Spring 1974

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V. DRUG TESTING PROCEDURES IN CRIME LABORATORIES

CARL R. PHILLIPS*

INTRODUCTION

In the trial of any drug case, the testimony of the chemist as an expert witness is crucial but limited. Seldom does the chemist's testimony serve to magnify or diminish the seriousness of the charge, but the prosecution of the case cannot be complete without his testimony. Even though the chemist is employed by the police department or other governmental agency and is brought into the trial at the request of the prosecuting attorney, he must maintain his detachment from the case. Ideally the function of the expert witness is simple and objective: either the suspected substance is found to be a drug as charged, or it is not. However, the lack of understanding of modern scientific analytic methods by the majority of attorneys often leads to an unproductive courtroom situation. Frequently the prosecutor asks questions which will make the witness appear to be an absolute expert in the field and his analysis beyond reproach, while the defense attorney asks questions with the hope of making the expert appear to be incompetent and his analysis without value. In fact, the questions asked by both sides usually have little relevance to the validity of the analysis and yield no information about the actual merits and limitations of the tests performed.

In order to improve the understanding of the chemist's testing procedures and facilitate more relevant questioning by both prosecuting and defense attorneys, the analytical theories and methods used for drug identification in the Indianapolis Police Department Crime Laboratory and most other crime laboratories are explained in the following paragraphs. The information presented here is only intended to provide a general survey of testing methods. Since the complexity of these procedures is too great to allow a comprehensive coverage which is completely understandable to someone with no scientific background, interested attorneys will hopefully use the references indicated for further assistance in handling drug cases. These sources provide more complete definitions of terms, diagrams

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of instrument construction and explanations of scientific principles involved in the various testing procedures described in this article.

**Spectroscopic Methods**

A spectroscopic analysis involves making a determination of the energy or change in energy caused by the interaction of electromagnetic radiation with a molecule or atom.\(^1\) When light of a known frequency and wavelength (monochromatic) is allowed to pass through a substance, the energy of the light beam will undergo a change. This change results from changes in the energy states of the molecules or atoms in the substance. As the wavelength of the excitation light is altered, the amount of energy that is either absorbed or emitted will also change. Across a range of excitation wavelengths, an atom or molecule will produce a characteristic spectrum dependant upon the observed energy changes. By studying this spectrum, spectroscopists are able to determine the chemical composition of the substance through which the light beam was passed.\(^2\)

The electromagnetic radiation ranges encountered in spectroscopic analyses are shown in Table 1.

*Ultraviolet-Visible Spectroscopy*

This method of spectroscopy utilizes the principle that radiation is absorbed when valence (least strongly held) electrons are raised from a low energy state (ground state) to a higher energy state (excited state).\(^3\) An analogy could be made to lifting a brick from the floor (ground state) to a table (excited state). The energy required to lift the brick from one level to the other would be analogous to the radiant energy required to raise an electron from its ground state to an excited state.\(^4\)

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1. For those unfamiliar with the concept of atoms and molecules, a basic knowledge sufficient for the purposes of this article may be obtained by consulting any textbook in freshman college chemistry. See, e.g., T. Brown, *General Chemistry* (2d ed. 1968); C. Keenan & J. Wood, *General College Chemistry* (3d ed. 1966); W. Masterson & E. Slowinski, *Chemical Principles* (2d ed. 1969).


4. See Appendix I, part A.
TABLE I

<table>
<thead>
<tr>
<th>ELECTROMAGNETIC RADIATION</th>
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<tbody>
<tr>
<td>X-rays</td>
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<tr>
<td>Ultraviolet</td>
</tr>
<tr>
<td>Visible</td>
</tr>
<tr>
<td>Infrared</td>
</tr>
<tr>
<td>Microwaves</td>
</tr>
<tr>
<td>Radio Waves</td>
</tr>
</tbody>
</table>

**Higher Energy**

- Higher energy of radiation.

**Longer Wavelength**

- Longer wavelength of radiation.

**Spots**

- Shorter wavelength.

**Laser**

- Laser frequencies.

**Resonance Spectroscopy**

- Resonance spectroscopy.

**Fluorescence**

- Fluorescence spectroscopy.

**Atomic Distances**

- Atomic distances measured.

**Intrinsic Transitions**

- Inner electron transitions.

**Scoping**

- Scoping procedures.

**Resonance Spectroscopy**

- Resonance spectroscopy.

**Atomic Energy**

- Atomic energy levels.

**Molecular Field**

- Molecular field.

**Rotation of Molecules**

- Rotation of molecules.

**Rotation of Parts**

- Rotation of parts.

**Rotation of Atoms**

- Rotation of atoms.

**Rotation of Molecules**

- Rotation of molecules.

**Infrared**

- Infrared spectroscopy.

**Microwaves**

- Microwaves spectroscopy.

**Resonance**

- Resonance spectroscopy.

**Fluorescence**

- Fluorescence spectroscopy.

**Atomic Energy**

- Atomic energy levels.

**Intrinsic Transitions**

- Inner electron transitions.

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**Atomic Distances**

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in spectroscopy, the electron transitions observed require the presence of a chromophoric group on the molecule. A chromophoric group is a part of a molecule which absorbs ultraviolet or visible light. Usually a chromophoric group includes one or more atoms which are bonded by two or three bonds to an adjacent atom rather than the usual one bond. An ultraviolet or visible spectrum is produced by plotting the wavelength of light versus the absorption of energy by a solution of a drug.

Theoretically, small differences in the wavelength of maximum and minimum absorptions could be observed for chromophoric groups attached to slightly different molecules. Unfortunately, however, the energy states of the electrons in these groups are coupled to a variety of vibrational and rotational states of the whole molecule. This coupling of electronic, vibrational and rotational states leads to very broad bands of absorption of ultraviolet or visible radiation instead of single sharp bands so that the effects of differences in the non-chromophoric part of the molecule are minimized. Both amphetamine and methamphetamine, for example, have a group of identical peaks in the ultraviolet spectrum and therefore cannot be absolutely distinguished by this method alone. In fact, the mono-substituted benzene ring chromophoric group is such a common entity in drug molecules that well over fifty common drugs will have spectra similar to amphetamine and methamphetamine.

5. The radiation range most commonly used in ultraviolet spectroscopy is from 200 to 400 nanometers (nm). This is equivalent to $2 \times 10^4$ to $4 \times 10^4$ centimeters (cm). The visible range of radiation is from 400 to 700 nm, or $4 \times 10^4$ to $7 \times 10^4$ cm. (See Table 1.)

6. The phenyl group $C_6H_5\ldots$ is an example of a chromophoric group. This group has six carbon atoms bonded together in a six-member benzene ring by the equivalent of three double bonds and three single bonds. Energy transitions for the electrons in the double bonds give rise to a series of absorption peaks centered at around 257 nm. In contrast, the cyclohexyl group $C_6H_{10\ldots}$ which also has a six-member ring of carbon atoms, has all single carbon-carbon bonds and no double bonds. The cyclohexyl group is not a chromophoric group since it does not absorb energy in either the 200-400 nm ultraviolet region or in the visible range.

7. See Appendix I, part B for additional information on the ultraviolet-visible spectrum. Appendix II provides a schematic drawing and brief description of an ultraviolet-visible spectrometer.

8. Parts of a molecule will be stretching, bending and rotating relative to the remainder of the molecule with a set of particular energies. These movements are referred to as the vibrational and rotational modes, and the energies of these movements are the vibrational and rotational states.

9. The peaks of amphetamine and methamphetamine are centered at around 257 nm. Both of these drugs have a single benzene ring $(C_6H_5)$ as the chromophoric group and differ by only one methyl group $(CH_3)$ on a remote nitrogen atom.
Furthermore, no general rules may be formulated regarding the statistical value of an ultraviolet spectrum since the factors underlying such rules would be too variable and complex.  

Examination of the expert witness concerning ultraviolet-visible spectroscopy should be centered upon his knowledge of the underlying theory and applicability of the method to the particular drug or drug mixture in question, and not on useless generalities about whether the method is specific for all drugs in all cases. Ultraviolet spectroscopy is a very useful tool as a screening method to formulate a preliminary group of possible drugs because most diluents or excipients used to cut drugs to street levels do not greatly interfere with the spectrum. Lists of drugs and their absorption maxima are very useful for this purpose.

Fluorescence Spectroscopy

The ultraviolet-visible region of the electromagnetic spectrum is also used for fluorescence spectroscopy. Recalling the previously mentioned brick analogy, an absorptive transition occurs when energy is absorbed by raising the brick from the ground state to an elevated state. A fluorescence transition may be visualized as that energy which is emitted by lowering the brick to the ground state from the elevated state. Hence, fluorescence occurs when light is initially absorbed by a molecule and is then immediately emitted at either the same wavelength or a longer wavelength. Two spectra are routinely obtained in fluorescence spectroscopy: the excitation spectrum (energy emitted at a constant wavelength as a function of the wavelength of monochromatic excitation light) and the emission of fluorescence spectrum (energy emitted as a function of monochromatic emission light at a constant excitation wavelength). A fluorescence excitation spectrum is obtained by detecting a set wave-

10. To be meaningful, a general rule would have to consider simultaneously such variable factors as the peak maximum and minimum wavelengths, the general shape of the absorption curve, the relative degree of absorption (extinction coefficient), the number of peaks, and the shift in absorption maxima and minima with a change in the acidity (pH) of the solvent. Differentiation between acid solutions of such closely related drugs as morphine at 284 nm and heroin (diacetylmorphine) at 278 nm, is easily done, whereas differentiation between amphetamine and methamphetamine both at 257 nm would be virtually impossible.


length of light given off by the sample (electrons being lowered from the excited to the ground electronic states) and plotting the value of the energy emitted at that wavelength versus the wavelength of the light being used to irradiate the sample. A fluorescence emission spectrum is obtained by setting a particular value for the excitation wavelength and then plotting the energy emitted versus the wavelength of the emitted light.\textsuperscript{13}

Intensity differences are much greater for fluorescence spectroscopy than for ultraviolet-visible absorption spectroscopy; thus the method is much more sensitive. The major factor limiting the use of this method in crime laboratories is cost. At present, the only instruments available under $10,000 are “uncorrected” (single-beam) devices which do not allow direct comparison of spectra obtained on instruments in other laboratories. This method of spectroscopy has been principally used in the identification of LSD, which requires a higher degree of sensitivity than is possible by ultraviolet spectroscopy.\textsuperscript{14}

\textit{Infrared Spectroscopy}

This spectroscopic method measures the absorption of energy for the transition from one specific \textit{vibrational} energy state of a molecule to another.\textsuperscript{15} Atoms in a molecule are not held rigidly in one position, but are in constant motion by either stretching or bending the molecular bonds. The difference in energy between vibrational energy states is the same as that of infrared radiation.\textsuperscript{16} The region of the spectrum from 2.5 to 7 microns corresponds to stretching of molecular bonds, and the region from 7 to 14 microns and beyond corresponds to bending of molecular bonds.\textsuperscript{17}

The 7 to 14 micron region is very specific for individual molecules and is sometimes called the “fingerprint region.” Many mole-

\begin{itemize}
\item \textsuperscript{13} Additional information on the fluorescence spectrum is contained in Appendix I, part B.
\item \textsuperscript{14} Dal Cortiva, \textit{Identification and Estimation of Lysergic Acid Diethylamide by Thin-Layer Chromatography and Fluorometry}, 38 \textit{Analytical Chemistry} 1959 (1966).
\item \textsuperscript{16} The most commonly encountered range of wavelengths is from 2.5 microns to 14 or 40 microns ($2.5 \times 10^{-4} \text{ cm}$ to $1.4 \times 10^{-3} \text{ cm}$ or $4.0 \times 10^{-4} \text{ cm}$). (See Table 1.)
\item \textsuperscript{17} See Appendix I, part C for additional information on the infrared spectrum.
\end{itemize}
cules may have basically the same type of atoms bonded together so that little difference will be seen in the infrared region from 2 to 7 microns where the bonds are being stretched. But since very minor differences between molecules in their atomic arrangements cause drastic differences in the ways groups of atoms may be twisted or bent relative to one another, the fingerprint region will be unique for each molecule. In other words, since no two molecules will have the same set of bending mode absorptions, it is theoretically possible for any molecule to be identified through its fingerprint infrared spectrum if the pure material can be obtained.

The infrared spectrum is acquired in much the same manner as the ultraviolet-visible spectrum. A measurement is taken of the absorption of energy by the molecules as a function of monochromatic light wavelength in the infrared region of the electromagnetic spectrum. This absorption occurs in slightly broadened bands due to coupling of vibrational and rotational states of the molecule. Since the bands are not as broad, and the energy differences are relatively greater than for ultraviolet spectra, a much more specific determination is possible. Virtually all functional groups (segments of the molecules) will exhibit infrared activity making the infrared spectrum representative of the entire molecule, and not just of a few chromophoric groups as in ultraviolet-visible spectroscopy.

The principal shortcoming of infrared spectroscopy in identification of street drugs is that all of the excipients as well as the active component will exhibit an infrared spectrum. A separation procedure is therefore required to obtain the active component in 95 percent or better purity. Due to the wide variety and number of excipients, the small irreplaceable quantity of street mixture, and the often low percentage of the active component, the applicability of infrared spectroscopy in identification of street drugs is greatly limited.

A notable exception to this limitation, however, is in the comparison of commercial tablets and capsules, especially when the active component is present in a significant quantity as in barbi-

18. Appendix II provides a schematic drawing and brief description of an infrared spectrometer.

19. The active component in street heroin is usually around 5-10 percent, while that of LSD is around 0.02 percent.
tuates. Percentages of drugs and excipients in commercial products are carefully regulated, which means that the infrared spectrum of the mixture will be specific for both active components and excipients simultaneously. Comparison of a known dosage unit with the case sample may constitute a large part of the laboratory analysis. Several sources are available for direct comparison of purified drug spectra with known spectra when no standard is available to the crime laboratory.\textsuperscript{20}

\textit{Spot Tests}

Spot tests are in actuality visible spectroscopy. Instead of using a photocell as a detector in an instrument, the human eye is the detector. Absorption of light of various wavelengths in the very narrow visible region of the electromagnetic spectrum is observed as a color change when a drug is chemically reacted with another reagent.\textsuperscript{21} The observed change is due to formation of chromophoric groups which are of a much more limited variety than those of ultraviolet spectroscopy. Although many of the reaction products in spot tests are not actually known, this does not lower their value to the chemist since they are used principally as screening tests. Spot tests are of widely varying degrees of specificity, but combinations of several spot tests may be used to limit the possible drugs to a very small number.\textsuperscript{22} The value of a spot test in an overall analysis depends greatly upon the type of test, the degree of observation and the experience of the individual chemist.


\textsuperscript{21} E. CLARK, ISOLATION AND IDENTIFICATION OF DRUGS 123 (1969); INT. REV. PUB. NO. 341, METHODS OF ANALYSIS 136 (1967) (for confidential use of law enforcement officers and officials); I. SUNSHINE, HANDBOOK OF ANALYTICAL TOXICOLOGY 400 (1969).

\textsuperscript{22} Examples of spot tests and the observed colors are:

a.) Reaction of Marquis reagent (sulfuric acid-formaldehyde) with opium derivatives—purple; amphetamines—orange changing to brown; MDA—black.

b.) Reaction of Dille-Koppanyi (cobaltous acetate, isopropyl amine) with barbiturates—purple.

c.) Reaction of paradimethylaminobenzaldehyde with LSD—blue.

d.) Reaction of cobalt thiocyanate with cocaine—azure blue precipitate.

e.) Reaction of nitric acid with heroin—yellow turning to green; codeine—orange to yellow; morphine—orange-red to yellow.

CHROMATOGRAPHY

The chromatographic process is one of the most powerful methods of separation of closely related compounds available to the modern chemist. All chromatographic methods of analysis and separation are based upon the principle that the relative affinities of different types of molecules for other substances are unequal. Chromatography requires a stationary phase (material which does not move) and a mobile phase (material which moves over the stationary phase). Compounds are separated by being moved along the stationary phase by the mobile phase at different rates of speed. Gas, thin layer, paper and liquid modes of chromatography differ only in the apparatus, the types of mobile and stationary phases, and the means of detection. The underlying principles are the same for each method.

Thin layer chromatography uses a non-reactive glass, metal or plastic plate as a support for the stationary phase which is a dried powder (usually silica gel or alumina) placed on it in a "thin layer" (commonly 250 microns). A liquid solution of varied solvents is used for the mobile phase. A small quantity of the drug mixture in solution (around 1-5 microliters) is placed as a spot near the bottom of the plate on the thin layer and a known drug standard is placed in an adjacent spot. After drying, the bottom of the plate is placed in a closed chamber to be developed by allowing a liquid solvent or mixture of solvents to creep up the plate a predetermined distance by capillary action. As the solvent moves up the plate, the components of the drug mixture will separate. Compounds more strongly attached to the solvent (mobile phase) than to the thin layer (stationary phase) will move a greater distance up the plate. After the solvent front has reached either the top of the plate or a line scribed on the plate, the plate is removed and dried. Unless a naturally colored drug is encountered, which is rare, the separated circular spots corresponding to the pure components of the mixture will be invisible to the naked eye. The spots may be visualized by

23. Paper chromatography uses basically the same methods as thin layer chromatography, differing only by use of paper or impregnated paper as the stationary phase instead of the thin layer on a non-reactive support.

observing their fluorescence under ultraviolet light, charring them at high temperature, or reacting them with a sprayed reagent to form a visible chromophore.25

An \( R_f \) value, which is the decimal fraction of the distance that a specific component has traveled from the point of initial spotting to the final solvent front, can be calculated for each component.26 \( R_f \) values will be the same on any plate for the same compound under identical conditions. Thus if suspected heroin from a criminal case and known heroin are spotted side by side on the same plate, both heroin spots will travel the exact same distance up the plate. The \( \Delta R_f \) value for a drug may be varied over a wide range by changing either the mobile or stationary phase. Compounds of very close similarity may be easily separated by thin layer chromatography to give widely different \( R_f \) values. Proper technique in keeping the sample spots very small, prior equilibration of the development chamber, and absolute insistence on perfect matching of \( R_f \) values must be maintained for accurate thin layer chromatographic determinations.

Gas chromatography, or more precisely gas-liquid chromatography, employs a metal or glass tube packed with a non-volatile liquid coated on an inert solid powder as the stationary phase. An inert gas flowing through the column provides the mobile phase.27 For example, the stationary phase may be a high molecular weight silicone on crushed firebrick and the mobile phase may be nitrogen gas or helium gas.28 The analysis is conducted by injecting the drug sample into the gas stream through a rubber septum, separation of the sample as it passes through the stationary phase, and detecting the separate components of the sample as they emerge from the packed column with a suitable detector. A graph of the quantity of material detected versus time is then plotted to facilitate a determi-

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25. For example, a combination of heroin, methapryilene and quinine may be separated with an ammonia-methanol solution on a silica gel coated plate and visualized with an iodoplatinate spray to form three distinct spots of slightly different shades of blue color, one above another on the plate.


27. See generally A. Keulmans, Gas Chromatography (1957); A. Littlewood, Gas Chromatography (1962).

28. E. Clark, Isolation and Identification of Drugs 63 (1969); Cravey, Reed & Sedgwick, Gas Chromatography in Forensic Toxicology, 4 Am. Laboratory 63 (1972).
nation of the retention time (RT). The RT is the length of time from the point of injection until the maximum intensity for the peak of the eluted compound is reached. Different components will have varying RT's due to differences in their relative affinities for the stationary phase and their volatilities.

As with thin layer chromatography, very closely related compounds can be easily separated and differentiated by gas-liquid chromatographic testing. Thin layer is advantageous over gas-liquid chromatography because of more selectivity of the mobile phase, more rapid identification of several samples simultaneously, less costly equipment, and some degree of specificity in detection by observation of the visualized spots' colors. The advantages of gas-liquid over thin layer chromatography include more selectivity of the stationary phase, detection of components over a wider range of concentrations, and separation of more complex mixtures on a single mobile phase-stationary phase system by programmed temperature changes. Both of these chromatographic methods are extremely useful in the crime laboratory for identifying minute quantities of specific drugs in a wide variety of street dosage forms.

Liquid chromatography is a lesser used technique which is most often employed for preliminary purification of samples. A liquid solvent system serves as the mobile phase, and the stationary phase is a solid packed inside a metal, plastic or glass tube. Gravity flow is utilized in the simplest systems for forcing the liquid through the column, while more recent, but relatively expensive, systems employ a high pressure pump for this purpose. Commercial instruments now available may make liquid chromatography as popular as gas chromatography in the near future.

MISCELLANEOUS CHEMICAL TESTS

Microchemical Tests

These tests are made by reacting drugs with metallic reagents and observing the formation of minute crystals under a microscope. Very specific tests may be conducted due to a wide variety

30. See generally J. Kirkland, Modern Practice of Liquid Chromatography (1971).
of crystalline forms which may be observed for closely related drugs.\textsuperscript{32} A detailed theoretical discussion of condensation of ions and molecules to form crystals is too complex to be presented in an article of this nature. In general, however, the factors to be considered are the shapes of the molecular species, the number of bonds formed, orientations of bond forming groups on the molecules, relative intermolecular forces and relative sizes of the condensing species.\textsuperscript{33} Since the factors involved in the formation of crystals are complex, the scientist is prohibited from predicting the types of crystals formed in all but the simplest cases. Thus most crystal tests are made on a comparative basis with known materials. The technical skill and experience of the chemist in making his observations are crucial to microchemical testing.

\textit{Mass Spectrometry}

A mass spectrometer is an instrument capable of accurately measuring atomic masses. Mass spectrometers operate on the principle that molecules which are bombarded with high energy electrons will ionize (become positively or negatively charged) and/or break into characteristic ionic fragments which can be separated by electrical, magnetic or time-of-flight methods. Relative quantities of the ionic fragments (component parts of the molecule) are determined as a function of mass.\textsuperscript{34} Mass spectrometry is highly specific and provides direct information about the structure of a molecule. For most compounds, the mass spectrum is unique for that specific substance. In combination with a gas chromatograph, whereby pure compounds are first separated and then sequentially characterized by mass spectrometry, the GC-mass spectrometer is probably the most powerful tool for drug identification.

\textit{X-Ray Diffraction}

An X-ray powder diffraction pattern of a solid compound is unique to that compound in virtually all cases. The diffraction pattern (interference of electromagnetic waves) occurs as a function of

\textsuperscript{32} D-amphetamine and de-amphetamine, which have the same chemical structural formula but have different spatial orientations of atoms about one carbon atom in the molecule (stereoisomers), form very different crystals.

\textsuperscript{33} L. Berry \& B. Mason, Mineralogy 12 (1959).

the dimensions and arrangements of atoms in a solid crystalline structure. Pattern intensities are a function of the scattering power and arrangements of the atoms.\textsuperscript{35} It is unfortunate that X-ray diffraction has not been used to the extent of its potential applicability in drug analyses. Components of intractable mixtures can often be simultaneously determined by X-ray diffraction because the diffraction lines are very sharp and exist independently of other substances which may be present.

**Identification of Marijuana**

Analysis of marijuana, cannabis sativa,\textsuperscript{36} by a chemist is a special case which necessitates a microscopic morphological examination, and a chemical confirmation of the presence of tetrahydrocannabinol (THC).\textsuperscript{37} Claims are often made that only a botanist is qualified to testify in marijuana cases. However, marijuana is rarely encountered in the laboratory as a whole plant and consequently must be identified from the crushed fragments. A plant taxonomist is therefore no more capable than a trained, well-read chemist in making a morphological examination and much less capable when chemical tests are included in the analysis.

The parts of the marijuana plant most commonly encountered are crushed leaf fragments, stems, seeds and flowering tops. A marijuana seed displays the following characteristics under low power magnification: ovoid shape, lacy surface and a sharp longitudinal ridge circumscribing the seed.\textsuperscript{38} On the under-side of the leaves there is a profusion of fine non-glandular hairs. The upper side of the leaves is covered with unicellular, non-glandular hairs somewhat resembling "bear claws" which contain a cystolith (spheroidal calcium carbonate stone) enclosed in the base.\textsuperscript{39}

\textsuperscript{35} AM. SOC'Y FOR TESTING MATERIALS, X-RAY POWDER DATA FILE AND INDEX TO THE X-RAY POWDER DATA FILE (1959); L. BERRY & B. MASON, MINERALOGY 270 (1959). See also M. BUERGER, X-RAY CRYSTALLOGRAPHY (1942).

\textsuperscript{36} The proper name of marijuana is Cannabis Sativa L.: genus—cannabis; species—sativa; L.—for Linne", the Swedish botanist who devised the nomenclature system for botany.

\textsuperscript{37} A. CURRY, ADVANCES IN FORENSIC AND CLINICAL TOXICOLOGY 29 (1972); Butler, Duquenois-Levine Test for Marijuana, 45 J. ASS'N OFFICIAL AGRICULTURAL CHEMISTS 597 (1962); Pitt, The Specificity of the Duquenois Color Test for Marijuana and Hashish, 17 J. FOR. SCI. 693 (1972). See also U.S. TREAS. DEP'T, MARIHUANA, ITS IDENTIFICATION (1948).


\textsuperscript{39} Photomicrographs of a marijuana seed and crushed leaf fragments may be found in Appendix III.
A recent study comparing marijuana with other plant species which have cystolithic hair has shown that humulus japonica (hops) has cystolithic hairs which are somewhat similar to those of marijuana. Nevertheless, an experienced observer should be able to differentiate them by observing other characteristics of humulus japonica such as its two-armed unicellular hairs. Although many plant species exist which have cystolithic hairs that an untrained observer might find confusing, it is probable that a complete identification could be made by microscopic examination alone. Such examination would be possible if the crushed vegetation was observed at higher power (around 100X) to identify the glandular resin producing hairs, and the cystoliths were confirmed to be calcium carbonate by reaction with acid.

Chemists may largely circumvent problems related to a lack of training in plant taxonomy by confirming the presence of THC, which is unique to cannabis sativa. The most widely used test is the Duquenois-Levine or modified Duquenois Test. Duquenois tests are performed by extracting the resins from the dry vegetation with petroleum ether, evaporating the extract to dryness, adding the Duquenois reagent (vanillin, acetaldehyde and ethanol) to the dried extract, and adding concentrated hydrochloric acid to initiate the color-producing reaction. Development of a deep violet color indicates the presence of cannabinoids. Further confirmation by extraction of this violet color into a chloroform layer constitutes the Duquenois-Levine modified test. While many other substances which possess cystolithic hair are known to give positive Duquenois color tests, none of these substances have been found to yield positive modified Duquenois tests.

Presently, the combination of a modified Duquenois test and a microscopic examination must be considered a confirmation of marijuana. In the Indianapolis Police Department Crime Laboratory, thin layer chromatography is done in addition to the above two

tests as a further check of the presence of THC. This procedure eliminates the reliance on a color test alone which has been attacked at times as giving "false positives" with some materials.

The expert witness testifying in drug cases usually finds that an inordinate amount of his time is devoted to defending his analysis of marijuana. This is doubtless due to the large number of mistaken ideas perpetuated in over 2000 literature references on cannabis. 43 Minor taxonomical differences in plants may vary according to environmental growing conditions as much as from the parental strain of the seed. 44 Although marijuana imported from India has been medically specified due to its higher potency over the American marijuana, the two plants are indistinguishable taxonomically. 45 There is also no significant difference in psychoactive content of male and female plants. Male plants are removed from cultivated fields earlier than female plants because they mature faster. Because of the longer growing periods, female plants yield a higher total THC content. Consequently, many previous references as well as the United States Dispensatory have incorrectly stated that the male plant has no psychoactivity. Additional confusion has arisen due to four separate systems of nomenclature used to describe the active component of cannabis, THC. 46 From a legal viewpoint, however, the only identification necessary for the expert witness in court is marijuana, cannabis sativa. Questions regarding the sex of the

44. A. Curry, Advances in Forensic and Clinical Toxicology 31 (1972).
45. Id. at 33; C. Joyce & S. Curry, The Botany and Chemistry of Cannabis 11 (1970).
American marijuana (cannabis sativa americana) was used in the pharmacopeia of the United States until 1942, when it was made illegal. Indian marijuana (cannabis sativa indica) was imported until World War I.
46. A. Curry, Advances in Forensic and Clinical Toxicology 33 (1972).
Two isomers of THC exist: 1.) Δ⁴-trans-tetrahydrocannabinol, Δ⁴'-trans-tetrahydrocannabinol, (D)-(-) Δ⁴' tetrahydrocannabinol, or more simply Δ⁴' -tetrahydrocannabinol (or Δ⁴' ) are all names for the principle isomer in natural cannabis; and 2.) Δ⁴'(t)-trans-tetrahydrocannabinol, Δ⁴'-trans-tetrahydrocannabinol, or more simply Δ⁴' -tetrahydrocannabinol (or Δ⁴' ) are names for the isomer which is more easily prepared synthetically. C. Joyce & S. Curry, The Botany and Chemistry of Cannabis 93 (1970); Mechoulam & Gaoni, The Absolute Configuration of Δ⁴' -tetrahydrocannabinol, The Major Active Constituent of Hashish, 12 Tetrahedron Letters 1109 (1967). The Δ¹(t) -THC isomer is also found in natural cannabis, but in less than 1 percent of the quantity of Δ⁴' -THC. The two isomers differ only in the location of one carbon-carbon double bond in the structure, and both isomers are psychoactive.
plant, the so-called American and Indian types of marijuana, and varying nomenclatures are irrelevant, and may be misleading.

**General Laboratory Procedure and Analysis**

A general procedure for analyzing a street drug might proceed stepwise by:

1. carefully logging in both the evidence and request form from the narcotics officer;
2. maintenance of the evidence under lock until it is moved for analysis;
3. recording the markings and descriptions of materials submitted;
4. weighing the suspected drugs;
5. spot testing and ultraviolet spectroscopy for a tentative identification;
6. confirmation of identity by:
   
   a. a minimum combination of two or more thin layer chromatographic, gas-liquid chromatographic and/or microchemical determinations, and
   b. infrared spectroscopy, X-ray diffraction or mass spectrometry;
7. resealing and marking the evidence inside and outside for identification;\(^{47}\)
8. maintenance of the evidence under lock until it is returned to the narcotics agent;
9. typing and filing of completed report forms on the analysis; and
10. logging out the evidence to the narcotics agent.

Steps 1, 2, 3, 7, 8, 9 and 10 are necessary to maintain a secure chain of evidence while the sample is in the custody of the laboratory. Confirmation of a suspected drug's identity in step 6 may vary

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\(^{47}\) In the Indianapolis Police Department Crime Laboratory, heat sealed plastic bags are used to seal the actual drug samples, and permanent type evidence tags and staples are used to seal the outer envelope.
widely depending upon the type of sample and judgment of the individual chemist. Any minor discrepancy in one test must be explainable on a sound theoretical basis, and a substitute test should be run to avoid the explained incongruance. Commercial dosage forms may be tentatively identified through the size, shape, color and markings on the tablet or capsule, and their identity confirmed by the methods outlined in steps 5 and 6.

A decision on the validity of the overall test results can only be made by the expert witness because the procedures are too complex to enable nonscientists to make a true judgment. A common misconception is that scientific methods are absolute; therefore, one test should unambiguously identify a compound. However, identification of sub-microscopic molecules requires indirect measurements of a variety of properties. An analogy of trying to identify a horse through a small opening in a solid fence could be made. For example, a black horse might be identified by singly observing such characteristics as: parts of the body (nose, eyes, tail, feet, etc.), color of hair, noises and odor. Some combination of these observations would then be used to arrive at a final identification in the same manner as a combination of chemical observations is used to make a chemical identification. This analogy points out vividly the fallacy in the defense tactic of going through the series of tests made one by one and asking if each is specific for that compound, i.e., even though a black horse cannot be identified by either its nose or eyes or color or whinny alone, the combination of all these parameters will unambiguously identify it. Most chemical analyses are made on the exclusionary principle. One test eliminates all possible compounds but one finite set, a second test eliminates a majority of this set, and so forth. Probabilities are not useful in establishing the validity of analyses because either prejudicially large or small values may be realistically assigned for any test, even though the combined test made could yield a unique result or a general set of results. No workable standard method of analysis can be established for drugs in crime laboratories because the variety of excipients and the variability of sample size and conditions are too extreme.

CONCLUSION

An attempt has been made to introduce and summarize for the practicing attorney the types of drug tests performed in most modern crime laboratories. Attorneys could not be expected to ask penetrating technical questions with the limited information presented here. The purpose of this article was rather to guide the line of cross-examination by enabling the attorney to judge for himself whether the expert witness is able to give an acceptable explanation of his testing methods and whether the expert has an understanding of the methods used. If the witness is qualified to testify in court on chemical analyses, then he should be able to explain his own testing methods in a simple and logical manner. No witness can be expected to be an authority on every insignificant question of fact, and no purpose of justice is served by asking such questions. If questions of competence do arise, then it is the obligation of both the prosecution and the defense to obtain other expert opinions. Unfortunately, the field of criminalistics has a long tradition of untrained "cookbook chemists" serving as expert witnesses. This unprofessional approach is inexcusable and can hopefully be terminated by more relevant questioning by attorneys.

APPENDIX I

BASIC SCIENTIFIC PRINCIPLES USED IN SPECTROSCOPY

Part A: Electron Energy Transitions in a Single Atom

In an isolated atom the ground (lowest) and excited (higher) energy states of an electron have precise and unique values. When radiant energy or light is absorbed by the atom an absorptive transition occurs. As is illustrated in Figure 1, the energy is increased to raise it from the ground state to an excited state. Since both the ground and excited states are of very precise energies, the change in energy ($\Delta E$) also has a very precise value.
An emission or fluorescence transition occurs when an electron which has been raised to an excited state returns to the ground state. (See Figure 1.) The energy is given off as radiant energy, and again since the excited and ground states both have precise values, $\Delta E$ is a precise value and light of only wavelength $\lambda$ is observed. (See Figure 2.) In neon and mercury vapor fluorescent lights, for example, the electrons are raised to excited states by an electrical discharge. Thereafter the electrons drop to the ground state and release the energy as light. Since neon and mercury atoms have completely different ground and excited states, the light emitted is of different energies, i.e., neon produces orange light and mercury produces blue-white light.

The wavelength of light is related to the energy of light by the equation $\lambda = hc/E$ where $\lambda$ is the wavelength in nanometers (nm),
h is Planck's constant \((6.6242 \times 10^{-34} \text{ erg-sec})\), \(c\) is the speed of light \((2.9979 \times 10^{10} \text{ nm/sec})\), and \(E\) is the energy in ergs. In the diagrams within this appendix, \(\lambda^o\) is the value of the wavelength of the light at either the maximum in absorption or the maximum in transmission.

**Part B: Change of Electron Energy States in Molecules**

Theoretically, molecules might be expected to have precise energy states as are observed in atoms. However, since atoms are attached to other atoms in molecules, the electronic energy states are interrelated (coupled) to a large set of vibrational and rotational energy states of only slightly different energies. Further broadening of these states due to interaction with solvent molecules will be observed when liquid solutions are used. Thus, as is shown in Figure 3, both the ground and excited electronic energy states exist as bands of energies rather than single precise values.

**Figure 3**

![Figure 3](image)

**Figure 4**

![Figure 4](image)
Irradiating a molecular sample and plotting the energy absorbed versus wavelength when electrons are raised from the ground electronic states to the excited electronic states gives rise to the ultraviolet-visible spectrum shown in Figure 4. Because of the energy bands, the absorptive transition in molecules consists of a large series of overlapping transitions over a definite range of energies centered at some maximum value $\lambda^\circ$. In the case of heroin, for example, $\lambda^\circ$ has a value of 278 nm.

The fluorescence transition likewise consists of many separate overlapping transitions. As is seen from Figure 5, the fluorescence emission spectrum is somewhat similar to an ultraviolet-visible absorption spectrum except that the light measured is that which is emitted by the molecule. Contrariwise, ultraviolet-visible spectroscopy measures that light which is absorbed. When electrons are raised to excited states most of them immediately drop back to the ground state, i.e., only a few more absorptive transitions than fluorescence transitions occur. Due to the design limitations of ultraviolet-visible spectrometers, these instruments are only capable of measuring the net absorbed energy. Fluorescence spectrometers, on the other hand, are able to detect the more numerous isolated fluorescence transitions, and are therefore more useful for Part

C: Change of Vibrational Energy States in Molecules

Both the ground and excited vibrational energy states of a molecule, shown in Figure 6, arise entirely from within the ground electronic energy state. The two vibrational states consist of bands of energy due to coupling of the vibrational states with many rotational states.
In infrared spectroscopy, the absorptive transition occurs when infrared radiation raises the energy of the molecule from the ground vibrational state to an excited vibrational state. Irradiating a sample with infrared radiation and plotting the energy absorbed versus the wavelength gives rise to the infrared spectrum shown in Figure 7. Since the energy bands are much narrower and the difference in energy $\Delta E$ relative to the band widths is much greater than for electron transitions, a narrow absorption peak is observed. The presence of many active vibrational states in molecules produces a large number of infrared absorption peaks.

The emission transition occurs when the process is reversed. Rather than given off as radiant energy, much of the energy for the emission transition is dissipated to surrounding molecules as they collide with one another. Thus this transition is not a useful one for instrumental analysis.
APPENDIX II

INSTRUMENTATION USED IN INFRARED AND/OR ULTRAVIOLET-VISIBLE SPECTROSCOPY

Figure 8

A. Light Source
B. Sample Solution
C. Reference Solution
D. Chopper and Mirrors
E. Scanning Monochromator
F. Detector
G. Recorder

Produced by The Berkeley Electronic Press, 1974
A. Polychromatic (multi-energy) light is produced by a light source and split into two beams. Infrared light is generated by a Nernst glower, a ceramic rod heated to white heat. Ultraviolet light is generated by a hydrogen discharge lamp, and visible light is produced by a heated tungsten filament.

B. One light beam passes through the sample, and the other passes through the reference solution or pure solvent. Samples may be in the form of a gas, a pure liquid, a solid dispersed in an inorganic salt or dissolved in solution with a suitable solvent.

C. Rotating mirrors chop the light beams into segments so that alternately light from the sample, then light from the reference, passes to the detector. After leaving the chopper, the two light beams are condensed to a single pathway to the detector.

D. Prior to detection of the light at the detector, the light is separated into its component parts by the monochromator. The monochromator distributes the light so that light of approximately only one energy, *i.e.*, wavelength, passes on to the detector at any given time.

E. The detector compares the energy of the monochromatic light from the sample with the energy of the same monochromatic light from the reference beam and produces an electrical signal to drive the recorder.

F. A graph is plotted by the recorder of the percentage of light absorbed by the sample relative to the amount of light which has passed through the reference solution versus the wavelength of the monochromatic light. The recorder is synchronized with the scanning speed of the monochromator to produce a continuous value of the absorbance of the sample with the changing wavelength of the light.
Figure 9 displays the upper (darker) surface of a marijuana leaf magnified approximately 80X. The cystolithic hair resembling “bear’s claws” are clearly visible. Figure 10 displays both a marijuana seed and leaves magnified to approximately 15X. In particular, note the characteristic veining on the surface of the seed and the finer non-glandular hair on the lighter surface of the leaves. Higher magnification will show even greater detail of these characteristics.