.1 μg. Thirty-five TLC systems reported in the literature for opiate analysis were classified according to their resolving power.

INTRODUCTION

Heroin (diacetylmorphine, DAM) is prepared by the diacetylation of morphine, an alkaloid of opium. Depending on the purity of the starting material (morphine base *vs.* raw opium) and the manufacturing process involved, the heroin produced may contain some other opium alkaloids such as paraverine, thebaine, noscapine and codeine (natural alkaloids) or acetylcodeine and 6-monoacetylmorphine (synthetic alkaloids resulting from the acetylation step). In addition to these eight opiates, heroin at street level could contain other interfering substances such as methadone, cocaine, caffeine, ephedrine and quinine. Thus information relating to the source, trafficking and distribution patterns of the illicit heroin trade could be deduced from a detailed analysis of the product.

Thin-layer chromatography (TLC) is the most popular screening method for opiates[1–15]. However, an assessment of published solvent systems carried out in this laboratory demonstrated their unsuitability for simultaneously identifying the above eight opiates and five adulterants as they were designed specifically to identify a selected number of opiates and adulterants.

In this paper we report a TLC solvent system that is capable of separating the above 13 components. The TLC system was tested on samples of illicit heroin and the results were confirmed by gas chromatography (GC). The GC data was used for grouping the samples according to their chemical composition.

0021-9673/86/$03.50

EXPERIMENTAL

The solvents used were of analytical-reagent grade and were not purified further. Opiates and other chemical standards were obtained from the United Nations Division on Narcotic Drugs, Vienna. Caffeine was purchased locally. Samples for TLC and GC were prepared in chloroform–methanol (9:1) solution.

TLC was performed on Merck pre-coated plates (20 × 20 cm, aluminium backed; silica gel 60 GF_{254}, 0.2 mm thickness) and developed in chloroform–*n*-hexane–triethylamine (9:9:4). The developed plates were examined under UV light (254 and 366 nm) and treated with spray reagents.

The GC analysis was carried out on a Hewlett-Packard HP 5880A gas chromatograph equipped with a flame ionization detector. The glass column (6 ft. × 0.2 mm I.D.) was packed with 3% OV-210 (unsilanized) on 100–120-mesh Chromosorb W. The column oven was temperature programmed from 190°C (15 min) to 270°C (5 min) at 8°C min^{-1}. The carrier gas was nitrogen (25 ml min^{-1}). The injector and detector temperatures were 270 and 300°C, respectively. Prior to GC analysis, the column was conditioned overnight at 270°C and 45 ml min^{-1}.

Two batches of illicit heroin samples were analysed. The first batch of four samples (A–D) was seized overseas whereas the second batch of 21 samples (Nos. 1–21) was seized locally (Malaysia).

RESULTS AND DISCUSSION

The chemical composition of a sample of street heroin can usually be classified into four groups:

(i) natural opium alkaloids (*e.g.*, morphine, codeine, noscapine, papaverine and thebaine), some of which may pass unchanged through the extraction, acetylation and purification procedure;

(ii) synthetic opium alkaloids [*e.g.*, 6-monoacetylmorphine (MAM), diacetylmorphine and acetylcodeine];

(iii) diluents (*e.g.*, caffeine, quinine, sugar and talc), which are added for bulk; and

(iv) adulterants (*e.g.*, methadone, cocaine and ephedrine), which produce added pharmacological effects.

The chemical composition of heroin intercepted in the illicit drug traffic and at consumer level varies substantially, depending on factors such as the geographical source, the manufacturing process used (extraction, acetylation and purification) and the distribution pattern (international and local). Thus a detailed chemical analyses of a sufficient number of samples would contribute towards providing data relating to their origin and distribution pattern. As a preliminary analytical method, a TLC system that is capable of resolving the components of an illicit heroin sample would contribute towards monitoring illicit opiate traffic.

The TLC systems reported in literature were evaluated for their suitability for analysing illicit heroin and are listed in Table I. These systems were assessed using a mixture of the eight opiates and five adulterants and were classified according to their ability to resolve the major opiates (without interference from the other components of the standard mixture) in the following manner: (i) urine (morphine and

TABLE I

ASSESSMENT OF PUBLISHED TLC SYSTEMS FOR OPIATE ANALYSIS

Abbreviations: Acet = acetone; amyOH = amyl alcohol; Bz = benzene; $CHCl_3$ = chloroform; cycHex = cyclohexane; Diox = dioxane; DEA = diethylamine; EtOAc = ethyl acetate; Et_2O = diethyl ether; EtOH = ethanol; H_2O = water; iPrOH = isopropanol; MeOH = methanol; MeCN = acetonitrile; nBu_2O = *n*-butyl ether; NH_3 = ammonia solution; nHex = *n*-hexane; Pyr = pyridine; t-amyDH = *tert.*-amyl alcohol; Tol = toluene. Numbers: 1 = morphine; 2 = 6-monoacetylmorphine; 3 = diacetylmorphine; 4 = codeine; 5 = acetylcodeine; 6 = noscapine; 7 = papaverine; 8 = thebaine; 9 = ephedrine; 10 = quinine; 11 = methadone; 12 = caffeine; 13 = cocaine.

Ref.	*Solvent system*	*Opiate and adulterants*	*Interfering substances**	*Category***
1	EtOAc–cycHex–Diox–MeOH–H_2O–NH_3 (50:50:10:10:1.5:0.5)	1, 2, 4, 6, 8 3 7	None 5, 12 13	(i)
	EtOAc–cycHex–MeOH–NH_3 (70:15:10:5)	1–5, 7, 8 6	None 11, 13	(i)
	EtOAc–cycHex–Diox–MeOH–H_2O–NH_3 (50:50:10:10:0.5:1.5)	1, 6, 7	2–5, 8, 9 None	–
	EtOAc–cycHex–NH_3–MeOH–H_2O (70:15:2:8:0.5)	1, 2, 4–7 3	None 8, 12	(i)
2	Bz–Diox–EtOH–NH_3 (50:40:5:5)	1, 4, 7, 8 2 3 6	None 10 5 13	(i)
	MeOH–NH_3 (100:1.5)	1 2 6	4 3, 5, 8, 11 7	–
	EtOH–HOAc–H_2O (6:3:1)	1 2, 4 5 6	3, 12 None 8 7, 10	–
3	Diox–$CHCl_3$–EtOAc–NH_3 (60:25:10:5)	1–5, 7, 8 6	None 13	(i)
	EtOAc–Bz–MeCN–HN_3 (25:30:40:5)	1–4, 6, 7 5	None 8, 12	(i)
	MeCN–$CHCl_3$–EtOAc–NH_3 (40:30:25:5)	1–4, 6, 7 5	None 8	(i)
	EtOAc–Bz–MeCN–NH_3 (50:30:15:5)	1–3, 6, 7 4 5	None 10 8, 12	–

(*Continued on p. 366*)

TABLE I (*continued*)

Ref.	*Solvent system*	*Opiate and adulterants*	*Interfering substances**	*Category***
	$EtOAc–nBu_2O–NH_3$ (60:35:5)	1	4, 10	–
		2	3, 8	
		5	12	
		6, 7	None	
4	$EtOAc–iPrOH–NH_3$ (40:30:3)	1, 4, 5, 6	None	(i)
		2	3, 8, 10	
		7	11	
5	$CHCl_3$–MeOH (9:1)	1	9	–
		2	4, 11	
		3	5	
		6	7	
		8	None	
	$nBu_2O–Et_2O$–DEA (45:45:10)	1, 3, 6	None	–
		2	7	
		4	10	
		5	8	
6	Bz–EtOAc–MeOH–NH_3 (80:20:6.5:0.1)	1	2, 4, 9, 10	–
		3	5, 8	
		6, 7	None	
	nBuOH–HOAc–H_2O (35:3:10)	1	4	–
		2	8, 13	
		3	5	
		6	7	
	nHex–EtOAc–NH_3 (60:40:0.1)	1	2–5, 7–13	–
		6	None	
	$nBuOH–nBu_2O–NH_3$ (25:70:2)	1	4, 9	–
		2	3, 5, 8, 12	
		6, 7	None	
7	EtOAc–MeOH–NH_3 (85:10:5)	1	9	–
		2	3, 8	
		4, 5, 6	None	
		7	11	
8	$CHCl_3$–cycHex–DEA (8:10:3)	1	4, 12	–
		2, 3, 5, 7	None	
		4	12	
		6	8	
	$CHCl_3–EtOH–Acet–NH_3$ (20:20:5:1)	1, 2, 4	None	(i)
		3	5, 8, 12	
		6	7, 13	
	$CHCl_3$–MeOH–DEA (16:3:1)	1	None	–
		2	3, 4	
		5	12, 13	
		6	7, 8, 10, 12	

TABLE I (*continued*)

Ref.	*Solvent system*	*Opiate and adulterants*	*Interfering substances**	*Category***
9	MeOH–nBuOH–Bz–H_2O (60:15:10:15)	1 2 6 8	4 3, 5, 10, 13 7 None	–
	EtOH–Pyr–Diox–H_2O (50:20:25:5)	1 2 3 4 6	11 None 5, 10 8 7	–
	t-amyOH–nBu_2O–H_2O (80:7:13)	1 6, 7	2–5, 8, 13 None	–
10	Tol–Acet–EtOH–NH_3 (20:20:3:1)	1, 4, 6, 7 2 3	None 8, 12 5	(i)
11	$CHCl_3$–Et_2O–MeOH–NH_3 (75:25:5:1)	1 2–5 6 8	9 None 7, 13 11, 12	–
12	nHex–$CHCl_3$–DEA (50:30:7)	1, 3–8 2	None 12	(ii)
13	Tol–Acet–EtOH–DEA (30:60:7:3)	1, 2, 4, 7 3	None 5, 8, 12	(i)
14	Tol–Acet–EtOH–DEA (45:45:7:3)	1, 2, 4, 7 3 6	None 5, 8 11, 13	(i)
	cycHex–Bz–DEA (70:25:10)	1 3, 4 5	2, 10, 12 None 6, 8	–
	$CHCl_3$–Acet (9:1)	1 6 7	2–5, 8–11 None 13	–
15	Tol–Acet–EtOH–NH_3 (40:40:6:2)	1, 4, 7 2 3 6	None 10 5, 8 13	(i)
	Tol–EtOAc–DEA (70:20:10)	1, 4, 6 2 3	None 9, 12 5, 7, 8, 9	(i)

* The components not listed here do not interfere.
** See text for explanation.

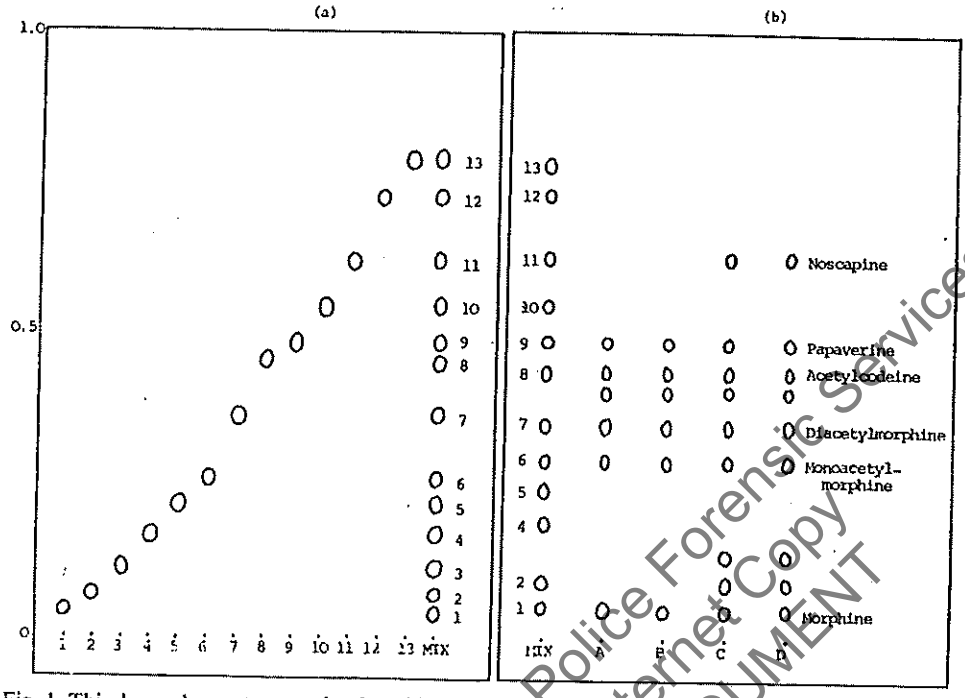

Fig. 1. Thin-layer chromatogram developed in chloroform–*n*-hexane–triethylamine (9:9:4): (a) individual standards and mixture of standards; and (b) seized heroin samples (A–D) and mixture of standards. 1 = Morphine; 2 = quinine; 3 = ephedrine; 4 = codeine; 5 = caffeine; 6 = 6-monoacetylmorphine; 7 = diacetylmorphine; 8 = acetylcodeine; 9 = papaverine; 10 = thebaine; 11 = noscapine; 12 = cocaine; 13 = methadone.

codeine); (ii) opium (morphine, codeine, noscapine, papaverine and thebaine); (iii) unadulterated heroin (the above five opiates, MAM, DAM and acetylcodeine); or (iv) adulterated heroin (the above eight opiates and possibly the five adulterants caffeine, cocaine, methadone, ephedrine and quinine).

Of the 35 systems listed in Table I, only 15 could be classified according to the categories listed, of which 14 were capable of resolving the opiates in urine (codeine and morphine) and only one of resolving the opium alkaloids. The remaining 20 systems could not be classified because of interference from the other components used in the evaluation. None of the reported systems were able to resolve the 13 test components. However, the information provided in Table I can be used for selecting a suitable solvent system if some of the opiates and adulterants are excluded from the analysis of an illicit heroin sample.

Fig. 1a shows the TLC of the eight opiates and five adulterants run individually and in a mixture using the new solvent system, chloroform–*n*-hexane–triethylamine (9:9:4). Table II shows the colours observed with the use of Marquis, acidified iodoplatinate and Dragendorff reagents. As the opiates and adulterants are visible under UV light (254 nm) and quinine is strongly fluorescent (light blue) at 366 nm, all 13 components were first located under UV light and subsequently identified on the basis of their R_F values and the colour produced with the spray reagents. The limit of detection of the individual components in the mixture was 0.1 μg.

TABLE II

COLOUR REACTIONS OF OPIATES AND ADULTERANTS WITH DIFFERENT REAGENTS

Compound	*Iodoplatinate reagent*	*Dragendorff's reagent*	*Marquis reagent*
Morphine	Blue	Orange	Violet
6-Monoacetylmorphine	Dark blue	Orange	Black
Diacetylmorphine	Dark blue	Orange	Black
Codeine	Blue	Red-orange	Dark blue
Acetylcodeine	Purple	Orange	Black
Noscapine	Purple	Orange	Green-black
Papaverine	Purple-brown	Orange	Maroon
Thebaine	Red-brown	Orange	Orange-red
Caffeine	Yellow	No colour	Light brown
Cocaine	Green-black	Pink	No colour
Methadone	Green-brown	Pink	Orange-red
Quinine	Black	Pink	No colour
Ephedrine	Brown	No colour	Yellow-brown

The applicability of this system was demonstrated by the analysis of two batches of illicit heroin samples. Fig. 1b shows the TLC of a mixture of the opiates and adulterants (excluding quinine) and the four overseas illicit samples (A–D) run under the same conditions. The individual components of the samples were identified by comparison with a standard mixture and are listed adjacent to sample D. The TLC system also resolved three other components that currently remain unidentified. None of the five adulterants was present in the illicit samples. The TLC analysis was confirmed by GC using an OV-210 column. Fig. 2a shows the chromatogram of the standard mixture of opiates with caffeine as the adulterant and Fig. 2b is the chromatogram of sample D. The peaks were identified from the retention times of the components of a standard mixture. All the opiates identified by TLC were also observed in GC, with the exception of morphine. Morphine and the three unidentified components were probably adsorbed by the column packing material and therefore eluted slowly.

A qualitative comparison of the chemical composition of the samples (A–D) in Fig. 1b suggests a classification into two groups: A + B and C + D, the latter group containing noscapine and other interfering substances. This qualitative assessment is supported by semi-quantitative GC data based on relative peaks areas. The relative peak-area percentages of the GC opiate peaks in each sample are shown in Table III. The data represent the mean of four analyses per sample and show the similarity in opiate composition between samples A and B or C and D.

The GC and TLC procedures mentioned above were also applied to a batch of 21 heroin samples that were seized locally. The TLC of a representative selection of the samples is shown in Fig. 3a, together with the standard mixture. The analysis indicated that all samples contained acetylcodeine and MAM but only some samples contained DAM. The absence of DAM from some samples could be due to its total breakdown during storage or shipment. None of the samples contained any other unidentifiable components. The TLC analysis was confirmed by GC. Fig. 3b illus-

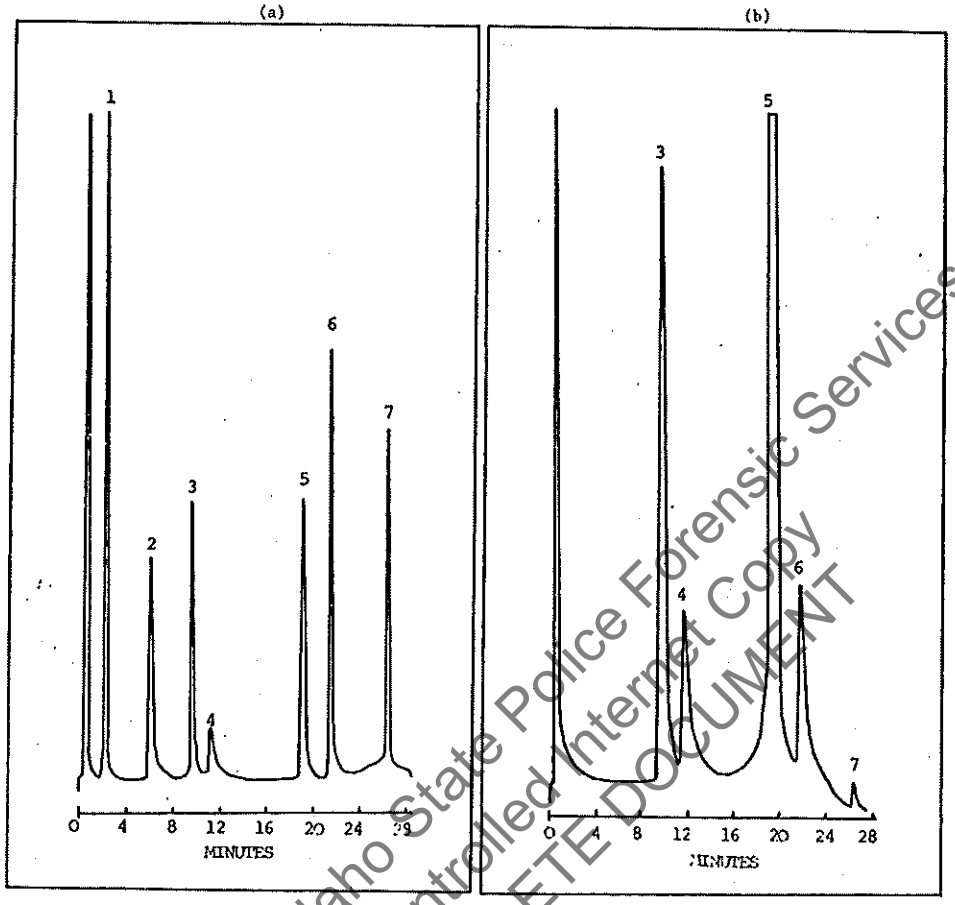

Fig. 2. Gas chromatogram using an OV-210 column: (a) mixture of opiates and caffeine; and (b) seized heroin sample D. See text for conditions. 1 = Caffeine; 2 = codeine; 3 = acetylcodeine; 4 = 6-monoacetylmorphine; 5 = diacetylmorphine; 6 = papaverine; 7 = noscapine.

trates this by showing the gas chromatogram of one of the samples (No. 11). The peaks were identified by comparison with the chromatogram of a standard mixture (Fig. 2a).

Semi-quantitative analysis of the 21 samples based on peak-area percentages

TABLE III

RELATIVE OPIATE COMPOSITION OF SEIZED HEROIN (A–D) BASED ON GC PEAK AREAS

Sample	*Relative percentage*				
	Acetylcodeine	*MAM*	*Heroin*	*Papaverine*	*Noscapine*
A	7.9	2.1	89.3	0.3	—
B	7.7	3.2	88.2	1.0	—
C	7.5	4.2	85.7	1.6	1.8
D	7.3	4.6	84.8	1.5	1.7

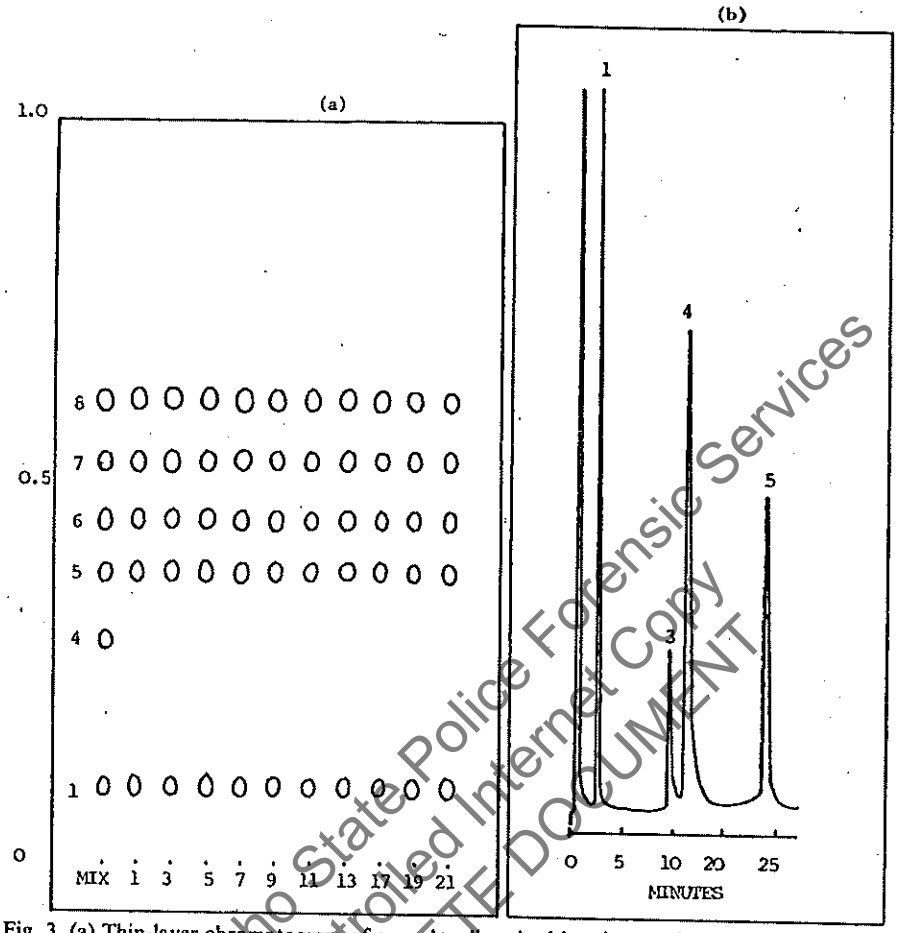

Fig. 3. (a) Thin-layer chromatogram of some locally seized heroin samples. See Fig. 1 for identification. (b) Gas chromatogram of one of the locally seized heroin samples (No. 11). See text for conditions and Fig. 2 for identification.

TABLE IV

GROUPING OF ILLICIT HEROIN SAMPLES BASED ON GC ANALYSIS

Values given are relative peak-area percentages.

Set	*Group*	*No. of samples*	*Caffeine*	*Acetylcodeine*	*MAM*	*DAM*	*MAM + DAM*
A	1	6	23.9 ± 1.5	7.9 ± 1.7	t	68.8 ± 2.7	–
	2	3	21.3 ± 2.3	15.0 ± 1.5	t	63.7 ± 0.9	–
	3	2	59.1 ± 2.1	6.6 ± 0.7	34.4 ± 1.5	ND*	–
B	4	2	32.3 ± 0.8	8.9 ± 0.1	–	–	58.8 ± 0.7
	5	2	37.3 ± 1.0	8.5 ± 1.5	–	–	54.3 ± 0.6
	6	2	42.9 ± 0.2	8.0 ± 2.0	–	–	49.1 ± 1.2
C	7	2	–	11.4 ± 0.9	t	88.6 ± 0.9	–

* ND = Not detected.

permitted the classification of 19 samples into seven groups. Two samples could not be classified because of their widely differing compositions. The seven groups shown in Table IV are divided into three sets according to the nature of the comparison. In set A the samples were grouped on the basis of their relative (and individual) opiate and caffeine compositions. The samples in set B could not be classified in a similar manner because of the varying amounts of MAM and DAM. However, if it is assumed that MAM originated from the breakdown of DAM, then the samples can be grouped according to their combined DAM and MAM composition. In set C the two samples have similar opiate compositions without any adulterant.

A comparison of the overseas and local samples (Figs. 1b and 3a) shows the former to contain morphine, DAM, MAM, acetylcodeine, noscapine, (in some) papaverine and other unidentified components. In contrast, however, the local samples contained fewer opiates and only caffeine as the adulterant. The absence of noscapine and papaverine from the latter suggests that the extraction procedures utilized in the illicit preparation were more effective. Caffeine was added subsequently, probably at the source of manufacture.

ACKNOWLEDGEMENTS

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REFERENCES

1 K. K. Kaistha and J. H. Jaffe, *J. Pharm. Sci.*, 61 (1972) 679.
2 E. G. C. Clark, *Isolation and Identification of Drugs*, Vol. 1, Pharmaceutical Press, London, 1978.
3 J. A. Steele, *J. Chromatogr.*, 19 (1965) 300.
4 A. A. Masoud, *J. Pharm. Sci.*, 65 (1976) 1584.
5 C. C. Clark, *J. Forensic Sci.*, 21 (1976) 418.
6 A. L. Misra, R. J. Potani and S. J. Mulé, *J. Chromatogr.*, 71 (1972) 554.
7 K. G. Blass, R. J. Thibert and T. F. Draisey, *J. Chromatogr.*, 95 (1974) 75.
8 T. A. Gough and P. B. Baker, *J. Chromatogr. Sci.*, 20 (1982) 289.
9 S. J. Mulé, *J. Chromatogr.*, 39 (1969) 302.
10 B. F. Engelke and P. G. Vincent, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 538.
11 R. A. Van Welsum, *J. Chromatogr.*, 78 (1973) 237.
12 R. D. Budd, *Clin. Toxicol.*, 16 (1980) 61.
13 H. Huizer, *J. Forensic Sci.*, 28 (1982) 32.
14 F. Mari, E. Bertol and M. Tosti, *Bull. Narcot.*, 34 (1982) 37.
15 H. Wagner, S. Bladt and E. M. Zgainski, *Drogen Analyse*, Springer, Berlin, 1984.

REFERENCES

Pharmacognosy, 7th Ed., V.E. Tyler, et al, Lea & Febiger, Phil., Penn., 1976.

Identification Procedures of Drugs of Abuse, N.H. Choulis, European Press, Ghent, Belgium, 1977.

A Textbook of Pharmaceutical Analysis, 3rd Ed., K.A. Connors, John Wiley & Sons, New York, 1982.

Handbook of Spectrophotometric Data of Drugs, I. Sunshine, Ed., CRC Press, Inc., Boca Raton, Fla., 1981.

Drug Fate and Metabolism, Vol 1 to 4, E.A. Garrett and J.L. Hirtz, Eds., Marcel Dekker, Inc., New York, 1983.

Handbook of Chromatography, Vol 1 and 2, R.N. Gupta, Ed., CRC Press, Inc., Boca Raton, Fla., 1981.

GLC and HPLC Determination of Theurapeutic Agents, Parts 1 to 3, k. Tsugi, Ed., Marcel Dekker, Inc., New York, 1979.

Analysis of Drugs and Metabolites by GC-MS, Vol. 1 to 5, B.J. Gudzinowicz and M.J. Guczinowicz, Marcel Dekker, Inc., New York, 1978.

Instrumental Data for Drug Analysis, Vol. 1 and 2, T. Mills III, et al, Elsevier, New York, 1984.

Analysis of Drugs, Analytical Manual, S.P. Sobol and R.A. Moore, U.S. Government Printing Office, Washington, D.C., 1974.

Clarke's Isolation and Identification of Drugs, 2nd Ed., A.C. Moffat, Ed., The Pharmaceutical Press, London, 1986.

Handbook of Analytical Toxicology, I. Sunshine, Ed., CRC Press, Cleveland, 1969.

Toxicology, Vol. 2, C.P. Stewart and A. Stolman, Eds., Academic Press, New York, 1961.

Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, et al, Eds., Mach Printing Co., Easton, Penn, 1980.

Clinical Laboratory Science, Section B: Toxicology, I. Sunshine, Ed., CRC Press, W. Palm Beach, Fla., 1978.

Methods of Analysis of the A.O.A.C., 12th Ed., W. Horwitz, Ed., A.O.A.C., Washington, D.C., 1975.

Spot Tests in Organic Analysis, F. Feigl, Elsevier Pub., Amsterdam, 1966.

Modern Microcrystal Tests for Drugs, C.C. Fulton, Wiley-Interscience, New York, 1969.

Gas Chromatographic Retention Indices of Toxicologically Relevant Substances on SE-30 or OV-1, 2nd Ed., R. Ardrey, Ed., V.C.H. Publishers, Weinheim, Germany, 1985.

Drug Analysis by Gas Chromatography, D.B. Jack, Academic Press, Orlando, Fla., 1984.

Phenethylamines, RCMP Understudy Training, G.J. Kupferschmidt, Government of Canada Document, 1980.

Micro Color Reaction Manual, RCMP Understudy Training, G.J. Kupferschmidt, Government of Canada Document, 1980.

AMPHETAMINE AND METHAMPHETAMINE

I. Spot Tests

Marquis quick orange to brown, plus water lime green. Strong bluish green fluorescence. Secondary amines - quick deep blue color for secondary amines such as methamphetamine.

II. TLC *Run standards of each suspected compounds*

1. 1.5 mls NH_4OH (Conc.)/100 mls.
2. $CHCl_3$, 50/Cyclohexane, 40/Diethylamine, 10 (Separates methamphetamine from ephedrine.

Visualize: Fluorescamine-primary amines such as amphetamine, fluoresce lime green while secondary amines, such as methamphetamine, appear as dark spots under long wave U.V. (enclosure).

Iodoplatinate-

3. Toxi-Lab TLC

III. GC *Run standards of each suspected compound*

Apiezon L & 2% KOH
OV 1, SE 30, OV 101, & DB 1
OV 17 & DB 17

Can inject sample plus acetic anhydride diluted in a solvent such as ethyl acetate into GLC amphetamine and methamphetamine derivative on column. The retention time of the derivative is different from the underivatized compound. The derivatives tail less.

IV. IR (With methamphetamine can end up with scans in between d Methamphetamine HCl and dl Methamphetamine HCl) *d Meth HCl and dl Meth HCl have slightly different IR scans. dl Meth HCl may look like d Meth HCl sometimes.*

Amphetamine:
Mandelate salt (enclosure) excellent for amphetamine.
Amphetamine or Methamphetamine.

1. Lumpology (run IR on shiny crystals or lumps of material).
2. Acid-base shake out.
   (a) CH_2Cl_2 (enclosure).
   (b) Pet Ether (Various procedures enclosed - one procedure separates amphetamine and methamphetamine from ephedrine and caffeine).

Methamphetamine only

1. Dissolved in CH_2Cl_2, filter, recrystallize with pet ether.
2. PIT derivative (see enclosure)
   Only works on dl Methamphetamine. Do gold chloride in H_3PO_4 microcrystalline test first.
3. Celite column. *(enclosure) Either run your own standard for purposes of comparison or use a very well accepted standard for comparison such as the Georgia state crime Lab FT-IR file.*

V. GC-MS (if available)

VI. Microcrystalline tests

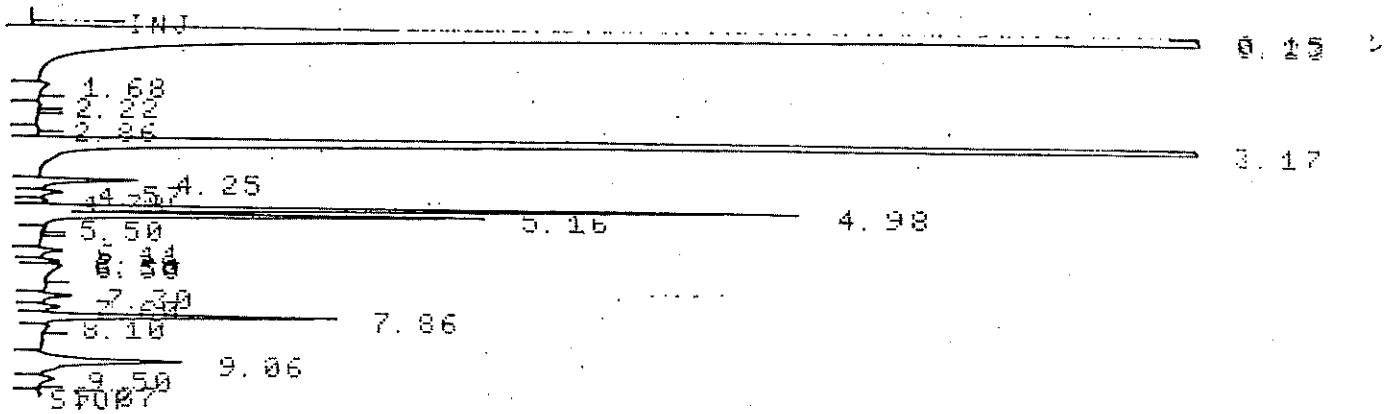
Amphetamine: Volatility tests (enclosure)

1. Gold chloride in H_3PO_4 (swords & stars)
2. Platinic chloride (chicken tracks)

Methamphetamine: Direct test

1. Gold chloride in H_3PO_4 (enclosure) (tricky)
   Run standards until you are very familiar with results.

VII. Quantitate
Preferably on Apiezon L & 2% KOH. Either internal standard method or external standard method. Only report as approximate value - do not require quality control.



AREA %

RT	TYPE	AREA	
.15		378057	7.607
.23	*M	4991343	82.12
1.68	T	1475	.029 68
2.22	T	398	.008 008
2.86		247	.004 97
3.17		342955	6 9
4.25	T	8755	.176 0
4.57	TM	2155	.043 36
4.78	TM	543	.011 94
4.98	TM	55744	1.122
5.16	TM	37209	.748
5.50	TM	499	.010 04
6.11		1926	.038 75
6.38	M	1367	.027 51
6.50	M	3268	.065 75
7.30		2495	.050 2
7.60	M	1611	.032 41
7.86	M	26072	.524 6
8.10	M	870	.017 51
9.06		20740	.417 3
9.50	M	1810	.036 42
9.87	I	365	.007 .44

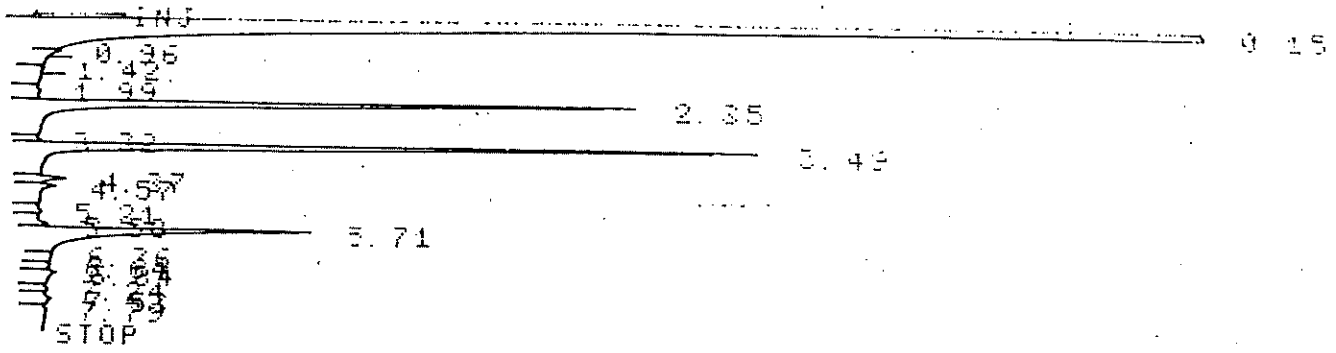
HP 3380A
DLY OFF STOP 10 REJECT OFF
MV/M .30 ATTN 16

Figure 1

Chromatogram-Column A

80-180 ^{o}C at 20 ^{o}C/min
Initial Hold - 1 min
Final Hold - 5 min

Phenyl-2-propanone Reaction Mixture



AREA %

RT	TYPE	AREA	%
.15	*	4206151	95.12
.96	T	259	.005
1.42	T	77	.001
1.99	T	182	.004
2.35	TM	65923	1.491
3.32	TM	414	.009
3.49	TM	69979	1.583
4.37	TM	3261	.073
4.57	TM	3072	.069
5.21	TM	1306	.029
5.58	TM	2178	.049
5.71	TM	42929	.971
6.36	TM	3804	.086
6.64	TM	2453	.055
6.84	TM	5345	.120
7.24	TM	2384	.053
7.53	TM	4000	.090
7.79	ITM	8065	.182

HP 3380A
DLY OFF
MV/M .01

STOP 10
ATTN 16

REJECT OFF

Figure 2

Chromatogram-Column A

80-180 ^{o}C at 20 ^{o}C/min
Initial Hold - 1 min
Final Hold - 5 min

Retention time	Compound
2.35	Phenyl-2-propanone
3.49	Isopropylaniline
5.71	N-formylamphetamine

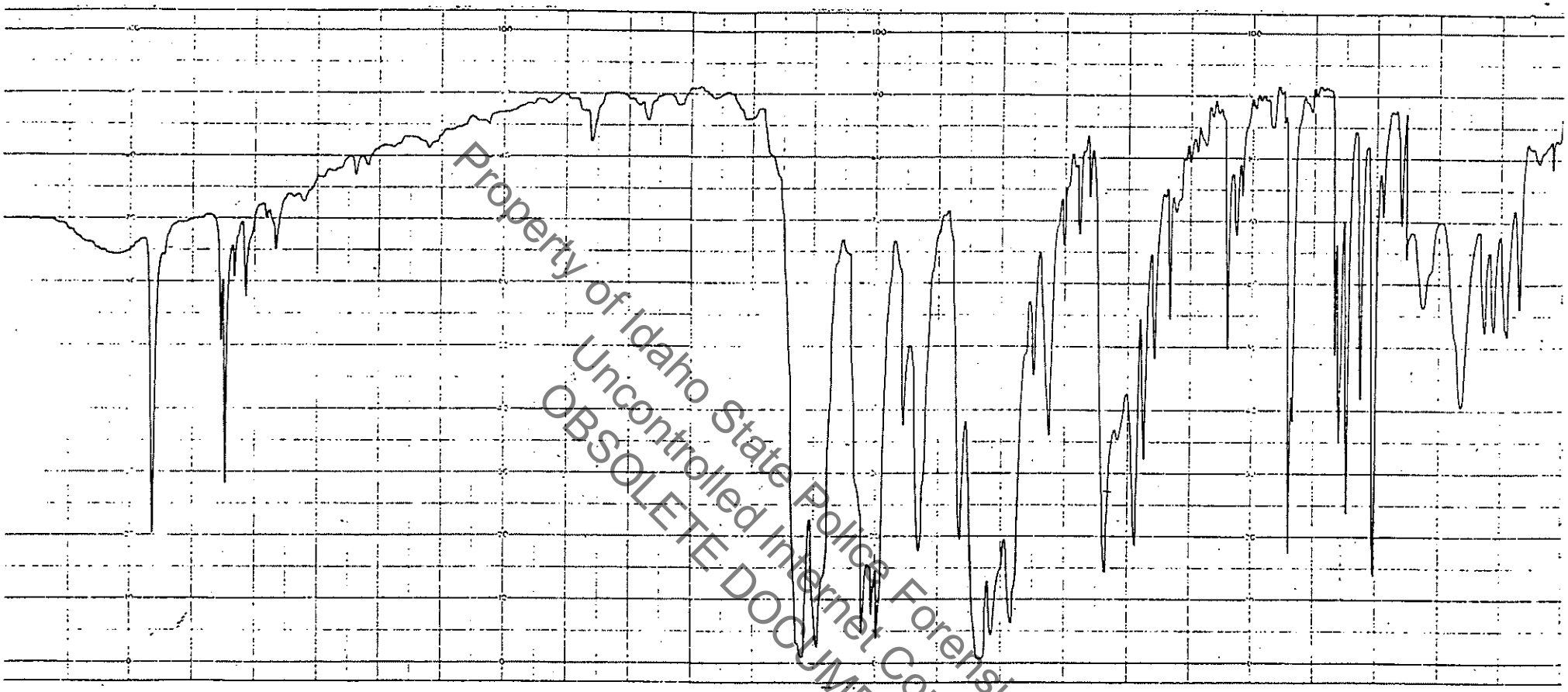
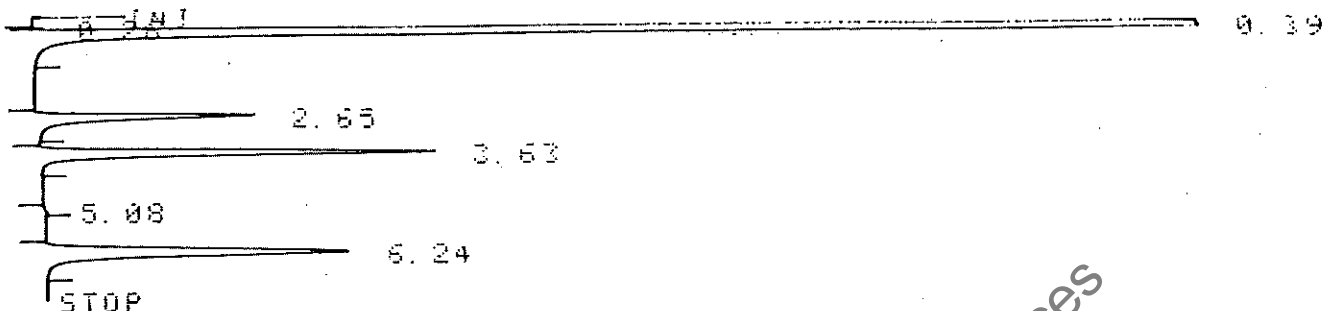


Figure 3

Infrared Spectra
of
Phenyl-2-propanone 2,4-dinitrophenylhydrazine
derivative

3800 - 400 cm^{-1}



AREA %

RT	TYPE	AREA	
.26		1135	018 16
.39	*M	5651914	90.44
2.65		128395	2.055
3.63		223013	3.569
5.08		1411	.022 58
6.24		243256	3.893

HP 3380A
DLY OFF STOP 15 REJECT OFF
MV/M 1.00 ATTN 64

Figure 4

Chromatogram-Column B

150-180 ^{o}C at 10 ^{o}/min
Initial Hold - 1 min
Final Hold - 5 min

Retention time	Compound
2.65	Amphetamine
3.63	Phenyl-2-propanone
6.24	Isopropylaniline

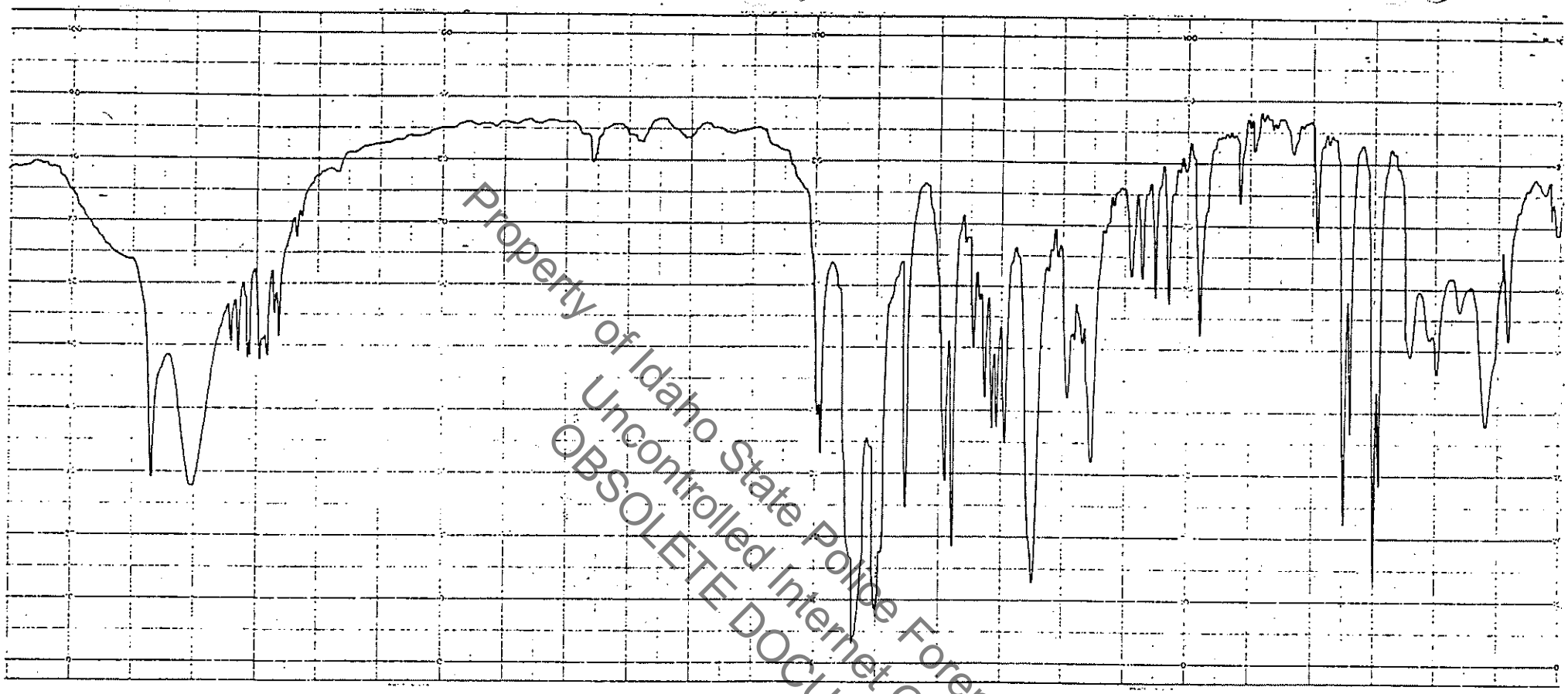


Figure 5

Infrared Spectra
of
Amphetamine phenylisothiocyanate
derivative

3800 - 400 cm^{-1}

NOTES ON THE TEST TUBE METHOD FOR SEPARATION OF AMPHETAMINE OR METHAMPHETAMINE FROM EPHEDRINE OR CAFFEINE

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Illinois Bureau of Identification
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Introduction

Problems were encountered in the separation and crystallization of particularly methamphetamine when mixed with ephedrine or caffeine. The critical part of the procedure is to allow the test tube to set for 5 to 10 minutes after the hydrochloride salt forms. The following procedure with the minor changes is recommended.

Procedure

1. Follow the procedure of Stinson and Berry Microgram, Vol. VII, No. 4, April 1974.

2. For the usual white cross tablets - grind to powder one or two tablets, usually two. Shake NaOH and powder in corked test tube.

3. Add the NaOH, Hexane, distilled water with clean, individual pasteur pipets. Also use a clean pipet to transfer the Hexane layer.

4. Each time a shaking is necessary, use a corked test tube. Spin down each time using centrifuge.

5. After Hydrochloride Salt has been formed, spin down, set tube aside for 5 to 10 minutes to allow crystals to form on those spun to sides of test tube.

6. Pour off Hexane and allow the Hydrochloride Salt to dry in the test tube. A warm but not hot oven should be used.

7. KBR pellet can be formed by scraping the dried powder from the tube with a spatula.

Results

1. Resulting IR's are very sharp.

2. Procedure has been successful for suspected Amphetamine, Methamphetamine, MDA, and Methylphenidate. Other related compounds, which are soluable in Hexane, may also work.

Identification of Impurities in Illicit Methamphetamine Samples

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Three impurities have been identified in illicit methamphetamine samples. One of these, *N*-formylmethamphetamine (*N*-methyl-*N*-(α-methylphenethyl)formamide), is an intermediate in the synthesis of methamphetamine by the Leuckart reaction. The remaining 2, *N*,α,α′-trimethyldiphenethylamine and α-benzyl-*N*-methylphenethylamine, are by-products of methamphetamine synthesis. Ultraviolet, infrared, nuclear magnetic resonance, and gas chromatographic-mass spectral techniques were used to identify these compounds. The data obtained are discussed and synthetic pathways are postulated to explain the presence of each compound in samples of methamphetamine.

One of the most frequently abused drugs in the United States is methamphetamine, a stimulant popularly known as "speed." The methamphetamine used in the illicit trade has been obtained both by diversion of legitimately manufactured material and by synthesis in illicit or clandestine laboratories.

Methamphetamine produced in clandestine laboratories often contains impurities arising from incomplete reaction and inadequate purification of intermediates and the final product of the synthesis. The purpose of this paper is to describe 3 such impurities which have been identified in illicit methamphetamine samples.

Knowledge of impurities in methamphetamine is important to the Drug Enforcement Administration for several reasons. One is that the impurities could have additional harmful effects on the methamphetamine user. They should therefore be identified so that these added potential hazards of methamphetamine use can be publicized and treatment provided if necessary.

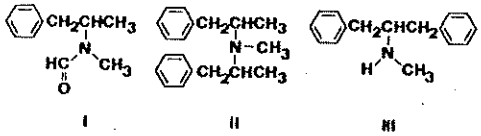
A second reason is that knowledge of impurities in methamphetamine can provide useful intelligence to law enforcement officials concerning illicit methamphetamine production. Information on synthetic methods used to produce methamphetamine, including necessary chemicals and equipment, may be obtained. With knowledge of the materials being used for methamphetamine synthesis, law enforcement officials can monitor production and sale of commercially available precursors of methamphetamine. This could lead to detection of clandestine laboratories.

In addition, the presence or absence of specific impurities can aid in identifying methamphetamine samples which are of common origin and in distinguishing between samples of legitimate and illicit manufacture.

Finally, awareness of possible impurities in methamphetamine is important to the forensic chemist performing sample analysis because of possible interference with the analytical technique being used.

I. Detection of Impurities in Methamphetamine Samples

The 3 impurities which have been identified are shown in Fig. 1. These impurities were encountered during the analysis of a number of illicit methamphetamine samples received from various locations throughout the United States. Components in addition to methamphetamine were detected by thin layer (TLC) and gas-liquid chromatography (GLC) of the samples. Quantitative analysis also suggested the presence of impurities. Depending on the nature of the impurity and the method of quantitation, methamphetamine assays were either significantly below 100% or, in some cases, above 100%. The erroneously high percentages were



I II III

FIG. 1—Impurities in illicit methamphetamine samples: *N*-formylmethamphetamine (I), *N*,α,α′-trimethyldiphenethylamine (II), and α-benzyl-*N*-methylphenethylamine (III).

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obtained by ultraviolet (UV) analyses of samples containing an impurity with a UV spectrum similar to that of methamphetamine but with greater absorptivity.

The 3 impurities were identified by UV, infrared (IR), nuclear magnetic resonance (NMR), and combined gas-liquid chromatography-mass spectrometry (GLC-MS) techniques. The methods used to isolate and identify each compound as well as the manner in which each can occur in methamphetamine samples as a result of synthesis are described below. Since the NMR and mass spectra which will be discussed have many features in common with those of methamphetamine, NMR and mass spectra of methamphetamine have been included for comparison (Figs. 2 and 3, respectively).

A. *N*-Formylmethamphetamine

Formation in Synthesis of Methamphetamine

One method which has been used for production of illicit methamphetamine is the Leuckart synthesis (1), shown in Fig. 4. This synthesis involves reaction of methyl benzyl ketone with methylamine and formic acid (or *N*-methylformamide) to give *N*-formylmethamphetamine as an intermediate. Hydrolysis of this intermediate with hydrochloric acid produces methamphetamine. Incomplete hydrolysis can result in the presence of the formyl compound as an impurity in the methamphetamine.

Isolation and Identification

N-Formylmethamphetamine was isolated as an oil by both preparative GLC and TLC of methamphetamine samples as described under *Experimental*. The oil was identified by the data given below.

Ultraviolet and Infrared Spectra.—UV analysis produced a spectrum typical of a nonconjugated monosubstituted benzene compound.

An IR spectrum of the compound (Fig. 5) indicates the presence of an amide carbonyl by an intense band at 1665 cm^{-1}. A monosubstituted benzene ring is suggested by bands at 705 and 750 cm^{-1}.

Mass Spectrum.—A mass spectrum of the material (Fig. 6) shows a molecular ion at m/e 177 with a base peak at m/e 86 due to loss of benzyl from the molecular ion. Other fragment ions were observed at m/e 118, 91, and 58.

Nuclear Magnetic Resonance Spectra.—NMR analysis with time averaging was carried out on a sample extracted from a TLC spot and sub-

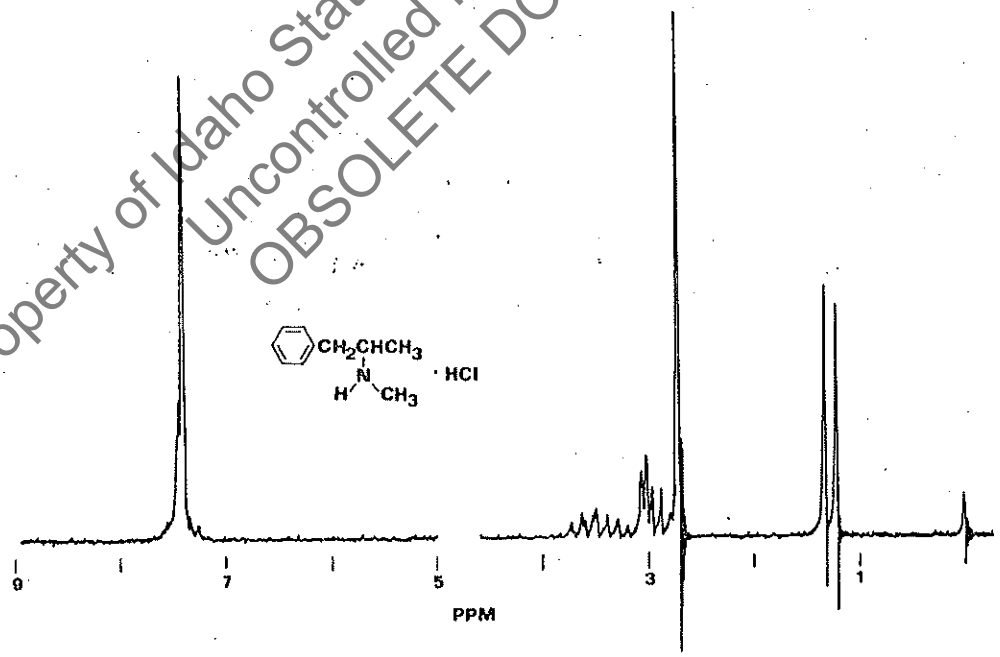

FIG. 2—NMR spectrum of methamphetamine hydrochloride in deuterated water.

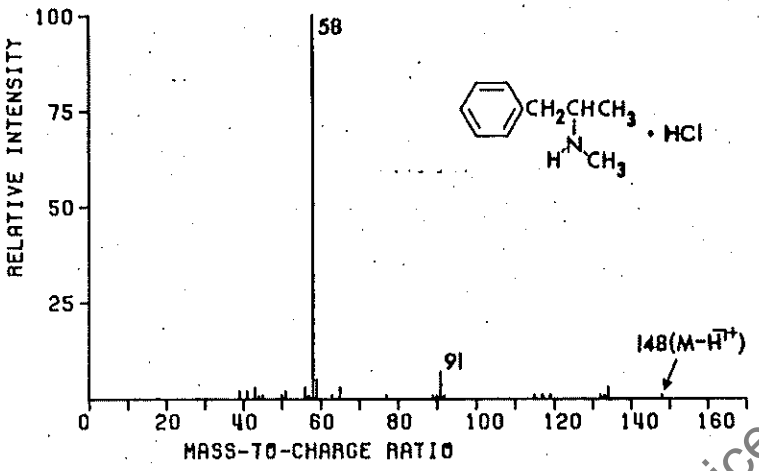

FIG. 3—Mass spectrum of methamphetamine hydrochloride.

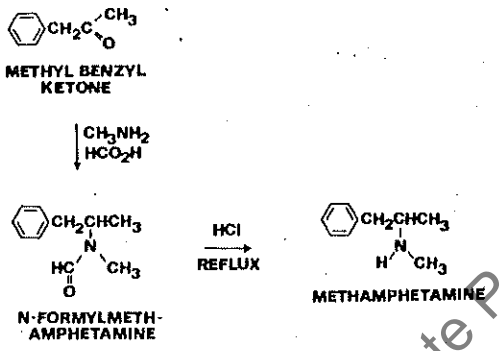

FIG. 4—Leuckart synthesis of methamphetamine from methyl benzyl ketone.

sequently dissolved in carbon tetrachloride. (A spectrum of the material in deuterochloroform was also obtained.)

The spectrum of the carbon tetrachloride solution (Fig. 7) shows the presence of 2 rotational isomers of *N*-formylmethamphetamine arising from hindered rotation about the bond between the carbonyl carbon and nitrogen. The chemical shifts of formyl, methine, and C-methyl protons for one rotational isomer of *N*-formylmethamphetamine differ from those of the corresponding protons in the other isomer. The isomers were shown to be present in a ratio of approximately 2:1 by integration. The predominant isomer produces a singlet at 7.65 ppm (δ) due to the formyl proton, a multiplet centered at 3.71 ppm for methine, and a doublet at 1.28 ppm for C-methyl, whereas the other isomer shows a singlet, multiplet, and doublet at 7.82, 4.61, and 1.15 ppm, respectively, for the same protons. (Although the 2 overlapping C-methyl doublets appear as only 3 peaks in the spectrum of *N*-formylmethamphetamine in carbon tetrachloride, shown in Fig. 7, 4 peaks were observed for a deuterochloroform solution.) Other peaks include a singlet at 6.91–7.30 ppm due to the phenyl group and a multiplet at 2.66–2.87 ppm for the methylene and N-methyl protons.

Cis and *trans* isomers of formamides have been detected previously by NMR and in some cases the preferred configuration has been established (2–7). In a study of unsymmetrically *N,N*-disubstituted formamides, LaPlanche and Rogers (2) have demonstrated that the preferred isomer has the larger substituent on nitrogen *trans* to the carbonyl oxygen. These same investigators also studied a series of aliphatic *N*-monosubstituted formamides (3). In this case the preferred isomer was found to have the alkyl substituent on nitrogen *cis* to the carbonyl oxygen, although the percentage of this isomer decreases as the substituent becomes more bulky. Other investigators (5, 6) have shown that, for formanilide, the predominant form depends on the concentration of the solution being observed. The preferred isomer has the phenyl substituent *cis* to the carbonyl oxygen in concentrated solution and *trans* in dilute solution.

Further work is planned to determine which is the preferred configuration for *N*-formylmethamphetamine.

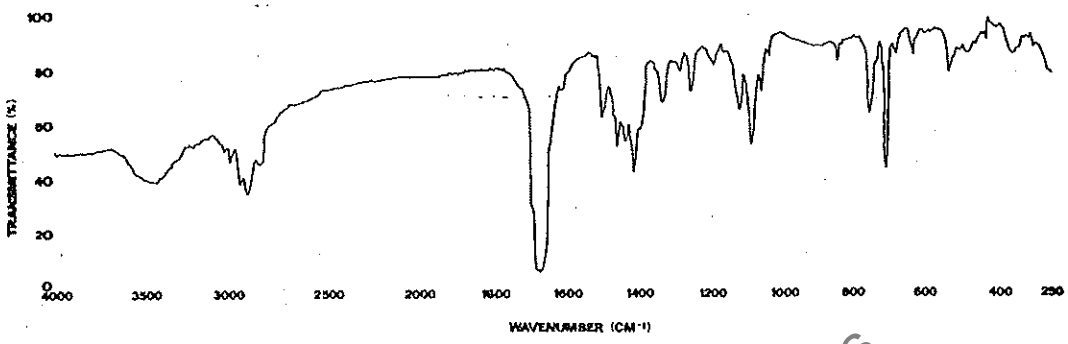

FIG. 5—IR spectrum of *N*-formylmethamphetamine.

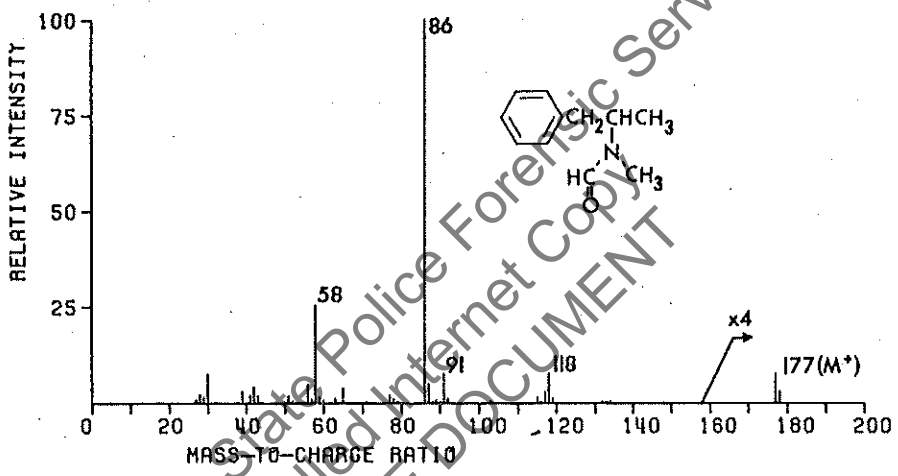

FIG. 6—Mass spectrum of *N*-formylmethamphetamine.

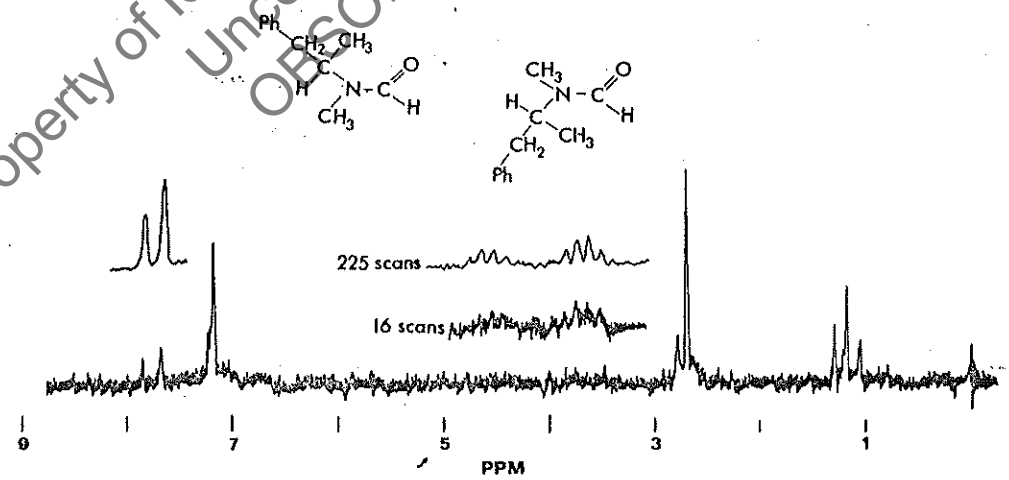

FIG. 7—NMR spectrum of *N*-formylmethamphetamine in carbon tetrachloride.

Synthesis

In order to confirm the identity of this impurity, it was synthesized by the Leuckart reaction. This method was used for the synthesis because, in addition to supplying *N*-formylmethamphetamine as a standard for purposes of identification, it would provide support for the synthetic pathway proposed to explain the presence of this compound in illicit methamphetamine samples. (*N*-Formylmethamphetamine has been reported to be produced by the Leuckart reaction of methyl benzyl ketone with *N*-methylformamide (8), but it has apparently not been isolated from this reaction. It has been prepared previously by formylation of methamphetamine (9, 10).)

The procedure used for the synthesis consisted of heating methyl benzyl ketone with formic acid and methylamine for 7 hr at 160–170°C. The resulting mixture was poured into water and the yellow oil which separated was isolated by ether extraction.

Analysis of the oil by GLC-MS showed that it contained the desired *N*-formylmethamphetamine as one major component. Another major component was a tertiary amine by-product of the Leuckart synthesis of methamphetamine. (This by-product is another of the impurities identified in illicit samples and will be discussed later.)

N-Formylmethamphetamine was separated from the product mixture by GLC on a 3% OV-1 column at 190°C. Its IR, NMR, and mass spectra were identical to those of the material found in illicit samples of methamphetamine.

The presence of *N*-formylmethamphetamine in illicit methamphetamine samples has recently been reported by LeBelle *et al.* (10). The present work confirms the findings of these investigators.

B. *N*,α,α′-Trimethyldiphenethylamine

Formation in Synthesis of Methamphetamine

N,α,α′-Trimethyldiphenethylamine can be obtained as a by-product in the Leuckart synthesis of methamphetamine from methyl benzyl ketone. Formation of tertiary amines as by-products in Leuckart syntheses of secondary amines is a common occurrence (1). As shown in Fig. 8, this by-product can be formed by further reaction of the intermediate *N*-formylmethamphetamine with methyl benzyl ketone and formic acid.

N,α,α′-Trimethyldiphenethylamine can also be obtained as a by-product in the synthesis of methamphetamine by catalytic hydrogenation of a mixture of methyl benzyl ketone and methylamine.

Isolation and Identification

N,α,α′-Trimethyldiphenethylamine was isolated as its hydrochloride salt by column chromatography of methamphetamine samples as described under *Experimental*. The data used to identify the salt are given below.

Ultraviolet and Infrared Spectra.—A UV spectrum of the amine hydrochloride was characteristic of a nonconjugated monosubstituted benzene.

The IR spectrum (Fig. 9) indicates the presence of a tertiary amine salt by a series of broad bands between 2300 and 2700 cm^{-1}. A monosubstituted benzene ring is suggested by intense bands at 708 and 750 cm^{-1}.

Mass Spectrum.—A mass spectrum of the material (Fig. 10) shows a peak at m/e 266 due to loss of hydrogen from the molecular ion. A fragment ion appears at m/e 252 from loss of a methyl group with the base peak at m/e 176

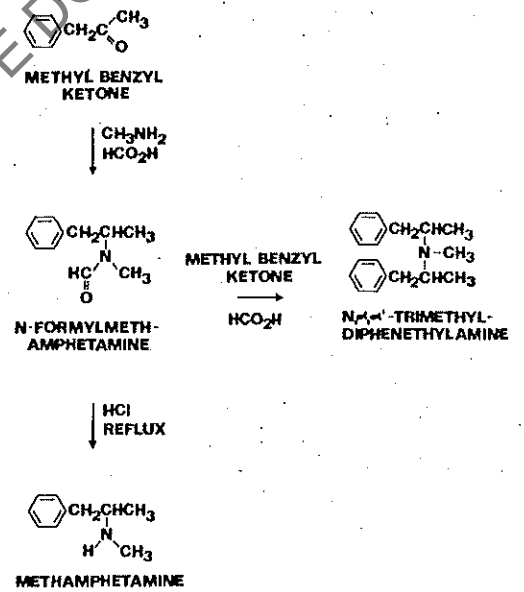

FIG. 8—Leuckart synthesis of methamphetamine showing formation of tertiary amine by-product, *N*,α,α′-trimethyldiphenethylamine.

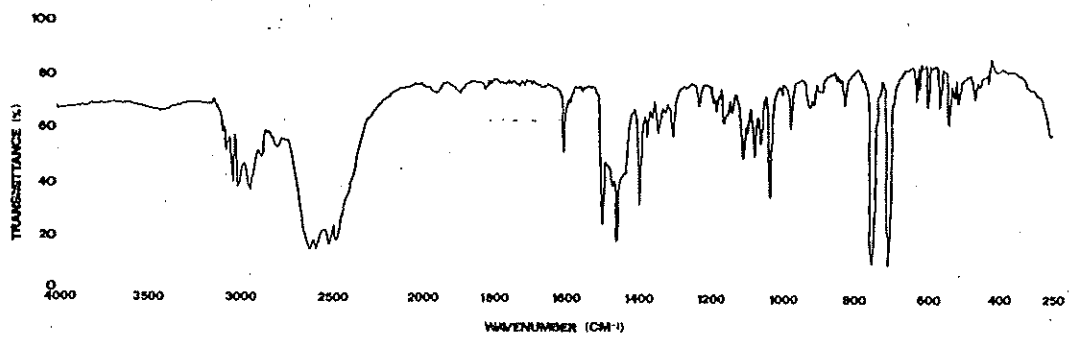

FIG. 9—IR spectrum of *N,α,α'*-trimethyldiphenethylamine hydrochloride.

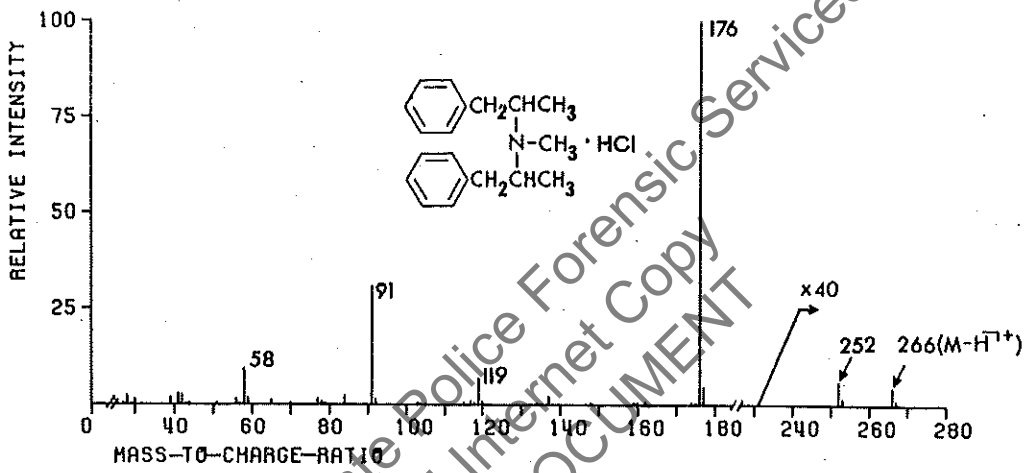

FIG. 10—Mass spectrum of *N,α,α'*-trimethyldiphenethylamine hydrochloride.

from loss of benzyl. Accurate mass measurement of the 176 ion produced a value of 176.138. The most plausible structure agreeing with the measured mass is the fragment with an elemental composition of $C_{12}H_{18}N$ obtained by loss of benzyl from the molecular ion (actual mass 176.144). Intense ions at *m/e* 119, 91, and 58 are also present.

Nuclear Magnetic Resonance Spectra.—NMR analysis was performed on both deuterium oxide and deuterochloroform solutions of the amine hydrochloride. (A single extraction with an equal volume of chloroform is sufficient to remove the amine hydrochloride from water, even if the solution is acidified.) The spectrum of the deuterium oxide solution is shown in Fig. 11. The doublet at 1.25 ppm is due to the 2 C-methyl groups. The N-methyl and methylene groups produce a multiplet at 2.51–3.49 ppm. The methine and phenyl protons absorb at 3.50–4.31 and 7.20–7.70 ppm, respectively.

The spectrum of the deuterochloroform solution of *N,α,α'*-trimethyldiphenethylamine hydrochloride differed from that of the deuterium oxide solution, showing a multiplet instead of a doublet for the C-methyl groups. The extra peaks may be due to the presence of diastereoisomers as observed by NMR for a salt of a similar tertiary amine, *N*-methyldi(α-phenethyl)-amine (11). Another possibility is restricted rotation, also detected by NMR for another tertiary phenethylamine, *N-tert*-butyldiphenethylamine (12).

Synthesis

Since, as previously noted, *N,α,α'*-trimethyldiphenethylamine was obtained along with *N*-formylmethamphetamine by the Leuckart reaction of methyl benzyl ketone with formic acid and methylamine, it was possible to confirm its identity.

Received January 23, 1974.

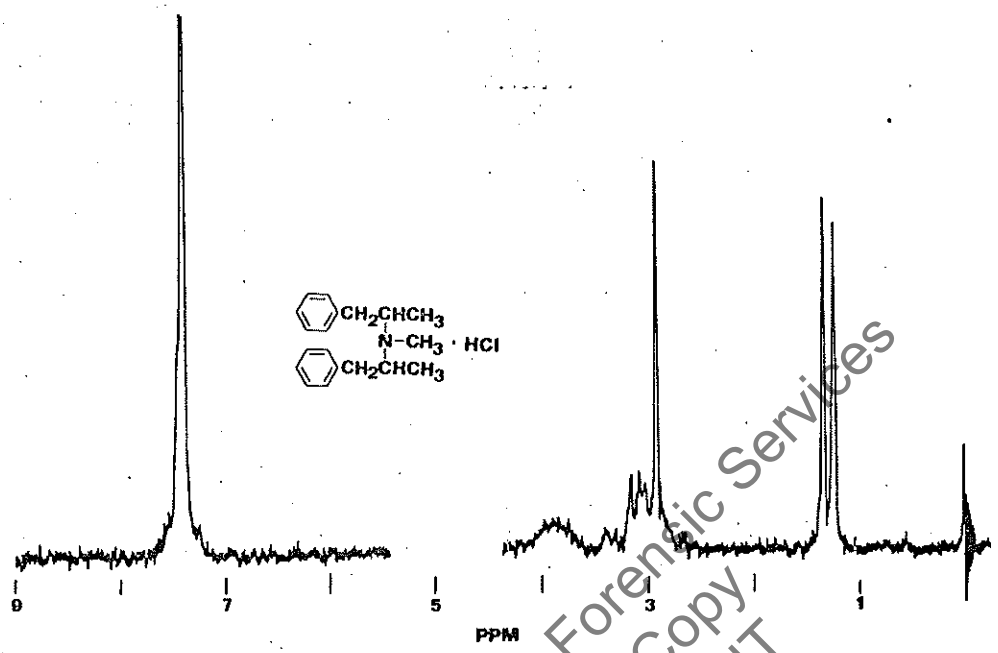

FIG. 11—NMR spectrum of *N,α,α'*-trimethyldiphenethylamine hydrochloride in deuterated water.

The tertiary amine was separated from the product mixture as its hydrochloride salt by column chromatography in the manner described for isolation of this compound from methamphetamine samples (see *Experimental*). Its IR, NMR, and mass spectra were identical to those of the material found in the samples.

The formation of *N,α,α'*-trimethyldiphenethylamine as a by-product in the Leuckart reaction supports one synthetic pathway postulated to explain its presence in illicit samples of methamphetamine. (No previous report of isolation of *N,α,α'*-trimethyldiphenethylamine from the Leuckart reaction of methyl benzyl ketone with formic acid and methylamine was found. Both *meso*- and *dl*-diastereoisomers of the diphenethylamine have been prepared by 2 methods (13): (*a*) reaction of 3,4,5-trimethyl-1,6-diphenyl-1-oxa-4-azahexane with phenylmagnesium bromide; and (*b*) condensation of methyl benzyl ketone with amphetamine followed by reduction of the resulting imine and *N*-methylation.)

This paper was presented at the Symposium on Forensic Sciences, 87th Annual Meeting of the AOAC, Oct. 9-12, 1973, at Washington, D.C.

C. α-Benzyl-*N*-methylphenethylamine

Formation in Synthesis of Methamphetamine

α-Benzyl-*N*-methylphenethylamine can occur as a by-product of methamphetamine synthesis by the sequence, shown in Fig. 12, which begins with a synthesis of methyl benzyl ketone from phenylacetic acid. (Synthesis of α-benzyl-*N*-methylphenethylamine from phenylacetic acid as shown in Fig. 12 has been reported by Aonuma and co-workers (14).)

Preparation of methyl benzyl ketone from phenylacetic acid has been found to give dibenzyl ketone as a by-product (14, 15). Methyl benzyl ketone and dibenzyl ketone can be separated by fractional distillation.

If the 2 ketones are not separated and the mixture of ketones is used for methamphetamine synthesis, dibenzyl ketone will react in a manner analogous to that of methyl benzyl ketone to give α-benzyl-*N*-methylphenethylamine, along with the desired methamphetamine.

Support of this synthetic route to α-benzyl-*N*-methylphenethylamine was recently obtained from a seizure of a clandestine methamphetamine laboratory by the Drug Enforcement Administration. The methyl benzyl ketone being

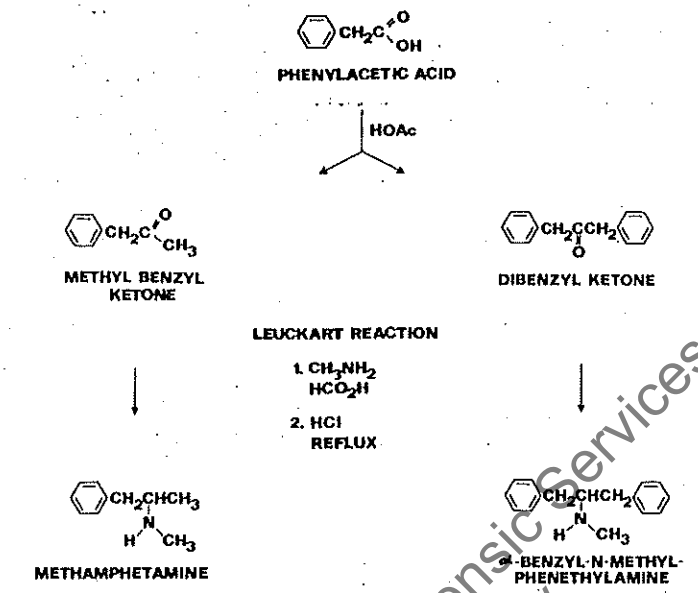

FIG. 12—Synthesis of methamphetamine and α-benzyl-*N*-methylphenethylamine from phenylacetic acid.

used for methamphetamine synthesis was being produced in the laboratory from phenylacetic acid. As anticipated, the seized methamphetamine was found to contain α-benzyl-*N*-methylphenethylamine as an impurity.

Isolation and Identification

α-Benzyl-*N*-methylphenethylamine was isolated as its hydrochloride salt by column chromatography of methamphetamine samples as described under *Experimental*. The salt was identified by the data given below.

Ultraviolet and Infrared Spectra.—The UV spectrum of the amine hydrochloride was similar to the spectra of the first 2 compounds described, i.e., typical of a nonconjugated monosubstituted benzene.

The IR spectrum (Fig. 13) suggests a secondary amine salt by a single intense band at 2460 cm^{-1}, a series of strong bands between 2700 and 3000 cm^{-1}, and absorption at about 1600 cm^{-1}. The presence of a monosubstituted benzene ring is indicated by bands at 700 and 750 cm^{-1}.

Mass Spectrum.—The mass spectrum of α-benzyl-*N*-methylphenethylamine hydrochloride (Fig. 14) shows, as the most intense peak in the molecular ion region, a peak at m/e 224 due to loss of hydrogen from the molecular ion. The base peak at m/e 134 arises from loss of benzyl from the molecular ion. Other intense ions appear at m/e 119 and 91.

Nuclear Magnetic Resonance Spectrum.—NMR analysis was performed on a deuterium oxide solution of the hydrochloride salt. The spectrum (Fig. 15) has many features in common with that of methamphetamine hydrochloride (Fig. 2), including the absorption patterns and chemical shifts for phenyl, methine, and methylamino protons. Distinguishing features of the spectrum of α-benzyl-*N*-methylphenethylamine are the absence of a C-methyl doublet and the occurrence of a single doublet for the methylene protons instead of a pair of doublets as observed for methamphetamine. In addition, integration showed the number of phenyl and methylene protons to be twice that in methamphetamine.

II. Screening of Methamphetamine Samples for Impurities

Two chromatographic procedures have been developed for rapid screening of methamphetamine samples for the 3 impurities described in this paper. Since it is possible that all 3 impurities could be present in a single sample of methamphetamine, the method used for screening should allow detection of all components of a

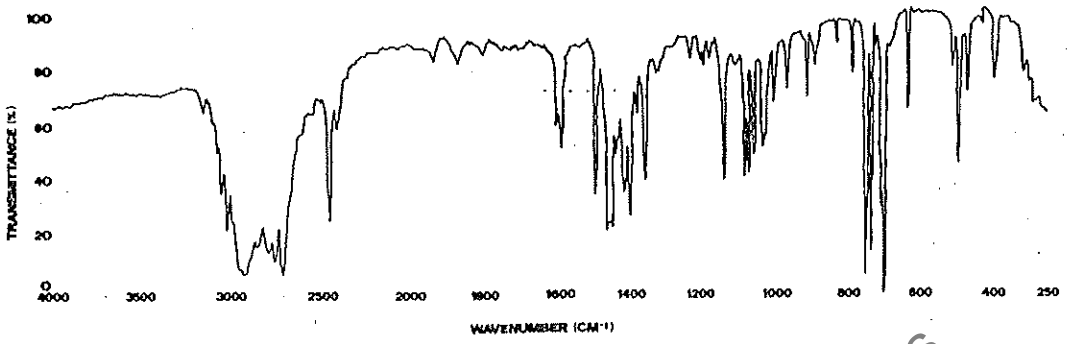

FIG. 13—IR spectrum of α-benzyl-*N*-methylphenethylamine hydrochloride.

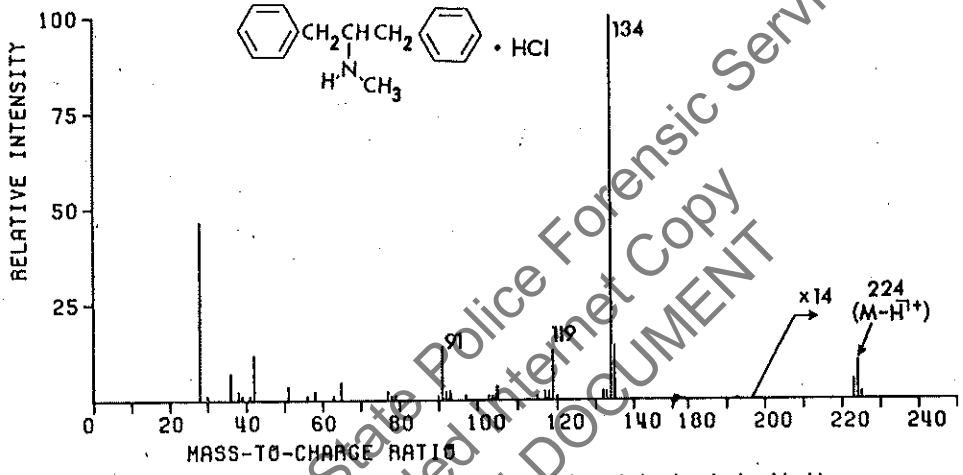

FIG. 14—Mass spectrum of α-benzyl-*N*-methylphenethylamine hydrochloride.

mixture containing the 3 impurities and methamphetamine. A mixture of the 4 compounds was separated by both TLC and GLC.

TLC data are given in Table 1 and GLC data are given in Table 2. Separation of the 4 compounds by TLC was achieved with 3 solvent systems: 1, 2, and 4. GLC data show separation on 2 columns: 10% OV-101 and 3% OV-25.

III. Experimental

A. Apparatus and Materials

Instruments

(a) *Ultraviolet spectrophotometer.*—Cary Model 14.

(b) *Infrared spectrophotometer.*—Perkin-Elmer Model 457.

(c) *Mass spectrometer.*—Hitachi Perkin-Elmer RMU-6L single-focusing, 90° magnetic sector mass spectrometer interfaced to Perkin-Elmer 900 gas chromatograph by single-stage Watson-Biemann fritted glass separator. Operating parameters: 70 ev electron energy, 4400 v accelerating potential, source temperature 220°C, helium carrier gas, separator temperature 185°C.

(d) *Nuclear magnetic resonance spectrometer.*—Japan Electron Optics Laboratory (JEOL) C-60HL 60 MHz unit; probe temperature 25°C, signal-to-noise ratio 50:1. Chemical shifts are reported in ppm δ.

(e) *Data system.*—Digital Equipment Corporation PDP-12 Data System with RK08 random access disk storage interfaced to mass spectrometer and NMR spectrometer using appropriate software for on-line high and low resolution mass spectrometry and NMR time averaging. During acquisition of GLC-MS data, mass spectrometer was computer-controlled to take 140 scans from m/e 20 to 450 at 7 sec/scan with 7 sec delay between scans.

(f) *Gas chromatographs.*—(*1*) *For trapping.*—

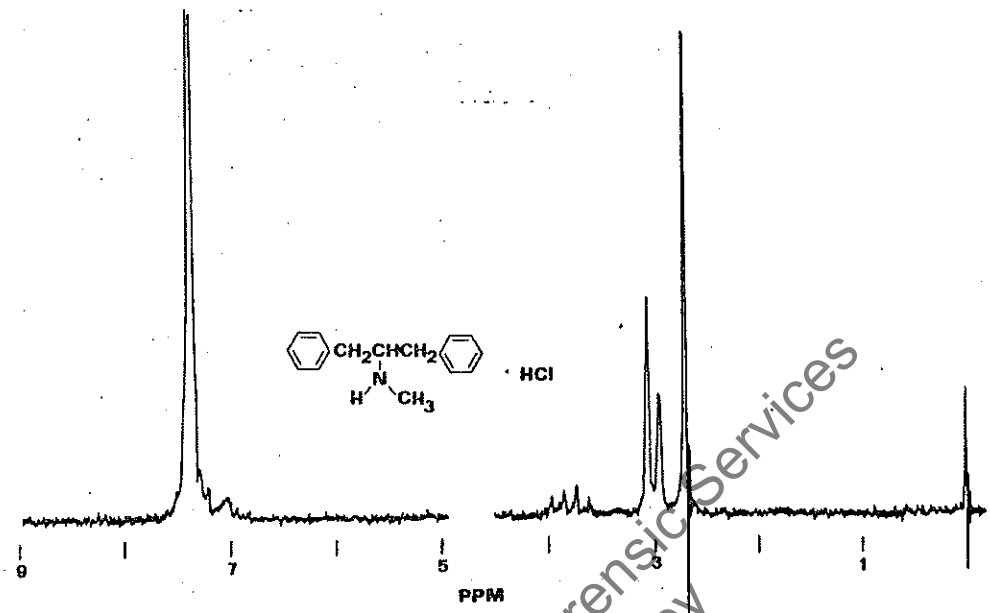

FIG. 15—NMR spectrum of α-benzyl-*N*-methylphenethylamine hydrochloride in deuterated water.

Perkin-Elmer Model 990 (Perkin-Elmer Corp., Norwalk, Conn. 06852), with flame ionization detector equipped with 50:1 stream splitter. Operating parameters: 6′ × 0.08″ id glass column packed with 3% OV-1 on 100–120 mesh Chromosorb W (HP) at 190°C with nitrogen as carrier gas. Temperatures of injection port and detector were 250 and 270°C, respectively. (2) *For screening.*—Packard Model 7400 (Packard Instrument Co., Inc., Downers Grove, Ill. 60515). Operating parameters: 4′ × 0.16″ id glass column packed with 10% OV-101 on 100–120 mesh Chromosorb W (HP) and 6′ × 0.16″ id glass column packed with 3% OV-25 on 100–120 mesh Gas-Chrom Q. Column temperature was programmed with initial column temperature of ca 175°C and initial hold of 5 min with program rate of 5°/min; final temperature was 260°C with final hold of 5 min; injection and detector temperatures were 250 and 230°C, respectively. A flame ionization detector was used for both instruments with nitrogen carrier, hydrogen, and air flow rates of ca 60, 50, and 500 ml/min, respectively. (All phases were obtained from Applied Science Laboratories, P.O. Box 440, State College, Pa. 16801.)

(g) *Melting point apparatus.*—Mettler FP2 with FP21 micro furnace.

Chromatographic Materials

(a) *Thin layer chromatography.*—(*1*) *Plates.*—Precoated with silica gel GF, 2000 μm thickness on 20 × 20 cm glass plates and 250 μm thickness on 100 × 25 mm glass plates (Analtech, Inc., Newark, Del. 19711). (*2*) *Solvent systems.*—*See* Table 1. (*3*) *Visualization reagents.*—(*i*) Acidified iodoplatinate, prepared by adding 250 ml 4% aqueous potassium iodide and 5 ml concentrated HCl to 10 ml 10% aqueous platinum chloride and diluting to 500 ml with water; (*ii*) *p*-dimethylaminobenz-

Table 1. R_f values for TLC separation[a] of methamphetamine and impurities

Compd	Solvent system[b] 1	2	3	4
Methamphetamine	0.21	0.40	0.18	0.03
N-Formylmethamphetamine	0.66	0.73	0.63	0.43
N,α,α′-Trimethyldiphenethylamine	0.52	0.81	0.83	0.68
α-Benzyl-*N*-methylphenethylamine	0.45	0.67	0.64	0.27

[a] Developed plates were visualized under shortwave UV light and by spraying successively with acidified iodoplatinate and *p*-dimethylaminobenzaldehyde reagents (10).

[b] 1 = chloroform-methanol (9+1)
2 = ammonia-saturated chloroform-methanol (20+1)
3 = ethanol-dioxane-benzene-ammonium hydroxide (5+40+50+5)
4 = ethyl acetate-benzene-ammonium hydroxide (60+35+5)

Table 2. Retention times (min) for GLC separation of methamphetamine and impurities

Compd	Column 10% OV-101	Column 3% OV-25
Methamphetamine	<3.0	<3.0
N-Formylmethamphetamine	3.3	6.6
α-Benzyl-*N*-methylphenethylamine	8.2	11.0
N,α,α'-Trimethyldiphenethylamine	12.4	13.9
Hexacosane internal standard	22.7	17.5

aldehyde, prepared from 2 g *p*-dimethylaminobenzaldehyde and 100 ml ethanol-concentrated HCl (1+1).

(b) *Column chromatography.*—(*1*) *Packing*—Celite 545, acid-washed (Johns-Manville, Manville, NJ. 08835). (*2*) *Columns.*—Glass, 22 × 300 mm with 45 mm delivery stem (Kontes Glass Co., Vineland, NJ. 07360).

Reagents

All chemicals and solvents were reagent grade unless indicated otherwise.

Standards

(a) *Methamphetamine hydrochloride.*—K & K Laboratories, Plainview, N.Y. 11803.

(b) *Hexacosane internal standard.*—Applied Science Laboratories, Inc.

B. Isolation of Impurities from Methamphetamine Samples

N-Formylmethamphetamine

N-Formylmethamphetamine was isolated as an oil by both preparative GLC and TLC of methamphetamine samples. A column of 3% OV-1 on Chromosorb W (HP) at 190°C was used for GLC trapping. For TLC, a $CHCl_3$ solution of a methamphetamine sample containing *N*-formylmethamphetamine as an impurity was applied to a TLC plate and developed with $CHCl_3$-methanol (9+1). Good separation of methamphetamine and its formyl derivative was obtained with this solvent system. *N*-Formylmethamphetamine was extracted from the TLC plate with $CHCl_3$.

Tertiary and Secondary Amine Impurities

Both amines were isolated by column chromatography of methamphetamine samples on Celite 545.

N,α,α'-Trimethyldiphenethylamine. — A methamphetamine sample (ca 100–300 mg) containing the tertiary amine impurity was dissolved in 3 ml 0.5*N* HCl and mixed with 5 g Celite 545. The mixture was packed into a glass chromatographic column. Elution with water-washed $CHCl_3$ yielded the amine impurity in the first 50 ml of eluate. After the solvent was evaporated, *N*,α,α'-trimethyldiphenethylamine HCl remained as an off-white powder. (This tertiary amine impurity can also be isolated from a sample of methamphetamine HCl by extraction of an aqueous solution of the sample with $CHCl_3$.)

α-Benzyl-N-methylphenethylamine.—A methamphetamine sample (ca 100–300 mg) containing the secondary amine impurity was dissolved in 0.5*N* HCl and chromatographed in the manner described for isolation of the tertiary amine. The first 50 ml of eluate again contained the impurity. Evaporation of solvent left α-benzyl-*N*-methylphenethylamine HCl as a white crystalline powder, mp 194.7–197.5°C (literature 191–192°C (14)).

Characterization of Impurities

The spectral data characterizing the 3 compounds are described under the discussion of each compound. Methanol solutions of the compounds were used for UV analyses. IR spectra were obtained as KBr disks. Samples for NMR analysis were dissolved in deuterium oxide, deuterochloroform, or carbon tetrachloride, as noted.

C. Leuckart Reaction of Methyl Benzyl Ketone with Methylamine and Formic Acid

The procedure followed was adapted from procedures of Crossley and Moore (16) and Novelli (17). Methylamine 40% in water, 93.1 g, 1.2 moles) and formic acid (88%, 62.9 g, 1.2 moles) were placed in a 1 L 3-neck round-bottom flask equipped with a thermometer and down-directed condenser. The solution was heated in an oil bath. Water began distilling when the solution temperature had exceeded 100°C. Heating was continued until the solution was 180–190°C. It was kept at this temperature for ca 15 min while a colorless liquid continued to distill. (Approximately 100 ml distillate was collected, some of which was probably *N*-methylformamide.)

The solution was cooled to room temperature and methyl benzyl ketone (Aldrich, 97%, 40.3 g, 0.3 mole) was added. The resulting pale yellow mixture was returned to the oil bath and heated 7 hr at 160–170°C. During this time gas evolved and several ml distillate was collected.

After the reaction mixture, consisting of 85 ml golden-yellow liquid, was cooled to room temperature, it was diluted with 170 ml water. The gold oil which separated was isolated by ether extraction of the aqueous mixture. The ether extract was dried (Na_2SO_4) and the ether was removed with a rotary evaporator. The oil, after being dried 16 hr over Drierite in a vacuum desiccator, weighed 37 g.

Analysis of Product by Gas-Liquid Chromatography–Mass Spectrometry

The oil was analyzed by GLC-MS on a 6′ × 0.08″ id glass column packed with 3% OV-1 on 100–120 mesh Chromosorb W (HP) at 230°C with the GLC-MS-computer system described above. The analysis showed that the oil contained the expected *N*-formylmethamphetamine as 1 major component. Another major component was *N*,*α*,*α*′-trimethyldiphenethylamine, a by-product formed by further reaction of *N*-formylmethamphetamine with methyl benzyl ketone and formic acid. The oil was also found to contain the starting material, methyl benzyl ketone, and a fourth unidentified component. (The mass spectrum of this fourth component was discovered to be identical to that of an impurity in an illicit sample of methamphetamine. This same illicit sample also contained *N*-formylmethamphetamine and *N*,*α*,*α*′-trimethyldiphenethylamine as impurities.) The 4 components were eluted in the following order: methyl benzyl ketone, *N*-formylmethamphetamine, the unidentified component, and *N*,*α*,*α*′-trimethyldiphenethylamine.

Isolation of Components of Product Mixture

N-Formylmethamphetamine was separated from the product mixture by preparative GLC and *N*,*α*,*α*′-trimethyldiphenethylamine was separated as its hydrochloride salt by column chromatography in the manner described for isolation of these compounds from illicit methamphetamine samples. IR, NMR, and mass spectra of both compounds were identical to those of the materials isolated from the illicit samples of methamphetamine.

D. Screening of Methamphetamine Samples for Impurities

Thin Layer Chromatography

A mixture of methamphetamine hydrochloride and the 3 compounds identified as impurities in illicit methamphetamine was dissolved in methanol and applied to 100 × 25 mm TLC plates precoated with silica gel GF (250 μm thickness). The plates were developed with the 4 solvent systems listed in Table 1 and visualized under 254 nm UV light and by spraying successively with acidified iodoplatinate and *p*-dimethylaminobenzaldehyde reagents. The mixture of 4 compounds was successfully separated with 3 of the 4 solvent systems tried, 1, 2, and 4 (*see* Table 1 for R_f values).

Gas-Liquid Chromatography

A methanolic solution of methamphetamine hydrochloride, the 3 impurities, and an internal standard, hexacosane, was subjected to GLC on 2 columns, 10% OV-101 and 3% OV-25, with temperature programming from 175 to 260°C as described previously. GLC retention times (Table 2) show separation of all components on both columns.

Acknowledgment

The authors thank Joseph Hirata for translating the article by Aonuma *et al.* (ref. 14) from Japanese.

References

(1) Moore, M. L. (1949) *Org. React.* 5, 301–330
(2) LaPlanche, L. A., & Rogers, M. T. (1963) *J. Amer. Chem. Soc.* 85, 3728–3730
(3) LaPlanche, L. A., & Rogers, M. T. (1964) *J. Amer. Chem. Soc.* 86, 337–341
(4) Cockerill, A. F., & Rackham, D. M. (1970) *Tetrahedron Lett.* No. 45, 3953–3956
(5) Bourn, A. J. R., Gillies, D. G., & Randall, E. W. (1964) *Tetrahedron* 20, 1811–1818
(6) Suzuki, I., Tsuboi, M., Shimanouchi, T., & Mizushima, S. (1960) *Spectrochim. Acta* 16, 471–478; thru (1960) *Chem. Abstr.* 54, 17053c
(7) Enomoto, N., & Kondo, M. (1972) *Bull. Chem. Soc. Jap.* 45, 2665–2668; thru (1973) *Chem. Abstr.* 78, 29118r
(8) Mastagli, P., & Bièvre-Gallin, G. de (1947) *C.R. Acad. Sci.* 224, 1290–1292; thru (1947) *Chem. Abstr.* 41, 5853h
(9) Cavallito, C. J., & Gray, A. P. (1970) U.S. Patent 3,489,840, Jan. 13; thru (1970) *Chem. Abstr.* 72, 90091u
(10) LeBelle, M., Sileika, M., & Romach, M. (1973) *J. Pharm. Sci.* 62, 862
(11) Solladié-Cavallo, A., & Solladié, G. (1972) *C.R. Acad. Sci. Ser. C* 275, 1053–1055; thru (1973) *Chem. Abstr.* 78, 42725f
(12) Asahara, T., Seno, M., Tanawa, S., & Den, N. (1969) *Bull. Chem. Soc. Jap.* 42, 1996–2005; thru (1969) *Chem. Abstr.* 71, 90954g
(13) Schmitt, J., Comoy, P., Boitard, J., Meingan, J. P., LeMeur, J., & Briot, M. (1966) *Chim. Ther.* No. 2, 71–77; thru (1966) *Chem. Abstr.* 65, 15255a
(14) Aonuma, S., Hama, T., & Kametani, T. (1955) *Jap. J. Pharm. Chem.* 27, 607–611; thru (1956) *Chem. Abstr.* 50, 13292g
(15) Herbst, R. M., & Manske, R. H. (1943) *Org. Syn. Coll.* 2, 389–391
(16) Crossley, F. S., & Moore, M. L. (1944) *J. Org. Chem.* 9, 529–536
(17) Novelli, A. (1939) *J. Amer. Chem. Soc.* 61, 520–521

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METHAMPHETAMINE
PIT DERIVATIVE

1. Dissolve sample in water. If sample was obtained from a lab raid, use dilute H_2SO_4 and wash with CH_2Cl_2. (To remove phenylacetic acid and P-2-P.)

2. Make aqueous basic (drop of NH_4OH)

3. Extract with pet ether

4. Filter through cotton

5. Reduce volume (preferably over a steam bath) Heating drives off Methylamine.

6. Place melting point tube into PIT and place it in pet ether.

7. Let solution stand. White ppt. should form within 15 minutes.

8. Suck off liquid.
   Wash with more pet ether.
   Suck off pet ether and use blotter paper to remove all of the liquid.

MIXTURES
SEPARATION OF AMPHETAMINE
& METHAMPHETAMINE

AMPHETAMINE

↓

CH_2Cl_2

↓

PPT out w/ Mandelic acid

↓

Wash with basic water to wash out excess Mandelic acid

↓

PIT derivative or HCl salt of METHAMPHETAMINE

Infrared Method for Distinguishing Optical Isomers of Amphetamine

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THE NECESSITY to identify optically active drugs occurs frequently in forensic science. Quite often such drugs are identified as to the basic drug in question without determining the optical sign and indeed many drugs are found in both licit and illicit markets as only one isomer. Amphetamine and some other drugs, however, occur in the drug trade as *d*-, *dl*-, and *l*-isomers. A simple microcrystalline test (*1*) will distinguish *dl*-amphetamine from the *d*- or *l*-isomers but cannot distinguish *d*- from *l*-. The *d*- or *l*-isomers are distinguished by mixing the sample with the proper proportion of standard *d*- or *l*-amphetamine and observing a positive test for *dl*-amphetamine. The polarimeter will, of course, distinguish the isomers but this instrument is not available in many laboratories. A gas–liquid chromatographic method has also been developed (*2*) using *N*-trifluoroacetyl-(*l*)-prolyl chloride to form diastereoisomeric derivatives with *d*- and *l*-amphetamine. This author has developed a simple method by which three distinct infrared spectra can be produced for *d*-, *dl*-, and *l*-amphetamine as the *d*-mandelate salts.

EXPERIMENTAL

A water solution of any amphetamine salt (10–50 mg) is made basic and the amphetamine extracted into methylene chloride. The methylene chloride is passed through anhydrous sodium sulfate into a small beaker and concentrated to *ca.* 2 ml by heating on a steam bath. A saturated solution of *d*-mandelic acid in methylene chloride is added several drops at a time until the amphetamine is neutralized as determined by a drop of solution on pH paper. The beaker is then covered for several minutes, allowing the *d*-mandelate salt to crystallize and the solution is filtered using suction and the crystals washed with a small portion of methylene chloride. After drying, a KBr disk of the crystals is prepared and the infrared spectra are run.

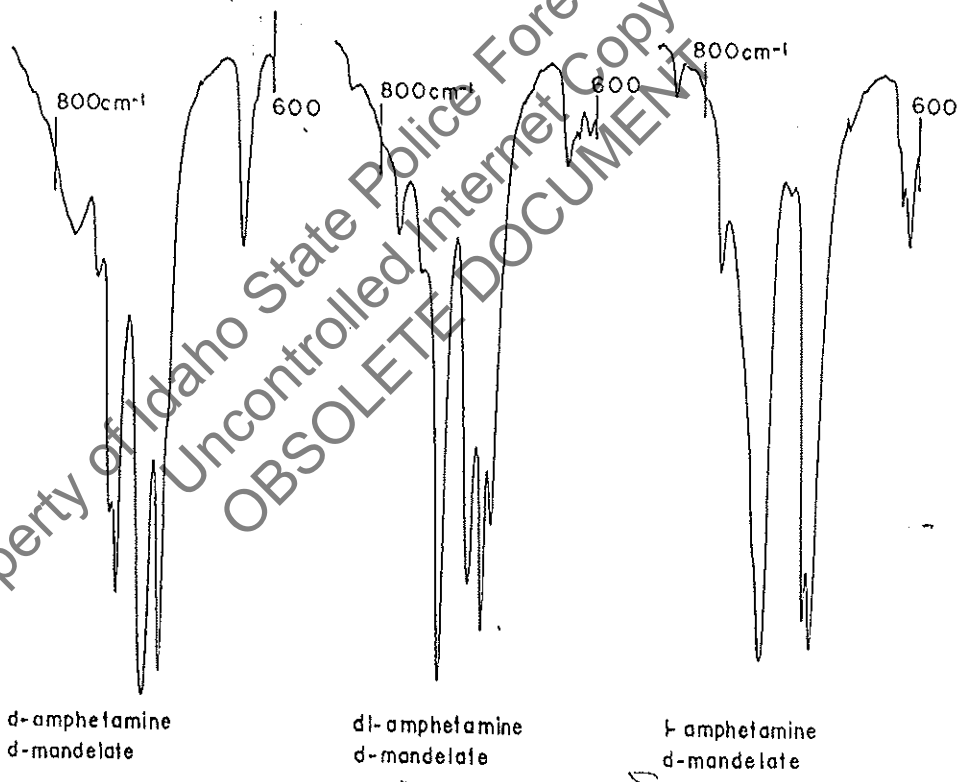

Figure 1. Infrared spectra of amphetamine *d*-mandelate salts in KBr between 800 and 600 cm^{-1}

RESULTS AND CONCLUSION

The resulting spectra of the different isomers show several differences, the greatest being in the 800–600 cm^{-1} region as illustrated in Figure 1. The differences in the three spectra are certainly sufficient to distinguish *d*-, *dl*-, and *l*-amphetamine. This method has been used successfully on several samples of illicit amphetamine tablets. Only impurities precipitated by mandelic acid will interfere and none have been encountered in samples. Other optically active drugs can probably be distinguished using *d*-mandelic or other acids.

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(1) Methods of Analysis of the A.O.A.C. 10th ed., 1965, William Horwitz, Ed., p 597.
(2) Clyde E. Wells, *J. Ass. Offic. Anal. Chem.*, 53, 113–115 (1970).

ACID BASE SHAKEOUT

Dissolve in dilute acid (can wash with solvent), make basic extract into pet ether or CH_2Cl_2 . Dry solvent through Na_2SO_4.

CH_2Cl_2 Extract:

Add small amount HCl directly or in methanol. Recrystallize with pet ether. May have to boil down with CH_2Cl_2 a few times to prevent two phases. Recrystallize with pet ether.

Pet Ether Extract:

Bubble HCl through the extract. Collect crystals.

(8)

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SEPARATION AND IDENTIFICATION OF AMPHETAMINE OR METHAMPHETAMINE IN COMBINATION WITH EPHEDRINE OR CAFFEINE

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Introduction

"White cross" tablets containing the following mixtures are being encountered in our laboratory: amphetamine with caffeine, amphetamine with ephedrine, and methamphetamine with ephedrine. The following rapid extraction procedure results in the isolation of the controlled substance and the subsequent identification by infrared spectroscopy.

Procedure

Grind up 1 tablet and place in a test tube. Add 0.5N NaOH and extract with an equal volume of hexane. Transfer hexane to a clean test tube. Wash the hexane 3 times with distilled water to remove the ephedrine or caffeine. Transfer the washed hexane layer to a clean test tube. Form the hydrochloride salt by bubbling HCl gas through the hexane. (The HCl gas can be obtained by withdrawing the vapors over conc. HCl with a disposable pipet equipped with a bulb). Precipitate the hydrochloride salt by centrifuging this mixture. Decant the hexane and dry the residue. Obtain the IR spectrum of the residue using a KBr pellet. (Chloroform may be used to transfer the residue to the mortar, followed by drying). Compare with standard amphetamine HCl (or methamphetamine HCl).

Remarks

This procedure is also suitable for routine analyses of suspected amphetamine tablets, but omitting the 3 water washes. In amphetamine samples containing relatively large amounts of caffeine, this one extraction procedure results in IR spectra clearly recognizable as being of amphetamine HCl (although slightly impure). Usually in the case of caffeine mixtures, one water wash is sufficient.

(9)

NOTES ON THE TEST TUBE METHOD FOR SEPARATION OF AMPHETAMINE OR METHAMPHETAMINE FROM EPHEDRINE OR CAFFEINE

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Introduction

Problems were encountered in the separation and crystallization of particularly methamphetamine when mixed with ephedrine or caffeine. The critical part of the procedure is to allow the test tube to set for 5 to 10 minutes after the hydrochloride salt forms. The following procedure with the minor changes is recommended.

Procedure

1. Follow the procedure of Stinson and Berry Microgram, Vol. VII, No. 4, April 1974.

2. For the usual white cross tablets - grind to powder one or two tablets, usually two. Shake NaOH and powder in corked test tube.

3. Add the NaOH, Hexane, distilled water with clean, individual pasteur pipets. Also use a clean pipet to transfer the Hexane layer.

4. Each time a shaking is necessary, use a corked test tube. Spin down each time using centrifuge.

5. After Hydrochloride Salt has been formed, spin down, set tube aside for 5 to 10 minutes to allow crystals to form on those spun to sides of test tube.

6. Pour off Hexane and allow the Hydrochloride Salt to dry in the test tube. A warm but not hot oven should be used.

7. KBR pellet can be formed by scraping the dried powder from the tube with a spatula.

Results

1. Resulting IR's are very sharp.

2. Procedure has been successful for suspected Amphetamine, Methamphetamine, MDA, and Methylphenidate. Other related compounds, which are soluable in Hexane, may also work.

CELITE COLUMN

Whether macro or micro, celite should be fluffy after addition of aqueous phase. Fluffy mixture must be packed down in the column.

As long as the aqueous phase retains the methamphetamine, adequate aqueous phase is present. The aqueous phase can be $_4$NHCl or 1 ml conc. HCl + 16.5g sodium chloride in 100 ml. The methamphetamine can be placed on the column in the aqueous phase or in water washed diethyl ether or chloroform. The methamphetamine can be eluted with water washed chloroform. The methamphetamine is eluted as the HCl salt. It can be recrystallized from the $CHCl_3$ with pet ether or heptane

36.531

Characteristics of Microchemical Tests for Barbiturates—Continued

Barbiturate	Crystal Form	Dichroism or Pleochroism	Remarks
Methylphenobarbital (5-Diethyl-1-methylbarbituric acid)	Dark needles, small to large, and splint blades.	Dichroism black to brown.	Good birefringence with crossed nicols.
5-Methyl-5-phenylbarbituric acid (Rutonal®)	Red-brown irregular platy forms appear after free acid is pptd.	Gradually strongly dichroic rods or blades.	Test fairly sensitive for dil. soln.
Phenobarbital (5-Ethyl-5-phenylbarbituric acid)	Soon crystallizes in little dark grains; also a few larger red blades and dark splinter-rods in clusters.	—	*Free acid may also crystallize out.*
Probarbital (5-Ethyl-5-isopropylbarbituric acid)	Scattered iodine-reaction crystals form in various jagged shapes, color dark brown to black dichroism, or red-black with but little dichroism.		Free acid thrown out, forming long rods with pointed ends.
Secobarbital (5-Allyl-5-(1-methylbutyl) barbituric acid)	Crystallizes in plates or elongate and rectangular but mostly distorted into any shape after 1 hr.	Light yellow to orange or red dichroism by polarized light.	Distinctly birefringent.
Sodium Pentobarbital (Sodium 5-ethyl-5-(1-methylbutyl) barbiturate)	Crystallizes quickly in great numbers of small red-brown plates.	Minute light-colored flakes exhibit dichroism; dark brown or black to yellow.	—
Talbutal (5-Allyl-5-sec-butylbarbituric acid)	Amorphous ppt crystallizes in large needles and dichroic blades, lighter to deeper brown, in dendrites; then gray-black curled sheaves of threads.	—	Excellent test. Both types of crystals have good birefringence.
Vinbarbital (5-Ethyl-5-(1-methyl-1-butenyl) barbituric acid)	Multitudes of small dark crystals, tiny grains and rods with dichroism brown to black. In quite dil. soln possible to get good small crystals, little dark rods with dichroism red to black, and small plates tending to be square, generally appearing red but with same dichroism, and with square extinction (not diagonal).		Very sensitive.

* This drug has barbiturate-type formula (although there is only one N) but is central nervous stimulant instead of depressant.

36.533 *Identification*

(a) *Direct test.*—Add drop of reagent to little of powd solid or crushed tablet and spread out on slide with little stirring. Do not stir to homogeneity as local concns and dilns will assist crystn. Let stand to evap. to higher acid conen if necessary for crystal formation.

(b) *Volatility test.*—Place small amt of substance or crushed tablet in depression of cavity slide, add drop 5% NaOH soln, and stir briefly. Place very small drop of reagent on thin slide, invert over cavity slide, and let stand. As crystals appear, examine with inverted slide in place. After observing crystals or after 1 hr or more exposure, if only few or no crystals form, reinvert thin slide with hanging drop, and let stand for gradual evapn of H_2O from reagent drop. Examine for crystals. Compare with descriptions, 36.534.

36.534 *See page 714.*

For Synthetics—Official Final Action

36.535 *Reagents*

(a) *Acetic acid.*—Dil. 6 ml HOAc to 100 ml with H_2O.

(b) *Ammoniacal nickel acetate soln.*—Mix 1 vol. 5% $Ni(OAc)_2.4H_2O$ soln with 1 vol. NH_4OH (2 + 3). Use clear supernatant.

(c) *Ammoniacal silver nitrate soln.*—*See* 36.525(a).

(d) *Ammonium thiocyanate soln.*—*See* 36.525(c).

(e) *Barium hydroxide soln.*—Satd aq. soln.

(f) *Benzaldehyde.*—NF quality.

(g) *Bismuth iodide soln.*—*See* 36.525(d).

(h) *Bromide-bromate soln.*—Dissolve 0.3 g $KBrO_3$ and 1.2 g KBr in H_2O, and dil. to 100 ml.

(i) *Glycerol-alcohol mixture.*—(1 + 1).

(j) *Gold bromide in hydrochloric acid soln.*—*See* 36.525 (g).

(k) *Gold chloride soln.*—*See* 36.525(h).

(l) *Iodine-potassium iodide soln.*—*See* 36.525(j).

(m) *Lead acetate soln.*—Dissolve 5 g $Pb(OAc)_2.3H_2O$ in H_2O and dil. to 100 ml.

(n) *Lead triethanolamine soln.*—Add 1 ml triethanolamine (tech. 90% is satisfactory) to soln of 1 g $Pb(OAc)_2.3H_2O$ in 20 ml H_2O. Slight turbidity does not interfere.

(o) *Magnesia mixture.*—Dissolve 5.5 g $MgCl_2.6H_2O$ and 14.0 g NH_4Cl in H_2O. Add 13.05 ml NH_4OH and dil. to 100 ml with H_2O.

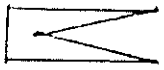
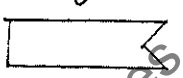
(p) *Mercuric chloride soln.*—*See* 36.525(l).

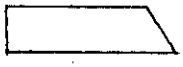
(q) *Mercurous nitrate soln.*—Dissolve 15 g $HgNO_3.H_2O$ in mixt. of 90 ml H_2O and 10 ml HNO_3 (1 + 9). Store in dark, amber bottle contg small globule of Hg.

(r) *Nitric acid.*—(1 + 1).

Differentiation of d-methamphetamine from dL-methamphetamine using gold chloride in H_3PO_4 directly.

d-methamphetamine

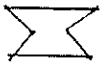
1. should have a number of good-sized crystals shaped  or 

2. should have some plates shaped  ,  etc.

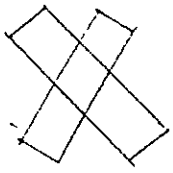
no perfect rectangles

3. Not many plates consisting of larger plates

dL-methamphetamine

1. Should have a number of good-sized crystals shaped  these fill to  which may finally fill to  the area shown by the dotted lines may be observed under crossed-polars.

2. Should have some larger crystals which are perfect and also some crystals which are not perfect in lengths.

3. tends to form larger plates in X's: 

36.534 *Characteristics of Microchemical Tests for Sympathomimetics*

Sympathomimetic	Reagent	Test	Description of Crystals
		Volatile Substances	
dl-Amphetamine	Gold chloride in dild phosphoric acid	direct or volatility	Very irregular plates, with irregular blade-arms especially after evapn; square if perfect.
	Platinic chloride in dild phosphoric acid	volatility	Irregular blades and needles, very low birefringence; after evapn, characteristic plates with narrow irregular arms of blades.
d-Amphetamine	Gold chloride in dild phosphoric acid	direct or volatility	Long yellow rods and blades; with evapn, some crystals as with *dl* may form.
	Platinic chloride in dild phosphoric acid	volatility	Long needles, often bent, very little birefringence, after some evapn, long rectangular blades. (*l*-Ephedrine in direct test gives similar crystals which are more sol.; it is less volatile and does not normally form crystals in hanging drop.)
Epinephrine	Sodium tetraphenylboron	volatility	$MeNH_2$ liberated; birefringent X's or 4-arm crystals; also thick blades with central rib, pointed ends, positive elongation.
Isoproterenol	Sodium tetraphenylboron	volatility	Isopropylamine liberated; plates tending to nonregular hexagons; no birefringence where plates lie flat but there are rods which are birefringent.
d- and *dl*-Methamphetamine (*d*- and *dl*-Desoxyephedrine)	Gold chloride in dild phosphoric acid	direct or volatility	Long blades and jointed crystals, fairly high birefringence.
	Platinic chloride in dild phosphoric acid	volatility	Grains with sharp edges which aggregate in chains and short prisms. Birefringent.
d-Methamphetamine	Bismuth iodide in dild sulfuric acid	volatility	Drops, long orange splinters, blades, needles; also deep red angular grains (red prisms only after evapn).
dl-Methamphetamine	Bismuth iodide in dild sulfuric acid	volatility	Drops, crystg in orange-red prisms with conspicuously slanting ends; inclined extinction ca 20°; also "mossy" formation of grains and some large deep red grains.
		Slightly Volatile Substances	
dl-Ephedrine (racephedrine)	Gold chloride in dild phosphoric acid	direct or volatility	Irregular plates based on the square growing along diagonals in 4 arms; some birefringent, some not.
	Bismuth iodide in dild sulfuric acid	volatility	Orange rods or sticks, short and stubby, some plates; more irregular plates on evapn.
l-Ephedrine	Gold chloride in dild phosphoric acid	direct or volatility	Long needles or splinters and long jointed forms, strong birefringence.
	Bismuth iodide in dild sulfuric acid	volatility	Long brownish-orange needles, often branching or in sheaves; also, especially with evapn, orange irregular blades.
Pseudoephedrine	Gold chloride in dild phosphoric acid	direct or volatility (2 hr)	Thin branching sticks, many like combs; some broaden to blades or spear-head plates; very high birefringence.
Phenylpropanolamine	Gold chloride in dild phosphoric acid	direct	Plates and blades of extremely high birefringence, elongate hexagonal or diamonds, very bright colors. Branch into 4 or 6 irregular arms.
		volatility (2 hr)	After definite drying, pyramidal grains to blades and plates with irregular arms, very birefringent.
Phenmetrazine	Gold chloride in dild phosphoric acid	direct or volatility	Rectangular plates joined in jagged arms or strongly birefringent crystals, often in X forms very characteristic.
	Bismuth iodide in dild sulfuric acid	volatility	Orange-red blades, usually pointed ends, often in rosettes; also with needles in branching aggregates; also red prisms.

I. Stimulant

A. Phenmetrazine.

B. Phendimetrazine.

C. Related drug substances.

II. Color tests

A. Marquis: non-purple.

B. Lieberman's: No reaction.

III. Ultraviolet spectrophotometry

A. Place portion of sample in 0.02N HCl.

B. Run UV scan with solvent reference cell 360-200.

C. Examine charts - note peaks.

D. Compare to known charts, reference materials or known substance.

IV. Infrared spectrophotometry

A. Prepare KBr window - neat sample.

B. Compare to known library reference spectrum or known substance.

Note: Depending on sample, may have to do an aqueous solvent extraction or celite/solvent column extraction for a cleanup.

V. Gas liquid chromatography

A. Extract sample in appropriate solvent.

B. Inject GC

1. Isothermal: OV-17 or SE-30, between 120°C and 190°C.

2. Programmed: OV-17 or SE-30, 100 to 200°C

C. Examine charts - note peaks.

D. Compare to charts of known substance under same conditions.

VI. TLC

A. Note systems in Clarke or Sunshine

B. Toxi-Lab

ISOLATION AND IDENTIFICATION OF MAJOR PRODUCTS AND REACTANTS OF CLANDESTINE AMPHETAMINE LABORATORIES

David K. Eaton and Glenn C. Harbison

INTRODUCTION

In the past several years, the north central counties of Texas have experienced a large increase in the number of operating clandestine amphetamine labs. According to the Texas Narcotics Officers Association approximately 60% of the illicit amphetamine in the U.S. is manufactured in Texas. Although the reaction is more involved than that for methamphetamine, the current state laws regarding the manufacture of controlled substances places amphetamine in a lower penalty group, while the street price for the two drugs is approximately the same. The number of labs is escalating rapidly, and more cases are seen each month.

Approximately 70% of the clandestine labs utilize the Leukart reaction to produce amphetamine. This reaction is a reductive amination of a ketone, in this case phenyl-2-propanone, by a reaction with formamide and formic acid. The product formed is not the amphetamine, but the N-formylamphetamine. The formyl group is then cleaved through a simple acid hydrolysis step with concentrated HCL. After neutralization with base, the amphetamine is removed by extraction with ether or other organic solvent. The amphetamine base is converted to the HCL salt by bubbling HCL gas through the solvent, and isolated by vacuum filtration. As the main starting material, phenyl-2-propanone is difficult to obtain legally, many labs also synthesize their own. In fact, many labs have started producing only phenyl-2-propanone, as no state statute regarding the legality of the manufacture of phenyl-2-propanone currently exists.

The majority of clandestine labs in this area of Texas synthesize phenyl-2-propanone by a reaction of phenylacetic acid and acetic anhydride, with anhydrous sodium acetate. The phenylacetic acid and acetic anhydride react in an acid-anhydride exchange reaction to form the mixed anhydride, benzyl methyl anhydride, which undergoes a 1,3 C-O shift and decarboxylation to form the phenyl-2-propanone. The reaction also forms other ketones, which make isolation and identification of the phenyl-2-propanone difficult.

The objective of this study is to offer varied methods and procedures for the identification and quantitation of amphetamine as well as its precursor, phenyl-2-propanone. These methods have been tested in our laboratory and were found to facilitate an otherwise difficult analysis for a laboratoty which does not have GC-MS capabilities. Several novel methods of analysis will be introduced, including the use of isopropylaniline as a GC internal standard. This compound was chosen because it is similar in boiling point and is a structural isomer of amphetamine. The compound also has retention times for both columns which did not coincide with the other coumpounds. Isopropylaniline is also a non-volatile liquid which allowed accurate weighing. The derivatization of phenyl-2-propanone and amphetamine to form easily analyzed solids will also be discussed.

EXPERIMENTAL

A Tracor 560 dual column gas chromatograph with flame ionization detectors was used for separation of the reaction mixtures. Two different columns were utilized in order to achieve the best separation of the different reactions. Column A was a 6' X 2mm ID packed with 3% OV-1 on Chromosorb W. Column B was a 6' X 2mm ID packed with 10% Carbowax 6000/5% KOH on Chromosorb W. The latter is a basic column, and is well suited for the separation of amines and other organic bases. Helium at 40 ml/min was used as the sheath gas, and the detector air and H_2 flow rates were adjusted for

maximum detector sensitivity. Column temperatures were optimized for each analysis, and are given for each separate chromatogram. Isopropylaniline at 2 mg/ml in $CHCl_3$ was used as an internal standard.

A Beckman Model 4250 IR spectrophotometer was used for final identification of the compounds of interest. Due to the difficulty of isolating the pure liquid compounds, the phenyl-2-propanone and amphetamine were converted to their solid derivatives by the reaction with 2,4-dinitrophenylhydrazine and phenylisothiocyanate respectively. The procedures are as follows:

Phenyl-2-propanone - In a small test tube, 2 drops of the phenyl-2-propanone were added to the 2,4 dinitrophenylhydrazine reagent. This reagent was prepared by dissolving 0.4 g of 2,4-dinitrophenylhydrazine in 3 ml of conc. H_2SO_4. Three ml of water were added with stirring until the solid dissolved. This solution was then diluted with 10 ml of 95% ethanol. Upon addition of the propyl-2-propanone, a bright yellow precipitate formed immediately. The precipitate was washed twice with cold methanol and isolated on filter paper. The methanol was allowed to evaporate and the precipitate was washed with hexane and allowed to dry.

Amphetamine - The amphetamine base was extracted into approximately 20 ml of hexane from the aqueous solution. Two drops of pure phenylisothiocyanate was added and the reaction was allowed to stand for 30 minutes, or until the white precipitate formed. The precipitate was isolated on filter paper and washed twice with hexane and allowed to dry.

The derivatives were then combined with KBr and pressed into KBr pellets. All other solutions were prepared with the respective pure compounds and disposable glassware and plasticware were used when possible to prevent contamination.

RESULTS AND DISCUSSION

The first step in analyzing a clandestine lab was defining which reaction was

being performed. The odor of the mixture was one of the best indicators. Once identified, the contents were weighed and an aliquot was taken and quantitated and the various components identified. As each reaction required a somewhat different clean-up procedure, each will be discussed separately.

1. Phenyl-2-propanone

The phenyl-2-propanone reaction has the pungent odor of acetic anhydride as well as the acetic acid formed during the reaction. This reaction also may have a solid yellow residue in the bottom of the reaction vessel after cooling. The residue is unreacted phenylacetic acid and sodium acetate. The liquid portion is usually reddish-brown to almost black.

Quantitation by GC was performed as a preliminary indication of the products within the reaction mixture. A known quantity of the reaction mixture was added to a separatory funnel with a small quantity of sat. Na_2CO_3 and 10 ml of 2 mg/ml isopropylaniline in chlorform. The mixure was swirled gently(Caution CO_2 evolved) and then shaken with frequent venting. Additional base was added until the aqueous layer was basic to pH paper. The organic layer was dried over Na_2SO_4 and analyzed by GC. Sample chromatograms are shown in Figure 1 and 2.

The final identification was by IR spectrophotometry. An aliquot was removed and treated with 6N NaOH. The mixture was swirled carefully, and the reddish organic layer allowed to float to the top. Additional base washs with 6N NaOH and sat. Na_2CO_3 removed the acidic reactants and bi-products, such as phenylacetic acid, acetic acid and acetic anhydride. The organic layer contains the phenyl-2-propanone and other organic bi-products. As mentioned previously, this reaction is not specific for phenyl-2-propanone, and the easiest method of purification of the phenyl-2-propanone was simple distallation. The majority of the other bi-products have boiling points higher than phenyl-2-propanone. The distallate between 190 and 210 ^{o}C was collected and washed with water in a separatory

funnel. The resulting pale yellow organic layer was dried over Na_2SO_4 and the 2,4-dinitrophenylhydrazine derivative prepared as discussed previously. The KBr pellet was prepared by our normal procedure. The resulting IR spectra is shown in Figure 3.

2. N-formylamphetamine

The reductive amination reaction to form N-formylamphetamine was recognized by the clear, reddish-brown reaction mixture and the lack of any solid material within the reaction flask. There was also little odor which characterize the other reactions. Quantitation by GC followed a similar procedure as that used for phenyl-2-propanone, and the chromatogram is shown in Figure 2.

N-formylamphetamine may be isolated by washing an aliquot in a separatory funnel with water and sat. Na_2CO_3. The reddish, brown organic layer will be fairly pure N-formylamphetamine. Final identification by IR may be of the pure N-formylamphetamine on a NaCl plate, or the N-formylamphetamine may be converted to the amphetamine through an acid hydrolysis and then converted to the phenylisothiocyanate derivative and pressed into a KBr pellet.

3. Amphetamine

The conversion of N-formylamphetamine to amphetamine was a simple acid hydrolysis of the amide with HCl. The reaction was recognized by the odor of HCl and the reddish-purple color. Quantitation of amphetamine followed a procedure similar to that of phenyl-2-propanone, except that column B was used. Conditions and a sample chromatogram are given in Figure 4. As noted, the Carbowax 6000/5% KOH allowed good separation of the reaction components and the internal standard. For final identification, an aliquot was removed and neutralized with base. Extraction with $CHCl_3$ removed the amphetamine base. The organic layer was extracted with 1N H_2SO_4. This removes the amphetamine as the sulfate salt, and leaves the phenyl-2-propanone and other neutral organics within the $CHCL_3$. The aqueous

layer was made basic with 6N NaOH and extracted with hexane followed by the formation of the phenylisothiocyanate derivative. The IR spectra is shown in Figure 5.

DISCUSSION

The following methods and materials have been found to be very useful for the identification and quantitation of the desired compounds. These methods, however, are for particular reactions and many different reactions exist for the production of both phenyl-2-propanone and amphetamine. The basic principles outlined previously may facilitate the develop of similar schemes for different reaction mixtures.

Techniques for the detection and identification of amphetamines and amphetamine-like substances

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ABSTRACT

This paper reviews analytical techniques for the detection and identification of amphetamines and amphetamine-like substances in non-biological samples. It shows the wide range of methods available, from simple testing procedures to the use of the most powerful instruments available in analytical chemistry. The following techniques are discussed: colour tests, microcrystal tests, ultraviolet spectrophotometry, fluorescence spectrometry, infra-red and Raman spectrophotometry, thin-layer chromatography, gas chromatography and high performance liquid chromatography.

Introduction

Amphetamines are stimulants of the central nervous system and have become a major class of drugs of abuse. The many closely related amphetamines present a challenge to the forensic chemist who must design analytical procedures to distinguish and identify the individual drugs. "Street drugs" are seldom pure; they are usually encountered as mixtures with cutting agents, excipients and other related drugs. In some situations it is necessary to identify optical isomers of amphetamines. By using multiple analyses and combining different methods, large amounts of analytical data are gathered. However, there will not be much improvement in analytical information unless discriminative power is improved through new or better methods of analysis.

This article reviews common methods used in the analysis of amphetamines from simple inexpensive methods to advanced instrumental analysis. The methods used to identify the individual drugs in street drug

samples are discussed briefly. Articles on the forensic analysis of drugs of abuse [1 – 3] and a textbook on amphetamines and related stimulants [4] have been published elsewhere.

Colour tests

Colour tests of different types are the most common form of preliminary drug screening. Commercial drug detection kits for field use are based on colour tests. The law enforcement agents who use the kits may not always understand the forensic chemistry involved; some may be under the misapprehension that the kit is a drug identification kit instead of a drug screening kit. Colour tests do not identify a drug but serve to narrow down the list of drugs possibly present in a sample. Clarke [5] has drawn up a comprehensive list of colour tests and has also developed a technique using microdrops.

Colour tests are not specific for a single drug but may give useful hints about chemical groups in a molecule. Amphetamine gives an orange colour with Liebermann's reagent (sulphuric acid and nitrous acid) and so will many compounds containing a monosubstituted benzene ring. With Marquis' reagent (sulphuric acid and formaldehyde), amphetamine gives an orange-brown colour as do phenformin (an antidiabetic) and other drugs. If no colour is observed with Marquis' reagent, it is fairly reasonable to assume that amphetamine is not present or is present in quantities below the detection limit.

The colour formation may be influenced by many factors, such as the reaction time, temperature, the stability and concentrations of the reactants and the presence of dyes or other substances giving colour reactions.

The assignment of colour to the result of a colour test is subjective; standardization of the colour assignment procedure would decrease the ambiguities associated with the interpretation of the colour. Velapoldi and Wicks [6] carried out a study of colour tests. The colours produced were assigned numbers and descriptions corresponding to colours in the ISCC-NBS central colour charts using colour chips and evaluating the colour lightness, saturation and hue. They concluded that a positive identification of a pure drug according to the colour produced with a single reagent is difficult and probably incorrect, even if the interpretation is by a trained investigator. They suggested that seven reagents would be necessary for a reasonable, multi-reagent testing scheme that would decrease the number of false positives and increase specificity. The use of multiple reagents in a logical fashion will help in sorting out the possible drug classes and in eliminating others.

The use of multiple colour tests generates large amounts of data that have to be interpreted. Johns and others [7] presented the colour test results

in tabular form, which facilitated manual or computer searches. The colour test results were assigned numerical codes. Given reliable and reproducible colour test results, the numerical code system can be time-efficient for handling large amounts of colour test data.

Colour tests work well with pure drugs. In spite of the improvements discussed above, however, the lack of specificity and ability to discriminate between chemically closely related compounds limit the colour tests to screening, which gives only preliminary and presumptive evidence. Law enforcement agencies that contemplate using colour test kits as field tests for amphetamines and other drugs would be well advised to plan with the forensic chemistry laboratory that is going to follow up the field screening tests with definite drug identification. It would be wise for law enforcement agencies to be very cautious about taking legal action on the basis of results obtained by colour test kits before a drug has been definitively identified.

Microcrystal tests

Microcrystal tests are not suited to systematic drug screening but are an excellent means of confirming results. They are performed under the microscope, mixing solutions of the sample with test reagent and observing the crystals formed. The tests vary in specificity. There is no systematic identification system, and it takes both time and skill to learn the tests. Closely related crystals may be reported as false positives and problems in getting the crystals to form at all may give false negative results.

Clarke [5] has listed many microcrystal tests. One example is a test to differentiate between related compounds such as amphetamines, phenmetrazine and phendimetrazine that give different crystal forms with picrolonic acid. Fulton [8] has written an extensive book on microcrystal tests. One of their particular uses is to distinguish optical isomers of a compound. Differentiation between *d*-, *l*- and the racemic *dl*-form of amphetamine can be made on the basis of microcrystal tests.[1]

Related to microcrystal tests are the crystallographic tests to determine such crystal optic properties as the refractive index and the extinction of the crystal. A simple but useful test to detect small amounts of amphetamine in a mixture is to put the mixture in alkaline solution. The amphetamine base is volatile, and it can be trapped in a hanging drop of diluted hydrochloric acid. The amphetamine hydrochloride is allowed to crystallize, and the crystals are observed between crossed polars under the microscope. The test can also be scaled up to a preparative isolation procedure, and the amphetamine hydrochloride can be analysed using spectrophotometry or chromato-

[1] Interested readers may refer to the works of Clarke [5] and Fulton [8].

graphy. When doing the colour or microcrystal tests, it may also be convenient to do traditional wet-chemistry ion tests for sulphate, phosphate and chloride.

Ultraviolet spectrophotometry

The ultraviolet (UV) absorption spectrum contains information about the chemical functional groups involved in electronic transition. It is not, however, specific enough to provide proof of identity for single drugs such as amphetamine. On the other hand, if a sample does not show any absorption in the 200 to 400 nm range, it is reasonable to conclude that the sample contains no amphetamine or that the quantity of amphetamine is too small to be detected.

More data may sometimes be obtained using multiple solvents with different polarities. Differences in solute-solvent interactions may give recognizable shifts in the ultraviolet absorption spectra and help to distinguish between compounds. Clarke [5] has listed ultraviolet absorption data for many amphetamines, most recorded in 0.1 N sulphuric acid. Recording ultraviolet absorption spectra at different pH may provide more information about compounds containing ionisable groups. The *Drug Identification Atlas* [9] contains ultraviolet absorption spectra recorded at pH 2 and pH 12 for many amphetamines. Ultraviolet spectrophotometry may be used to determine the quantity of amphetamines, provided that there are no other compounds in the sample that would interfere on the absorption band used for quantitation.

Amphetamines have rather low specific absorptivities in the ultraviolet region, however, and it may be better to use chromatographic methods to determine the quantity of amphetamine in street drug samples. Gill and others [10] used a diode array rapid-scanning spectrophotometer and found that the differential spectra made it easier to recognize benzenoid spectra which would otherwise be masked by a broad background absorption in a mixture. They also reported differential spectra to be of value in distinguishing amphetamine from benzphetamine and ephedrine from phenelzine.

Fluorescence spectrometry

Fluorescence spectrometry may be much more sensitive than ultraviolet spectrophotometry, and the characterization of a compound by spectra for excitation and for emission may give high specificity [11], although strongly fluorescent impurities may interfere. It is possible to make strongly fluorescent derivatives of amphetamines. Nix and Hume [12] reported on a procedure for the determination of amphetamine as a fluorescing lutidine derivative. The reported detection limit for the amphetamine derivative was

250 ng/ml in the cuvette. The method was claimed to be highly specific for amphetamine. However, phenethylamine and methamphetamine also formed fluorescent derivatives and might interfere if present. A fluorescamine derivative of amphetamine was studied by de Silva and Strojny using a xenon arc energy source [13] and later a dye laser as energy source [14] for the determination of amphetamine as the fluorescamine derivative.

Infra-red and Raman spectrophotometry

Organic molecules such as amphetamines absorb infra-red radiation under transitions between rotational/vibrational energy levels in the electronic ground state of the molecule. The fundamental, overtone and combination infra-red absorption bands produced give very characteristic spectra, ensuring good selectivity and capable of identifying a pure compound in almost all cases. Amphetamines in mixtures may have to be isolated to obtain good infra-red spectra. For screening purposes, infra-red spectrophotometry may yield good results provided that not too many interfering compounds are present and that the amphetamine concentration is reasonably high. Polymorphism may occur in some compounds and give slightly different spectra. There are collections of infra-red spectra of many amphetamines in Clarke [5] and the *Drug Identification Atlas* [9]. Optical isomers of amphetamines may be distinguished by infra-red spectrophotometry as isomeric mandelate derivatives [1, 15].

Computer-aided chemistry with digital storage of the spectra and rational programmes for search, identification and quantitation may be of great help to the forensic chemist handling and documenting large amounts of spectrophotometric data. Moss and others [16] used numerical taxonomy techniques in an attempt to classify the infra-red spectra of amphetamines and other drugs of abuse.

Raman and infra-red spectrometry are complementary techniques. Antisymmetric vibrations and polar groups are most easily studied by the infra-red technique, while symmetric vibrations and nonpolar groups are best studied by Raman, and the sample preparation is very simple. At the empirical level, both techniques provide excellent "fingerprint" spectra for qualitative identification of molecules [17].

Thin-layer chromatography

Thin-layer chromatography (TLC) is a low-cost and very versatile technique, and the choice of plates, stationary or mobile phases and visualization means is large. TLC may be run qualitatively with many samples in parallel, two-dimensionally, quantitatively or as a preparative technique. The chromatographic data may be documented as Rf values, by

comparison with standard drugs, or by preserving the plates with varnish, photocopying the plate or photographing it in colour. An improved version of TLC is high performance thin-layer chromatography (HPTLC). The favourable cost and effectiveness ratio for TLC makes it well suited for forensic chemistry laboratories that cannot afford expensive analytical instruments. To get the maximum amount of analytical information from the TLC plate, the chromatographic data should be interpreted by a forensic chemist.

TLC systems

Many types of TLC systems have been used to test for amphetamines. Much information can be found in Clarke [5] and the *Drug Identification Atlas* [9]. The various systems are described below.

Stead and others [18] noted that the use of a single TLC system was of little help in identifying an unknown compound; more information could be gained using multiple TLC systems. However, if the systems had correlated chromatographic properties, multiple systems would not give much further information. They stressed the evaluation of the discriminating power of a TLC system, the discriminating power being defined as the probability that two drugs selected at random can be separated by a TLC system. Twenty-nine TLC systems were evaluated and 4 systems were selected for basic drugs:

1. Methanol-ammonia (100 : 1.5)
2. Cyclohexane-toluene-diethylamine (75 : 15 : 10)
3. Chloroform-methanol (9 : 1)
4. Acetone

Silica gel was dipped in 0.1 M KOH and dried. For screening for basic drugs using TLC the chloroform-methanol system was best. The increase in the discriminating power for the combination of TLC systems for basic drugs was shown to be 0.860 for system 1 alone, 0.962 for systems 1 and 2, 0.988 for systems 1, 2 and 3 and 0.993 for all four systems. The spray reagents for basic drugs were put on to a plate; the reagents were, sequentially: ninhydrin solution, FPN solution (iron-III-chloride, perchloride acid, nitric acid in water), Dragendorff's reagent and acidified iodoplatinate. Marquis' reagent was put on another plate. Stead and others have pointed out that by standardizing TLC systems, chromatographic data can easily be transferred from one laboratory to another. They list Rf values for 794 drugs on eight TLC systems.

O'Brien and others [19] have used TLC to differentiate amphetamine and its major hallucinogenic derivatives with two solvent systems: *(a)* ethylacetate-methanol-water-ammonia (95 : 3.5 : 1.5 : 0.75); and *(b)* acetone-ammonia (100 : 0.5). The developed silica gel plates were

sequentially exposed to formaldehyde vapour, dipped into Mandelin's reagent, viewed under 366 nm UV radiation and dipped into a modified Dragendorff's reagent. Gallic acid or chromotropic acid were used as confirmatory reagents for methylenedioxy groups.

Bailey and others [20] reported the identification of the N-methylated analogs of hallucinogenic amphetamines using TLC, UV, infra-red, mass spectrometry (MS), nuclear magnetic resonance (NMR) and melting points. Six TLC systems were used to separate N-methylamphetamines. The developed plates were examined under 254 nm UV radiation and sprayed with ninhydrin or chromatropic acid.

Vinson and others [21] proposed the TLC system ethylacetate-methanol-ammonia (100 : 18 : 1.5) for the general screening of street drugs and the TLC system methanol-ammonia (100 : 1.5) as a confirmatory system for basic drugs. As a single visualization reagent they propose TCBI (N-2,6-trichloro-p-benzoquinoneimine).

Loh and others [22] chromatographed in less than five minutes the dansyl derivatives of amphetamine and methamphetamine in several TLC systems using 3 × 3 cm polyamide plates.

Cartoni and others [23] presented chromatographic data for amphetamines on silica, cellulose, polyamide and alumina developed in butanol-formic acid-water (20 : 1 : 2). A two-dimensional method was also reported on silica with the first TLC solvent system as before, the second system being n-amylalcohol-5 N ammonia (1 : 1). Four visualization reagents were used.

Brown and others [24] proposed a TLC screening system for street drugs using silica plates and ethylacetate-n-propanol-28 per cent ammonium hydroxide (40 : 30 : 30) as solvent system. The developing time was 70 minutes. The chromatograms were visualized at 254 nm UV radiation, using iodoplatinate spray for the amphetamines.

Genest and Hughes [25] analysed 2-amino-1-(2,5-dimethoxy-4-methyl)phenylpropane (STP) and other amphetamines in three different TLC systems. Sundholm [26] described a rapid TLC method for the separation of amphetamines and other drugs using a horizontal developing chamber with HPTLC plates, silica gel G 60 F_{254}, 10 × 10 cm. One half of the plate was immersed in 0.1 M $KHSO_4$ and dried. The developing solvent was methanol containing 0.0125 M KBr to reduce tailing, run simultaneously from both sides. Visualization was performed by UV, ninhydrin and iodoplatinate. The effect of various KBr concentrations on acidic silica and untreated silica was studied. The correlation factor was 0.63, and the discrimination power was 0.94 for Rf with untreated silica versus Rf with acidified silica at 0.01 M KBr. The compounds studied were divided into four groups according to their retention characteristics in the different systems. The first group contained amines with high basicity such as amphetamine with higher Rf value on the acidic silica than on the untreated

silica. The studied compounds could be nicely arranged into four groups. However, compounds such as amphetamine and ephedrine, with similar basicities, were not well resolved.

Eskes [27] reported a TLC procedure for the differentiation of the optical isomers of amphetamine and methamphetamine. The samples spotted on a silica plate were overspotted with an optically active reagent, and the plate was developed as usual. The optically active reagents used were N-trifluoroacetyl-L-prolylchloride (TPC) or N-benzyloxycarbonyl-L-prolylchloride (ZPC). Three developing solvents were used: chloroform-methanol (197:3), hexane-chloroform-methanol (10:9:1) and hexane-ethylacetate-acetonitrile-diisopropylether (2:2:2:1). Marquis' reagent was used for visualization.

Operation of TLC screening data

TLC may be used as the primary screening procedure to determine the Rf values. Searching lists of Rf values, and matching other chromatographic and spectrophotometric data in order to identify the unknown compound may be greatly facilitated by a rational computer system. McLinden and Stenhouse [28] reported a chromatography system for drug identification based on three types of TLC data from different solvents; two were gas chromatography (GC) data and one a UV parameter. A drug on a TLC plate was classified with reference to a series of standard drugs with defined zones that were coded alphabetically.

Gas chromatography

Since the introduction of GC as a method of separating and identifying sympatomimetic amines in 1962 [29, 30], numerous reports on the qualitative and quantitative determination of amphetamines and related compounds have been published. GC is today one of the most widely used methods of analysing these drugs. The technique is reliable, highly selective and very sensitive. The flame ionization detector (FID) is used as a universal detector. Selective detection of compounds containing nitrogen is offered by the nitrogen-phosphorus selective detector (NPD), and the electron capture detector (ECD) can be used after the formation of a suitable derivative.

Stationary phases

Many stationary phases have been used for gas-liquid chromatography (GLC) of amphetamines [31, 32]. Moffat and others [33] compared eight different stationary phases (SE-30, Apiezon L/KOH, OV-17, Carbowax

20 M/KOH, Carbowax 20 M, CDMS, DEGS/KOH, DEGS) and concluded that a low polarity phase such as SE-30 should be chosen as the preferred liquid phase for GLC of basic drugs. The retention index values of 62 basic drugs were reported. In a later study Moffat [34] compiled retention index values of 480 drugs on SE-30, which showed that the majority of amphetamines and amphetamine-like substances were separated. Moffat's paper reported retention indices arranged in alphabetical order of drug name and in ascending order of retention index. These data provide valuable information for drug identification. Huber and others [35] reported on retention index values for 43 stimulant drugs on four stationary phases (OV-101, OV-225, Apiezon L, PEG 20 M) and the correlation between retention index values on these four phases and the mass spectra of the drugs. The use of retention index values and temperature-programmed gas chromatography, which is very useful for drug screening, was discussed by Perrigo and Peel [36]. They reported retention index values of 289 compounds, including amphetamines, for SE-30, as well as retention index values for common compounds on OV-7 and OV-17.

These papers demonstrate that a non-polar phase such as SE-30 is the most popular liquid phase for the analysis of basic drugs. A large amount of data in the form of retention index values is available from the literature. However, some peak tailing of amphetamines occurs on SE-30, and there is also considerable use of KOH-treated stationary phases,[2] such as Apiezon/KOH and Carbowax 20 M/KOH.

Formation of derivatives

Derivative formation is often used as an aid to identification. The derivative formed with a particular reagent indicates the functional groups that an unknown drug may contain, and the retention characteristics of the derivatives provide additional data. Another important reason for the formation of derivatives is to increase the sensitivity of the analysis, either by improving the chromatographic behaviour of a drug or by forming derivatives which makes it possible to use a high sensitivity detector. Derivatives can be formed in a tube or on-column by injecting the sample solution along with the reagent onto the column.

Primary amines may be converted to Schiff's bases by aldehydes or ketones. Brochmann-Hansen and Baerheim Svendsen. [29] chromatographed the acetone and butanone derivatives of sympathomimetic amines. Schiff's bases were later studied by several groups.

Beckett and others [32] chromatographed a number of Schiff's bases and acyl derivatives on both SE-30 and Carbowax 20 M/KOH in order to

[2] The KOH coating of the support is done in order to reduce adsorptive effects.

identify stimulant drugs. O'Brien and others [37] formed trifluoroacetamide derivatives with trifluoroacetic anhydride to provide symmetrical peaks for the GLC analysis of amphetamine and phentermine on a OV-1 column.

In a screening of amphetamines Jain and others [38] chromatographed free bases on an Apiezon L/KOH column and trifluoroacetamide derivatives on a OV-17 column. On-column trifluoroacetylation was employed.

On-column derivatization was also used by Brettell [39] to form trifluoroacetyl derivatives of amphetamine analogues. MBTFA was used as the reagent and Carbowax 20 M/KOH as a stationary phase. Sharp symmetrical peaks were obtained.

On-column acylation with N-acylimidazoles as reagents has also been reported [40]. To eliminate interferences with amphetamine and methamphetamine from ephedrine and phentermine Budd and Leung [41] chromatographed the trifluoroacetamide derivatives on a SP-2510-DA column and on a SP-1240-DA column. These columns eliminated interferences with amphetamine, methamphetamine and n-propylamphetamine from ephedrine and β-phenetylamine and provided satisfactory separation between amphetamine and methamphetamine.

Optical isomers

Optical isomers can be separated by GLC by reacting a racemate or an unknown optical isomer with a chiral reagent to yield a diastereoisomeric mixture that may be separated on a normal GC column. Another approach is to resolve the racemate on a chiral phase. Several papers have been published on the separation of optical isomers of amphetamines by GLC [42–45].

Capillary columns

Recent years have been a period of innovation with regard to capillary gas chromatography. A new generation of glass capillary columns and fused silica columns has been created that combines high separation power with good column stability, flexibility and load capacity. The chemical bonding of stationary phases has been shown to increase the stability of the stationary phases compared with conventionally coated films. Modern gas chromatographs are designed for capillary columns and different injectors permit on-column injection, split injection and splitless injection of the sample. Important contributions to the analysis of amphetamines by capillary gas chromatography have been published in recent years.

Kinberger and others [46] used a 25-m fused silica capillary column deactivated with Carbowax 20 M and with SP 2100 as a stationary phase to

analyse underivatized stimulants. The gas chromatograph was equipped with a flame ionization detector and a split injector.

Schepers and others [47] compared retention index values for various drugs on three capillary columns with the corresponding values on packed columns.

Plotczyk [48] discussed considerations for optimizing on-column and splitless injection as part of a study of system discrimination and reproducibility.

A nitrogen detector and a 10-m glass capillary column coated with SP-2250 were used by Pettitt [49] for the rapid screening of drugs of abuse.

Alm and others [50] installed two differently coated columns in a common split-splitless injector and connected the column ends to an NPD and to an FID. Fused silica columns were used with immobilized stationary phases. One 11-m-long column with SE-54 as stationary phase was connected to an FID and the other, a 10-m-long column with OV-215 as stationary phase, was connected to an NPD. The relative retention times from both detector signals were calculated from two internal standards by use of a Basic program. The values obtained were then compared to empirically determined values of individual substances, and the names of compounds identified were printed on the chromatogram. This screening method was combined with a quantitative determination of the drugs. Amphetamine was quantitatively determined on the SE-54 column connected to the FID. With this dual-column system, considerable and accurate chromatographic information could be obtained from a single run. By using an auto-sampler in combination with the Basic program, both qualitative and quantitative analysis could be performed routinely.

Mass spectrometry

Mass spectrometry in combination with gas chromatography (GC-MS) is a very powerful analytical tool, but few laboratories have the resources to use GC-MS as a screening method. The principal use of this technique is in the confirmation of the identity of a drug tentatively identified by other techniques. MS of amphetamines has been described in several articles [32, 35, 51] as well as MS of their N-trimethylsilyl derivatives [52] and their N-mono-trifluoroacetyl derivatives [39].

High performance liquid chromatography

High performance liquid chromatography (HPLC) is a technique of major importance for the analysis of drugs of forensic interest. It is particularly suitable for drugs that can be troublesome to analyse by gas

chromatography because they are thermally degradeable, non-volatile or polar. The technique is non-destructive, and compounds can be isolated for identification by other methods. Several detectors are available such as the UV detector, the fluorimetric detector and the electrochemical detector; UV detection is most commonly employed in amphetamine analysis. The forensic applications of HPLC to the analysis of amphetamines have been briefly reviewed [53, 54], and a textbook on HPLC in forensic chemistry has been published [55].

Column solvent systems

Several HPLC systems have been reported for the analysis of amphetamines.

An important contribution was published by Jane [56]. The author used a 25 cm × 4.6 mm ID column packed with 6 µm silica (Partisil) and methanol-2 N ammonia-1 N ammonium nitrate (27 : 2 : 1) as a mobile phase. A wide range of drugs of abuse were examined, and relative retention times of 27 amphetamine-type stimulants were tabulated. The majority of the stimulants were separated using this system. A variation in the retention of the compounds was affected by changes in either the methanol-water ratio, the concentration of ammonia or the concentration of ammonium nitrate. Detection was made by UV absorption at 254 nm and quantitation by measurements of peak heights. Good column stability was obtained.

Reversed phase chromatography on µ-Bondapak C_{18} was reported by Twitchett and Moffat [57]; 30 compounds including amphetamine and methylamphetamine were studied, but the column efficiency for basic drugs was poor. Later Twitchett and others [58] chromatographed the same 30 compounds on a microparticulate cation-exchange column. Tolerable efficiency for most drugs was obtained but the authors observed that the column life was rather short.

To reduce peak tailing in reversed-phase HPLC, Gill and others [59] examined a series of eluents containing amines as part of the buffer system. Large improvements in peak shape were demonstrated, and practical guidelines for the selection of suitable amine additives were given.

Lurie [60] used reversed-phase ion-pair chromatography for the analysis of drugs of forensic interest. The optimum resolution of amphetamines was with µ-Bondapak C_{18} and methanol-water-acetic acid (20 : 79 : 1) with 0.02 M methane-sulphonic acid as counter-ion at pH 3.5. Detection was made by UV absorption at 254 nm.

Flanagan and others [61] chromatographed basic drugs on a silica column with non-aqueous ionic eluents and reported retention data of a variety of compounds. A Spherisorb 5 silica column was used, and the eluent

was methanol-hexane (85 : 15) containing perchloric acid. The non-aqueous ionic systems showed high efficiency, stability and reproducibility and gave long column-life.

Multi-detection and multi-column systems

The identification of drugs using HPLC with dual wavelength UV detection was reported by Baker and others [62]. The column was connected to a 254-nm detector then in series to a 280-nm detector enabling the absorbance ratio of the drugs at 254 nm to be determined. Three column solvent systems were used: a μ-Bondapak C_{18} with methanol-water (2 : 3) pH 7 with phosphate buffer, a μ-Porasil column with methanol-2 N ammonia-1 N ammonium nitrate (27 : 2 : 1) and a μ-Porasil column with dichloromethane and ammonia; 101 drugs were tested and only 9 per cent of the drugs could be distinguished using relative retention times alone, while when both the retention times and absorbance ratios were used, 95 per cent of the drugs could be distinguished.

Isocratic multi-column HPLC was reported by Wheals [63] as a technique for the qualitative analysis of basic drugs. Three columns were studied, a column packed with silica (Si), a mercaptopropyl bonded phase (SH) and a column packed with an aliphatic strong cation exchanger (SCX). The eluent was methanol-2 N ammonia-1 N ammonium nitrate (27 : 2 : 1); 161 drugs, including amphetamines, were studied. The retention sequence was found to parallel closely the order of increasing basicity of the drugs.

Although HPLC has not been extensively applied to stimulant drugs, these reports show that several systems can be used to separate and identify them. The system devised by Jane [56] is particularly well documented in the literature. Retention data used in combination with data obtained from either stopped-flow UV scanning or multiple wavelength monitoring can provide a very effective method of characterizing compounds. Both column technology and instrumentation have been greatly improved during the last years. The new generation of diode-array spectrophotometers used as HPLC detectors represent a significant improvement. These detectors provide simultaneously chromatographic and spectral data on drugs.

Concluding remarks

A number of methods are available for the detection and identification of amphetamines, from simple testing procedures to the use of the most powerful instruments available in analytical chemistry. Simple and low-cost screening techniques can be used in many cases, however, and it is not always necessary to depend on sophisticated instruments. The strategy for solving a particular problem will depend on the expertise and experience built up in

the laboratory, as well as on the time and the facilities available. It is, however, not advisable to rely only upon non-separatory methods, because street drugs are seldom pure.

At present IR and UV spectroscopy are the most frequently used spectroscopic methods, and TLC and GLC are the work-horses of the separation methods. HPLC has also much to offer in terms of separation power, selectivity and reproducibility. The information derived from chromatographic and spectroscopic analyses confirms the identity of a drug.

Forensic drug identification and quantitation has progressed rapidly during the last years. Large improvements both in instrumentation and separation technology have been seen. This progress will certainly continue, and analysts must keep themselves up-to-date not only with scientific literature but also with developments in analytical instrumentation.

References

1. P. B. Baker and C. F. Phillips, "The forensic analysis of drugs of abuse", *The Analyst*, vol. 108, 1983, pp. 777–807.
2. T. A. Gough and P. B. Baker, "Identification of major drugs of abuse using chromatography", *Journal of Chromatographic Sciences*, vol. 20, 1982, pp. 289–329.
3. T. A. Gough and P. B. Baker, "Identification of major drugs of abuse using chromatography. An update", *Journal of Chromatographic Sciences*, vol. 21, 1983, pp. 145–153.
4. J. E. Caldwell, ed., *Amphetamines and Related Stimulants: Chemical, Biological, Clinical and Sociological Aspects* (Boca Raton, Florida, CRC Press, 1980).
5. C. G. C. Clarke, *Isolation and Identification of Drugs*, vol. I, II (London, The Pharmaceutical Press, 1969 and 1975).
6. R. A. Velapoldi and S. A. Wicks, "The use of chemical spot test kits for the presumptive identification of narcotics and drugs of abuse", *Journal of Forensic Sciences*, vol. 19, 1974, pp. 636–654.
7. S. A. Johns, A. A. Wist and A. R. Najam, "Spot tests: a colour chart reference for forensic chemists", *Journal of Forensic Sciences*, vol. 24, 1979, pp. 631–649.
8. C. C. Fulton, *Modern Microcrystal Tests for Drugs* (New York, Wiley-Interscience, 1969).
9. *Drug Identification Atlas*, vol. I–V (Ottawa, Agriculture Canada, Race Track Division).
10. R. Gill, T. S. Bal and A. C. Moffat, "The application of derivative UV-visible spectroscopy in forensic toxicology", *Journal of Forensic Science Society*, vol. 22, 1982, pp. 165–171.
11. H. H. Willard and others, *Instrumental Methods of Analysis*, 6th ed. (New York, D. von Nostrand Co., 1981), pp. 6–9.

12. C. R. Nix and A. S. Hume, "A spectrophotofluorometric method for the determination of amphetamine", *Journal of Forensic Sciences*, vol. 15, 1970, pp. 595 – 600.

13. J. A. F. de Silva and N. Strojny, "Spectrofluorometric determination of pharmaceuticals containing aromatic or aliphatic primary amino groups as their fluorescamine (Fluram) derivatives", *Analytical Chemistry*, vol. 47, 1975, pp. 714 – 718.

14. N. Strojny and J. A. F. de Silva, "Laser induced fluorimetric analysis of drugs in biological fluids", in *Lasers in Chemical Analysis*, G. M. Hieftje, J. C. Travis and F. E. Lytle, eds. (Clifton, New Jersey, Humana Press, 1981), pp. 225 – 236.

15. J. A. Heagy, "Infrared method for distinguishing optical isomers of amphetamine", *Analytical Chemistry*, vol. 42, 1970, p. 1459.

16. W. W. Moss, F. T. Posey and P. C. Peterson, "A multivariate analysis of the infrared spectra of drugs of abuse", *Journal of Forensic Sciences*, vol. 25, 1980, pp. 304 – 313.

17. J. G. Grasseli, M. K. Snavely and B. B. Bulkin, *Chemical Applications of Raman Spectroscopy* (New York, John Wiley & Sons, 1981), pp. 2 – 4.

18. A. H. Stead and others, "Standardised thin-layer chromatographic systems for the identification of drugs and poisons", *The Analyst*, vol. 107, 1982, pp. 1106 – 1168.

19. B. A. O'Brien, J. M. Bonicamp and D. W. Jones, "Differentiation of amphetamine and its major hallucinogenic derivatives using thin-layer chromatography", *Journal of Analytical Toxicology*, vol. 6, 1982, pp. 143 – 147.

20. K. Bailey and others, "Identification of the N-methylated analogs of the hallucinogenic amphetamines and some isomers", *Journal of the Association of Official Analytical Chemists*, vol. 58, 1975, pp. 62 – 69.

21. J. A. Vinson, J. E. Hooyman and C. E. Ward, "Identification of street drugs by thin-layer chromatography and a single visualization reagent", *Journal of Forensic Sciences*, vol. 20, 1975, pp. 552 – 556.

22. H. H. Loh and others, "Mini thin-layer chromatography III. A rapid and sensitive method for the estimation of amphetamine and methamphetamine", *Journal of Chromatography*, vol. 68, 1972, pp. 289 – 293.

23. G. P. Cartoni, M. Lederer and F. Polidori, "Some chromatographic and electrophoretic data for amphetamine-like drugs", *Journal of Chromatography*, vol. 71, 1972, pp. 370 – 375.

24. J. K. Brown, L. Shapazian and G. D. Griffin, "A rapid screening procedure for some street drugs by thin-layer chromatography", *Journal of Chromatography*, vol. 64, 1972, pp. 129 – 133.

25. G. Genest and D. W. Hughes, "Chromatographic methods for the identification of the new hallucinogen, 4-methyl-2,5-dimethoxy-α-methylphenethylamine, and related drugs", *The Analyst*, vol. 93, 1968, pp. 485 – 489.

26. E. G. Sundholm, "More economical use of high performance thin-layer plates for chromatographic screening of illicit drug samples", *Journal of Chromatography*, vol. 265, 1983, pp. 293 – 300.

27. D. Eskes, "A procedure for the differentiation of the optical isomers of amphetamine and methamphetamine by thin-layer chromatography", *Journal of Chromatography*, vol. 117, 1976, pp. 442 – 444.

28. V. J. McLinden and A. M. Stenhouse, "A chromatography system for drug identification", *Forensic Science International*, vol. 13, 1979, pp. 71 – 79.

29. E. Brochmann-Hansen and A. Baerheim Svendsen, "Separation and identification of sympathomimetic amines by gas-liquid chromatography", *Journal of Pharmaceutical Sciences*, vol. 51, 1962, pp. 938 – 941.

30. K. D. Parker, C. R. Fontan and P. L. Kirk, "Separation and identification of some sympathomimetic amines by gas chromatography", *Analytical Chemistry*, vol. 34, 1962, pp. 1345 – 1346.

31. D. E. van Zwol, "Gas chromatographic separation and identification of some sympathomimetic amines in use in anorexigenic drugs", *Journal of Chromatography*, vol. 24, 1966, pp. 26 – 31.

32. A. H. Beckett, G. T. Tucker and A. C. Moffat, "Routine detection and identification in urine of stimulants and other drugs, some of which may be used to modify performance in sport", *Journal of Pharmacy and Pharmacology*, vol. 19, 1967, pp. 273 – 294.

33. A. C. Moffat, A. H. Stead and K. W. Smalldon, "Optimum use of paper, thin-layer and gas-liquid chromatography for the identification of basic drugs. III Gas-liquid chromatography", *Journal of Chromatography*, vol. 90, 1974, pp. 19 – 33.

34. A. C. Moffat, "Use of SE-30 as a stationary phase for the gas-liquid chromatography of drugs", *Journal of Chromatography*, vol. 113, 1975, pp. 69 – 95.

35. J. F. K. Huber, E. Kenndler and G. Reich, "Quantitation of the information content of multi-dimensional gas chromatography and low-resolution mass spectrometry in the identification of doping drugs", *Journal of Chromatography*, vol. 172, 1979, pp. 15 – 30.

36. B. J. Perrigo and H. W. Peel, "The use of retention indices and temperature-programmed gas chromatography in analytical toxicology", *Journal of Chromatographic Sciences*, vol. 19, 1981, pp. 219 – 226.

37. J. E. O'Brien and others, "Determination of amphetamine and phentermine in biological fluids", *Journal of Chromatographic Sciences*, vol. 10, 1972, pp. 336 – 341.

38. N. J. Jain and others, "Rapid screening and confirmation of amphetamine, methamphetamine, methadone, and methadone metabolite in urine by gas/thin layer chromatography", *Journal of Chromatographic Sciences*, vol. 14, 1976, pp. 293 – 295.

39. T. A. Brettell, "Analysis of N-mono-trifluoroacetyl derivatives of amphetamine analogues by gas chromatography and mass spectrometry", *Journal of Chromatography*, vol. 257, 1983, pp. 45 – 52.

40. G. Brugaard and K. E. Rasmussen, "Quantitative gas-liquid chromatography of amphetamine, ephedrine, codeine and morphine after on-column acylation", *Journal of Chromatography*, vol. 147, 1978, pp. 476 – 480.

41. R. D. Budd and W. I. Leung, "Gas-liquid chromatographic separation of trifluoroacetamide amphetamine drugs", *Journal of Chromatography*, vol. 179, 1979, pp. 355–356.

42. C. E. Wells, "Collaborative study of the gas liquid chromatographic method for determination of stereochemical compositions of amphetamine", *Journal of the Association of Official Analytical Chemists*, vol. 55, 1972, pp. 146–148.

43. R. W. Souter, "Gas chromatographic resolution of enantiomeric amphetamines and related amines: I. Structural effects on some diastereomer separation", *Journal of Chromatography*, vol. 108, 1975, pp. 265–274.

44. J. Gal, "Stereochemistry of metabolism of amphetamines: use of (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride for GLC resolution of chiral amines", *Journal of Pharmaceutical Sciences*, vol. 66, 1977, pp. 169–172.

45. J. H. Liu and others, "Approaches to drug sample differentiation. III: A comparative study of the use of chiral and achiral capillary column gas chromatography/mass spectrometry for the determination of methamphetamine enantiomers and possible impurities", *Journal of Forensic Sciences*, vol. 27, 1982, pp. 39–48.

46. B. Kinberger, A. Holmén and P. Wahrgren, "Determination of underivatized CNS-stimulants and methadone in urinary extracts by glass capillary gas chromatography", *Journal of Chromatography*, vol. 207, 1981, pp. 148–151.

47. P. Schepers and others, "Applicability of capillary gas chromatography to substance identification in toxicology by means of retention indices", *Journal of Forensic Sciences*, vol. 27, 1982, pp. 49–60.

48. L. L. Plotczyk, "Application of fused-silica capillary gas chromatography to the analysis of underivatized drugs", *Journal of Chromatography*, vol. 240, 1982, pp. 349–360.

49. B. C. Pettitt, "Rapid screening for drugs of abuse with short glass capillaries and a nitrogen selective detector", *Journal of High Resolution Chromatography & Chromatography Communications*, vol. 5, 1982, pp. 45–47.

50. S. Alm and others, "Simultaneous gas chromatographic analysis of drugs of abuse on two fused silica columns of different polarity", *Journal of Chromatography*, vol. 254, 1983, pp. 179–186.

51. J. R. Reisch and others, "Mass spectra of derivatives of phenylalkylamines", *Journal of Pharmacy and Pharmacology*, vol. 20, 1968, pp. 81–86.

52. R. T. Coutts and others, "A note on the synthesis and gas chromatographic mass-spectrometric properties of N-(trimethylsilyl)-acetates of amphetamine and analogs", *Journal of Chromatographic Sciences*, vol. 17, 1979, pp. 350–352.

53. B. B. Wheals, "Forensic aspects of high-pressure liquid chromatography", *Journal of Chromatography*, vol. 122, 1976, pp. 85–105.

54. B. B. Wheals and I. Jane, "Analysis of drugs and their metabolites by high performance liquid chromatography", *The Analyst*, vol. 102, 1977, pp. 625–643.

55. I. S. Lurie and I. D. Wittver, *High Performance Liquid Chromatography in Forensic Chemistry* (New York, Marcel Dekker, 1983).

56. I. Jane, "The separation of a wide range of drugs of abuse by high-pressure liquid chromatography", *Journal of Chromatography*, vol. 111, 1975, pp. 227–233.

57. P. J. Twitchett and A. C. Moffat, "High-pressure liquid chromatography of drugs. An evaluation of an octadecylsilane stationary phase", *Journal of Chromatography*, vol. 111, 1975, pp. 149–157.

58. P. J. Twitchett, A. E. P. Gorvin and A. C. Moffat, "High-pressure liquid chromatography of drugs. An evaluation of a micro-particulate cation-exchange column", *Journal of Chromatography*, vol. 120, 1976, pp. 359–368.

59. R. Gill, S. P. Alexander and A. C. Moffat, "Comparison of amine modifiers used to reduce peak tailing of 2-phenylethylamine drugs in reversed phase high-performance liquid chromatography", *Journal of Chromatography*, vol. 247, 1982, pp. 39–45.

60. I. S. Lurie, "Improved isocratic mobile phases for the reversed phase ion-pair chromatographic analysis of drugs of forensic interest", *Journal of Liquid Chromatography*, vol. 4, 1981, pp. 399–408.

61. R. J. Flanagan and others, "High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents", *Journal of Chromatography*, vol. 247, 1982, pp. 15–37.

62. J. K. Baker, R. E. Skelton and C.-Y. Ma, "Identification of drugs by high-pressure liquid chromatography with dual wavelength ultraviolet detection", *Journal of Chromatography*, vol. 168, 1979, pp. 417–427.

63. B. B. Wheals, "Isocratic multi-column high-performance liquid chromatography as a technique for qualitative analysis and its application to the characterisation of basic drugs using aqueous methanol solvent", *Journal of Chromatography*, vol. 187, 1980, pp. 65–85.

I. Depressants:

A. Phenobarbital

B. Amobarbital

C. Pentobarbital

D. Secobarbital

E. Butabarbital (usually seen in combination with analgesics)

F. Phenytoin (hydantoins)

G. Glutethimide

II. Color Tests:

A. Liebermans - no reaction or faint yellow color.

B. Dille-Koppanyi - violet ring.

C. Zwikker's - purple, violet or lavender lower layer; phenobarb usually gives a negative test. A bright blue color may be aspirin; this may mask the purple color of a barbiturate. A green lower layer may be a thiobarbital.

III. Ultraviolet spectroscopy - dissolve sample in .01N NaOH and run UV peak at 240 nm. Make sample acidic with small amount of concentrated HCl run UV again - peak at 240 nm disappears. This method does not differentiate barbs.

IV. Thin Layer chromatography

A. Toxi-lab gives rapid separation.

B. Other systems available, see Clarke.

V. Microcrystal tests

A. Wagenaars reagent

B. Davis reagent

VI. Gas chromatography

A. Isothermal, OV-17 or SE-30 between 160°C and 190°C.

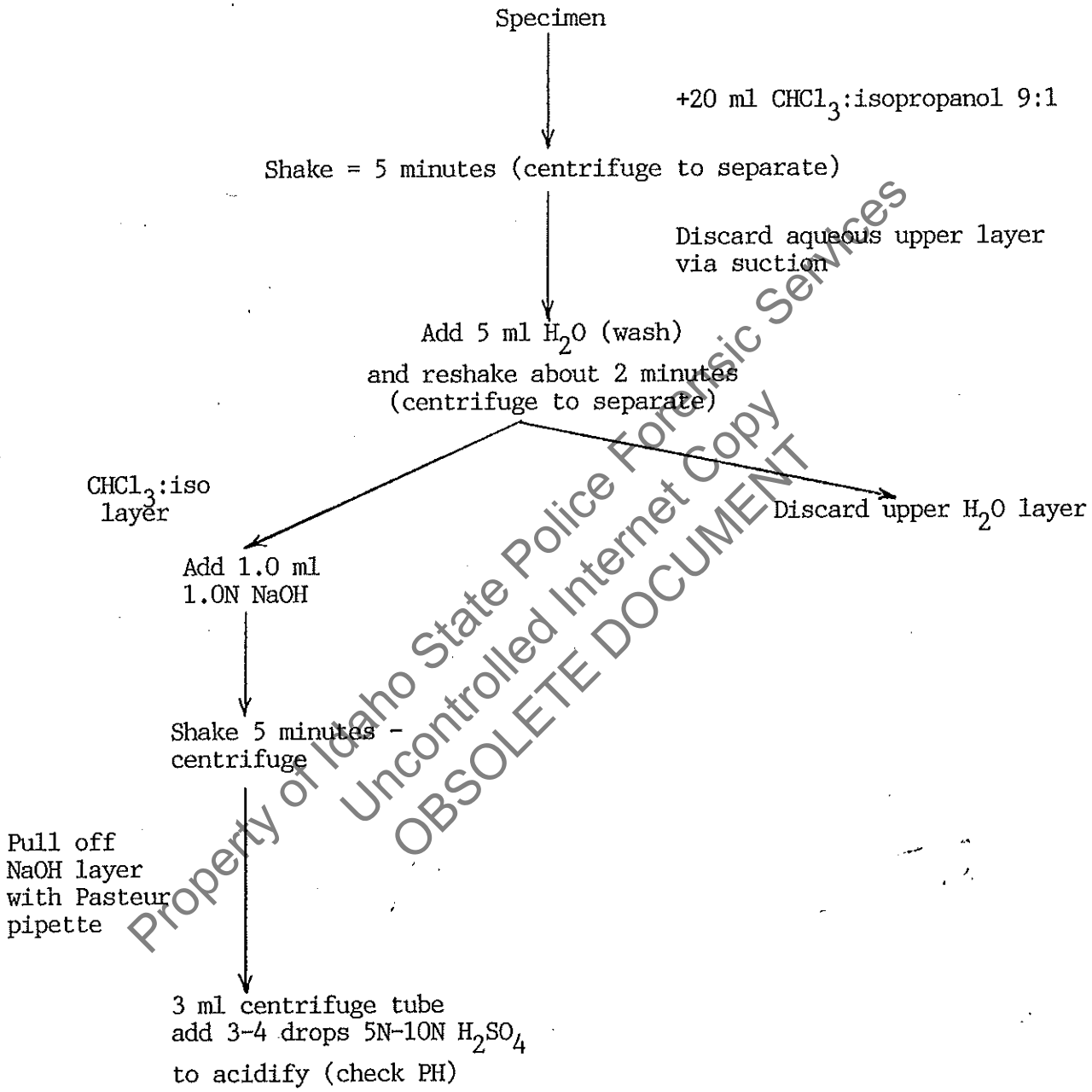
B. Can also refer to specialized methods in toxicology section of this manual and/or Clarke.

VII. Infrared

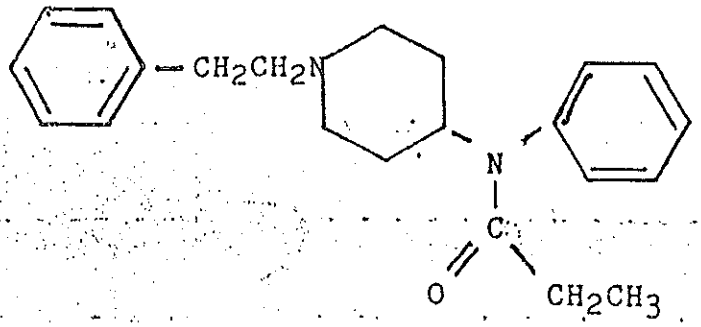
A. KBr pellet

GLC Barbiturate Extraction Procedure

Specimen: Urine, blood, gastric, bile



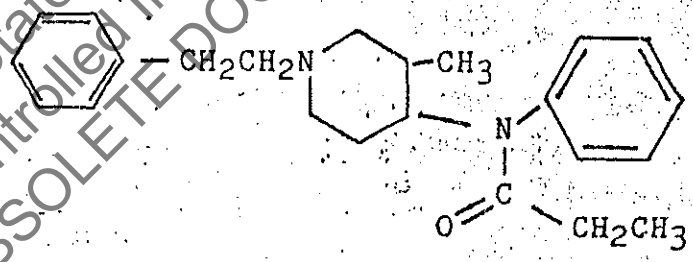
Then add ≅ 100 ul $CHCl_3$:isopropanol and Vortex.
Centrifuge to separate. Inject lower layer.



DESIGNER DRUGS

PRESENTED BY THE DEA SAN FRANCISCO

FIELD DIVISION



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DESIGNER DRUGS ARE A WAY OF WORKING AROUND AN EXPLICIT LEGAL DEFINITION OF A DRUG.

DESIGNER DRUGS ARE DRUGS MADE IN IMITATION OF KNOWN DRUGS AND HAVE THE SAME ACTION

BUT HAVE DIFFERENT CHEMICAL STRUCTURES TO AVOID:

1. PATENTS,
2. CONTROLLED SUBSTANCES ACT,
3. PROBLEMS IN SOURCE OF SUPPLY: I.E. OPIUM FOR HEROIN.

AT PRESENT, WE HAVE PROBLEMS WITH THREE TYPES OF DESIGNER DRUGS:

FENTANYLS (HEROIN SUBSTITUTES)

MEPERIDINES (HEROIN SUBSTITUTES)

AMPHETAMINES (MIND ALTERING SUBSTANCES)

THESE THREE TYPES ARE DISCUSSESD ON THE FOLLOWING PAGES.

FENTANYLS

FENTANYL AND ITS ANALOGS ARE DANGEROUS; CAN EVEN BE FATAL.

THEY HAVE THE POTENTIAL FOR ARRESTING THE CENTRAL NERVOUS SYSTEM AND CAUSING DEATH UPON INHALATION OF EVEN AN INFINITESIMAL AMOUNT.

THEY ARE ABSORBED THROUGH THE EYES, NOSE, EARS, MOUTH, AND SMALL CUTS. SINCE THEY ARE VERY FAT SOLUBLE, IT IS PROBABLE THAT THEY CAN BE ABSORBED THROUGH THE SKIN.

THE USUAL CAUSE OF DEATH WOULD BE BY DEPRESSION OF RESPIRATION. THE VICTIM MIGHT NOT BE AWARE HE IS NOT BREATHING. WHEN THE POSSIBILITY EXISTS OF THE PRESENCE OF SUCH COMPOUNDS, THE "BUDDY SYSTEM" SHOULD BE IMPLEMENTED. WATCH YOUR ASSOCIATES AND WATCH YOURSELF FOR PROPER BREATHING. IF A PERSON IS OVERDOSED, PROVIDE RESUSCITATION AND RUSH TO EMERGENCY MEDICAL AID. NARCOTIC ANTAGONISTS SUCH AS NALOXONE SHOULD BE ADMINISTERED.

IF A SUSPECTED FENTANYL LABORATORY MUST BE ENTERED, YOU MUST WEAR PROTECTIVE CLOTHING, GLOVES, FACE MASK, ETC., SO THAT NO DUST CAN TOUCH YOUR SKIN OR BE INHALED. CLOTHING SUCH AS THAT USED TO DECONTAMINATE RADIOACTIVE SITES IS RECOMMENDED. IN MANY SUCH LABS THE FUMES WILL BE SO BAD YOU WILL HAVE TO WEAR FULL SELF-CONTAINED BREATHING DEVICES TO WORK IN THE AREA. USE GREAT CAUTION BEFORE USING FANS TO VENTILATE THE LAB; YOU MUST NOT RISK BLOWING PARTICLES OF FINISHED PRODUCT INTO THE SURROUNDING AREA.

FENTANYL AND ITS ANALOGS

NAME	SCHEDULE	DATE	POTENCY	APPROXIMATE DOSE
FENTANYL	SCHEDULE II	10-70	80 TIMES MORPHINE	125 MICROGRAMS
ALPHA METHYL-FENTANYL	SCHEDULE I	09-81	300 TIMES MORPHINE	30 MICROGRAMS
SUFFENTANYL	SCHEDULE II	05-84	400 - 800 TIMES MORPHINE	25 MICROGRAMS
ALFENTANYL	SCHEDULE I	08-84	20 TIMES MORPHINE	500 MICROGRAMS
3-METHYL-FENTANYL	SCHEDULE I	04-85	1000 TIMES MORPHINE	10 MICROGRAMS
LOFENTANYL	NOT SCHEDULED		4000 TIMES MORPHINE	2.5 MICROGRAMS

FENTANYL WAS FIRST REPORTED IN THE PATENT LITERATURE BY THE BELGIAN PHARMACEUTICAL COMPANY JANSSEN PHARMACEUTICAL IN 1965.

IN 1968, FENTANYL WAS INTRODUCED CLINICALLY IN THE UNITED STATES AS AN INTRAVENOUS ANESTHETIC/ANALGESIC AGENT UNDER THE TRADE NAME "SUBLIMAZE." FENTANYL IS ALSO MARKETED IN A MIXTURE WITH A TRANQUILIZER UNDER THE TRADE NAME "INNOVAR." THIS MIXTURE IS USED FOR POSTOPERATIVE PAIN AND STRESS.

SUFENTANYL IS SOLD AS AN INTRAVENOUS ANALGESIC UNDER THE TRADE NAME "SUFENTA." AT LEAST TWO OTHER FENTANYL ANALOGS--ALFENTANYL AND LOFENTANYL--WILL PROBABLY BE INTRODUCED INTO CLINICAL MEDICINE IN THE U.S. WITHIN THE NEXT FEW YEARS. ALFENTANYL IS SOLD IN EUROPE UNDER THE TRADE NAME "RAPIFEN."

THE DILUENTS FOR STREET DOSES OF THE FENTANYL COMPOUNDS HAVE BEEN MANITOL AND LACTOSE. IN ADDITION, 3-METHYLFENTANYL IS SOMETIMES MIXED WITH COCAINE AND SOLD AS COCAINE FOR SNORTING.

IT HAS BEEN REPORTED THAT COCAINE IS AT TIMES BEING SOLD IN SEPARATE PACKETS AS A POTENTIAL ANTIDOTE FOR FENTANYL OVERDOSE.

SOME PRECURSORS FOR FENTANYL ANALOGS

N-(4-PIPERIDINYL) ANILINE

2-PHENYL-1-BROMOETHANE (FOR FENTANYL ONLY)

1-PHENYL-2-BROMOPROPANE (FOR ALPHA METHYLFENTANYL ONLY)

PROPIONIC ANHYDRIDE

N-(1-PHENETHYL)-PIPERIDINE-4-ONE

ANILINE

N-(1-PHENETHYL-4-PIPERIDINYL) ANILINE

PHENETHYLAMINE

*METHYL ACRYLATE

*METHYL METHACRYLATE (FOR 3-METHYLFENTANYL ONLY)

*WARNING: THESE MATERIALS ARE HAZARDOUS VAPORS WHICH WILL CAUSE CRYING AND CAN BE INJURIOUS AT HIGH CONCENTRATIONS. THE VAPORS FORM EXPLOSIVE MIXTURES IN AIR AND CAN REACT VIOLENTLY WITH MANY CHEMICALS.

REAGENTS

SODIUM BOROHYDRIDE

p-TOLUENESULFONIC ACID

SODIUM CARBONATE

POTASSIUM IODIDE

HYDROCHLORIC ACID

SODIUM METHOXIDE

SODIUM HYDROXIDE

SOLVENTS

TOLUENE

METHANOL

ACETONITRILE

ETHYL ETHER

POSSIBLE PROFIT MARGIN

ILLICIT MANUFACTURE OF

3-METHYLFENTANYL

FOR AN INVESTMENT OF $150 IN CHEMICALS 500 GRAMS OF 3-METHYLFENTANYL COULD BE PRODUCED. THIS IS THE EQUIVALENT OF 500,000,000 MICROGRAMS.

THIS IS THE EQUIVALENT OF 50,000,000 DOSAGE UNITS--
(1 DOSE = 10 MICROGRAMS).

A DOSAGE UNIT OF 3-METHYLFENTANYL HAS BEEN SELLING AT THE SAME PRICE AS HEROIN.

AT A VERY CONSERVATIVE PRICE OF $10 A SPOON, THIS MEANS YOUR $150 INVESTMENT WOULD YIELD $500,000,000 ON THE STREET.

THE ABOVE FIGURES WERE PREPARED BY THE DEA WESTERN FIELD LABORATORY.

MPPP AND MPTP

DEMEROL WAS FIRST PRODUCED BY THE GERMANS IN WORLD WAR II AFTER THE ALLIES EFFECTIVELY SHUT OFF THEIR SUPPLIES OF OPIUM.

THE COMPOUND MPPP WAS PRODUCED TO IMITATE THE EFFECTS OF DEMEROL. THE COMPOUND MPTP WAS INADVERTENTLY PRODUCED WHEN OVERHEATING IN THE PREPARATION OF MPPP OCCURRED. THIS IS THE SUBSTANCE BELIEVED TO CAUSE PARKINSON'S DISEASE.

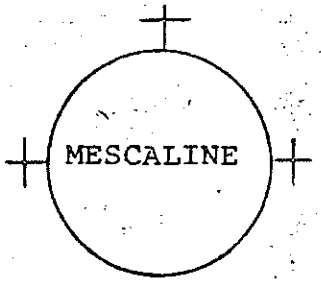
IN OCTOBER 1984 A CLANDESTINE PCP LABORATORY WAS SEIZED BY DEA IN BROWNSVILLE, TEXAS WHICH HAD AN INTERMEDIATE TO PROVIDE ANOTHER MEPERIDINE TYPE DRUG, PEPAP. IN NOVEMBER 1984, PEPAP WAS FOUND IN EXHIBITS FROM HAYWARD, CALIFORNIA, WHICH HAD BEEN SOLD AS METHAMPHETAMINE. THE OPERATOR OF THE LAB IN BROWNSVILLE ADMITTED MAKING MPPP AND PEPAP AND SELLING THEM IN THE SAN FRANCISCO AREA. THE OPERATOR OF THE LAB HAS PARKINSON'S DISEASE AS A RESULT OF HIS EXPOSURE IN THE LABORATORY.

IF OVERHEATED, PEPAP BREAKS DOWN TO PEPTP. IT IS BELIEVED THIS MATERIAL WILL ALSO CAUSE PARKINSON'S DISEASE.

MPPP IS EFFECTIVE AT AROUND 3000 MICROGRAMS. PEPAP IS EFFECTIVE AT AROUND 1500 MICROGRAMS.

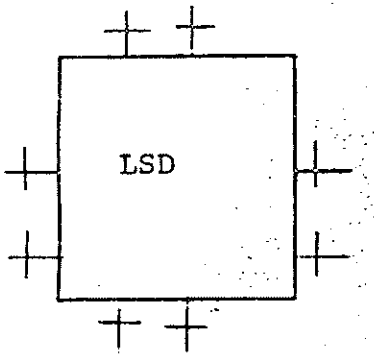
MPPP AND PEPAP WILL BE CONTROLLED AS SCHEDULE I SUBSTANCES ON AUGUST 12, 1985, UNDER DEA'S EMERGENCY POWERS.

TO ACHIEVE THF ECTS OF

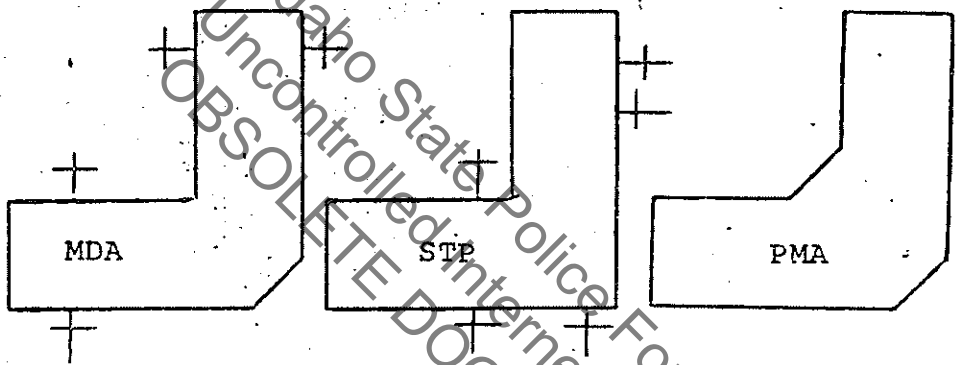


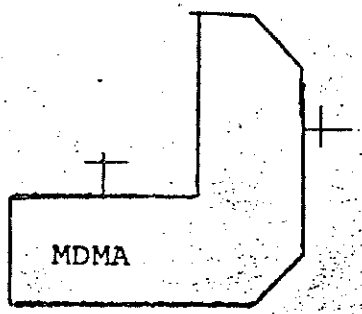
CROSSES INDICATE DIFFERENCES IN MOLECULAR STRUCTURE

(LSD FAMILY)



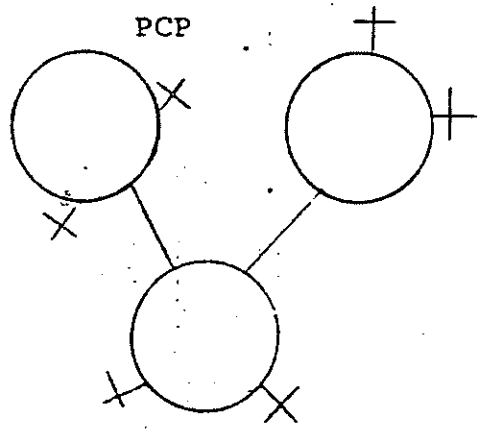
(AMPHETAMINE FAMILY)



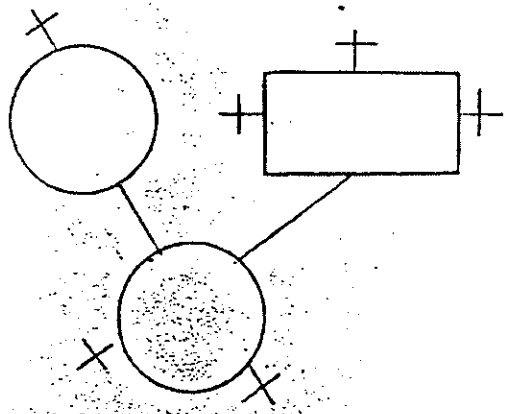


ALL OF THE COMPOUNDS ON THIS PAGE ARE SCHEDULE I

(PCP FAMILY)



PCT



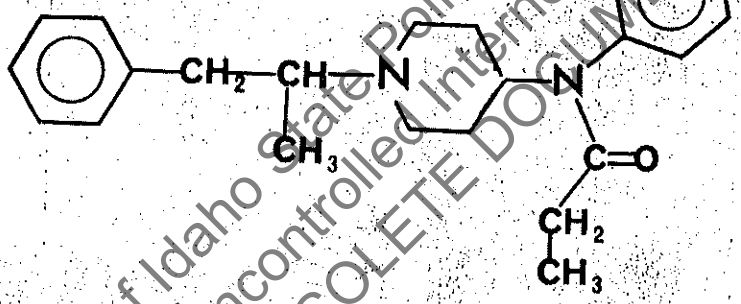
DEA LABORATORY NOTES

CHINA WHITE

α-Methyl Fentanyl

A. Allen, D. Cooper, T. Kram
Special Testing and Research Laboratory
Drug Enforcement Administration

A methyl homolog of Fentanyl has been identified in several exhibits received from California. The specific compound is α-methyl fentanyl, the structure and chemical name of which are shown below. It has been encountered as the racemic hydrochloride salt. The purpose of this note is to provide analytical data to assist other laboratories in the identification of this material.

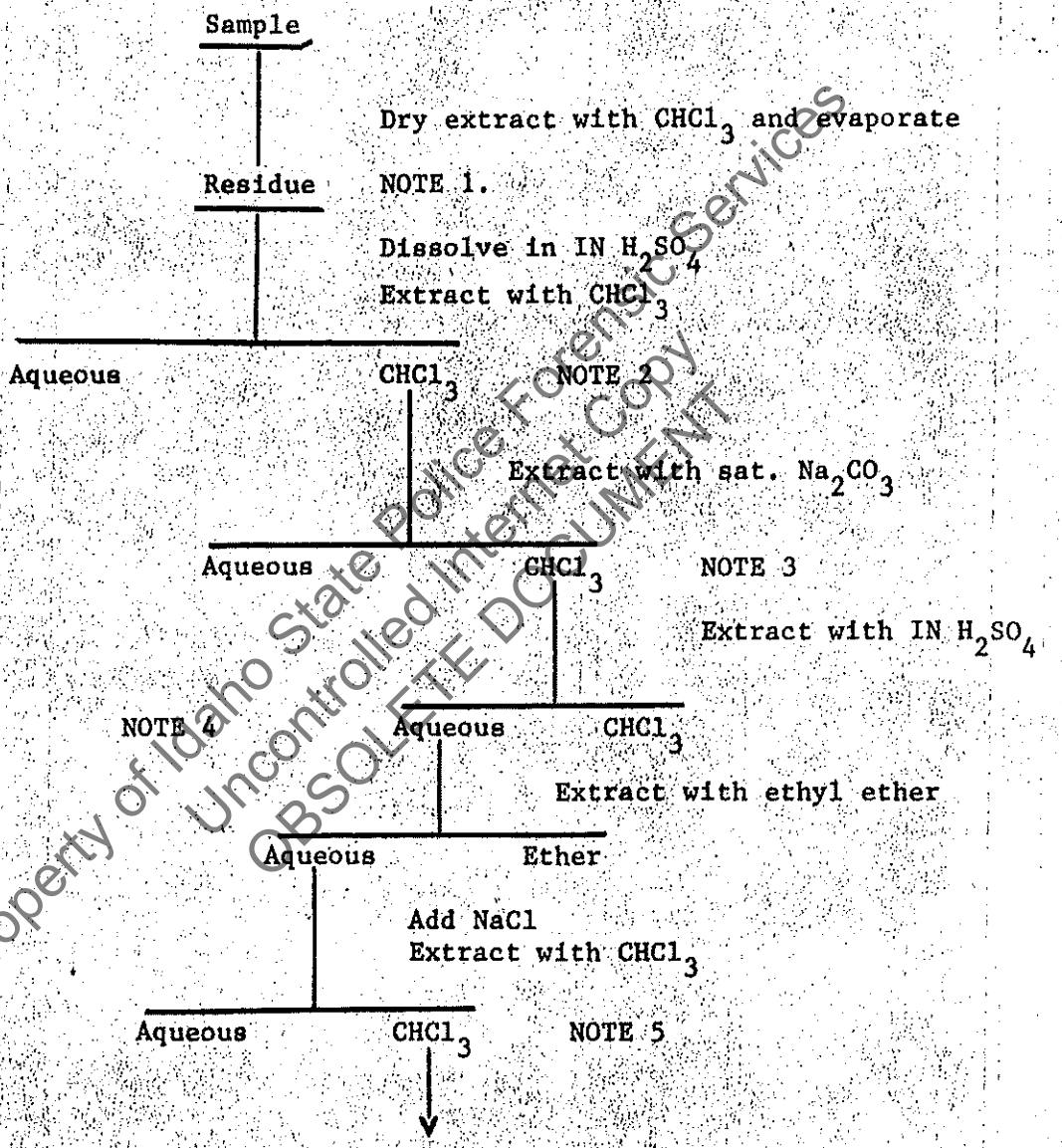


α-methyl fentanyl
1-(1-methyl-2-phenylethyl)-4-(N-propanilido) piperidine

Because α-methyl fentanyl is effective at doses in the microgram range, it is present at low levels in evidence exhibits. In addition, several synthetic by-products are also present. Finally, the solubilities of this compound and its associated impurities are such that isolation of the drug is extremely difficult and not quantitative. For these reasons, obtaining enough pure drug from illicit samples for NMR or IR analysis is unlikely unless large amounts of sample are available. Gas chromatography/mass spectrometry is the method of choice for the positive identification of this compound.

EXTRACTION

The following flow chart depicts the extraction scheme developed to isolate "pure" α-methyl fentanyl.



Note 1: This residue is typically contaminated with precursors and by-products.

Note 2: If α-methyl fentanyl is present as the hydrochloride salt, then greater than 90% of the drug will be extracted with chloroform. If no ion pair is present, then only about 50% will be extracted.

Note 3: This step removes the ion pair, allowing the drug to be extracted from acid.

Note 4: Due to the limited solubility of α-methyl fentanyl in aqueous media, there is only about 50% recovery in this step.

Note 5: The chloroform extract at this point will contain essentially pure α-methyl fentanyl, but only about 20% of the original amount. The preceding extraction with ether is necessary to remove N-propanalide, one of the major impurities encountered in exhibits.

COLOR TESTS: Marquis Reagent - weak orange

CRYSTAL TESTS: None

THIN LAYER CHROMATOGRAPHY: Under the following conditions, α-methyl fentanyl can be separated from its by-products, and has an R_f of approximately 0.60.

Solvent: Hexane:isopropyl alcohol:diethylamine:30:3:0.5

Substrate: Whatman K6 silica gel

Detection: Iodine vapors

GAS CHROMATOGRAPHY:

Column: 3% OV-1 on Gas Chrom Q 100/120 mesh

Column Temp: 255°

Carrier Gas: Nitrogen

Using the above conditions on a packed 6 ft. column, α-methyl fentanyl has a retention time of 6.1 minutes (0.54 relative to triacontane). It will be separated from its associated impurities and is linear at concentrations from 0.2 to 2 mg/ml.

NMR/IR/MS:

NMR, IR, and mass spectra are shown in Figures I - III, respectively. The NMR spectrum was obtained on a 200 MHz instrument, the IR on a grating instrument, and the electron impact mass spectrum was obtained using a GC/MS (quadrupole) under normal operating conditions. These techniques will differentiate among the positional isomers.

No molecular ion was obtained in the EI mass spectrum. Table I lists the most intense ions and their approximate relative intensities.

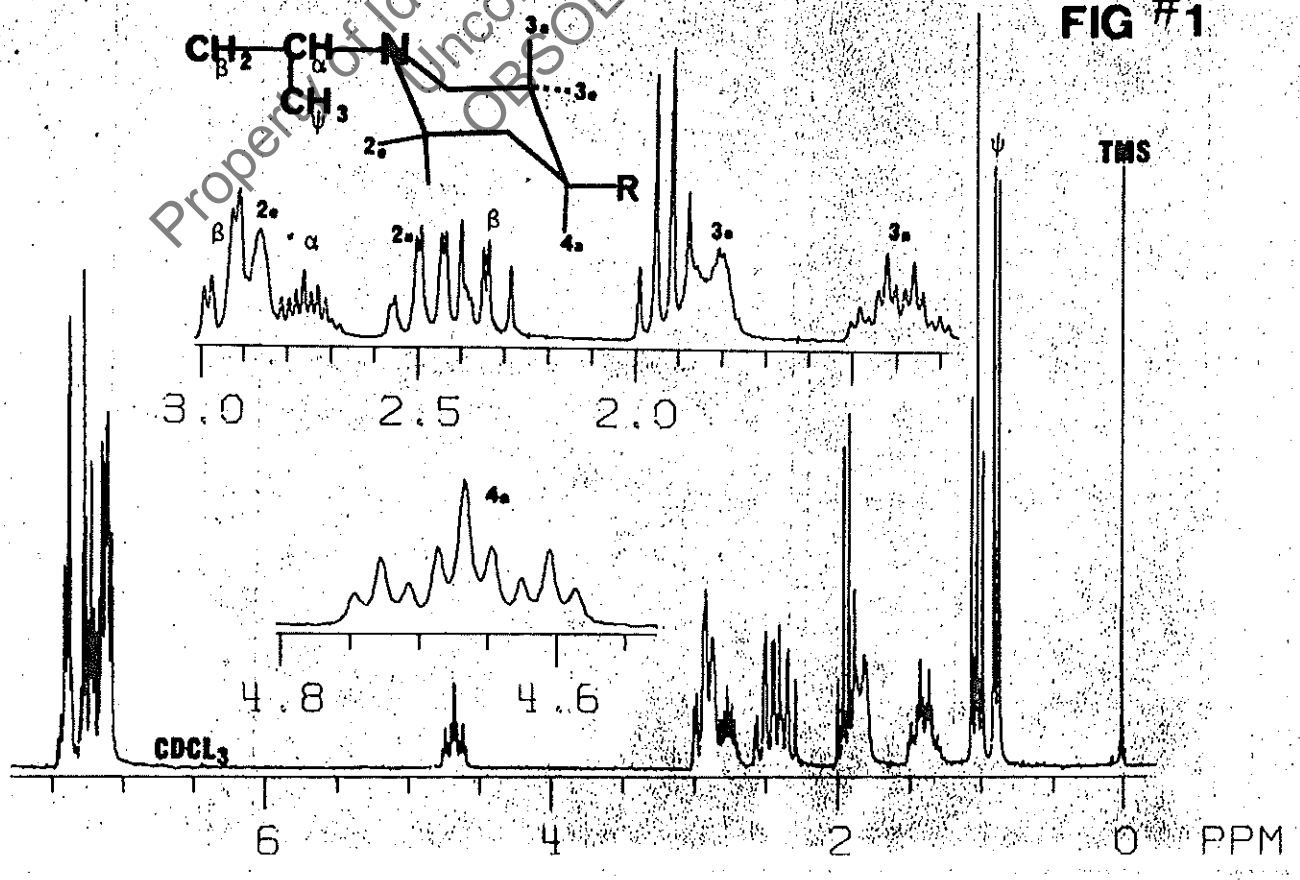
Table I

m/z	RI
259	100%
56	76%
57	64%
91	60%
110	44%
58	32%
146	22%

DISCUSSION

There are several positional isomers of α-methyl fentanyl which can exist. The chromatographic systems presented in this note will not differentiate among these isomers. The spectrometric data, however, will provide definite proof of the presence of this specific compound.

FIG #1



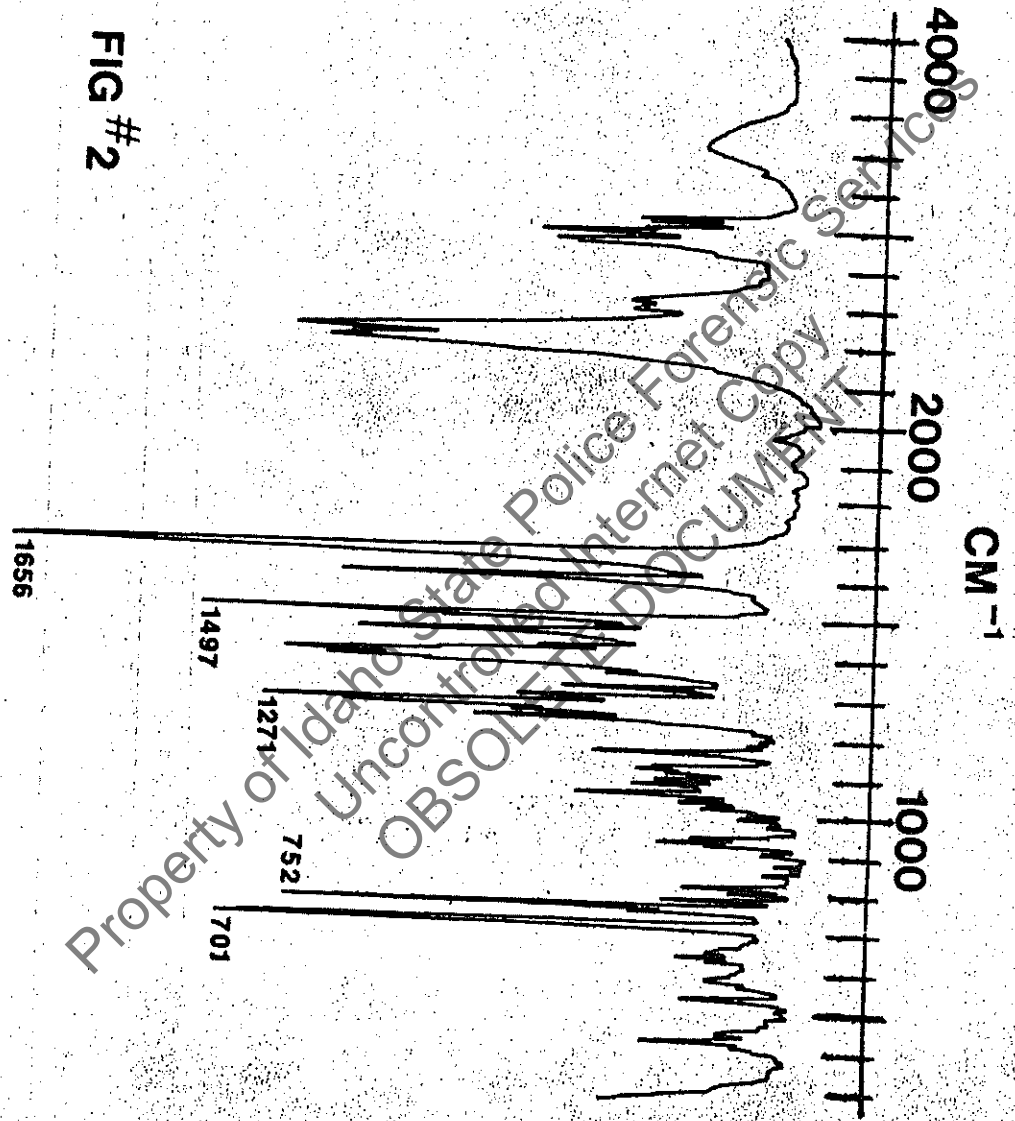


FIG #2

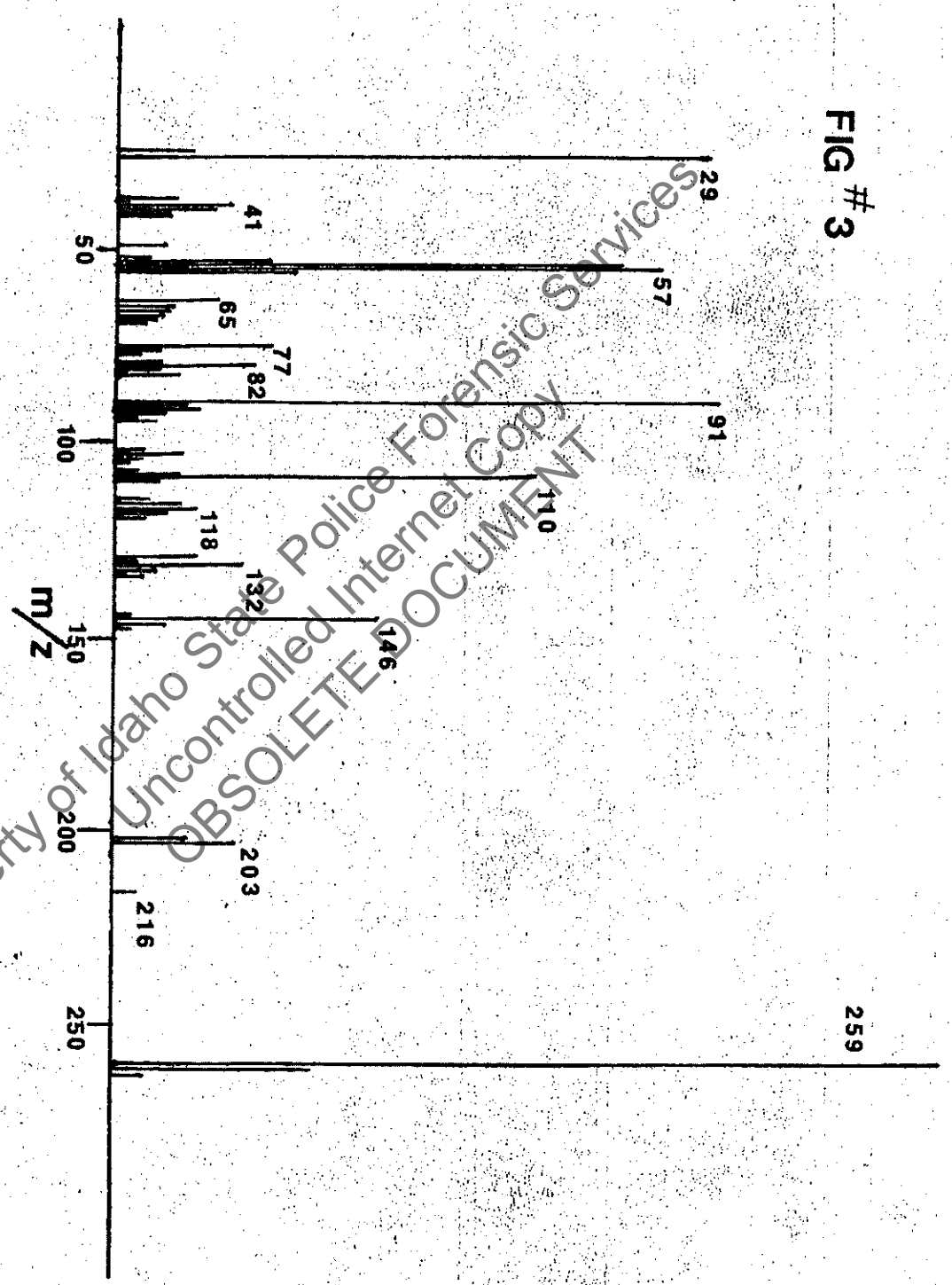


FIG #3

From Spring 1981 NWAFS

DEA LABORATORY NOTES

DATE

DRUG TYPE

METHODOLOGY

25 µg dose for humans — (orally)
5 min. onset - death in 15 min. —
use gloves

CLEANUP FOR IR
α-METHYL FENTANYL

by

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FORENSIC CHEMIST
WESTERN REGIONAL LABORATORY
DRUG ENFORCEMENT ADMINISTRATION

The new drug α-methyl fentanyl (MICROGRAM - March 1981) is usually found at low levels in small amounts of sample containing many by-products and other impurities. The illicit sample used for this paper contained 1.9% α-methyl fentanyl HCl, 0.6% propionanilide, 1.2% of other chloroform-soluble compounds, and the remainder lactose.

A simple cleanup procedure has been developed to produce purified α-methyl fentanyl HCl from small samples in about ten minutes. The extraction of a 100 mg. sample (1.9 mg. α-methyl fentanyl HCl) gave enough drug to produce a good IR spectrum in the normal manner without beam condenser (Fig. 1), so recovery must be fairly complete. A 30 mg. sample gave more than sufficient drug for IR using a beam condenser (or slower scan without beam condenser). The spectrum of the sample shows only a slight impurity, the strongest impurity peak being at 1540 wave numbers. The ammonium band (around 2500) varies from one sample (or standard) to the next depending on the degree of exchange of chloride and the bromide of the KBr. This is often seen in other drugs (phencyclidine HCl, etc.).

GLC data is presented here showing standards of α-methyl fentanyl with other drugs and normal hydrocarbons for reference. The strongest peaks seen by GLC on a chloroform extract of an illicit sample are propionanilide and α-methyl fentanyl with eight or more small peaks in between. There is also a significant amount of an unknown impurity which shows in the infrared spectrum of the chloroform-soluble portion of the samples, but does not show up on GLC.

CLEANUP METHOD

To a 7 ml. glass-stoppered test tube add a sample (100 mg. or less), 1 ml. HCl (approximately 0.5%) 1 ml. NaCl (sat. solution), 2 ml. petroleum ether. Mix well, pipette off the petroleum ether (discard), add 2 drops conc. ammonium hydroxide, add 2 ml. petroleum ether and mix well.

Transfer the petroleum ether layer to a second test tube containing 1 drop of sulfuric acid in 1 ml. of water. Mix well and pipette off the petroleum ether (discard). Add 1 ml. NaCl (sat. solution) and 2 ml. methylene chloride. Mix well, pipette off the methylene chloride, dry it through sodium sulfate, and evaporate, adding a few drops of petroleum ether when nearly dry to precipitate α-methyl fentanyl HCl.

All steps of this cleanup are rapid and mixing can be done by strong shaking of the stoppered tube since emulsion problems do not occur. This method should work for many other drugs, especially tertiary amines.

PERKIN-ELMER ®

CHART NO. 283-1259

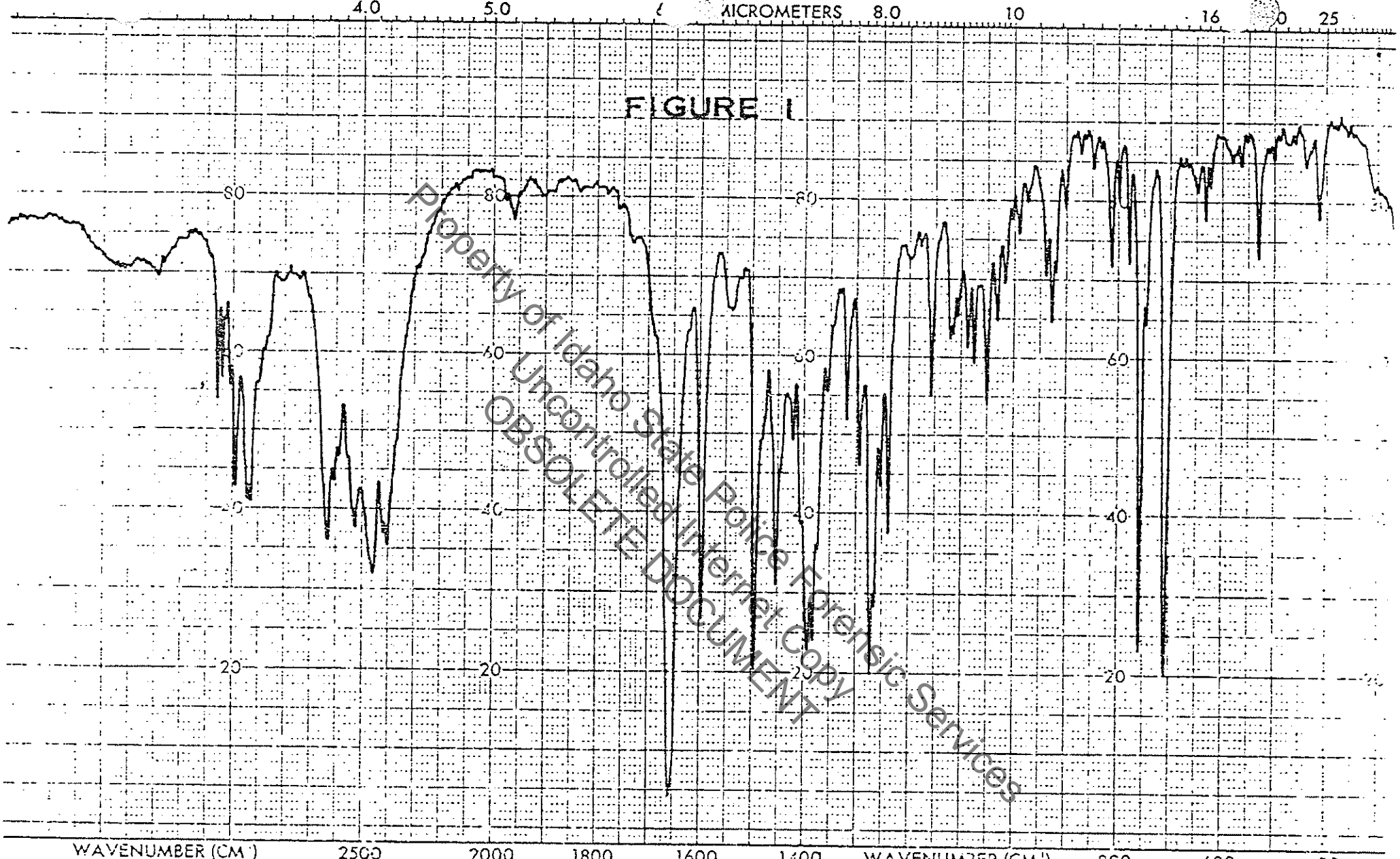
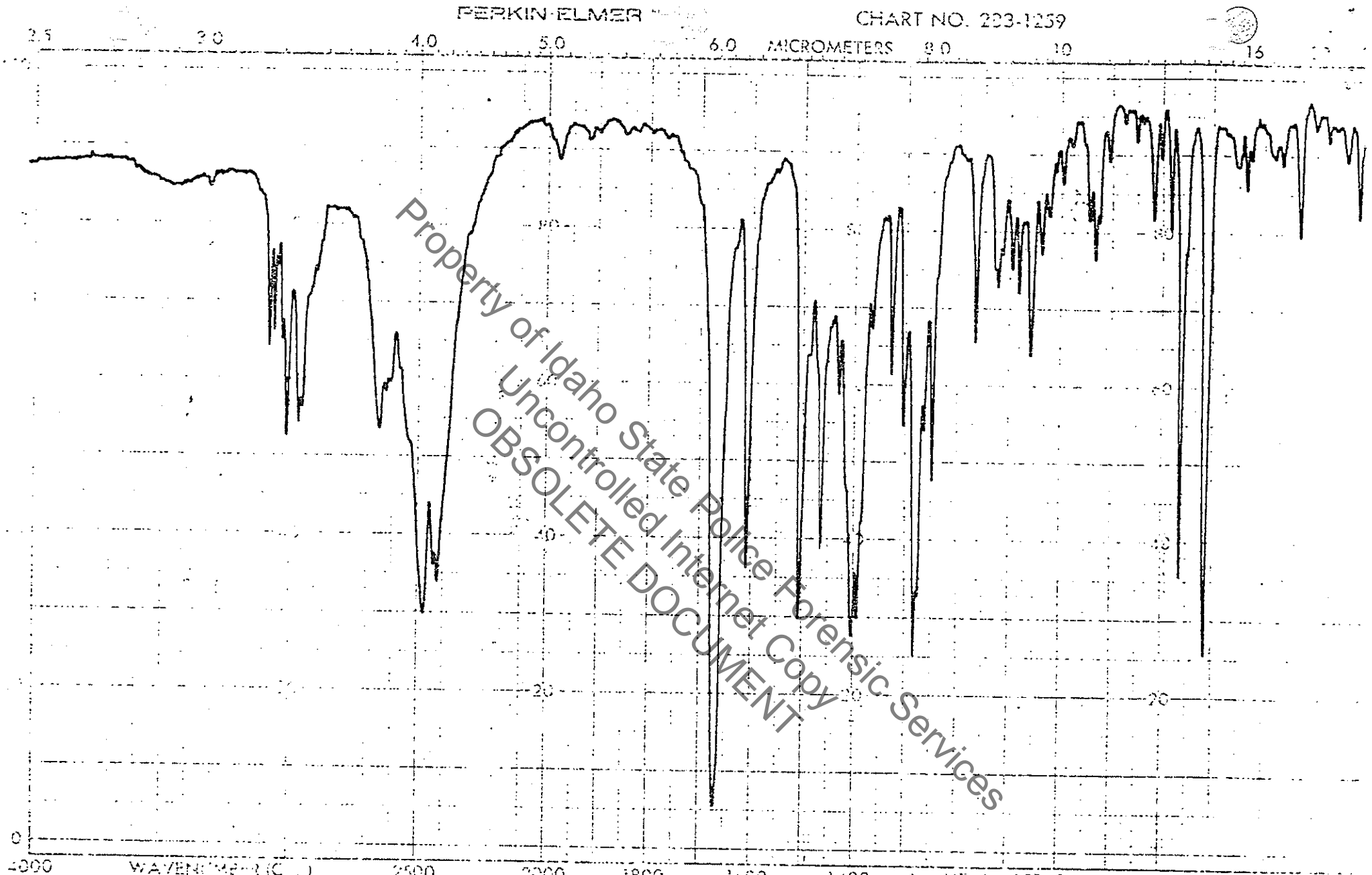
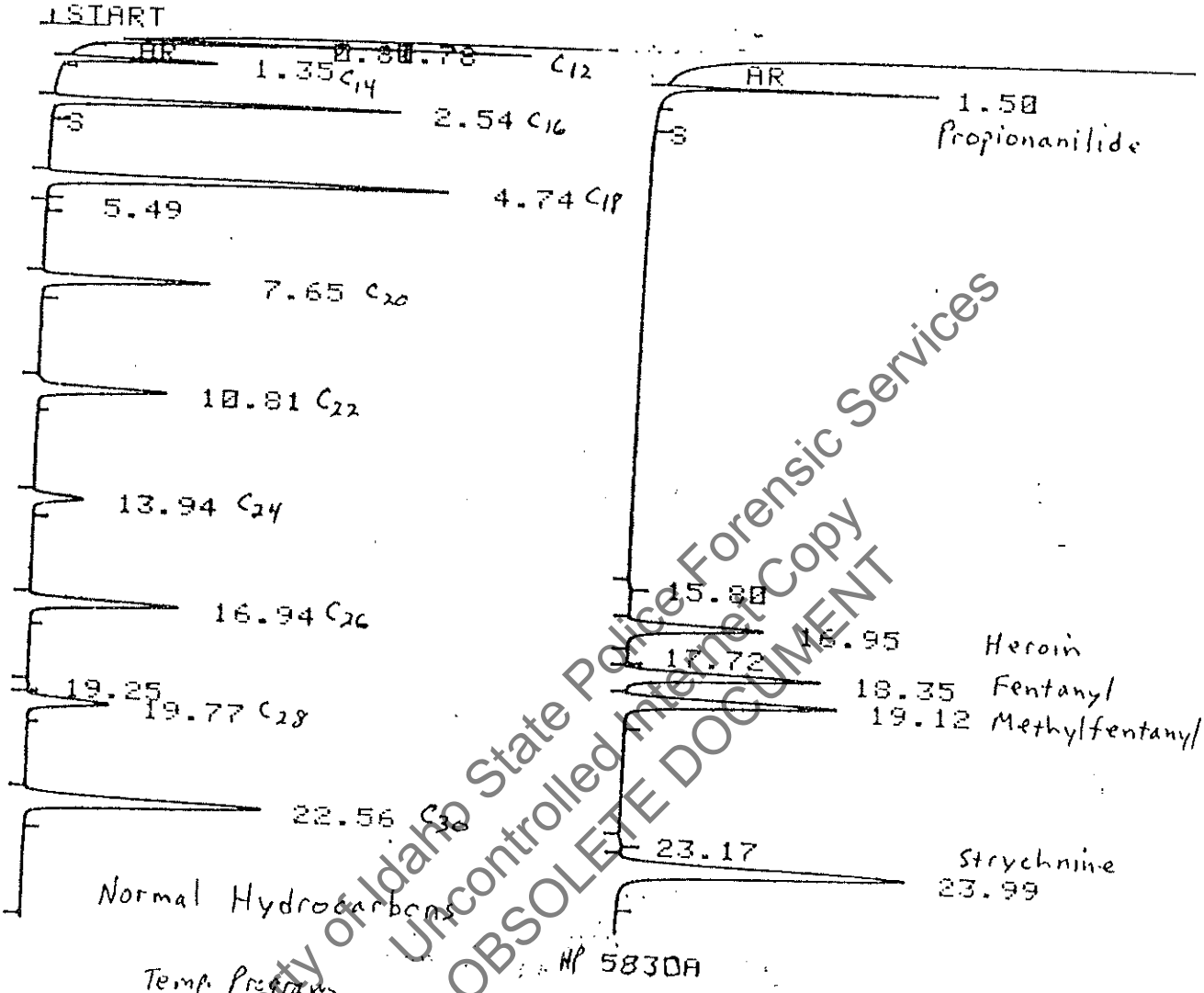


FIGURE 1

ABSCISSA	ORDINATE		
EXPANSION	EXPANSION	SCAN TIME 6	REP. SCAN / SINGLE BEAM
SUPPRESSION	% T / ABS	RESPONSE 1	TIME DRIVE / PRE-SAMPLE CHOP
		SLIT PROGRAM N	OPERATOR JMT DATE 7/14/
SAMPLE 4-methylfentanyl HCl in loading sample	REMARKS	SOLVENT KBr	CELL PATH
ORIGIN		CONCENTRATION	REFERENCE air





Temp. Program

TEMP1	180°	180
TIME1		2.0 min.
RATE		5.00 °/min.
TEMP2	275°	
TIME2		5.0 min.
INJ TEMP	260	260
FID TEMP	345	345
OVEN MAX	350	

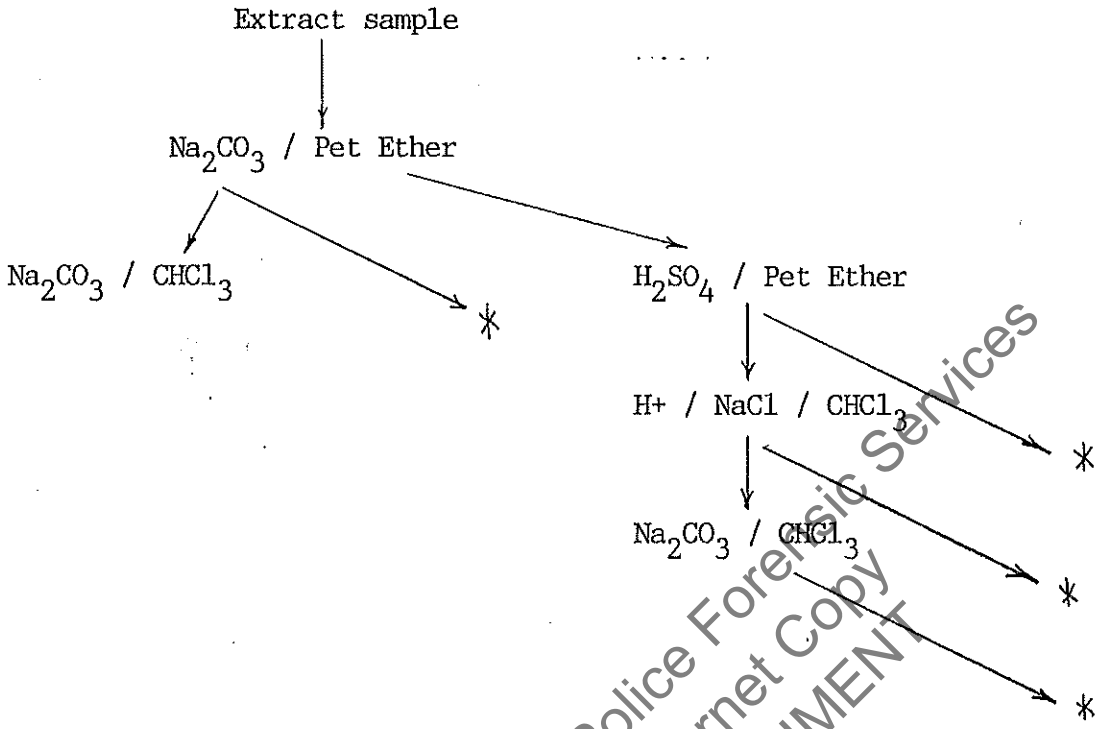
CHT SPD	0.50
ATTN 2↑	12
FID SGNL	A
SLP SENS	1.00
AREA REJ	-
FLOW A	60
FLOW B	59
OPTN	0
1.0 AREA REJ	1000

3% OV-1
length 6 ft.

GLC DATA

3% OV-1
1.8m x 4mm ID
Hydrogen [illegible] ml/min
Air

EXTRACTION OF FENTANYLS



* At these points different fentanyl analogs can be isolated. You can allow the organics to evaporate and then reconstitute the substance or do micro cleanup.

FENTANYL TOXICOLOGY

10 ml or 10 mg of specimen +
10 ml saturated borate buffer +
21 ml n-butyl chloride:ether (3:1)

↓

mix & centrifuge

↓

aqueous ← → organic

aqueous: discard

organic: add 3 ml 1N H_2SO_4

↓

mix & centrifuge

↓

aqueous ← → organic

aqueous: make basic with concentrated NH_4OH

organic: discard

↓

mix & centrifuge

↓

aqueous ← → organic

aqueous: discard

organic: evaporate to dryness
reconstitute with MeOH

BLOOD & URINE

2.5 ml sample

2.5 ml saturated borate buffer

10 ml CH_2Cl_2

↓

mix & centrifuge

aqueous ← → organic

discard

evaporate to dryness

reconstitute with MeOH

BENZODIAZEPINES & DERIVATIVES; Chlordiazepoxide (Librium)
Diazepam (Valium)
Flurazepam (Dalmane)
Prazepam (Centrax)
Oxazepam (Serax)

Diazepam: (base)
1. Place powder on top of a plug of cotton in a pipet.
2. Extract with ethyl ether
3. Dry down & run IR scan

Others: (HCl)
1. Place powder on top of plug of cotton in a pipet.
2. Extract with CH_2Cl_2 - reduce volume
3. Recrystallize with pet ether/hexane
4. Run IR scan (beware of polymorphism)

Sometimes it may be necessary to do an acid wash to clean-up a pharmaceutical.

ACKNOWLEDGEMENT

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REFERENCES

1 D. J. Greenblatt, M. Divoll, D. R. Abernethy, H. R. Ochs and R. I. Shader, *Clin. Pharmacokin.*, 8 (1983) 233.
2 D. J. Greenblatt, M. D. Allen, B. J. Noel and R. I. Shader, *Clin. Pharmacol. Ther.*, 21 (1976) 497.
3 H. Koler-Schmidt and G. Bohn, *Forensic Sci. Int.*, 22 (1983) 243.
4 R. J. Bastiani, R. C. Phillips, R. S. Schneider and E. F. Ullman, *Am. J. Med. Technol.*, 39 (1973) 211.
5 J. M. Clifford and W. F. Smyth, *Analyst (London)*, 99 (1974) 241.
6 D. M. Hailey, *J. Chromatogr.*, 98 (1974) 527.
7 K. Verebey, D. Jukofsky and S. J. Mule, *J. Anal. Toxicol.*, 6 (1982) 305.
8 J. A. F. De Silva, I. Bekersky, C. V. Puglisi, M. A. Brooks and R. E. Weinfeld, *Anal. Chem.*, 48 (1976) 10.
9 J. A. F. De Silva and I. Bekersky, *J. Chromatogr.*, 99 (1974) 447.
10 M. Divoll and D. J. Greenblatt, *J. Chromatogr.*, 222 (1981) 125.
11 R. Jochemsen and D. D. Breimer, *J. Chromatogr.*, 227 (1982) 199.
12 R. J. Wong, *J. Anal. Toxicol.*, 8 (1984) 10.
13 W. J. Adams, P. A. Bombardt and J. E. Brewer, *Anal. Chem.*, 56 (1984) 1590.
14 M. M. A. de Bruyne, A. Sinnema and A. M. A. Verweij, *Forensic Sci. Int.*, 24 (1984) 125.
15 G. L. Lensmeyer, C. Rajani and M. A. Evenson, *Clin. Chem.*, 28 (1982) 2274.
16 U. R. Tjaden, M. T. H. A. Meeles, C. P. Thys and M. Van Der Kaay, *J. Chromatogr.*, 181 (1980) 227.
17 M. D. Osselton, M. D. Hammond and P. J. Twitchett, *J. Pharm. Pharmacol.*, 29 (1977) 460.
18 D. M. Hailey, *J. Chromatogr.*, 98 (1974) 527.
19 A. G. Howard, G. Nickless and D. M. Hailey, *J. Chromatogr.*, 90 (1974) 325.
20 R. Riva, G. Tedeschi, F. Albani and A. Baruzzi, *J. Chromatogr.*, 225 (1981) 219.
21 M. Divoll and D. J. Greenblatt, *J. Chromatogr.*, 222 (1981) 125.
22 J. D. Wittwer, *J. Liq. Chromatogr.*, 3 (1980) 1713.
23 H. Schuetz and V. Westenberger, *J. Chromatogr.*, 169 (1979) 409.
24 H. Schütz, *Benzodiazepines — a Handbook*, Springer, Berlin, Heidelberg, New York, 1982.
25 D. J. Berry and J. Grove, *J. Chromatogr.*, 80 (1973) 205.
26 M. Hasegawa and T. Matsubara, *Chem. Pharm. Bull.*, 23 (1975) 1826.
27 A. C. Moffat, *J. Chromatogr.*, 110 (1975) 341.
28 E. Kováts, *Helv. Chim. Acta*, 41 (1958) 1915.
29 R. E. Ardrey and A. C. Moffat, *J. Chromatogr.*, 220 (1981) 195.

or three compounds and a few data on the chromatographic behaviour of several benzodiazepines and their benzophenones simultaneously with different techniques have been reported[15,22–24].

The purpose of this work was to examine the possibility of screening nineteen benzodiazepines currently available in Italy by different chromatographic techniques (TLC, GLC and HPLC) and to establish the sensitivity limits for each substance.

EXPERIMENTAL

Standards and chemicals

Pure chemical standards of chlordiazepoxide, clonazepam, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam and temazepam were obtained from Hoffman-La Roche (Nutley, NJ, U.S.A.). Bromazepam, camazepam, chlordesmethyldiazepam, clobazam, clorazepate, desmethyldiazepam, flurazepam, lormetazepam, medazepam, pinazepam and prazepam were obtained from commercial products by methanol extraction.

Benzophenones were obtained by acid hydrolysis of the parent benzodiazepines according to Berry and Grove[25].

Azinphos-methyl{phosphorodithioic acid O,O-dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4*H*)-yl]methyl ester} was employed as an internal standard (I.S.) in HPLC.

Methylclonazepam, obtained by methylation of clonazepam with diazomethane[26] was used as the internal standard for high-resolution capillary gas chromatography (HRGC).

Other chemicals and reagents were of UV grade.

Thin-layer chromatography

Pre-coated silica gel 60 F_{254} fluorescent plates (20 cm × 20 cm) (Merck) were used.

Five eluents were tested for the analysis of benzodiazepines: (A) benzene–2-propanol–30% ammonia solution (85:15:1); (B) ethyl acetate–methanol–30% ammonia solution (85:10:5); (C) toluene–acetone–ethanol–30% ammonia solution (45:45:7:3); (D) chloroform–acetone (90:10); and (E) ethyl acetate–cyclohexane–30% ammonia solution (50:40:0.1).

R_F values according to Moffat[27] were calculated only for eluents D and E, which gave better separations, using diazepam, clonazepam, pinazepam and oxazepam as reference compounds for eluent D and diazepam, lorazepam, pinazepam and bromazepam for eluent E.

Two eluents were also tested for the analysis of benzophenones: (D) Chloroform–acetone (90:10) and (F) benzene–acetic acid (97:3).

After development for 10 cm the plates were observed under UV light and sprayed with Dragendorff and Bratton–Marshall reagents[25].

Gas–liquid chromatography

A Carlo Erba Fractovap 4200 gas chromatograph was used. Retention indices (RI)[28] were calculated in the isothermal mode (240, 260 and 280°C) on three different packed columns (2 m × 4 mm I.D.) for benzodiazepine standards: (a) 3% GP 2100

DB on 100–120 mesh Supelcoport; (b) 1% Dexsil 300 on 100–120 mesh Chromosorb W; and (c) 3% SE-30 on 100–120 mesh Chromosorb W.

The injector and flame ionization detector temperatures were set 10°C above the oven temperature. Helium was used as the carrier gas at a flow-rate of 30 ml/min.

High-resolution gas chromatography

A Carlo Erba Fractovap HRGC gas chromatograph equipped with both split–splitless and on-column injectors, flame ionization and electron-capture detectors (10 mCi Ni-63, operated in the pulsed mode) and a temperature programmer was used.

The study of gas chromatographic mobility was performed on three different columns: (a) a 25-m glass capillary column with a 0.4 μm film of SE-54 stationary phase, using hydrogen as carrier gas at a flow-rate of 3 ml/min, temperature programming (from 100 to 220°C in the ballistic mode, hold for 1 min at 220°C, then heat to 270°C at 7°C/min; finally hold at 270°C for 15 min) and a detector temperature of 300°C; (b) a wide-bore SPB-5 glass capillary column (30 m × 0.75 mm I.D.) with bonded SE-54 stationary phase in the isothermal mode at 220°C using helium as the carrier gas at a flow-rate of 5 ml/min; and (c) wide-bore SPB-1 glass capillary column (30 m × 0.75 mm I.D.) with bonded SE-30 stationary phase, using helium at a flow-rate of 5 ml/min as the carrier gas, temperature programming (hold for 2 min at 200°C, heat to 285°C at 4°C/min, then 10 min in the isothermal mode) and a detector temperature of 300°C.

High-performance liquid chromatography

A Perkin-Elmer 3B liquid chromatograph equipped with LC 75 autocontrol set at 254 nm was employed.

The chromatographic separation of benzodiazepines and benzophenones was performed in the reversed-phase mode using a Perkin-Elmer 3-μm HS C_{18} column (7 cm long) in the isocratic mode with two different elution systems:

(a) methanol–water (70:30) at a flow-rate of 0.5 ml/min and

(b) 5 m*M* phosphate buffer (pH 6)–methanol–acetonitrile (57:17:26) at a flow-rate of 1.5 ml/min.

RESULTS AND DISCUSSION

Thin-layer chromatography

R_F distributions (means of ten replicate analyses) of all benzodiazepines for the five eluents investigated are shown in Fig. 1.

Of the eluent systems tested, D and E gave the best chromatographic separations.

Tables I and II give mobility data for benzodiazepines (only for the chosen eluents) and for benzophenones (eluents D and F).

Table II gives R_F values with two different detection techniques because some of the compounds show two or three spots detectable with UV light, whereas Bratton–Marshall reagent does not react with all of them (*e.g.*, flunitrazepam and desmethyldiazepam).

The results show that TLC allows the easy identification of benzodiazepines

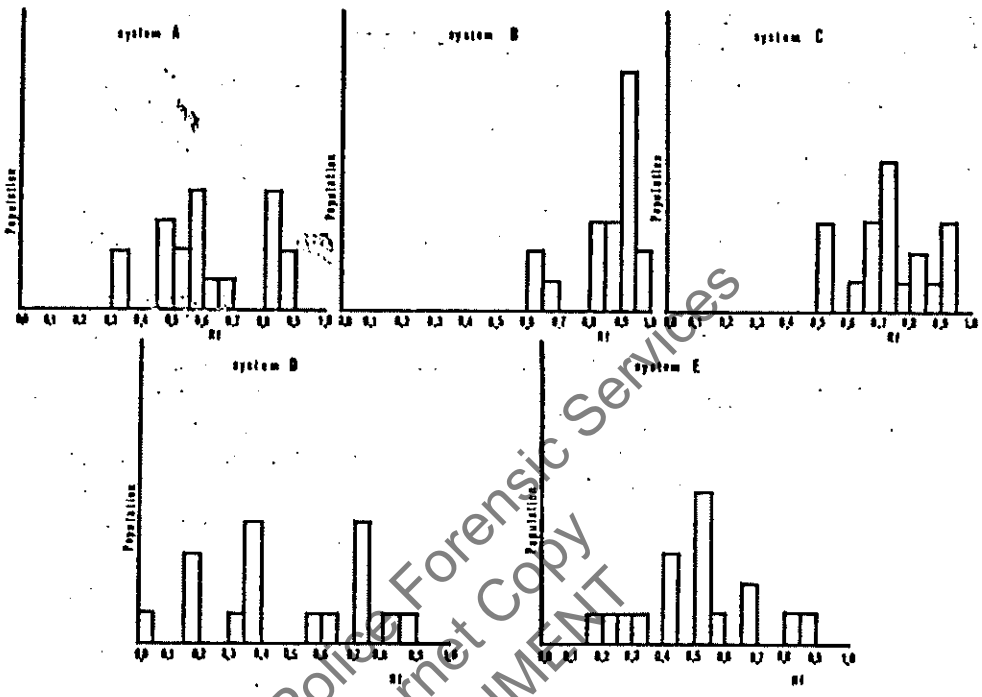

Fig. 1. Frequency distributions of R_F values of nineteen benzodiazepines using eluent systems A, B, C, D and E (see text).

TABLE I

R_F VALUES FOR BENZODIAZEPINES USING ELUENTS D AND E

Detection with Dragendorff reagent and UV light.

Compound	R_F	
	D	*E*
Bromazepam	0.20	0.19
Camazepam	0.75	0.32
Chlordesmethyldiazepam	0.39	0.51
Chlordiazepoxide	ND*	ND
Clobazam	0.72	0.52
Clonazepam	0.40	0.42
Clorazepate	0.38	0.55
Desmethyldiazepam	0.37	0.56
Diazepam	0.75	0.70
Flunitrazepam	0.74	0.52
Flurazepam	0.00	ND
Lorazepam	0.20	0.25
Lormetazepam	0.60	0.45
Medazepam	0.76	0.68
Nitrazepam	0.35	0.45
Oxazepam	0.17	0.26
Pinazepam	0.86	0.89
Prazepam	0.82	0.83
Temazepam	0.62	0.53

* ND = not determined.

TABLE II

R_F VALUES FOR BENZOPHENONES USING ELUENTS D AND F

Compound	R_F			
	D		*F*	
	UV light	*Bratton–Marshall*[25]*	*UV light*	*Bratton–Marshall*[25]*
Bromazepam				
Camazepam	0.60		0.06	
	0.96		0.25	
			0.87	
Chlordesmethyldiazepam				
Chlordiazepoxide	0.93	0.93 (v)	0.24	0.55 (v)
			0.55	
Clobazam	0.64		0.07	
Clonazepam	0.88	0.88 (p)	0.35	0.35 (p)
	0.91		0.73	0.73 (p)
				0.92 (p)
Clorazepate	0.92	0.92 (v)	0.53	0.53 (v)
Desmethyldiazepam	0.93	0.93 (v)	0.21	0.51 (v)
			0.51	
Diazepam	0.97		0.82	0.53 (v)
Flunitrazepam	0.88	0.88 (p)	0.36	0.36 (p)
	0.95		0.67	
Flurazepam	0.93	0.93 (v)	0.61	0.61 (v)
Lorazepam	0.90	0.94 (v)	0.18	0.69 (v)
	0.94	0.98 (v)	0.69	
Lormetazepam	0.62		0.05	0.05 (v)
	0.96		0.87	
Medazepam	0.88		0.19	0.84 (v)
	0.96		0.24	
			0.84	
			0.97	
Nitrazepam	0.00	0.88 (p)	0.00	0.30 (r)
	0.88		0.30	0.70 (p)
	0.96		0.70	
Oxazepam	0.93	0.93 (v)	0.20	0.52 (v)
	0.99		0.24	0.90 (v)
			0.52	
			0.90	
Pinazepam	0.96	0.96 (v)	0.85	0.54 (v)
				0.85 (v)
Prazepam	0.75		0.10	0.54 (v)
	0.97		0.91	
Temazepam	0.65		0.06	0.06 (v)
	0.96		0.25	0.53 (v)
			0.85	

* p = pink; r = red; v = violet.

with the systems chosen when chromatography of benzophenones is carried out too, notwithstanding its low resolving power compared with GLC and HPLC. In this instance it is possible to discriminate between those groups of compounds not completely resolved with only one chromatographic system (eluent D or E). For example, flunitrazepam and clobazam migrate with similar R_F values in both eluents D and

TABLE III

GC RETENTION INDICES

Compound	*This work*			*Literature data*	
	3% GP SP 2100 DB	*Dexsil 300*	*3% SE-30*	*Ref. 29*	*Ref. 23*
Bromazepam	Neg.*	2893	Neg.	2663	2670
Camazepam	3111	3191	2946	ND	ND
Chlordesmethyldiazepam	ND**	ND	2592	ND	ND
Chlordiazepoxide	2689	2636	2808	2530	2845
Clobazam	2773	2797	2559	ND	2660
Clonazepam	Neg.	3174	Neg.	2885	2965
Clorazepate	2701	2759	Neg.	ND	2655
Desmethyldiazepam	2794	2740	2502	2496	2555
Diazepam	2561	2641	2448	2425	2490
Flunitrazepam	2803	2883	2607	2645	2645
Flurazepam	2841	2960	2790	2785	2800
Lorazepam	ND	2601	2430	2402	2440
Lormetazepam	ND	ND	2491	ND	ND
Medazepam	Neg.	2871	2270	2226	2285
Nitrazepam	Neg.	3111	2746	2750	2830
Oxazepam	2503	2529	2354	2336	2380
Pinazepam	2683	2691	2529	ND	ND
Prazepam	2756	2868	2656	2641	2715
Temazepam	ND	ND	ND	ND	2630

* Neg. = no peak between 1200 and 3200.
** ND = not determined.

TABLE IV

HRGC RETENTION INDICES

Compound	*Stationary phase*		
	SPB-1	*SPB-5*	*SE 54*
Bromazepam	2510	2662	2658
Camazepam	2935	3036	3059
Chlordesmethyldiazepam	2552	2647	2700
Chlordiazepoxide	2703	2882	2855
Clobazam	2557	2605	2650
Clonazepam	2814	2879	2945
Clorazepate	ND*	ND	2588
Desmethyldiazepam	2426	2549	2591
Diazepam	2434	2492	2531
Flunitrazepam	2577	2661	2707
Flurazepam	2703	2809	2926
Lorazepam	2391	2466	2484
Lormetazepam	2603	2717	2753
Medazepam	ND	2674	ND
Nitrazepam	2718	2820	2865
Oxazepam	2314	2386	2436
Pinazepam	2505	2572	2604
Prazepam	2619	2685	2712
Temazepam	2517	2637	2684

* ND = not determined.

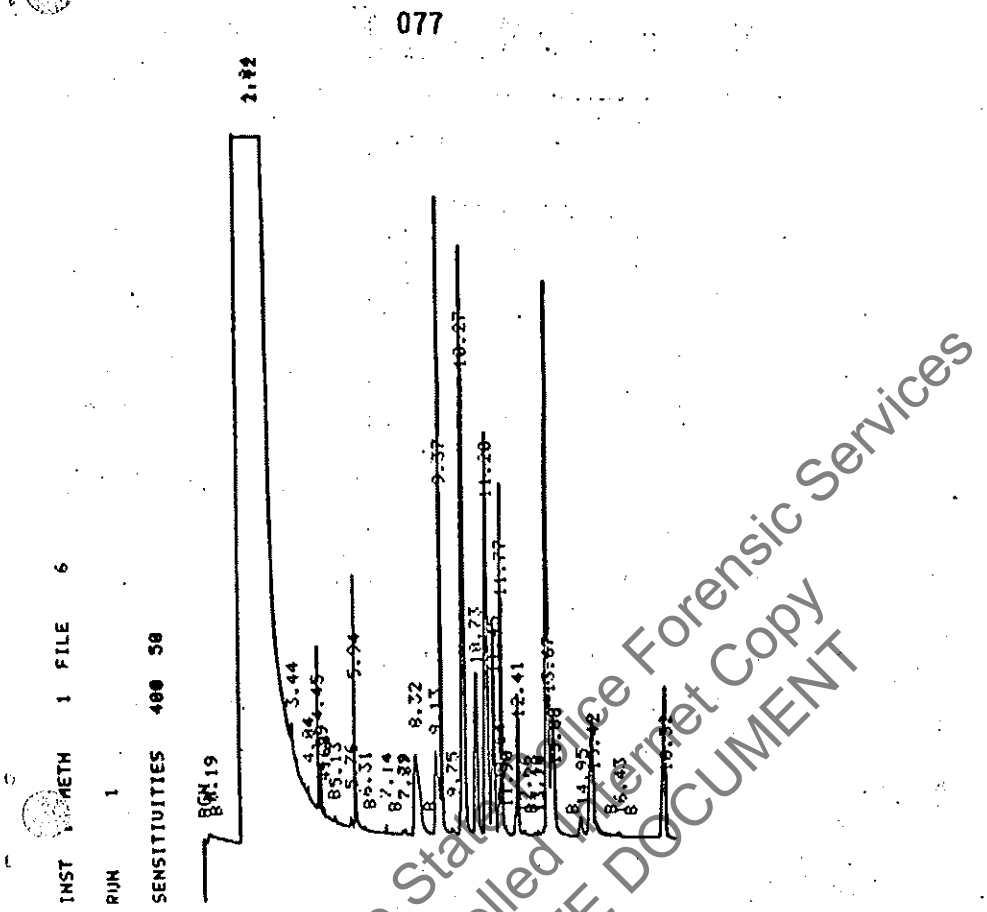

Fig. 2. Gas chromatographic separation of sixteen benzodiazepines on an SE-54 capillary column. Retention times: oxazepam 4.45, lorazepam 5.94, clorazepate 8.32, pinazepam 9.37, clobazam 10.27, bromazepam 10.73, temazepam 11.20, chlordesmethyldiazepam 11.45, flunitrazepam and prazepam 11.77, lormetazepam 12.41, chlordiazepoxide 13.67, nitrazepam 13.88, flurazepam 14.95, clonazepam 15.42 and camazepam 18.32 min.

E, but they give different benzophenones and in this way they can be identified after acid hydrolysis with system D or F.

Moreover, Dragendorff reagent is able to detect all benzodiazepines except lorazepam and clorazepate. Bratton–Marshall reagent gives different coloured spots with all benzophenones except camazepam and clobazam.

TLC analysis can also be used as in inexpensive screening technique when acute intoxication is suspected[25].

Gas–liquid and high-resolution gas chromatography

Retention indices (RI) obtained on packed and capillary columns are reported in Tables III and IV.

Table III shows our results and values obtained by Ardrey and Moffat[29] and Schuetz and Westenberger[23] using SE-30.

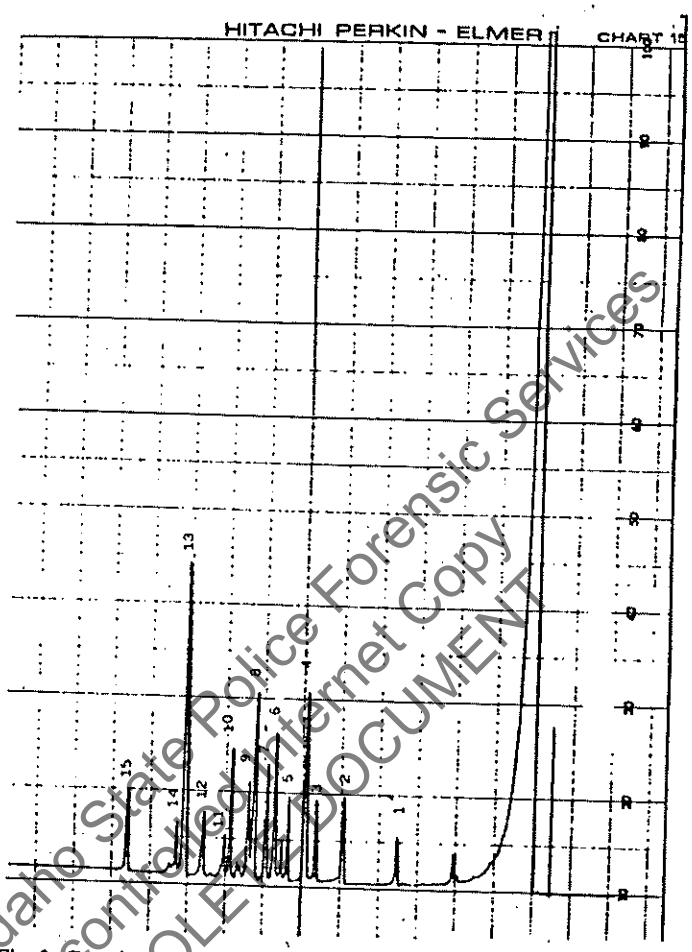

Fig. 3. Gas chromatographic separation of fifteen benzodiazepines on an SPB-1 capillary column. 1 = Oxazepam; 2 = lorazepam; 3 = desmethyldiazepam; 4 = diazepam; 5 = pinazepam; 6 = bromazepam; 7 = temazepam; 8 = chlordesmethyldiazepam; 9 = clobazam; 10 = flunitrazepam; 11 = lormetazepam; 12 = prazepam; 13 = flurazepam; 14 = nitrazepam; 15 = clonazepam.

For several benzodiazepines our results agree with the literature data; however, in some instances a variation more than 50 retention index units between the three sets of data was observed.

Retention indices obtained on the capillary column (SPB-1) were in agreement with those obtained on SE-30 under our analytical conditions. However, the packed column cannot allow the complete separation of all of the standards tested, particularly when using 3% GP and SE-30. The use of Dexsil 300 permits lower analytical temperatures with less pyrolytic destruction of the compounds.

In contrast, using a capillary column a complete separation is obtained and especially with SPB-5 in the isothermal mode the rapid identification of benzodiazepines is possible. The use of temperature programming and the high resolving power of SE-54 and SPB-1 (Figs. 2 and 3) allow a complete separation even when the

TABLE V

HPLC RESULTS OBTAINED USING ELUENTS a AND b

Compound	*Relative retention time**	
	a	*b*
Bromazepam	0.717	0.277
Camazepam	1.403	1.085
Chlordesmethyldiazepam	ND**	ND
Chlordiazepoxide	1.008	0.462
Clobazam	0.755	0.582
Clonazepam	0.684	0.406
Clorazepate	1.032	0.598
Desmethyldiazepam	1.040	0.589
Diazepam	1.198	0.879
Flunitrazepam	0.725	0.510
Flurazepam	2.050	1.329
Lorazepam	0.772	0.417
Lormetazepam	0.902	0.644
Medazepam	3.104	0.393
Nitrazepam	0.703	0.380
Oxazepam	0.802	0.390
Pinazepam	1.168	1.308
Prazepam	1.979	2.130
Temazepam	0.914	0.538

* Relative to azinphos-methyl.
** ND = not determined.

retention indices differ by less than 5 units. Moreover, SPB-1 permits complete resolution without tailing peaks for more polar compounds.

Using a capillary column, the detection limit with the flame-ionization detector was about 1–2 ng, whereas with the electron-capture detector 1 ng of each benzodiazepine gave a nearly full-scale response (>95%) on the 1.0-mV recorder.

Our data confirm the reliability of the use of retention indices especially on a capillary column with temperature programming, because of the good reproducibility (standard deviation) <2% (five replicate analyses).

High-performance liquid chromatography

Retention time relative to azinphos-methyl are reported in Table V for eluent systems a and b.

Complete separation of the compounds was achieved with eluent a. Nevertheless, with eluent b it is possible to carry out screening using a mixture that is easy to prepare and commonly employed for washing and preserving reversed-phase columns. UV detection allows a detection limit as low as 10 ng, which can be decreased further by shifting the wavelength to 220 nm, but then the background increases when analysing biological extracts.

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ANALYSIS OF BENZODIAZEPINES

I. CHROMATOGRAPHIC IDENTIFICATION

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SUMMARY

The chromatographic mobilities of nineteen benzodiazepines were determined by thin-layer, gas–liquid, high-resolution gas–liquid and high-performance liquid chromatography. The results were correlated with literature data.

INTRODUCTION

The number and frequency of requests made to toxicologists for analyses of blood and plasma samples for benzodiazepines are increasing dramatically, and therapeutic monitoring and screening of drug addicts are of interest in preventive medicine and forensic science[1,2]; these substances are also frequently found in judicial exhibits. Therefore, a reliable method for the identification of these compounds in biological samples and confiscated drugs is necessary.

Immunochemical techniques (EMIT and RIA)[3], generally employed as screening tests, are able to identify the presence of this class of compounds at microgram levels, but they do not discriminate between different commercial benzodiazepines; moreover quantitative analysis is difficult because the responses of different benzodiazepines and their metabolites towards the antigen-antibody reaction (EMIT-dau) are different[4]. The use of more specific techniques is thus necessary for identifying benzodiazepines and their metabolites accurately for correct pharmacological monitoring and for forensic purposes.

Thin-layer chromatography (TLC) has been proposed[5,6], but most workers prefer reliable methods based on gas–liquid chromatography (GLC)[7–11] and high-performance liquid chromatography (HPLC)[12–16]. The latter method is suitable for thermally labile substances such as some benzodiazepines. GLC techniques generally involve hydrolytic steps, owing to the formation of the same benzophenones from several different benzodiazepines[17,18], so that the individual parent drugs cannot be readily identified; however, some workers have separated intact benzodiazepines by GLC[19–21].

Published methods for benzodiazepine analysis have usually involved only two

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GENERAL UNKNOWNS ANALYSES

Occasionally, cases are submitted to the laboratory where no specific drug or drugs are suspected, or the criminalist, during analysis, detects the presence of additional components in the sample that are not typical of the drugs commonly encountered. These cases are referred to as "General Unknowns".

The following represents the suggested general analytical methods to employ when analyzing samples for the presence of controlled substances in those cases.

A minimum of spot tests and at least one TLC test should be employed, although additional analytical methods may be necessary.

I. SCREENING TESTS (SPOT TESTS)

The main purpose of the spot test is to narrow the list of substances possibly present in any given unknown. The literature lists dozens of spot tests, all of which are not necessarily pertinent. The following three references consolidate the diverse information available on spot tests. The first two sources are available in a hard copy or looseleaf notebook, and have not been reproduced for this manual.

A. ISOLATION AND IDENTIFICATION OF DRUGS, E.G.C. Clarke.
B. MICRO COLOUR REACTIONS, (looseleaf), G. J. Kupperschmidt.
C. SPOT TESTS: A COLOR REFERENCE FOR FORENSIC CHEMISTS, S. H. Johns, A. A. Wist, A. R. Najam (J.F.S. July 1979).

In general, the following screening tests may be employed to screen for the majority of controlled substances. It should be noted that neither a positive or negative reaction with these tests must not be considered conclusive for the presence or absence of a specific substance.

A. Marquis - for amphetamines and related substances, natural and synthetic opiates.
B. Cobalt Thiocyanate - for the "caines", PCP and related compounds, phenothiazines, tricyclic antidepressants and related compounds, some opiates.
C. Dille-Koppanyi - for barbiturates.

Other screening tests may be employed depending on the nature of the sample and the results of the previously listed tests. These may be located in the three references listed.

II. TABLET AND CAPSULE IDENTIFICATION

The following represent suggested references to be utilized for determining the possible contents of unknown capsules or tablets that are submitted.

A. Physicians' Desk Reference.
B. Poison Control Center - 378-2121.
C. Microgram, vol. XIII, no. 2 (Feb. 1980).
vol. XIII, no. 9 (Sept. 1980).
vol. XIV, no. 12 (Dec. 1981).
vol. XV, no. 11 (Nov. 1982).
D. Canadian Journal of Forensic Science, vol. 18, no. 1 (March 1985).
E. Misc. sources included.

III. TLC SCREENING

The included references contain suggested TLC systems to use when screening for general unknowns.

A dry extract with $CHCl_3$: MeOH (approx. 1:1 or 1:2) is capable of placing most substances in solution. However, if a more specific extraction scheme is desired to determine whether a component - ie. an acid, base, neutral, or amphoteric substance, Clarke's Isolation and Identification of Drugs is an excellent reference for the appropriate techniques and for Rf values.

An additional TLC screening method involves the use of the TOXI-LAB system. The directions for use of that system is found with each.

IV. GC SCREENING

The included references contain suggested GC systems to use when screening for general unknowns. GC screens should be run on samples extracted in the same manner as for TLC screening. The Boise lab has two wide bore capillary columns for drug screening - DB-1 and DB-17. The KOVATS RETENTION INDICES that are listed in the references may refer to packed or capillary columns equivalent to these columns (SE-30, OV-1, OV-101, OV-17, etc.). It has been found that the RETENTION INDEX of substances on these columns is sufficiently close to those on DB-1 and DB-17 to be used for screening purposes. The equation for determining the KOVATS RETENTION INDEX is included. The retention times of the substances are usually calculated as their adjusted or net retention time or the retention time as measured from the solvent front. The Instrumental Data for Drug Analysis, however, list Retention Indices as a function of the absolute retention time.

Other GC references not included as photocopies are:

A. Clarke's Isolation and Identification of Drugs.
B. Instrumental Data for Drug Analysis.
C. The International Association of Forensic Toxicologists Gas Chromatographic Retention Indices of Toxicologically Relevant Substances on SE-30 or OV-1.
D. The Retention Index Library on the Leading Edge computer in the Boise lab.

V. OTHER SCREENING METHODS

In some circumstances, the search systems using the database libraries on the FT-IR in the Boise Forensic Section and on the GC-MS in the Boise Toxicology Section and on the GC-MS in the Boise Toxicology Section may be utilized to obtain an idea of the possible class or structure of an unknown. While the GC/MS does not require a sample that has been "cleaned-up" prior to analysis, the FT-IR should be employed when the sample contains a single component or when a second component is present in very low concentrations relative to the first. The number of components in a mixture is usually determined with TLC or GC techniques.

GC & TLC

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A CHROMATOGRAPHY SYSTEM FOR DRUG IDENTIFICATION

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Summary

A classification system is described for drugs using thin-layer chromatography (TLC), gas–liquid chromatography (GLC) and ultraviolet (UV) spectrophotometry. The TLC classifications are based on division of the plate into zones relative to a set of mixed drug standards. The GLC classification is based on Kovats' retention indices. The procedure for classifying drugs is presented, together with a list of over 200 classified drugs.

Introduction

With the vast array of drugs now available on both the licit and illicit market, identification of specific drugs has become a problem. Most laboratories dealing in drug analysis identify drugs by measuring certain parameters and matching these to parameters of known drugs. This process has two distinct problems which our laboratory has overcome.

The first problem is what techniques are to be used and how they are to be measured. TLC is used as the primary technique in most laboratories and the measurement made is the R_f value. GLC usually follows in which retention times are measured, sometimes relative to certain standard compounds (drugs [1] or alkanes [2]). UV spectra of drugs are quickly done and hence are often used. The system described uses six parameters to describe a drug, three are TLC data in different solvents, two are GLC data on different columns, and, lastly, a UV parameter is used.

The second problem is that of searching lists of parameters in order to match those parameters of the drug whose identity is in question. Several methods are available ranging from card index systems to computer searches. This laboratory uses a computer search.

Experimental

Materials and instrumentation

Gas chromatography was performed on either a Packard 7400 or a Hewlett-Packard 5720 using 6 ft by 0.25-inch I.D. glass columns packed with

3% OV-101 or OV-17 on Chromosorb W. Detection was by flame ionization, and nitrogen was the carrier gas.

UV spectra were made on either a Shimadzu UV-200 or Unicam SP-800 spectrophotometer.

Merck pre-coated silica-gel $60F_{254}$ TLC plates without any treatment were used. All tanks were saturated by the inclusion of a 20 cm × 20 cm sheet of chromatography paper dipping into the solvent.

The I_2 in methanol spray was made by dissolving 2 g of iodine in 100 ml of methanol. For the copper chloride spray solution 20 g of $CuCl_2$ were dissolved in 100 ml of water. The modified Ludy-Tenger reagent was prepared as follows. Mix 300 g of KI with 50 g bismuth oxycarbonate, 150 ml conc. HCl and 1000 ml water. Stand for about two months until dark. Mix 100 ml of this solution with 100 ml conc. HCl and 5 g of bismuth carbonate. Dilute 20 ml of this solution with 10 ml conc. HCl and 70 ml water for spraying.

Method

Thin-layer chromatography

The three TLC systems used (T1, T2 and T3) were as follows: T1, chloroform–methanol (9:1); T2, methanol–conc. ammonia (100:1.5); T3, chloroform–acetone (9:1). With all three systems drugs are first located by viewing under UV light (254 nm) and then by spraying in turn with I_2 in methanol, modified Ludy-Tenger, I_2 in methanol and copper chloride.

The method of classifying a drug consists of reference to a series of standard drugs which define zones. These zones are given in alphabetical code as in Fig. 1. A drug which lies clearly between two reference standards is assigned two similar letters AA, BB, *etc.* If the drug does not resolve itself clearly between the reference standards its classification contains two consecutive letters AB, BC, *etc.* Any drug which fails to show up under the spraying conditions used is given the classification XX on T1, T2 and T3.

Gas–liquid chromatography

Using the method of Kovats [3] retention indices for columns using the stationary phases OV-101 and OV-17 were obtained. All retention indices for OV-101 quoted in the file were determined in this laboratory and these are, with some exceptions, in general agreement with those reported by Moffat [4] for his OV-1 column. Where possible the retention indices of Kazyak [2] are used for the OV-17 column, otherwise they were determined in this laboratory. Checks carried out against Kazyak's figures generally gave reasonable agreement. −500 is used to denote compounds that have a retention index of less than 1000 or where no peak is obtained.

Wavelengths of the maximum absorption in 0.1 *M* HCl were determined at this laboratory and generally showed good agreement with data

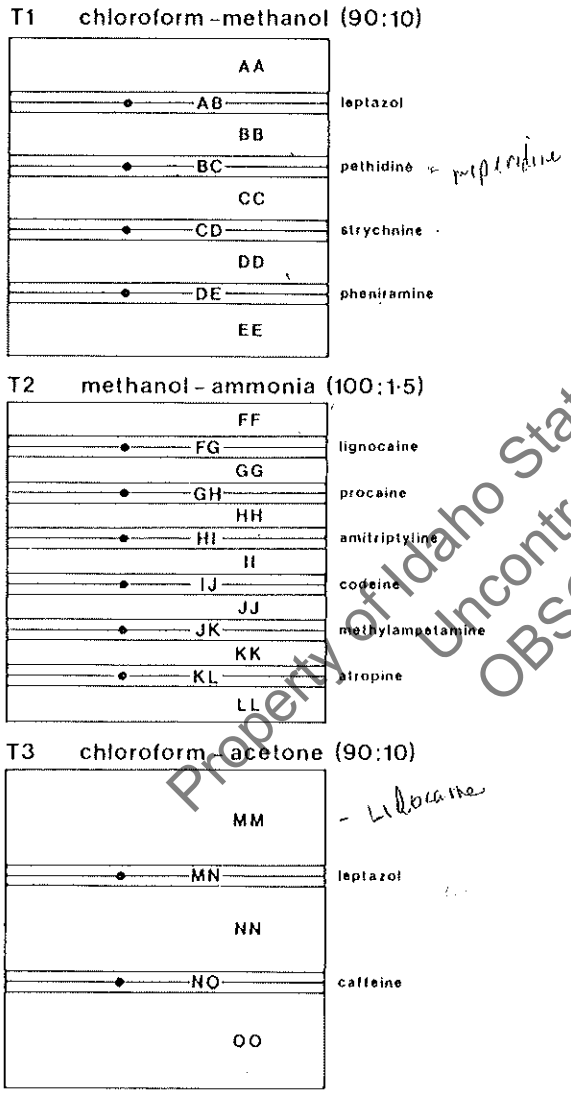

Fig. 1. Classification of drugs by TLC using a system of zones.

published by Clarke [1]. —50 is used to denote a drug that has no absorption maximum between 350 and 200 nm.

Discussion

Having drugs classified in the way described facilitates easy searching with a computer, card index or other manual systems. Our laboratory used a manual system until recently when the acquisition of a computer provided

TABLE 1

File searches for one to six parameters of a drug

Parameters used	Average No. of matches from file
T1	48.9
T1 and T2	14.4
T1, T2 and T3	12.3
OV-101 and OV-17	10.2
T1, T2 and UV	2.1
T1, T2 and OV-101	1.9
T1, T2, T3, OV-101	1.8
T1, T2, T3, OV-101 and OV-17	1.4
T1, T2, OV-101 and UV	1.1
T1, OV-101 and UV	1.1
T1, T2, T3, OV-101, OV-17 and UV	1.0

Ten drugs were randomly selected from the file and using their data the file was then searched for similar data using the parameters outlined. The number of drugs with similar data was averaged to produce the above table.

As described in the experimental section, for TLC two letters describe a drug's position on a plate in a certain solvent system. The program needs to match only one of these letters to either letter on the file for a parameter match. At times (especially when two different letters are used to describe a drug's position) this will produce quite a number of possibilities for drugs. However, the authors believe the possibility of missing the correct drug due to experimental error is a less desirable choice than a few extra possibilities.

Kovats' method of measuring retention indices seems now to be a common practice in drug work [2, 4, 5]. Again this is a desirable form in which to have data to facilitate computer searches. It has been pointed out that these retention indices can vary according to a number of conditions [5, 6] namely column temperature, flow-rate, purity of the mobile and stationary phases, activity of the support, column efficiency, sample size, retention measurement and calculations. Without resorting to special conditions to reduce this variability a large error factor must be used when data files are searched so as not to exclude possible drugs. In practice this error factor seems to lie within ±50 retention index units for most drugs. In our experience some drugs fail to give a reproducible retention index within this range between various operators and laboratories. For this reason and again to be on the safe side the authors use a window of ±100 units when searching the file.

In searching the UV data an error factor of ±2 nm is used.

The computer program allows file searches given from one to all six parameters of a drug. Naturally the more parameters there are the fewer matches are returned (see Table 1). In practice, it is unusual to revert to all

TABLE 2

Drug data file

DRUG DATA FILE

DRUG	T1	T2	T3	OV101	OV17	UV
ACEPIFYLLINE	EE	LL	OO	-500	-500	271
ACEPROMAZINE	CC	JJ	OO	2850	3270	242
ADENINE	DE	HH	OO	-500	-500	263
ALPRENOLOL	DE	JJ	OO	1840	2080	270
AMETHOCAINE	CC	GG	OO	2280	2580	230
AMITRIPTYLINE	BB	HH	OO	2220	2540	238
AMPHETAMINE	EE	JJ	OO	1130	1300	257
AMYLOBARBITONE	XX	XX	XX	1760	2010	-50
AMYLOCAINE	AA	GG	NN	1590	1800	233
ANTAZOLINE	EE	KK	OO	2320	2820	242
APOMORPHINE	DC	GG	OO	-500	-500	272
ASPIRIN	XX	XX	XX	1340	1460	229
ATROPINE	EE	KL	OO	2250	2610	257
AZACYCLONAL	EE	LL	OO	2220	2720	258
BARBITONE	XX	XX	XX	1530	1800	-50
BEMEGRIDE	XX	XX	XX	1410	1660	-50
BENZAMINE	DD	HH	OO	1820	2080	231
BENZOCAINE	AA	FF	OO	1560	1920	226
BENZPHETAMINE	AA	GG	NN	1860	2120	258
BENZTROPINE	EE	LL	OO	2310	2640	258
BENZYDAMINE	DD	JJ	OO	2410	2830	306
BIPERIDEN	BB	GG	OO	2300	2650	257
BR-DIMETHOXYAMPHETAMINE	EE	JJ	OO	1830	2150	294
BROMODIPHENHYDRAMINE	CC	HH	OO	2170	2500	230
BROMPHENIRAMINE	DD	JJ	OO	2090	2470	265
BRUCINE	DD	LL	OO	3560	500	265
BUPHENINE	EE	GH	OO	2350	2770	274
BUPIVACAINE	AB	FF	NN	2360	2640	259
BUTOBARBITONE	XX	XX	XX	1680	1940	-50
CAFFEINE	BB	GG	NO	1830	2270	272
CAMBENDAZOLE	BB	GG	OO	-500	-500	324
CARBAMAZEPINE	BB	FF	NN	2340	2860	285
CARBINOXAMINE	DD	II	OO	2060	2440	264
CHLORAZEPATE	BB	GG	NN	2760	3200	238
CHLORCYCLIZINE	BB	HH	OO	2240	2580	232
CHLORDIAZEPOXIDE	AB	GG	OO	2580	3120	244
CHLOROQUINE	EE	JK	OO	2660	3000	221
CHLORPHENIRAMINE	DD	JJ	OO	2030	2280	265
CHLORPHENTERMINE	EE	II	OO	1350	1520	266
CHLORPROMAZINE	BB	HI	OO	2540	2900	255
CHLORPROPAMIDE	BB	FF	OO	-500	-500	233
CINCHOCAINE	CC	GG	OO	2740	3100	245
CINCHONIDINE	CC	II	OO	2600	3090	236
CINCHONINE	CC	II	OO	2580	3070	236
CLEMIZOLE	AA	FG	NN	2690	3140	275
CLONAZEPAM	BB	GG	NN	2920	3670	270
COCAINE	BB	GG	NN	2240	2620	233
CODEINE	DD	JJ	OO	2380	2860	285
COLCHICINE	BB	GG	OO	-500	-500	247
CYCLIZINE	BB	HH	OO	2010	2390	224
CYCLOBARBITONE	XX	XX	XX	1980	2360	-50
CYPROHEPTADINE	BB	HH	OO	2400	2760	223

(continued overleaf)

OV-101 OV-17

DESIPRAMINE	DE	JJ	OO	2220	2720	250
DESMETHYL DIAZEPAM	BB	FF	NN	2510	3130	237
DEXTROMORAMIDE	AA	FG	NO	2940	3480	258
DEXTROPROPOXYPHENE	BB	GG	NN	2210	2500	257
DI-IODOHYDROXYQUINOLINE	BB	FF	OO	500	500	262
DIAMORPHINE	BB	II	OO	2600	3080	278
DIAZEPAM	AA	FG	MM	2440	2920	241
DIBENZEPIN	CC	HH	OO	2420	3000	221
DICYLOMINE	BB	GG	NN	2110	2320	50
DIETHYLPROPION	BB	FG	NN	1490	1710	252
DIHYDROCODEINE	DE	JK	OO	2350	2870	284
DIPHENHYDRAMINE	CC	HH	OO	1880	2150	258
DIPROPHYLLINE	CC	GG	OO	500	500	271
DIPYRONE	BB	FF	OO	500	500	258
DISOPYRAMIDE	DD	JJ	NN	500	500	263
DOXEPIN	CD	HI	OO	2340	2610	291
DROPERIDOL	BB	FF	OO	500	500	246
EPHEDRINE	EE	JK	OO	1360	1590	257
ETHAMIVAN	BB	GG	NN	1930	2220	280
ETHOHEPTAZINE	EE	JJ	OO	1860	2120	256
ETHOPROPAZINE	BB	HI	OO	2270	2700	248
ETHYL MORPHINE	CC	IJ	OO	2400	2900	285
FENFLURAMINE	DD	II	OO	1220	1350	263
FLUFENAMIC ACID	BB	FF	MM	1090	2360	50
FLUOPROMAZINE	BB	GG	OO	2220	2540	256
FLURAZEPAM	BB	GG	OO	2800	3240	239
FRUSEMIDE	BB	FF	OO	500	500	342
GLUTETHIMIDE	AA	FF	MM	1830	2420	50
GLYCERYL GUAIACOLATE	BB	FG	OO	500	500	272
HALOPERIDOL	BB	FF	OO	3020	3520	220
HARMAN	CC	GG	OO	1980	2400	257
HEXOBARBITONE	XX	XX	XX	1870	2210	50
HEXOCYCLIUM METHYLSO4	EE	LL	OO	500	500	257
HYDROMORPHONE	DE	KK	OO	2580	3120	280
HYDROXYEPHEDRINE	EE	KK	OO	500	500	222
HYOSCINE	BB	GG	OO	2280	2750	257
IMIPRAMINE	CC	II	OO	2240	2580	250
INDOMETHACIN	BB	FF	OO	2680	3140	50
IPRONIAZID	BB	GG	MM	1630	1980	268
ISOCARBOXAZID	AA	FF	MM	1960	2160	50
ISOPENTYLAMINE	EE	KL	OO	500	500	50
LEPTAZOL	AB	GG	MN	1600	2000	50
LIGNOCAINE	AA	FG	MM	1860	2220	263
LORAZEPAM	BB	GG	OO	2430	2970	232
MEBEVERINE	BB	HH	NN	500	500	220
MECLOFENAMIC ACID	BB	FF	NN	2420	2830	50
MECLOFENOXATE	BB	KL	OO	1790	2100	224
MECLOZINE	AA	GG	MM	3030	3490	231
MEFENAMIC ACID	EE	FF	MM	2480	2610	50
MEPHENESIN	BB	FG	OO	500	500	270
MEPHENTERMINE	EE	KK	OO	1230	1400	258
MEPIVACAINE	BB	GG	NN	2090	2380	263
MEPROBAMATE	XX	XX	XX	1800	2200	50
MEPYRAMINE	CC	II	OO	2250	2620	239
MESCALINE	EE	KK	OO	1700	2020	269
METHADONE	DD	II	OO	2170	2440	259

(continued on facing page)

OV-101 OV-17

METHAPYRILENE	CD	HH	NN	1980	2340	238
METHAQUALONE	AA	FG	MM	2150	2820	233
METHOCARBAMOL	BB	FG	OO	500	500	272
METHYL PHENIDATE	CC	GG	OO	1810	2060	258
METHYL PHENOBARBITONE	XX	XX	XX	1770	2210	-50
METHYLAMPHETAMINE	EE	JK	OO	1170	1340	257
MORPHINE	EE	IJ	OO	2400	2930	285
NALORPHINE	CC	GG	OO	2570	3050	286
NAPHAZOLINE	EE	LL	OO	2100	2530	281
NARCEINE	DE	HI	OO	500	500	277
NICOTINAMIDE	CD	GG	OO	500	500	260
NICOTINE	BB	HH	OO	1340	1540	260
NICOTINIC ACID	EE	FF	OO	500	500	260
NIKETHIMIDE	BB	GG	NN	1530	1840	263
NITRAZEPAM	BB	FG	NN	2740	2990	279
NORHARMAN	CC	GG	OO	2100	2480	247
NORPETHIDINE	EE	KK	OO	1740	2080	257
NORPROPOXYPHENE	EE	JK	OO	2440	2800	254
NORPROPOXYPHENE AMIDE	AA	FF	NO	2580	3000	251
NORTRIPTYLINE	DE	JK	OO	2240	2500	230
NOSCAPINE	AA	GG	NN	3240	3920	312
OPIPRAMOL	CC	HH	OO	500	500	253
ORCIPRENALINE	EE	II	OO	500	500	277
ORPHENADRINE	CC	GG	OO	1950	2250	265
OXAZEPAM	BB	GG	MM	2350	2820	236
OXETHAZAINE	BB	LL	OO	500	500	258
OXPRENOLOL	DE	II	OO	1910	2220	220
OXYPHENBUTAZONE	BB	FF	MM	500	500	-50
OXYPHENCYCLIMINE	AA	FF	MM	2540	3080	-50
PAPAVERINE	AA	GG	NN	2840	3480	250
PARACETAMOL	CC	FF	OO	1780	2120	243
PENTAZOCINE	DE	GG	OO	2260	2620	278
PENTOBARBITONE	XX	XX	XX	1770	2040	-50
PERICYAZINE	CC	GG	OO	3340	3930	268
PETHIDINE	BC	HH	OO	1740	2000	257
PHENACETIN	BB	FF	MM	1700	2040	243
PHENAZOCINE	BB	GG	OO	2720	3220	262
PHENCYCLIDINE	CC	HH	OO	1880	2120	262
PHENELZINE	AA	FF	MM	1330	1560	257
PHENIRAMINE	DE	II	OO	1840	2130	262
PHENMETRAZINE	DD	HH	OO	1440	1670	257
PHENOBARBITONE	XX	XX	XX	1990	2390	-50
PHENOTHIAZINE	AA	GG	OO	2030	2430	251
PHENTERMINE	EE	IJ	OO	1140	1270	258
PHENYLBUTAZONE	AA	FF	MM	2350	2850	-50
PHENYLEPHRINE	LL	JK	OO	500	500	272
PHENYLETHYLAMINE (B)	EE	JJ	OO	1100	1320	257
PHENYLPROPANOLAMINE	EE	II	OO	1310	1570	257
PHENYTOIN	XX	XX	XX	2400	2920	50
PHOLCODINE	DD	JJ	OO	3170	3880	283
PHTHALYLSULPHATHIAZOLE	EE	FF	OO	500	500	280
PIMOZIDE	BB	FF	OO	500	500	275
PINDOLOL	EE	II	OO	2260	2750	263
PIPERAZINE	AA	LL	OO	500	500	50
PIPRADROL	CD	II	OO	2170	2540	258
PRACTOLOL	EE	II	OO	500	500	243
PRILOCAINE	AA	GG	NN	1800	2170	222

(continued overleaf)

OV-101 OV-17

PROCAINE	CD	GH	OO	2000	2410	227
PROCHLORPERAZINE	BB	II	OO	2970	3480	254
PROCYCLIDINE	CC	II	OO	2220	2510	257
PROLINTANE	CD	II	OO	1640	1800	257
PROMAZINE	CC	II	OO	2310	2740	252
PROMETHAZINE	BB	HH	OO	2280	2740	250
PROPIOMAZINE	BB	HH	OO	2740	3220	242
PROPRANOLOL	DE	HH	OO	2150	2610	289
PROPYLHEXEDRINE	DD	KK	NN	1160	1270	-50
PROTRIPTYLINE	EE	KK	OO	2300	2660	292
PSEUDOEPHEDRINE	EE	JK	OO	1360	1620	256
PYRROBUTAMINE	BB	HH	OO	2430	2830	226
QUINALBARBITONE	XX	XX	XX	1820	2090	-50
QUININE	DD	HH	OO	2760	3300	250
RESERPINE	AA	FF	NN	-500	-500	267
SALBUTAMOL	EE	II	NN	-500	-500	224
SALICYLAMIDE	BB	FF	MM	1470	1780	236
STRYCHNINE	CD	KK	OO	3150	3760	255
SUCCINYLSULPHATHIAZOLE	EE	FF	OO	-500	-500	280
SULPHADIAZINE	BC	FF	OO	-500	-500	242
SULPHADIMIDINE	BB	FF	OO	-500	-500	242
SULPHAFURAZOLE	CC	FF	OO	-500	500	264
SULPHAGUANIDINE	EE	FF	OO	-500	500	264
SULPHAMETHIZOLE	CC	FF	OO	-500	-500	267
SULPHAMETHOXAZOLE	BB	FF	OO	-500	-500	265
SULPHAPYRAZOLE	EE	FF	OO	-500	-500	-50
SULPHASALAZINE	EE	FF	OO	-500	-500	-50
SULPHASYMAZINE	BB	FF	OO	-500	-500	252
TACRINE	EE	JJ	OO	2160	2700	241
THEBAINE	BC	II	OO	2520	2600	282
THEOBROMINE	CC	GG	OO	1910	2460	272
THEOPHYLLINE	BB	FF	OO	2040	2560	270
THIABENDAZOLE	BB	GG	NN	2080	2540	302
THIAMINE	EE	LL	OO	-500	-500	246
THIOPROPAZATE	BB	GG	OO	3480	-500 *	254
THIORIDAZINE	CC	II	OO	3120	3660	262
THIOTHIXENE	BC	II	OO	-500	-500	229
THYMINE	CC	GG	OO	-500	-500	265
TOLAZOLINE	EE	LL	OO	1510	1880	256
TOLBUTAMIDE	BB	FF	OO	-500	-500	229
TRANYLCYPROMINE	CC	GG	NN	1210	1430	264
TRIFLUOPERAZINE	BB	HH	OO	2720	3030	256
TRIMIPRAMINE	BB	GH	OO	2200	2580	250
TRIPELENNAMINE	DD	HH	OO	1960	2200	238
TRIPROLIDINE	DD	II	OO	2260	2580	230
TRYPTAMINE	EE	KK	OO	1730	2140	278
TYRAMINE	EE	JK	OO	-500	-500	220
URACIL	DD	GG	OO	-500	-500	258
YOHIMBINE	CC	GG	OO	3260	3790	220

NOTE:

-500 REFERS TO COMPOUNDS WITH RETENTION INDICES OF <1000 OR UNOBTAINABLE

-50 REFERS TO COMPOUNDS WITH NO ABSORPTIONS BETWEEN 350 and 220 nm IN .1m HCl

XX SPOT NOT LOCATED ON T.L.C.

* RETENTION INDICES >4000

of the six parameters to establish the identity of the drug in question. In some instances the use of TLC systems T1 and T2 and GLC system OV-101 will provide sufficient information to identify the drug.

The system of drug identification has evolved in this laboratory over a number of years and has proved extremely useful. No claim is made as to the discriminating power of the GLC columns or the TLC systems used and it is likely that using more discriminating solvent systems and stationary phases the method of identification could be even more selective [7].

Acknowledgements

The authors are indebted to their colleagues in the Forensic Chemistry Division for assistance in obtaining TLC and GLC data and the Director of the Government Chemical Laboratories for approval to publish this paper.

References

1 E. G. C. Clarke, Isolation and Identification of Drugs, Vols. 1 and 2, The Pharmaceutical Press, London 1969 and 1975.
2 L. Kazyak and R. Permisohn, J. Forensic Sci., 15 (1970) 346.
3 E. Kovats, Helv. Chim. Acta, 41 (1968) 1915.
4 A. C. Moffat, J. Chromatogr., 113 (1975) 69.
5 D. Courtot, Brit. J. Sports Med., 10 (1976) 143.
6 X. Guardino *et al.*, J. Chromatogr., 118 (1976) 13.
7 A. C. Moffatt, A. H. Steard and K. W. Smalldon, J. Chromatogr., 90 (1974) 19.

from INSTRUMENTAL METHODS OF DRUG ANALYSIS

GAS CHROMATOGRAPHY

The gas chromatography data are presented in the monographs in the form of Kovats indices calculated by the following formula:

$$I = 100\left(2\,\frac{\log T_D/T_X}{\log T_Y t_X} + X\right)$$

where

- I = Kovats index
- T_D = Retention time of the drug
- T_X = Retention time of an even numbered normal hyrocarbon whose carbon number is X
- T_Y = Retention time of an even numbered normal hydrocarbon whose carbon number is Y where $Y = X + 2$

and

$$T_X \leq T_D \leq T_Y$$

Each retention index is presented in the monograph with the temperature at which its T_D, T_X, and T_Y values were measured. All retention times were measured from the time of injection. A Hewlett-Packard 5830A gas chromatograph was used with an 18850A terminal, FID detector, and a 4′ × 1/4″ column of 3% OV-1 on Chromosorb WHP 80/100 mesh. The carrier gas was nitrogen at a flow rate of 32 ml/min. Large samples of drugs were used (10–100 micrograms) for injection. At these levels, a moderate change in the amount injected does not change the retention time significantly. To reproduce the results obtained in this book, the same temperature, column packing, carrier gas, gas flow rate, and column length should be used. A table of retention indices appears in Appendix B.

"Look-Alike"Preparations Encountered by Wisconsin Department of Justice- Crime Laboratory Bureau

(Note: First entries in each category correspond to legitimate product being copied)

DESCRIPTION	MARKINGS	CONTENTS	SCH.
Black capsule	"18-875"	Amphetamine	II
	"F-9031"	Caffeine	NO
	"RX 102"	Caffeine	NO
	"0147"	Phentermine	IV
	"817"	Ephedrine, Phenylpropanolamine, Caffeine	NO
	"335"	Ephedrine, Caffeine	NO
	"127"	Pseudoephedrine, Caffeine	NO
	"AHS"	Mephentermine, Caffeine	NO
	"RJS"	Ephedrine, Phenylpropanolamine, Caffeine	NO
	"DEX"	Pseudoephedrine, Caffeine	NO
	Unmarked	Ephedrine, Caffeine	NO
	Unmarked	Caffeine	NO
	Unmarked	Phenylpropanolamine, Caffeine	NO
Green and clear capsule with green and white granules	"SKF D92"	Amphetamine/Amobarbital	II
	"127"	Pseudoephedrine, Caffeine	NO
	"815"	Ephedrine, Phenylpropanolamine, Caffeine	NO
	"975"	Caffeine	NO
	Unmarked	Caffeine	NO
	Unmarked	Ephedrine, Caffeine	NO
Brown and clear capsule with orange and white granules	"SKF E14"	Amphetamine	II
	"RX101"	Caffeine	NO
	"BTP L19"	Ephedrine	NO
	"127"	Pseudoephedrine, Caffeine	NO
	"AHS"	Caffeine	NO
	Unmarked	Caffeine	NO
	Unmarked	Ephedrine, Caffeine	NO
Blue and clear capsule with blue and white granules	"BMP 147"	Phentermine	IV
	"SKF H76"	Phenobarbital	IV
	"BTP L17"	Ephedrine, Caffeine	NO
	"127"	Pseudoephedrine, Caffeine	NO

Continued from page 1			
	"813"	Ephedrine, Phenylpropanolamine, Caffeine	NO
	"975"	Pseudoephedrine, Caffeine	NO
	Unmarked	Ephedrine, Phenylpropanolamine, Caffeine	NO
White and clear capsule with green, orange and white granules	"SKF J66"	Amphetamine, Prochlorperazine	II
	"127"	Pseudoephedrine, Caffeine	NO
Yellow capsule	"18-904"	Phentermine	IV
	"0147"	Phentermine	IV
	"172"	Phentermine	IV
	"Ⓡ"	Phentermine	IV
	"860"	Phentermine	IV
	"13-807"	Caffeine	NO
	"819"	Ephedrine, Phenylpropanolamine, Caffeine	NO
	"RJ8"	Pseudoephedrine, Caffeine	NO
Clear capsule with white powder	Unmarked	"Haysma"	NO
	Unmarked	Ephedrine	NO
White oblong tablet, half-scored, with green speckles	"71" or "Lemmon"	Phendimetrazine	III
	Unmarked	Caffeine	NO
	Unmarked	Ephedrine, Phenylpropanolamine, Caffeine	NO
	Unmarked	Ephedrine, Caffeine	NO
Salmon tablet	"BI 62"	Phenmetrazine	II
	"BT 72"	Pseudoephedrine, Caffeine	NO
	Unmarked	Phenylpropanolamine, Caffeine	NO
Green triangular half-scored tablet	"SKF 093"	Amphetamine, Amobarbital	II
	"165"	Caffeine	NO

Orange triangular, half-scored tablet	"SKF A92" Unmarked Unmarked	Amphetamine Caffeine Ephedrine, Caffeine	II NO NO
White, medium, round, half-scored tablet	"W/C 230" Unmarked	"Tedral" Ephedrine, Theophylline, Phenobarbital	NO NO

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Division of Law Enforcement Services
Crime Laboratory Bureau

MICROGRAM, VOL. XIII, NO. 2 (FEBRUARY 1980)

A folded paper packet containing about 0.4 gram of a white, crystalline substance purported to be cocaine, but containing cyclizine HCl, an antinauseant.

Forty white tablets bearing the "Lemmon 714" logo containing methaqualone HCl, thioridazine and diphenhydramine. Although each of these compounds has been reported in counterfeit "Lemmon Quaaludes" on various occasions, this is the first instance known to DEA of all three being present in the same tablets.

A quart oil can with a screw-cap jar inside, the annular space between the jar and the wall of the can being filled with plaster of Paris. The lid of the jar was secured to the lid of the can with epoxy resin so that the can appeared to be intact when the lid was screwed down. The jar contained 14 grams of bulk methaqualone HCl.

Additionally, the laboratory continues to see a large number of amphetamine "Look-Alikes," most of which have been described in past issues of Microgram.

The Baltimore City Police Department Laboratory Division recently analyzed a submission containing a mixture of white heroin 7.5% with methaqualone 2.5%. The exhibits also contained mannitol, dextrose, starch and quinine, and were packaged in glassine bags.

"Look-Alike Preparations"

DEA is publishing the following list of "Look-Alike" preparations because of the wide distribution of these products and the considerable interest evident among Microgram readers. The list contains only those products that have been analyzed in the DEA Special Testing and Research Laboratory. Readers should be aware that the products may contain either one or any combination of the drugs listed. There is no quality control of "Look-Alikes."

Capsule Color - Opaque Black

Markings	Drugs Identified
RX 102	Caffeine
AHS	Caffeine
335	Caffeine, Pseudoephedrine HCl
F-9031	Caffeine
Balance Pan Logo plus F-9031	Caffeine
K-200	Caffeine, Phentermine
17-865	l-Ephedrine SO4 and poppy seeds
18-789	Pseudoephedrine HCl and Caffeine
975	Caffeine
SK+K	Caffeine
BT logo plus 18-845	dl-Ephedrine HCl and Caffeine
Balance pan logo plus 817	Caffeine and Phenylpropanolamine HCl

P-200	Caffeine
1167	Ephedrine
RJS	Ephedrine, Caffeine
DEX	Pseudoephedrine HCl and Caffeine
127	Pseudoephedrine HCl and Caffeine
TUTAG	Phendimetrazine
172	Phentermine
"W" in box logo	Phentermine
0147	Phentermine
J-818	Phentermine
888	Phentermine
18-876	l-Ephedrine SO4 and Caffeine

Capsule Color - Opaque Black/White

Markings	Drugs Identified
AHS	Caffeine
19-731	Caffeine
1168	l-Ephedrine HCl and Caffeine

Capsule Color - Opaque Black/Red

Markings	Drugs Identified
K-200	Caffeine
AHS	Caffeine
30 30	Phentermine
127	Caffeine and Poppy Seeds

Capsule Color - Opaque Yellow

Markings	Drugs Identified
Balance Pan logo plus 13-807	Caffeine
RJ8	Caffeine, Pseudoephedrine
16-907	Caffeine, dl-Ephedrine HCl, l-Ephedrine SO4, Poppy Seeds (combinations of above)
BT logo plus 18-894	dl-Ephedrine HCl and Caffeine
617	Pseudoephedrine and Caffeine
172	Phentermine
WRL	Phentermine
860	Phentermine
J-818	Phentermine
0185 5000	Phentermine
0147	Phentermine
R in diamond logo	Phentermine
H.S. 364	Phentermine
822-A	Phentermine
725	Phentermine
RJS	Phentermine
Pennwalt logo plus 18-904	Phentermine

Capsule Color - Opaque Blue

Markings	Drugs Identified
697	Phentermine

Capsule Color - Opaque Brown/White

Markings	Drugs Identified
1168	Caffeine

Capsule Color - Opaque Gray/Yellow

Markings	Drugs Identified
K-200	Caffeine

Capsule Color - Clear White

Markings	Drugs Identified
555166	Phendimetrazine
697	Phendimetrazine

Capsule Color - Clear Yellow

Markings	Drugs Identified
RG	Phendimetrazine

Capsule Color - Clear Pink

Markings	Drugs Identified
97209-63	Caffeine

Capsule Color - Opaque White/Clear With Pellets

Markings	Drugs Identified
675	Pseudoephedrine HCl and Caffeine
975	Phenylpropanolamine HCl and Caffeine

Capsule Color - Brown/Clear with Pellets

Markings	Drugs Identified
SK+K	Caffeine
AHS	Caffeine
RX 101	Caffeine
K-200	Caffeine
127	Phenylpropanolamine HCl and Caffeine, OR pseudoephedrine HCl and Caffeine
BTP and L19	Phenylpropanolamine HCl and Caffeine
CCD	Phendimetrazine

Capsule Color - Brown/Clear with Pellets

Markings	Drugs Identified
725	Phendimetrazine
0147	Phendimetrazine, Phentermine
H.S. 364	Phendimetrazine
R in diamond logo	Phendimetrazine
172	Phendimetrazine, Phentermine
822-A	Phendimetrazine
WRL	Phendimetrazine
46193	Phentermine
W in box logo	Phentermine

Capsule Color - Green/Clear with Pellets

Markings	Drugs Identified
RX 103	Caffeine
AHS	Caffeine
SK+K	Caffeine
092	l-Ephedrine SO4 and Caffeine
127	Phenylpropanolamine HCL and Caffeine
BTP plus L14	Phenylpropanolamine HCl and Caffeine
518	Phenylpropanolamine HCl and Pseudoephedrine HCl and Caffeine
0147	Phentermine, Phendimetrazine
46193	Phentermine

Capsule Color - Blue/Clear with Pellets

Markings	Drugs Identified
697	Caffeine
975	Caffeine
127	Phenylpropanolamine HCl and Caffeine, OR Pseudoephedrine HCl and Caffeine
BTP and L17	Phenylpropanolamine HCl and Caffeine
WRL	Phentermine
0147	Phentermine, Phendimetrazine
172	Phentermine
46193	Phentermine
BMP and 147	Phentermine

SELECTED REFERENCES

Constituents of Cannabis sativa L., XVII. A Review of the Natural Constituents, Journal of Natural Products, 43 #2, 169-234, March/April 1980.

Identification of the constituents of Cannabis sativa L. Part of a series of papers.

"Look-Alike Preparations"

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Capsule Color - Opaque Black

Markings	Drugs Identified
RX 102	Caffeine
AHS	Caffeine
335	Caffeine, Pseudoephedrine HCl
F-9031	Caffeine
Balance Pan Logo plus F-9031	Caffeine
K-200	Caffeine, Phentermine
17-865	l-Ephedrine SO4 and poppy seeds
18-789	Pseudoephedrine HCl and Caffeine
975	Caffeine
SK+K	Caffeine
BT logo plus 18-845	dl-Ephedrine HCl and Caffeine
Balance pan logo plus 817	Caffeine and Phenylpropanolamine HCl
P-200	Caffeine
1167	Ephedrine
RJS	Ephedrine, Caffeine
DEX	Pseudoephedrine HCl and Caffeine
127	Pseudoephedrine HCl and Caffeine
TUTAG	Phendimetrazine
172	Phentermine
"W" in box logo	Phentermine
0147	Phentermine OR Phendimetrazine
J-818	Phentermine
888	Phentermine
18-876	l-Ephedrine SO4 and Caffeine
"18-858"	Caffeine, Ephedrine + Phenylpropanolamine
"SMR 22"	Caffeine + Ephedrine
"1S875"	Caffeine, l-Ephedrine SO4 + Phenylpropanolamine (MFR:FRYE)
"TM" + "AKS" in box + "18-789"	Caffeine + Phenylpropanolamine
"1010"	Caffeine, l-Ephedrine SO4 + Phenylpropanolamine (MFR:FRYE)
"C8858"	Caffeine, l-Ephedrine SO4 + Phenylpropanolamine
"18C658"	Caffeine, l-Ephedrine SO4 + Phenylpropanolamine
"C8658"	Caffeine + Phenylpropanolamine
"MO/L.E"	l-Ephendrine HCl + Caffeine
"RUS"	Caffeine + l-Ephedrine SO4
"335"	Caffeine, Ephedrine + Phenylpropanolamine

Capsule Color - Opaque Black (Continued)

2W in box + "18-845"	Caffeine, Ephedrine, Phenylpropanolamine
BT logo = "10-845"	Caffeine, Poppy Seeds, l-Ephedrine SO4, Phenylpropanolamine
SV logo + "18-985"	Caffeine, l-Ephedrine SO4, Phenylpropanolamine
Square/triangle logo + "16-870"	Caffeine, Ephedrine, Phenylpropanolamine (MFR:FRYE)
SV logo + "18-850"	Caffeine, Ephedrine, Phenylpropanolamine
SV logo + "18-789	Caffeine, Ephedrine, Phenylpropanolamine

Capsule Color - Opaque Black/White

Markings	Drugs Identified
AHS	Caffeine
19-731	Caffeine
1168	l-Ephedrine HCl and Caffeine

Capsule Color - Opaque Black/Red

Markings	Drugs Identified
K-200	Caffeine
AHS	Caffeine
30 30	Phentermine
127	Caffeine and Poppy Seeds
"ST 22"	Caffeine + Ephedrine

Capsule Color - Opaque Yellow

Markings	Drugs Identified
Balance Pan logo plus 13-807	Caffeine
RJ8	Caffeine, Pseudoephedrine
16-907	Caffeine, dl-Ephedrine HCl, l-Ephedrine
	Poppy Seeds (combinations of above)
BT logo plus 18-894	dl-Ephedrine HCl and Caffeine
617	Pseudoephedrine and Caffeine
172	Phentermine
WRL	Phentermine
860	Phentermine
J-818	Phentermine
0185 5000	Phentermine
0147	Phentermine
R in diamond logo	Phentermine
H.S. 364	Phentermine
822-A	Phentermine
725	Phentermine
RJS	Phentermine
Pennwalt logo plus 18-904	Phentermine

Capsule Color - Opaque Yellow (Continued)

"RJS"	Caffeine, 1-Ephedrine S04 + Phenylpropanolamine
Balance pan logo + "819"	Caffeine + Phenylpropanolamine
"18-907"	Ephedrine + Caffeine
"RUS"	Caffeine, 1-Ephedrine S04 + Phenylpropanolamine
SV logo + "18-985"	Caffeine + 1-Ephedrine S04
"18-906"	Phenylpropanolamine, 1-Ephedrine S04 + Caffeine
"RVJ"	1-Ephedrine S04 + Caffeine
"C RJ8"	Caffeine, Ephedrine + Phenylpropanolamine
"C 8074"	Caffeine, Ephedrine + Phenylpropanolamine
BT logo + "10-984"	Caffeine, Phenylpropanolamine, 1-Ephedrine HCl
Square/triangle logo + "16-807"	Caffeine, Phenylpropanolamine, 1-Ephedrine HCl (MFR:FRYE)
Square/circle/"d" logo + "812"	Caffeine, Phenylpropanolamine, 1-Ephedrine S04

Capsule Color - Opaque Blue

Markings	Drugs Identified
697	Phentermine

Capsule Color - Opaque Brown/White

Markings	Drugs Identified
1168	Caffeine

Capsule Color - Opaque Gray/Yellow

Markings	Drugs Identified
K-200	Caffeine

Capsule Color - Clear White

Markings	Drugs Identified
555166	Phendimetrazine
697	Phendimetrazine

Capsule Color - Clear Yellow

Markings	Drug Identified
RG	Phendimetrazine

Capsule Color - Clear Pink

Markings	Drugs Identified
97209-63	Caffeine

Capsule Color - Opaque White/Clear With Pellets

Markings	Drugs Identified
675	Pseudoephedrine HCl and Caffeine
975	Phenylpropanolamine HCl and Caffeine
"528"	Pseudoephedrine + Caffeine
"697"	Pseudoephedrine + Caffeine

Capsule Color - Brown/Clear With Pellets

Markings	Drugs Identified
SK+K	Caffeine
AHS	Caffeine
RX 101	Caffeine
K-200	Caffeine
127	Phenylpropanolamine HCl and Caffeine, OR Pseudoephedrine HCl and Caffeine
BTP and L19	Phenylpropanolamine HCl and Caffeine
CCD	Phendimetrazine
725	Phendimetrazine
0147	Phendimetrazine, Phentermine
H.S. 364	Phendimetrazine
R in diamond logo	Phendimetrazine
172	Phendimetrazine, Phentermine
822-A	Phendimetrazine
WRL	Phendimetrazine
46193	Phentermine
W in box logo	Phentermine
"591-N"	Phendimetrazine
"DELO" + "L19"	l-Ephedrine

Capsule Color - Green/Clear with Pellets

Markings	Drugs Identified
RX 103	Caffeine
AHS	Caffeine
SK+K	Caffeine
092	l-Ephedrine SO4 and Caffeine
127	Phenylpropanolamine HCl and Caffeine
BTP plus L14	Phenylpropanolamine HCl and Caffeine
518	Phenylpropanolamine HCl and Pseudoephedrine and Caffeine
0147	Phentermine, Phendimetrazine
46193	Phentermine
Balance pan logo + "815"	Caffeine + Phenylpropanolamine

Capsule Color - Blue/Clear with Pellets

Markings	Drugs Identified
697	Caffeine
975	Caffeine
127	Phenylpropanolamine HCl and Caffeine, OR Pseudoephedrine HCl and Caffeine
BTP and L17	Phenylpropanolamine HCl and Caffeine
WRL	Phentermine
0147	Phentermine, Phendimetrazine
172	Phentermine
46193	Phentermine
BMP and 147	Phentermine
"BNP" and '247"	Ephedrine, Caffeine and Phenylpropanolamine (MFR:FRYE)

double-scored white tab → "npi" — almost entirely l-epiedrine

20|20
white pill - pink and blue speckles
d, l - ephedrine caffeine

D|E
white tablet w/blue specks
caffeine

Blue oblong tabs "Entex LA" phenylpropanolamine and caffeine"

4/86 P. Server
small cunnarted pink capsules ephedrine

pink-small → ephedrine caffeine

Canadian Journal of Forensic Science Vol 18 No 1 March 85

Table 3
^{1}H NMR CHEMICAL SHIFT (δ)* DATA FOR STANDARD AND "LOOK-ALIKE" PREPARATIONS

Sample	C^1-H	C^2-H	C^3H_3	N-CH_3	C^1-H	C^2-H	C^3H_3
1 (a)	5.14(d)	3.56(m)	1.16(d)	2.79(s)			
(b)	4.76(d)	2.78(m)	0.85(d)	2.46(s)			
2 (a)							
(b)					4.98(d)	3.70(m)	1.20(d)
					4.51(d)	3.15(m)	0.96(d)
3 (a)	5.16(d)	#	1.16(d)	2.84(s)	5.00(d)	#	1.22(d)
(b)	4.74(d)	2.77(m)	0.85(d)	2.46(s)	4.51(d)	3.13(m)	0.97(d)
4 (a)	5.25(d)	#	1.20(d)	2.92(s)	5.08(d)	#	1.25(d)
(b)	4.81(d)	2.79(m)	0.84(d)	2.50(s)	4.55(d)	3.12(m)	0.97(d)
5 (a)	5.16(d)	#	1.16(d)	2.84(s)	5.08(d)	#	1.21(d)
(b)	4.76(d)	2.79(m)	0.86(d)	2.47(s)	4.51(d)	3.15(m)	0.98(d)

* Protons attached to corresponding carbons, s = singlet, d = doublet, m = multiplet
a) Salt in D_2O
b) Free base in $CDCl_3$
1- ephedrine
2- phenylpropanolamine
3- Standard mixture (ephedrine, phenylpropanolamine and caffeine). δ values for N-CH_3's of caffeine 3.89, 3.41 and 3.24 in D_2O and 4.00, 3.58, 3.41 in $CDCl_3$
4- Black capsule
5- Pink tablet
\# masked by caffeine peaks

The NMR methods, especially ^{1}H NMR, are fairly rapid and specific. Moreover, major impurities or excipients may be identified. For example, in the case of the tablets analyzed, dextrose was found to be present. Dextrose resonance peaks appeared in the D_2O spectrum, but when the free base was liberated and extracted into $CDCl_3$, the dextrose remained in the aqueous layer. The procedure has proven extremely useful as a rapid screening method to distinguish these counterfeits from actual Narcotic, Controlled or Restricted drugs submitted as police exhibits.

APPENDIX

"Look-Alike Preparations"

Capsule Color – Opaque Black

Drugs Identified	*Markings*
Caffeine	RX 102, AHS, F-9031, Balance Pan Logo plus
Caffeine, Pseudoephedrine HCl	F-9031, 975, SK + K, P-200
Caffeine, Phentermine	335, 18-789, DEX, 127
l-Epherdrine SO4 and poppy seeds	K-200
dl-Ephedrine HCl and Caffeine	17-865
	BT logo plus 18-845

Drugs Identified	*Markings*
Caffeine and Phenylpropanolamine HCl	Balance pan logo plus 817
Ephedrine	1167
Ephedrine, Caffeine	RJS, "SMR 22"
Phendimetrazine	TUTAG
Phentermine	172, "WL" in box logo, J-818, 888
Phentermine OR Phendimetrazine	0147
l-Ephedrine SO4 and Caffeine	18-876, "RUS"
Caffeine, Ephedrine + Phenylpropanolamine	"18-858", SV logo + "18-850", "335", 2W in box + "18-845", SV logo + "18-789"
Caffeine, l-Ephedrine SO4 + Phenylpropanolamine (MFR:FRYE)	"IS875", "1010"
Caffeine + Phenylpropanolamine	"TM" + "AKS" in box + "18-789", "C8658"
Caffeine, l-Ephedrine SO4 + Phenylpropanolamine	"C8858", "18C658", SV logo + "18-985"
l-Ephedrine HCl + Caffeine	"MO/L.E"
Caffeine, Poppy Seeds, l-Ephedrine SO4, Phenylpropanolamine	BT logo + "10-845"
Caffeine, Ephedrine, Phenylpropanolamine (MFR:FRYE)	Square/triangle logo + "16 870"
Capsule Color - Opaque Black/White	
Caffeine	AHS, 19-731
l-Ephedrine HCl and Caffejne	1168
Capsule Color - Opaque Black/Red	
Caffeine	K-200, AHS
Phentermine	30 30
Caffeine and Poppy Seeds	127
Caffeine + Ephedrine	"SST 22"
Capsule Color – Opaque Yellow	
Caffeine	Balance Pan logo plus 13-807
Caffeine, Pseudoephedrine	RJ8
Caffeine, dl-Ephedrine HCl, l-Ephedrine	
Poppy Seeds (combination of above)	16-907
dl-Ephedrine HCl and Caffeine	BT logo plus 18-894
Pseudoephedrine and Caffeine	617
Phentermine	172, WRL, 860, J818, 0185 5000, 0147, R in diamond logo, H.S. 364, 822-A, 725, RJS, Pennwalt logo plus 18-904
Caffeine, l-Ephedrine SO4 + Phenylpropanolamine	"RJS", "RUS", Square/circle/"d" logo + "812", "18-906"
Caffeine + Phenylpropanolamine	Balance pan logo + "819"
Ephedrine + Caffeine	"18-907"
Caffeine + l-Ephedrine SO4	SV logo + "18-985"
l-Ephedrine SO4 + Caffeine	"RVJ"
Caffeine, Ephedrine + Phenylpropanolamine	"C 8074", "C RJ8"
Caffeine, Phenylpropanolamine, l-Ephedrine HCl	BT logo + "10 984"
Caffeine, Phenylpropanolamine, l-Ephedrine HCl (MFR:FRYE)	Square/triangle logo + "16-807"
Capsule Color - Opaque Blue	
Phentermine	697
Capsule Color - Opaque Brown/White	
Caffeine	1168

Drugs Identified	*Markings*
Capsule Color - Opaque Gray/Yellow	
Caffeine	K-200
Capsule Color - Clear White	
Phendimetrazine	555166, 697
Capsule Color - Clear Yellow	
Phendiametrazine	RG
Capsule Color - Clear Pink	
Caffeine	97209-63
Capsule Color - Opaque White/Clear with Pellets	
Pseudoephedrine HCl and Caffeine	675
Phenylpropanolamine HCl and Caffeine	975
Pseudoephedrine + Caffeine	"528", "697"
Capsule Color - Brown/Clear with Pellets	
Caffeine	SK + K, AHS, RX 101, K-200
Phenylpropanolamine HCl and Caffeine, OR Pseudoephedrine HCl and Caffeine	127
Phenylpropanolamine HCl and Caffeine	BTP and L19
Phendiametrazine	CCD, 725, H.S. 364, R in diamond logo, 822-A, WRL, "591-N"
Phendimetrazine, Phentermine	0147, 172
Phentermine	46193, W in box logo
l-Ephedrine	"DELO" + L19"
Capsule Color - Green/Clear with Pellets	
Caffeine	RX 103, AHS, SK + K
l-Ephedrine SO4 and Caffeine	092
Phenylpropanolamine HCl and Caffeine	127, BTP plus L14
Phenylpropanolamine HCl and Pseudoephedrine and Caffeine	518
Phentermine, Phendimetrazine	0147
Phentermine	46193
Caffeine + Phenylpropanolamine	Balance pan logo + "815"
Capsule Color - Blue/Clear with Pellets	
Caffeine	697, 975
Phenylpropanolamine HCl and Caffeine, OR Pseudoephedrine HCl and Caffeine	127
Phenylpropanolamine HCl and Caffeine	BTP and L17
Phentermine	WRL, 172, 46193, BMP and 147
Phentermine, Phendimetrazine	0147
Ephedrine, Caffeine and Phenylpropanolamine.. (MFR: FRYE)	"BNP" and "247"

REFERENCES

1. Noggle, F. Taylor Jr., *Assoc. Off. Anal. Chem.*, *63* No. 4, (1980), p. 702.

2. Levy, G.C. and Nelson, G.L., Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, John Wiley & Sons, Inc., (1972), p. 31.

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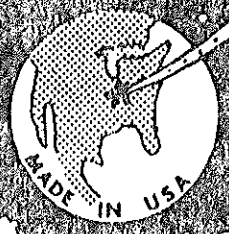

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*caf: caffein ph: phenylpropanolamine es: ephedrine sulph.

Johnny Bob: Have a 'lude.
Driver: Thanks, pal. You a Deadhead? Good? Yeah. All right.
Johnny Bob: Listen, I hate these rat sacks. How about dropping me off here?
Driver: I don't know about that, Injun brother. We're way up in the hills. Let you off here you might get jumped by psychopaths—don't know what they might do. Why don't you just string along with the rest of us until I drive off the cliff? I let you out first if you decide then you don't want to. (*Johnny Bob looks hard at driver.*) I can see you're one of the stubborn ones, all right. Your mind's made up and you're not about to change it. Well, you know what's best for you. Being an Indian and all, I can't expect to properly understand you. Well, I respect you just the same. (*He slowed the car to a stop.*) Don't none of the rest of you get out. I'd hate to shotgun you down and blow my state of mind to jagged shards. Stay right there where you are. Help yourself to the bar. It's free all night. (*Johnny Bob steps out and the driver pulls away. Turning back to face the Injun on the road, he lifts his beaded leather hat in a farewell salute.*) Sorry you couldn't come along for the ride, Injun! Don't worry about a thing! (*Johnny could hear his agent shout.*)
Agent: (*fading*) Let me out! I'm his agent. He needs me. (*The taillights fade shortly before the last whisper of the Grateful Dead. Johnny turned and began to walk slowly back down the canyon road.*)

Right there is where the credits would have been rolled in a real Hollywood movie. But it was not a real Hollywood movie. It was a rehearsal. Developmental, experimental improvisation, scriptwriting and casting. He had been written out. Because he was an Indian, the driver had said. Maybe it was a car theft as well with the possibility of a little kidnapping and murderous intent. The possibility could not be ruled out. It may have been a joke. "Maybe they'll come back and give me a ride to the hotel." Johnny stumbled on dejectedly until he came to a lighted road, which led to a bigger road upon which taxicabs for hire presented themselves. The cabs were quick and unfamiliar with the practice of picking fares off the street. A worthy game. Johnny stalked carefully, watched and waited, and when the moment was right pulled one in with the effortless grace of his forest-born ancestors.

He swelled with a small, hard pride. Then, prodding at this knotted emotion, he discovered not an insubstantial emotion but an assortment of diminutive liquor bottles containing assorted premixed cocktails. Happily that early Christmas morning he opened a Mr. Boston Margarita.

"My one weakness," he mumbled and drank the bottle. □

This issue marks the HIGH TIMES debut of veteran investigative journalist **Michael Dorgan.** Born and raised in

Wisconsin, the cheese capital of our nation, Michael and his lovely wife Lydia (who's French, by the way) now call San

Who's high?

Francisco home. Beginning his career as a cub reporter for the nationally known *Capital Times*, Michael then went on to work for the notorious *Madison Press Connection*. He now eks out a living spewing spitout for the California News Bureau. These last few months have found him sucking up rice balls and incense as he doggedly stalks the Krishna Connection.

After seeing *Carrie* and *The Shining* and reading all those spooky books, we figured we'd better send *two* people over to interview Stephen King. Smart cookies that we are, we dispatched newlyweds **Martha Thomases** and **John Robert Tobbel.** We got two reporters for the

price of one—and, talk about icing on the cake, got them both back alive! Which was really good for them, too, 'cause they've just started putting out a great new nonfiction humor mag called *Comedy*, and, as everybody knows, it's real hard to put out a humor magazine if you're dead. Ha, ha!

"Whoever said 'Stone walls do not a prison make' never spent any time at the federal penitentiary," groans **R. David Holdaway,** who contributed this month's story on hash smuggling. Holdaway, or he's known to his friends, 00785M, has spent the last year in jail and he figures he's got four more to grind out before h released. We're sure he'd love hearing from any and all H.T. readers. No samples please.

Combining gun fetishism with a flai for the Orient is manly **Paul Kirchner,**

creator of the infamous Dope Rider. Though Kirchner's work has appeared in numerous magazines throughout the years, he calls *Heavy Metal* home, and regularly feeds them the outpourings of his depraved imagination. An old HIGH TIMES favorite, welcome back Dope Rider.

Capt. Bo Redux: At first we thought the big shots were having sport with us. Cruel bastards, they'd done it before, taunting and teasing us about the return of **Robert Martin Sacks,** former HIGH TIMES vice-president and production manager and the only man ever to be gang raped by a group of heavily armed Catholic high school girls. Having devoted nearly all his adult life to the alternative press, and hailed by many as the world's leading authority on printing dope, Sacks stands a veritable King Kong in the rarefied realm of drug journalism. With his unavailed technical knowledge of magazine production and the even-mindedness of the old Mahatma, it's wonderful to have the Captain back. ☐

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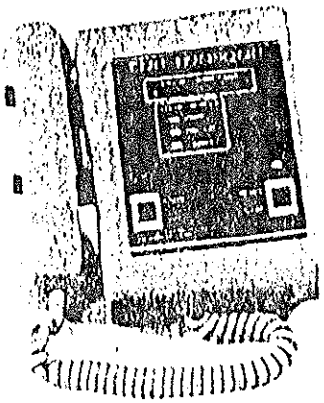

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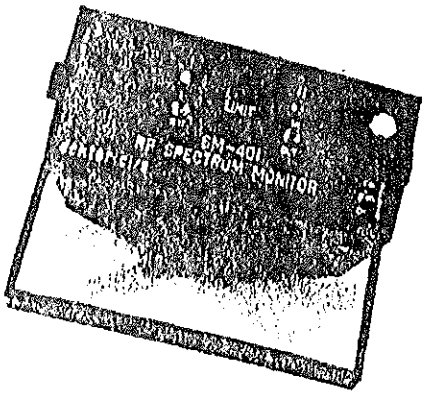

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continued from page 71

Krishna members.

The *San Francisco Chronicle* has estimated ISKCON's holdings at $50 million, but many observers consider that figure extremely conservative. Some estimates run into the hundreds of millions.

ISKCON's worldly empire is overseen by the Governing Board Commission (known as the GBC) composed of 24 members, 11 of whom are the spiritual masters appointed by Prabhupada shortly before his death (or "disappearance," as devotees call it) in 1977.

The appointment of 11 successors was viewed by many observers as curious if not downright suspicious. Traditionally, transmission of leadership in such a movement is from one old man to another, the successor having been prepared for his task over many years, usually decades. Now suddenly there were 11 successors, most of them young and relatively inexperienced.

According to religion expert Lowell Streiker, executive director of the Freedom Counseling Center in Burlingame, California, which counsels families disrupted by cults, it was in Prabhupada's choice of successors that many of ISKCON's current problems have their roots. He blames the successors for what he terms the "Americanization" of the movement.

"They introduced pragmatic standards; started playing the good old American money game," says Streiker. "Those who brought in the most money were the most spiritual. Rewards and discipline were not equal for the successful (at making money) and the unsuccessful." In short, Streiker claims the movement now has "too many half-baked leaders, without age and without experience."

Half-baked, perhaps, but powerful nonetheless. Only the 11 spiritual masters have authority to initiate new devotees, and the devotees' devotion to their spiritual master appears to be nearly total.

So if a spritual master like Hansadutta says a holocaust is coming, it's time to load the rifles. And if their trigger fingers tremble, devotees can find strength from that passage in the *Bhagavad Gita* where Krishna admonishes the warrior Arjuna:

> Fall not into degrading weakness, for this becomes not a man who is a man. Throw off this ignoble discouragement, and arise like a fire that burns all before it. □

e Evangelists Billion—Bucks

The television ministry has gradually developed its own panoply of superstars, who make the rounds from program to program, testifying to their colorful experiences of agony and redemption. Entertainers Graham Kerr [aka the Galloping Gourmet] and Johnny Cash clock a lot of satellite time, and electric parishioners tune in breathlessly for any panel that includes former Nixon hatchetman Charles Colson and former Black Panther firebrand Eldridge Cleaver. In addition to evangelical programs, Armstrong reports, "we also run a few programs which don't compromise our beliefs, like 'Lassie.'"

In 1979 the nationwide electronic church grossed, by its own reckoning, around $3 billion. And of that, less than a quarter was amassed by the "big six" preachers like Falwell, Humbard, Roberts and Herbert Armstrong. The amount of cold cash changing hands even among relatively bush-league TV prophets is therefore incalculable, especially considering that accountants for religious outfits only have to answer to God and their own consciences.

The federal Securities and Exchange Commission is not eager to look into religious financing. In 1973 it charged the Humbard outfit with fraud totaling $12 million, and wound up in such a sordid First Amendment morass that it was glad to settle for repayment of the funds in question—effected largely through the sale of a Humbard-owned girdle factory. Last year, California state authorities started looking into Herbert Armstrong's electronic episcopate—whereupon Armstrong ordained that henceforth all donations were to be mailed to his home in Arizona, safe from the philistine revenuers of California.

e drag) has big smile, swinging tail and recorded hip- the freeway

Microgram
Vol XV No 11
Nov. 1982

OBSERVATION OF QUAALUDE PRINTING CHARACTERISTICS FOR POSSIBLE DRUG CONTENT

Jennifer Y. Price
Criminalist I, Drug Chemist
Charlotte Mecklenburg Crime Laboratory

OBJECTIVE:

There are large quantities of Quaalude tablets representing several clandestine operations circulating through North Carolina. Many of the counterfeit Quaaludes do not contain the controlled drug methaqualone. The most common substitutes in this area are diazepam and doxylamine. Several undercover agents have requested a field procedure for determining rip-off tablets from those containing methaqualone. Therefore the following information was collected to aid the agents.

BACKGROUND:

Since January 1980, the Laboratory has analyzed Quaaludes representing eighteen (18) different counterfeit LEMMON 714 presses and four (4) presses representing counterfeit RORER 714 tablets. Counterfeit variations such as LMN 300, LEMON 714, LENNON 714, 714, and RORER 714 have also appeared recently to add confusion. There are obvious visual differences in the printing size and shape of the tableting machines. It has been observed that the drug content of tablets from an individual press will seldom change.

PROCEDURE:

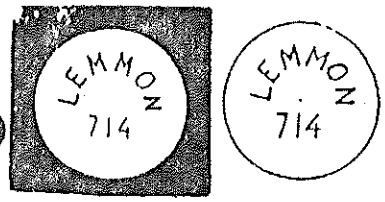
The ability to visually determine the possible drug content of Quaaludes requires a current knowledge of printing characteristics for tableting machines encountered in the area. Included in this paper are photographs of the tablets, handdrawn diagrams with information on drug content, and letter and digit printing variations from the standard authentic pharmaceutical products. The flow charts can be used to determine the drug and the North Carolina State Controlled Substance Act schedule. A key is available with descriptions to demonstrate characteristics used in the classification system.

RESULTS AND DISCUSSIONS:

The tablets discussed in this paper appear only in the Charlotte area. Each geographic area will have different counterfeit suppliers but the basic idea can be applied to other areas with very little effort. Several of the tablets have appeared only once and are considered rare. An update of data will be necessary for new additions. The process of identification may appear involved but presently the majority of the tablets here include only four or five presses.

Std. Methaqualone

LEMMON Co. Authentic Product



Reference for printing in following examples

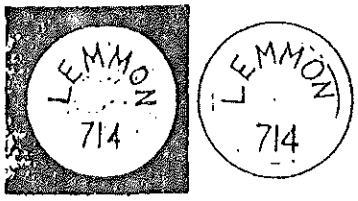
Drug Content

NC: Not Controlled
C-IV: NC State Schedules of Controlled Substances

Characteristics Common to LEMMON Tablets

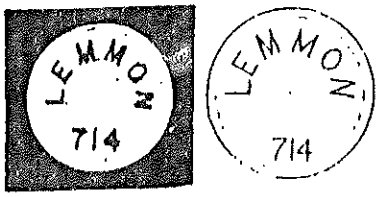
White, round, flat, single score, beveled edges, diameter 13.0 - 13.5mm, thickness 4.5 - 5.0 mm, monogram LEMMON 714 on one side.

a. Methaqualone: C-II Diazepam: C-I



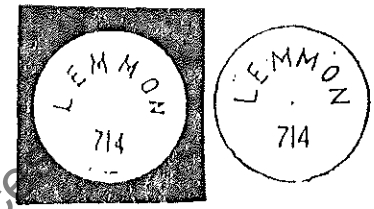
Letters: Larger in Size
Digits: Larger in Size
7 - Curved Stem
4 - Closed at Top

e. Methaqualone C-II



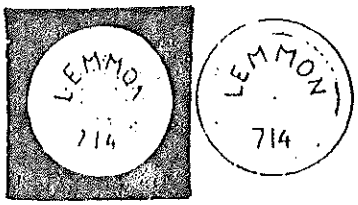
Letters: Same Size, Thicker
Digits: Slightly larger
7 - Curved Stem
4 - Closed at Top

d. Diazepam: C-IV



Letters: Approximately Same Size
0 - Narrow Width
Digits: 7 - Curved Stem
4 - Closed at Top

b. Methaqualone: C-II

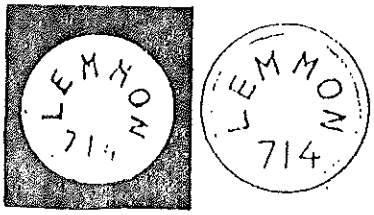


Letters: Smaller in Size
M - Shallow Middle Depre
Digits: Smaller in Size
4 - Angle Opening at Top

f. Methaqualone: C-II

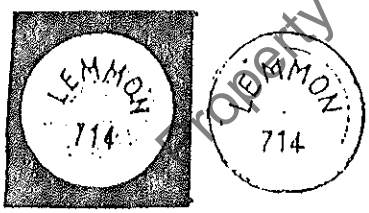
Letters: Shorter, Slightly Smaller
E - Equal Arm Lengths
Digits: Smaller in Size

h. Diazepam: C-IV



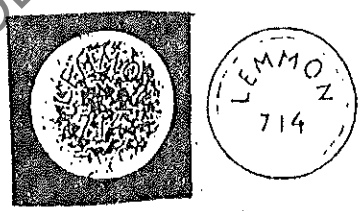
Letters: Larger, Cover More Than Half Circle Thick Print
Digits: Larger

c. Methaqualone: C-II



Letters: Slightly Larger in Size
E - Equal Arm Lengths
Digits: 7 - Curved Stem
1 - Has Top Appendage
4 - Closed at Top

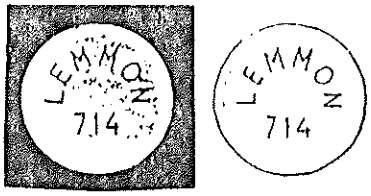
j. Methaqualone: C-II



Letters: Same Size
Digits: Same Size

* Speckled, offwhite color

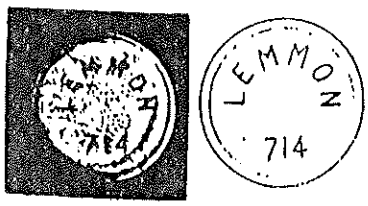
i. Doxylamine: -NC



Letters: Approximately Same Size
Digits: Same Size
1 - Has Top Appendage

* Tablet Considerably Thicker

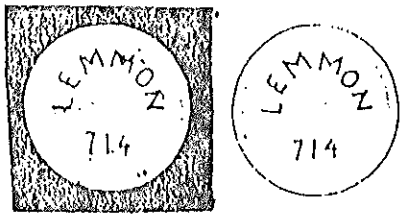
k. Methaqualone: C-II



Letters: Smaller, Shallow Print Off Center
Digits: 7 - Curved Stem

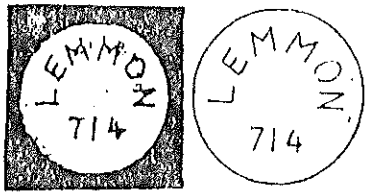
* Slightly Speckled

m. Methaqualone: C-II



Letters: Same Size
M-Wider
Digits: 4-Angle Opening at Top
* Diameter Larger: 15mm

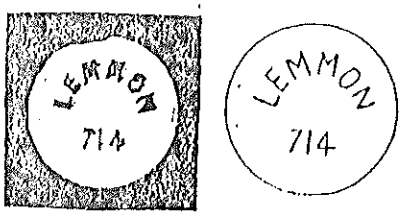
o. Diazepam: C-IV



Letters: Larger in Size
Digits: 4-Angle Opening

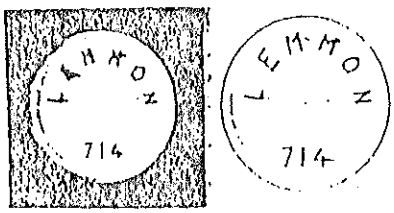
* Slick Finish

r. Methaqualone: C-II



Letters: Thicker, Compact
E-Equal Arm Length
Digits: 4-Closed Top

p. Diazepam: C-IV

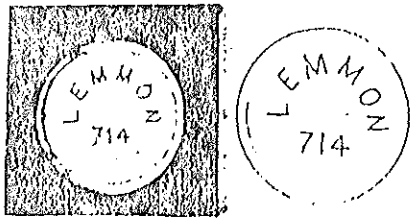


Letters: Smaller
Close to Edge
Digits: Smaller

q. Methaqualone: C-II

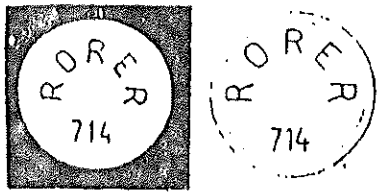
Letters: Smaller, Thick
Compact
M-Deep Middle Depression
Digits: Smaller
7-Curved Stem
4-Closed Top

s. Methaqualone: C-II



Letters: Smaller, Shallow
M-Deep Middle
Depression
Digits: Smaller
7-Curved Stem
4-Closed Top
* Speckled, off white col

Methaqualone



William H. RORER, Inc.
Authentic Product

Reference for printing in following examples

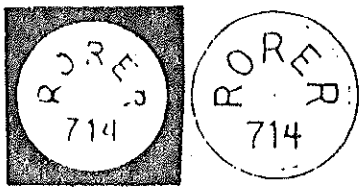
Characteristics Common to RORER Tablets:

White, round, flat, single score
beveled edges,
thickness 4.0 - 5.0mm,
diameter 13.0 - 13.5mm,
monogram RORER 714 on one side.

Drug Content

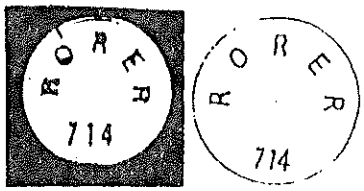
NC: Not Controlled
C-IV: NC State Schedules of Controlled Substances

b. Methaqualone: C-II



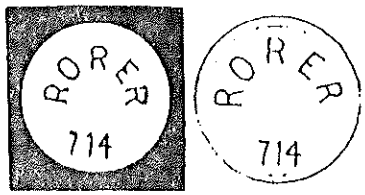
Letters: Larger in Size
Digits: Larger in Size
7 - Curved Stem
4 - Parallel Top Openin:

Methaqualone: C-II



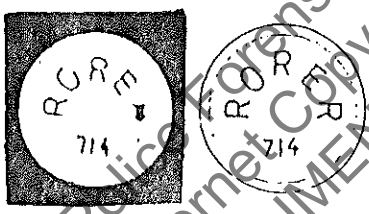
Letters: Smaller in Size
E - Equal Arm Lengths
Digits: Smaller in Size
4 - Closed at Top

Diazepam: C-IV



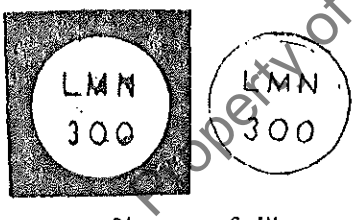
Letters: Deep Print
Approximately Same Size
Do not Complete Half Circle
Digits: 4 - Closed at Top

a. Thioridazine: -NC

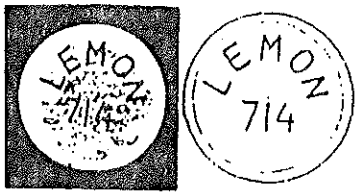


Letters: Approximately Same Size
E - Equal Arm Lengths
Digits: Smaller in Size
1 - Has NO Top Appendage
4 - Has Perpendicular Top Opening

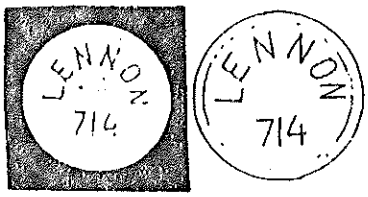
COUNTERFEIT VARIATIONS



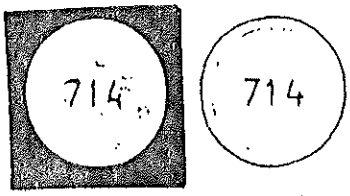
Diazepam: C-IV



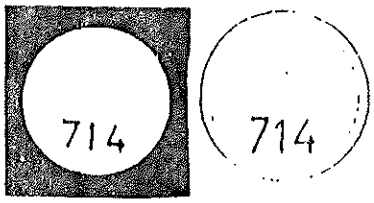
Doxylamine: - NC



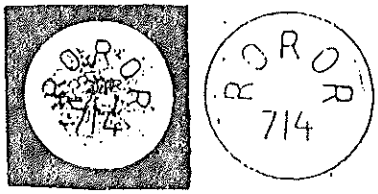
Doxylamine: -NC



Methapyrilene: - NC



Acetaminophen: - NC



Acetaminophen: - NC

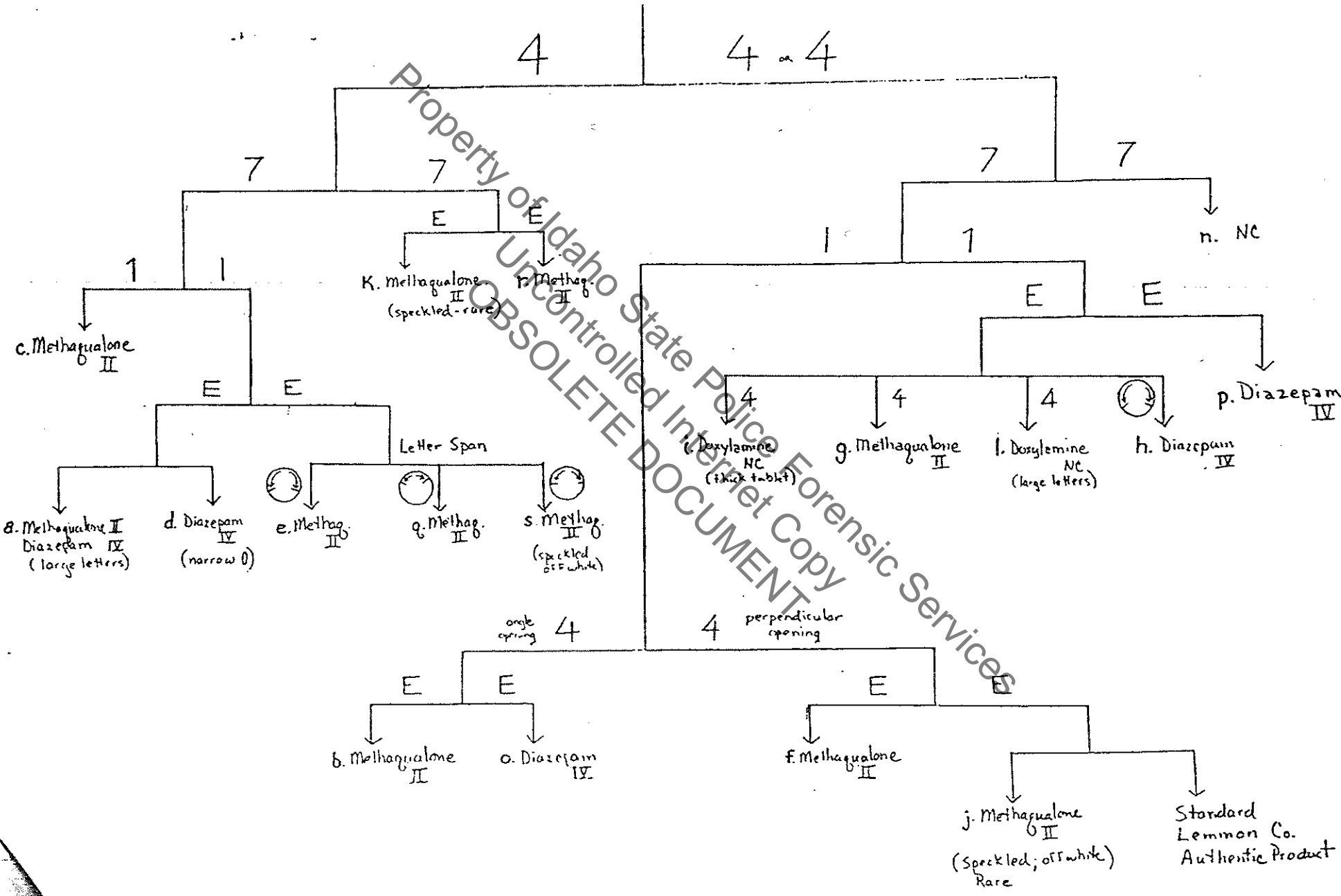
KEY FOR PRINTING CHARACTERISTICS

E - equal arm lengths ✓
E - unequal arm lengths
M - shallow middle depression
M - deep middle depression ✓
0 - narrow width

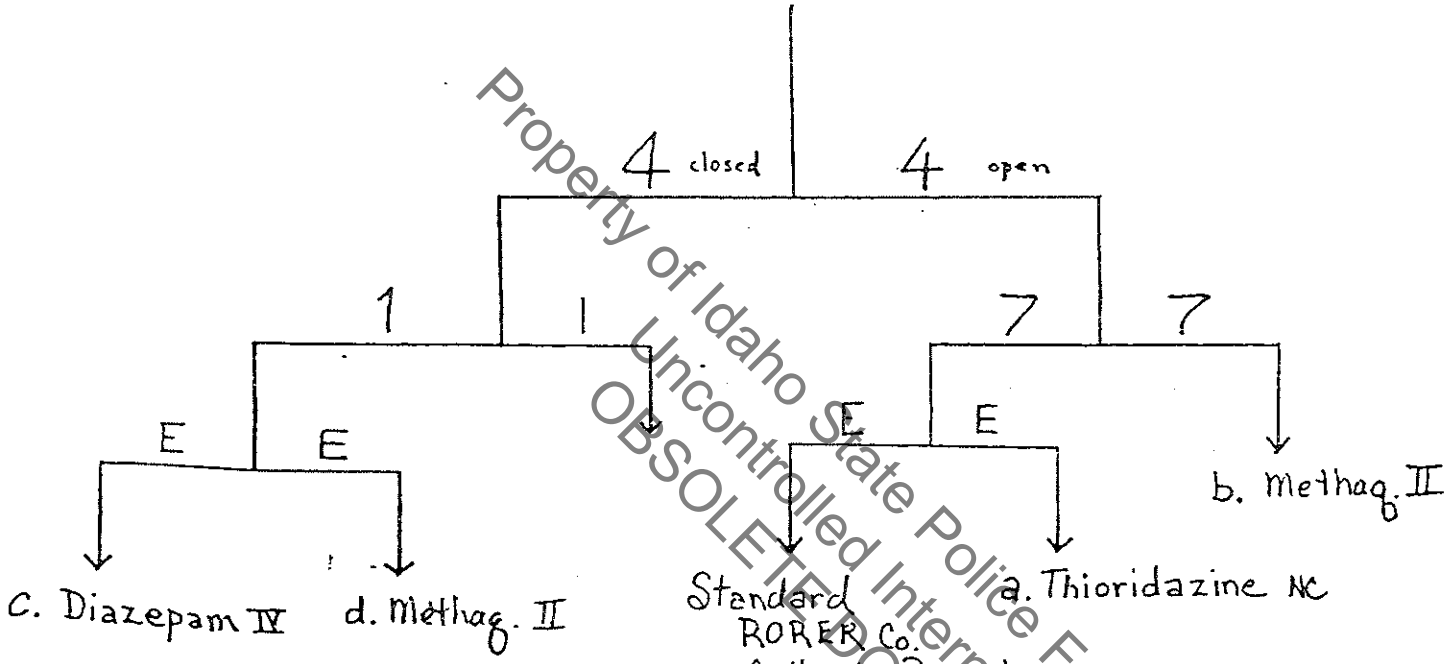
 - letters cover half circle surface ✓

7 - straight stem ✓
7 - curved stem
1 - no top appendage ✓
1 - top appendage
4 - closed four ✓
4 - open four ✓
4 - perpendicular opening
4 - angle opening

LEMMON 714



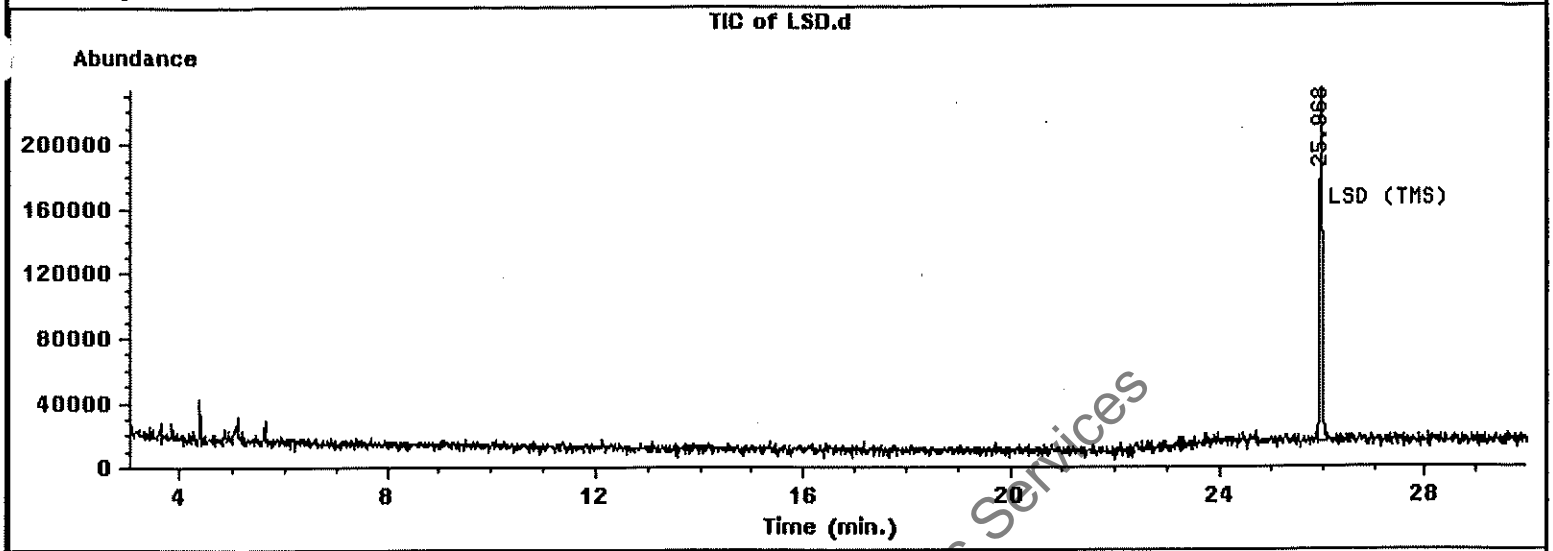
RORER 714

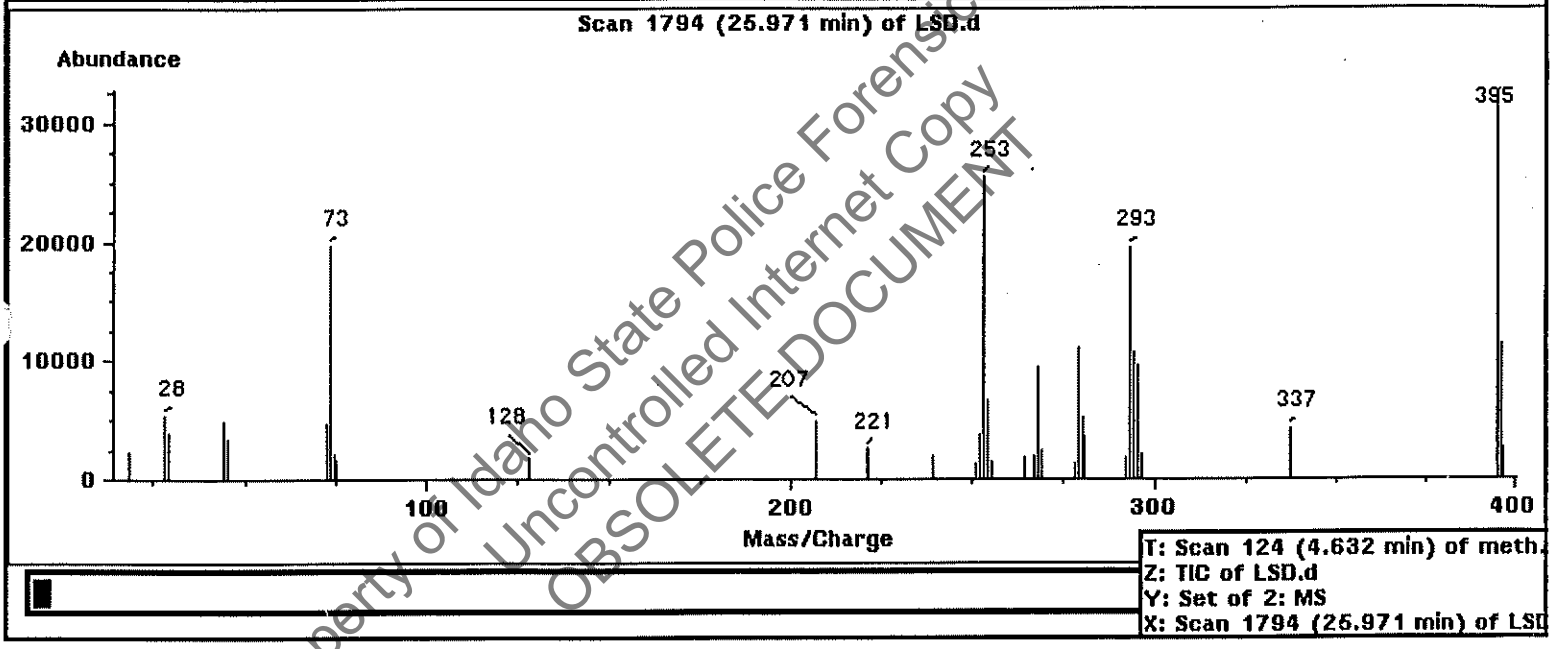


DataAnalysis

Files Graphics Integration Reports Spectra LibrarySearch Help

directory /chem/msd





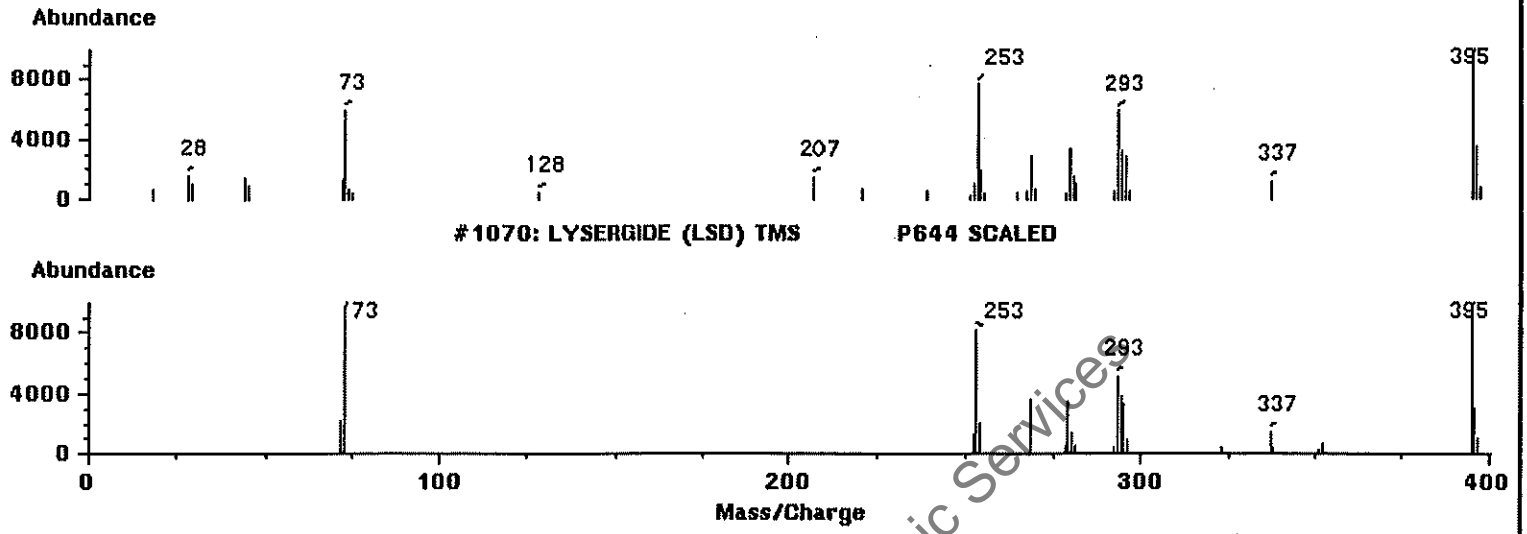
T: Scan 124 (4.632 min) of meth.
Z: TIC of LSD.d
Y: Set of 2: MS
X: Scan 1794 (25.971 min) of LSD

DataAnalysis

Files Graphics Integration Reports Spectra LibrarySearch Help

directory /chem/msd

Scan 1794 (25.971 min) of LSD.d SCALED

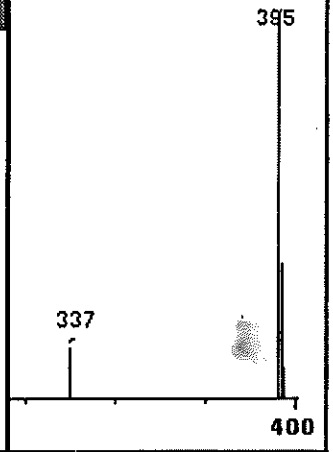


#1070: LYSERGIDE (LSD) TMS P644 SCALED

Graphics Results

Compound Name	Lib	Entry #	Quality
LYSERGIDE (LSD) TMS	P644	1070	95
DICLOFENAC-M (HO-) -H2O AC	P588	1212	7

Send to printer | Display Diff | Exit | Help



T: Scan 1794 (25.971 min) of LSD
Z: null.
Y: TIC of LSD.d
X: Set of 2: MS

RL

August 29, 1994

MEMORANDUM

TO: Boise Drug Analysts

FROM: MiChell and Donna

SUBJECT: **LSD ON GCMS**

We have finally completed a conclusive method for LSD.

FOR PAPER ONLY
-Place at least two squares in a test tube.
-Add methanol to cover sample completely.
-Soak overnight.
-Remove methanol and concentrate to smallest quantity in insert.
-Run on LSD method.

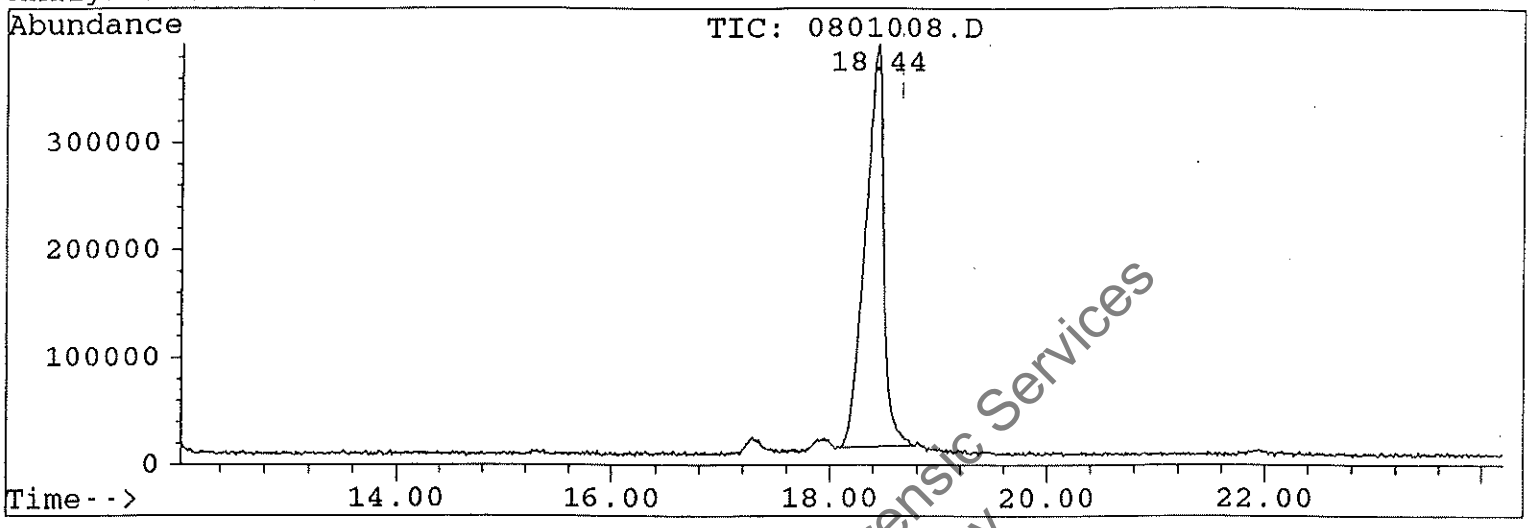
OTHER LSD SAMPLES
-In test tube, cover sample with concentrated NH_4OH.
-Soak at least 10 minutes.
-Transfer NH_4OH to new test tube and add methylene chloride.
-Mix, centrifuge and transfer extract into GCMS insert.
-Air dry to dryness and redilute with methanol (small quantity).
-Run on LSD method.

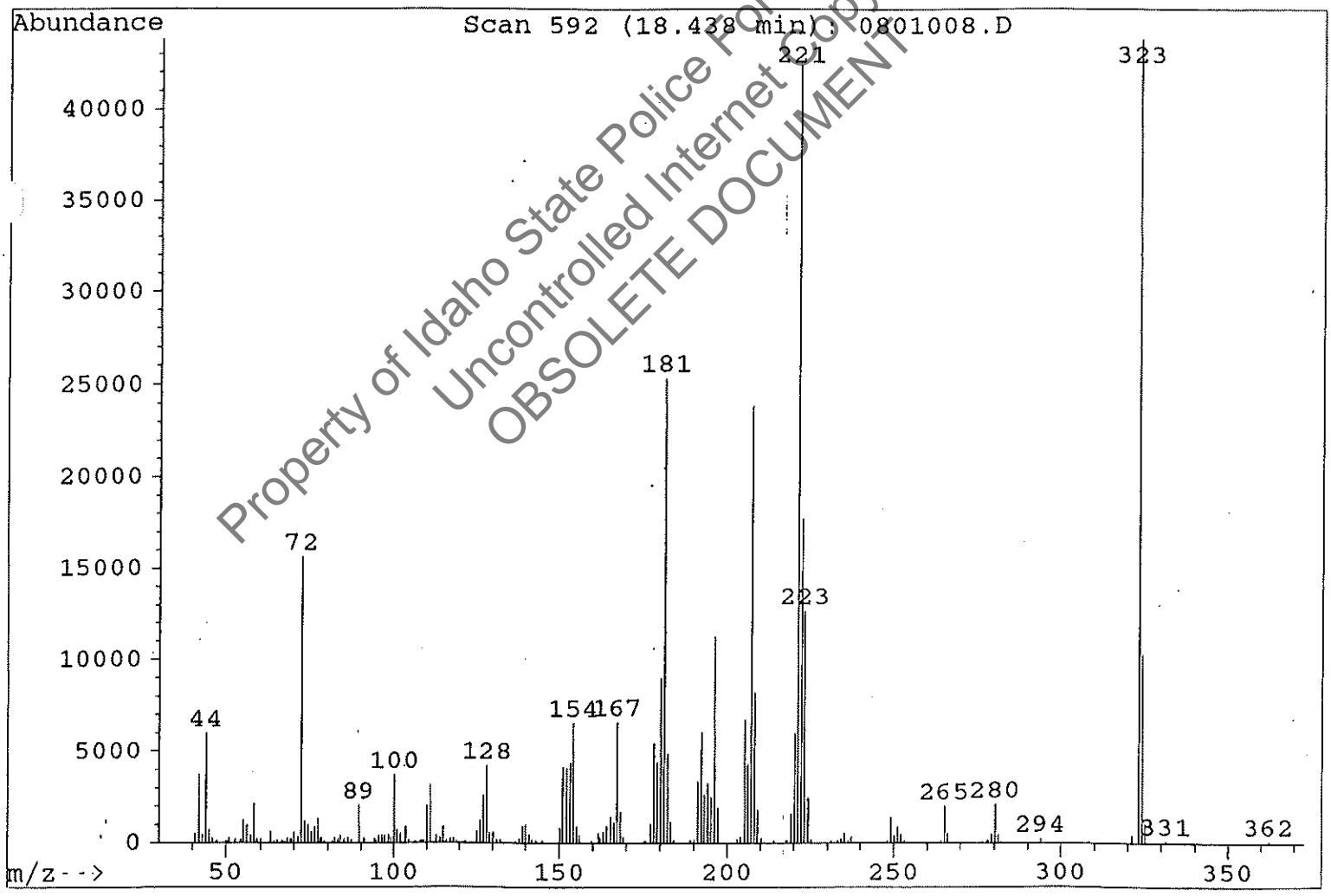
GCMS INFORMATION
Method: LSD.m
Column: 12m HP Ultra 2.
Temperature: 100-280 at 25/min with 15 min hold.
Splitless
Clean liner

** Retention times should be within .10 of the standard retention time.

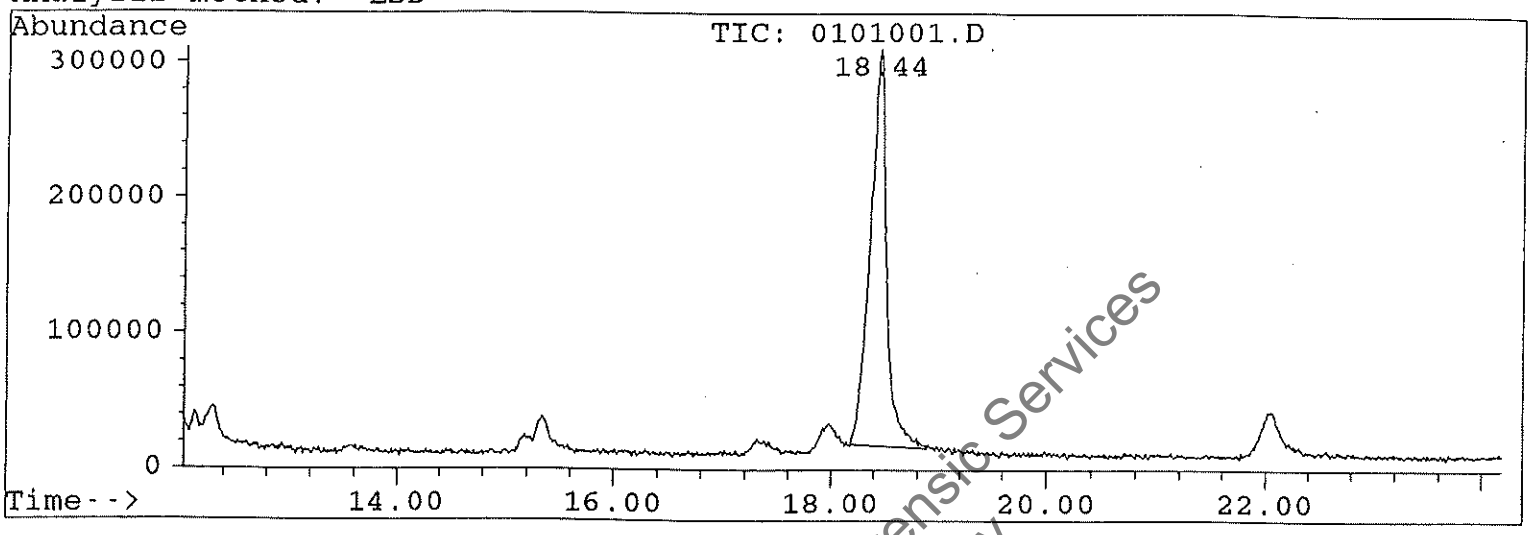
We will only be running LSD samples the last week of each month. No rush LSD's will be done.

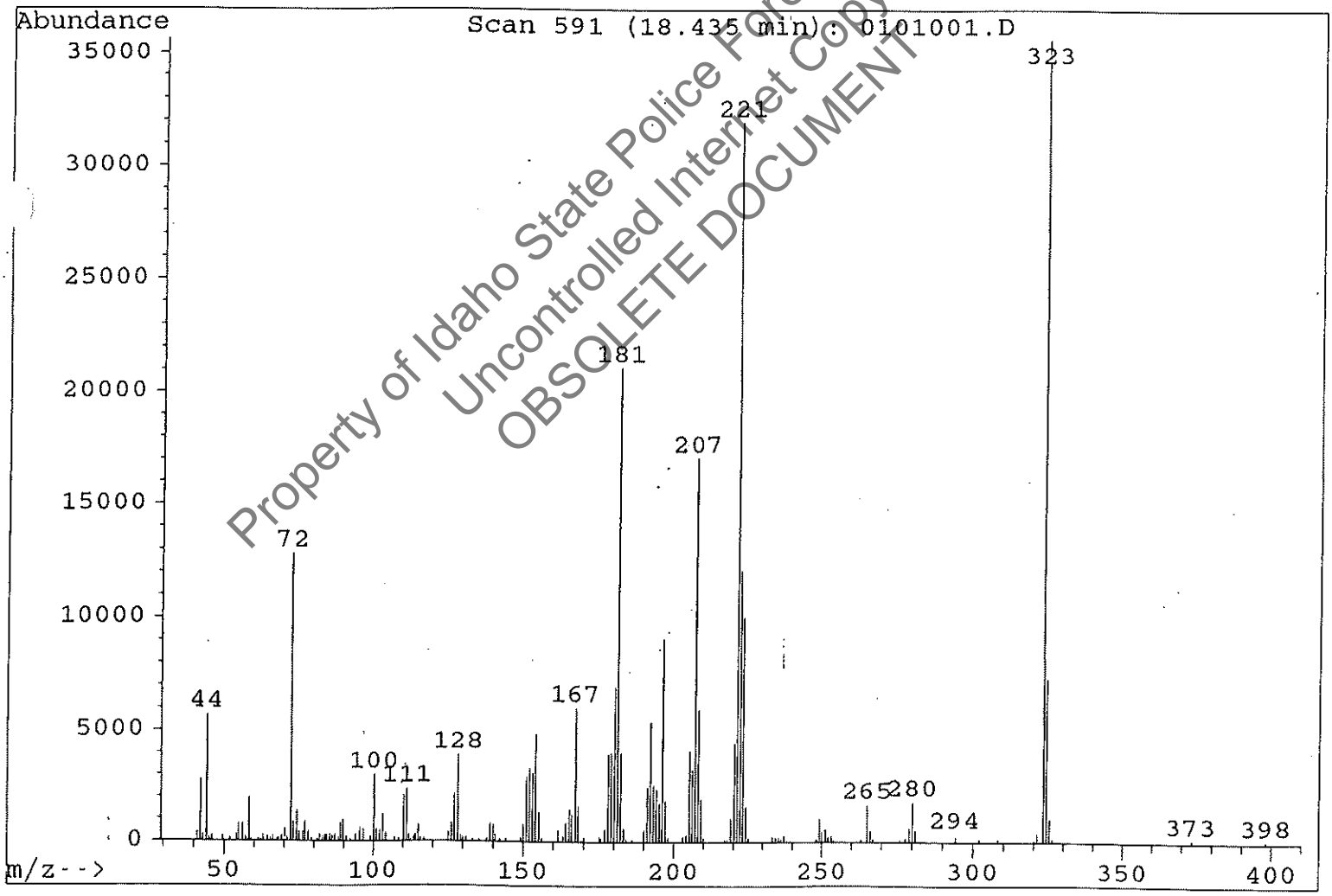
Name:
Date: 26 Aug 94 12:11 pm Vial number 8
Identifying Information: LSD- ~~REALLY~~ DILUTE
SPLITLESS MODE
HP 5890 GC, 5971 MSD, Column Information: HP-1, 12.5M
0.2 mm ID, crosslinked dimethylsilicone, 0.33 um film thickness
Data file location: C:\HPCHEM\1\DATA\AUG2694\0801008.D
Analysis method: LSD





Name:
Date: 26 Aug 94 8:52 am Vial number 1
Idehtifying Information: BROWN PAPER- 5 SQUARES
MEOH OVERNIGHT, SPLITLESS MODE
HP 5890 GC, 5971 MSD, Column Information: HP-1, 12.5M
0.2 mm ID, crosslinked dimethylsilicone, 0.33 um film thickness
)ata file location: C:\HPCHEM\1\DATA\AUG2694\0101001.D
Analysis method: LSD





LSD

I. Lysergic Acid Diethylamide (LSD) - Submitted forms may be of the following:

A. Powders (many colors - usually bright).

B. Tablets (many colors, sizes, shapes - usually bright colored).

C. Capsules (many colors and sizes).

D. Mushroom material.

E. Window panes (gelatin squares - many forms).

F. Blotters (paper soaked in or spotted with LSD solution).

Note: Window panes and blotter forms are often cleverly disguised and may be taped, hidden or represented as something else.

II. Screening

A. Look for discoloration on tabs, paper, etc., using visible and U.V. lights.

B. Color test: p-DMAB (P-Dimethylaminobenzaldehyde): LSD and other indoles give purple color.

III. Thin layer chromatography

A. Prepare silica gel plate with unknown and known.

B. Run in appropriate systems

1. Systems especially selected by DEA

(a) Acetone

(b) Dimethyl formamide-benzene (2:13)

(c) 1,1,1 Trichloroethane - methanol (9:1)

The closest available relative to LSD (besides iso-LSD) is LAMPA. Ideally, the thin-layer system(s) used should differentiate LSD from LAMPA. System (b) above may achieve this separation, especially if E. Merck's "High Performance" Silica Gel plates are used. (Spot stds of LSD and LAMPA and a mixture of the 2 on the plate besides your unknown). If this does not work, try NH_4OH sat'd $CHCl_3$ (1 part) + acetone (2 parts) using 2 developments. (See reprint Microgram Vol. VII, No. 12, pg. 151).

2. Refer to Clarke, system T-1 and T-9.

C. Observe spots under UV light, note fluorescence.

D. Visualize with P-Dimethylaminobenzaldehyde and HCl.

IV. Infrared spectrophotometry

In the past, IR scans on LSD purified from case materials rarely matched the standard, so this technique is not in routine use. Various clean-up techniques can be found in the literature.

LSD

I. Lysergic Acid Diethylamide (LSD) - Submitted forms may be of the following:

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Note: Window panes and blotter forms are often cleverly disguised and may be taped, hidden or represented as something else.

II. Screening

A. Look for discoloration on tabs, paper, etc.

B. Check long wave and short wave ultraviolet fluorescence.

III. Color Test

A. P-DAMB (P-Dimethylaminobenzaldehyde): purplish

IV. Thin layer chromatography

A. Prepare silica gel plate with unknown and known.

B. Run in appropriate system

1. Refer to Clarke, system T-1 and T-9

2. Other possible solvent systems:

(a) Acetone - chloroform

(b) Ammonium hydroxide - ethanol - trichlorethane

(c) Trichloroethane - methanol

C. Observe spots under UV light

D. Visualize with P-Dimethylaminobenzaldehyde and HCl

V. Ultraviolet spectrophotometry

A. Place in 0.02N HCl solvent

1. Window pane and blotter may go directly into 0.02N HCl.

2. Other forms usually require aqueous neutral, acid, and then basic extraction into chloroform - before going into 0.02N HCl.

B. Run UV scan with solvent reference cell.

C. Examine and compare to known charts, reference material or known standard (peak 310).

D. Remove both cells and expose to long and short wave UV light for approximately 15-20 minutes - noting fluorescence while in UV chamber.

E. Re-run UV scan.

F. Note degradation of peak and peak shift - compare to known charts.

VI. Infrared spectrophotometry

A. Cleanup of sample

1. Solvent extraction of suspect material (chloroform, dichloromethane, etc.).

(a) Direct evaporation of KBr and then prepare winkow.

(b) Prepare KBr window and then evaporate solvent on window.

(c) Direct evaporation of solvent on ATR crystal.

2. Aqueous phase extraction (neutral, acid, and then basic) into a solvent (chloroform or dichloromethane).

3. May run on beam condenser.

B. Examine and compare to known reference spectrum or known standard.

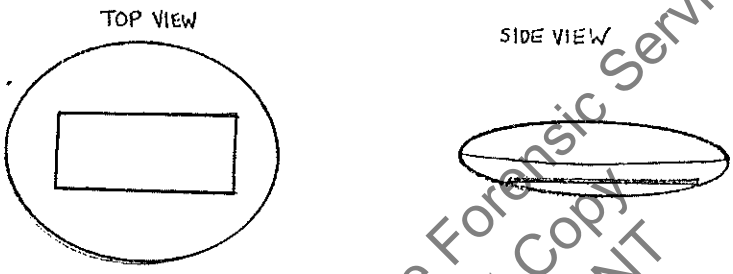
VII. Comments and observations concerning potential problem areas.

A. LSD fluorescence may be quenched or masked by other materials.

p-DMAB
FLYING SAUCER

When using p-DMAB to visualize LSD and psilcyn/cybin the following is safer than spraying the reagent.

1. Prepare a sat'd solution of p-DMAB in Pet Ether. (Evaporates off)
2. apply p-DMAB to plate with cotton or Q-tip
3. place plate in a watch plate that has a squirt of conc. HCl in it and place an additional plate over the top.
4. Allow to sit until spots visualize.



LSD
IR Procedure

1. Extract with CH_2Cl_2 from a basic soln. (NH_4OH)
2. Run through an alumina column
3. Run through CH_2Cl_2/MeOH - watch for LSD band
4. Reduce volume to 1 ml
5. Run an IR using a very small amount of KBr and a pin hole in a card.
6. If the above IR needs clean-up, do so on a TLC plate

Clinical Laboratory Science, Section B: Toxicology, I. Sunshine, Ed., CRC Press, W. Palm Beach, Fla., 1978.

Methods of Analysis of the A.O.A.C., 12th Ed., W. Horwitz, Ed., A.O.A.C., Washington, D.C., 1975.

Spot Tests in Organic Analysis, F. Feigl, Elsevier Pub., Amsterdam, 1966.

Modern Microcrystal Tests for Drugs, C.C. Fulton, Wiley-Interscience, New York, 1969.

Gas Chromatographic Retention Indices of Toxicologically Relevant Substances on SE-30 or OV-1, 2nd Ed., R. Ardrey, Ed., V.C.H. Publishers, Weinheim, Germany, 1985.

Drug Analysis by Gas Chromatography, D.B. Jack, Academic Press, Orlando, Fla., 1984.

Phenethylamines, RCMP Understudy Training, G.J. Kupferschmidt, Government of Canada Document, 1980.

Micro Color Reaction Manual, RCMP Understudy Training, G.J. Kupferschmidt, Government of Canada Document, 1980.

SYNOPSIS OF "IDENTIFICATION OF PEYOTE BY MAJOR NON-PHENOLIC ALKALOIDS"

By Phillip Lua and Paul Labish, Criminalists

Criminalistics Laboratory, Alameda County Sheriff's Department

I. EXTRACTION SCHEME

.1-.5 g peyote + 15 ml 10% HCl → 15 min in boiling water bath → Filter (with 10 ml H_2O wash if more than .2 g peyote used) → Adjust to pH 11 or higher with conc. NaOH → Extract w/ 50 ml ethyl ether → Evap to 10 ml

If it is desired to obtain a large quantity of extract for use as a reference extract and more than .5 gram of plant material is used, increase heating time, wash, and extractant volumes proportionately.

II. CHROMOTROPIC ACID TEST

Evaporate to dryness 1/3 of ether extract in test tube, add approx. 50 mg chromotropic acid and 3 ml conc. sulfuric acid, heat 3 - 5 minutes in boiling water bath. Purple red color is positive presumptive test for peyote.

III. TLC METHOD FOR STANDARDIZING A NON-PHENOLIC PEYOTE ALKALOID EXTRACT

Streak extract remaining after chromotropic acid test on glass plates coated with Silica Gel G. Run plate in chloroform: n-butyl alcohol: conc. NH_4OH (50:50:2.5), remove NH_4 with draft and careful heating, spray with ninhydrin reagent on band ends. After mescaline develops (3-5 min. in ordinary daylight, follow with Iodoplatinate-Dragendorff Reagent.

Approximate Rf's: Mescaline 0.46, Anhalonine 0.66, Lophophorine, 0.89; unknown substance with strong yellow fluorescence under UV light, 0.94; peyophorine, 0.96.

Color reactions of silica gel scraped from unsprayed portions of alkaloid bands are given in Table 3 of three tables copied from the article.

UV maximae of alkaloids eluted in 3-1/2 ml. 0.1N HCl:

Mescaline: 269 mμ - Anhalonine: 276 mμ - Lophophorine: 274.5 mμ

Keep standardized peyote extract in solvent such as methanol for optimum preservation.

The above method (chromotropic acid test of extract plus TLC-color-UV) is considered forensically adequate for standardization of a peyote extract. However, the following alternative, purely TLC-IR method of standardization may be preferred by some workers: Scrape off the unsprayed silica gel areas containing the presumed mescaline, anhalonine and lophophorine and transfer the material from each of the alkaloid bands to individual Pasteur pipets with small wads of glass wool in the necks. Elute the material in each pipet with 2 - 3 ml of $CHCl_3$:MeOH:conc. NH_4OH (80:20:1). Evaporate the eluates to dryness on steam bath, immediately remove from steam bath when dry, dissolve in small quantity of $CHCl_3$ and coat silver chloride disks drop-wise with the solutions. Fig's 1, 2, 3 show IR traces obtained as described above. Anhalonine and lophophorine recovered by TLC were purer than our available standards as shown by IR spectrophotometry and TLC.

IV. TLC METHOD FOR ROUTINE IDENTIFICATION OF PEYOTE USING A STANDARDIZED EXTRACT

Streak extract remaining after chromotropic acid test in two adjacent ½" long streaks. Apply standard extract over second streak, overlaying the unknown, and alone in a third adjacent streak. Use Eastman Chromatogram 6061 Silica Gel sheets, solvent system: 25 ml ethylene dichloride shaken with 1 ml conc. NH_4OH, after 1 min. standing use the organic phase. Remove NH_3 from sheet, observe under UV. Spray as described above. The approximate relative Rf with respect to lophophorine of the non-phenolic alkaloids are:

Mescaline (base of band after spraying with ninhydrin - .20; anhalonine - .65, unknown substance with strong yellow fluorescence under UV light which itself reacts with the Iodoplatinate-Dragendorff Rgt. to give a bluish band - .84; lophophorine - 1.0; peyophorine - 1.2.

By scaling down the conditions of extraction, the chromotropic test and spotting in tight spots, as little as 20 - 30 mg of peyote can be run by this method.

RECIPES

Iodoplatinate Spray Reagent: Dissolve 0.25 gram of platinic chloride and 5 grams of KI in sufficient water to make 100 ml.

Modified Dragendorff Spray Reagent: Solution A: .85 gram bismuth subnitrate, 10 ml glacial acetic acid and 40 ml water. Solution B: KI, 40 grams, 100 ml water.

The reagent is prepared by mixing 5 ml of solution A with 5 ml solution B, 20 ml glacial and 100 ml water.

Iodoplatinate-Dragendorff Spray Reagent: This spray is made by mixing equal parts of the iodoplatinate and the modified Dragendorff reagents.

ACKNOWLEDGEMENT

The authors express gratitude to John Thornton and John Murdock of the Contra Costa County Sheriff's Office, Criminalistics Laboratory, for reference literature, samples of reference alkaloids and cacti. We also thank Dr. Govind J. Kapadia of Howard University, College of Pharmacy, Washington, D. C., and Dr. Henry M. Fales of National Heart Institute, Bethesda, Maryland, for the samples of reference alkaloids which were not available from commercial sources.

BIBLIOGRAPHY

1. Stig Agurell, Cactaceae Alkaloids I, 1969, Lloydia, 32, 2, 206-216
2. Stig Agurell, Cactaceae Alkaloids X, Alkaloids of Trichocereus Species and Some Other Cacti, 1971, 34, 2, 183-187. Lloydia
3. Govind J. Kapadia, Henry M. Fales, Peyophorine, A Tetrahydroisoquinoline Cactus Alkaloid Containing an N-Ethyl Group, J. Pharm. Sci., 1968, 57, 11.
4. J. Lundstrom, S. Agurell, J. Chromatog, 1967, 30, 271.

TABLE I

NON-PHENOLIC ALKALOIDS OF CACTI CONTAINING MESCALINE

Cacti	Non-Phenolic Alkaloids
Lophophora Williamsii	Mescaline Anhalonine Anhalinine Lophophorine N-Methylmescaline N-Acetylmescaline 3, 4 - Dimethoxyphenethylamine
Trichocereus Bridgesii	Mescaline 3-Methoxytyramine 3, 4 - Dimethoxyphenethylamine
Trichocereus Cuzcoensis	Mescaline 3-Methoxytyramine
Trichocereus Fulvilanus	Mescaline
Trichocereus Macrogonus	Mescaline 3-Methoxytyramine 3, 4 - Dimethoxyphenethylamine
Trichocereus Pachanoi	Mescaline 3, 4 - Dimethoxyphenylethylamine 3-Methoxytyramine
Trichocereus Pasacana	Mescaline
Trichocereus Schickendantzii	Mescaline
Trichocereus Taquimbalensis	Mescaline 3, 4 - Dimethoxyphenethylamine 3-Methoxytyramine
Trichocereus Terschreckii	Trichocereine Mescaline
Trichocereus Validus	Mescaline
Trichocereus Werdermannianus	Mescaline 3, 4 - Dimethoxyphenylethylamine 3-Methoxytyramine

TABLE 2

RESULTS OF CHROMOTROPIC ACID TEST AND TLC OF SEVERAL PURPORTED MESCALINE CONTAINING CACTI AND OTHERS

Cactus	Chromotropic Acid Test	Mescaline*
Lophophora Williamsii	Strong reaction within 2-4 minutes	Positive
Trichocereus Bridgesii	Negative	Positive
Trichocereus Pacanoi	Negative	Positive
Trichocereus Pascana	Negative	Negative
Trichocereus Macrogonus	Negative	Positive
Trichocereus Grandiflorus	Negative	Possible Trace
Trichocereus Terezensis	Negative	Negative
Trichocereus Spachianus	Negative	Negative
Trichocereus Peruvianus	Negative	———
Carnegia Gigantea	Negative	Possible Trace
Pelecyphora Aselliformis	Weak reaction within 20-30 minutes	Negative

The following were negative in the chromotropic acid test and negative for mescaline: Gymnocalycium Gibbosum, Gymnocalycium Andreae, Gymnocalycium Hossei, Gymnocalycium Monvillei, Gymnocalycium Zegarras, Gymnocalycium Quehlianum, Gymnocalycium Deesianum, Gymnocalycium Denudatum, Gymnocalycium Venturianum, Coryphantha Dainiocereus

*As indicated in routine TLC procedure by reaction with ninhydrin and R_f

TABLE 3

COLOR TEST OF MAJOR NON-PHENOLIC PEYOTE ALKALOIDS

Test	Mescaline	Anhalonine	Lophophorine
Marquis	orange	lavender → green (1 min) → blue	lavender → green (1 min) → blue
Mecke	yellow-orange	yellow green (15 sec) → gray (10 sec) → black (1 min) → black violet	yellow green (15 sec) → gray (10 sec) → black (1 min) → black violet
Frohde	Brown	blue	blue
Mandelin	brown	reddish-pink	reddish-pink
Conc. HNO_3	red		

Thin-layer chromatography of the peyote alkaloids*

The peyote cactus, *Lophophora williamsii* (Lem. ex SD.) Coult. (T.) (syn. *Anhalonium lewinii* Hennings), contains, besides the narcotic mescaline, a number of other bases, which are derivatives of phenylethylamine or tetrahydroisoquinoline[1,2]. The use of peyote ("mescal buttons") by the natives of Mexico as a hallucinogenic drug has lately also spread to other countries. Until the recent paper by McLAUGHLIN AND PAUL[3] apparently no thin-layer or paper chromatographic procedures for the rapid identification of *Lophophora* bases had been published.

In this note several thin-layer chromatographic systems suitable for the separation and identification of the peyote alkaloids are described.

Methods and materials

Thin-layer chromatography was carried out as described earlier[4] on silica gel coated glass plates (20 × 20 cm, 0.25 mm layer) except that the coated plates were dried overnight at room temperature. For details regarding solvent systems, see Table I.

The base fraction from a peyote cactus (fresh wt. *ca.* 100 g–0.4 g alkaloids) was

TABLE I

R_F VALUES × 100 OF PEYOTE ALKALOIDS

Silica Gel G chromatoplates with the following solvent mixtures:
(A) chloroform–ethanol–diethylamine (85:5:10 by vol.)
(B) chloroform–ethanol–diethylamine (85:10:5)
(C) chloroform–ethanol–conc. NH_3 (85:15:0.4)
(D) chloroform–*n*-butanol–conc. NH_3 (50:50:2.5)
(E) pyridine–conc. NH_3 (90:10)

Alkaloid	*Solvent system*					*Colour*[a]
	A	*B*	*C*	*D*	*E*	
Phenolic						
Anhalamine	11	20	—	—	40	purple
N-Methyltyramine	31	31	—	—	32	yellow
Tyramine	34	33	—	—	42	yellow
Anhalonidine	39	51	—	—	51	purple
Hordenine	51	56	—	—	60	yellow
Anhalidine	55	65	—	—	72	purple
Pellotine	63	70	—	—	69	purple
Non-phenolic						
N-Methylmescaline	—	—	22	20	25	yellow
Mescaline	—	—	24	31	36	brown
Anhalinine	—	—	30	41	48	yellow
O-Methylanhalonidine	—	—	33	45	50	yellow
Anhalonine	—	—	45	58	56	yellow
Lophophorine	—	—	68	80	72	blue gray
N-Acetylmescaline	—	—	82	95	68	pale brown

[a] Colour with *o*-dianisidine reagent.

* Supported by the Swedish National Research Council. The technical assistance of Miss B. BURELL is appreciated.

isolated by chloroform extraction[5]. The evaporated chloroform extract was dissolved in 100 ml chloroform and passed through a 2 × 15 cm column of acid Celite (15 g Celite 545 and 4 ml 0.5 *M* H_3PO_4). The column was washed with 200 ml chloroform to remove non-basic compounds. The alkaloids were eluted with chloroform saturated with ammonia[6]. A solution of the alkaloids in methanol was applied to a column (1 × 20 cm) of Amberlite IRA 400 (OH) ion-exchange resin. The column was washed with 100 ml of 30 % aqueous methanol to yield the non-phenolic alkaloids. The phenolic alkaloid fraction was obtained by elution with 200 ml of a solution of 120 ml methanol, 60 ml water and 20 ml glacial acetic acid.

Alkaloids were located by the use of an *o*-dianisidine reagent (equal volumes of 0.5 % *o*-dianisidine in dilute HCl and 10 % $NaNO_2$ in water) or iodoplatinate reagent[7].

Reference alkaloids were kindly supplied by Drs. A. BROSSI, Hoffman-La Roche Inc., and G. KAPADIA, Howard University, or isolated or synthesized according to known procedures (*cf.* ref. 1).

Results and discussion

The thin-layer chromatographic behaviour of the peyote alkaloids in several solvent systems and their colour reactions with the dianisidine reagent are recorded in Table I. This reagent produces a red colour with phenolic tetrahydroisoquinolines and a yellow or brown, fading colour with non-phenolic alkaloids.

Solvent system A was found to be must suitable for the separation of phenolic alkaloids and system D for non-phenolic alkaloids. With the exception of solvent system E, no system was found to resolve satisfactorily both phenolic and non-phenolic alkaloids.

Details of thin-layer and gas chromatographic separation of peyote alkaloids will be published at a later date.

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1 H. G. BOIT, *Ergebnisse der Alkaloidchemie bis 1960*, Akademie-Verlag, Berlin, 1961, pp. 13–24, 210–215.
2 A. HOFMANN, *Planta Med.*, 12 (1964) 341.
3 J. L. MCLAUGHLIN AND A. G. PAUL, *Lloydia*, 29 (1966) 315.
4 S. AGURELL, *Acta Pharm. Suecica*, 2 (1965) 357.
5 E. LEETE, *J. Am. Chem. Soc.*, 88 (1966) 4218.
6 *Pharmacopea Nordica, Editio Suecica*, Vol. I, Apotekarsocietetens Förlag, Stockholm, 1964, pp. 74–75.
7 E. STAHL, *Dünnschichts-Chromagraphie*, Springer-Verlag, Berlin, 1962, p. 506.

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EXTRACTION OF MESCALINE FROM PEYOTE

Mescaline is extracted as other amines:

Chop two buttons and put in beaker with 50 ml. of water. Add one or two drops of concentrated HCl to make acidic (this forms a water soluble amine salt).

Heat mixture to boiling for 10 minutes, stir and mash.

Cool, filter through paper or glass wool into graduated cylinder, make basic with NaOH and extract with two 10 ml. portions of benzene (watch for emulsion) a $CHCl_3$. Evaporate solvent on hot plate, when approximately 4 ml. remain, add a few drops of concentrated HCl (acid converts it to salt making nonvolatile). Take down to dryness, this residue contains mescaline. An alternative to ppt. out the mescaline is to leave it soluble in solvent, this facilitates analysis by gas chromatograph, thin layer chromatograph and crystalline tests.

Some mescaline will also be extracted by simply chopping a portion of the button and allowing it to sit in $CHCl_3$, this can then be analyzed by G.C. or T.L.C.

Crystal Tests: Reagents added directly to sample.

1. Gold chloride
2. Platinic chloride
3. Wagner's (first dissolved in dilute HCl)

Color Test: Concentrated HNO_3: deep red

Wagner's: brown ppt.

Marquis: yellow with H_2SO_4 - deep orange then fades with HCHO

T.L.C. System: (both given good separation)

1. NH_4OH : MeOH (1.5 : 100)
2. *$CHCl_3$: MeOH : Acetic acid (75 : 20 : 5)

* by good separation of two components extracted.

G.C. : OV - 17 Column

1. Column temperature - 190° C
   Injection temperature - 240° C
   Flame temperature - 250° C
   Flow rate - 0.3 reading
   Chart speed - 0.5 inch/min.
   Atten. range - 64 x 100

2. Column temperature - 180° C
Injection temperature - 240° C
Flame temperature - 250° C
Flow rate - 0.3 i
Chart speed - 0.5 in./min.
Attn. - range - 64 x 100.

Retention time for mescaline measured from $CHCl_3$ (solvent) peak front edge;

System 1) 3.30 min.
System 2) 5.04 min.

Refer to case FR-D-172-3

BNDD LABORATORY NOTES

DATE March 14, 1973 - 43 -

NO.

DRUG TYPE Hallucinogen

METHODOLOGY Infra-Red

EXTRACTION OF MESCALINE FROM PEYOTE BUTTONS

TERRY A. DAL CASON
Forensic Chemist
Bureau of Narcotics and Dangerous Drugs
Chicago Regional Laboratory

The following system of extractions is provided as an alternative to the overnight Soxhlet extraction. The mescaline, recovered as the hydrochloride salt, is quite suitable for infra-red spectroscopy. Identification can be completed in less than five hours.

PROCEDURE

The buttons, if not received as dry material, should be dried until hard and brittle. One to five buttons are placed in a mortar and ground to a powder. The powdered buttons should then be placed in a beaker and 15-20mls of ETOH added/button. Place the beaker on a steam bath for 15-20 minutes. Then filter the ethanol solution into another beaker. Repeat the ethanol extraction on the steam bath three times combing the filtered ethanol solutions and evaporating them to dryness on the steam bath.

15-20mls of 1.N HCl is then added to the residue and the solution is filtered into a separatory funnel. The acid solution is then extracted with three 30ml portions of ether, the ether being discarded.

Add aqueous sodium hydroxide to the acid solution until a pH of 11 or greater is reached. Extract this solution with three 30ml portions of ether and discard the aqueous solution. The ether layer is extracted with two 20ml aliquots of 1.0N HCl and the ether is discarded. The acid solution is extracted three times with 30ml aliquots of chloroform and the chloroform discarded.

Carefully add Na HCO_3 until a saturated solution results (pH approx. 8) and extract this with three 25ml portions of $CHCl_3$ and combine the $CHCl_3$ extracts. The $CHCl_3$ extracts are re-extracted with three 15ml portions of 0.5N NaOH, discarding the NaOH. Filter the $CHCl_3$ through filter or phase separation paper into a beaker. Add 2-3 drops of concentrated HCl, and evaporate on a steam bath.

Wash the residue in the beaker with acetone twice. The resulting mescaline hydrochloride will remain as a light tan to off white powder suitable for I.R. identification.

EXTRACTION OF MESCALINE FROM PEYOTE AND SUBSEQUENT INSTRUMENTAL ANALYSIS

BY

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PURPOSE: To develope a relatively simple procedure for the extraction of Mescaline from the other Phenolic and Non-Phenolic Peyote Alkaloids.

APPARATUS AND REAGENTS:

1. Various beakers, test tubes
2. 250 ml. Separatory funnel
3. Variable speed blender
4. 6N NaOH
5. $CHCl_3$
6. Methanol
7. HCl (Conc.)
8. Glass Wool
9. TLC Plates (Silica Gel G, Glass, 20 x 20 cm., 250 microns, E. Merck).
10. Ethyl Acetate: Methanol: NH_4OH (17:2:1) (Davidow Development Solvent) (Ref. 4).
11. Acidic Iodoplatinate Spray Reagent (4 ml. of 10% Platinum Chloride solution added to 12 gms. of Potassium Iodide in 100 ml. of H_2O. Add 1.0 ml. Conc. HCl).
12. Ultra - Violet Spectrophotometer (Acta CIII), Gas Chromatograph (Bendix 2600), Infra-Red Spectrophotometer (Pye Unicam SP-1000)
13. Potassium Bromide (IR Grade)
14. Press for making KBr pellets
15. Mescaline HCl Standard (From Applied Science Laboratories).

PROCEDURE:

1. Place 3 or 4 Peyote Buttons in 10 ml. of H_2O. Add 5 ml. of 6N NaOH. Let soak for about 10 minutes.
2. Place in blender and liquify at lowest speed setting. This usually takes 3-5 minutes.
3. Pour mixture into a 250 ml. separatory funnel. Add 50 ml. of $CHCl_3$. Shake gently. If an emulsion forms, add more $CHCl_3$.
4. Filter the $CHCl_3$ through glass wool into a beaker. Evaporate to about 0.5 ml.
5. Using a template, mark about 10-15 points on a TLC plate. Spot the samples. Also spot 1 or 2 Mescaline standards.
6. Develope for approximately 30 minutes, or until solvent travels 10 cm. (Use about 200 ml. of development solvent).
7. Remove TLC plate and dry thoroughly. Spray only the Mescaline Standard (s). Cover the rest of the TLC plate. Mescaline appears as a purple spot with an Rf. of 0.31.
8. Gently, mark a line through the Mescaline Standard, and extend it across the TLC plate.
9. Scrape the unsprayed Silica Gel G, which contains the Mescaline

from the Peyote, from the glass plate (about 0.5 cm. on each side of the line) and dissolve in 5 ml. of H_2O.

10. Add 2 ml. of 6N NaOH. Add 10 ml. of $CHCl_3$. Shake for 1 minute.
11. Filter the $CHCl_3$ through glass wool into a beaker. (Remove 0.5 ml. of the $CHCl_3$ extract and place in a test tube.)
12. Add 1 drop of Conc. HCl and 1.0 ml. of Methanol to the $CHCl_3$ in the beaker and stir. Evaporate to dryness.
13. Dissolve residue in 5.0 ml. of Methanol. Filter through glass wool into a beaker and evaporate to dryness. The resulting powder is Mescaline HCl.
14. Dissolve a small portion of the powder in Methanol and scan on a U.V. Spectrophometer. Compare to standard Mescaline HCl Spectrum (See attached U.V. Spectrums)
15. Evaporate the 0.5 ml. from step 10 above to about 50 µl. Inject into a Gas Chromatograph. Inject Mescaline Standard and compare the retention times. (See attached G.C. Tracings)
16. Place a portion of the powder into a mortar and add about 100 mgs. of KBr. Grind thoroughly and make a KBr pellet with a suitable press. Record the I. R. Spectrum and compare to the Mescaline HCl standard. (See attached I.R. Spectrums)

DISCUSSION:

The Mescaline HCl was used as purchased from Applied Science Laboratories. Excellent results can be obtained from 3 or 4 Peyote Buttons. TLC provides a very simple and fast method for separating the Mescaline from other Peyote Alkaloids. Extraction from the Silica Gel G gives essentially pure Mescaline. This is confirmed by U. V. and G.C.. I. R. indicates that the Mescaline (HCl) from the Peyote matches the Mescaline HCl standard except for a few subtle differences. The Mescaline (HCl) from Peyote gives two small shoulders at 818 and 870 cm^{-1} that do not appear in the Mescaline HCl standard. Also the two doublets at 775 and 785 cm^{-1} and 1460 and 1470 cm^{-1} are not as well defined in the Peyote extract as they are in the standard.

Additional Peyote Alkaloids can be analyzed by scraping the appropriate spots from the TLC plate and extracting.

Total analysis time is 3 hours or less.

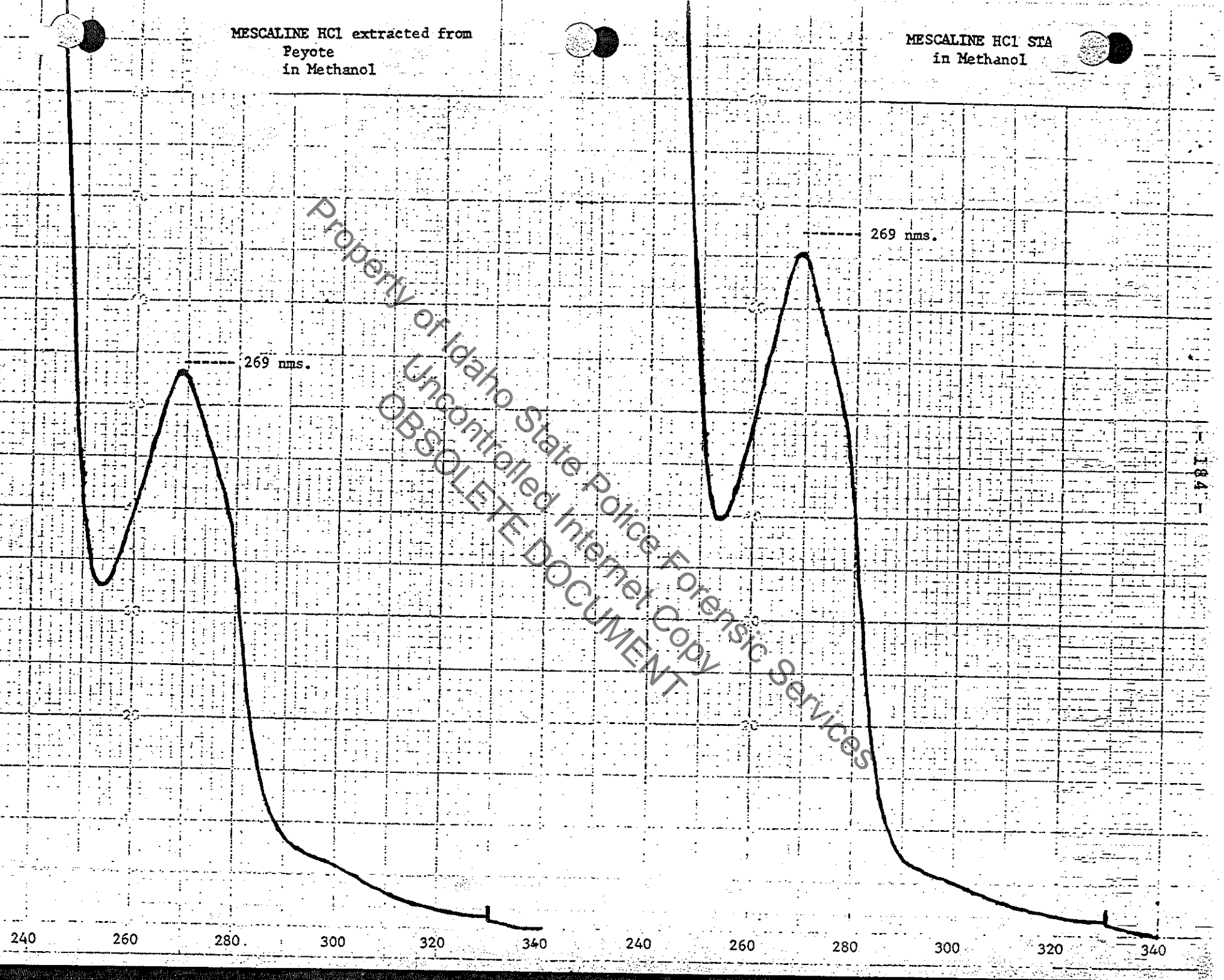
REFERENCES:

1. BNDD Manual Analysis of Drugs, Pgs 75-82.
2. Clarke, E. G. C., Isolation And Identification of Drugs, The Pharmaceutical Press. London, 1971, Pgs. 404-405.
3. Dal Cason, Terry A. Microgram. Vol. VI, No. 3, Pg. 43.
4. Davidow, B., PhD, Nicolo Li Petri, B.S., and Babington Quame, M. S., A Thin-Layer Chromatographic Screening Procedure for Detecting Drug Abuse, The American Journal of Clinical Pathology, Vol. 50, No. 6, Pgs. 714-719.
5. Levine, J. Microgram. Vol. 1, No. 3, Pgs 28-29 (January 1968).
6. Smith, R. Martin, PhD, and Frank C. Dolejsi, Jr. Microgram, Vol. IV, No. 10, Pgs. 138-139; 142 (November 1971).

May 15, 1975 John J. Barbara

MICROGRAM, VOL. VIII, No. 12 (December, 1975).

MICROGRAM, VOL. VIII, No. 12 (December, 1975).



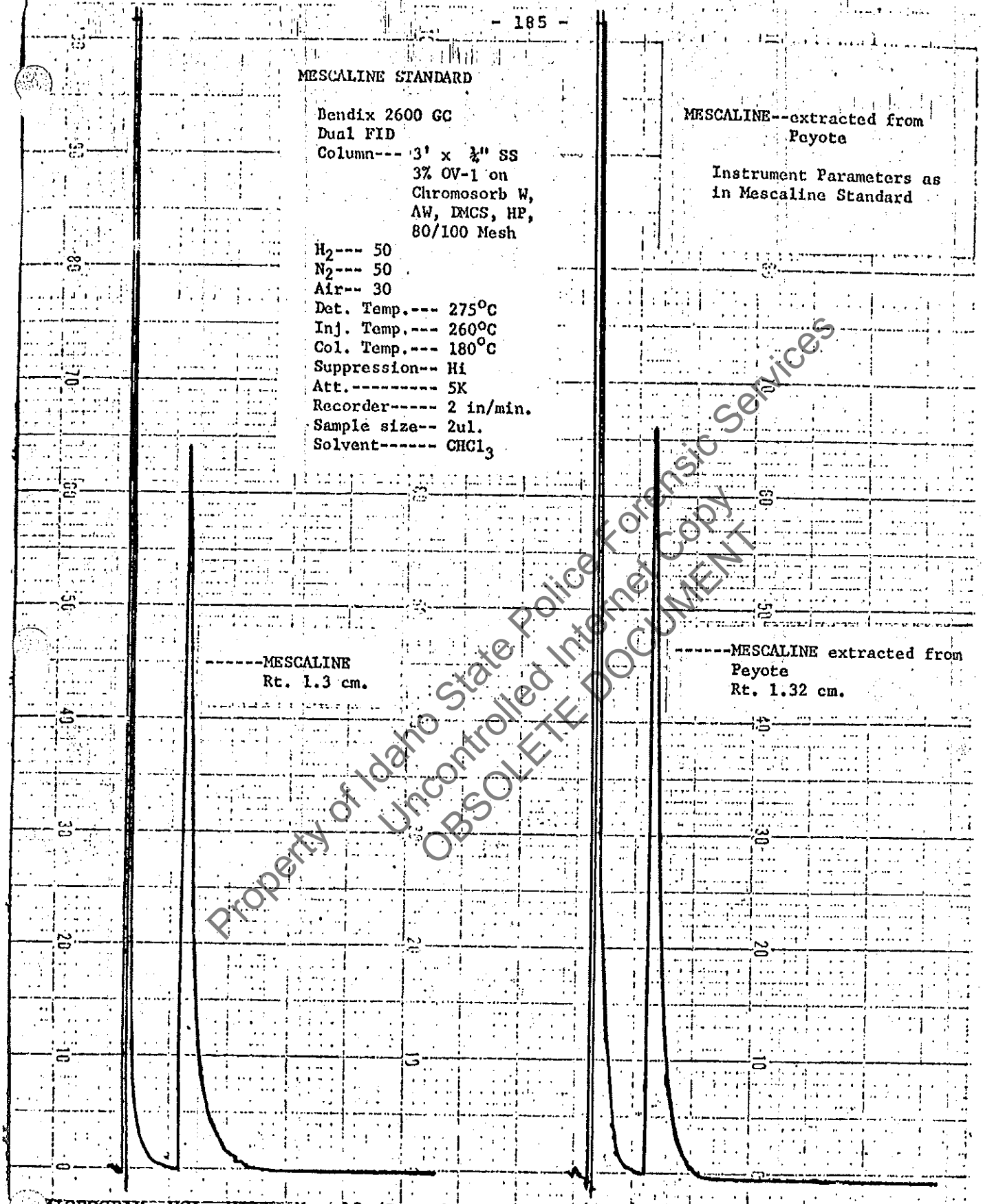
MESCALINE STANDARD

Bendix 2600 GC
Dual FID
Column--- 3' x ⅛" SS
3% OV-1 on
Chromosorb W,
AW, DMCS, HP,
80/100 Mesh

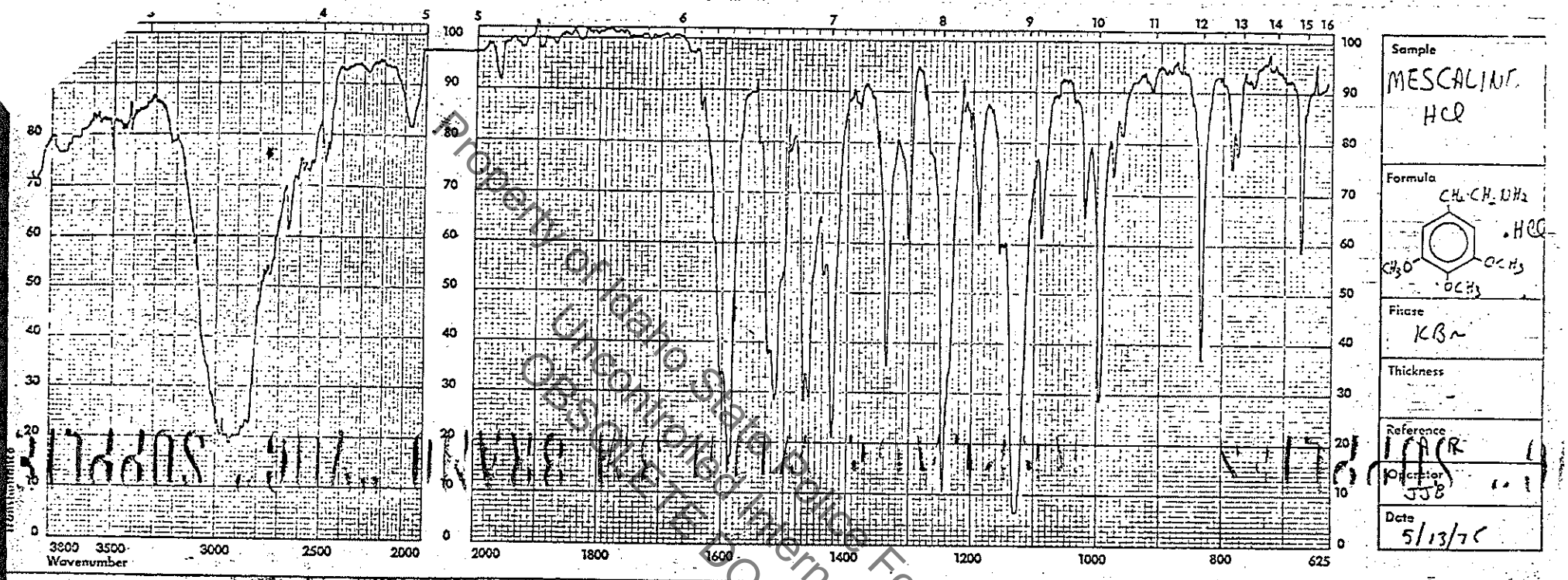
H_2--- 50
N_2--- 50
Air-- 30
Det. Temp.--- 275°C
Inj. Temp.--- 260°C
Col. Temp.--- 180°C
Suppression-- Hi
Att.---------- 5K
Recorder----- 2 in/min.
Sample size-- 2ul.
Solvent------ $CHCl_3$

MESCALINE--extracted from Peyote

Instrument Parameters as in Mescaline Standard

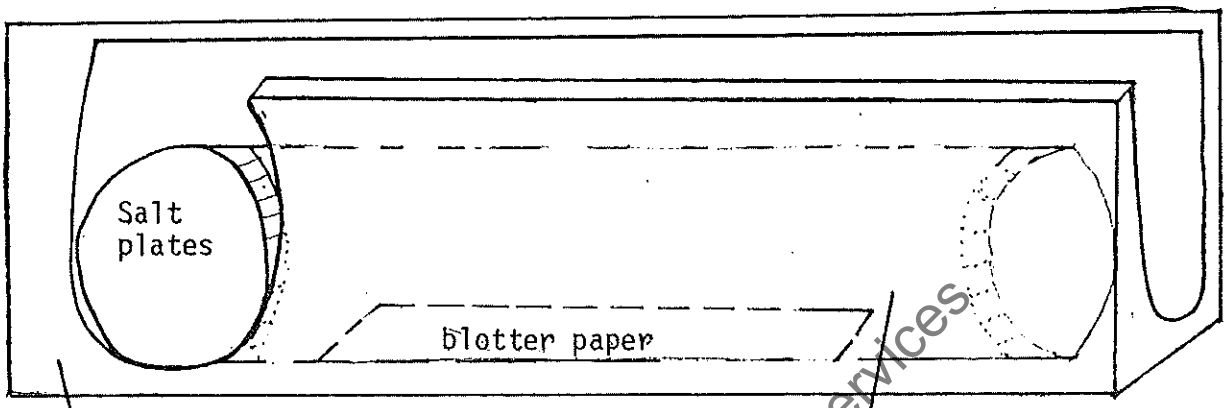


MICROGRAM, VOL. VIII, No. 12 (December, 1975).



MICROGRAM, VOL. VIII, No. 12 (December, 1975).

Do-it-yourself
GAS CELL



wooden holder
(can be made of any material)
to hold tube in beam

Melting point tube glass vial

RUN IR ON SLOW
CUT DOWN SLIT TO 2

This is especially good for use in analyzing materials from lab raids.
e.g. methylamine & other organics

MIXTURES
SEPARATION SCHEME

DRY EXTRACTION WITH:

1. Pet ether (will dissolve the least)
2. Ethyl ether
3. CH_2Cl_2
4. CH_3Cl
5. CH_3/MeOH 3:1 (best @ dissolving)

This procedure will indicate: What the substance is soluble in & suggests clean-up scheme.

What TLC system to use to separate

Psilocin Extraction Procedure

Robert E. Burroughs
Denver Police Laboratory

1. Cut up the mushrooms and dry them in an oven at 90 - 100 C.

2. Grind the dried mushrooms to a powder using a mortar and pestle.

3. *Wash the mustrooms with chloroform to remove fatty material.

4. Soak the mushrooms in 0.1N H_2SO , with occasional stirring for at least thirty minutes. (a magnetic stirrer hot plate is helpful)

5. Collect acidic solution by filtration, and * wash (1 to 1) with chloroform.

6. Make the acidic solution basic using 25% sodium hydroxide to a pH of ten (10).

7. Extract the now basic solution with chloroform (1 to 1).
   a. Psilocin extracted into chloroform.

8. *Wash the chloroform with saturated sodium borate solution (1 to 1).

9. Dry the chloroform through anhydrous sodium sulfate, and filter.

10. Concentrate the chloroform (sample is now ready for GC, and GCMS), add heptanes (3:1) and take to dryness using low heat and dry air. (the greenish powder should be ready for I.R.)

11. *Recrystallization may be required again using heptanes and chloroform (3:1).

12 *If the material does not produce an acceptable IR it can be re-dissolved in 0.1 N H_2SO), washed ($CHCl_3$), made basic, re-extracted, dried, and recrystallized.

*Optional steps

Notes: (1) An emulsion can form during any of the above extraction steps. (centrifuging, salting, or ethanol may help in breaking emulsions). Any precipate formed should be left behind and not taken on to the next step.

(2) With small samples all washes can be back extracted, enhancing psilocin recovery.

MUSHROOMS
GC CLEAN-UP

1. Wash material with ether.
2. Grind material in MeOH
3. Filter with Buchner & glass fiber filter paper.
4. Evaporate MeOH over steam with air.

TLC

n-BuOH sat'd water:HOAc (9:1) is an excellent system. R_f=.82

PSILOCYN EXTRACTION

1. Dry mushrooms if needed.
2. Grind mushrooms to powder (.5 - 3.0 grams)
3. Place powder in folder filter paper and rinse with approximately 50 ml. of $CHCl_3$. Discard $CHCl_3$.
4. Dry powder and place in folded filter paper.
5. Pour approximately 50 ml. of .1N H_2SO_4 over powder and collect in flask.
6. Transfer liquid to two 50 ml. (25 ml. in each) centrifuge tubes and wash with equal volume of $CHCl_3$. Mix and centrifuge briefly.
7. Transfer aqueous to two 50 ml. centrifuge tubes (approximately 20 ml. in each tube).
8. Make basic with 1N NaOH (add approximately 2 ml.) - check pH.
9. Extract with equal volumes of CH_2Cl_2. Mix and centrifuge briefly.
10. Aspirate aqueous.
11. Transfer extract to 50 ml. beaker and boil to 1 - 2 ml.
12. Dry thru $NaSO_4$ column and spot for TLC and evaporate remainder on KBr for IR. shoot full on GCMS. ↓ over

NOTE: For TLC I use T2 - spray first with Fast Blue BB - red spot then with p-DMAB - blue spot.

T1 7.5 drops NH_4OH 1.5:100
25 ml MeOH

10-14-97
T1 7.0 drops NH_4OH
10 ml MeOH

PSILOCYN EXTRACTION

1. Dry mushrooms if needed.

2. Grind mushrooms to powder (.5 - 3.0 grams)

3. Place powder in folder filter paper and rinse with approximately 50 ml. of $CHCl_3$. Discard $CHCl_3$.

4. Dry powder and place in folded filter paper.

5. Pour approximately 50 ml. of .1N H_2SO_4 over powder and collect in flask.

6. Transfer liquid to two 50 ml. (25 ml. in each) centrifuge tubes and wash with equal volume of $CHCl_3$. Mix and centrifuge briefly.

7. Transfer aqueous to two 50 ml. centrifuge tubes (approximately 20 ml. in each tube).

8. Make basic with 1N NaOH (add approximately 2 ml.) - check pH.

9. Extract with equal volumes of CH_2Cl_2. Mix and centrifuge briefly.

10. Aspirate aqueous.

11. Transfer extract to 50 ml. beaker and boil to 1 - 2 ml.

12. Dry thru $NaSO_4$ column and spot for TLC and evaporate remainder on KBr for IR.

NOTE: For TLC I use T1 - spray first with Fast Blue BB - red spot then with p-DMAB - blue spot.

THE WEBER TEST
A Color Test for the Presence of Psilocin in Mushrooms

The popularity of hallucinogenic fungi has increased in the past few years. Along with the increased popularity, we see an increase in mushroom possession cases and mushroom buys by narcotics agents. Some of these mushrooms will be hallucinogenic, but many will be bogus. In the Weber State College Crime Lab, over the past three years, the ratio of controlled vs. non-controlled mushrooms has been about 50/50.

Those of you who work with identification of controlled mushrooms know that it is no simple task. There are several factors that make such identification difficult:

1. There are many varieties of mushrooms, literally thousands of different species, of which only a few are hallucinogenic and not all of these are in the same genus.

2. Even with fresh mushroom samples, genus and species identification can be a real challenge, unless one is well-versed in the identification and classification of mushrooms. When samples are submitted to the laboratory in a dried, crushed, and/or frozen state, botanical identification is almost impossible.

3. Psilocin and psilocybin are light and heat sensitive and can decompose very quickly if handled improperly.

4. Most labs use analytical methods to identify controlled mushrooms. These methods can be challenging when trying to identify the controlled components in hallucinogenic mushrooms.

Using a gas chromatograph to identify psilocybin and psilocin with an FID or hot wire detector can be very tricky. GC/MS is an excellent and quick procedure, but not all labs are fortunate enough to possess one. Sample clean-up and preparation for TLC, IR, or UV can take several hours. Because of these factors, much valuable lab time can be wasted on the analysis of bogus mushrooms.

At Weber State College Crime Lab, we have devised a simple color test to distinguish non-controlled mushroom samples in approximately two minutes. It is a preliminary test, and not designed as a complete analytical procedure. A negative test result, however, should eliminate any need for further testing.

The chemicals used react with psilocin, which is typically present in the hallucinogenic mushrooms in much smaller quantities than psilocybin. Some literature reports that there may be species of hallucinogenic mushrooms that contain only psilocybin, but, in our test, we have not found any "magic mushrooms" that do not contain at least some psilocin. This may be due to the fact that psilocybin hydrolyzes to psilocin very easily, and thus is usually present if psilocybin is present.

Over the past two years, the Weber State College Crime Lab has tested all mushroom samples that have been submitted, with very good results. In no case did a sample test negatively with the color test and subsequently show the presence of psilocin by TLC and IR.

Recently, the mushroom collection of Brigham Young University was tested with this color test (a total of 55 different species) as well as numerous unidentified species collected from our local environment, with no false positive or false negative results being received.

The procedure is a very simple one, consisting of a two-part chemical addition to a small fragment of a mushroom.

Step 1: Make fresh daily, a 0.1% solution of Fast Blue B or Diazo Blue B (o-dianisidine, tetrazotized) by dissolving .01 grams in 10ml distilled water. Two to three drops of this solution is added to a sample of mushroom, at room temperature. The solution will turn red if psilocin is present.

Step 2: One to two drops of concentrated hydrochloric acid is added to the (red) solution of mushroom sample and Fast Blue B reagent. In the presence of psilocin, the solution will change from red to blue in color.

If psilocin is not present, no color is obtained or, in a few incidents, a pink or orange color will appear with no change in the HCL addition. Where colors were obtained, they were not confused with those of a positive test for psilocin.

We, at Weber, feel that this is, and can be used as, a valid preliminary screening test for the presence of psilocin in mushrooms.

Thanks to B.Y.U. Botany Department for mushroom samples, as well as the article by John Kearns of the Spokane, Washington Lab on the "Isolation and Identification of Psilocin from Psilocybe Mushrooms".

Paper presented to the Northwest Association of Forensic Scientists: May 4, 1984 in Coeur d'Alene, Idaho by Allen Steve Garrett, Steven R. Clemens, and James H. Gaskill.

WEBER STATE COLLEGE

LABORATORY OF CRIMINALISTICS

OGDEN, UTAH 84408
801-626-6147

7 January 1984

Lt. G. K. Matsuda
Editor, NWAFS Newsletter
Oregon State Police
222 S. W. Pine Street
Portland,
Orgeon 97204

Lt. Matsuda:

Since this paper was presented in May 1984 at Coeur d'Alene, there have been requests to our laboratory for a write-up of the procedure. We hope that you will see fit to publish this paper in the upcoming edition of the newsletter.

Kathleen T. Ledford

Kathleen T. Ledford, Criminalist

for James H. Gaskill, Lab Director

EXTRACTION AND PURIFICATION OF PSILOCYN FROM HALLUCINOGENIC MUSHROOMS:

PRINCIPLE:

A sample of finely ground suspect mushroom is treated with acetic acid to extract any psilocybin and psilocyn present. The solution is then heated to effect complete dephosphorilation of the psilocybin to psilocyn. The psilocyn is then extracted, crystalized and identified via FTIR.

SPECIMEN REQUIREMENTS:

A sample as small as two (2) grams should yield enough psilocyn for identification.

REAGENTS AND EQUIPMENT:

1) Mortar and pestle
2) Extraction vessel and associated glassware
3) Buchner funnel
4) Glass wool
5) Glacial acetic acid
6) Ammonium hydroxide
7) Diethyl ether
8) Sodium sulfate
9) Chloroform
10) n-Heptane
11) KBr

PROCEDURE:

1) Grind the dried mushroom pieces (2 - 10 grams) to a fine powder.

2) Add this powder to 100 ml of dilute acetic acid in a suitable beaker.

3) Adjust the pH to 4 with glacial acetic acid and let stand for one hour.

4) Place the beaker in a boiling water bath until the temperature of the acid solution reaches 70°C (only takes a few minutes).

5) Bring the solution back to room temperature by cooling under running water.

6) Filter the solution using suction filtration and glass wool.

7) Adjust the pH to 8 with concentrated ammonium hydroxide and promptly extract with two 50 ml portions of diethyl ether.

8) Dry the ether extracts with sodium sulfate, filter (glass wool) and evaporate to dryness without the use of heat.

9) A crusty dark green residue indicates the presence of crude psilocyn. Add approximately 10 ml of chloroform/n-Heptane (1:3), mix well and transfer to another evaporation dish, let dry. The off-white residue that remains should be a fairly clean sample of psilocyn and can now be run on the FTIR.

EXTRACTION AND PURIFICATION OF PSILOCYN FROM HALLUCINOGENIC MUSHROOMS:

COMMENTS:

The prompt extraction in Step 7 is necessary as psilocyn may decompose at a pH greater than 7.

REFERENCE:

1) An Aqueous-Organic Extraction Method for the Isolation and Identification of Psilocyn from Hallucinogenic Mushrooms, by John F. Casale, Journal of Forensic Sciences, Volume 30, Number 1, January 1985, page 247.

Hallucinogenic Mushrooms of the Pacific Northwest

by Michael W. Beug, Ph.D.
The Evergreen State College

The hallucinogenic mushrooms of the Pacific Northwest are as yet incompletely known. As our knowledge of the mycoflora of the Pacific Northwest improves, I believe that we will find psilocybin in a total of roughly 20 species in at least four genera.

Most hallucinogenic mushrooms are in the genus Psilocybe and chromatographically they can be roughly divided into two groups -- those with chromatographic patterns resembling Psilocybe semilanceata, "Liberty Caps," and those resembling Psilocybe cyanescens.

Psilocybe semilanceata is probably the most widely collected indigenous hallucinogenic species. It is found in somewhat boggy pasture and wet grasslands. It contains psilocybin but not psilocin. Psilocybin levels we have measured by HPLC are between 6.2 mg/g and 12.8 mg/g dry weight. This species is one of the most consistent producers of psilocybin and shows the least variation in level from one species to the next. Reports of psilocin in this species result from breakdown of the psilocybin during extraction. Cromatographically similar species include a woodland mushroom, Psilocybe pelliculosa (1.2 to 7.1 mg/g dry weight psilocybin) and two grassland species, Psilocybe liniformans var. americana (6.5 mg/g to 12.8 mg/g dry weight psilocybin) and an unnamed species (no psilocybin). The three grassland species are easily confused by collectors.

Psilocybe cyanescens is characterized by containing both psilocybin and psilocin plus other indoles. Psilocybin levels range from 1.5 to 15.5 mg/g while psilocin levels range from 0.6 to 9.6 mg/g dry weight. I have never found Psilocybe cyanescens or the chromatographically similar species in either pastures or woodlands. They prefer cultivated areas, particularly where bark mulch or sawdust mulch has been used. The chromatographically similar species include Psilocybe baeocystis (1.5 to 8.5 mg/g psilocybin and 0 to 5.9 mg/g psilocin); Psilocybe stuntzii (0 to 3.6 mg/g psilocybin and 0 to 0.6 mg/g psilocin); and Psilocybe cyanofibrillosa (0 to 2.1 mg/g psilocybin and 0.4 to 1.4 mg/g psilocin).

Other psilocybes analyzed include P. coprophila, P. montana, and P. inquilina. None of these have been found to contain either psilocybin or psilocin.

Of the many species of Panaeolus in the Pacific Northwest, two have been found to contain psilocybin. The most common species, P. subbalteatus, occurs on well-manured lawns and around horse stables. It contains 1.6 to 6.5 mg/g psilocybin. The other active species is as yet unnamed and contains 0 to about 4 mg/g psilocybin. It is only found on horse manure compost.

At least one species of Conocybe contains psilocybin. One sample of the small, grass-inhabiting species, Conocybe cyanopus, was found to contain 9.3 mg/g dry weight psilocybin.

Other workers using TLC have detected psilocybin in Pluteus salicinus.

Psilocybe or Stropharia cubensis is frequently cultivated in the Pacific Northwest. It is a semitropical species that grows well on rye grain or on straw compost. It contains 0.7 to 13.3 mg/g dry weight psilocybin and 0 to 2.9 mg/g psilocin.

Chemical Analysis. Fresh mushroom samples were freeze-dried, sealed in plastic and stored at -60°C until analysis. After grinding to a fine powder, 250 mg portions were extracted at room temperature for 12 hours in 7 ml methanol, filtered through a 0.5 µm Teflon filter, rinsed with additional methanol and diluted to exactly 10 ml. Analysis was performed on a Waters 200 series high performance liquid chromatograph at 254 nm with a 30 cm x 3.9 mm µ-Bondapak C_{18} column (particle size 10 µm) using 75% water -25% methanol containing 0.05 M heptanesulfonic acid adjusted to pH 3.5 with acetic acid (Waters Pic B-7 reagent) at 2 ml/min flow rate. Results were confirmed with TLC on 5 x 20 cm silica gel plates developed with butanol-acetic acid-water (12:3:5) and visualized with freshly prepared Ehrlich's reagent (10% p-dimethylamino-benzaldehyde in conc. HCl). Ten other solvent systems and numerous other spray reagents were also evaluated.

d-PROPOXYPHENE (Darvon)
IR PROCEDURE

1. Add water to ground tablet in tt.
2. Filter through cotton plugged pipet
3. Make basic with drop of NH_4OH
4. Extract with Pet Ether - Suck-up with pipet to mix.
5. Pipet off Pet Ether into another tt
6. Add water (equal volume) to wash
7. Filter Pet Ether through cotton
8. Evaporate down - tease until crystals form or
   - run on salt plates in CH_2Cl_2
9. Run IR scan

THIS PROCEDURE CAN BE USED FOR MANY PHARMACEUTICAL DRUGS

REFERENCES

Pharmacognosy, 7th Ed., V.E. Tyler, et al, Lea & Febiger, Phil., Penn., 1976.

Identification Procedures of Drugs of Abuse, N.H. Choulis, European Press, Ghent, Belgium, 1977.

A Textbook of Pharmaceutical Analysis, 3rd Ed., K.A. Connors, John Wiley & Sons, New York, 1982.

Handbook of Spectrophotometric Data of Drugs, I. Sunshine, Ed., CRC Press, Inc., Boca Raton, Fla., 1981.

Drug Fate and Metabolism, Vol 1 to 4, E.A. Garrett and J.L. Hirtz, Eds., Marcel Dekker, Inc., New York, 1983.

Handbook of Chromatography, Vol 1 and 2, R.N. Gupta, Ed., CRC Press, Inc., Boca Raton, Fla., 1981.

GLC and HPLC Determination of Theurapeutic Agents, Parts 1 to 3, k. Tsugi, Ed., Marcel Dekker, Inc., New York, 1979.

Analysis of Drugs and Metabolites by GC-MS, Vol. 1 to 5, B.J. Gudzinowicz and M.J. Guczinowicz, Marcel Dekker, Inc., New York, 1978.

Instrumental Data for Drug Analysis, Vol. 1 and 2, T. Mills III, et al, Elsevier, New York, 1984.

Analysis of Drugs, Analytical Manual, S.P. Sobol and R.A. Moore, U.S. Government Printing Office, Washington, D.C., 1974.

Clarke's Isolation and Identification of Drugs, 2nd Ed., A.C. Moffat, Ed., The Pharmaceutical Press, London, 1986.

Handbook of Analytical Toxicology, I. Sunshine, Ed., CRC Press, Cleveland, 1969.

Toxicology, Vol. 2, C.P. Stewart and A. Stolman, Eds., Academic Press, New York, 1961.

Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, et al, Eds., Mach Printing Co., Easton, Penn, 1980.

INTRODUCTION

Gas chromatography offers the analyst a way to separate and identify components of mixtures. It is applicable over the wide range of samples seen in the crime laboratory. This method coupled with other tests can be used to identify a particular compound. In certain cases this technique may be coupled with mass spectrometry, whereby this technique would serve as a confirmatory test alone.

At present chromatogrphs are equipped with only flame-ionization or nitrogen-phosphorus detectors. The theory and use of gas chromatography are covered very well in the first two refernces. The analyst should study these, and other references available, before embarking on laboratory work. The following articles of this section may be a starting point as well.

Be sure to acquaint yourself with the instrument manuals for the chromatographs that are being used. Within the frame-work of drug analysis there are a relative few types of column packing materials used. The references address this topic also. The gas chromatographs are coupled with strip-chart recorders and integrators. Manuals exist for these also and should be studied by the anayst. Syringe maintenance is covered by the manufacturer's flyers sent with each syringe.

GENERAL INFORMATION ON REACTIONS TO SPECIFIC DRUGS

The following outlines some general color reactions to a few of the specific reagents used within the laboratory system.

METHOD OF ANALYSIS

Samples of unknown composition may be dissolved in a suitable organic solvent and injected onto the instruement. Papers in the following set, address retention indices and their application to qualitative analysis. Casework will usually involve gathering retention time or response factor data, and by comparison, deriving a list of compounds to check against. If GLC is used as a screening tool this method would be sufficient to continue to more specific testing. A better use of the method would be to inject a known standard during the time of analysis. Should the GLC method be used as the confirmatory test, this comparison to a known standard must be used. In some cases derivitization may be used. Columns that are used to derivitize should not be used for quantitative analysis. References address derivitizing agents and their use.

Quantitative analysis involving the GLC requires weighing out a known amount of sample and dissolving this in a known volume of suitable solvent. The follwoing formulas are used to determine the concentration of the unknown using either an internal or external standard.

Internal Standard:

$$\%\ \text{Unknown} = \frac{\text{Peak Area Sample}}{\text{Peak Area Standard}} \times \frac{\text{Peak Area (std) in Std Soln}}{\text{Peak Area (std) in Sample Soln}} \times \frac{\text{Concentration of std}}{\text{weight of sample}} \times 100\%$$

External Standard:

$$\%\ \text{Unknown} = \frac{\text{Peak Area Sample}}{\text{Peak Area Standard}} \times \frac{\text{Concentration of Standard}}{\text{Concentration of Unknown}} \times 100\%$$

Chromatograms developed in the analysis of casework that are usedto reach conclusions must be kept in the case file.

Paul Schepers,[1] Drs; Jaap Wijsbeek;[1] J. P. Franke,[1] Ph.D.; and R. A. de Zeeuw,[1] Ph.D.

Applicability of Capillary Gas Chromatography to Substance Identification in Toxicology by Means of Retention Indices

REFERENCE: Schepers, P., Wijsbeek, J., Franke, J. P., and de Zeeuw, R. A., "Applicability of Capillary Gas Chromatography to Substance Identification in Toxicology by Means of Retention Indices," *Journal of Forensic Sciences*, JFSCA, Vol. 27, No. 1, Jan. 1982, pp. 49-60.

ABSTRACT: Three capillary columns, set up in a routine screening system, were tested in temperature-programmed runs. A narrow-bore fused silica capillary, Carbowax-deactivated and with a methylsilicone liquid phase, was found to be unstable at higher temperatures, giving irreproducible results and retention indices that varied considerably from those obtained on packed columns. The two other columns, a wide-bore glass capillary and a narrow-bore fused silica capillary, were polysiloxane-deactivated and had a dimethylpolysiloxane liquid phase. Although both showed good stability, reproducibility, and load capacity, retention indices for various drugs still showed discrepancies as compared to corresponding values on packed columns.

KEYWORDS: toxicology, chromatographic analysis, drug identification, systematic drug screening, capillary gas chromatography, retention indices

Gas-liquid chromatography (GLC) has proven to be an indispensable tool in screening for the presence of drugs in systematic toxicological analysis (STA). Owing to the work of Moffat and co-workers [*1,2*], it is now generally accepted that dimethylsilicone stationary phases like SE-30 and OV-1 provide optimum discriminating power. In addition, it has been shown that measurement of the retention indices [*3*] is the technique of choice for substance characterization as well as for the compilation of gas chromatographic data in a data bank and the exchange of those data between different laboratories [*2,4-11*]. The retention index (RI) of a substance on a given stationary phase can be considered a physical parameter of reasonable constancy, provided the method is adequately standardized. The interlaboratory standard deviation of measurement is usually between 15 and 20 RI units [*2,10,12*]. Although RI's are temperature-dependent [*10*], it has been shown that those obtained in a temperature-programmed run are usually in good agreement with those determined under isothermal conditions [*7,8*].

So far, almost all RI data for toxicological analysis have been obtained on conventional, packed, glass columns, with an inner diameter of 2 to 5 mm and with the stationary phase coated on relatively inert supports such as Chromosorb. Although open capillary columns were introduced in 1957, their use in toxicology has remained rather limited, probably

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[1]Research associate, research associate, senior research associate, and professor of toxicology, respectively, Department of Toxicology, State University, Groningen, The Netherlands.

because of their high cost, fragility, and limited load capacity. However, recent years have been a period of innovation with regard to column technology, resulting in a new generation of glass capillary columns and the so-called fused silica and fused quartz columns, which combine high separation power with good column stability, flexibility, load capacity, and so on [*13,14*]. These newer capillary columns are made from high purity materials that are relatively inert to susceptible solute molecules and exhibit a smooth surface. Although the content of total metal oxides is less than 1 ppm, to obtain optimum results deactivation of the wall surface is still necessary, the most common procedures being treatment with polyethylene glycol (Carbowax) or silylation. A second advantage of deactivation procedures is that they improve the wettability of the wall surface for the stationary phase. Although various deactivation procedures have been described [*14,15*], it should be realized that their performance requires a considerable amount of experience. For that reason, most toxicological laboratories would have to buy their capillary columns from commercial sources.

The present study was undertaken to evaluate the applicability of some of the new capillary columns for toxicological screening purposes. We chose to work with temperature-programmed runs to diminish analysis time, and special attention was paid to column stability, day-to-day reproducibility, load capacity, and the degree of agreement between RI's determined on capillary and packed columns with similar stationary phases.

Materials and Methods

The drugs used in this investigation were obtained from commercial suppliers and were used as received. All were dissolved in methanol to give solutions of approximately 0.5 mg/mL, of which 1-μL aliquots were injected. Straight chain alkanes (C_{11} to C_{32}) were used as references for the calculation of retention indices [*3*], with each dissolved in hexane methanol (99:1) to give a solution of about 0.5 mg/mL. Aliquots of 1 μL were injected into an HP 5880 gas chromatograph (Hewlett-Packard) with a splitless capillary injection system. Injections were performed with a HP 7671 A automatic injector. The columns and their operating conditions were as follows:

1. A Carbowax® 20 M-deactivated fused silica narrow-bore capillary column coated with methylsilicone fluid [*16*] was obtained from Hewlett-Packard. The column was 12 m in length and had an internal diameter of 0.20 mm, a film thickness of 0.12 μm, a coating efficiency of 72%, 4200 theoretical plates per meter (C_{15}), and a capacity ratio of 6.1. The maximum operating temperature was given as 280°C. During the first 20 days of this study, the temperature program of the oven was 2 min at 120°C, 8°C/min to 260°C, and then 8 min at 260°C; the injector and detector temperatures were 275°C. During the second part, the program was 2 min at 100°C, 8°C/min to 250°C, and then 15 min at 250°C, with the injector and detector at a temperature of 250°C.

2. The polysiloxane-deactivated glass wide-bore capillary column, which was coated with CP-Sil 5, a dimethylpolysiloxane phase prepared from SE-30 [*17*], was obtained from Chrompack (Middelburg, The Netherlands). The column was 25 m in length and had an inner diameter of 0.49 mm, a film thickness of 1.14 μm, a coating efficiency of 90%, 2060 theoretical plates per meter (C_{14}), and a capacity ratio of 8.6. The upper temperature limit for isothermal use is given as 325°C and 350°C for temperature programming. With the injector and detector temperatures at 275°C, the temperature program for the oven was 2 min at 100°C, 8°C/min to 275°C, and then 15 min at 275°C.

3. The polysiloxane-deactivated fused silica narrow-bore capillary column coated with CP-Sil 5, a dimethylpolysiloxane phase prepared from SE-30 [*17*], was also obtained from Chrompack. The column was 12 m in length and had an inner diameter of 0.22 mm, a film thickness of 0.45 μm, a coating efficiency of 95%, 5160 theoretical plates per meter, and a capacity ratio of 7.0. The upper temperature limits were as under Condition 2. With the injector and detector temperatures at 300°C, the temperature program for the oven was 2 min

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Results

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[*18,19*]. Methylsilicon
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temperatures of the in
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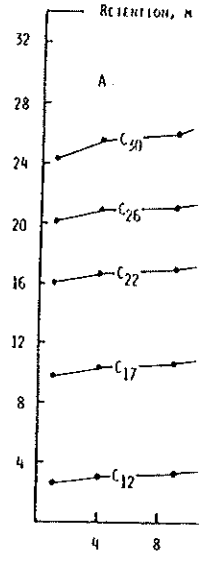

FIG. 1—*Retention beh*
silica column in temperat
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detector, and maximum o

ity, and limited load capacity. However, recent years h regard to column technology, resulting in a new generat e so-called fused silica and fused quartz columns, wh ith good column stability, flexibility, load capacity, and y columns are made from high purity materials that te molecules and exhibit a smooth surface. Although t ss than 1 ppm, to obtain optimum results deactivation y, the most common procedures being treatment wi silylation. A second advantage of deactivation procedur y of the wall surface for the stationary phase. Althou e been described [14,15], it should be realized that the ble amount of experience. For that reason, most to o buy their capillary columns from commercial sources ken to evaluate the applicability of some of the ne ereening purposes. We chose to work with temperatur lysis time, and special attention was paid to colum load capacity, and the degree of agreement betwee cked columns with similar stationary phases.

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used silica narrow-bore capillary column coated with from Hewlett-Packard. The column was 12 m lo 0.20 mm, a film thickness of 0.12 μm, a coating effi- er meter (C_{15}), and a capacity ratio of 6.1. The max- as 280°C. During the first 20 days of this study, the min at 120°C, 8°C/min to 260°C, and then 8 min at atures were 275°C. During the second part, the pro- 250°C, and then 15 min at 250°C, with the injector

wide-bore capillary column, which was coated with prepared from SE-30 [17], was obtained from ds). The column was 25 m in length and had an in- ess of 1.14 μm, a coating efficiency of 90%, 2060 capacity ratio of 8.6. The upper temperature limit 350°C for temperature programming. With the in- , the temperature program for the oven was 2 min 5 min at 275°C.

lica narrow-bore capillary column coated with CP- ared from SE-30 [17], was also obtained from th and had an inner diameter of 0.22 mm, a film of 95%, 5160 theoretical plates per meter, and a e limits were as under Condition 2. With the in- the temperature program for the oven was 2 min

120°C, 8°C/min to 300°C, and then 12 min at 300°C. Helium was used throughout as carrier gas and the precolumn pressure was set such that the C_{32} alkane reference had a retention time of not more than 35 min. The temperature programs were chosen so that a early straight line was obtained when the carbon numbers of the reference alkanes were plotted versus their retention times. Flame ionization detectors were used.

The capillary data given in the figures represent the results of single determinations; those n the table represent the means of at least three determinations, with the individual obser- ations differing by not more than ±5 RI-units. The P column data in the figures were ob- rained on packed SE-30 or OV-1 columns and were taken from Ref *12*.

Results

The first column tested was a fused silica wall-coated open tubular column with an inter- nal diameter of 0.20 mm [*16*]. The stationary phase is described by the manufacturer as a methylsilicone fluid comparable to SE-30 and OV-1. Deactivation is achieved by thermally degrading Carbowax 20 M and feeding the pyrolysis products through the columns. The col- umn is then extracted with solvents, but unextractible material remains on the column, resulting in substantial deactivation and increased wettability for the stationary phase [*18,19*]. Methylsilicone columns treated in this way are claimed to be thermally stable up to 280°C.

When first testing these columns during routine day-to-day operation for 24 h per day, seven days a week, it soon became apparent that the retention times of the reference alkanes were not constant (see Fig. 1A). The increase in retention time suggested that the column was becoming less polar with time of operation. This was presumed to be the result of the temperatures of the injection port and the detector being too high (both at 275°C), resulting in bleed-off of the Carbowax deactivation material at both ends of the columns. After 20 days of operation we then shortened each of the two ends 20 cm and lowered the injector and

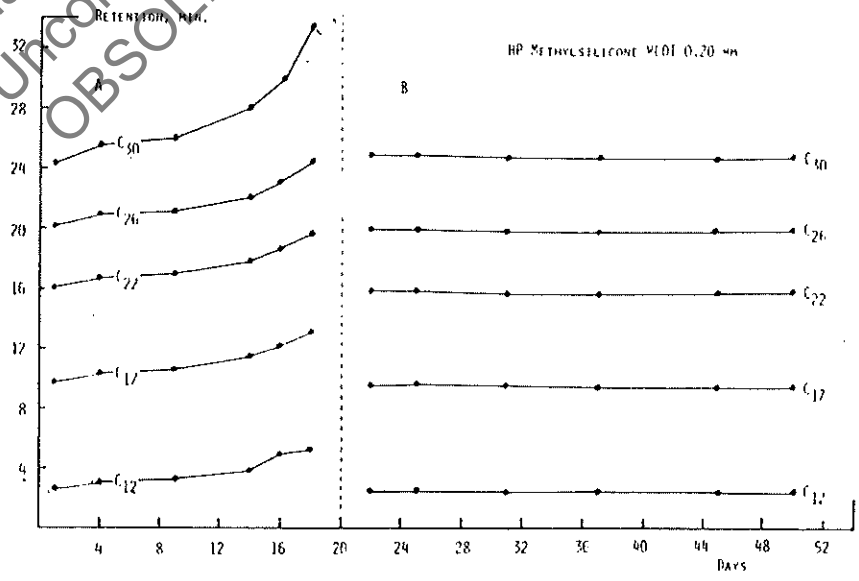

FIG. 1—*Retention behavior of reference alkanes on a Carbowax-deactivated methylsilicone fused silica column in temperature-programmed runs:* (A) *injector and detector temperatures 275°C, maximum oven temperature 260°C;* (B) *after removing 20 cm from both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

detector temperatures to 250°C. The maximum temperature of the oven was also reduced to 250°C. As shown in Fig. 1B, after these changes had been made the alkane retention times remained constant for the rest of the testing procedure.

Figures 2 and 3 show the time versus retention behavior of a selection of drugs, expressed in terms of RI. Different tendencies can be observed. During the first 20 days the 5,5-disubstituted barbiturates showed declining RI's, which seemed to be less pronounced with the *N*-methylated derivatives hexobarbital and metharbital. Other substances, such as caffeine, benzocaine, bromisoval, bemegride, and acetylsalicylic acid, yielded fairly constant RI's, whereas some benzodiazepines with higher RI's showed some variation. Even after we lowered the injector and detector temperatures, RI's decreased for most substances, with some of the barbiturates and clonazepam giving somewhat more pronounced decreases.

Comparison of RI's measured on capillary columns with those obtained on normal packed columns (P in Figs. 2 and 3) clearly indicate marked differences. The 5,5-disubstituted barbiturates all have lower RI's on packed columns; the 1,5,5-trisubstituted barbiturates have quite comparable values, as do caffeine, benzocaine, and bemegride; the diazepines show some variations, with bromisoval and acetylsalicylic acid having higher RI's on packed methylsilicone columns than on Carbowax-deactivated ones.

The CP-Sil 5 wide-bore glass column was treated with polysiloxane according to the procedure of Houtermans and Boodt [*17*]. The upper temperature limit for the deactivation material is claimed to be 350°C. The stationary phase is dimethylpolysiloxane prepared specially from normal SE-30 and is stable to at least 325°C [*17*]. Its retention characteristics are similar to those of SE-30 or OV-1.

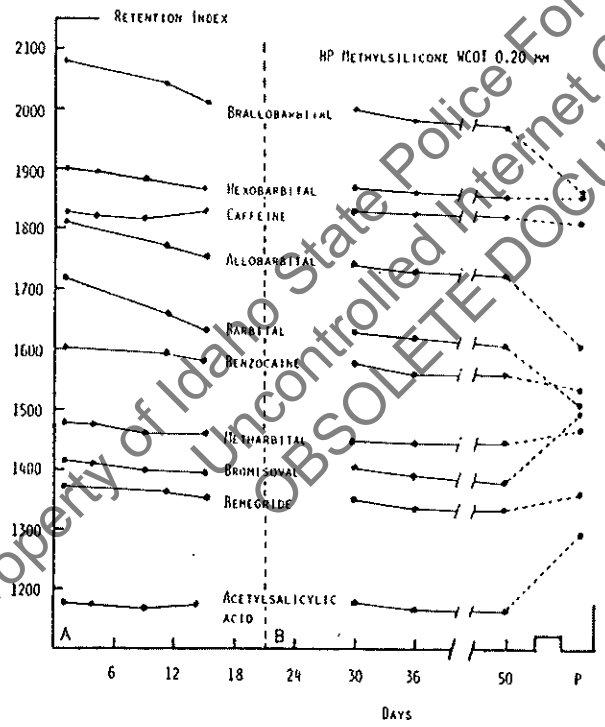

FIG. 2—*Retention index as a function of time of some drugs on a Carbowax-deactivated methylsilicone fused silica column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P): (A) injector and detector temperatures 275°C, maximum oven temperature 260°C; (B) after removing 20 cm at both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

FIG. 3—*Retention i methylsilicone fused silic OV-1 column (P): (A) in (B) after removing 20 cm temperatures 250°C.*

As can be seen in Fi entire test period of 6(drugs is depicted in Fi variations staying with pounds, and never exc RI's on packed SE-30 nitrazepam and diazepa ity gave higher values o other substances showe

The third column, wh was a fused silica narrow above. The stationary ph quate load capacity for t tion, the RI's of the refe on the wide-bore CP-Sil agreement with those me 6 and in Table 1. How discrepancies with those glass CP-Sil 5 capillary. I the narrow-bore fused sili

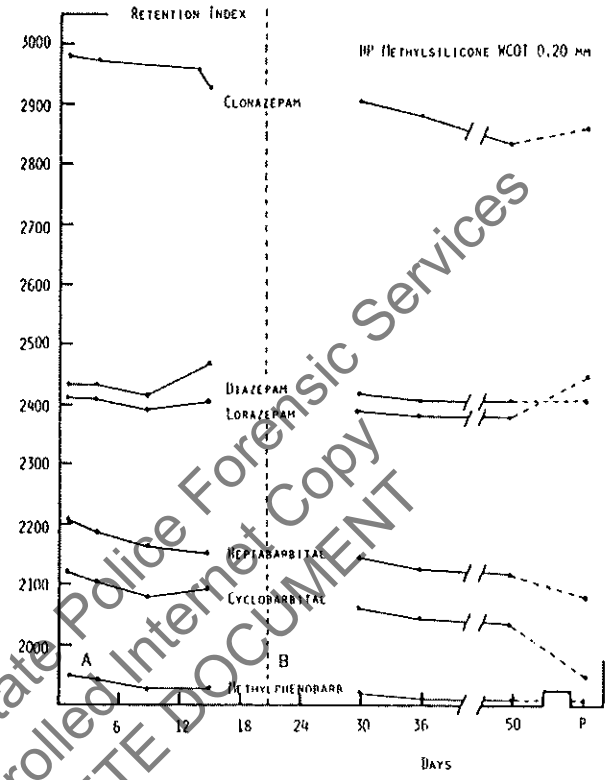

FIG. 3—*Retention index as a function of time of some drugs on a Carbowax-deactivated methylsilicone fused silica column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P): (A) injector and detector temperatures 275°C, maximum oven temperature 260°C; (B) after removing 20 cm from both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

As can be seen in Fig. 4, retention times of the reference alkanes were constant over the entire test period of 60 days of continuous operation. The time versus RI of a selection of drugs is depicted in Figs. 5 and 6. It can be observed that RI's are relatively constant, the variations staying within acceptable limits of ±5 RI units (open circles) for most compounds, and never exceeding ±10 units. The majority of substances tested showed higher RI's on packed SE-30 or OV-1 columns, with the exception of two benzodiazepines, nitrazepam and diazepam. This trend was also found with some 120 other drugs: the majority gave higher values on packed columns, sometimes amounting to more than 40 RI's, but other substances showed the reverse (see Table 1).

The third column, which could only be tested during the latter part of our investigations, was a fused silica narrow-bore CP-Sil 5 capillary, deactivated with polysiloxane as described above. The stationary phase had a relatively large layer thickness of 0.45 μm to ensure adequate load capacity for biological samples. Over a period of four weeks of continuous operation, the RI's of the reference alkanes showed excellent reproducibility, comparable to that on the wide-bore CP-Sil 5 column. The RI's of the drugs investigated also showed good agreement with those measured on the wide-bore column, as is demonstrated in Figs. 5 and 6 and in Table 1. However, RI's determined on this fused silica capillary showed some discrepancies with those on packed columns similar to those mentioned for the wide-bore glass CP-Sil 5 capillary. Figure 7 depicts an actual chromatogram taken from case work on the narrow-bore fused silica CP-Sil 5 capillary, illustrating the excellent separation efficiency

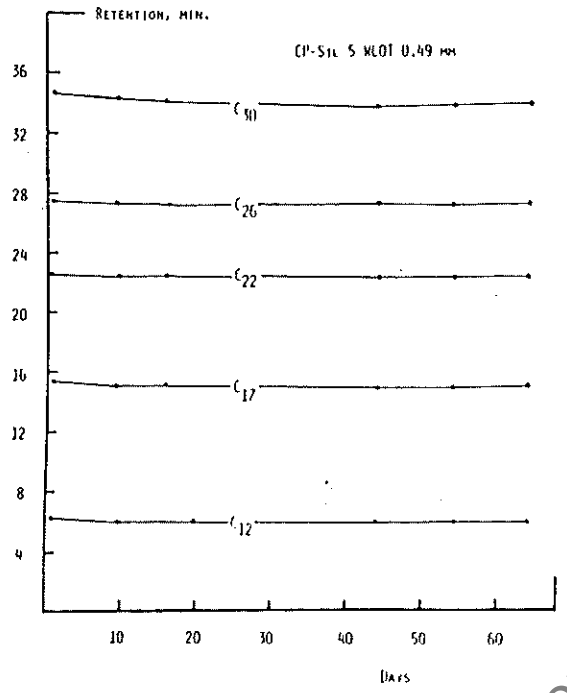

FIG. 4—*Retention behavior of reference alkanes on a polysiloxane-deactivated dimethylpolysiloxane (CP-Sil 5) glass capillary column in temperature-programmed runs.*

(note that acetylcodeine and 6-monoacetylmorphine show almost baseline separation in this programmed run), the narrow peak shape, and the nearly flat baseline.

Discussion

This investigation has clearly demonstrated that the maximum operation temperature of the Carbowax-deactivated column was a rather critical factor to separation efficiency. Although the temperatures were kept within the limitations recommended by the manufacturer, high temperatures at the injector and detector ports apparently caused significant bleedoff of the deactivation material in the adjoining column ends. By lowering the maximum oven temperature and that of the injector and detector to 250°C, the bleedoff could be virtually eliminated. It should be noted, however, that this temperature is too low to obtain full, effective use for STA because of the prolonged analysis time required. Moreover, the RI's obtained on this type of capillary column were found to be quite different from those obtained on comparable packed columns. These discrepancies were more pronounced in certain drug classes yet did not show a clear and predictable pattern. This might have been due to the fact that the Carbowax deactivation material and the methylsilicone liquid phase acted as a mixed stationary phase, interacting differently with certain components than did methylsilicone alone. In view of their thermal instability, which substantially affects both retention behavior and RI, plus the observed discrepancies with RI's measured by packed columns, Carbowax-deactivated methylsilicone capillaries cannot be recommended for general use in STA.

Both polysiloxane-deactivated capillary columns showed excellent stability as well as reproducibility at temperatures up to 300°C and thus were quite effective in STA, permitting rapid screening (within 45 min) for components with RI's up to 3400. The CP-Sil 5 col-

1900
1800
1700
1600
1500
1400
1300
1200

FIG. 5—*Retention ind*
methylpolysiloxane (CP-Sil
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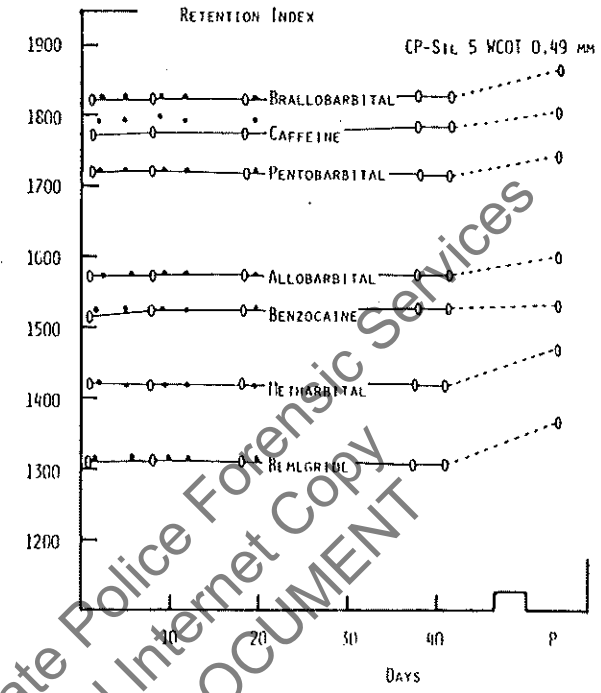

FIG. 5—*Retention index as a function of time of some drugs on polysiloxane-deactivated methylpolysiloxane (CP-Sil 5) capillary column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P). Open circles represent data from a wide-bore glass capillary; closed circles represent data for a narrow-bore fused silica capillary.*

umn produced excellent chromatograms both in this study and in intermittent case work and appeared to have adequate load capacity. Even when overloading did occur, which happened occasionally during case work, there was no residual detrimental effect on column performance. The fused silica column was more flexible and easier to handle than the glass column. Purchased at a cost of about $200, the former has now been in continuous operation for three months and provides excellent value for the money.

Although at first sight there seems to be a fairly good agreement between RI's measured on the CP-Sil 5 capillaries and those reported on the comparable SE-30 or OV-1 packed columns, deviations do occur, possibly because the deactivation material and the dimethylpolysiloxane coating may act as a mixed stationary phase. Of the 120-odd substances studied, 16 gave differences of more than 40 RI units. This has an important impact on the use of RI data compilations. The presently available RI compilations have all been obtained on packed columns, and the above results indicate that it may be unwise to carry out STA on capillary columns and then use a packed column data base for identification.

Yet, the highly increased separation efficiency, reproducibility, stability, and flexibility of the fused silica CP-Sil 5 columns argue that it may be worthwhile to set up a separate capillary column data base. It will be clear, however, that, before starting such an endeavor, additional investigations must be performed on a much larger selection of drugs and over a longer period of time and spread over different institutions to evaluate interlaboratory variations. On the other hand, it should be realized that the manufacture of capillary columns, especially of the newer fused silica types, is undergoing a process of rapid development, so that even better deactivation and coating techniques may become available in the near future. Thus, presently available capillary columns and the capillary materials being developed need to be evaluated further.

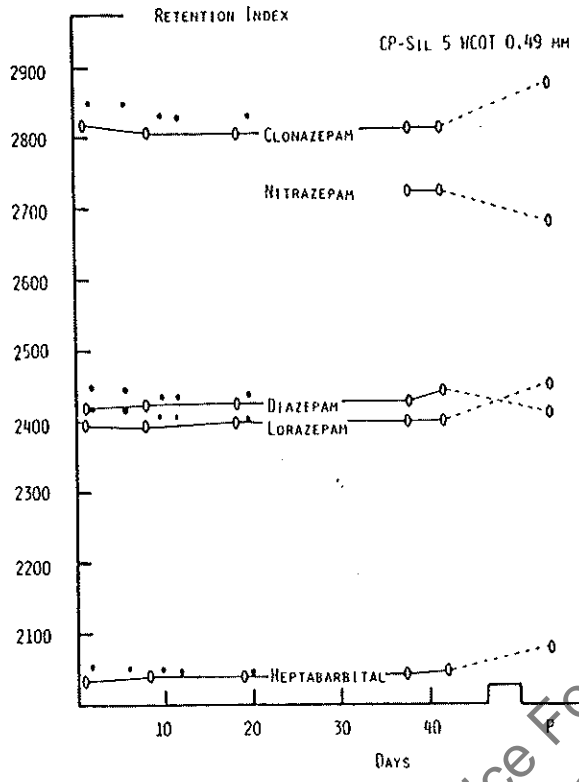

FIG. 6—*Retention index as a function of time of some drugs on polysiloxane-deactivated methylpolysiloxane (CP-Sil 5) capillary column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P): Open circles represent data from a wide-bore glass capillary; closed circles represent data for a narrow-bore fused silica capillary.*

TABLE 1—*Comparison of gas-liquid chromatographic retention indices on methylsilicone-packed columns and capillary columns of compounds of toxicological interest.*

Compound	Retention Index		
	Capillary Column		Packed Column,[a]
	Wide Bore CP-Sil 5	Narrow Bore CP-Sil 5	SE-30 or OV-1
Allobarbital	1577	1577	1605
Amethocaine	2218	...	2230
Amidopyrine	1900	1903	1895
Amitriptyline	2195	...	2205
Amphetamine	1105	...	1105
Amobarbital	1696	1698	1720
Antazoline[b]	2295	...	2350
Aprobarbital	1592	1598	1620
Atropine	2184	...	2190
Barbital	1469	1467	1495
Bemegride[b]	1309	1314	1365
Benzocaine	1526	1528	1535
Brallobarbital	1828	1828	1860
Bromodiphenhydramine	2148	...	2155

TABLE I—*Continued.*

Compound	Retention Index		
	Capillary Column		Packed Column,[a] SE-30 or OV-1
	Wide Bore CP-Sil 5	Narrow Bore CP-Sil 5	
Buphenine[b]	2519	...	2315
Butacaine	2445	...	2460
Butabarbital	1637	...	1655
Butethamate	1742	...	1750
Butobarbital	1642	1646	1660
Caffeine	1780	1796	1810
Carbinoxamine	2067	...	2060
Chlorcyclizine	2232	...	2215
Chlorpromazine	2499	...	2465
Cinchonine	2585	...	2575
Clomipramine	2419	...	2415
Clonazepam[b]	2813	...	2860
Cocaine	2191	...	2195
Codeine	2376	...	2385
Cyclizine	2017	...	2020
Cyclobarbital	1950	1952	1960
Desipramine	2241	...	2250
Diamorphine	2630	...	2615
Diazepam	2426	2439	2410
Dimethoxanate[b]	1990	...	2030
Diphenhydramine	1857	...	1870
Diphenylpyraline	2101	...	2100
Dipipanone	2490	...	2470
Doxepin	2226	...	2210
Ephedrine	1337	...	1355
Ethinamate	1352	1348	1360
Ethoheptazine	1848	...	1860
Ethopropazine	2378	...	2355
Fluphenazine	3035	...	3045
Glutethimide	1820	1818	1830
Guanethidine	0000	...	0000
Heptabarbital	2041	2047	2080
Hexobarbital	1841	1843	1855
Hydroxyzine	2867	...	2850
Hyoscine	2310	...	2300
Imipramine	2222	...	2220
Iproniazid[b]	1531	...	1580
Isocarboxazid	1926	...	1950
Isothipendyl	2268	...	2260
Levallorphan	2348	...	2350
Lignocaine	1869	...	1870
Lorazepam[b]	2399	2411	2450
Malathion	1920	1922	1900
Meclozine	3034	...	3045
Mephenesin	1531	1533	1545
Meprobamate	1762	1752	1790
Mepyramine	2225	...	2220
Methadone	2150	...	2150
Methapyrilene	1974	...	1985
Methaqualone	2142	...	2115
Metharbital[b]	1421	1417	1470
Methoin	1786	...	1795
Methotrimeprazine	2532	...	2515
Methylamphetamine	1163	...	1155
Methylphenobarbital	1875	1880	1905

TABLE 1—*Continued.*

Compound	Retention Index: Capillary Column, Wide Bore CP-Sil 5	Retention Index: Capillary Column, Narrow Bore CP-Sil 5	Retention Index: Packed Column,[a] SE-30 or OV-1
Morphine	2423	...	2435
Naphazoline[b]	1993	...	2065
Nialamide[b]	1673	...	1500
Nicotine	1328	...	1345
Nicotinyl alcohol[b]	1092	...	1150
Nikethamide	1515	1536	1510
Nitrazepam[b]	2724	...	2675
Nortriptyline	2211	...	2215
Noscapine[b]	3154	3170	3100
Orphenadrine	1932	...	1935
Papaverine	2815	...	2805
Parathion	1946	1947	1925
Pentobarbital	1721	1720	1745
Pethidine[b]	1739/2490	...	1765
Phenelzine	1266	...	1340
Phenindamine	2147	...	2160
Pheniramine	1799	...	1810
Phenobarbital	1938	1939	1960
Phensuximide	1618	...	1630
Phenylbutazone	2368	...	2375
Phenylpropanolamine	1291	...	1305
Phenyramidol	1957	...	2010
Phenytoin	2308	...	2330
Piperidolate	2347	...	2325
Piperocaine	1984	...	1975
Pramoxine	2275	...	2290
Primidone[b]	2202	...	2250
Procaine	2007	...	2010
Procyclidine	2177	...	2170
Promazine	2326	...	2305
Promethazine	2276	...	2270
Propiomazine	2736	...	2725
Propranolol	2141	...	2150
Propyphenazone	1917	...	1925
Prothipendyl	2343	...	2330
Protriptyline	2246	...	2230
Pyrrobutamine	2428	...	2430
Quinine	2796	...	2785
Secobarbital	1768	...	1790
Strychnine	3115	...	3115
Thenyldiamine	1992	...	2010
Theophylline[b]	1947	...	2105
Thiopentone	1846	...	1855
Thioridazine[b]	3116	...	3180
Tranylcypromine	1195	...	1210
Trimipramine	2228	...	2205
Tripelennamine	1974	...	1980
Triprolidine	2250	...	2250
Yohimbine[b]	3168	...	3290

[a]Data taken from Ref *12*.

[b]Substance showing a difference of more than 40 RI units between RI measured on capillary column and on packed column.

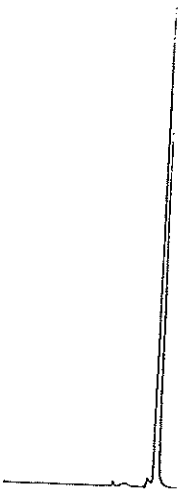

FIG. 7—*Chromatogram of ar ane (CP-Sil 5) fused silica capill chromatographic trace between 14.98; a = acetylcodeine, 20.66. not identified.*

References

[1] Moffat, A. C., Stead, A. H Gas-Liquid Chromatograph *Journal of Chromatography*

[2] Moffat, A. C., "Use of S Drugs," *Journal of Chroma*

[3] Kovats, E., "Gas-Chroma Retentionsindices aliphatisc *Acta*, Vol. 41, 1958, pp. 19

[4] Kazyak, L. and Permisot Chromatography," *Journal*

[5] Finkle, B. S., Cherry, E. Poisons, Drugs and Hum *Chromatographic Science*, V

[6] Caddy, B., Fish, F., and Capillary Columns. II: Peak of Central Nervous System S

[7] Nau, H. and Biemann, K Chromatography-Mass Spec *Analytical Chemistry*, Vol. 4

[8] Peel, H. W. and Perrigo, E icological Analysis," *Canadi*

[9] Post, D., "Gaschromatograp mophorer Basen," *Beitrage*

[10] Berniger, H. and Möller, K von Arzneimitteln," *Archives*

[11] Ramsey, J. D., Lee, T. D., Retention Indices of 296 No Toxicological Analysis," *Jour.*

[12] Moffat, A. C., Finkle, B. S., Retention Indices of Compo

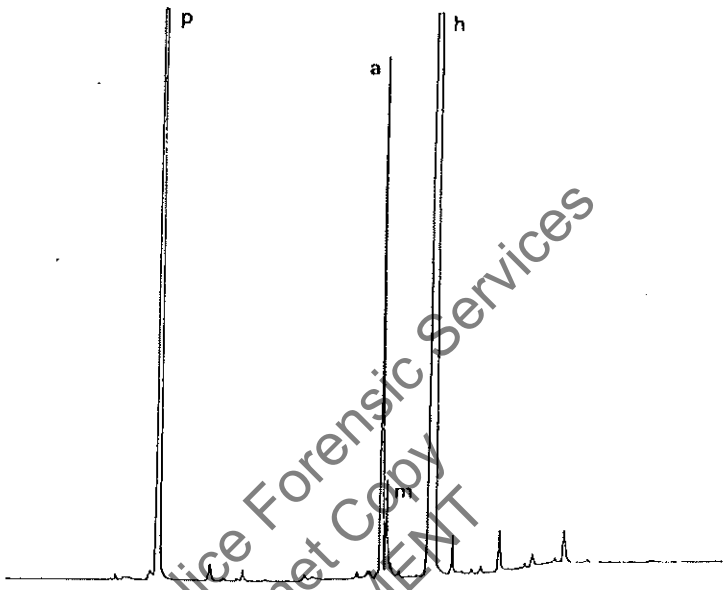

FIG. 7—*Chromatogram of an illicit heroin sample on a polysiloxane-deactivated dimethylpolysiloxane (CP-Sil 5) fused silica capillary column in a temperature-programmed run. Only that portion of the chromatographic trace between 12 and 32 min is presented. Retention time, in minutes:* p = *procaine, 14.98;* a = *acetylcodeine, 20.66;* m = *6-monoacetylmorphine, 20.80;* h = *heroin, 22.01; other peaks not identified.*

References

[1] Moffat, A. C., Stead, A. H., and Smalldon, K. W., "Optimum Use of Paper, Thin-Layer and Gas-Liquid Chromatography for the Identification of Drugs. III. Gas-Liquid Chromatography," *Journal of Chromatography*, Vol. 90, 1974, pp. 19–33.

[2] Moffat, A. C., "Use of SE-30 As a Stationary Phase for the Gas-Liquid Chromatography of Drugs," *Journal of Chromatography*, Vol. 113, 1975, pp. 69–95.

[3] Kovats, E., "Gas-Chromatographische Charakterisierung organischer Verbindungen. Teil I: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone," *Helvetica Chimica Acta*, Vol. 41, 1958, pp. 1915–1932.

[4] Kazyak, L. and Permisohn, R., "Retention Indices for Compound Identification by Gas Chromatography," *Journal of Forensic Sciences*, Vol. 15, 1970, pp. 346–353.

[5] Finkle, B. S., Cherry, E. J., and Taylor, D. S., "A GLC-Based System for the Detection of Poisons, Drugs and Human Metabolites Encountered in Forensic Toxicology," *Journal of Chromatographic Science*, Vol. 9, 1971, pp. 393–419.

[6] Caddy, B., Fish, F., and Scott, D., "Chromatographic Screening for Drugs of Abuse Using Capillary Columns. II: Peak Identification on Support Coated Open Tubular Columns for a Series of Central Nervous System Stimulant Drugs," *Chromatographia*, Vol. 6, 1973, pp. 293–300.

[7] Nau, H. and Biemann, K., "Computer-Assisted Assignment of Retention Indices in Gas Chromatography-Mass Spectrometry and Its Application to Mixtures of Biological Origin," *Analytical Chemistry*, Vol. 46, No. 3, March 1974, pp. 426–434.

[8] Peel, H. W. and Perrigo, B., "A Practical Gas Chromatographic Screening Procedure for Toxicological Analysis," *Canadian Society of Forensic Science Journal*, Vol. 9, 1976, pp. 69–74.

[9] Post, D., "Gaschromatographischer Beitrag zur Analytik pyridin-chromophorer und phenylchromophorer Basen," *Beitrage zur gerichtlichen Medizin*, Vol. 34, 1976, p. 219.

[10] Berniger, H. and Möller, M., "Retentionsindices zur gaschromatographischen Identifizierung von Arzneimitteln," *Archives of Toxicology*, Vol. 37, 1977, pp. 295–305.

[11] Ramsey, J. D., Lee, T. D., Osselton, M. D., and Moffat, A. C., "Gas-Liquid Chromatographic Retention Indices of 296 Non-Drug Substances on SE-30 or OV-1 Likely to Be Encountered in Toxicological Analysis," *Journal of Chromatography*, Vol. 184, 1980, pp. 185–206.

[12] Moffat, A. C., Finkle, B. S., Müller, R. K., and de Zeeuw, R. A., "Gas-Liquid Chromatographic Retention Indices of Compounds on SE-30 or OV-1 Likely to Be Encountered in Toxicological

Analysis," *TIAFT Bulletin Special Issue: GC Retention Indices*, The International Association of Forensic Toxicologists, Aldermaston, Berkshire, England, 1981, in press.

[13] Freeman, R. R., Ed., *High Resolution Gas Chromatography*, Publication 5950-3562, Hewlett-Packard, Avondale, PA, 1979.

[14] Jennings, W., "Evolution and Application of the Fused Silica Column," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 3, No. 12, Dec. 1980, pp. 601-608.

[15] Neu, H. J. and Heeg, F. J., "Surface Modification of Soft-Glass Capillaries for Gas Chromatography by Treatment with Water Vapour," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 3, No. 11, Nov. 1980, pp. 537-544.

[16] Dandeneau, R. and Zerenner, E. H., "An Investigation of Glasses for Capillary Chromatography," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 2, No. 6, June 1979, pp. 351-356.

[17] Houtermans, W. J. M. and Boodt, C. P., "Increased Thermostability of Non-Polar Wall-Coated Open Tubular Columns," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 2, No. 5, May 1979, pp. 249-250.

[18] Aue, W. A., Hastings, C. R., and Kapila, S., "On the Unexpected Behaviour of a Common Gas Chromatographic Phase," *Journal of Chromatography*, Vol. 77, 1973, pp. 299-307.

[19] Cronin, D. A., "The Proportion of Stable Glass Capillary Columns Coated with Carbowax 20 M," *Journal of Chromatography*, Vol. 97, 1974, pp. 263-266.

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Evaluation of I
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Drug Deaths

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A PRACTICAL GAS CHROMATOGRAPHIC SCREENING PROCEDURE FOR TOXICOLOGICAL ANALYSIS[1]

H. W. PEEL[2] and B. PERRIGO[3]

ABSTRACT

A gas chromatographic screening procedure for toxicological analysis is described, which uses a precise temperature program and applies a Kovats'-type of retention index in order to ensure reproducibility of data. Data is presented for 78 compounds. Effects of concentration and flow rate changes are discussed.

RÉSUMÉ

Une méthode générale d'analyse toxicologique par chromatographie gazeuse est ici décrite. Elle consiste en une programmation thermique précise et d'un index de retention du genre KOVATE afin d'assurer la reproductibilité des données. L'effet des changements de concentration et des taux de débit sont discutés et des données sur 78 composés sont présentées.

This study was undertaken in order to develop an effective screening procedure of samples of blood, urine and tissue for a moderate sized toxicology laboratory. Once the presence of a particular compound is indicated, the forensic toxicologist can then carry out further qualitative analysis and quantitation. There has been a number of excellent references on gas chromatography screening procedures reported which include a single column approach(1), a multi column approach(2), and a temperature programmed method(3). In developing a system which could offer a reproducible screening program with a wide range, three parameters were considered. They were: a) column characteristics, b) the application of retention indices to monitor column life and inter-batch column material, and c) a temperature programmed system to enlarge the scope of analysis.

The report by Moffat et al which studied the discriminating power of eight stationary phases for 62 basic drugs indicated that the SE-30 column gave the most uniform frequency distribution pattern of elution(4). The refinements of using retention indices which were introduced to gas chromatography by Kovats(5), were applied to toxicological problems by Kazyack and Permisohn(1) using isothermal conditions. An excellent review, recently published by Moffatt(6), shows the retention indices of 480 compounds on isothermal SE-30 columns.

The method and data presented here is used in conjunction with thin layer chromatographic procedures to screen extracts for the presence of drugs and poisons.

APPARATUS AND MATERIAL

1 **Gas Chromatograph.** Hewlett Packard, Model 5711A, dual column, FID, temperature programmer, equipped with 3% SE-30 (Ultraphase) on Chromosorb W 80/100 (AWDMS) in a glass column 6 feet and 2mm I.D. The carrier gas of nitrogen which had a flow rate of about 32 mls/min at 130° was carefully regulated so that the C-20 hydrocarbon standard eluted from the programmed run at a constant time of 12.7 (± 0.1 minutes). The design of the glass lined injection port was such that the column head is influenced by the changing oven temperature. The injection port temperature was 150°, the detector temperature was 300°. The total temperature

1 This paper was presented at the annual meeting of the Canadian Society of Forensic Science, October 15 in Toronto, Ontario.
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program was: initial — 130° for 2 minutes; program rate at 8°/min to 290°; final — 290° for 8 minutes. An attenuation setting of 640 or 1280 generally gave a half scale deflection for the standards.

2 **Hydrocarbon Calibration Standards.** Aliphatic straight chain hydrocarbons (C11 to C31) were prepared in ethanol in a concentration of 200 mg%. Various mixtures of 5 or more hydrocarbons were prepared in similar concentrations, so that data could be gathered with a single injection. A mixture containing the hydrocarbons C11 to C24 was used for monitoring purposes.

3 **Drug Standards.** The drug compounds were prepared as the free drug in a concentration of 200 mg% in ethanol.

METHOD

Preparation of the Calibration Graph

One microlitre (μl) volumes of the hydrocarbon calibration standards were analyzed by the temperature program using C20 (n-eicosane) as an internal standard. This was accomplished by drawing a 1 microlitre volume of the C20 standard solution, wiping the syringe and taking a second microlitre volume of the other hydrocarbon standards in a mixture of about 5 compounds, for simultaneous injection. The retention times were measured in minutes and the relative retention times to C-20 calculated. A calibration graph was prepared — relative retention time versus carbon number. Carbon number is the number of carbon atoms in the hydrocarbon standard multiplied by 100. Thus, C12 is given a carbon number of 1200. (See figure 1).

Determination of Carbon Numbers for Drugs

One μl volumes of the drug standards were similarly injected with the C-20 internal standard. The retention times were observed and the relative retention times calculated. The carbon number for the drug was determined using the calibration graph. Data for acid and neutral compounds, and for basic and neutral compounds, was gathered from two separate SE-30 columns (one for acid/neutral; one for basic/neutral).

Application to Casework Material

A 2 μl volume of extract (of base/neutral or acid/neutral) is injected with the C-20 internal standard. The retention time and relative retention time is recorded and compared with appropriate calibration graph. (See Figures 1 and 2). Extraction procedures are described in Figure 3. The extraction for basic compounds using butyl chloride is adapted from the procedure of Foerster and Mason(7).

RESULTS

Figure 1 describes the calibration graph obtained for a number of basic and neutral compounds. Figure 2 describes the calibration graph obtained for a number of acid and neutral compounds. Table I describes the chromatographic data for basic and neutral compounds. Table II describes the chromatographic data for acidic and neutral compounds.

DISCUSSION

The temperature program described above shows that at least 50 drugs can be resolved in 30 minutes. The program contains a 2 minute hold at 130° to facilitate more volatile drugs and an 8 minute hold after 290°, to insure elution of the heavier background compounds. Extracts of basic type compounds are run on a separate column from that for acidic type compounds in order to prevent the formation of salts. Neutral compounds are run on either column. Since the same program is used for either the 'acid' or 'base' column, the carbon numbers are essentially applicable to each column. It was found useful to make a single injection of a hydrocarbon standard mixture at least once weekly in order to monitor the program.

The data described in Figures 1 and 2 and Tables I and II are the result of at least 3 determinations on at least 2 different columns. The standard error of the carbon numbers is between 3 and 10, depending on the portion of the program from where the drug elutes. With reference to Figures 1 and 2, the standard error of the carbon number is about 5 in the linear portion of the graph. In the end portions of the graph, the error is generally 8-10. For example, the carbon number for lidocaine is 1865 ±5 (linear

portion); the carbon number for strychnine is 3120 ±10 (non-linear portion). Most of the compounds elute in the linear portion from 1600 to 2400.

A brief study was made to evaluate the effects of gas flow rate on retention times. The elution time of C-20, which was 12.7 minutes, was used as the reference. Variations of ±20 seconds did not change the retention times of the hydrocarbon standards to a significant degree. The average error of the retention time values, as described in Table III.

A change in flow which causes an elution time of 12.39 minutes for C-20, results in a standard error of 0.007 for retention which means a change of 5-10 in the carbon number of 2000. This indicates that only minor errors should occur to carbon numbers if a moderate change in flow rate occurs.

In order to evaluate any changes in retention values in the temperature program which may occur due to concentration effects, a brief study was carried out to evaluate the significance of these effects. The data in Tables I and II was gathered using about 2 μg of drug per injection. The results in Table IV describe changes in carbon number values due to concentration effects of compounds eluting at different portions of the program. Thus, the therapautic, toxic and lethal concentrations of many drugs would be detected using this method.

The above method describes an effective screening procedure for drugs and poisons, providing a precise temperature program facility is available. Although quantitation is possible using the temperature program, the data reported can be used to determine a suitable isothermal temperature for quantitative analysis.

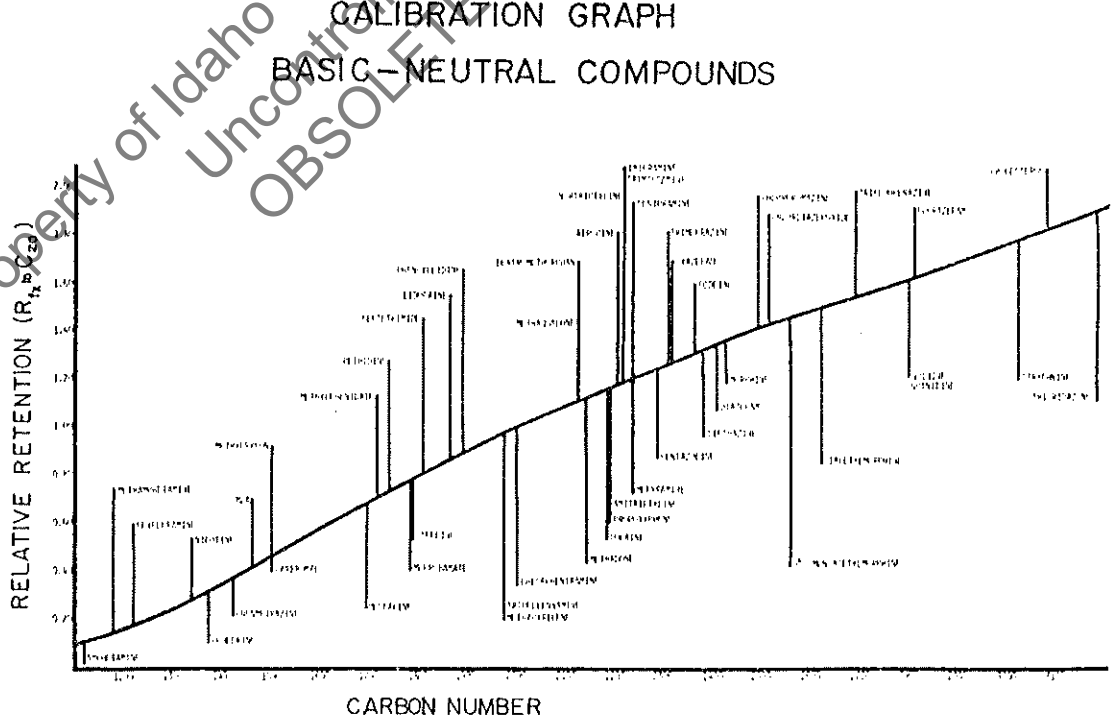

FIGURE 1

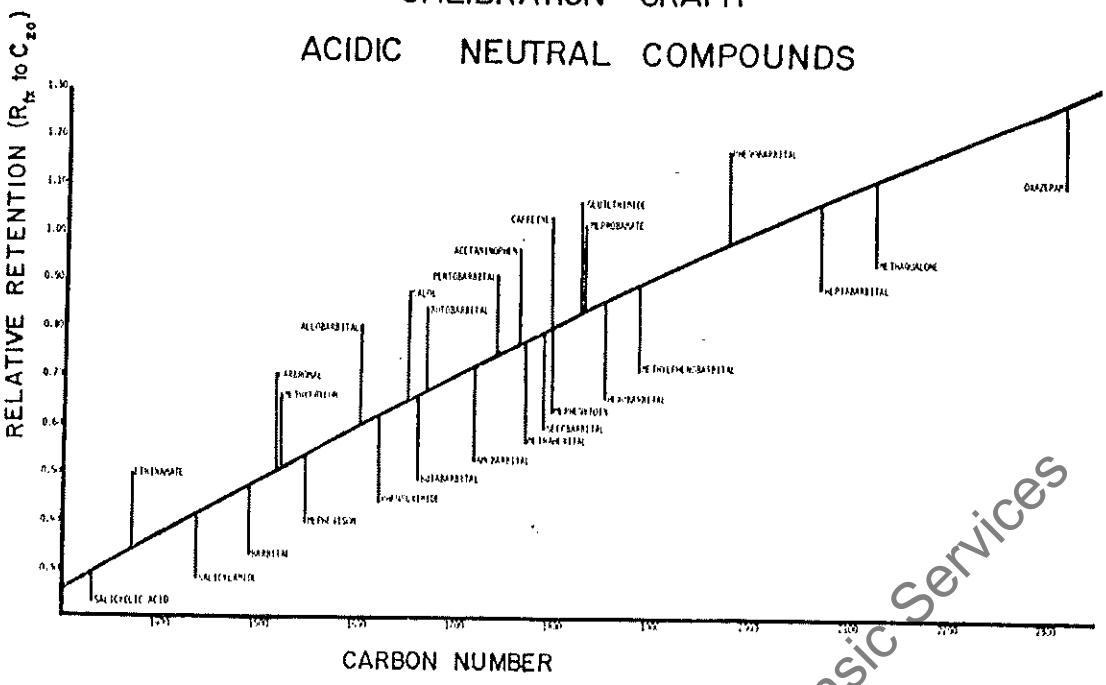

FIGURE 2

FIGURE 3

EXTRACTION PROCEDURES

BASIC/NEUTRAL COMPOUNDS

Blood, urine (5ml) + NH_4OH (0.1ml)

n-butylchloride (2x5ml)

aqueous

pH to 8.5

$CHCl_3$/isopropanol (4:1), (60µl)

AMPHOTERIC

organic

0.2N H_2SO_4 (5ml)

organic

filter, evaporate

reconstitute, ethanol (100µl)

NEUTRAL

aqueous

aerate

NH_4OH (0.8ml)

$CHCl_3$ (75µl)

BASIC

FIGURE 3

ACIDIC/NEUTRAL COMPOUNDS

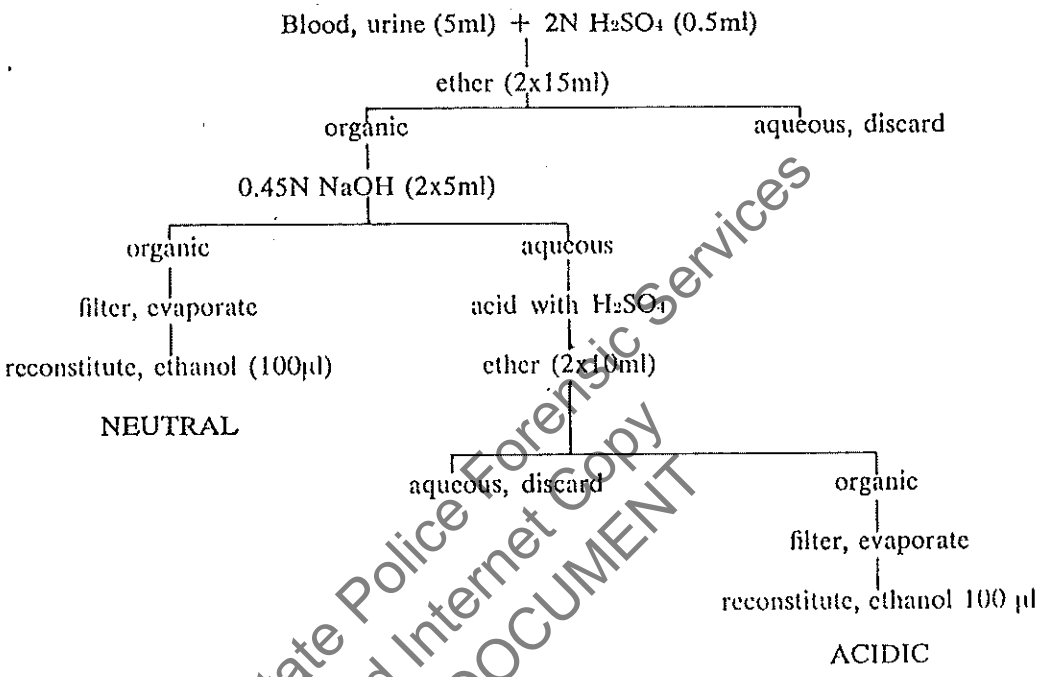

TABLE I

CHROMATOGRAPHIC DATA FOR BASIC AND NEUTRAL COMPOUNDS

DRUG	Carbon Number	DRUG	Carbon Number
AMITRIPTYLINE	2190	MESCALINE	1695
AMPHETAMINE	1118	METHADONE	2140
ATROPINE	2202	METHAPYRILENE	1975
CAFFEINE	1790	METHAQUALONE	2123
CARBROMAL	1501	METHAMPHETAMINE	1175
CHLORDIAZEPOXIDE	2510	METHYLPHENIDATE	1715
CHLORPROMAZINE	2490	METHYPRYLON	1500
CHOLESTEROL	3080	MORPHINE	2433
CHLORPHENIRAMINE	2000.	NICOTINE	1335
COCAINE	2187	NORTRIPTYLINE	2215
CODEINE	2360	OXAZEPAM	2318
DESIPRAMINE	2236	O^{6}MONOACETYLMORPHINE	2552
DEXTROMETHORPHAN	2125	PENTAZOCINE	2286
DIACETYLMORPHINE	2620	PETHIDINE	1740
DIAZEPAM	2405	PHENCYCLIDINE	1890
DIETHAZINE	2380	PHENMETRAZINE	1420
EPHEDRINE	1370	PROPOXYPHENE	2190
FENFLURAMINE	1220	QUINIDINE	2794
FLURAZEPAM	2808	QUININE	2797
GLUTETHIMIDE	1810	STRYCHNINE	3120
IMIPRAMINE	2215	THIORIDAZINE	3180
LIDOCAINE	1865	TRIFLUOPERAZINE	2685
MDA	1460	TRIMEPRAZINE	2305
MEPROBAMATE	1785	TRIMIPRAMINE	2215
MEPYRAMINE	2235	TRIPELLENNAMINE	1975

TABLE II

CHROMATOGRAPHIC DATA FOR ACIDIC AND NEUTRAL COMPOUNDS

DRUG	Carbon Number	DRUG	Carbon Number
ACETAMINOPHEN	1760	METHAHEXITAL	1770
ALLOBARBITAL	1604	METHYLPHENOBARBITAL	1885
AMOBARBITAL	1718	METHYLSALICYLATE	1180
BARBITAL	1490	METHYPRYLON	1520
BUTABARBITAL	1660	NORDIAZEPAM	2480
BUTOBARBITAL	1667	PENTOBARBITAL	1740
CHLORPROPAMIDE	1830	PHENOBARBITAL	1975
ETHINAMATE	1370	PHENSUXIMIDE	1620
ETHOSUXIMIDE	1220	PHENYLBUTAZONE	2370
HEPTABARBITAL	2067	PHENYTOIN	2460
HEXOBARBITAL	1850	SALICYLAMIDE	1435
MEPHENISON	1545	SALICYLIC ACID	1330
MEPHENYTOIN	1796	SALOL	1650
MEPROBAMATE	1830	SECOBARBITAL	1788

TABLE III

EVALUATION OF FLOW RATE CHANGE

C-20 Elution Time (Min)	Average Error (C11-C24)*
12.39	0.007
12.47	0.004
12.66	0.001
12.70	—
12.78	0.004
13.01	0.009

* *(all injection volumes were 2μl)*

TABLE IV

EFFECT OF CONCENTRATION

Drug	Amount Injected (μg)*	Carbon Number
NICOTINE	0.4	1329
	0.4	1329
	2	1327
	2	1327
	10	1325
	10	1326
AMOBARBITAL	0.1	1710
	0.1	1697
	2	1712
	10	1713
	15	1721
	15	1711
MORPHINE	0.2	2425
	0.2	2423
	2.5	2417
	2.5	2425
	10	2433
	10	2432

* *(all injection volumes were 2μl)*

REFERENCES

(1) Kazyack, L. and Permisohn, R., J. Forensic Sci., 15, 346 (1970).
(2) Finkle, B. S., Cherry, E. J. and Taylor, D. M., J. Chrom. Sci. 9, 393 (1971).
(3) Nau, H. and Biemann, K., Anal. Chem. 46, 426 (1974).
(4) Moffat, A. C., Stead, A. H. and Smalldon, K. W., J. Chrom. 90, 19 (1974).
(5) Kovats, E., Helv. Chim. Acta. 41, 1915 (1958).
(6) Moffat, A. C., J. Chrom. Sci. 113, 69 (1975).
(7) Foerster, E. H. and Mason, M. F., J. Forensic Sci., 19, 155 (1974).

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Applicability of Capillary Gas Chromatography to Substance Identification in Toxicology by Means of Retention Indices

REFERENCE: Schepers, P., Wijsbeek, J., Franke, J. P., and de Zeeuw, R. A., **"Applicability of Capillary Gas Chromatography to Substance Identification in Toxicology by Means of Retention Indices,"** *Journal of Forensic Sciences*, JFSCA, Vol. 27, No. 1, Jan. 1982, pp. 49–60.

ABSTRACT: Three capillary columns, set up in a routine screening system, were tested in temperature-programmed runs. A narrow-bore fused silica capillary, Carbowax-deactivated and with a methylsilicone liquid phase, was found to be unstable at higher temperatures, giving irreproducible results and retention indices that varied considerably from those obtained on packed columns. The two other columns, a wide-bore glass capillary and a narrow-bore fused silica capillary, were polysiloxane-deactivated and had a dimethylpolysiloxane liquid phase. Although both showed good stability, reproducibility, and load capacity, retention indices for various drugs still showed discrepancies as compared to corresponding values on packed columns.

KEYWORDS: toxicology, chromatographic analysis, drug identification, systematic drug screening, capillary gas chromatography, retention indices

Gas-liquid chromatography (GLC) has proven to be an indispensable tool in screening for the presence of drugs in systematic toxicological analysis (*STA*). Owing to the work of Moffat and co-workers [*1,2*], it is now generally accepted that dimethylsilicone stationary phases like SE-30 and OV-1 provide optimum discriminating power. In addition, it has been shown that measurement of the retention indices [*3*] is the technique of choice for substance characterization as well as for the compilation of gas chromatographic data in a data bank and the exchange of those data between different laboratories [*2,4–11*]. The retention index (RI) of a substance on a given stationary phase can be considered a physical parameter of reasonable constancy, provided the method is adequately standardized. The interlaboratory standard deviation of measurement is usually between 15 and 20 RI units [*2,10,12*]. Although RI's are temperature-dependent [*10*], it has been shown that those obtained in a temperature-programmed run are usually in good agreement with those determined under isothermal conditions [*7,8*].

So far, almost all RI data for toxicological analysis have been obtained on conventional, packed, glass columns, with an inner diameter of 2 to 5 mm and with the stationary phase coated on relatively inert supports such as Chromosorb. Although open capillary columns were introduced in 1957, their use in toxicology has remained rather limited, probably

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because of their high cost, fragility, and limited load capacity. However, recent years have been a period of innovation with regard to column technology, resulting in a new generation of glass capillary columns and the so-called fused silica and fused quartz columns, which combine high separation power with good column stability, flexibility, load capacity, and so on [*13,14*]. These newer capillary columns are made from high purity materials that are relatively inert to susceptible solute molecules and exhibit a smooth surface. Although the content of total metal oxides is less than 1 ppm, to obtain optimum results deactivation of the wall surface is still necessary, the most common procedures being treatment with polyethylene glycol (Carbowax) or silylation. A second advantage of deactivation procedures is that they improve the wettability of the wall surface for the stationary phase. Although various deactivation procedures have been described [*14,15*], it should be realized that their performance requires a considerable amount of experience. For that reason, most toxicological laboratories would have to buy their capillary columns from commercial sources.

The present study was undertaken to evaluate the applicability of some of the new capillary columns for toxicological screening purposes. We chose to work with temperature-programmed runs to diminish analysis time, and special attention was paid to column stability, day-to-day reproducibility, load capacity, and the degree of agreement between RI's determined on capillary and packed columns with similar stationary phases.

Materials and Methods

The drugs used in this investigation were obtained from commercial suppliers and were used as received. All were dissolved in methanol to give solutions of approximately 0.5 mg/mL, of which 1-μL aliquots were injected. Straight chain alkanes (C_{11} to C_{32}) were used as references for the calculation of retention indices [*3*], with each dissolved in hexane methanol (99:1) to give a solution of about 0.5 mg/mL. Aliquots of 1 μL were injected into an HP 5880 gas chromatograph (Hewlett-Packard) with a splitless capillary injection system. Injections were performed with a HP 7671 A automatic injector. The columns and their operating conditions were as follows:

1. A Carbowax® 20 M-deactivated fused silica narrow-bore capillary column coated with methylsilicone fluid [*16*] was obtained from Hewlett-Packard. The column was 12 m in length and had an internal diameter of 0.20 mm, a film thickness of 0.12 μm, a coating efficiency of 72%, 4200 theoretical plates per meter (C_{15}), and a capacity ratio of 6.1. The maximum operating temperature was given as 280°C. During the first 20 days of this study, the temperature program of the oven was 2 min at 120°C, 8°C/min to 260°C, and then 8 min at 260°C; the injector and detector temperatures were 275°C. During the second part, the program was 2 min at 100°C, 8°C/min to 250°C, and then 15 min at 250°C, with the injector and detector at a temperature of 250°C.

2. The polysiloxane-deactivated glass, wide-bore capillary column, which was coated with CP-Sil 5, a dimethylpolysiloxane phase prepared from SE-30 [*17*], was obtained from Chrompack (Middelburg, The Netherlands). The column was 25 m in length and had an inner diameter of 0.49 mm, a film thickness of 1.14 μm, a coating efficiency of 90%, 2060 theoretical plates per meter (C_{14}), and a capacity ratio of 8.6. The upper temperature limit for isothermal use is given as 325°C and 350°C for temperature programming. With the injector and detector temperatures at 275°C, the temperature program for the oven was 2 min at 100°C, 8°C/min to 275°C, and then 15 min at 275°C.

3. The polysiloxane-deactivated fused silica narrow-bore capillary column coated with CP-Sil 5, a dimethylpolysiloxane phase prepared from SE-30 [*17*], was also obtained from Chrompack. The column was 12 m in length and had an inner diameter of 0.22 mm, a film thickness of 0.45 μm, a coating efficiency of 95%, 5160 theoretical plates per meter, and a capacity ratio of 7.0. The upper temperature limits were as under Condition 2. With the injector and detector temperatures at 300°C, the temperature program for the oven was 2 min

at 120°C, 8°C/min to 300°C, and then 12 min at 300°C. Helium was used throughout as carrier gas and the precolumn pressure was set such that the C_{32} alkane reference had a retention time of not more than 35 min. The temperature programs were chosen so that a nearly straight line was obtained when the carbon numbers of the reference alkanes were plotted versus their retention times. Flame ionization detectors were used.

The capillary data given in the figures represent the results of single determinations; those in the table represent the means of at least three determinations, with the individual observations differing by not more than ±5 RI-units. The P column data in the figures were obtained on packed SE-30 or OV-1 columns and were taken from Ref *12*.

Results

The first column tested was a fused silica wall-coated open tubular column with an internal diameter of 0.20 mm [*16*]. The stationary phase is described by the manufacturer as a methylsilicone fluid comparable to SE-30 and OV-1. Deactivation is achieved by thermally degrading Carbowax 20 M and feeding the pyrolysis products through the columns. The column is then extracted with solvents, but unextractable material remains on the column, resulting in substantial deactivation and increased wettability for the stationary phase [*18,19*]. Methylsilicone columns treated in this way are claimed to be thermally stable up to 280°C.

When first testing these columns during routine day-to-day operation for 24 h per day, seven days a week, it soon became apparent that the retention times of the reference alkanes were not constant (see Fig. 1A). The increase in retention time suggested that the column was becoming less polar with time of operation. This was presumed to be the result of the temperatures of the injection port and the detector being too high (both at 275°C), resulting in bleed-off of the Carbowax deactivation material at both ends of the columns. After 20 days of operation we then shortened each of the two ends 20 cm and lowered the injector and

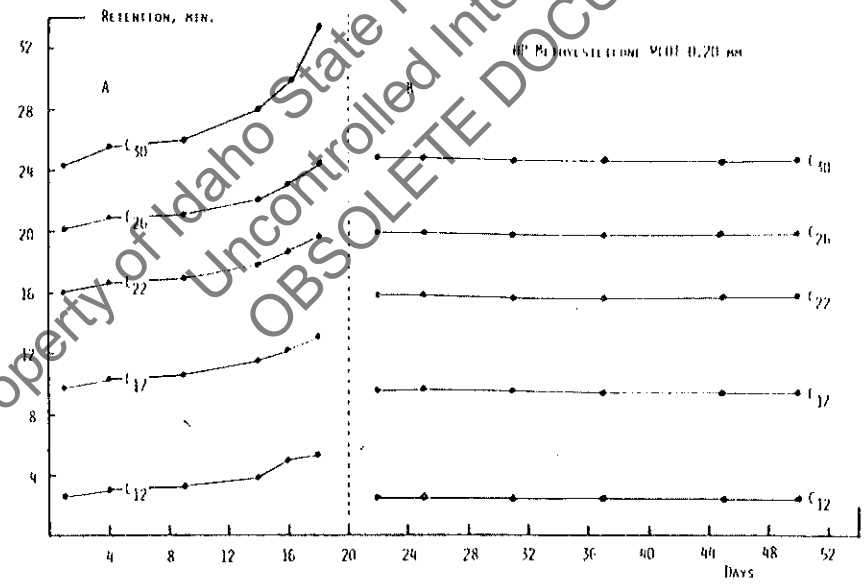

FIG. 1—*Retention behavior of reference alkanes on a Carbowax-deactivated methylsilicone fused silica column in temperature-programmed runs:* (A) *injector and detector temperatures 275°C, maximum oven temperature 260°C;* (B) *after removing 20 cm from both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

detector temperatures to 250°C. The maximum temperature of the oven was also reduced to 250°C. As shown in Fig. 1B, after these changes had been made the alkane retention times remained constant for the rest of the testing procedure.

Figures 2 and 3 show the time versus retention behavior of a selection of drugs, expressed in terms of RI. Different tendencies can be observed. During the first 20 days the 5,5-disubstituted barbiturates showed declining RI's, which seemed to be less pronounced with the *N*-methylated derivatives hexobarbital and metharbital. Other substances, such as caffeine, benzocaine, bromisoval, bemegride, and acetylsalicylic acid, yielded fairly constant RI's, whereas some benzodiazepines with higher RI's showed some variation. Even after we lowered the injector and detector temperatures, RI's decreased for most substances, with some of the barbiturates and clonazepam giving somewhat more pronounced decreases.

Comparison of RI's measured on capillary columns with those obtained on normal packed columns (P in Figs. 2 and 3) clearly indicate marked differences. The 5,5-disubstituted barbiturates all have lower RI's on packed columns; the 1,5,5-trisubstituted barbiturates have quite comparable values, as do caffeine, benzocaine, and bemegride; the diazepines show some variations, with bromisoval and acetylsalicylic acid having higher RI's on packed methylsilicone columns than on Carbowax-deactivated ones.

The CP-Sil 5 wide-bore glass column was treated with polysiloxane according to the procedure of Houtermans and Boodt [17]. The upper temperature limit for the deactivation material is claimed to be 350°C. The stationary phase is dimethylpolysiloxane prepared specially from normal SE-30 and is stable to at least 325°C [17]. Its retention characteristics are similar to those of SE-30 or OV-1.

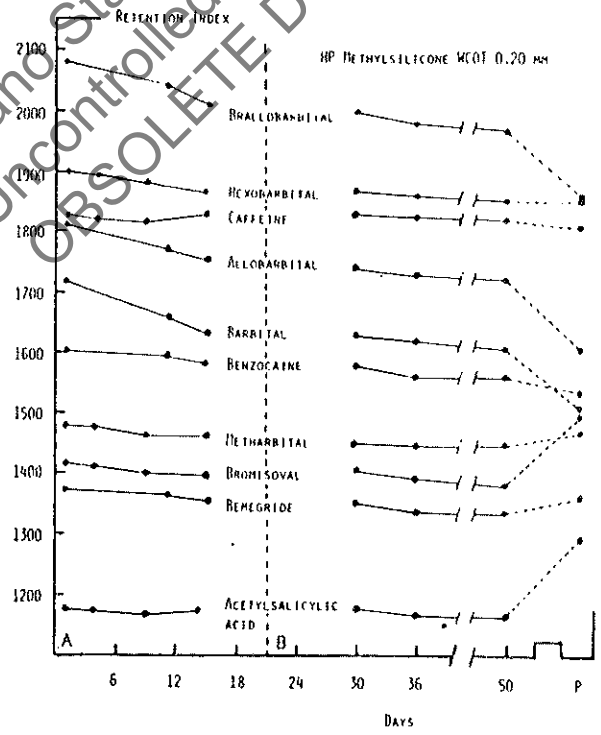

FIG. 2—*Retention index as a function of time of some drugs on a Carbowax-deactivated methylsilicone fused silica column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P): (A) injector and detector temperatures 275°C, maximum oven temperature 260°C; (B) after removing 20 cm at both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

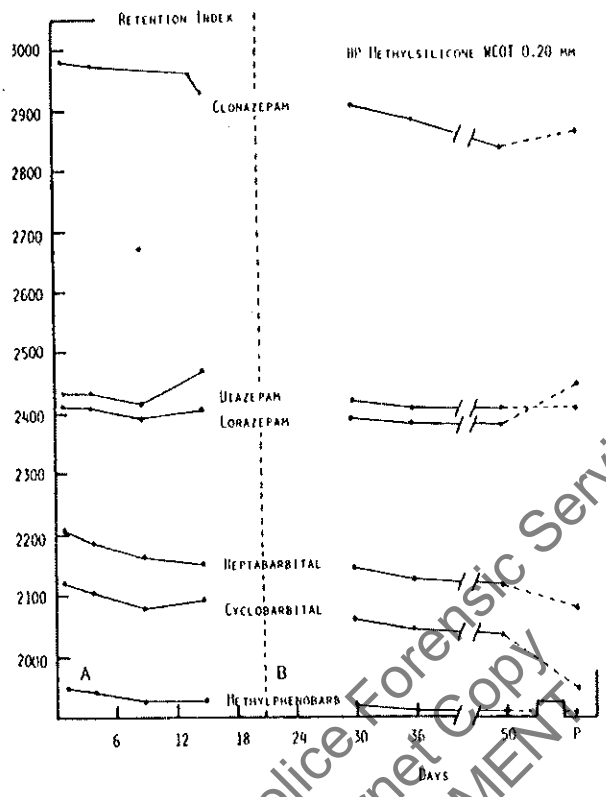

FIG. 3—*Retention index as a function of time of some drugs on a Carbowax-deactivated methylsilicone fused silica column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P); (A) injector and detector temperatures 275°C, maximum oven temperature 260°C; (B) after removing 20 cm from both ends of the column, with injector, detector, and maximum oven temperatures 250°C.*

As can be seen in Fig. 4, retention times of the reference alkanes were constant over the entire test period of 50 days of continuous operation. The time versus RI of a selection of drugs is depicted in Figs. 5 and 6. It can be observed that RI's are relatively constant, the variations staying within acceptable limits of ±5 RI units (open circles) for most compounds, and never exceeding ±10 units. The majority of substances tested showed higher RI's on packed SE-30 or OV-1 columns, with the exception of two benzodiazepines, nitrazepam and diazepam. This trend was also found with some 120 other drugs: the majority gave higher values on packed columns, sometimes amounting to more than 40 RI's, but other substances showed the reverse (see Table 1).

The third column, which could only be tested during the latter part of our investigations, was a fused silica narrow-bore CP-Sil 5 capillary, deactivated with polysiloxane as described above. The stationary phase had a relatively large layer thickness of 0.45 μm to ensure adequate load capacity for biological samples. Over a period of four weeks of continuous operation, the RI's of the reference alkanes showed excellent reproducibility, comparable to that on the wide-bore CP-Sil 5 column. The RI's of the drugs investigated also showed good agreement with those measured on the wide-bore column, as is demonstrated in Figs. 5 and 6 and in Table 1. However, RI's determined on this fused silica capillary showed some discrepancies with those on packed columns similar to those mentioned for the wide-bore glass CP-Sil 5 capillary. Figure 7 depicts an actual chromatogram taken from case work on the narrow-bore fused silica CP-Sil 5 capillary, illustrating the excellent separation efficiency

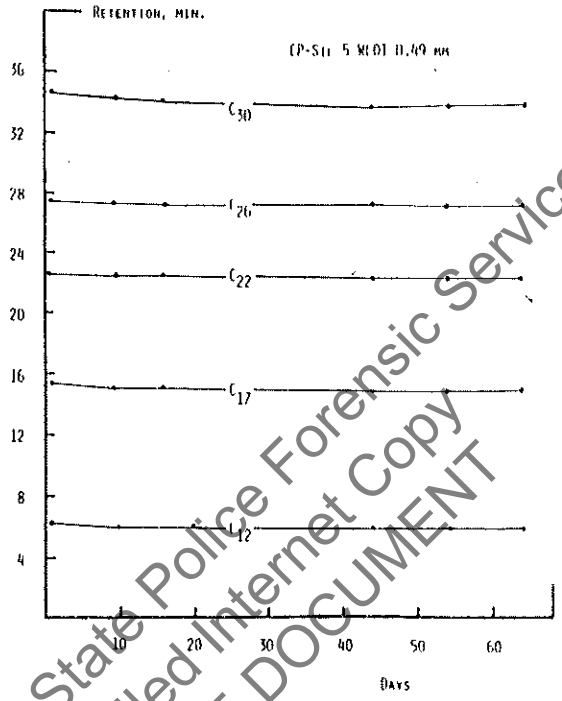

FIG. 4—*Retention behavior of reference alkanes on a polysiloxane-deactivated dimethylpolysiloxane (CP-Sil 5) glass capillary column in temperature-programmed runs.*

(note that acetylcodeine and 6-monoacetylmorphine show almost baseline separation in this programmed run), the narrow peak shape, and the nearly flat baseline.

Discussion

This investigation has clearly demonstrated that the maximum operation temperature of the Carbowax-deactivated column was a rather critical factor to separation efficiency. Although the temperatures were kept within the limitations recommended by the manufacturer, high temperatures at the injector and detector ports apparently caused significant bleedoff of the deactivation material in the adjoining column ends. By lowering the maximum oven temperature and that of the injector and detector to 250°C, the bleedoff could be virtually eliminated. It should be noted, however, that this temperature is too low to obtain full, effective use for STA because of the prolonged analysis time required. Moreover, the RI's obtained on this type of capillary column were found to be quite different from those obtained on comparable packed columns. These discrepancies were more pronounced in certain drug classes yet did not show a clear and predictable pattern. This might have been due to the fact that the Carbowax deactivation material and the methylsilicone liquid phase acted as a mixed stationary phase, interacting differently with certain components than did methylsilicone alone. In view of their thermal instability, which substantially affects both retention behavior and RI, plus the observed discrepancies with RI's measured by packed columns, Carbowax-deactivated methylsilicone capillaries cannot be recommended for general use in STA.

Both polysiloxane-deactivated capillary columns showed excellent stability as well as reproducibility at temperatures up to 300°C and thus were quite effective in STA, permitting rapid screening (within 45 min) for components with RI's up to 3400. The CP-Sil 5 col-

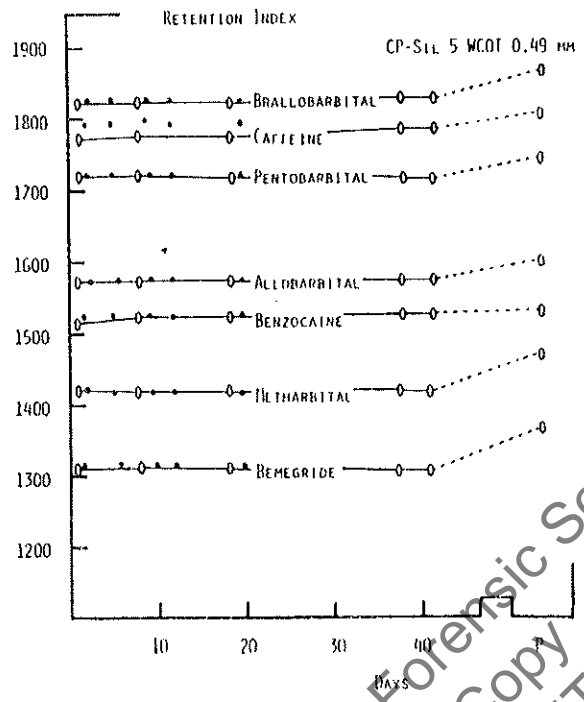

FIG. 5—*Retention index as a function of time of some drugs on polysiloxane-deactivated methylpolysiloxane (CP-Sil 5) capillary column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P): Open circles represent data from a wide-bore glass capillary; closed circles represent data for a narrow-bore fused silica capillary.*

umn produced excellent chromatograms both in this study and in intermittent case work and appeared to have adequate load capacity. Even when overloading did occur, which happened occasionally during case work, there was no residual detrimental effect on column performance. The fused silica column was more flexible and easier to handle than the glass column. Purchased at a cost of about $200, the former has now been in continuous operation for three months and provides excellent value for the money.

Although at first sight there seems to be a fairly good agreement between RI's measured on the CP-Sil 5 capillaries and those reported on the comparable SE-30 or OV-1 packed columns, deviations do occur, possibly because the deactivation material and the dimethylpolysiloxane coating may act as a mixed stationary phase. Of the 120-odd substances studied, 16 gave differences of more than 40 RI units. This has an important impact on the use of RI data compilations. The presently available RI compilations have all been obtained on packed columns, and the above results indicate that it may be unwise to carry out STA on capillary columns and then use a packed column data base for identification.

Yet, the highly increased separation efficiency, reproducibility, stability, and flexibility of the fused silica CP-Sil 5 columns argue that it may be worthwhile to set up a separate capillary column data base. It will be clear, however, that, before starting such an endeavor, additional investigations must be performed on a much larger selection of drugs and over a longer period of time and spread over different institutions to evaluate interlaboratory variations. On the other hand, it should be realized that the manufacture of capillary columns, especially of the newer fused silica types, is undergoing a process of rapid development, so that even better deactivation and coating techniques may become available in the near future. Thus, presently available capillary columns and the capillary materials being developed need to be evaluated further.

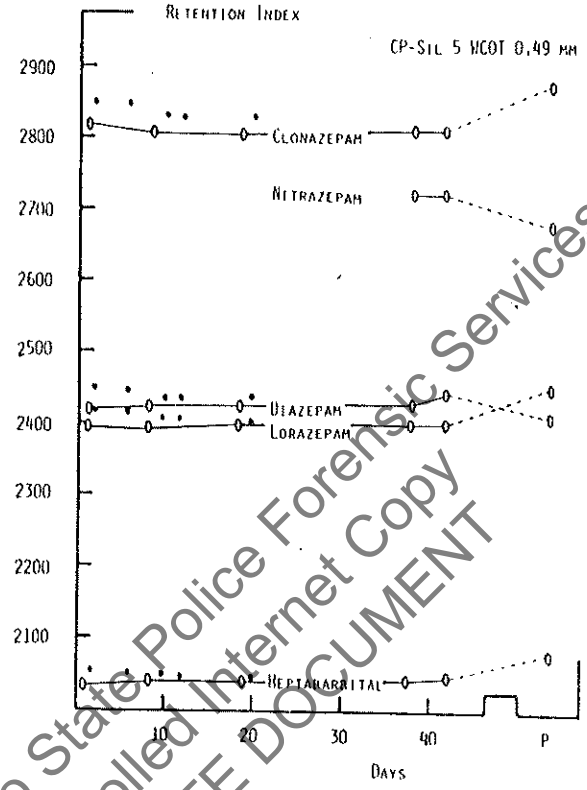

FIG. 6—*Retention index as a function of time of some drugs on polysiloxane-deactivated methylpolysiloxane (CP-Sil 5) capillary column and comparison with corresponding RI obtained on packed SE-30 or OV-1 column (P). Open circles represent data from a wide-bore glass capillary; closed circles represent data for a narrow-bore fused silica capillary.*

TABLE 1—*Comparison of gas-liquid chromatographic retention indices on methylsilicone-packed columns and capillary columns of compounds of toxicological interest.*

Compound	Retention Index		
	Capillary Column		Packed Column,[a] SE-30 or OV-1
	Wide Bore CP-Sil 5	Narrow Bore CP-Sil 5	
Allobarbital	1577	1577	1605
Amethocaine	2218	...	2230
Amidopyrine	1900	1903	1895
Amitriptyline	2195	...	2205
Amphetamine	1105	...	1105
Amobarbital	1696	1698	1720
Antazoline[b]	2295	...	2350
Aprobarbital	1592	1598	1620
Atropine	2184	...	2190
Barbital	1469	1467	1495
Bemegride[b]	1309	1314	1365
Benzocaine	1526	1528	1535
Brallobarbital	1828	1828	1860
Bromodiphenhydramine	2148	...	2155

TABLE 1—*Continued.*

Compound	Retention Index		
	Capillary Column		Packed Column[a]
	Wide Bore CP-Sil 5	Narrow Bore CP-Sil 5	SE-30 or OV-1
Morphine	2423	...	2435
Naphazoline[b]	1993	...	2065
Nialamide[b]	1673	...	1500
Nicotine	1328	...	1345
Nicotinyl alcohol[b]	1092	...	1150
Nikethamide	1515	1536	1510
Nitrazepam[b]	2724	...	2675
Nortriptyline	2210	...	2215
Noscapine[b]	3154	3170	3100
Orphenadrine	1932	...	1935
Papaverine	2815	...	2805
Parathion	1946	1947	1925
Pentobarbital	1721	1720	1745
Pethidine[b]	1739/2490	...	1765
Phenelzine	1266	...	1340
Pheniindamine	2147	...	2160
Pheniramine	1799	...	1810
Phenobarbital	1938	1939	1960
Phensuximide	1618	...	1630
Phenylbutazone	2368	...	2375
Phenylpropanolamine	1291	...	1305
Phenyramidol	1957	...	2010
Phenytoin	2308	...	2330
Piperidolate	2347	...	2325
Piperocaine	1984	...	1975
Pramoxine	2275	...	2290
Primidone[b]	2202	...	2250
Procaine	2007	...	2010
Procyclidine	2177	...	2170
Promazine	2326	...	2305
Promethazine	2276	...	2270
Propiomazine	2736	...	2725
Propranolol	2141	...	2150
Propyphenazone	1917	...	1925
Prothipendyl	2343	...	2330
Protriptyline	2246	...	2230
Pyrrobutamine	2428	...	2430
Quinine	2796	...	2785
Secobarbital	1768	...	1790
Strychnine	3115	...	3115
Thenyldiamine	1992	...	2010
Theophylline[b]	1947	...	2105
Thiopentone	1846	...	1855
Thioridazine[b]	3116	...	3180
Tranylcypromine	1195	...	1210
Trimipramine	2228	...	2205
Tripelennamine	1974	...	1980
Triprolidine	2250	...	2250
Yohimbine[b]	3168	...	3290

[a]Data taken from Ref *12*.
[b]Substance showing a difference of more than 40 RI units between RI measured on capillary column and on packed column.

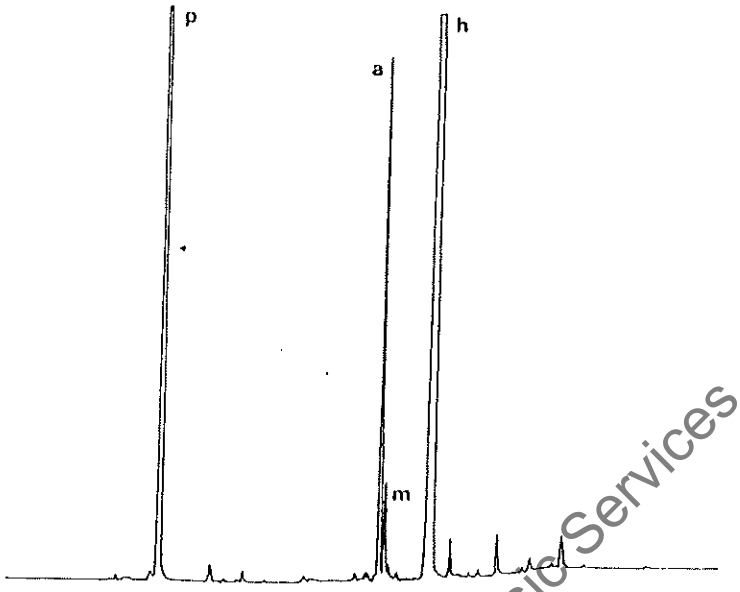

FIG. 7—*Chromatogram of an illicit heroin sample on a polysiloxane-deactivated dimethylpolysiloxane (CP-Sil 5) fused silica capillary column in a temperature-programmed run. Only that portion of the chromatographic trace between 12 and 32 min is presented. Retention time, in minutes:* p = *procaine, 14.98;* a = *acetylcodeine, 20.66;* m = *6-monoacetylmorphine, 20.80;* h = *heroin, 22.01; other peaks not identified.*

References

[1] Moffat, A. C., Stead, A. H., and Smalldon, K. W., "Optimum Use of Paper, Thin-Layer and Gas-Liquid Chromatography for the Identification of Drugs. III. Gas-Liquid Chromatography," *Journal of Chromatography*, Vol. 90, 1974, pp. 19–33.

[2] Moffat, A. C., "Use of SE-30 As a Stationary Phase for the Gas-Liquid Chromatography of Drugs," *Journal of Chromatography*, Vol. 113, 1975, pp. 69–95.

[3] Kovats, E., "Gas-Chromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone," *Helvetica Chimica Acta*, Vol. 41, 1958, pp. 1915–1932.

[4] Kazyak, L. and Permisohn, R., "Retention Indices for Compound Identification by Gas Chromatography," *Journal of Forensic Sciences*, Vol. 15, 1970, pp. 346–353.

[5] Finkle, B. S., Cherry, E. J., and Taylor, D. S., "A GLC-Based System for the Detection of Poisons, Drugs and Human Metabolites Encountered in Forensic Toxicology," *Journal of Chromatographic Science*, Vol. 9, 1971, pp. 393–419.

[6] Caddy, B., Fish, F., and Scott, D., "Chromatographic Screening for Drugs of Abuse Using Capillary Columns. II: Peak Identification on Support Coated Open Tubular Columns for a Series of Central Nervous System Stimulant Drugs," *Chromatographia*, Vol. 6, 1973, pp. 293–300.

[7] Nau, H. and Biemann, K., "Computer-Assisted Assignment of Retention Indices in Gas Chromatography-Mass Spectrometry and Its Application to Mixtures of Biological Origin," *Analytical Chemistry*, Vol. 46, No. 3, March 1974, pp. 426–434.

[8] Peel, H. W. and Perrigo, B., "A Practical Gas Chromatographic Screening Procedure for Toxicological Analysis," *Canadian Society of Forensic Science Journal*, Vol. 9, 1976, pp. 69–74.

[9] Post, D., "Gaschromatographischer Beitrag zur Analytik pyridin-chromophorer und phenylchromophorer Basen," *Beitrage zur gerichtlichen Medizin*, Vol. 34, 1976, p. 219.

[10] Berniger, H. and Möller, M., "Retentionsindices zur gaschromatographischen Identifizierung von Arzneimitteln," *Archives of Toxicology*, Vol. 37, 1977, pp. 295–305.

[11] Ramsey, J. D., Lee, T. D., Osselton, M. D., and Moffat, A. C., "Gas-Liquid Chromatographic Retention Indices of 296 Non-Drug Substances on SE-30 or OV-1 Likely to Be Encountered in Toxicological Analysis," *Journal of Chromatography*, Vol. 184, 1980, pp. 185–206.

[12] Moffat, A. C., Finkle, B. S., Müller, R. K., and de Zeeuw, R. A., "Gas-Liquid Chromatographic Retention Indices of Compounds on SE-30 or OV-1 Likely to Be Encountered in Toxicological

Analysis," *TIAFT Bulletin Special Issue: GC Retention Indices*, The International Association of Forensic Toxicologists, Aldermaston, Berkshire, England, 1981, in press.

[13] Freeman, R. R., Ed., *High Resolution Gas Chromatography*, Publication 5950-3562, Hewlett-Packard, Avondale, PA, 1979.

[14] Jennings, W., "Evolution and Application of the Fused Silica Column," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 3, No. 12, Dec. 1980, pp. 601–608.

[15] Neu, H. J. and Heeg, F. J., "Surface Modification of Soft-Glass Capillaries for Gas Chromatography by Treatment with Water Vapour," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 3, No. 11, Nov. 1980, pp. 537–54d.

[16] Dandeneau, R. and Zerenner, E. H., "An Investigation of Glasses for Capillary Chromatography," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 2, No. 6, June 1979, pp. 351–356.

[17] Houtermans, W. J. M. and Boodt, C. P., "Increased Thermostability of Non-Polar Wall-Coated Open Tubular Columns," *Journal of High Resolution Chromatography and Chromatography Communications*, Vol. 2, No. 5, May 1979, pp. 249–250.

[18] Aue, W. A., Hastings, C. R., and Kapila, S., "On the Unexpected Behaviour of a Common Gas Chromatographic Phase," *Journal of Chromatography*, Vol. 77, 1973, pp. 299–307.

[19] Cronin, D. A., "The Proportion of Stable Glass Capillary Columns Coated with Carbowax 20 M," *Journal of Chromatography*, Vol. 97, 1974, pp. 263–266.

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GUIDELINES FOR TESTING

The analyst who is just being introduced to microcrystalline analysis should run standards along with casework to satisfy himself that any crystals that are formed are similar in nature to the known, and the reagent is working.

Place a small amount (appoximately 1 to 2 mg.) of the unknown compound on a microscope slide. Add the particular reagent to the sample and observe crystal formation at 20 to 25X objective under the microscope. If the reagent must remain under the microscope for any length of time, cover the sample with a coverglass to protect the objective.

If a number of different reagents or samples are used at one time, make an identifying mark on the slides. General procedures covering reagents, samples, and techniques are given in Modern Microcrystal Tests for Drugs.

GUIDELINES FOR TESTING

Analysts who have little or no experience with spot test procedures should follow this procedure during the initial periods of testing.

Ensure yourself that you have sufficient sample for testing as well as other tests that may be required. Place a drop or two of reagent in the well of a spot test plate, than add a small amount (approximately 1 mg.) of the unknown to the well. Compare results, to those known for a specific compound. If you have some idea of the unknown, run standard samples in separate wells and check to see that comparable reactions or color changes are observed using the same reagents.

Colors observed using particular reagents should be noted in the laboratory notes associated with a particular case.

METHOD OF ANALYSIS

When TLC plates are developed for a particular case, some guidelines must be followed. These are outlined below.

Solvents used for TLC development should be made fresh at at the time of use. Thin layer plates must have the drug standard used for comparison, spotted on the plate along with any unknowns. Migration distances should be roughly the same (owing to some edge discrepancies). Spots should be located using UV light prior to visualization, after development. If a visualizing reagent is used, color changes should be noted in laboratory case file, and standards should develop the same as unknowns. Copies of thin layer plates must be kept in case files, along with information gathered in the development and visualization of plates.

Plates should be sprayed with reagents in a properly operating chemical vapor hood. After use be sure to rinse chemicals from sprayers and discard used solvents.

TABLE OF CONTENTS FOR MICROCRYSTAL TESTS

INTRODUCTION

Microcrystal tests are a method to provide the analyst with more specific tests to determine the presence of a particular drug. The purpose of these tests is to deal with small amounts of material and determine chemical composition.

This section deals with the microcrystalline test reagents used within the laboratory system to identify a number of compounds. In the hands of a qualified analyst these tests could very well be used to conclusively identify compounds. However with the limited use of this method here, more specific procedures must be used to conclusively identify unknowns.

Prior to actual testing or laboratory work, the analyst should spend time acquainting himself with the polarizing microscope, the literature, and the following article on optics.

breaks down (or is destroyed) when exposed to bright light. The instant or so of the absence of sight on entering a darkened area from a more brightly lit one, is due to the time required for the visual purple to be built up in the retina.

Vitamin A is one of the main constituents of visual purple. We have all heard that the eating of carrots will improve our eyesight -- and this is based on the fact that carrots are high in vitamin A content, which will work to improve our night vision if we are vitamin A deficient (there are also a number of other sources of vitamin A).

The Optic Nerve

All nerve endings form a part of larger units, to form a neuron or nerve cell. The rods and cones, being nerve endings, are no exception. The rods and cones have very short axons (the attached nerve fiber) which synapse with integrating (longitudinal and bipolar) cells in the mid retina, and they in turn synapse with ganglion cells in the superficial retina. The ganglion cell axons traverse the surface of the retina (accumulating posteriorly) actually form the optic nerve. The optic nerve, acting somewhat like a fiber optic bundle, exits the eye at the rear of the eyeball, and this is the reason that we have a "blind spot" at that point (an image passing through this blind spot will completely disappear from our sight).

The optic nerves (the one from the left and the one from the right eye) converge a short distance behind the eye, with their fibers partially intertwining (see Figure 1-2). They proceed directly back through the center of the brain to the occipital lobes, where the left and right optic nerves again split, forming thousands of smaller nerve bundles called visual radiations and disappear into the visual cortex of the occipital lobes. It is at this point that the light stimulation of our rods and cones is changed into images that can be "seen" by our brain.

Eye Focus and Regulation of Light

By again making a comparison between the eye and a camera: if your film (the rods and cones of the fovea and macula lutea) is bad, your picture will be bad -- or nonexistent. By the same token, if your lens system (the cornea and lens of the eye) will not come into sharp focus at film plane, or if your diaphragm (the iris) will not open or close to the proper f-stop, your picture will be out of focus, or over or under exposed.

Proper vision is dependent upon the lens system of the eye (the cornea and lens combine to make up this system) having the ability to bend incoming light rays in such a way that they come to sharp focus on the fovea. This bending of the light rays, which we call refraction, is brought about by two curved transparent surfaces -- the cornea and lens -- and the curvature and thickness of these determine whether or not we see normally, or are near or farsighted (other sight problems can result from matters that we shall touch upon later).

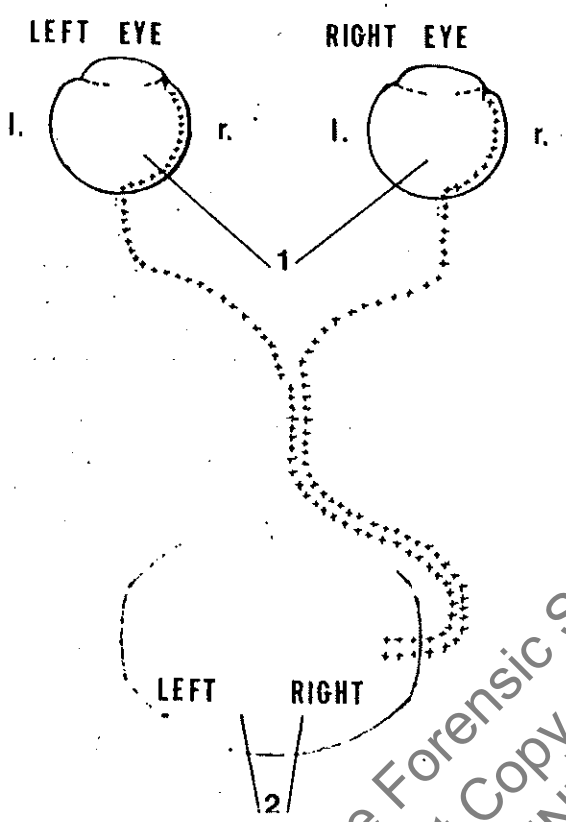


FIGURE 1-2

Shown, is the means of light sensor impulse transmission to the occipital cortex of the brain.

Those light sensors from the right side of either eye (the nasal retina of the left eye and the temporal retina of the right eye) and the path of their corresponding impulses are indicated by a series of plus signs (+).

The light sensors from the left side of either eye (the temporal retina of the left eye and the nasal retina of the right eye) and the path of their impulses are shown by a series of dots (.).

The optic nerve from either eye carries both the left and right impulses, however, the impulses from the left and right retinas are separated enroute to the occipital cortex of the brain, so that the left and right signals arrive at the proper side of the occipital cortex.

The Lens and Cornea

The major portion of light bending is done by the cornea of the eye, which has substantially a fixed curvature and thickness (essentially, a fixed-focus lens). The lens of the eye fine-tunes the focusing -- its thickness and curvature being able to adjust (see Figure 1-3). The focusing of the lens is done almost without conscious awareness, but allows us to focus on either near of distant objects. The lens is made thicker or thinner (which also changes its curvature) by contracting or relaxing the ciliary muscles located about its circumference -- these muscles, needless to say, are quite small in size. Where the eye is to be focused on objects in excess of about 20 feet distant, the ciliary muscles are not called upon for any work at all, but for close work over long periods of time, they may be overworked.

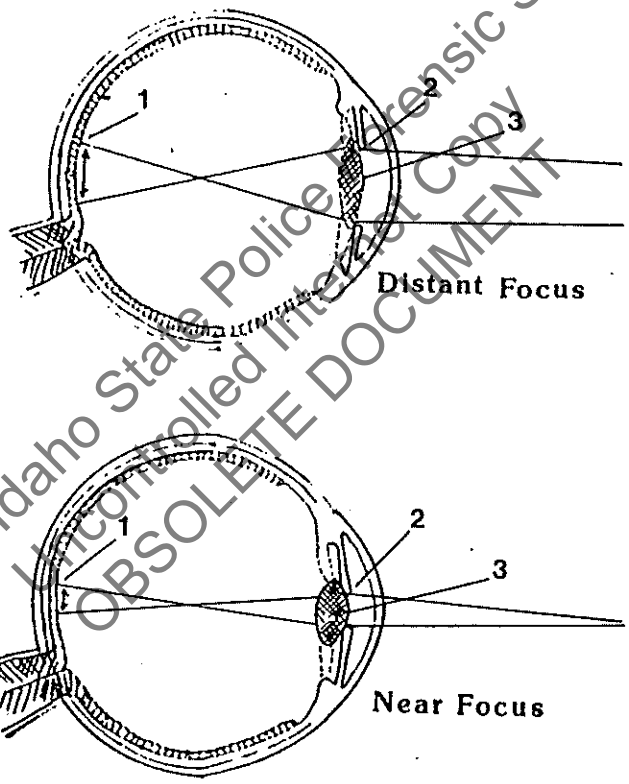


FIGURE 1-3

When focus is on a distant object (in excess of 20 feet distant), the lens is flattened, the iris dilated, and the light projection on the retina covers a greater area.

When focused on close objects (as when reading), the lens becomes more oval in shape, the iris contracted in size, and the resulting area where light is directed on the retina is considerably lessened in size.

Recent advances in eye surgery include a procedure called radial keratotomy, for correction of nearsightedness -- and which can decrease myopia (nearsightedness) and occasionally eliminate the need for eye glasses or contact lenses. The procedure involves the making of some 4 to 16 deep incisions in the cornea of the eye, radially, which weakens it, causing it to become somewhat flattened. The procedure was developed, based on research findings in Japan then the Soviet Union, and is now performed in this country. Lens implant surgery is also of fairly recent origin, and involves the removal of the lens of the eye, which is replaced by a plastic one of fixed focus. Persons suffering from cataracts -- a clouding of the lens of the eye -- now are able to have this type of surgery, instead of the more traditional surgery which removed the lens and then required the use of thick cataract eyeglass lenses, or special contact lenses.

Incidentally, farsightedness is referred to as hyperopia, whereas, nearsightedness is called myopia. 20/20 vision is the normal visual acuity of the eye that can distinguish characters 1/3" in diameter at a distance of 20 feet.

The Pupil and Iris

Effective focus, as well as the ability to adjust to varying light conditions, are also dependent on the diameter of the hole through which light enters the eye -- the pupil. The diameter of this opening is controlled (almost automatically) by the muscles of the iris, with the opening becoming wider in diameter under dim lighting conditions, and constricting (becoming smaller in diameter) in bright light. The muscle which comprises the iris contains pigment which gives us our eye color (blue, brown, hazel, etc.), with the actual pupil having a black appearance. The fully opened pupil, as would be the case in dim light, illuminates more than 15 times more retinal surface than when constricted in bright light. The depth of field -- the distance between the near point and far point of sharp focus -- is greater with a pupil that is restricted in size, as opposed to a dilated pupil. This depth of field phenomena will be dealt with later in this writing, with particular reference to camera lenses.

Eye Protection and Structural Support

There are two outer layers that protect the eye. The outermost layer is quite tough, and is called the sclera. It is what is referred to as the "white of the eye". Beneath the sclera is another layer, called the choroid, which contains the blood vessels that service the sclera and other parts and structures of the eyeball. Both the sclera and the chorois, of course, have openings to allow for the pupil. The cornea is actually a sort of specialized extention of the sclera, and the iris an extension of the choroid.

In order for the eye to retain its shape, as well as to provide a somewhat frictionless functioning of the moving parts, the eye contains two trapped reservoirs of fluid. The fluid contained in these two reservoirs is called the aqueous humor and the vitreous humor. The small space between the cornea and the lens is the anterior chamber, filled with the clear and watery, aqueous humor. The larger, posterior chamber, is filled with the vitreous humor. (See Figure 1-1).

Muscles Controlling Eye Movement

In addition to the muscles within the eye -- which are quite diminutive in size -- that control the opening and constricting of the pupil and the lens shape, there are also a number of somewhat larger muscles that control the vertical and lateral movement of the eyeball, as well as to coordinate and make this movement in unison.

There are six muscles attached to either eyeball, attached at the top, bottom and sides. These muscles work as a team -- to move laterally, vertically, and obliquely. It also might be noted that one of our eyes is the dominant of the two. The dominate eye (master eye) movements are always followed by that of the other.

Also to be considered, would be the eyelids. The eyelids serve as protection for the eye, and are controlled by opening and closing muscles -- all of which are outside the eye proper. The muscles controlling the eyelids function both voluntarily and involuntarily.

Lubrication, Etc.

The tears provide moisture for lubrication, without which the eyelid (or its inner lining, the conjunctiva) would scrape against the eyeball -- which would be uncomfortable, to say the least. The tears also provide a cleansing action -- not merely because of the water which rinses and washes the eye, but because tears also contain a mild germicidal solution called lysozyme, which acts against bacteria and microbes.

Tears are produced by the lacrimal glands, located above the eyeball and just below the eyebrow, on the temple side as opposed to the nose side. Tears are discharged from several ducts and are spread over the eyeball by blinking. The conjunctival sac, located inferiorly and superiorly between the eyeball and eyelid, acts as a collecting pool for the tears. The tears are then drained down a duct into the nasal cavity (this is why one's nose runs when one cries).

Additionally, there is a drainage network, located at the point of connection between the cornea and iris of the eye, and which serves to keep the fluid space filled by the aqueous humor in balance. This area is drained by way of conduits (microscopic in size) called the canals of Schlemm (so help me, I didn't make that term up). If drainage is obstructed, it can cause a build-up of pressure. Such a build-up occurs in glaucoma, which can result in impairment or loss of vision.

Other Common Defects

One of the more common defects which result in the eye not functioning as it should is astigmatism. This is a defect of the lens system, causing the light rays to fail to meet at the normal focal point, and resulting in blurred or imperfect vision, normally due to corneal irregularity.

Most of us have heard of a condition called detached retina. In such a condition, the retina is separated from the underlying tissue, usually due to a fluid build-up behind the retina (this may be blood or other fluid). Treatment for such a condition is dependent upon the cause of the separation -- retinal bleeding due to diabetic retinopathy, for instance, may require the use of a lazer beam to seal the blood vessels

which are hemorrhaging. In cases involving fluid (other than blood) build-up behind the retina, pressure may be increased within the eyeball to push the retina back into its proper place. A detached retina can result in blindness (light and colors may be seen, but no focus is possible). Recently, a technique has been developed by which the retina is actually tacked to the underlying tissue by tacks or pins -- resulting in partial vision (though usually not permitting one to read or see adequately -- one just doesn't fall over things as readily as before such an operation.

CONCLUSION (Part I)

While the foregoing does not completely cover the sum total of information I have concerning the eye, it comes close. The good Lord has seen fit to bless the more fortunate of us with one good wife (or husband, as the case may be), one good dog, and one reasonably useable pair of eyes. It is up to us to nurture and care for these blessings -- for, rarely are we invited back for a "second helping."

No reason was seen for going into such matters as a visual fields examination, or pressure tests which might show indications of glaucoma, nor was it thought necessary to include a coverage of the instruments and/or equipment used in performing such tests.

The majority of those reading this dissertation will probably recall previous exposure to information of a similar nature presented to us in our school years. Most likely, it did not make any lasting impression (it didn't in my case). Now, however, when our livelihood is virtually dependent on our having reasonably good eyesight, it might warrant a bit of study and review.

This first portion (Part I) has been included as a very necessary part of the discussion of the subject of Optics. The language used is essentially the language of the layman, and where I have used terminology that is not quite in character with such an approach, such terms have been defined. The same general approach will be taken in other portions of this writing, for should you need to dredge up any portion of this material at some later date, it will most likely be in connection with the making of an explanation to laymen. By the same token, should the information be needed in a discussion with professionals from any of the fields that may be touched upon, it is hoped that the coverage given here will be such that some benefit will be realized from a perusal of this material.

BASIC OPTICS - PART II (AN OVERALL VIEW)

Here, we shall be discussing those lenses, prisms, mirrors, and the like, which were briefly alluded to in the Introduction. Logic would indicate that we should begin with the more simple and less complicated, and progress to some point where (should we proceed further) we'd be getting in over our ears. As I wish to preserve the illusion that I am a somewhat logical individual, I shall proceed in this manner -- attempting to repress my natural tendency to stray too far afield just to smell the roses.

MAGNIFIERS

Simple magnifiers, or magnifying lenses, may be put to a multitude of uses. Whenever the unaided eye has difficulty in reading fine scales, inspecting small parts, searching an area for small imperfections or looking for trace evidence, the examination or comparison of fingerprints, or things of like nature, magnifiers may be brought into play to provide what we shall refer to as low-power magnification (a higher degree of magnification may be needed at some later point but, at this stage, we will not be making an attempt to count the hairs on a gnat, or have a need to examine cell structure).

The Power of a Magnifier

For one to determine the power, or degree of magnification (expressed in the number of diameters of magnification), of a given lens or magnifier, divide the focal length of the lens into ten inches: P = 10/FL. (Should you be working in the metric system, divide into 254mm -- the metric equivalent of ten inches.)

As you can see, we are dealing solely with height or width increase (not area), and are using diameters of enlargement as the power determining factor. The distance of ten inches is assumed to be the distance between the eye and object being viewed which will afford as close comfortable vision as possible. It follows, therefore, that if ten inches is taken to be normal vision, an object located but one inch from the eye would appear ten times as large, were the eye able to focus on it. The purpose of the magnifier is to make such close vision possible. Since a lens having a focal length of one inch would make an object appear ten times as large, we should designate such a lens as 10X (or a ten power lens).

A lens (or lens system) such as a fingerprint magnifier, the objective or ocular lens of a microscope, and others, is usually identified by its power (diameters of magnification). Camera lenses and projection lenses, on the other hand, are normally designated by their focal length.

Field of View

As the power of a magnifier increases, the lens diameter and field of view decreases. This means that things within the field of view at lower power (lower magnification) -- things near the outer edges of the

field of view -- may no longer be in the field of view at a higher magnification, due to the constricting of the field as magnification increases. At 5X, the field of view is about 1.5 inches in width, while at 10X, the field only covers about half an inch. For this reason, it is usually better to use a relatively low power to scan an area, then increase the magnification to permit more detailed study once the object of our search has been located.

Working Distance

The working distance of a lens, or optical system, is the distance between the lens (objective lens, or lens nearer the object being viewed) and the object being viewed, when the lens is in proper focus. This sounds as if the working distance and the focal length amount to the same thing, which is true under most circumstances, however, there are other factors which may have an effect on this.

Normally, the greater the degree of magnification, the shorter the working distance. With microscopes, should additional magnification be desired, the power of the ocular (eyepiece) can be changed by substituting an ocular of a higher power -- leaving us with about the same working distance between objective lens and the specimen, but also giving us greater magnification. (This added magnification is great, however, it is only an increase in overall image size -- there is no increase in the amount of detail. What we have achieved is empty, or hollow, magnification. If we desire better resolution -- increased detail -- we must do so by increasing the power of the objective lens, with the accompanying shortened working distance.)

Depth of Field

The depth of field is the distance between the near and far points at which a magnifying (or reducing) lens remains in sharp focus. With an increase in magnification comes a corresponding decrease in depth of field. With photographic equipment, the depth of field when focused on distant objects is far greater than when focusing for close-ups. While we are still on the subject of photographic equipment, I might also add that the camera lens system is fitted with an iris diaphragm that makes possible an increase or decrease in the size of the lens opening, commonly referred to as f-stops. A decrease in the size of the lens opening serves two purposes: (1) it decreases the amount of light permitted to pass through the lens (requiring an exposure of longer duration for a given amount of light to be delivered to the film); and, (2) it also increases the depth of field -- earlier models of the Bauch & Lomb bullet comparison microscope were equipped with such an iris diaphragm behind the objective lenses (accomplishing both 1. and 2., above).

The foregoing phenomena will be covered a bit more fully later in this writing, when we discuss photographic applications of the study of optics. The depth of field (depth of focus) phenomenon, as well as all other phenomena we may touch upon, will apply equally to micro and macroscopic viewing, projection and photographic applications -- it's just that certain of these phenomena are most apparent under certain conditions than others.

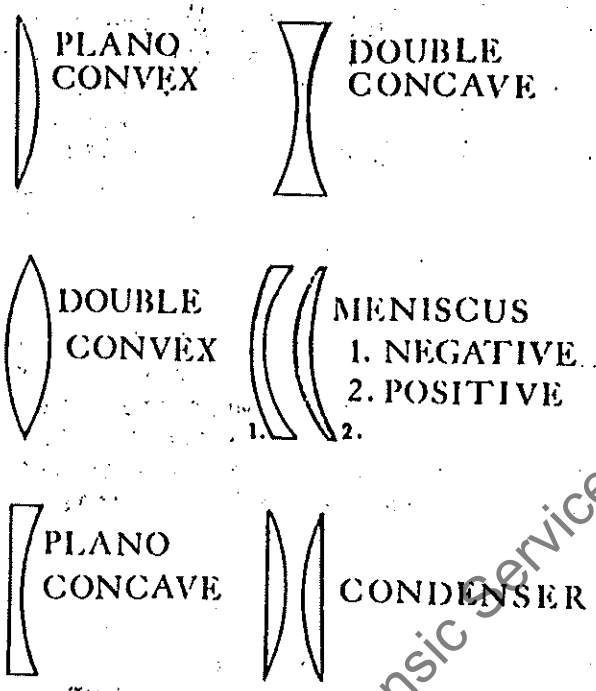


FIGURE 2-1

Single element lenses. Plano convex lens has one outward curving face and one flat face -- acts as a magnifier or condensing lens, and has a positive FL. Double convex lens has two outward curving faces. Curvature may be symmetrical (same degree for each face) or one face may have greater curvature than the other -- a magnifier or condensing lens, having a positive FL. Plano concave lens has one flat and one inward-curving face. Used as a reducing or dispersing lens and has a negative FL. Double concave lens has both faces curving inward. Faces may be symmetrical or one face may have a greater degree of curve than the other. A reducing or dispersing lens, with a negative FL. The meniscus (concave convex lens has one outward and one inward curving surface. If the outward curve is greater it will have a positive FL. If the inward curve is greater, FL will be negative. Condenser lens (condenser lens system) projects light into a concentrated beam, and is used in projectors, spotlights, substage microscope lighting, etc. The combined FL of the lenses divided by the number of lens elements equals the focal length of the lens system. Such lenses are normally set in quite close proximity to each other.

Lenses and Lens Systems

Figure 2-1 illustrates the various lens shapes, and may be of use in identifying basic lens types. The basic (or simple)lens is normally the double convex lens. It is used in hand magnifiers, which we usually refer to as magnifying glasses. Such a lens normally gives considerable distortion, especially at the outer edges.

A double lens system may be composed of two plano convex lenses, usually with flat sides outward (although other lens configurations might be used equally in such a lens system). These lenses are normally separated from each other by some little distance so that the system

will focus properly -- the amount of separation being determined by experimentation, on an optical bench. The doublet lens is essentially two lenses cemented together, and may consist of a double convex lens cemented to a double concave or plano concave lens. The triplet is a three lens system, or three element lens, with three lenses being cemented together, and may consist of a double concave sandwiched between two double convex lenses. These are usually referred to as multi-element lenses, rather than lens systems -- the term lens system, being usually in reference to all of the lenses (either single, or multi-element) in an optical device, or at least a portion of such a device.

Multi-element lenses, such as just mentioned, may be referred to as optically corrected lenses. Such lenses improve resolution (sharpness of detail) of the image and correct for chromatic (color) and other aberrations (a failure to produce an image that is the exact duplicate of the object viewed). The simple (double convex) lens will focus various colors of the spectrum at different points or distances from the lens (see Figure 2-2). The visible spectrum runs from violet on one end, through blue, green, yellow, and orange to red at the other end. On the outer ends of the spectrum are ultraviolet (UV) and infrared (IR), which are beyond visible range. Multi-element lenses giving chromatic correction are called achromatic, or color corrected lenses.

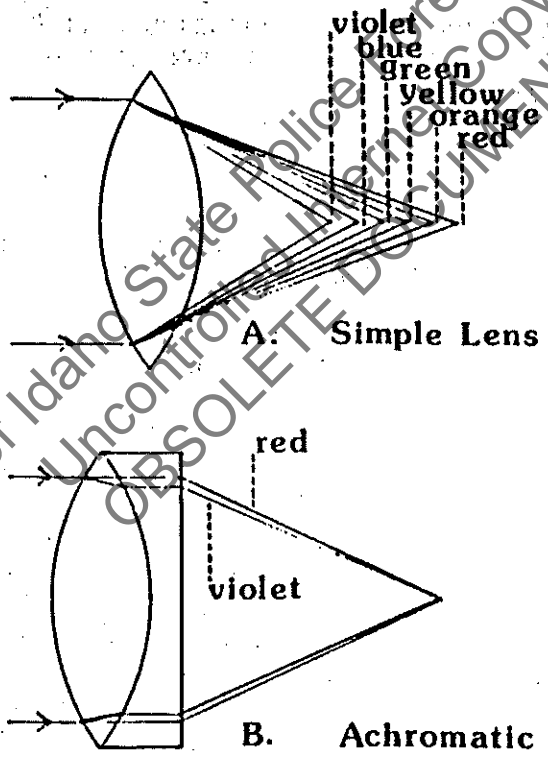


FIGURE 2-2

Achromatic (color corrected) lenses. In the upper illustration is shown a simple lens focusing different colors at different distances from the lens, with violet focusing nearer and red farthest away. The other colors of the spectrum fall between these points. In the lower illustration is shown the color-correcting effect of the achromat, which uses two lenses of different types of glass, cemented together. Normally, one lens is of flint glass and the other lens element is of crown glass. Achromatic lenses also correct for certain other aberrations.

It might be well to note, that such multi-element lenses are composed of elements made from different types of glass -- one may be of flint glass and another of crown glass. An achromatic lens may correct for other aberrations beside color.

Proper Viewing

For proper viewing through a lens, or lens system, the eye must be reasonably close to the viewing lens (the distance for proper viewing, being called eye relief, will be touched upon later in this writing). This applies, whether we are using a simple magnifier, microscope, pair of field glasses, telescopic rifle sights, or whatever. The farther the eye is from the lens, the more light is received from the edges of the lens (as opposed to the center of the lens, which is in sharp focus). Should the eye be too far from the lens, light rays from the lens may miss the eye altogether. Also, if the eye is positioned farther away from the lens than might be considered normal, the wide field that is possible normally, cannot be seen -- you are effectively restricting yourself to only the central portion of the image. The distance between the eye and the lens (as mentioned previously) is called eye relief.

It should also be noted that the greater the distance between the eye and the lens, the greater the magnification will be. Magnification in excess of that normally obtained by a lens of a given focal length can be achieved in this manner.

PRISMS

Since the object of this portion of my dissertation is to cover (at least to a limited extent) the major components of an optical system, we cannot conclude without touching upon some of these other components. We shall, therefore, begin by a brief coverage of prisms -- what they are, their intended purpose/s, and how they fit into an optical system.

A prism, by definition, is a polyhedron with two faces that are polygons in parallel planes, with the other faces being parallelograms, and is composed of glass, crystal, or quartz crystal -- a transparent body bounded in part by two plane faces that are not parallel, and which are used to bend or disperse a beam of light.

Prisms have a number of uses in optical systems. While instruments such as the spectrograph use prisms to break up the light emanating from a flame (burning specimen) to record the various parts of the light spectrum present in that flame -- and, hence, identify the chemical composition of the minute specimen being burned -- our interest is with other visual applications of the prism (though, a spectrograph is an optical instrument). The uses of a prism/s in such optical equipment as we are discussing, is primarily to redirect a light beam. Prisms are frequently used in series combinations to artificially increase draw tube length, and provide a shorter, more compact, instrument than would be possible were we dependent on the distance separating the lenses (ocular and objective) only. Figure 2-3 illustrates how prisms are used for this purpose.

Prisms are used in such optical equipment as periscopes and range finders, as well as binoculars, telescopes, the viewing system of reflex cameras, and similar equipment. Often mirrors (particularly, front

surface mirrors) are used in many of the applications where prisms might also be used. This is done, for the most part, for reasons of economy. The prism, however, is a much more versatile item and has a number of applications which cannot be duplicated by mirrors.

MIRRORS

Mirrors are used in a variety of optical instruments. While prisms are used to redirect light which may actually pass through the prism/s, mirrors are used to reflect and redirect light. In optical equipment, normally, front surface mirrors are used, which means that the light is reflected from the outer (front) surface, rather than having to pass through the glass of the mirror to be reflected from the silvered rear surface -- which would cause aberrations due to imperfections in the glass, or its refractive or reflective indices.

Specifically ground and polished mirrors having curved surfaces (concave or convex curvature) are used extensively in telescopes used for the study of astronomy. Such mirrors are used to magnify an image, which is then reflected back for viewing through a lens system.

Both mirrors and prisms are presently being used, and have been used in the past, in a variety of types of optical instruments and equipment. The single lens reflex (SLR) camera uses prisms to redirect the image from the lens to the viewfinder -- in the past mirrors were used for this same purpose, however, the mirror had to be taken out of the way when the shutter was snapped to expose the film (the prism not only can redirect the image to the viewfinder, but can remain stationary and permit straight-through light transmission to the film. Mirrors and prisms are also used in a variety of projection equipment, for rear projection, overhead projector, and other uses.

Mirrors may be either flat (plain) mirrors, or of a concave or convex configuration, often being used to achieve magnification (being, in effect, lenses). A fair portion of this information will be touched upon later under APPLICATIONS.

THE DRAW TUBE

The draw tube is, quite simply, the housing used to separate the ocular and objective lenses, also serving to close out extraneous (outside) light, which could adversely affect proper viewing. Draw tube length, however, borders on being a critical factor. Lenses can be brought into critical focus at more than one point (hence, the use of bellows extension or extension tubes in photography, which make it possible to record an image at 1:1 -- image the same size as the object being focused upon -- and, of course, points in between). The ideal draw tube length is such that when lenses are used in combination (an objective of 50X and an ocular of 10X, for instance), they produce an image which is the product of the powers of the lens combinations (50 x 10, or 500X). With a draw tube having a longer or shorter length, however, the lens combination would not produce these results.

BASIC OPTICS - PART III (APPLICATIONS)

Under the above heading, we shall continue with our discussion of lenses, mirrors, prisms, and lens separation (the distance between two lenses or lens systems), attempting to relate our observations to specific bases. Hopefully, in so doing, this will help the reader to better understand the equipment with which he will be working in his daily pursuits and will assist in obtaining the full capabilities of the equipment being used.

PHOTOGRAPHIC APPLICATIONS

The Lens

We have all heard such terms as normal lens, telephoto lens, and wide angle lens, used in conversations relative to some form of photographic application. Most of us understand in general terms what each of these terms make reference to and what may be accomplished by using them. Here, we shall dispense with such general terminology and attempt to get down to specifics.

The Normal Lens

The normal lens produces an image that is essentially what would be seen by the viewer, had he been standing where the camera was positioned at the time a specific photograph was taken. The focal length of a normal lens should equal the diagonal measurement of the film frame. With 35mm cameras, the normal lens has a focal length of 50mm -- this being the diagonal measurement of the film frame (for all practical intents and purposes). Some 35mm cameras may be equipped with a lens of 55mm, which would still be considered as a normal lens. By the same token, a camera using the 4X5 inch format would need a lens with a focal length of about 165mm.

The Telephoto Lens

A telephoto lens gives greater enlargement than does a normal lens, and to achieve this it must have a longer focal length. For 35mm camera use, should we wish to determine which telephoto lens to use for a given distance, we must multiply the distance in feet (camera to subject) by two, and this will give us the focal length of the desired lens in millimeters. Say that a person was some 100 feet distant and we wished to photograph him, filling the frame with his image, we should need a lens having a focal length of 200mm. We are normally limited to the use of whatever lenses we may have at our disposal, so, should we have but a 150mm lens, we would not get quite the enlargement needed to fill the full frame. Zoom lenses, or lenses that can be brought into focus at any desired degree of magnification within a given range (say 70-200mm) might prove ideal for certain types of telephoto photography.

(For work, such as in courts of law, where a photograph is required to give as nearly as possible a view as would be seen by the observer, the normal lens is almost a must. The telephoto lens will give us an image that shortens the foreground, and gives the impression that two objects

are located much closer together than is the actual case. A telephoto lens, however, may be of considerable benefit in taking portraits, as it allows the camera to be sufficiently distant from the subject that overexposure of the negative from flash will not be experienced.)

The Wide Angle Lens

Wide angle lenses have shorter focal lengths than do normal lenses. Should you have a camera equipped with a 50mm normal lens, and should you substitute a 35mm lens, you will be using a wide angle lens (a lens giving less than 1X magnification). It might be well to mention that zoom lenses may be purchased with continuously variable focal lengths that include both wide angle and telephoto (for instance, one designated 35 - 80mm).

While one can easily see why a need for a telephoto lens may occasionally occur, one often wonders why he might need a wide angle lens. If you have ever been in a situation where you wished to photograph something across a narrow street and were unable to move back far enough to get your whole subject within frame (for instance, the front of a building), you would understand. A bit of caution on the use of wide angle lenses is in order, however -- the greater the degree of wide angle effect, the more distorted the outer edges of the resulting photograph will be.

Should you wish to determine the actual power of a photographic lens, merely divide the FL of your normal lens into the FL of the lens in question. If the normal lens is one of 50mm and you are using a telephoto lens having a 100mm FL, 100/50 = 2 (2X). Similarly, a 35mm lens would give a power of .70 (.7X) or not quite 3/4 of your normal image.

One-to-One Photography

There will be times when you will desire to produce an image on your film of the same size as the object being photographed (once developed, this film will become your negative). Such cases may arise when you are attempting to reproduce a fingerprint or a signature in exact size as the original. Normally, a camera having a larger format than the 35mm format will be needed (for instance the 4x5 inch or 2 1/4 X 3 1/4 format, even one with an 8x10 format). The camera set-up for such photography is determined by the formula 1:1 2 x FL for lens-to-subject and lens-to-filmplane distance. By shooting at 1:1, a latent fingerprint impression and a rolled impression can be produced at the same degree of magnification, which will make the darkroom work a more simple matter. (The 1:1 technique also permits you to have an enlarged image on your negative, making enlarged prints of better quality -- greater detail, or resolution.)

Aperture

The term aperture refers to the size of the opening of the iris diaphragm located behind the lens. The size of the aperture (f-stop setting) determines the amount of light permitted to pass through the lens -- and ultimately delivered to the film. Aperture also refers to the lens itself -- disregarding the use of the iris diaphragm. The optimum lens would be one having a lens diameter equal to its focal length, and would be designated as an f 1.0 lens.

As most cameras are equipped with an iris diaphragm that permits the photographer to control the amount of light reaching the film, a few words should be said about f-stops. My camera, for instance, is equipped with an f-2 lens, and has f-stops ranging from f-2 (the wide open lens), down through f-2.8, f-4, f-5.8, f-8, f-11, to the smallest opening, which is f-16. View cameras and press cameras often are equipped with lenses that stop down to as small an opening as f-32. Decreasing the f-stop decreases the amount of light delivered at film plane, but it also serves to increase the depth of field (which means that with a lens stopped down all the way, nearly everything will be in focus -- regardless of its distance away from, or proximity to, the camera). There are times, however, when we might not wish for such great depth of field (usually for artistic purposes, where we might want our subject to be in sharp focus but have a blurred background). In such cases, we will open the diaphragm to its widest point and focus only on the subject.

Shutter Speed

Proper exposure of photographic film is dependent on the amount of light permitted to reach the film and the duration of the exposure (cumulative light exposure). By again making reference to my own camera, the shutter speeds run from "B" (bulb), through exposures of 1 second, 1/2 second, 1/4 second, 1/15, 1/30, 1/80, 1/125, 1/500, and ending with an exposure of but 1/1000 second. The faster the shutter speed, the less light permitted to reach the film, and adjustments must be made by opening the diaphragm wider to ensure the proper amount of light reaching the film.

Shutters, generally, are of two types: 1) the diaphragm type shutter -- a mechanical shutter located behind the lens of the camera, and 2) the focal plane shutter, which consists of a piece of opaque fabric with narrow slits cut in it that move across the film plane under spring tension -- much on the order of a roller-type window shade (wider slits in the fabric giving slightly longer exposures than narrower ones). The focal plane shutter type is used for high speed photography for stop-action shots for sporting events, and the like.

Many modern cameras (mine included) have a built-in light metering system for exposure, which allows one to preset his film type (for film speed) and shutter speed. One has then but to adjust the f-stop until the meter indicator falls within a given area and the camera is then set for proper film exposure. As slower film (that requiring a longer exposure to light in order to react properly) will not react in the same manner as a faster film, the ASA rating of the film must be considered in making exposure computations.

Black and white film is easier to work with in the darkroom, and with it one can correct for over or under exposure (to quite some degree) without resorting to the use of either bleachers or intensifiers. Basically, one can correct for just about everything except for poor focus or blur due to movement. (A friend of mine once took a photo of a cathedral in Europe, but being closer to the base of that building and more distant from the tops of the spires, the towers seemed drawn together, because of the angle involved -- a perspective problem which you may have noted on looking down a set of railroad rails. I was able to straighten his towers out on a print I made in the darkroom, and he's never found out how I did it. It was merely a matter of stopping down the projection lens of the enlarger so that maximum depth of field might be obtained, and tilting the easel so that the base of the building on the projection was closer to the enlarger than the tower tops.)

Photographic Film

While a great deal could be said about the various photographic films, their different film speeds and applications, we will not go into all that. Countless good books exist on all phases of photography, including the applications of different films, and darkroom work -- including some of the so-called "darkroom magic". These topics are too involved and really do not warrant being touched upon in this writing, anymore than a coverage of double-exposure techniques, painting with flash, long exposure techniques, etc., would be applicable -- though, all may be considered as matters involving applied optics.

Basically, an emulsion containing photo-sensitive (light sensitive) chemicals is applied as a coating to the clear acetate film strip, in the manufacture of photographic film. These chemicals react when exposed to light, and in the darkroom, various chemical baths serve to develop the exposed film by dissolving away those portions of the emulsion not affected by light (greyed areas are only partially dissolved away, and areas of intense light are left untouched). The development is followed by the film being put through a fixing bath to stop any further development. These procedures produce a negative (light reversal) image on the film. In the printing process, a reversal is again brought about, when light is projected through the negative upon chemically sensitized paper, and a positive print is made.

In the above, we have strayed considerably from a strict discussion of optics, and have moved into the area of chemistry (photo-chemistry). It does, however, fall within an area which we might call applied optics.

LONG RANGE VIEWING

The Simple Telescope

What was at one time referred to as a "spyglass", mariners glass, or telescope, is merely a telescope in its more simple form. Such an instrument consists of an objective (viewing) lens and an ocular (eyepiece), mounted at opposite ends of a long draw tube. This tube consists of two or more sections of cylindrical tubing, of such diameters that those of the smaller diameter may be slid inside those whose diameters are larger. This telescoping action enables adjustment for focus, by permitting the objective and ocular lenses to be moved closer together or farther apart (such things as telescoping tripod legs, or telescoping fishing rods, get their names from the action of these early telescopes and their draw tubes). Today's microscopes, spotting scopes, and certain types of telescopes, also have what is referred to as a draw tube, although, there is no telescoping action and the lengths of such tubes are fixed.

Binoculars

The term binocular indicates that we are discussing an instrument designed for the use of both of the user's eyes. Were the instrument designed for the use of but one eye, it would be called a monocular. A pair (or set) of binoculars, as we know them, is essentially a matched pair of monocular telescopes mounted side by side, in such a manner as to permit the user to look through one with his left eye and the other with his right. It might also be well to remember that a single-barreled microscope (or other normally monocular system) may be fitted with a binocular eyepiece, to permit more comfortable viewing.

Binoculars (also called field glasses) are marked to reflect their magnification power. A pair of binoculars marked 7x35 has an overall magnification of 7X, and the diameter of the objective (front) lens is 35mm. The lens diameter is important, as this is what determines the amount of light passing through to reach the eye. If binoculars are to be used under poor lighting conditions, a lens of greater diameter -- such as the 50mm lens -- is desirable.

The field of view (usually specified in degrees -- the viewing angle) may vary considerably among binoculars having the same magnifying power and lens diameter. For the viewing of action sports, the user might prefer 7x35 wide-angle glasses, as opposed to 7x35 all-purpose binoculars.

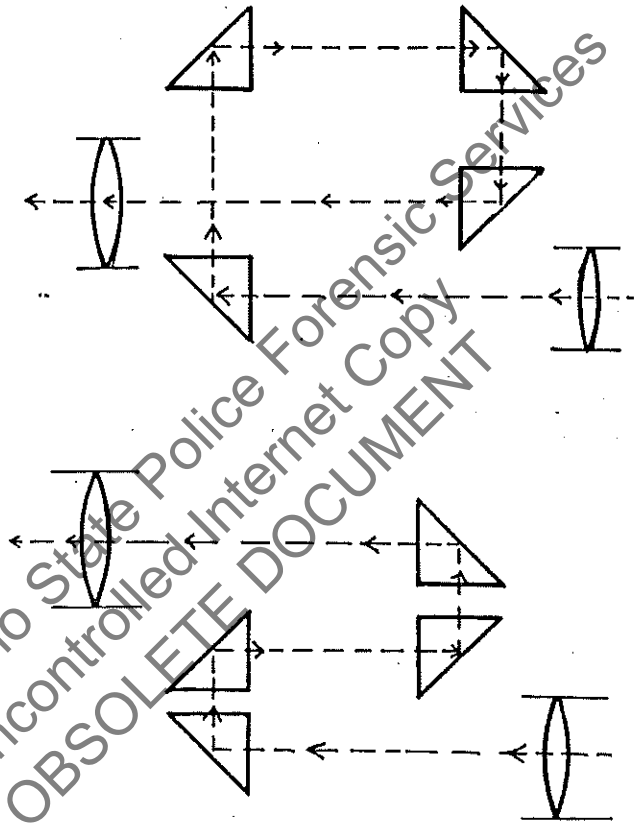


FIGURE 2-3

Shown here are two of the possible systems (arrangements of prisms) that might be used to artificially increase the length of the draw tube. While the ocular and objective lenses are fairly close together, the optical distance is more than tripled by using prisms to redirect the light path. (Arrows indicated the line-of-sight -- actual light path would be in the opposite direction).

For more distant viewing, 10x50 binoculars might be selected -- such binoculars, giving greater magnification and detail than the less powerful 7X glasses. One must remember, however, that as the power of the lenses is increased, the user's ability to hold the instrument steady is decreased. With glasses of higher magnification, it is frequently advisable to use a tripod to steady the instrument -- this is especially true with equipment such as the high-powered spotting scopes (monocular telescopes used for the spotting of the strike of a bullet on a target, or for viewing games at considerable distance).

The mariners telescope, which has been described earlier, was quite long -- even when "telescoped-down". Modern binoculars and spotting scopes, however, are able to keep their total length to a minimum by artificially increasing draw tube length by the proper use of prisms (see Figure 3-1), as well as more highly developed lens grinding techniques and the development of more highly sophisticated multiple-element lens design.

Riflescopes

The riflescope, or telescopic sighting apparatus for use with a firearm (scopes are also adaptable for use with handguns -- even crossbows), is nothing new on the scene. Back in the days of cap-and-ball shooting, many "buggy rifles" of the muzzle loading type, were equipped with telescopic sights (sometimes referred to as "glass sights" -- fixed sights being called "iron sights", and adjustable sights being known under a variety of names depending on their type). These early riflescopes, however, usually extended the full length of the barrel of the weapon, and the draw tube was a glass cylinder of fixed length.

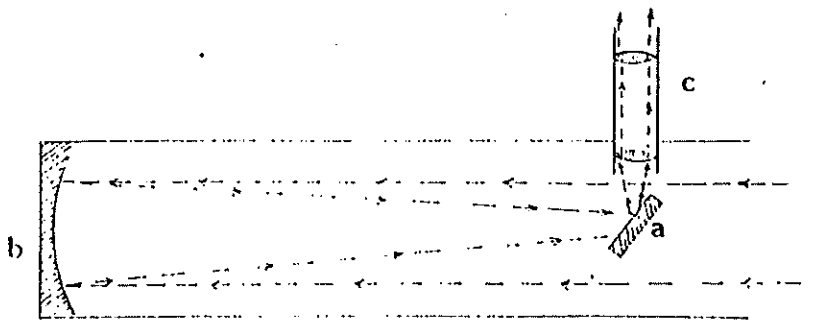
The modern riflescope is mounted on the weapon (usually on top of the receiver) parallel to the longitudinal axis of the bore, and produces an enlarged image of the target, permitting more accurate sighting (not necessarily more accurate shooting -- accuracy in shooting is dependent on the ability of the shooter to hold, squeeze the trigger smoothly, control of breathing, and even the effects of heartbeat). Many modern scopes are of variable power to permit different degrees of magnification, and allow the shooter to adapt to different shooting conditions (range, target size, and field of view).

Riflescopes (telescopic sights), if one desires to use them, need not only be selected on the basis of the degree of desired magnification, the lighting conditions which the shooter may encounter, the type and size of cross-hairs, post, or other sighting reference, but the type and caliber of the weapon one wishes to use the sighting equipment with, all have a definite bearing. Eye relief for a telescopic sight suitable for use with a handgun would not be suitable for use with a big bore game rifle, or vice versa. The ability of the sighting equipment to withstand shock is not nearly the problem with a caliber .22 rifle as with a rifle chambered for .30-06 cartridges.

The type of mounting device, too, must be considered. One may wish to be able to use the normal sights with which the rifle is equipped, on occasion, rather than to be strictly limited to the use of the telescopic sights. Also, some types of mount may cause problems with the normal ejecting or loading of ammunition. Hinged-type mounts may be used, but they must be able to be repositioned exactly, each time they are moved.

Telescopes

Telescopes used in the study of astronomy differ in their construction from the types of instruments discussed thus far. Such telescopes, generally, have a barrel or tube, which is fairly large in diameter. The objective lens (if such is used) is mounted at the front of the tube, and focuses on a concave mirror at the rear of the tube. The mirror reflects its image forward again to a smaller mirror positioned somewhere within the tube, and is then deflected at an angle to the lens system of the eyepiece or ocular lens. A high degree of magnification is thus obtained, and the overall length of the instrument is kept to a workable minimum. (See Figures 3-1 and 3-2).



A. MIRROR
B. CONVEX MIRROR
C. EYEPIECE

FIGURE 3-1

Shown is the path of light from a distant object to the concave mirrored surface at the rear of the telescope (b), reflected to another mirror back near the forward end (a) and then directed to the eyepiece (c) which is also mounted near the forward end of the telescope. The magnification of the eyepiece provides additional (hollow) magnification, but resolution is not improved.

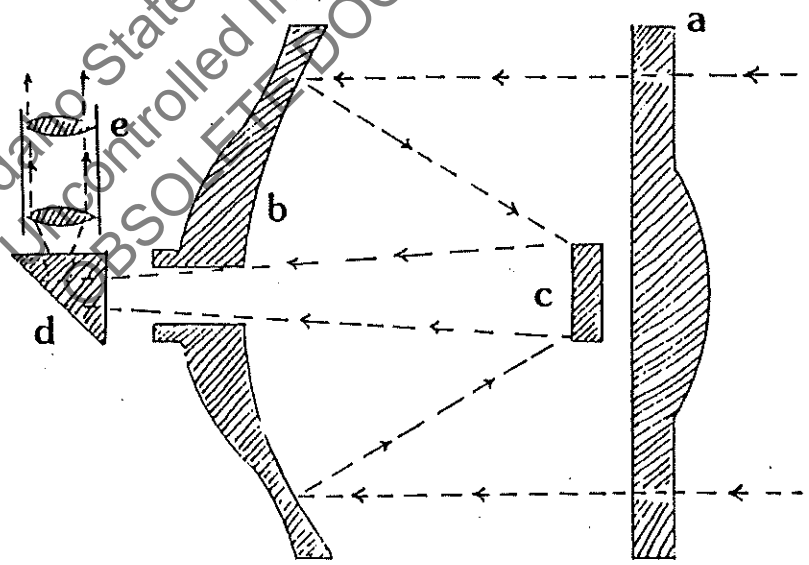


FIGURE 3-2

Illustration of the Schmidt-Cassegrain optical system, another system using mirrors and lenses in an astronomical telescope. Light passes through the lens (a), is reflected from two mirrors (b and c), then passes through the prism (d) and the eyepiece (e). Both Figure 3-1 and Figure 3-2 are of reflector telescopes, as opposed to a refractive system using lenses only.

PROJECTION EQUIPMENT

Most of us are somewhat familiar with motion picture or still (transparency or slide) projectors -- possibly, even with opaque projection equipment, or overhead projectors. We shall not dwell at any great length on this topic, however, a few comments need be made -- in particular, comments regarding processes that are (and are not) shared with other optical equipment and optical systems.

Image Reversal

With any single lens (or single element) system, a point is reached in positioning the lens in relation to the object to be viewed (or projected) where image reversal occurs. If the reader has ever had occasion to view the image on the ground glass screen of a view camera, it has been noted that this image is upside-down. The same image reversal takes place in regard to single-lens-reflex (SLR) camera viewing, however, the image is again reversed by introduction of a prism or mirror into the act. With field glasses or riflescopes, this second reversal is brought about by the ocular lens. As motion picture and slide projectors have one-element lens systems, the material to be projected (the transparency) must be placed between the lens and light source in an upside-down position, in order for the projected image to be upright on the screen after being reversed in passing through the lens.

With rear projection equipment, not only must the transparency be placed in the equipment in this upside-down manner, but the projected image must be directed at a mirror and back to the rear of the screen, so that the image when viewed from the front of the screen will not have left-right reversal. (The reasons for using rear projection equipment are several: 1) The projector need not be placed some distance out in front of the screen, where its placement could cause problems. 2) Less space is required, as we are halving the projector to screen distance by using a mirror to redirect the projected image. 3) It permits the use of projection equipment, under conditions where -- due to the area where the viewing audience is situated being too well lit -- the more conventional projection equipment would not be acceptable.)

Mirrors and Prisms

While both mirrors and prisms have been mentioned earlier in this writing, our coverage was primarily along the lines of their reflective quality, why front surface mirrors are more desirable for optical purposes, and things of similar nature. We have also made brief comment regarding their use in image reversal, and this is one of the matters I wish to cover at this point.

When you look into a mirror, the person staring back at you is a left-right-reversal, or reversed image, of yourself. When you raise your right hand, the person in the mirror raises his left hand. We have become accustomed to this sort of reversed image, and have no problem with performing such tasks as combing our hair, shaving, or any of a number of things, using the reversed image as a guide for our movements. Were two mirrors placed at right angles, however, and were we to position ourselves at some point where the angle formed by these two mirrors was bisected by an imaginary line, something entirely different would take

place -- when we raised our right hand, that person in the mirror would also raise his right hand -- performing such a simple task as combing one's hair under such conditions could cause major problems (can you envision setting up such a devise in your local tavern -- everyone in the place would be convinced that they were inebriated).

Another thing we don't give any thought to is the location (relative location) of that mirror image. When one stands in front of a mirror the virtual image is located as far behind the mirror as we are in front of it. Should you wish to photograph your reflection in a mirror, and the distance to the mirror is ten feet, your camera must be focused at a distance of twenty feet if you are to get the photograph you intended -- otherwise, you will only get a blurred reflection.

Prisms, when used as image reflectors, perform similarly to mirrors. To go more deeply into the matter here would serve no beneficial purpose, and would tend to make matters more involved than would be warranted.

BASIC OPTICS - PART IV (MICROSCOPES)

The material preceding this portion was presented in order that the reader might gain some understanding of the components of an optical system, before we began exploration of the more complex subject of microscopes. The microscope is one of the basic instruments of most types of laboratories -- in particular, those laboratories devoted to forensic, or criminal investigative pursuits. In the forensic laboratory, use of a microscope (in one or more of its forms) is mandatory in firearms and/or tool mark examination, hair and fiber study, questioned document work, or any of the fields relating to the examination of biological specimens. Often times, the microscopist can identify chemicals, or chemical compounds, by the microscopic examination of crystalline structure. Microscopes are used by the metalurgist, the biologist, the zoologist, the various medical service personnel, and in more other scientific areas than one might conveniently name. The types of microscope may differ, based on the type of performance required of it, yet the basic principles remain much the same. Reflected lighting will be needed for the examination of opaque specimens, while other types of specimen will require the use of transmitted light. The basic principles will not change, only the application of those principles.

The worker worthy of his pay must understand and respect his tools. He must know their capabilities and limitations, and must understand how to care for them and maintain them. The firearms technician or artificer who subscribes to the statement: 'Never use force -- just use a slightly larger hammer', is referred to as a "Blacksmith" (which is an unwarranted slur to those engaged in true blacksmithing). We cannot afford to have a person with such an attitude, manhandling delicate and expensive equipment such as a microscope.

For many routine tasks, one needs only to have a generally understanding of the microscope and the routines he must follow to obtain the needed results. All tasks, however, may not be routine in nature, and to ensure that one is capable of obtaining optimum results, one must have at least a marginal understanding of the components of the instrument and how these components interact to bring about the results desired.

There are certain things that any given piece of equipment cannot be expected to do. By the same token, there might also be a number of things of which that equipment is capable, and of which we are not aware. Without some understanding of how and why a piece of equipment works, we can't begin to realize either its full potential or its limitations.

THE SIMPLE MICROSCOPE

The simple microscope is, essentially, nothing more than a magnifier (although, normally, containing more than one lens element). Such a unit will provide macroscopic viewing, giving an enlargement of but a few diameters, however, that slight magnification will permit certain work to be performed with vastly greater accuracy and with far less time waste (the fingerprint glass used by the fingerprint examiner or fingerprint classifier, and the dissecting microscope used by the biologist or medical technician, are good examples of simple microscopes). By definition, we might also include such items as the jewelers loupe, or even the hand-held magnifying lens, under this heading but, of course, such one element lenses tend to give a perceptible amount of distortion as we approach the outer edges of the lens in our viewing. A significant amount of distortion is also brought about when such a lens is not held completely parallel to the surface being viewed.

Magnifiers of the type we are discussing under the heading of simple microscopes, are normally of relatively low magnification power -- twenty diameters (20X) or less, with fingerprint magnifiers being of only 2.5X to 3.5X, normally.

THE COMPOUND MICROSCOPE

The compound microscope differs from the simple microscope in that it has two separate lens systems. The objective lens being located at the lower end of the draw tube nearest the specimen to be examined, while the ocular lens (the viewing lens or eyepiece) is mounted at the other end of the tube. The objective lens magnifies the specimen a given amount, while the second lens system, the eyepiece, gives additional magnification to the image formed by the objective lens. The image seen through the eyepiece, or ocular lens, is the product of the magnifying power of both the objective and ocular lenses. Therefore, if the objective lens has a power of 10X and the ocular has a power of 5X, total magnification would be 50X.

Were we to change the power of the objective to 5X and the power of the ocular to 10X, we would still wind up with 50X (fifty diameters magnification). A decided difference would exist, however. The objective furnishes the initial amount of magnification, which determines just how much detail will be observed. Enlargement brought about by the ocular lens, however, merely serves to enlarge this initial image, giving empty or hollow magnification to the image first obtained. Therefore, were we to employ a 10X objective and a 10X ocular, we would achieve a total magnification of 100X. By the same token, were we to use a 20X objective and a 5X ocular, we would still get 100X but much more detail would be obtained in our final image.

A simple example of this principle can be seen by a comparison to the photographic enlarger, used to change a photograph from the size appearing on the negative to one of much greater size for the

photographic print. While the print may be of greatly increased size, detail that was not present on the negative will not be present on the resulting print (the larger the negative format, the more detail being recorded).

Shown in figure 4-1 is a drawing of a compound microscope, indicating the various parts and controls. The simple compound microscope will not have a fine adjustment knob (nor will the shop microscope -- which is also without the stand, stage, etc., and which may be used in a machine shop for inspection of work), but in most other respects will be the same as the laboratory microscope. Microscopes referred to as student, laboratory, or research microscopes, however, are all much the same -- with the research microscope being dignified by that title, as it is a more expensive piece of equipment.

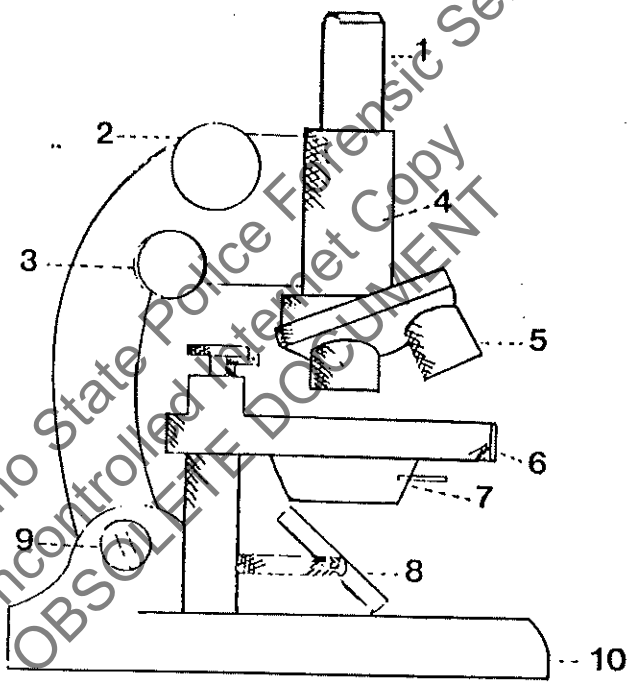


FIGURE 4-1

The conventional compound microscope, designed for monocular viewing. This same type of microscope could be set up for binocular viewing with the addition (or substitution) of a binocular eyepiece (see Figure 4-2). The parts of the microscope are: the eyepiece or ocular lens (1), coarse adjustment knob (2), fine adjustment knob (3), body tube or draw tube (4), revolving nosepiece with objective lenses (5), stage (6), substage condenser (7), mirror (8), inclination joint (9), and microscope base (10).

The two lens systems and the barrel or draw tube are usually mounted on a stand (or base) provided with a stage to support the specimen. There is normally a mechanism beneath the stage that permits regulation of the amount of light reflected up by the mirror from some convenient light source (this may be eliminated in some microscopes that have built-in light sources, and microscopes designed purely for use with reflected lighting and used for such purposes as bullet comparison or metalurgical work, involving only opaque specimens). A condenser lens system may be used for better light concentration and control. Where a direct light source is provided in lieu of the mirror arrangement, the incandescent bulb may be dimmed by use of a rheostat. Where this is the case, however, the dimmed light contains a much higher yellow and red concentration, so it may be better to adjust the amount of light by use of a diaphragm or filters (I won't get into color temperature and its assorted effects, as this is designed to be a more basic writing. Color temperature variation -- or imbalance, as with two light sources used with a comparison microscope -- can be the ruination of color microphotographs).

MICROSCOPE TYPES

In our discussion of the compound microscope thus far, coverage has been directed principally to the biological microscopes (in rather broad and non-technical manner). Such microscopes are usually used with relatively high-power lens systems (higher powered objective lenses -- oculars are generally of 5X or 10X magnifications), and transmitted light. Admittedly, the coverage given has been quite basic and unsophisticated, but basic information is what is wished to be presented. Should we get technical, even one narrow phase of what we intend to cover might well fill an entire volume.

The stereoscopic, or Greenough type, microscope is essentially two microscopes set side-by-side, providing one microscope for each of the viewer's eyes. Such an arrangement permits three dimensional viewing, much as would be the case were you able to view the specimen with your unaided eyes. Even a pencil mark or pen stroke has depth and is not flat and in the same plane as the paper it is placed upon, and the stereo microscope enables you to see that depth. Stereo microscopes are generally set up with optics permitting relatively low order magnifications, and find their chief uses in crime laboratory work in questioned document examination, hair and fiber examinations of certain types, and some firearm and tool mark work. The three dimensional effect is quite useful in determining which line is uppermost when two pen strokes intersect, and things of that nature. Such an instrument is also quite useful when it comes to viewing powder particles, certain types of tool marks, or in the examination of clothing. (See figure 4-3).

The comparison microscope is the main tool of the hair and fiber examiner and the firearms/tool mark examiner. It is illustrated in figure 4-4. The questioned document examiner might also make use of such an instrument in line with certain types of counterfeiting determinations or comparisons of rubber stamp impressions. The type of stage employed, or the type of lighting used will vary, depending on the use to which the instrument will be put, however, the bridge arrangement will be essentially the same in all cases.

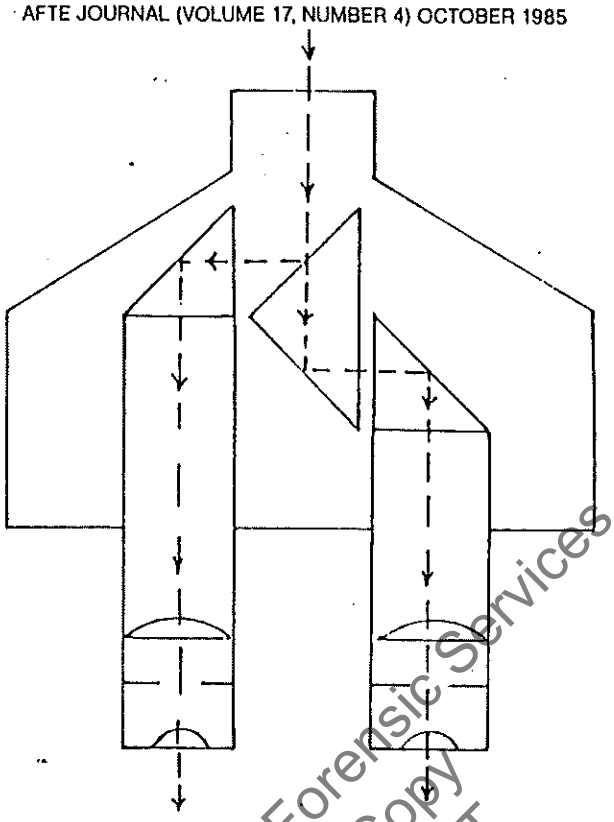


FIGURE 4-2

Drawing a binocular eyepiece for a monobjective microscope. This type of ocular system permits viewing with both eyes. Arrows indicate the path of light from the object viewed through the prism system of the eyepiece. (One eyepiece is of fixed focus, and your initial focus is done through that eyepiece. The other eyepiece has a focus adjustment which enables you to bring both eyes into sharp focus and accommodate for any discrepancies between the left and right eye.)

The comparison microscope is, for all practical intents and purposes, two microscopes mounted in close proximity to each other, with the upper portions being coupled together by a prism bridge that enables the viewer to examine two specimens (one mounted on either microscope stage) in side-by-side, super-imposed, or other position. (In most cases, you are not seeing a true super-imposition, but rather the left portion of the specimen appearing on the left stage abutted against the right portion of the specimen appearing on the right stage.) In looking through the eyepiece/s, one might observe a fine vertical line, roughly dividing the field of view into two half circles. The specimen mounted on the left microscope stage will be observed to the left of this dividing line, and anything on the right stage will appear to the right of the dividing line. The lens systems of either the stereo microscope or the comparison microscope must be in carefully matched pairs to ensure exactly the same degree of magnification being developed for each side.

With the comparison microscope, instead of the stages being fixed and focus obtained by movement of the tubes and lenses, the tubes, lenses and bridge remain stationary and the stages supporting the specimens are moved upward or downward to achieve proper focus. The stages may be moved independently of each other, both for focus and to position the

specimen vertically or laterally. The upper portions of the comparison microscope may only be moved upward or downward in unison, because of the connecting bridge. The comparison microscope used by the firearms/tool mark examiner, in most cases, is provided only with lighting of the direct (reflected) type, whereas, that for use of the hair and fiber examiner is usually set up for use of transmitted light.

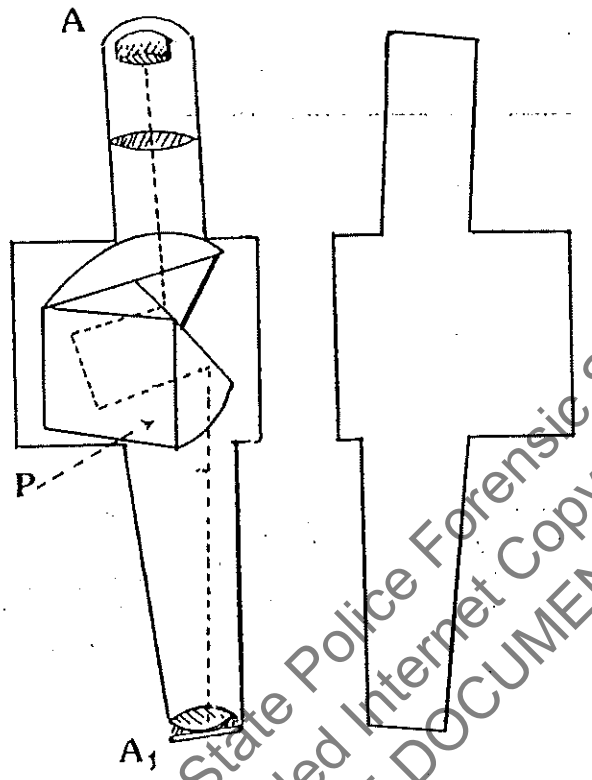


FIGURE 4-3

The Greenough binocular microscope (also called the stereo microscope). Basically, it is two microscopes having a common focal point -- one microscope provided for either eye. Focus is done as with the binocular eyepiece, to ensure that both eyes are receiving an image in sharp focus. Both the binocular eyepiece and the stereo microscope are provided with a means of adjustment for interpupillary distance, as different individuals may have eyes set wider apart or closer together. The unit is mounted on a stand and is usually designed for low power viewing.

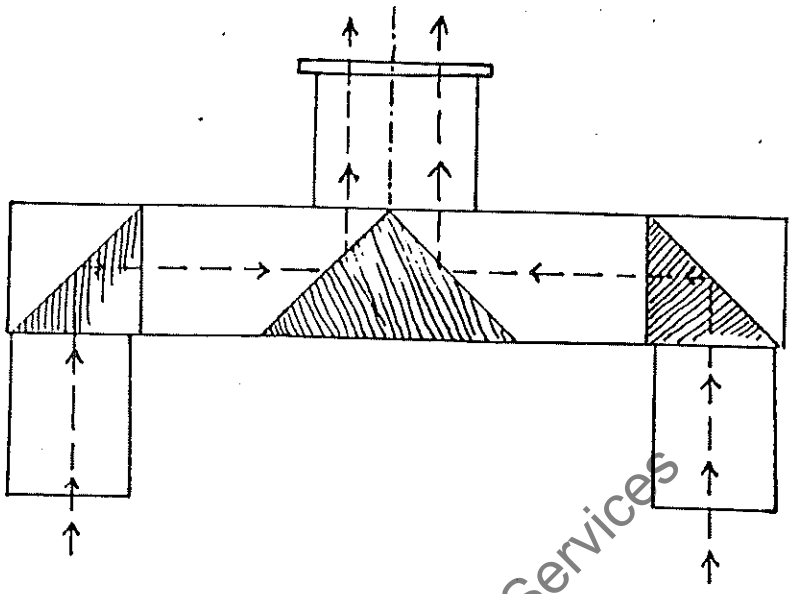


FIGURE 4-4

The prism-bridge system of a comparison microscope. Looking through the eyepiece, a fine vertical line will be observed. The image to the left of this line is from the left microscope stage, while the image on the right is from the right stage. Such a unit may be used to connect two microscopes (this was commonly the case some 40 years ago) in tandem, or as a complete unit -- if horizontal tube length is sufficient. The unit may also be equipped with a binocular eyepiece, to reduce eye strain. Other possible refinements to this unit would include a moveable base line (the vertical line that separates the images from right and left stages), which is accomplished by a shifting of the prisms, and left and right stages can be made to move in unison for tracking to the left, right, or forward or backward. The single prism beneath the eyepiece in this drawing is usually two prisms, properly positioned to achieve the proper angle, in modern comparison microscopes.

PREPARATION FOR VIEWING

The Biological Microscope

Most materials may not be examined with the microscope as they occur in nature. For viewing under the microscope, the specimen must be sufficiently thin to transmit light -- excessive thickness may also tend to obscure details. Because of the wide variety of specimens that one might wish to examine, different techniques must be used in preparing the specimens for viewing. What will follow will be but general directions, and the reader will be referred to other writings for additional techniques, mounting materials, and procedures.

Many temporary preparations will show more detail after being mounted in a drop of water and covered by a thin cover glass. The cover glass is essential to prevent the liquid from getting onto the surface of the objective of the microscope as well as to give a flat surface for more uniform viewing. As water evaporates rapidly, such other liquids as glycerine may be used in mounting a specimen for viewing. When water mounts are used, it is often to one's advantage to add a drop of water to the edge of the cover glass periodically to replace water lost by evaporation. (It also might be noted that as the water evaporates, the cover glass is drawn down toward the slide and can result in crushing or damaging the specimen).

For permanent preparations, the specimen is mounted in some transparent material beneath a cover glass (the specimen is, of course, placed on a slide initially). Permanent mounting may require dehydrating by means of alcohol, dioxin, or some other chemical suitable for such work. Canada Balsam or Gum Damar are among the usual mounting media. Mounting in glycerine jelly is often done, when dehydrating the specimen might cause damage.

One might select material for examination from a mass of material by means of forceps, or when dealing with a liquid preparation a capillary pipette may be used. If plant or animal tissue is to be placed in water and a specimen selected for examination, it can usually be teased apart by the use of dissecting needles to obtain a specimen of a size and thickness suitable for use. Materials may be separated by filtering, and sedimentation is still another common means of separating materials to be examined. A microtome of either the hand, rotary, or sliding types may be used to section material for viewing -- the material being first prepared by freezing, or being set in paraffin.

As this article concerns optics theory, rather than the techniques that may be used, the above shall have to suffice. While staining techniques may be employed to enhance a specimen, I shall refer the reader to other sources for coverage of this as well.

The Stereoscopic Microscope

The stereoscopic microscope is used primarily for the viewing of opaque objects, and reflected lighting techniques must be used in such cases. Specimen mounting does not usually present any problem. The microscope set up for stereo viewing, in its older form, was equipped with pairs of objective lenses that had to be changed in order to go from one degree of magnification or another. They are more commonly found today with a revolving or rotating nosepiece to make changes in magnification an easier matter, or the instrument may be set up for zoom viewing where any desired degree of magnification within its range may be selected. A variety of mounts and bases are available for use with this equipment.

As for preparation of the specimen to be examined, transparent or semi-transparent materials are prepared and examined much the same as with the compound microscope. Opaque specimens may be set up for viewing, as circumstances may dictate. One often needs to use a bit of ingenuity in devising means of mounting a specimen, staining it, or preserving it, and this will apply to any of the types of microscope equipment one may be using.

The Comparison Microscope

Regardless of the microscope type one may be using, it is assumed that a manual of instruction accompanied the instrument, and that one is not attempting to use the instrument for the first time blindly. Each make and model of microscope will have slight differences from others -- many in the way of refinements. One should always check through such a manual before using the equipment, in order to avoid damage to the equipment or to the specimen material to be examined. As regards the comparison microscope, the following is given as general information; specific details of this procedure may vary with different makes and/or models of equipment:

1. Set the bridge assembly to the height desired. This may be accomplished, in most cases, by loosening the set knob and raising or lowering the bridge assembly. This may be done manually or mechanically by some sort of rack and pinion assembly, depending on the manufacturer of the equipment with which we are dealing.

2. If the two stages of the microscope are adjustable by a dovetail adjustment in addition to rack and pinion height adjustment, the dovetail adjustment should be set at the same height for both stages. There may be times when you will wish to deviate from this procedure, but for the time being, let's let this statement stand.

3. Select the objective lenses you wish to use, making certain you have a pair matched for magnification purposes. With some microscopes, the objectives are mounted on a revolving nosepiece and it is merely a matter of rotating the nosepiece until lenses of the same magnification are positioned under each tube. Some of the older models require manual removal of lenses already mounted, and screwing in of others.

4. Mount your specimens (questioned and known) in the appropriate holders and look both from the side and from the front to roughly position the specimens under the objective lenses. (Note: Holders are provided for the mounting of bullets and/or cartridge cases on the comparison microscope stages designed for firearms examination. If working with tool mark specimens, it may be to advantage to remove the flat portion of the stages which contains the means of mounting either bullets or cartridge cases, and replace it with a flat, rectangular stage which can be fabricated if not provided with the equipment. Mount tool mark specimens on these flat stages with modeling clay.

   If the comparison microscope is designed for use in hair and fiber comparison, specimens are mounted as one would with the biological microscope).

5. Adjust the lighting for both stages so that it is directed on the specimens from the same angle and relative distance. Set the light at maximum brightness, and adjust the light for each stage so that the specimens are well illuminated. (For hair and fiber work, adjust light as you would for the biological microscope.)

6. Determine the approximate working distance of your objectives, and set the distance between objectives and specimens accordingly.

7. If your microscope has binocular eyepieces, adjust the interpupillary setting until spacing coincides with the separation between your eyes. At this point, an image from both stages should be visible.

8. Bring the images into sharp focus by adjusting the stage-focusing knobs. The mechanical stage adjustments may be made by operating the proper controls to move the stage forward, backward, or to either side. Should your equipment be provided with a field selector control, center the reference line (vertical dividing line) so that an equal portion of each side is in view. If your equipment has binocular eyepieces for viewing, focus with the fixed eyepiece, then close that eye and adjust the other eyepiece so that both are in focus for your eyes.

9. The equipment is now set up, and by manipulating the appropriate controls, specimens may be moved forward, backward, or to either side -- or rotated in either the horizontal or vertical planes -- so that comparison may be made between the known and questioned specimen. (Note: If your objectives are equipped with iris diaphragms, make all adjustments with diaphragms open to their widest point. Diaphragms may be stopped down later for light balance or for slight changes in depth of focus.)

The foregoing should serve to give some sort of understanding to microscopes to even the most uninitiated. It will give no one more than a rudimentary bit of background information, but this writing is meant to cover the basics. This is the foundation, and you will have to progress from here.

PART V, which follows and concludes this writing, will continue with additional information concerning microscopes, accessories for microscopes, microscope lighting, and a few of those formulas that both you and I have been dreading.

BASIC OPTICS - PART V (MICROSCOPES - CONTINUED)

In our continuing discussion of the microscope, let us start this portion with a short coverage of microscope lenses and matters connected thereto. It may seem like we are breaking the flow of the writing, to tackle microscope lenses at this point, but this information is needed here in order to avoid unnecessary backtracking as we progress.

MICROSCOPE LENSES

Limits of Magnification

There is no upward limit to the magnification possible with the microscope -- only a limit to the useful magnification. The limitation is not one of magnification but of resolving power, the ability of the microscope to make visible a usable amount of fine detail. When a point is reached where a specimen being viewed becomes blurred or indistinct because of the lack of sufficient resolving power of the lenses, any additional magnification would serve only to make the image larger in size. No additional detail would be shown, and the larger we might make the image the less distinct it would become. When we have exceeded the limits of useful magnification, additional enlargement is "empty magnification", serving no purpose whatever.

Resolving Power

Microscope resolving power is dependent on the design and construction of the objective lens. A lens capable of making use of a large angular cone of light coming from the specimen being observed will have better resolving power than a lens to which the cone of light is more limited. The objective lens that picks up the larger cone of light will have measurably more detail visible in the viewed image.

Because of the phenomenon of diffraction, the image of a point object is not a point but is a small circular spot of light surrounded by rings of light. Sir George Airy made investigations of this effect back in 1834, and demonstrated that the distribution of light in such a pattern is such that the radius of the first dark ring is a measure of the resolvable separation in the image. This pattern of concentric rings has become known as the "Airy disc". This separation having the radius of the first dark ring is defined as h in the following equasion:

$$h = 0.61 \lambda / N.A.$$

h, being the ring separation and λ being the wavelength of light (about 0.0005mm or 0.00002") and N.A. the "Numerical Aperture" of the objective lens.

Numerical Aperture (N.A.)

The quantity N sin U is called the Numerical Aperture or N.A. of an objective, so (by definition) the formula:

$$N.A. = N \sin U$$

U being the angle of light entering the lens, and N the refractive index in the object space.

The N.A. is normally engraved or stamped on the body of an objective lens, since this is an important feature of that lens. The higher the N.A. the more complex and costly the lens system. When purchasing a microscope, it would be to one's advantage to ensure that N.A.s are consistent with the price one might be paying.

An indication of the fineness of detail h which can be resolved is shown in the formula:

$$h = 0.61 \lambda / N.A.$$

showing that the fineness of detail that can be resolved is inversely proportional to the N.A. of the objective.

As this formula shows, there are three ways to increase the resolving power -- to decrease the resolvable separation h.

a. Decrease the wavelength λ
b. Increase the angle U in the object space.
c. Increase the index N in the object space.

The wavelength λ can be decreased by going toward the violet, or shorter end of the light spectrum, by use of filters. By special techniques, it is possible to extend into the ultraviolet and further lower the resolvable separation.

The angle U can be increased toward 90° which is the theoretical maximum, but this can be done only so far. The theoretical maximum N.A. would be 1.00, however, the 0.95 N.A. apochromat is about the highest value of U practicable. N.A.'s higher than 0.95 can be achieved by the use of immersion fluids.

The last method of decreasing resolvable separation would be to increase N, the index in the object space, which may be brought about by the use of "immersion objectives", where a fluid is used between the slide and front lens of the objective lens system. Oil is the normal immersion fluid (having an N 1.52), however, water (N 1.33) and/or monobromonapthalene (N 1.66) have been used to some degree. While using immersion fluids, objectives as high as 1.60 N.A. have been made possible, the practical limit is about 1.40 N.A.

Depth of Focus

Depth of Focus (Depth of Field, in photography terminology) is the measurable range above and below the object being viewed in which other objects are in sharp focus. This depth of focus will vary noticeably between objectives of different N.A and may be determined by the formula

$$d = \frac{\lambda \sqrt{N^2 - (NA)^2}}{(NA)^2}$$

where d is the depth of focus for photomicrography. Visually, further depth can be added, as the eye is capable of accommodating to some degree (your eye will see more than can be recorded on the camera film). The visual depth of focus may be determined by the formula

$$d' = d + \frac{250}{M^2}$$

where M is the magnification of the microscope. This is based on the assumption that the human eye can accommodate on an image 250mm distant.

As the foregoing deals with theory, suffice it to be said that as the magnification and N.A. of a lens (objective) increases, the depth of focus decreases, with the visual depth of focus being only slightly greater than would be the case with photomicrography. This is the reason that microscopes using high powered objectives must have very finely controlled focusing actions.

Aberrations

Aberrations (distortions) are inherent in all lenses and a perfect lens simply does not exist. These aberrations exist to a greater or lesser degree, and are dependent on the ability of the lens designer and how great the lens design problem may be. Lenses have a spherical (ball shaped) surface/s, and such a shape does not permit the forming of a perfect image. The designer will use various combinations of lens shapes and choices of glass, and is generally able to balance one defect against another by including an element with an opposite defect to the one he is attempting to correct for. The result in the end comes near permitting the perfect image, but never quite reaches that goal.

The main aberrations in an image formed by a spherical lens surface are as follows:

1. Spherical Aberration -- occurs when the outer portions of the lens has more power than the inner portion. The designer will attempt to counteract this by arranging a series of convergent and divergent lens elements, to minimize the variation in focal power and aperture.

2. Astigmatism -- is a defect where a marginal point object is drawn out to form two separate line images at different distances from the surface of the lens. Like curvature of field, a general off-axis image deterioration is apparent, however, an astigmatic image cannot be brought into sharp focus except for detail either parallel or perpendicular to a radius of the field, which is not the case with curvature of field.

3. Coma -- is where different concentric circular zones of the surface of the lens give different magnification to an off-axis image.

4. Distortion -- is the defect (aberration) which causes the sides of a square object to be imaged as a curve. Inward curvature of the image is called "cushion distortion" and the opposite effect is called "barrel distortion", where curvature is outward.

5. Field Curvature -- refers to a condition where a flat surface is imaged as a curved (bowed) image, as marginal portions of the image are brought into focus at a different distance than the more centrally located portions of the image. This means that when the center portion is in sharp focus the outer areas are out of focus, and vice versa.

6. Chromic Aberration -- is the condition where light of a short wavelength is brought into focus closer than light of a longer wavelength, causing different colors to focus at different lens to object distances. This condition may be controlled by using combinations of different types of glass for the different elements of the lens system.

7. Lateral Color -- is a chromic difference in magnification, and results in light of one color being imaged at a higher magnification than light of another color. This causes an off-axis image of a point object to be spread out into a tiny spectrum of color.

Aberrations of the Microscope

Of the aberrations referred to in the foregoing, spherical aberration should be understood (if none of the others) and the means of control known by the microscopist. It is evidenced by a loss of contrast and a sort of hazy appearance of the observed image. If you are using good quality equipment, this is usually brought about by use of the wrong cover glass thickness. The proper cover glass thickness is engraved on the side of the barrel of the objective, and that cover glass thickness should be used in order to achieve the best results.

Astigmatism, if present in the off-axis image, is a design characteristic and cannot be controlled by the microscopist. On-axis, it is usually due to less than quality workmanship in assembling the objective lens system.

The remarks applying to off-axis astigmatism also apply to off-axis coma. On-axis coma, however, is normally the result of mistreatment of some of the lens elements.

Distortion is usually under pretty good control in the microscope, and is not normally a cause for concern.

Field Curvature is a problem of long-standing, and one that is almost impossible to eradicate completely in microscope design. Lenses currently available commercially have gone a long way toward eliminating this defect, but the problem is one that is still with us. It is a design problem and one that nothing that can be done by the microscopist will get rid of it.

Chromic Aberration is not normally present to any great degree in a well made microscope, however, lateral color may be. Where lateral color exists, it is usually due to the use of the wrong combination of objective and eyepiece.

Apochromatic Objectives

A good many years in the past, it was found that an objective lens system combining fluorite with glass lenses was superior to the achromats which were composed of glass elements only. This improved type of lens was referred to as an apochromat or a fluorite lens. The apochromats are better than the achromats in correcting for both spherical and chromatic aberration, and are also usually higher in N.A. and have better resolving power.

Ocular Lenses or Eyepieces

The ocular lens, as previously noted, is merely a magnifier which enlarges the image formed by the objective. The most commonly used, and least costly, is the Huygenian eyepiece. It was developed by a Dutch scientist, Christian Huygens, back in the 17th century (the 1600's). Ol' Christian did good work, as his brain-child is still more widely used than any of the other oculars in this day and age. The Huygenian eyepiece performs well with the flat-field series of low power achromats, but has some lateral color problems when used with the higher power achromats or apochromats. It does not have a very large field of view, and eye-relief is a bit short. Its generally lower cost and the fact that it gives an acceptable performance has contributed to its continuing acceptance.

The Huygenian eyepiece is quite simple in construction, being made up of but two simple lenses. Light passing through the first lens (called the field lens) is spread out into a spectrum of color with red on the one end and blue on the other. Lateral color correction, however, is achieved by the proper spacing between the two lenses, which draws the various colors of the spectrum together, converging on the retina of the eye as a color free image.

The widefield eyepiece is now coming into more and more general use, having been originally designed for use with stereo microscopes. While a bit more costly, it gives a larger field of view and more comfortable

eye-relief. From the standpoint of lateral color correction, it is similar to the Huygenian eyepiece.

The Hyperplane and Compensating oculars are quite similar to the Huygenian, but for the fact that the lens nearest the eye is a doublet (two elements). Lateral color correction is better with these lenses than with the first two mentioned.

Any of the above ocular lenses are suitable for use with the binocular microscope, though the widefield eyepiece has significant advantages.

MICROSCOPE ILLUMINATION

In its more primitive form, the biological microscope is not fitted with a substage condenser to better focus the light directed through the specimen slide by the mirror. The mirror itself will have one flat surface and one concave one, and in the earlier forms of the microscope, that was it. When I was in what our educational system now refers to as junior high school, I came into possession of such an instrument. There may be modestly priced microscopes and inexpensive microscopes, but this was a cheap microscope, lacking all but the bare essentials. In spite of everything, however, I managed to achieve results that I would not now think possible with such a crude piece of equipment. Dark field effect (though quite unreliable) was even pulled off, by using the concave side of the substage mirror; leaving this naive juvenile believing that he had stumbled across the discovery of the century -- as the manual accompanying this little optical abortion made no reference to this effect, and I being quite ignorant that such an effect existed.

As the biological microscope was the first microscope type covered in the previous portion of this article, we shall start there in our discussion of microscope lighting.

The Substage Condenser

For objectives having a power higher than 10X, the mirror by itself is not large enough to provide the illuminating cone of light needed to fill the aperture of the objectives lens. By introducing a suitable lens or lens system between the mirror and the specimen slide, however, we are able to deliver the required light on target. Such lenses are called substage condenser or Abbe condensers. Although other types of substage condensers exist (and will be touched upon), they are generally referred to as Abbe condensers -- unless the speaker wishes to be more specific.

The Abbe Condensers are not normally either chromatically or spherically corrected, however, they serve their purpose fairly well for most normal work. Their purpose, as previously stated, is to direct light through the slide and specimen at an angle sufficiently large to fill the aperture of the objective lens of the microscope. Abbe condensers are usually furnished in two numerical apertures (N.A.'s): 1.25 N.A., having two lenses, the top lens being removable, and 1.40 N.A., which is made up of a three lens system.

The condenser mounts are made to fit into the substage from below and are provided with an iris diaphragm to control the amount of light permitted to enter the condenser and the angle of the light cone emitted. They are also fitted with a carrier to hold a blue glass filter or a dark ground stop.

The blue filter (which is of glass, because of the heat involved -- gelatin would melt) is used with artificial, or incandescent, light to make the emitted light more in line with daylight -- similar filters are used in color photography, where daylight film is being used indoors (another type of filter being used when indoor film is to be used in daylight.) The dark field stop on the Abbe condenser will be touched upon when we discuss the Dark Field Illumination.

Achromatic condensers are a bit more sophisticated than Abbe condensers, and are corrected for two colors as well as being spherically corrected for two zones. It is recommended that such condensers be used where it is a requirement that a sharp image of the light source be directed into the specimen plane, with the beam being free from color fringes.

Theory and Technique (Transmitted Light)

When an object being examined under the microscope has two transparent regions of different refractive index, the light passing through the area of contact will be bent and the shape of the material is observed from this bent light or retraction image. Refractive index may be determined by this means -- the material is immersed successively in fluids of varying known index until the boundary line between them disappears. When the material is of different index, there is normally a bright line surrounding the specimen -- the Becke line. If the becke line moves in toward the specimens as the microscope is raised, the specimen is of higher refractive index than the surrounding field.

Should a specimen contain planes that reflect light, it may be seen, partially, by the reflection images. Tissue cultures and other transparent materials are visualized as a combination of refracted and reflected images, and with Kohler illumination, little detail is to be seen. Under such conditions, one has little choice but to resort to staining the specimen.

When different parts of the observed image exhibit different colors, then the specimen is being seen by means of its color images. Many dyes are used for staining in microscopy, and this is the usual method used to make material visible. The use of colored filters between the lamp and the microscope may increase contrast and detail observable in colored, or dyed, specimens. Filters of complementary color increase contrast and filters of about the same color as the specimen tend to reveal detail.

Portions of coherent light waves tend to interfere with each other, causing bright and dark regions referred to as diffraction patterns. The observed image is the result of these patterns.

Certain materials which either fluoresce or are affected by polarized light will also give specific color images which aid in visualization.

Oblique Light

When the light from the mirror is directed so that it passes through only one side of the condenser (usually attained by swinging the mirror to one side), the specimen is then illuminated by oblique light. An oblique light attachment is available as a part of the substage for research microscopes. By turning the control knob the obliqueness of the light can be changed through an arc of 90°. The principal use of oblique light is for the study of diatoms.

Dark Field Illumination

The condenser used for dark field observation has a dark stop located centrally. This effectively prevents any direct light rays from entering the objective. The cone of light thus produced is focused onto the specimen, which appears as if it were a self-luminous body on a black background. Small bright objects are more readily seen against a dark background than small dark objects against a light background. The dark field technique makes visible objects too small to be observed under bright field conditions. The more intense the illumination, the smaller are the particles which may be seen.

Polarized Light

Light (visible light) is energy of a band of wavelengths that are visible to the human eye. On the two opposite ends of this visible band are ultra-violet and infra-red. Sound waves are of much lower frequency than are light waves, and radio waves, X-ray, etc. are of much higher frequency. Light waves, as with other energy waves, are transmitted as transverse waves, meaning that they vibrate at right angles to the direction of light transmission. If light energy were being transmitted vertically, the transverse waves would be in the horizontal plane -- although, in any direction as long as it would be at 90° from the vertical (essentially, any direction within a 360° heading). By the use of polarizers, however, we can restrict this vibration to one single direction. If two polarizers are inserted in the optical beam in such a manner that they are at right angles to each other, visible light will be extinguished (crossed polarizers). Should we insert a crystalline object between these crossed polarizers, however, the crystalline object will appear bright against a dark background. This type of illumination is quite useful in the study of crystalline chemical compounds. As entire texts are devoted to polarized light microscopy, we shall not attempt to go any deeper into the matter here.

Fluorescence Illumination

A fluorescent object is one which when illuminated by light of one color will emit light of another color. Frequently, specimens can be identified based on the coloration of the fluorescence. Illumination is normally from an ultra-violet or blue-violet source. Again, to go into any detailed explanation of this phenomenon here would not be profitable, and the matter would be best pursued in texts devoted to such techniques.

Phase Contrast

Phase contrast is basically a method of illumination in which a portion of the light is treated differently from the rest of the light, causing an interference and, thereby, causing a transparent object to become visible. This too is a specialized technique best learned of from a more comprehensive text.

MICROSCOPE ACCESSORIES

Accessories for the microscope are so numerous that it is almost useless to even touch upon the subject. We shall, however, make mention of a few of the more common accessories, with the understanding that this will only scratch the surface in some areas and leave others not touched upon at all.

The Mechanical Stage

The mechanical stage is an accessory. Sure, most research microscopes are equipped with them, but it is an accessory, none-the-less, and can be fitted to less expensive instruments not already so equipped. It is merely a mechanism for moving the specimen slide either vertically or laterally on the stage of the microscope, and not only makes it a more easy matter to position a specimen for viewing, but enables one to index the location of some specific point for future reference.

The Binocular Eyepiece

The binocular eyepiece is normally considered an accessory as well, and is used to replace the monocular eyepiece by mere substitution. The binocular eyepiece has been discussed earlier in this writing.

The Demonstration Eyepiece

A demonstration eyepiece is an attachment, or accessory, which converts the microscope to an instrument having two (or more) eyepieces so that more than one person can observe a specimen simultaneously.

Counting Equipment

One of the better known pieces of counting equipment is the haemacytometer, used in the counting of blood cells. It is divided off into squares and filled with diluted blood, then the cells in one square are counted, and the total cells present can then be estimated.

Another method uses a ruled reticule inserted in the eyepiece, to accomplish essentially the same thing as the haemacytometer.

Micrometers

A scale or reticule may be placed in the eyepiece of the microscope, then calibrated against a standard stage micrometer, permitting accurate measurement of a very fine degree. The screw micrometer, the eyepiece reticule, filar micrometers, and, of course, the stage micrometer, are all types of such equipment. Such measuring equipment can be used with essentially all types of microscopes -- the biological microscope, the shop microscope, comparison microscopes, etc.

MICROSCOPE LIGHTING

While we have discussed, to some extent, substage microscope lighting, we have yet to deal with reflected light illumination. Transmitted light works well for transparent or semi-transparent specimens, but it is a bit difficult to shine a light through such opaque material as pistol or rifle bullets, cartridge cases, or metal specimens.

Various means exist for vertical illumination of a specimen, and such devices are used extensively in metallurgical work. The Firearms Examiner, the Tool Mark Examiner, the Questioned Documents Examiner, and others, are more in need of lighting equipment that will direct the

illumination from an angle to better show detail caused by depressions or furrows in a specimen. In the past, both cold light (light from a fluorescent tube) and incandescent light have been used, and to good effect. In the recent past, however, light from an incandescent light source has been directed through a fiber optic bundle to the specimen. By "piping in" the light in this manner, one can separate the incandescent light sources from the immediate vicinity of the specimen, and eliminate the problems caused by heat.

One problem that may be occasioned by the use of fiber optic lighting is vibration. The incandescent light source must have an electric fan for cooling, and the vibration of the electric motor for the fan can cause noticeable vibration. I have found that this problem can be minimized by keeping the light source, or lamp housing, on some other surface having no physical contact with either the microscope or the fixture it is sitting upon. The manufacturers of such lighting equipment have devised bundles that are quite flexible and which absorb much of the vibration so that it does not affect the microscope to such a great degree, however, if you are working under any great amount of magnification, the slightest vibration is all that it takes to foul things up. Complete isolation is your best bet.

Balance of Lighting

When you may be using two separate light sources, such as would be the case with the comparison microscope, the light must not only strike the specimens at the same relative angle, but the lighting must be balances -- the same general degree of brightness. Where possible, it is best to adjust your lighting by use of an iris diaphragm. When light is balanced by use of rheostats, you could run into problems with photography, due to the changing of color temperature.

CONCLUSION

While I have attempted to cover the greater part of the basics of the field of optics, I realize that I have but scratched the surface. There has been much of a basic nature that has been left out of this article, as there was just not the space to include it all. The field of optics is not only a complex one, it can be broken down into so many different areas, no one of which could be given adequate coverage in a short article such as this.

If you use a microscope in your daily pursuits, however, it is almost mandatory that you have knowledge of at least the basics of the optical theory involved, and the same would apply to any of the other optical equipment one might use. Not only will such basic knowledge enable you to realize some of the capabilities and limitations of your equipment, but it will enable you to better explain the equipment and its use should you be called upon to do so -- and in courts of law, this can happen. In courts of law, if one does not know the answer to a specific question, he should admit that he does not have that information -- too many "I don't know's", however, can plant the seed in the minds of the court and jury that perhaps you don't know your field of expertise as well as you might.

I would advise retaining this material for review from time to time. I would also advise the reader to compile a brief glossary of terminology to accompany this material -- to include such terms as Virtual Image, Aerial Image, and the like. The time may come when you may have need of such information.

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PAM'S

JOY OF COOKING AND OTHER CULINARY FEATS WITH APPENDICES PERTAINING TO WITCHCRAFT AND SHAMANISM

VOLUME 1. BUCKETOLOGY

PREFACE

This manual was developed as a criteria for accreditation by the Association of Crime Laboratory Directors. The manual covers the topics relating to analysis methods used within the State of Idaho Crime Laboratory System. It is written to be a training manual for the incoming criminalist as well as a reference to the analyst already at the bench.

The format consists of an introduction, a guidelines and/or methods section, pertinent reagent data, and references. The introduction gives the reader a basis from which to start, and the references are listed for more concentrated study. For the incoming criminalist, these are to be used to develop a sound background in the method. The guidelines and reagent data are more specific to our particular system.

An analytical manual accompanies this manual, and deals with classes of drugs or a particular drug analyzed by the laboratory.

VISUALIZATION REAGENT RECIPES

The following information lists the reagents presently being used to visualize compounds within the laboratory system. Should unusual unknowns or chemicals be found the references list systems and reagents that may be used.

IODOPLATINATE SPRAY ACIDIFIED

.25 grams platinic chloride and 5 grams of KI in sufficient H_2O to produce 100 ml. Add 2.0 ml. concentrated HCl to the 100 ml.

Or)

Take 2.5 ml. of 10% ageous platinic chloride and add 5 grams of KI and sufficient H_2O to produce 100 ml. Add 2 ml. of conc. HCl.

FLUORESCAMINE SPRAY

1. 5 mg/100 ml in acetone
   Journal of Chromatography Vol 104 (1973) 202

2. 50 mg/100 ml in acetone
   Journal of Forensic Sciences Vol 21, No 1, Jan 1976 154

Keep in refrigerator

p-DMAB-TS (for indoles)

To a cool solution of 65 ml. of Conc. H_2SO_4 in 35 ml. of H_2O,
Add 125 mg. of p-DMAB.
Dissolve, add 1-2 drops of $FeCL_3$-TS.

Handbook of Analytical Toxicology, pg. 408
Sunshine, 1969

Ninhydrin Visualization Reagent

.3% (300 mg.) solution in acetone or chloroform.

Isolation and Identification of Drugs, pg. 802
Clark

Mercurous Nitrate Spray for Barbiturates

5.0 ml. conc. HNO_3 in 66 ml. water.
To this add, 13.0 g. of $HgNO_3$

ETHANOLIC p'DMAB

Dissolve 2 g. of p-DMAB in 50 ml. ethanol, make up to 100 ml. with HCl.

Reference: Handbook of Analytical Toxicology
Sunshine, 1969

IODOPLATINATE SPRAY

Dissolve 0.25 grams of platinic chloride and 5 grams of KI in sufficient water to make 100 ml.

If alkaline solvents are used on TLC plates, use Acidified Iodoplatinate spray.
Add 2 ml. of HCl to 100 ml. of above spray.

Isolation and Identification of Drugs, pg. 801
EGC Clarke

DRAGENDORFF'S REAGENT (470 ml): 2.6 grams of bismuth subnitrate in 120 ml. water plus 30 ml. glacial acetic acid are added to 24 grams potassium iodide in 60 ml. of water. The mixture is diluted with 200 ml. of water and 50 ml. glacial acetic acid. It is important that the potassium iodide is completely dissolved before the bismuth subnitrate - water - acetic acid mixture is added.

DUQUENOIS REAGENT

1. Dissolve 5 drops of Acetaldehyde and 0.4 grams vanillin in 20 ml. of 95% alcohol.

or) 1.5 ml. of acetaldehyde and 12 grams of vanillin in 600 ml. of 95% alcohol

or)

2. Dissolve 12 drops acetaldehyde (fresh) and 1 g. vanillin in 50 ml. alcohol.

AOAC Methods of Analysis, 11th Ed. 1970, pg. 705

IRS Manual, pg.136

Ethanolic p-DMAB Spot Test

Dissolve 2 g. of p-dimethylaminobenzaldehyde in 50 ml. ethanol, qs to 100 ml. with HCl.

REFERENCE: Handbook of Analytical Toxicology, Sunshine, 1969

Steriod TLC Visualizing Spray

2,3,5-Triphenyl-2H-tetrazolium chloride freshly prepared 1:1 mixutre of 4% reagent solution in CH_3OH and 1M NaOH.

Heat.

Reference: J.T. Baker Catalogue

REAGENT RECIPES

The following recipes are for reagents presently being used within the laboratory system. A more definitive treatise relating to drugs is Fulton's Modern Microcrystal Tests for Drugs.

Reagents should be made up and maintained in glassware and in most cases should be kept away from plastic or rubber products during shelf storage and use. Each reagent should be properly labeled with reagent name, and date that it was made. If discoloration of reagents occurs or it does not react as it should to a standard drug , then the reagent should be remade. Normal shelf-life for these reagents should not exceed 6 months.

5% Platinic chloride in diluted Phosphoric Acid

Dissolve 1 gram $H_2P+C16 \cdot 6H_20$ in 20 ml. H_3PO_4 (1+3) or 15 grains $H_2P+Cl_6 \cdot 6H_20$ in 1914 ml. H_3PO_4 (1+3)

Methods of Analysis A.O.A.C., 10th Ed., Pg. 597

$HAuBr_4$ in (2 + 3) H_2SO_4 (30)

$HAuCl_4 \cdot 3H_2O$ crystals, 1 gram; HBr 40%, 1.5 ml.; (2 + 3) H_2SO_4 (30) (Enough (2 + 3) H_2SO_4 (2 parts concentrated H_2SO_4 + 3 parts H_2O) to bring total volume to 30 ml.)

Modern Microcrystalline Tests for Drugs
Fulton pp. 383 - 386

$HAuBr_4$ in HOAc - (2+3) H_2SO_4 (60)

Mix equal volumes of glacial acetic acid and $HAuBr_4$ in (2+3) H_2SO_4 (30)

$HAuBR_4$ in (2+3) H_2SO_4 (30)

$HAuCl_4 \cdot 3H_2O$ crystals, 1 gram; HBR 40%, 1.5 ml.; (2+3) H_2SO_4 (30) (Enough (2+3) H_2SO_4 (2 parts conc. H_2SO_4 + 3 parts H_2O) to bring total volume to 30 ml.)

Modern Microcrystalline Tests for Drugs,
Fulton pp. 383 - 386

Gold Chloride in dil. phosphoric acid
(for sympathomimetics)

1 g $HAuCl_4 \cdot 3H_2O$ dissolved in 20 ml. H_3PO_4(1+2)

Methods of Analysis A.O.A C., pg. 597

Gold Chloride in diluted Phosphoric Acid

Dissolve 1 gram $HAuCL_4 \cdot 3H_2O$ in 20 ml. H_3PO_4 (1+2):
15 grains $NAuCl_4 \cdot 3H_2O$ in 19.4 ml. of H_3PO_4(1+2)

Methods of Analysis, A.O.A.C. 10th Ed., p. 597

Wagenaar's Reagent (for microcrystalline tests)

Add ethylenediamine to a 5% solution of copper sulfate until the solution becomes a dark violet in color.

IRS "Methods of Analysis", pg. 137

Iodine-Potassium Iodide Solution
(For Microcrystalline test on barbiturates)

Dissolve 5 g. I and 80 g. KI in enough H_2O (78 ml.) to make 100 ml. Dilute with 2 parts by volume of H_3PO_4 (not less than 85%).

Methods of Analysis of the A.O.A.C., New Ed., pg. 711

REAGENT RECIPES

The following recipes are for spot test reagents used by the laboratory system.

FROEHDE'S REAGENT

Dissolve 50 milligrams of molybdic acid or sodium molybdate in 10 ml. hot concentrated sulfuric acid.
Takes a while to dissolve.

IRS Manual, Pg. 136

MARQUIS'S REAGENT

Add 8-10 drops of 40% formaldehyde solution to 10 ml. pure concentrated sulfuric acid. (approximately 1 ml. formaldehyde/20 ml. H_2SO_4) (Make up frequently)

Methods of Analysis for Alkaloids, etc.
Internal Revenue Service, Publication 341, pg. 136

SANCHEZ REAGENT

1. A saturated aqueous solution of furfural slightly acidified with acetic acid.

   Methods of Analysis, I.R.S. Publication #341, pg. 124

2. | | |
   |---|---|
   | Furfural (freshly distilled) | 2.5 ml. |
   | Ethanol 95% | 22.5 ml. |
   | Glacial acetic acid | 75 ml. |

   Microgram, Vol. V, No. 5, P. 49

3. 10 drops furfural
   10 ml. glacial acetic

Spot Tests in Organic Analysis, Pg. 249
Feigl

RUYBAL'S REAGENT FOR COCAINE AND PROCAINE

A) Cobalt thiocyanate (2% aqueous solution)

B) Phosphoric Acid Syrupy H_3PO_4 (85%)

C) Platinum Chloride 1 gram $H_2PtCl_6 \cdot 6H_2O$ in 20 ml. H_3PO_4(1+3)

Reagent: Mix 9 parts A, 3 parts B, 1 part C

To use: Allow crystals to settle out, but do not remove from the reagent. Take 3 drops of supernatant reagent, add to sample, then add 2 drops of water and stir.

Microgram, Feb. 1972 and Feb. 1973

MECKE'S REAGENT:

Dissolve 0.5 grams selenious (selenous) acid in 50 ml. concentrated H_2SO_4.

IRS Manual, Pg. 136

SCOTT'S REAGENT

#1 2% Cobalt Thiocyanate dissolved in water and then diluted 1:1 with 96% glycerine

#2 Concentrated HCl

#3 $CHCl_3$

PROCEDURE

Step 1: Place a small amount of suspected cocaine in a test tube, add 5 drops Solution #1 and shake. If cocaine is present, a blue color develops at once. If a blue color is not seen, add more sample. If a blue color still does not develop, the sample does not contain cocaine.

Step 2: Add 1 drop of Solution #2 and shake. The blue will disappear and a clear pink solution is seen. If all the blue does not disappear, add a second drop (no more) of HCl and shake.

Step 3: Add several drops of Solution #3 (chloroform) and shake. The $CHCl_3$ layer will develop an intense blue color if cocaine is present.

Microgram, November, 1973

LIEBERMANN'S REAGENT

Dissolve 10 grams of KNO_2 in sufficient concentrated H_2SO_4 to produce 100 ml.

CAUTION: KNO_2 reacts violently with the H_2SO_4; MIX VERY CAUTIOUSLY.

Isolation and Identification of Drugs, Clarke, p. 801

ZWIKKERS:

A. .5% $CuSO_4$: .781 grams $CuSO_4 \cdot 5H_2O$ per 100 ml.

B. 5% by volume pyridine in $CHCl_3$

Methods of Analysis, I.R.S. Manual No. 341, p. 107

DILLE-KOPPANYII TEST

1. Cobalt acetate (cobaltous acetate, tetrahydrate) 0.1 g. dissolved in 100 ml. of absolute methanol plus 0.2 ml. of glacial acetic acid.
2. Isopropylamine - 5 ml. dissolved in 95 ml. of absolute methanol.

IRS MANUAL, pg. 106

MAYER'S REAGENT:

To 0.5 grams of mercuric chloride in 50 ml. of water, add just sufficient potassium iodide to redissolve the scarlet precipitate first produced. (First crystals produce precipitate which goes into solution as you swirl. Keep adding KI. Eventually get orange-red heavy precipitate. Keep adding KI. Eventually will clear to light straw color).

IRS Manual, pg. 136

SECONDARY AMINES

A) 1% solution of sodium nitroferricyanide to which 10% by volume of acetaldehyde is added.
Mixture must be freshly prepared.
Keep in refrigerator

B) 2% solution of sodium carbonate.

Spot Tests in Organic Analysis, pg. 251
Feigl

dibenzyl ketone

DEA LABORATORY NOTES

A FIELD TEST FOR PHENYL-2-PROPANONE

(= phenylacetone)

Wilmer O. Kiser
Southeast Regional Laboratory

Since the time Phenyl-2-propanone (P-2-P, Phenylacetone) was classified as a controlled substance, there has been a drastic increase in the number of clandestine P-2-P synthesis laboratories. Investigation and seizure of these laboratories has often been hampered by lack of a sensitive field test for P-2-P. Although the odor of P-2-P is very intense and recognizable at very low concentrations, magistrates and judges are generally reluctant to accept such identification as grounds for a search warrant.

A test for aliphatic ketones in Feigl's Spot Tests in Organic Analysis (1) has been modified to give a very sensitive test for P-2-P.

Reagents required:

Methanol

0.5% m-dinitrobenzene in methanol (Reagent I)

Saturated solution of potassium hydroxide in methanol (Reagent II)

Sample Preparation:

Liquids: No preparation; add 4 or 5 drops liquid to test tube and proceed.

Paper Towels, etc.: If contamination appears to be strong, tear off a small piece (ca 1/4" square) and place directly in test tube.

If contamination is not so strong, wet a piece of the questioned item with methanol then squeeze 4 or 5 drops of the methanol into the test tube.

Table Tops, Glassware, etc.: Drop a small amount of methanol on the object, then transfer (by dropper, pouring, etc.) 4 or 5 drops of the methanol to the test tube.

Testing:

Into one test tube place sample to be tested (see above). Into another test tube place 4 or 5 drops of methanol (Reagent Blank). To each test tube add 5 drops Reagent I. Mix. To each test tube add 10 drops Reagent II. Mix.

Interpretation:

Formation of purple color in sample tube within ca 5 seconds constitutes a positive test. The purple color progresses slowly to brown. The reagent blank produces a pink color; other negatives produce colors from pink to brownish orange.

Results of this test with some selected compounds are as follows:

Reagent Blank	Negative
Acetone	Negative
Amphetamine	Negative
Benzaldehyde	Negative
Cyclohexanone	Positive
Diazepam	Negative
Methadone	Negative
Methamphetamine	Negative
Methaqualone	Negative
Methylethylketone	Positive
N-Methylformamide	Negative
Phenylacetic Acid	Negative
Phenyl-1-propanone	Positive
Phenyl-2-propanone	Positive

REFERENCES

[1]Feigl, F. and Anger, V., Spot Tests in Organic Analysis, Seventh English Edition, Elsevier, New York, 1966, pp 206-207.

(3)

COLOR TEST FOR PHENCYCLIDINE AND METHADONE

Paul Zelonis
Forensic Chemist
Drug Enforcement Administration
Southwest Regional Laboratory

OBJECTIVE

To develop a field test possessing sensitivity and high specificity for use in powders, solvents, and plant material.

INTRODUCTION

This test was developed at the request of narcotics agents who desired a reliable field test for phencyclidine and its analogs. The commercial field test previously utilized by agents had failed on occasion to indicate the presence of phencyclidine and its analogs in powdered samples. Laboratory evaluation of the phencyclidine field test previously utilized was found to satisfactorily detect only the pure drug, and not the crude, unrefined material commonly encountered.

The Zelonis Test for phencyclidine and its analogs has enabled agents to successfully test varied materials at clandestine laboratory sites ranging from laboratory glassware residues and solvents, to treated plant material. This test can be utilized for field testing powders and liquids for methadone.

REAGENTS

Solution A - 1 gram Platinum Chloride (reagent grade)
20 ml water (deionized)
5 ml Glacial Acetic Acid

Solution B - 2 grams Cobaltous Thiocyanate
50 ml water (deionized)
50 ml Glycerin

Solution C - Benzene

Solutions A and B should be aged 24 hours, and shaken prior to use.

METHODOLOGY

1) Add 3 drops of Solution A to a test tube containing 5-10 mg of powder, 0.5-1.0 ml liquid, or 100-200 mg of plant material.

2) Add 5 drops (0.25 ml) of Solution B and shake for 5 seconds.

3) Add 10 drops of Solution C and shake vigorously for 5 seconds.

   (A) The top benzene layer will turn a clear blue color for powders and liquids containing Phencyclidine or its analogs, and Methadone.

   (B) It is only necessary to wet the plant material and turn the test tube on a horizontal plane to note the blue color.

DISCUSSION

The volumes of Solutions B and C cited are approximations and are not critical for obtaining satisfactory results. If desired, larger volumes of Solutions B and C can be utilized when testing plant material without increasing the volume of Solution A. After utilizing the prescribed methodology with 120 controlled and non-controlled substances, it was found that the clear blue color also developed with methadone and dibucaine. A blue cloudy opaque benzene layer also developed with propoxyphene, bromodiphenhydramine, and PCC, a phencyclidine intermediate. Larger amounts of powder sample (100 mg) will turn the phencyclidine intermediate (PCC) from a cloudy blue to a distinct green within 10 minutes. For screening purposes, it should be noted that phencyclidine and methadone do not develop a Marquis reaction, which maybe used to differentiate them from propoxyphene and bromodiphenhydramine.

Although Solution A enhances the specificity of the test, it can be omitted without seriously jeopardizing the degree of specificity. Although this modification of the test does not have the same specificity of the recommended three reagent procedure, it is superior to other phencyclidine field test presently in use.

One ounce of reagent grade Platinum Chloride is sufficient for the preparation of approximately 5000 test. The sensitivity of the three reagent procedure is 0.5 mg of Phencyclidine powder.

CONCLUSION

No problems have been encountered by the Los Angeles Clandestine Laboratory group utilizing the recommended three reagent test. The test has gained agent acceptance and referral as being suitable for use by law enforcement officiers.

Retention Indices for Compound Identification by Gas Chromatography *

*Leo Kazyak, B.S.*** *and Robert Permisohn, B.S.***

Progress in gas chromatography over the past deca probably best demonstrated in the remarkable number variety of applications of this technique. Although Jame Martin (1) had described the separation of fatty acids b chromatography twenty years ago, most applications i decade that followed were limited to more volatile subst such as low molecular weight petroleum hydrocarbons, alc aldehydes, and aromatics. However, during the early 1960' success of VandenHeuvel and his associates (2) with gas matographic separations of steroids and alkaloids (3) was followed by success in separations of drugs (4, 5, 6). Ap tions in analytical toxicology were published as early as (7). What followed during the next few years is well kno everyone in the field of analytical chemistry as diverse such as sugars (8), pesticides (9), metals (10), and ba (11) were characterized or identified by gas chromatogr separations.

Paradoxically, generally accepted practices for reporting seem to defy standardization. Many are frustrated in attempts to reproduce published retention times until the ready to compromise and settle for an approximation. N theless, some qualitative and reliable reference data shou

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possible to obtain with a technique characterized by excellent reproducibility; otherwise, everyone applying gas chromatography applications will be confronted with the time-consuming task of compiling his own reference information.

The difficulty in duplicating reported data can be attributed frequently to variations in operating parameters. Small changes in conditions can be responsible for some rather profound effects; e. g., a temperature change of only one degree can produce as much as a five percent shift in retention time. Since differences can be observed in the performance of columns prepared similarly—the eddy diffusion coefficient (Van Deemter equation) tends to vary between columns—attempts to adjust conditions so that retention times can be reproduced often serve to increase frustrations. Nevertheless, identification of components in a chromatogram is essential to the toxicologist. Moreover, this consideration assumes greater proportions at a time when attention is focused on computerized, automated procedures. If computers are to be intrusted with the task of compound identification by gas chromatography, the approach must be as impersonal as possible and independent of variation. Personal experience and interpretation cannot be utilized except to conceive and perfect programs intended to accomplish the identification.

Results are optimum when conditions approach the ideal, and in the case of gas chromatography, the distribution isotherm is linear, i. e., the distribution of a component in one phase is always proportional to the concentration of that component in the other phase. Peak symmetry is the most obvious manifestation of a linear isotherm. However, skewed peaks are all too familiar to those engaged in toxicological applications where the variety of compounds is so great and their properties so divergent. This does not necessarily reduce the value of gas chromatography or limit its application. Notice in Fig. 1 the effect of concentration on peak geometry and retention time. If concentration is plotted against the logarithm of the retention time, the plot is linear and can be extrapolated to the intersection of the ordinate; i. e., infinite dilution (zero concentration). In this way the retention time becomes independent of concentration, although other conditions will continue to exert their effects.

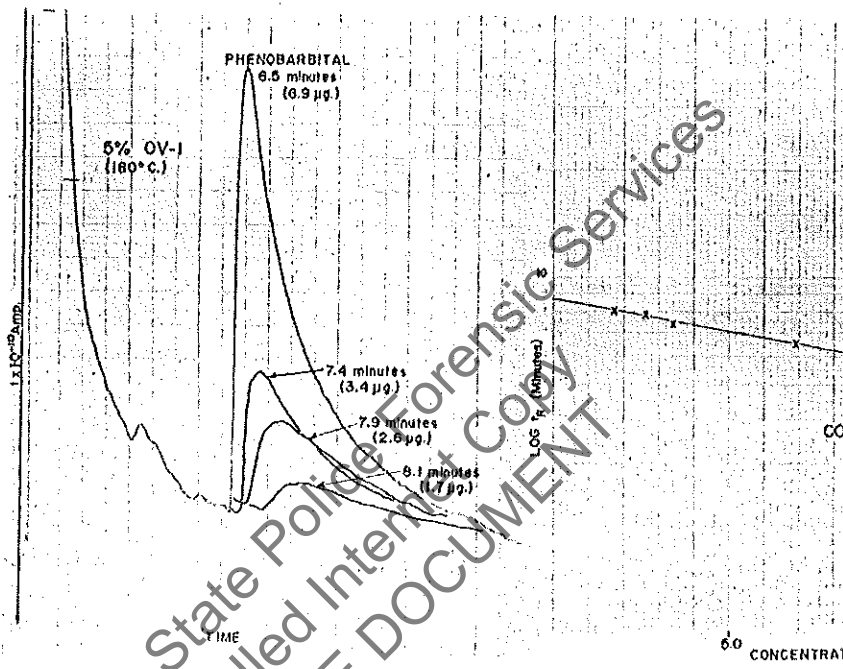

Fig. 1—Effect of concentration on retention time.

If the retention time is related to some standard compou group of compounds, the influence of operating paran variation can be reduced. Thus, temperature and flowrate tuations affect the standard as well as the other compo being chromatographed, and the effect of the changes are mized. Relative retention data (Fig. 2) are now preferre many for reporting gas chromatographic information. proper selection of a compound to serve as a standard, how is critical, since ratios near unity are usually more accur reproduced. The nearer the retention time of the standard that of the compound in question, the more likely are the pounds to be affected to the same extent. If the reference pounds are non-ideal in that peak symmetry cannot alway attained, their usefulness may be limited. Furthermore, r ence compounds must be readily available and sufficiently fied to provide unambiguous data. The decision to avoid an trary or subjective approach to reporting of retention times

to the investigation of a system proposed by Kováts (12) that has gained considerable attention since it was first introduced several years ago.

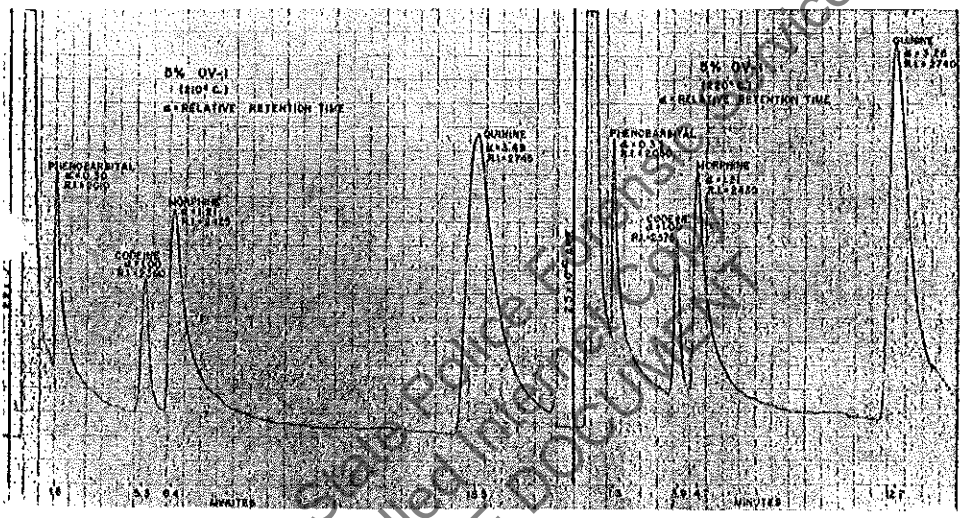

Fig. 2—Comparison of relative retention time to retention index at different temperatures.

Kováts' Index, or Retention Index as it is now called, is based on the relationship of carbon number to the logarithm of the retention time (or retention volume), demonstrated first by James and Martin (1). If the logarithms of the retention times for members of a homologous series of organic compounds, such as are shown in Fig. 3, are plotted against their respective carbon number, the resultant plot is linear. This finding has proven useful in relating or identifying unknown members of a homologous series as well as for predicting retention times of compounds in such a series. Comparative characteristics of different column liquid phases are evident from this kind of plot, viz. QF–1 and OV–1 or OV–17. The greater amount of QF–1 liquid phase at 115° C. produces retention times for the aliphatic hydrocarbons comparable to less OV–1 or OV–17 at 180° C. This difference reflects the greater polarity of the QF–1 (fluorosilicon polymer).

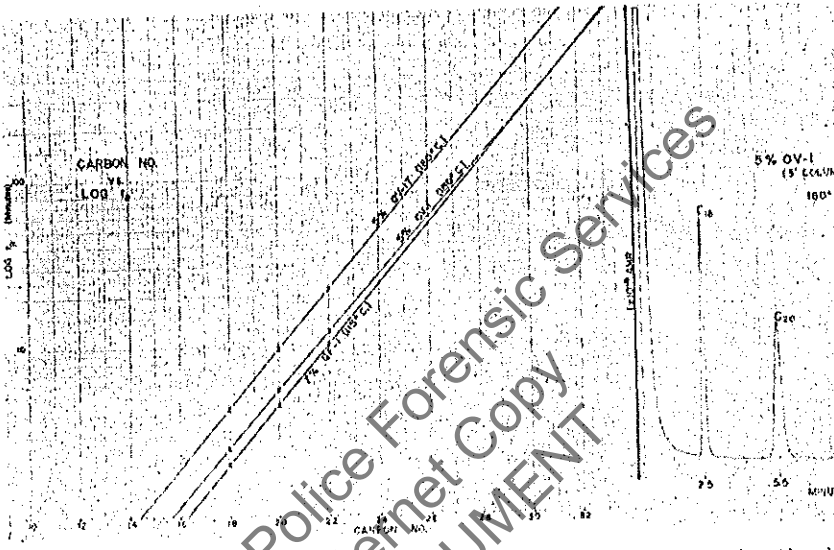

Fig. 3—Relationship of carbon number to log t_R (retention time)

The relationship of carbon number to the log of the ret time applies only to members of a homologous series, an restriction could severely limit the usefulness of such a l toxicological applications where comparatively few comp can be grouped in any kind of homologous series. Ho Kováts has proposed that compounds be related to a s series for the purpose of identification. To express this re ship, the abscissa of a carbon number vs. log of retentio plot is converted to retention index as in Fig. 4. Instea carbon number of twenty the abscissa now reads a retent dex of 2000 for the C_{20} hydrocarbon. Thereafter, any com is assigned a retention index related to this plot (or a s plot) by the log of its retention time. To identify a peak temperature or flowrate, it is necessary only to chromat a mixture of two or three aliphatic hydrocarbons to be construct the corresponding plot and determine the re index, e. g., phenobarbital in Fig. 4. The apparent adv is that regardless of the choice of liquid phase concent

temperature, or flowrate, retention data compiled in this manner are constant and can be standardized to give accurate information.

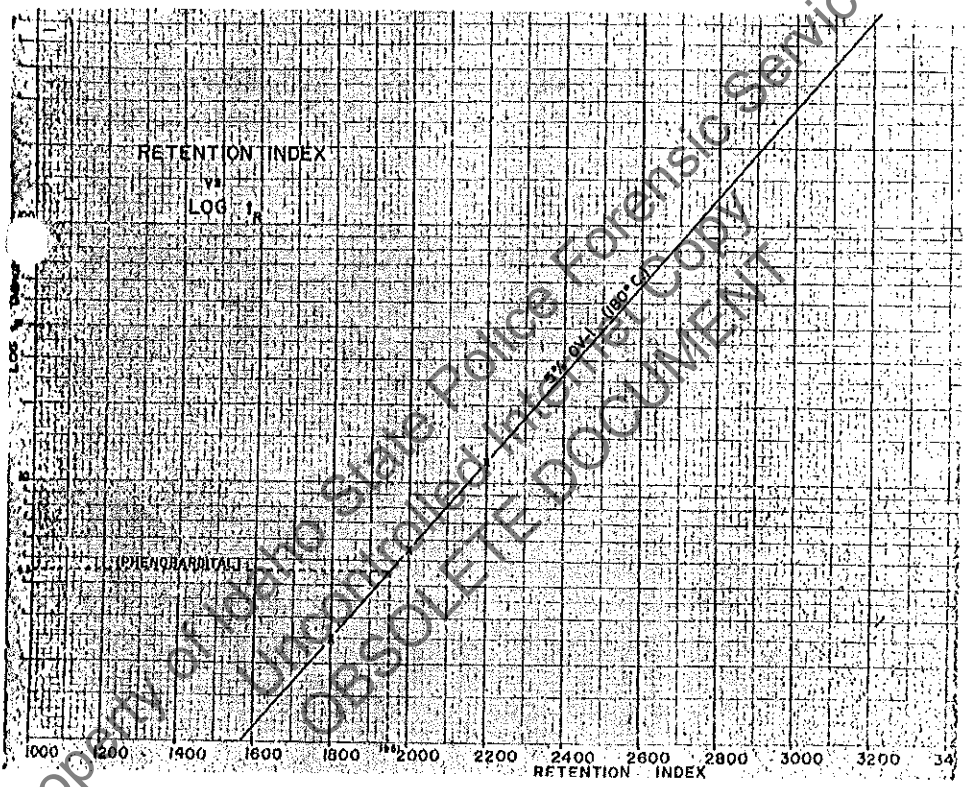

Fig. 4—Retention index plot.

Drugs, insecticides, and alkaloids have been compiled (Table I) on three columns, OV–1, OV–17, and QF–1. If retention indices are obtained on more than one column the reliability of the identification is greater. In effect, the three columns provide a tridimensional matrix for more definitive identification.

TABLE I

COMPOUND	RETENTION INDEX* OV-1	OV-17	OF-1
ACETAMINOPHEN	1810	2190	2270
ACETOPHENETIDIN	1660	2035	2425
ACETYLSALICYLIC ACID	1260	1480	1808
ACRIFLAVINE	2280	2650	0
ALORAMIC (THIOPENTAL)	1950	2300	2150
AMBODRYL	2150	2465	2460
9-AMINOACRIDINE	1950	2425	2830
AMITRIPTYLINE	1990	2300	2600
AMPHETAMINE	1118	1390	1410
AMYTAL	1725	1985	2350
ANILERIDINE	2845	2440	3550
ANTIPYRINE	1830	2310	2930
ANTISTINE	2330	2820	3060
ARTANE	2230	2590	2470
ATARAX	2840	2100	3110
ATROPINE	2175	2555	2700
AZACYCLONOL	2210	2700	2510
BARBITAL	1495	1780	2105
BENADRYL	1855	2135	2110
BENACTYZINE	2230	2665	2640
BENZYLAMINE	33	1210	33
BONAMINE	3000	3480	3390
BRUCINE	3280	3740	4480
BUCLIZINE	3285	3720	3630
BUTISOL	1780	1955	2280
CAFFEINE	1810	2265	2530
CANTHARIDIN	1490	1870	2930
CAPIOSIAMINE	2775	3210	0
CARBROMAL	1500	1790	2020
CARPHENAZINE (PROKETAZINE)	0	3150	0
CELONTIN	1595	1960	2260
CHLORBENSIDE	2050	2440	2400
CHLORCYCLIZINE	2215	2535	2240
CHLORDANE	2520	2180	2330
CHLOROQUINE	2575	2885	3345
CHLORPHENTERMINE	1320	1530	1610
CHLORPROMAZINE	2440	2890	3090
CHLORTRIMETON	2000	2265	2315
CINCHONIDINE	2625	3065	3260
CINCHONINE	2575	2550	3230
COCAINE	2375	2760	2885
CODEINE	2385	2820	2890

COMPOUND	RETENTION INDEX* OV-1	OV-17	OF-1
BETA- 3,4-DIMETHOXY -PHENETHYLAMINE	1540	1850	2070
DIMETHRIN	1210	1430	1770
N,N-DIMETHYL ANILINE	1030	1260	1310
DIMETHYLPHENETHYLAMINE	1160	1270	1350
DIONIN	2415	2110	2990
DIPHENYLPYRALINE	0	2765	0
DORIDEN	1825	2420	2535
ECIVLUREA (MOSIAL)	1360	1680	1820
EPHEDRINE	1350	1590	1680
ETHOXYQUIN (SANTOQUIN)	2800	3260	3120
N-ETHYL AMPHETAMINE	1210	1360	1390
N-ETHYL BENZYLAMINE	1120	1290	1300
FLUPHENAZINE (PROLIXIN)	0	3590	0
FORMISTAL	2220	2625	2620
HEROIN	2615	3050	3135
HOMATROPINE	2045	2440	2565
HYDRASTINE	2975	3680	4030
HYDRASTININE	1590	2400	2790
HYOSCYAMINE	2225	2580	2695
IPRAL	1550	1850	2175
IPRONIAZID	1700	1890	0
ISONIAZID	1630	1900	2305
LIBRIUM	2520	2650	2735
LISTICA	1740	2090	2245
LOBELINE	1780	2090	2260
L.S.D. (LYSERGIDE)	2465	0	0
MARPLAN (ISOCARBOXAZID)	1960	2160	0
MEBUTAMATE	1865	1859	2690
MECLIZINE	3050	3490	0
MELLARIL	3110	0	0
MEPAZINE	2550	3040	2940
MEPHENOXALONE	2120	2680	3260
MEPHENTERMINE	1240	1440	33
MEPROBAMATE	1790	2180	2630
MESANTOIN	1795	2185	2525
MESCALINE	1690	2030	2290
METHAMPHETAMINE	1170	1340	1420
METHADON	2170	2465	2465
METHAPYRILENE	1970	2290	2300
METHOPROMAZINE	2500	3010	2990
METHOXYCHLOR	2410	2850	2840
METHYL DIMETHOXYAMPHETAMINE (STP)	1620	1900	2220

COMPOUND	RETENTION INDEX OV-1	OV-17	QF-1	COMPOUND	OV-1	OV-17	QF-1
PENTAZOCINE	2265	2620	2610	THONZYLAMINE	2045	2585	2520
PENTOBARBITAL	1750	2030	2385	TOFRANIL	2220	2605	2570
PENTOTHAL (THIOPENTAL)	1850	2185	2360	TRASENTINE	2190	2580	2590
PERPHENAZINE	2200	2730	2510	TRANILCYPROMINE	1210	1430	1530
PHENCYCLIDINE (PCP)	1870	2140	2050	TRIMETHYLPHENETHYLAMINE	1240	1390	1440
PHENELZINE	0	1610	0	TRIDIONE	1100	1240	1570
PHENDIMETRAZINE	1440	1660	1700	TRIFLUPROMAZINE	2210	2580	0
ALPHA-PHENETHYLAMINE	1010	1230	93	TRIPROLIDINE	2250	2715	2540
BETA-PHENETHYLAMINE	1120	1310	1450	TRYPTAMINE	1710	2140	2320
PHENMETRAZINE	1930	1670	1720	TUAMINE	33	960	1130
PHENOBARBITAL	1950	2365	2705	VALMID	1360	1680	1770
PHENTERMINE	1130	1310	1390	WARFARIN	1460	1630	1890
PHENYLPROPANOLAMINE	1310	1500	1670	YOHIMBINE	3290	3790	0
PHENYL-2-PROPANONE	1310	1340	1520				
1-PHENYL-N-PROPYLAMINE	1130	1305	1810				
PHENYLTOLAMINE	2010	2250	2280				
PHYSOSTIGMINE	1810	2270	2940				
PICROTOXIN	2205	2990	3840				
PIPRADROL	2150	2570	247				
PIRACETAM	1030	1160	33				
PLAQUENIL	2660	2460	0				
PRIMAQUINE	2105	2755	0				
PROMIDONE	2250	2815	3240				
PROCAINE	1995	2410	2610				
PROMAZINE	2295	2765	2580				
PROMETHAZINE	2260	2700	2680				
PROPINAL	1920	2300	2530				
N-PROPYL AMPHETAMINE	1330	1460	1490				
PROPYL HEXEDRINE	1170	1270	33				
PYRIBENZAMINE	1960	2300	2290				
PYRIDOXAMINE	2000	2170	0				
PYRROBUTAMINE	2430	2830	0				
QUINIDINE	2760	2535	3190				
QUININE	2755	2530	3500				
SALICYLAMIDE	1460	1780	2070				
SALICYLIC ACID	1330	1550	0				
SANGUINARINE	2880	3990	3760				
SANTONINE	1945	2640	3425				
SCOPOLAMINE	2285	2440	3025				
SECONAL	1775	2070	2420				
SEVIN	1490	1780	1900				
SOMA (CARISOPRODOL)	1850	2250	2610				
SPARTEINE	1765	1985	2785				
STRIATRAN	33	1270	2000				
STRYCHNINE	3040	3760	3940				
SURITAL	1890	2240	2410				
TENUATE	1480	1705	1780				
TETRACAINE	2305	2600	2915				
THEBAINE	2925	3085	2930				
THIANTOIN	2145	2900	3225				
THIOPROPAZATE	0	2845	0				

* '0' INDICATES RETENTION INDEX HAS NOT BEEN DETERMINED.
'33' INDICATES NO RETENTION TIME, OR RETENTION INDEX IS BELOW 1000.

To demonstrate some applications, results from a cor
program based on this approach are included. Figure 5 sho
chromatogram of a residue from urine containing metham
mine that had been chromatographed on OV–1 and C
Several possibilities are indicated for the first peak. Am
mine, probably produced as an intermediate of methamphet
metabolism, was later confirmed by mass spectrometry wi
LKB 9000 gas chromatograph-mass spectrometer. The cor
was programmed to select those compounds in the comp
with indices in the range of ± 30 units of the index
unknown peak. The indices given in parentheses refer
compound selected, and are as they appear in the comp
(i. e., OV–1, OV–17, and QF–1), and can be compared to

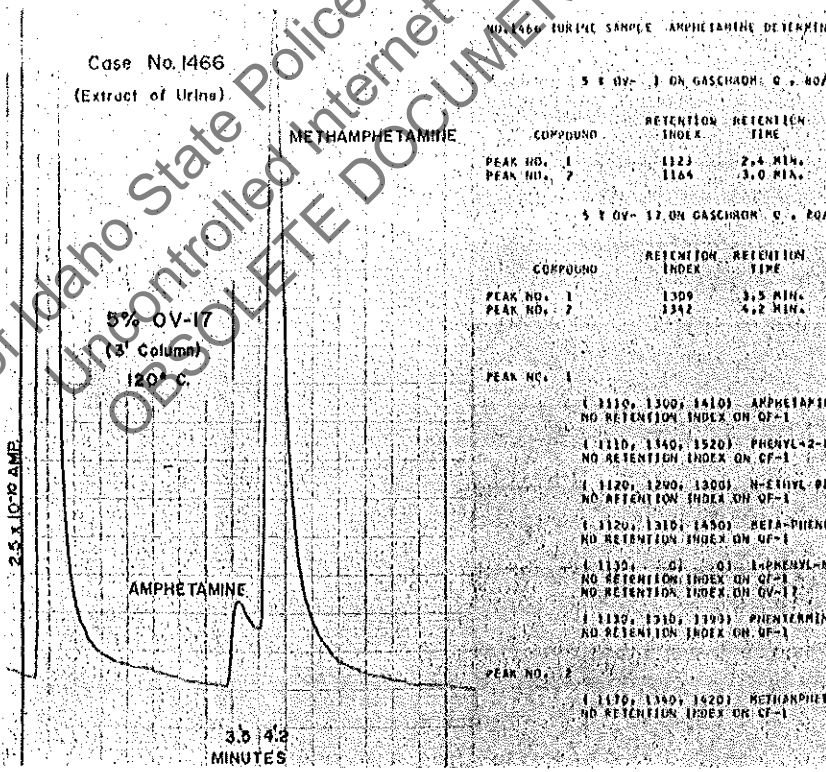

Fig. 5—Chromatogram and computer printout in an analysis of a
extract containing methamphetamine and another component.

of the unknown peaks indicated in the upper part of the computer printout. Identification of the second peak was limited to methamphetamine.

With retention indices from three columns, selectivity was considerably improved for a barbiturate identification (Fig. 6) where only secobarbital was selected. The second peak represents artificial data added to the chromatogram by increasing the retention time of the first peak two-tenths of a minute to test the selectivity of the computer program and the retention indices.

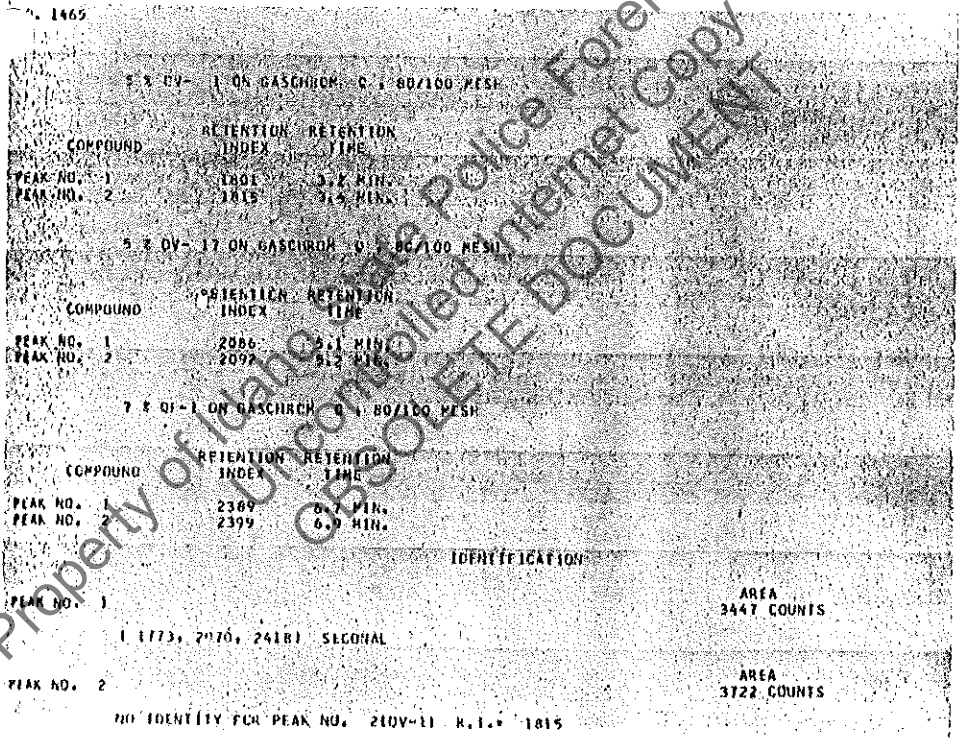

Fig. 6—Identification of secobarbital by retention indices and computer evaluation.

Summary

Operating parameters and column differences complicate standardization of gas chromatographic data based on retention time alone. Retention indices provide reference data independent

of these influences so that standardization is possible. Wi
standard indices from three columns that have different
acteristics of separability, significant improvement in iden
tion of compounds of toxicological importance is attainal

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REFERENCES

1. James, A. T., and J. P. Martin. Biochem. J., 50, 679 (1952).
2. VandenHeuvel, W. J. A., Sweeley, C. C., and E. C. Horning. J.
Chem. Soc., 82, 3481 (1960).
3. Lloyd, H. A., Fales, H. M., Highet, P. F., VandenHeuvel, W.
and W. C. Wildman. J. Amer. Chem. Soc., 82, 3791 (1960).
4. Fales, H. M., and J. J. Pisano. Anal. Biochem., 3, 337 (1962)
5. Kazyak, L., and E. C. Knoblock. Anal. Chem., 35, 1448 (196
6. Parker, K. D., Fontan, C. R., and P. L. Kirk. Anal. Chem.,
(1962).
7. Parker, K. D., and P. L. Kirk. Anal. Chem., 33, 1378 (1961)
8. VandenHeuvel, W. J. A., and E. D. Horning. Biochem. Biophy
Commun., 4, 399 (1961).
9. Watts, J. O., and A. K. Klein. J. Assoc. Offic. Agr. Chemists,
(1962).
10. Ross, W. D. and G. Wheeler, Jr. Anal. Chem., 36, 266 (1964)
11. Mitruka, B. M., Norcross, N. L., and M. Alexander. Appl. Mic
16, 1093 (1968).
12. Kováts, E. Helv. Chim. Acta., 41, 1915 (1958).

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REFERENCES

Basic Gas Chromatography, H.M. McNair and E.J. Bonnelli, Varian Aerograph, 1967.

Gasic Gas Chromatography, H.M. McNair, A.C.S. Audio Course, Washington, D.C., 1984.

Chromatography, 3rd Ed., E. Heftmann, Ed., Van Nostrand Reinhold Co., New York, 1975.

Clarke's Isolation and Identification of Drugs, A.C. Moffat, Ed., Pharmaceutical Press, London, 1986.

Analysis of Drugs, Analytical Manual, S.P. Sobol and R.A. Moore, U.S. Government Printing Office, Washington, D.C., 1974.

Gas Chromatographic Retention Indices of Toxicologically Relevant Substances on SE-30 or OV-1, 2nd Ed., R. Ardrey, Ed., V.C.H. Publishers, Weinheim, Germany, 1985.

Organic Functional Group Analysis by Gas Chromatography, T.S. Ma and A.A. Ladas, Academic Press, London, 1976.

Drug Analysis by Gas Chromatography, D.B. Jack, Academic Press, Orlando, Fla., 1984.

Handbook of Derivatives for Chromatography, K. Blau and G.S. King, Heyden & Son, Ltd., London, 1978.

REFERENCES

Thin Layer Chromatography, V.W. Rodwell, A.C.S. Audio Course, Washington, D.C., 1975.

Chromatography, 3rd Ed., E. Heftmann, Ed., Van Norstrand Rheinhold, Co., New York, 1975.

Clarke's Isolation and Identification of Drugs, A.C. Moffat, Ed., Pharmaceutical Press, London, 1986.

Clinical Laboratory Science, Section B: Toxicology, Vol. 1, I. Sunshine, Ed., CRC Press, W. Palm Beach, Fla., 1978.

Analysis of Drugs, Analytical Manual, S.P. Sobol and R.A. Moore, U.S. Government Printing Office, Washington, D.C., 1974.

Drug Analysis by Chromatography and Microscopy, E. Stahl, Ed., Ann Arbor Science Pub., Inc., 1973.

REFERENCES

Polarized Light Microscopy, W.C. McCrone, L.B. McCrone, and J. G. Delly, Microscope Publications, Chicago, 1984.

Modern Microcrystal Tests for Drugs, C.C. Fulton, Wiley-Interscience, New York, 1969.

Handbook of Chemical Microscopy, Vol. 1, E.M. Chamot, John Wiley and Sons, Inc., New York, 1958.

Crystals and the Polarizing Microscope, N.H. Hartshorne and A. Stuart, Edward Arnold Ltd., London, 1970.

Methods of Analysis, I.R.S., A.P. Mathers, U.S. Government Printing Office, Washington, D.C., 1970.

Official Methods of Analysis of the A.O.A.C., 10th Ed., W. Horwitz, Ed., A.O.A.C., Washington, D.C., 1965.

REFERENCES

Qualitative Analysis by Spot Tests, F. Feigl, Elsevier Publishing, Amsterdam, 1939.

Spot Tests in Organic Analysis, F. Feigl, Elsevier Publishing, Amsterdam, 1966.

Clarke's Isolation and Identification of Drugs, A.C. Moffat, Ed. The Pharmaceutical Press, 1986.

REAGENT REMAKE SCHEDULE

In most cases, reagents for spot tests have a shelf-life of approximately 6 months. There are some exceptions and those are outlined below.

Reagents for spot test should be made and kept in glass labware. Reagent bottles should be clean, labeled, and dated as to time of makeup. Avoid contact of reagents with plastic or rubber products during storage and dispensing as much as possible. This will lengthen shelf-life.

Shelf-life for any reagent should not exceed 6 months, and some reagents should be remade much more often. The following reagents should be made up at least bimonthly:

Marquis
Mecke
Froehde
Secondary Amine
Ruybal
Dille-Koppanyi

If discoloration of reagents occurs, or after testing against standards, the reaction is not as expected, reagents must be remade. After make-up, reagents must be tested against known compounds, and give the required reactions, before they may be placed out for use.

TABLE OF CONTENTS FOR SPOT TESTS

INTRODUCTION

Many drugs give distinctive color reactions when particular chemical reagents are brought in contact with them. These reactions may signify a particular class of compounds or may be specific to a relative few.

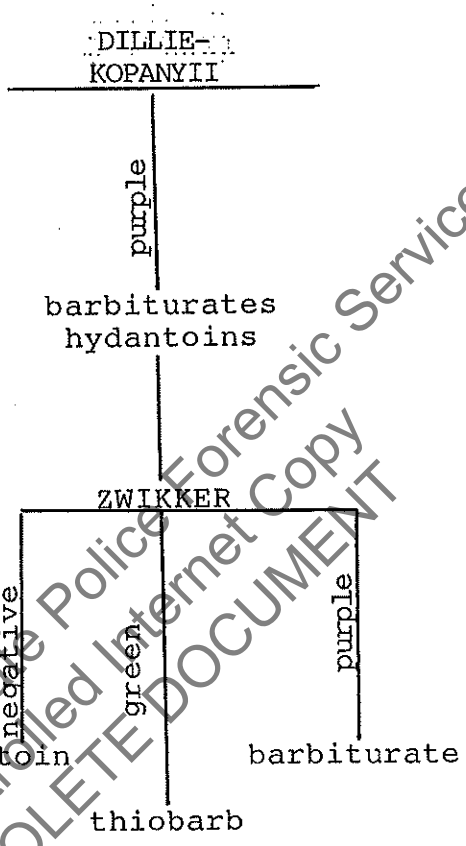
This section addresses spot test reagents used within the laboratory, to narrow down the number of controlled chemicals that may be in submitted samples. These tests are not confirmatory in themselves, and must be used in conjunction with more specific procedures. But they offer the analyst clues as to what is present and ultimately, ways to differentially separate mixtures of compounds.

Before actual testing or laboratory work begins the analyst should spend time reviewing the listed references. These references offer specific protocol to follow and background on this particular testing method.

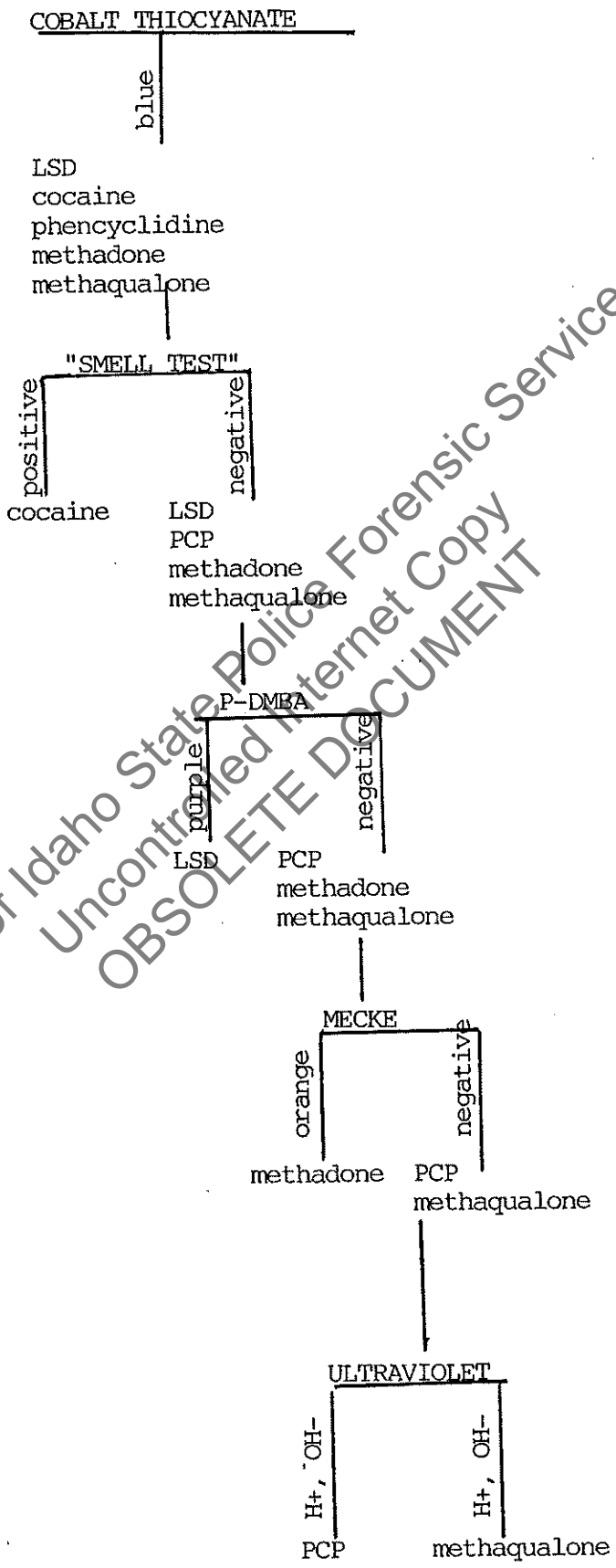
SCHEME FOR TESTING

The following is a guideline for the analyst involving a testing scheme for a particular compound using various spot test reagents.

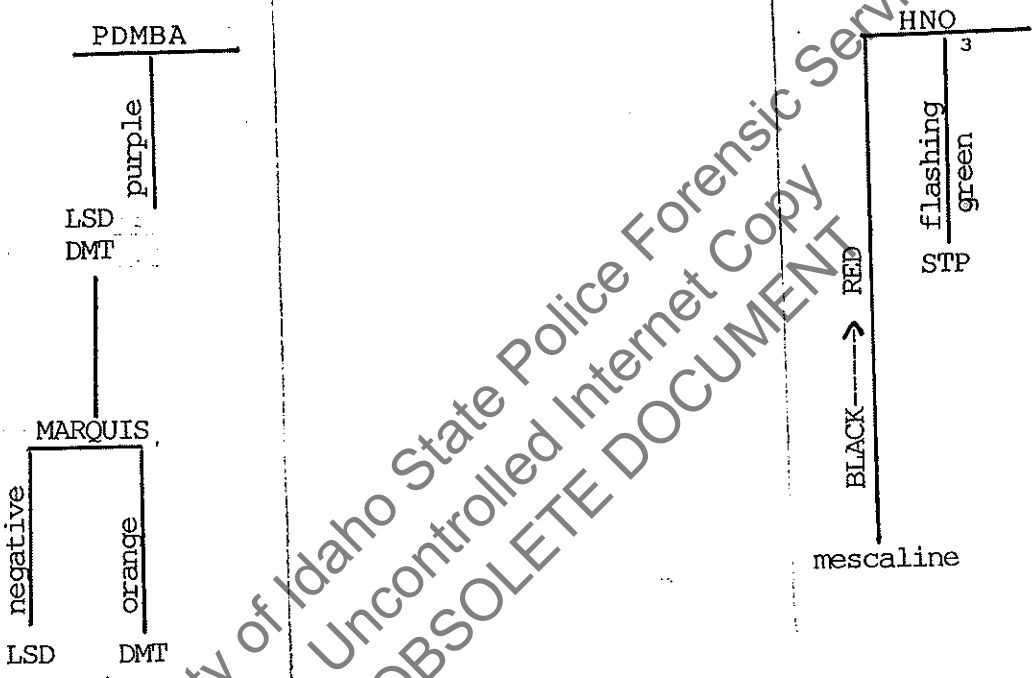
CONTROLLED SUBSTANCE
SPOT TEST
ANALYSIS SCHEME



CONTROLLED SUBSTANCE
SPOT TEST
ANALYSIS SCHEME



CONTROLLED SUBSTANCE
SPOT TEST
ANALYSIS SCHEME



CONTROLLED SUBSTANCE SPOT TEST ANALYSIS SCHEME

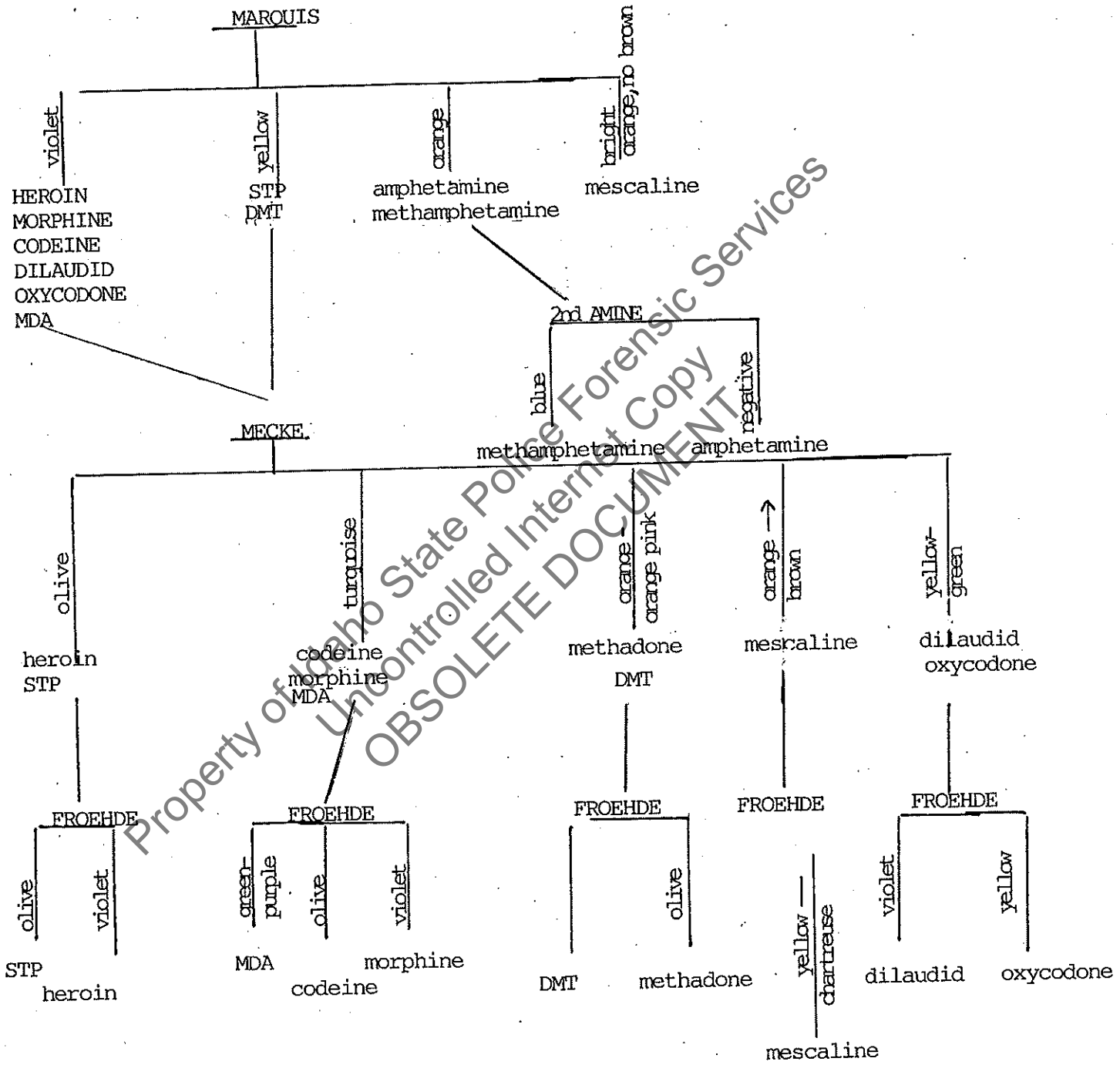


TABLE OF CONTENTS FOR THIN LAYER CHROMATOGRAPHY

INTRODUCTION

Thin layer chromatography is a technique used to separate drugs from one another or from other components in a mixture. It is applicable to biological samples, pharmaceutical formulations, and unknown liquids and powders. As used by our laboratory system, it is primarily a screening technique. However instances will arise when this method is the sole technique used to identify a particular compound. An added feature of TLC, is that of being able to recover a separated compound for further analysis, should it be needed.

This section covers thin layer chromatography as used within the laboratory. The references offer a starting point for the analyst to begin to study this technique. Because of the breadth of this topic, the theory and technique will be left to the reader.

The analyst should research the TLC systems to use when a particular compound is suggested by previous testing. The last four references offer information in this area, for both classes of compounds and particular drugs.