CONTROLLED SUBSTANCE TRAINING MANUAL

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Revision History

Revision #	Description of Changes
1	Original version transferred into qualtrax
2	Removed 6.4.3.2, corrected formatting section 4.4 of Appendix I, changed 1.7
3	Changed 1.0, 1.6, 1.8, 4.42,5.48, 6.31, 6.4.3.2, 17.1, appendix G pg 100, added 4.43, 4.45, 5.47, dropped 8.1.3

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1.0 Introduction

This manual is designed to give the Trainee, with no experience in the field of forensic chemistry, the tools necessary to analyze evidence for the presence of controlled substances. After a general overview of the laboratory and safety procedures the typical trainee will start with the sections needed to perform marijuana analysis. At the completion of marijuana training, a competency test and mock court for marijuana may be conducted. Upon successful completion of the mock court, the trainee may be signed off to begin supervised cases in marijuana analysis only. Once that is completed, the other sections can be mastered. No particular order of completion is required, although trainees may find going in the following order will make it easier. If a trainee has experience, then some sections may progress faster than others. Some of the extractions /procedures in this training manual might be for general knowledge only. Always refer to the current version of the analytical method for approved procedures.

1.1 Where applicable the Trainer will provide the trainee with practice samples so that the techniques can be mastered.

1.2 At the discretion of the trainer, the trainee can begin working on case samples as the "Hands of the Analyst" (HOA). Since the case always belongs to the trainer, they will observe and verify each step of the analysis up until the case is signed and submitted for review. It will be noted in the case record any case where the trainee conducted any part of the analysis. The number of HOA cases will depend on the abilities of the Trainee and the comfort level of the Trainer/Technical Leader.

1.3 At the end of most sections there is a test. The expectation is 100% correct answers on each test but not necessarily on the first attempt. Any incorrect answers may be corrected by discussion with the Trainer/ Technical Leader. At the trainer's discretion further study or practice may be required until mastery of the subject can be demonstrated.

1.4 A competency test will be given to the trainees when they are ready. Failure of any part of the competency test will necessitate the test to be rerun in part or in whole.

1.5 When all parties agree it is appropriate, a mock court will be held. The trainee will be evaluated on both content and presentation. The "Case" presented will use the results from the competency test.

1.6 After the successful completion of the mock court then a minimum of 5 marijuana and then 5 solid dosage supervised cases will be analyzed. The exact number of cases will be determined by the Trainer and Technical Leader on a case by case basis. The stated number may change if difficulties arise.

1.7 Technical Review training can begin any time after completion of the trainee's supervised cases. Training will consist of reviewing at least 100 cases, evenly split between the three laboratories, covering as many different types of samples and cases as possible. The trainee will be looking

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for any errors in the case record, and the trainer will be looking for any differences between what the trainee does or does not find and what the actual reviewer finds.

1.8 Sections 15 and 16 of this training manual will not be completed by all trainees and are not required to be considered as fully trained.

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2.0 Roles and Responsibilities

2.1 Supervisor

The supervisor in coordination with the Technical Lead, Trainer, and the Trainee will set up a schedule outlining expectations for the trainee and will then monitor the progress of the trainee.

2.2 Technical Lead

The Technical Lead (TL) will appoint a Trainer and monitor the trainee's progress. The TL will report to the supervisor any delays to the training schedule. The TL will be available to the trainer to answer any questions. The TL will review results from the exercises, arrange and grade competency tests, and mock courts.

2.3 Trainer

The Trainer will provide the trainee with the materials necessary to complete training. Materials include but are not limited to samples, equipment and most importantly knowledge. They will keep the TL up to date on the progress of the trainee.

2.4 Trainee

In order to get the most out of this training, it is important for trainees to realize that the information provided through this manual is only a portion of what will be needed in order to not only become proficient but also to become a productive member of the team. It is incumbent on the trainee to utilize not only the information presented but also knowledge learned in school and/or from previous work experience. The use of outside sources of information is encouraged as are questions.

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3.0 General Laboratory

3.1 Background and Theory
The trainee should start their training with a fundamental understanding of good laboratory practices learned from classes at school.

3.2 Objectives, Principles, and Knowledge
To gain and hone the skills necessary to be able to conduct analysis and testify to the results for the justice system

3.3 Health and Safety Hazards
NA

3.4 Universal Precautions should be practiced at all times
PPE is provided, but it is up the trainee to protect themselves.

3.5 Reading and Practical Exercises

3.5.1 Complete ISPFS Core Training
3.5.2 Read section 28B, pg 533-540 of Analytical Chemistry by Skoog/ West/ Holler, 6th edition.
3.5.3 Review Controlled Substances Analytical methods
3.5.4 Demonstrate competence in using laboratory balances by performing monthly checks
3.5.5 Review and demonstrate understanding of Uncertainty of Measurement and how it is calculated at ISPFS

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4.0 Testimony Training

4.1 Background and Theory

At its heart, Forensic Science applies scientific theory and analysis to items of evidence. The end goal of this analysis is to be able to present the results in a court of law. The forensic scientist must not only be competent in performing the analysis but must be able to explain and defend what they did and why they did it. It is assumed that their audience does not possess scientific knowledge, as such, the forensic scientist must be able to explain complex principals and procedures in layman's terms.

4.2 Objectives, Principles, and Knowledge

Through observation of actual testimony, discussions with the trainer, preparations for and participating in mock court, the trainee will gain familiarity with presenting testimony in court.

4.3 Health and Safety Hazards

NA

4.4 Reading and Practical Exercises

4.4.1 Observation of court testimony, at least three times and in different disciplines if possible.

4.4.2 Prepare for and attend their own mock courts. Preparations should include but not be limited to, presenting to a jury, proper court attire, practice answering the most commonly asked questions. Attend other mock courts if possible.

4.4.3 Read Idaho code section 37-2700

4.4.4 Prepare a CV

4.4.5 Attend a testimony class (not required)

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5.0 Marijuana

5.1 Background and Theory

5.1.1 Marijuana, also known as hemp and cannabis, is one of the more commonly abused controlled substance in Idaho. Possession and sale of marijuana has been prohibited by Federal law in the United States since 1937. Any plant material or the resin or any derivative thereof, regardless of form which contains any of the chemical substances classified as tetrahydrocannabinols is presumed to be "marijuana" as defined by Idaho code. The main psychoactive ingredient of the marijuana plant, delta-9-tetrahydrocannabinol or (THC), is contained in all parts of the plant except for the roots and possibly the seeds. Very young marijuana plants often contain little or no THC. A synthetic form of tetrahydrocannabinol called Dronabinol is used to treat nausea associated with chemotherapy. This form is sold under the trade name Marinol and is packaged as gelatin capsules containing the THC suspended in sesame oil. Dronabinol is controlled separately from tetrahydrocannabinol and marijuana.

5.1.2 In the United States, Canada, and Mexico, the dried crushed tops and leaves are rolled into cigarettes and smoked. In modern times, hash oil is produced by soaking the plant material in solvents to extract the THC. The solvent is removed from the plant material and evaporated, leaving behind an oil that is usually very high in THC content. This oil is commonly used in edible products.

5.1.3 Marijuana plants have distinct physical characteristics and THC is easily identified using chemical or instrumental analysis.

5.1.4 In the majority of cases the Idaho State Police laboratories use a combination of microscopic and chemical testing to identify marijuana.

5.2 Objectives, Principles, and Knowledge

5.2.1 At the end of this module the trainee will be able to independently analyze plant material and pipes for the presence of marijuana using the chemical and microscopic methods only.

5.2.2 Analysis of non-marijuana plant material and edibles can only be done after the completion of the other modules in this training manual.

5.3 Health and Safety Hazards

No additional health and safety hazards.

5.4 Reading and Practical Exercises

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5.4.1 Read Idaho Code sections 37-2701,37-2705 d(27), 37-2732B, 37-2733, and 37-2734

5.4.2 Using the Idaho Drug Statutes and find where marijuana, THC and Dronabinol are listed.

5.4.3 Read the listing for Marinol (Dronabinol) in the Physician's Desk Reference (PDR). Another source may be used if the PDR is not available.

5.4.4 Read the marijuana section of the "*Drug Identification Bible, 2010 edition*", or equivalent.

5.4.5 Read Analytical Method section 7.

5.4.6 Read Appendix A.

5.4.7 Read and complete TLC section (6) up until the exercises. The exercises will be completed after marijuana training is completed.

5.4.8 Exercises:

5.4.8.1 Extract a sample of marijuana with petroleum ether and place equal amounts of the extract into five test tubes and evaporate. Place an equal amount of Duquenois reagent into each of the test tubes. To the first test tube add 10 drops concentrated HCl. To the second add 1 drop concentrated HCl. To the third add one drop of 9 parts concentrated HCl diluted with 1 part water. To the fourth add one drop of 5 parts concentrated HCl diluted with 1 part water. To the fifth add one drop of 1 part concentrated HCl diluted with 1 part water. Add an equal amount of chloroform to each test tube. Does the Duquenois-Levine reaction appear to be very dependent on acid concentration? Explain.

5.4.8.2 Take a marijuana sample and divide it equally among three test tubes. Extract one sample with petroleum ether. Extract one sample with methanol. Evaporate the extracts and perform the Duquenois-Levine on these extracts. At the same time perform the rapid Duquenois-Levine test on the third vegetation sample. Are there any color differences between the three different tests? If so, explain why these color differences could have arisen.

5.4.87.3 Perform TLC and DL tests on fresh samples of dry coffee, olivetol, and Patchouli oil. What do the results indicate about these testing methods?

5.4.8.4 Draw the 8 possible isomers of THC. Indicate, by name only, which one(s) are referenced in your readings as being naturally occurring.

5.4.9 After consultation with the Trainer and the Discipline Lead, analyze a number of samples via "hands of the analyst".

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6.0 Thin Layer Chromatography

6.1 Background and Theory

6.1.1 Thin layer chromatography (TLC) is an analytical technique that often offers a quick and easy separation of chemical compounds. The forensic chemist uses this technique primarily for screening drug samples and for isolating active constituents from solutions containing two or more compounds. TLC is a physicochemical separation method. The thin separatory layer (stationary phase) is usually placed on a support plate of glass. After dissolving a small portion of the sample mixture with an appropriate solvent, the solution is applied, as a spot, at the starting point of the plate.

6.1.2 The thin separatory layer (stationary phase) is usually placed on a support plate of glass. After dissolving a small portion of the sample mixture with an appropriate solvent, the solution is applied, as a spot, at the starting point of the plate. After the "spots" have dried, the plate is placed into a TLC tank containing a suitable solvent (mobile phase or solvent system). The TLC tank is immediately covered and separation takes place as a result of capillary migration (development process). As the mobile phase moves over the adsorbed spot, the equilibrium is shifted and constituents present in the spot may be desorbed. The more tightly adsorbed compounds are desorbed to a lesser extent than the more loosely absorbed ones. A new equilibrium is established as the re-dissolved compounds are carried to the edge of the spot, where they come into contact with fresh adsorbent. Throughout this process, the composition of the mobile phase is continuously altered by the interchange of compounds between the adsorbent and the mobile phase. Whenever two compounds adsorb at the same site, the compound that is more strongly adsorbed, will displace the other. The displaced compound will then form a spot further away from the origin. The more similar the adsorptive properties of two compounds are, the more difficult it is to separate them. Compounds having nearly identical properties cannot be separated under most TLC conditions.

6.1.3 At the termination of the development process, the plate is removed from the TLC tank, air-dried and visualized (detection process). Under given conditions of temperature, solvent system and type of adsorbent, the chromatographic behavior of sample constituents is described in terms of “Rf” values. The Rf value is a characteristic of a particular substance and is described as the ratio of the distance traveled by the constituent to the distance traveled by the solvent. This can be expressed as follows:

$$Rf = \frac{\text{distance the (spot center) of the constituent traveled from origin}}{\text{distance the solvent front (mobile phase) traveled from origin}}$$

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6.1.4 Distance for calculating Rf values are usually measured in centimeters. Since Rf values are a function of a number of variables, they should be considered only as guideline values.

6.2 Objectives, Principles, and Knowledge

6.2.1 This technique is used by ISPFS for the analysis of marijuana and occasionally for other analytes as well.

6.2.2 At the end of this module the trainee will not only be able to perform the analysis but should have a basic understanding of chromatographic principles.

6.3 Health and Safety Hazards

6.3.1 Normal precautions apply to the handling of the various solvents and developing sprays that can be employed.

6.4 Reading and Practical Exercises

6.4.1 Appendix B

6.4.2 Clark, Isolation and Identification of Drugs, 3rd edition, vol.1, pg 392-424.

6.4.3 Exercises:

6.4.3.1 Compile a list of the TLC visualizing agents most commonly used in drug analysis. Also indicate the applications, advantages, disadvantages, potential hazards, and safety considerations of each agent.

6.4.3.2 Evaluate by a TLC system suggested by literature:

Mixture of LAMPA (LSMP) and LSD

6.4.3.3 Document your answers by photographing the TLC plates and indicating approximate location of the spots. Also indicate the developing solvents used and the mode of visualization.

6.4.4 TLC Test

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7.0 Extraction Techniques

7.1 Background and Theory

In the field of forensic chemistry, the main purpose of an analysis is the identification of the substance in question. For this reason, each active ingredient of a mixture must be isolated from other sample constituents. There are different ways to achieve this, one of the simplest being by use of the varying solubility properties of chemical compounds. Solubility is the capacity of two or more substances to form spontaneously, without chemical reaction, a homogeneous molecular (or colloidal) dispersion. More specifically, the solubility of a solid in a liquid refers to the concentration that is reached when a fixed amount of liquid has dissolved the entire solid it can hold at equilibrium (at a specific temperature). The solubility of solids in liquids range from very low to very high values. Because of this range of solubilities, the word "soluble" does not have a precise meaning. There is usually an upper limit to the solubility of even the most soluble solid, while even the least soluble would yield a few dissolved crystals per liter of solution. Table 1 below lists descriptive terms for varying degrees of solubility with corresponding (but indefinite) solubility ranges.

Determining the exact solubility of a substance is not required for drug analysis; however, knowing the approximate solubility properties of substances is extremely useful for the separation of constituents in simple drug mixtures.

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Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very Soluble	Less than 1
Freely Soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly Soluble	From 30 to 100
Slightly Soluble	From 100 to 1000
Very Slightly Soluble	From 1000 to 10,000
Practically Insoluble or Insoluble	More than 10,000

Table 1 - Descriptive Solubilities

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7.2 Objectives, Principles, and Knowledge

The trainee will understand extractions and how to employ them to help identify substances.

7.3 Health and Safety Hazards

Various solvents, acids, and bases are utilized in performing extractions, their inherent hazards should be understood and Personal Protective Equipment (PPE) used when handling them.

7.4 Reading and Practical Exercises

7.4.1 *Microgram*, Vol. XVI, No. 1, Jan 1983, "The ANOR Extraction Procedure"

7.4.2 Appendix C

7.4.3 Obtain a phenobarbital and lactose mixture from your training instructor. Extract half the mixture with ether and water. In which solvent do you expect to find the phenobarbital? Evaporate the correct solvent and verify the phenobarbital by IR. Extract the remaining portion of the mixture with chloroform and water. Again, isolate the phenobarbital and verify by IR. Explain any differences you may find in the IR spectrums.

7.4.4 Obtain a methamphetamine HCl, secobarbital, caffeine and sugar mixture from your training instructor. Devise and conduct a separation of all four individual components. Obtain an IR of each individual component. Run the three organic soluble compounds on the GC/MS to verify completeness of isolation.

7.4.5 Extraction Test

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8.0 Gas Liquid Chromatography

8.1 Background and Theory

8.1.1 In 1942, Martin and Synge developed a partition method of chromatography, in which the solute (material to be chromatographed) is partitioned between a stationary liquid phase absorbed on an inert support and a moving liquid, which is only partially miscible with the stationary phase. At that time, they pointed out that the moving liquid phase could be replaced with a gaseous one. Martin and James exploited this suggestion and, in 1952, published a paper that marks the birth of gas chromatography. Development of the technique was rapid, with the petroleum industry, in particular, playing a large part in its advance.

8.1.2 Gas-liquid chromatography (GLC) is a method of separating the components of a volatile mixture by distributing (partitioning) them between a stationary liquid phase and a moving gaseous phase. The stationary phase is loaded into a tubular column and gas is passed through the system. The sample is placed at the head of the column, vaporized, and passed down the column by the carrier gas. At the column exit is a device for detecting the solute as it is eluted from the column. The signal from this detector is amplified and displayed on a data system.

8.2 Objectives, Principles, and Knowledge

It is expected that the trainee will possess a basic knowledge of GC's and how they work. This section should be a review of materials presented in classwork.

8.3 Health and Safety Hazards

The trainer should cover the basic health hazards of a GC, i.e. heated zones, hydrogen carrier gas, electric shock etc.

8.4 Reading and Practical Exercises

8.4.1 Clark, Isolation and Identification of Drugs, 3rd edition, vol. 1, pg 425-499.

8.4.2 Appendix D

8.4.3 GC Test

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9.0 Mass Spectrometry

9.1 Background and Theory

9.1.1 Mass Spectrometry (MS) is an important analytical technique for the identification of chemical compounds. In the mass spectrometer, the sample to be analyzed is vaporized and ionized yielding gaseous-charged particles with unique masses. These charged particles are separated, collected, and measured. The instrument produces a record known as the mass spectrum that records the abundance of the charged species at each mass to charge ratio (m/z). The position and abundance of the m/z values provide qualitative information about the compound.

9.1.2 A mass spectrometer, regardless of type or manufacturer, consists of a sample inlet system, an ionizing source, a mass filter to separate the ions by m/z ratio, and a detector.

9.1.3 The use of a gas chromatograph as a sample introduction device for the mass spectrometer is widely accepted in the forensic science field. The only real limitation on sample types amenable to this system is that the sample components of interest must pass intact through the gas chromatograph. The gas chromatograph portion of the inlet system does not differ from the gas chromatograph used in analytical work in the laboratory except in the treatment of the column effluent; therefore, all the precautions applicable to gas chromatography in general also apply to this system.

9.2 Objectives, Principles, and Knowledge

An overview/ review of basic Mass Selective Detector is covered in this section.

9.3 Health and Safety Hazards

None

9.4 Reading and Practical Exercises

9.4.1 MS Fundamentals (Agilent Multimedia Course).

9.4.2 Appendix E

9.4.3 Watch an autotune and try to identify what part of the instrument is being adjusted for each screen shown.

9.4.4 Compare the mass spectra of heroin, morphine, and codeine. Prepare a narrative explaining the differences and/or similarities.

9.4.5 MS test

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10.0 FTIR

10.1 Background and Theory

10.1.1 Infrared spectrophotometry (IR) is one of the most reliable instrumental techniques used for the identification of drugs. Almost every chemical compound, regardless of its phase (i.e., liquid, solid, gas), produces a different infrared spectrum. Consequently, an infrared spectrum can generally be assumed to be specific for a particular compound. IR is regarded as an indispensable tool for qualitative analysis. If a drug sample contains more than one constituent, preliminary treatment of the sample is usually necessary in order to isolate each constituent in a fairly pure state for IR analysis. Fortunately, with the advent of computer data stations for data manipulation, the spectral contributions of one or more components in a mixture can sometimes be digitally subtracted leaving an acceptable spectrum of a single component.

10.1.2 The unit of wavelength in the infrared region of the electromagnetic spectrum is most commonly expressed in either wavelength (given in microns or micrometers) or wave numbers, with wave numbers being the most popular. One micron (μ) = one micrometer (μm) = 10^{-6} meters = 10^{4} angstroms (Å) = 10^{-4} cm. Wave numbers are defined as the number of waves per centimeter and have the units of reciprocal centimeters (cm^{-1}).

10.1.3 The overall infrared region extends for 0.78 to 1000 μm (12,800 to 10 cm^{-1}). This region is subdivided into three categories: near, middle and far. Different literature references give different values for the wavelength ranges each of these regions cover. In forensic chemistry, we are chiefly interested in the mid-infrared region from 2.5 – 25μ or 4000 - 400 cm^{-1}. This region is divided into the "group frequency" region of 4000 – 1300 cm^{-1} (2.5 – 8 μ), and the "fingerprint" region of 1300 – 650 cm^{-1} (8 – 15.4μ). In the group frequency region the principal absorption bands may be assigned to vibration units consisting of only two atoms of a molecule; that is, units which are more or less dependent only on the functional group giving the absorption and not on the complete molecular structure.

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10.2 Objectives, Principles, and Knowledge

An overview/ review of basic Infrared Detector is covered in this section.

10.3 Health and Safety Hazards

None

10.4 Reading and Practical Exercises

10.4.1 Silverstein, R.M., Bassler, C.G., and Morrill, T.C., *Spectrometric Identification of Organic Compounds*, chapter on Infrared Spectrophotometry

10.4.2 Clarke, E.G.C., Isolation and Identification of Drugs, 3rd edition, vol. 1, pg 328-345.

10.4.3 Appendix F

10.4.4 Read the applicable ISP AM section~~SOP's~~ for Infrared Analysis and discuss with training instructor.

10.4.5 Demonstrate to your training instructor the proper calibration and operation procedures for the Infrared Spectrophotometer.

10.4.6 Prepare an IR spectrum of each of the following substances:

10.4.6.1 Procaine hydrochloride (using a KBr pellet, if possible)

10.4.6.2 Pseudoephedrine hydrochloride

10.4.6.3 Pseudoephedrine Base and compare with 10.4.6.2, describe similarities and differences.

10.4.7 Perform the tutorial on software operation that came with your IR instrument.

10.4.8 Display , print and compare the same compound spectrum in both %T and Absorbance modes..

10.4.9 IR Test

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11.0 Spot Tests

11.1 Background and Theory

Spot tests are color tests used for rapid screening of samples to determine what drugs may or may not be present. A spot test is usually performed by placing one or two drops of reagent in a depression of a spot plate, adding small amount of sample and observing the color produced. Some tests, e.g., the modified Duquenois and Scott tests require the use of a test tube instead of a spot plate. The color produced during a spot test is usually indicative of a class of compounds (e.g., Dille-Koppanyi turns purple with barbiturates). The first appearance of a color is frequently the most important. A weak response may fade, and samples containing sugars will char on standing in the presence of reagents made with sulfuric acid (e.g., Marquis). The drug to be tested must be soluble in the reagent in order to produce a color. For example, diazepam and methaqualone base are both insoluble in water; therefore, in order to obtain a proper response for these compounds, it is necessary that a drop of methanol be added prior to the addition of the testing reagent, Cobaltous Thiocyanate.

11.2 Objectives, Principles, and Knowledge

11.2.1 The reagents used in spot tests can be classified as either general or special. General reagents give different colors for different types of compounds.

11.2.2 Special reagents yield one color for a particular class of compounds. For example, the Sanchez reagent yields red with primary aromatic amines such as procaine and benzocaine.

11.2.3 Special Tests

11.2.3.1 Modified Duquenois (Duquenois-Levine) Test

This color test can be used to detect marijuana plant resins. See the marijuana SOP.

11.2.3.2 Secondary Amine Test

This test involves the use of two reagents to distinguish secondary amines, such as methamphetamine, from other amphetamines.

11.2.4 Reagent Preparation

11.2.5 Directions for preparation of the commonly used spot test reagents can be located in Clarke's Isolation and Identification of Drugs or other sources.

11.3 Health and Safety Hazards

Most color test reagents consist of strong acids or bases and/or toxic chemicals. Standard precautions apply to their handling and use.

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11.4 Reading and Practical Exercises:

11.4.1 Clarke, E.G.C., Isolation and Identification of Drugs, 3rd edition, vol. 1, pg 279-300

11.4.2 Prepare the spot test reagents assigned by the instructor. Then use these reagents to test the reference standards supplied by the instructor.

11.4.3 Spot test quiz

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12.0 Cocaine

12.1 Background and Theory

12.1.1 Archaeological artifacts show that the use of coca was widely accepted in ancient cultures of South American Indians. Paintings on pottery, ornaments depicting pictures and symbols of the coca bush and its leaves, as well as sculptured wood and metal objects dating as far back as 3000 BC on the coast of Ecuador indicate the use of coca in both civil and religious rituals. Relatively recent studies of the antiquity of the use and cultivation of coca indicate that the coca plant is native to the eastern Andes Mountains. Until this day, the natives in the area continue the custom of chewing coca.

12.1.2 A French chemist, Angelo Mariani, introduced Europe to the coca leaf by importing tons of coca leaves and using an extract from them in many products such as his "Coca Wine." Cocaine, as obtained from the coca leaves, was first discovered by Gaedecke in 1855 and rediscovered by Niemann in 1859, at which time he gave the compound the name cocaine. The local anesthetic properties of cocaine were demonstrated first by Wohler in 1860; however, it was not used medically until 1864 as a topical anesthetic in the eye.

12.2 Objectives, Principles, and Knowledge

Gaining knowledge about one of the common and important drugs seen in the ISPFS laboratories.

12.3 Health and Safety Hazards

None

12.4 Reading and Practical Exercises

12.4.1 Appendix I
12.4.2 Drug Identification Bible, section on Cocaine.
12.4.3 Prepare a sample of "crack" cocaine from cocaine hydrochloride.
12.4.4 Cocaine Test

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13.0 Amphetamines

13.1 Background and Theory

The most commonly encountered stimulants are the amphetamines. These compounds are widely used and abused, primarily for their stimulant effect; however, many people have been introduced to the amphetamines because of their anorectic effect. Amphetamines are habituating rather than addictive and abusers develop a tolerance for the compounds.

13.2 Objectives, Principles, and Knowledge

The solid dosage controlled substance most commonly encountered by ISPFS are the amphetamines.

13.3 Health and Safety Hazards

None for this section

13.4 Reading and Practical Exercises

13.4.1 Appendix H
13.4.2 Drug Identification Bible, Amphetamines section.
13.4.3 Obtain Pseudoephedrine HCl, pseudoephedrine base, and ephedrine, IR's and compare.
13.4.4 Amphetamines Test

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14.0 Hallucinogens

14.1 Background and Theory

Hallucinogens fall into three general categories - 1) compounds containing the indole skeleton, 2) compounds containing the phenethylamine skeleton and 3) phencyclidine derivatives.

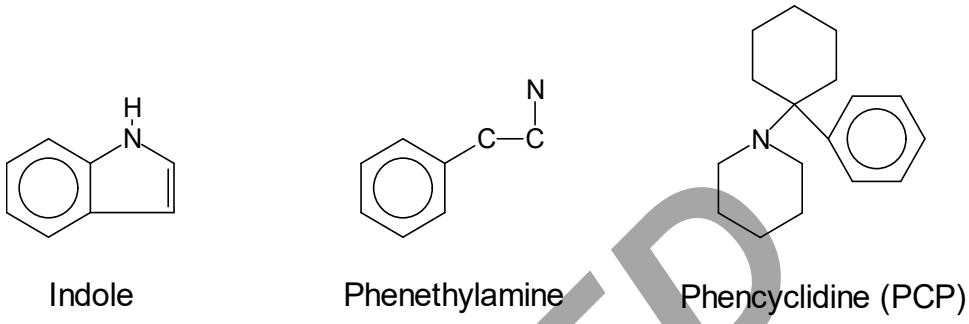

Figure 1

There are several groups within the "indole" category, including ergot alkaloids, synthetic cannabinoids, and tryptamines.

14.2 Objectives, Principles, and Knowledge

An overview of the wide field that is the hallucinogens.

14.3 Health and Safety Hazards

Most of the hallucinogens are very potent. Extra care must be observed when handling reference materials of these substances.

14.4 Reading and Practical Exercises

14.4.1 Appendix I

14.4.2 Analyze LSD and LAMPA via GC/MS, and TLC. Which techniques differentiated LSD from LAMPA and describe the differences.

14.4.3 List the mass spectral differences between psilocyn and bufotenine.

14.4.4 Read the ISP SOP and list the requirements for reporting Psilocyn and/or psilocybin was detected on a lab report.

14.4.5 List the substituted phenethylamines that are controlled under Idaho Statutes.

14.4.6 Hallucinogen Test

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15.0 Methamphetamine Quantitation

15.1 Background and Theory

Quantification of the purity of samples containing methamphetamine is only performed on Federal cases.

15.2 Objectives, Principles, and Knowledge

To develop and demonstrate the skills to accurately determine the purity of methamphetamine samples

15.3 Health and Safety Hazards

Large amounts of chloroform are used in this procedure thus all extractions must be done in a hood. Preparation of the samples for analysis can produce a significant dust issue. Contact with large quantities of methamphetamine cannot be avoided but every effort must be made to minimize exposure.

15.4 Reading and Practical Exercises

15.4.1 Read Methamphetamine Quant method.

15.4.2 Demonstrate the ability to generate a valid calibration curve using the GC/MS

15.4.3 Demonstrate ability to generate precision and accuracy data within acceptable limits using test samples provided by the Technical Leader.

15.4.4 Demonstrate competency using split samples from at least one sample run.

15.4.5 Successfully complete a competency test.

15.4.6 Successfully complete a mock court.

15.4.7 Supervised cases will consist of at least one sample run.

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16.0 Clandestine Laboratories

16.1 Background and Theory

16.1.1 Although by definition any illegally produced drug would come from a clandestine laboratory, the vast majority of cases in Idaho have involved the production of methamphetamine.

16.1.2 With the exception of iodine and phosphorus the analysis of clan lab samples are covered by the other sections of this manual.

16.2 Objectives, Principles, and Knowledge

The difficulty of analyzing clan labs is not the actual chemical analysis but rather in explaining the results and articulating how all of the results either do or do not support the charge of manufacturing. That is the objective of this training.

16.3 Health and Safety Hazards

The analysis of phosphorus involves the use of a Bunsen burner and adding chloroform to a very hot sample, caution is advised.

16.4 Reading and Practical Exercises

16.4.1 Make and analyze a phosphorus standard using the method.

16.4.2 Identify Iodine using the method.

16.4.3 Read "Syntheses & Analysis of P-2-P, Amphetamine, and Methamphetamine, Vol 1& 2. 1996. M. Kalchik, R. Ely

16.4.4 Identify and explain, as you would to a jury, the two common methods of manufacturing methamphetamine.

16.4.5 Identify and explain, as you would to a fellow chemist, the two common methods of manufacturing methamphetamine.

16.4.6 Complete the written test.

16.4.7 Mock court.

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17.0 Technical Review

17.1 Background and Theory

The last section of training is in technical review. This section can be started at anytime after completion of the last supervised cases are completed.

17.2 Objectives, Principles, and Knowledge

After sufficient experience is gained from performing their own casework reviewing other analyst's cases will act as the polish to finish off the trainee's training.

17.3 Health and Safety Hazards

NA

17.4 Reading and Practical Exercises

17.4.1 Review at least one hundred cases where the trainee performs the review and notifies the trainer of any errors they detect.

17.4.2 The trainer then does the review and discusses any differences.

17.4.3 The one hundred or more cases should be a mixture of types of cases with varying degrees of complexity, from different analysts, and from the three laboratories.

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18.0 Appendix A

Idaho State Police

Forensic Laboratory Training Manual

Marijuana Analysis

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MARIJUANA ANALYSIS TRAINING PROGRAM

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1.0.0 INTRODUCTION AND BACKGROUND

1.1.0 Marijuana, also known as hemp and cannabis, is one of the more commonly abused controlled substance in Idaho. Possession and sale of marijuana has been prohibited by law in the United States since 1937.

Idaho Statute (37-2701(s)) defines marijuana as follows: "Marijuana" means all parts of the plant genus Cannabis, regardless of species, whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture or preparation of such plant, its seeds or resin. It does not include the mature stalks of the plant unless the same are intermixed with prohibited parts thereof, fiber produced from the stalks, oil or cake made from the seeds of the plant or the achene of such plant, any other compound, manufacture, salt, derivative, mixture or preparation of the mature stalks (except the resin extracted therefrom or where the same are intermixed with prohibited parts of such plant), fiber, oil, or cake or the sterilized seed of such plant which is incapable of germination. Evidence that any plant material or the resin or any derivative thereof, regardless of form, contains any of the chemical substances classified as tetrahydrocannabinols shall create a presumption that such material is “marijuana” as defined and prohibited herein.

The exceptions in the legal definition are present because there are some legitimate uses for certain parts of the marijuana plant. Fibers from the stalk are used to make rope, twine, canvas, cloth and hats. A rapid drying oil that is used in the arts and as a commercial substitute for linseed oil can be extracted from the seeds. This oil is also valuable in soap making. The oil cake is used as cattle feed. The seeds are sterilized and used as a constituent in commercial birdseed mixtures. Dilute solutions of the pollen extracts are occasionally used to develop antigens in those persons who exhibit allergic manifestations to the plant or pollen.

The main psychoactive ingredient of the marijuana plant, □9 -Tetrahydrocannabinol (pronounced delta-9-Tetrahydrocannabinol) or (THC), is contained in all parts of the plant except for the roots and possibly the seeds. Some literature reports that there is no THC in the seeds - other literature reports finding THC in the seeds in very minute amounts. THC is most abundant in the resin that is secreted by the plant. Very young marijuana plants often contain little or no THC. Tetrahydrocannabinol is controlled separately from marijuana. A synthetic form of Tetrahydrocannabinol called Dronabinol is used to treat nausea associated with chemotherapy. This form is sold under the trade name Marinol and is packaged as gelatin capsules containing the THC suspended in sesame oil. Dronabinol is controlled separately from tetrahydrocannabinol and marijuana.

Marijuana is cultivated the world over. Its culture is presumed to have originated in China from whence it spread. It presently grows wild or is cultivated in North and South America, Asia, India, Africa and small quantities are produced in some European countries. Although the plant is indigenous to many areas of the world, environmental factors govern the extent of growth and are responsible for many morphological modifications of the plants.

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In northern climates, hemp usually grows to a considerable height and produces more fiber than that grown in southern latitudes where the plant is usually of the dwarf variety. The shorter summers of the northern latitudes can sometimes prevent the seeds from ripening fully. During World War II when our supplies of Manila hemp were aborted, the Treasury Department licensed several farmers in both southern and northern tier states to produce hemp, which was so vital to the war effort. While the longer growing season of the southern states permitted the harvest of a seed crop, the quality of the fiber was such as to render it unsuitable for making rope. Thus, the southern seed crop was sold to farmers in the north where the colder climate was conducive to a hardier and firmer fiber.

Hemp was grown in the New England Colonies for fiber used in making homespun. It was also grown in the Virginia and Pennsylvania colonies and cultivated at a very early date in the settlements of Kentucky and Tennessee from whence it spread to Missouri and westward with the settlers. It is not known when the plant was introduced to the Southwest and Mexico, but probably along with the early Spanish settlers.

Formerly, the majority, if not all, the imports of cannabis into the United States were from India where hemp was largely cultivated for smoking purposes. The menace of the habit, which its culture made possible, led the Indian authorities to impose drastic restrictions on its production, hence the supply of hemp required by the United States had to be sought elsewhere. Thus, the domestic industry mostly in Kentucky and the Illinois River valleys come into being. The early cultivation of hemp in the United States was of the small European variety but this was replaced around 1850 by the larger Chinese variety. A great deal of hemp was also produced in Russia, formerly a principal source for American importation. The use of hemp fiber in the manufacture of rope in this country has been replaced almost entirely by Abacca or Manila fiber derived from a species of the banana plant. With the development of synthetics, especially nylon, the use of Abacca has declined.

The glandular hairs of the plant produce a sticky and somewhat viscous resin. This resin is found on many areas of the plant, however, it is most abundant on the reproductive parts. Moreover, certain plants seem to produce more resin than others. Whether this high resin content is due in part to ecological conditions or a built-in defensive mechanism or a genetic characteristic of the plant has not been determined. However, there seems to be a definite correlation between altitude, moisture and temperature, and the quantity of resin produced. Some botanists maintain that plants grown in hot, dry climates exude more resin as a protection against moisture loss, particularly where it involves female flowers; thus insuring favorable conditions for the propagation of the species. There tends to be some credence in this theory since the hemp grown in the hot and dry climates of the Himalayas, certain parts of Africa and the arid slopes of the Andean mountains of South America are noted for their relatively high resin production.

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The true origin of the name marijuana is lost in antiquity. Gray attributes a Greek derivation of the word "Cannabis" to be from the Persian name "Kanab". Other authors cite many words from many languages as the possible genesis of the word "marijuana". History tells us that the murderous frenzy of the Malays, characterized by running "amok" was the result of the habitual use of hashish. Hashish is the unadulterated resin collected from the flowering tops of the marijuana plants. It is also reported that the Mohammadan leaders, opposing the Crusaders, utilized the services of individuals while under the influence of hashish to commit secret murders. The frenzy produced by the drug led these persons to be called "haschischin", "hashihash" or "hashishi" from which the modern English word "assassin" is derived.

The flowering tops, leaves, and small stems are gathered, dried, and usually smoked in a pipe or as a cigarette. Its use in cigarettes is the method most often chosen. Sometimes the resin is expressed or obtained by rolling the pods between the hands or "carpets" and then eaten. It has been reported that the Egyptians gathered the resin by donning leather jackets and walking through a field of shoulder high plants. The sticky resin that adhered to the jacket was then scraped off and utilized in the usual manner. However, the credibility of this tedious method is lacking in standard references.

In the United States, Canada, and Mexico, the dried crushed tops and leaves are rolled into cigarettes and smoked. In India and Central Asia, the raw resin is extracted from the tops and kneaded into sticks or mixed with various spices and called Charos or Dawamesk which is either smoked or eaten. The leaves are also powdered and mixed with spices, honey or water and the concoction, referred to as Bhang is eaten or drunk. In North Africa, the dried crushed tops are mixed with tobacco and smoked in pipes. The user in Tunis refers to this as "takrouri" while the Moroccans call it "Kif". In the Eastern Mediterranean and around the Gulf of Arabia the raw resin from the flowering tops is reduced to powder for smoking (called Chira) or is expressed and kneaded into sticks for eating (hashish). While in the same area the flowering tops are soaked in butter and water and mixed with almonds and honey, then eaten in the form of cakes. The Turks call the mixture 'Madjun" 'Magoun" or "Esrar". In South Africa smoking "Djamba" or "Dagga", a mixture of crushed leaves and flowering tops seems to be the method of choice. In modern times, hash oil is produced by soaking the plant material in solvents to extract the THC. The solvent is removed from the plant material and evaporated, leaving behind an oil that is usually very high in THC content.

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Δ^9 - THC Content in Different Forms of Marijuana

Form	Δ^9 - THC Range
Marijuana Leaves	0.2 - 3 %
Marijuana Flowering Tops	3 - 20%

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Sinsemilla	3.5 - 4.5 %
Compressed Marijuana (pressed cake)	2.5 - 4 %
Hashish	0.1 - 14 %
Hash Oil	0.5 - 45%

From the early 1900's to 1937, many pharmaceutical preparations containing resin extracts of cannabis were readily available and were promoted extensively as analgesics and sedatives. Clinicians, however, soon learned that these preparations, rather than contributing to the treatment of clinical disorders, actually manifested their symptoms and caused such untoward side effects as to preclude their use. Shortly after the passage of the Marijuana Tax Act of 1937, the Food and Drug Administration declared these preparations to be without medical utility and they were removed from the market place.

Scattered stands of wild hemp are reported each year throughout the United States. It is abundant as a wild plant in many localities, often growing along hedgerows, riverbanks and roadsides. The plants are indigenous to many areas and are adaptable to almost every type of soil and climatic conditions except those in the extreme northern latitudes. The heaviest infestation tends to follow the Corn Belt in the states of Iowa, Kansas, Nebraska and Missouri while the lowest level of infestation occupies an area from Indiana eastward through New England with the exception of Maine where very little growth occurs. Moderate growth occurs in the Virginias, Tennessee, Kentucky and Ohio while scattered growth occurs along the southern tier of states.

There are many problems with controlling the wild growth including; (1) the lack of recognition of the plants by the land owners, (2) the tendency of the plant to grow in small widely scattered stands and its ability to adapt to many types of habitats, (3) the resistance of the mature plants to herbicides and (4) the production of viable seeds over a ten to twelve week period from mid July to mid October.

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2.0.0 TAXONOMY

2.1.0 The discipline concerned with the classification and naming of living things is called taxonomy. The table below lists the taxonomic classification of marijuana. (NOTE: An alternate group of classification categories may be encountered. That group consists of Kingdom; Phylum or Division; Subphylum; Class; Order; Family; Genus; and Specific Name.)

Category	Taxon
Kingdom	**Plant**
Division	**Spermatophyta** (seed plant)
Class	**Angiospermae** (flowering plants)
Subclass	**Dicotyledons** (dicots) 31,874 species
Order	**Urticales** (elms, mulberries, nettels, and hemps) 1,753 species
Family	**Cannabinacae** (hops and marijuana) 3 species
Genus	**Cannabis**
Species	**Sativa**

Individual plants are identified by giving genus and species names. Hence, the full botanical name for marijuana is “Cannabis sativa L”. The “L” refers to the botanist who first classified marijuana (Carlus Linnaeus).

Because there is only one species in the genus, Cannabis is known as a monotypic genus. There is debate among botanists as to whether there is more than one species of cannabis. A botany professor at Harvard University by the name of Dr. Schultes believes that there are several species including Cannabis sativa, Cannabis indica and Cannabis ruderalis (which is sometimes spelled ruberalis). He bases his belief on physical inconsistencies between plants. These inconsistencies include branching differences, broadness and thickness of leaves and overall plant shape and appearance. However, most experts believe that most of the variations between plants are neither adequate nor specific and are actually brought on by growing conditions. These botanists believe that indica, ruberalis, as well as americana are all agronomic varieties of Cannabis sativa. Most references agree that the different kinds of Cannabis interbreed and produce viable offspring, which is an indication that they are from the same species.

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The early federal and state statutes that controlled marijuana used the name Cannabis Sativa L. Because of the species disagreement among botanists, several defendants were acquitted because the court felt that the prosecution did not prove the substance in question was actually controlled. Below is a list of three early rulings on the Federal level supporting the single species view.

1. U.S. vs. John Moore (E.D.Pa. No 69-137) 330 Fed. Supp. 684 (1970)

2. U.S. vs. Eric Honeyman, et al., (71-1035-RHS) Northern District, California, (1972)

3. U.S. vs. Mitchell Rothberg, et al., (7-CR-164) 351 Fed. Supp. 1115, Eastern District, New York (1972)

This legal argument is now mute because the statutes have been rewritten to define marijuana as plants in the genus Cannabis. It is important that the examiner be aware of this argument however, because even though it is now groundless, it may still be brought up in court.

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3.0.0 PHYSICAL CHARACTERISTICS

3.1.0 Despite much variation among individual plants, marijuana is so distinct from all others that it can be recognized at all stages of growth by its botanical characteristics. Marijuana plants grow from 1 to 5 meters high. When planted for the production of hemp fiber, the stalks are crowded and without foliage except near the top. Wild growing plants on the contrary have numerous branches. The size of individual plants is primarily determined by growing conditions.

The marijuana plant is an annual - it dies after seed production and is dependent upon seed for survival of the species. The life cycle can be as little as two months or as long as eight months. Marijuana is a full sun plant that requires both the proper duration and wavelengths of light for photosynthesis and reproduction to occur. Marijuana is adaptable however, and will grow in the shade, although more slowly. High temperatures cause the plant to wilt due to water loss and, as a result, resin production usually increases to prevent dehydration. While marijuana can adapt to heat, exposure to a hard frost or several days of repeated light frost could kill the plant.

3.2.0 LEAVES:

The leaf of any plant consists of a blade and a leaf stem or petiole. The leaf is classified by the characteristics of the blade. If the leaf blade is undivided, the leaf is classified as simple. If the leaf blade is divided into distinct parts called leaflets, the leaf is classified as compound. There are two types of compound leaves, palmately compound in which the leaflets are all attached at the tip of the petiole, or pinnately compound in which the leaflets arise along the sides of a central stalk. The edges of the leaf or leaflet can either be serrated (toothed) or without serrations.

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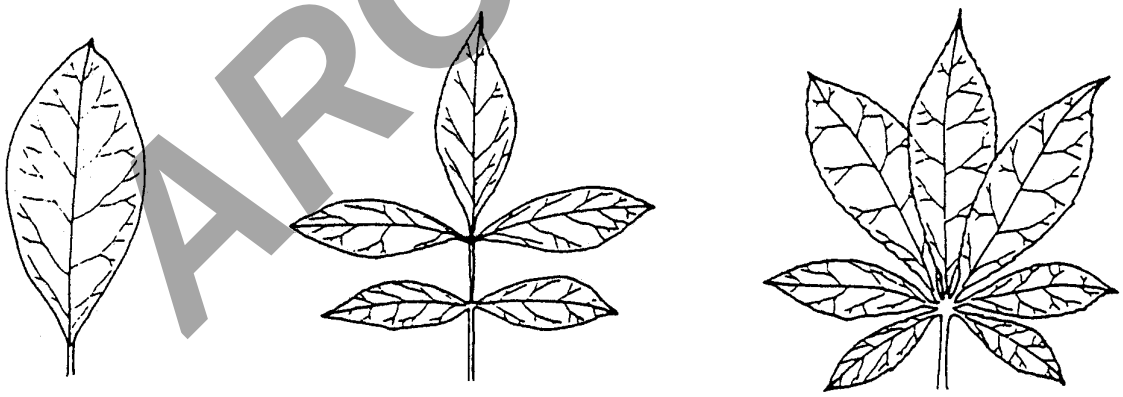

Figure 1 - Leaf Forms

Leaves can either be opposite or alternate in terms of their point of attachment to the stem. This point of attachment is known as the node. If only one leaf is attached to a node, the leaves are said to be alternate. If two leaves are found on a node, the leaves are oppositely arranged.

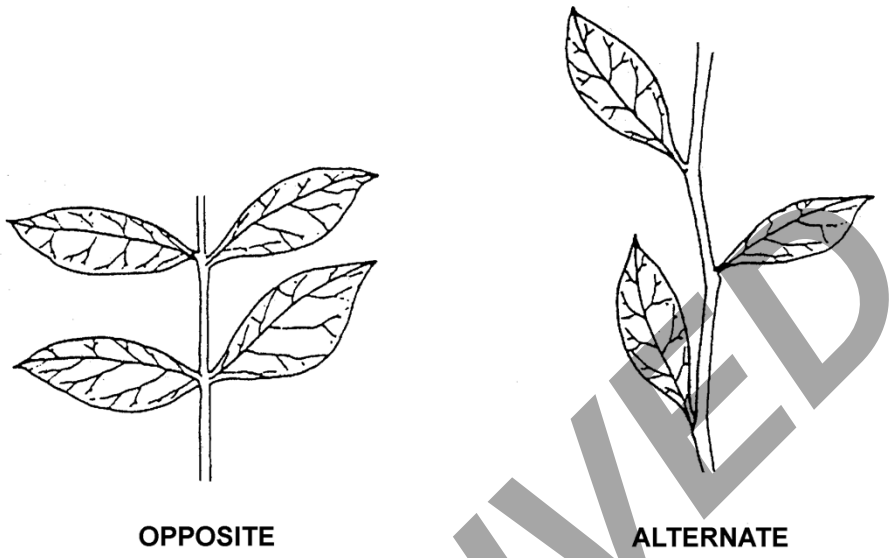

Figure 2 - Leaf Arrangements

Finally, leaves and leaflets are described in terms of their shape.

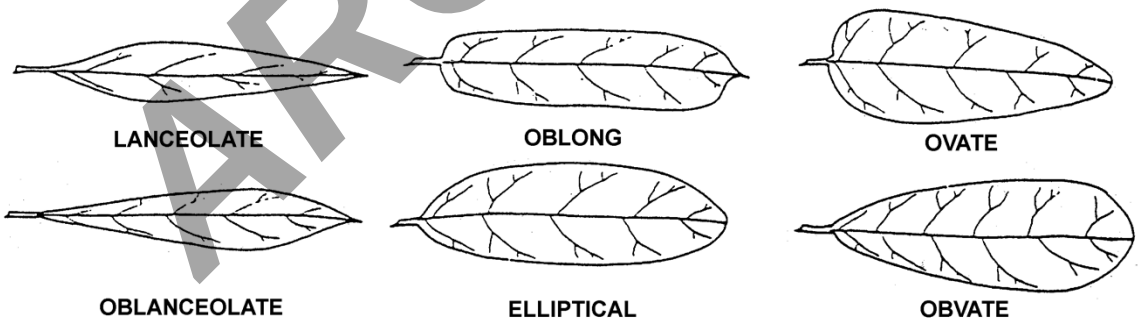

Figure 3 - Leaf Shapes

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The leaves of the marijuana plant are its most distinctive feature and are easily recognizable. Marijuana leaves are palmately compound and usually have 3 to 11 finger-like leaflets. The leaf is usually composed of an odd number of leaflets. The number of leaflets that a leaf possesses generally increases up the stem while the size of the leaves becomes progressively smaller toward the top of the plant. In other words, the lower leaves have fewer leaflets but are larger. The leaf attachments of marijuana are generally opposite near the bottom and alternate near the top. The leaflets are lanceolate in shape (i.e. 6 or so times as long as they are wide and widest below the middle) with a narrow wedge shaped base and a drawn out pointed tip. The leaflets are serrated and the teeth are sharp and pointed toward the tip of the leaflet. On large leaflets, the serrations can have serrations. The petiole or leaf stem has a groove and can be described as u-shaped.

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Figure 4 - Marijuana Leaf

The veins of the leaf are best seen on the lower surface. There is a major vein (the midrib) that runs from the petiole to the tip of the leaflet. There are other veins extending from the midrib to the point of a serration. A tiny vein branches from these veins and leads to the deepest indentation of the adjacent notch. The upper side of each leaflet is deep green in color and the lower side is lighter green.

3.3.0 HAIRS:

Many plants have hairs or trichomes over various parts of the plant. Trichomes have several functions, such as protection against dehydration or predation. Two basic types of trichomes are found on plants. Those that secrete substances (referred to as glandular) and those, which don't secrete substances (referred to as non-glandular). Both glandular and non-glandular trichomes can be composed of one cell (unicellular or monocellular) or have more than one cell (multicellular).

Cannabis plants have non-glandular, single-celled cystolithic hairs. These hairs, which are shaped somewhat like a bear claw, are short and fat due to calcium carbonate deposits called cystoliths in the base of the hair. Cystolithic hairs are found on the upper surface of the leaf pointing to the tip of the leaflet and most other parts of the plant. Dilute hydrochloric acid will cause bubbles of carbon dioxide to be freed from these hairs. Non-glandular, single-celled covering hairs are found on the lower surface of the leaf pointing to the tip of the leaf. They are longer and more profuse than the cystolithic hairs. **This combination of cystolithic trichomes on the upper leaf surface and covering trichomes on the lower leaf surface is a characteristic that is unique to marijuana.** A plant that does not show cystolithic hairs is not marijuana.

Multicellular glandular hairs are also found on the lower surface of the leaf. These glandular hairs secrete a resin that spreads over the surface of the leaf and also on various parts of the plant's flowers. This resin is thought to reduce moisture loss and protect the plant against predators. This resin contains the active ingredient tetrahydrocannabinol (THC). The glandular hairs look like glistening globules on the surface of the leaf. There are two types of glandular hairs: Stalked, which are found mainly on the seed hulls and usually not fount intact, and the sessile (without a stalk) hair, which is found mainly on the lower surface of the leaves.

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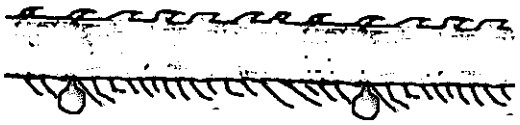
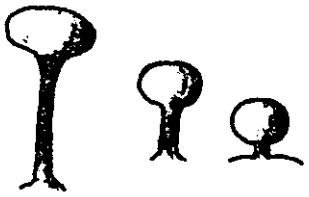
Cystolithic hairs - found on the tops of the leaves and most other parts of the plant.

Upper surface of leaf



Lower surface of leaf



Glandular hairs - found on the lower surface of the leaves but mostly around the flowers.

Soft covering hairs found on the lower surface of the leaves

Figure 5 - Plant Hairs

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3.4.0 STEMS:

The term “stalk” is a legal or vernacular term, which is defined by Webster as “the main stem of an herbaceous plant often with its independent parts”. Botanists consider the correct term to be “stem” and define it as “the major supporting structure in plants, to which buds, leaves, and flowers are attached at regular intervals at points called nodes”. In forensic work and in dealing with the legal system both terms will be used. It is important to know that botanists may take exception.

The “stalk” of the marijuana plant is angular with lengthwise fluting. The green outer covering contains tough fiber and is covered with cystolithic hairs that curve upwards with their tips pressing against the stem. Beneath the outer covering is a layer of woody material and within the woody material is the pith. The center is usually hollow. Rather inconspicuous nodes occur on the stalk at intervals of 4 to 20 inches and from these spring the leaves and branches - a branch immediately above each leaf.

The plant branches at the nodes. The branch attachments of marijuana are generally opposite near the bottom of the plant with each pair situated almost at right angles from those above and below them. Near the top of the plant the branch arrangement becomes alternate instead of opposite.

3.5.0 FLOWERS:

The flower of a plant is believed to be a grouping of highly modified leaves. In many plants, both the male and female sex organs are contained in the same flower. The typical flower is composed of four such groupings or whorls. The outermost whorl is composed of the sepals that is collectively called calyx. The sepals are usually green and are easily seen on flowers that aren't completely open such as the green objects surrounding partially opened rose petals. The next whorl proceeding toward the center are the petals, collectively known as the corolla. The next most inner whorl is composed of the stamens, which can be thought of as the male flower parts. Each stamen is composed of a long stalk called the filament, which is terminated by an anther. The anther is a pollen sack, which is made up of chambers that contain the pollen. The final innermost whorl is composed of a pistil, which can be thought of as the female flower parts. A pistil is composed of a swollen lower portion or ovary, which contains the ovules. Attached to the ovary is a stalk-like style, which expands at the tip. The expanded tip is called a stigma and is the receptive surface for the pollen.

Marijuana is “dioecious” i.e., the male and female flowers are borne on separate plants. Since marijuana is dioecious, not all flower parts are contained in the flowers of individual plants. Some parts are in the male flowers and some are in the female flowers.

The male inflorescence is loosely arranged, much branched and many-flowered, standing out from the leaves, with individual flowering branches. Each flower usually has five sepals and five stamens. The flowers, which are usually white, green and yellow, are covered with hairs. Some of these hairs are resin producing. The stamens hang freely from the flower. Each stamen consists of a short slender filament that leads to the anther. The anthers open lengthwise from the tip downwards to release the pollen that is carried by the wind to female flowers.

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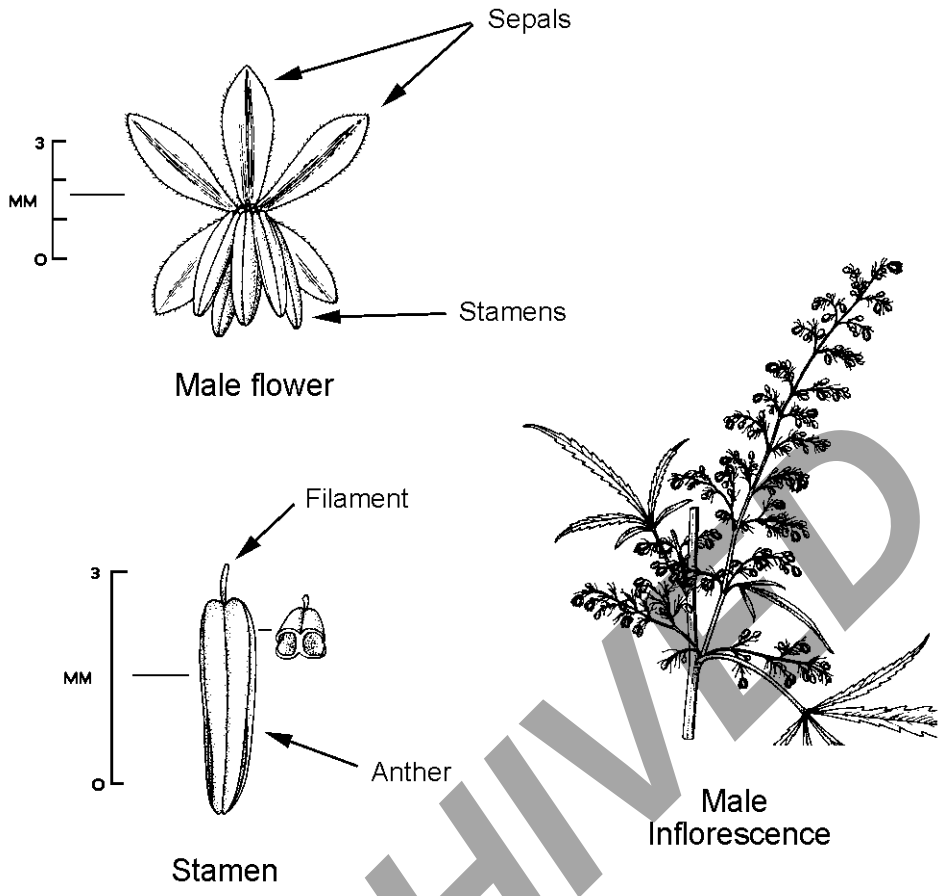

Figure 6 - Male Flower and Inflorescence

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The female inflorescence does not project beyond the leaves. They are compact, short and few-flowered. The flowers occur in pairs that grow at the joints of the leaves. At the center of each flower is the pistil. The ovary contains one ovule. The flower also has a small green organ, sometimes called a bract, sometimes a calyx, which completely enwraps the ovary forming a tubular “sheath”. Out of this sheath project the stigmas. After pollination the stigmas quickly fall off. The sheath increases in size as the ovary matures into the fruit and the ovule becomes the seed.

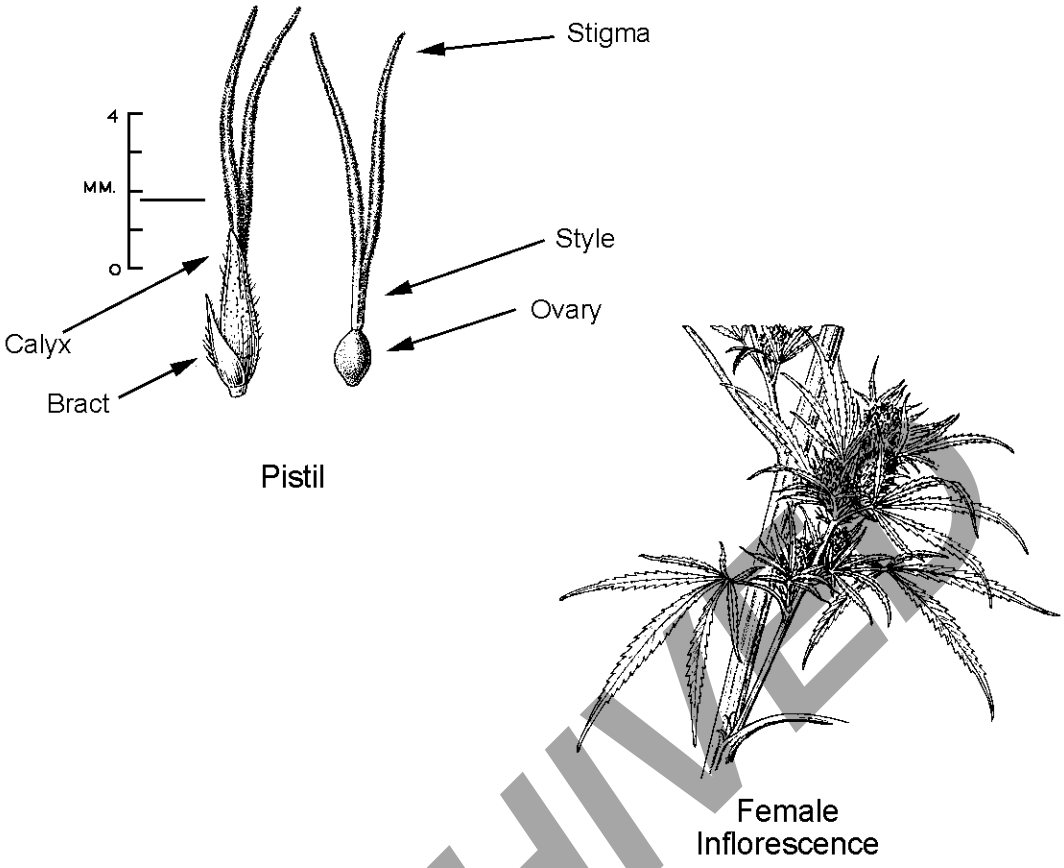

Figure 7 - Female Flower and Inflorescence

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Marijuana plant sexes cannot be differentiated with certainty until flowers appear. Female plants tend to be shorter and have more limbs than the male. The female plants appear leafy to the top with many leaves surrounding the flowers while the male plants appear thinly leafed near the flowering limbs. The male flower develops about three to four weeks ahead of the female flower.

There are two theories regarding what determines the sex of a marijuana plant. The first is that sex is determined by physiological stimuli at some stage after fertilization. The second is that sex is determined by inheritance of the XY type. The best explanation is probably a combination of the two. Initially, sex is determined by inheritance. The final production of flowers on an individual plant is influenced by the environment that may override the inherited sex. It has been reported that the ratio of male to female plants can be influenced by exposure of seeds to ultraviolet light, by air temperature, by carbon monoxide concentration, by the age of pollen and the stigma, and by nitrogen concentration in the soil. Unfertilized female plants occasionally produce a few male flowers. This condition is described as “monoecious”. The offspring of these monoecious plants will be mainly female.

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Resin is more abundant on the female plant. It is postulated that the copious resin protects the female flower and plant while the seed is developing. The male plant, on the other hand, dies after giving off pollen and does not need to be protected as long. Usually the development of glandular hairs stops on the male flowers when pollination occurs.

3.6.0 SEED:

The fruit in marijuana is technically an achene, i.e. it contains a single seed with a hard shell tightly covered by the thin wall of the ovary, the whole being regarded in practice as a "seed". The seed is 3-5 mm in diameter - about the size of a large kernel of wheat. It is ovoid in shape and has been described as resembling tiny melons. The surface is divided into two "halves" by a rather sharp ridge round the greatest circumference. The surface color may vary from a greenish-yellow to brown and it is frequently somewhat mottled. The surface is covered with characteristically peculiar lacy markings. The interior of the seed is white and oily and resembles coconut meat.

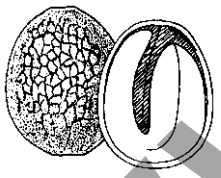

Figure 8 - Marijuana Fruit

Marijuana seeds germinate in 3-7 days. The first structure to emerge from the seed is the radicle or embryonic root. The radicle will eventually develop into a taproot that will have numerous lateral roots growing from it. The first leaves to emerge from the seed are termed cotyledons. There are usually two cotyledons but there can be three. They are slightly unequal in size, oblanceolate shaped and rounded at the base. The cotyledons have trichomes on the upper leaf surface and no trichomes on the lower leaf surface. About 2 cm above the cotyledons the first true leaves develop.

3.7.0 SINSEMILLA:

Occasionally, the term Sinsemilla is applied to marijuana. "Sinsemilla" is Spanish for "without seeds". Sinsemilla is a "high potency" marijuana that is produced by removing the male marijuana plants before they can pollinate the female plants. This causes the female plants to produce more THC rich resin in its flowering buds.

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3.8.0 GLOSSARY OF BOTANICAL TERMS

Achene - A dry, one-seeded fruit with a firm close fitting wall that does not split open at maturity.

Alternate - Located singly at a node, as leaves on a stem; situated between

other parts, as stamens between petals.

Annual - A plant that completes its development in one year or one season and then dies.

Anther - The pollen containing part of a stamen, usually consisting of two sacs.

Axel - The angle formed between two organs, as between a leaf and stem.

Maxillary (axile) - In the axis; designating flowers borne in the axils of leaves, and ovules or seeds produced III the angles formed by partitions n the ovary of a compound pistil.

Bract - A leaf on a flower, located just below the flower or flower cluster at the base

Bracteole (bractlet) - A secondary bract, often very small.

Calyx - The outermost series of flower parts; the sepals of a flower considered as a group

Cannabinacea - the hemp family

Cannabis sativa - Hemp plant or marijuana.

Capitate - Like a head; in a dense, more or less rounded cluster.

Compound - Composed of two or more parts; compound leaves have two or more leaflets.

Cotyledon - The first leaf or leaves developed in the seed. These leaves are present in the seed and they may or may not enlarge and become green when the seed germinates. Often food materials are stored in them.

Cystoliths - A calcified deposit at the base ofa hair.

Dioecious - The flowers are unisexual and the male flowers are on one plant and the female flowers are on a separate plant.

Embryo - The rudimentary plant within a seed.

Epidermis - The outer tissues of a plant.

Fertile - Capable of reproducing, as a stamen producing viable pollen or a carpel producing ovules.

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Filament - The stalk of a stamen.

Fruit - A ripened ovary.

Flower - An aggregation of highly modified leaves that make up the reproductive structure of certain plants.

Gland - A secretory hair or other part that produces nectar of some other liquid.

Glandular hair - A hair that produces a resin.

Herb - A plant that dies at the end of a growing season and is not woody stemmed.

Hypocotyl - The part of a seedling below the cotyledons and above the radicle.

Inflorescence - A flower arrangement or cluster.

Lanceolate - Lance shaped.

Leaflet - One of the divisions of a compound leaf

Midrib - The main or central vein of a leaf.

Monoecious - Having separate staminate and pistillate flowers on the same plant.

Morphology - The study of external plant structures and their shapes and forms.

Node - The point of a stem where leaves or branches are attached.

Ob - A prefix meaning inverted, as in "oblanceolate," upside down lanceolate and broadest above the middle.

Opposite - (leaves) in pairs, one on either side of the node; (stamens) inserted in front of petals and thus opposite them.

Ovary -- The basal portion of a pistil containing one or more ovules.

Ovate - Egg-shaped, the broadest part below the middle.

Ovule - The structure that becomes a seed after fertilization.

Palmate - Compound leaves in which leaflets radiate from a common point, like the fingers of the hand.

Perennial - A plant that continues to live year after year.

Perianth - A collective term for the calyx.

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Pericarp - The ovary wall in the fruiting stage.

Petiole - The stalk of a leaf.

Phenotype - The external, manifest, or visible characters of an organism, as contrasted with its genetic constitution (the genotype).

Pinnate - Resembling a feather in the leaflets are on each side of a stem.

Pistil - The organ of a flower which bears ovules and later seeds. It is composed of ovaries, stigma, and style.

Pistillate - A flower with only pistils; a female flower or plant.

Pollen - Minutes spores produced by the anther of a stamen.

Rapine - A seam that joins the two halves of a seed.

Radicle - The part of the seedling which becomes the root.

Root - The absorbing, usually underground part of a plant, without nodes.

Seed - A mature ovule consisting of an embryo and a surrounding protective coat.

Seminal root - The first or primary root produced by a seedling.

Sepal - The outer set of floral leaves (part of the calyx).

Sessile - Lacking a stalk, as some leaves and flowers.

Simple fruit - derived from a single flower and a single pistil

Simple leaf - having the blade in one piece

Simple pistil - consisting of a single carpel.

Sinsemilla - Spanish for "without seeds". When applied to marijuana - very mature female plants that have not been pollinated.

Stamen - The pollen producing part of a flowering plant, consisting of an anther and a filament or stalk.

Staminate - Bearing stamens and consequently male; usually used in reference to unisexual flowers or plants.

Stem - The major supporting structure in plants, to which buds, leaves, and flowers are attached at regular intervals at points called nodes.

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Stigma - The part of a pistil on which pollen adheres and germinates, generally terminal in position, and often enlarged.

Style - The stalk-like part of some pistils, connecting the stigma and the ovary.

Taproot - A stout, tapering main root from which arise smaller, lateral branches.

Taxonomy - The study of plant classification.

Trichome - A plant hair.

Variety - A reproducing, natural population of genetically related individuals.

Vascular - Containing conductive tissues.

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4.0.0 MICROSCOPIC ANALYSIS OF MARIJUANA

4.1.0 One of the tests performed on suspected marijuana samples submitted to the ISP laboratory is a microscopic examination. Marijuana displays a set of microscopic features that are unique. No other vegetation displays exactly the same appearance under the microscope. It is the view of many analysts that marijuana can be conclusively identified by microscopic examination alone. A positive microscopic examination is required for the identification of marijuana in the ISP laboratory. However, in this laboratory the microscopic examination must be confirmed by additional chemical tests. These tests will be discussed in a later section.

The microscopic examination is performed by viewing a representative sample of the questioned vegetation at 10 to 50 times magnification with a stereo microscope. For a positive identification to be made, leaf material must be present and that leaf material must have cystolithic hairs on one side and profuse covering hairs on the other side. Both types of hairs must in general be pointed towards the tips of the leaves (or if only leaf fragments are present, they must be pointed in the same direction). **Any other characteristics and plant parts that are also present (including vein structure, leaf texture and color, stem material, flower parts, and seeds) should also be examined and considered before arriving at any conclusions.** There are some variations in the appearance of different samples of marijuana due to growing conditions and other factors, but all marijuana samples exhibit common characteristics which can usually be recognized quite easily. To be proficient at identifying marijuana microscopically requires much practice and experience in examining a wide variety of marijuana samples as well as a large number of non-marijuana samples with a microscope.

The stereomicroscope is a low power magnifying instrument which produces a three-dimensional image of a specimen. This is achieved using two separate optical paths showing two slightly different views of the specimen. These two different views allow the user's brain to create a three dimensional image. The viewed image is "erect", that is it is not inverted, which makes the microscope much easier to use. The stereomicroscope basically allows the user to see an image that is similar to that seen by the unaided eye, only magnified. Depending on the lighting, the user can see color, texture, shape, glossiness, and other useful characteristics.

The total magnification of a microscope is the product of the occular magnification and the objective magnification (total magnification = occular magnification x objective magnification). For the stereo microscope, the objective is a group of lenses built into the body of the microscope. The occulars are the eyepieces of the microscope and they usually have a fixed magnification printed on them. The total magnification of the microscope can be easily changed by installing occulars with different levels of magnification, or by varying the objective magnification via adjusting the zoom

knob.

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Most stereo microscopes in forensic labs are mounted on boom stands, allowing a large distance between the microscope head and the heavy base of the stand. This allows the examination of large or bulky objects. Stereomicroscopes also usually have a large working distance. The working distance is the clearance between the upper surface of the object being viewed and the lowest edge of the objective of the microscope. The practical working distance generally decreases with an increase in total magnification.

There are two types of lighting that are commonly used: point source and annular. Point source lighting is from a single lamp and allows shadows to form behind high points in the specimen which allows a greater depth awareness of the specimen. Annular or ring lighting is created by a “ring” of light that completely encircles the specimen. This tends to cancel out shadows and provide even illumination across the entire field of view. Bifurcated fiber optic lights can be used as one- or two-point light sources to illuminate the specimen in a variety of ways.

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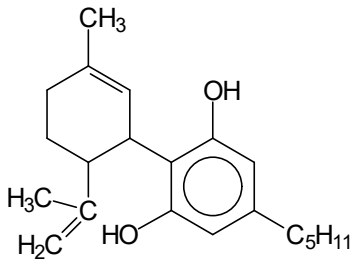
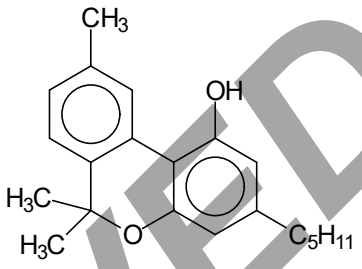
5.0.0 CHEMISTRY AND CHEMICAL TESTING OF MARIJUANA

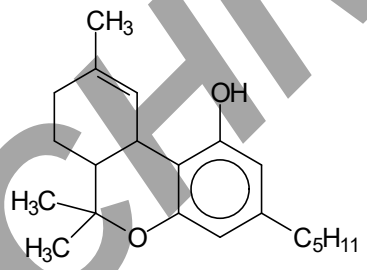
5.1.0 There are over 400 compounds in the marijuana plant, including cannabinoids, amino acids, proteins, sugars, hydrocarbons, steroids and terpenes. Forensic scientists are concerned chiefly with the cannabinoids. Cannabinoids are a group of structurally similar compounds which usually contain 21 carbon atoms. Of the 61 known cannabinoids three are most abundant. These three are; cannabinol (also known as CBN), cannabidiol (CBD) and tetrahydrocannabinol (THC). Tetrahydrocannabinol is considered to be the most pharmacologically active.



Cannabidiol (CBD)



Cannabinol (CBN)



Tetrahydrocannabinol (THC)

Figure 5 - 1 Common Cannabinoids

In attempting to assign chemical names to the cannabinoids, several numbering systems have been used. This has resulted in more than one name being assigned to each compound. The two most common numbering systems are the “Dibenzopyran” system and the “Monoterpene” system.

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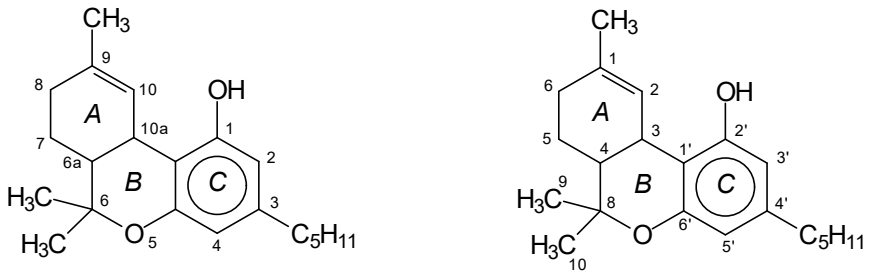
A = terpene ring
B = pyran ring
C = phenolic ring



Δ^9 - THC

Dibenzopyran System

Δ^1 - THC

Monoterpenoid System

Figure 5 - 2 Different Numbering Systems

As can be seen in figure 5-2 the delta (Δ) refers to the position of the double bond in the terpene ring. The compound in figure 5-2 can be called either Δ⁹ - THC or Δ¹ - THC. The dibenzopyran numbering system has become the most common and will be used for the rest of this discussion.

To further complicate matters, there are theoretically 8 possible isomers for THC. The double bond in the terpene ring could occur between C_8 and C_9. The resulting compound would be Δ⁸ - THC (or Δ⁶ - THC in the monoterpene numbering system). Different isomeric forms can also arise due to the geometry around the bond that joins the two asymmetric centers -- C_{10a} and C_{6a}. There are two possible cis arrangements and two possible trans arrangements. Analysis of coupling constants of the protons at these centers indicates that the true geometry between these two carbon atoms to be trans. The absolute configuration at both asymmetric centers -- C_{10a} and C_{6a} -- in naturally occurring THC is “R”. Polarimetry shows that naturally occurring THC is levo. Therefore the name (-) Δ⁹ -trans-tetrahydrocannabinol has been assigned to naturally occurring Δ⁹ THC. The Δ⁸ variety occurs naturally at about 1/100 the concentration of the Δ⁹ variety. The name assigned to the natural Δ⁸ form is (-) Δ⁸ -trans-tetrahydrocannabinol.

In living plants, THC and most other cannabinoids are present predominately in the form of their carboxylic acid derivatives and to a lesser degree in their "neutral" form. Δ^9-THC carboxylic acid occurs in two isomeric forms as illustrated in Figure 5-3. Approximately 95% of THC in fresh marijuana occurs in its acid forms.

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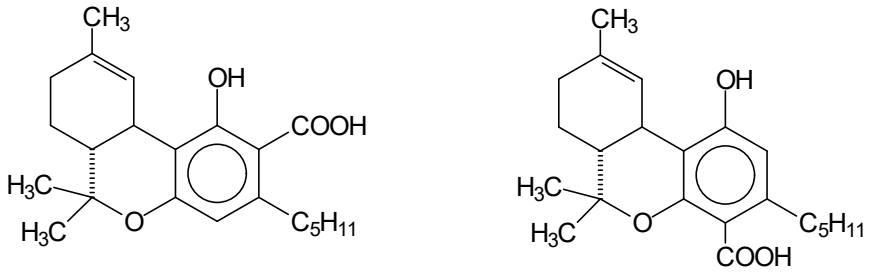

Figure 5 - 3 THC Acids

Both isomeric forms are converted on decarboxylation to Δ^9 - THC. Literature reports that most marijuana contains the A form. It would also seem to be the preferred molecular structure considering that steric hindrance from adjacent groups is minimal in comparison to the B form. However, the fact that the B form has been found in a few marijuana samples indicates the possibility that different variants of cannabis may have slightly different biosynthetic pathways.

Decarboxylation of the acidic cannabinoids is suspected to begin when marijuana is harvested and continues rather slowly on storage. The acid cannabinoids decarboxylate in solvent media especially when exposed to daylight. Under the influence of heat, decarboxylation occurs instantly and completely.

At room temperature, Δ^9-THC is oxidized to cannabinol at the rate of about 3-5% per month. This conversion is hastened at elevated temperatures. Δ^9-THC also slowly isomerizes to Δ^8-THC which decomposes at a lower rate. The decomposition of cannabinoids stored in solvent media is also hastened by light. Various literature references indicate that CBD condenses into THC. Analysis of old samples shows relatively large amounts of CBN and CBD with very little THC so the conversion of CBD to THC must not be nearly as efficient as the conversion of THC to CBN.

5.2.0 CHEMICAL TESTS FOR MARIJUANA

Chemical tests resulting in color production are widely used to test for the presence of marijuana, more specifically, for the cannabinoids produced by marijuana.

5.2.1 Duquenois Test:

The most popular color test is the modified Duquenois-Levine test. The Duquenois test was first reported in 1938; modifications which increase the specificity of the test for cannabinoids have resulted in renaming the test the "modified Duquenois-Levine" test. Various literature articles[15, 16, 17] have explored the possibility of obtaining false-positives using the modified version of the Duquenois-Levine test. The authors of these articles subjected various chemical compounds and plant materials to the

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test in an effort to obtain the same color formation as that obtained with marijuana. As a result of these studies, it appears that, when properly used, the modified Duquenois-Levine test can furnish presumptive evidence for the presence of marijuana or a marijuana product. The reagent will not only react with THC, but it will also react with CBD and/or CBN to provide the same blue-purple color. Combining this chemical test with a careful examination of the morphology of the sample plant material can definitely serve as a reliable screen for marijuana

The procedure for performing the Modified Duquenois-Levine test is given below:

Duquenois Reagent -- 2 grams vanillin, 2 ½ ml acetaldehyde in 100 ml ethyl alcohol (This reagent may be kept for some time in glass-stoppered bottles. Place approximately 1/4 gram of dry crushed sample in a test tube and extract with about 1 ml. of petroleum ether.

1. Transfer the petroleum ether to another test tube evaporate to dryness.
2. Add ~5 drops of Duquenois reagent.
3. Add ~ 5 drops of concentrated hydrochloric acid and mix. The presence of marijuana is indicated by an indigo-violet shade.
4. Add ~ ½ ml. of chloroform and stir. The indigo violet color produced by the action of the reagents on marijuana will partition into the chloroform layer.

NOTE: No strict rule need be observed with respect to amounts of sample or reagents. Those given are convenient. The test succeeds with a much smaller amount of vegetation. With differences in sample size, it is a good idea to adjust the amounts of reagent accordingly.

Below is a proposed mechanism for the Duquenois reaction. There may be other possible mechanisms.

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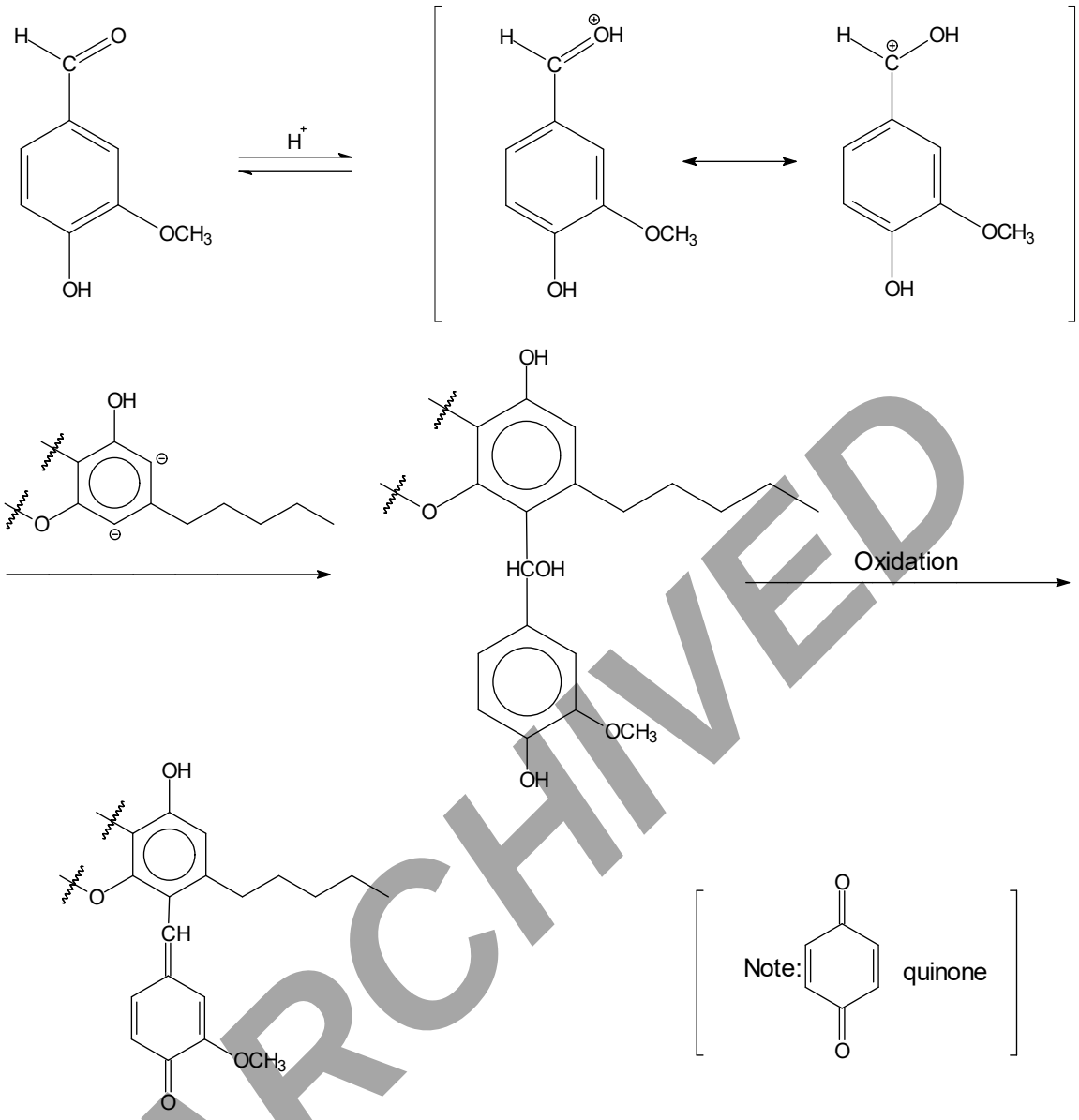

Figure 9 - Duquenois Reaction

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The petroleum ether extract removes the cannabinoids from potentially interfering substances. A non-polar solvent is used because many of the compounds present on the plant material are insoluble in this type of solvent. The cannabinoids by virtue of the n-amyl side chain of the phenolic ring are soluble in non-polar solvents.

Vanillin is protonated under acidic conditions. This is why the Duquenois reagent is not mixed with acid until the test is conducted. The protonated aldehyde group of vanillin acts as an electrophile which attacks the phenolic ring of the cannabinoids. The para-hydroxy group of vanillin is not disturbed in the initial attack on the cannabinoid. The intermediate product resulting from the initial attack then undergoes further oxidation to form a highly conjugated quinone-like compound. Conjugated compounds are usually colored.

The exact role of the acetaldehyde in the reaction in unknown. The reaction will proceed without it, albeit more slowly. Its role is probably as an oxidizing agent. Since it is an aldehyde like vanillin, it can also substitute at the ortho and para positions of the phenolic ring of the cannabinoids and then undergo further condensation.

The reaction is acid catalyzed and the final colors are pH dependent. This is the reason why a range of colors is considered positive for the cannabinoids and why it is important to always use a consistent amount of Hydrochloric Acid when performing the Duquenois test. The range of colors is also dependent on the relative proportions of the cannabinoids which are present in the sample.

The Duquenois test is designed to detect molecules containing structures similar to the cannabinoids. Like any other color test, it reacts with the chemical moiety and not just a specific compound. The Duquenois reagent does react with other phenolic and terpene derivatives but the product, if colored, may not be in the correct color range. There may, however, be materials which produce a color which cannot be distinguishable from the color produced by the cannabinoids. The fact that this possibility exists does not necessarily mean that there are other plant materials which produce a similar color or that the color will be soluble in chloroform.

5.2.2 There are several variations of the Duquenois Test:

Duquenois-Negm: The original Duquenois test where the Duquenois reagent and Hydrochloric acid were added to the evaporated plant extract to form the purple color.

Modified Duquenois-Levine: The Levine modification to the Duquenois-Negm test is the addition of the chloroform.

Physical tests used to identify THC in suspected marijuana samples vary from TLC to to GC/MS to IR. TLC is widely used in forensic laboratories because of the availability of many solvent systems, supports, and sensitive visualizing reagents. There is little doubt that a mass spectrum, an infrared spectrum, or a nuclear magnetic resonance spectrum will unequivocally identify THC, as well as CBN and CBD .

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5.3.0 THIN LAYER CHROMATOGRAPHY:

Thin Layer Chromatography (TLC) is a very common analytical technique that has many applications. It will be covered in greater depth in a later phase of the forensic scientist training program. For now, a brief introduction is all that is necessary to use this technique for marijuana analysis.

Thin Layer Chromatography is a technique that incorporates a solid stationary phase and a liquid mobile phase to effect the separation of the constituents of a mixture. A TLC plate is prepared by coating a glass plate with a thin layer of an adsorbent material. Silica gel is a commonly used stationary phase. A small amount of the sample to be analyzed is placed near the lower edge of the TLC plate. The plate is then placed in a closed chamber called a development chamber that contains a selected liquid or solvent. The solvent slowly travels up the plate by capillary action. This rising solvent serves as the mobile phase. As the solvent moves past the sample spot, the components of the sample will become distributed between the solid stationary phase and the liquid mobile phase. Those components with the greatest affinity for the mobile phase will travel up the plate faster, and hence farther, than those that have a greater affinity for the stationary phase. When the solvent front, has moved a sufficient distance (usually almost the entire height of the plate) the development of the plate is complete. The plate is removed from the chamber and allowed to dry.

Often the various components of the sample cannot be seen with the naked eye. A number of methods are available to "visualize" them. Often a chemical reagent is sprayed on the plates. This reagent reacts with the various components of the sample forming colored spots.

The distance a compound moves up a TLC plate can be assigned a numerical value known as the R_f value. This value is defined as the distance traveled by the compound divided by the distance traveled by the moving liquid phase. The actual R_f value for a given compound can vary due to a number of different factors including: humidity, relative concentration of components in the developing solvent, thickness of the stationary phase, chamber saturation, etc. Because of the variability of the R_f values, questioned samples are developed on the same plate alongside an authentic standard. If both the sample and the standard travel the same distance up the plate, they can be tentatively identified as being the same. It must be cautioned that such an identification cannot be considered definitive, for the possibility exists that other substances can migrate the same distance up the plate when chromatographed under similar conditions. Thus, a single TLC cannot by itself provide an absolute identification. It must be utilized in conjunction with other testing procedures to prove absolute identity.

In years past, many forensic laboratories, used a combination of two TLC procedures using two different developing solvents to identify THC. The two solvent systems separate the various components in the samples differently. The combination of the two solvent systems greatly enhance the specificity of

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identification. An authentic □9 - THC standard is run alongside the sample in these tests. The samples are visualized with either Fast Blue B ((3,3'-dimethoxybiphenyl - 4,4' - bisdiazonium chloride also known as ortho-dianizadine) or Fast Blue 2B (4 - benzoylamino - 2,5 - diethoxybenzenediazonium chloride). Both of these reagents react with the cannabinoids to give unique colors. Literature reports that very few compounds will react with these reagents to give the exact same color as THC. Those compounds that do give the same color will not migrate the same distance on the TLC plates as THC. Therefore, the two different developing solvents in combination with the specific visualizing reagents makes the dual TLC identification procedure highly specific for identifying □9 - THC.

Marijuana identification at ISP is accomplished via microscopic examination backed up by a single TLC procedure and a Duquenois-Levine test. For samples that cannot be identified as marijuana via a microscopic examination, □9 - THC is identified by a combination of two TLC systems and Duquenois-Levine test.

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5.4.0 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS):

Gas Chromatography/Mass Spectrometry (GC/MS) is a very common instrumental technique that has many applications. It is considered state of the art for many forensic analyses. Both gas chromatography and mass spectrometry will be covered in depth in a later phase of the forensic scientist training program. For now, a brief introduction is necessary to use this technique for marijuana analysis.

A GC/MS is really two analytical instruments attached in sequence. The first instrument is the gas chromatograph. Like all forms of chromatography, gas chromatography employs a mobile phase and a stationary phase. A tube known as a "column" contains the stationary phase. In the instruments used in this laboratory for THC analysis, the stationary phase is a very viscous liquid (typically made of cross-linked polymerized methyl or phenyl-methyl siloxane compounds) bonded to the inside walls of the column. The mobile phase is an inert gas such as Helium flowing through the column. This mobile phase is often called the "carrier gas". The sample is dissolved in a solvent and injected on one end of the column. The column is contained within an oven. The temperature within the oven is raised to a point where the components within the sample mixture vaporize. The components are carried through the column by the carrier gas. As they move through the column, they continuously partition between the mobile phase and the stationary phase. The amount of time it takes a given component to travel through the column depends on how much time it spends in the stationary phase, and the gas flow rate. The amount of time a component spends in the stationary phase depends on a variety of factors including the component's vapor pressure, molecular weight, polarity, and atomic makeup. Different chemical compounds will travel through the column at different rates. The amount of time between the point where a compound is injected on one end of the column and when it elutes from the other end of

the column is called the retention time. In most instances, compounds can be tentatively identified by comparing their retention times with the retention times of known standards.

In the typical GC/MS system, compounds pass from the end of the GC column into the mass spectrometer. The compounds initially enter an area of the mass spectrometer known as the ion source where they are bombarded by electrons. The neutral molecules are ionized to form a variety of products, including positive ions. Initially the ions consist of whole molecules with a single electron missing. This is know as the "molecular ion". Loss of an electron often destabilizes the molecule causing it to fragment into both neutral and charged species. While fragmentation can occur by breaking any bond in the molecule, the bond cleavage tends to occur at certain preferred locations, giving rise to a reproducible distribution of ions which is unique for most compounds.

The positive ions are electronically ejected from the ion source through a series of electronic "lenses" where they are focused into the mass filter.

The quadrupole filter consists of four parallel electrodes (rods) held in a square array. To each diagonally paired set of rods a combination of radio frequency (rf) and dc voltage is applied. One pair receives an rf voltage and positive dc voltage, and the other pair receives an rf voltage with an 180^{o} phase shift and a negative dc voltage. These voltages create an electrostatic field that causes the ions to oscillate as they travel along the space between the rods. Only ions with the proper mass to charge ratio will have a stable trajectory through the quadrupole array at a given voltage and rf combination. The voltage and rf values are "ramped" through a range of appropriate values to sequentially pass ions with ascending or descending (depending on the model of instrument) mass to charge ratios.

Once ions are ejected from the mass filter, they are quantitatively detected by an electron multiplier which creates an amplified signal. This signal is fed to an appropriate data handling system.

The data handling system depicts the data in the form of a bar graph in which each bar represents a mass of ion detected, with the height of the bar being proportional to the ion's abundance. This graph is known as a mass spectrum. The majority of chemical compounds give unique mass spectrums. In most instances, comparing the mass spectrum and GC retention time of an unknown compound with those of a known compound run on the same instrument under identical conditions is enough for absolute identification of a unknown compound. Some isomers and diastereoisomers have similar or identical ion distributions and retention times. These instances are not a problem in THC analysis and will be covered later in the training program.

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6.0.0 ~~MISCELLANEOUS~~ Additional Considerations

6.1.0 The purpose of this section is to cover the information what was not covered in the other sections. ~~For this reason, it will appear as a sort of hodgepodge of information.~~

Cigarettes that appear to have gotten wet or smell like organic chemicals may contain PCP and should be subjected to further analysis.

Crack cocaine is sometimes mixed with the vegetation in cigarettes. If small white masses are noticed during the microscopic exam, the sample should be subjected to further analysis.

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19.0 Appendix B

Idaho State Police

Forensic Laboratory Training Manual

Thin Layer Chromatography

1.0.0 Background

Thin layer chromatography (TLC) is an analytical technique that often offers a quick and easy separation of chemical compounds. The forensic chemist uses this technique primarily for screening drug samples and for isolating active constituents from solutions containing two or more compounds. TLC is especially applicable to drugs that cannot be analyzed by gas chromatography (GC). LSD, for example, may degrade on some GC columns; but is easily detected by TLC and sufficient quantities of the drug can be separated and isolated by TLC for further confirmatory techniques.

TLC is a physicochemical separation method. Since the technique is identical to the function of a micro column, the theoretical aspects of column adsorption chromatography are also applicable to TLC. The thin separatory layer (stationary phase) is usually placed on a support plate of glass. After dissolving a small portion of the sample mixture with an appropriate solvent, the solution is applied, as a spot, at the starting point of the plate, i.e., at the "origin." All necessary drug standards are spotted at the origin (and, occasionally, a combination of the sample and standard solutions applied as a single spot to verify chromatography resolution). After the "spots" have dried, the plate is placed into a TLC tank containing a suitable solvent (mobile phase or solvent system). The TLC tank is immediately covered and separation takes place as a result of capillary migration (development process). As the mobile phase moves over the adsorbed spot, the equilibrium is shifted and constituents present in the spot may be desorbed. The more tightly adsorbed compounds are desorbed to a lesser extent than the more loosely absorbed ones. A new equilibrium is established as the redissolved compounds are carried to the edge of the spot, where they come into contact with fresh adsorbent. Throughout this process, the composition of the mobile phase is continuously altered by the interchange of compounds between the adsorbent and the mobile phase. Whenever two compounds adsorb at the same site, the compound that is more strongly adsorbed, will displace the other. The displaced compound will then form a spot further away from the origin. The more similar the adsorptive properties of two compounds are, the more difficult it is to separate them. Compounds having nearly identical properties cannot be separated under most TLC conditions.

At the termination of the development process, the plate is removed from the TLC tank, air-dried and visualized (detection process). Under given conditions of temperature, solvent system and type of adsorbent, the chromatographic behavior of sample constituents is described in terms of "Rf" values. The Rf value is a characteristic of a particular substance and is described as the ratio of the distance traveled by the constituent to the distance traveled by the solvent. This can be expressed as follows:

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Rf = distance the (spot center) of the constituent traveled from origin

distance the solvent front (mobile phase) traveled from origin

Distance for calculating Rf values are usually measured in centimeters. Since Rf values are a function of a number of variables, they should be considered only as guideline values.

2.0.0 Absorbents

Many adsorbents are used in TLC. These include silica gel, alumina, diatomaceous earth (kieselguhr), cellulose, magnesium silicate (florisil), ion exchange resins, and polyamide powder. These adsorbents may be purchased with or without either a binder (5-15% calcium sulfate, starch, or carboxymethylcellulose) and/or an inorganic fluorescent substance. The adsorbent is applied to a backing as a uniform coating. The most common support is a glass plate, but other supports such as plastic sheets and aluminum foil are also used.

Silica gel is the most popular adsorbent used by forensic chemists. It is slightly acidic in nature and works quite well for separating alkaloids.

Today, commercially available pre-coated TLC plates are widely used in forensic laboratories. Their popularity may be attributed to their high degree of coating uniformity, convenience, and moderate cost. Commercially available TLC plates come in a number of variations in order to facilitate their use for different drugs or different classes of drugs. Each variation is designated by the use of a specific suffix highlighted on the carton label. The suffixes "60", "90", or "150" indicate the mean pore diameter in angstroms. The suffix "G" indicates a calcium sulfate (gypsum) binder and "HL" a silicon dioxide/aluminum oxide binder. The designation "F" indicates a fluorescent indicator is present in the adsorbent, and the subscript number (i.e., F_{254}) gives the excitation wavelength for viewing the quenching of a fluorescent background. The letter "P" is the code designation for preparative thin layer chromatography, while "R" indicates a specially purified adsorbent. "RP" indicates a silanized gel for reverse-phase work. It is very important to select the proper type of pre-coated plate for a particular drug group.

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3.0.0 Variables Which Affect TLC Rf Values and Separation

A number of factors can affect the reproducibility of Rf values and resolution so actual results may vary from literature values and may also vary from run to run. For this reason, it is preferable to run actual standards alongside unknown samples rather than make identifications based on calculated Rf values.

Some of the factors that can affect the reproducibility of Rf values and separation quality are:

1. The relative humidity and ambient temperature of the TLC system,
2. The degree of activation or the moisture content of the adsorbent,
3. The moisture content in the solvent system,
4. Accidental contamination of absorbent or solvent system,
5. Thickness of the adsorbent layer,
6. Presence of impurities (including undesirable commercial ingredients such as binders and preservatives) in the adsorbents or solvents used,
7. Possible reaction between the sample spot on the TLC plate and the solvent system, which may be further aggravated in the presence of the adsorbent material,
8. Sample degradation,
9. Variation in the properties between adsorbent batches,
10. The pH of the adsorbent and/or solvent system,
11. Chamber design or the degree to which equilibration is achieved and maintained within the chamber, and
12. The drying conditions of the TLC plate.

Of the above twelve factors, the most commonly encountered are contamination and extraneous water in the adsorbent. The finely divided adsorbent provides a large surface area, which can rapidly pick up any organic vapors in the atmosphere. These organic contaminants are often observed as a dark band at the solvent front, and are rarely serious enough to cause problems when monitoring the movement of different components with the solvent front. Another form of contamination that may be encountered is oil from the hands caused by careless handling of the plates before development. Oil

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from the hands can be transferred to the edges and back of the plates and dissolved by the solvent system. As the solvent moves up the plates, the oil is deposited throughout the adsorbent.

The amount of water present in the adsorbent, the sample, and/or the solvent system is probably the most important variable in TLC. The presence of excessive moisture can lead to distortion of the TLC separations, resulting in non-reproducible results. Moisture is present in the adsorbent in three forms: (1) water of constitution, (2) water of hydration, and (3) free water. The free water can be removed from the adsorbent by heating the thin layer plate to 105°C for one hour (preferably in a forced draft oven). Water in the free state is not necessarily detrimental to the chromatographic separation. It should be recognized, however, that the water level must be consistent from plate to plate. Water of hydration, if calcium sulfate is the binder, can be removed by drying at 180°C for one hour. The water of constitution can be removed by heating to 450°C - 500°C for several hours. In most cases, the water of hydration and the water of constitution are allowed to remain while the free water is removed by drying at 105°C for one hour. While always a potential problem, the moisture content of TLC plates is rarely found at levels that necessitate pre-drying of the plates prior to routine use.

Extraneous moisture can be introduced to the plate by blowing the dust from the plate, resulting in moisture from the breath being condensed onto the plate. Water may also be introduced at the point of sample application if the plate is not dried long enough to allow the water to evaporate after it has condensed from the atmosphere. The developing solvent may also contain water.

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4.0.0 Solvents

Suggested Reading Reference (2) lists the most common TLC systems used for isolating numerous drugs. For each TLC system, all of the required conditions are described, e.g., the type of plate to use, sample preparation prior to spotting, solvent system, equilibration time, development distance, and the visualization method. Additional solvent systems applicable to drug analysis can be found elsewhere in the literature.

On infrequent occasions, external conditions, such as insufficient humidity, high temperature, etc., may lead to chromatography where component Rf values are consistently higher or lower. To a limited extent, this can be corrected by modifying the solvent system. For most drug applications, reducing the polarity of the solvent system will reduce the Rf values, and increasing the polarity of the solvent system will increase the Rf values. Care should be exercised when attempting to modify a solvent system since resolution may become adversely affected.

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5.0.0 Visualization of the Developed Chromatogram

The first two steps in performing thin layer chromatography are spotting and development, but results cannot be evaluated without visualization. Spray reagents, which visualize certain chemical groups, are quite common in most analytical laboratories. They are suitable for detecting the drug in question, but since most of these reagents react with certain organic groups rather than with particular classes of drugs, considerable caution should be used in assigning specificity to their use. For example, ninhydrin, a general reagent for the detection of primary amines, is not specific for amphetamines, since positive reactions can occur from any primary amine. Therefore, it is important to establish the identification of a TLC spot on the basis of a sample/standard comparison (i.e., the color and Rf values with those of known drugs spotted on the same plated on which the unknowns were spotted.)

In addition to the chemical visualization method mentioned above, physical detection methods can also be used with many drugs. For example, quinine and LSD fluoresce naturally when they are exposed to long-wavelength UV light. Using their fluorescent properties, it is possible to detect them without chemically altering the sample. Some pre-coated plates are available with fluorescent indicators, which can be used to detect substances absorbing at particular wavelength. For example, sodium fluorescein fluoresces when exposed to UV light of 254 nm wavelength. Therefore, substances absorbing this wavelength will contrast sharply by appearing dark while quenching the greenish-yellow fluorescing background. After the spots have been visualized, they are compared with the proper reference standards and controls.

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6.0.0 Elution of TLC Spots

A secondary advantage of TLC is that it permits elution of the migrated spots from the TLC plate for further analysis. Although this is now being superseded by preparative high performance liquid chromatography (HPLC), the method of TLC spot elution warrants mentioning because of its simplicity and because it can serve as an alternative.

As mentioned above, LSD can be isolated in sufficient quantities by streaking a sample extract onto a TLC plate and developing with the proper solvent system. The developed streak (conforming to the Rf for standard LSD) is scraped from the plate, transferred onto filter paper, and washed with a solvent to retrieve the LSD for further confirmation by a technique such as infrared spectrophotometry (IR). This identification can be accomplished within the time span of about an hour. (Because LSD free base is subject to slow degradation, elution and final identification should be performed immediately and without delay.)

7.0.0 Readings

1. Clark, Isolation and Identification of Drugs, 3rd edition, vol.1, pg 392-424.

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20.0 Appendix C

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Forensic Laboratory Training Manual

Extraction Techniques

1.0.0 SOLUBILITY

In the field of forensic chemistry, the main purpose of an analysis is the identification of the substance in question. For this reason, each active ingredient of a mixture must be isolated from other sample constituents. There are different ways to achieve this, one of the simplest being by use of the varying solubility properties of chemical compounds. Solubility is the capacity of two or more substances to form spontaneously, without chemical reaction, a homogeneous molecular (or colloidal) dispersion. More specifically, the solubility of a solid in a liquid refers to the concentration that is reached when a fixed amount of liquid has dissolved the entire solid it can hold at equilibrium (at a specific temperature). The solubility of solids in liquids range from very low to very high values. Because of this range of solubilities, the word "soluble" does not have a precise meaning. There is usually an upper limit to the solubility of even the most soluble solid, while even the least soluble would yield a few dissolved crystals per liter of solution. Table 1 below lists descriptive terms for varying degrees of solubility with corresponding (but indefinite) solubility ranges.

Determining the exact solubility of a substance is not required for drug analysis; however, knowing the approximate solubility properties of substances is extremely useful for the separation of constituents in simple drug mixtures.

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Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very Soluble	Less than 1
Freely Soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly Soluble	From 30 to 100
Slightly Soluble	From 100 to 1000

Very Slightly Soluble	From 1000 to 10,000
Practically Insoluble or Insoluble	More than 10,000

Table 2 - Descriptive Solubilities

2.0.0 DRY EXTRACTIONS

Dry extraction (sometimes referred to as direct extraction) is one of the simplest separation techniques known. It is based on the premise that the substance of interest in a sample mixture is soluble in a specific solvent while all other components of the sample are insoluble. A dry extraction procedure is part of the qualitative scheme and involves titration of a portion of a simple powder with a specific solvent, filtration, collection of the extract, and evaporation of the extract to dryness. The resulting residue would be the constituent of interest, in a fairly pure state, available for specific identification studies.

Generally, samples of cocaine (base or HCl salt) intermixed with any sugar and/or inorganic bicarbonates can be dry extracted with chloroform to separate the cocaine from its diluents. Although cocaine is very soluble in methanol, it would not be a suitable extracting solvent for these mixtures. Since sugars are sparingly soluble in methanol, a dry extraction of the sample powder would not only remove the cocaine, but it would dissolve some of the sugar as well and ultimately provide an impure product.

The most common organic solvents used for dry extractions are chloroform, methylene chloride, ethyl ether, and methanol. These solvents must be used in an anhydrous state. Prolonged standing of partially filled solvent containers may allow moisture to accumulate to the point where its use may introduce separation difficulties in the dry extraction process.

3.0.0 CRYSTALLIZATIONS

Crystallization is a method commonly used for isolation and purification of one or more substances. In crystallization, a substance containing impurities is dissolved in just sufficient solvent to give a saturated or nearly saturated solution close to the boiling point of the solvent. If necessary, the hot solvent is filtered and then cooled rapidly with occasional stirring. Crystals of the desired substance will precipitate out with only a minor amount of impurities (compared to original sample powder) because the cooled saturated solution formed is not saturated with the impurities, which, therefore, remain in solution.

If only one compound is separated from its impurities, the process is called a simple recrystallization. If two or more pure compounds are separated from a mixture, such a process is termed fractional recrystallization. Recrystallization should be repeated as many times as necessary in order to reach the desired level of purity.

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The effectiveness of crystallization is determined by two important factors:

1. The solvent power, and
2. The crystallization temperature coefficient.

The power of the solvent is expressed as the mass of solute that can be dissolved in a given mass of pure solvent at a specified temperature. The crystallization temperature coefficient is an indication of how "powerful" a solvent is at different temperatures. The "power" thus determines the volume of solvent to be used, while the temperature coefficient determines the yield.

Selection of the proper solvent is based on several conditions. For inorganic substances, water is the ideal solvent since it is inexpensive and readily available. For organic substances, a variety of liquid ketones, ethers, chlorinated hydrocarbons, or aromatics can be used. A mixture of these can be used when a substance is highly soluble in one and only slightly soluble in the other.

Other factors to consider for proper solvent selection are volatility, viscosity, flammability, toxicity, flash point, carcinogenic properties, availability, reactivity of solvent/solute, and purity. Purity is extremely important since a contaminated solvent can inhibit a substance from crystallizing or impart an undesirable coloring to the crystals.

The development of different crystalline forms (needles, rods, etc.) is caused by the formation of different crystal habits or by polymorphism. Different crystal habits are produced when the environment of the growing crystal affects its external shape without changing its internal structure. This phenomenon is caused by such circumstances as super-saturation at different points in the solution, proximity of one crystal to another, cooling rate, degree of agitation, size and number of nuclei.

Polymorphism occurs when the same compound exists in one or more crystalline and/or amorphous forms. Different polymorphic forms are produced, depending on such factors as:

1. The surface on which the crystals are formed
2. The type of nuclei introduced
3. The temperatures at which spontaneous crystallization occurs
4. The type of solvent used.

Polymorphic forms of a compound's crystals can be distinguished by melting points, light microscopy, X-ray diffraction, infrared spectroscopy, and other techniques.

Although crystallization is an excellent method for the production of pure substances, it is a tedious and time-consuming procedure. The success of this technique depends largely on the care taken when

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scratching the surface, adding the proper crystalline form of the nuclei (also known as “seed,” “germ,” or “submicron”), controlling the cooling rate, etc. There are three common techniques of inducing crystallization:

1. Leaving the sample open to the environment (dust sometimes induces crystallization)
2. Placing the sample in a refrigerator (cooling)
3. Placing the sample in a desiccator (hygroscopic reasons).

When time and sample size permit, the sample should be divided into three different portions, and all three techniques attempted simultaneously. Then, the portion exhibiting the best crystallization can be selected for final use. Once crystallization begins, the surface of the container can be scratched (and the “germs” dispersed).

4.0.0 PARTITIONING AND DISTRIBUTION

The extraction or removal of a constituent of a solid mixture by contact with a suitable solvent or by transferring the substance from one liquid to another (immiscible) liquid is called distribution. The distribution of a solute between two immiscible liquids is called partitioning.

The distribution law governs the partitioning of a solute between two immiscible solvents. This law is expressed in terms of solute concentrations (C) in each liquid phase, independent of the total amount of solute (A) present. If the solute A is allowed to distribute itself between water and an organic phase, the resulting equilibrium can be expressed as:

$$A_W \leftrightarrow A_O$$

At equilibrium, the ratio of phase concentrations is constant and is termed the partition coefficient or distribution coefficient (K).

$$K = \frac{(A\ \text{organic})}{(A\ \text{aqueous})} \quad \text{or} \quad K = \frac{C_O}{C_W}$$

Where C_O = concentration of A in organic and C_W = concentration of A in aqueous phase.

The values of C can be determined experimentally and plotted as C_O vs. C_W, in which the slope equals K. This plot is called the partition or distribution isotherm and should be linear. (However, there are exceptions; e.g., the formation of a dimer between the solute and one of the solvents.)

For a simple or single extraction in which K is known for a particular system, one can determine the fraction of the solute in each phase at equilibrium, since:

p = fraction of the solute in the organic phase

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q = fraction of the solute in the aqueous phase

so that,

$$p = \frac{\text{amount of solute in the organic phase}}{\text{total amount of solute}}$$

Solute amounts (moles, etc.) can be expressed in terms of concentration and volumes, as follows:

Solute in the organic phase = $(C_O)(V_O)$, where V_O = volume of organic phase

Total solute = $[(C_O)(V_O)] + [(C_W)(V_W)]$, where V_W = volume of aqueous phase.

To simplify, substitute volume ratios expressed in terms of U, as follows:

$$U = \frac{V_O}{V_W}$$

Since p + q = 1 by definition and $U = V_O/V_W$, one can express p and q in terms of K and U:

$$p = \frac{KU}{KU+1} \quad \text{and} \quad q = \frac{1}{KU+1}$$

5.0.0 LIQUID - LIQUID EXTRACTIONS

The selective power of the extracting process for separating and purifying drugs is vastly expanded when two immiscible solvents are employed simultaneously in a liquid-liquid partitioning or “shake-out” procedure. In this process, a drug dissolved in one solvent (usually the aqueous phase) may be separated from accompanying impurities by shaking out with a second immiscible solvent (the organic phase) in which the substance sought is quite soluble, but the impurities are not. Removal of the organic phase containing the desired compound with impurities retained in the aqueous layer isolates the desired component in a purified solution. In other instances, the impurities may be in the organic phase that is initially removed and the compound of interest retained in the aqueous layer. The classical apparatus used in accomplishing this segregation of immiscible phases is the separatory funnel. Other extracting devices such as the mixer-extractor-separators, liquid-liquid extractors, super-critical fluid extractors, etc., are commercially available.

The shakeout procedure has been widely exploited for the qualitative and quantitative separation of drugs. Its use is dependent upon the availability of two convenient immiscible solvents in which the substance sought shows a distribution coefficient considerably different from that of accompanying impurities or secondary products.

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Theoretically, a single extraction for the quantitative recovery of a substance is feasible when K is 10 or greater (and accompanying components partition coefficient values are small). In practice, however, even if the distribution coefficient is extremely large, a single extraction will not quantitatively extract the substance of interest. What causes this deviation from the theoretical is that part of the organic phase (containing some of the substance) remains in the aqueous phase within the separatory funnel because of the impossibility to achieve complete segregation of the separatory phases. Also, another contributing factor is that the organic phase will adhere to the surface of the separatory funnel.

To achieve a quantitative recovery, the extraction process of the original phase (usually the aqueous phase) must be repeated with fresh portions of the organic phase (after removing the first extract and washing with solvent as needed). The extracts and washings are combined for quantitative measurement.

One can determine the number of extractions needed by determining p and q values. If one calculates the progress of the extractions, the following conclusions are reached in the Nth extraction:

1. Fraction of the total extracted in the Nth extraction = Fraction of the total left after (N-1) extraction times p;

   written as: $pq^{(N-1)}$

2. Total fraction extracted = $\sum_{N=1}^{N} pq^{(N-1)}$

3. Fraction remaining = q^N

A more efficient extraction is achieved by performing several extractions rather than a single one utilizing the same total volume of extractant.

The factor determining the success of a separation by extraction if the separability factor B, where $B = K_1 / K_2$.

The more B deviates from unity, the more feasible the separation. Although the most important factor in the separation of two substances is the difference between their distribution ratios, there are other factors to consider:

1. Nature of the solvent -- solubility of solute in it (polar, non-polar, etc.)

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2. Ease of recovery of solute from solvent
3. Miscibility of the two phases
4. Relative specific gravity
5. Viscosity
6. Tendency to form emulsions
7. Toxicity and flammability

Acid-base extractions are commonly employed in liquid-liquid partitioning to isolate a compound (or a group of similar compounds) from a complex matrix and to retrieve the isolated compound in the form of a concentrate for further study. While performing the extraction procedure, the retention of a compound in acid or basic solution provides some general clues about the compound. For example, most basic drugs are soluble (and retained) in acid solution and insoluble in basic solution. Most acidic drugs are soluble (and retained) in basic solutions and insoluble in acid solution. Neutral drugs (as well as a few basic drugs) are soluble, as well as extractable, from either acid or basic solutions. There are a number of qualitative schemes devised for the extraction of different types of drugs. These schemes should only be considered as a guide since a forensic chemist must usually modify a selected scheme in order to accommodate the nature of the sample and the resolution of a specific problem.

To bring two immiscible solvents into close contact and to establish the equilibrium distribution of the solute sought, one must shake the solvents in a separatory funnel. The purpose of the shaking is to temporarily emulsify the phases so as to increase the interface at which the exchange or partitioning of the solute occurs. However, long or violent agitation may cause the two phases to form a stable emulsion.

Stable emulsions are one of the main problems encountered when conducting acid-base extractions. As a rule, alkaline solutions emulsify more readily than acidic solutions. For an emulsion to break, sedimentation and coalescence of the droplets of the dispersed phase must occur. The settling will decrease as the viscosity decreases, the density difference decreases, and the drop size decreases.

In systems where emulsions tend to occur, one may use one or more of the following techniques to limit emulsion formation or stability:

1. Give special attention to the method of agitation;
2. Choose solvents which will not react chemically with the solutes;
3. Choose liquids with large interfacial tensions;
4. Filter to remove any solid material before extraction;

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5. Increase the relative volume of the organic solvent;

6. Filter the emulsion through some porous substance to induce coalescence.

6.0.0 READINGS

1. Microgram, Vol. XVI, No. 1, Jan 1983, “The ANOR Extraction Procedure”

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21.0 Appendix D

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Gas / Liquid Chromatography

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1.0.0 Background

In 1942, Martin and Synge developed a partition method of chromatography, n which the solute (material to be chromatographed) is partitioned between a stationary liquid phase absorbed on an inert support and a moving liquid, which is only partially miscible with the stationary phase. At that time, they pointed out that the moving liquid phase could be replaced with a gaseous one. Martin and James exploited this suggestion and, in 1952, published a paper that marks the birth of gas chromatography. Development of the technique was rapid, with the petroleum industry, in particular, playing a large part in its advance.

Gas-liquid chromatography (GLC) is a method of separating the components of a volatile mixture by distributing (partitioning) them between a stationary liquid phase and a moving gaseous phase. The stationary phase is loaded into a tubular column and gas is passed through the system. The sample is placed at the head of the column, vaporized, and passed down the column by the carrier gas. At the column exit is a device for detecting the solute as it is eluted from the column. The signal from this detector is amplified and displayed on a data system.

Any of the permanent gases, with the exception of oxygen, can be used as the mobile phase. From a theoretical standpoint, the lighter gases are more desirable. Hydrogen is to be avoided as a carrier gas because it is explosive and, in some instances, reactive with the solute. Helium is an excellent gas for GLC, but its use, because of cost, is restricted to situations involving certain types of detectors. Nitrogen is the carrier gas normally used in packed GC column chromatography, and helium is the preferred carrier gas in capillary GC column chromatography.

2.0.0 COLUMN SUPPORTS AND PHASES

The heart of any gas chromatograph is its column. The column tube can be made from a variety of materials including metals and glass. Glass is preferred for its chemical inertness. If the tube is filled with a liquid-coated solid support, it is called a “packed” column; if the inner wall of a small diameter tube is coated with the liquid, it is called an open tubular or capillary column.

In order to eliminate "active sites" on the various supports, great care is taken in their manufacture. Such sites are usually -Si-OH, onto which the solute can be adsorbed, leading to poor peak symmetry, possible irreversible adsorption, and even decomposition of the solute.

The rate at which the solute molecules pass through the column depends upon two factors:

1. Their affinity for the stationary phase

2. Their vapor pressure at the column temperature employed

The vapor pressure of solutes increases with increasing column temperature, thereby causing the solutes to elute faster because of less residual time in the stationary phase. A mixture of solutes of widely varying volatilities can thus all be eluted from the column in a single run by starting at a low column temperature and then raising it. This technique is known as temperature programming.

When a solute is distributed between two phases under a given set of conditions (temperature and pressure), the ratio of the concentration of the solute on the stationary phase (C_L) to the concentration of the solute in the mobile phase (C_M) is referred to as the distribution or partition coefficient, K, and is expressed as $K = C_L/C_M$. Separation of two solutes can occur only when their K values are different. A separation that cannot be achieved on a nonpolar stationary phase may be possible on a polar phase if the two solutes are of different polarity. As a general rule, "like dissolves like." Hence, on a polar stationary phase, a solute of greater polarity will be more strongly retained (lower K value) than a solute of lower polarity. In addition to being a good solvent for the solutes, the stationary phase should be:

1. Thermally stable

2. Nonvolatile at the temperature used

3. Of low viscosity (in order to form thin films on the support)

For drug analysis, the most common liquid phases in use are the silicone gums and fluids. OV-1 and SE-30 are silicone gums and OV-101 and CD-200 are silicone fluids. All four liquid phases are methyl silicones whose active groups are 100% methyl groups. As a result, all four are nonpolar phases and all are considered chromatographically equivalent.[1] OV-17, which is a more polar phase, consists of 50:50 methyl silicone: phenyl silicone, which means that 50% of its active groups are phenyl and 50% are methyl groups. Generally, as the phenyl content increases, the polarity increases. Furthermore, substitution of more polar groups, such as -C≡N or $-CH_2CH_2CF_3$, creates an even more polar liquid phase. Note that on a nonpolar column, the individual GLC peaks will emerge according to their boiling point, while on columns prepared with various polar

[1]Liquid phases termed equivalent or identical may sometimes reflect dissimilarities due to different polymerization grades or the presence of impurities.

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phases, the individual components will be retarded according to the interaction between their polar groups and the active groups of the particular phase.

3.0.0 COLUMN EFFICIENCY

The efficiency of a particular GC column is dependent upon the substance being chromatographed, the molecular diffusion of the substance in the mobile phase and mass transfer in the stationary phase, the operating conditions of the GC (such as flow rate and temperature), the quality of the packing material, the uniformity of the packing within the column and column and system geometry.

A measure of the efficiency of a column is obtained by calculating the number of theoretical plates, *n*, in the column with the equation:

$$n = 16\left(\frac{t_R}{W_b}\right)^2 = 5.54\left(\frac{t_R}{W_h}\right)^2$$

t_R = retention time of the substance (seconds)

W_b = the width of the base of the peak obtained by extrapolating the relatively straight sides of the peak to the baseline (seconds)

W_h = peak width at half height (seconds)

Note that *n* obviously will vary with the length of the column. To compare the efficiency of columns of different lengths, one can determine the height equivalent to one theoretical plate by ratioing the column length (L) to the number of theoretical plates *n* as follows:

HETP = h = L/*n*

4.0.0 GC DETECTORS

In principle, it is possible to make use of any physical difference between the pure carrier gas and carrier gas plus solute as a method of detection of the solute. About 40 different detectors have been described in the literature. One of the most commonly used detectors in forensic laboratories is the flame ionization detector (FID). This detector contains a hydrogen flame, the resistance of which is monitored by means of polarizing electrodes. The introduction of the solute into the flame increases the ion concentration in the flame, thus resulting in a decrease in the resistance of the flame with a corresponding increase in ion current. This current is then amplified and recorded. Others that are

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sometimes used include the nitrogen-phosphorus detector (NPD) for its specificity, and the electron-capture detector (ECD) for its high sensitivity to halogen atoms.

5.0.0 SOLVENT EFFECTS

Ideally, a nonpolar solvent should be used to dissolve the sample; however, ideal solvents are rare in GLC because of the differences in solubility and polarity between sample and solvent. Almost any solvent can be used as a vehicle to introduce a compound or mixture of compounds onto a gas chromatographic column. The only limiting criteria is that the desired compound(s) be soluble in that solvent and that the solvent selected be compatible with the GC detector. This requirement, for example, eliminates the use of halogenated solvents with electron capture detectors. With the use of packed columns and flame ionization detectors, gas chromatography is fairly immune to the ill effects of small amounts of moisture or particulate matter injected into the instrument. Over long periods of time, however, these effects may accumulate. Continual injection of particulate matter, such as insoluble sugars, creates a buildup of material at the very beginning of the column that serves to promote the breakdown of sensitive compounds. The combustion of halogenated solvents at the FID detector forms mineral acids (e.g., hydrochloric acid) that are corrosive to the hardware of the detector. The use of certain solvents will gradually "strip" the column of its stationary phase, reducing the level of coating and its effectiveness as a chromatographic medium. Although these problems may not be evident during the short period of time that a gas chromatograph is used during an analysis, they constitute maintenance problems that must eventually be attended to in order to ensure continued efficient use of the instrument.

6.0.0 INTERPRETATION

Chromatographic techniques alone are sometimes sufficient for an analyst to identify the components in a mixture with a high degree of certainty, or at least to narrow down the range of possibilities. Such identifications are based on the fact that for a given column under fixed operating conditions a particular solute is eluted by a definite volume of carrier gas. When the carrier gas is flowing at a constant rate, the retention of the solute can be expressed in terms of the retention time. The retention time of the sample solute is then compared to the retention time, under the same chromatographic conditions, of a solute of known identity. Since the use of one stationary phase is not sufficient to state with a high degree of certainty that a given component is identical to the compound of know identity, it is necessary to repeat the analysis on a stationary phase of different polarity in order to be reasonably certain of the identification. Supplementary methods such as infrared, ultraviolet, nuclear magnetic resonance, and mass spectroscopy may be combined with GLC to provide a more positive identification.

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Although GLC can provide quantitative information about a sample, a number of sources of error can occur, and these must be minimized in order to increase the accuracy of this technique. Such error sources include:

1. Adsorption problems

2. Measurement of the amount of solute injected

3. Measurement of the instrument responses to a solute

Adsorption of the solute can occur on "active sites" of the inert support. Preferential adsorption of one of the sample components will render the quantitative analysis meaningless. Adsorption can be detected by plotting detector response against sample size for diminishing sample sizes. If adsorption effects are present, the plot will cross the abscissa at a point representative of the amount of the solute lost. Adsorption effects can be minimized by using a large sample size and "saturating" the column with the solute prior to beginning the quantitative analysis.

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7.0.0 QUANTITATIVE ANALYSIS

In GLC, comparative analysis is the basis for single and multiple component quantitative analysis. Three GLC quantitative techniques used in forensic laboratories are presented below.

7.1.1 PEAK AREA MEASUREMENT

Peak area (as well as peak height) is proportional to the amount of solute present. Today's modern gas chromatographs are equipped with on-line computing integrators that will electronically determine peak area as well as compute the potency for each individual peak desired. Furthermore, computing integrators monitor and control the gas chromatographs, even correcting for drifting baseline and overlapping and/or asymmetrical peaks.

7.1.2 DIRECT COMPARISON OF PEAK AREA

This method is sometimes referred to as the "dilute and shoot" method. To determine the concentration of a component in a sample, quantitative solutions of the sample and its corresponding authentic standard are prepared. A specific volume, usually 1-3 µL, of each solution is injected into the GLC and the resulting area for the sample component peak is compared directly to the ratio of the area for the authentic standard peak versus its solution concentration. The concentration of the component (comp) in the sample is then derived proportionately as follows:

$$\frac{\text{Peak Area}_{comp}}{\text{Concentration}_{comp}} = \frac{\text{Peak Area}_{std}}{\text{Concentration}_{std}}$$ therefore

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$$\%\ \text{Comp. in Sample} = \left(\frac{\text{Peak Area}_{\text{comp}}}{\text{Peak Area}_{\text{std}}}\right)(\text{Concentration}_{\text{std}})(\text{Dilution})\left(\frac{100}{\text{Wt. of Sam.}}\right)$$

Obviously, sample size is a major factor that affects peak area (as well as peak height). Consequently, the method of sample injection is critical in this quantitative method since the exact size or volume of injection must be measured. To minimize this major source of human error, all peak area measurements are performed in duplicate in order to check reproducibility of injection size with chromatographic response (i.e., peak area).

In addition to sample size, however, there are instrumental factors that will affect peak area (as well as peak height) if not precisely controlled. Factors that affect chromatographic response are fluctuations in carrier gas flow rate, fluctuations in detector or column temperatures, lack of uniformity of column packing material, dirty detectors, etc. The true advantage of the GLC quantitative method is that it is simple and rapid.

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7.2.0 THE INTERNAL STANDARD METHOD

7.2.1 SELECTING THE INTERNAL STANDARD

The term “internal standard” refers to a measured amount of a pure constituent that is equally transferred to the quantitative solutions of the sample and the corresponding authentic standard. Ideally, the structure of the internal standard should closely resemble the molecular structure of the component being quantitated or at least be in the same chemical class, so that it can “mimic” the detector response of the component and precisely compensate for minor column or other instrumental variations. Unfortunately, ideal internal standards are rare. As an alternative, a number of solid organic hydrocarbons have been successfully used as internal standards for the quantitative GLC analysis of various drugs. Some of the hydrocarbons used are:

1. n-Tetradecane $C_{14}H_{30}$
2. n-Docosane $C_{22}H_{46}$
3. n-Tetracosane $C_{24}H_{50}$
4. Octacosane $C_{28}H_{58}$
5. Triacontane $C_{30}H_{62}$

The use of solid hydrocarbons as internal standards has become quite popular because of their availability, high purity, and relative ease in handling. However, if one was to search the literature, there are other compounds used as internal standards, such as Benzopinocolone, Squalene, Cholesterol, etc.

The selection of an internal standard should meet the following requirements:

1. The compound selected must not be present in the sample.
2. Its GLC chromatographic peak should be completely resolved.
3. It should elute close to the resolved component peak being quantitated.
4. The ratio of its peak area to the area of the component peak should be close to unity.

7.2.2 The major advantage of this quantitative method is that the exact size of injection need not be known which eliminates a major human error. In addition, this method nullifies the effects of instrumental variations since the internal standard will be influenced by these variations to the same extent as the component of interest.

In short, this quantitative procedure involves the addition of equivalent amounts of an internal standard to the sample and authentic standard solutions. After chromatographing and measuring the peak area, the ratio of the peak area of the sample (i.e., component of interest) to the peak area of its internal standard is compared to the ratio of the peak area of the authentic standard to its internal standard. This can be expressed as

$$\frac{A_{smpl}}{A_{is/smpl} \times C_{smpl}} = \frac{A_{std}}{A_{is/std} \times C_{std}}$$ therefore

$$\%spl = \left(\frac{A_{spl}}{A_{std}}\right)\left(\frac{A_{is/std}}{A_{is/spl}}\right)(C_{std})(\text{Dil fac})\left(\frac{100}{\text{Spl Wt}}\right)$$

A_{spl} = peak area of sample component

A_{std} = peak area of authentic standard

$A_{is/std}$ = peak area of internal standard in the authentic standard solution

$A_{is/spl}$ = peak area of internal standard in the sample solution

C_{std} = concentration of the standard solution

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Dil fac = dilution factor

% spl = percentage of the component in the sample

Obviously, the internal standard method will take a little longer to perform than the direct comparison method described above. However, its overwhelming advantages make it the method of choice in GLC.

8.0.0 CAPILLARY COLUMN CHROMATOGRAPHY

Since 1957, open tubular columns (OTC) coated with a thin film of a liquid phase were found to provide enhanced resolution and greater sensitivity in comparison to packed GC columns. The adaptation and development of these columns led to what is now known as capillary column chromatography or more precisely "wall coated open tubular (WCOT) column chromatography."

WCOT columns provide two major advantages:

1. Increased column efficiency
2. A decrease in pressure drop per unit length of column (in comparison to packed columns)

The significance of these two advantages is that it has led to the production of WCOT columns having a very high number of theoretical plates that account for the enhanced resolution and increased sensitivity achieved. The fused silica WCOT columns with bonded (or polymerized) stationary phases of various film thickness have been known to provide over a 500-fold increase in sensitivity in comparison to conventionally packed columns, and they provide high quality chromatograms that are characterized by sharp narrow peaks with baseline resolution.

Because of the small amount of stationary phase in WCOT columns, the size of the injection must also be kept small.

8.1.0 SPLIT MODE

In this mode, only a small portion of the vaporized, mixed injection is allowed to enter into the column and the remainder is vented through a vent port (a design feature on the injector). This split ratio is controlled by balancing the carrier gas flow rate to the vent flow rate (which is controlled by a needle valve). If the vent flow rates are adjusted within a range of 25 mL/min to a corresponding column flow rate of 1 mL/min, the useful vent: column split ratio will be 25:1. This means that 25 parts of the injection will be vented and only 1 part (which corresponds roughly to 4% of the injection) is allowed to pass on to the column. A vent: column ratio of 100:1 means that only 1 part of the injection (roughly 1%) is allowed to pass on to the column.

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Split injection can provide acceptable and reproducible results. However, it should be recognized that for mixtures containing components of widely varying volatility, discrimination in favor of the more volatile components must be considered as a possibility.

8.2.0 SPLITLESS MODE

In this mode, approximately 90% of the sample injection passes onto the column. With only the carrier gas flowing at a rate of about 1 mL/min, the vaporized injection is allowed to pass into the column. The solute concentrates at the head of the column because of "solvent effects;" meanwhile, solvent vapor passes through the column (what also permits concentration of the solute band at the head of the column is condensation which occurs due to the low temperature at which the column is maintained prior to commencing temperature programming). At a preselected time (about 1 minute) after injection, a valve on the injector is turned on which alters the injection flow to permit purging of the injector system of any residual vapor.

The splitless mode requires care in the selection of the solvent medium and chromatographic parameters and is more difficult to optimize and control than the split mode; however, it permits far greater sensitivity to be achieved.

Considering that WCOT column chromatography is used to detect quantities of components at trace levels, the forensic chemist should be acquainted with the following terms relating to specific levels of sensitivity and detection:

Milligram	mg	10^{-3}g
Microgram	mcg (μg)	10^{-6}g
Nanogram	ng	10^{-9}g
Picogram	pg	10^{-12}g
Femtogram	ftg	10^{-15}g
Attogram	atg	10^{-18}g

Note that the level of detection at the attogram level, fentogram level, and mid-picogram level can only be achieved with capillary column gas chromatographs equipped with an electron capture detector (ECD). The flame ionization detector (FID) can detect components at the high picogram level and the microgram level.

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9.0.0 READINGS

Clark, Isolation and Identification of Drugs, 3rd edition, vol. 1, pg 425-499.

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22.0 Appendix E

Idaho State Police

Forensic laboratory Training Manual

Mass Spectrometry

1.0.0 Background

Mass Spectrometry (MS) is an important analytical technique for the identification of chemical compounds. In the mass spectrometer, the sample to be analyzed is vaporized and ionized yielding gaseous-charged particles with unique masses. These charged particles are separated, collected, and measured. The instrument produces a record known as the mass spectrum that records the abundance of the charged species at each mass to charge ratio (*m/z*). The position and abundance of the *m/z* values provide qualitative information about the compound.

A mass spectrometer, regardless of type or manufacturer, consists of a sample inlet system, an ionizing source, a mass filter to separate the ions by mass/charge ratio, and a detector.

2.0.0 MS Inlet Design

Chromatographic interfaces are applicable to a wide range of sample types. They are probably the most convenient and efficient means of introducing samples into the mass spectrometer. Mixed or impure samples can be purified and introduced into the analyzer by this means.

The use of a gas chromatograph as a sample introduction device for the mass spectrometer is widely accepted in the forensic science field. The only real limitation on sample types amenable to this system is that the sample components of interest must pass intact through the gas chromatograph. The gas chromatograph portion of the inlet system does not differ from the gas chromatograph used in analytical work in the laboratory except in the treatment of the column effluent; therefore, all the precautions applicable to gas chromatography in general also apply to this system.

Modern GC/MS instruments utilizing a capillary column in the GC allow the column effluent to be introduced directly into the ionization source of the mass spectrometer. Older models of GC/MS instruments, as well as those currently so designed, that use a packed column in the GC have column effluent flow rates far beyond the pumping capacity of mass spectrometers. These instruments used a variety of devices to enrich the effluent with sample, or remove excess carrier gas. The most popular of these devices are the fritted glass separator and the jet separator.

Several interfaces have been introduced in recent years that allow the effluent of an HPLC to be introduced into a mass spectrometer. Some of these interfaces produce a charged analyte with little or

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no fragmentation. The recent introduction of bench top MS systems with MS/MS capability allows the isolation of the charged analyte and subsequent fragmentation producing valuable structural information.

3.0.0 Ionization and Fragmentation Process

Upon entering the mass spectrometer, the samples must be ionized. Two methods widely in use today are electron impact ionization (EI) and chemical ionization (CI).

3.1.0 In electron impact ionization (EI), the sample molecules are ionized by bombardment with electrons produced by electrically heating a metal filament to the temperature at which it emits electrons. The energy of electrons generally determines the degree of fragmentation of the sample molecules. At low energy levels (approximately 10-15 eV), little fragmentation occurs in most molecules, thus leaving the molecular ion (M+) abundant. At electron energies above 20 eV, extensive fragmentation generally occurs. Most EI/MS studies are conducted at 70 eV in order to avoid the effects of slight variations in electron energies. Low eV studies are generally employed only where the molecular ion is very unstable at 70 eV.

3.2.0 In chemical ionization (CI), an entirely different process occurs. Reagent gas molecules (e.g., methane, ammonia, isobutane) are introduced into the ionization chamber, wherein they are partially ionized by relatively high-energy electrons (approximately 100 - 500 eV). Sample molecules introduced into the ionization chamber are ionized by collision with the ionized reagent gas. This process generally results in the production of fewer fragment ions than in EI.

Fragmentation processes in CI are generally characterized by hydride abstractions, proton additions, or neutral losses of small molecules such as water, ammonia, etc., from the protonated parent molecule. These simpler fragmentation routes make the spectra much easier to interpret than those obtained by EI. Quite often in CI, quasi-molecular ions (M+1) are obtained, thereby easily identifying the molecular weight of the compound in question.

Thus, CI is a valuable tool in determining molecular weights, but is generally less effective than EI in elucidation of molecular structure. The two ionization processes often produce two completely different sets of positive ions from the same compound. This, therefore, gives two sets of physical parameters by which the compound can be characterized and this, with the addition of GC retention time data, is often sufficient to unambiguously establish the identity of a compound.

4.0.0 Analyzer Design

Four basic types of mass spectrometers are known by the design of their mass filter:

1. Magnetic sector
2. Time of flight

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3. Radio frequency (including quadrapole and ion trap)

4. Fourier transform: ion cyclotron (FTMS or ICR)

4.1.0 Magnetic Sector MS

Magnetic sector instruments separate the ions by extracting them from the ionizer, then accelerating them down a curved tube by means of a high potential (approximately 4 Kv) electrical field. Positioned along the curved tube is a powerful magnet designed to produce a wedge-shaped (sector) magnetic field. When the sample is extracted from the ionizer, it consists of a mixture of all the ions of various masses produced in the ionization process. As these ions are accelerated toward the magnetic sector, the potential V, applied to the entrance slits, controls their velocities. Subsequently the ions are diverted into circular paths by a magnetic field parallel to the slits and perpendicular to the ion beam. The ion velocity in the magnetic field is given by the equation $zV=(1/2)mv^2$. A stable, controllable magnetic field separates the components of the total ion beams according to momentum. By this means, the individual ion beams are separated spatially and each has a unique radius of curvature (ion trajectory), *r*, according to it mass/charge (*m/z*) ratio. Only ions of a single *m/z* value will have the proper trajectory leading to the exit slit ahead of the detector.

By varying the accelerating potential and/or the strength of the magnetic field, each ion in turn can be focused on the detector, thereby enabling the recorder to produce a complete mass spectrum of a sample.

4.2.0 Time of Flight MS

Time-of-flight (TOF) mass spectrometers separate the ions by measuring the time required by each ion to travel the length of a straight tube 30 to 100 centimeters long. In the TOF mass spectrometer, sample ionization occurs for a short time interval, and then the ionizing voltage is turned off. The ions produced during this interval are then accelerated as an "ion packet" down the tube by means of an accelerating voltage (approximately 3 Kv). Since there is no further energy input to the ion packet after acceleration, the ion packet spreads out as it drifts down the length of the tube. This separation of the ions is a function of ion velocity derived from the kinetic energy imparted by the accelerating potential $zV=(1/2)mv^2$. Because all ions have essentially the same energy at this point, their velocities are inversely proportional to the square roots of their masses. As a result, ions with different *m/z* ratios spatially separate as they travel down the flight tube. Ions of high velocity (low *m/z* ratios) speed on ahead and arrive at the detector before the heavier ions of lower velocity (high *m/z* ratios). Thereby, groups of ions are formed which sequentially impact the ion detector.

4.3.0 Quadrapole MS

Quadrapole instruments are the most commonly used mass spectrometers for forensic drug analysis today.

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Molecules from the inlet system, i.e., the gas chromatograph, enter the ion source where they are bombarded with electrons that are emitted from a hot filament. The neutral molecules are ionized to form a variety of products, including positive ions. The positive ions are generally used in routine forensic analysis because they predominate over the other species present by several orders of magnitude. While ionization can occur at any bond in the molecule, it does occur at certain preferred locations, giving rise to a distribution of ions which is reproducible and which constitutes a fingerprint of the original molecule.

The positive ions are electrically extracted from the ion source and injected into the quadrupole mass filter where they are separated according to their mass.

The governing principle of the quadrupole filter is based on alternating electric fields applied to four electrodes (rods) held in a square array. To each diagonally paired set of rods a combination radio frequency (rf) and dc voltage of increasing amplitude is applied. One pair receives an RF voltage and a positive dc voltage, and the other pair receives and RF voltage with an 180^0 phase shift and a negative dc voltage. These voltages give rise to an electrostatic field that gives bounded oscillations to an ion fragment of the selected mass to charge (*m/z*) ratio and unbounded (collected and discharged on rods) oscillations to all ions of different *m/z*.

The mass of the ions transmitted is directly proportional to the voltage applied to the quadrapole filter. Since a linear ramp voltage is applied to the filter, the output of the mass numbers will also be displayed linearly. The separation between mass 19 and 20 is, for example, identical to that between masses 600 and 601.

Correct assignment of mass number for each mass peak displayed is far easier on a linear mass spectrum than a quasi-logarithmic mass spectrum as obtained with a sector type mass spectrometer. This advantage is particularly important in case of chemical ionization MS, where one is apt to find long stretches of blank space on the spectrum where no mass peaks appear. This advantage is of little consequence in modern instruments that employ digital data analysis systems.

The ions passing through the quadrapole filter are quantitatively detected by an electron multiplier, amplified, and the resulting signal is fed to an appropriate data handling system.

4.4.0 Ion Trap MS

A new technique for mass analysis was introduced to mass spectrometers in the early 1980s. The quadrapole ion trap or Paul trap, named after its inventor Wolfgang Paul, is rapidly gaining wide acceptance in the field of chemistry, due largely to the intense research programs studying its function and applications.

Beam-type scanning mass spectrometers (e.g., quadrapole mass filter and magnetic sector instruments) operate on the principle of mass-selective stability. That is, ions that have a small range of *m/z* values

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maintain “stable” trajectories throughout the analyzer and can pass to an ion detector. The mass spectrum is collected by “scanning” the analyzer field so that the window of stable *m/z* values is sequentially swept across the entire *m/z* range of interest. The ratio of the width of the transmitted *m/z* window to the total width of the *m/z* range of interest determines the fraction of time during a scan that any given ion can be transmitted. This ratio can be considered the “duty cycle” of a scanning beam type instrument, and is typically 1%. Therefore, for a continuous ionization method >99% of all ions generated are lost simply because of the small magnitude of the duty cycle.

Ion traps utilize a principle that has been termed “mass selective instability.” The ion trap consists primarily of two hyperbolic shaped end cap electrodes bracketing a ring electrode. A sine-wave signal is applied to the ring electrode. When a potential exists between the ring electrode and the end caps, a quadrapole electric field is created within the electrodes because of their hyperbolic shape. As ions enter the trap the oscillating electrical field captures the ions in a “pseudo-potential well.” The ions begin to move toward one side of the trap (sliding down the side of the well) that, if allowed to continue for a sufficiently long period, the motion away from the center of the ion trap would result in acceleration of the ion to one of the end-cap electrodes. However, if the potential is alternated at a sufficiently high frequency, an ion present at the center of the ion trap with a low initial kinetic energy cannot reach an electrode before it is repelled, and thus becomes “trapped.”

Under a fixed set of conditions ions of different *m/z* values have different trapping well depths. They also undergo motion with a unique set of frequencies. This characteristic allows the quadrapole ion trap to be used as a mass spectrometer rather than simply as an ion storage device. A variety of frequencies characterize the motion of a particular *m/z* ion in a quadrapole ion trap. By selectively adding energy to a specific *m/z* ion by manipulating the so called “fundamental *z*-dimensional secular frequency” that ion can be ejected through a hole in the end-cap electrode of the ion trap, thus giving rise to the term “mass selective instability.”

Current instrument designs have adopted a sequential approach to ion generation and filtering. The ion source generates the ions to be studied and the ion trap collects and stores the ions until an optimum number of ions have been collected (approximately 10^6 ions). The trap then ejects the ions sequentially for detection and analysis. When a strong ion source exists the duty cycle of the ion trap is only a few percent, in the same range as a scanning beam instrument. Conversely, with a weak ion source the accumulation time to fill the trap increases and hence the duty cycle increases. For example, if the ion trap can be filled in 1 ms and the mass analysis time is ~50 ms, the duty cycle is but a few percent. However, if the ion accumulation rate for an analyte ion of interest is sufficiently low to require an ion accumulation time of up to 1 s, a duty cycle as high as 95% is obtained. Because of this potential for a very high duty cycle, ion trap mass spectrometers can produce full scan spectra from much smaller samples than quadrapole or magnetic sector instruments.

In some ion trap instruments, sample molecules are fragmented inside of the ion trap instead of in a separate ion source. In these types of instruments, collisions between ionized fragments and neutral

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molecules occur. These collisions sometimes result in a form of chemical ionization where the neutral molecules are ionized by the charged ions instead of electrons. The resulting mass spectrum is an electron impact spectrum superimposed on a chemical ionization spectrum (which can include ions of higher molecular weights than the original molecule). Because this phenomenon is concentration dependent (i.e. the more molecules in the trap, the more collisions take place) the actual mass spectrum obtained with these instruments varies with the amount of sample introduced into the instrument.

5.0.0 Technical Concerns

Before attempting to interpret a mass spectrum, the operator should evaluate the data as to usability of the spectrum. The problem most frequently encountered is that of sample impurity, readily detected by observing the mass spectra at various points throughout the chromatogram. If one or more fragment ion peaks in the mass spectrum appear, disappear, or change intensities relative to the other ion fragments during production of the sample peak, the spectrum is not that of a pure compound.

Another problem that reduces the value of a mass spectrum is that of molecular ion (M+) instability. If M+ is very small or absent, it may be overlooked, causing the wrong molecular weight to be assigned to the sample compound and making spectrum interpretation impossible. Several methods of identifying M+ are available e.g.; lowering the ionizing voltage to increase the relative abundance of M+, examination of the molecular ion cluster for the presence of M+ +1, M+ +23, M+ -1, etc., or checking for irrational losses (3 to 13 amu) from the peaks at highest amu. Use of chemical ionization rather than electron impact ionization simplifies the assignment of molecular weight.

Careful plans should be formulated before running a sample on the mass spectrometer. All available sample information should be studied (e.g., microscopic, chemical, GC retention data, etc.). Samples should be at least partially “cleaned up” to prevent contamination of the ion source.

6.0.0 Reading

MS Fundamentals (Agilent Multimedia Course), Agilent Company

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23.0 Appendix F

Idaho State Police

Forensic Laboratory Training Manual

Infared Spectrophotometry

1.0.0 Background

Infrared spectrophotometry (IR) is one of the most reliable instrumental techniques used for the identification of drugs. Almost every chemical compound, regardless of its phase (i.e., liquid, solid, gas), produces a different infrared spectrum. Consequently, an infrared spectrum can generally be assumed to be specific for a particular compound. Whereas ultraviolet spectrophotometric (UV) analysis is widely accepted as a quantitative tool, IR is regarded as an indispensable tool for qualitative analysis. However, similar to UV analysis, if a drug sample contains more than one constituent, preliminary treatment of the sample is usually necessary in order to isolate each constituent in a fairly pure state for IR analysis. Fortunately, with the advent of computer data stations for data manipulation, the spectral contributions of one or more components in a mixture can sometimes be digitally subtracted leaving an acceptable spectrum of a single component.

The unit of wavelength in the infrared region of the electromagnetic spectrum is most commonly expressed in either wavelength (given in microns or micrometers) or wave numbers, with wave numbers being the most popular. One micron (μ) = one micrometer (μm) = 10^{-6} meters = 10^{4} angstroms (Å) = 10^{-4} cm. Wave numbers are defined as the number of waves per centimeter and have the units of reciprocal centimeters (cm^{-1}).

The overall infrared region extends for 0.78 to 1000 μm (12,800 to 10 cm^{-1}). This region is subdivided into three categories: near, middle and far. Different literature references give different values for the wavelength ranges each of these regions cover. For our purposes, we will use the ranges given in the following table.

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Region	Wavelength Range, □	Wave number Range, cm^{-1}	Frequency Range, Hz
Near	0.78 to 2.5	12,800 to 4000	3.8×10^{14} to 1.2×10^{14}

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Middle	2.5 to 25	4,000 to 400	1.2×10^{14} to 1.2×10^{13}
Far	25 to 1000	400 to 10	1.20×10^{13} to 3.0×10^{11}

In forensic chemistry, we are chiefly interested in the mid-infrared region from 2.5 – 25μ or 4000 - 400 cm^{-1}. This region is divided into the "group frequency" region of 4000 – 1300 cm^{-1} (2.5 – 8 μ), and the "fingerprint" region of 1300 – 650 cm^{-1} (8 – 15.4μ). In the group frequency region the principal absorption bands may be assigned to vibration units consisting of only two atoms of a molecule; that is, units which are more or less dependent only on the functional group giving the absorption and not on the complete molecular structure.

2.0.0 IR Instrumentation

The ISPFS laboratories use interferometer based instruments. With interferometer instruments, repeated measurements are taken of many wavelengths more or less at the same time and an interferogram (i.e., an output of intensity versus mirror displacement) is generated which is converted to an IR spectrum via a mathematical (Fourier) transformation. This type of instrument is generally known as a Fourier Transform Infrared (FTIR) Spectrophotometer.

There have been major advances in the development of Fourier Transform Infrared (FTIR) spectrophotometers over the past decade. Present FTIR instruments produce an IR spectrum on a computer monitor in a few seconds. Current trends in instrument development are toward smaller, more powerful instruments with the newest generation of instruments adding Raman capabilities to the FTIR bench. Due to the higher energy throughput and increased detector sensitivities of the current generation of instruments a broad spectrum of sampling devices have been introduced.

3.0.0 Sample Preparation Techniques

The type of sample preparation required depends on the nature of the sample. Most samples submitted to forensic laboratories are usually powdered material. Two options are available for powdered samples including:

1. Dispersion in potassium bromide (KBr) and pressing into a transparent KBr disc;
2. Direct analysis via a FTIR with a ATR attachment

Of the above options, KBr dispersion is the sample preparation technique least commonly utilized. When the KBr pellet is utilized, the sample must be finely ground to prevent radiation scattering. Some compounds absorb more strongly than others and the optimum sample concentration may vary from 0.1% to 3%. A general recommendation for preparation of a 1.2 cm KBr pellet is 1 mg of sample to 200 mg KBr, however the proper concentration is best determined by trial.

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Solids, liquids and opaque materials (such as plastic and rubber) can be analyzed via Attenuated Total Reflectance (ATR). The ATR technique depends upon the fact that a beam of light that is internally reflected from the crystal surface passes a short distance beyond the reflecting boundary and then returns to the crystal as part of the process of reflection. (See Figure 1). If a sample of lower refraction index than the transmitting medium is brought in contact with the reflecting surface, the light passes through the material to a depth of a few microns producing an absorption spectrum. This technique is also referred to as Multiple Internal Reflectance (MIR).

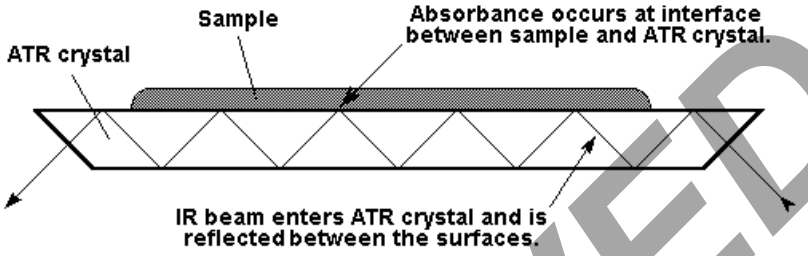

Figure 10

4.0.0 Unknown Sample identification

With a little experience, most scientists will be able to recognize the infrared spectra of commonly encountered substances. Beyond the familiar substances however, identifying a compound from its spectrum usually requires comparing the spectrum to a group of reference spectra. Most IR operating systems are capable of comparing an unknown spectrum to reference spectra in libraries. Many libraries of infrared spectra are available. Library searching is not always a perfect process. Many factors can affect the success of a library search and even cause differences between a sample and a known standard ran on the same instrument.

The quality of the unknown spectrum greatly affects how well that spectrum will match a reference spectrum.

Too much sample results in complete absorbance of the radiation at some wavelengths and leads to broad flat absorbance bands that cause the peak locations to not be accurate. Too little sample leads to a weak spectrum where noise and the absorbance's of, H_2O and CO_2 are large in relation to the absorbance from the sample. A good rule of thumb is to try to dilute the sample so that the most intense band gives ~10% transmittance.

Correction of badly sloping baselines is sometimes necessary to obtain good library search results.

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Most reference (standard) spectra and libraries are of pure compounds. Trying to library search a spectrum from a multi-component sample may not give a good match. However the search results may identify one of the components, which can then be digitally subtracted. The subtraction result can then be searched to identify other components in the sample.

Standard spectra or the spectra in a library may have been collected at a different resolution than the sample spectrum. To assure good matches, the unknown spectrum may have to be acquired at higher or lower resolutions (or the resolution digitally changed) to correlate better with the reference spectra.

Many compounds exhibit polymorphism. That is, as they solidify they may form into different shapes of crystals. These different crystal formations will put different strains on some bonds in the molecule and cause a shifting of peak locations. Diazepam and some of the barbiturates are examples of compounds that exhibit polymorphism.

When comparing a spectrum to reference spectra, it is important to know how the reference spectra were collected. Most forensic libraries obtained IR spectra primarily from samples dispersed in KBr. The KBr-crystal matrix may affect the position of the observed group frequencies of a sample. This could make the correlation of published group frequencies and observed group frequencies difficult, if the spectra in the reference were collected via ATR. In these instances, it is necessary to use an in house library consisting of spectra generated using the ATR.

As discussed above, there are occasions where a compound does not match the reference spectrum of a known standard of that compound. There are also occasions when an unknown spectrum matches the reference spectrum of a different compound. In general, infrared spectrometry is a very discriminating technique and these occasions are rare. One instance where this does occur is in a homologous series. For example, infrared spectrometry cannot readily distinguish between the n-hydrocarbons tetradecane, pentadecane and hexadecane. Another class of compounds that infrared spectrometry cannot always distinguish is enantiomers. For example, the IR spectra of d-ephedrine and l-ephedrine are indistinguishable. However, a racemic mixture of d & l-ephedrine recrystallized from a solvent can easily be distinguished from the spectra of the single isomers. Caution should be exercised however, because if d and l ephedrine are simply mixed together and not dissolved and recrystallized from a solvent, they will give an IR spectrum indistinguishable from the single isomer spectra.

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5.0.0 Infrared Interpretation

Having a computer to compare an unknown spectrum to a reference saves a lot of time and greatly reduces the occasions when a chemist must interpret spectra. However it is still desirable to have a basic competency in IR interpretation, as samples are submitted that do not match any of the reference spectra. Because of the array of information that an infrared spectrum provides, it is almost impossible to explain every feature of an infrared spectrum. The interpretation of infrared spectra is a skill, which a forensic chemist can develop after years of practice. Rarely are the IR spectrum used to identify certain

functional groups in an unknown. This information could be used in conjunction with other instrumental data such as a mass spectrum, to identify a sample. This training program is not designed to make a chemist into an in expert at spectral interpretation. The following paragraphs give a little background on some of the important spectral features that may be of interest to a forensic chemist.

The region of 3700 cm^{-1} to 3100 cm^{-1} is usually associated with NH and OH stretching vibrations. The NH_2 group gives rise to two bands in this region because of symmetric and asymmetric stretch. (For example, anthranillic acid, a methaqualone precursor, demonstrates a typical NH_2 stretching.) Codeine displays a sharp singlet around 3515 cm^{-1}, which is indicative of the OH group at position six in the codeine molecule. A good example of hydrogen-bonded OH groups and non-bonded OH groups are the common sugars such as lactose, glucose, etc. It should be emphasized that the water molecule has absorption bands in this region and that KBr readily absorbs water.

The 3100 to 3000 cm^{-1} region is generally associated with aryl and olefin CH stretch. These bands are usually weak and may be nonexistent; e.g., benzocaine shows little if any absorption in this region while benzphetamine hydrochloride demonstrates a recognizable band between 3100 and 3000 cm^{-1}.

The 3000-2700 cm^{-1} IR region is associated with aliphatic CH stretch; however, many compounds common to the forensic laboratory are amine salts, which tend to obscure the information in this region. Stearic acid and many of the sugars show representative CH stretching in this area.

Primary amine salts, e.g., amphetamine HCl, show strong absorption between 3200 and 2800 cm^{-1}. O-toluidine HCl, a primary aromatic amine salt, displays characteristic bands around 2800 cm^{-1} and 2600 cm^{-1}. Secondary amine hydrochlorides, such as amphetamine hydrochloride, exhibit strong multiple absorption bands between 3000 and 2700 cm^{-1}. At still smaller wave numbers, tertiary amine hydrochlorides absorb between 2700 and 2330 cm^{-1}, as demonstrated by diphenhydramine hydrochloride.

The IR region 2300-1900 cm^{-1} is associated with triple bonds. The C≡N stretch of 1-piperidinocyclohexanecarbonitrile (PCC) readily demonstrates the usefulness of this region.

Carbonyl compounds absorb strongly within the 1900-1550 cm^{-1} region. Compounds of forensic interest include amides, esters, and a few anhydrides. Ketones have CO stretch around 1715 cm^{-1} (e.g., methadone and tropinone). Acid carbonyls usually absorb in the 1720 to 1680 cm^{-1} region, but they have a tendency to be somewhat unreliable. For example, aspirin's acid carbonyl occurs around 1750 cm^{-1}, N-acetylanthranillic acid has a 1700 cm^{-1} band, and ecogonine's carbonyl is found around 1690 cm^{-1}. Resonance weakens the C-O bond and thus lowers the absorbing frequency.

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The 1695 to 1630 cm^{-1} region is normally assigned to amide carbonyls; meprobamate and acetaminophen (both N-substituted amides), and methaqualone (a di-substituted amide) shows carbonyl absorbances of 1695, 1650 and 1670 cm^{-1} respectively.

Esters absorb strongly near 1740 cm^{-1} and 1200 cm^{-1} because of the C=O and C-O stretch, respectively. Acetyl codeine, having a single ester group, shows strong 1735 cm^{-1} and 1240 cm^{-1} bands. On the other hand, heroin and cocaine show two strong bands in the 1700 cm^{-1} region, indicative of two carbonyl absorptions, and both also exhibit the 1200 cm^{-1} band.

Beyond the 1600 cm^{-1} region, assignment of absorptions becomes more difficult due to the skeletal bending and deformations occurring within the molecule. Nonetheless, there are a few regions where assignments may be made with some consistency. The 1400 cm^{-1} region is usually characterized by CH_2 and asymmetric CH_3 deformations. Absorptions in the middle to high 1300 cm^{-1} region usually arise from CH_3 symmetric deformations. The doublet caused by two methyl groups on the same carbon is well exemplified by phentermine, which exhibits two strong peaks around 1390 and 1370 cm^{-1}, respectively.

Although the 1200 cm^{-1} region has been discussed in conjunction with esters, this region is also common to most C-O stretch; i.e., a strong band in the 1200 cm^{-1} region usually indicates a C-O bond of some type within the molecule; for example the IR spectrum of *p*-dimethoxyamphetamine exhibits a strong absorption around 1250 cm^{-1}, while that of amphetamine shows an absence of bands in this region. MDA may also be compared to amphetamine to demonstrate the effect of the C-O stretch.

The 900-700 cm^{-1} region may be useful in determining the substitution on aromatic rings such as benzene. A single substitution on a benzene ring leaves five adjacent hydrogen atoms, which give rise to two strong bands, one around 750 cm^{-1} and the other around 690 cm^{-1}. PCP and cocaine are typical mono-substituted compounds. Ortho substitution, as characterized by N-acetylanthranilic acid, shows a band around 750 cm^{-1}. The presence of two bands around 690 cm^{-1} and 780 cm^{-1}, respectively, usually indicates meta substitution, while para substituted compounds demonstrate a band around 820 cm^{-1}.

Chlorphentermine hydrochloride isomers can have the chlorine in the ortho, meta, or para position, and the IR of each of these compounds clearly parallels the above discussions. The 745 cm^{-1} band in each chlorphentermine arises from the C-Cl stretch and illustrates the caution that must be applied to the use of the 900-700 cm^{-1} region. In fact, it is strongly recommended that the 2000-1700 cm^{-1} region be used only in conjunction with the 900-700 cm^{-1} region. Overtones of aryl ring vibrations occur in the 2000-1700 cm^{-1} area; however, because of the relatively low concentration of sample, these bands may not be seen whenever the KBr pressed-disc technique is used.

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6.0.0 Required Reading

1. Silverstein, R.M., Bassler, C.G., and Morrill, T.C., Spectrometric Identification of Organic Compounds, chapter on Infrared Spectrophotometry

2. Clarke, E.G.C., Isolation and Identification of Drugs, 3rd edition, vol. 1, pg 328-345.

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24.0 Appendix G

Idaho State Police

Forensic Laboratory Training Manual

Stimulants

1.0.0 Background

The most commonly encountered stimulants are the amphetamines. These compounds are widely used and abused, primarily for their stimulant effect; however, many people have been introduced to the amphetamines because of their anorectic effect. Amphetamines are habituating rather than addictive and abusers develop a tolerance for the compounds. Amphetamines (also called phenylisopropylamines or phenethylamines) have the general structure shown in Figure 1.

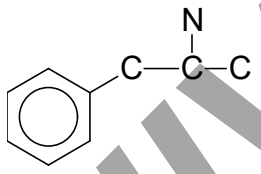

Figure 1

The different compounds in this family arise from: (1) substitutions on the propane chain; (2) substitutions on the nitrogen atom; (3) substitutions on the benzene ring; (4) combinations of one or more of (1) through (3). Pages 627 and 628 of reference 3 give the structures of some of the more common of these compounds. In general, substitutions on the propane chain and the nitrogen atom affect the stimulant properties of the compounds while substitutions on the benzene ring give hallucinogenic properties. With a couple of exceptions, the ring substituted compounds will be addressed in the hallucinogen section.

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1.1.0 Amphetamine and Methamphetamine

Two of the most frequently encountered stimulants in the forensic laboratory are Methamphetamine and Amphetamine, which are Schedule II controlled substances. Both of these compounds have one chiral center and hence there are two possible stereoisomers for each as shown in Figure 2.

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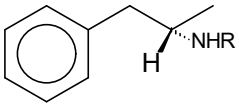
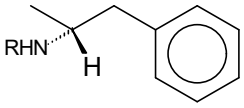
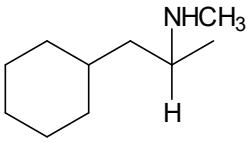


2S configuration
R=H, d-Amphetamine
R=CH3, d-Methamphetamine



2R configuration
R=H, l-Amphetamine
R=CH3, l-Methamphetamine



Propylhexedrine

Figure 2

The dextro forms are approximately 10 times more potent than the levo forms. The levo form of methamphetamine works well as a bronchadialator and, until recently, could be purchased over the counter in Vick's inhalers. Benzedrex brand inhalers contain Propylhexedrine that is a Schedule IV controlled substance when not in the inhaler form.

1.2.0 Ephedrine, Pseudoephedrine, Phenylpropanolamine and Cathine

The 1-Hydroxy-phenethylamines have two chiral centers so there are four possible structures for each as shown in Figure 3.

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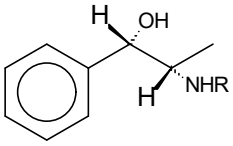
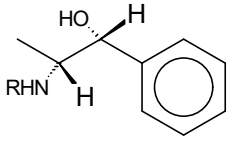
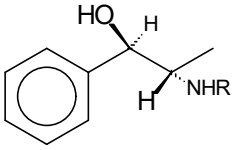


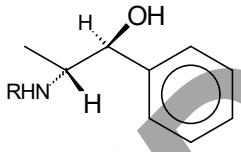
1S,2S configuration
R=H, Cathine
R=CH3, d-Pseudoephedrine



1R,2R configuration
R=H, l-Norpseudoephedrine
R=CH3, l-Pseudoephedrine



1R,2S configuration
R=H, l-Norephedrine
aka l-Phenylpropanolamine
R=CH3, l-Ephedrine

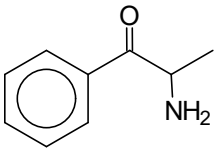


1S,2R configuration
R=H, d-Norephedrine
aka d-Phenylpropanolamine
R=CH3, d-Ephedrine

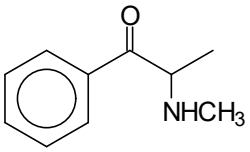
Figure 3

Ephedrine is a naturally occurring stimulant found in the Ma Huang (Ephedra vulgaris) plant. d-Pseudoephedrine is a decongestant and l-Ephedrine is a bronchadialator. Both can be found in many over the counter cold preparations. Older methods of manufacturing methamphetamine including those that start with phenylacetone yield a mixture of dextro and levo forms. Reduction of d-Pseudoephedrine or l-Ephedrine yields the more potent dextro-Methamphetamine. For this reason there is a great demand by illicit methamphetamine manufacturers for ephedrine and pseudoephedrine. In an attempt to reduce the clandestine manufacture of methamphetamine, certain laws have been enacted to control the possession of "bulk" ephedrine and pseudoephedrine. Controls have also been placed on preparations having ephedrine as the only active ingredientThe 1S,2S configuration known as cathine is a Schedule IV controlled substance. Cathine is found in various European drug preparations, but is rarely encountered in the United States.

1.3.0 Cathinone and Methcathinone



Cathinone



Methcathinone

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Figure 4

Cathinone is a naturally occurring stimulant found in the Khat plant. Khat is an evergreen shrub that grows at high altitudes in East Africa and on the Arabian Peninsula. After the leaves of the Khat plant are picked, the cathinone rapidly converts into cathine. Methcathinone is synthetically produced from the oxidation of ephedrine or pseudoephedrine. Care must be taken in the analysis of cathinone and methcathinone. The use of a too alkaline reagent during extraction will convert the cathinones into their corresponding 1-Hydroxy-phenethylamines. In addition, the retention times and mass spectra of the cathinones are very close to those of the corresponding 1-Hydroxy-phenethylamines. There are several derivatives of cathinone with a variety of substitutions, at the ring structure, at the two positions off of the amine or at the tail end of the carbon chain.

2.0.0 ANALYTICAL APPROACH

2.1.0 Extraction/Separation:

Amphetamines can be separated from excipients by routine acid-base extraction techniques. Nitrogen substituted amphetamines can also be isolated by ion-pairing techniques.

2.2.0 Gas Chromatography:

The compounds presented in this section are generally quite volatile; therefore, their Gas Liquid Chromatography (GLC) retention times are fairly short. In addition, these compounds "tail" badly in capillary systems with dirty injectors. In general, the greater the polarity of the column, the better the peak symmetry. Either a methylsilicone (OV-1) or a 5% phenyl methylsilicone (OV-5) capillary column will provide suitable resolution and peak shape.

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2.3.0 Mass Spectrometry:

Mass Spectrometry (MS) fragmentation patterns of these compounds are similar. The molecular weights are generally less than 160 with the phenyl ring comprising approximately half of weight. The electron impact mass spectral base peak usually includes the nitrogen and its substituents -- m/z 58 for methamphetamine and ephedrine, m/z 44 for amphetamine and phenylpropanolamine, etc. Molecular ions are usually weak or absent. Chemical ionization mass spectrometry gives more information about the identity of these compounds, since the M+1 ion is quite intense (in most cases).

2.4.0 Derivatizations:

In order to improve chromatographic properties, separate isomers and make the mass spectrums more distinguishable, a number of derivatization techniques can be performed. References 13 -16 give

several examples. A derivatization technique that was employed in this laboratory involves adding acetic anhydride to the free base amine to create the acetyl derivative of the original compound as shown in Figure 5.

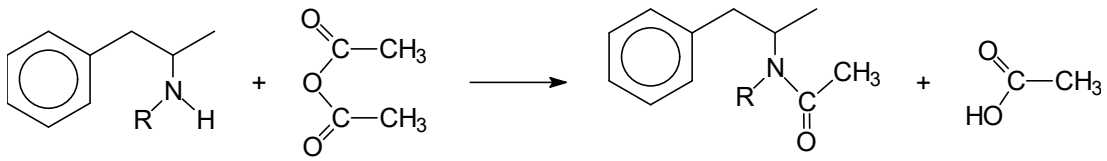

Figure 5

The addition of the acetyl group adds 42 amu's to the weight of several key ions in the mass spectrum.

2.5.0 Infrared Spectrophotometry:

Because of the volatility of their free bases, infrared spectra of the amphetamines are generally run as the acid salts. Some of the most useful acid salt forms are the hydrochloride, tartrate, and oxalate. The hydrochloride salts of some amphetamines, when ground with KBr, absorb large quantities of moisture from the atmosphere, thus giving rise to broad, intense absorbance bands in the region above 2500 cm-1. This problem can be avoided by using the tartrate or oxalate salts; however, the intense absorbance bands of the tartrate or oxalate moieties then become the dominant features in a spectrum. Another technique, which can be used to avoid the moisture problem, is derivative formation. One of the most useful of the derivatizing reagents for primary and secondary amines is phenylisothiocyanate (PIT). The amphetamine PIT derivatives are nonhygroscopic, and the infrared spectra of the derivatives furnish positive identification of the original amine. An added benefit of this derivative is that the IR spectra of the d- or l- enantiomers of the amphetamines differ from those of the racemic mixture.

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25.0 Appendix H

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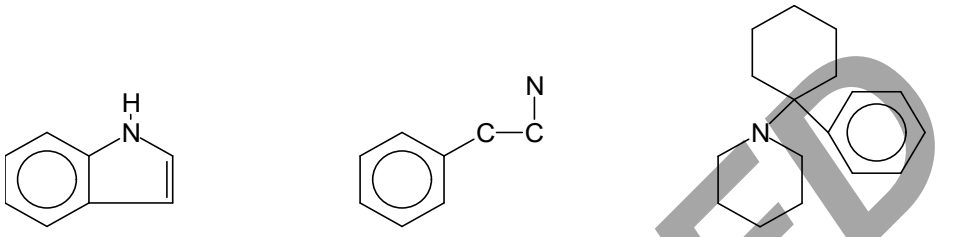
Hallucinogens

1.0.0 Background

Hallucinogens fall in to three general categories - 1) compounds containing the indole skeleton, 2) compounds containing the phenethylamine skeleton and 3) phencyclidine derivatives.



Indole Phenethylamine Phencyclidine (PCP)

Figure 11

There are several groups within the "indole" category, including ergot alkaloids, and tryptamines.

2.0.0 LSD and other ergot alkaloids

d-Lysergic Acid Diethyl amide Tartrate (LSD) was first synthesized in 1938 by Arthur Stoll and his collaborator, Albert Hofmann, both of the Sandoz Laboratories, Basel, Switzerland. It was not until 1943 that Hofmann, as a result of an accidental ingestion of some of the derivatized material, recognized the potent effects of LSD. Stabilized in the form of tartrate salt, as little as 0.025 mg of LSD was found to cause hallucinations – distortion of perception.

The starting material for the synthesis of LSD is d-lysergic acid, a compound obtained by alkaline hydrolysis of alkaloids produced by a fungus on certain species of rye and other grains and on morning glory and Hawaiian wood rose seeds. From these materials, a mixture of compounds, called ergot, can be extracted. Lysergic acid and other alkaloids isolated from ergot are known as ergot alkaloids. The chemical structures of LSD and lysergic acid contain the indole nucleus, which is also found in the tryptamines.

LSD has two asymmetric carbon atoms in its molecular structure (which are denoted by stars in the structure of LSD in Figure 2). Because of this, there are four possible stereoisomers; d- and l-LSD and d- and l-iso-LSD. Of these four, only d-LSD has a high degree of physiological activity.

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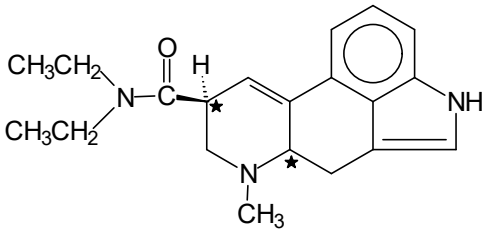
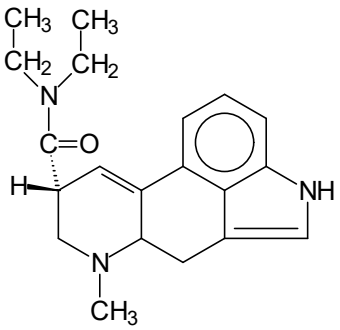


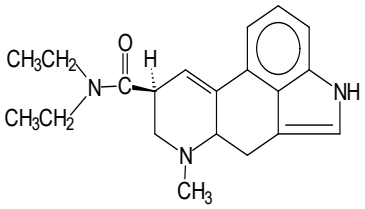
LSD



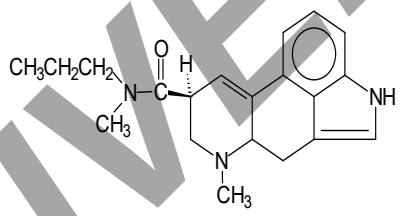
iso-LSD

Figure 12

A compound known as LAMPA (lysergic acid N-methyl-N-propylamide) is structurally very similar to LSD. LAMPA has a mass spectrum and chromatographic properties that are very similar to LSD. The structure of LAMPA is given in Figure 3.



LSD



LAMPA

Figure 13

LAMPA also has two asymmetric carbons so four stereoisomers are possible d- and l-LAMPA and d- and l-iso-LAMPA.

In the illegal market, LSD is usually found deposited on thick paper known as blotter paper which is usually divided by perforations into numerous squares about ¼" per side. LSD is occasionally found intermixed or deposited on strips of gelatin (and cut into squares known as "windowpanes"). In addition, it has been found in the form of very small tablets known as "microdots" or "barrels", and occasionally mixed into various food preparations.

The small quantity of LSD present in dosage preparations presents some analytical difficulties. In tablet preparations, the drug is extensively diluted with sugars, starches, binders, lubricants, gelatin, and other excipients. It is not uncommon to find the active ingredient representing only 0.1% of the total dosage weight.

2.1.0 Screening tests

Tests that can be used to check for the presence of LSD include spot tests and UV fluorescence. A spot test widely used for the detection of compounds with an indole nucleus employs the use of Van Urks reagent (refer to section on spot tests for

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preparation of Van Urks reagent). In the presence of an ergot alkaloid, this light yellow reagent produces a violet to purple color. Note that LSD tablet preparations frequently contain dyes that can obscure this color or produce a false color reaction.

LSD will fluoresce under long-wave UV light. A quick screening test for LSD can be accomplished by spotting a methanol extract of the suspected sample on a TLC plate, placing it under long-wave UV light and looking for a fluorescent "spot". Occasionally, dyes from the blotter paper or tablet preparations will also fluoresce. LSD is so highly fluorescent that in many instances, the fluorescence from dyes is insignificant by contrast. Development of the TLC plate will separate the LSD from these compounds.

2.2.0 Extractions

In most instances LSD can be dry extracted from the sample by simply soaking the evidence in a small amount of methanol for about 10 minutes. In certain cases, methanol-soluble excipients may interfere with the analysis procedure. This will most likely be a problem with TLC. To circumvent this problem, the sample may be subjected to a double extraction procedure in which a sample portion is dissolved in 2% citric or tartaric acid solution and extracted with chloroform. These extracts containing the impurities are discarded. The acidic solution is rendered alkaline with solid sodium bicarbonate and the liberated LSD base is extracted with chloroform. After evaporating the extract, the resulting reside is redissolved with a few drops of chloroform and subjected to analysis.

2.3.0 GC/MS

For a number of years, the low volatility of LSD and its tendency to decompose at high temperatures precluded the use GLC in the analysis of LSD. With the advent of inert GC injectors and capillary columns coupled with high sensitivity mass spectrometers, analysis of LSD via GC/MS has become common. Low polarity columns such as methyl-silicon work the best, but high GC temperatures are still necessary. Great care must be taken when analyzing LSD via GC/MS as LAMPA gives a mass spectrum that is very similar to LSD and, depending on GC conditions, a retention time that is near that of LSD.

Introduction into the MS can also be accomplished via a solid probe. Cleanup of the sample prior to MS analysis is necessary, however techniques such as MS/MS can greatly reduce the amount of cleanup.

Hybrid techniques such as chemical ionization with ammonia reagent gas coupled with MS/MS have been developed to lower the levels of detectability of LSD. These techniques are especially useful for toxicology analyses.

2.4.0 TLC

A great deal of difficulty has been reported in separating LSD from LAMPA by TLC. Developing solvents that contain acetone - such as 100% acetone or a 1 to 1 ratio of

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acetone and chloroform can separate the two compounds. Visualization of the TLC plates can be accomplished by fluorescence under long-wave UV or by spraying the plates with Van Urks reagent.

2.5.0 IR

FTIR can and has been used for the identification of LSD: however, a rigorous cleanup procedures such as preparative TLC is required. Frequently, special techniques such as the use of micropellets are necessary because of the small amount of LSD present in dosage forms.

2.6.0 HPLC

HPLC gives the best chromatography for LSD. LSD is highly fluorescent making the UV fluorescent detectors found on many HPLC's well suited for detecting LSD. These two factors make HPLC a very sensitive method for detecting LSD, and the method of choice for quantitating LSD. Because UV fluorescent detectors cannot distinguish between LSD, iso-LSD and LAMPA, chromatographic conditions must be carefully selected.

Adequate quantities of LSD can be isolated for IR or solid probe MS by collecting the eluantes from HPLC.

LSD is highly fluorescent in acid solution. Although a fluorescence spectrum is not specific for LSD, if the excitation and emission spectra of a sample solution corresponds to those of standard LSD, one may strongly suspect LSD to be present.

3.0.0 Tryptamines

The tryptamines are structurally similar to Serotonin and Melatonin that are naturally present in the human body. The most commonly encountered compounds within the tryptamine category include Psilocyn and Psilocybin which are found in mushrooms of the *Psilocybe* genus, and Bufotenine which is found in the skin of *Bufo Marinus* the Australian cane toad, and in the parotid glands on the back of *Bufo alvarius* toads, as well as the seeds of the genus *Anadenanthera* (*piptadenia*) such as *piptadenia peigrina*, and the bark of the genus *Banisteriopsis* (*Malpighiacae*).

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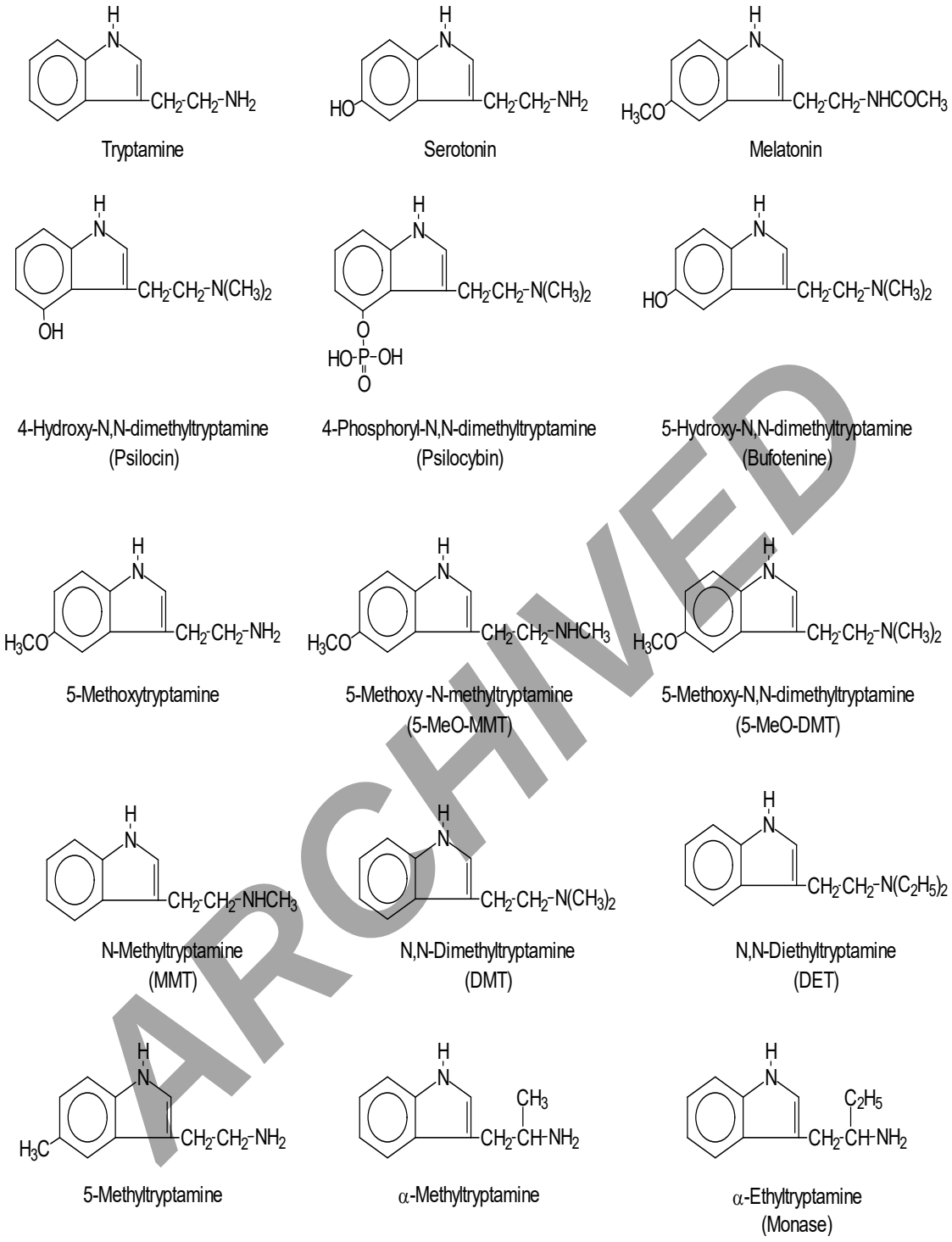

Figure 14

Figure 4 gives the structures of the more common tryptamines.

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3.1.0 Spot tests

A spot test widely used for the detection of compounds with an indole nucleus employs the use of Van Urks reagent. In the presence of a tryptamine, this light yellow reagent produces a violet to purple color.

3.2.0 GC/MS

GC/MS is generally suitable for identifying the tryptamines, although some care must be taken as some of the tryptamines (such as Psilocyn and bufotenine) produce similar mass spectra.

Reference 8 gives more information as well as mass spectral data for some common tryptamines.

4.0.0 Psilocyn and Psilocybin

Psilocyn and Psilocybin are found in mushrooms of the Psilocybe genus. The word Psilocybe comes from the Greek work "psilos" which means bald head. Common species include *Psilocybe cubensis*, *Psilocybe cyanescens, Psilocybe mexicana* and *Psilocybe tampanensis*. The dried mushrooms contain 0.2-0.4% psilocybin and trace amounts of Psilocyn. Psilocybin is a stable water-soluble compound. Psilocyn is water insoluble and easily oxidized. Psilocyn is 1.5 times more potent then psilocybin. A typical dose is 4 to 8 mg. corresponding to about 2 grams of dried mushrooms.

4.1.0 Spot tests

Van Urks reagent can be added directly to dry mushroom material. Due to the low Psilocyn and psilocybin content present in mushrooms, several minutes may elapse before a faint violet to purple color forms.

4.2.0 Extraction

One half to one gram of mushrooms should be crushed or ground and soaked in a minimal amount of methanol for at least five minutes (NOTE: Attempts to concentrate extracts via heating usually result of decomposition of the Psilocyn and psilocybin and should be avoided if possible). The addition of ~~a~~ one to three drops of ammonium hydroxide will assist in the extraction as will gentle heating. After soaking for a suitable period of time the methanol is decanted and filtered through a Pasteur pipette with a cotton plug. Acid base extractions may also be employed via the analytical method. The solution is ready for analysis.

4.3.0 GC/MS

The heat of the GC injector cleaves the phosphoryl ester of psilocybin turning it into Psilocyn. Hence, Psilocyn and psilocybin give identical results when analyzed via GC/MS.

4.4.0 TLC

TLC using ammonical methanol, as a developing solvent will distinguish Psilocyn from psilocybin. Van Urks reagent makes a suitable visualizing agent.

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2 5.0.0 Substituted Amphetamines and Phenethylamines

Drugs of the ring substituted amphetamine and phenethylamine class have a high potential for abuse and occur frequently on the illicit market. While some of these compounds such as Mescaline are naturally occurring, most are synthetic and constitute a major portion of the "Designer Drugs" on the market. These compounds are often synthesized in an attempt to circumvent controlled substance statutes. Recently, analog laws have been introduced in an attempt stifle this activity. In general, to be declared an analog, the substance must have a chemical structure which is substantially similar to a controlled substance, and: A) …which has a … effect on the central nervous system substantially similar to … a controlled substance, or B) …which the individual represents to have a … effect on the central nervous system substantially similar to … a controlled substance.

As shown in Figure 5, there are many possible sites on the phenethylamine structure where substitution can take place, and often more than one of these sites have substitutions on them. Methoxy-substituted amphetamines possess both stimulant and hallucinogenic properties.

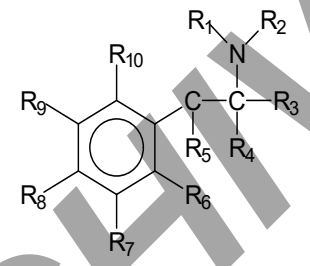

Figure 15

Substituted amphetamines having one, two, or three methoxy groups have all been encountered. Other variations include the addition of a bromine on the ring, e.g., 4-bromo-2,5-dimethoxyamphetamine (Bromo-STP or DOB), the inclusion of methyl group on the ring, e.g., 4-methyl-2,5-dimethoxyamphetamine (STP or DOM), the addition of a methyl group on the nitrogen, e.g., 2-methoxy-N-methylamphetamine (methoxyphenamine), or the shortening of the alkyl side chain to two carbons instead of three, e.g., 3,4,5-trimethoxyphenethylamine (mescaline). Closely related are compounds having oxygen attached to adjacent aromatic carbons and having the oxygen joined by a methylene group, e.g., 3,4-methylenedioxyamphetamine (MDA). Figure 6 shows the structure of some of the more common compounds in this class.

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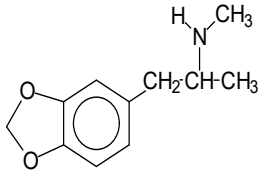
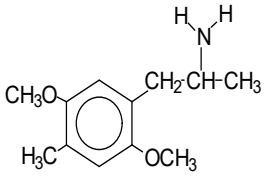
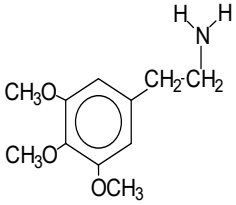


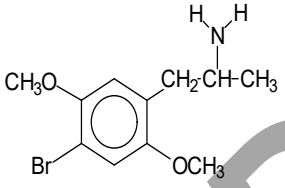
3,4-Methylenedioxymethamphetamine
(MDMA)



2,5-Dimethoxy-4-methylamphetamine
(STP, DOM)



3,4,5-Trimethoxyphenethylamine
(Mescaline)



4-Bromo-2,5-dimethoxyphenethylamine
(Nexus)

Figure 16

5.1.0 Extraction

Since all the substituted amphetamines are strong amines, they can be isolated from neutral and acidic impurities by the usual acid-base extraction techniques. The free bases occur as volatile, oily liquids, making isolation as a salt or a less volatile derivative a necessity.

Presumptive tests that can be used to determine the presence of a methoxy-substituted amphetamine include spot tests (such as the Marquis Test), GLC, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), and UV spectroscopy. Since there is overlap of the responses of the various methoxy-substituted amphetamines, additional instrumental tests may be necessary to determine the identity of the positional isomer present.

5.2.0 GC/MS

MS has several drawbacks when used to identify methoxy-substituted amphetamines. The spectra obtained are weak, and amphetamines with the same molecular weight exhibit very similar fragmentation patterns. MS alone may not allow identification of the substitution pattern. The molecular ions are of low intensity, and most ring-substituted amphetamines have a base peak of *m/z* 44 while most N-methylamphetamines have a base peak of *m/z* 58.

5.3.0 IR

Infrared Spectrophotometry (IR) permits the unequivocal identification of all the substituted amphetamines. Both the free bases and salts can be used for the identification. Since even the salts tend to be hygroscopic, considerable difficulty is frequently encountered in obtaining a KBr dispersion disc suitable for use. The easily

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prepared and less hygroscopic phenylisothiocyanate derivatives are suitable for overcoming this obstacle.

6.0.0 PCP and its Analogs

The scheduling of the animal tranquilizer phencyclidine (PCP) as a controlled substance in 1970 brought to the illicit market a number of analogs reported by Maddox, et. al., to give responses in animals similar to those produced by PCP. One such compound in 1-[1-(2-thienyl) cyclohexyl] piperidine (TCP), in which the thienyl group has replaced the phenyl group in PCP. Another analog is PCE, in which N-ethyl has replaced the piperidine ring in PCP. (see Figure 7).

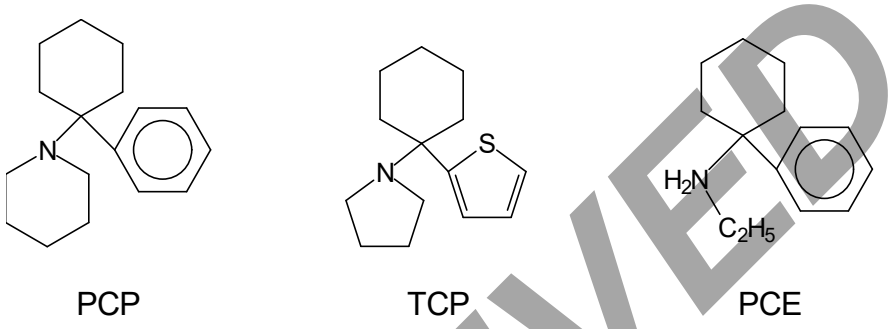

Figure 17

6.1.0 Extraction

Since PCP and its analogs are basic, they can be isolated from neutral and acid impurities by the usual acid-base extraction techniques. Phencyclidine hydrochloride is very soluble in $CHCl_3$ and can be easily extracted by $CHCl_3$ from an aqueous solution containing chloride ions. The free bases of PCP and its analogs are either liquids or low melting solids. Their HCl salts are stable solids but are hygroscopic to a varying degree.

Screening tests that can be used to detect the presence of PCP or its analogs include UV, TLC, and GLC.

6.2.0 TLC

A wide variety of solvent systems and absorbents have been used for the TLC identification of PCP and its analogs.

6.3.0 GC/MS

Phencyclidine and its analogs are also sufficiently thermally stable to be identified by GLC on a wide variety of columns. Injection of the free base is preferable because of some decomposition upon injection of the HCl salts. Those analogs having the thiophene moiety are less thermally stable than those having the phenyl moiety. The EI mass spectra of phencyclidine and its analogs are very informative. They can be used to confirm a suspected structure, as well as to elucidate the structure of uncharacterized

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analogs of PCP. The molecular ions vary in intensity but can be assigned, nonetheless. Other prominent mass fragments include M - 83 in the thiophene series and M - 77 in the phenyl series, resulting from loss of the respective unsaturated moieties. All members of the thiophene series give a strong peak at m/z 97, while those of the phenyl series give a strong peak at m/z 91. These are characteristic of the thiophene and benzene derivatives. Phencyclidine and its analogs that contain the cyclohexyl moiety also give a strong M-43, resulting from the loss of a C_3H_7 radical portion of this ring.

6.4.0 IR

The IR spectra of both the free bases and the hydrochloride salts can be used for identification. Both are capable of distinguishing closely related isomers and homologs.

Because of the large number of PCP analogs that can and have been prepared, multiple instrumental tests may be necessary to establish the complete identity of the compound present. These tests include MS and IR.

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26.0 Appendix I

Idaho State Police

Forensic Laboratory Training Manual

Cocaine

1.0.0 HISTORY

Archaeological artifacts show that the use of coca was widely accepted in ancient cultures of South American Indians. Paintings on pottery, ornaments depicting pictures and symbols of the coca bush and its leaves, as well as sculptured wood and metal objects dating as far back as 3000 BC on the coast of Ecuador indicate the use of coca in both civil and religious rituals. Relatively recent studies of the antiquity of the use and cultivation of coca indicate that the coca plant is native to the eastern Andes Mountains. Until this day, the natives in the area continue the custom of chewing coca.

A French chemist, Angelo Mariani, introduced Europe to the coca leaf by importing tons of coca leaves and using an extract from them in many products such as his "Coca Wine." Cocaine, as obtained from the coca leaves, was first discovered by Gaedecke in 1855 and rediscovered by Niemann in 1859, at which time he gave the compound the name cocaine. The local anesthetic properties of cocaine were demonstrated first by Wohler in 1860; however, it was not used medically until 1864 as a topical anesthetic in the eye.

2.0.0 TAXONOMY

The French botanist Joseph de Jussieu made the first taxonomical reference to coca[2] in 1750. He assigned the plants to the genus **Erythroxylum**. Later, Lamarck, another French botanist, published six new species, including the famous **Erythroxylum coca** Lamarck, in 1786. Today, the full taxonomical classification is:

CATEGORY	TAXON
Division (phylum)	**Spermatophyta**
Class	**Dicotyledons** (shrubs and trees)
Order	**Geraniales**
Family	**Erythroxylaceae**

[2]Note that other literature sources seem to credit Patrick Brown as the founder of the genus **Erythroxylum** (in 1756).

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Genus	**Erythroxylum**
Species One	**Erythroxylum coca** Lamarck
Variety of Species One	**Erythroxylum coca var. ipadu** (Plowman)
Species Two	**Erythroxylum novogranatense** (Morris) Hieronymus
Variety of Species Two	**Erythroxylum novogranatense var. truxillense** (Rusby) Plowman

The spelling of the genus name **Erythroxylum** is considered a hybrid of Greek and Latin. Linnaeus changed the spelling of the generic name to **Erythroxylon**, a pure Latin form. Nevertheless, botanists consider **Erythroxylum** as the proper spelling since it was derived in accordance with the rules of nomenclature.

Excluding the wild species of coca, all cultivated coca plants are currently considered to belong to two distinct species of **Erythroxylum** including one variety within each of the species. Specific areas of cultivation for each follows:

1. **Erythroxylum Coca Lam. (**or **E. coca)** -- This is the most important species from which almost all-commercial cocaine is derived. This species is native to the eastern Andes extending from Ecuador south to Bolivia. This was the first type of cultivated coca to be collected and studied by Europeans and was referred to as Huanuco or Bolivian coca after the main areas of cultivation. Like many plants that have been cultivated for thousands for years, E. coca is now rarely found in a truly wild state.

A particular variety of E. coca that warrants a brief mention since it has gained some recent notoriety is the coca of the Amazon Valley that, until today, continues to be cultivated on a very small scale by a number of Indian tribes. It belongs to the species of E. coca, but differs from the typical Andean species and is described as a variety, i.e., **Erythroxylum coca**, var. ipadu.

2. **Erythroxylum novogranatense** (Morris) Hieronymus -- This is the second species[3] of cultivated coca and is commonly known as Colombian coca. This species is cultivated throughout the mountains of Colombia. The species name "novogranatense" refers to the geographical origin of the plant, Nueva Grenad, the old colonial name for Colombia. There appears to be little cocaine production based upon this species.

[3]Older scientific publications (prior to 1976) refer to the studies of Morris who described this type of coca plant as a variety of E. coca. The German botanist Hieronymus was the first to recognize it as a distinct species.

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During the early 1900s, the Europeans created a cocaine industry on one of the major islands of Indonesia (i.e., Java). Dutch farmers adapted the coca species **Erythroxylum novogranatense** to their soil and climate using modern agricultural techniques. Commonly referred to as "Java" coca, its total alkaloidal content was found to be higher than Colombian coca; however, its true cocaine content was found to be much lower than Colombian coca. This variation in alkaloidal content is attributed to changes in climate, soil, and possibly other environmental conditions. The Java coca industry practically collapsed just prior to World War II because of declining prices for coca leaves from other countries.

3. **Erythroxylum novogranatense var. truxillense** (Rusby) Plowman -- This is one recognized and distinct variety within the species **Erythroxylum novogranatense**. It is well known as Trujillo coca[4] since it is cultivated near the city of Trujillo in northern Peru on the slopes of the Andes (as well as the nearby desert coast of Peru). This variety of (Colombian) coca is particularly rich in methyl salicylate (oil of wintergreen) and other flavoring compounds that are used in the production of coca-flavored beverages. (Coca-Cola is a popular beverage that incorporates decocainzed extracts of Trujillo coca in its preparation.)

3.0.0 MORPHOLOGICAL AND ECOLOGICAL CHARACTERISTICS

Bolivian (or Huanuco) coca leaves are usually large and thick, broadly elliptic in shape, pointed at the apex, and dark green in color. The underside of the leaves has two lines (parallel) to the midrib. These leaves are not considered characteristic of this species since they are present to a degree in the other species of coca. The leaves are usually deciduous after the current season's growth. The plant grows as a small to medium size bush usually planted in rows on large, hillside plantations. This species thrives on moist, cool mountain climates.

Colombian coca can be distinguished from Bolivian coca by its smaller, narrower, thinner, bright yellowish-green leaves, which are usually rounded at the apex. Contrary to the Bolivian plant, the Colombian coca plant holds its leaves on the branches after a season's growth. This habit gives Colombian coca a larger, bushier, and robust appearance. In addition, Colombian coca prefers the hotter, drier climate and thrives at lower elevations. Dried Colombian leaves also have a more noticeable odor of methyl salicylate than Bolivian leaves.

Trujillo coca is morphologically similar to Colombian coca. Trujillo coca has smaller, narrower, and slightly thicker leaves that are darker green at maturity.

Refer to Table 1 for additional characteristics for both varieties of **Erythroxylum coca** and **Erythroxylum novogranatense**.

The genus **Erythroxylum** is the only natural source of the alkaloid cocaine and related compounds. Almost all commercial coca leaves and cocaine are derived from the species **Erythroxylum coca** Lam.;

[4]Sometime referred to as Truxillo or Peruvian coca.

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Erythroxylum novogranatense (Morris) Hieronymus, because of its quick adaptability to different climates, is most likely the dominant species that is illegally cultivated for the illicit drug trade.

SPECIES OF COCA AND THEIR CHARACTERISTICS

Genus, Species	**ERYTHROXYLUM COCA**		**ERYTHROXYLUM NOVOGRANATENSE**	
Varieties	**coca**	**ipadu**	**novogranatense**	**truxillense**
Origin	montaña region of eastern Andes; Ecuador, Peru, and Bolivia, mainly between 500 - 1500m	western Amazon of Brazil, Colombia, and Peru	Colombia, Venezuela and Central America, Sierra Nevada de Santa Marta and rugged mountains of Cauca and Hucha	desert coast of Peru and in adjacent arid valley of the Rio Maranon, Truxillo region on the north coast of Peru
Description of plant and/or leaves	pointed leaves, parallel longitudinal lines on leaf undersides	tall, spindly shrub with long weak branches and relatively large elliptical leaves which are blunt or rounded at the apex; flowers have a shorter flusher pedicel and a markedly denticulate staminal tube	large bush plant with small, narrow, thin, and bright yellow-green leaves which are rounded	up to 3m tall with multiple trunks reaching 4 cm in diameter; branches are dense erect and spread leaves narrowly elliptical to oblong-lanceolate 20-65 mm long; medium to light green above pale green to glossy green beneath and midrib fluted with slight medial ridge

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		only short styled morphs		
Odor	grassy or haylike		wintergreen	wintergreen
Climate	favorable tropical environment with high rainfall, moderate temperatures and well drained mineral rich soils; moist cool	does not like intense heat or poorly drained soils, short-lived	hot, seasonably dry habitat resistant to drought	has been cultivated in arid, desert climate and wet montaña habitat of Colombia; even more tolerant to drought; prefers desert conditions
Adaptability	very little	very little	will survive under a wide range of environmental conditions; Resistant to drought	
Means of propagation	seeds	cuttings	seeds	seeds
Commercial uses	most important commercial species providing by far the largest supply of coca leaves and cocaine; 95% of Peru's crop	used for chewing	illegal in Colombia; grown illegally for coca chewing and cocaine production	principal variety used in beverage industry owing to its high content of essential oils and flavors-several hundred tons exported to N.Y. for preparation of extracts, used in making Coca-Cola
% Alkaloids	0.5 - 1.0	unknown	1.0 - 2.5	1.0 - 2.5
% Cocaine of Total alkaloid content	70 - 90	very little	20 - 50	20 - 50

Table 3

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Note that the genus **Erythroxylum** also includes a number of wild species of coca (reportedly about 200), most of which contain minimal quantities of cocaine and some of which contain no detectable quantities of cocaine.

4.4.0 CHEMISTRY

4.1.0 Coca leaves contain three basic groups of alkaloids, most of which are present in the form of esters. The content of these alkaloids will vary depending on the particular species of coca used, age of the plant, where it is grown, how it was cultivated, and when the leaves were picked. A higher total alkaloid content but with a smaller cocaine content reportedly occurs when the leaves are picked at an early stage of development; the reverse occurs when the leaves are picked when fully developed. Another important factor affecting the variation of an alkaloidal extract is the manner in which the alkaloids were refined from the leaves. Listed in specific groups are some of the popular alkaloids found in coca leaves:

Derivatives of Ecgonine

a. *l*-Cocaine (Methylbenzoylecgonine)

b. cis- and/or trans-Cinnamoylcocaine[5] (cis- and/or trans-Methylcinnamoylecgonine)

c. α and/or β-Truxillenes (Methyl α and/or β-truxilloylecgonine or α and/or β-cocaine)

d. Methylecgonine (Ecgonine methyl ester)

e. Methylecgonidine

Derivatives of Tropine (and ψ-Tropine)[6]

a. Tropococaine (Benzoyl-ψ-tropeine or ψ-tropine benzoate)

Derivatives of Hygrine

a. Cuscohygrine

b. Hygroline

c. Hygrine

In addition to *l*-cocaine, cinnamoylcocaine and the truxillenes are considered the most important coca alkaloids for pharmaceutical use. Hygrine and its related alkaloids are reported to be present in significantly large quantities in both varieties of **E. novogranatense** (i.e., Colombian and Trujillo coca).

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[5]Cinnamylcocaine and cinnamoylcocaine have been used interchangeably in the literature.

[6]Esters of tripine are called tropeines. ψ is the Greek symbol for pseudo.

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Hygrine is a tertiary aminoketone, hygroline is the secondary alcohol related to hygrine, and cuscohygrine is a diaminoketone containing two N-methyl groups.

The pharmaceutical industry produces pure cocaine semi-synthetically. The ecgonine conversion process employed eliminates the need to separate cocaine from related ecgonine alkaloids and produces a much greater yield of cocaine. Briefly, the procedure involves:

1. Extracting the coca alkaloids previously mentioned from dried coca leaves and isolating the mixture of ecgonine alkaloids.

2. Converting the group of alkaloids to ecgonine via hydrolysis with dilute hydrochloric acid:

Cocaine ⟶ *l*-Ecgonine•HCl + MeOH + Benzoic Acid

Cinnamoylcocaine ⟶ *l*-Ecgonine•HCl + MeOH + Cinnamic Acid

Truxillene ⟶ *l*-Ecgonine•HCl + MeOH + Truxillic Acid

(Tropococaine is excluded since it would hydrolyze to pseudotropin and not ecgonine.)

3. Isolating and purifying the ecgonine and converting it to its free base.

4. Converting the ecgonine to *l*-cocaine by benzoylating the ecgonine with benzoic anhydride to benzoylecgonine followed by methylation with methyl iodide.

An alternate commercial process converts the isolated alkaloids to ecgonine methyl ester (or methyl ecgonine) by hydrolyzing the alkaloids in methyl alcohol-hydrogen chloride. The ester is isolated and benzoylated directly to *l*-cocaine. Either industrial process provides the following approximate yield:

Dried Coca Leaves	⟶	Cocaine HCl
145 kgs		1 kg

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In clandestine cocaine laboratories in South America, the crude cocaine process also seeks to eliminate most of the coca alkaloids except cocaine. The cured process is an extensive operation. It generally consists of:

1. a “pasta” laboratory where all the coca alkaloids are extracted from a batch of coca leaves and converted to a water soluble (sulfate salt) pasty material called “pasta” or “sulfate” or coca paste;

2. a “base” laboratory (optional) where the coca paste is treated to remove most undesirable substances and to provide cocaine base;

3. a “crystal” laboratory where the cocaine base is converted to cocaine hydrochloride.

Very often, however, the illicit laboratory “cook” abbreviates or overlooks a critical step of the process and causes other alkaloids, impurities, and cocaine decomposition products to become part of the final product. The approximate yield from this illicit process can be summarized as follows:

Dried Coca Leaves	→	Coca Paste	→	Coca Base	→	Cocaine HCl
240 kgs		1.4 kg		0.9 kg		1 kg

If chemical conditions become too acidic or basic, cocaine can degrade, as illustrated in Figures 1 and 2, and the degradation products may recombine to form new products. For example, benzoic acid and methyl alcohol will combine to yield methyl benzoate, a sweet smelling, volatile liquid. Similarly, cinnamoylcocaine and truxilline can decompose, producing products that can recombine to form new products, such as

Chemical Structure of Cocaine

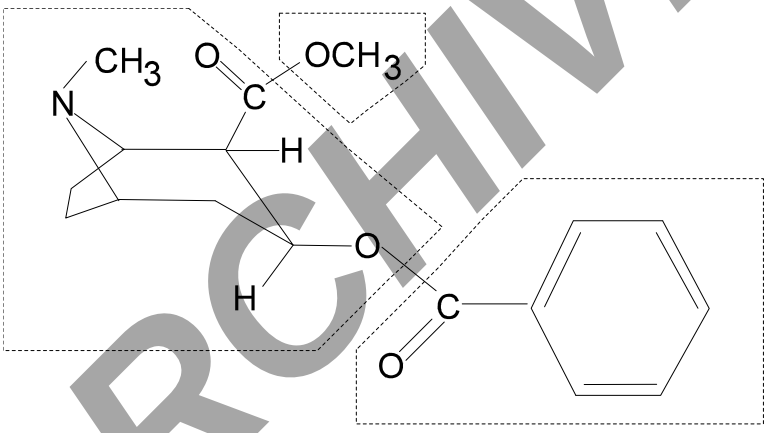

Figure 18

The cocaine molecule is made up of three building blocks. If chemical conditions become too acidic or too basic the cocaine molecule readily breaks down into these three pieces.

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Decomposition of Cocaine

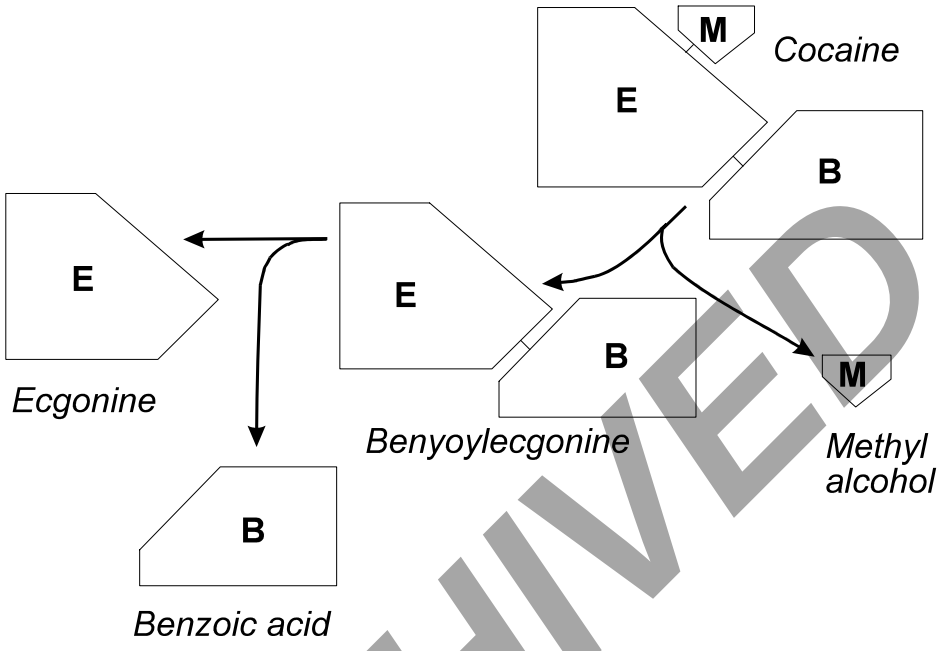

Figure 19

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methyl cinnamate and methyl truxillate. Contrary to earlier studies, benzoylecgonine, ecgonine, pseudococaine, and cocaethylene are believed to be produced during the refining process and are not naturally occurring in the coca plant.

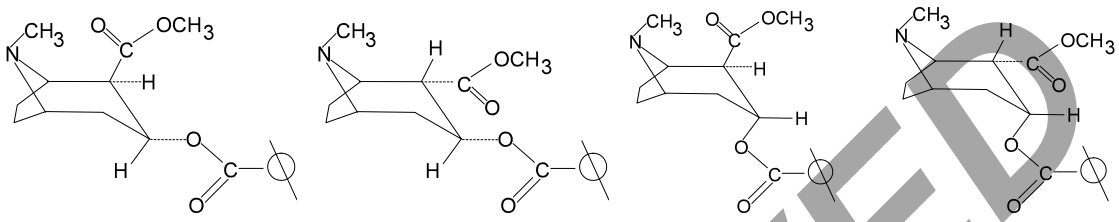
4.2.0 Synthesis of Cocaine

In 1923, using 2-carbomethoxytropanone as an intermediate, Willstatter prepared (±) cocaine along with pseudococaine. At least four synthetic methods have been reported for the synthesis of 2-carbomethoxytropanone. These syntheses range from the difficult cyclization of 1-methyl-2,5-dicarboethoxypyrrolidine, to the simplest, at least in principle, reaction of succindialdehyde, methylamine, and β-ketoglutaric acid to yield the target compound 2-carbomethoxytropanone. Willstatter's synthesis in 1923 involved the formation of the dipotassium salt of monomethyl-β-ketoglutarate, which was then reacted with methylamine and succinaldehyde to obtain the 2-carbomethoxytropanone. The methyl esters of (±) pseudoecgonine and (±) ecgonine are formed by the reduction of 2-carbomethoxytropanone using a sodium-mercury amalgam; fractional crystallization can be used to separate the two ecgonine methyl esters. The final step in the reaction scheme is between the (±) ecgonine methyl ester and benzoyl chloride to yield (±) cocaine.

4.3.0 Stereochemistry

During the period 1898 to 1923, Willstatter and his collaborators were able to obtain pseudococaine by transformation of (-) cocaine and by total synthesis. At the time of this early work, stereochemistry was in its infancy. Based on chemical studies regarding ecgonine and tropine, Willstatter incorrectly assigned pseudococaine a structure that has both the C-2 carbomethoxy group and the C-3 benzoyloxy group axial. Instead, later studies show that pseudococaine has both the carbomethoxy group at C-2 and the benzoyloxy group at C-3 in equatorial position.

COCAINE ψ-COCAINE ALLOCOCAINE ψ-ALLOCOCAINE



Two other cocaines, allococaine and allopseudococaine, were synthesized and characterized by Findley in the 1950s. Again, problems arose with the nomenclature used in representing the stereochemistry of these compounds. Using Findley's assignments, the carbomethoxy group at C-2 and the benzoyloxy group at C-3 were equatorial-axial, respectively, for allococaine and axial-axial for allopseudococaine. More recent nomenclature places allococaine and cocaine with the same stereochemistry at the C-2 position, that is, the carbomethoxy group is axial. Pseudococaine and allopseudococaine have the same spatial relationship at the C-2 position; viz., the carbomethoxy group is equatorial. Thus, the cocaine molecule has four asymmetrical centers which give rise to eight stereoisomers arranged as four diastereoisomeric pairs -- *d,l*-cocaine, *d,l*-pseudococaine, *d,l*-allococaine, and *d,l*-allopseudococaine.

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5.0.0 SAMPLE ANALYSIS

The detection and identification of cocaine through acceptable analytical methods generally presents no problems; however, in most instances, pure cocaine is not presented for analysis. Even when diluents and adulterants have not been added to the cocaine, the sample usually contains impurities resulting from the original isolation of the alkaloid. For example, cis- and trans-cinnamoylcocaine are usually present in illicit cocaine samples. Benzoylecgonine may be present in many cocaine samples and should be eliminated by a basic extraction since its UV is similar to that of cocaine. Note that the detection of benzoyl ecgonine (as well as ecgonine) by UV, GLC, or TLC can be difficult because of the polar nature of the acid group at C-2.

A second complication in the analysis of cocaine samples results from the presence of adulterants. Many of the "caines," such as benzocaine, lidocaine, procaine, and tetracaine, are commonly encountered.

From a practical standpoint most of the analyses conducted at the ISP Laboratory will utilize capillary GC/MS for the identification of cocaine. The high resolution capabilities of capillary GC will easily separate cocaine from most common adulterants and diluents.

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