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STATE OF IDAHO

DEPARTMENT OF HEALTH AND WELFARE

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I.R. methodology for methamphetamine and phentermine used in the central lab.

making HCl salt

1. Acid base extraction method: The methamphetamine or phentermine is extracted from a basic aqueous solution into CH_2Cl_2 and this solution can then be dried through a sodium sulfate column in a pasteur pipette. Add a very small drop of concentrated HCl in .5 to 1 milliliter of methanol, or add HCl in methanol to the CH_2Cl_2 drop by drop, until pH 2, or bubble HCl vapor through the solution until you are satisfied all the sample has been converted to the hydrochloride salt. Reduce the solution to a small volume, add more CH_2Cl_2 and reduce in volume again. The purpose of this step is to get rid of water, HCl, and methanol. If when you add petroleum ether, two phases are formed, go through the CH_2Cl_2 volume reduction phase again. Recrystallize with petroleum ether by adding it to the CH_2Cl_2 solution when reduced to a small volume. If necessary reduce to a small volume and add more petroleum ether. Let the crystals grow before decanting or filtering for I.R. This procedure may give different scans for d-methamphetamine and dl-methamphetamine. Be prepared for scans inbetween. Leaving in beam for a couple of hours can enhance scan occasionally.

2. Use the hexane - H_2O method.

3. Extract methamphetamine HCl directly with CH_2Cl_2, filter through sodium sulfate, and recrystallize with petroleum ether.

4. A celite column method as outlined in the white DEA manual the Analysis of Drugs or in "Minnibennies Purification for IR" separation IV. This will separate methamphetamine from amphetamine and phentermine.

5. Chloroplatinic derivative of methamphetamine.
(To clean up where other methods don't work or to separate from phentermine).

   a. Shake out into organic solvent and dry down to decrease sample size.

   b. Add five drops 0.1N HCl.

   c. Add 3-4 drops 5% aqueous platinic chloride add enough to form all the precipitate possible.

   d. After crystals have formed filter (with suction).

   e. Wash crystals with three or four drops 0.1NHCl then two to three drops methanol (be careful not to dissolve crystals).

   f. Run on KBr.

   g. To regenerate methamphetamine extract (if you don't like to run complexes) add several drops of NH_4OH extract methamphetamine

I

I. Introduction: why this conference.
1. each lab tends to go its own way without feedback to other labs - learn techniques aren't always shared
2. source manual of commonly used techniques and procedures
3. since each case we use apply methods where they seem to be useable.
4. we eventually we could have a meaningful manual that covers all our routine analyses.

IV Discussion of topics not related to subject at hand (very brief) not often all labs doing things linked up at one time

II Discussion of amphetamine a. Boise
b. Coeur d' Alene
c. Pocatello

Points to include methodology, parameters, solvent systems TLC, developers etc.

III Discussion of methamphetamine

II

Boise - Amphetamine

1. Quick report is included so you'll know reasoning behind our analysis. Saves us a lot of time on reports in analysis - less
no type written report
no quant unless needed for a special reason.
- what quant & why

2. For our quick report we tend to do things which are reasonable quick, don't require prior separation and almost always would Gives strong probability and nature of substances - sufficient for preliminary hearings.

3. For our final analysis we do an I.R. if feasable within context of the situation. If we can't get an I.R. except by MMVLSA special procedures - we go for a variety of tests.

4. Now you can why our analysis is split into two parts. Following our procedure is SA-DLA procedure from about 1972 - so some things have certainly changed.

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1. Spot tests: Marq -
Mecke -
Mecke - why
Frohde -

2. TLC very straight forward used Fluor.
Marquis yellow-orange amph.
a. works well - needs basic ph. It works well with it
b. simple
c. doesn't interfere with other drugs. a word about sources on fluorescamine
(1) article from Trad Chem - general background
doesn't fluor. with many of the things it does high sensitivity for general reading only
(2) (a) article from Journ of Forensic Science
ignore 5mg/10ml - works through 5ng/100ml
(b) lists 525 drugs tells which it reacts with & causes fluorescence such as procaine

III

3. GLC with Apiezon L + KOH

a. originally needed column for quant of amph + meth — unacylated amphet. (??) ran at too low temp or 0.1% acylation is often incomplete for meth. KOH reduces tailing, can run directly at 150°C.

b. found to be such a good column in terms of sensitivity, reproducibility, & separation, that we use it for routine qualitative analysis. Other labs with a smaller number of GLC may not have room for it.

end of quick report of 90% to 95% of all amphetamine analysis

First comment

1. Most of us would think it for an I.R.-sample size providing. procedure — I.R. on mandelate salt

1. extract ground up sample into 1N H_2SO_4

2. may want to filter & then wash with $CHCl_3$ to eliminate caffeine — shouldn't be necessary but has helped with crystals — loaded with caffeine. Can also go through hexane — H_2O wash.

3. make basic, extract with CH_2Cl_2 — through Na_2SO_4 column reduce volume add mandellic acid in CH_2Cl_2 not too much, it supposedly inhibits formation of crystals.

4. tricks

1. reduce solution to volume of mandelic acid in CH_2Cl_2 if only small amount amph present

2. catch crystals directly in KBr where only small amount of crystals

3. add Et_2O to increase number of crystals.

4. Prepurify with 1.5 NaOH — Hexane — H_2O

5. Some other amines will crystallize out with mandellic acid

6. Ordered l-mandelic acid by accident seems purer than one of mandelic acid I use it.

5. I report as amphetamine not ... dl amphetamine

(margin notes:) we use this method because it can differentiate d, l & dl. because it works so well! — separate crystals for IR — Using pH

IV

2. I.R. by extracting amphetamine from .5N NaOH into hexane which is then washed 3x which eliminates ephedrine & caffeine only works with but does not separate amphetamine methamphetamine phentermine, etc.

papers on this on quick & explanation, note there are two papers the second is a modification of the first I recommend that you follow the second.

3. Mandelamines Purification for F.P. - Ann Bradley will discuss

4. Vol. test I copied HCl - do both other labs have failed? If not don't should have Goes into these same chicken scratch tests.

How we do volatility tests

1. put sample material on clean slide
2. add 5% NaOH
3. place clear cover on microscope slide
4. invert over heat to catch vapors on test slide
5. turn over to view in microscope.

So many volatile impurities Both tests excellent for common drugs. can cross test but so good. Ann B & I then had some luck with which interfere chicken tracks produced by Platinic chloride Hg very sensitive not all amph. makes such similar crystals.

6. Finally more GLC: short 5m wall amph short sample + 20% acetic anhydride in pyridine, DMF, or ethyl acetate. Cayenne + DMF supposed to enhance acylation, but ethyl acetate works fine not such bad solvents front. Ace acetic anhydride can change characteristics of column momentarily.

b. 20% OV-17 at 160°C handy if you happen to have this column set up otherwise do for another purpose

V

conclusion: for final reports we prefer I & if possible, if not possible, I prefer 3 different techniques in combination, TLC, GLC & microcrystal tests because each one should test different properties. Acid/base columns still may be measuring same properties same for TLC. TLC - GLC combinations much more powerful but things similar enough to give some GLC-like columns may give same TLC also. Probably a different microcrystal test. Entirely different approach. I like two methods using GLC & TLC only.
run through DEA methodology
Cocaine - amph.

Ecy - amph.

Methamph.

Mehm and.

1. Spot test: Marquis
Fast's methamine test

2. TLC : Methamph. reacts bests with Dragendorffs may take up to one hour
2. Phentermine - very weakly reactive at all

3. GLC Apiezon L + tol - already covered

4. Microcrystal tests using gold chloride in H_3PO_4 direct. I show diagram
a. d meth - clothespins
b. dl meth - X's or clothespins stack head to head.
c. my diagram was what I observed - you may see it differently. By looking for these subtleties improve specificity of test - most clandestine meth is dl meth

this is much more important than differentiating d, l, & dl

5 to 10% — I R sheet goes along with this

VI

final analysis on separate sheet

1. (IR): the CH_2Cl_2 + HCl in MeOH recrystallized with pet ether is the way we try to recrystallize any base that we don't have another specific I.R. method for. read so people in Boise etc. can make comments and/or criticisms

2. .5 N NaOH + hexane + used to separate methamphetamine from ephedrine or caffeine

3. simple, quick procedure, works quite often
read

4. read self explanatory

(6). read then ask for discussion and/or comment: sort of a [illegible] methodology let Ann discuss

(5.) Variations in I.R. of methamphetamine

1. properly prepared dl meth gives different scan than [illegible] may have to warm or set in beam for awhile to get into most stable form

2. I — d meth HCl
II — dl meth HCl I made
III — dl meth HCl I made and left in I.R. beam for 11 hrs.
slight changes at 1465, 1365, 1000 to 1080 peaks at 700 & 750 change relative intensity slightly - this change very minor

∴ d meth is quite different from 1000 to 1110 & also from 1400 to 1500

VII

IV & V commercial d-l meth HCl
IV = scanned immediately after recrystallization
V = a scan obtained by heating overnight at 70°C
differences from 1400 to 1300 in fingerprint region
also at 1570 to 1630
& 1000 to 1120

warmed scan of d-l meth HCl from both sources virtually identical except for concentration differences.

Importance of d & d-l meth HCl differences doesn't legally matter but you should be aware of these differences
1. optical isomers can give different scans
2. heating to convert to most stable crystalline form.

Fi

2. More GLC - already discussed

3. Quant if you do quant too many possibilities to trust it except in commercial preparations

then Coeur d'Alene

then Pocy

Order of material on analysis of ~~methamphetamine~~ amphetamine, methamphetamine, phentermine, etc.

1. Copy of quick report used by Boise lab.

2. Procedure for amphetamine in central lab, a brief outline

3. outline of ~~DEA~~ San Fran. DEA ~~lab~~ procedures for amphetamine 1972

4. article from Jour. of Chem. ~~Ed~~ 51 1104 (1975) 201-204 mentions reactions of various drugs to Fluorescamine.

5. article from Jour. of Forn. Science, Jan 1976, vol 21, No 1, p154; more complete description of reaction of various drugs to Fluorescamine.

6. ~~An~~ article from Microgram VII, No. 2, Feb 1974 describes use of aprizen L + 2% tow as liquid phase for GLC of amphetamine, methamphetamine, etc.

7. ~~Art~~ article concerning the forming of mandelate salts of amphetamine for I.R.

8. Microgram ~~article~~ article concerning, ~~formation~~ purification of ~~HCl salt~~ of amphetamine & methamphetamine for I.R by Hexane - H_2O wash Microgram, April, 1974

9. More relative on using Hexane - H_2O to purify amphetamine etc. for I.R. Microgram, April, 1975

10. ~~Purification Meth~~ Minibennies purification for I.R., DEA

11. Microcrystal test for amph. & methamphetamine

12. Procedure often followed in the central Lab for analysis of methamphetamine, phentermine, etc. an outline

13. Outline of DEA methodology for ~~methods~~ chrysophanic in 1972.

14. Microcrystal test for methamphetamine using gold chloride direct

15. IR methodology for methamphetamine, used in the central lab

16. 5 IR scans of methamphetamine HCl

(3)

DEA
San Fran ~~BNDD~~

AMPHETAMINE Approx 1972

Identification (including optical activity)

A. Melting point (of the d-mandelate salts)

B. Color and microscopic examination - Marquis, gold chloride (volatility) platinic chloride (volatility) are used. Optical crystallography (also used for diluents), platinic chloride (direct), barium chloride test for sulfate, gold chloride (direct) are used by a minority of chemists.

Assay

A. Ultraviolet assay by direct dilution in acid and filtration. Solution sometimes washed with ether to remove interfering peak.

Most of their chemists now quantitate

TLC ~~GLC~~ GLC

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1 (2)

Procedure often followed in the Central Lab for the analysis of amphetamine.

A. For preliminary analysis:
   1. Spot tests: Marquis
      Feigl's secondary amine test
      Mecke
      Froede

   2. TLC: Either basic extract or a methanolic extraction is spotted on a plate and ran in Clark's T_1 (methanol 100/NH_4OH, 1.5). The plate is visualized by a series of sprays: Fluorescamine, Iodoplatinate, Dragendorff's. (Dragendorff's I use mainly)

   3. GLC: 10% Apiezon l + 2.5% potassium hydroxide useful for both quantitation and qualitative identification.

B. Additional analysis for a final report.
   1. If there is enough material, we always try for an I.R. First we try to recyrstallize as a salt of mandelic acid, perhaps with some preliminary cleanup to remove caffeine and/or ephedrine. Can catch crystals right on KBr in pasteur pipette. Might try the hexane - H_2O cleanup and formation of the hydrochloride salt.

   If that fails:
   2. Volatility microcrystalline tests (not very successful on the common junky crosstops)
      a. Gold chloride in phosphoric acid.
      b. Platinic chloride in phosphoric acid (more sensitive- may work more often).

   3. More GLC: 3% OV17 3 feet column with and without acylation to observe peak shift. (Take up sample plus acetic anhydride and inject)
      :20% OV17 6 feet column without acylation

Journal of Chromatography, 104 (1975) 201–204

CHROM. 7864

Note

Thin-layer chromatography of 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine and other phenethylamine derivatives

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Many phenethylamine derivatives (amphetamine drugs) are used medicinally for therapeutic treatment. Illicit manufacture and use of many of these derivatives have increased greatly in recent years. The relative ease with which chemical groups can be manipulated on the basic phenethylamine nucleus (moiety) has resulted in the synthesis and use of many new hallucinogenic substances, including such drugs as 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MMDA). Substances such as these are a problem to the forensic analyst who must have the capability to screen for them. There are often not sufficient differences of physico-chemical properties to readily distinguish popular phenethylamine drugs by chromatographic means.

The following study was carried out to develop a method that could be used to screen for MDA and MMDA in thin-layer systems already in use in our laboratory. As with other current methods using thin-layer chromatography (TLC) as a screening technique, a sequential spraying pattern is carried out[1,2]. Indeed, the appearance of a particular color with a specific spray reagent, in conjunction with an accurate R_F value, can often serve to distinguish a particular substance from other chromatographically similar compounds. The use of a gallic acid spray, adapted from a procedure reported by DeMayo *et al.*[3], served to distinguish MDA from MMDA. The application of the fluorogenic reagent fluorescamine is described for the general detection of phenethylamine substances.

EXPERIMENTAL

Apparatus

The chromatography tanks used were glass, generally of the size 23 × 12 × 23 cm, with glass tops sealed with starch glycerin paste. The tanks were lined with filter paper. Analtech silica gel G plates (250 μm) were activated for 20 min at 115° before use (available from Mandel Scientific Co., Montreal, Canada).

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Reagents

Developing solvents. (I) Methanol–ammonia (100:1.5); (II) Benzene (distilled)–dioxane (distilled)–ethanol–ammonia (150:120:15:15); (III) Chloroform (distilled)–cyclohexane (distilled)–diethylamine (50:40:10).

Fluorescamine spray. Fluorescamine (Fluram®, Roche Diagnostics, Vaudreuil, Canada), 5 mg/100 ml in acetone.

Ninhydrin spray. Ninhydrin 0.1% (w/v) in acetone is prepared fresh.

Gallic acid spray. Gallic acid 1.0% in sulphuric acid–ethanol (1:1). Store in the dark.

Potassium iodoplatinate (acidified) spray. Platinic chloride (0.25 g) and potassium iodide (5 g) are mixed with water to produce 100 ml. Hydrochloric acid (2 ml) is added to this solution.

Drug standards. Solutions of either the free base or salt were prepared in a known concentration of approximately 5 mg/ml in ethanol or methanol.

Procedure

Approximately 50 μg of each drug were spotted and run. The running distance of each plate was 15 cm. Codeine was run on each plate as an internal standard. A reference mixture of amphetamine, benzphetamine and mescaline was included on most of the plates to monitor the tank condition. The plates obtained from using solvent III were sprayed with a 5% ethanolic solution of hydrochloric acid prior to commencing the sequential spraying pattern to remove the effects of the residual diethylamine. After each run, the plate was air dried, sprayed with fluorescamine spray and viewed under ultraviolet light. All developed spots were noted. The plate was then oversprayed with ninhydrin spray, warmed at 70° for approximately 5 min and observed for any spot development. The plate was again oversprayed with gallic acid spray, warmed again for 10 min at 70° and observed. The plate was finally oversprayed with acidified potassium iodoplatinate spray for general development. After each spray sequence, the running distances of the spots were measured and the R_F and $R_{codeine}$ (R_F relative to codeine) values calculated. The values obtained for the phenethylamine substances and some other hallucinogens are given in Table I.

The sensitivity of detection of some of the drugs with ninhydrin spray, fluorescamine spray and gallic acid spray was determined by diluting the appropriate stock solutions and running the drug on the thin-layer plate in developing solvent I or II. These results are given in Table II.

The data in Table I show that it is often difficult to resolve MDA and MMDA using only R_F determinations. The inclusion of gallic acid spray in a sequential spraying pattern readily distinguishes these two compounds from each other with very good sensitivity. MDA appears as a green spot and MMDA appears as a blue spot. The other phenethylamine derivatives did not develop any interferences with this reagent.

A number of other TLC solvent systems had previously been investigated, but were discarded for reasons of poor resolution, poor sensitivity and difficult handling procedures. The three solvent systems used in this study are useful for many other drugs/poisons and generally give good reproducibility. A second spray reagent for MDA and MMDA was also considered —chromotropic acid, 1.0% in sulphuric acid–ethanol (1:1). A similar chromotropic reagent has been previously investigated by

TABLE I

TLC DATA

Substance
MDA
MMDA
Mescaline
Amphetamine
Methamphetamine
Phenethylamine
Ephedrine
Phenylephrine
Benzphetamine
Phenmetrazine
Phendimetrazine
Chlorphentermine
Dimethyltryptamine
Diethyltryptamine
PCP
LBJ
Codeine

* S.D.codeine is

DeMayo *et al.*, respectively, and the gallic acid sp

RESULTS AND

The R_F v running distan usually from th

TABLE II

DETECTION LI

Substance
MDA
MMDA
Mescaline
Amphetamine
Methamphetamin
Phenethylamine
Ephedrine
Phenylephrine

TABLE I

TLC DATA

Substance	*Developing solvent*								
	I			*II*			*III*		
	R_F	$R_{codeine}$	$S.D._{\cdot codeine}$*	R_F	$R_{codeine}$	$S.D._{\cdot codeine}$*	R_F	$R_{codeine}$	$S.D._{\cdot codeine}$*
MDA	0.50	1.01	0.04	0.43	1.20	0.05	0.42	1.8	0.1
MMDA	0.55	0.98	0.04	0.41	1.10	0.05	0.40	1.7	0.1
Mescaline	0.46	0.81	0.02	0.19	0.57	0.05	0.26	1.10	0.09
Amphetamine	0.61	1.08	0.01	0.46	1.27	0.04	0.41	1.8	0.2
Methamphetamine	0.56	0.96	0.01	0.47	1.22	0.08	0.46	2.0	0.1
Phenethylamine	0.53	0.96	0.07	0.37	0.96	0.01	0.04	0.18	0.05
Ephedrine	0.48	0.85	0.04	0.26	0.71	0.05	0.13	0.60	0.04
Phenylephrine	0.46	1.81	0.03	0.03	0.09	0.02	0.05	0.20	0.03
Benzphetamine	0.78	1.37	0.03	0.76	2.3	0.3	0.71	3.0	0.2
Phenmetrazine	0.60	1.06	0.07	0.50	1.40	0.07	0.38	1.7	0.2
Phendimetrazine	0.02	0.02	0.01	0.01	0.04	0.01	0.18	0.9	0.2
Chlorphentermine	0.62	1.10	0.05	0.51	1.42	0.09	0.41	1.8	0.2
Dimethyltryptamine	0.59	1.04	0.01	0.48	1.34	0.04	0.21	0.89	0.04
Diethyltryptamine	0.65	1.15	0.03	0.61	1.70	0.08	0.31	1.33	0.03
PCP	0.71	1.27	0.05	0.82	2.30	0.20	0.72	3.1	0.3
LBJ	0.76	1.33	0.07	0.70	2.0	0.20	0.52	2.2	0.2
Codeine	0.57	1.00	—	0.36	1.00	—	0.24	1.00	—

* $S.D._{\cdot codeine}$ is the standard deviation of $R_{codeine}$.

DeMayo *et al.*[3]. The colors obtained with MDA and MMDA were pink and purple, respectively, and somewhat easier to differentiate than the gallic acid spray. However, the gallic acid spray was found to be more sensitive.

RESULTS AND DISCUSSION

The R_F values (and subsequent $R_{codeine}$ values) were obtained by measuring the running distances obtained from three separate tanks of the same solvent system, usually from three separate plates within that tank. Thus, most of the values recorded

TABLE II

DETECTION LIMITS (μg) OF SRAYS

Substance	*Fluorescamine*	*Ninhydrin*	*Gallic acid*
MDA	0.05	20	1
MMDA	0.05	10	1
Mescaline	0.05	5	
Amphetamine	0.05	20	
Methamphetamine	0.5	20	
Phenethylamine	0.05	5	
Ephedrine	3.0	10	
Phenylephrine	0.1	5	

in Table I are the result of nine values. In addition, similar data have been obtained separately in another laboratory.

The application of fluorescamine as a spray was investigated and found to be very useful. After spraying, the plate is viewed under ultraviolet light. Generally the phenethylamines are visible as green fluorescing spots or as dark-blue absorbing spots. The intensity of the latter may be increased by exposing the plate to a vapor of ammonia prior to spraying. Table II shows the detection limits obtained by the fluorescamine spray on some of the drugs.

ACKNOWLEDGEMENT

The authors wish to express their appreciation to the Health Protection Branch, Health and Welfare, Canada for supplying some of the standard substances.

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(5)

Studies on Fluorescamine: Part I—Applications of Fluorescamine in Forensic Toxicological Analysis

We use at concentration of 5mg/100ml and its sensitivity is just fine

Weigele and co-workers [*1,2*] reported the structure and synthesis of the reagent 4-phenylspiro [furan-2(3H), 1'-phthalan]-3,3'-dione (fluorescamine), which reacts with substances containing primary amino groups to yield highly fluorescent products. Recently, Undenfriend and co-workers [*3–5*] published a paper describing the use of fluorescamine in biochemical analysis. This reagent was of interest because of its possible use to improve the detection and differentiation of amphetamine and its relatives in biological samples and solid dosage forms. The main subject of this paper is to present data which clearly demonstrate the use of fluorescamine in forensic toxicological analysis of amphetamine and differentiation from methamphetamine. This reagent also has potential application for analysis of other drugs containing a primary amine group.

Two compounds of primary interest to the law enforcement effort are amphetamine and methamphetamine, although a few other amphetamine-like drugs are also federally controlled. The physiological effects of these two drugs are similar [*6–8*], methamphetamine having a slightly greater stimulant effect on the central nervous system. Amphetamine and methamphetamine are both optically active compounds. The dextro isomers are about twice as active as the recemic mixtures, however all isomers are under Federal control and are listed in Schedule II of Public Law 91-513.

An excellent review of existing methods for analysis of amphetamine analogs has been published [*9*]. Miles and Schenk [*10*] made use of the natural fluorescence of phenylethylamines to assay these compounds in pharmaceutical preparations. Few attempts have been made to form fluorescent derivatives of amphetamine and related substances.

In this work, fluorescamine is used to form fluorescent derivatives. It has also been found very simple to further analyze the fluorescent derivative formed in the spot test by thin-layer chromatographic analysis.

Methods and Materials

Fluram™ (fluorescamine) was purchased from Roche Diagnostics, Division of Hoffman-La Roche, Inc., Nutley, N.J. The reagent is supplied in vials containing 100-mg fluorescamine crystals. Fluram is stable at room temperature in both solution and powder form; thus, refrigeration is not recommended by the manufacturer.

The fluorescamine is prepared into a working solution by dissolving 50 mg of fluorescamine in 100 ml of acetone. Fluorescamine has minimal solubility in water

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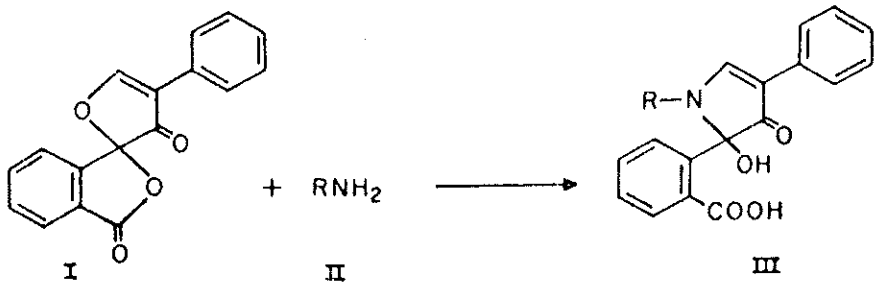
and it will decompose in the presence of water. In the assay, excess reagent is hydrolyzed to water-soluble nonfluorescent products [3].

In Fig. 1, fluorescamine (I) reacts with primary amines (II) to form intensely fluorescent substances (III), providing the basis for a rapid and highly sensitive assay for compounds containing a primary amine group, such as amino acids, primary amines, peptides, and proteins [3]. The reagent (I) does not react with secondary or tertiary amines. Thus, it provides a quick method to distinguish different types of amines; this has been found to be very helpful to distinguish between amphetamine and methamphetamine. Amphetamine is a primary amine and yields an intensely blue-green fluorescent product in the fluorescamine test and methamphetamine does not yield a fluorescent product.



I II III

FIG. 1—*The reaction between Fluram®* (I) *and a primary amine* (II).

The spot test procedure used was to add to a tile-welled spot plate two drops of borate buffer, pH 9.0, and check the fluorescence. All fluorescence examinations were performed in a Chromato-Vue® box (manufactured by Ultra-Violet Products, Inc., San Gabriel, Calif.,) using the long wave (366 nanometer) for excitation. In the spot test the fluorescence is so strong that it can be detected in room light with a small hand ultraviolet (UV) source for excitation. Then a small amount of the exhibit sample was added to the borate buffer and the fluorescence was determined. It is important to check the cleanliness of the plate and the natural native fluorescence of the substance for proper interpretation of the results. The test was completed by adding one drop of the fluorescamine acetone solution and checking the long-wave fluorescence. A positive test was the formation of a very intense blue-green fluorescent product. A negative test was one that did not form a fluorescent product or had fluorescence of another color due to the native fluorescence of the substance.

Quite often it was found to be helpful to analyze the intensely blue-green product further; this was done by thin-layer chromatography (TLC). Two to three microlitres of the spot test were applied to a silica gel thin-layer chromatogram which did not contain any fluorescent material. Standard fluorescent products were prepared from known substances and also applied to the chromatogram. The chromatograph was then developed in a chloroform:methanol system (90:10). After development the fluorescent products were located with the aid of long-wave UV light and the R_f values compared to that of standard compounds. Since very small amounts of the aqueous reaction mixture were required for thin-layer chromatography analysis, there were no problems with drying of the chromatogram before chromatography.

Amphetamine present in urine specimens (10 ml) was isolated by extraction at pH 11 with two volumes of chloroform:isopropanol (3:1) [11]. The solvent extracts were treated with 0.10 ml of acidified methanol (0.1 mol sulfuric acid per litre of methanol) and evaporated. The residue was redissolved in 50 μl of methanol for application to a thin layer plate

lone)

Simultaneously, in duplicate, drug free urine and amphetamine-containing urine (drug-free urine that was "spiked" with amphetamine ranging from 0.10 to 30.0 μg per 10 ml urine) specimens were prepared. The duplicates were then spotted on separate TLC plates, coated with a 250-μm layer of silica gel without fluorescent indicator. The TLC plates were developed in ethyl acetate:methanol:concentrated ammonium hydroxide (170:20:10 by volume) [*12*]. The plates were removed when the solvent migrated between 18 and 19 cm and dried with warm air from a hair drier type of air blower. Then the plates were dried 10 min in an oven at 75°C to remove residual ammonia. Residual ammonia interferes with the ninhydrin spray; it does not interfere with the fluorescamine reagent.

One TLC plate was developed with ninhydrin and the duplicate was developed with fluorescamine. When the fluorescamine test was applied to detection of amphetamine on thin-layer chromatograms, the fluorescamine-acetone solution (50 mg%) was first sprayed onto the dried chromatogram and then oversprayed immediately with the pH 9.0 borate buffer. The fluorescent produce was visualized and located with long-wave UV light (366 nm).

Results and Discussion

Theoretically, the fluorescamine test should yield positive results only with compounds containing a primary amine group. In order to verify this and evaluate possible interfering compounds, many common drugs which are seen as exhibits in the forensic chemistry laboratory were tested. The standard compounds contained in this laboratory consist of a combination of pure drugs, tablets, and capsules. The chemical constituents of the trade name compounds can be found in standard references [*13,14*]. About 10% of the standard compounds tested yielded a positive fluorescamine test; these results are contained in Table 1. From the molecular structures of the compounds examined, it was concluded that a primary amine was a requirement for obtaining a positive fluorescamine test.

Many compounds have native fluorescence; it is important to note this by determining the presence of fluorescence at the stage of addition of borate buffer, before the addition of fluorescamine. It is important to run blanks with this test because it is extremely sensitive and very small contamination will yield false positive results.

Amphetamine and methamphetamine can be distinguished easily by the fluorescamine test. Both of these compounds give the same colors in the Marquis, Meckes, and Froehdes spot tests, and the UV spectra of these compounds are indistinguishable. However, the fluorescamine test is positive with the primary amine, amphetamine, and negative with the secondary amine, methamphetamine.

Sometimes amphetamine preparations have caffeine or other components which interfere with the UV spectrum. Under this circumstance it is convenient to spot 2 to 3 μl of the positive fluorescamine spot test on a thin-layer chromatogram and compare with standard fluorescent derivatives. The chromatographic mobility of the fluorescent derivative of amphetamine has been found to be unique. No other standard compound (see Table 1) has been found to form a fluorescent derivative with fluorescamine and then migrate the same to that to amphetamine fluorescent derivative. The amino acids tyrosine, phenylalanine, and histidine have been studied, as well as Aldomet® (α-methyldihydroxyphenylalanine). All four of the fluorescent derivatives of these compounds remained at the origin in the chloroform:methanol (90:10) mobile phase, while the amphetamine fluorophore moved at an R_f of 0.30.

Often it is necessary to perform urinalysis to determine amphetamine abuse. The classic method of detection of amphetamine in urine is extraction and TLC analysis with ninhydrin spray. The generally accepted minimal detection limit of ninhydrin reagent spray for this

99. Dianabol® (methandrostenolone) –

TABLE I—*Examination of standard drugs with the fluorescamine test*

Drug	Test	Drug	Test
1. Acetylcodeine	–	49. Caffeine (PCP)	–
2. Amesec® Caps (aminophylline compound)	–	50. Cafilon® (calcium lactate)	–
3. Aminophylline + Phenobarbital Tablets	–	51. Calcium cyclobarbital	–
4. Aminopyrine	–	52. Camoquin® HCl (amodiaquin)	–
5. Aminosalicylic acid	–	53. Camphocodine®	–
6. Amobarbital	–	54. Carbarsone® (N-carbamoylarsanilic acid)	+
7. *dl*-Amphetamine	+	55. Carbinoxamine maleate	–
8. *d*-Amphetamine sulfate	+	56. Carisoprodol	–
9. Sodium Amytal®	–	57. Cascara sagrada	–
10. Amphojel® (aluminum hydroxide)	–	58. Chloracetophenone	–
11. Ampicillin trihydrate	+	59. Chloramphenicol	–
12. Ampicillin	+	60. Chloromycetin® powder (chloramphenicol)	–
13. Anacin®	–	61. Chloral hydrate	–
14. Anahist®	–	62. Chloropyrilene	–
15. Analexin-AF®	–	63. Chloroquinediphosphate	–
16. Ananase® (plant protease concentrate)	–	64. Chloroquine phosphate	–
17. Ansional®	–	65. Chlorpheniramine	–
18. Antipyrine	–	66. Chlortetracycline HCl	–
19. Anturane®	–	67. Chlor-Trimeton® (chlorpheniramine maleate)	–
20. Apresoline® HCl (hydralazine HCl)	–	68. Cholan® (dehydrocholic acid)	–
21. Ascorbic acid	–	69. Chrysarobin	–
22. Atarax® (hydroxyzine HCL)	–	70. Cibalgin®	–
23. Atraxin® (meprobamate)	–	71. CKD Tablet® (ephedrine HCl)	–
24. Atropine sulfate	–	72. Clarmil	–
25. Auramin®	–	73. Cocaine	–
26. Aureomycin®	–	74. Codeine	–
27. Aventyl® HCl (nortriptyline HCl)	–	75. Colchicine	–
28. Barbital	–	76. Combid®	–
29. Belladona	–	77. Compazine®	–
30. Bellergal® Tablets	–	78. Co-Pyronil®	–
31. Bellergal® Spacetabs®	–	79. Cortancyl® (prednisone)	–
32. Benadryl®	–	80. Coricidin D®	–
33. Bendectin®	–	81. Cortisone acetate	–
34. Benemid® (probenecid)	–	82. Creatine	–
35. Benzedrine® (amphetamine sulfate)	+	83. Crystoids® (hexylresorcinol)	–
36. Benzocaine (ethyl aminobenzoate)	–	84. Crystoserpine® (reserpine)	–
37. Beta-Chlor® (chloral betaine)	–	85. Cyclandel	–
38. Bicillin® (benzathine penicillin G)	–	86. Cyclobarbital	–
39. Binoctal® (amobarbital, secobarbital)	–	87. Dalmane® (flurazepam HCl)	–
40. Bismuth subnitrate	–	88. Daprisal®	+
41. Bromanautine®	–	89. Dapsone® (4′, 4′-sulfonyldianaline)	+
42. Brovarin®	–	90. Darvon® (propoxyphene HCl)	–
43. Brucine	–	91. Darvon® Compound-65	–
44. Butabarbital	–	92. Darvo-Tran®	–
45. Butalbital	–	93. DBI® (phenformin HCl 5)	–
46. Butazolidin® (phenylbutazone)	–	94. Declomycin® (demethylchlortetracycline HCl)	–
47. Cafergot® (ergotamine tartrate	–	95. Delta-Cortef® (prednisolone)	–
		96. Dexamyl®	+
		97. Dexedrine® (*d*-amphetamine sulfate)	+
		98. Diabinese® (chlorpropamide)	–
		99. Dianabol® (methandrostenolone)	–

Drug	Test
101. Digitalis	–
102. Digitoxin	–
103. Digoxin	–
104. Dihydrostreptomycin polymix	–
105. Diiodohydroxyquin	–
106. Dilantin® (diphenylhydantoin)	–
107. Dimetane® (brompheniramine maleate)	–
108. Dimetapp®	+
109. Dioctyl sodium	–
110. Diphenhydramine	–
111. Diphenylhydantoin with phenobarbital	–
112. Disophrol Chronotab®	–
113. Diuril®	–
114. Doloran® (allobarbital)	–
115. Donnatal®	–
116. Doriden® (glutethimide NF)	–
117. Dormopan® (hexobarbital)	–
118. Doxidan®	–
119. Dramamine® (dimenhydrinate)	–
120. Drixoral®	–
121. Ducolax® (bisacodyl)	–
122. Edrisal®	+
123. Elavil® HCl (amitriptyline HCl)	–
124. Ephedrine HCl	–
125. Ephedrine sulfate	–
126. Ergotrate® maleate (ergonovine maleate)	–
127. Erythrocin® (erythromycin ethylsuccinate)	–
128. Erythromycin-base Film Tab®	–
129. Erythromycin stearate (Ilotycin®)	–
130. Erythromycin-tan®	–
131. Eskatrol® Spansule®	+
132. Estinyl® (ethinyl estradiol)	–
133. Ethylmorphine	–
134. Ferrous sulfate	–
135. Fiorinal®	–
136. Flagyl®	–
137. Floraquin® vaginal tablets (diiodohydroxyquin)	+
138. Flurazepam HCl	–
139. Gantrisin® (sulfisoxazole)	+
140. Glutethimide	–
141. Grifulvin V® (griseofulvin microsize)	–
142. Griseofulvin	–
143. Gynergen® (ergotamine tartrate)	+
144. Halotestin® (fluoxymesterone)	–
145. Hashish	–
146. Helozid	–
147. Heptabarbital	–
148. Heroin (diacetylmorphine)	–
149. Hetrazan® (diethylcarbamazine citrate)	–
150. High Grelan® (secopyrabital)	–
151. Homatropine hydrobromide	–
152. Hygroton® (chlorthalidone)	–
153. Hyminol® (methaqualone)	–
154. Hyoscyamine or stramonium	–
155. Ilosone® (erythromycin estolate)	+
156. Immenoctal® (amobarbital)	–
157. Indocin® (indomethacin)	–
158. Ismelin® sulfate (guanethidine sulfate)	–
159. Isopropyl meprobamate	–
160. Isordil® (isosorbide dinitrate)	–
161. Kafe Soft® (caffeine)	–
162. Kemicetine® (chloramphenicol)	+
163. Ketamine	–
164. Leukeran® (chlorambucil)	–
165. Librium® (chlordiazepoxide HCl)	–
166. Lidocaine HCl (injection, USP 2%)	–
167. Lincocin® (lincomycin HCl monohydrate)	–
168. Lomotil® (diphenoxylate HCl with atropine sulfate)	–
169. Lysergic acid diethylamide	–
170. Mandelamine® (methenamine mandelate)	–
171. Mannitol (Korean)	–
172. Maple Tablet (maple preparation)	–
173. MDA (methylenedioxyamphetamine)	+
174. Marezine® (cyclizine)	–
175. Meclizine	–
176. Meconic acid	–
177. Medroxyprogesterone acetate	–
178. Mellaril® (thioridazine)	–
179. Mephenesin	–
180. Mephobarbital	–
181. Mepivacaine HCl injection 1% (carbocaine)	–
182. Meprobamate	–
183. Meprophen® (meprobamate)	–
184. Meridon	–
185. Merthiolate® (thimerosal)	–
186. Methadone	–
187. Methamphetamine	–
188. Methapyrilene	–
189. Methaqualone	–
190. *dl*-Methionine and vitamin B_1	+
191. Methylcellulose	–
192. Methylhexabarbital	–
193. 3-Monoacetylmorphine	–
194. Mycostatin® (nystatin USP)	+
195. Myleran® (busulfan)	–
196. Mylicon®	–
197. Mysoline® (primidone)	–
198. Naron® (cyclopyrabital)	–

Drug	Test	Drug	Test
295. Tetracycline	–	311. Tylenol® (acetaminophen)	–
296. Tetrex® (tetracycline phosphate)	–	312. Urecholine® (bethanechol chloride)	–
297. Thebaine	–	313. Valium® (diazepam)	–
298. Theophylline	–	314. Vallestril® (methallenestril)	–
299. Thianphenicol	–	315. Vasodilan® (isoxsuprine HCl)	–
300. Thorazine® (chlorpromazine)	–	316. Vigosan®	–
301. Tigan® (trimethobenzamide HCl)	+	317. Vistaril® (hydroxyzine pamoate)	–
302. Titralac® (calcium carbonate)	–	318. Vitamin B_{12}	+
303. Tofranil® (imipramine HCl)	–	319. Vitamin K (synthetic)	–
304. Tranquinal® (meprobamate)	–	320. Warfarin	–
305. Triacetyloleandomycin	+	321. Wyamine sulfate® (mephentermine)	–
306. Trilafon® (perphenazine)	–		
307. Tri-Span®	+	322. Wyanoids®	–
308. Tropacocaine HCl	–	323. Zactirin®	–
309. Tuinal® (sodium amobarbital and sodium secobarbital)	–	324. Zarontin® (ethosuximide)	–
310. Tuss-Ornade®		325. Zyloprim (allopurinol)	–

limit of ninhydrin and fluorescamine. It has been found that fluorescamine is a more sensitive method for the detection of amphetamine on thin-layer chromatograms. These results are tabulated in Table 2. The detection limit of amphetamine in urine samples is increased 100 times when the only parameter varied is the method of detection, ninhydrin or fluorescamine.

Other physiological chemicals containing primary amino groups normally present in drug-free urine extracts will also react with fluorescamine. This was evidenced in the non-drug-containing urine (Table 2). As many as eight well-separated fluorescent areas were routinely observed. These naturally occurring substances containing primary amino groups all have mobilities considerably less than that of amphetamine in the developing solvent system used; thus, the naturally occurring compounds do not interfere with the analysis.

While this paper was in the process of publication another has appeared on the use of fluorescamine in amphetamine detection in urine [*16*]. These authors have also stressed the increased detection limit of fluorescamine compared to ninhydrin. Klein et al quantitated the amphetamine level by extracting the silica gel area containing the amphetamine fluorophore and analyzed the extract in a microfluorimeter. They also reported that after amphetamine was made visible by fluorescamine spray, the plate could be sprayed with other common identification reagents with no interference. Accordingly, it is possible to substitute fluorescamine for ninhydrin in a routine battery of sprays for drug abuse screening in urine samples.

Summary

This paper describes some applications of the fluorescamine spot test to forensic toxicological analysis. The fluorescamine test only reacts with primary amines; thus, this test makes a clear-cut distinction between amphetamine and methamphetamine. Previous common spot tests used reacted the same with these two amines. Fluorescamine is 100 times more sensitive in detecting amphetamine extracted from urine on thin-layer chromatograms than ninhydrin. Thus, it is a more sensitive method of detecting amphetamine abuse in urinalysis screening programs.

Drug	Test
199. NegGram® (nalidixic acid)	–
200. Nembutal® (sodium pentobarbital)	–
201. Neomycin	+
202. Nicotinic acid	–
203. Nitrofurantoin	–
204. Nitroglycerin	–
205. Norflex® (orphenadrine citrate)	–
206. Norgesic®	–
207. Norinyl® (norethindrone with mestranol)	–
208. Noludar® (methyprylon)	–
209. Novahistine® LP tablets	–
210. Novatophen® (neocinchophen)	–
211. Optalidon® (allyisobutylbarbital)	–
212. Opium (raw)	–
213. Oretic® (hydrochlorthiazide)	–
214. Ornade® Spansule®	+
215. Oxsoralen® (methoxsalen)	–
216. Paraflex® (chlorzoxazone)	–
217. Pavatrine® HCl	–
218. Pavron®	–
219. Penicillin G potassium	–
220. Penicillin, phenoxymethyl potassium	–
221. Pentobarbital	–
222. Periactin® HCl (cyproheptadine HCl)	–
223. Peritrate® (pentaerythritol tetranitrate)	+
224. Persantine® (dipyridamole)	–
225. Phenaphen®	–
226. Phencyclidine	–
227. Phenergan®	–
228. Phenformin HCl 5	–
229. Phenobarbital	–
230. Phenobarbital, ephedrine, and theophylline	–
231. Phenylpropanolamine HCl	+
232. Physostigmine salicylate	–
233. Pilocarpine nitrate	–
234. Pival® (pindone)	–
235. Placidyl® soft capsules (ethchlorvynol)	–
236. Polycillin® (ampicillin trihydrate)	–
237. $KMnO_4$® tablet	–
238. Povan® (pryvinium pamoate)	–
239. Prednisolone	–
240. Prednisone	–
241. Preludin® (phenmetrazine HCl)	–
242. Premarin®	–
243. Pre-Sate® (chlorphentermine HCl)	–
244. Primaquine phosphate	–
245. Pro-Banthine® (propantheline bromide)	–
246. Procaine hydrochloride	+
247. Promacetin® (acetosalfone sodium)	–
248. Pronestyl® (procainamide HCl)	–
249. Propadrine® (phenylpropanolamine)	+
250. Propadrine HCl®	+
251. Propoxyphene HCl	–
252. Prostaphlin® (sodium oxacillin)	–
253. Provest® Daypak (birth control)	–
254. Pyridium® (phenazopyridine HCl)	–
255. Pyridoxine HCl (vitamin B_6)	–
256. Quinacrine HCl	–
257. Quinidine sulfate	–
258. Quinine sulfate	–
259. Rarical	–
260. Rela® (carisoprodol)	–
261. Rhubarb	–
262. Riboflavin (vitamin B_2)	–
263. Ritalin® HCl (methylphenidate HCl)	–
264. Robaxin® (methocarbamol)	–
265. Romilar® (racemethoxphan HBr)	–
266. Rotoxamine	–
267. Salol® (phenyl salicylate)	–
268. Sand	–
269. Sansert® (methysergide maleate)	–
270. Saridon® (isopropyl antipyrine)	–
271. Scopalamine hydrobromide	–
272. Secobarbital	–
273. Sedes® (hexobarbital)	–
274. Sedalin® (pyrabital)	–
275. Sernylan® (phencyclidine HCl)	–
276. Sinequan (10 mg)	–
277. Sintrom® (acenocoumarol)	–
278. Sodium bicarbonate	–
279. Sodium chloride and sodium bicarbonate	–
280. Sodium salicylate	–
281. Sparine® (pronazine HCl)	–
282. Stelazine® (trifluoperazine)	–
283. Streptomycin	+
284. Strychnine	–
285. Sudafed® (pseudoephedrine HCl)	–
286. Sulfadiazine	–
287. Surfak® [calcium bis-(dioctyl sulfosuccinate)]	–
288. Syntho Tab	–
289. Surgex® (pipradral)	–
290. Talwin® (pentazocine)	–
291. Tapazole® (methimazole)	–
292. Tenuate® Dospan® (diethylpropion HCl)	–
293. Terramycin® (oxytetracycline)	–
294. Tessalon® (benzonatate)	–

TABLE 2—*Relative detection of amphetamine isolated from urine with ninhydrin and fluorescamine on thin-layer chromatograms.*

Amphetamine*, μg	Ninhydrin	Fluorescamine
30.0	+	+
20.0	±	+
10.0	−	+
5.0	−	+
0.5	−	+
0.25	−	+
0.1	−	±
0	−	−

*Amount of amphetamine added to 10 ml of control, non-drug-containing urine.

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Microgram, VII, No 2
Feb, 1974 (6)

LABORATORY NOTES

DATE

NO.

DRUG TYPE

METHODOLOGY

The Determination of Amphetamine and Methamphetamine Preparations by Gas Chromatography

by Stanley Blasof and Jack Fasanello
Northeast Regional Laboratory
Drug Enforcement Administration

A gas chromatographic procedure is presented for the quantitative determination of Amphetamine and Methamphetamine. This procedure, which primarily involves a direct methanolic dilution of the sample preparation and the use of naphthalene as an internal standard, can be utilized for the analysis of the above drugs when they appear alone or in combination with each other, or other interfering substances.

Introduction

The quantitative determination of amphetamine mixtures by ultraviolet analysis has presented some difficulty. Current separation techniques often prove difficult, time consuming and incomplete. Interferences from other components within the sample mixture becomes a problem due to the relatively low absorbtivity of amphetamine.

The successful separation of amine drugs on alkaline GC columns has been previously reported (1) (2) (3). To quantitate amphetamine and/or methamphetamine a GC procedure employing a 10% Apiezon L + 2% KOH column is presented.

Experimental

Apparatus

Gas Chromatograph:	Packard Model 804 equipped with a flame ionization detector.	
Column:	Borosilicate glass. Length - 6' I.D. - 2mm	
Packing:	10% Apiezon L plus 2% KOH (Analabs, North Haven, Connecticut) on Chromasorb WHP 80-100 Mesh.	
Operating Conditions:	Column Temperature	145°C
	Inlet Temperature	300°C
	Detector Temperature	210°C
	Carrier Gas	Nitrogen
	Flow Rate	40 ml./min.
	Range	1 x 10-9 amps

Preparation of Standard Solutions:

a. Internal Standard - prepare a stock solution by accurately weighing and transferring 400-600 mgs. of naphthalene into a 250.0 ml. volumetric flask and dilute to volume with methanol.

b. Reference Standard - accurately weigh portions of the reference standards equivalent to 3-5 mgs. of amphetamine and/or methamphetamine and transfer into a 10.0 ml. volumetric flask. Pipet 1.0 ml. of the internal standard solution into the flask and dilute to volume with methanol.

Procedure

Sample Preparation - Uniformly mix the entire sample powder. Time delay pellets must be thoroughly ground and passed through a 60 mesh seive. Accurately weigh and transfer a portion of the sample mixture equivalent to 3.0 mgs. of amphetamine to a 10.0 ml. volumetric flask. Add 1.0 ml. of internal standard solution and dilute to volume with methanol. Preparations containing time delay pellets should be shaken on a mechanical shaker for 15 minutes to insure all amphetamine is in solution.

Calculations:

One microliter of the standard and sample solutions are injected into the gas chromatograph (See Fig. 1). The concentration of amphetamine and/or methamphetamine is calculated using the following formula:

$$\% \text{ amphetamine} = \frac{H_{SP}}{H_{ST}} \times \frac{H_{IST}}{H_{ISP}} \times \frac{C_{STD}}{WT.SP} \times 100$$

H_{SP} = height of sample peak
H_{ST} = height of standard peak
H_{IST} = height of internal standard peak in standard solution
H_{ISP} = height of internal standard peak in sample solution
C_{STD} = concentration of standard
WT.SP = weight of sample used

Results and Discussion

The ratio of peak height to concentration was found to be linear over the range of 0.1 - 1 mg. per ml. of methamphetamine and amphetamine. Other concentrations were not determined.

This procedure has been utilized routinely by this laboratory for the past year for both illicit as well as commercial preparations[1]. Illicit

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1. Phenolic amines are not eluted from this column.

preparations of amphetamine or methamphetamine containing interfering by products resulting from the synthesis were accurately quantitated by this procedure. An example of an unusual clandestine preparation which this procedure readily resolved corsisted of a mixture of amphetamine, methamphetamine and ephedrine. The amphetamine and methamphetamine were quantitated at 145°C; the ephedrine content was estimated [2] using a column temperature of 165°C (See Fig. 2).

The amphetamine content in "Eskatrol" capsules was also quantitated by this procedure. (Eskatrol TDC's is a commercial preparation consisting of a mixture of amphetamine and prochlorperazine.)

The standard solutions employed are stable indefinitely when refrigerated. Therefore, most sample preparations can be determined rapidly and accurately by direct dilution, addition of internal standard and subsequent injection of the sample and standard solutions into the gas chromatograph. An additional advantage of this procedure is that sample handling is maintained at a minimum and derivatization is eliminated.

It should be noted that this laboratory has concurrently been using a Carbowax 20M + 2% KOH column for the analysis. Amphetamine and methamphetamine exhibit a reversal of elution order when chromatographed on this column, thus providing an excellent qualitative check. We have also used the Carbowax column for quantitative analysis, but prefer the Apiezon column because the separation is better and analysis time is shorter. Further qualitative proof may be obtained using the Schiff base-derivative technique.[4]

Reference

1. Parker, K.D., Fontan, C.R., Kirk, P.L., Analytical Chemistry, 34, 1345 (1962).

2. Beckett, A.H., Rowland,M., Journal of Pharmacy and Pharmacology, 1964, 16.

3. Chromatography Lipids Newsletter, Vol. VI, No. 3.

4. Brochmann-Hanssen, E., and Svendsen, A.B., Journal of Pharmaceutical Science, 51, 938 (1962).

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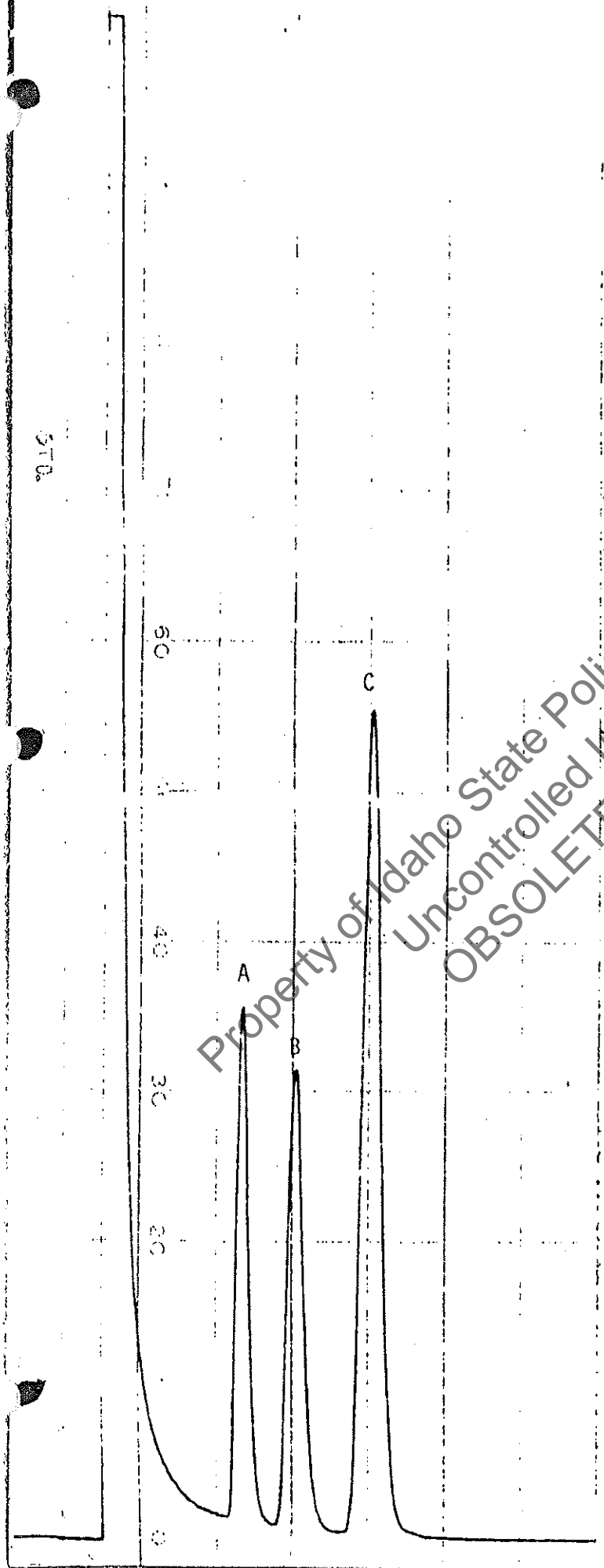
2. Some decomposition was noted for ephedrine.

FIGURE #1

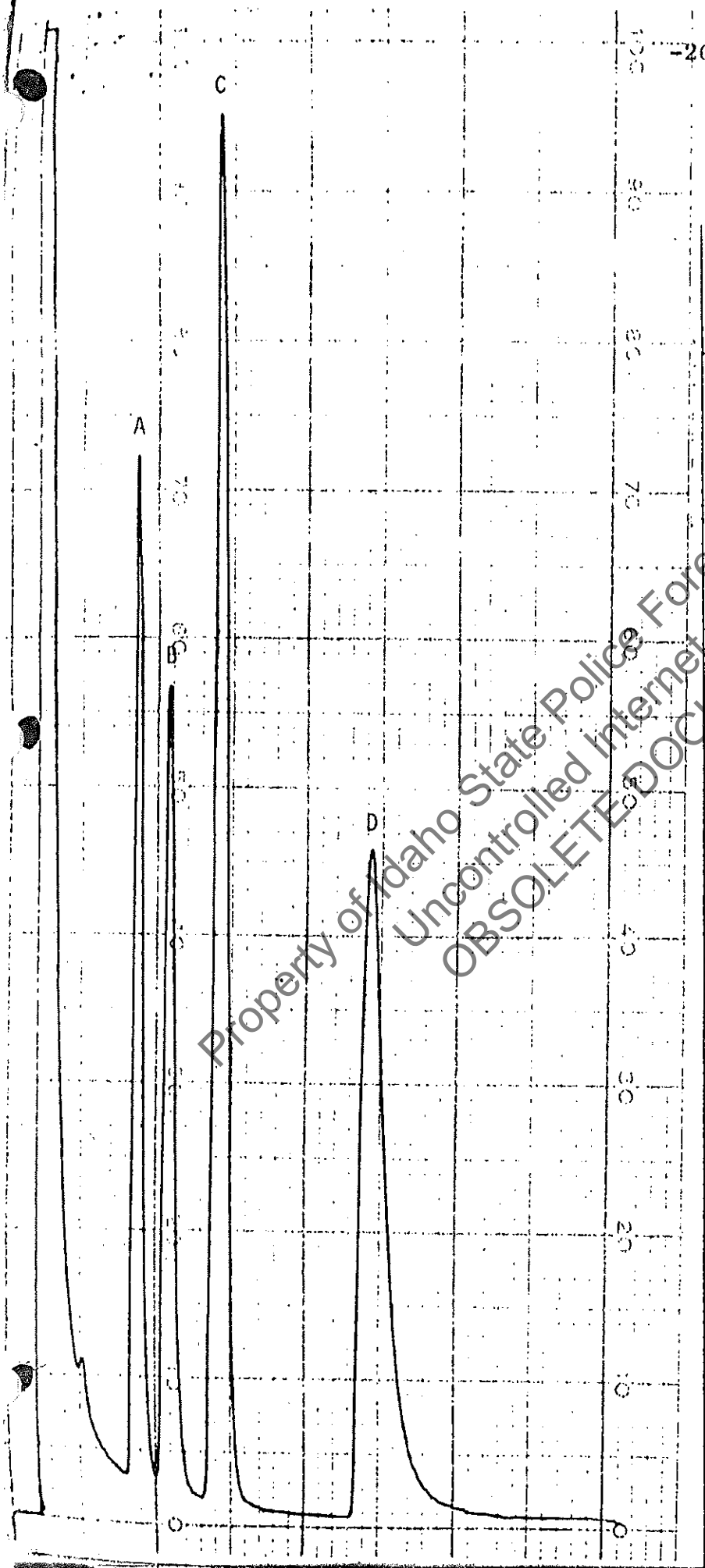
STANDARD SOLUTION AT 145°C



	Compound	Retention Time Relative to Naphthalene
A	Amphetamine	0.5
B	Methamphetamine	0.7
C	Naphthalene	1.0

FIGURE #2

AMPHETAMINE, METHAMPHETAMINE, NAPHTHALENE AND EPHEDRINE AT 165°C



	Compound	Retention Time Relative to Naphthalene
A	Amphetamine	0.5
B	Methamphetamine	0.7
C	Naphthalene	1.0
D	Ephedrine	1.8

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.

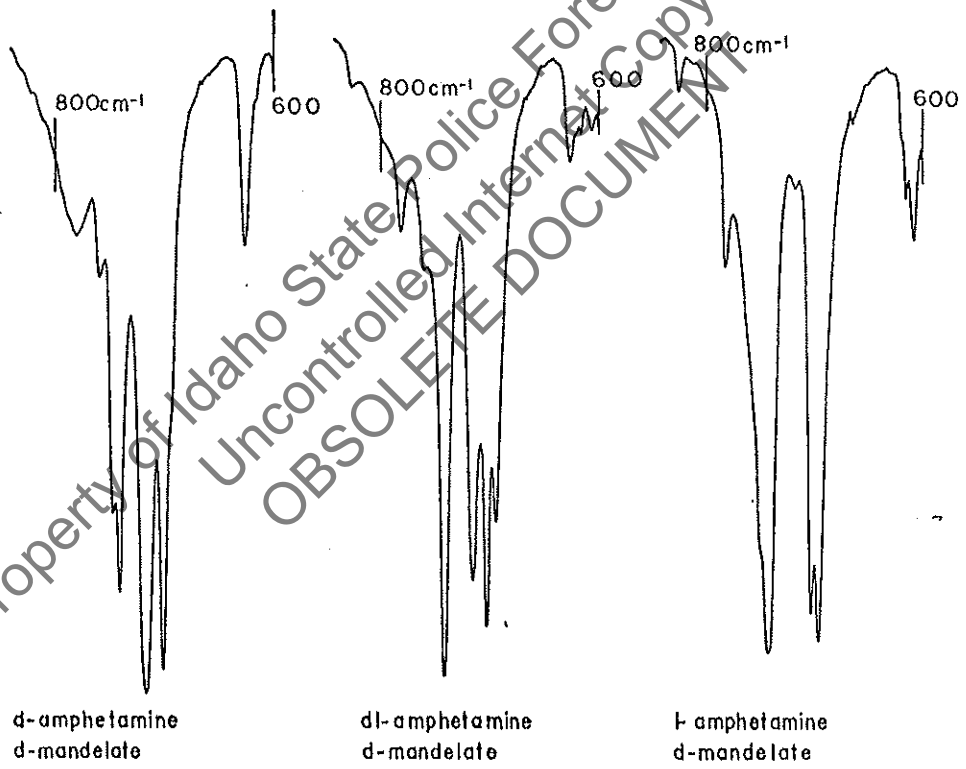

Figure 1. Infrared spectra of amphetamine *d*-mandelate salts in KBr between 800 and 600 cm^{-1}

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.

RECEIVED for review April 30, 1970. Accepted July 15, 1970.

(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).
(3) Chouhs, N. H., J. Pharm. Sci. 54, 1267 (1965).

(8)

Vol. ED No. 4-51- From Microgram April, 1974

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.

(10)

Mini bennies Purification for I.R, DEA

1. Amphetamine

2. Desoxyephedrine

3. Diphenhydramine

4. Caffeine

5. Ephedrine

6. Meprobamate

7. Amphetamine, caffeine

7A. Acetaminophen, diphenhydramine

8. Aspirin, ephedrine

9. Brücine, ephedrine

10. Chloramphenicol, pemoline ~~~~

11. Caffeine, diphenhydramine

12. Caffeine, ephedrine

13. Caffeine, desoxyephedrine

14. Desoxyephedrine, phentermine (amphet)

15. Ephedrine, strychnine

16. Acetaminophen, caffeine, diphenhydramine

17. Amphetamine, caffeine, pemoline 1 mg level

18. Amphetamine, caffeine, 1,3-diphenylisopropylamine

19. Caffeine, ephedrine, phentermine

20. Caffeine, ephedrine, erythromycin

21. Caffeine, ephedrine, thonzylamine

22. Caffeine, diphenhydramine, ephedrine

23. Acetaminophen, caffeine, diphenhydramine, ephedrine

24. Caffeine, diphenhydramine, ephedrine, thonzylamine

25. Caffeine, desoxyephedrine, ephedrine, phentermine

[illegible]

7. $CHCl_3$ wash from acid (caffeine) then CH_2Cl_2 wash from base followed by V (amphetamine).

7A. II (hexane diphenhydramine); I start with CH_2Cl_2 followed by $CHCl_3$ (acetaminophen).

8. Dry extract with $CHCl_3$ (aspirin) then $CHCl_3$:CH_3OH 3:2 (ephedrine).

9. I petroluem ether (brucine), ether (brucine), $CHCl_3$:CH_3OH (ephedrine). It may be necessary to put the brucine through II.

10. CH_2Cl_2 dry extract (chloramphenicol), $CHCl_3$ extract from acid solution evaporate to dryness, residue may have to be washed with ether to remove impurities (pemoline).

11. II hexane (diphenhydramine) then extract with $CHCl_3$ (caffeine).

12. I ether, CH_2Cl_2 and $CHCl_3$ (all caffeine), $CHCl_3$:CH_3OH (ephedrine).

13. II hexane (desoxyephedrine) then extract with $CHCl_3$ (caffeine), i.e. desoxyephedrine precipitate out using 0.1N HCl in ether.

14. IV $CHCl_3$ (desoxyephedrine), column retains (phentermine).

15. Same as #9-brucine.

16. II hexane (diphenhydramine), I CH_2Cl_2 (caffeine) use 2nd $CHCl_3$ extract (acetaminophen).

17. III neutral (caffeine), basic (amphetamine), acid (pemoline). Pemoline residue may have to be washed with ether.

18. $CHCl_3$ extract from HCl solution, then CH_2Cl_2 extract from basic solution followedby V (amphetamine). Evaporate $CHCl_3$ extract then II hexane (1,3-diphenylisopropylamine), then extract with CH_2Cl_2 (caffeine).

19. II hexane (phentermine), I ether (caffeine), $CHCl_3$:CH_3OH (ephedrine).

20. I erythromycine is usually present as the propionate which comes out with the petroleum ether extract (use larger volume), then proceed as in 19.

21. Same as #19 hexane (thonzylamine).

22. Same as #19 hexane (diphenhydramine).

23. I CH_2Cl_2 (caffeine), use 2nd $CHCl_3$ extract (acetaminophen), $CHCl_3$: CH_3OH (ephedrine). Use TLC to locate diphenhydramine-varies from petroleum ether to CH_2Cl_2 extract. Use II - hexane (diphenhydramine) on this extract (evaporated) or on another portion of tablets.

24. Use I as in #23 and II on appropriate extracts (evaporated) for separation of diphenhydramine and thonzylamine.

25. I for ephedrine, caffeine -II for phentermine, desoxyephedrine, then IV on hexane extract.

MINIBENNIES
PURIFICATION FOR IR

Most of the separation techniques listed are used only to extract an ingredient for IR in a reasonably pure form and are not quantitative.

SEPARATION I

Finely ground tablet is packed in small column and dry washed with petroleum ether, then ether, CH_2Cl_2, $CHCl_3$, $CHCl_3$:CH_3OH 3:2, CH_3OH. Use about 2ml each. TLC on each fraction with iodoplatinate spray to locate separated unknowns.

SEPARATION II

Ground tablet material is partitioned between basic water and hexane (3ml each). Hexane layer is washed thoroughly with 5ml water.

SEPARATION III

Ground tablet mixed with water and extracted with $CHCl_3$ from neutral, then basic, then acid solution.

SEPARATION IV

Ground tablets are mixed with 4N HCl celite, or hexane from separation II is poured onto 4N HCl celite column. Column is eluted with $CHCl_3$.

SEPARATION V

A basic water solution of the tablets is extracted with CH_2Cl_2. The CH_2Cl_2 is dried through Na_2SO_4 and d-mandelic acid in CH_2Cl_2 is added. The volume is reduced by boiling and amphetamine mandelate precipitates out. Too much mandelic acid will prevent the amphetamine from precipitating. Most other amines will not precipitate.

36.528 *Characteristics of Microchemical Tests for Alkaloids and Related Amines—Continued*

Alkaloid	Reagent	Description of Crystals
Narceine (*194*)	Iodine potassium iodide, or zinc potassium iodide	1:400. Blue, radiating needles, sometimes with yellow dichroism.
	Platinic chloride	Beautiful feathery rosettes develop in all solns.
Nicotine (*204*)	Mercuric chloride	Radiating, transparent blades form in presence of slight excess of H_2SO_4; feather-like blades form in presence of HCl.
	Mercuric chloride-sodium chloride	Radiating, transparent blades.
Noscapine (*194*) (l-Narcotine)	Potassium hydroxide or ammonium hydroxide	1:200. White, amorphous ppt that crystallizes slowly; dense rosettes of needles.
Papaverine (*205*)	Zinc chloride	Thin, rectangular plates in excess HCl.
Physostigmine (*206*)	Lead iodide	1:100. Radiating, serrated plates.
	Gold bromide in HCl	1 mg in 1 drop H_2O. Brown, dendritic aggregates.
Pilocarpine (*192*)	Platinic chloride	Crystals form slowly; layers of thin, yellow, triangular plates of delicate structure.
Procaine (*205*)	Platinic chloride	Spherical crystals of radiating branches.
	Gold chloride and HCl	Irregular, radiating branches.
Quinidine (*197*)	Potassium iodide	Small, triangular crystals in great numbers; best in 1:1000 diln; sol. in excess reagent.
Quinine (*197*)	Disodium phosphate	Silvery, sheaf-like crystals.
Racephedrine (*207*) (dl-Ephedrine)	Bismuth iodide in dild sulfuric acid	1:200. Large orange plates and red prisms and grains.
Scopolamine (*203*) (Hyoscine)	Gold chloride	Clusters of pale yellow, transparent blades, with coarse, saw-toothed edges form immediately on shaking slide. Crystals grow to large size in 1:200 soln.
Sparteine (*204*)	Gold chloride	Large numbers of blade-like crystals varying in size according to concn.
Strychnine (*208*)	Platinic chloride	Crystals form immediately in clusters and singly in small, wedge-shaped needles that move about field.
	Potassium cadmium iodide	Silvery masses, slowly forming rosettes.
Yohimbine (*189*)	Sodium carbonate	In 1:1000 soln heated to 50°. Fine needles in sheaf-like bundles and rosettes.

For Barbiturates (209)—Official Final Action

(*See also* 36.536.)

36.529 ***Reagent***

Iodine-potassium iodide soln.—Dissolve 5 g I and 80 g KI in enough H_2O (ca 78 ml) to make 100 ml. Dil. with 2 parts by vol. of H_3PO_4. Prep. dild reagent every 2–3 weeks.

36.530 ***Identification***

Dissolve little barbiturate in drop H_2O on slide. If present as Na salt, it dissolves readily; if present as acid, add little droplet 1% NaOH on stirring rod and mix. Add 1 full drop reagent and let stand until crystn occurs (immediate with some compds, 0.5–1 hr with secobarbital). Free acid may fail to crystallize. With I reaction crystals are easily distinguished by their color, often coupled with strong dichroism. Det. birefringence with polarizing microscope. Cover glass is usually not needed but may be used for observation at high magnification and when slide stands >1 hr; on standing, KI may crystallize as square, colorless, isotropic crystals.

Note crystals formed and compare characteristics with description, 36.531.

36.531 *See* pages 712–713.

For Sympathomimetics (207)—Official Final Action

36.532 ***Reagents***

(a) *Bismuth iodide in diluted sulfuric acid soln.*—*See* 36.525(e).

(b) *Gold chloride in diluted phosphoric acid soln.*—Dissolve 1 g $HAuCl_4.3H_2O$ in 20 ml H_3PO_4 (1 + 2).

(c) *Platinic chloride in diluted phosphoric acid soln.*—Dissolve 1 g $H_2PtCl_6.6H_2O$ in 20 ml H_3PO_4 (1 + 3).

(d) *Sodium tetraphenylboron soln.*—Aq. soln (1 + 20).

Barbiturate	Crystal Form	Dichroism or Pleochroism	Remarks
Metharbital (5,5-Diethyl-1-methylbarbituric acid)	Dark needles, small to large, and splinter-like 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.*

36.531 *Characteristics of Microchemical Tests for Barbiturates—Continued*

Barbiturate	Crystal Form	Dichroism or Pleochroism	Remarks
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 red to black 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 concn 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. 99% 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).

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 of 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 rod prisms.

(12)

STATE OF IDAHO

DEPARTMENT OF HEALTH AND WELFARE

BUREAU OF LABORATORIES, 2220 Old Penitentiary Road, Boise, ID 83702

Procedure often followed in the Central Lab for the analysis of methamphetamine, phentermine, etc.

A. Fore preliminary analysis:

1. Spot tests: Marquis
   Feigl's secondary amine test (for methamphetamine)

2. TLC: Same extraction and solvent system as amphetamine.
   Fluorescamine: No reaction (may appear as dark spots under shortwave UV).
   Iodoplatinate: May react with methamphetamine, very weak reaction if any with phentermine.
   Dragendorff's: Should react with methamphetamine, (within one hour in small amounts) very weak reaction if any with phentermine.

3. GLC: 10% Apiezon L + 2.5% KOH.

4. Gold chloride in phosphoric acid direct microcrystalline test.
   Saturated phosphoric acid direct on dry methamphetamine - gives squares and rectangles

B. For a final report:

1. IR: methodology we have used is mentioned separately.

2. More GLC: OV 17, 3% 3 ft. column, with and without acylation (take up sample into syringe and also 20% acetic anhydride in ethyl acetate, pyridine, or dimethylformamide).

3. If needed, quantitation on home-made methamphetamine can be done on the apiezon column. Use UV only for commercially manufactured preparations.

(13)

SF DEA
41972

DESOXYEPHEDRINE

Identification

A. Infrared Spectrum - usually as the hydrochloride salt recrystallized from methylene chloride-petroleum ether. Different spectra are obtained for d and dl isomers.

B. Microscopic and color tests - Platinic chloride (volatility), gold chloride (direct and volatility) are used. Optical Crystallography used by a minority of chemists.

Assay

A. Ultraviolet assay by direct dilution or shakeout.

now GLC

Optical Activity

A. Polarimeter - on concentrated solution.

B. Melting Point for dl-isomer

C. Crystal form - Dimuth Iodide

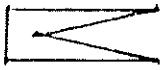
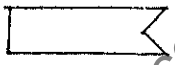
Diluents

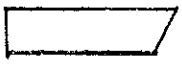
A. Infrared spectrum on chloroform insoluble portion.

B. Optical Crystallography

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

d-methamphetamine

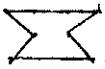
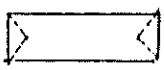
1. should have a number of good-sized crystals shaped  or 

2. should have some plates shaped   etc.

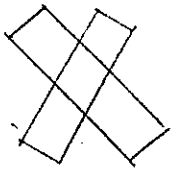
no perfect rectangles

3. Not many clusters 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 distinguishable under crossed-polars.

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

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

Infrared Method for Distinguishing Optical Isomers of Amphetamine

James A. Heagy

U. S. Bureau of Narcotics and Dangerous Drugs, Box 36075, 450 Golden Gate Ave., San Francisco, Calif. 94102

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.

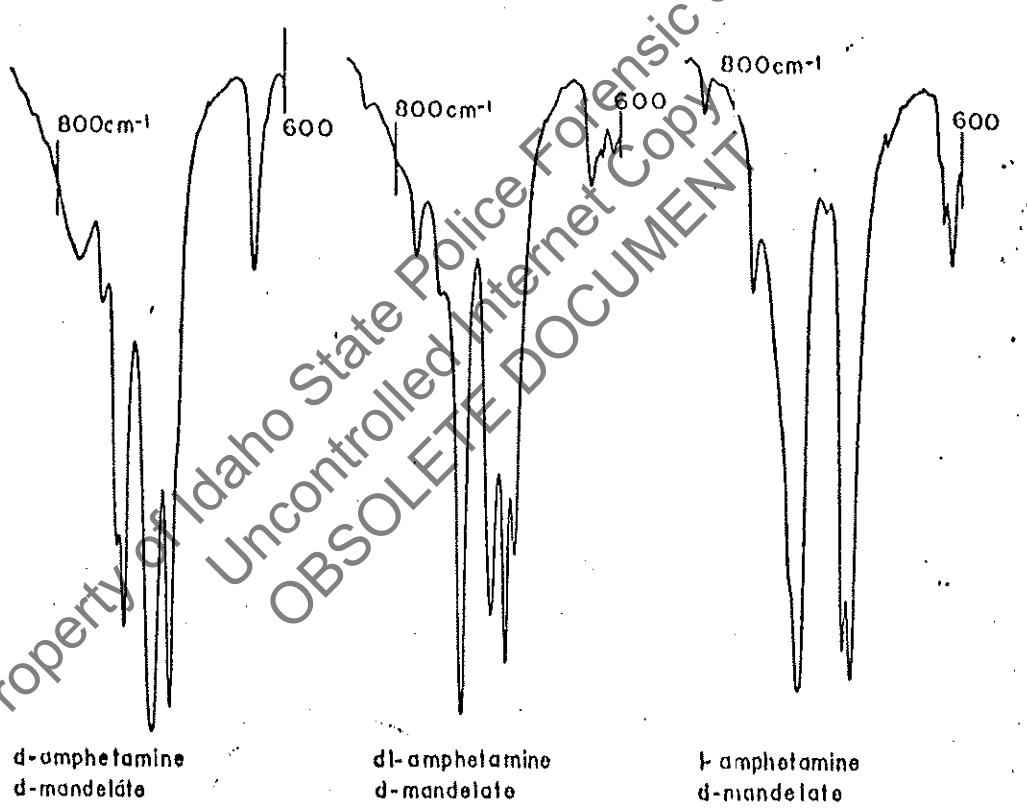


Figure 1. Infrared spectra of amphetamine *d*-mandelate salts in KBr between 800 and 600 cm^{-1}

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.

RECEIVED for review April 30, 1970. Accepted July 15, 1970.

(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).
(3) Chaulia, N. H., J. Pharm. Sci. 54, 1367 (1965)

COCAINE

Background Information-
DEA Booklet for purification process, use, etc.

Screening tests-
Cobalt thiocyanate 2% aq - (Reference: IRS Methods of Analysis)
Modified by using stannous chloride to remove false positives caused by procaine. But cocaine base does not react, so add HCl to all negatives.

False positive with phenazocine (antipyrine) (1)
Microgram Vol 6, No 1 pg 14 (Jan 1973)

Richard Ruybal's modification of cobalt thiocyanate reagent
Microgram Vol 5, No 3 pg 28 (March 1972) (2)

Carolyn Ruybal's Modification of the above reagent
Microgram Vol 6, No 2 pg 28 (Feb 1973) (3)

Scott's Test
Microgram Vol 6, No 11, pg 179 (Nov. 1973) (4)

Lorch advocates FPN, for phenothiazine, in addition to Scott's (5)
Microgram Vol 7, No. 8, pg 100 (Aug 1974)

Lorch: Non-phenothiazine false positives with Scott's test (6)
Microgram Vol 7, No 11 page 129 (Nov. 1974)

Prall: List of false positives with Scott's test, detected with Marquis (7)
Microgram Vol 8, No 9 pg 130 (Sept 1975)

Bleach test
Microgram Vol 7, No 6 pg 68 (June 1974) (8)

Methylbenzoate test
Microgram Vol 8, No 1, page 10 (Jan 1975) (9)
Also Bulletin on Narcotics Vol 27, No 2 (Apr-June 1975)

For Procaine: Sanchez test - positive with procaine or benzocaine (3)
Microgram Vol 5, No 5 pg 51

Pocatello also uses Liebermann's, Mayer's and Wagner's for general screening.

TLC:

			Comments
A.	Reference: Microgram Vol 5, No 5, May 1972, Page 51 (10)		
	(1) Cyclohexane	5	procaine, cocaine, benzocaine
	Chloroform	4	
	Diethylamine	1	
	(2) Ethylacetate	6	procaine, cocaine benzocaine
	Benzene	3.5	
	Ammonium hydroxide	1	
B.	Reference: Microgram Vol 7 No. 10 pg 122 (Oct 1974) (11)		
	Ethyl acetate	60	Paper gives Rf's: cocaine, tetracaine procaine lidocaine, piperocaine, benzocaine
	Dimethyl formamide	20	
	Dioxane	10	
	Acetic Acid	10	
C.	Reference Microgram Vol 7, no 10 page 124 (Oct 1974) (12)		
	Methylcyclohexane	3	cocaine, lidocaine (Apply as base, not as HCl salt)
	$CHCl_3$	5	
D.	Reference Microgram Vol 8, No 1, page 4 (Jan 1976) (13)		
	Spray plate with 0.5 N NaOH and dry at 140° C		Benzocaine, lidocaine cocaine, procaine
	Develop in methyl acetate		
	(Can be done without spraying plate but tailing occurs)		
E.	Reference Analytical Manual by Sobol and Moore ("The White Book")		
	1. Chloroform	25	
	Dioxane	60	
	Ethyl acetate	10	
	Ammonium hydroxide	5	
	2. Chloroform	40	Separate cocaine, procaine well
	Ethyl acetate	10	
	Ammonium hydroxide	10 drops	
F.	Chloroform	9	Separates cocaine procaine. slight streaking
	MeOH	1	
G.	Chloroform saturated with NH_4OH	18	Separates cocaine, procaine well, high Rf.
	MeOH	1	
H.	Cyclohexane	80	Supposed to be good for cocaine, procaine, PCP
	Benzene	16	
	Isopropylame	4	
I.	Clarke's T_1		See picture (14)
J.	4% diethylamine in Benzene		" "

Microcrystal tests

1. Platinic chloride - 5% aq. (Reference: Methods of Analysis of AOAC Eleventh Edition 1970 pg 709, 710). Gives feathers. Does not work if lidocaine present, or some other diluents.

2. Gold chloride - 5% aq. - dissolve powder in 0.1 NHCl, add drop of 5% gold chloride - floating feathers.
Ref. EGC Clarke - Isolation and Identification of Drugs
Note: Pocatello reminds us l-cocaine and d,l-cocaine give different microcrystal forms. (15)

3. Gold bromide - Reference: Modern Microcrystal Tests for Drugs - Fulton, page 385 #4 (See Pam's write up for recipe in plain English). Almost immediately a large number of irregular blades form which appear green with low birefringence. After standing 1-2 minutes see small x's, usually in a different plane (focus down). This test works even in the presence of lidocaine. (Sometimes called Ruybal's Gold Bromide because Richard Ruybal first described the use of this reagent on cocaine at the American Academy meeting in Dallas). (16)

4. Sodium acetate: 20% aq. (Ref: "Isolation and Estimation of Cocaine in the Presence of Tetracaine, Procaine, Benzocaine, and Amphetamine" by Milos and Porto, Alcohol and Tax Laboratory, IRS, New York). Supposed to form cocaine base crystals, unaffected by procaine, tetracaine, benzocaine, and amphetamine. (17)

5. Ecgonine with phosphomolybdic acid (1 gram phosphomolybdic acid in 20 ml of 1:1 HNO_3). Reference-same as #4 above. See paper for a discussion of the test. (17)

Note: I am unaware of anyone having used #4 and #5 above, and so am not sure if they work . . . they are listed for sake of completeness and in case you are desperate.

GLC -

Microgram Vol IV No. 3 pg 29. (March 1971) (18)
6' 3% OV-1 on Chromosorb WHP at 210°C

Microgram Vol IV No. 6 pg 60 (July 1971) (19)
Procaine, cocaine, PCP 6' 3% OV-17 Chromosorb WHP at 230°

Microgram Vol V No 5 pg 51 (May 1972) (10)
Procaine, cocaine, benzocaine 3% OV-1, OV-25, 210° 140° respectively

Microgram Vol V No 12 pg 140 (Dec 1972) (20)
Procaine, cocaine, benzocaine
Internal std of docosane, dissolve sample 9/1 ($CHCl_3$:MeOH)
3% OV-17, 6' Chromosorb WHP, 210°

Microgram Vol 9 No 2 pg 18 (Feb. 1976) (21)
Table of retention values on OV-17, 6' on Gas Chrom Q

Journal of Chromatographic Science Vol 9, July 1971 page 393
Finkle's Table of Relative Retention times on SE-30, 2' and 6'

Journal of Pharmaceutical Sciences Vol 63, No 12 Dec. 1974 pg 1963. (22)
Derivatization of Cocaine w/trimethylanilinium hydroxide.

Currently most people shoot on SE-30 (or OV-1 or -101) and/or OV-17, at temperatures appropriate to column length and GC performance.
Example: (a). 3' OV-17 at 205° (Boise prefers to SE-30, OV-1 or -101 because less tailing) OV-17

(b) 2' 3% OV-17 at 225°C and 250°C used by Pocatello

For detection of hydrolysis by-products benzoylecgonine and ecgonine (23)
Journal of Chromatography 101 (1974) pg 215-218.

IR

Microgram Vol 2, No 2 pg 47 June 1969
Cocaine ion-pairing extraction from $CHCl_3$

Microgram Vol 5, No 7 July 1972 pg 80
Ion pairing - use phase separating paper

Microgram Vol 3 No 3 May 1970, pg 89, and Vol III No. 4 June 1970, pg 121
Celite column: cocaine and procaine - requires sample containing 5-15 mg cocaine

Microgram Vol 4, No 3, March 1971, pg 29
Celite column: cocaine, caffeine and procaine

Microgram Vol 5, No 5, May 1972 pg 49
Celite column: cocaine, procaine, benzocaine

Microgram Vol 7, No 6 page 70 (June 1974)
Cocaine extracted from $NaHCO_3$ with CCl_4

Microgram Vol 7 No 8, page 96 (Aug. 1974)
Clark's & Allen's paper on cocaine - lidocaine by Celite column. Rick says this did not work for him.

I. Quick cleanup if cocaine HCl + sugars; or cocaine-procaine mix (Does not work if lidocaine present)

Make a column using a pasteur pipette (unconstricted) with glass wool and place a portion of the sample in it. Run CH_2Cl_2 through the samples fast. If you wish, dry the CH_2Cl_2 through a separate column of Na_2SO_4. Put the CH_2Cl_2 on a steam bath to reduce its volume, and precipitate using pet ether. Dry crystals and run on KBr disc. (This method depends on the fact that procaine HCl is not very soluble in CH_2Cl_2 esp. in a short time).

II. Acid-base shake out.
As noted in the early microgram papers, cocaine-HCl will ion-pair, that is it will be extracted into $CHCl_3$ if the HCl concentration is 2.8N to 4N. If you shake it out on the basic side, it's best to use $NaHCO_3$ or Na_2CO_3 to avoid hydrolysis in very basic solutions. After shaking with $CHCl_3$ or CH_2Cl_2, dry the organic phase through Na_2SO_4, reduce its volume and dry directly on KBr. Do not try to make the HCl salt by adding HCl and MeOH because of risk of hydrolysis.

III. Cobalt-thiocyanate derivative for cocaine+lidocaine
(Reference: Naylor et al from Bullet - Sn of Midwest Assocation).

IV. Platinic chloride derivative for cocaine + lidocaine.
Joe Powers' method.

V. Spot from TLC plate extracted with dilute acid. Make basic keeping in mind the precautions in II, above.

VI. Celite column - see U.V. section, reference #1. Follow directions given in that paper. Also see Fred's column chromatography notes.

VII. "Lumpology"

If your cocaine is lumpy or contains 2 kinds of material which you can separate under the stereoscope try running an IR direct on the lumps (or whatever component your screening tests indicate may be cocaine).

VIII. Alumina column

As a last resort you might try alumina. Make a microcolumn of alumina and wet it with CH_2Cl_2. Apply your sample in CH_2Cl_2 or ether. Wash with ether or CH_2Cl_2 to remove the contaminants (esp dyes). Elute using progressively more polar solvents. The cocaine will come off as the base, not HCl salt. Two cautions: different batches of alumina behave differently -- you may have to try different ones to get one that works properly. Also this procedure is far from quantitative; bad recoveries are to be expected.

UV-

1. Paper by Nakamura and Parker "Assay of Cocaine in the Presence of Procaine and Quinine by Column Chromatography" Journal of Chromatography Vol 52 (1970) pg 107-110.

2. Rick's Micro Adaptation of the above
   A. Column prep: Mix 1 gram Celite + 0.5 ml of (1M KNO_3 in 0.1 N HCl) Put in column on top of glass wool, tamp down, and put glass wool over top.

   B. Sample prep: Dissolve sufficient sample in 0.5 ml of (1M KNO_3 in 0.1 N HCl) to give absorbance of approximately 0.5A. (Is about 0.14 mg cocaine HCl/ml when 0.25 ml of solution put on column). Apply 0.25 ml of sample solution to column.

   C. Elution: Use 9 ml of water-washed $CHCl_3$ on column. Catch in 10 ml volumetric containing 1 ml of MeOH and 1 drop of HCl; bring to volume with $CHCl_3$. Scan on U.V.

Quantitation-

1. By external std on GLC

2. By internal std on GLC - have used methadone as internal std.--need to show none present originally.

3. By U.V. using celite column (if no lidocaine). Or Pocatello uses EtOH if no interfering substances present.

4. "Back seat" method - see separate sheet-- for narcotics officers.

Isomer problem

Microgram Vol 9, No 5 page 66 (May 1976) (35)
Review isomer problem, suggest micro X-tal test

Microgram Vol 9, No 8, page 107 (Aug 1976) (36)
Offer of small amount of d,l cocaine.

Microgram Vol 10, No 4, page 52 (April 1977) (37)
Use of salt of tartaric acid to give different IR's.

Microgram Vol 11, No. 3 page 41 (March 1978) (38)
Preparative TLC and mixed melting points

Microgram Vol 11, No 3 page 45 (March 1978) (39)
Philosophical discussion of wording of law - "derivative".

Also see latest Board of Pharmacy list. (40)
JOURNAL OF CHROMATOGRAPHY Vol 152 (1978) 589-591 - TLC of derivatives separate isomers
Scientific Sleuthing Vol 3 No 3.
Reference to People vs. Harper, 562 P2nd 1112 (Colo. 1977)
Colorado Supreme Court said their statute includes natural and synthetically produced cocaine. The chemists' testimony was admissible to identify cocaine even though his tests did not distinguish the two forms.

Stability problems

1. Discoloration effects of diluents
   Journal of Forensic Sciences Vol 19, No. 4 (868-872) (41)

2. Stability in dilute H_2SO_4
   Microgram Vol 6, No 9, page 135 (Sept. 1973) (42)

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.

Microgram Vol 5, No 3
March 1972
(2)

BNDD LABORATORY NOTES

DATE February 8, 1972

NO. 31

DRUG TYPE

METHODOLOGY

COLOR TEST TO DIFFERENTIATE BETWEEN COCAINE AND PROCAINE

Richard Ruybal
Forensic Chemist
Bureau of Narcotics and Dangerous Drugs
Dallas Regional Laboratory

Using the present cobalt thiocyanate reagent, blue colors are obtained with both Cocaine and Procaine as well as with many other drugs. The Sanchez reagent gives a positive reaction with Procaine and other primary amines.

As screening field tests, especially to the agent in the field, the combination of the two reagents affords no real indication that Cocaine is present.

The following reagent is submitted for a color test to differentiate between Cocaine and Procaine in illicit samples. Other drugs were also tested and results are noted.

Stock Solutions

A. Cobalt Thiocyanate - a 2% solution in H_2O

B. Phosphoric Acid - Syrupy H_3PO_4

C. Platinum Chloride - 1 gram $H_2PtCl_6 \cdot 6H_2O$ in 20 ml H_3PO_4 (1+3)

Reagent

Mix by volume 9 parts of A, 3 parts of B, and 1 part of C for test reagent.

Test

Place small amount of sample on a spot plate, add 3-4 drops of reagent and stir with glass rod.

DRUG	RESULTS
1. Cocaine	Blue, flaky ppt. remaining undissolved.
2. Procaine	No ppt. or blue color forms.
3. Benzocaine	No ppt. or blue color forms.
4. Butacaine	Green color forms, but fades away.
5. Dibucaine	Slight green color and ppt. forms, but fades away.
6. Lidocaine	Blue, flaky ppt. remaining undissolved.
7. Mepivicaine	Blue ppt. forms, but fades away.
8. Tetracaine	Blue color forms, but fades away.
9. Quinine	Green ppt. and color remaining undissolved, but different from Cocaine.
10. Methapyrilene	Green ppt. and color, but fades away.
11. Heroin	Blue ppt. and color, but fades away.
12. Methadone	Blue ppt. and color, but fades away.
13. Demerol	Blue ppt. and color, but fades away.
14. Phencyclidine HCl (PCP)	Blue-green ppt. remaining undissolved.

The only similarity of any of the above drugs tested to Cocaine was Lidocaine. It was the only other one that formed a blue, flaky ppt. which remained unchanged. All others forming blue ppts. or blue colors lost all blue color within a few minutes leaving either a pink-orange or a yellowish color. Quinine formed a green ppt. and color which is quite easily discernible from Cocaine.

The drugs listed above were also tested with a mixture of Stock Solutions (A) and (B) in a ratio of 9.3. The drugs that gave a blue, flaky ppt. in addition to Cocaine and Lidocaine were Dibucaine, Mepivicaine, Heroin, Methadone, and PCP. Demerol gave a blue color.

Microgram Vol 6, No 2 (Feb 1973)

(3)

BNDD LABORATORY NOTES

DATE

NO. 52

DRUG TYPE

METHODOLOGY

COLOR TEST TO DIFFERENTIATE BETWEEN COCAINE AND LIDOCAINE

Carolyn N. Ruybal
Forensic Chemist
Bureau of Narcotics and Dangerous Drugs
Dallas Regional Laboratory

The reagent for differentiation between cocaine and procaine described by Mr. Ruybal in BNDD Laboratory Notes in Microgram Vol. 5 No. 3 March 1972 is successful for differentiating cocaine from all the "caines" except lidocaine.

The following preparation of this reagent is submitted as a color test to differentiate between cocaine and lidocaine. It is simply a dilution of the "Ruybal" reagent 3 parts to 2 parts water. Lidocaine will not give a color reaction.

STOCK SOLUTIONS:

A. Cobalt Thiocyanate - a 2% solution in H_2O.

B. Phosphoric Acid - syrupy H_3PO_4.

C. Platinum Chloride - 1 gm H_2PtCl_6-$6H_2O$ in 20 ml H_3PO_4 (1+3)

REAGENT:

By volume mix 9 parts A and 3 parts B. Add 1 part C and mix well. Add 9 parts distilled water and mix. When these stock solutions are first mixed the reagent is clear orange in color. Let stand at least 5 to 7 days. A reddish brown precipitate is formed which settles to the bottom and the supernatant is a clear pink color. The reagent is then ready for use. The precipitate should not be removed or separated from the reagent.

TEST:

Place a small amount of sample on a spot plate, add 2-3 drops of reagent.

RESULTS:

Cocaine gives a blue flaky precipitate.

Procaine, benzocaine, butacaine, dibucaine, lidocaine, methapyrilene, heroin and demerol produce no color with this reagent. Methadone forms a slight blue color on standing. Phencyclidine HCl and antipyrine also give a blue color similar to cocaine.

Antipyrine (phenazone) can be differentiated from cocaine with Mandelin's reagent. Antipyrine gives an intense blue-green color whereas cocaine gives a slight red color which quickly fades.

DISCUSSION:

The first approach to modifying this reagent for cocaine-lidocaine differentiation was to dilute the original Ruybal Cobalt Platinum Reagent with a freshly made 5% solution of stannous chloride. But when distilled water was substituted for the stannous solution the reagent worked equally well.

When preparing this reagent it would appear that a 1% solution of cobalt thiocyanate solution in water could be used in lieu of the final dilution step. This was tried with no success. It is important to mix the reagent in the manner described.

When using the reagent for the test use only the pink portion (supernate) of the reagent with the precipitate present but settled to the bottom. Agitation of the reagent before the test will not affect its performance but is undesirable as the dispersed red particles of precipitate make it harder to see the color reaction of the cocaine.

Positive results were obtained with this reagent on samples containing as low as 5 percent cocaine.

The final dilution with water may be done either at the time of mixing the original solution, as described above, or later. Some of an aged original Ruybal cobalt platinum solution was diluted approximately 2 weeks later in the ratio 3 parts reagent plus 2 parts water. The final diluted mixture performed successfully for differentiating cocaine from lidocaine when used immediately

after dilution and when used again 3 weeks later. It is important when diluting the aged reagent in this manner to agitate well first to disperse the precipitate before measuring the aliquot to be diluted. The precipitate must always be present in the reagent.

An alternate approach to performing this test is to add 3 drops of the original Ruybal cobalt platinum solution to the sample on a spot plate. At this point both cocaine and lidocaine give a blue precipitate. Add 2 drops of water and stir. The lidocaine precipitate will dissolve. The cocaine precipitate will partially dissolve but the larger blue flakes of the cocaine precipitate remain.

Microgram Vol. 8, No. 9 (Sept 1975)

DEA LABORATORY NOTES

DATE June 11, 1975

NO.

DRUG TYPE Cocaine

METHODOLOGY Color Tests (Field Test)

AN ADDITIONAL SCREENING TEST FOR COCAINE

Josef D. Prall
Forensic Chemist
South Central Regional Laboratory
Drug Enforcement Administration

OBJECTIVE

To develop a series of field test reagents for cocaine, readily available to federal, state, and local agents, which will screen out false positive tests with the Scott Field Test for cocaine (1).

BACKGROUND

A sample purchased as cocaine submitted to this laboratory for analysis gave a positive reaction to all three steps of the Scott Field Test for cocaine. Further analysis by gas chromatography/mass spectrometry and infrared spectroscopy showed the sample to be diphenhydramine. Lorch (2,3) has also encountered similar difficulties with suspected cocaine samples which were found to be phencyclidine and phenothiazine derivatives. Lorch suggested using a second test with the "FPN Reagent" (4). However, since the Scott test is primarily intended as a field test and the FPN Reagent is not readily available to agents in the field, an additional test using a reagent which is readily available was deemed advisable. It was found that the Marquis Reagent gives such a test. In addition to the false positives reported by Lorch, several additional substances were found to give false positives; these too were screened out by the Marquis test.

REAGENTS

(1) Scott Field Test (1)

Solution #1 — 2% cobaltous thiocyanate dissolved in water and then dilute 1:1 with 96% USP Glycerine (use 5 drops).

Solution #2 — Concentrated hydrochloric acid (use 1 drop, 2nd drop if necessary -- no more).

Solution #3 — Chloroform.

(2) Marquis Reagent -

A 4-5% solution of 40% formaldehyde in concentrated sulfuric acid, also packaged and sold commercially as "Marquis Reagent" used as a test for opium alkaloids, heroin, and amphetamines.

METHOD

The three step Scott Field Test is performed on a portion of the sample as previously described by Scott (1). If a positive result is obtained on all three parts, a second portion is added to Marquis Reagent. In general:

Positive Scott, Positive Marquis (Some Color Reaction)

- Indicates presence of some compound other than cocaine, although it does not rule out the presence of cocaine.

Positive Scott, Negative Marquis (No Color Reaction)

- Indicates presence of cocaine, and absence of the compounds listed below in Table 1 which give a positive Marquis Test.

A positive reaction to the Scott Field Test involves

a blue solution in Step 1, a pink solution in Step 2, and a blue extract in the chloroform layer in Step 3. It must be emphasized that the solution should be blue in Step 1; a pink solution with blue specks should not be taken as a positive for this step.

Based on this criterion, some of the compounds which Lorch reported as giving positive reactions to Step 1 were instead found to be negative. This may be a function of the particular form of the compound used for the test; i.e., free base, hydrochloride salt, or other salt. Note the reactions for the various forms of cocaine, diphenhydramine and doxylamine in Table 1. It might be expected that certain salts, especially the hydrochlorides, of other drugs might give positive Scott tests; for example, phenyltoloxamine, which is similar in structure to diphenhydramine.

Combinations of certain drugs may of course give the same results to both tests as cocaine hydrochloride; for example, a mixture of antipyrine and carbinoxamine maleate.

REFERENCES

1. Scott, L. J., Jr., Microgram, Vol. 6, No. 11, Nov. 1973

2. Lorch, S. K., Microgram, Vol. 7, No. 8, August 1974

3. Lorch, S. K., Microgram, Vol. 7, No. 11, November 1974

4. Clarke, E. G. C., "Isolation and Identification of Drugs", The Pharmaceutical Press, London, U. K. (1969).

TABLE 1

	SCOTT			MARQUIS
Steps	1	2	3	
Cocaine HCl	+	+	+	NR
Cocaine Base	-	+	+	NR
Chlorpromazine HCl	+	+	+	Pink to Red to Violet
Diphenhydramine HCl	+	+	+	Yellow to Orange to Brown
Diphenhydramine Base	SP	NC	+	Orange to Brown
Diphenhydramine Salicylate	NR	+	+	Yellow to Orange to Brown
Diphenhydramine Theoclate	NR	SP	+	Yellow to Orange to Brown
Doxylamine HCl	+	+	+	Yellow to Dirty Pink to Light Purple
Doxylamine Succinate	+	+	-	Yellow to Dirty Pink to Light Purple
Diphenylpyraline HCl	+	+	+	Yellow to Orange to Brown
Dibucaine	+	+	-	NR
Tetracaine HCl	+	+	-	NR
Carbinoxamine Maleate	+	+	-	NR
Promazine HCl	+	+	-	Pink to Red to Dark Red
Dimethindine	+	+	-	Purple
Pyrilamine Maleate	+	+	-	Purple
Homochlorcyclizine HCl	+	+	-	Pale Yellow to Pale Green
Methapyrilene	+	+	-	Purple
Tripelennamine HCl	+	+	-	Orange-brown to Brown
Lidocaine HCl	SP	NC	+	Faint Pink
Phencyclidine HCl	SP	NC	+	NR
Phenyltoloxamine Citrate	SP	+	+	Wine Red
Promethazine HCl	SP	NC	+	Pink to Red to Violet
Pyrathiazine	Slow SP	NC	+	Pink to Red to Violet
Pyrathiazine HCl	Slow SP	NC	+	Pink to Red to Violet
Prochlorperazine Edisylate	SP	+	-	Pink to Red to Violet
Pheniramine Maleate	SP	+	-	NR
Orphenadrine Citrate	SP	NC	+	Orange
Chlorcyclizine	NR	NC	-	Pale Yellow
Cyclizine HCl	NR	NC	NC	NR
Brompheniramine Maleate	NR	NC	NC	NR
Methoxyphenamine HCl	Pale Blue w/Lg.Amt.	NC w/5 drops HCl	+	Wine Red
Perphenazine	NR	NC	NC	Pink to Red to Violet

NR - No Color Reaction
NC - No Change from Previous Step
SP - Blue Specks in Pink Solution

TABLE 1 (Continued)

	SCOTT			MARQUIS
Steps	1	2	3	
Diethylpropion	SP	NC	+	NR
Benzocaine	NR	NC	NC	NR
Procaine HCl	NR	NC	NC	NR
Antipyrine	NR	Flashes Blue then Pink	+	NR

NR - No Color Reaction
NC - No Change from Previous Step
SP - Blue Specks in Pink Solution

Microgram Vol 7, No 11 (Nov. 1974)
(6)

SPECIFICITY PROBLEM WITH THE COCAINE-SPECIFIC FIELD TEST II. NON-PHENOTHIAZINE FALSE POSITIVES AND THE SEPARATION OF PHENCYCLIDINE - PROMAZINE COMBINATIONS

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OBJECTIVE

We have found that certain non-phenothiazine drug combinations will give the "highly specific" field test (1) for cocaine, and that the FPN test (2) will not screen for these false positives.

BACKGROUND

Since our previous report (3) our laboratory has received three more cases of phencyclidine (PCP) - phenothiazine combinations which gave the cocaine-specific field test color sequence and a positive FPN test for phenothiazines. The phenothiazine was identified as promazine by gas chromatography and gas chromatography-mass spectrometry. As reported earlier promazine gives steps one and two of the cocaine-specific field test (3).

The test was run on standard samples of the following drugs and combinations of drugs:

TABLE 1

	Color after step 1	Color after step 2	Color in $CHCL_3$	FPN
1) Phencyclidine	blue	pink	clear	-
2) Promazine	blue	pink	clear	+
3) Cocaine	blue	pink	blue	-
4) 1 + 2	blue	pink	blue	+
5) Dibucaine	blue	pink	clear	-
6) Methapyrilene	blue	pink	clear	-
7) 1 + 5	blue	pink	blue	-
8) 1 + 6	blue	pink	blue	-
9) 5 + 6	blue	pink	clear	-
10) 2 + 5	blue	pink	blue*	+
11) 2 + 6	blue	pink	blue*	+

*depends on the relative amounts of each component.

It is important to note that combinations #7 and 8 give a false positive for cocaine, but cannot be screened out with the FPN test, as neither compound is a phenothiazine.

SEPARATION OF PCP - PROMAZINE COMBINATIONS

Dissolve mixture in 2.8 N HCl and extract with an equal volume of chloroform. Wash chloroform two times with fresh 2.8 N HCl. Extract PCP from chloroform with an equal volume of 0.1 N sulfuric acid. A U.V. spectrum of the 0.1 N sulfuric acid extract will reveal a promazine peak at 252 mμ with a 269 mμ shoulder representing the PCP (promazine 252 mμ E 1% 1 cm 1122, PCP 269 mμ E 1% 1 cm 9.2). Add three drops of concentrated HCl to the sulfuric acid extract, extract with an equal volume of chloroform and dry. The resulting extract will give an IR spectrum clearly recognizable as that of phencyclidine HCl.

(1) Scott, L.J., Jr., "Specific Field Test for Cocaine", Microgram, 6:11, Nov. 1973, pages 179-181.

(2) Clarke, E.B.C., "Isolation and Identification of Drugs", The Pharmaceutical Press, London, U.K. (1969).

(3) Lorch, S.K., "Specificity Problem With the Cocaine Specific Field Test, and Its Solution", Microgram, 7:8, Aug. 1974, pages 100-101.

The author wishes to thank Dr. Fathi M. Saad, Chief, Warren Regional Laboratory, Bureau of Laboratories, Michigan Department of Public Health, for the G.C.-Mass Spectrographic identification of the promazine.

9/10/74

Microgram Vol 7, No 8
(aug. 1974)
(5)

SPECIFICITY PROBLEM WITH THE COCAINE SPECIFIC FIELD TEST, AND ITS SOLUTION

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OBJECTIVE

We have found that certain drug combinations will give the "highly specific" field test (1) for cocaine. An extra test is suggested for screening out these false positives.

REAGENTS

Solution #1 (1) 2% cobaltous thiocyanate dissolved in water and then dilute 1:1 with 96% USP Glycerine (use 5 drops).

Solution #2 (1) Concentrated hydrochloric acid (use 1 drop, 2nd drop if necessary -- no more).

Solution #3 (1) Chloroform.

FPN Reagent (2) 5 ml of 5% w/v solution of ferric chloride in water.
45 ml of 20% w/w perchloric acid
50 ml 50% w/w nitric acid

BACKGROUND

A purported cocaine sample submitted to this laboratory gave a positive field test for cocaine. However, further analysis showed it to be a combination of phencyclidine (PCP) and a phenothiazine; our first test, a UV spectrum, led us to believe it was promethazine, but we were unable to finally identify it as such.

The field test run on promethazine alone failed to give a blue color in solution 1, and a pink color in solution 2 but gave a blue color in the chloroform layer. Combinations of PCP, dibucaine or methapyrilene (which as reported earlier by Scott (1), gave blue with solution #1 and pink on addition of concentrated HCl), with promethazine gave the full cocaine specific color sequence (Table 1). Butacaine was not tested as we had no available standard. Two other phenothiazines tested, chloropromazine and promazine, gave the proper color sequence when in combination with promethazine.

METHOD FOR DISTINGUISHING FALSE POSITIVES

We find we can improve test specificity by performing a fourth test. Add a few drops of FPN solution (2) to another portion of the sample. FPN indicates the presence of phenothiazines, and gives a short term orange, pink or red color, depending on which of the many phenothiazines it is. If one of these colors is observed, there is a strong indication of a combination of the nature described above, and further testing is indicated. FPN tests on cocaine, PCP, dibucaine and methapyrilene alone are negative.

It should be noted that the phenothiazine in the actual case was not promethazine, but probably another phenothiazine as yet unidentified.

TABLE 1

		Color after Step 1	Color after Step 2	Color in $CHCl_3$	FPN
1)	Cocaine	blue	pink	blue	-
2)	Phencyclidine	blue	pink	clear	-
3)	Dibucaine	blue	pink	clear	-
4)	Methapyridene	blue	pink	clear	-
5)	Chloropromazine	blue	pink	clear	+
6)	Promazine	blue	pink	clear	+
7)	Perphenazine	pink	pink	clear	+
8)	Prochloroperazine	pink	pink	clear	+
9)	Promethazine	pink	pink	blue	+
10)	1 + 9	blue	pink	blue	+
11)	2 + 9	blue	pink	blue	+
12)	3 + 9	blue	pink	blue	+
13)	4 + 9	blue	pink	blue	+
14)	5 + 9	blue	pink	blue	+
15)	6 + 9	blue	pink	blue	+

1. Scott, L.J., Jr., Microgram, Vol. 6, No. 11, Nov. 1973.

2. Clarke, E.B.C., "Isolation and identification of Drugs," The Pharmaceutical Press, London, U.K. (1969).

DEA LABORATORY NOTES

DATE November 2, 1973

NO. 68 -179-

DRUG TYPE COCAINE

METHODOLOGY Color Test

(4)

from Microgram. Vol 6, No 11, (Nov. 1973)

SPECIFIC FIELD TEST FOR COCAINE

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OBJECTIVE

To develop a rapid, sensitive, highly specific color test for cocaine.

BACKGROUND

The use of cobalt thiocyanate reagent for the presumptive detection of cocaine is of limited reliability since many other compounds give the same reaction as cocaine. Lidocaine, procaine, PCP, tetracaine, benzocaine as well as cocaine give a blue precipitate with this reagent. The following test for cocaine has been emperically developed and successfully tested several hundred times by both chemists and agents. The "Ruybal" reagent[1] and its modification[2] are very useful in the hands of chemists, but field personnel often have experienced difficulty in their interpretation. The method proposed herein is almost impossible to misinterpret, and is highly sensitive and specific.

REAGENTS

Solution #1: 2% cobaltous thiocyanate dissolved in water and then diluted 1:1 with 96% USP Glycerine(*)

Solution #2: Concentrated Hydrochloric Acid

Solution #3: Chloroform

Other: Small disposible test tubes

(*) Available from K&K Laboratories, Plainview, N.Y.

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.

DISCUSSION

Only cocaine, from the following list of compounds, will give the results outlined above. The following chart illustrates how the test can select cocaine from other compounds listed.

	Blue Color with Solution #1	Pink After Solution #2 Added	Blue $CHCl_3$ Layer From Solution #3
Cocaine	yes	yes	yes
Phencyclidine	yes	yes	no
Dibucaine	yes	yes	no
Butacaine	yes	yes	no
Methapyrilene	yes	yes	no

The following compounds do not produce a blue color with Solution #1

Antipyrine
Procaine
Benzocaine
Mepivacaine
Prilocaine
Beta Eucain
Aminopyrine
Quinine
Methadone
Tetracaine
Lidocaine
Heroin
Demerol
Sugars and starches

Instructions to agents who use this procedure specifies that they are to use 5 drops solution 1, 1 or 2 drops (not more) of solution 2 and 5 drops solution 3. The amount of solution 1 is not critical, nor is the amount of solution 3. However, the ratio of solution 1 to solution 2 is critical. If excess concentrated Hydrochloric Acid is added to solution 1 after the blue color has developed with cocaine, a blue rather than pink solution will result; this blue will not extract into the $CHCl_3$ layer. If excess cocaine is used with solution 1, then it is sometimes necessary to add 2 drops of concentrated Hydrochloric Acid, no more should be used.

Consequently, if one adheres to the 5 drops + 1 drop + 5 drops of the three solutions 1, 2 and 3 respectively, the test will function easily and well. This test has not failed to detect cocaine as low as 1% cocaine in some cases. This system has been used in this laboratory on approximately 150 cocaine samples over a period of about 5 months, and about 50 to 75 times by agents on an experimental basis over a period of about 4 months.

No extensive shelf life tests have been run, but the solution #1 has been stored at room temperature under laboratory conditions for a period of six months with no detectable deterioration or loss of effectiveness.

REFERENCES

(1) Richard Ruybal, Microgram Vol. 5, No. 3, 1972
(2) Carolyn N. Ruybal, Microgram Vol. 6, No. 2, 1973

Microgram Vol 7, No 6 (June 1974) (8)

LABORATORY NOTES

DATE JUNE 4, 1974 -68-

NO.

DRUG TYPE

METHODOLOGY

A "NEW" FIELD TEST REAGENT

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INTRODUCTION

Agent Park Kaestner of the Kansas City, Missouri DEA Task Force recently described the use of laundry bleach as a field test reagent for procaine. It has been used in the Kansas City area in an undercover capacity.

Laundry bleach, commonly sold in grocery stores as Purex or Chlorox, is an aqueous solution of sodium hypochlorite, usually 5.25% by volume. As such, in an alkaline state, it is a strong oxidizing agent. A literature search of available material revealed nothing on sodium hypochlorite as a color test for organic compounds of interest in our field of endeavor.

PROCEDURE

Spot tests were performed in the usual manner using spot plates and comparing the colors developed with a standard. Purex solution purchased from a grocery store was used.

RESULTS AND DISCUSSION

Sodium hypochlorite, nicknamed "doper's reagent", when tried on a number of controlled and non-controlled drugs including some of the "caines" behaved much like the Sanchez Test for certain primary amines. "Doper's reagent" gave a chocolate brown with procaine and benzocaine. It was flaky in appearance. The following drugs were tested with "doper's reagent" and no color developed:

Heroin HCl, Cocaine (HCl and base), MDA HCl, PCP HCl, Methadone HCl, Demerol, common barbiturates, Codeine (phosphate and base), Amphetamine sulfate, Methamphetamine HCl, Mescaline sulfate, LSD, Chlordiazepoxide (Librium), Diazepam (Valium), Phenacetin, Acetanilid, Carbromal, Propoxyphene (Darvon), Phenyl propanolamine, Ephedrine sulfate, Mepivicaine, Dibucaine, Hexylcaine, Quinine, Caffeine, Mannitol, Saccharine, Lactose, Dextrose, Starch, Aspirin, Boric Acid, Stearic Acid, and Talcum.

Listed below are color reactions obtained with other drugs not previously mentioned:

Methapyrilene HCl-Light green-flaky
Lidocaine HCl-orange brown, oily, floating drops
Lidocaine sulfate-orange brown, oily, floating drops

Stovaine HCl-orange, oily drops-on standing
Salicylamide-greenish brown on standing
Salicylic Acid-brown on standing
Oxytetracycline-greenish yellow (chartreuse)
Sulfamerazine-greenish orange, then greenish yellow
Sulfadiazine-greenish orange, then greenish yellow
Chloroprocaine-orange
DMT-instant orange, then brown
DET-instant orange, then brown
Para amino benzoic acid-brown-flaky
Meta amino benzoic acid-brown-flaky
Morphine sulfate-brown-flaky

In conclusion, it would seem that sodium hypochlorite would have some usefulness as an easily obtained field test reagent for suspected procaine in cocaine-procaine mixtures; cobalt thiocyanate being used to check for cocaine. DMT, DET, benzocaine, and morphine sulfate should also be kept in mind. If the Marquis is negative for opium alkaloids, then morphine can be eliminated. The test does not work well with brown heroin-procaine mixtures. Since it gives the chocolate brown color with the hydrolysis product (or metabolite) of procaine, it could possibly be used in TLC drug screening procedures as a color development spray for p-amino benzoic acid.

The use of standards for color comparison with the unknown drug is encouraged whenever possible.

(9)

Microgram Vol. VIII, No1
Jan. 1975

A SIMPLE FIELD TEST FOR COCAINE NOT RELYING ON COBALT THIOCYANATE

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OBJECTIVE

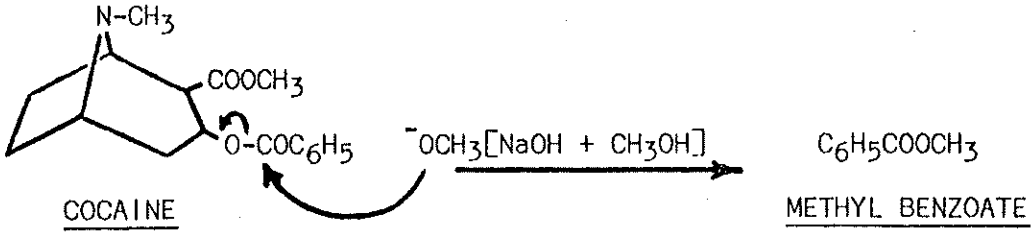
Our purpose was to improve the sensitivity and specificity of present methods of cocaine detection in a simple, easily interpreted field test.

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 section 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. 1, No. 3, pp. 26-27 (1974)

Microgram Vol 5, No 5
May 1972
(10)

DATE

NO. 37

DRUG TYPE Mixture

METHODOLOGY

Analysis of Cocaine, Procaine, Benzocaine Mixtures

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Introduction

This laboratory frequently receives illicit cocaine samples which have been adulterated with synthetic local anesthetics and sugar. Two local anesthetics commonly encountered are benzocaine and procaine.

Since cocaine, benzocaine and procaine have similar UV absorption characteristics, a means of separating all three components has been investigated. Milos and Porto[2] devised a procedure for the simultaneous spectrophotometric determination of cocaine and procaine mixtures. Canaff[3] separates cocaine from procaine or tetracaine by using a 2.0N HCl chromatographic column. Moore[4] separates the same mixture utilizing a 0.1N HNO_3 column.

The method below is a modification of Moore's procedure which can be utilized to quantitate and identify cocaine in the presence of benzocaine and/or procaine.

Method

Apparatus

Chromatographic Column
UV Spectrophotometer

Reagents

Nitric Acid - 0.1N
Sulfuric Acid - 0.1N
Chloroform - Reagent Grade
Ethyl Ether - Reagent Grade
Ammonia - conc.
Celite 545 - acid washed

Procedure

Pack a pledget of fine glass wool into the base of a chromatographic column (25 cm long, 2 cm diameter) with a tamping rod. Place 2 grams of diatomaceous earth (Celite 545, acid washed)* into a 100 ml beaker. Add 1 ml 0.1 Nitric Acid, and mix with a spatula until homogeneous. Transfer the mixture to the column, and tamp moderately to compress the material into a uniform mass.

* Johns - Manville, New York, New York

Into another 100 ml beaker weigh accurately a portion of sample equivalent to about 15-20 mgs cocaine. (Illicit cocaine samples are often damp and lumpy, therefore, sample mixture should be carefully ground before proceeding). Add 2 mls 0.1N Nitric Acid to the sample and swirl to wet powder thoroughly. To this solution add 3 grams of diatomaceous earth and mix until fluffy. Quantitatively transfer this mixture to the column and tamp moderately. Place a pledget of glass wool on top of the packing.

The benzocaine is eluted from the column into a 200 ml volumetric flask with 200 ml water washed ether. Dilute the flask to volumn with ether. Evaporate a 20 ml aliquot to dryness on a steam bath. Dissolve the residue in a known volume of 0.1N H_2SO_4 to give a final concentration of approximately 10 mcg/ml. Read the absorbance of the solution at 226 mμ and compare against a standard solution of benzocaine.

The cocaine may then be eluted by passing 100 mls water washed $CHCl_3$ into a 100 ml volumetric flask. Dilute flask to volume with $CHCl_3$. Evaporate a 10 ml aliquot to dryness on a steam bath. Dissolve residue in 0.1N H_2SO_4 to give a final concentration of approximately 15 mcg/ml. Read the absorbance of the solution at 233 mμ and compare against standard cocaine.

Procaine is eluted from the column into a 250 ml beaker by passing 100 mls of ammoniacal chloroform (prepared by vigorously mixing 2 ml concentrated ammonium hydroxide with 100 ml $CHCl_3$ and allowing the phases to separate) followed by 100 mls water saturated chloroform. Remove a 20 ml aliquot and evaporate to dryness on a steam bath. Dissolve the residue in 0.1N H_2SO_4 to give a final concentration of approximately 15 mcg/ml. Read the absorbance of the solution at 228 mμ and compare against standard procaine.

Identification

Methods most frequently used for the qualitative determination of mixtures of this type are infrared spectroscopy and thin layer or gas chromatography.

A - Infrared Spectroscopy

To obtain spectra for benzocaine and procaine, the remainder of the stock solutions eluted form the column are evaporated to dryness. The residue may then be deposited as a film on a sodium chloride window.

The cocaine that is eluted from the column must be re-extracted in order to obtain a satisfactory IR curve.

Evaporate the $CHCl_3$ stock solution to about 20 ml. Transfer to a 60 ml separatory funnel and shake with 20 mls 4% ammonium hydroxide. Evaporate the $CHCl_3$ extract on a steam bath (prior to doing so add 1 drop conc. HCl to solution). A KBr disk may now be prepared in the usual manner.

B - Thin Layer Chromatography

Mix a portion of powdered sample with methanol and apply to a silica gel G plate (Analtech). Use the solvent systems mentioned below and observe spots by spraying with iodoplatinate spray (prepared by mixing 0.25 gms Platinic Chloride and 5 grams Potassium Iodide in 100 ml water).

Solvent System A[5] - Cyclohexane: Chloroform: Diethylamine 5: 4: 1
Solvent System B[6] - Ethyl Acetate: Benzene: Ammonium Hydride (6:3.5:1)

	(System A) Rf	(System B) Rf
Cocaine	0.65	0.68
Procaine	0.34	0.60
Benzocaine	0.26	0.41

C - Gas Chromatography

Sample is dissolved directly in methanol and injected under the following conditions:

Instrument	Packard #7360	
Column	3% OV1	3% OV25
Temperature	210°C	140°C
Carrier Gas	Nitrogen	Nitrogen
Carrier Flow	40ml/min	25ml/min
Detector	Flame Ionization	Flame Ionization
Air Flow	500ml/min	500ml/min
Hydrogen Flow	30ml/min	25ml/min

The following results were obtained:

Retention Time Cocaine	5.9 min	4.7 min
Retention Time Procaine	4.3 min	2.4 min
Retention Time Benzocaine	0.7 min	0.5 min

Discussion

Cocaine samples should be subject to qualitative tests before quantitative analysis is performed. The presence of procaine or benzocaine may be detected using the following spot tests:

1 - Sanchez reagent (prepared by mixing 2.5 ml freshly distilled furfural, 22.5 ml 95% EtOH and 75 ml glacial acetic acid). A red color is obtained in the presence of benzocaine or procaine.

2 - p-dimethylaminobenzaldehyde (1 gm in 50 ml EtOH and 50 ml conc. hydrochloric acid). An intense yellow color is obtained in the presence of benzocaine or procaine.

Furthermore, chromatographic screening tests should be performed prior to quantitation to detect the presence of benzocaine and/or procaine. In cases where no benzocaine is present, the ether wash will not be necessary during the quantitation.

Quantitative results of better than 95% cocaine have been obtained using this procedure. Results obtained for the benzocaine and procaine ranged between 92 and 94%. The results obtained on simulated mixtures are tabulated as follows:

	mgs added			mgs found		
	Benz.	Pro.	Coc.	Benz.	Pro.	Coc.
1.	9.8	10.1	10.4	9.3	9.2	10.1
2.	10.0	10.3	20.6	9.2	9.8	19.6
3.	15.1	14.8	15.4	14.0	14.1	14.9

References

1. E.G.C. Clarke, Isolation and Identification of Drugs Pharmaceutical Press, London, 1969

2. C. Milos, P. Porto, Spectrphotometric Determination of Cocaine in the Presence of Procaine and Benzocaine, IRS Publication, Alcohol and Tabacco Tax Laboratory, New York, N.Y.

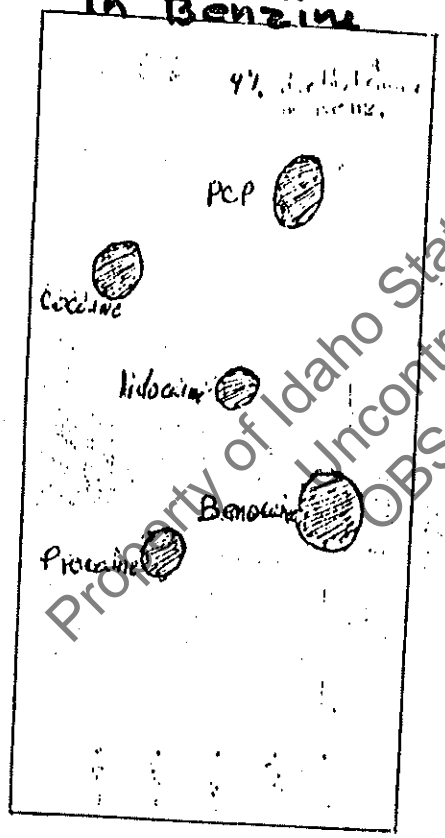
3. R.F. Canaff, Ion Pairing Chromatographic Separation and Determination of Cocaine, Microgram Vol III, #4, 121 June 1970.

4. J. Moore, The Analysis of Cocaine in Illicit Preparations Microgram Vol III, #3, 89-95, May 1970.

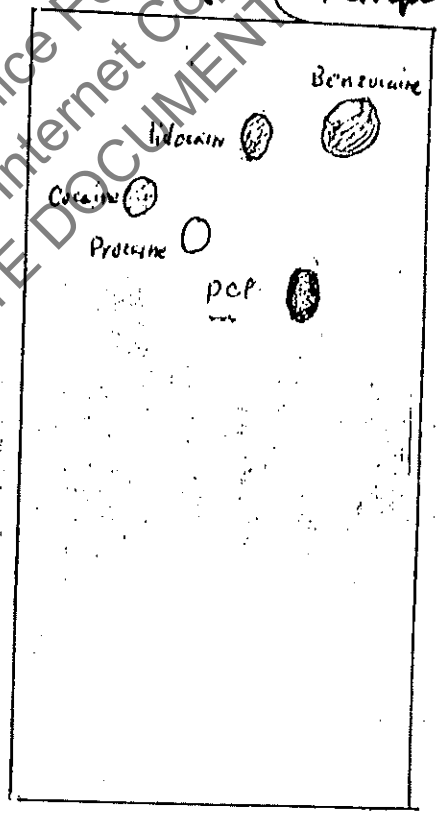
5. Sunshine, Handbook of Analytical Toxicology, Chemical Rubber Company 1969, Reference #1A.

6. IRS Publication #341, June 1967, page 94, system S4

4% diethylamine in Benzine



TLC (7 drops NH_4OH in 10 ml CH_3OH)



Sept 73

OTHER MICROCHEMICAL TESTS

and dl-Amphetamine - 1% HCl (volatility) gives "peacock feather" like crystal forms with low birefringence.

Ethylamphetamine - AuCl in dilute H_3PO_4 (volatility) gives single forms, fans, clusters and sheafs of <u>rods</u> with considerable rectangular-like internal structure. Birefringence colors.

ndimetrazine - AuCl in dilute H_3PO_4 (volatility) gives curved needles and rods in extensive branching systems with moderate birefringence.

henhydramine - AuCl in dilute H_3PO_4 (volatility) gives very small white rods with slanted ends (= narrow parallelograms).

ocaine - AuCl in H_2O (direct) gives bright birefringent needles and rods of regular shape alone, in clusters and in Xs.

aine - AuCl in H_2O (direct) gives rough, irregular forms with moderate to low birefringence.

haqualone - dissolve sample in drop of MeOH + add drop of 5% Sodium Carbonate which gives diamonds, hexagons, parallelograms and trapezoid forms with bright birefringence.

in - dissolve in 5% HCl + add drop of 10% Sodium Acetate which gives grey & colored birefringent hexagons.

* *

in dil.H_3PO_4 = 1g. $HAuCl_4 \cdot 3H_2O$ in 20ml. H_3PO_4(1+2)

in H_2O = 1 g. $HAuCl_4 \cdot 3H_2O$ in 20 ml. H_2O

dry PtBr = 1g. platnium chloride in 10ml of 40% HBr; add 10ml. conc. H_2SO_4 + 10ml. H_2O

dry PtBr = 5g. $H_2PtCl_6 \cdot 6H_2O$ and 10g. NaBr in 100ml. H_2O. allow to age 48 hours

Cocaine in the presence of Lidocaine

~~Ruybal's~~ Ruybal's "Gold Bromide"

from: Modern Microcrystal Tests for Drugs, Fulton.
page 385 #4

Reagent: $HAuBr_4$ in $2H_3PO_4$ - 1 (2+3) H_2SO_4

In plain English, you make it up this way:

50 ml.

$HAuCl_4 \cdot 3H_2O$ crystals ..... 1.0 GRAM

HBr (40%) ..... 1.5 ml.

Dilute above with

~~2 vol. of conc. H_2SO_4 to 3 vol. H_2O~~

{ 12 ml. of conc. H_2SO_4 dil. with 18 ml. of H_2O }

(23)

Reprinted from

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CHROM. 7751

Note

Gas chromatographic detection of ecgonine and benzoylecgonine in cocaine

J. M. MOORE

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(Received June 24th, 1974)

Cocaine hydrochloride is used in medicine as a topical anesthetic. It also has widespread use as a stimulant in the illicit drug market. During recent analytical investigations into illicitly manufactured cocaine, it was discovered that gas chromatographic (GC) methodology to detect the primary cocaine hydrolysis products was lacking. Cocaine can be hydrolyzed in dilute acid to benzoylecgonine, ecgonine, benzoic acid and methanol[1].

The detection of cocaine hydrolysis products is important in the quality control of pharmaceutical cocaine. A number of papers have described the detection of benzoylecgonine and ecgonine in pharmaceutical cocaine and also in botanical and biological samples[2–7]. These procedures utilized primarily paper and thin-layer chromatographic techniques. Fish and Wilson[8] detected benzoylecgonine in urine by methylation with diazomethane prior to GC analysis. However, in this procedure ecgonine was not determined.

GC was selected as an identification tool for ecgonine and benzoylecgonine because it was believed it would surpass present methodology in sensitivity, speed, and specificity. Due to the highly polar and amphoteric nature of the two degradation products, it was determined that their separation from cocaine by liquid–liquid extraction techniques prior to GC analysis was impractical. Subjection of the sample to direct GC analysis was also difficult due to the very poor chromatographic behavior of ecgonine and benzoylecgonine. Therefore, this study describes a procedure whereby both hydrolysis products are silylated prior to GC analysis using N,O-bis(trimethylsilyl)acetamide (BSA). Both ecgonine silyl and benzoylecgonine silyl possess very good chromatographic properties. The derivatized samples are chromatographed on 10% OV-101 on Chromosorb W-HP and 3% OV-25 on Gas-Chrom Q, using temperature programming. Using this procedure ecgonine and benzoylecgonine can be detected in uncut cocaine samples at levels less than 0.1% and 0.3%, respectively.

EXPERIMENTAL

Reagents and chromatographic materials

The BSA silylating reagent used in this study was supplied by Pierce (Rockford, Ill., U.S.A.). The 10% OV-101 on Chromosorb W-HP (100–120 mesh) and 3% OV-25 on Gas-Chrom Q (100–120 mesh) stationary phases were obtained from Applied Science Labs. (State College, Pa., U.S.A.).

Standards

The cocaine hydrochloride used in this study was supplied by S. B. Penick (New York, N.Y., U.S.A.). Hexadecane, eicosane, tetracosane and triacontane internal standards were obtained from Applied Science Labs. Benzoylecgonine and ecgonine were supplied by the Drug Enforcement Administration.

Apparatus

A Packard Model 7400 gas chromatograph was used for all chromatography.

Sample analysis

A 25-mg sample of cocaine hydrochloride is placed in a 1-ml glass-stoppered test tube and 500 μl of BSA is added. The tube is stoppered loosely and heated at 75° for 10 min with occasional agitation. After derivatization is complete, 3–4 μl of the solution are injected into the gas chromatograph using the following parameters:

Column:	Coiled glass, 4 ft. × 4 mm I.D. (OV-101) Coiled glass, 6 ft. × 4 mm I.D. (OV-25)
Stationary phase:	(a) 10% OV-101 on Chromosorb W-HP, 100–120 mesh (b) 3% OV-25 on Chromosorb Q, 100–120 mesh
Carrier gas:	Nitrogen, 60 ml/min
Detector:	Flame ionization
Air:	500 ml/min
Hydrogen:	50 ml/min
Injection temperature:	275°
Detector temperature:	275°
Column temperature:	Temperature programmed initial temperature: 180° (OV-101), 170° (OV-25) initial hold: 10 min (OV-101), 5 min (OV-25) program rate: 3°/min (OV-101 and OV-25) final temperature: 260° final hold: 5 min
Sensitivity:	3×10^{-9} a.f.s. (OV-101 and OV-25)
Chart speed:	0.2 in./min (OV-101 and OV-25)

RESULTS AND DISCUSSION

Fig. 1 illustrates a gas chromatogram on OV-101 of cocaine hydrochloride containing 3% benzoylecgonine and 1% ecgonine following BSA treatment. Since temperature programming was used, two internal standards were desirable. It is also apparent from Fig. 1 that the detection level for ecgonine is lower than that for benzoylecgonine. However, benzoylecgonine can still be detected at levels considerably less than 0.3%.

In order to investigate the effect the moisture content of cocaine samples would have on the silylation process, water was added to the sample shown in Fig. 1 and to a commercial cocaine hydrochloride standard at a 20% w/w level. The presence

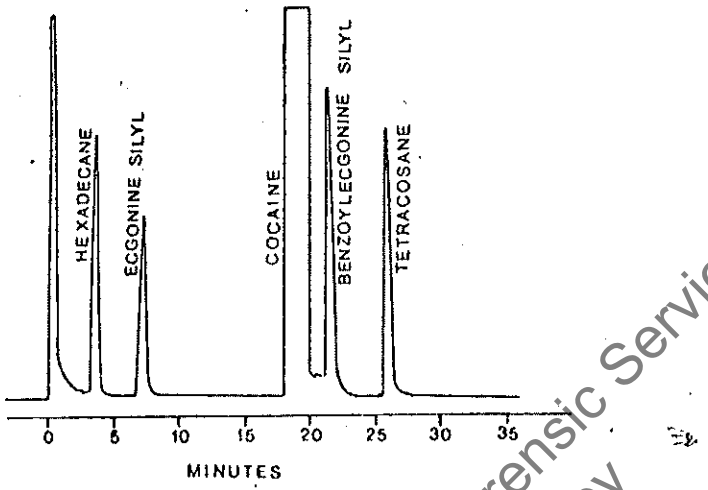

Fig. 1. Gas chromatogram of cocaine hydrochloride containing 1 % ecgonine and 3 % benzoylecgonine on OV-101 following BSA treatment. See GLC parameters under *Sample analysis*.

of moisture causes no detectable cocaine hydrolysis during the derivatization process. Additionally, the silylation of benzoylecgonine and ecgonine was not affected noticeably. The silylation of both ecgonine and benzoylecgonine is rapid and the BSA solution is stable for at least several hours.

In addition to using BSA derivatizing reagent and OV-101 stationary phase, other silylating compounds and chromatographic stationary phases of varying polarity were investigated. The other silylating reagents included N-trifluoroacetylimidazole and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), the fluorinated analog of BSA. Of these only BSTFA proved of any value. The only advantage in using BSTFA, instead of BSA, was that a slight increase in resolution of the benzoylecgonine and cocaine chromatographic peaks was noted. This was probably due to the relative insolubility of cocaine in BSTFA. However, because of this insolubility, and subsequent loss in benzoylecgonine sensitivity using BSTFA, BSA was selected as the reagent of choice.

In addition to OV-101, 3 % OV-1 stationary phase was investigated. However, the resolution between cocaine and benzoylecgonine was less than desirable on this column. More polar phases were also investigated. These included OV-17, OV-25, OV-210, and OV-225. On OV-17, cocaine and benzoylecgonine silyl had the same retention times. When OV-210 and OV-225 were used in conjunction with temperature programming, a rather unstable baseline resulted. OV-25 proved to be the most suitable of the polar stationary phases. When using this phase the elution order of cocaine and benzoylecgonine silyl were reversed when compared to OV-101 (see Table I). The OV-25 was used in this study only as a confirmation for the presence of ecgonine and benzoylecgonine in cocaine. OV-101 was the column of first choice because the resolution, sensitivity and retention times of cocaine and its hydrolysis products were the most favorable. Table I lists retention times of cocaine, benzoylecgonine silyl, ecgonine silyl and internal standards on OV-101 and OV-25. All internal standards were chromatographed using separate chloroform solutions.

The procedure given in this paper is a rapid and sensitive method for the de-

TABLE I

RETENTION TIMES OF COCAINE, ECGONINE SILYL, BENZOYLECGONINE SILYL AND INTERNAL STANDARDS ON OV-101 AND OV-25 STATIONARY PHASES

GLC operating parameters for both columns are given in the text under *Sample analysis*.

Compound	*Retention time (min)*	
	OV-101	*OV-25*
Hexadecane internal standard	3.8	—
Ecgonine silyl	6.7	2.8
Eicosane internal standard	—	5.2
Cocaine	19.7	25.8
Benzoylecgonine silyl	22.2	24.5
Tetracosane internal standard	26.7	—
Triacontane internal standard	—	30.2

tection of small amounts of ecgonine and benzoylecgonine in cocaine. It offers the added advantage of being readily adaptable to quantitative work. The procedure is useful not only in pharmaceutical quality control but in the detection of contaminants in illicitly manufactured cocaine.

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Microgram Vol 2, No 2 pg 47
June 1969

(24)

IDENTIFICATION OF COCAINE BY INFRA-RED SPECTROSCOPY

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The spectrogram obtained from the infra-red spectroscopic examination of compounds isolated in a pure or nearby pure state is one of the most substantive methods of examination. It is a very important aid to the forensic chemist now that the courts and defense attorneys are beginning to challenge the identification of alkaloids, such as cocaine, by means of crystal and color tests alone.

Cocaine is ordinarily separated from most of its adulterants and/or diluents by extracting it as the hydrochloride salt with chloroform from a solution or suspension in 1+9 Hcl. The filtered chloroform extract is then evaporated on the steam bath to obtain the cocaine salt relatively pure. No difficulty was ordinarily encountered in obtaining the characteristic identifying crystals with the platinic chloride or gold chloride reagent solution. However, the cocaine hydrochloride crystals isolated this way were found not to give infra-red spectrograms which, for forensic purposes, sufficiently matched that given by the authentic.

It was theorized that the above difficulty was due to the acid hydrolysis, however much or slight, of the cocaine at steam bath temperatures due to the hydrochloric acid dissolved in the water solubilized in the chloroform. When the chloroform extract was first dried with excess anhydrous sodium sulfate, decanted, and then evaporated no such deterioration of the cocaine occurred and excellent infra-red spectrograms were obtained.

Microgram Vol 5
No. 7 July, 1972
(25)

DATE January 6, 1972 -80-

NO.

DRUG TYPE Narcotic

METHODOLOGY Infrared Identification

RAPID SEPARATION OF COCAINE FROM ADULTERANTS SUCH AS PROCAINE AND QUININE, AND SUBSEQUENT INFRARED IDENTIFICATION

Roger G. Fuelster
Forensic Chemist
CHICAGO REGIONAL LABORATORY, BNDD

Previous methods of separation of cocaine from adulterants involved lengthy column chromatography. The following procedure is not only rapid but yields a highly pure product.

PROCEDURE

Place sample in a small separatory funnel. Add approximately 15ml 2.8 normal hydrochloric acid. Extract with an equal volume of chloroform. Pass the extract through Whatman Phase Separating Paper #1PS. Evaporate the chloroform to dryness and dry at 105°C for 10 minutes. Obtain the IR spectra in the usual manner.

SUMMARY

This method effectively removes traces of hydrochloric acid which would otherwise hydrolize the cocaine during the final evaporating and drying stages.

from Bullet-Sn Vol 3 #2, July 1975

(30)

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

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

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

Cocaine/Lidocaine Mixture

Rapid separation/Infrared Identification
(11.73386 minutes)

Dissolve sufficient sample in minimum amount 0.1N HCl to provide 5 mg cocaine.

Precipitate cocaine by dropwise addition of 5% Platinic Chloride (H_2PtCl_6) until ppt. ceases.

With disposable pipet transfer ppt and supernatant to a little, tiny buchner funnel and, w/vacuum, remove liquid.

Wash 3X w/ 3ml portions of 0.1NHCl, followed by 3X 3 ml portions of methanol.

Continue to dry the precipitate with suction, for

Three or four minutes - cover buchner with dry filter paper if air is wet.

When sufficiently dry, press with KBr.

Compare with spectrum prepared from known cocaine.

Also works with methamphetamine separation from phentermine but use methanol sparingly as the methamphetamine is slightly methanol soluble.

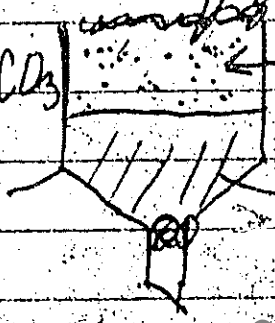
Joe Power - DEA

Colum Chromatograph

Celite 545 aqu. stationary bed

D. ion Pairing
must use a chlorinated solvent
to elute drug from column

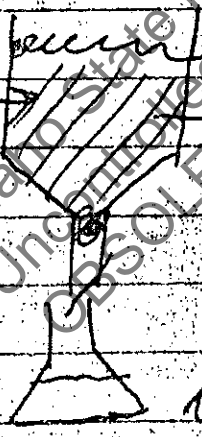
Drug mix
2 ml 0.5N $NaHCO_3$
3g Celite
4 ml 0.2 N HNO_3
+6g Celite



sugars & starch

procaine & quinine

2 ml 0.2N
H_2SO_4 + 3g Celite



cocaine

caffeine

mix of
cocaine
procaine
Caffeine
Quinine
Dextrose
lactose
corn starch

(17)

ISOLATION AND ESTIMATION OF COCAINE IN THE PRESENCE OF TETRACAINE, PROCAINE, BENZOCAINE AND AMPHETAMINE

By

Charles Milos and Philip V. Porto

Alcohol and Tobacco Tax Laboratory

Internal Revenue Service

New York, New York

Isolation and Estimation of Cocaine in the Presence of Tetracaine, Procaine, Benzocaine and Amphetamine

By

Charles Milos and Philip V. Porto

Illicit samples of cocaine are generally adulterated with sugar and procaine. These present no problem to the analyst (1). Some samples with low cocaine content may contain all of the components listed in the title of this paper. With this type of sample the problem is twofold -- detecting the cocaine and isolating it from the other components. Sometimes the second objective must be accomplished before the first can be established.

Since existing methods failed to resolve this problem, a new procedure was needed. An extraction method was devised which makes use in part of some of the techniques in existing methods (1).

Experimental

Chloroform completely removes benzocaine from aqueous acid solutions. Separation of tetracaine from procaine, amphetamine and cocaine is effected by first dissolving their free bases in a mixed solvent consisting of equal volumes of ethyl ether, ethyl acetate plus 20% by volume of isobutyl alcohol. Extraction of the mixed solvent with a citric acid-phosphate buffer of pH 6.25 removes the latter three components, leaving the tetracaine

in the solvent. By altering the pH of the buffer to 4.5 the cocaine can be removed by extraction with chloroform. Traces of tetracaine remaining in cocaine thus separated cause no interference with the usual cocaine tests.

Preliminary Examination of Sample

1. Dissolve a small portion of sample in 1 or 2 drops of 1:1 hydrochloric acid on a microscopic slide. Add a drop of platinum chloride reagent (1g platinum chloride in 20 ml. of water) and examine the slide at once under the microscope, using the low power objective. If appreciable amounts of cocaine are present (5% or more), characteristic feathery crystals form. In a brief period of time they become modified beyond recognition. When relatively large amounts of benzocaine or amphetamine are present these crystals may not be observed. When this occurs place another portion of sample on a microscope slide, add several drops of a 20% aqueous solution of sodium acetate, and after several minutes observe for free base cocaine crystals. These crystals are characteristic and procaine, tetracaine, amphetamine and benzocaine do not interfere.

2. Add concentrated nitric acid to a portion of sample on a spot plate. Formation of a yellow to light brown color indicates tetracaine. Treat another portion with Marquis' reagent. A yellow to orange color indicates amphetamine.

3. Place a few milligrams of dry sample on a microscopic slide and while observing under low power, add a drop of 1:1 hydrochloric acid. Cocaine, procaine, tetracaine and amphetamine dissolve rapidly. Benzocaine dissolves very slowly.

4. To another portion of sample on a spot plate, add several drops of Sanchez reagent (2). A red to purple color indicates benzocaine or procaine. NOTE: Each 100 ml. of Sanchez reagent contains 2.5 ml. of freshly distilled furfural, 22.5 ml. of 95% ethyl alcohol and 75 ml. of glacial acetic acid.

5. To several milligrams of dry sample on a spot plate add a few drops of cobalt thiocyanate, stannous chloride reagent. The formation of a blue precipitate indicates cocaine. NOTE: This reagent contains equal volumes of a 2% aqueous solution of cobalt thiocyanate and a 5% stannous chloride in 10% hydrochloric acid (3).

Method

Reagents:

0.5 Molar citric acid

0.5 Molar disodium phosphate

Buffer solution -- To 40 ml. of 0.5 molar disodium phosphate add 15 ml. of 0.5 molar citric acid and mix.

Mixed solvent ---- To 40 ml. of ethyl ether add 40 ml. of ethyl acetate and 20 ml. of isobutyl alcohol and mix.

Separation Procedure

1. Dissolve a known weight of sample (approximately 0.1 g.) in 10 ml. of approximately 0.1 N hydrochloric acid and quantitatively transfer to a separatory funnel. Extract four times with chloroform using 15, 10, 10, and 5 ml. portions. If a quantitative determination of benzocaine is desired, combine and wash the chloroform with 5 ml. of water in a separator. Transfer the solvent to a tared dish and evaporate to dryness on a steam bath.

2. To the acidic aqueous solution add dilute ammonium hydroxide (10%) until just alkaline to litmus and extract three times with 20 ml. portions of solvent. Combine the solvent in a separator, wash with 5 ml. of water and after complete separation of solvent, discard the lower aqueous layer. Extract the solvent three times with 10 ml. portions of prepared buffer and combine the buffer in a separator. For a quantitative estimation of tetracaine wash the mixed solvent with 10 ml. of water and transfer the solvent to a tared dish. Evaporate to dryness on a steam bath. The residue is free base tetracaine.

3. To the combined buffer add 3 ml. of 0.5 molar citric acid, mix and extract with chloroform as already described for benzocaine. Wash the combined chloroform with 5 ml. of water and transfer the chloroform to a tared dish. Evaporate to dryness on a steam bath. The residue is free base cocaine.

Analytical Data

Table 1

	Milligrams Added					Milligrams Found		
S.	B	T	P	A	C	B	T	C
1.	23	19	50	10	4.7	22.6	19.7	4.4
2.	12	15	20	12	8.0	12	14.8	7.7
3.	13	22.5	30		15.0	13	22.8	14.5
4.		9.5	21	5	2.8		9.3	2.3
5.	10	20	30	12	9.0	9.7	19.6	8.4
6.					8.2			7.6

S--Sample

B--Benzocaine

T--Tetracaine HCl

P--Procaine HCl

A--Amphetamine HCl

C--Cocaine HCl

Table 1 shows results obtained by the proposed method with simulated mixtures. All of the results were obtained gravimetrically.

Discussion

Failure of the preliminary tests to disclose the presence of cocaine should not be taken as conclusive evidence of its absence. Further explorations should be conducted to either verify or negate the preliminary findings. Some samples of illicit cocaine are predominately mixtures of local anesthetics containing small amounts of cocaine. By the proposed method a large number of these diluents can be removed. However, if the cocaine residue is contaminated and the usual tests do not give a clear-cut decision with respect to its presence or absence, part of the residue should be reserved for preparing and identifying the derivative ecgonine. Use of the original sample may introduce complications which can be avoided by using part of the cocaine residue. The reaction of ecgonine with phosphomolybdic acid reagent (1g. phosphomolybdic acid in 20 ml. of 1:1 nitric acid) is so sensitive that the concentration of ecgonine should be kept in the neighborhood of about 15 parts per million. With high concentrations of ecgonine an innumerable number of crystals are formed. These adhere to each other and the characteristics of the individual crystal cannot be discerned. To prepare this derivative heat a dilute slightly acidic solution of cocaine to the boiling point. Add 10% sodium hydroxide solution until alkaline and continue the heating for a minute. Cool and transfer to a separator. Extract several times with ml. portions of chloroform and discard the chloroform.

Acidify with acetic acid and extract once with about 15 ml. of ethyl acetate. Transfer the aqueous layer to a dish and heat to expel the dissolved solvent. Test a few drops and either dilute or concentrate, depending on the results of the test.

In the samples shown in the preceeding table, the tetracaine residue was examined for cocaine and none was detected. Small amounts of procaine were present when the amount in the sample was large.

Some cocaine remains in the final buffer. By altering the pH the cocaine can be completely removed, but invariably it is contamined with procaine.

The proposed method gives results of sufficient accuracy for routine analysis.

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$$w = (f_1 w_1 + f_2 w_2 + \ldots f_n w_n) \quad \text{(Eq. 9)}$$

f_1 is the weight fraction of particles of weight, etc. For spherical particles:

$$w = \frac{\pi\rho}{6} \sum_1^n f_i d_i^3 \quad \text{(Eq. 10)}$$

the case where particles are the same size, Eq. 10 comes:

$$w = \frac{\pi\rho d^3}{6} \quad \text{(Eq. 11)}$$

For Eqs. 10 and 11 to be equivalent:

$$d^3 = \sum_1^n f_i d_i^3 \quad \text{(Eq. 12)}$$

Substituting the result from Eq. 8 into Eq. 12 gives = 96.2 μm. Hence, the particle-size distribution iven in the example, which was determined by trial nd error, is equivalent to the calculated value of $d_{(R)}$ f 96 μm. This distribution is typical of the kind of sult encountered in practice. Use of such a particle-ze distribution for all active ingredients would allow safety margin for ingredients P and Q but, in the se of R, would necessitate achieving a truly random ixture to fulfill the desired tolerance range of 10%.

In practice, a random mix is not always achieved nd it may be desirable to introduce an additional margin for the lowest concentration drug, R. ey *et al.* (1) did this in effect by setting the cal-lated effective mean particle-size limit for R as the aximum particle-size limit for the mixture. Alter-tively, the coefficient of variation used in Eq. 7 ld be set at a lower value than that corresponding the specified tolerance range of ±10%. For exam-le, instead of 3.333%, a C_v value of 2.5% could be sed which would give $d_{(R)}$ from Eq. 7 equal to 79 m. An equivalent particle-size distribution corre-onding to this value of $d_{(R)}$ would contain a consid-rable fraction above the proposed maximum limit of 3 μm (1) while still incorporating a safety margin to low for the occurrence of nonrandomized mixing.

In conclusion, converting the particle-size limit to an equivalent particle-size distribution increases e utility of the calculations and provides a more nvenient guideline in the practical situation. Addi-onally, a particle-size distribution of a drug ob-ined on recrystallization or precipitation or after illing can be tested for its suitability with regard to ntent uniformity by evaluating $\Sigma f_i d_i^3$ and compar-g this value with the value of d^3 derived from Eq. 2 r 7.

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(22)

Definitive GLC Method of Identifying Cocaine

Keyphrases □ Cocaine—definitive GLC identification □ Trimethylanilinium hydroxide—on-column methylation of cocaine, GLC identification □ GLC—identification, cocaine

To the Editor:

The identification of underivatized cocaine by GLC can be misinterpreted and erroneously reported as pentazocine, levorphanol, or methaqualone when using programmed or isothermal temperatures on 7% OV-17[1]. TLC can also pose problems and lead to the report of a false positive for methadone instead of cocaine (1). Many laboratories are combining mass spectrometry with GLC to provide a more definitive instrumental method for identifying drugs such as cocaine (2); however, many laboratories cannot afford a mass spectrometer and, therefore, more definitive GLC methods of analysis are desirable.

In view of these problems encountered when employing GLC or TLC as a means of identifying cocaine, we wish to report a novel, definitive GLC method of identifying cocaine *via* an on-column GLC reaction under methylation reaction conditions that is applicable to confirming the presence of cocaine in various legitimate and illegitimate dosage forms. In our laboratory we have routinely used trimethylanilinium hydroxide in methanol as a methylating reagent for GLC analysis of anticonvulsant drugs in body fluids (3, 4). We anticipated that this methylating reagent would have an interesting on-column

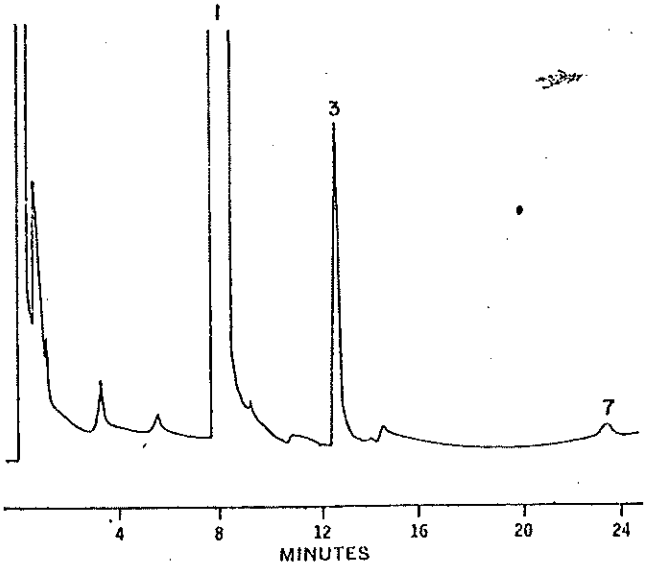

Figure 1—*Characteristic chromatogram representing an on-column reaction of cocaine (1.5 μg) and trimethylanilinium hydroxide (no time lapse after adding the methylating reagent to cocaine). See Table I for identification of the peaks.*

[1] In our laboratory, these drugs have retention times similar to cocaine under programmed and isothermal conditions and are extracted concurrently with cocaine at basic pH.

Table I—Relative GLC Retention Times of Cocaine and Some Commonly Abused Drugs after On-Column Reaction[a]

Drug	R_t, min
Cocaine[b,c]	Peak 1 (8.0), *N,N*-dimethylaniline Peak 2 (10.4), possible Hoffman elimination product Peak 3 (12.5), ecgonidine methyl ester Peak 4 (13.9), under investigation Peak 5 (14.2), ecgonine methyl ester (under investigation) Peak 6 (17.2), under investigation Peak 7 (23.3), cocaine
Amphetamine	8.3
Benzocaine	15.7, 16.8, 17.6, 18.2
Codeine	27.5
Heroin	24.1, 27.2
Levorphanol	21.4
Lidocaine	16.6, 18.5
Methaqualone	23.2
Morphine	27.5
Pentazocine	21.9
Phencyclidine	18.2
Phenobarbital	12.8, 14.3, 14.5, 18.4
Procaine	17.5, 20.8, 21.7, 23.1
Quinine	34.0, 37.0
Secobarbital	15.4

[a] Under programmed temperature GLC conditions. [b] The number of chromatographic peaks seen after on-column reaction depends on the concentration of cocaine and the time lapse between addition of the methylating reagent and its on-column injection (see Figs. 1–3). [c] Similar results can be obtained on 3% OV-17 by reducing the nitrogen flow rate from 60 to 30 ml/min and changing the programmed column conditions from 50–250° (10°/min) to 50–250° (8°/min).

action with an unusual bicyclic diester tertiary amine structure such as cocaine, through possible ester cleavage and methylation, as well as serve as a definitive confirmatory method by converting it into one or more identifiable derivatives.

All GLC injections were made on a dual-channel instrument[2] equipped with four hydrogen flame detectors. The 1.83-m (6-ft) U-shaped glass columns (2 mm i.d.) were packed with 7% OV-17 on 80–100-mesh Chromosorb W[3]. Operating temperatures were: injector port, 275°; column (isothermal), 250°; column, 50–250° (programmed at 10°/min); and detector, 275°. Flow rates (milliliters per minute) were: nitrogen, 60; air, 300; and hydrogen, 40. Instrumental attenuation was 8×10^{-10}. Under these conditions, reference standard underivatized cocaine had retention times of 23.3 and 2.9 min under programmed and isothermal column temperatures, respectively.

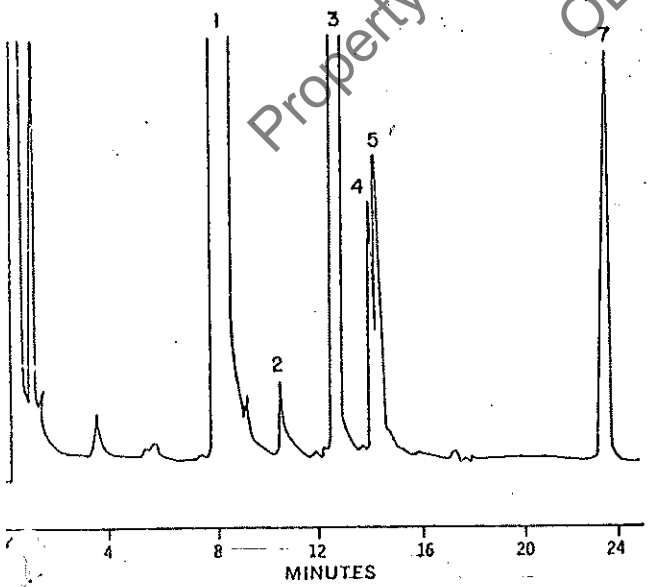

Figure 2—*Chromatogram representing an on-column reaction of cocaine (21 μg) and trimethylanilinium hydroxide (no time lapse after adding the methylating reagent to cocaine). See Table I for identification of the peaks.*

The reaction of cocaine with trimethylanilinium hydroxide solution was initially studied by adding 50 μl of a 2 *M* methanolic trimethylanilinium hydroxide solution[4] (3) to 0.15 mg of cocaine and immediately injecting 0.5 μl (1.5 μg) into the chromatograph under programmed temperature conditions. The resulting chromatogram is illustrated in Fig. 1. Peak 1 is *N,N*-dimethylaniline, a product derived from trimethylanilinium hydroxide during the methylation reaction.

Utilizing a preparative gas chromatograph, we trapped peak 3 and obtained its mass spectrum. Peak 3 was identified as ecgonidine methyl ester (molecular ion, *m/e* 181; base peak, *m/e* 152). Its mass spectrum is identical to the corresponding methylated and trapped reference standard ecgonidine[5]. Ecgonidine methyl ester (peak 3) appeared to be the most characteristic product of on-column reaction between cocaine and trimethylanilinium hydroxide and was observed at all concentrations regardless of the length of time elapsing between addition of the methylating reagent and its on-column injection (peak 3 in Figs. 1–3).

Figure 2 is a representative chromatogram depicting the cocaine–trimethylanilinium hydroxide reaction using a larger amount of cocaine (21 μg), in which case the cocaine–trimethylanilinium hydroxide solution was injected immediately after adding the methylating reagent to cocaine. Mass spectral analysis of peak 2 indicates it to be a potential double Hoffman elimination product, although its structural determination is inconclusive at this time.

Figure 3 represents the chromatogram of the same cocaine–trimethylanilinium hydroxide solution after standing at room temperature for 24 hr. An increase in the concentration of peak 2 (possible Hoffman elimination product) occurred when cocaine was allowed to stand in the alkaline trimethylanilinium hydroxide solution for 1–24 hr (compare Figs. 2 and 3). Peaks 4 and 6 are presently under investigation, and peak 7 is cocaine.

Based on preliminary mass spectral analysis, peak 5 appears to be the ecgonine methyl ester derivative (molecular ion, *m/e* 199; base peak, *m/e* 152). Therefore, to minimize the number of products formed, in-

[2] Varian 2100.

[3] OV-17 and Chromosorb W were obtained from Applied Science Laboratories, Inc., State College, Pa. The 7% OV-17 was prepared in our laboratory using conventional methods (2.1 g OV-17/30 g Chromosorb W).

[4] Trimethylanilinium hydroxide (0.1 *M* in methanol) (trimethylphenylammonium hydroxide) was obtained from Eastman Kodak Co., Eastman Organic Chemicals Division. Trimethylanilinium hydroxide (2 *M* in methanol) was prepared in our laboratory by concentrating 100 ml of 0.1 *M* reagent to 5 ml.

[5] K & K Laboratories, Inc., Plainview, N.Y.

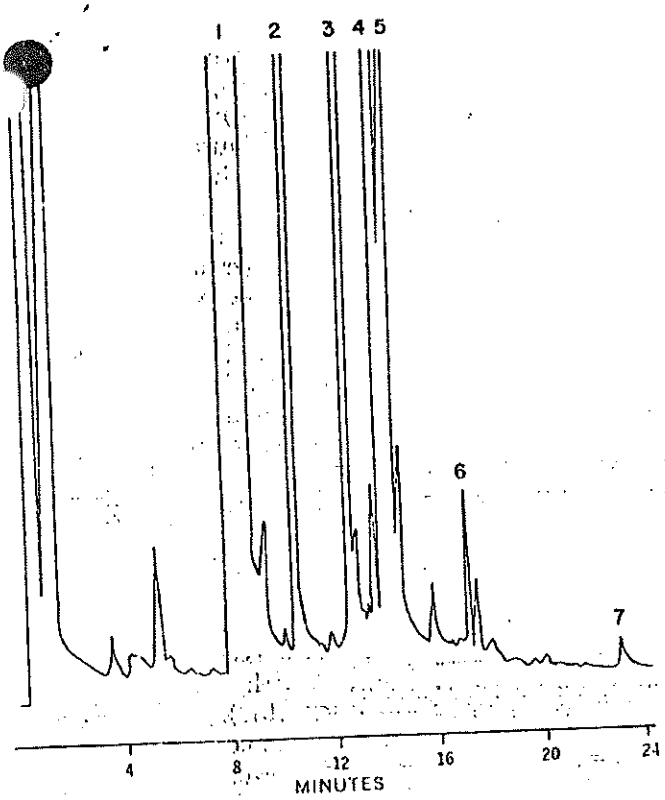

Figure 3—*Chromatogram representing an on-column reaction of cocaine (21 μg) and trimethylanilinium hydroxide (24-hr elapse after adding the methylating reagent to cocaine). See Table I for identification of the peaks.*

jection of the solution should be performed immediately after adding the methylating reagent to the suspected cocaine residue.

We also wish to report the relative GLC retention times of other commonly abused drugs frequently extracted at alkaline pH ranges (pH 8–10) which could possibly interfere with the confirmation of cocaine by this method. These values are reported in Table I and represent the relative retention times of the product(s) produced using the same on-column reaction conditions performed with cocaine. Of the drugs examined, phenobarbital is the only one that could possibly interfere with the characteristic ecgonidine methyl ester peak (peak 3) under programmed temperature conditions. However, there is no interference between cocaine and phenobarbital when underivatized cocaine is analyzed under isothermal temperature conditions.

To test this method further, we analyzed a simulated street sample containing 6% cocaine hydrochloride, 19% quinine, and 75% dextrose. This analysis was carried out by performing a conventional alkaline extraction (pH 10) of 2.7 mg of the sample with chloroform–isopropanol (3:1), evaporation of the solvent, addition of 50 μl of trimethylanilinium hydroxide to the residue, and immediate injection of 1 μl. The resultant chromatogram resembled Fig. 1, with no interference from quinine.

In summary, we believe this method will be of value as a definitive confirmatory screening test for cocaine after first tentatively identifying underivatized cocaine using isothermal or programmed GLC temperature conditions.

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(33)

ASSAY OF COCAINE IN THE PRESENCE OF PROCAINE AND QUININE BY COLUMN CHROMATOGRAPHY

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(Received June 18th, 1970)

SUMMARY

A column chromatography method is presented for the separation and quantitation of cocaine in the presence of procaine, quinine and lactose. Separation procedures are described to afford purification of cocaine for infrared absorption spectrum examination.

INTRODUCTION

Illicit cocaine is examined in the forensic laboratories as a fine, white powder, invariably occurring as a hydrochloride salt; occasionally crude-appearing brown samples have been examined. Adulteration and dilution are effected by illicit dealers with procaine and lactose respectively; and less frequently, quinine is used as an adulterant. The dilution has been mostly in the range of 10 to 60%.

The rapid detection tests for cocaine are described in the U.S. Treasury Manual[1]. The preferred methods are in the use of cobalt thiocyanate test and in the formation of the characteristic microcrystals with platinic chloride. These tests afford a rapid and convenient procedure for the presumptive identification of cocaine.

Procaine is detected by the Sanchez color test[1] for cyclic aromatic amines resulting in a vivid red color. Quinine possesses an intense blue fluorescence under UV light, and is readily characterized by placing a sample preferably in an acid solution under fluorescent light.

Thin-layer method for chromatography is recommended if materials other than those mentioned, *i.e.*, procaine, quinine, and lactose are suspected. COMER AND COMER[2] have reviewed a number of papers describing the use of thin-layer chromatography (TLC) for the separation of different kinds of drugs. He has listed some sixteen different TLC systems for separating cocaine from a number of local anesthetics and

from a number of analgesics-antipyretics. In addition, this paper offers a simple TLC system using Eastman Chromatogram sheets for separating cocaine from procaine and quinine. We have not hitherto encountered adulterants other than procaine and quinine in the illicit samples.

YOUNG[3] reported a chemical method for the determination of cocaine in the presence of procaine in which cocaine is hydrolyzed to yield methanol as a product. Methanol is distilled and is measured colorimetrically, using the well-known permanganate oxidation procedure. The method proved time-consuming for forensic work and a number of chloroform shake-out methods[1] were adapted to provide a final residual product suitable for volumetric titration. This procedure proved to be not only laborious but was attended by losses in recovery simply because of its many manipulative steps.

The manual[1] also describes a determination of cocaine, procaine, and tetracaine, employing a direct reading of the three components in alkaline solution and calculating the amounts by simultaneous equation.

The method is beset by the errors introduced by overlapping curves and these errors have been compensated by the introduction of experimental factors. The errors introduced for cocaine determination become more pronounced when proportionally more adulterant is present in the sample than cocaine.

The present method employs the principle of ion pair formation and its extraction by partition chromatography. This principle is discussed by HIGUCHI *et al.*[4] and it has been adapted by LEVINE and co-workers[5–8] for the separation of a large number of pharmaceutical amines.

Among the common anions tested, including chloride, phosphate and sulfate, nitrate proved to be the most efficient for quantitative purposes. Potassium nitrate in HCl solution is used as a stationary phase and chloroform for elution. HCl acts to retain quinine and possibly other basic materials[6] which may be present in the cocaine sample. The eluant from the column is acidified and measured by UV absorption.

PROCEDURE

Mix 4 g of Celite 545 (Johns Mansville) with 2 ml of 1 *M* KNO_3 in 0.1 *N* HCl and transfer to a column, such as used by LEVINE[6]. Tamp the mixture on a pad of fine glass wool. Pipette 1 ml of aliquot of solution containing 50 mg of sample in 1 *M* KNO_3 in 0.1 *N* HCl onto the surface of the column. Irrigate the column with 45 ml of chloroform saturated with water (spectro-grade solvent preferred); collect the eluant in a 50-ml volumetric flask containing 5 ml of methanol and 5 drops of concentrated HCl. Bring the solution to 50 ml mark with chloroform and read its UV spectrum from 340 to 255 nm, max. at 275 nm, using chloroform as a reference.

Prepare a quantitative standard by dissolving 10 mg of cocaine·HCl in 50 ml chloroform containing 5 ml methanol and 5 drops HCl. Absorbance at 1%/1 cm was 32.5 using a Cary 15 spectrophotometer under these conditions. Calculate % cocaine, as hydrochloride, using the following equation:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \frac{10}{50} \times 100 = \% \text{ cocaine} \cdot \text{HCl}$$

aim for A = .5 (.15 mg/ml)

Clean-up for IR examination

Shake the chloroform eluant saved from the assay with 10 ml of water. Discard the chloroform phase. Adjust the pH of the aqueous extract with dilute NH_4OH just to alkalinity (observe white precipitate) and extract with 50 ml chloroform twice. Filter the chloroform extract into a beaker and then treat the filtrate with sodium sulfate to remove water. Filter and evaporate the extract to dryness. Press the resulting crystalline material into KBr for IR spectrum examination for cocaine base.

DISCUSSION

While most forensic laboratories do not quantitate fillers or adulterants present in illicit narcotic samples, procaine and quinine can be eluted from the column using triethylamine in chloroform according to a procedure by LEVINE[6]. 1 *N* HNO_3 or 1 *M* KNO_3 can be used in the column *per se* if procaine is the only adulterant in the illicit cocaine.

In the clean-up procedure, there is the hazard of hydrolyzing cocaine to benzylecgonine and methanol in the presence of water and heat[9]. Therefore, a desiccant such as sodium sulfate is used to dry the chloroform extract prior to evaporation over a hot steam bath.

For the clean-up, 10 mg of cocaine can be recovered by the method. If sufficient amount of material cannot be recovered, *ca.* 100 mg of sample can be passed through the column to recover enough material for IR examination.

On occasions, this laboratory has analyzed brown colored cocaine powder preparations containing procaine as an adulterant. The interference due to the presence of chloroform-soluble colored component was removed by washing the sample in the column, under standard conditions, with 50 ml of water-saturated 1,1-dichloroethane, which has been used in the clean-up of brown heroin[10]. The cocaine which was eluted subsequently with chloroform yielded a UV curve similar to those obtained with standard cocaine; recovery values of cocaine standards under standard conditions are shown in Table I.

TABLE I

RECOVERIES OF COCAINE STANDARDS

mg added	*mg recovered*	*% recovery*
15.00	14.89	99.2
12.00	11.90	99.2
10.00	9.95	99.5
8.00	7.91	99.1
5.00	4.92	98.9

HEAGY[11] reported that cocaine can be purified sufficiently for IR determination by its relative solubility in methylene chloride over procaine which is sparingly soluble in this solvent at room temperature. To a sample of illicit cocaine mixed with procaine, just enough methylene chloride is added to the mixture to dissolve cocaine. The extract is filtered immediately and evaporated to dryness. The residue is recrystallized with petroleum ether and pressed into KBr for IR spectrum reading.

TABLE II

TLC OF COCAINE, PROCAINE AND OTHER COMPOUNDS OF FORENSIC INTEREST

Solvent mixture: 40 ml chloroform, 10 ml ethyl acetate, and 10 drops conc. NH_4OH. Chromatography sheet: Eastman Chromatogram.

Compounds	*Typical* R_F
Cocaine	0.84
Procaine	0.45
Quinine	0.05
Heroin	0.34
Codeine	0.11
Morphine	0.00
LSD	0.34
Mescaline	0.05
STP	0.13
PCP	0.89
Nupercaine	0.45
Benzocaine	0.67
Tetracaine	0.11
dl-Amphetamine	0.00
DMT	0.10

In addition to those TLC solvent systems listed by COMERS AND COMERS[2] the authors suggest a very simple, rapid procedure using a solvent mixture containing 40 ml chloroform, 10 ml ethyl acetate and 10 drops of ammonium hydroxide. Eastman chromatosheets are cut to approximately 1½ in. and 4 in. and irrigated in the solvent mixture in a small bottle accommodating this sized sheet. The spots are revealed by using iodoplatinate reagent. Table II shows the typical R_F values obtained for cocaine, procaine, quinine and other forensically important compounds.

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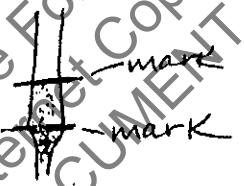
Sm Southcombe 5-6-77

Cocaine

.200grams (200mg) 1/5 of a gram

1. Place comparable amount in a plugged pipet - compare with known amount
2. Flick with finger to shake ~~shown~~ down into pipet - tap lightly
3. Mark with a "Sharpie" the top of the powder and the bottom interface with the glass wool.



4. Place 2 droppers full of methylene chloride into pipet - let drain into waste beaker.
5. At this point, you may place draining pipet over a spot test well - let a few drops fall into the well and let dry. Add Cobalt thiocyanate reagent to well and observe reaction. — OR Scott's test (3 stage)
6. Compare remainder of powder with previous marks - make decision on %.

Example
- remaining powder approx 50%

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DEA LABORATORY NOTES

DATE October 26, 1976

NO.

DRUG TYPE Cocaine

METHODOLOGY Salt Formation

PURIFICATION OF COCAINE BY SALT FORMATION

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OBJECTIVE

To provide a simple purification of cocaine mixed with other caines.

BACKGROUND

Cocaine is often mixed with other drugs, commonly with lidocaine and procaine. Cocaine and lidocaine can be easily separated with aqueous platinic chloride.1 However, there is no simple method for separating cocaine from lidocaine and procaine.

METHOD

The cocaine sample is dissolved in water and made basic with ammonia. The caines are then extracted with dichloromethane. Evaporate off the solvent and dissolve the residue in a minimum amount of acetone. Add an equal volume of Di-p-toluoyl-(-)-tartaric acid solution (about 50mg per ml of acetone). Vigorously stir and scratch the bottom of the beaker with a glass capillary tube until crystals form (2-30 sec.) (salt of cocaine and TTA). If no crystals form, concentrate the solution and try again. A small amount of pet ether may also help to initiate crystallization.

Once crystals of the salt form, they are extremely insoluble and can be washed with acetone. The cocaine can be recovered from the salt by dissolving the salt in dilute ammonia and extracting the cocaine with dichloromethane. The purity of the cocaine residue is indicated by how quickly the oily residue solidifies when stirred with a glass rod. The more quickly it solidifies the purer the cocaine.

PURIFICATION OF COCAINE BY SALT FORMATION

METHOD - continued

If the cocaine is not purified enough, a simple micro alumina column clean up can be used for further purification. To a disposable pipet plugged with cotton add about one inch of chromatographic aluminum oxide (alumina) powder. Wet the alumina with one milliliter of dichloromethane. Dissolve the cocaine residue in a minimum amount of dichloromethane and add the solution to the column. Elute the cocaine with dichloromethane. Collect the eluate in several beakers. Collect one to two milliliters in the first beaker, about three to five in the second, and about five in the third beaker. Evaporate off the solvent. Most of the cocaine will be in the second beaker.

DISCUSSION

Cocaine has been purified from a mixture of cocaine, procaine, and lidocaine hydrochlorides by the formation of the salt of (-)-cocaine and (+)-Di-p-toluoyl-(-)-tartaric acid. The other optical isomer, (-)-Di-p-toluoyl-(+)-tartaric acid, also reacts with (-)-cocaine but not as quickly. The Di-p-toluoyl-l-tartaric acid from Aldrich is the monohydrate and is soluble in acetone. However, the Di-p-toluoyl-d-tartaric acid is anhydrous and as such is insoluble in acetone. To dissolve add a drop of water to the acetone. Di-p-toluoyl-(-)-tartaric acid is available from Aldrich, Fluka, and Pfaltz & Bauer chemical companies.

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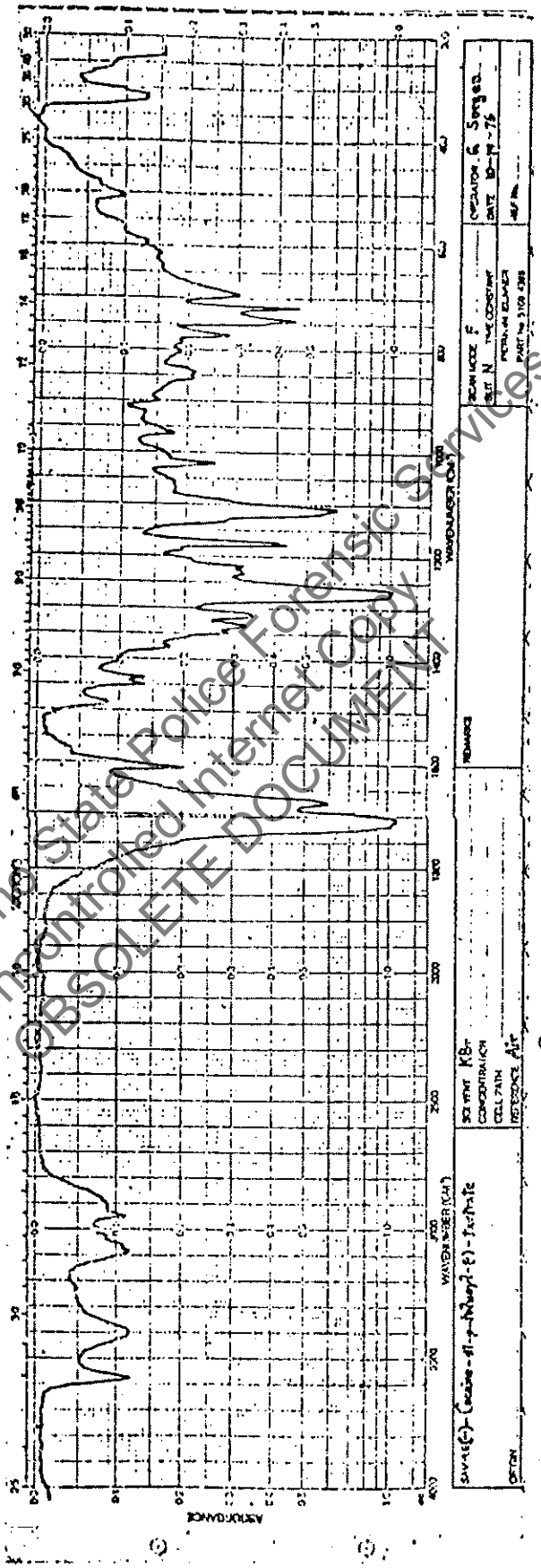
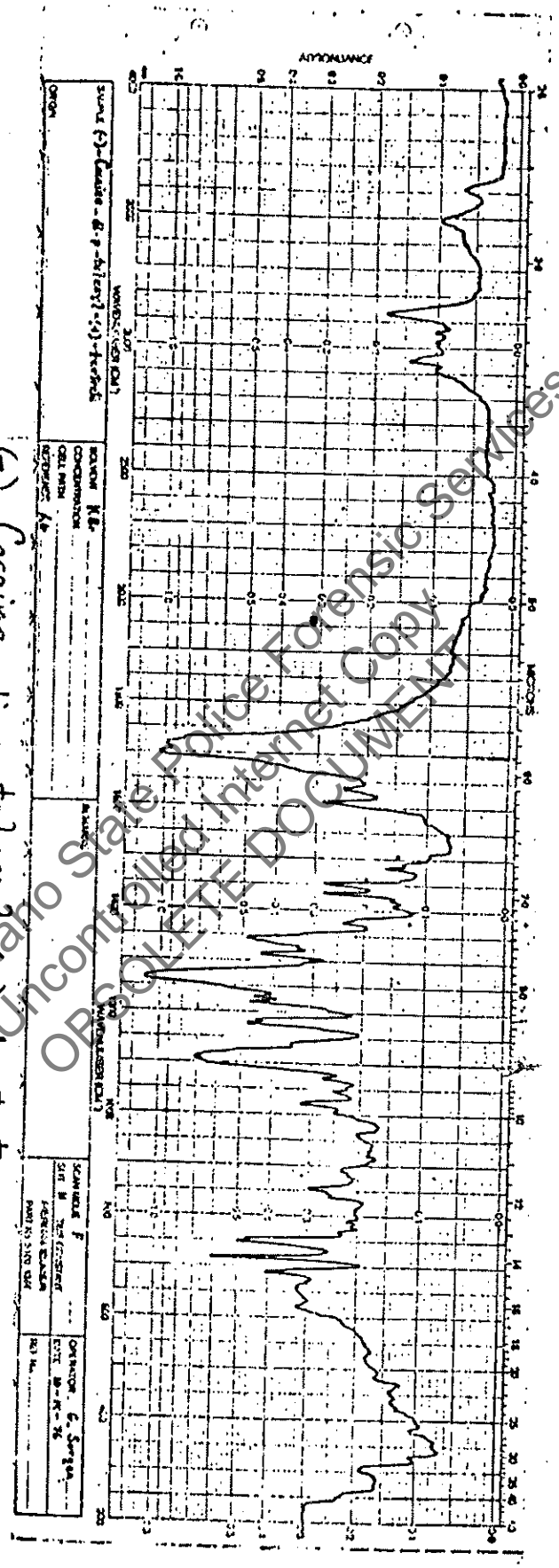


(-)- Cocaine - di-p-toluoyl-(-)-tartrate



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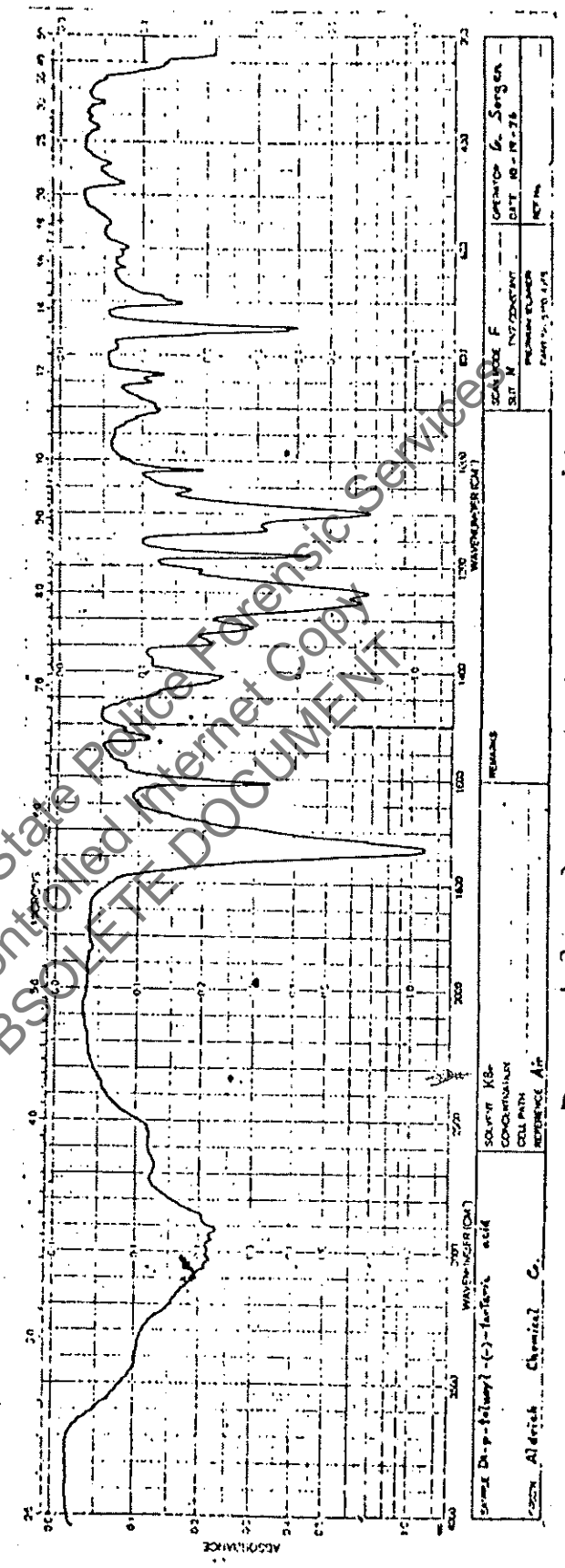

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DEA LABORATORY NOTES

DATE

NO.

DRUG TYPE

METHODOLOGY

ISOLATION AND DETERMINATION OF l, d, or dl-COCAINE IN SMALL AMOUNTS OF ILLICIT SAMPLES

by

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OBJECTIVE

To devise a method for the determination of the d, l, or dl isomer of cocaine by using prep-TLC and mixed melting points under the following sample conditions:

1. The total sample quantity is very small i.e. as little as 50mg.
2. The percentage of cocaine is very low i.e. as low as 3.3%.
3. The cocaine is found singly or in combination with other drugs.

BACKGROUND

Recently, the analysis of cocaine has received special attention in order to determine its isomeric form. The isomeric form is generally determined by use of a polarimeter or a mixed melting point determination.

When sufficient quantities of sample are available and the percentage of cocaine high enough, the isomeric determination is readily

accomplished. However, in the absence of such quantities and with low percentages, the task is far more difficult. This is especially true in the presence of other drugs. Under these conditions it is difficult to obtain information concerning the isomeric form of cocaine by use of conventional means.

This paper presents a method which allows such a determination under the stated sample conditions. It employs prep-TLC and mixed melting points of the sample and procedural standard l-cocaine.

REAGENTS AND APPARATUS

Chloroform, Methanol, NH_4OH (concentrated), petroleum ether, anhydrous sodium sulfate, Pasteur type pipets, Thomas Hoover Capillary Melting Point Apparatus, glass wool, and TLC plates (Silica Gel GF, 250 microns thick from Analteck, Inc. Newark, Delaware).

PROCEDURE

Dry extract the sample* with approximately 0.5ml of methanol and spot across two or more TLC plates using a Pasteur type pipet. Standard cocaine may be spotted next to the sample for a rate of flow (R_f) comparison. Develop the plates in an appropriate solvent system which separates cocaine from other components in the sample.

Dry the plates thoroughly on a warm hot plate aided by a gentle flow of air. Under short wave u.v. light locate and outline the cocaine area.

Prepare a mini column by packing glass wool (1cm) in a 15cm Pasteur type pipet. Add anhydrous sodium sulfate (1cm) on top of the glass wool.

Scrap off the outlined cocaine area from the dried plates. This may be conveniently done by using a flat head type spatula. Transfer the scraping to the mini column on top of the anhydrous sodium sulfate. The transfer to the mini column can be greatly facilitated by use of a vacuum. This mini column preparation is essentially that described in Microgram, Vol. IX, No. 9, pp. 130-135 (1).

Prepare an eluting solvent by adding 2-3 drops of methanol to 15ml of chloroform saturated with concentrated NH_4OH.

Elute the cocaine from the mini column using 1-2ml of the eluting solvent. Collect the eluate in a mortar dish and take to dryness on a warm hot plate aided by a gentle flow of air.

After drying thoroughly, scrape the residue thus forming a powder. Add 1-2 drops of petroleum ether and take to dryness. The sample is now ready for a melting point determination.

Determine and compare the melting points of the sample, procedural standard l-cocaine and a mixture of the two.

* Standard l-cocaine must be treated in the same manner as the sample and may be done concomitantly with the sample. This is referred to as the procedural standard l-cocaine.

RESULTS AND DISCUSSION

Substance	Melting Points
Sample Cocaine	92 - 95°
Sample plus procedural standard l-cocaine	92 - 95°
Procedural standard l-cocaine	92 - 95°
Non-procedural standard l-cocaine	95 - 97°
Literature values	
l-cocaine (3)	96 - 98°
dl- cocaine (2)	79 - 80°

The usefulness of this method can be readily seen whenever the total sample quantity and percentage of cocaine is small enough to obviate use of the polarimeter.

The presence of other drugs in samples limited by quantity and low percentages of cocaine can, and may often dictate the use of prep-TLC as the only feasible means of separation for isomeric determination.

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James Conrad Roberson

(39)

Introduction

In the great tradition of the cannabis variety debate, we now see a rise in the question of isomers of cocaine in forensic analysis. The following outlines the legal ramifications of that question and analytical steps taken to avoid the problem.

Legal

On January 14, 1977, Mr. John Partain petitioned (Civil Action No. C77-67A) the United States District Court in Atlanta to overturn his prior conviction of possession of cocaine in violation of the Georgia Controlled Substance Act, Section 79A-807, Schedule II, b4. That Act is patterned after the Federal Schedule and reads as follows:

"(4) Coca leaves, any salt, compound, derivative, or preparation of coca leaves, and any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of these substances, but not including decocainized coca leaves or extractions which do not contain cocaine or ecgonine."

The sample consisted of a trace of cocaine on balance pans whose presence was established by Mr. William Price of the Georgia State Crime Laboratory using UV, TLC, GC, and mass spectra. Dr. Robert Shapiro of the University of Colorado testified that this evidence was insufficient to differentiate naturally occurring l-cocaine from d-cocaine, pseudococaine, allococaine, allopseudococaine, or alpha cocaine. The Georgia State Crime Laboratory contended that mass spectra would differentiate cocaine from its diastereomers and that d-cocaine would be covered by the Controlled Substance Act as a derivative of l-cocaine.

According to the definitions (1, 2, 3) of derivative, it must have the same basic structure as and must be theoretically derivable from the parent compound. The tropane structure is common to both d- and l-cocaine. The following are theoretical pathways from l- to d-cocaine.

(4)

1.

l-cocaine
↓ 6N HCL
↓ Reflux
l-ecgonine
↓ Kiliani Reagent
↓ (H_2CrO_4 - H_2SO_4 - H_2O)
Tropanone
↓ Potassium Metal
Tropanone Potassium
(Enol salt form)
↓ Dimethylcarbonate
(+) α,β - 2-Carbomethoxy Tropanone
↓ Na·Hg - H_2SO_4
↓ or Aluminum Isopropoxide
(+) α,β - 2-Carbomethoxy Tropine
↓ Benzoyl Chloride
d,l-cocaine, d,l-Pseudococaine

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2.

l-cocaine
↓ H_3O+
l-ecgonine
↓ chromic acid
tropanone
↓ sodium metal
tropanone sodium
↓ CO_2
sodium tropanone-2-carboxylate
↓ Na-Hg or Na-alcohol
d-ecgonine
↓ 1. CH_3OH
↓ 2. benzoyl chloride
d-cocaine (and 3 other isomers)

Literature ("Introduction to Stereochemistry" by Kurt Mislow) was quoted to indicate that IR or mass spectra would differ between diastereomers. The D.E.A. laboratory in Miami has compared IR and mass spectra of cocaine and pseudococaine and found differences. Pseudococaine is the diastereomer most likely to be spectrally similar to cocaine and is the only other isomer manufactured commercially.

Probably the most important legal point was simply the rarity of isomers other than l-cocaine. Dr. Shapiro admitted that if a person wished to obtain these isomers he would have to synthesize them himself by a difficult chemical procedure. Therefore it is unreasonable to consider their possible presence in a sample in this country.

In his order of August 26, 1977, Judge Newell Edenfield denied the petition of Civil Action No. C77-67A.

Analytical

Mixture melting point[6] and NMR spectra[7] are methods of differentiating cocaine and its diastereomers that are fully documented in the literature. The multiplet at 5.27 δ (ppm) in the NMR spectrum is characteristic of cocaine free base. The following clean-up procedure is very useful.

1. Wash sample powder with 5 to 10 portions of 1,4-dioxane (removes lidocaine).
2. Wash sample powder with 2 or 3 small portions of acetone (removes procaine or tetracaine).
3. Dissolve sample in 0.1NHCl, add $NaHCO_3$ to pH 7, and extract cocaine base with $CHCl_3$.
4. Evaporate $CHCl_3$ and dissolve residue in $CDCl_3$ for NMR spectrum.

Very little cocaine is lost in the dioxane wash, but the acetone wash removes a good deal of cocaine and should be avoided if possible. An alternative procedure is to dissolve the sample in 0.1N boric acid and extract the cocaine with $CHCl_3$, leaving the procaine in the acid solution.

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From: The Georgia State Crime Laboratory
959 East Confederate Avenue, S. E.
Atlanta, Georgia 30316

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COMPREHENSIVE LIST OF CONTROLLED SUBSTANCES

The Uniform Controlled Substances Act, Title 37, Chapter 27, Section 37-2414, Idaho Code, states that the Board shall revise and republish the schedules semi-annually for two years from the effective date of this act and thereafter annually. Therefore, pursuant to the mandate of that section, the Executive Secretary of the Idaho State Board of Pharmacy hereby orders the annual publication of the schedules of controlled substances. Chemical or trade names follow generic names in parentheses.

SCHEDULE I. (a) The controlled substances listed in this section are included in schedule I.

(b) Any of the following opiates, including their isomers, esters, ethers, salts, and salts of isomers, esters, and ethers, unless specifically excepted, whenever the existence of these isomers, esters, ethers and salts is possible within the specific chemical designation:

(1) Acetylmethadol; (Acemethadone, Amidolacetate)
(2) Allylprodine; (Alperidine, NIH-7440, Ro2-7113)
(3) Alphacetylmethadol; (see Acetylmethadol)
(4) Alphameprodine; (NU-1932)
(5) Alphamethadol;
(6) Benzethidine;
(7) Betacetylmethadol; (see Acetylmethadol)
(8) Betameprodine; (NU-1932)
(9) Betamethadol; (Betametadol)
(10) Betaprodine; (NU-1779)
(11) Clonitazene;
(12) Dextromoramide; (Palfium, Jetrium, Pyrrolamidol, R-B75, SKF-d, 5137)
(13) Diampromide;
(14) Diethylthiambutene; (Themalon)
(15) Difenoxin;
(16) Dimenoxadol; (NIH-7577, Lokarin)
(17) Dimepheptanol; (Methadol, Pangerin, Amidol, NIH-2933, Amidalgon)
(18) Dimethylthiambutene; (Kobaton, Ohton, Skikiton, Takaton)
(19) Dioxaphetyl butyrate; (Amidalgon, Spasmoxale)
(20) Dipipanone; (Fenpidon, Pamedon, Pipadone, 378C48, HOECHST 10805)
(21) Ethylmethylthiambutene; (Emethibutin, 1C50, NIH-5145)
(22) Etonitazene;
(23) Etoxeridine; (Atenorax, Atenos, Cargetidine)
(24) Furethidine;
(25) Hydroxypethidine; (Bemidone)
(26) Ketobemidone; (Ketogan, Cliradon)
(27) Levomoramide;
(28) Levophenacylmorphan; (NIH-7525, Ro4-0288)
(29) Morpheridine;
(30) Noracymethadol;
(31) Norlevorphanol; (NIH-7539)
(32) Normethadone; (Ticarda, HOECHST 10582, Mepidon, Deatussan, Normedon, Phenyldimazone Veryl)
(33) Norpipanone; (HOECHST 10, 495, Hexalgon, Orfenso)
(34) Phenadoxone; (CB-11, Hepagin, Heptalgin, Heptalin, Heptan, Heptone, Heptazone, Supralgin, HOECHST 10, 600
(35) Phenampromide;
(36) Phenomorphan; (NIH-7274)
(37) Phenoperidine; (Lealgin)

(38) Piritramide; (A65, Dipidolor)
(39) Proheptazine; (Proheptazone)
(40) Properidine; (Gevelina, Ipropethidine, Isopedine, Spasmodolosina)
(41) Propiram;
(42) Racemoramide; (R-610)
(43) Trimeperidine; (Promedol).

(c) Any of the following opium derivatives, their salts, isomers and salts of isomers, unless specifically excepted, whenever the existence of these salts, isomers and salts of isomers is possible within the specific chemical designation:

(1) Acetorphine;
(2) Acetyldihydrocodeine; (Acetylcodone)
(3) Benzylmorphine; (Peronine)
(4) Codeine methylbromide; (Eucodin)
(5) Codeine-N-Oxide; (Genocodeine, Codeigene)
(6) Cyprenorphine;
(7) Desomorphine; (Dihydrodesoxymorphine-D, Permonid)
(8) Dihydromorphine; (Paramorfan)
(9) Drotebanol;
(10) Etorphine, except hydrochloride salt; (M99, M183)
(11) Heroin; (Diacetylmorphine)
(12) Hydromorphinol;
(13) Methyldesorphine;
(14) Methyldihydromorphine; (Metopan)
(15) Morphine methylbromide; (Morphosan)
(16) Morphine methylsulfonate;
(17) Morphine-N-Oxide; (Genomorphine)
(18) Myrophine; (Leucodinine, Myricodine, Peronine myristate)
(19) Nicocodeine;
(20) Nicomorphine; (Vilan)
(21) Normorphine; (Desmethymorphine)
(22) Pholcodine; (Ethnine, Glycodine, Memine, Codylin, Hibernyl, Pectolin, Prodromine, Weifacodine)
(23) Thebacon.

(d) Any material, compound, mixture or preparation which contains any quantity of the following hallucinogenic substances, their salts, isomers and salts of isomers, unless specifically excepted, whenever the existence of these salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) 3,4-methylenedioxy amphetamine;
(2) 5-methoxy-3, 4-methyldenedioxy amphetamine; (MDA)
(3) 3,4,5 -trimethoxy amphetamine;
(4) Bufotenine; (Mappine)
(5) Diethyltryptamine; (DET)
(6) Dimethyltryptamine; (DMT)
(7) 4-methyl-2,5-dimethoxyamphetamine; (STP, DOM, DMA)
(8) Ibogaine;
(9) Lysergic acid diethylamide; (LSD)
(10) Marihuana;
(11) Mescaline;
(12) Peyote;
(13) N-ethyl-3-piperidyl benzilate;
(14) N-methyl-3-piperidyl benzilate;
(15) Psilocybin;
(16) Psilocyn;
(17) Tetrahydrocannabinols; (THC); (Synthetic equivalents of the substances contained in the plant, or in the resinous extractives of Cannabis, and/or synthetic substances, derivatives, and their isomers with similar chemical structure and pharmacological activity;)
(18) 2,5-dimethoxyamphetamine (2,5-dimethoxy-a-methylphenethylamine: 2,5-DMA);
(19) 4-bromo-2,5-dimethoxyamphetamine (4-bromo-2,5-dimethoxy-a-methylphenethylamine: 4-bromo-2,5,DMA);

(20) 4-methoxyamphetamine (4-methoxy-a-methylphenethylamine; paramethoxyamphetamine, PMA).

(21) Thiophene analog of phencyclidine (1-(1-(2-thienyl) cyclohexyl) piperidine).

(e) Any material, compound, mixture or preparation which contains any quantity of the following substances having a depressant effect on the central nervous system, including its salts, isomers, and salts of isomers wherever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) Mecloqualone.

SCHEDULE II. (a) Schedule II shall consist of the drugs and other substances, by whatever official name, common or usual name, chemical name, or brand name designated, listed in this section.

(b) Substances, vegetable origin or chemical synthesis. Unless specifically excepted or unless listed in another schedule, any of the following substances whether produced directly or indirectly by extraction from substances of vegetable origin, or independently by means of chemical synthesis, or by a combination of extraction and chemical synthesis:

(1) Opium and opiate, and any salt, compound, derivative, or preparation of opium or opiate, excluding naloxone and its salts, and naltrexone and its salts, but including the following:

1. Raw Opium;
2. Opium extracts;
3. Opium fluid extracts;
4. Powdered opium;
5. Granulated opium;
6. Tincture of opium;
7. Codeine; (methylmorphine)
8. Ethylmorphine;
9. Etorphine hydrochloride;
10. Hydrocodone;
11. Hydromorphone; (Dilaudid)
12. Metopon;
13. Morphine; (Papine, H-M-C #1 & #2)
14. Oxycodone; (Percodan, Percobarb, Nucodan)
15. Oxymorphone; (Numorphan)
16. Thebaine.

(2) Any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of the substances referred to in paragraph (b) (1) of this section, except that these substances shall not include the isoquinoline alkaloids of opium.

(3) Opium poppy and poppy straw.

(4) Coca leaves and any salt, compound, derivative, or preparation of coca leaves, and any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of these substances, but not including decocainized coca leaves or extractions which do not contain cocaine or ecgonine.

Methylbenzoylecgonine (Cocaine).

(5) Concentrate of poppy straw (the crude extract of poppy straw in either liquid, solid or powder form which contains the phenanthrine alkaloids of the opium poppy).

(c) Any of the following opiates, including their isomers, esters, ethers, salts, and salts of isomers, whenever the existence of these isomers, esters, ethers and salts is possible within the specific chemical designation:

(1) Alphaprodine; (Nisentil HcL, Nisintil, Prisilidene)
(2) Anileridine; (Leritine, Nipecotan, Alidine, Apodol)
(3) Bezitramide;
(4) Dihydrocodeine; (Codhydrine, Dehacodin, DF118, DH-Codeine, Didrate, Hydrocodone, Dihydrin, Parazone, Hydrocodin, Paracodin)
(5) Diphenoxylate;

(6) Fentanyl; (R4263, Phentanyl, Pentanyl, Sublimaze)
(7) Isomethadone; (Liden, Isoadanone)
(8) Levomethorphan;
(9) Levorphanol; (Aromarine, Dromoran, Levo-Dromoran, Levorphan)
(10) Metazocine; (NIH 7539)
(11) Methadone; (Amidone, Dolophine, Methadon, Methajade)
(12) Methadone--Intermediate, 4-cyano-2-dimethylamino-4, 4-diphenyl butane;
(13) Moramide--Intermediate, 2-methyl-3-morpholino-1, 1-diphenyl propane-carboxylic acid;
(14) Pantopon (Hydrochlorides of opium alkaloids);
(15) Pethidine; (Demerol, Isonipecaine, Meperidine, Mepergan, Demerol APAP)
(16) Pethidine--Intermediate--A, 4-cyano-1-methyl-4-phenylpiperidine;
(17) Pethidine--Intermediate--B, ethyl-4-phenyl-piperidine-4-carboxylate;
(18) Pethidine--Intermediate--C, 1-methyl-4-phenylpiperidine-4-carboxylic acid;
(19) Phenazocine; (NIH-7519, SKF 6574, Narphen, Prinadol)
(20) Piminodine; (Alvodine, Anopridine, Cimadon, Pimadine, NIH 7590, WIN-14098)
(21) Racemethorphan;
(22) Racemorphan; (Citarin, Methorphinan, Levodromoran

(d) Stimulants. Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system:

(1) Amphetamine, its salts, optical isomers, and salts of its optical isomers;
1. Dextroamphetamine/amphetamine; (Biphetamine)
2. Amphetamine Sulfate; (Benzedrine).
3. Dextroamphetamine sulfate; (Dexedrine, Dextro-Amphetamine Phosphate, Dextro-Amphetamine Sulfate, Amphetamine HCl, D.A.S., Synatan, Desarex, Dex-Sule).

Combinations:
1. Dextroamphetamine sulfate & amobarbital; (Amodex, Dexamyl, Dexobarb, Daprisal, Obocell).
2. Dextroamphetamine sulfate & prochlorperazine; (Eskatrol, Bamadex, Appetrol, Amvicel, Amvicel X).

(2) Methamphetamine, its salts, isomers, and salts of its isomers; (Desoxyn, Fetamin).
(3) Phenmetrazine and its salts; (Preludin).
(4) Methylphenidate. (Ritalin HCl).

(e) Depressants. Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a depressant effect on the central nervous system, including its salts, isomers, and salts of isomers whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) Methaqualone; (Quaalude, Parest, Sopor, Optimil, Somnafac-200, Forte-400)
(2) Amobarbital; (Amytal)
(3) Secobarbital; (Tuinal)
(4) Pentobarbital; (Nembutal)

SCHEDULE III. (a) Schedule III shall consist of the drugs and other substances, by whatever official name, common or usual name, chemical name, or brand name designated, listed in this section.

(b) Stimulants. Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system, including its salts, isomers, and salts of its isomers, (whether optical or geometric), and salts of such isomers whenever the existence of such salts, isomers, and salts of

isomers is possible within the specific chemical designation:

(1) Those compounds, mixtures, or preparations in dosage unit form containing any stimulant substances listed in schedule II which compounds, mixtures, or preparations were listed on August 25, 1971, as excepted compounds under C.F.R. Sec. 308.32, and any other drug of the quantitive composition shown in that list for those drugs or which is the same except that it contains a lesser quantity of controlled substances.

(2) Benzphetamine; (Didrex)

(3) Chlorphentermine; (Pre-Sate)

(4) Clortermine; (Voranil)

(5) Mazindol; (Sanorex)

(6) Phendiametrazine; (Apidex, Edrisal, Genegesic, Mediatric, Thora-Dex #1 & #2, Ropledge, Bacarate, Banobese, Bontril PDM, Melfiat)

(c) Depressants. Unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a potential for abuse associated with a depressant effect on the central nervous system:

(1) Any compound, mixture or preparation containing:

i. Amobarbital; (Amytal)

ii. Secobarbital; (Tuinal, Efed)

iii. Pentobarbital; (Nembutal, Carbrital),

or any salt thereof and one or more other active medicinal ingredients which are not listed in any schedule.

(2) Any suppository dosage form containing:

i. Amobarbital; (Alurate)

ii. Secobarbital;

iii. Pentobarbital; (Emersert)

or any salt of any of these drugs and approved by the Food and Drug Administration for marketing only as a suppository.

(3) Any substance which contains any quantity of a derivative of barbituric acid or any salt thereof

(4) Chlorhexadol; (Lora, Mecoral, Medodorm)

(5) Glutethimide; (Doriden, Dormtabs, Rolathimide)

(6) Lysergic acid;

(7) Lysergic acid amide; (LSD)

(8) Methyprylon; (Noludar)

(9) Phencyclidine; (Sernylan)

(10) Sulfondiethylmethane;

(11) Sulfonethylmethane; (Trional, Ethyl Sulfonal)

(12) Sulfonmethane; (Sulfonal)

(d) Nalorphine; (N-Allyl Nor Morphine, N Alline, Norfin, NANM)

(e) Narcotic Drugs. Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation containing limited quantities of any of the following narcotic drugs, or any salts thereof:

(1) Not more than 1.8 grams of codeine, or any of its salts, per 100 milliliters or not more than 90 milligrams per dosage unit, with an equal or greater quantity of an isoquinoline alkaloid of opium, and

(2) Not more than 1.8 grams of codeine, or any of its salts, per 100 milliliters or not more than 90 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts;

1. Codempiral
2. Empirin Compound #1, #2, #3, and #4
3. APC with Codeine
4. Copavin
5. Anexia with codeine
6. Phenaphen with codeine

(3) Not more than 300 milligrams of dihydrocodeinone, or any of its salts, per 100 milliliters or not more than 15 milligrams per dosage unit, with a fourfold or greater quantity of an isoquinoline alkaloid of opium;

(4) Not more than 300 milligrams of dihydrocodeinone, or any of its salts, per 100 milliliters or not more than 15 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts;

(5) Not more than 1.8 grams of dihydrocodeine, or any of its salts, per 100 milliliters or not more than 90 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts;

(6) Not more than 300 milligrams of ethylmorphine, or any of its salts, per 100 milliliters or not more than 15 milligrams per dosage unit, with one or more ingredients in recognized therapeutic amounts;

(7) Not more than 500 milligrams of opium per 100 milliliters or per 100 grams, or not more than 25 milligrams per dosage unit, with one or more active, nonnarcotic ingredients in recognized therapeutic amounts; (Paregoric).

(8) Not more than 50 milligrams of morphine, or any of its salts, per 100 milliliters or per 100 grams with one or more active, nonnarcotic ingredients in recognized therapeutic amounts.

(f) The board may except by rule any compound, mixture, or preparation containing any stimulant or depressant substance listed in subsections (b) and (c) of this section from the application of all or any part of this act if the compound, mixture, or preparation contains one or more active medicinal ingredients not having a stimulant or depressant effect on the central nervous system, and if the admixtures are included therein in combinations, quantity, proportion, or concentration that vitiate the potential for abuse of the substances which have a stimulant or depressant effect on the central nervous system.

SCHEDULE IV. (a) Schedule IV shall consist of the drugs and other substances, by whatever official name, common or usual name, chemical name, or brand name designated, listed in this section.

(b) Depressants. Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances, including its salts, isomers, and salts of isomers whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) Barbital; (Barbital Sodium, Veronal)
(2) Chloral betaine; (Beta-Chlor, Somilan)
(3) Chloral hydrate; (Noctec, Somnos, Felsules)
(4) Chlordiazepoxide; (Librium, Libritabs)
(5) Clonazepam; (R05-4023, Clonopin)
(6) Clorazepate; (Tranxene)
(7) Dextropropoxyphene; (Darvocet-N50, N100, Darvon Compound, Dolene AP-65)
(8) Diazepam; (Valium)
(9) Ethchlorvynol; (Placidyl)
(10) Ethinamate; (Valmid)
(11) Flurazepam; (Dalmane)
(12) Mebutamate;
(13) Meprobamate; (Equinal, Miltown, Kesso-bamate, Deprol, Meprospan)
(14) Methohexital; (Brevital Sodium)
(15) Methylphenobarbital;
(16) Oxazepam; (Serax)
(17) Paraldehyde; (Paral)
(18) Petrichloral; (Periclor)
(19) Phenobarbital; (Eskabarb, Luminal, Phenobarbital Sodium)

(c) Fenfluramine - Any material, compound, mixture, or preparation which contains any quantity of the following substances, including its salts, isomers (whether optical, position, or geometric), and salts of such isomers, whenever the existence of

such salts, isomers, and salts of isomers is possible:

(1) Fenfluramine.

(d) Stimulants. Unless specifically excepted or unless listed in another schedule, any material, compound, mixture, or preparation which contains any quantity of the following substances having a stimulant effect on the central nervous system, including its salts, isomers (whether optical, position, or geometric), and salts of such isomers whenever the existence of such salts, isomers, and salts of isomers is possible within the specific chemical designation:

(1) Diethylpropion; (Ro-diet)

(2) Phentermine; (Rolaphent, Adipex-P, Fastin, Ionamin)

(3) Pemoline (including organometallic complexes and chelates thereof. (Cylert)

(e) The board may except by rule any compound, mixture, or preparation containing any depressant substance listed in subsection (b) of this section from the application of all or any part of this act if the compound, mixture or preparation contains one or more active medicinal ingredients not having a depressant effect on the central nervous system, and if the admixtures are included therein in combinations, quantity, proportion, or concentration that vitiate the potential for abuse of the substances which have a depressant effect on the central nervous system.

SCHEDULE V. (a) Schedule V shall consist of the drugs and other substances, by whatever official name, common or usual name, chemical name, or brand name designated, listed in this section.

(b) Narcotic drugs containing nonnarcotic active medicinal ingredients. Any compound, mixture, or preparation containing any of the following limited quantities of narcotic drugs or salts thereof, which shall include one or more nonnarcotic active medicinal ingredients in sufficient proportion to confer upon the compound, mixture, or preparation, valuable medicinal qualities other than those possessed by the narcotic drug alone:

(1) Not more than 200 milligrams of codeine per 100 milliliters or per 100 grams; (Cosanyl, Cosadeine, Cheracol, TH&C, Robitussin AC, Histadyl Ec)

(2) Not more than 100 milligrams of dihydrocodeine per 100 milliliters or per 100 grams;

(3) Not more than 100 milligrams of ethylmorphine per 100 milliliters or per 100 grams;

(4) Not more than 2.5 milligrams of diphenoxylate and not less than 25 micrograms of atropine sulfate per dosage unit; (Lomotil)

(5) Not more than 100 milligrams of opium per 100 milliliters or per 100 grams.

NOTE: The Drug Enforcement Administration computer list of controlled substances is in excess of 525 pages in length and contains about 7,000 drug products in various dosage forms. This list is on file in the office of the Idaho Board of Pharmacy.

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Discoloration Effect of Diluents in Contraband Cocaine

In the course of police investigations of confiscated drug samples, cocaine mixtures of various shades from white to brown have been encountered and analyzed at the Crime Laboratory of the New York City Police Department. Slight discoloration of cocaine mixtures is within reason, in view of the reported discoloration of lactose [*1,2*] and of lactose-amphetamine [*3-5*] upon storage.

Recently a very interesting case involving cocaine was resubmitted to the laboratory, wherein a drastic change in the physical appearance of the confiscated evidence was observed. The evidence, originally a tan powder, was analyzed in July 1972. It contained aspirin tablets along with the contraband cocaine powder. The other diluents present were not analyzed at that time. This case was reanalyzed in February 1973 and at that time the powder was brown in color, as compared to the original tan powder. Finally, during a court trial in June 1973, the evidence was opened and found to be a dark tarry substance and the aspirin tablets were no longer present. Due to the discrepancy in the physical appearance of the evidence from the time of the original analysis to its appearance in court, the court had reservations in accepting the evidence unless a reasonable scientific explanation was furnished for this change. Since the trial was in progress, the court permitted a maximum of two days to conduct the related experiments with the evidence in question so that an explanation for this drastic change could be ascertained.

This paper presents the experimental work done with evidence in conjunction with the studies on the discoloration effects of known cocaine and the common diluents associated with it. The interaction of the diluents, which causes discoloration and tarry transformation, is also discussed.

Experimental

Studies were conducted in two parts: The first part deals with the analysis of the evidence and the second part deals with a study on the discoloration of various combinations of diluents commonly associated with contraband cocaine.

Analysis of Evidence

1. Positive cocaine identification was determined from the alkaline chloroform extraction of the water-soluble part of the tarry substance. Identification was made by

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[1]Chemist, police officer, and police officer, respectively, New York City Police Department Crime Laboratory, New York, N.Y.

color and microcrystalline tests [6,7]. Additional confirmation was obtained on a gas chromatograph/mass spectrometer (GC/MS).

2. The tarry substance was acidic and had the odor of acetic acid. The presence of acetate ions was determined by $FeCl_3$ test and ethyl acetate tests [8,9].

3. 5-hydroxymethyl furfural and related compounds are the degradation products of lactose, especially when the spray-dried process lactose is used [1,2]. Identification of these furfural compounds was made from the benzene extraction of the tarry substance in acid media. The McCance method [10] and Sanchez reaction [7] were used for this identification.

4. The tarry substance dissolved in methanol giving a brown solution and leaving a white crystalline solid. The solid was filtered, purified by further washings with methanol, and identified as lactose. Lactose identification was made by primary screening test for reducing sugars with benedict's solution, followed by X-ray diffraction and GC/MS identification.

5. All organic nitrogeneous compounds were isolated from the tarry substance by repeated extractions with water and chloroform in acid and alkaline media. The residual tarry substance was submitted to the classical sodium fusion test, and presence of nitrogen in that tarry substance was detected.

6. Furfural derivatives were isolated by benzene extraction of the tarry substance in an acidic medium. The cocaine was then extracted with benzene in sodium bicarbonate media. The extracted cocaine was passed through an acid-washed celite column and quantitation was done using a Perkin-Elmer 350 spectrophotometer [11,12].

Discoloration Due to Diluents

Procaine, benzocaine, tetracaine, lidocaine, quinine, and sugars such as lactose are the most common diluents mixed with cocaine. On occasion other chemicals, such as aspirin, have been found with the other diluents. A 5-hydroxymethyl furfural and other furfural derivatives are also present as contaminants when spray-dried lactose is used as a cutting agent [1,2].

Studies on discoloration were conducted using USP-grade lactose and hydrochlorides of cocaine, procaine, benzocaine, tetracaine, lidocaine, and quinine. The thermal stability of each of the compounds was studied by heating them up to four hours in sealed plastic bags on a steam bath. Each was found thermally stable and no color change nor browning was observed.

Experiments on the discoloration effects of various combinations of these compounds, which under normal storage conditions at ambient temperatures would take long periods of time, were conducted. To increase the rate of reaction and to study the discoloration effect in a short period of time the samples were heated on a steam bath.

Table 1 shows the discoloration effect due to the interaction of lactose with those amines which are commonly used as cutting agents with cocaine. Tables 2 to 4 show the effect of other contaminants, such as aspirin, furfural, and moisture, on the lactose-amine reaction resulting in the brown tar formation.

Discussion and Results

The results of this study indicate that lactose, if mixed with cocaine and stored for a long period of time, will not react and give discoloration. However, some discoloration may occur due to the condensation reactions of the degradation products of lactose, such as 5-hydroxymethyl furfural and other furfural derivatives which are associated with lactose due to its spray-drying process [1,2]. If other amines, commonly used for cutting cocaine, are present in addition to lactose, browning on storage is likely to occur due to

TABLE 1—*Browning due to lactose-amine interaction. Heating on steam bath was done for 3 h in heat-sealed plastic bags.*

Reactants	Ratio[a]	Discoloration
Lactose + procaine	7:1	gradual discoloration finally resulting in dark brown color in 3 h
Lactose + benzocaine	7:1	very slow and slight discoloration observed in 3 h
Lactose + tetracaine	7:1	no discoloration observed
Lactose + lidocaine	7:1	very slow and slight discoloration observed in 3 h
Lactose + quinine	7:1	no discoloration observed
Lactose + cocaine	7:1	no discoloration observed

[a] Ratio of 7:1 was chosen since it was the most realistic ratio pertaining to the evidence in question.

TABLE 2—*Effect of furfural on browning. Heating on steam bath was done for 3 h in heat-sealed plastic bags.*

Reactants	Ratio	Discoloration
Lactose + furfural	7:1	gradual and slight browning only
Lactose + procaine + furfural	7:1:1	immediate red coloration which turns to a brown tarry substance
Lactose + benzocaine + furfural	7:1:1	immediate red coloration which turns to a brown tarry substance
Lactose + tetracaine + furfural	7:1:1	gradually turns brown
Lactose + lidocaine + furfural	7:1:1	gradually turns brown
Lactose + cocaine + furfural	7:1:1	gradual and slight browning only

TABLE 3—*Effect of acetate ions on browning. Heating on steam bath was done for 3 h in heat-sealed plastic bags.*

Reactants	Ratio	Discoloration
Lactose + aspirin + CH_3COOH	7:1:1	no color change
Lactose + procaine + CH_3COOH + aspirin	7:1:1:1	starts browning almost immediately, 35 min to a dark tarry substance, no procaine detected
Lactose + procaine + aspirin + 1 drop of water	7:1:1	starts browning almost immediately, 35 min to a dark tarry substance, no procaine detected after the physical change[a]
Lactose + procaine + CH_3COOH	7:1:1	starts browning almost immediately, 35 min to a dark tarry substance, no procaine detected after the physical change
Lactose + cocaine + aspirin	7:1:1	no change
Lactose + benzocaine + aspirin	7:1:1	browning starts slow, benzocaine still present
Lactose + tetracaine + aspirin	7:1:1	slight browning

[a] An acetic-acid-like odor was detected.

TABLE 4—*Effect of moisture on lactose-amine reaction. Heating on steam bath was done for 3 h in heat-sealed plastic bags.*

Reactants	Ratio	Discoloration
Cocaine + lactose + aspirin (ovendried)	7:1:1	no discoloration
Cocaine + lactose + aspirin + moisture	7:1;1	no discoloration
Procaine + lactose + aspirin (ovendried)	7:1:1	slow discoloration towards browning
Procaine + lactose + aspirin + moisture	7:1:1	starts turning brown within 5 min and completes tar formation in 30 min, no procaine found present after tar formation[a]

[a] An acetic-acid-like odor was detected.

lactose-amine reaction, commonly known as the Milliard reaction [1].[2] The extent of browning would depend on the reactivity of amines with lactose (Table 1). Procaine, (and other primary amines), is very reactive because it requires a low order of initiation energy and exhibits autocatalytic qualities once the reaction has begun [1]. The furfurals associated with lactose will also react with the amines, as can be seen from Table 2. It has been further reported that certain ions such as acetates, stearates, etc catalyze the Milliard reaction [2]. Table 3 indicates the effect of acetate ions on the Milliard reaction. Aspirin brings about the same catalytic effect as that of acetates, since on hydrolysis it releases acetic acid. Moisture has been found to be an equally important factor in bringing about the Milliard reaction, as can be seen from Table 4.

The evidence in question, after transformation, was found to contain lactose, acetate ions, cocaine, furfural derivatives, and moisture. Quantitation of the evidence indicated that the amount of cocaine found in the tarry substance is in agreement with the amount of cocaine reported in the original analysis and, as previously stated, known mixtures of cocaine and lactose do not result in discoloration (Table 1). Therefore, it can be concluded that cocaine was not involved in the formation of the tarry substance. A sodium fusion test on the isolated tarry substance indicated the presence of nitrogen. In view of the presence of nitrogen in the isolated tarry substance, the experimentation with the common diluents (Table 3), and the similarity in the physical appearance between the evidence and the known lactose-procaine mixture, it was deduced that the most probable reaction which resulted in the tar formation was that of procaine with lactose.

The original evidence, which was analyzed in July 1972, was a tan powder which turned brown by February 1973 during storage. This indicates the initiation of the lactose-procaine browning reaction. The tar formation, which occured in a relatively short period of time between February and June 1973, could be explained by the catalytic effect of acetates resulting from the decomposition of aspirin during storage.

The hydrolysis of procaine in dilute acetic acid did not occur. However, the effects of moisture and acetate ions on the mechanism of browning due to the lactose-procaine reaction, and the isolation and identification of the end products formed, are presently under study and will be presented in a subsequent communication.

Summary

Discoloration in contraband drugs may occur on storage. The extent of this discoloration depends on the time of storage and the type of cutting agent present with

[2] The Milliard reaction is a Schiff base reaction.

the drug. In the investigation of the evidence in question the main cause of the brown tarlike transformation of the tan powder was attributed to the procaine-lactose reaction, which was catalyzed by the acetic acid due to the hydrolysis of the aspirin present in the contraband seizure. The self-condensation of the degradation products of lactose, such as 5-hydroxymethyl furfural, can also contribute to the discoloration.

Comments

The explanation for the brown tarry transformation of the confiscated cocaine powder, as a result of the experimental work detailed herein, was accepted by the court. The confiscated cocaine was then admitted as evidence and subsequent convictions were obtained.

Acknowledgments

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Note

Thin-layer chromatographic procedure for the differentiation of the optical isomers of cocaine

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Medicinally used cocaine is prepared from the leaves of the coca plant (*Erythroxylon coca*, Lamarck) by extracting the total alkaloids and converting them to *l*-ecgonine by acid hydrolysis. The isolated *l*-ecgonine is esterified with methanol and benzoic acid to produce the natural product, *l*-cocaine[1]. In 1923 a method for the synthesis of *dl*-cocaine was published[2]. The racemic mixture was separated into the *dextro* and *levo* isomers of cocaine with tartaric acid[3]. Probably all of the currently available illicit cocaine is prepared from coca leaves and therefore is the natural isomer *l*-cocaine. However, there is the possibility of the occurrence of synthetic *dl*- or *d*-cocaine in illegal drugs and, for legal reasons, it may be necessary for the forensic chemist to be able to distinguish between the optical isomers. The following method was developed for this purpose and makes possible the routine analysis of small samples of illicit cocaine and the determination of the optical isomer or isomers present in the sample. Cocaine was hydrolysed to ecgonine and then esterified with the enantiomeric 2-octanols to give the necessary derivatives. The resulting diastereoisomers may be distinguished by thin-layer chromatography (TLC).

EXPERIMENTAL

Materials and methods

The *dl*-cocaine was prepared by benzoylation of *dl*-methylecgonine[2] and the *d*-cocaine by resolution of *dl*-cocaine[3]. The *l*-cocaine and the benzenesulfonyl chloride were obtained commercially (E. Merck, Darmstadt, G.F.R.) as well as the *d*-2-octanol, *l*-2-octanol and *dl*-2-octanol (Fluka, Buchs, Switzerland).

The diastereomeric derivatives were prepared in 0.3 ml Reacti-Vials® provided with magnetic stirrers (Pierce Chem. Co., Rockford, Ill., U.S.A.) according to the procedure of Brewster and Ciotti[3].

TLC separation of the isomers was carried out on non-activated pre-coated silica gel plates of thickness 0.25 mm (E. Merck) with methanol as the developing solvent.

Procedure

Approximately 0.5 mg of cocaine base or hydrochloride was placed in a 0.3 ml

screw-cap vial and 30 μl of 2 *N* hydrochloric acid were added. The vial was capped and heated for 30 min in a heating block maintained at 120°. The cap was removed and the heating continued for about 20 min to evaporate the solvent completely. The residue was allowed to cool to room temperature, then 30 μl of pyridine were added, followed by 3 μl of *d*-2-octanol and the vial was placed in a small dish with water and ice. After cooling for some minutes 1 μl of benzenesulfonyl chloride was added. The vial, provided with a magnetic stirrer, was capped and stirred slowly (60 rpm) for 30 min while the solution was kept cold. Then 30 μl of 2 *N* ammonia were added, followed by 100 μl of ether and the mixture was stirred rapidly for a short time.

Then 2 μl of the ether layer were placed on the starting line of the TLC plate. The plates were developed in cylindrical glass vessels (without prior equilibration) with methanol until the solvent front had reached a height of 15 cm. The plates were air dried at room temperature and sprayed with acidified iodoplatinate[4]. The two reference spots were obtained by using the same procedure with *l*-cocaine and *d*-2-octanol and with *l*-cocaine and *l*-2-octanol or, more simply, with *l*-cocaine and *dl*-2-octanol.

RESULTS AND DISCUSSION

The method for the preparation of esters with benzenesulfonyl chloride in pyridine[3] was found to be the most convenient, giving a high yield in a short time. The optically active alcohols, menthol, borneol, 2-methyl-1-butanol and 2-octanol, were used to prepare esters of *dl*-ecgonine in this way. The TLC separation was best when the diastereoisomers prepared from 2-octanol were used. The R_f values of the diastereoisomers of 2-octanyl-ecgonine are shown in Table I. After spraying with acidified iodoplatinate[4] the spots were intense blue-green, discrete and well defined. In case of double spots of equal amounts of the diastereoisomers, the spot with the high R_f value was always distinctly smaller than the other spot.

TABLE I

SEPARATION OF DIASTEREOISOMERS OF 2-OCTANYLECGONINE BY THIN-LAYER CHROMATOGRAPHY

Ester prepared from	R_f
l-Ecgonine and *d*-2-octanol	0.36
l-Ecgonine and *l*-2-octanol	0.28
l-Ecgonine and *dl*-2-octanol	0.28, 0.36
d-Ecgonine and *d*-2-octanol	0.28
d-Ecgonine and *l*-2-octanol	0.36
d-Ecgonine and *dl*-2-octanol	0.28, 0.36
dl-Ecgonine and *d*-2-octanol	0.28, 0.36
dl-Ecgonine and *l*-2-octanol	0.28, 0.36

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Proc. Manual

TLC METHOD TO SEPARATE LSD FROM LYSERGIC ACID METHYL PROPYL AMIDE

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OBJECTIVE

Because IR identification of LSD in small amounts can be difficult, a method for TLC identification, including separation from lysergic acid methyl propyl amide (LAMPA) is desired.

BACKGROUND

The separation of LSD from LAMPA by thin layer chromatography has been the subject of recent investigation (Microgram VIII, No. 6, and J. Association of Offic. Anal. Chem. Volume 56, (1973), 88). This laboratory has tried both methods suggested by these authors: (1) Formation of trimethylsilyl derivatives of LSD and LAMPA, followed by separation on Merck Silica Gel F-254 plates using acetone as the solvent; (2) Impregnating pre-coated silica gel G plates with ethyl acetate: N,N-dimethylformamide: ethanol (13: 1.9: 0.1), letting them dry, then spotting samples and developing for 16 cm in the same solvent. The results were not satisfactory in our hands.

Using the former method, we could not form the TMS derivative using the conditions given and in the latter case, the separation achieved was not adequate. This may have been due to the difference between the Brinkmann plates used by those authors and the Merck plates available to us.

An alternate TLC method is proposed.

APPARATUS

Chromatography tank lined with filter paper for optimum saturation.

Thin layer plates; (5 x 20 cm, precoated 0.25 mm, Merck Silica Gel 60 F-254). These plates are scored and broken to 5 x 10 cm prior to use, then oven dried.

PROCEDURE

Extract the sample and standards into CH_2Cl_2 or similar organic solvent before spotting. Do not use methanol, as the spots must be kept small. Apply spots of LSD standard, mixed LSD standard and LAMPA standard, the sample extract and a mixed spot of sample + LAMPA standard. (LAMPA is available from Applied Science, Cat. No. 01810.)

Prepare the following solvent; chloroform freshly saturated with concentrated NH_4OH (1 part) + acetone (2 parts). Moisten the paper lining of the tank with the solvent and develop the plate to its full height, 10 cms. This takes about 20 minutes. Remove the plate, dry it briefly, examine the spots under UV light. If desired, repeat the development one more time and again dry the plate.

PROCEDURE (continued)

Locate the spots using UV light and spray with p-DMAB spray to produce purple color.

RESULTS

The first pass moves the LSD spot ahead of the LAMPA spot by about 3 mm (measured between the two centers). The second pass improves the separation to about 4 mm, with a total distance traveled of approximately 55-60 mm. If iso-LSD is present, it runs slower than LAMPA and does not interfere.

DISCUSSION

1. The chloroform component of the solvent should be freshly saturated with ammonia and the solvent fresh in the tank. The same solvent may be used for the second development, if one is needed, but then it should be discarded.

2. As with any TLC procedure, care should be taken to not overload the plate. Total amounts should be kept as small as possible, consistent with visualization using p-DMAB spray.

3. Often the separation appears better when the plate is viewed from the back side.

4. Tank geometry seems to have an influence on the quality of separation. Best results were obtained with these short plates in rather short, round jars, in which they fit at an angle.

5. Each chemist has his/her own standard for what constitutes separation between two close spots. Some may find one development sufficient; others may decide two passes are needed.

6. Merck improved their silica gel plates in 1971-72. This separation cannot be achieved using the older type plates. The improved ones carry the designation, Silica Gel 60 F-254.

7. This laboratory does not rely on one TLC system alone for the identification of LSD. The other systems in use are 1,1,1 Trichloroethane/Methanol (9/1) and Benzene/Dimethylformamide (13/2), which were recommended by James A. Heagy, San Francisco Regional DEA Laboratory.

PROC. Manual

LSD (Lysergic acid diethylamide)

Analytical

Sample preparation for TLC:
(Applies to blotter acid, tablets, window panes)

Short Method: One hit either ground up or cut into small pieces; add a small amount of methanol or CH_2Cl_2 and shake, spot the solvent.

Long Method: Cut or grind material; add 0.1N H_2SO_4 - shake for 15 minutes; make slightly alkaline with Na_2CO_3, extract with ether; concentrate in volume in hot water bath under N_2. Spot this extract.

TLC Systems: (Use three for confirmatory identification)
1. 1,1,1 Trichloroethane/methanol (9/1)
2. Benzene/Dimethylformamide (13/2)
3. $CHCl_3$ saturated with NH_4OH/acetone (1/2) See attached paper by Bradley.

It is advisable to spot lysergic acid methyl propyl amide (LAMPA) in addition to LSD.

Visualize with p-DMAB spray.

Attached is an IR preparation method.

LSD - Micro-Pelleting for IR

1. Using a centrifuge tube, place sample in 2.0 ml. ~~NH_2SO_4~~ 0.1 N H_2SO_4 (for 3 hits or less)
2. Vortex 1 min.
3. Make basic with concentrated ~~ion~~ NH_4OH.
4. Add 1 ml. $CHCl_3$ - spectro or nano grade.
5. Vortex 1 min.
6. Centrifuge.
7. Remove organic layer to 5.0 ml centrifuge.
8. Repeat steps 4 through 7 3X.
9. Reduce in volume to 1/4-1/2 ml. under N_2.
10. Spot on TLC plate.
11. Rinse centrifuge tube with 1/4 ml $CHCl_3$ and vortex.
12. Spot same area.
13. System for TLC - $CHCl_3$ 9/MeOH, 1 (Same grade $CHCl_3$).
14. Scrape off LSD band from plate into mortar and grind with pestle.
15. Use disposable pipet, plug with glass wool.
16. Test on vacuum - see if plug holds.
17. Vacuum silica gel into pipet.
18. Elute LSD with 0.1N H_2SO_4 in 3-4 ml. aliquots. Pipet plugs up; may be necessary to stir silica gel with long pipet and force .1N H_2SO_4 through. Catch in 15 ml centrifuge tube.
19. Add NH_4OH til basic.
20. Extract with 0.5 ml $CHCl_3$.
21. Vortex.
22. Centrifuge.
23. Transfer organic to 5.0 ml centrifuge tube.
24. Repeat 20-23 3X.
25. Reduce to dryness under N_2.
26. Place dry tube in vacuum desiccator for 15 min.

27. Remove and add 1/2 ml. $CHCl_3$.

28. Drop on 5 mg KBr - mortar & pestle - let dry.

29. Rinse tube with 0.5 ml. $CHCl_3$.

30. Vortex.

31. Drop on KBr.

32. Place mortar in vac. des. for 15 min.

ORIGINAL

PHENCYCLIDINE

BACKGROUND

-late 1950's developed for use as an anesthetic, but too many adverse side effects resulted in it being discontinued for human use in 1967; still available as a veterinary immobilizing agent under the brand name "SERNYL" (Parke, Davis, and Co.)

-first reported illicit use was in mid-1960's on the West Coast; it was known as the "PeaCe Pill" or "angel dust" which was sprinkled on parsley and smoked.

-became a Schedule III drug on 4/6/69, then placed in Schedule II 2/24/78

-TCP placed in Schedule I in 1975; PCE (cyclohexamine) and PHP placed PHP = PCPy in Schedule I 10/25/78; PCC controlled not as a drug itself, but as an immediate precursor/intermediate in the manufacture of PCP. (C-II)

MANUFACTURE

(1) -PCP most commonly produced through nitrile intermediate, PCC, which is formed in high yield (90-98%) and is frequently found in the final product; the entire process for producing PCP takes only about 4 hours.

(1) -NaCN or KCN is used in this process and is converted to HCN in the presence of acid; ventilation is important if one is involved in any clandestine lab seizures.

(1) -the other process often leaves piperidine as an impurity which can often be recognized by its odor.

PHYSICAL PROPERTIES

(1) -refer to list of structures for PCP and PCP analogs.

(14) -PCP

a) hydrochloride salt $C_{17}H_{25}N$-HCl MW 279.86 mp 230-231 C
soluble in MeOH, EtOH, $CHCl_3$, CH_2Cl_2, H_2O
sparingly soluble in dilute HCl
insoluble in hexane, ether

b) free base $C_{17}H_{25}N$ MW 243.41 mp 46-46.5 C
soluble in $CHCl_3$,
insoluble in hexane, ether

(8), (19) -TCP

a) hydrochloride salt $C_{15}H_{23}NS$-HCl MW 285.85 mp 233-236 C
soluble in MeOH, EtOH, $CHCl_3$, H_2O
insoluble in hexane, ether

b) free base $C_{15}H_{23}NS$ MW 249.40
soluble in $CHCl_3$, hexane, ether

SPOT TESTS PCP

(8) Marquis---no rxn., effervesces; rule out carbonates by addition of dilute HCl

(8) Meckes---no rxn., effervesces

Froehdes---no rxn., effervesces

(8) Mandelins---no rxn., effervesces

Wagners---pos. rxn. for alkaloids

Mayers---white ppt.

Cobalt thiocyanate---slow, faint blue ppt.

Ruybals---blue ppt.

(2) Scotts---PCP is negative, but can get false positives when PCP in combination with some drugs.

(3) Zelonis---clear blue benzene layer; works well on plant material, but best to extract material first with $CHCl_3$ or CH_2Cl_2, and take to dryness before adding reagents.

Sodium nitroprusside---some samples give pos. rxns. for secondary amines.

TCP

(8),(14),(19) Marquis---effervesces, gray-orange, then slowly to gray-green

Meckes---effervesces, orange or yellow/green, then to yellow-blue/green, then to deep blue

Mandelins---green, effervesces

Zelonis - ⊕

(5) KETAMINE

Marquis---no rxn.

Cobalt thiocyanate---blue ppt.

Wagners---pos. rxn. for alkaloids

Liebermans---slow purple

Zelonis ⊖

Ruybal's ⊖

2° any amine ⊖ !!

Cyclohexanone

2° any amine ⊖ !!

Zelonis ⊖

Ruybal's ⊕

TLC

(4) 1) Butyl ether:diethyl ether:diethylamine (45:45:10)

(4) 2) Benzene:diethylamine (95:5) May be useful for Cocaine + PCP also.

(8,9) 3) Chloroform:methanol (9:1) - Rf's vary from paper to paper

(5) 4) Clarke's T_1

(5) 5) Hexane:benzene:diethylamine (75:25:10)

(5) 6) Chloroform:dioxane:ethyl acetate:ammonium hydroxide (25:60:10:5)

(6) 7) Benzene:acetone:pyridine (16:8:1)

(7) 8) Davidows

(7) 9) Acetone:chloroform (1:1)

(7) 10) Acetone:chloroform (65:35)

11) Ethyl acetate:methanol:ammonium hydroxide:water (29:1:0.25:0.5)

12) Same as (11) with ammonium hydroxide removed

	(1)	(2)
PCP	0.91	0.64
THC	0.61	0.41
CBD	0.69	0.52
CBN	0.47	0.30
DET	0.30	0.15
DMT	0.18	0.09

	(4)	(5)	(6)
Ketamine	0.72	0.55	0.86
PCP	0.59	0.90	0.92

	(7)	(8)	(9)	(10)
PCP	0.24	0.86	0.23	0.32
TCP	0.49	0.82	0.45	0.49
PCC	0.70	--	--	--
PHP	--	0.75	0.05	0.13
PPP	--	0.80	0.29	0.40
Piperidine	0.00	--	--	--

Rf's for (3)			
0.17	0.43	0.37	0.57
0.33	0.67	0.70	
0.82	0.91		
0.10	0.43		
0.25			
--			

11) PCP = 0.90
12) PCP = 0.20

by eliminating ammonium hydroxide in(12) PCP can be separated from more neutral drugs with same Rf as (11)

GC

(9) 1) 3% OV-17 on Gas Chrom Q 100/120 DMCS, 6' x ¼" x 2mm (id)
column = 170 C, inj. port = 250 C, detector = 270 C
Nitrogen flow rate = 30 ml/min

(7) 2) 3% OV-101, all other parameters same as (1) Better system for analysis

(10) 3) 3% OV-17 on Gas Chrom Q 100/120, 6' x 1/8" (id)
column = 90 C(3 min hold) to 250 C(2 min hold) @ 10 C/min
injector = 275 C, detector = 300 C, nitrogen = 60 ml/min

(12) 4) 3% OV-17, 6' column @ 170 C, no other parameters given

(12) 5) 3% OV-1, 6' column @ 170 C, no other parameters given

(min.)	(1)	(2)
PCP	6.52	6.70
TCP	6.47	6.48
PHP	4.83	4.94
PPP	4.06	4.36

	(3)	(4)	(5)
Piperidine	0.96	--	--
PCC	9.62	0.63	0.63
PCH	10.49	0.78	0.73
PCP	16.48	4.40	3.83

(11) -PCH (phenylcyclohexene) is a degradation product of PCP by the elimination of piperidine; caused by high injection port temps. mainly, but some breakdown does occur on-column; degree of degradation is proportional to the amount of sugars present in the sample and also on the salt form of PCP; the HCl salt breaks down more than the free base and is not consistent in the amount that degrades from one injection to the next; the free base is more stable and has a more consistent response.

UV

	0.1N HCl (nm) -- in order of size			
PCP	262	257	268	252
(9, 13) PHP	262	257	268	252
(13) PPP	261	256.5	268	252
(9, 14) TCP	232			
(17) PCE	262	257	268	236
Ketamine	270	277	263	

(15) -the free base form of PCP has almost no absorbance in the UV region, so it should be converted to the HCl salt before running the scan.

(18) -PCP/LSD mixture -- expose solution to longwave UV to decrease the absorbance of LSD so that PCP curve may be seen.

MICROCRYSTAL TESTS

(19)
1) $HAuBr_4$-HOAc-(2+3)H_2SO_4 cg
2) $HAuBr_4$-2H_3PO_4-(2+3)H_2SO_4 cg
3) HOAc (1 drop) + $HAuCl_4$ (5% aqueous)

-(1) -- worked best for PCP/marihuana sample; chloroform extract evaporated on the slide with a 50ul pipette.

-(3) -- also worked well, but (2) did not

Other crystal tests

(20) 10% KI (aqueous) , 10% NH_4SCN (aqueous) -- for PCP & TCP
(7) 10% HCl or 10% HOAc + 2% $KMnO_4$ -- for PCP, PHP, PPP
(21) 5% aqueous $HAuCl_4$ (volatility) -- for PCP and piperidine

CLEAN-UP

1) Direct extraction using $CHCl_3$, CH_2Cl_2, MeOH, EtOH, or H_2O and recrystallize with pet ether

(22) 2) Ion-pairing with $CHCl_3$ from dilute HCl

3) 0.1N HCl celite column - elute w/ H_2O washed $CHCl_3$

(23) 4) Alumina column

5) Prep TLC

6) Acid-base shakeout

7) For PCP & TCP refer to physical properties; free bases of each have different solubilities in hexane & ether

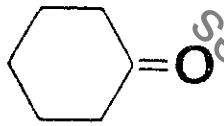
8) Cobalt thiocyanate derivative (ask Herbie)

-(2) useful for separating PCP from many other basic drugs; (6) can be used the same way with a few washes to carry the clean-up one step further.

-(3) & (4) good for cleaning up PCP from plant material; use water-washed solvents on celite; CH_2Cl_2 worked well for alumina, but you take your chances with each new lot no.

-(5) if you've fouled up everything else

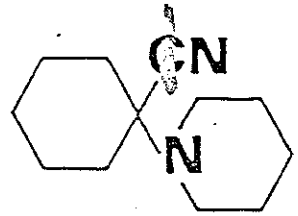
PCP THROUGH NITRILE INTERMEDIATE



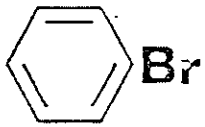
CYCLOHEXANONE

$NaHSO_3$

NH, NaCN



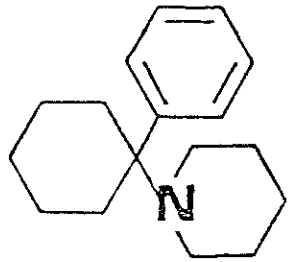
1-PIPERIDINOCYLO-
HEXANECARBONITRILE
(PCC)



BROMOBENZENE

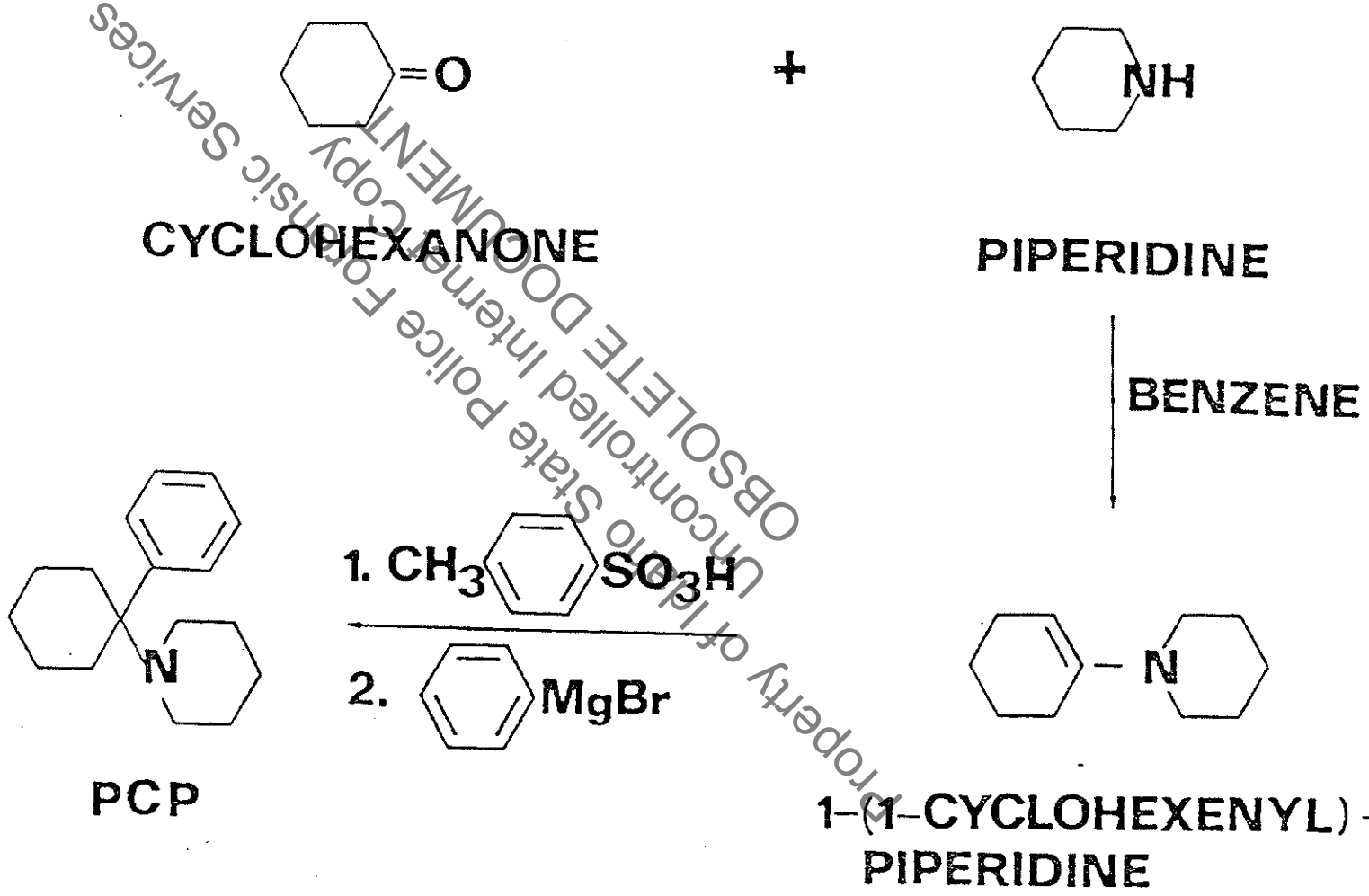
Mg

Et_2O

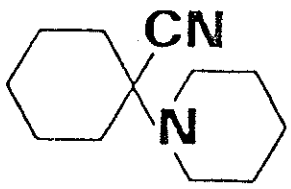


PCP

PCP THROUGH ENAMINE INTERMEDIATE

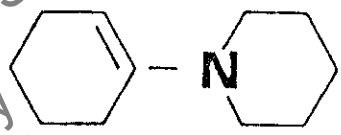


IMPURITIES IN ILLICIT PCP SAMPLES

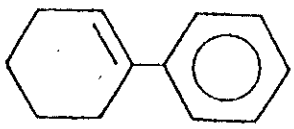


1-PIPERIDINOCYLO-HEXANECARBONITRILE

PCC

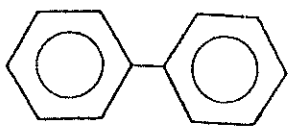


1-(1-CYCLOHEXENYL)-PIPERIDINE



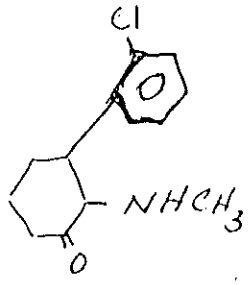
1-PHENYLCYCLOHEXENE

GC degradation product

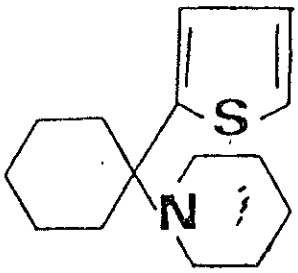


BIPHENYL

PCP ANALOGS

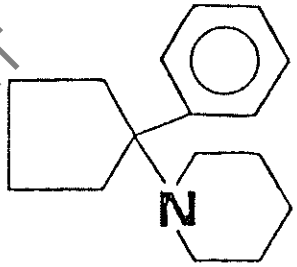


KETAMINE

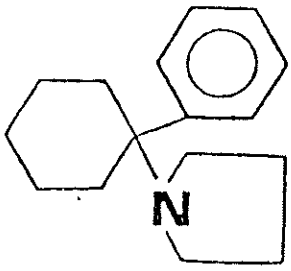


THIOPHENE, TCP

(Schedule I)

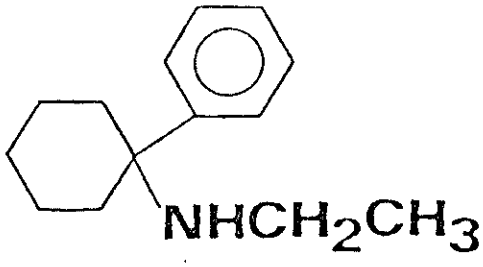


CYCLOPENTYL PPP



PYRROLIDINE, PHP

PCPy



N-ETHYL, PCE

CYCLOHEXAMINE

Phencyclidine and Some Analogues and Variants Thereof

1. 1-phenylcyclohexylamine
2. (1-phenylcyclohexyl)methylamine
3. (1-phenylcyclohexyl)dimethylamine
4. (1-phenylcyclohexyl)methylethylamine
5. Cyclohexamine - PCE - C-I
   (1-phenylcyclohexyl)ethylamine
6. (1-phenylcyclohexyl)diethylamine
7. (1-phenylcyclohexyl)isopropylamine
8. 1-(1-phenylcyclohexyl)pyrrolidine - PHP - PCPy - C-I
9. Phencyclidine
   1-(1- phenylcyclohexyl)piperidine
10. 1-(1-phenylcyclohexyl)morpholine
11. 2-[1-(1-amino)-cyclohexyl]thiophene
12. 2-[1-(1-dimethylamino)cyclohexyl]thiophene
13. 2-[1-(1-ethylmethylamino)cyclohexyl]thiophene
14. 2-[1-(1-ethylamino)cyclohexyl]thiophene
15. 2-[1-(1-diethylamino)cyclohexyl]thiophene
16. 2-[1-(1-isopropylamino)cyclohexyl]thiophene
17. 1-[1-(2-thienyl)cyclohexyl]pyrrolidine
    2-[1-(1-pyrrolidino)cyclohexyl]thiophene
18. 2-[1-(1-β-methylpiperidino)cyclyhexyl]thiophene
19. Thiophene Analog of Phencyclidine
    1-[1-(2-thienyl)cyclohexyl]piperidine
20. 1-[1-(2-thienyl)cyclohexyl]morpholine
21. 2-[1-(1-β-methyl-pyrrolidino)-cyclohexyl]-
    4-methyl thiophene

22. 2-[1-(1-piperidino)cyclohexyl]-4-methyl thiophene

23. 2-[1-(1-piperidino)cyclohexyl]-5-methyl thiophene

24. 2-[1-(1-dimethylamino)cyclohexyl]furan

25. 2-[1-(1-piperidino)cyclohexyl]furan

26. 2-[1-(1-piperidino)cyclohexyl]-5-methyl furan

27. 2-methylamino-2-phenylcyclohexanone

28. 2-ethylamino-2-phenylcyclohexanone

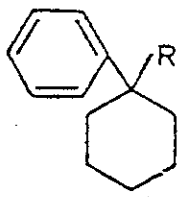
29. Ketamine
2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone

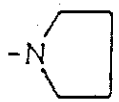
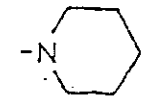
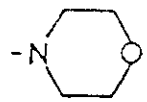
30 2-methylamino-2-(o-hydroxyphenyl)-cyclohexanone

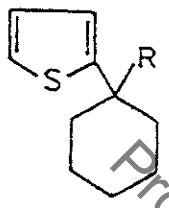
31. 2-methylamino-2-(m-hydroxyphenyl)-cyclohexanone

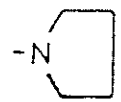
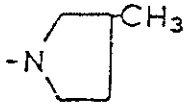
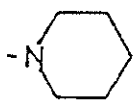
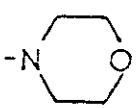
32. 2-amino-2(2-thienyl)cyclohexanon

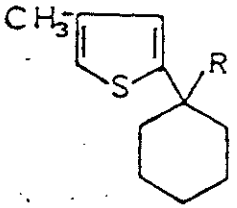
33. Tiletamine
2-ethylamino-2-(2-thienyl)cyclohexanone



1. $-NH_2$
2. $-NHCH_3$
3. $-N(CH_3)_2$
4. $-N(CH_3)(C_2H_5)$
5. $-NHC_2H_5$
6. $-N(C_2H_5)_2$
7. $-NH-CH(CH_3)-CH_3$
8. 
9. 
10. 



11. $-NH_2$
12. $-N(CH_3)_2$
13. $-N(CH_3)(C_2H_5)$
14. $-NHC_2H_5$
15. $-N(C_2H_5)_2$
16. $-NH-CH(CH_3)-CH_3$
17. 
18. 
19. 
20. 



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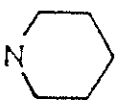
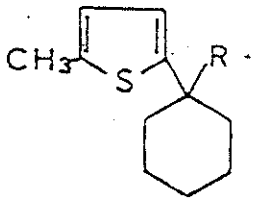
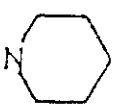
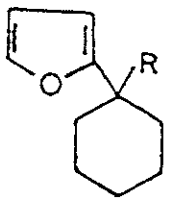
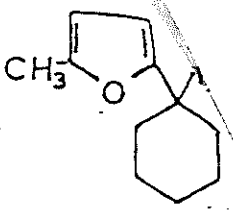
22. 

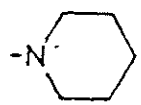


23. 



24. $-N(CH_3)_2$
25.



26. 

32. -NH_2

33. -NHC_2H_5

31. -$NHCH_3$

30. -$NHCH_3$

29. -$NHCH_3$

27. -$NHCH_3$

28. -NHC_2H_5

SPECIFICITY PROBLEM WITH THE COCAINE-SPECIFIC FIELD TEST II. NON-PHENOTHIAZINE FALSE POSITIVES AND THE SEPARATION OF PHENCYCLIDINE - PROMAZINE COMBINATIONS

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Division of Crime Detection
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Lansing, Michigan 48914

OBJECTIVE

We have found that certain non-phenothiazine drug combinations will give the "highly specific" field test (1) for cocaine, and that the FPN test (2) will not screen for these false positives.

BACKGROUND

Since our previous report (3) our laboratory has received three more cases of phencyclidine (PCP) - phenothiazine combinations which gave the cocaine-specific field test color sequence and a positive FPN test for phenothiazines. The phenothiazine was identified as promazine by gas chromatography and gas chromatography-mass spectrometry. As reported earlier promazine gives steps one and two of the cocaine-specific field test (3).

The test was run on standard samples of the following drugs and combinations of drugs:

TABLE 1

	Color after step 1	Color after step 2	Color in $CHCl_3$	FPN
1) Phencyclidine	blue	pink	clear	-
2) Promazine	blue	pink	clear	+
3) Cocaine	blue	pink	blue	-
4) 1 + 2	blue	pink	blue	+
5) Dibucaine	blue	pink	clear	-
6) Methapyrilene	blue	pink	clear	-
7) 1 + 5	blue	pink	blue	-
8) 1 + 6	blue	pink	blue	-
9) 5 + 6	blue	pink	clear	-
10) 2 + 5	blue	pink	blue*	+
11) 2 + 6	blue	pink	blue*	+

*depends on the relative amounts of each component.

It is important to note that combinations #7 and 8 give a false positive for cocaine, but cannot be screened out with the FPN test, as neither compound is a phenothiazine.

SEPARATION OF PCP - PROMAZINE COMBINATIONS

Dissolve mixture in 2.8 N HCl and extract with an equal volume of chloroform. Wash chloroform two times with fresh 2.8 N HCl. Extract PCP from chloroform with an equal volume of 0.1 N sulfuric acid. A U.V. spectrum of the 0.1 N sulfuric acid extract will reveal a promazine peak at 252 mμ with a 269 mμ shoulder representing the PCP (promazine 252 mμ E 1% 1 cm 1122, PCP 269 mμ E 1% 1 cm 9.2). Add three drops of concentrated HCl to the sulfuric acid extract, extract with an equal volume of chloroform and dry. The resulting extract will give an IR spectrum clearly recognizable as that of phencyclidine HCl.

(1) Scott, L.J., Jr., "Specific Field Test for Cocaine", Microgram, 6:11, Nov. 1973, pages 179-181.

(2) Clarke, E.B.C., "Isolation and Identification of Drugs", The Pharmaceutical Press, London, U.K. (1969).

(3) Lorch, S.K., "Specificity Problem With the Cocaine Specific Field Test, and Its Solution", Microgram, 7:8, Aug. 1974, pages 100-101.

The author wishes to thank Dr. Fathi M. Saad, Chief, Warren Regional Laboratory, Bureau of Laboratories, Michigan Department of Public Health, for the G.C.-Mass Spectrographic identification of the promazine.

9/10/74

(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.

LABORATORY NOTES

(4)

DATE March 28, 1974

NO.

DRUG TYPE Hallucinogens

METHODOLOGY Thin-layer Chromatography

SCREENING TEST FOR CANNABINOIDS, PHENCYCLIDINE, DIMETHYLTRYPTAMINE, DIETHYLTRYPTAMINE.

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INTRODUCTION

Recent samples of plant material, hashish and smoking paraphernalia have been found to contain mixtures of cannabinoids and phencyclidine. A screening test is needed, which can be run in conjunction with routine marihuana thin-layer chromatograms, for other possible hallucinogens.

REAGENTS

95% Petroleum Ether and 5% Absolute Ethanol solution.
Fast Blue B - 1 gram Fast Blue B salt and 100 ml H_2O.

Iodoplatinate - 10 ml of 10% Chloroplatinic acid, 250 ml of 4% Potassium Iodide diluted to 500 ml H_2O.

SOLVENT SYSTEMS

System #1 - 45% Butyl Ether, 45% Ethyl Ether, 10% Diethylamine

System #2 - 5% Diethylamine and 95% Benzene

TLC PLATES

Analtech - Uniplate precoated TLC plates, Silica Gel GF 250 microns

DATA

Rf values (using solvent front as 1.00)

Solvent System #1		Solvent System #2	
PCP	0.91	PCP	0.64
CBD	0.69	CBD	0.52
THC	0.61	THC	0.41
CBN	0.47	CBN	0.30
DET	0.30	DET	0.15
DMT	0.18	DMT	0.09

PROCEDURE

Routine extraction of suspected plant material and smoking paraphernalia using a solution of 95% Petroleum Ether and 5% Absolute Ethanol. Development of thin-layer chromatograms using solvent tank systems 1 and 2. Initial visualization after drying is with Fast Blue B spray for screening of cannabinoids, with subsequent spraying with Iodoplatinate to screen for presence of PCP, DMT or DET.

DISCUSSION

The use of the polar solvent Ethanol aids in elution of PCP, DMT and DET off plant material and smoking paraphernalia, which can be then readily detected using the two sprays. The visualization of PCP, DMT and DET are not effected by the initial Fast Blue B spraying, the use of Iodoplatinate has negligible affect on the prior cannabinoid visualization.

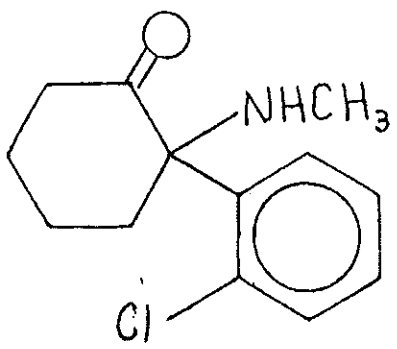
VEGETABLE MATTER COATED WITH A BARBITURATE AND A VETERINARY ANESTHETIC

BY

H. HARRIS, R. BEATUS, M. BIANCHI, T. CATALANO, AND E. MC DONNELL

We recently received a rather unusual sample which we feel should be brought to the attention of other laboratories around the country. The sample was vegetable matter shown to be parsley which also gave a positive Wagners test indicating possible presence of an alkaloid. The material was screened for phencyclidine (PCP) using thin layer chromatography with the T1 developing system (see table 3). One spot migrated, but did not match the known PCP standard. On the theory that this might be a PCP analogue, a small amount was placed in methanol and the solution examined using our gas chromatograph-mass spectrometer (GC-MS). Two distinct peaks were observed in the total ion scan at retention times of 3.4 and 3.8 minutes using our general program of 150-260C at 16/min using a 6'x2 mm I.D. glass column packed with 3% OV-1. The mass spectrum obtained for the second peak clearly indicated either amobarbital or pentobarbital. These two isometric barbiturates have very similar mass spectral fragmentation patterns, but can be differentiated by comparison with spectra of authentic samples of the two barbiturates run under identical conditions. This comparison indicated that our peak at 3.8 minutes was definitely pentobarbital (see table 1). Further confirmation was obtained from infra-red and thin layer chromatographic data.

The first peak (retention time 3.4 minutes) showed a mass spectrum (figure 1) rich in detail with a base peak at m/e 180 and a possible parent ion at m/e 239. Further, doublets in the ratio of about 3 :1 at m/e 180-182 and 209-211 suggested the presence of one chlorine in the molecule. Both computer and manual searches of the spectral reference libraries available to us failed to disclose a possible identification. Using the data we had gleened from the mass spectrum, a manual search of Clarke's "Isolation and Identification of Drugs" disclosed one compound which had the proper molecular weight, contained a chlorine atom, and whose structure appeared to fit reasonably well with the observed fragmentation pattern. This material is used primarily as a veterinary anesthetic with the common name ketamine. The chemical name is 2-(o-chlorophenyl -2- methylaminocyclohexane and has the structure shown below:

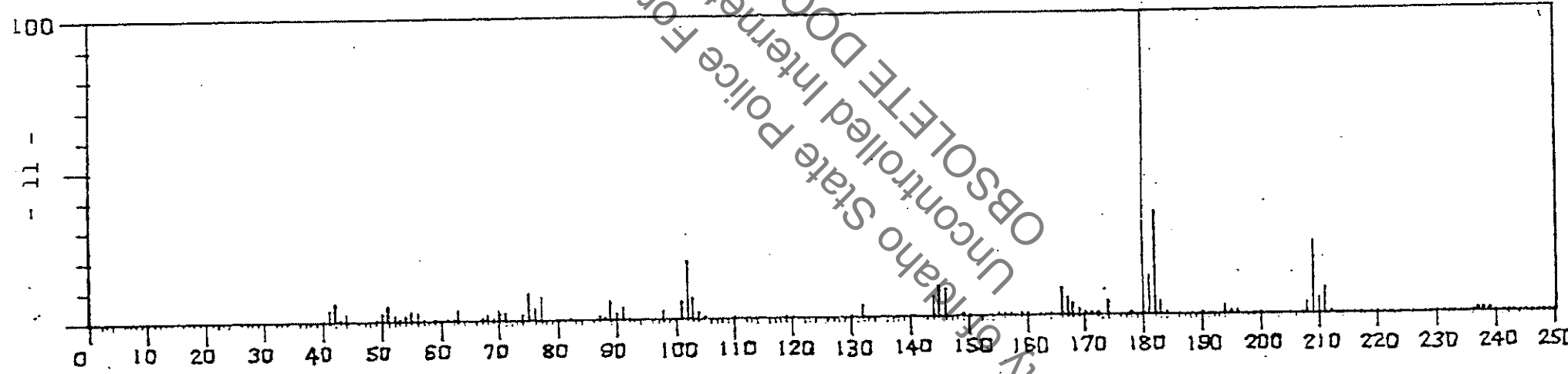


We were able to obtain an authentic sample of ketamine and confirm that the drug present along with pentobarbital was indeed ketamine. Ketamine does not appear in the subject index of "Microgram" and we have not seen it mentioned before in the forensic literature. The presence of barbiturate on vegetable matter is also very unusual in our experience. The "Physicians Desk Reference" in its section on Ketamine warns in a "Special Note": "Emergence reactions have occurred in approximately 12% of patients. The psychological manifestations vary in severity between pleasant dream-like states, vivid imagery, hallucinations, and emergence delerium".

We would like to alert other laboratories to look for this material. There are several points of similarity between ketamine and phencyclidine, i.e. both are primarily veterinary products and both appear to have mild hallucinogenic properties. In view of the very rapid rise of ilicit use of PCP one should be alert for a similar occurance for ketamine. Further data to aid in identification of Ketamine is given in Tables 2 & 3. It should be noted that Ketamine and methaqualone behave quite similarly with a number of spot tests and with several TLC systems. Therefore it is probably necessary to resort to IR, mass spectrometry or other more sophisticated methods to insure proper identification.

FIGURE 1

Mass Spectrum of Ketamine



KETAMINE

STD

TABLE 1

MASS SPECTRAL DATA ON BARBITUATES

	RATIOm/e 41:43	m/e 83:85	m/e 155:157
Amobarbital	1.5	2.0	0.2
Pentobarbital	0.8	1.0	0.45
Peak at R.T. 3.4	0.8	1.0	0.5

TABLE 2

PHYSICAL AND CHEMICAL PROPERTIES OF KETAMINE

Major Infra-Red bands of Ketamine Hydrochlpride in microns

2.95,3.4,3.5,3.55,5.8,5.85,6.85,7.0,7.85,8.1,8.6,

8.8,9.25,9.45,9.65,10.35,10.5,11.1,11.85,12.3,13.1

13.3,13.7,13.9

Spot Tests and Crystal Tests For Ketamine

Wagners (Alkaloid)	+
Marquis	-
Mandelin	-
Co Thiocyanate	+
+SnCl	-
Liebermann	Slow Purple
Permanganate	Crystals

TABLE 3

Thin Layer Chromatography Data

Compound	Solvent System T1	S2	S16
Ketamine	.72	.86(Gray)	.55(Br.)
Methaqualone	.70	.88(Br.)	.50(Purple)
Methylphenidate	.60	.83(Tan)	.55(Yellow)
Cocaine	.60	.89(Tan)	.67(Br.)
Phencyclidine	.59	.92(Purple)	.90(Purple)

T1 Methanol-Conc. Ammonia (100:1.5)
S2 Chloroform-Dioxane-Ethyl Acetate-Ammonia (25:60:10:5)
S16 Hexane-Benzene-Diethylamine (75:25:10)

Colors in () produced by Iodoplatinate spray

(6)

DIFFERENTIATION OF PCP, TCP, AND A CONTAMINATING PRECURSOR PCC, BY THIN-LAYER CHROMATOGRAPHY

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BACKGROUND

Analyses of drugs alleged to be mescaline or synthetic THC have often shown them to be phencyclidine (Sernyl, PCP). This base has also been encountered as a component in drug mixtures involving marijuana and LSD. Recently a pharmacologically related analog thienylcyclohexylpiperidine (TCP) has appeared in the place of, or in admixture with, PCP.

In the illicit synthesis of both PCP and TCP, the intermediate 1-piperidinocyclohexanecarbonitrile (PCC) is usually employed. This material has extraction properties that are extremely similar to those of both PCP and TCP, resulting in the preparation of a contaminated product. As a toxicological problem, this contaminant may contribute to the toxic aspects of these two drugs and its presence or absence might be of value in the treatment of cases of overdosage. From the forensic point of view, the ability to detect this precursor should be potentially valuable in two ways: 1. It will help establish the synthetic procedure employed in the manufacture of the drug in question, and 2. As PCC is not found as a contaminant in preparations intended for clinical application, its presence will establish the unlicenced nature of this manufacture.

Attempts to effect this analysis by conventional GLC procedures are thwarted by the thermal instability of PCC. Under ordinary chromatographic conditions, HCN is split out of the molecule resulting in the formation of the enamine cyclohexenylpiperidine. This process occurs during actual passage through the GLC column resulting in an inconsistant spectrum that depends upon column conditions, quantities injected, and the nature of the contamination of the injected sample. A further complication is the inherent instability of this enamine in that, if hydrolytic conditions are encountered, it further degrades to its two components piperidine and cyclohexanone. Both of these latter chemicals are invariably lost under the solvent peak on GLC analysis. Attempts to analyze these mixtures by conventional TLC procedures (activated plates, heated spot application) result in immediate and complete degredation of PCC to these same components.

OBJECTIVE

To provide a chromatographic procedure for the separation and localization of PCP, TCP, and PCC in a single extraction and

MATERIALS

TLC plates: E.Merck, 0.25 mm Silica Gel 60 prepared glass plates which have been deactivated by open-air storage for at least 48 hrs prior to use.

Solvent system: Benzene, acetone and pyridine, in the ratio of 16:8:1.

Visualization reagents: Ninhydrin (0.8% in acetone) and iodoplatinate (4 g chloroplatinic acid and 24 g KI in 1 l. water diluted, after standing, with 1 l. methanol).

PROCEDURE

Approximately one dosage unit of the drug to be analysed is suspended in four drops of anhydrous methanol, and broken up to a fine powder. No external heat is employed. An unactivated TLC plate is appropiately marked (origins located for at least four sample applications, i.e. reference PCP, reference TCP, reference PCC, and the unknown). The site of application of the unknown sample is wetted with a drop of the chromatographic solvent mixture. As soon as the apparent dampness has disappeared, but before the residual discoloration is also lost (the spot is still chalky white against a dull white background), about 5 ul of the methanolic extract is applied. Similar care is needed for the reference PCC solution. The solutions of the two standard drugs PCP and TCP may be applied in the conventional manner except that there must be no heating of the plate.

After air drying, the chromatogram is developed for about 10 cm, air dried until largely free of pyridine smell, then heated in an air oven at 110° for 10 min. While still hot it is sprayed with ninhydrin, and the developed colors noted. The plate is allowed to cool, and oversprayed with iodoplatinate. The following Rf's and colors should be observed:

Compound	Rf	Ninhydrin color:	Iodoplatinate color:
Piperidine (decomposition product of PCC)	0.00	purple	unchanged, fading to a bleached spot on standing
PCP	0.24	none	grey, perminent
TCP	0.49	purple	unchanged, fading to a bleached spot on standing
PCC	0.70	purple	inchanged, fading to a bleached spot on standing

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Division of Consolidated Laboratory Services
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Identification of 1-Piperidinocyclohexane Carbonitrile

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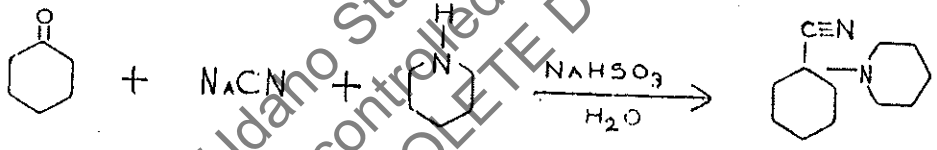
OBJECTIVE

To report occurrence of and provide analytical data for the identification of 1-piperidinocyclohexane carbonitrile, an intermediate in the preparation of phencyclidine (PCP).

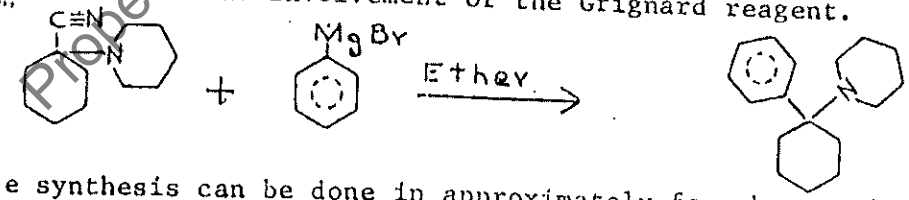
BACKGROUND

This laboratory has encountered in recent months a material present, not only in numerous exhibits of phencyclidine (powders, tablets and on plant material), but also in large quantities in pure form. The material was identified as 1-piperidinocyclohexane carbonitrile, an intermediate in the two-step preparation of phencyclidine. Although not readily available commercially, the intermediate is easily synthesized by the reaction:



The reaction is not only fairly rapid but produces the intermediate in good yield.

The subsequent reaction in the production of phencyclidine requires greater technique because of the involvement of the Grignard reagent.



The entire synthesis can be done in approximately four hours with substantial yields. However, when less than a stoichiometric amount of Grignard reagent is used, the final product will be a mixture of phencyclidine and the intermediate, as we have been encountering in the Northern Virginia area.

PROCEDURE

The initial sample of the intermediate encountered (a white powder) was in combination with phencyclidine. Thin layer chromatography ($CHCl_3$ - MeOH (9:1)) showed the presence of one other material (Rf - 0.82) in addition to phencyclidine (Rf - 0.43). In a benzene-methanol (3:1) solvent system, the second material had

an Rf of 0.74 as compared to an Rf of 0.46 for phencyclidine. The second material also developed with acidified iodoplatinate as did phencyclidine. Ultraviolet spectroscopy of the mixture showed no variations from the expected phencyclidine spectrum; however, the infrared spectrum of a basic extract of the sample showed differences from the standard phencyclidine spectrum, the most noteworthy being the addition of a -C≡N stretching band at 2220 cm^{-1}. On the gas chromatograph (5% OV-17; column temperature 180° C.), the relative retention time of the second material compared to phencyclidine was 0.11 (Figure 1). Solubility similarities precluded separation by solvent/solvent extraction, thus necessitating preparative thin layer chromatography. Using 1000 microns Silica Gel G plates in a $CHCl_3$-MeOH (9:1) solvent system, the intermediate was isolated and identified on the basis of infrared (Figure 2) and mass spectral characteristics (Figure 3). NMR (Figure 4) data was of little aid in structure determination due to similar methylene protons. The intermediate (M.P. 67.0-68.0°C.) does not absorb in the UV region. The identification was further confirmed by comparison to an independently synthesized authentic sample.

DISCUSSION

1-Piperidinocyclohexane carbonitrile continues to be seen in both the pure form (90-98 percent) and also in combination with phencyclidine. In some cases, the concentration of the intermediate is three to four times that of phencyclidine. Even though the intermediate is not controlled by federal or state codes, the identification of the intermediate in samples of phencyclidine has provided law enforcement officials with useful information as to possible common source of manufacture.

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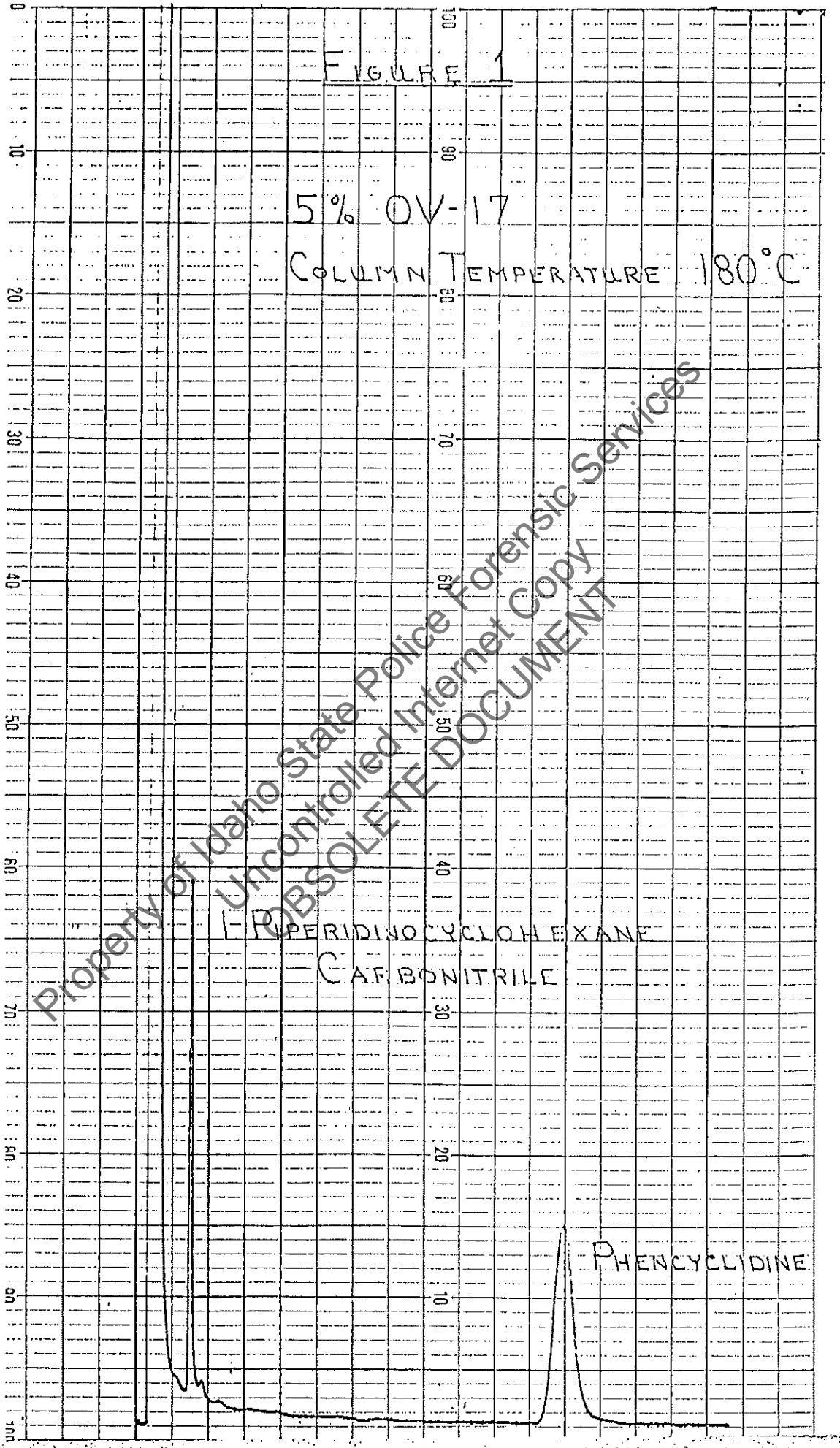

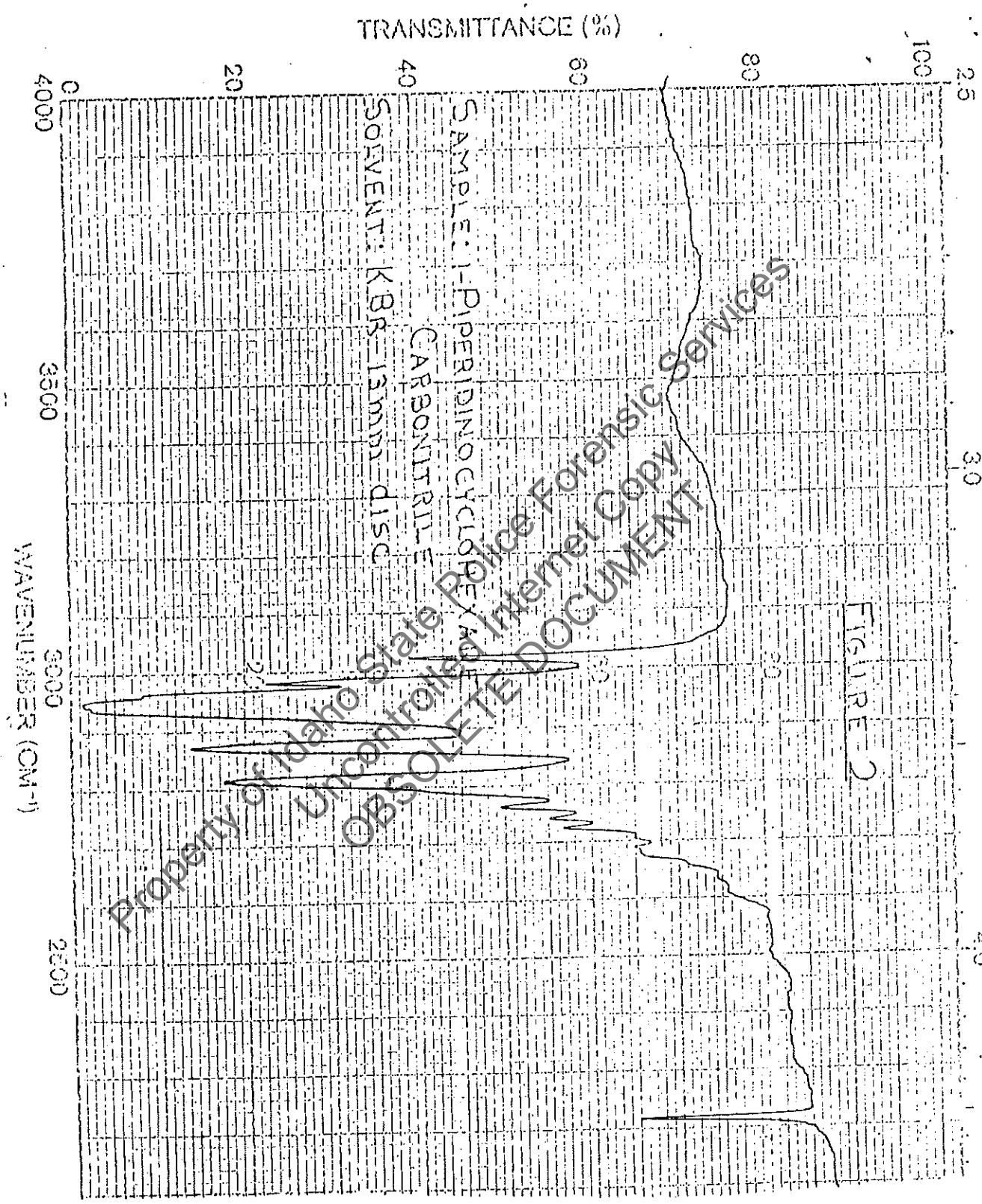

FIGURE 2

MICROGRAM, VOL. VIII, NO. 10 (October, 1975)

R. L. Epstein,[1] *Ph.D.; Philip Lorimer,*[1] *B.A.; and E. J. Sloma,*[1] *B.A.*

Identification of Phencyclidine-Related Drugs

The appearance of new drugs within the crime laboratory necessitates the development and improvement of analytical schemes for their detection. One class of particular interest is the phencyclidine-related drugs. The drugs studied are 1-(1-phenylcyclohexyl) piperidine (PCP), commonly known as phencyclidine, "Angel's Dust," or DOA [1]; the two homologs 1-(1-phenylcyclohexyl) pyrrolidine (PHP) and 1-(1-phenylcyclopentyl) piperidine (PPP); and an analog 1-(2[thienyl]cyclohexyl) piperidine (TCP).

The analytical procedures evaluated resulted in a positive identification of these drugs. They are microchemical tests, chemical ionization mass spectrometry (CIMS), thin-layer chromatography (TLC), and gas-liquid chromatography.

A comparison of the four drugs by chemical data is presented in Table 1. The ultraviolet (UV) spectrophotometric data, previously compiled [2,3], are listed in Table 2. As expected [4], only the thiophene analog (TCP) is markedly different. Infrared spectra are also available for each drug but identification by this method can be difficult with a sample that is highly adulterated or in trace quantities.

Experimental Methods

Approximately 3 mg of phencyclidine and the two homologs were placed on a glass slide with 10% aqueous hydrochloric acid or 10% aqueous acetic acid with 2% aqueous potassium permanganate. The crystal formations were observed on a compound microscope at ×400 and are depicted in Figs. 1 and 2.

The chemical ionization mass spectra were taken for all four drugs on a Dupont 21-490 single focusing mass spectrometer. The reagent gas was isobutane (99.9%). All drugs analyzed were admitted through direct probe. The instrument operating conditions were the same as described by Saferstein et al [5]. The data are tabulated in Table 3.

Thin-layer chromatography was conducted on 250-μm silica gel plates manufactured by Analtech, Inc., Newark, Del. All drugs were extracted as the free base and spotted with chloroform. After development, the drugs were visualized with 5% aqueous potassium iodoplatinate producing blue-gray spots. All chemicals and solvents were reagent grade and supplied by J. T. Baker, Phillipsburg, N.J. The following solvent systems were used with the resulting Rf × 100 values compiled in Table 4:

(A) cyclohexane:benzene:diethylamine (75:15:15),
(B) ethyl acetate:methanol:ammonium hydroxide (85:10:5),
(C) acetone:dimethylformamide:ammonium hydroxide (85:15:0.5).

Received for publication 7 April 1976; revised manuscript received 1 July 1976; accepted for publication 2 July 1976.

[1] Supervising forensic chemist, forensic chemist, and senior forensic chemist, respectively, New Jersey State Police, North Regional Laboratory, U.S. Highway 46, Little Falls, N.J. 07424.

TABLE 1—*Chemical data.*

Drug	Abbreviation	Empirical Formula (Free Base)	Molecular Weight (Free Base)	Molecular Weight (HCl Salt)	Source
1-(1-phenylcyclohexyl) piperidine (phencyclidine)	PCP	$C_{17}H_{25}N$	243.4	279.9	clandestine
1-(1-phenylcyclohexyl) pyrrolidine	PHP	$C_{16}H_{23}N$	229.4	265.9	Lot P*
1-(1-phenylcyclopentyl) piperidine	PPP	$C_{16}H_{23}N$	229.4	265.9	Lot P*
1-(1-[2-thienyl]cyclohexyl) piperidine	TCP	$C_{15}H_{23}NS$	249.3	275.8	Lot Q*

*Parke Davis & Co., Midland, Mich.

TABLE 2—*Ultraviolet spectra.*

Drug	Wavelength Maxima,[a] 0.1*N* HCl
PCP	262, 257, 268, and 252
PHP	262, 257, 268, and 252
PPP	261.5, 256.5, 268, and 252
TCP	232

[a] Strongest maxima are underlined.

(D) methanol:acetic acid (90:10),
(E) chloroform:methanol (90:10),
(F) chloroform:methanol (80:20),
(G) acetone:chloroform (50:50), and
(H) acetone:chloroform (65:35).

A Varian 2700 dual column gas chromatographic with flame ionization detectors at 270°C was used with two 6-ft (1.8-m) by ¼-in. (6.35-mm) outside diameter and 2-mm inside diameter glass columns from Analabs, Inc., North Haven, Conn. These columns were treated with dimethyldichlorosilane and packed with 3% OV-17 and 3% OV-101 on 100/120 mesh Gas Chrom Q. The column temperature was 170°C, and the injection port temperature was 250°C. A flow rate of 30 ml/min was used with medical-grade nitrogen as the carrier gas. The detector gases were H_2 (99.999%) and compressed air. All samples were injected as the free base with an 80[1-µl Hamilton syringe. A graphical presentation was presented on Hewlett Packard Corp. 3380 A integrator. The tabulated data are presented in Table 5 and Fig. 3.

Discussion

The crystal formations produced by PCP and PPP in 10% aqueous HCl and 2% $KMnO_4$ were indistinguishable. They can be described as violet, H-shaped plates, whereas PHP produced crystals also resembling violet, H-shaped plates, only thinner (Fig. 1). In 10% acetic acid and 2% $KMnO_4$, the crystals produced for phencyclidine and its homologs were somewhat dissimilar and can be used for differentiation. Again, PCP formed violet, H-shaped plates, but PHP formed orange, H-shaped plates. The PPP homolog produced definite, violet, X-shaped crystals by this test, as shown in Fig. 2.

The chemical ionization mass spectra for the drugs studied produced the protonated molecular ion (MH^+) in greatest abundance. Phencyclidine (PCP) with an *m/e* of 244 and the thiophene analog (TCP) having an *m/e* of 250 were easily distinguished from the two homologs PPP and PHP having an *m/e* of 230.

The molecular ion M^+ and the M^{-1} ion were also present. The homologs PHP and PPP (molecular weight 229.3) produced the same protonated molecular ions and molecular ions. However, the fragmented ions for PHP and PPP, *m/e* 159 and 165, respectively, allowed differentiation (Table 3).

A fragmented ion product of PCP (*m/e* 159) postulated by Hauber [6] as phenylcyclohexene was also present for PHP. Furthermore, the fragmented ion (*m/e* 145) produced by PPP probably corresponds to phenylcyclopentene and the *m/e* of 165, resulting from the fragmentation of the thiophene analog, is most likely thienylcyclohexene.

The alkaline TLC Systems A, B, C and the acidic solvent System D failed to produce adequate resolution. The best results were obtained with the neutral Systems E through H, which successfully resolved PCP, PHP, PPP, and TCP with excellent reproducibility. These systems were composed of various proportions of chloroform with methanol

TABLE 4—*Thin-layer chromatography Rf × 100 values.*

Drug	Solvent Systems							
	A	B	C	D	E	F	G	H
PCP	69	86	98	54	17	29	23	32
PHP	69	75	95	57	10	24	05	13
PPP	69	80	98	54	25	37	29	40
TCP	69	82	98	55	33	51	45	49

TABLE 5—*Gas chromatography data.*

Drug	OV-101 Gas Chrom Q		OV-17 Gas Chrom Q	
	Retention Time, min	Relative Time[a]	Retention Time, min	Relative Time[a]
PPP	4.36	0.65	4.06	0.62
PHP	4.94	0.74	4.83	0.75
PCP	6.70	1.00	6.52	1.00
TCP	6.48	0.97	6.47	0.99

[a] Based on phencyclidine (PCP).

(Systems E and F) and acetone with chloroform (Systems G and H) (Table 4). There was little difficulty in reproducing the chromatographic study of Shulgin [7] for separating PCP and TCP.

Gas chromatography (GC) completely resolved the homologs in the order of PPP, PHP, and PCP using either 3% OV-17 or 3% OV-101 (Table 5). The liquid phases chosen represent the divergent polarities as exhibited by their McReynold's constants [8]. Better resolution of PCP and TCP could be obtained by lowering the column temperature but these drugs can be easily distinguished by their UV spectra as well as by TLC, as previously discussed. A GC/MS interface could probably serve as an efficient tool for rapidly identifying these structurally related compounds.

Some of these phencyclidine-related drugs were studied by Bailey et al [9]; the study resulted in a separation scheme using absorption spectrometry, electron impact MS, protonated magnetic resonance spectra, and several TLC and GC techniques. The drugs of most interest to forensic laboratories, namely PCP, TCP, and PHP, were only partially resolved in the chromatography techniques described. In Bailey's study PPP was not included.

Conclusion and Summary

The analytical techniques presented allow the forensic chemist to readily differentiate phencyclidine (PCP) from the two homologs 1-(1-phenylcyclohexyl) pyrrolidine (PHP) and 1-(1-phenylcyclopentyl) piperidine (PPP) as well as the analog 1-(2-[thienyl] cyclohexyl) piperidine (TCP).

The UV spectra in dilute mineral acid for PCP, PHP, and PPP are indistinguishable, but the TCP spectrum is markedly different because of the thiophene moiety. The $KMnO_4$ crystal test using HCl can only suggest the presence of a phencyclidine-related drug, but acetic acid does offer more distinguishability.

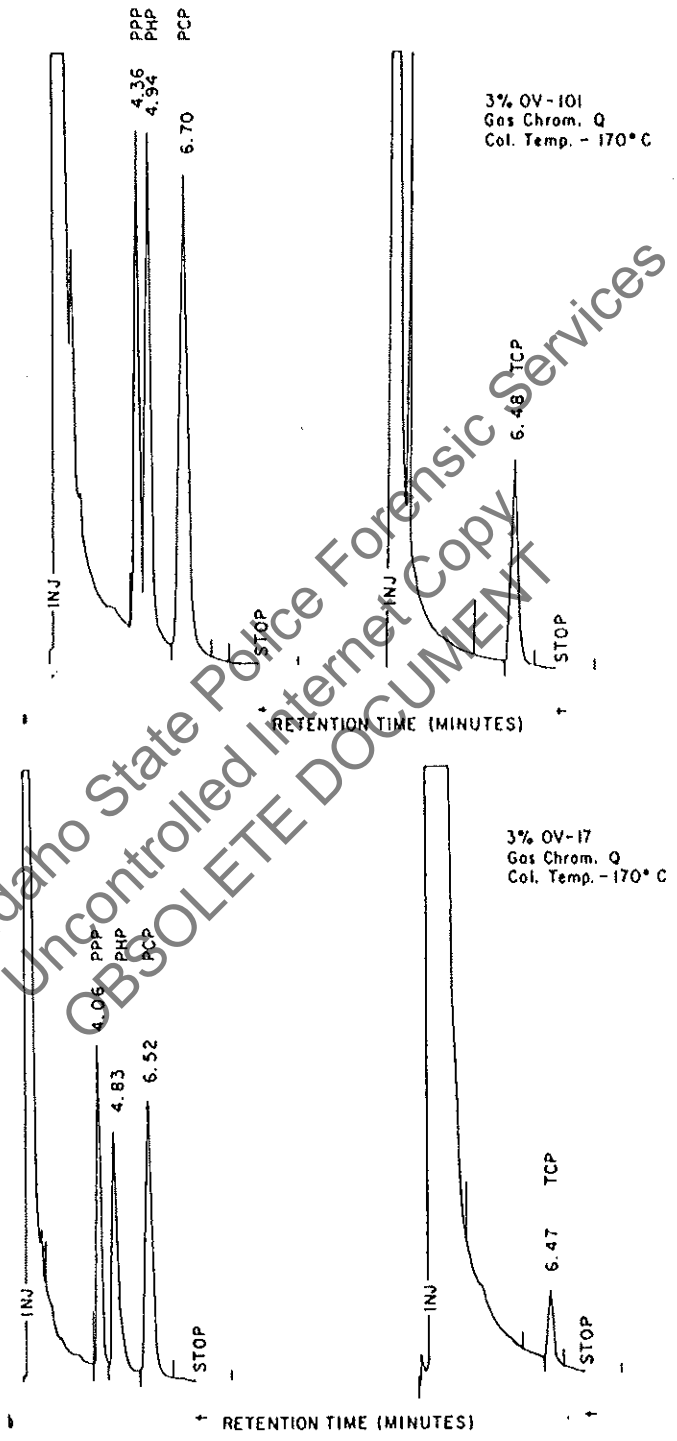

FIG. 3—*Gas chromatographic data.*

Chemical ionization mass spectra of all four drugs studied are different, except that the protonated molecular ion (MH^+) for the homologs PHP and PPP are the same. Both TLC and GC contribute to the separation and confirmation of these drugs after preliminary testing indicated their presence.

Acknowledgments

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(8)

ANALYSIS AND IDENTIFICATION OF 1-[1-(2-THIENYL)CYCLOHEXYL]PIPERIDINE (TCP)

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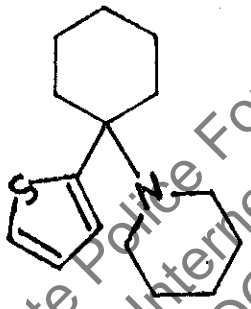

1-[1-(2-thienyl)cyclohexyl]piperidine (TCP), the thiophene analogue of phencyclidine (PCP) has been reported on the illicit drug market.[1,2,3] It was also encountered on several occasions in 1975 from northwest Wisconsin.

A search of the literature revealed a paucity of spectral data, although Heagy[4] reported the ultraviolet maximum and minimum and the infrared spectrum of the hydrochloride salt.

Since the occurrence of this drug appears to be on the increase nation wide, a method of analysis including spectroscopic methods of identification is hereby reported.

EXPERIMENTAL

Synthesis

1-[1-(2-thienyl)cyclohexyl]piperidine was synthesized according to Parell[5], purified by extraction and thin-layer chromatography, and subjected to the following examinations. This was the source of the spectra material in figures 1-3.

Physical Properties:

a. Hydrochloride Salt $C_{15}H_{23}NS \cdot HCl$ Mol. Wt. 285.85
   Appearance: fine, white, crystalline powder
   Solubility: soluble in chloroform and water, insoluble in hexane and ether
   Melting Point: 233-6° with transition at 182-3°.[5]

b. Free base $C_{15}H_{23}NS$ Mol. Wt. 249.40
   Appearance: viscous, light yellow oil
   Solubility: soluble in chloroform, ether, and hexane
   Boiling Point: 110-111° at 0.2 mm[5]

Color Tests

Color tests were performed on pure hydrochloride salts of both TCP and PCP for purposes of differentiation.

Color Reagent	TCP	PCP
Marquis	gas evolved - faint - grey-brown pink	gas evolved - faint salmon
Mecke's	gas evolved - orange	gas evolved
Mandelin's	gas evolved - green	gas evolved

All color reactions occur within one minute after reagent addition.

Extraction

Illicit samples were purified by adding a sample of the crude powder to 5 ml. of dilute NaOH. The free base just formed was converted to the chloroform insoluble sulfate by addition of 10 ml 0.5NH_2SO_4. Two 10 ml. chloroform extracts removed neutral and

acidic impurities. The aqueous phase was then rendered alkaline by the addition of ^{c}NaOH and extracted with 10 ml. of $CHCl_3$. The organic phase was dried with Na_2SO_4, filtered and evaporated to yield a pale yellow oil.

Gas Chromatography

Column Conditions: 200° C; N_2 flow 60 ml/min.

Column: 6 ft. x 2 mm i.d. 3% OV-101 on H.P. Chromosorb W (80-100 mesh)

Retention time relative to phencyclidine (PCP) - 1.0

Using the above conditions it was not possible to differentiate TCP from PCP.

Thin-Layer Chromatography (TLC)

Analytical TLC was carried out using commercial precoated silica gel GF plates (thickness 200 microns, available from Analtech, Inc., Newark, Delaware). $CHCl_3$, MeOH (9:1) was used as the eluting solvent. The spots were visualized with acidified iodoplatinate. Separation of TCP, PCP and 1-piperidinocyclohexane carbonitrile (PCC), the precursor to both of these compounds, was obtained.

Compound	Rf x 100
TCP	67
PCP	37
PCC	91

Preparative TLC was carried out using plates prepared in our laboratory. Silica gel GF-254 (Acc. to Stahl) supplied by Brinkman Instruments, Inc., Des Plaines, Illinois, was spread to a thickness of 0.5 mm on 20 x 20 cm. glass plates and oven dried at 110° C until used.

These plates, when used with $CHCl_3$, MeOH (9:1) as solvent,

were suitable for purification of street samples of TCP.

Ultraviolet Spectrophotometry

Purified TCP was run on a Perkin-Elmer 402 ultraviolet-visible spectrophotometer using 0.5N H_2SO_4 as solvent. (figure 1)

Infrared Spectrophotometry

Both the free base and hydrochloride salt of TCP were subjected to infrared spectrophotometry using a Perkin-Elmer 467 Grating Infrared Spectrophotometer (figure 2).

Gas Chromatography/Mass Spectrometry

GC/MS worked well as a means of identifying suspected TCP, (figure 3). Gas chromatography was performed on a 6 ft. x 2 mm i.d. 3% OV-101 on H.P. Chromosorb W (80-100 mesh) column at 210° with a helium flow of 45 ml/min. The transfer line between the gc and the mass spectrometer was kept at 225°.

A Hewlett-Packard 5930A dodecapole mass spectrometer with an electron energy of 70 e.v. was used. The ionization and analyzer areas were maintained at a temperature of 150°.

Comparison of the spectrum of TCP with that of PCP (see table 1) indicates the similarity in the structures of these two compounds.

NOTE: During the preparation of this manuscript Bailey, Gagne and Pike[6] published spectroscopic and chromatographic data on TCP and other analogs of phencyclidine.

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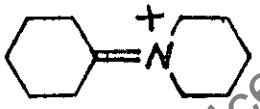
T A B L E 1

	TCP	Probable Ion Composition	PCP
m/e	249	M^+	243
	220	$(M-C_2H_5)^+$	214
	206	$(M-C_3H_7)^+$	200
	192	$(M-C_4H_9)^+$	186
	166		166
	165	(M-  $)^+$	---
	164	$(M-C_5H_{11}N)^+$	158
	149	$(M-C_6H_{14}N)^+$	143
	136	$(M-C_7H_{15}N)^+$	130
	123	$(M-C_8H_{16}N)^+$	117
	110	$(M-C_9H_{17}N)^+$	104
	97	$ArCH_2^+$	91
	84		84

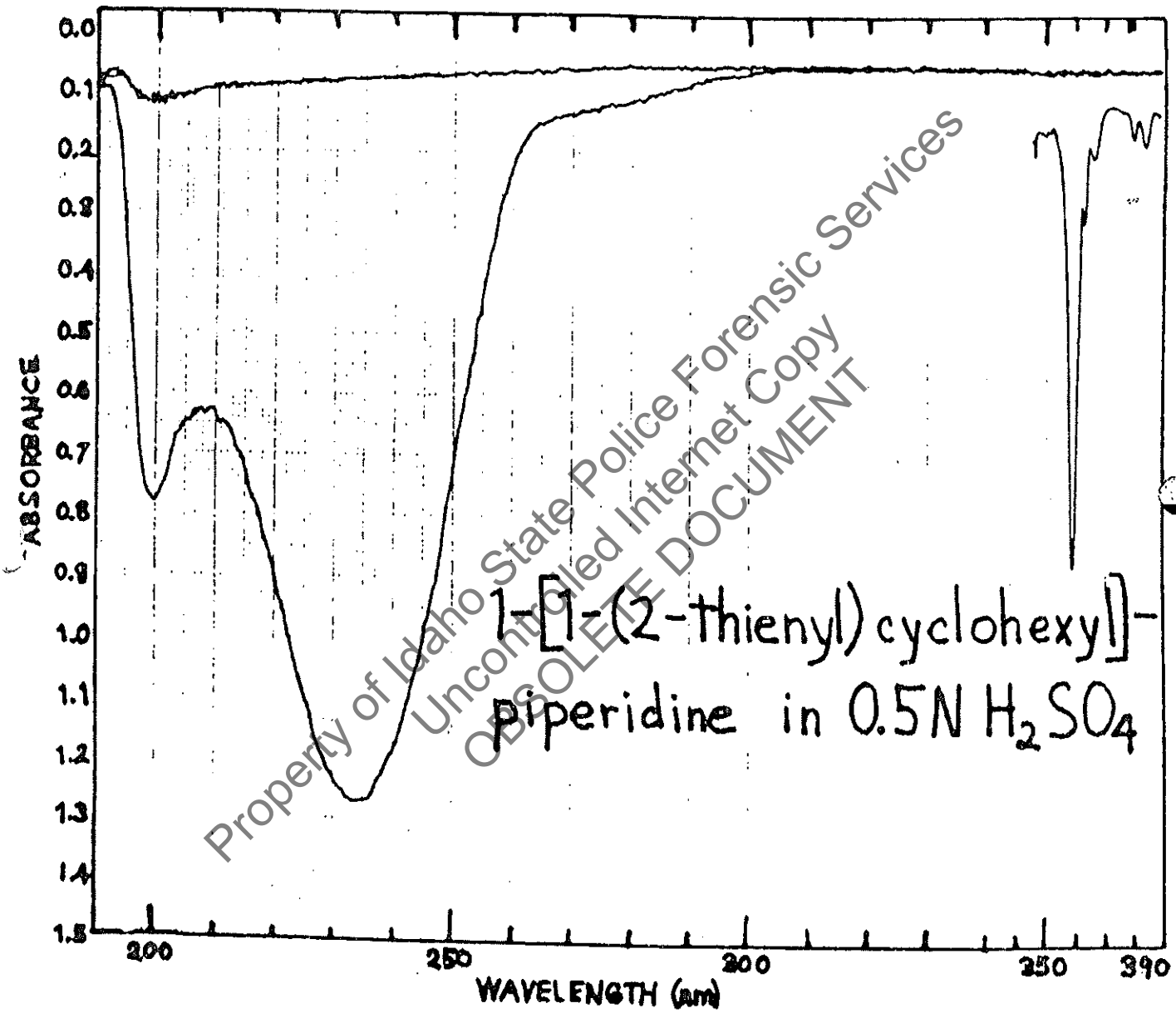
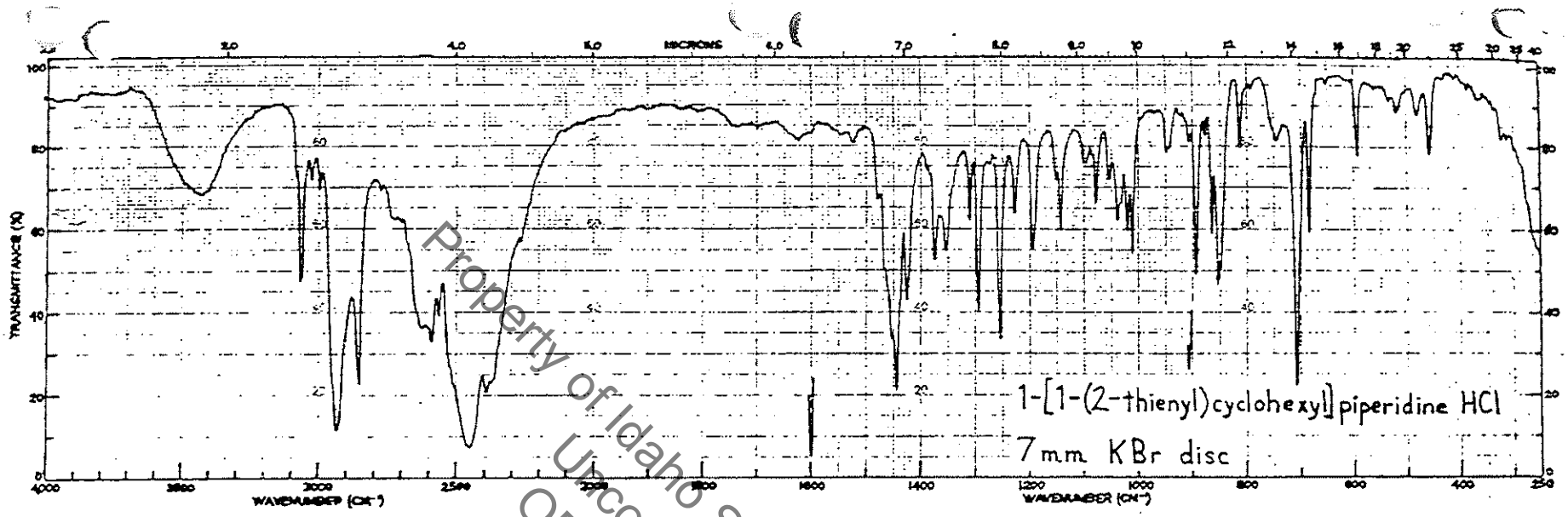


figure 1



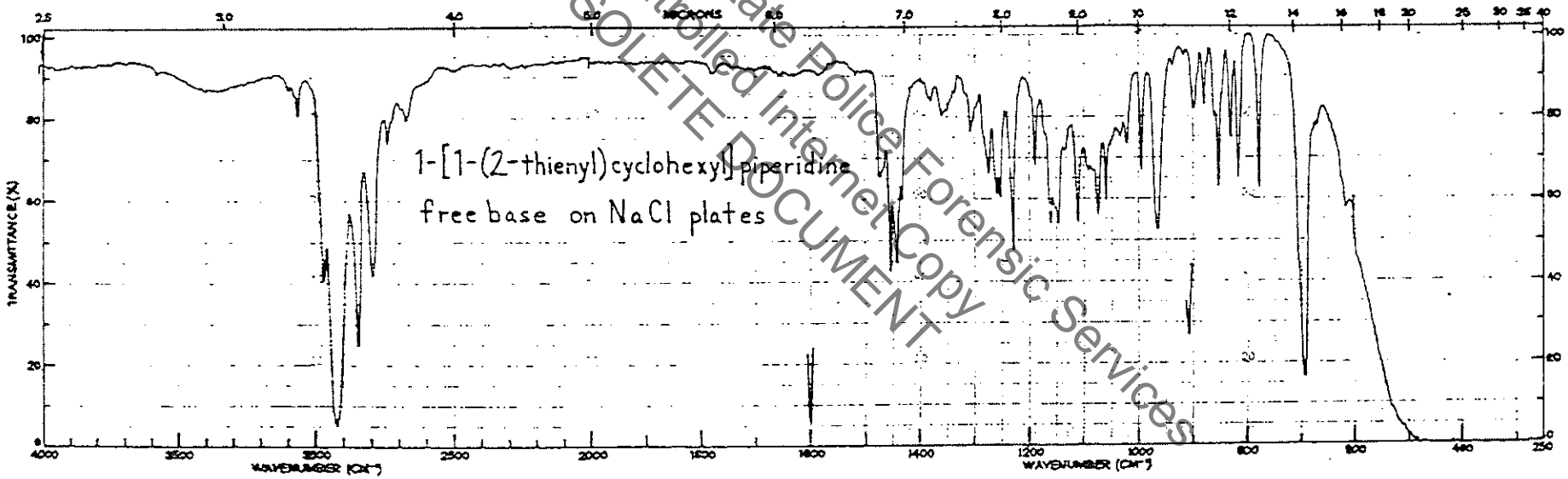
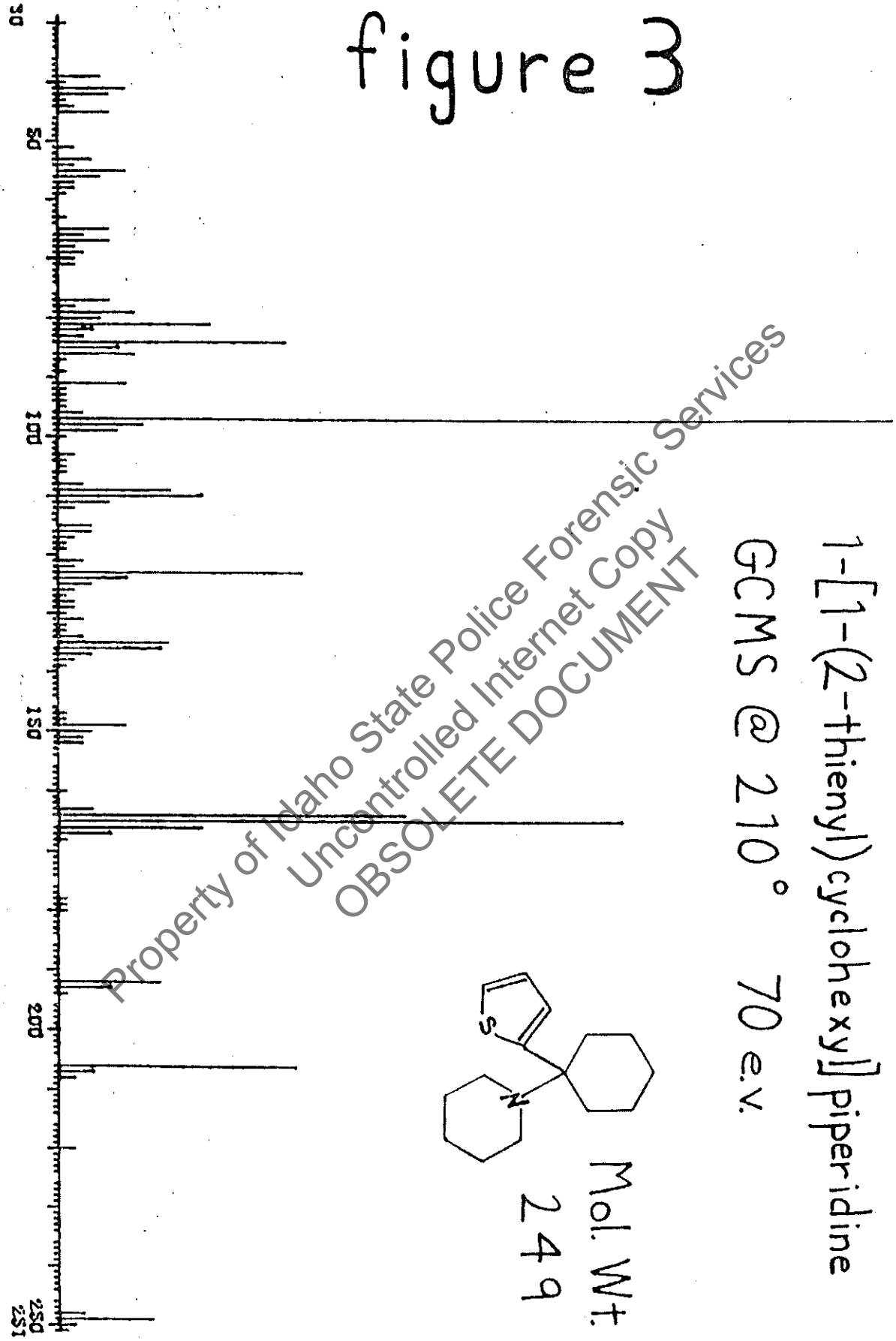


figure 2

figure 3



DRUGS

Identification of Some Analogs of the Hallucinogen Phencyclidine

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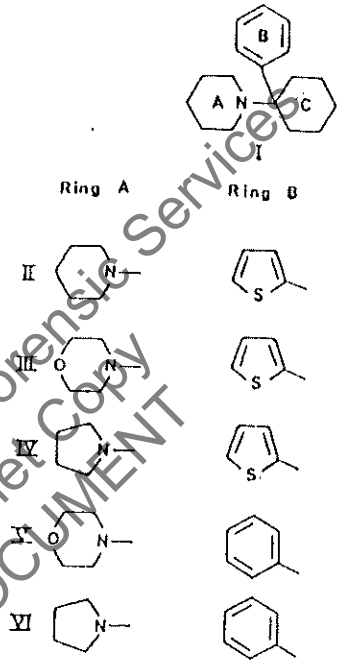
The drugs 1-[1-(2-thienyl)cyclohexyl]piperidine, 1-[1-(2-thienyl)cyclohexyl]morpholine, 1-[1-(2-thienyl)cyclohexyl] pyrrolidine, 1-(1-phenylcyclohexyl) morpholine, and 1-(1-phenylcyclohexyl) pyrrolidine are identified by spectroscopic techniques. The ultraviolet and proton magnetic resonance spectra of analogs are similar, but mass and infrared spectra are distinctly different, and reference spectra are provided. Gas-liquid and thin layer chromatographic systems for the analysis are discussed.

Phencyclidine (I) hydrochloride, 1-(1-phenylcyclohexyl)piperidine hydrochloride, is marketed as Sernylan,® an anesthetic used in veterinary medicine; it is also subject to abuse (1). It has appeared in preparations that are sold on the illicit market and which are said to contain lysergic acid diethylamide (LSD), cocaine, mescaline, or tetrahydrocannabinol rather than phencyclidine (2). Phencyclidine and its salts and derivatives are scheduled under the Narcotic Control Act in Canada and phencyclidine is designated a depressant in Schedule III of the Controlled Substances Act of the United States. However, analogs of phencyclidine have recently been encountered by narcotic control agencies (3, 4). There is obviously a need for methods which provide an unequivocal identification of these compounds.

This paper describes chromatographic and spectroscopic methods for identifying 5 analogs of phencyclidine: 1-[1-(2-thienyl)cyclohexyl] piperidine (II), 1-[1-(2-thienyl)cyclohexyl]morpholine (III), 1-[1-(2-thienyl)cyclohexyl]pyrrolidine (IV), 1-(1-phenylcyclohexyl)morpholine (V), and 1-(1-phenylcyclohexyl)pyrrolidine (VI).



Experimental

Compound I was obtained by recrystallization of an authentic sample supplied by the Division of Pharmaceutical Chemistry, Health and Welfare Canada. Compound II was made by the reaction of 2-magnesium bromothiophene on 1-(1-cyanocyclohexyl)piperidine; the latter intermediate resulted from the addition of the elements of hydrogen cyanide to the piperidine enamine of cyclohexanone. Compounds III-VI were obtained from the corresponding enamine of cyclohexanone by formation of its *p*-toluenesulfonic acid quaternary salt and subsequent attack by the appropriate aryl Grignard reagent. The compounds were purified as the hydrochloride salts by recrystallization from mixtures of isopropanol with ether. Melting points were measured on a Koffler hot stage and are uncorrected, and elemental analysis indicated that the salts were essentially anhydrous (Table 1). The spectra of free bases were recorded on the analog regenerated from the salt with Na_2CO_3 solution and extracted into $CHCl_3$; the $CHCl_3$ was removed by warming the solution under a stream of nitrogen. Thin layer chromatograms were developed 15 cm, in saturated tanks under ambient conditions, using precoated plates and sheets as received. They were examined under 254 nm ultraviolet (UV) light and sprayed with a solution of

Table 1. Melting point and analytical data for some analogs of phencyclidine as their hydrochlorides

Compound	Mp, °C	Found, %			Theoretical, %		
		C	H	N	C	H	N
II	200–203 subl.	62.98	8.43	4.91	63.02	8.46	4.90
III	162–165; 174	58.47	7.68	4.94	58.42	7.71	4.87
IV	187–188	61.87	8.09	5.17	61.85	8.16	5.15
V	188–190 subl.	68.05	8.63	4.96	68.19	8.58	4.97
VI	221–222 subl.	72.29	9.16	5.24	72.29	9.10	5.24

potassium iodoplatinate (5). Mass spectra were determined on a Hitachi Perkin-Elmer Model RMU-6L magnetic deflection instrument, operating at 160–180°C, ionization voltage 70 ev, and acceleration voltage 4–5 v. Samples were introduced via the probe. Infrared (IR) spectra were recorded on a Beckman IR 20A and UV spectra were recorded on a Beckman BD GT spectrophotometer. Proton magnetic resonance spectra were recorded on a Varian A-60A spectrometer. Gas-liquid chromatograms were obtained on a Hydro-flow Series 3000 instrument.

Results and Discussion

Mass Spectra

The compounds as salts give characteristic fragmentation patterns on electron impact; their normalized spectra are shown in Fig. 1. The molecular ions vary widely in relative intensity (12–60% of base peak) but can be assigned. All the compounds gave a brace of neighboring peaks corresponding to the molecular ion minus (a) the mass of the nitrogen heterocycle radical fragment and (b) the mass of the nitrogen heterocycle. This phenomenon is much more pronounced in the thiophene-containing series, one of these peaks usually being the base peak. As would be expected, cleavage of the aromatic moiety leads to a significant peak (M−83 in the thiophene series and M−77 in the phenyl series). The peak is more noticeable in the phenyl series, a reflection of the stronger aromaticity of the phenyl ring and the consequently greater stability of the phenyl radical. All members of the thiophene series give a strong peak at m/e 97 (70–90% of base peak) while those of the phenyl series give a strong peak at m/e 91 (80–95% of base peak). These are highly characteristic of the mass spectra of thiophene and benzene derivatives (6). All of the analogs and phencyclidine itself gave a prominent peak at M−43. In the thiophene series this varied in intensity (50–75% of base peak), while in the phenyl series this was the base peak in each case.

The mass spectra are very informative; they can distinguish between the compounds presented in this paper and could be used to elucidate the structure of uncharacterized isomers, analogs, and homologs of phencyclidine.

Ultraviolet Spectra

The wavelengths and molar absorptivities of the maxima in the spectra of the compounds (Table 2) closely resemble those of the corresponding aromatic moieties, enabling facile distinction between the 2 classes with reference to their aromatic substituent but not among analogs within each of these 2 classes.

Proton Magnetic Resonance Spectra

The spectra were examined as the free bases in $CDCl_3$ solution and as their hydrochloride salts in D_2O. The presence of a multiplet of absorbances (6.85–7.30 δ) due to the thienyl protons immediately distinguishes compounds containing this ring and their corresponding phenyl relatives whose phenyl protons give much simpler absorption (7.2–7.5 δ) at 60 MHz.

Infrared Spectra

IR spectra of the free bases (films between NaCl plates) and of the hydrochlorides (0.75% in KBr disks) are presented in Figs. 2 and 3. The spectra of phencyclidine have been published (5) but are included for comparison purposes.

Table 2. Ultraviolet data[a] for some analogs of phencyclidine

Compound	$\lambda_{max.}(\epsilon)$	$\lambda_{max.}(\epsilon)$	$\lambda_{max.}(\epsilon)$	$\lambda_{max}(\epsilon)$
I	251(301)	256(371)	261(413)	268(322)
II	232(7933)			
III	232(7944)			
IV	232(7837)			
V	251(288)	256(363)	261(416)	268(325)
VI	251(247)	256(327)	261(373)	268(293)

[a] Solutions of the hydrochloride in ethanol, $\lambda_{max.}$ in nm.

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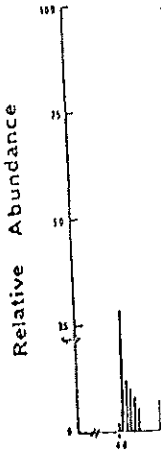

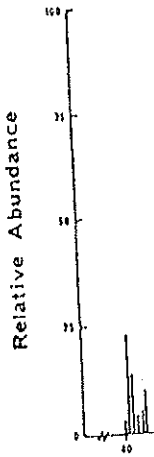

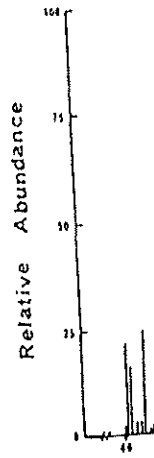

FIG. 1—Norm
HCl; III, 1-(1

...lorides

%
N
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4.87
5.15
4.97
5.24

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...1(413)	268(322)
...1(416)	268(325)
...1(373)	268(293)

...thanol, λmax. in nm.

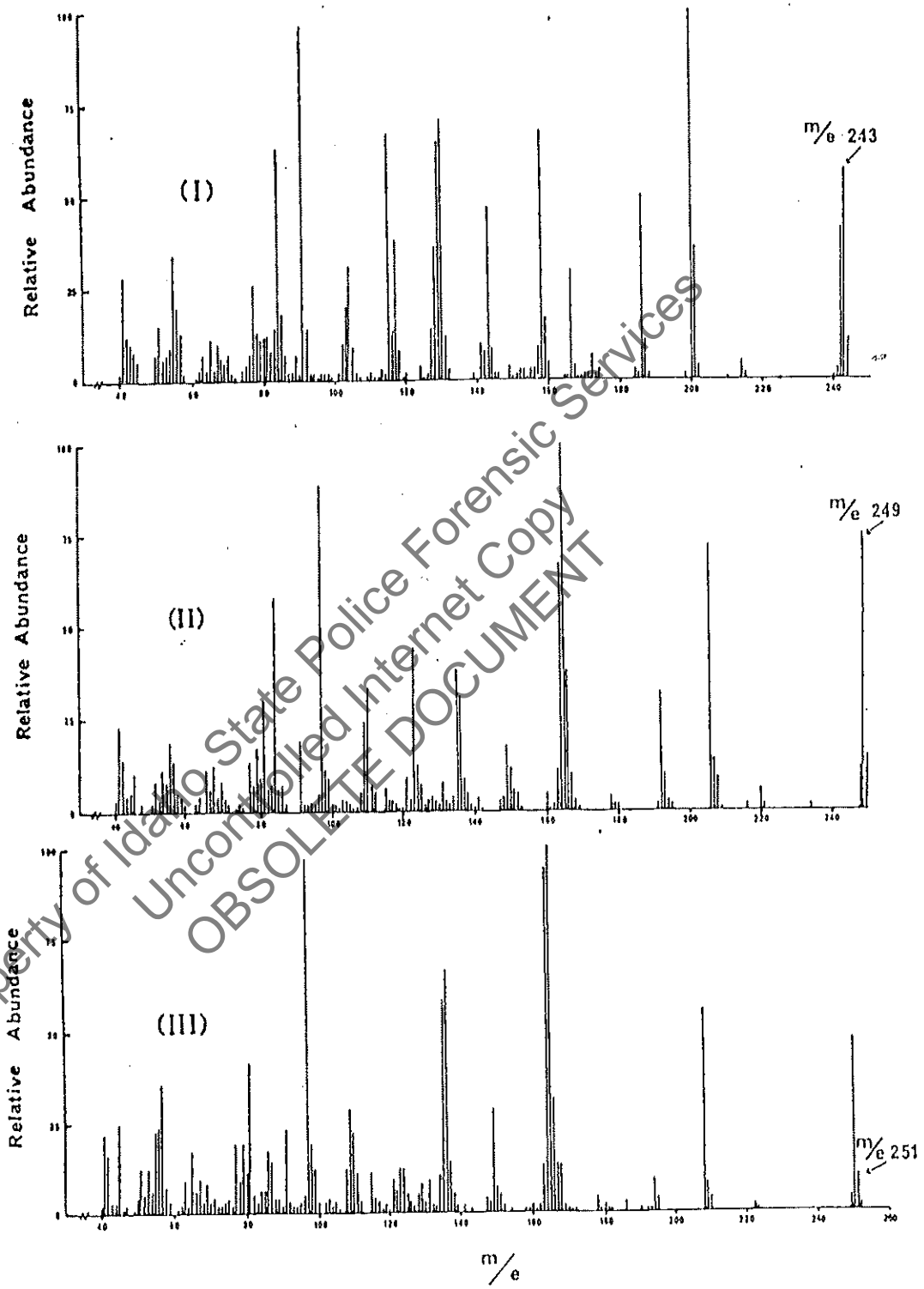


FIG. 1—Normalized mass spectra of I, 1-(1-phenylcyclohexyl)piperidine HCl; II, 1-[1-(2-thienyl)cyclohexyl]piperidine HCl; III, 1-[1-(2-thienyl)cyclohexyl]morpholine HCl; IV, 1-[1-(2-thienyl)cyclohexyl]pyrrolidine HCl; V, 1-(1-phenylcyclohexyl)morpholine HCl; and VI, 1-(1-phenylcyclohexyl)pyrrolidine HCl.

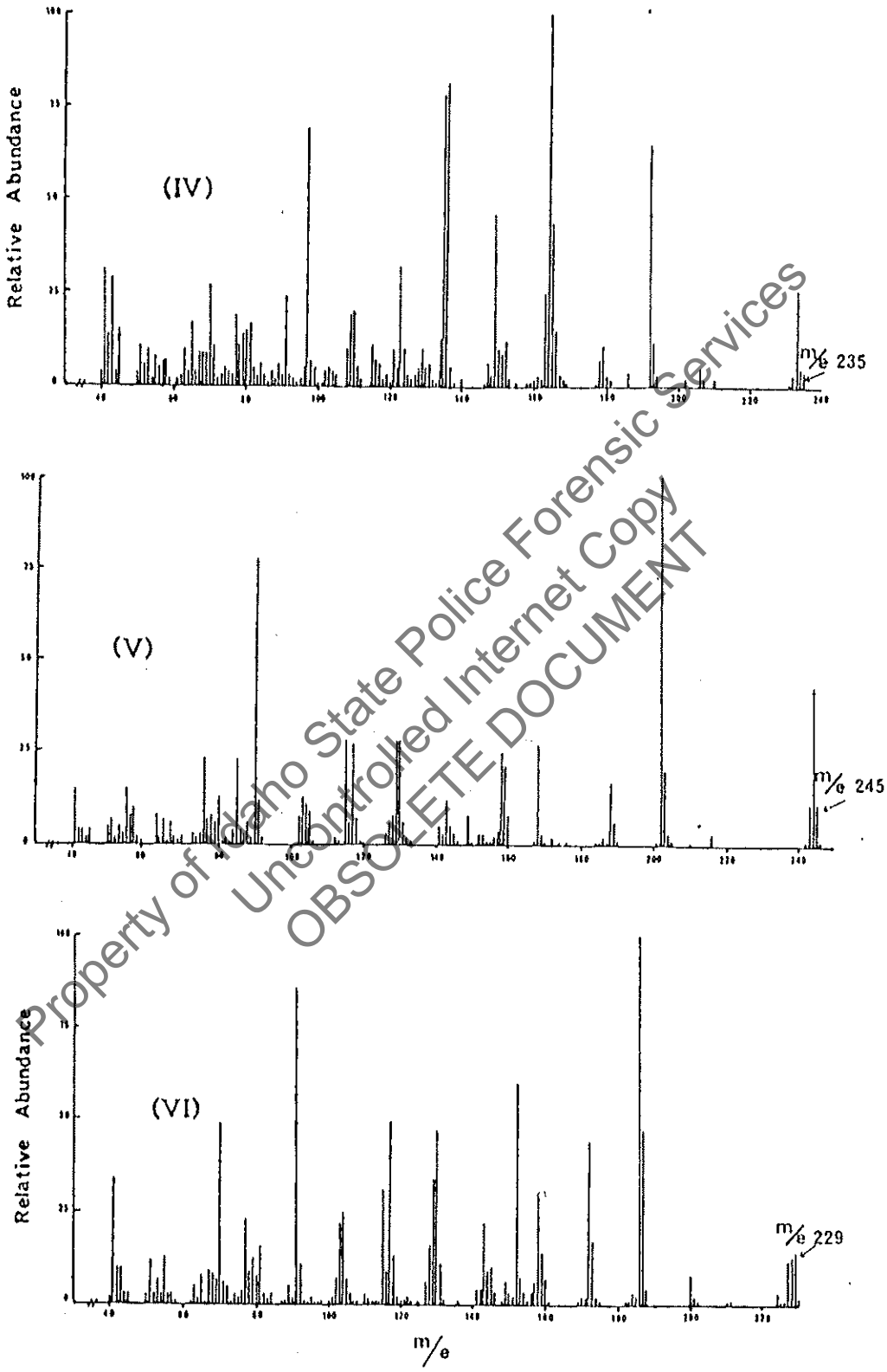
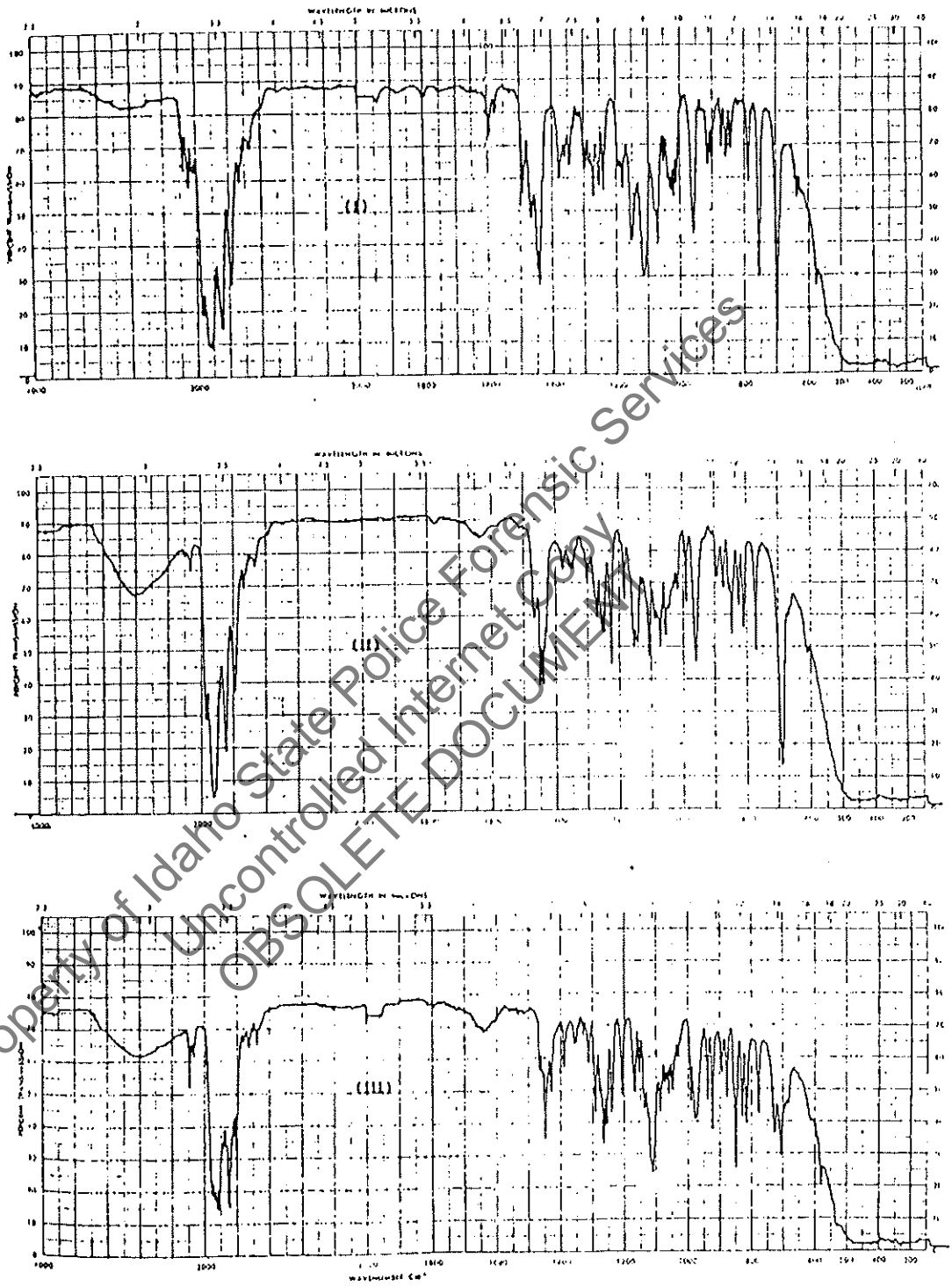


FIG. 2—IR sp
II, 1-[1-(2-thien

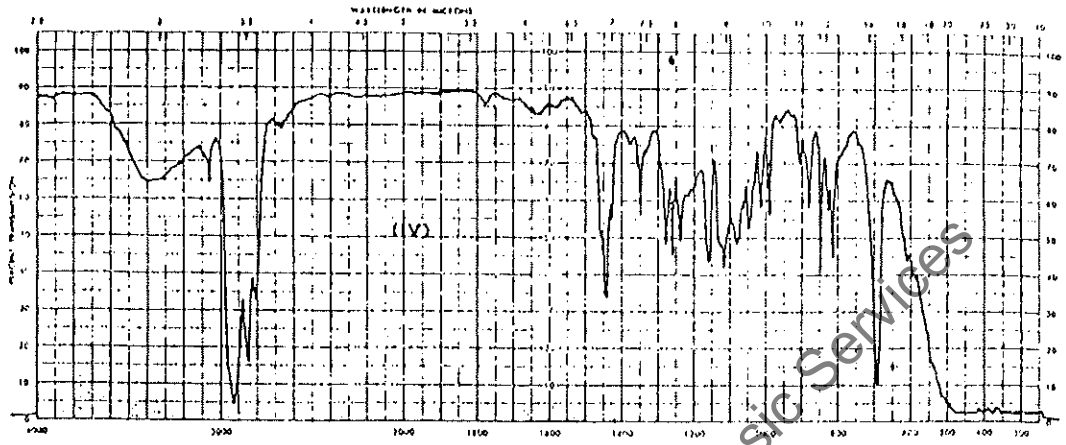


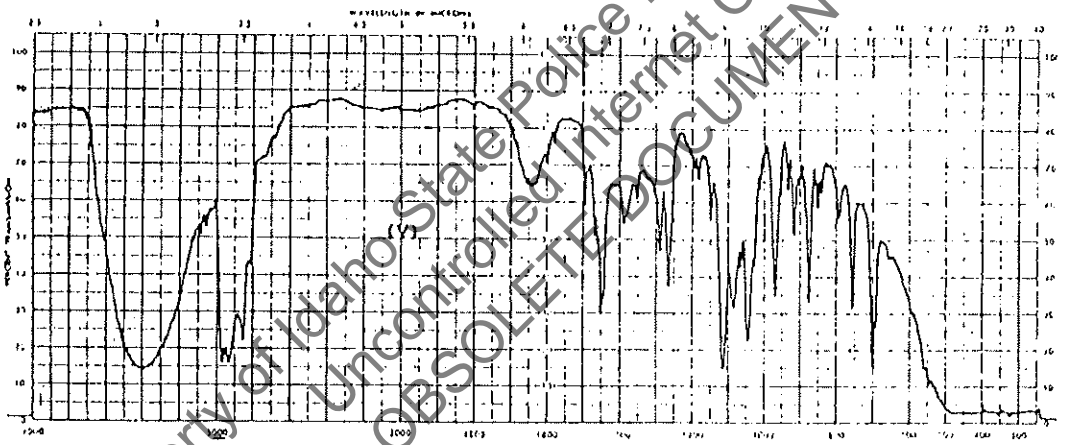
m/e 235

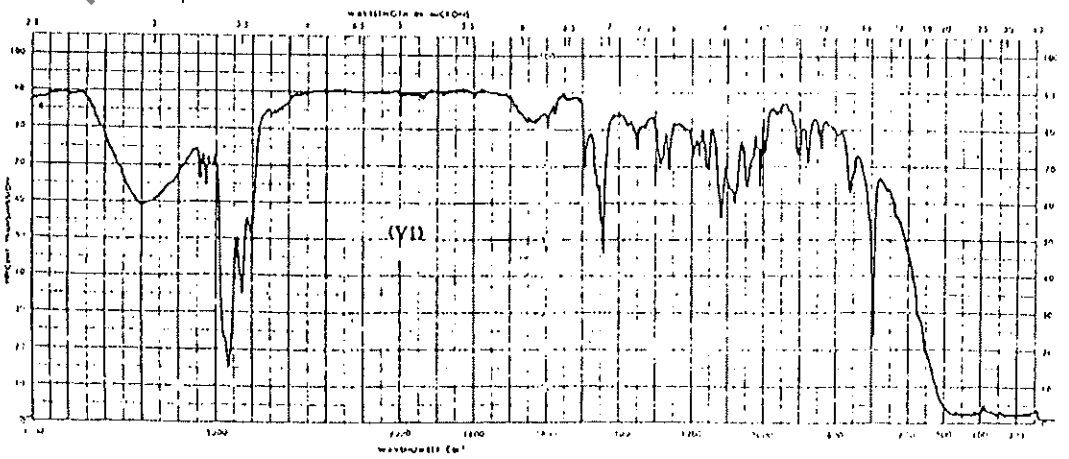
m/e 245

m/e 229

FIG. 2—IR spectra of some analogs of phencyclidine as their bases, NaCl film. I, 1-(1-phenylcyclohexyl)piperidine; II, 1-(1-(2-thienyl)cyclohexyl)piperidine; III, 1-[1-(2-thienyl)cyclohexyl]morpholine; IV, 1-[1-(2-thienyl)cyclohexyl]pyrrolidine; V, 1-(1-phenylcyclohexyl)morpholine; and VI, 1-(1-phenylcyclohexyl)pyrrolidine.







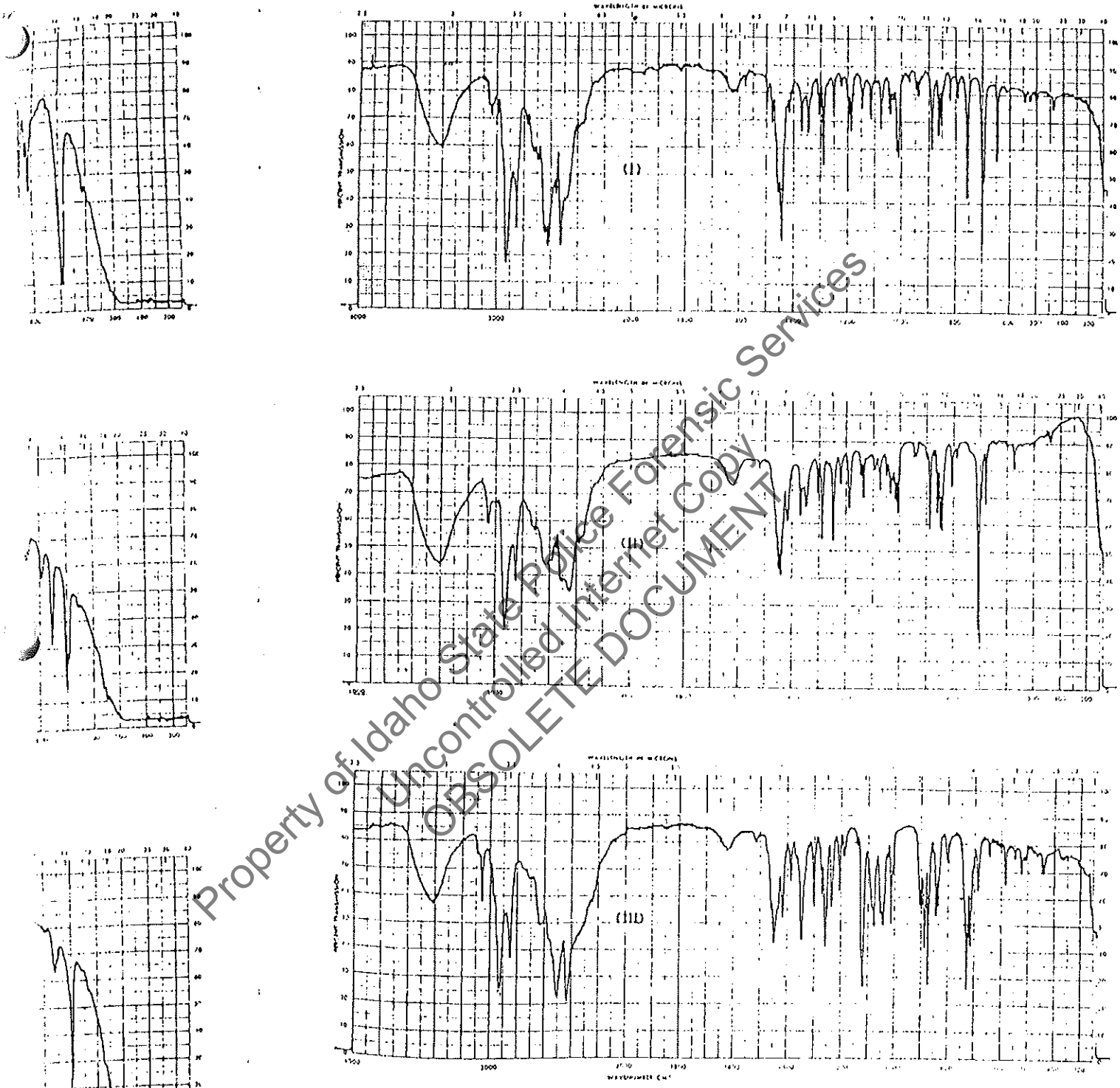
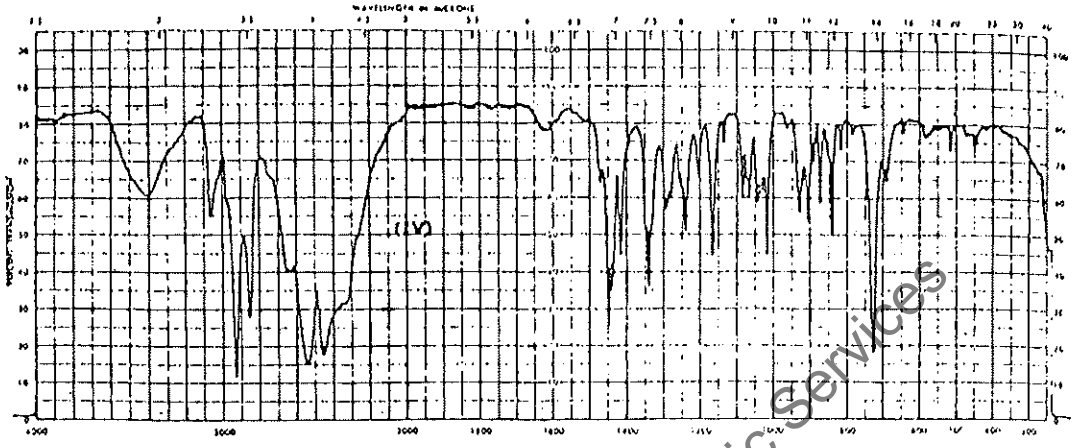
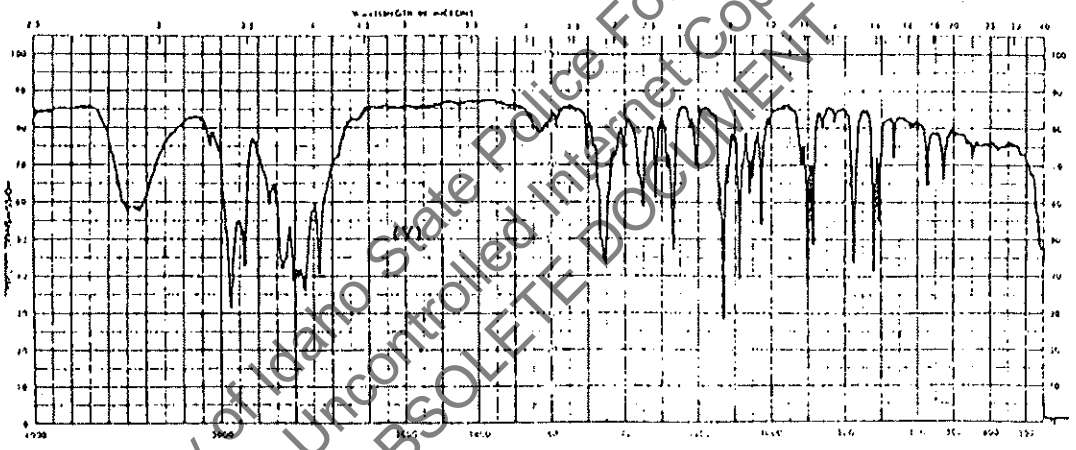
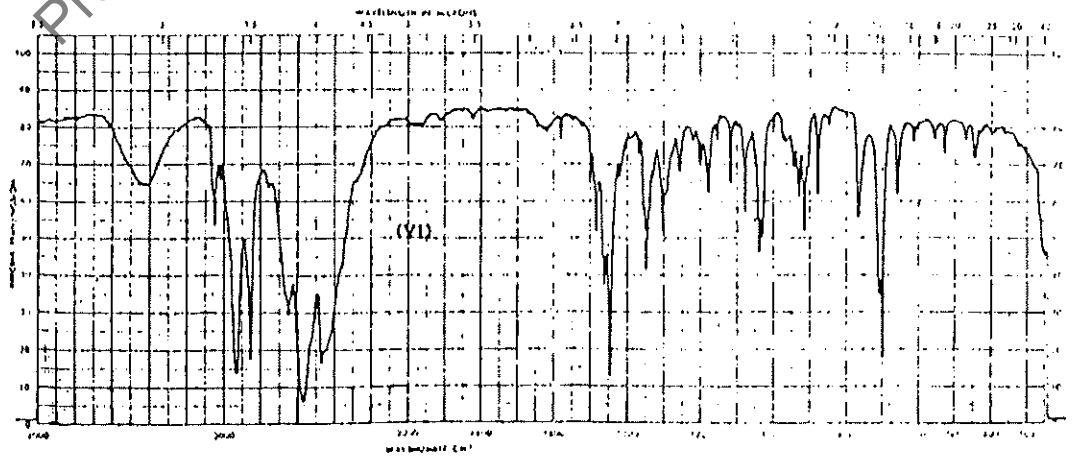
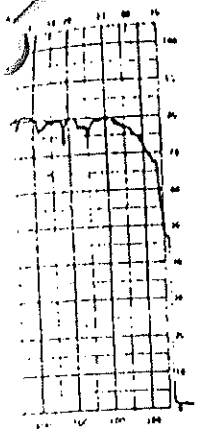


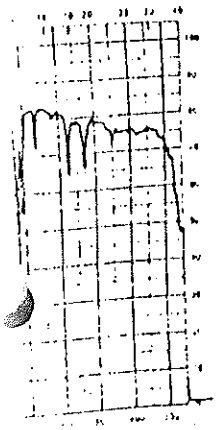
FIG. 3—IR spectra of some analogs of phencyclidine as their hydrochloride salts, KBr disks. I, 1-(1-phenylcyclohexyl)piperidine; II, 1-[1-(2-thienyl)cyclohexyl]piperidine; III, 1-[1-(2-thienyl)cyclohexyl]morpholine;; IV, 1-[1-(2-thienyl)cyclohexyl]pyrrolidine; V, 1-(1-phenylcyclohexyl)morpholine; and VI, 1-(1-phenylcyclohexyl)pyrrolidine.











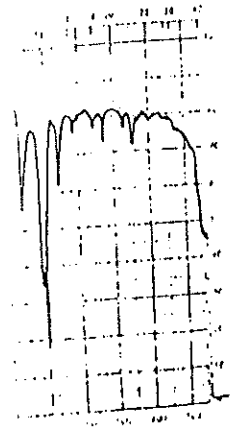


Table 3. Retention times (min) of some phencyclidine analogs for column packings and oven temperatures indicated[a]

Compound	3% OV-17			2.5% OV-225		5% OV-7		3% SE-30		
	225°C	200°C	175°C	175°C	150°C	200°C	175°C	150°C	125°C	100°C
I	4.0	7.4	19.5	5.2	14.6	2.6	5.0	13.5	4.3	16.5
II	4.0	8.8	19.3	5.2	14.9	1.1	1.5	2.6	1.0	2.0
III	5.2	10.7	30.3	10.6	32.0	1.1	1.5	2.6	1.1	2.0
IV	3.2	5.9	14.4	4.4	11.1	1.1	1.5	2.6	1.1	2.0
V	5.2	10.7	30.4	10.5	31.2	3.2	6.2	18.4	6.0	23.5
VI	3.2	5.9	14.5	4.3	11.2	2.2	3.9	10.0	3.3	11.8

[a] Columns were glass, 6′ long, injector 275°C, nitrogen flow 30 ml/min. Support material was 80–100 mesh Chromosorb W (HP), excepting 3% SE-30 when 60–80 mesh was used.

Table 4. R_f values (× 100) of some analogs of phencyclidine

System[a]	Plate[b]	I	II	III	IV	V	VI	LSD
A	Br.	82	85	79	72	78	58	21
A	Ea.	78	79	77	77	78	71	44
B	Br.	85	86	81	77	82	70	40
B	Ea.	70	75	75	68	73	67	60
C	Br.	82	85	84	76	84	68	76
C	Ea.	76	74	75	72	71	68	62
D	Br.	84	89	86	73	85	58	52
D	Ea.	79	81	77	76	79	63	62
E	Br.	12	30	79	18	73	12	88
E	Ea.	57	70	82	65	81	43	66
F	Br.	85	87	82	80	82	78	48
F	Ea.	74	71	75	71	75	74	58
G	Br.	24	42	65	26	63	12	21
G	Ea.	62	68	70	58	69	47	46
H	Br.	28	53	82	32	77	20	33
H	Ea.	64	72	74	64	75	55	51
I	Br.	4	8	66	8	42	5	22
I	Ea.	20	34	72	34	69	22	41

[a] A = ethyl acetate-cyclohexane-dioxane-methanol-water-ammonium hydroxide (50+50+10+10+1.5+0.5); B = ethyl acetate-cyclohexane-ammonium hydroxide-methanol-water (70+15+2+8+0.5); C = ethanol-5*N* ammonia (9+1); D = acetone-12*N* ammonia (99+1); E = chloroform-methanol (9+1); F = chloroform-acetone-triethylamine (5+4+1); G = 1,1,1-trichloroethane-methyl ethyl ketone-methanol (7+2+1); H = *n*-hexane-nitromethane-methanol (7+13+5); I = toluene-nitromethane-methanol (8+10+2).

[b] Br. = Brinkmann silica gel G glass plates and Ea. = Eastman Chromagram 6060 gel sheets with a fluorescent indicator.

The spectra of the hydrochlorides and the free bases are clearly distinct from one another and from those of closely related isomers and homologs (4).

Gas-Liquid Chromatography of Bases

The results obtained with several phases are presented in Table 3. The thiophene-containing analogs emerged more quickly and indistinguishably from the OV-7 and SE-30 columns than did the phenyl containing analogs, whereas with the OV-17 and OV-225 columns the phenyl and thienyl analogs were indistinguishable and the order of emergence was determined by the nitrogen heterocycle. The chromatograms, particularly of the thienyl analogs on OV-7 and SE-30 columns, are indicative of decomposition, and the nature of the emergent peaks remains to be investigated.

Thin Layer Chromatography

Nine systems were investigated (Table 4). LSD and phencyclidine were included in order to compare data for these compounds. The use of iodoplatinate spray reagent showed the compounds as magenta-colored spots.

Acknowledgments

The assistance of J. C. Ethier, D. Legault, and B. Lodge is appreciated.

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(3) Marshman, J. A. (Ed.) (1974) *Street Drug Analysis and Its Social and Clinical Implications*, Addiction Research Foundation of Ontario, Canada, p. 26

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(5) Beckstead, H. D., & French, W. N. (1971) *Some Analytical Methods for Drugs Subject to Abuse*, Department of National Health and Welfare, Ottawa, Canada

(6) Budzikiewicz, H., Djerassi, C., & Williams, D. H. (1964) *Interpretation of Mass Spectra of Organic Compounds*, Holden-Day, Inc., San Francisco, pp. 162–165 and 231–235

DEA LABORATORY NOTES

DATE

NO.

DRUG TYPE Phencyclidine

METHODOLOGY Gas Liquid Chromatography

GAS LIQUID CHROMATOGRAPHIC SCREENING PROCEDURE FOR COMPONENTS FOUND IN CLANDESTINE PHENCYCLIDINE REACTION MIXTURES

BY

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AND
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INTRODUCTION

Recently there have been numerous clandestine phencyclidine laboratories found operating throughout the country. Many of the samples originating from these laboratories are contaminated with starting materials, reaction intermediates and by-products. It is the scope of this paper to present a GLC temperature program that would enable the chemist to screen for these components. Since most of the PCP clandestine laboratories utilize the "carbonitrile intermediate" method, the data in this paper will reflect this synthetic route.

APPARATUS

Instrumentation: Hewlett-Packard 5840A Gas Chromatograph with a Flame Ionization Detector

Columns: 1) 6 ft. x 1/8 in. I.D. 3% OV-17 on 100/120 gas-chrom Q.
2) 6 ft. x 1/8 in. I.D. 10% OV-101 on 100/120 gas-chrom Q.

Parameters:

Carrier Gas:	Nitrogen @ approximately 60 ml/min.
Injector Temperature:	275°
Detector Temperature:	300°
Column Temperature:	Initial temperature of 90°c for 3 min. with a rate of 10°c/min for 16 min. Final temperature of 250°c for 2 min.

PROCEDURAL STANDARD

Starting Reagents: Cyclohexanone; Piperidine and Bromobenzene
Reaction Intermediate: 1-Piperidinocyclohexane Carbonitrile (1-PCC)
By-Products: 1-Cyclohexylpiperidine; Biphenyl; 1-Phenylcyclohexene;* 1-Phenylcyclohexanol; Phenol and 1-Phenylethanol.
Final Product: Phencyclidine (PCP) (See table 1 for structures)

The procedural standard is composed of the 11 individual components listed above with each being diluted to a concentration of approximately 1 mg/ml in methanol.

*Although listed as a byproduct, 1-phenylcyclohexene may be partially caused by the thermal degradation of PCP. [1,2]

PROCEDURE

A portion of the clandestine sample is dissolved in methanol and the solution is then injected into the GC using the above stated conditions. A chromatogram such as figure 1 is obtained and the retention times are compared to those of a procedural standard run under identical conditions (figure 2).

DISCUSSION

Comparison of the chromatograms in figures 1 and 2 indicate the possible presence of six or seven of the procedural standard components in the clandestine laboratory sample. The variations in retention times between the two chromatograms for four of these compounds (1-PCC, 1-phenylcyclohexene, 1-phenylcyclohexanol and PCP) fall within acceptable limits. Although the first two compounds eluting after the solvent front do not match the retention times of the procedural standard exactly, they have been identified as piperidine and cyclohexanone by "spiking" the sample with the respective standard (see figures 3 and 4). The retention times of the piperidine and cyclohexanone in the spiked sample likewise changed; this suggests that the retention times of these components can vary somewhat according to their concentrations in a sample.

Many of the smaller peaks in the sample have been ignored as being due to minor contaminants. Two of the peaks shown in the procedural standard, phenol and 1-phenylethanol, are rarely encountered in clandestine samples. They appear to be the results of an excessive use of phenyl magnesium bromide.

Since the 3% OV-17 column does not separate cyclohexanone and bromobenzene well, a second column, 10% OV-101, has been used to resolve these peaks (figures 5 and 6). In addition a better separation between 1-phenylcyclohexene and biphenyl is effected on the OV-101 column. Figure 7 is a clandestine laboratory sample "spiked" with piperidine and injected on the 10% OV-101 column. Again, as in figure 3, the "spiked" sample results in one peak which is indicative of piperidine. Although the OV-101 column separates cyclohexanone and bromobenzene, it now has the disadvantage that 1-PCC and biphenyl have the same retention time. We suggest the use of the OV-17 column as the primary column since it is often important to identify the presence of the reaction intermediate, 1-PCC.

The identity of each of the individual components in standard and sample was confirmed by GC-MS, and a subsequent paper will list the normalized mass spectra.

This two-column separation technique has been utilized in the North Central Regional Laboratory for the last year with very favorable results. The authors wish to express their gratitude to all members of the laboratory for suggestions as well as contributions of data obtained from the analysis of clandestine PCP samples.

REFERENCES

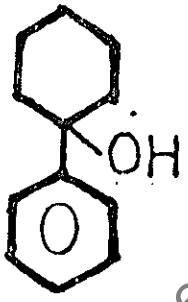
1. "Identification of Degradation Peak in Phencyclidine Gas Chromatography", by David Hauber, Microgram, Vol. VIII, No. 7, 1975, p. 100.

2. "Metabolites of Phencyclidine", by L.K. Wong and K. Biemann, Clinical Toxicology, 9 (4), 1976, pp. 583-591.

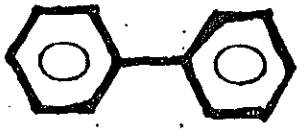
TABLE 1

COMPOUNDS FOUND IN PCP REACTION MIXTURES

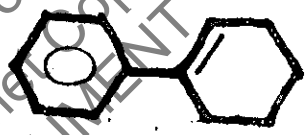
1-CYCLOHEXYLPIPERIDINE



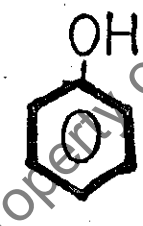
1-PHENYLCYCLOHEXANOL



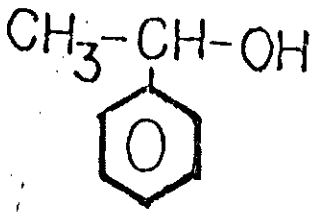
BIPHENYL



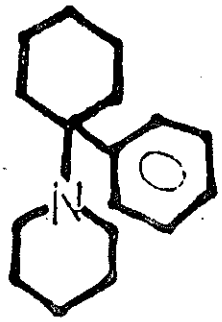
1-PHENYLCYCLOHEXENE



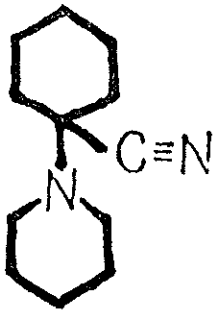
PHENOL



1-PHENYLETHANOL



PCP



PCC

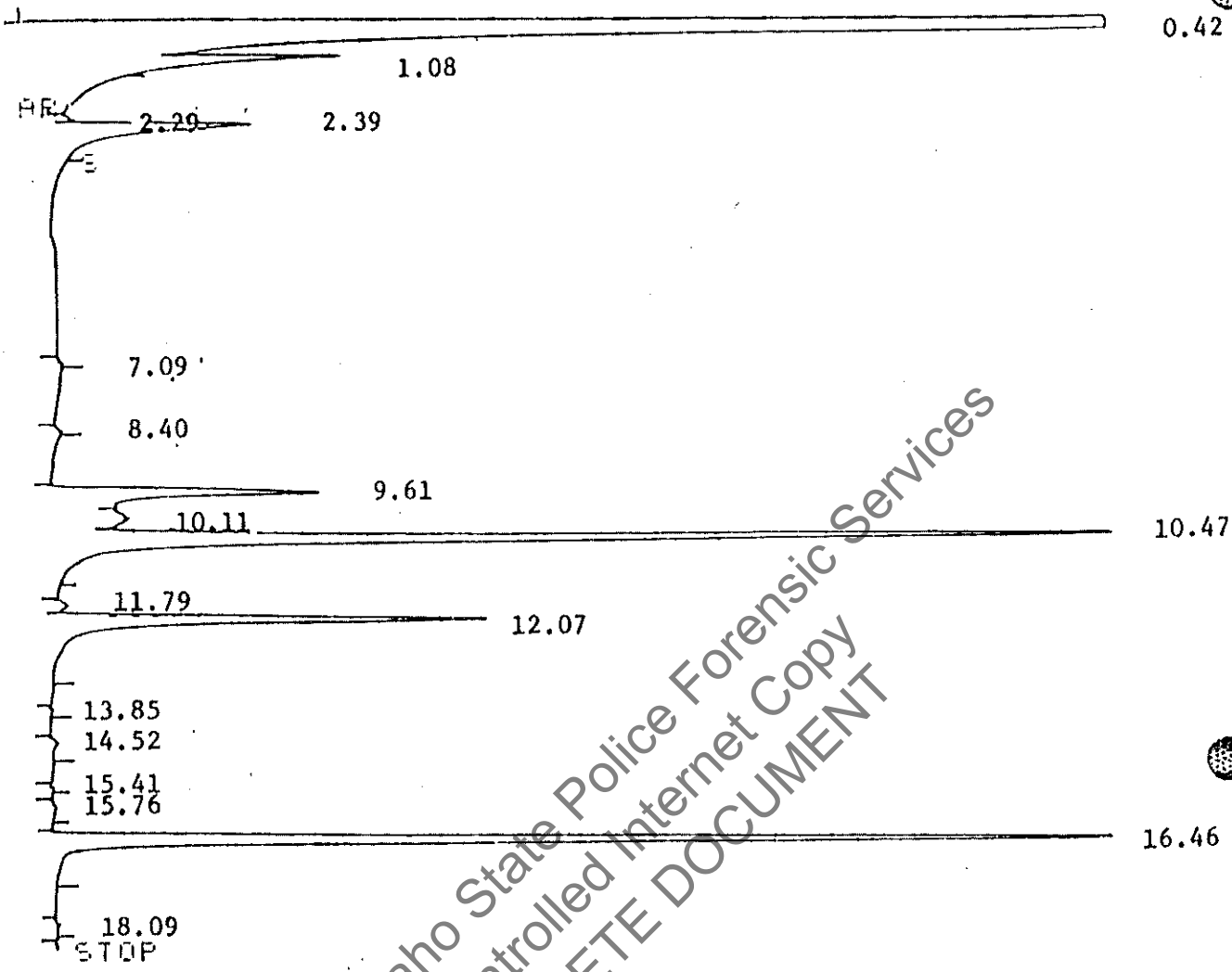


FIGURE 1 PCP CLANDESTINE LABORATORY SAMPLE (3% OV-17 Column)

RETENTION TIME IN MINUTES	COMPONENT PEAK
1.08	Piperidine
2.39	Cyclohexanone and/or Bromobenzene
9.61	1-PCC
10.47	1-Phenylcyclohexene
12.07	1-Phenylcyclohexanol
16.46	PCP

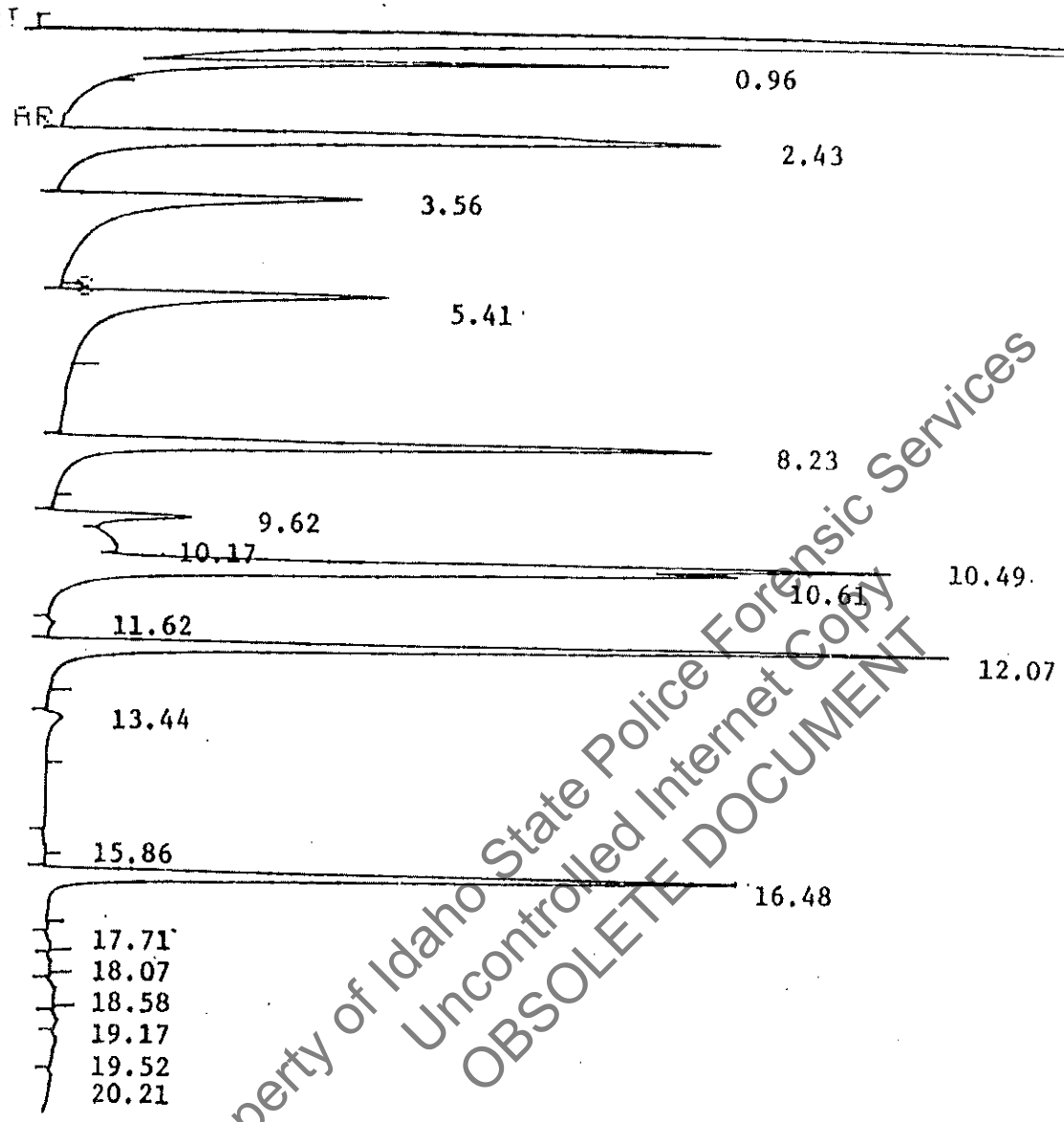


FIGURE 2 PROCEDURAL STANDARD (3% OV-17 Column)

RETENTION TIME IN MINUTES	COMPONENT PEAK
0.96	Piperidine
2.43	Cyclohexanone and Bromobenzene
3.56	Phenol
5.41	1-Phenylethanol
8.23	1-Cyclohexylpiperidine
9.62	1-PCC
10.49	1-Phenylcyclohexene
10.61	Biphenyl
12.07	1-Phenylcyclohexanol
16.48	PCP

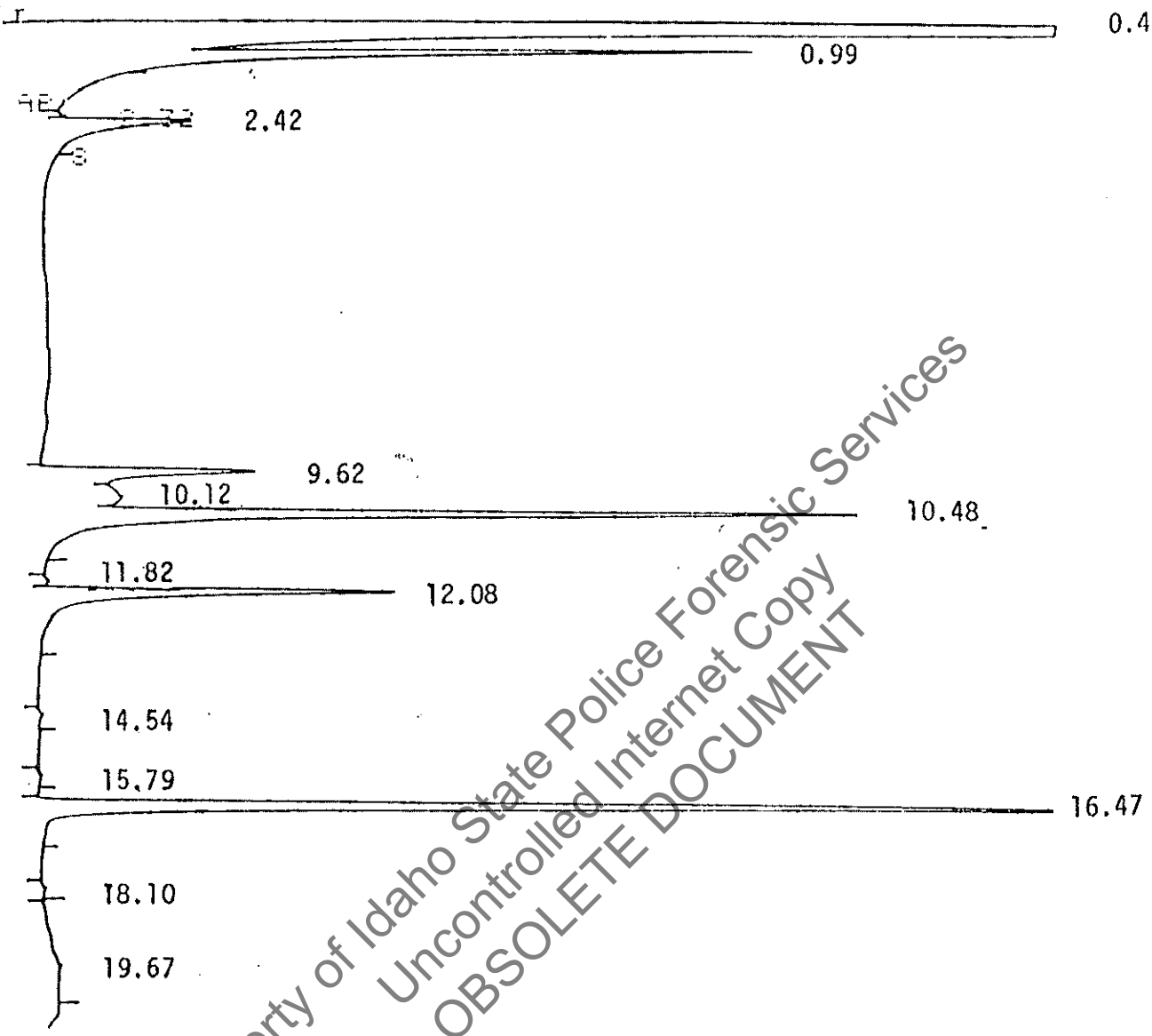


FIGURE 3 PCP CLANDESTINE LABORATORY SAMPLE SPIKED WITH PIPERIDINE (3% OV-17 Column)

RETENTION TIME IN MINUTES	COMPONENT PEAK
0.99	Piperidine
2.42	Cyclohexanone and/or Bromobenzene
9.62	1-PCC
10.48	1-Phenylcyclohexene
12.08	1-Phenylcyclohexanol
16.47	PCP

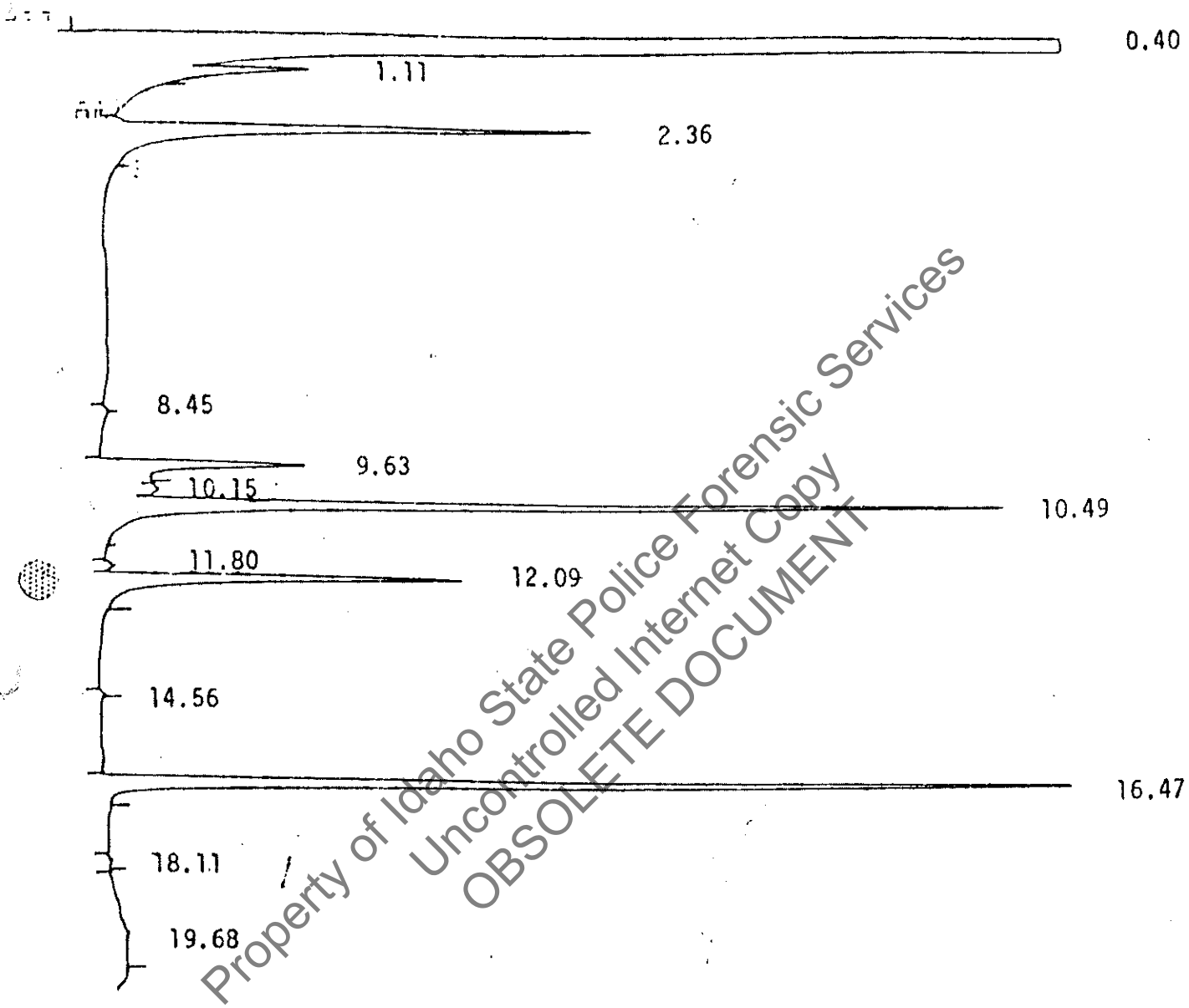


FIGURE 4 PCP CLANDESTINE LABORATORY SAMPLE SPIKED WITH CYCLOHEXANONE (3% OV=17 Column)

RETENTION TIME IN MINUTES	COMPONENT PEAK
1.11	Piperidine
2.36	Cyclohexanone and/or Bromobenzene
9.63	1-PCC
10.49	1-Phenylcyclohexene
12.09	1-Phenylcyclohexanol
16.47	PCP

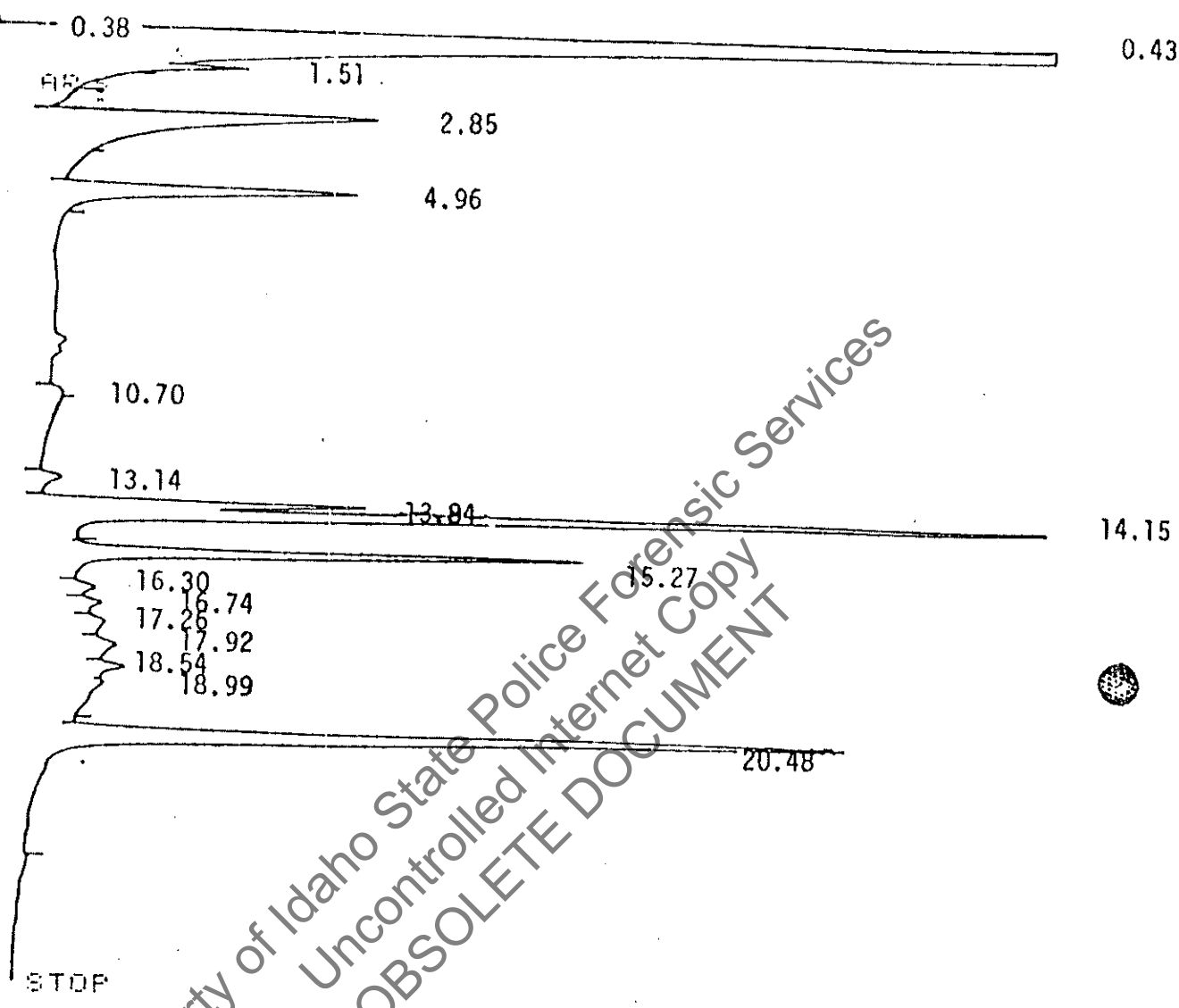


FIGURE 5 CLANDESTINE LABORATORY SAMPLE (10% OV-101 Column)

RETENTION TIME IN MINUTES	COMPONENT TIME
2.85	Piperidine
4.96	Cyclohexanone
13.84	1-PCC and/or Biphenyl
14.15	1-Phenylcyclohexene
15.27	1-Phenylcyclohexanol
20.48	PCP

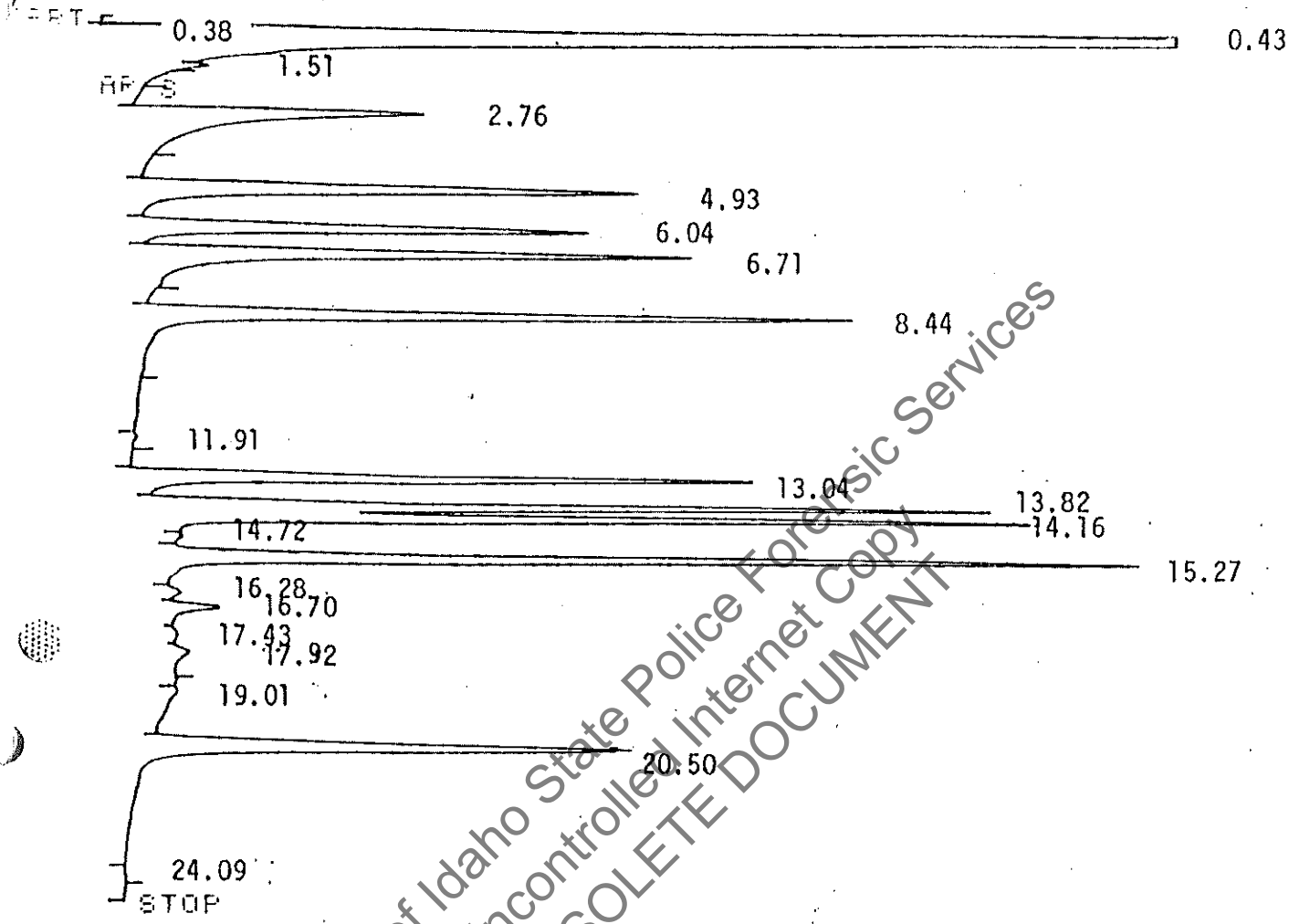


FIGURE 6 PROCEDURAL STANDARD (10% OV-101 Column)

RETENTION TIME IN MINUTES	COMPONENT PEAK
2.76	Piperidine
4.93	Cyclohexanone
6.04	Bromobenzene
6.71	Phenol
8.44	1-Phenylethanol
13.04	1-Cyclohexylpiperidine
13.82	1-PCC and Biphenyl
14.16	1-Phenylcyclohexene
15.27	1-Phenylcyclohexanol
20.50	PCP

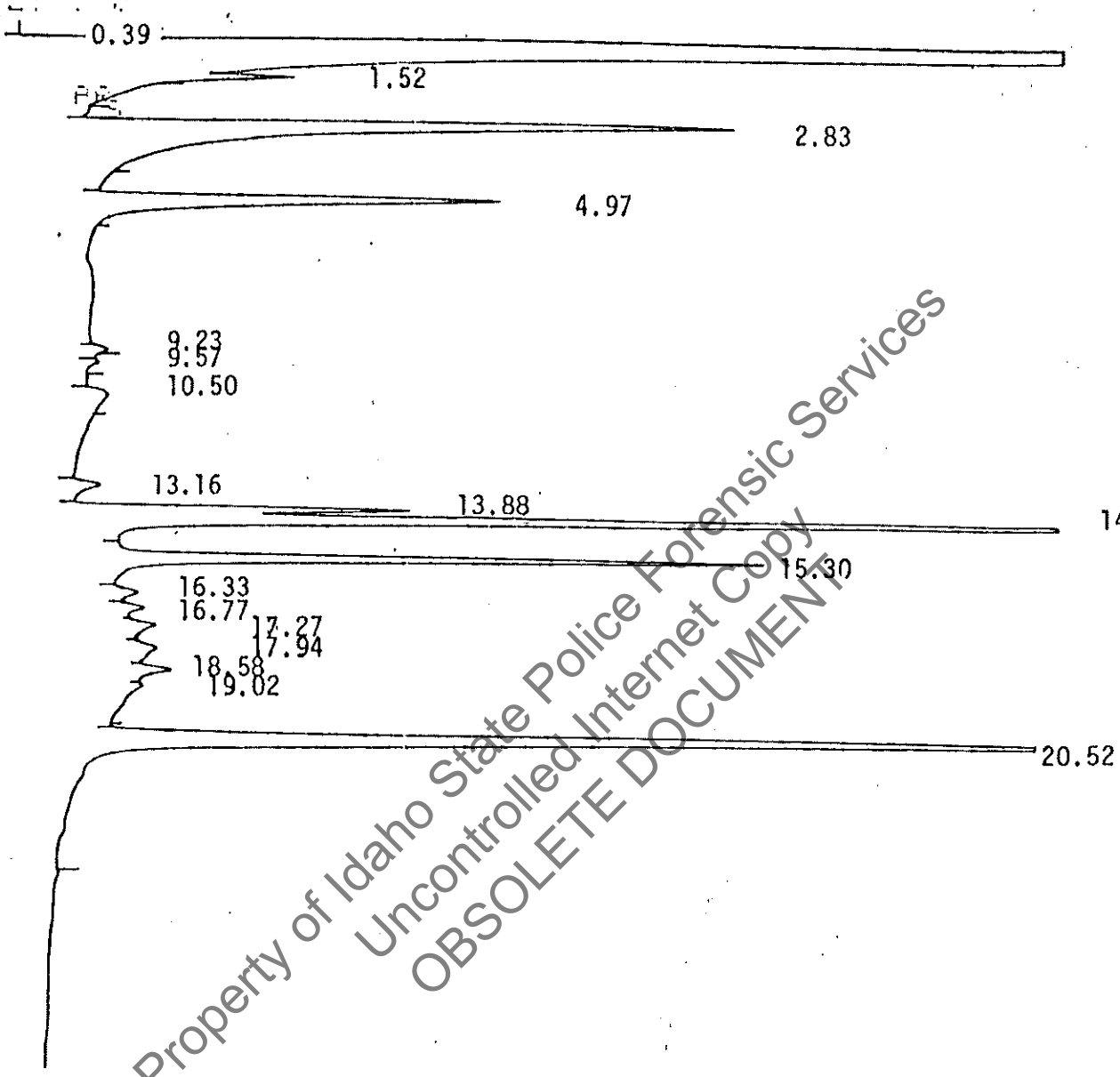


FIGURE 7 CLANDESTINE LABORATORY SAMPLE SPIKED WITH PIPERIDINE (10% OV-101 Column)

RETENTION TIME IN MINUTES	COMPONENTS PEAK
2.83	Piperidine
4.97	Cyclohexanone
13.88	1-PCC and/or Biphenyl
14.19	1-Phenylcyclohexene
15.30	1-Phenylcyclohexanol
20.52	PCP

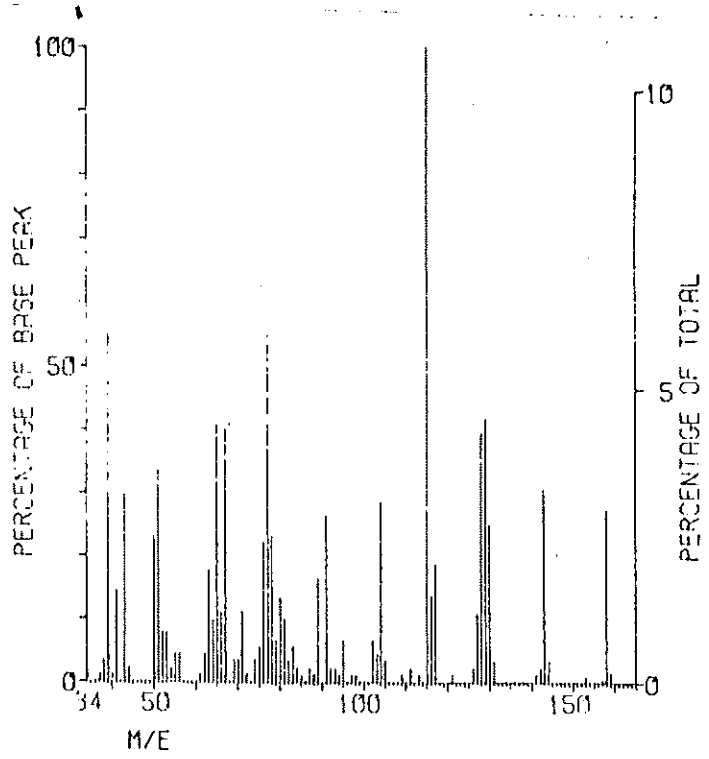
(17)

Identification of Degradation Peak in Phencyclidine Gas Chromatography

David Hauber, Kentucky State Police Crime Laboratory
Frankfort, Kentucky

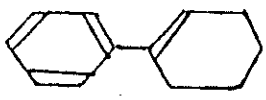
An extra peak in gas chromatograms of phencyclidine has been a constant occurrence in our lab with any PCP sample run at lower column temperatures. On our 5% SE-30 column (6 ft., M80-100), elution of PCP in 6 min. at constant temperature (250°C) will result in an extra peak at approximately 1.6 min. Elution of PCP in 5 min. on our 3% OV-1 column (8 ft., M80-100) at 200°C results in an extra peak at 1.8 min. The mass spectrum of this extra peak eluting from an OV-1 column (see figure) suggests phenylcyclohexene - a logical PCP degradation product resulting from the elimination of piperidine, which would be buried in the solvent elution. There is less degradation in systems with glass columns and glass-lined injection ports. Hot catalytic surfaces of injection ports (approximately 265°C) cause most of the degradation, but an elevated base line between the GC peaks suggests more degradation during the journey through the column. Some evidence exists for more degradation of the hydrochloride than the free base in certain glass systems.

This extra peak gives one more identifying mark in PCP gas chromatography, but the degradation precludes easy quantitation in many GC systems since the relative amounts of this degradation varies.



Computer normalized mass spectrum of the PCP degradation peak obtained from a Finnigan GCMS (Model 3100D).

Suggested structure:



12

STATE OF MICHIGAN

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1 - PIPERIDINOCYCLOHEXANE CARBONITRILE

A PHENCYCLIDINE PRECURSOR

by
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Crime Laboratory Scientist
Michigan Department of Public Health
Madison Heights Crime Detection Laboratory
Madison Heights, Michigan 48071

Our laboratory has recently received numerous submissions of phencyclidine (PCP) containing an impurity. GC/MS data of this impurity suggested that it might be 1 - Piperidinocyclohexane Carbonitrile (PCC), a precursor in the synthesis of PCP[1,2].

Concurrently we also received a fairly pure substance which was later proven to be PCC and exhibited the same GC/MS data as the impurity encountered in the above PCP submissions.

This substance was identified as PCC via its IR, UV, GC, GC/MS and conversion to PCP.

Discussion

The IR spectrum of the pure substance was identical to that of PCC[3] and showed a nitrile band at 2210 cm^{-1} as well as major bands at 2975,1447,1107,872 and 787 cm^{-1}.

As expected from its chemical structure, PCC did not show significant absorption in the UV region of 350-200 nm.

A GC/MS and a solid probe MS of PCC differed significantly due to the apparent loss of HCN (27 amu) on the GC collumn giving N-(1-cyclohexenyl) piperidine.

Gas chromatographic data of PCC using three different liquid phases at 170° are listed in Table 1.

(continued)

Conversion of PCC to PCP

Phenylmagnesium bromide was prepared in the usual manner[4] by placing 1.26 g of magnesium turnings, 5 ml of anhydrous diethyl ether and a crystal of iodine in a 100 ml round bottom flask, then adding 8.18 g (5.5 ml) of bromobenzene in 17 ml of anhydrous ether. The mixture was then refluxed until the Mg had completely dissolved. To the ethereal solution of phenymagnesium bromide was added 8.0 g of PCC in 50 ml of cyclohexane. After refluxing the mixture for 1 hr, the ether was distilled off and the remaining solution was extracted with 25 ml of 2.8 N HCl. The aqueous layer was separated and evaporated to yield a crude crystalline product. Subsequent purification and analysis of this product showed it to be PCP, thus confirming that the starting material was indeed PCC.

Table 1: Gas Chromatographic data using:

a) 3% OV-1, 6', 170°C

	R_t
PCC	0.63 min
1-phenylcyclohexene	0.73 min
PCP	3.83 min

b) 3% OV-17, 6', 170°C

PCC	0.63 min
1-Phenylcyclohexene	0.78 min
PCP	4.40

c) 3% Ultraphase, 5', 170°C

PCC	0.85 min
1-Phenylcyclohexene	0.92 min
PCP	4.50 min

References

1) V.H. Maddox, E.F. Godefroi, R.F. Parcell; J.Med. Chem, 8, 230 (1965)

2) A.T. Shulgin, D.E. MacLean, Clinical Toxicology, 9, 553 (1976)

3) R.D. Porter, Microgram, 8 (10), 151 (1975)

4) A.I. Vogel; A Textbook of Practical Organic Chemistry, Longman Group Limited, London, 1972, D756

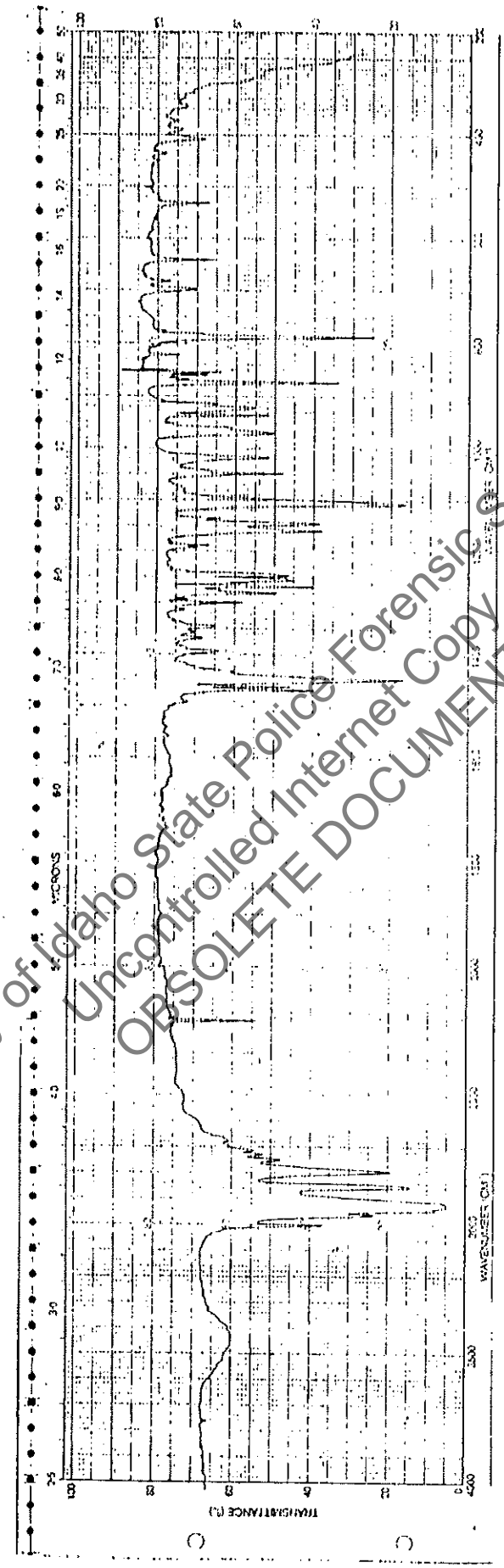


FIG 1. 1-PIPERIDINOCYCLOHEXANE CARBONITRILE

DEA LABORATORY NOTES

DATE

NO.

(13)

DRUG TYPE Hallucinogen

METHODOLOGY U.V, I.R., and G.C-M.S Identification

Identification of the PCP analogs,

(a) 1-(1-phenylcyclohexyl) pyrrolidine, and
(b) 1-(1-phenylcyclopentyl) piperadine.

Howard Dobres, Forensic Chemist
Mid-Atlantic Regional Laboratory

Compound (a) was encountered in a clandestine PCP laboratory in January, 1974 in Rockville, Maryland. It is believed to have been synthesized by substituting pyrrolidine for piperadine in the PCP synthesis. Compound (b) was alleged to have been synthesized, but not found, in the same laboratory.

EXPERIMENTAL:

Standard samples of 1-(1-phenylcyclohexyl) pyrrolidine monohydrochloride, Lot P, and 1-(1-phenylcyclopentyl) piperadine, Lot P, were supplied by Park-Davis & Company, Research and Development Division.

Samples were recrystallized from chloroform-methanol (plus a drop of conc. HCl) for the I.R. spectra.

Instruments used were the Beckman Acta-5 U.V. spectrophotometer, Perkin-Elmer 457 I.R. spectrophotometer, and Finigan 3000G.C-M.S with a 4ft. 3% O.V-1 column.

Operating conditions:

U.V.: 1-(1-phenylcyclohexyl) pyrrolidine hydrochloride-1.6 mg/3ml 0.1NHCl
1-(1-phenylcyclopentyl) piperadine base-1.2mg/3ml.

I.R.: 2% 1-(1-phenylcyclohexyl) pyrrolidine hydrochloride, and 1-(1-phenylcyclopentyl) piperadine hydrochloride-KBr pellets.
Scan speed - Fast
Slit - 7

(See attached spectra)

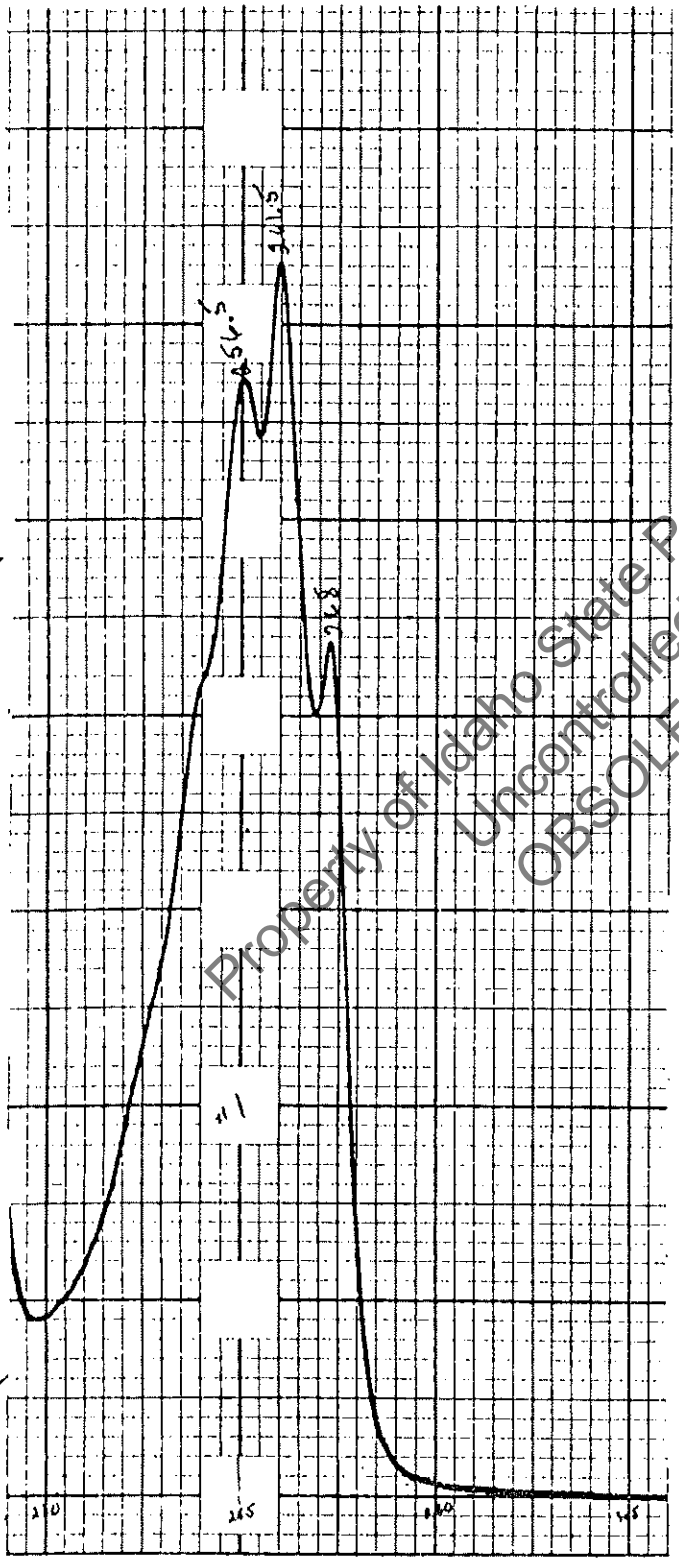
G.C-M.S - 1 mcg. of each compound.
Column temperature = 200°C
Carrier gas flow rate = 28 cc/min.
Senstivity 10^{-6}

IDENTIFICATION:

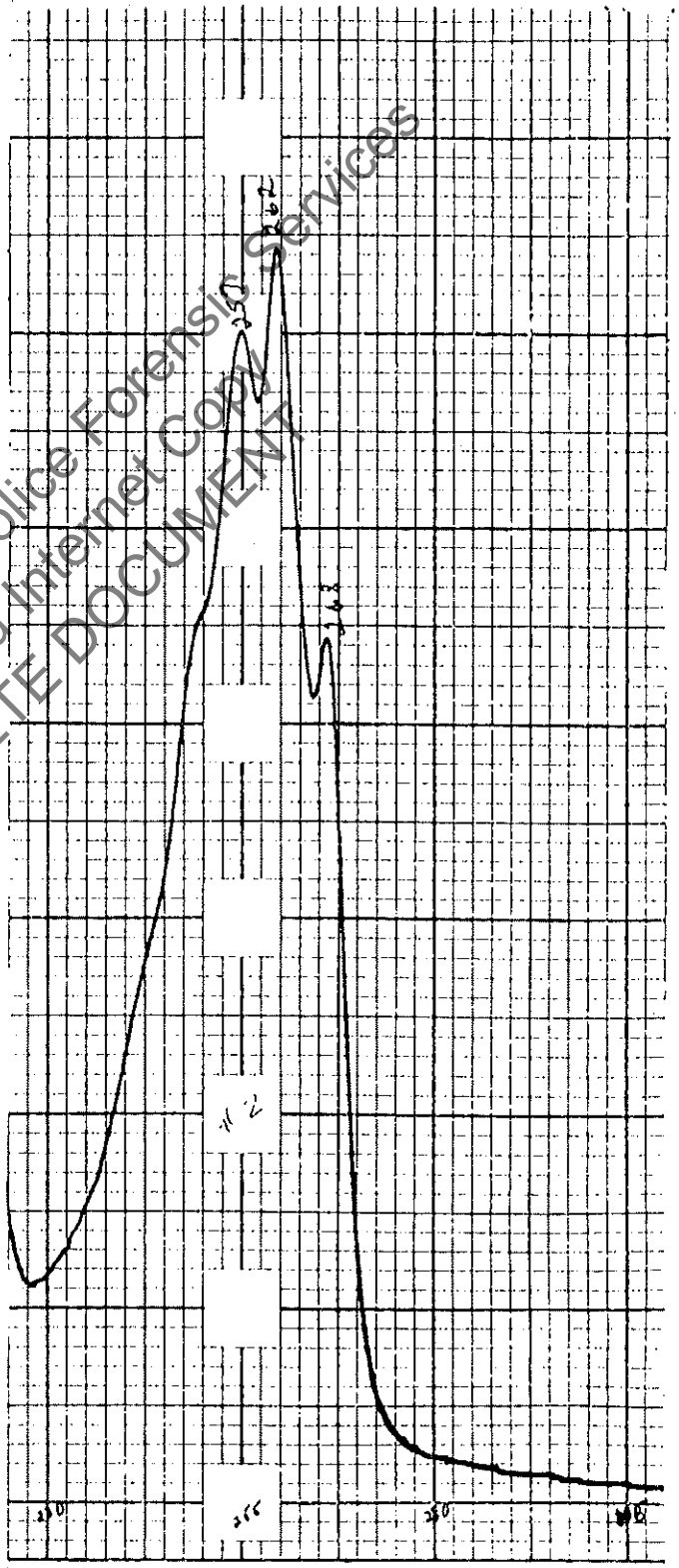
(a) 1-(1-phenylcyclohexyl) pyrrolidine
U.V.: max. 262nm, 257nm, 268nm, (0.1NHC1)
I.R. principal peaks: 1447, 700, 1353, 1040, 1031. 1305, 920, 769 cm^{-1}
G.C.-M.S - Parent peak - M/e = 229
Base peak - M/e = 186

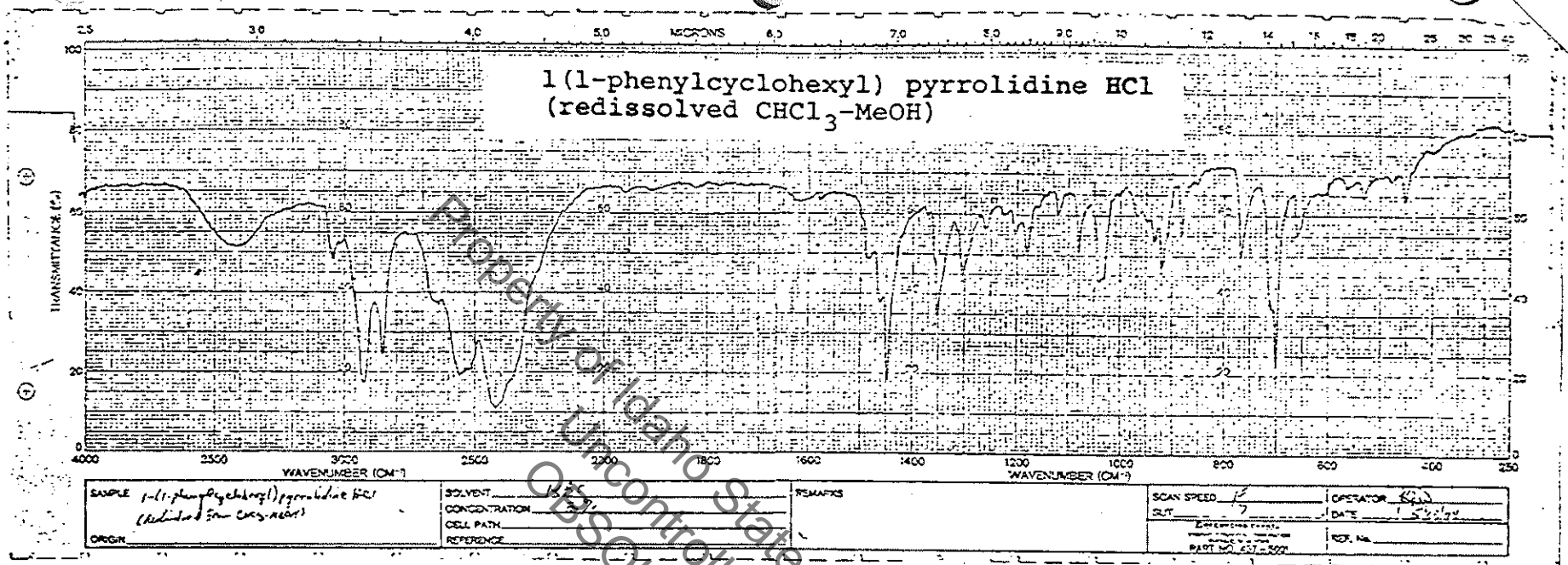
(b) 1-(1-phenylcyclopentyl) piperadine
U.V.: max. 261.5nm, 256.5nm, 268 nm
I.R. principal peaks: 707, 1453, 749, 1249, 1023, 765, 975, 1338 cm^{-1}
G.C. - M.S - Parent peak - M/e = 229
Base peak - M/e = 200

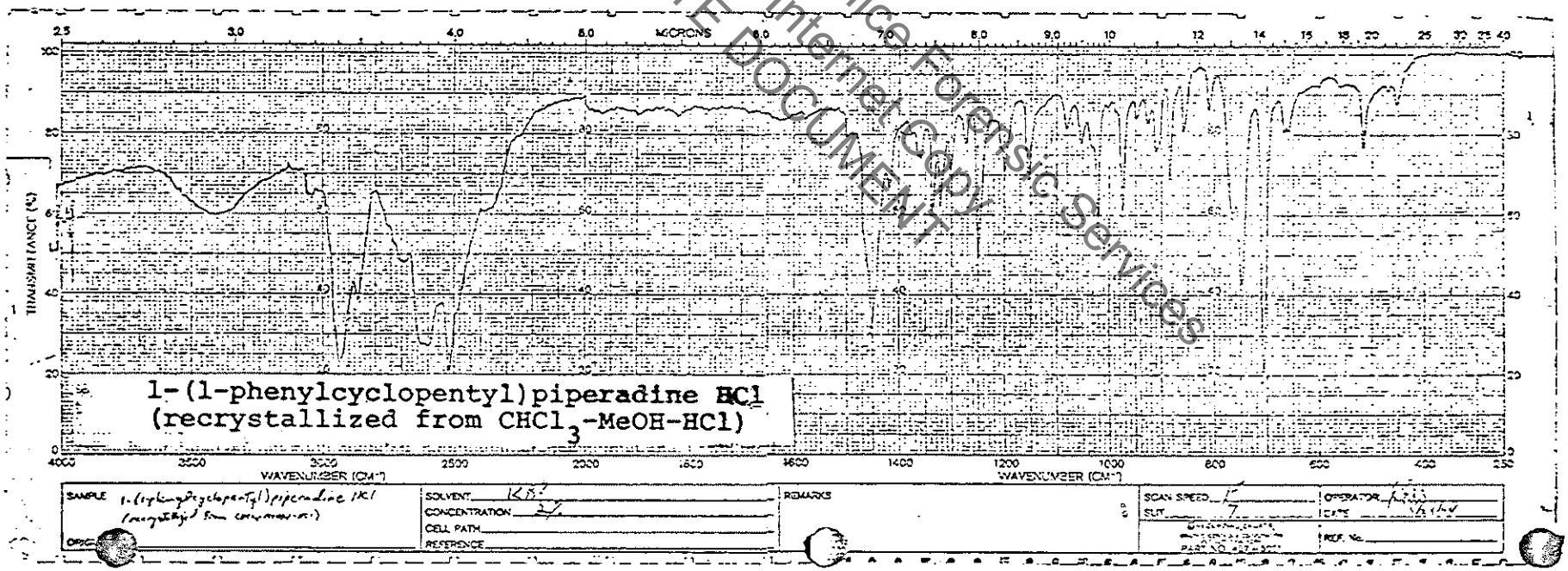
1-(1-phenylcyclopentyl) piperadine base 1.2 mg/3 ml



1-(1-phenylcyclohexyl) pyrrolidine hydrochloride 1.6 mg/3 ml







BNDD LABORATORY NOTES

DATE November 22, 1972 -122-

NO. 43

DRUG TYPE Hallucinogen

METHODOLOGY I. R. Identification

(14)

THIOPHENE ANALOG OF PCP

by

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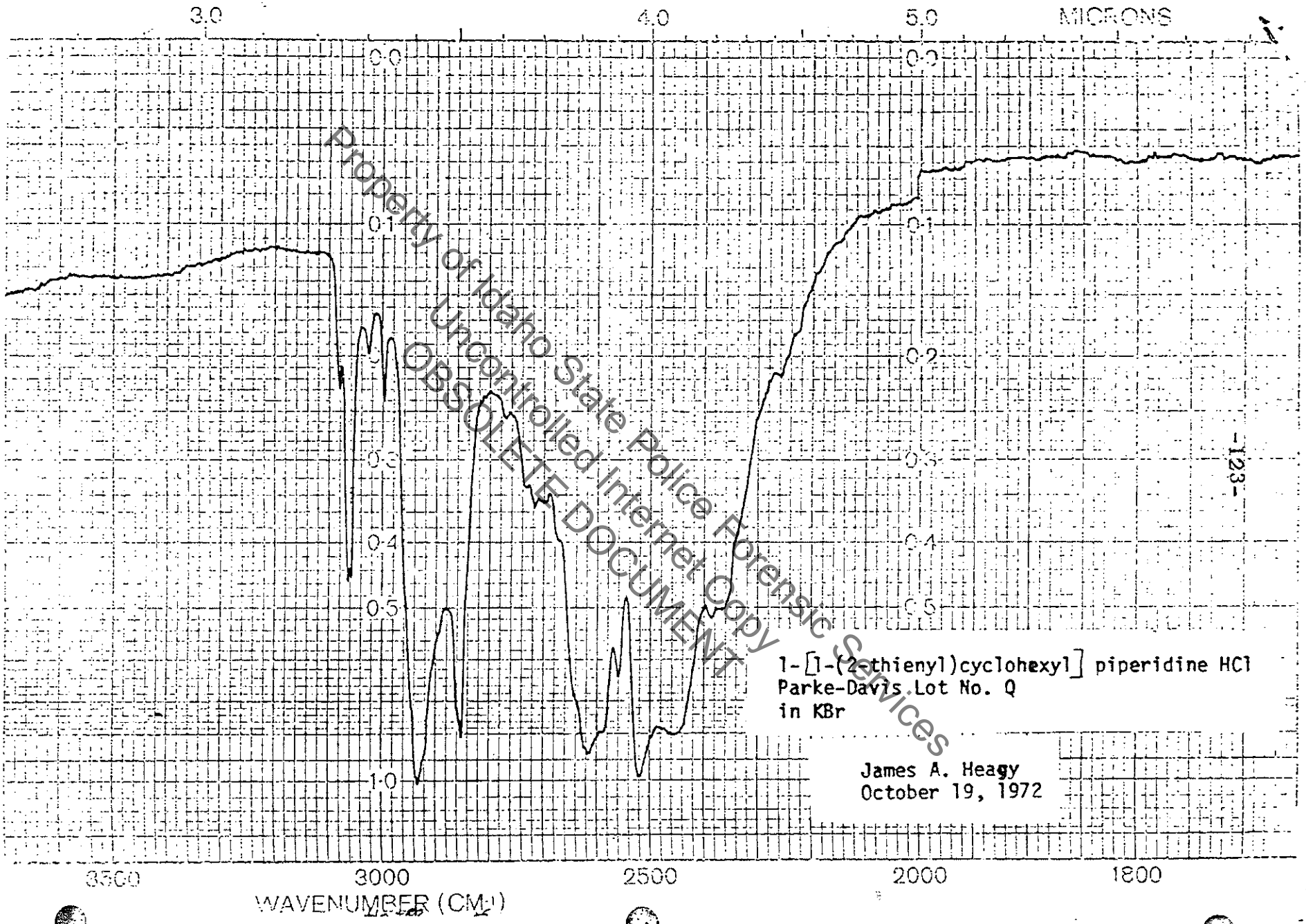
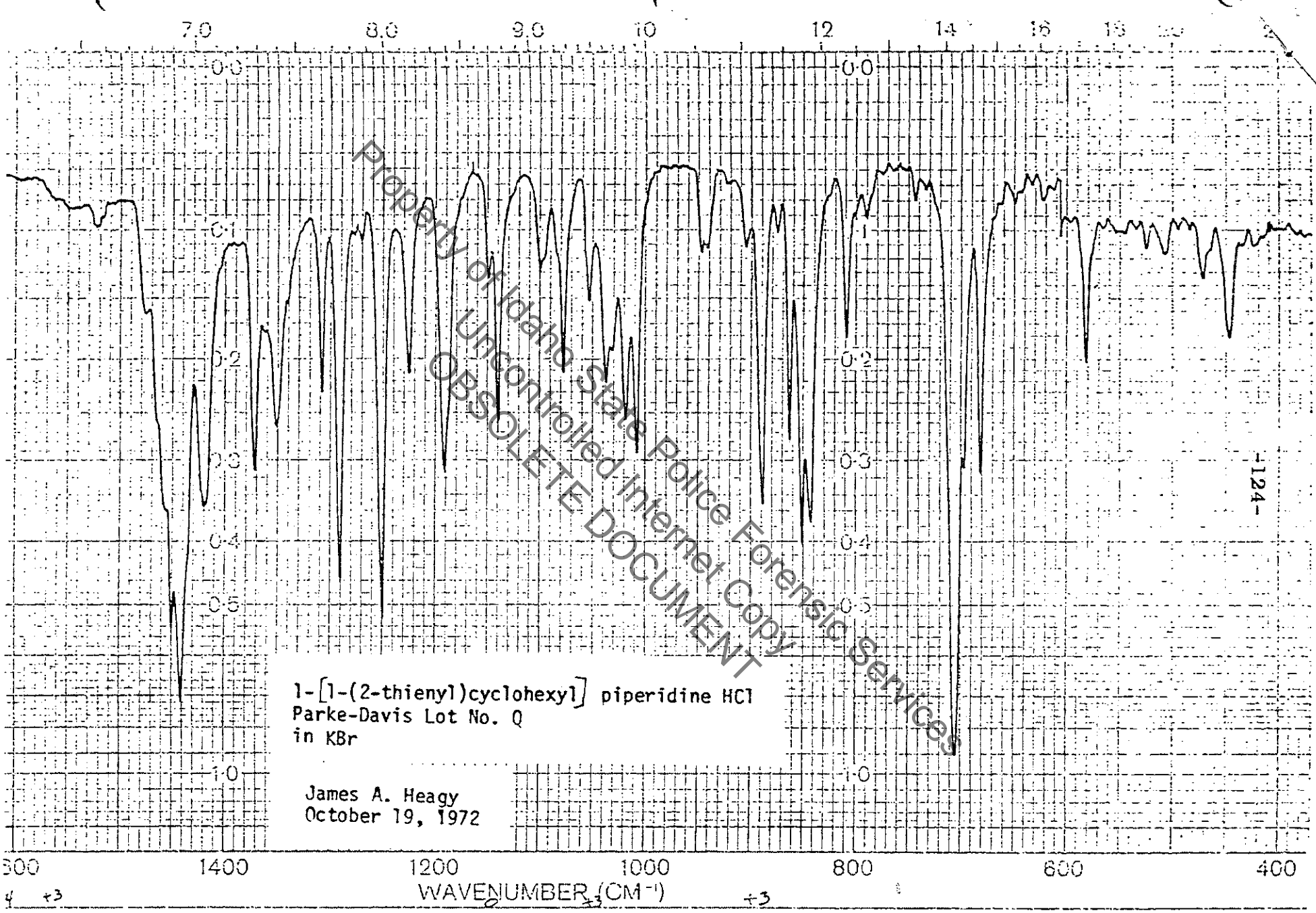
A new drug has recently appeared on the illicit market in several parts of the country. It has been identified as 1-[1-(2-thienyl) cyclohexyl] piperidine, is synthesized from 2-bromothiophene and piperidinocyclohexanecarbonitrile (CA 54:12159c) in a manner similar to the synthesis of phencyclidine. All samples encountered so far contain several amine impurities including, in some cases, large amounts of piperidine. A strong cyanide-like odor is also generally present.

Identification characteristics

U. V. max. 232.5 nm., min. 204 nm. (in 0.1 HCl)
I. R. peaks at 708, 1444, 1453, 1251, 1292, 854, 846 cm^{-1}.
(See attachment)
Marquis reagent: gas expelled, turns to gray-orange.
Mecke reagent: gas expelled, color turns to yellow-green, then blue-green, and slowly to deep blue.
Mass spectrum: Molecular ion is not present.





DEA LABORATORY NOTES

DATE OCT 23 1974

NO. 88

DRUG TYPE HALLUCINOGEN

METHODOLOGY UV SPECTRA OF PHENCYCLIDINE BASE

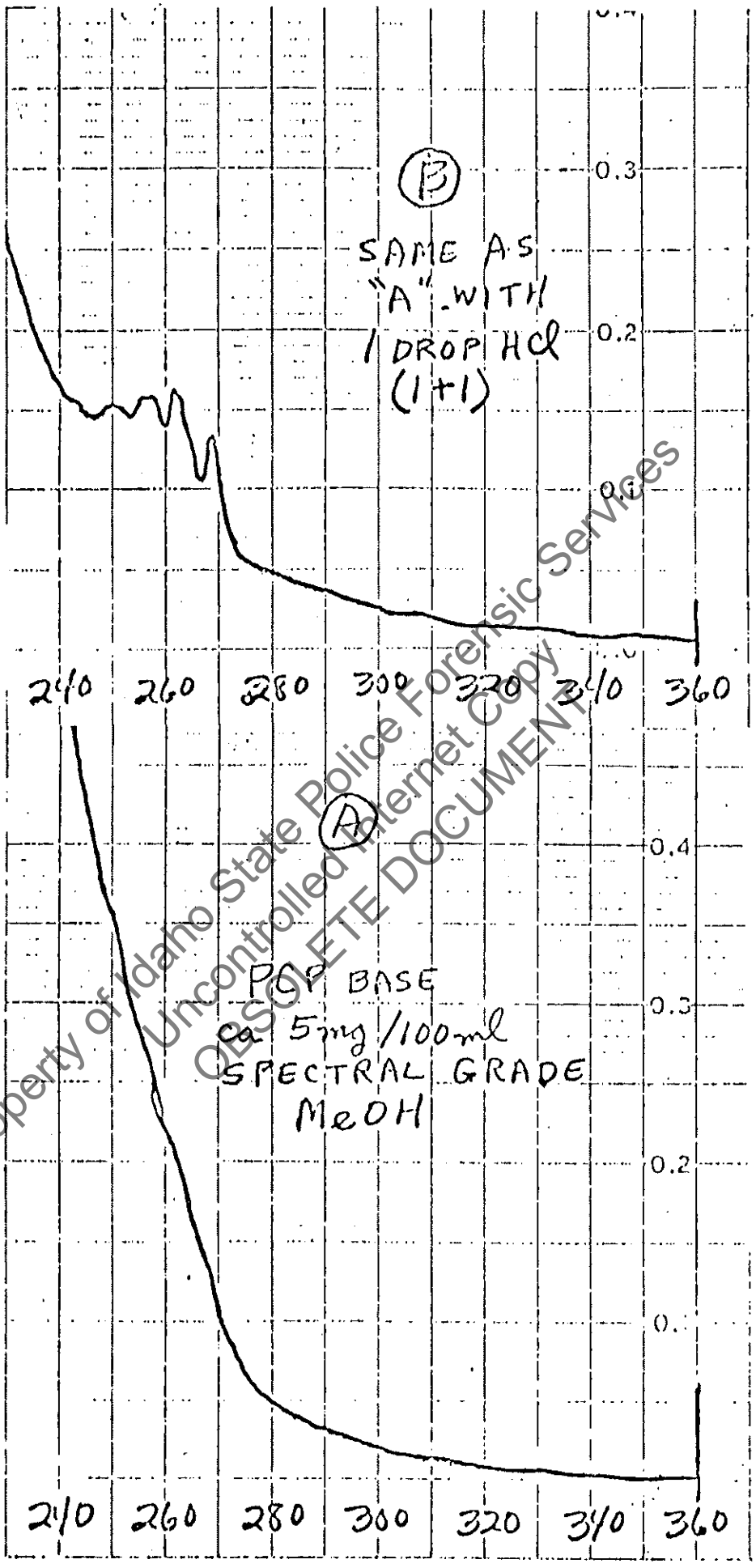
BY

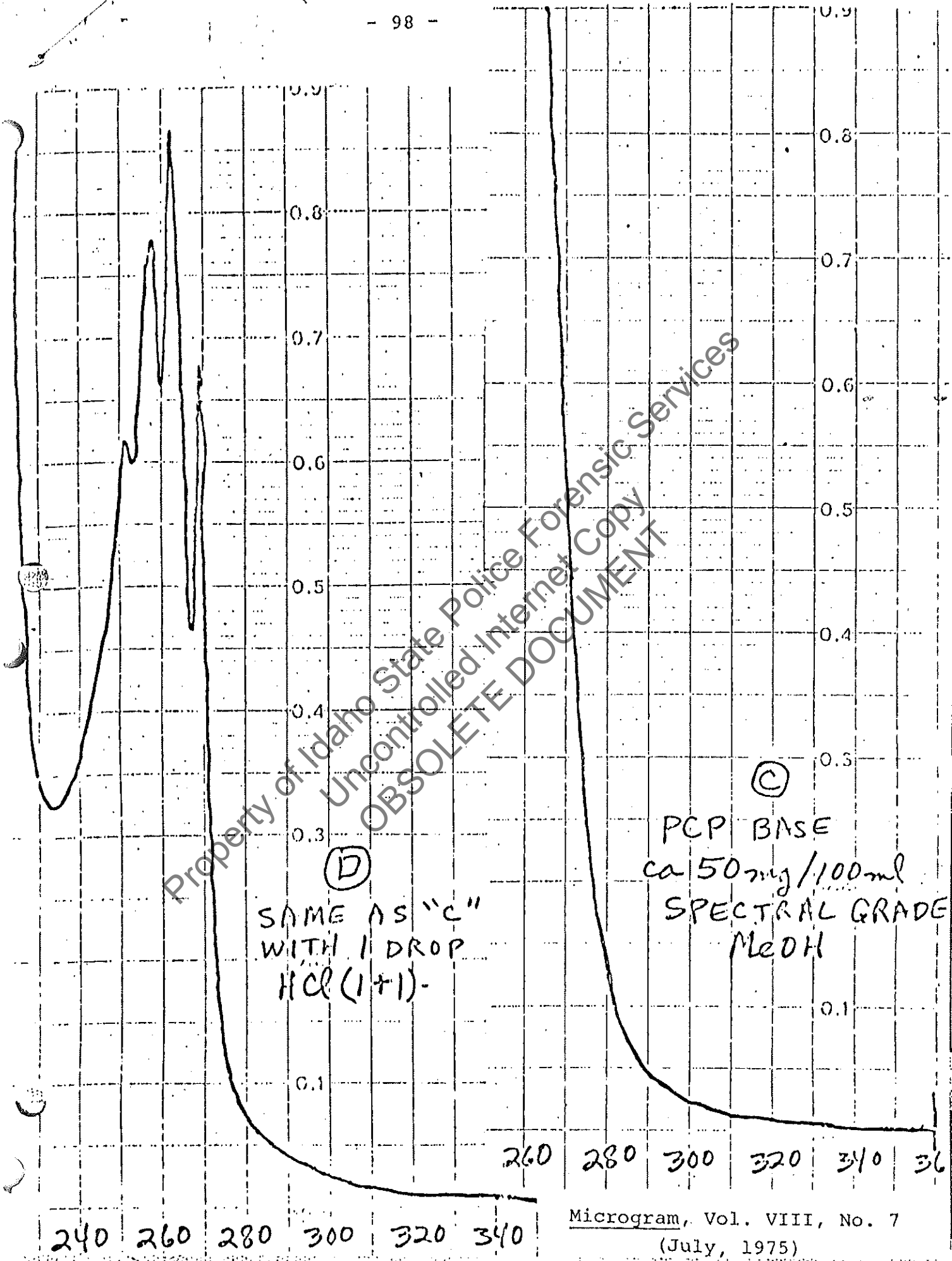
ROGER G. FUELSTER
FORENSIC CHEMIST, NORTH CENTRAL REGIONAL LABORATORY
DRUG ENFORCEMENT ADMINISTRATION

A recent sample received in the North Central Regional Laboratory consisted of rock-candy like light yellow chunks of material. Screening with acid CoSCN produced the pale blue of PCP. The material was then dissolved in Spectral Grade MeOH and a UV scan obtained. The scan was featureless (see attached scans A & C). Further analysis of the material by GC - MS and IR showed nearly pure PCP base.

The UV scans were then re-run in acidified spectral grade MeOH (see attached scans B&D), with the expected benzenoid curve of PCP.

It is apparent from these results that care must be exercised when using UV as a screening technique for unknowns. Curves must be run in acid as well as neutral solution. Although PCP is the only example of this behavior encountered to date, other organic bases may exhibit similar aberrations.

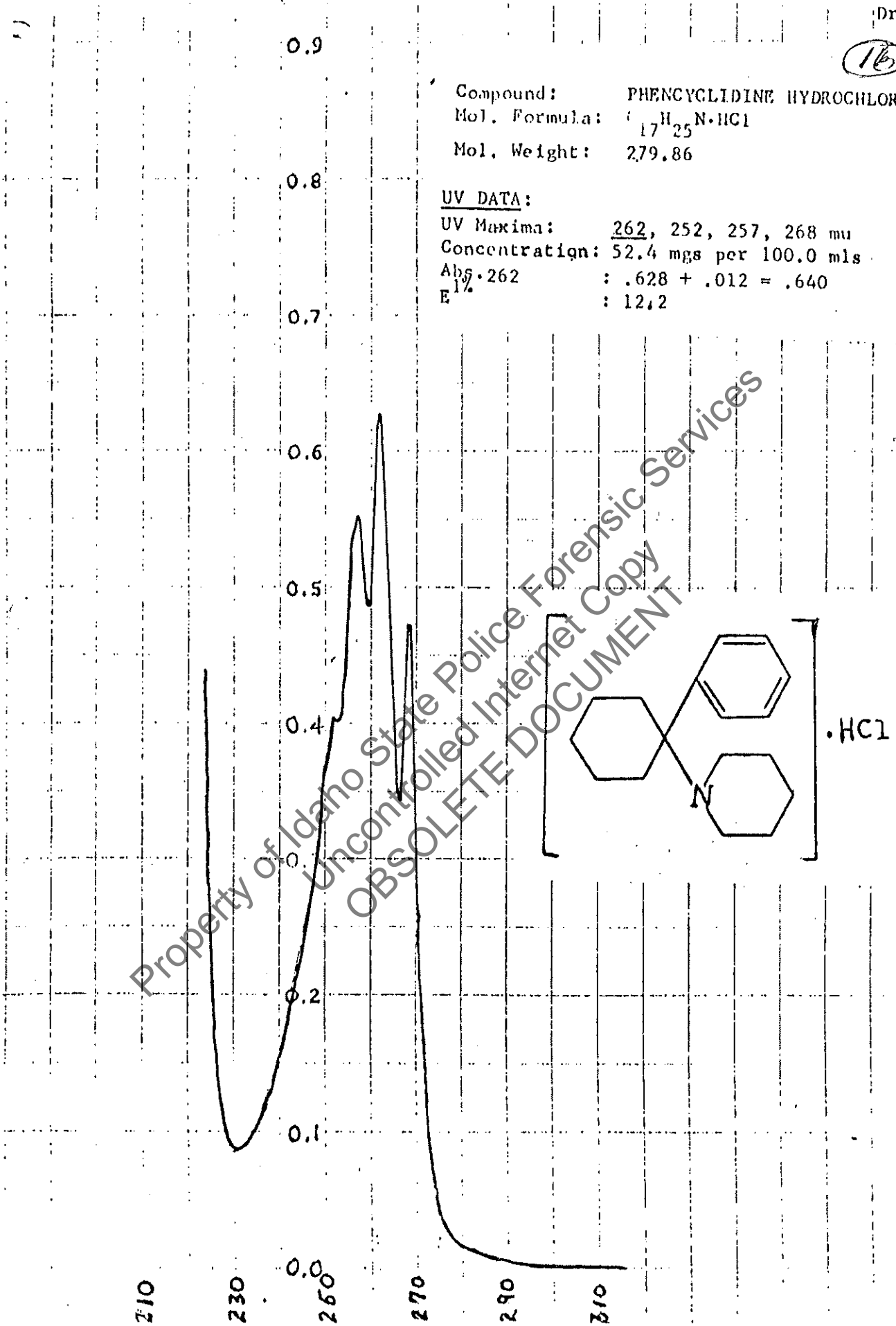




(16)

Compound: PHENCYCLIDINE HYDROCHLORIDE
Mol. Formula: $C_{17}H_{25}N \cdot HCl$
Mol. Weight: 279.86

UV DATA:
UV Maxima: 262, 252, 257, 268 mu
Concentration: 52.4 mgs per 100.0 mls
$Abs_{1\%} \cdot 262$: .628 + .012 = .640
$E^{1\%}$: 12.2



DRUG TYPE Hallucinogen

METHODOLOGY

The Identification of N-ethyl-1-phenylcyclohexylamine hydrochloride (Cyclohexamine)

by

R. P. Barron
Chemist
Special Testing and Research Laboratory
DRUG ENFORCEMENT ADMINISTRATION

This communication reports data utilized in the structural elucidation and confirmation of a recently encountered drug, N-ethyl-1-phenylcyclohexamine hydrochloride (Figure 1) on the illicit market. The compound is described in U.S. Patent 3,097,136 issued to Parke, Davis and Company[1] and included in "Psychotropic Drugs and Related Compounds[2]" under the names CI 440 and Cyclohexamine.

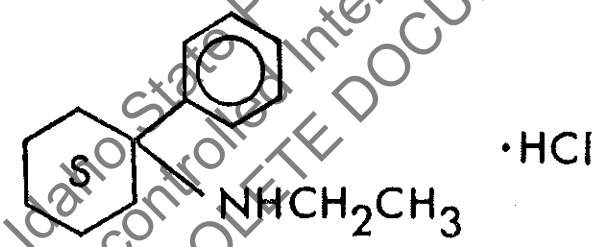


FIGURE 1

Identification Characteristics

UV: maxima at 236, 257, 262 and 268 millimicrons

IR: absorption peaks at 2940, 1582, 1463, 780, 701 and 624 cm^{-1} by KBr. See Figure 2 for spectrum.

MS: Molecular ion observed at m/e 203 and base peak at m/e 160. See Table 1 for spectrum.

NMR: See Figure 3 for spectrum.

(18)

AN OBSERVATION ON THE ULTRAVIOLET ABSORPTION CURVE OF MIXTURES OF LSD AND PCP

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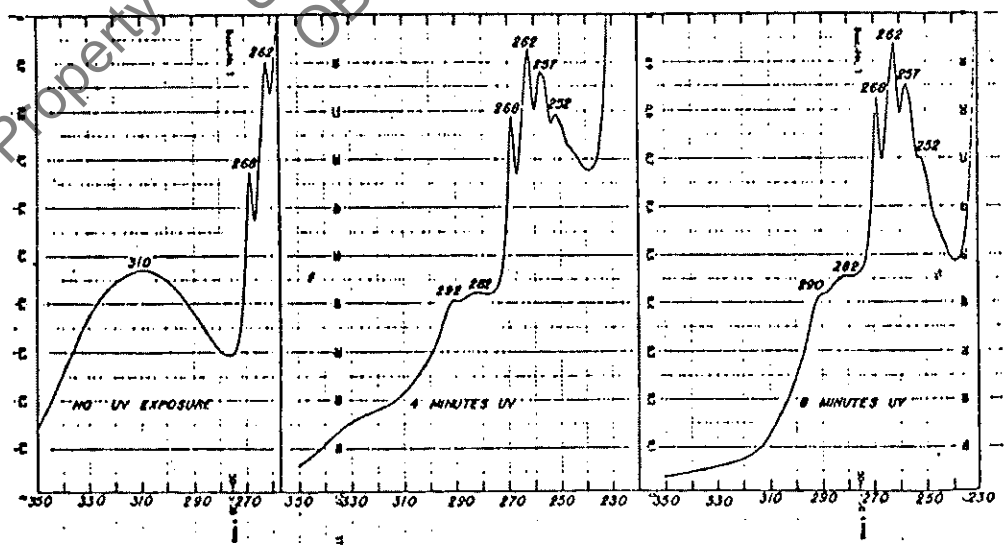
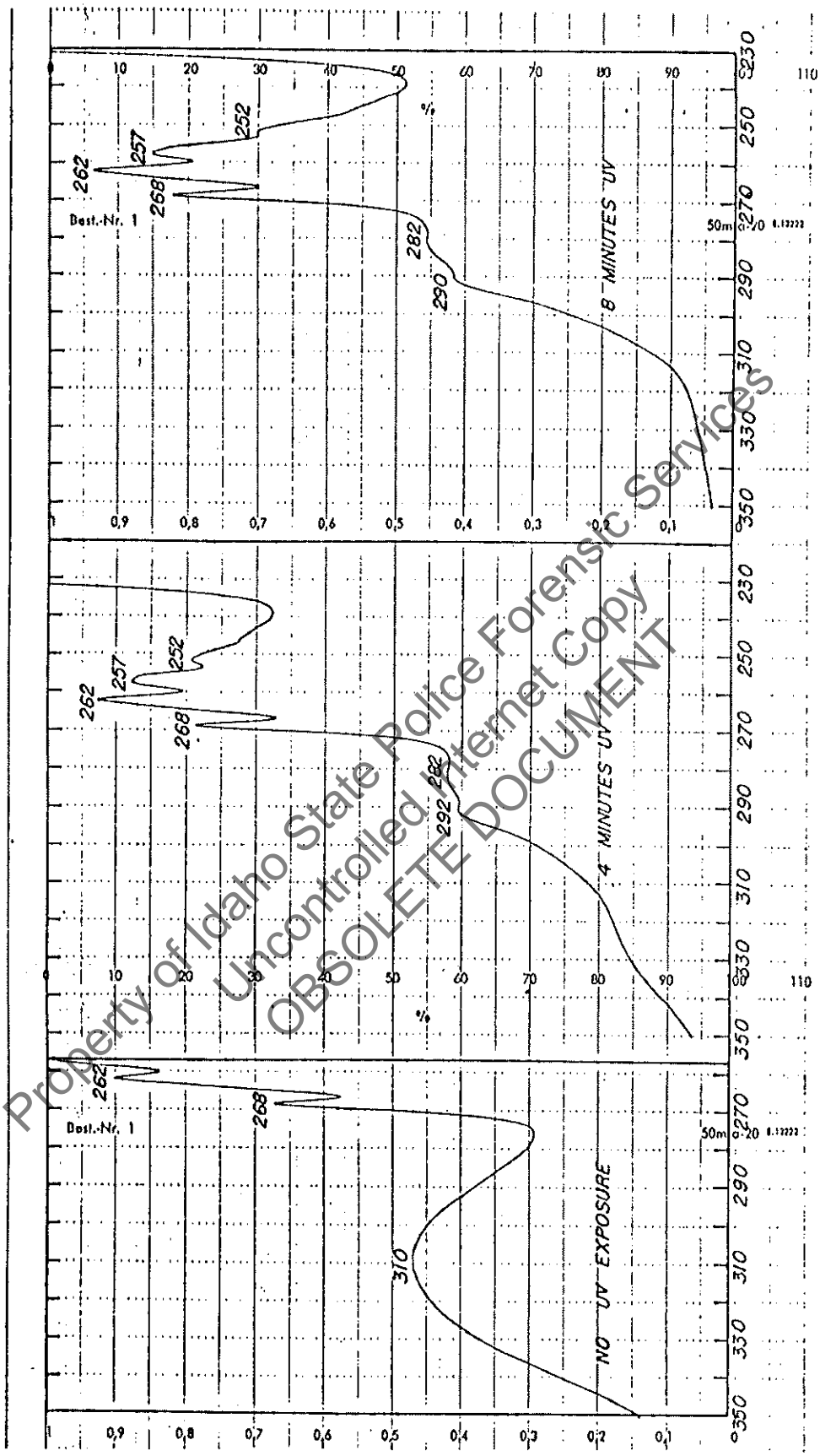
Submitted for examination were several tablets, light tan-pink in color, biconvex, diameter 6.4 mm, thickness 3.2 mm at center, average weight 160 mg.

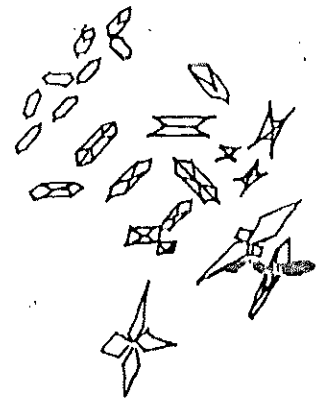
One of the tablets was powdered and extracted three times with 10 ml chloroform each. The combined extracts were filtered and evaporated to dryness at 25° C, by blowing air against the surface of the liquid in a porcelain dish. The residue was token up in 3 ml 0.1 N sulfuric acid and the UV spectrum was recorded with a Zeiss DMR 21. In addition to the LSD maximum at 310 nm, there were two peaks at 268 and 262 nm. The solution was exposed to long wave UV light for 4 min, and the UV scan was repeated. As expected, there was a lowering of the LSD maximum and a shift toward 292 nm. Unexpectedly, however, there occurred also a marked decrease in the LSD absorbance in the shorter wavelength UV range, resulting in the emergence of a complete PCP absorbance curve. This curve was brought out even better after an additional 4 min. UV exposure.

The spectral behavior of LSD after UV radiation offers a simple means of demonstrating the presence of PCP in preparations containing LSD.



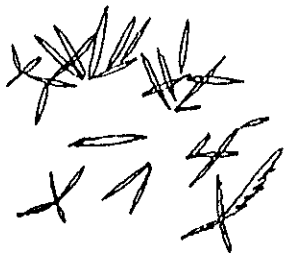


Phencyclidine HCl
$HAuCl_4$ 5% Aqueous (HOAc) soln. Cg.

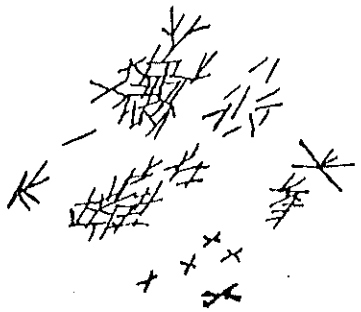


white-grey to light yellow.

Cocaine N.F.
$HAuCl_4$ 5% Aqueous Cg.

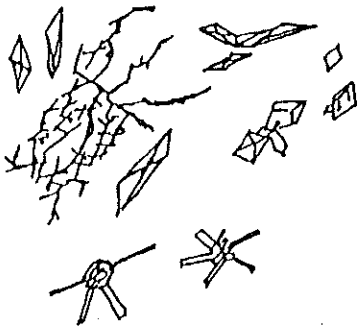


Cocaine HCl
$HAuCl_4$ 5% Aqueous Cg.

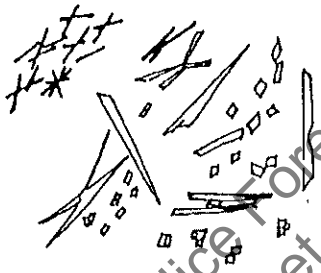


(RG)

Phencyclidine HCl - Cocaine HCl
$HAuCl_4$ 5% Aqueous (CHOAc) soln Cg.

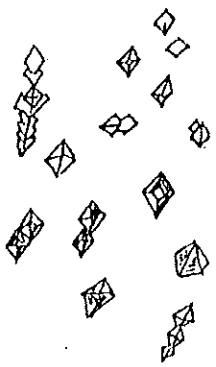


Phencyclidine HCl.
$HAuBr_4 - 2H_3PO_4 - (2:3)H_2SO_4$ Cg.



yellow-orange brown.

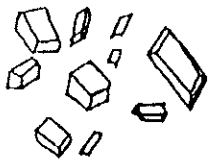
Phencyclidine HCl
$HAuBr_4$ - (HOAc) - $(2:3)H_2SO_4$



Pyrimada
yellow to

Phencyclidine HCl
$HAuCl_4$ - $(1:1)H_2SO_4$

clear.



Ic - clear to multi colored.

(20)

DEA LABORATORY NOTES

DATE

NO.

DRUG TYPE Thiophene Analog of Phencyclidine

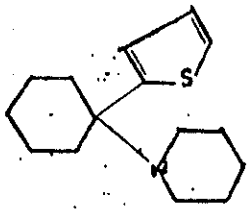
METHODOLOGY Microcrystalline, GC, TLC, UV, IR, X-RAY

PART I: ANALYTICAL DATA ON THE THIOPHENE ANALOG OF PHENCYCLIDINE

by Jose J. Alvarez

INTRODUCTION

Infrequently encountered controlled substances, especially those which are sporadically reported in the chemical literature, are difficult to analyze and identify routinely. If the usual color tests are employed and the concentration of the controlled substance is low, the controlled substance may be overlooked. An example of such a controlled substance is the thiophene analog of phencyclidine, 1- [1-(2-thienyl)cyclohexyl] piperidine (TCP):



$C_{15}H_{23}NS$ = 249.4

The following presentation on TCP was initiated to report the information in the literature and to report the results obtained during the laboratory investigations. Due to the length of one comprehensive paper, the study will be presented in two parts. This paper will cover microcrystalline tests, background, and various instrumental and chemical analyses; part two will cover the mass spectrometric analyses.

BACKGROUND

TCP is prepared in a manner similar to phencyclidine, PCP (1-5). It is synthesized from 2-thienylmagnesium bromide (prepared by reaction of 163 g 2-bromothiophene and 24 g Mg in 500 ml EtOH) and 1-piperidinocyclohexanecarbonitrile (4). The latter compound may be prepared as described by Kalir, et al. (5).

It appears that the synthesis was initiated to study its pharmacological activity, as related to PCP, which is used as a veterinary tranquilizer. Various papers have appeared in the literature in regards to its pharmacological activity. For brevity, some Chem. Abstr. references are cited (6). Kalir, et. al. (5) studied a series of N,N-substituted 1-arylcyclohexylamines and found TCP to be the most active in tests conducted for psychopharmacological activity. Also, to be noted is the presence of a positional isomer of TCP as reported by Mousseron, et. al. (7): 1-[1-(3-thienyl)cyclohexyl] piperidine.

TCP was added to the Controlled Substances Act in 1975 - as a Schedule One drug (8). It has been encountered on several occasions; Microgram has reported its appearance throughout the country, as input was received from the various laboratories (9).

EQUIPMENT AND REAGENTS EMPLOYED

Microcrystalline tests were performed with two reagents: 10% solution of KI in distilled water and 10% solution of NH_4SCN in distilled water. Both of these reagents are quite sensitive to PCP and TCP. Both reagents are very stable and can be used for a long period of time. The photomicrographs designated Figure 1 through Figure 4 were taken with the Nikon Microflex Model AFM (automatic photomicrographic attachment) Microscope, using ordinary transmitted light and a magnification of 150x.

Gas chromatographic data was obtained with the Perkin-Elmer 3920 Gas Chromatograph. Parameters are as follows: carrier gas - nitrogen @ 55 ml/min, injector and detector temperatures - 250 degrees C, chart speed - 1/2 in/min, GC interfaced with a Perkin-Elmer M-1 Computing Integrator, attenuation - x32, range - x10. Two columns were employed. Both were 6' x 1/4" glass columns; packing as follows: 3% OV-1 on 100/120 Gas Chrom Q and 10% OV-101 on 100/120 Chromasorb WHP. The oven temperatures employed for the columns were 180 and 270 degrees C, respectively.

Ultraviolet spectra were obtained with the Cary 15 UV Spectrophotometer (consult Figure 6 for absorbance and concentration data).

Infrared spectra (Figures 7-10) were obtained with the Perkin-Elmer 467 Grating Infrared Spectrophotometer.

The X-ray diffraction pattern was obtained with the Siemens X-Ray Diffraction System, which is equipped with a scintillation counter, using nickel-filtered copper radiation (Cu K<1 = 1.540 Angstroms). Recordings were obtained on a precision line recorder scanning at 1 cm/min.

The TCP standard was obtained from Parke, Davis & Company.

SOLUBILITY AND MELTING POINT

A. As the HCl salt - soluble in water, chloroform, methanol/ethanol

B. Melting points, as the HCl salt (as reported by the literature):
   1) 233 - 236 degrees C with transition at 182-3 degrees C (3,4)
   2) 230 - 235 degrees C (5)
   3) 200 - 203 degrees C subl. (2)

CHEMICAL COLOR TESTS

Several reagents were tested for chemical color sensitivity, but few produced a discernible color. Other possible tests were evaluated, but few were of total value because of the time and equipment involved (10).

The two color tests, used extensively for opium alkaloids/derivatives, are the preferential tests - the Marquis (10 drops formaldehyde solution in 10 ml conc. sulfuric acid) and the Mecke (0.25 g selenious acid in 25 ml conc. sulfuric acid), as reported by Heagy (1). Of the two, the Mecke's reagent is the most sensitive.

A. Marquis - effervescence, turns to a gray-orange and slowly to gray-green.
B. Mecke - effervescence, turns to a yellow-green, slowly to a yellow-blue/green, and then to a deep blue.

MICROCRYSTALLINE TESTS

Grind and mix a portion of the sample well; on a microscope slide place a small drop of distilled/deionized water. Dissolve a minute portion of the sample in the water and place a small drop of the reagent adjacent to the sample solution. Run the solution and the reagent drops together with a spatula. For best results, do not stir the mixture.

The crystal formation will occur within a minute; view the crystals through a microscope, preferably a polarizing microscope at 125x. As with all other microcrystalline tests, it is best to run a test with a standard right before or after the sample run. This should be done until enough familiarization is obtained with the particular crystal formations.

Figures 1 and 2 are the crystal formations of PCP and TCP, respectively, with NH_4SCN. Figures 3 and 4 are those of TCP and PCP, respectively, with KI. Of the two reagents, KI gave the more consistent crystal formation with the two compounds. With NH_4SCN, the PCP crystal formation was also consistent. Successful results have been obtained with PCP and TCP samples which were quantitated at three to five percent.

With NH_4SCN, the crystal formation of TCP was not consistent - the concentration is more critical. Aside from the crystal formation in Figure 2, the formation of long slender needles is prevalent. These needles are similar to Fulton's large, coarse, Class 1 needles (1). To enhance the proper microcrystal formation in low percentage TCP samples, a preliminary $CHCl_3$ dissolution was performed. This was done by dissolving a few milligrams of sample in $CHCl_3$, filtering the insolubles, and evaporating the $CHCl_3$ to dryness. The residue was then used for the microcrystalline work. This preliminary cleanup improved the consistency of the crystal formation.

GAS CHROMATOGRAPHY

The results of the gas chromatographic investigations are represented in Figure 5. The quantitation of PCP can be achieved successfully with an OV-1 column. There is noticeable decomposition, but negligible as compared to the OV-101. TCP is a compound difficult to quantitate by GC because of instability to the phases. Major decomposition occurs in both columns. These decomposition products will be discussed in part two of these TCP papers.

Bailey, et. al. investigated the employment of SE-30 and OV-17, 225, and 7 columns (2). They report decomposition in all four, particularly on OV-7 and SE-30.

THIN-LAYER CHROMATOGRAPHY

TLC investigations were not initiated in this laboratory.

The major work by Bailey, et. al. reports the investigations of nine solvent systems (2). Shulgin and Helisten have also investigated aspects of TLC analyses (12). Their work covered a differentiation of PCP, TCP, and the percursor, 1-piperidinocyclohexanecarbonitrile, using a benzene, acetone, and pyridine (16:8:1) solvent system.

ULTRAVIOLET SPECTRA

Figure 6 is the ultraviolet spectrum of TCP in the respective solvents. It is a strong absorber with a maxima at 232.5 nm (EtOH, 0.1N HCl). This is in good agreement with the works of Heagy (1) and Bailey, et. al. (2).

INFRARED SPECTRA

Figure 7 is the infrared spectrum of the Parke-Davis TCP hydrochloride standard. Figures 8-10 are spectra of a sample received at the laboratory.

Figure 8 represents the spectrum of the isolated TCP, converted to the HCl salt. It was isolated by dissolving the sample in $CHCl_3$ and filtering of the insoluble material. A 0.1N HCl extraction was performed on the $CHCl_3$ solution. The $CHCl_3$ layer was again filtered and $CHCl_3$ evaporated to approximately two ml. A mini-column of alumina was prepared and the two ml. were eluted with an additional ten ml. $CHCl_3$. The free base was converted to the HCl salt and pressed into a KBr pellet.

Figure 9 is the spectrum of the same TCP sample with a preliminary cleanup. The sample was dissolved in distilled water, made alkaline with sodium bicarbonate, and extracted with $CHCl_3$. The $CHCl_3$ extract was filtered and approx. five ml. of a 1% HCl solution of MeOH added; the mixture was taken to dryness. The HCl salt in a KBr pellet was scanned. The "impurity"/TCP infrared spectrum was obtained.

Figure 10 is the spectrum of the "impurity" in the sample. The 0.1N HCl solution or layer (obtained in the extractions to isolate the TCP for the spectrum of Figure 8) was made alkaline and extracted with $CHCl_3$. The HCl salt was prepared in the same manner as that for the spectrum of Figure 9. The infrared spectrum identifies piperidine as the "impurity".

X-RAY

Figure 11 depicts the diffraction pattern of TCP hydrochloride. It is presented in order of increasing 2Ø degrees versus peak intensity, as is usually obtained on the recorder. TCP hydrochloride was scanned from 3 to 40 2Ø degrees and that which is depicted in Figure 11 represents the area of interest for this compound. The 2Ø degrees of interest are as follows: 10.3, 14.0, 14.8, 15.4, 19.2, 20.7, 21.7, 22.8, 23.5, 24.5, 27.0, and 28.1. This is in good agreement with the X-ray study conducted by Berens (13).

ACKNOWLEDGMENTS

I would like to thank Mr. Carlos L. Castillo and Mr. Wayne Moody for their assistance in the drawings and in the photomicrographic work, respectively. Also, my sincere thanks to Mr. Buddy Goldston and Mr. Charles Teer for their valuable assistance and constructive criticism during the preparation of the paper.

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(5) Kalir, A.; Edery, H.; Pelah, Z.; Balderman, D.; and Porath, G., J. Med. Chem., 12(3), 473(1969)
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(8) Microgram, VIII(7), 101(1975)
(9) Microgram: V(10), 105(1972); VI(2), 16(1973); VI(6), 80(1973); VI(11), 168(1973); VII(5), 52 (1974); VII(10), 114(1974); VIII(5), 59, 60(1975); VIII(9), 126(1975); IX(1), 1(1976); and IX(4), 53(1976)
(10) Feigl, F., Spot Tests In Organic Analysis, Elsevier Publishing Co., 1966, New York
(11) Fulton, C.C., Modern Microcrystal Tests for Drugs, Wiley-Interscience, 1969, New York, p. 425 (1-02)
(12) Shulgin, A. T. and Helisten, C., Microgram, VIII(10), 149(1975) and VIII(11), 171(1975)
(13) Berens, L. J., personal communications

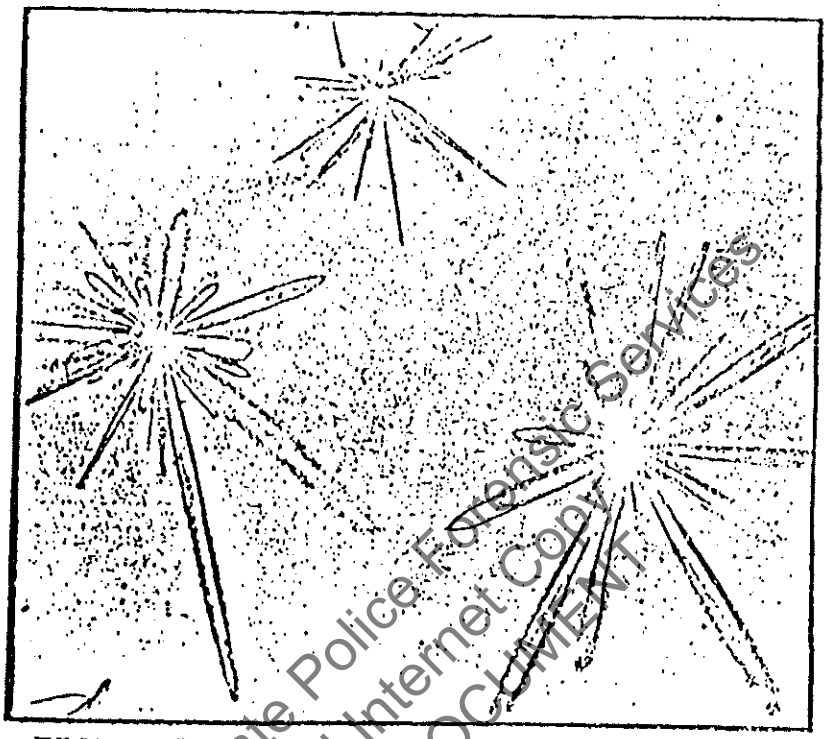


FIGURE 1: PCP/NH_4SCN 150×

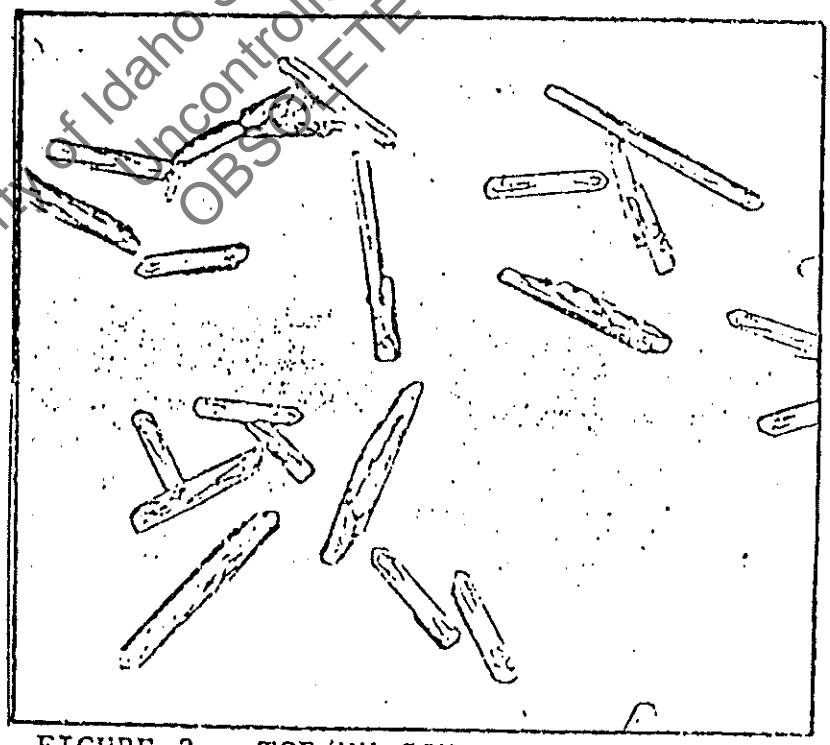


FIGURE 2: TCP/NH_4SCN 150×

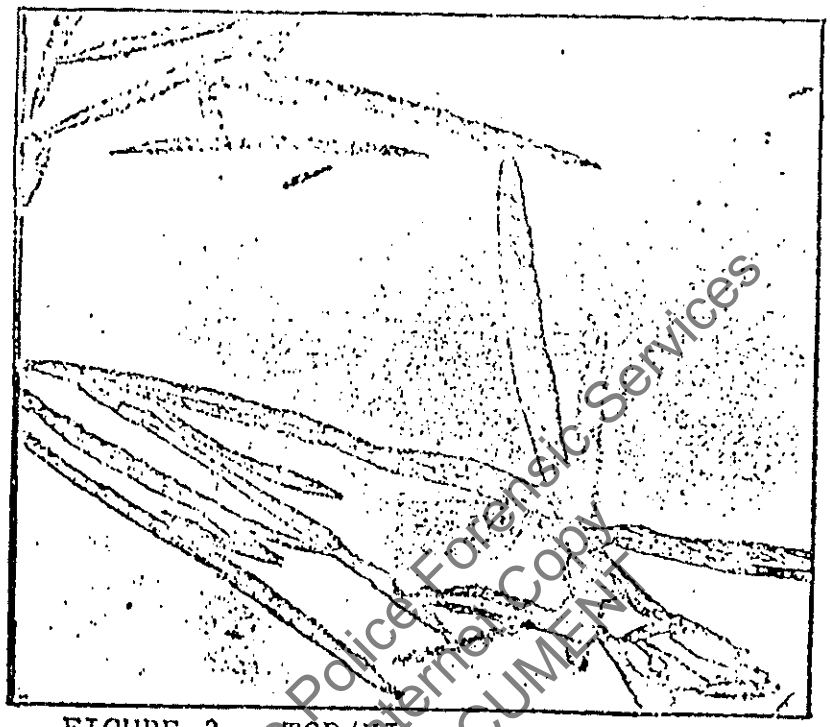


FIGURE 3: TCP/KI 150x

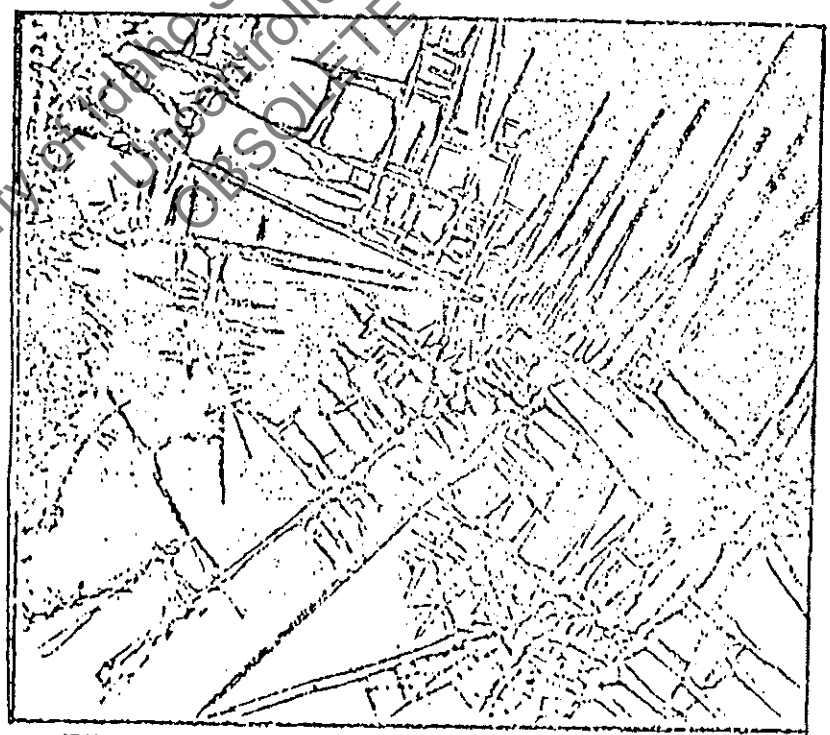


FIGURE 4: PCP/KI 150x

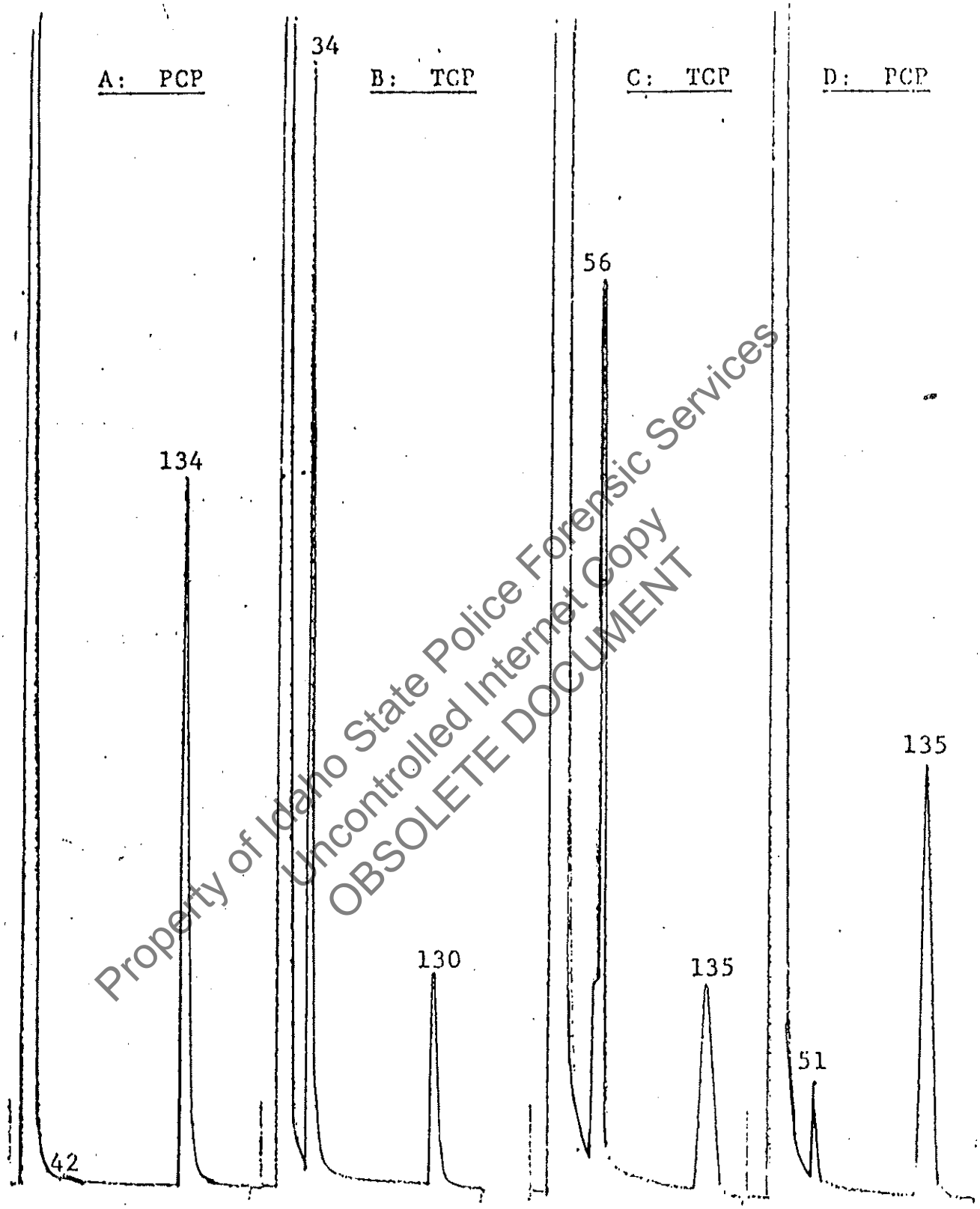
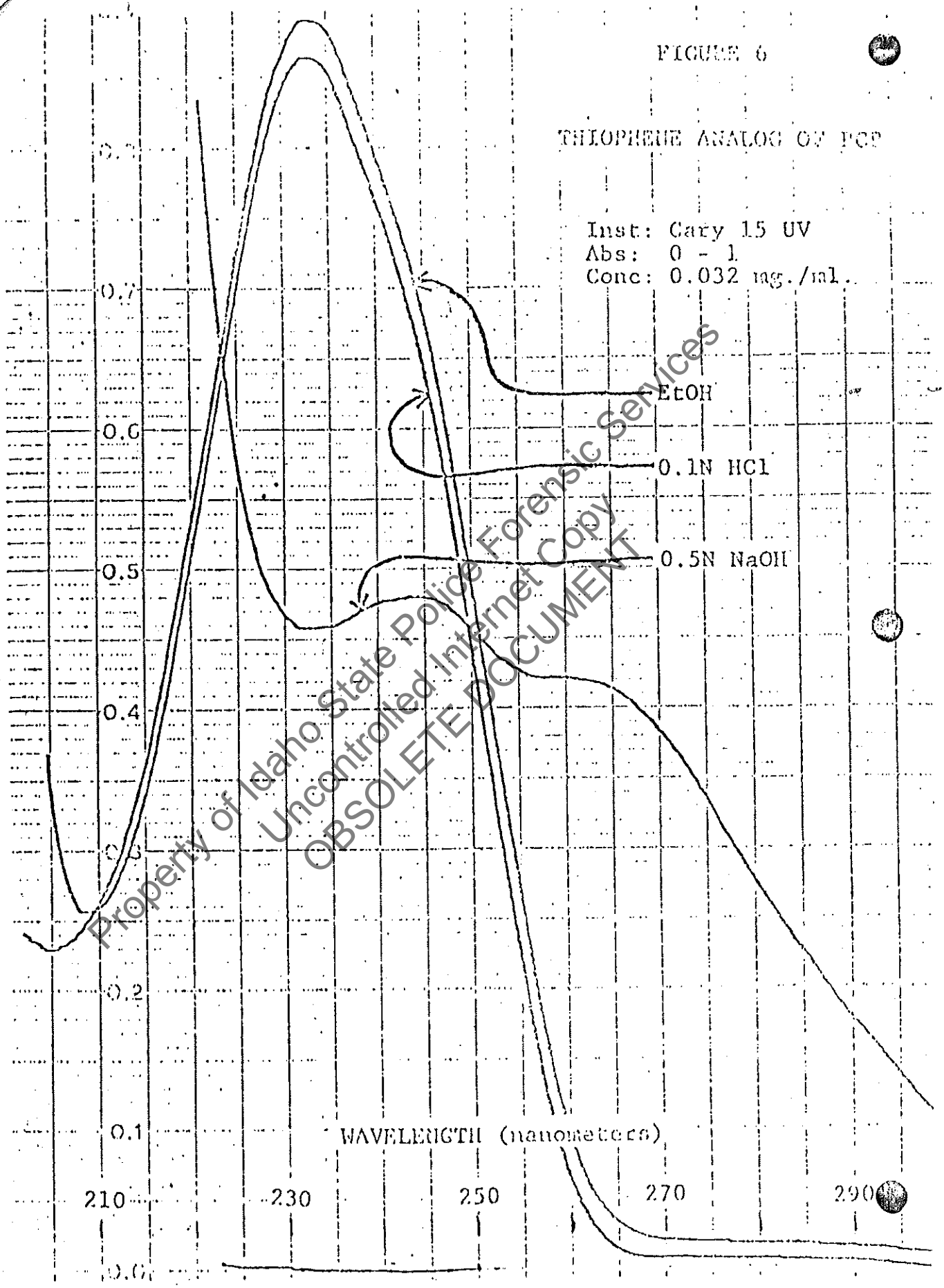


FIGURE 5: Chromatograms A and B were obtained with the 3% OV-1 column at 180 degrees C; C and D, with the 10% OV-101 at 270 degrees C. Numbers above the peaks denote the retention time in seconds.

FIGURE 6

THIOPHENE ANALOG OF PCP



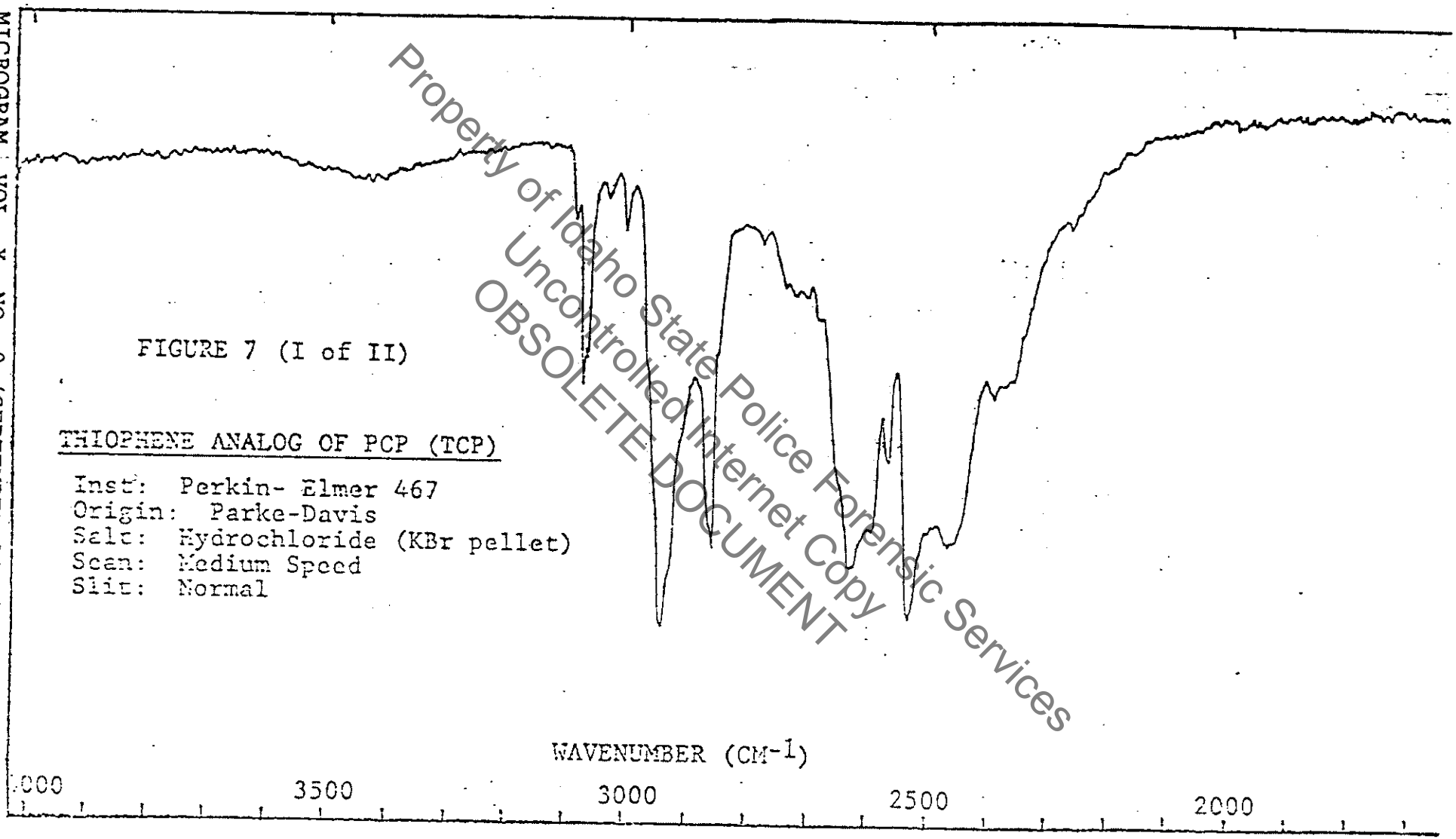


FIGURE 7 (I of II)

THIOPHENE ANALOG OF PCP (TCP)

Inst: Perkin- Elmer 467
Origin: Parke-Davis
Salt: Hydrochloride (KBr pellet)
Scan: Medium Speed
Slit: Normal

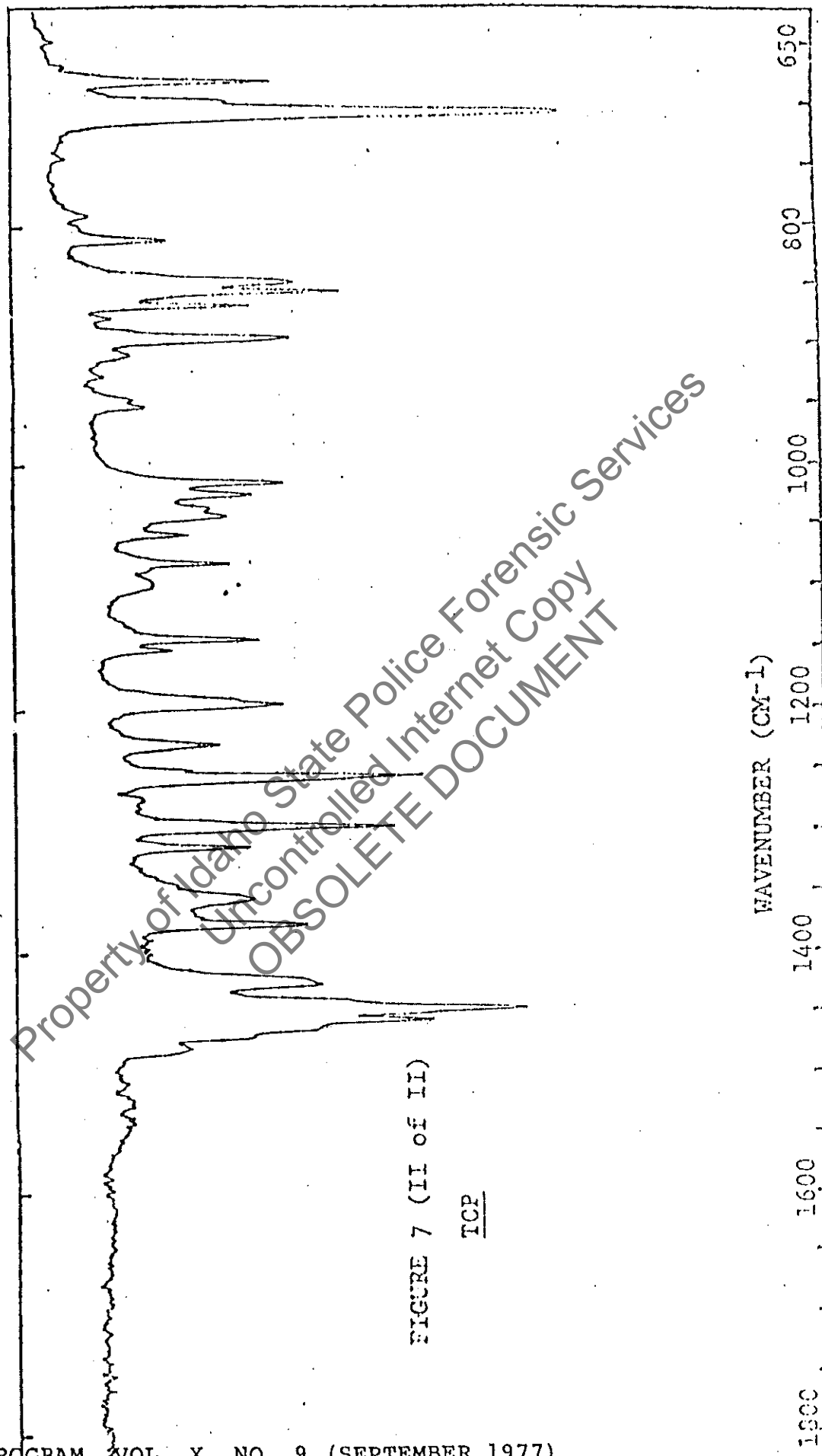


FIGURE 7 (II of II)

TCP

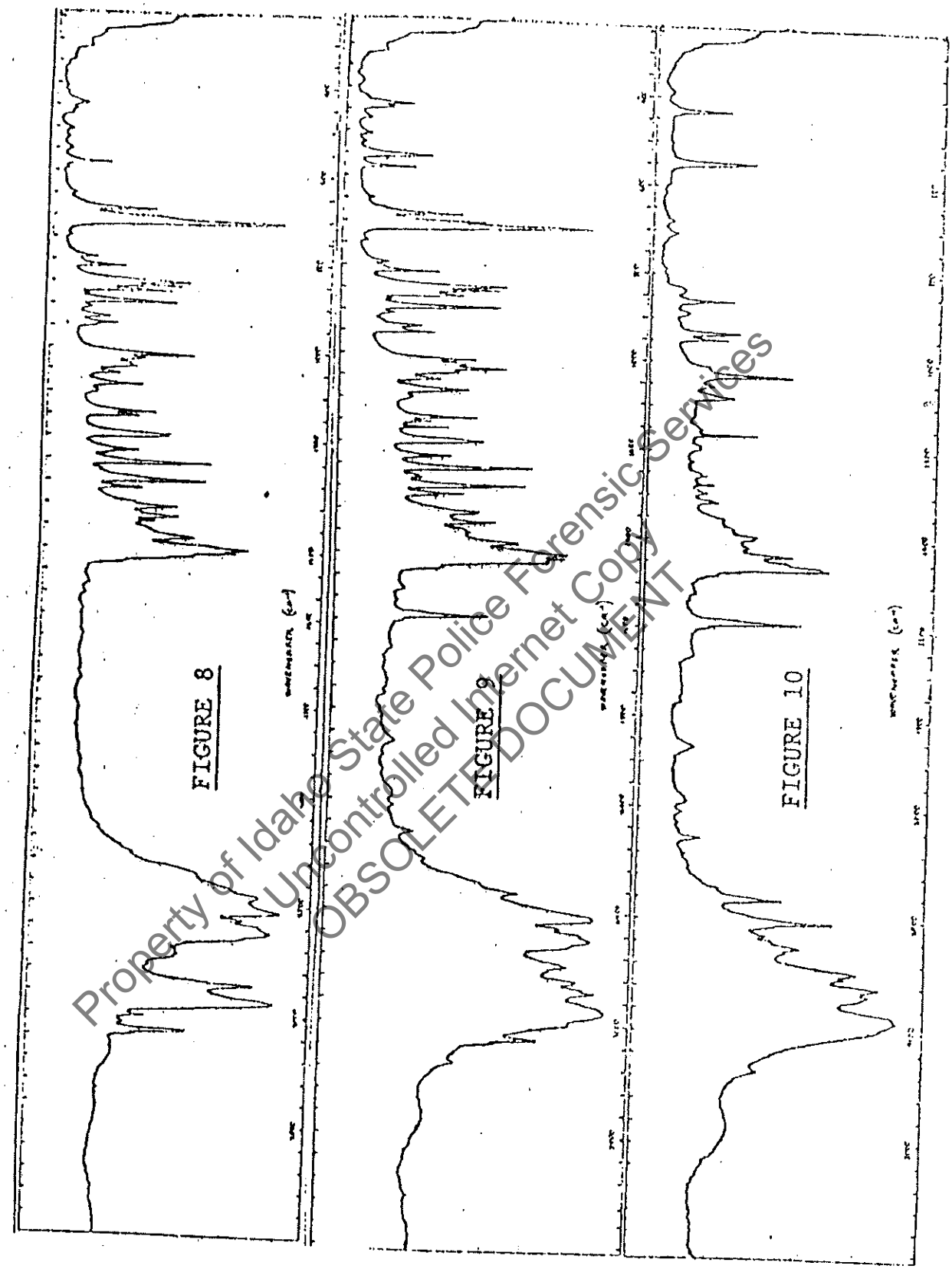


FIGURE 8

FIGURE 9

FIGURE 10

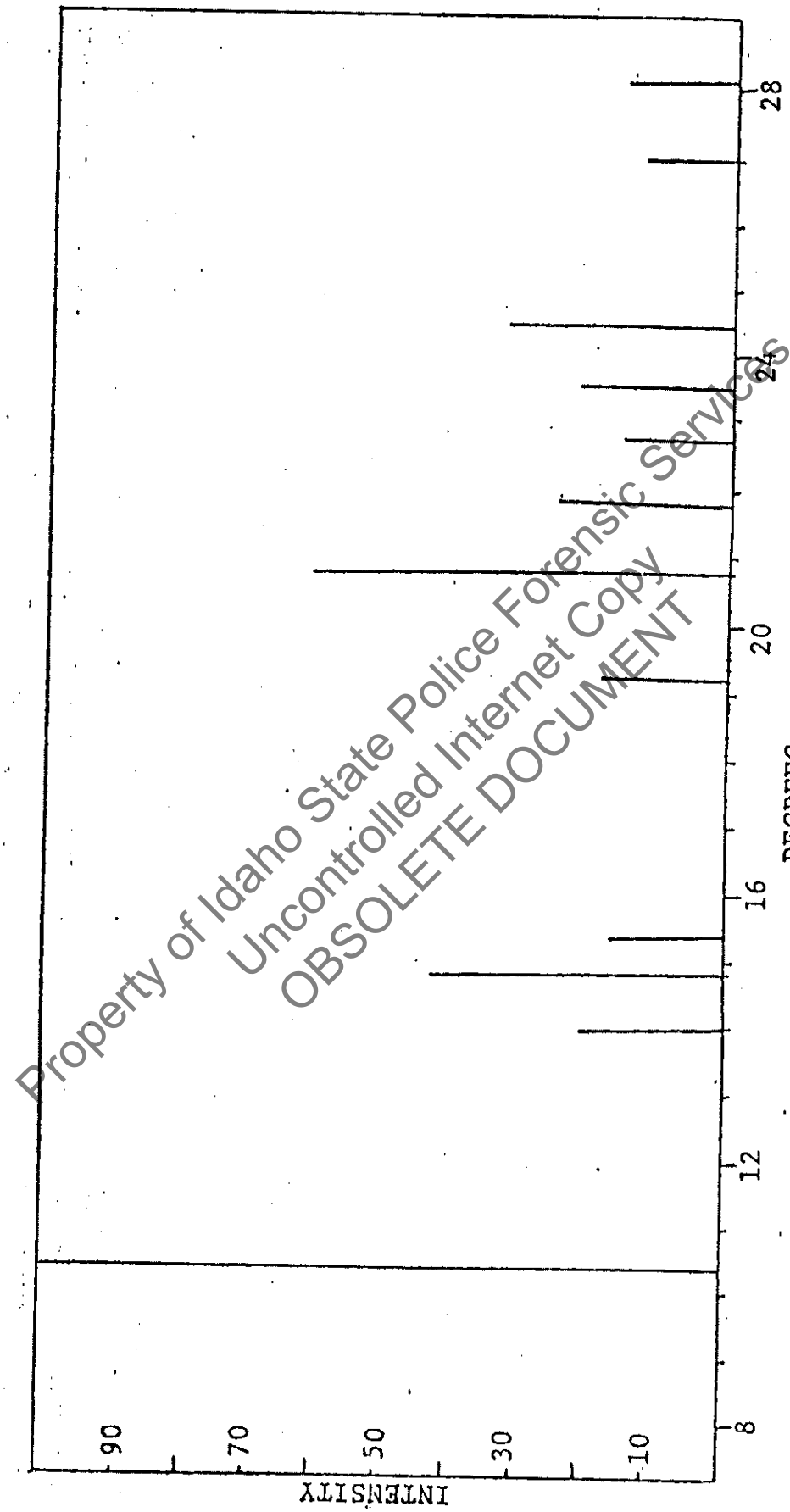


FIGURE 11: X-RAY DIFFRACTION PATTERN OF TCP·HCl

(21)

Identification of Mixtures of Phencyclidine Hydrochloride and Piperidine Hydrochloride

by

Victor A. Folen
Forensic Chemist
Special Testing and Research Laboratory

Several recent exhibits have been found to contain phencyclidine hydrochloride in combination with piperidine hydrochloride. In some of the mixtures phencyclidine was present both as the hydrochloride and as the free base. Piperidine was probably present as an unreacted precursor in the synthesis of phencyclidine. The material was either pale yellow or tan in color, and some of the mixtures were hygroscopic. Such samples gave no color with Marquis solution (conc. H_2SO_4-formaldehyde 10:1) but there was a rapid effervescence. There was no effervescence, however, with dilute HCl, ruling out the possible presence of carbonate.

Crystal Tests

For the tentative identification of phencyclidine, one of the routine crystal tests may be used. Dissolve a very small amount of sample (ca. 0.1 mg.) in 6-10% acetic acid on a microscope slide. Touch the solution to an adjacent drop of gold chloride solution (5% aq.). There is an immediate dense formation of microscopic droplets, followed by the gradual development of typical crystals[1], rectangular, envelope-shaped, and of low birefringence. If the solution in acetic acid is too concentrated, atypical crystals will result, being in the form of sheaves, or a branched, serrate mosaic, both types of low birefringence. The crystal test for piperidine involves volatization when treated with alkali.[1] Place a small amount of sample in a depression slide and add one-two drops of 10% NaOH. Place a microscope slide holding a drop of gold chloride solution (5% aq.) over the depression. Piperidine volatilizes quickly, with rapid crystallization in the drop of gold chloride solution of small, highly birefringent rods and clusters of three or four blades of unequal length.

Confirmation of the Presence of Phencyclidine and Piperidine

To separate the hydrochloride salt of phencyclidine from piperidine hydrochloride, advantage may be taken of their partitioning characteristics in water and chloroform.[2]

Dissolve about 200 mg. of sample in water in a separatory funnel. Extract phencyclidine hydrochloride three times using 10 ml. portions of chloroform, collecting the chloroform in a 50 ml. beaker; make the solution in the separatory funnel basic with Na_2CO_3. Extract piperidine base using three 10 ml. portions of chloroform, again collecting in a 50 ml. beaker. Add concentrated HCl (5-10 drops) to the beaker containing piperidine base and evaporate the contents of both beakers to dryness on a water bath under a stream of air. Obtain infra-red spectra for the isolated components.

Notes

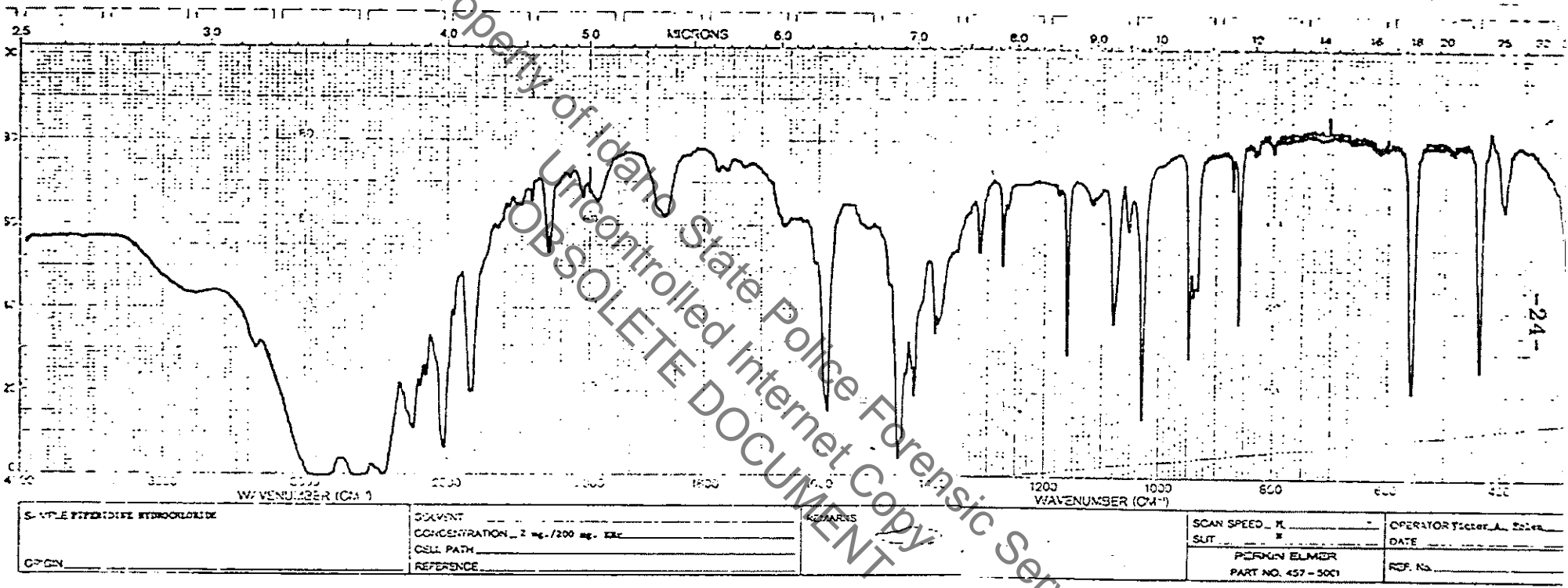
Attached is an infra-red spectrum for piperidine hydrochloride.

After evaporation of the chloroform from the extract containing piperidine hydrochloride, some moisture will persist, which can be removed by drying overnight in a dessicator.

References

1. Koles, Joseph E., Special Testing and Research Laboratory, BNDD - private communication.

2. Kram, Theodore C., Special Testing and Research Laboratory, BNDD - unpublished data.



SAMPLE PIPERIDINE HYDROCHLORIDE

OPCW

SOLVENT

CONCENTRATION 2 mg./200 mg. KBr

CELL PATH

REFERENCE

REMARKS

SCAN SPEED

SLIT

PERKIN ELMER
PART NO. 457 - 5001

OPERATOR Victor A. Soles

DATE

REF. No.

VI 511

22

DRUG PURIFICATION BY ION PAIR EXTRACTION

James M. White
Melvyn C. Kong
Orange County Sheriff's Department
Laboratory of Criminalistics
Santa Ana, California

Many drug preparations submitted to Forensic Laboratories require separation of the restricted substance present before identification and quantitation can be made by many of the conventional tests used for this purpose (e.g., Ultra-violet absorption, Infra-red absorption, crystal tests, etc.)

Methods routinely used to effect this separation include solvent-solvent extraction, column chromatography, thin-layer chromatography and gas chromatography.

Gas and thin-layer chromatographic separations are often disadvantageous because of analysis time and their inability to separate effectively the milli-gram quantities of substance often needed for subsequent analysis.

Column chromatographic methods, although highly selective, do not provide the rapid separation often needed in a high volume forensic drug laboratory.

Certain drugs form an ion pair in an HCl Solution. This ion pair is more soluble in chloroform than in the aqueous acid. Since aqueous acid and chloroform phases do not appreciably mix, this can provide an effective

means of separating certain drugs from adulterants, contaminants or other drugs. (See Analytical Chemistry, Vol. 39, No. 11, September 1967, pgs. 1283-1287).

Abstract

A Beckman DK-2A Spectrophotometer was used to quantitate recovery of drugs extracted under ion-pair and pH (saturated bicarbonate) conditions. Overall, bicarbonate extraction was found more efficient but less selective. The ion pair extraction is less efficient but more selective, lending itself to mixtures of drugs.

Procedure

I Ion Pair

The drugs to be evaluated were prepared in 10% HCl (v/v) solution to obtain an absorbance between .3 and .8. Five ml of this drug solution were pipetted into a 60 ml separatory funnel. Twenty five ml of chloroform were added; the mixture shaken for 1 minute and then allowed to separate for 2 minutes. The chloroform layer was drawn off and filtered. (Whatman No. 1 filter paper was used except for "LSD", "PCP "and "TCP "where glass wool was used). A second 25 ml of $CHCl_3$ was added and the process repeated. To the resulting 50 ml $CHCl_3$ was added 5 ml of 5% $NaHCO_3$, shaken for 1 minute and allowed to separate for 2 minutes. The $CHCl_3$ was drawn off to a 50 ml stoppered graduate cylinder. To this $CHCl_3$ was added 5 ml of 0.2 N

H_2SO_4 and shaken for 1 minute and allowed to separate for 2 minutes. This 0.2 N H_2SO_4 was then measured for absorbance in the appropriate ultraviolet region on the DK-2A.

II Bicarbonate Extraction

Similarly, after obtaining and recording an absorbance between 0.3 and 0.8, 5 ml of drug solution (0.2 N H_2SO_4) were pippetted into a 60 ml separatory funnel. The solution was neutralized to pH 8.4 with excess solid $NaHCO_3$. To the solution was added 25 ml $CHCl_3$, the mixture shaken for 30 seconds and allowed to separate for 15 seconds. The $CHCl_3$ was drawn off. Another 25 ml of $CHCl_3$ was added and the process repeated. To the approximately 50 ml of $CHCl_3$ was added 5 ml 0.2 N H_2SO_4. This mixture was shaken for 30 seconds and allowed to separate for 2 minutes. The 0.2 N H_2SO_4 was then measured for absorbance in the appropriate ultraviolet region of the DK-2A.

Calculations

The wavelength of maximum absorbance was chosen on both plots, original and extracted.

For single drugs $\frac{\text{height extracted peak}}{\text{height original peak}}$ = % recovery = recovery coefficient

For mixtures 1 : 1 $\frac{\text{height extracted peak}}{\text{height original solution peak} \times \frac{1}{2}}$ =% recovery = recovery coefficient

Standard Deviation

$$S = \sqrt{\frac{\Sigma (\bar{x} - x)^2}{N-1}} \qquad \bar{x} = \frac{\Sigma x}{N} \qquad N = \text{Number of trials}$$

Error Analysis

There appears to be a small amount of $CHCl_3$ loss by evaporation, absorption into filter paper and solubility into aqueous phase. Adjustments were not made for the loss. Inthe direct comparison of absorbances, care was taken so the baseline coincided for original and extracted solutions. The same procedure was used in zeroing the instrument each time and the same sample cells were balanced, so the difference attributed to correcting for absolute zero absorbance is negligible.

Results

Ion pair extraction will separate cocaine at about a 25% efficiency from tetracaine, procaine and benzocaine. Other methods must be used to separate hexylcaine or lidocaine from cocaine. Ion pair will not separate "PCP", "LSD" or "TCP" from each other. Ion pair will separate meperidine, cocaine and "PCP" from nicotine.

Bicarbonate extraction in general provided higher yields but less selectivity. Apparently, the only advantage of HNO_3 over HCl in ion pair extractions would be the increased yield of cocaine (50% vs 27%).

The complete testing of HCl, H_2SO_4, $NaHCO_3$, and $CHCl_3$ solutions used in the extractions provided valuable information, such as the fact that most of the benzocaine remains in the $CHCl_3$ phase in this ion pair extraction scheme.

Table 1: Extraction Recoveries: Ion Pair vs pH Extraction

	Ion Pair (10% HCl) Extraction		Bicarb Extraction	
Drug	$\bar{x}$	s	$\bar{x}$	s
Cocaine	.272	.011	.906	.028
Hexylcaine	.822	.041	-	-
Lidocaine	.227	.013	-	-
Tetracaine	Negligible *	-	-	-
Procaine	Negligible *	-	-	-
Benzocaine	Negligible **	-	-	-
Phencyclidine ("PCP")	.851	.028	.827	.027
LSD	.791	.059	.897	.006
1-[1-(2-thienyl) cyclohexyl] piperidine ("TCP")	.830	.059	.821	.020
Nicotine	Negligible *	-	-	-
Meperidine	.887	.017	.901	.016
Amphetamine	Negligible *	-	.903	.034
Codeine	-	-	.906	.028
Methamphetamine	-	-	.917	.017
Mescaline	-	-	.915	.016
Heroin	.938	.023	-	-

Table 2: Separation of Mixtures by Ion Pair Extraction

Mixture	Results
Cocaine/Hexylcaine	No separation
Cocaine/ Lidocaine	No separation
Cocaine/ Tetracaine	Separation 26.9% cocaine
Cocaine/Procaine	Separation 24.0% cocaine
Cocaine/ Benzocaine	Separation 23.6% cocaine
PCP/LSD	No separation
PCP/TCP	No separation
TCP/LSD	No separation
Meperidine/ Nicotine	Separation 80.6% meperidine
PCP / Nicotine	Separation 87.1% PCP
Cocaine/ Nicotine	Separation 23.6% cocaine
Amphetamine/ Nicotine	No separation

* Drug remains in 10% HCl

** 18% stays in 10% HCl, remainder in $CHCl_3$

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213-974-4611

23

PHENCYCLIDINE (PCP) ON MINT

J. W. Smith and G. P. Chasteen

December 1974

SOUTHCOMBE

An effective extraction procedure for PCP on mint has been found and is herein reported. This method is more complex than the method previously suggested by J. Smith, however, it is recommended due to the possibility of obtaining an infrared spectrum. The spectrum obtained is comparable to that of the primary standard.

PROCEDURE

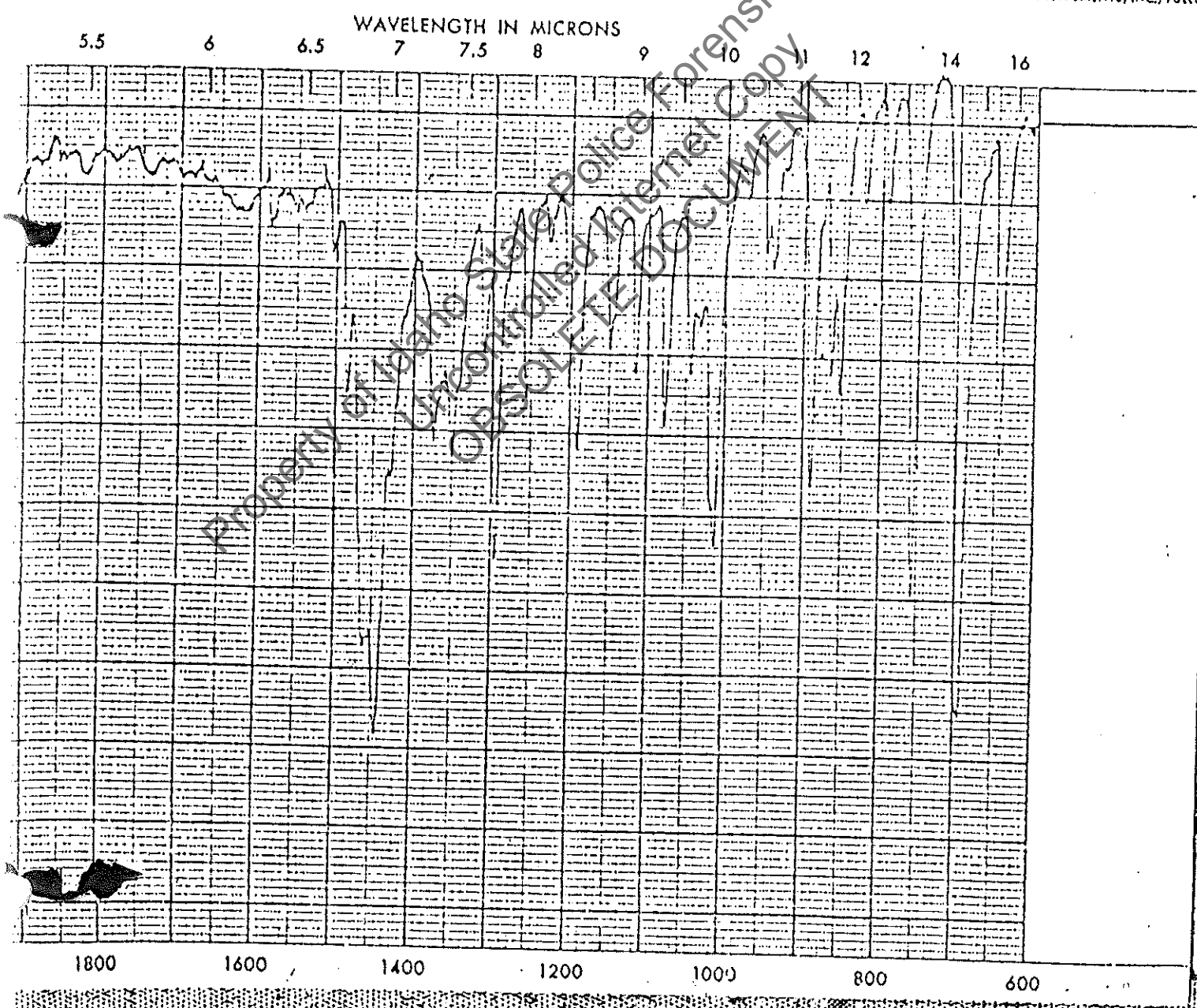
Place approximately 1 gram of plant material in a 13 X100 test tube. The method should work well with smaller amounts. Add enough conc. NH_4OH to wet the material, approx. 0.5 ml. Fill the remainder of the test tube with methylene chloride and thoroughly mix by shaking or stirring with a wooden stick. Remove the methylene chloride and extract the plant material again with methylene chloride. The methylene chloride solution will be green in color.

Prepare an alumina column in either a column or pipette (both have been used successfully). Run the methylene chloride through the column and wash with excess methylene chloride until the colored bands are well into the alumina. The methylene chloride should be either amber or colorless at this point.

Evaporate the methylene chloride to approximately 10 ml and extract with 0.1 N HCl. Obtain an ultraviolet spectrum on the acid layer. The UV spectrum should be fairly clean and typical for PCP.

Add Na_2CO_3 to make basic and extract with methylene chloride. Evaporate the methylene chloride adding approx. 2 ml methanol with 1 drop conc. HCl at first and again when near dryness. The previous mentioned infrared spectrum was run directly on this product.

Crystals, GC and TLC can also be run using this product. It will be noted that our present systems 3, 5, and 6 do not separate PCP and its thiophene analog ~~error~~ with the utilization of multiple development.



DEA LABORATORY NOTES

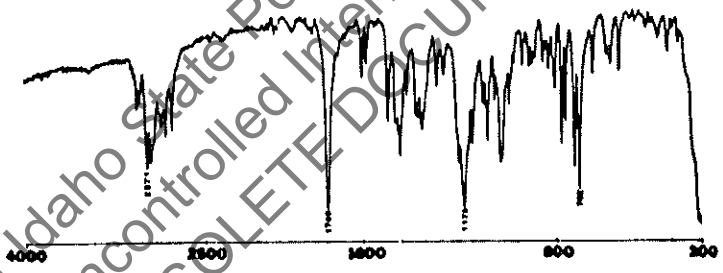
IDENTIFICATION OF PROPOXYPHENE IN MIXTURES CONTAINING APC OR ACETAMINOPHEN

Katherine T. Churchill
Southeast Regional Laboratory
Miami, Florida

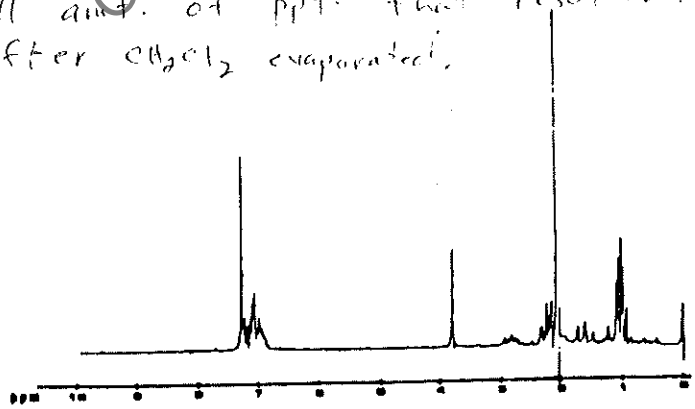
Dissolve sample in a small amount of 2N HCl (e.g. 1 capsule of Darvon Compound/10 ml 2N HCl) and filter. Make the filtrate strongly alkaline with 5N NaOH (approximately 30 ml). Allow the precipitated propoxyphene base to flocculate and settle out (approximately 45 minutes minimum). Filter and discard the filtrate. Wash the residue from the filter paper with methylene chloride, dry the methylene chloride by passing through anhydrous sodium sulfate. Evaporate to dryness, cool, and speed crystallization by scratching the beaker. The crystalline propoxyphene base produced may be used for IR or NMR and polarimetry.

IR and NMR spectra of the resulting crystalline propoxyphene base are presented.

Propoxyphene base is sol. in [illegible] ether — to remove any last contaminants I add pet. ether & remove it from the small amt. of ppt. that resulted. (Pet. ether added after CH_2Cl_2 evaporated.



IR Spectrum of Propoxyphene base



NMR Spectrum of Propoxyphene base

Pocatello

STATE OF IDAHO

DEPARTMENT OF HEALTH AND WELFARE

BUREAU OF LABORATORIES, 2220 Old Penitentiary Road, Boise, ID 83702
FORENSIC SECTION (208) 334-2231

SOUTHEASTERN IDAHO FORENSIC LABORATORY RECEIVED
DATE 3-11-85
TIME 8:00
BY

March 5, 1985

MEMO TO: Forensic Section

FROM: Richard D. Groff, Supervisor

SUBJECT: Sampling Technique to be Used in the Analysis of Solid Dosage Drug Analysis. (Please place in Policy Section of Methods Manual)

I. Do not use more than half of the sample. (The defendant should have an amount equal to what we used for analysis by an expert of his own choosing.)
   a. If it is obvious that, with the macro-techniques available in Idaho forensic labs, more than half will be used, consider forwarding the sample to the D.E.A.
   b. If all the material has to be manipulated during the examination, some way should be found to return at least half of the original specimen. For example, if residue off a spoon is extracted by washing it with methanol, then half the methanol extract should be dried in a glass vial and returned with the rest of the evidence.

II. Single samples - powder only.
   a. Under 1 gram: Mix powder thoroughly and take one representative sample, using your own best judgment as to amount.
   b. A gram or more: Take several portions from different locations in the sample to be mixed thoroughly and used for analysis. This should total .15 gram or more.

III. Multiple samples (powders, tablets, capsules and plant material.) For this sampling technique to be applicable, each item must have the same appearance. (For instance: In dealing with baggies of marihuana, each baggie should have the same general appearance as to contents, color, size, container, etc.)
   a. Determine the total net weight of the question material based on the tare weight of the packaging material from one or more items.
   b. For 1 to 2500 items, form a composite sample by combining portions of the square root of the number of items.
   c. For 26 to 2500 items, analyze composite sample of the square root of the total number of items. For 2500 items or more, analyze a composite sample of 50 items. If the number of items exceeds 125,000, analyze a composite sample of the cube root.

Richard Groff
Supervising Criminalist

PROCEDURES/IDEAS
from
DEA LABORATORY
SAN FRANCISCO
JANUARY 22-24, 1985

Susan C. Carre'
Pocatello Crime Lab

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.

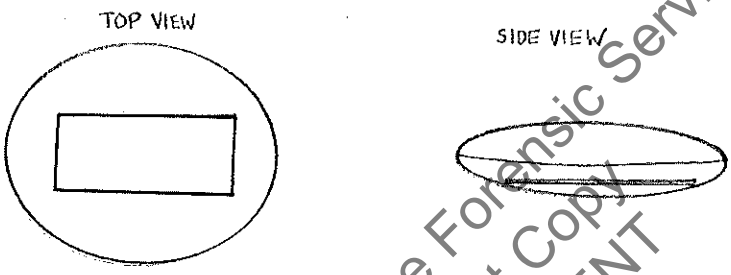
COCAINE
TARTARIC ACID

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

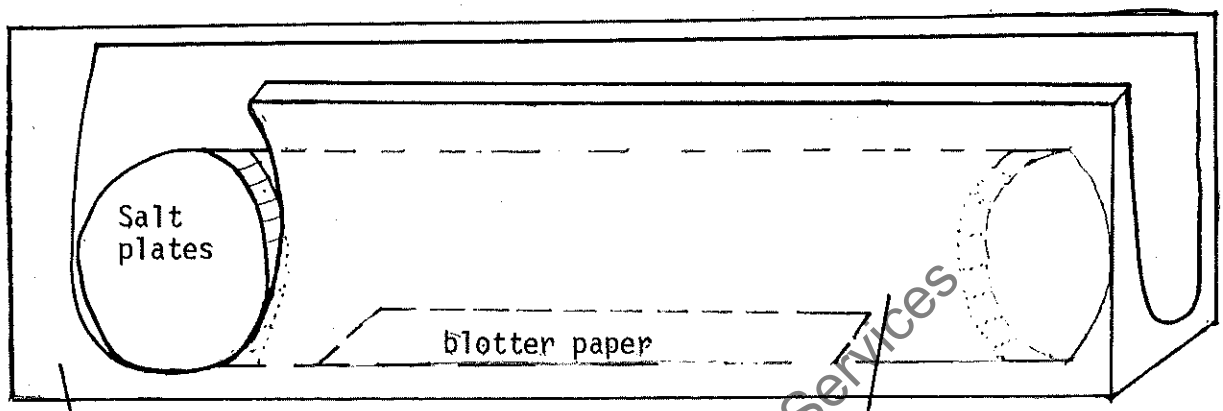
p-DMAB
FLYING SAUCER

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.



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

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

↓

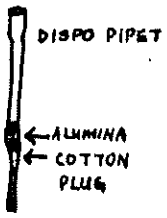
Evaporate-reduce volume

↓

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.

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

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.

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

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

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

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