Welcome to the 2021 IDS Forensic Science Education Series

- **Do today:** Attorneys – if you would like CLE credit, report your attendance to me using the link that I email you and I will submit your information to the bar. The State Bar will invoice you for $3.50 per hour of CLE credit at the end of the credit year. 60 min of general CLE credit is anticipated. You may only receive credit for attending the live (not recorded) version of the webinar.
- **Do later:** For participants who complete 10 or more webinars in this year's series and report their credit to me, you'll receive acknowledgement that you completed this course of forensic education. For this certificate, watching the recorded version is fine. Please report this attendance to me in Dec. 2021.
- I will email you the link to report your attendance to me.
- Questions? Email sarah.r.olson@nccourts.org

Edward G. Brown, Ph.D.

Dr. Brown obtained his Bachelor of Science degree in Chemistry from U.C. Berkeley in 1980 and his Doctoral degree in Organic Chemistry from U.C. Davis in 1988. Two post-doctoral chemistry research fellowships from University of Auckland, New Zealand, and Yale University furthered his academic studies in medicinal chemistry and analytical chemistry techniques through 1990.

Dr. Brown's productivity during his career as a medicinal and organic chemistry researcher in academics and while working at several pharmaceutical companies and other laboratories has led to his co-inventorship on ten US patents and his co-authorship on over thirty research articles and conference presentations.

Dr. Brown is also a patent agent who specializes in chemistry patent drafting and assists in patent litigation and prosecution issues for patent law firm clients in North Carolina and around the US.

In 1991, Dr. Brown began his consulting work as an expert witness in an LSD case. He has since continued to develop his expert witness practice throughout the intervening years, and to date, has assisted nearly 100 attorneys in law firms from North Carolina, Virginia, Maryland, Pennsylvania, and many other States, as well as in Canada and Great Britain on a large variety of drug cases and DWI/DUI cases, both at the State and Federal levels. He has testified in criminal drug cases and DWI/DUI cases as a Chemistry Expert in both Federal and State courts around the United States and has been deposed as a Chemistry Expert in a patent litigation case.

Drug Analysis: Methodology Used For Presumptive Tests and Common Errors Observed

Edward G. Brown, Ph.D.

Expert Chemistry Services
92 Cornerstone Drive #198
Cary, NC 27519
919-607-4626
Ed.Brown@ExpertChemistryServices.Com

NC Indigent Defense Services CLE

April 1, 2021

Today's Agenda

1. Presumptive Tests and Analysis Methods versus Confirmatory Tests and Analysis Methods in Drug Sample Testing:
   A. What are Presumptive Tests? What are Confirmatory Tests?
   B. What are some of the differences? In general, when are these tests used?
   C. Why are Presumptive Tests and Confirmatory Tests used when they are?

2. Presumptive Tests and Analysis Methods: Techniques for Initial Detection of Illegal Drugs and Related Materials:

   A. Color Tests, Microcrystalline Tests, Elisa Tests, UV and IR spectroscopy.
   B. How and why they are used.
   C. Possible false-positives and false-negatives.

3. Questions & Answers:

Presumptive Tests vs Confirmatory Tests in Drug Evidence Testing & Analytical Chemistry:

Presumptive Test Methods:

• In analytical and forensic sciences, a presumptive test is an analysis method which is used initially to quickly and easily establish:

• If a chemical sample of interest contains no substances which give positive test results. NOTE: Negative test results quickly rule out the need for further testing of the sample using more expensive or more time-consuming methods.

• If a chemical sample gives a positive test result, this indicates that the sample may contain one or more substances that require further analytical work to be performed.

•Presumptive testing can be performed in a lab setting or in the field using various commercially-available color-test kits which turn different colors with drugs, etc.

Confirmatory Test Methods:

• Tests that are required to confirm what chemicals are present in a sample are referred to as confirmatory tests. The rates of finding False Positives and False Negatives are much lower using Confirmatory Testing methods.

• These tend to cost more than simple presumptive tests, take more time to perform & review, require more training & are far more accurate and have fewer false positives.

Examples of Presumptive Testing Methods and Confirmatory Testing Methods :

Examples of Presumptive Test Methods:

Presumptive tests include color tests/spot tests, microcrystalline tests, ultraviolet spectroscopy (UV), infrared spectroscopy (IR), microscopic examinations, enzyme-linked immunosorbent assays (ELISAs), gas chromatography (GC) and thin layer chromatography (TLC).

These methods tend to have much higher rates of False-Positives and False Negatives than Confirmatory Tests.

Examples of Confirmatory Test Methods:

Fourier-Transformed Infrared Spectroscopy (FTIR), gas chromatography/mass spectrometry (GCMS) and liquid chromatography/ mass spectrometry (LCMS) Analysis Methods. For alcohol levels, GC quantitation is also used.

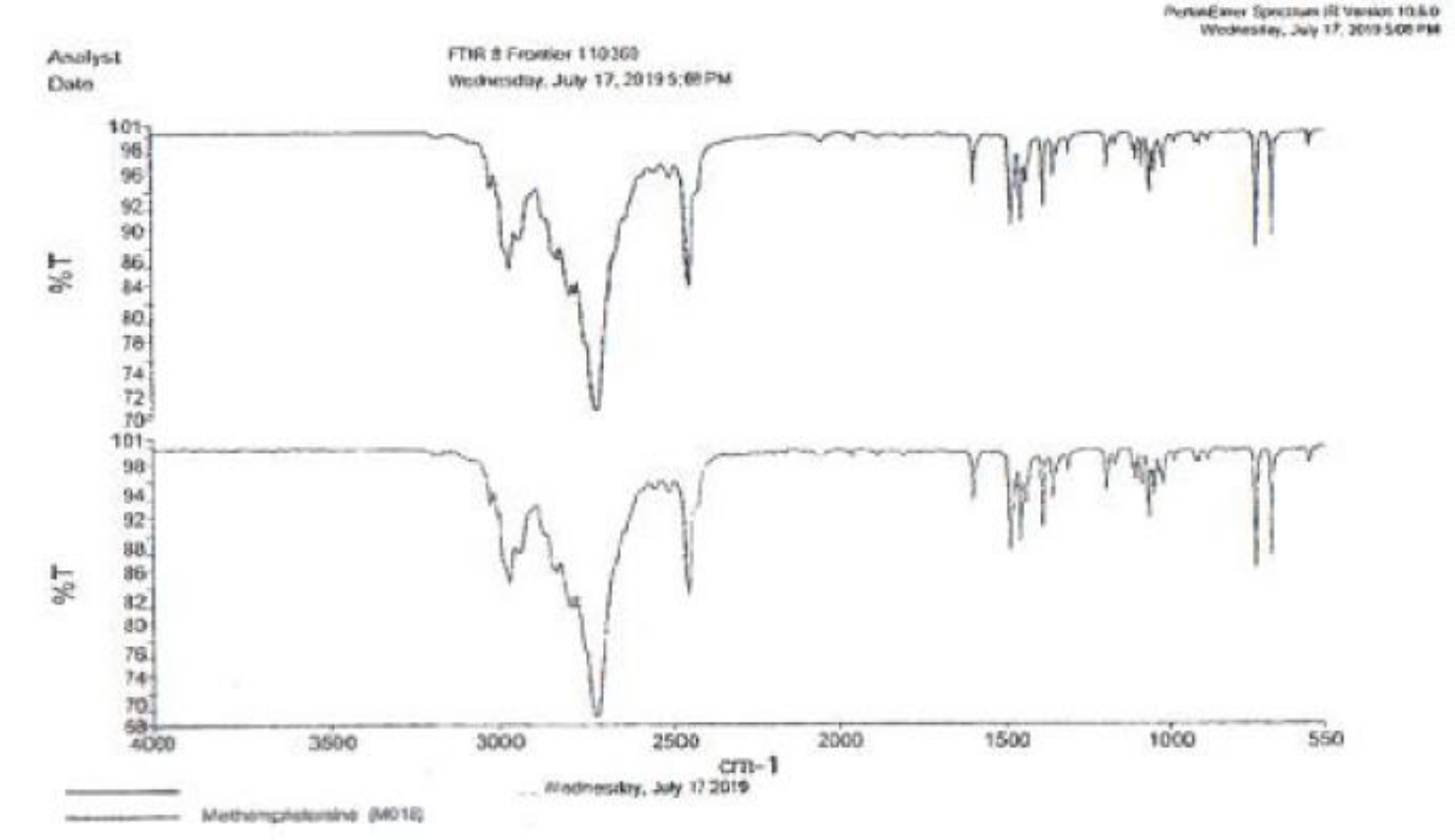
These methods tend to have much lower rates of False-Positives and False Negatives than Presumptive Tests.

Brief Summary of Confirmatory Testing Methods Used in Forensic Labs and Benefits of Use:

- In general, the instrumentation used for drug sample analysis and chemical identification include Fourier-Transform Infrared Spectroscopy (FTIR), Gas Chromatography/ Mass Spectrometry Methods (GCMS) and Liquid Chromatography/ Mass Spectrometry Methods (LCMS).
- Confirmatory testing is usually (but not always) performed secondarily to running one or more presumptive tests on an evidence sample. Confirmatory test methods usually have lower rates of false-positives than presumptive tests, although there are specific exceptions to this.
- Confirmatory tests require a laboratory to house the expensive, sophisticated equipment. This equipment requires frequent calibration and upkeep (usually weekly); operation of the equipment requires a great deal of operator training and updates on operator education.
- Confirmatory tests such as GCMS and LCMS can accurately detect extremely small (i.e., nanograms; a billionth of a gram) amounts of controlled substances in evidence samples when the instruments are well-tuned and calibrated.

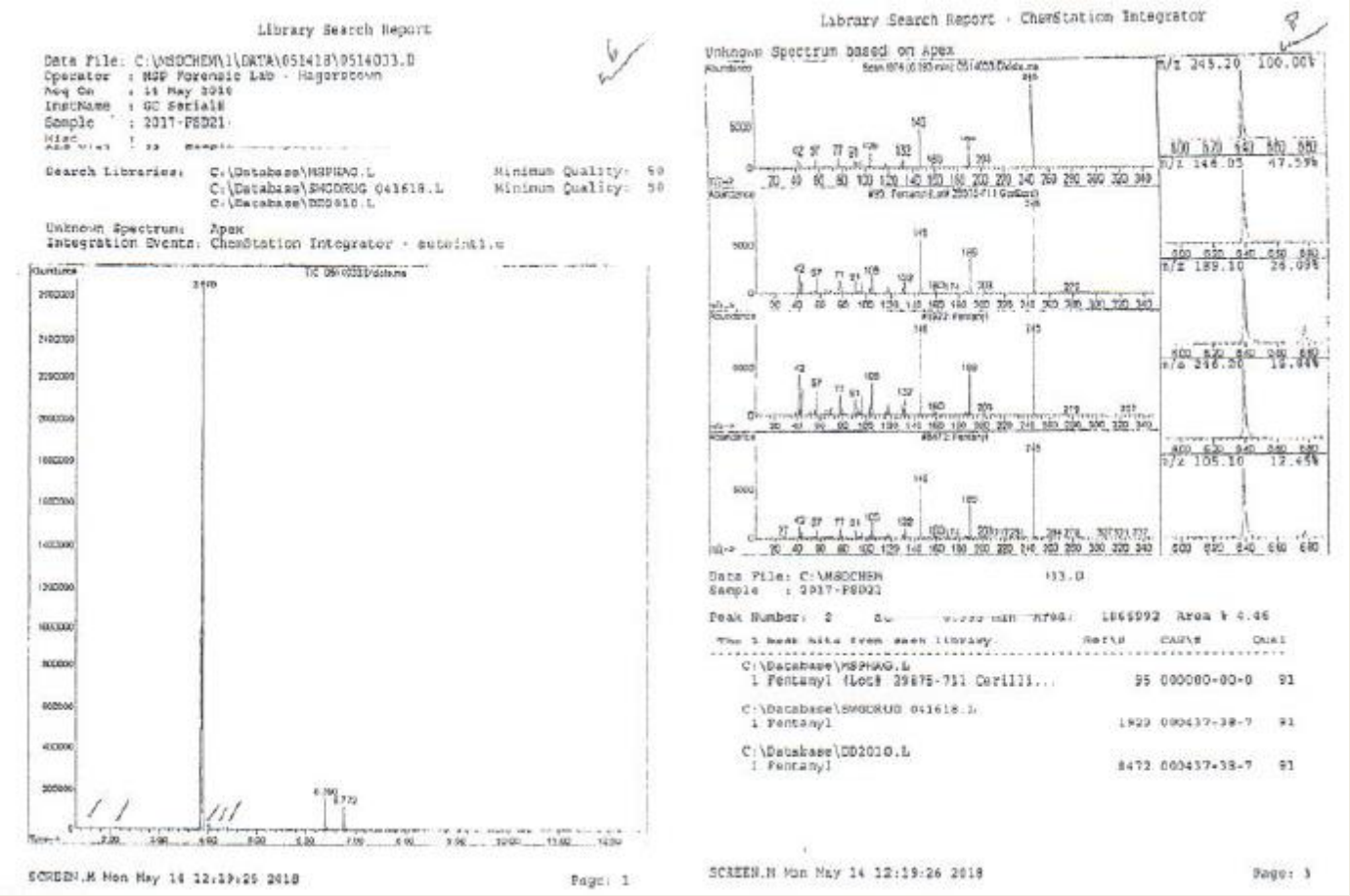
Example of Confirmatory Testing Printouts from Forensic Labs (FTIR):

FTIR Printout from an evidence sample:



Example of Confirmatory Testing Printouts from Forensic Labs (GCMS):

GCMS Printout from an evidence sample:



Library Search Report

Data File: C:\MSDCHEM\1\DATA\051418\0514033.D
Operator : NSP Forensic Lab - Hagerstown
Acq On : 14 May 2018
InstName : GC Serial#
Sample : 2017-PS021
Misc :

Search Libraries: C:\Database\NSPHAG.L Minimum Quality: 50
C:\Database\SWGDRUG 041618.L Minimum Quality: 50
C:\Database\DD2010.L

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - autoint1.e

SCREEN.M Mon May 14 12:19:25 2018 Page: 1

Library Search Report - ChemStation Integrator

Unknown Spectrum based on Apex

m/z 245.20 100.00%
m/z 146.05 47.59%
m/z 189.10 26.09%
m/z 216.20 12.44%
m/z 105.10 12.45%

Data File: C:\MSDCHEM 03.D
Sample : 2017-PS021

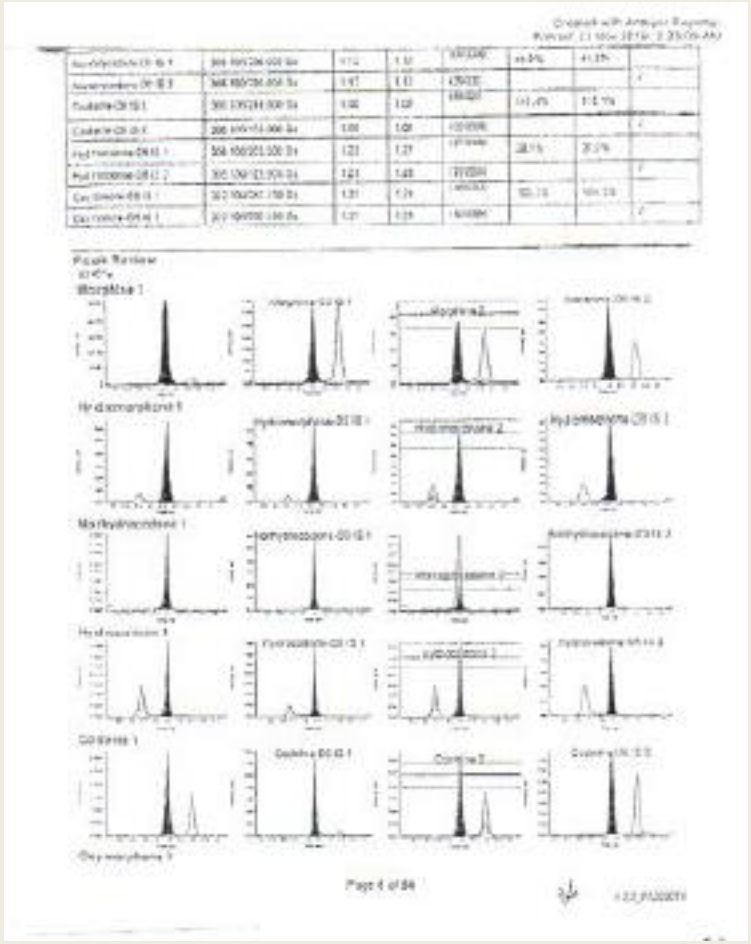
Peak Number: 2 Area % 0.46

The 3 best hits from each library:	Ref\#	CAS\#	Qual
C:\Database\NSPHAG.L			
1 Fentanyl (Lot# 29675-711 Cerilli...	95	000000-00-0	91
C:\Database\SWGDRUG 041618.L			
1 Fentanyl	1929	000437-38-7	91
C:\Database\DD2010.L			
1 Fentanyl	8472	000437-38-7	91

SCREEN.M Mon May 14 12:19:26 2018 Page: 3

Example of Confirmatory Testing Printout from Forensic Labs (LCMS):

LCMS Printout from an evidence sample:



Webinar on Confirmatory Testing Methods such as GCMS, LCMS and FTIR will be offered on May 6, 2021:

Stay Tuned

Presumptive Tests: Methods, Benefits & Limitations:

Examples of Presumptive Test Methods:

Examples of presumptive tests include color tests/spot tests, microcrystalline tests, ultraviolet spectroscopy (UV), infrared spectroscopy (IR), microscopic examinations, enzyme-linked immunosorbent assays (ELISAs) and thin layer chromatography (TLC).

Presumptive Test Benefits (Pros):

- Presumptive tests are relatively inexpensive & they are rapidly and easily performed.
- They can help to narrow or eliminate the need for further evidence sample testing.
- Many of these can help an analyst decide which analytical tests to perform next.
- Many are fairly sensitive & can be employed to detect various chemical & drug types.

Presumptive Test Limitations (Cons):

- Tests have higher risk of false positives or interpretations that lead to false positives.
- Cannot conclusively identify a substance; can only hint at identity possibilities.
- Most methods require the destruction of a small amount of an evidence sample.
- Require the use of chemical reagents which are hazardous to skin and eyes.
- Some of the test reagents require hazardous waste disposal methods to be employed.

Challenging admissibility of field drug test results: Examples of Case Law:

- *State v. Ward,* 364 N.C. 133 (2010) – visual inspection not enough, chemical test needed for an expert to testify to the identify of a substance
- *State v. Carter*, 237 N.C. App. 274 (2014)
- *State v. Pinnix*, 246 N.C. App. 190 (2016)
- *State v. Cobb*, 845 S.E.2d 870 (2020)
- *State v. Osborne*, 372 N.C. 619 (2020) "To be sure, much of the State's evidence identifying that rock-like substance as heroin, such as the field test results, might have been excluded had Osborne objected."

Presumptive Tests: Color Test Methodology:

Color Tests/ Spot Tests: How They Work:

- Chemical reactions between a few milligrams of an evidence analyte and a reagent indicator can create various colored stains or solutions; these colored materials can be compared to the color from that same type of analytical reagent reacting with a reference sample of a known drug or chemical. A negative control is also used.
- When a colored solution or stain is compared to the color produced by a reference sample's reaction with the same reagent, a similar color reaction can be a positive indication that the same reference chemical may be present in the evidence sample.
- Evidence samples with positive color comparisons are then retested using a more sensitive and selective confirmatory test.
- Samples of evidence that produce no color changes with test reagents are assumed to have no color-producing drugs or chemicals present. These samples can be set aside as negative test results.
- Quick and simple negative color test results can save a great deal of time and expense at a laboratory by reducing the work load spent on confirmatory methods.
- A photographic example of a color test being carried out is given on the next slide[16].

Presumptive Tests: Color Test Method Example:[1,16]

Presumptive Tests: Specific Color Test Methods:[1]

Different Color Test Methods Available:

- There are many different variations of color tests/spot tests used by analytical laboratories. Each test differs by the combination of chemicals used to create the reagent employed and each specific type of chemical combination of these reagents has been named after its inventor or has been named after the chemical employed.
- Each reagent type reacts with analytes to create various colors or stains; the colors depend upon chemical functionality present in each type of analyte (drug sample).

Examples of Color Test/ Spot Test Names and Reagents:[2-15]

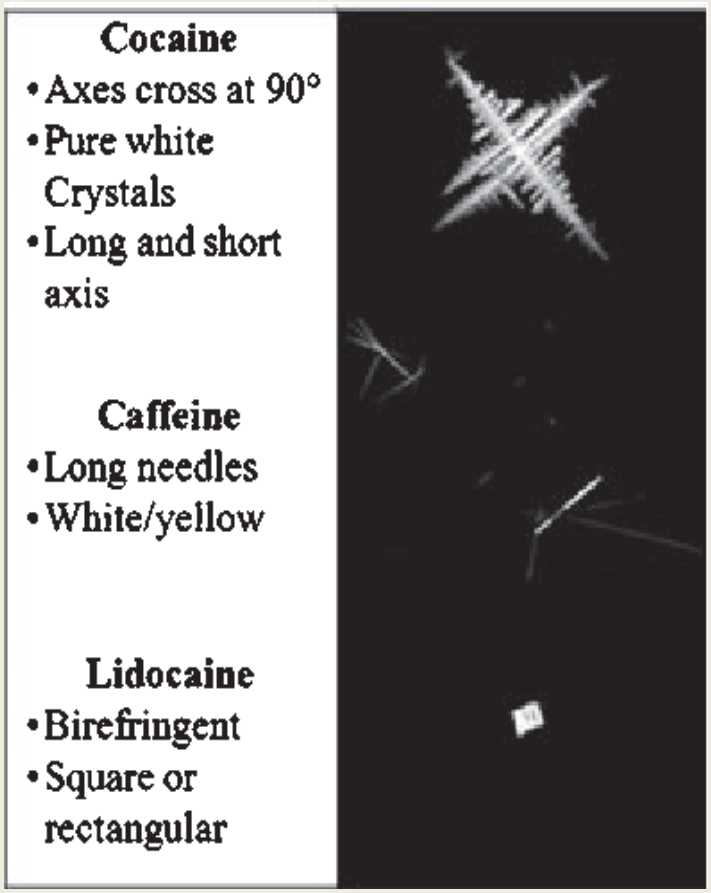
- Cobalt thiocyanate test, Dille-Koppanyi reagent, Duquenois-Levine reagent, Mandelin reagent, Marquis reagent, nitric acid test, para-dimethylaminobenz-aldehyde test, ferric chloride test, Froehde reagent, Mecke reagent, Zwikker reagent, and Simon's reagent (nitroprusside).
- Evidence samples with positive color comparisons to known chemical standards are then retested using a more sensitive and selective confirmatory test like GCMS.

Presumptive Tests: Microcrystalline Methods:[17]

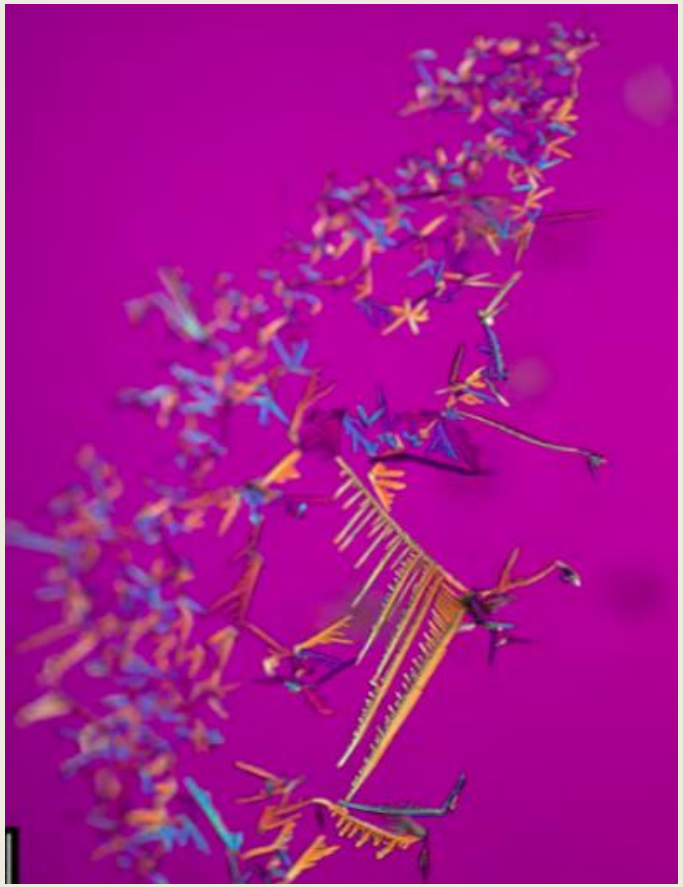
Microcrystalline Tests: How They Work:

- Chemical reactions occur between a few milligrams of an evidence analyte and a reagent indicator that usually contains a halide salt of gold, platinum or mercury.
- These chemical tests result in the formation of unique microcrystals of a given analyte complexed with a metal salt when a specific reagent is applied.
- The unique crystal formation is compared to microcrystals of a reference standard/control using a common light microscope.
- Microcrystals are compared based on shape, size, color, and spatial arrangement.
- Typical reagents containing metal salts include: Bromauric acid, Chlorauric acid, Chloroplatinic acid, Iodobismuth acid, Mercuric chloride, and Mercuric iodide.
- These are quick and simple tests which are relatively inexpensive to perform.
- The microcrystalline tests require the use of fairly pure samples of a drug otherwise impurities can interfere with the formation of crystals.
- Microcrystalline tests can be considered highly characteristic but are not specific enough to consider as a confirmatory test.
- Typically used for cocaine, heroin, phencyclidine, amphetamines, meth, etc.
- Examples of crystal forms are given on the next slides.

Presumptive Tests: Microcrystal Method Examples:



Gold chloride microcrystal test with cocaine hydrochloride, caffeine and Lidocaine.[18]



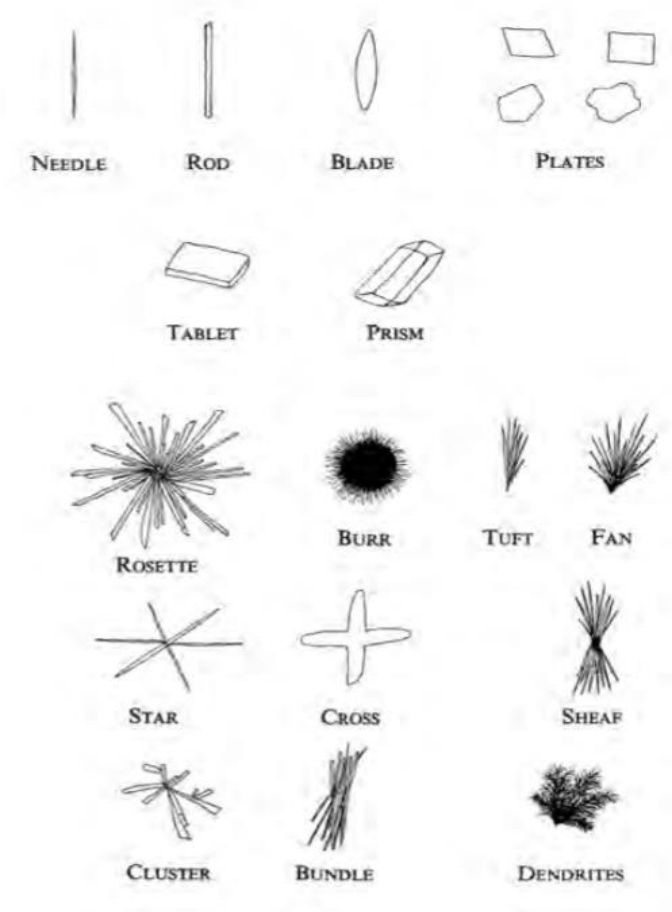
Chloroplatinic acid reagent with cocaine in 10% HOAc.[19]

Presumptive Tests: Microcrystal Method Examples:

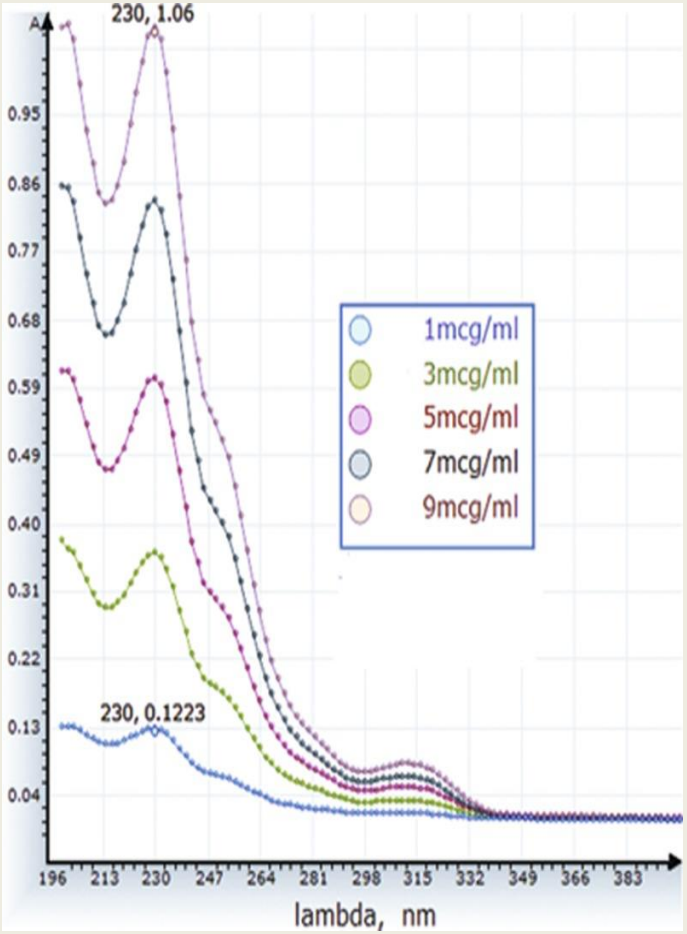
D-amphetamine in concentrated phosphoric acid with chloroplatinic acid reagent.[20]

Heroin with mercuric iodide and hydrochloric acid.[21]

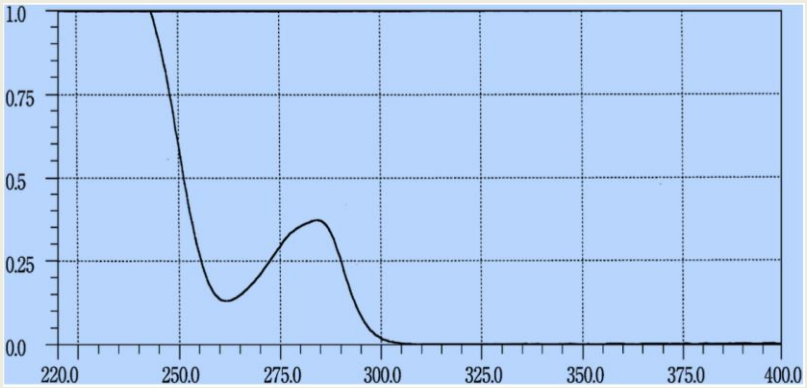
Presumptive Tests: Microcrystal Method Examples: Typical Test Results Reported Using Hand-Made Drawings of Crystal Shapes:



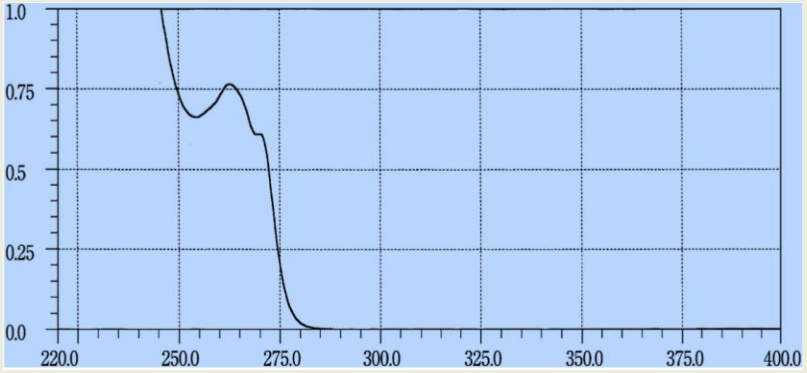
Presumptive Tests: UV Spectroscopy Examples:



Absorbance spectra of 5 standard solutions of diazepam (Valium) in phosphate buffer (pH 7.4).[22]



Codeine Phosphate UV Spectrum[23]



Lidocaine UV Spectrum[23]

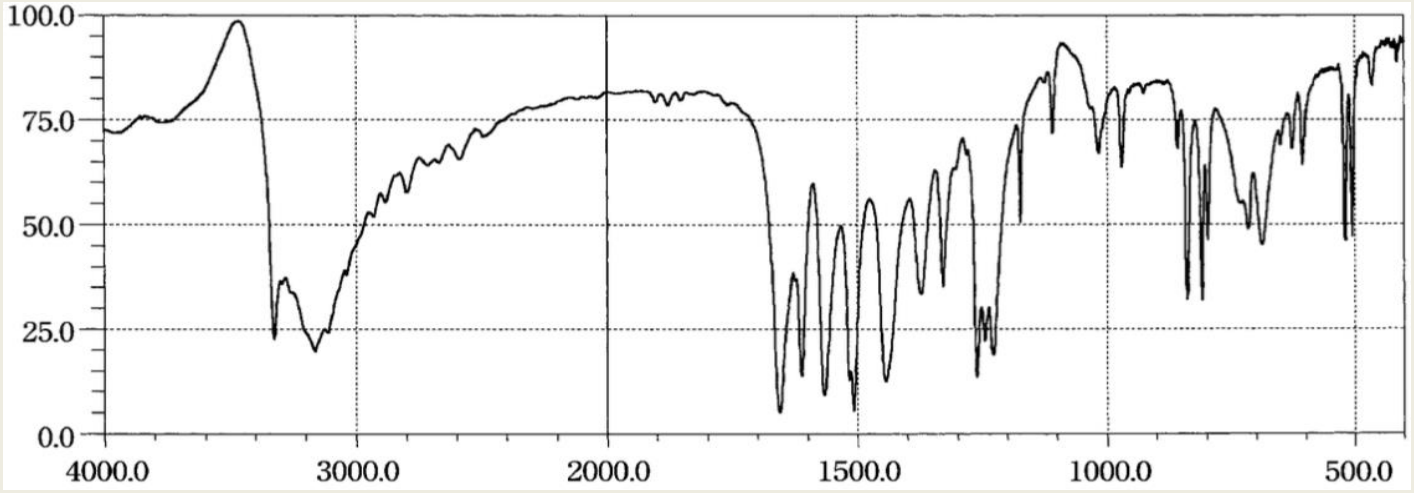
Presumptive Tests: Infrared Spectroscopy (IR)[1]

Infrared Spectroscopy (IR) and Fourier-Transformed IR (FTIR): How It Works:

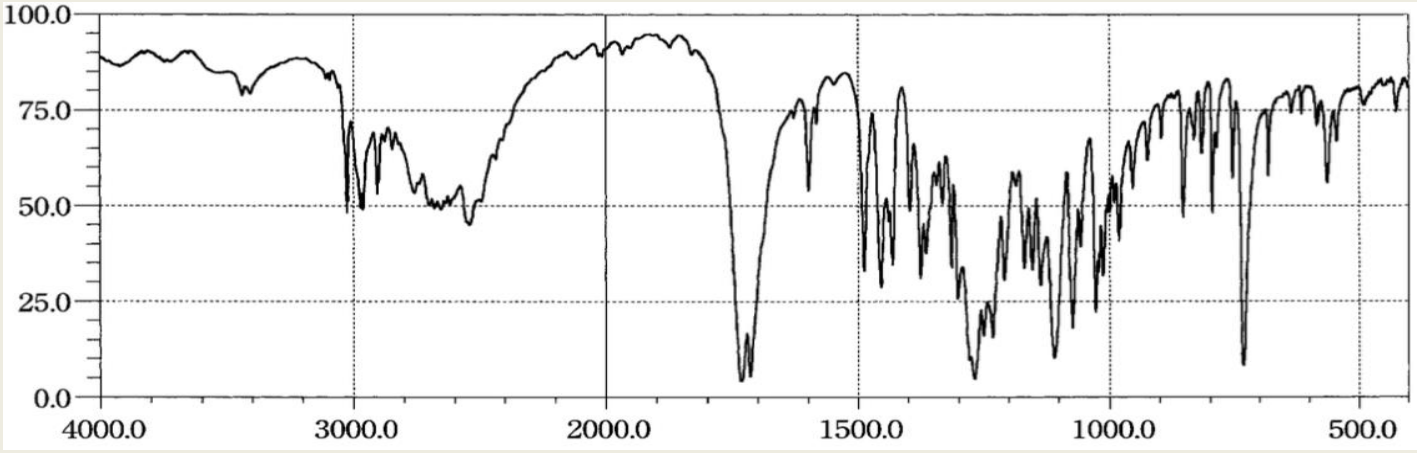
- Infrared (IR) spectroscopy is another highly discriminatory method and is based on the measurement of the amount of IR radiation which is absorbed or emitted by a sample as a function of the IR light wavelength passing through the sample.
- We sense light in this IR range as heat; the frequencies of IR radiation correspond with the vibrational frequencies between the atoms and groups in molecules.
- A spectrum is obtained by passing infrared radiation through a sample and determining the amount of the incident radiation (radiation that actually hits the molecule rather than passing through) that is absorbed at each IR frequency.
- The vibrational structure of a whole molecule determines what IR wavelength(s) are absorbed versus which pass through a sample.
- Interpretation of the spectra allows for determination of the types of molecular functional groups present in a sample.
- The IR spectrum of a pure compound provides a distinctive fingerprint which can be differentiated from the IR absorption pattern of other compounds.
- Drugs can be identified through databases (such as at http://webbook.nist.gov/)
- Examples of IR spectrophotometric analysis spectra are given on the next slides.

Presumptive Tests: IR Spectroscopy Examples:[24]

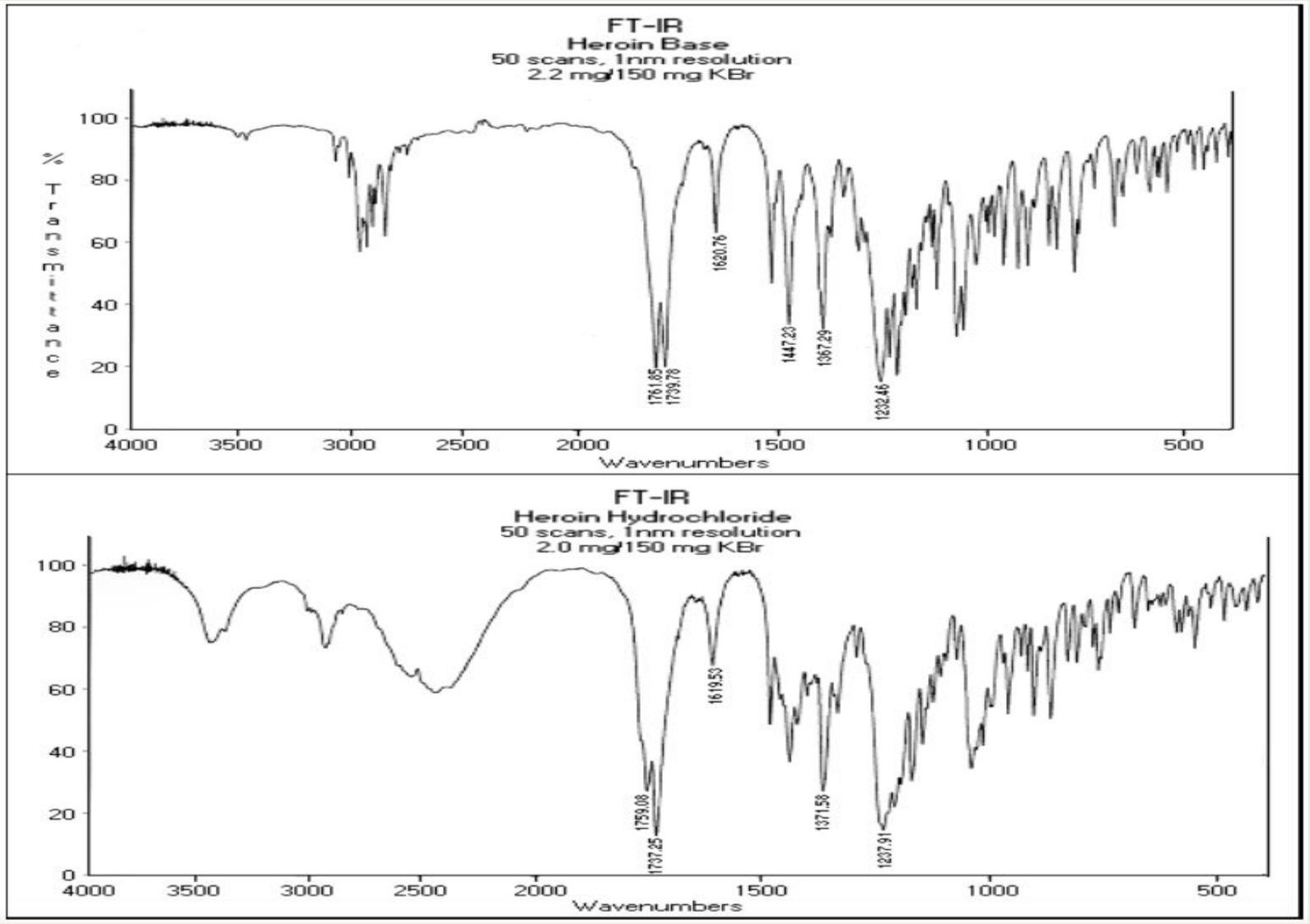
Acetaminophen IR Spectrum:



Cocaine Hydrochloride IR Spectrum:

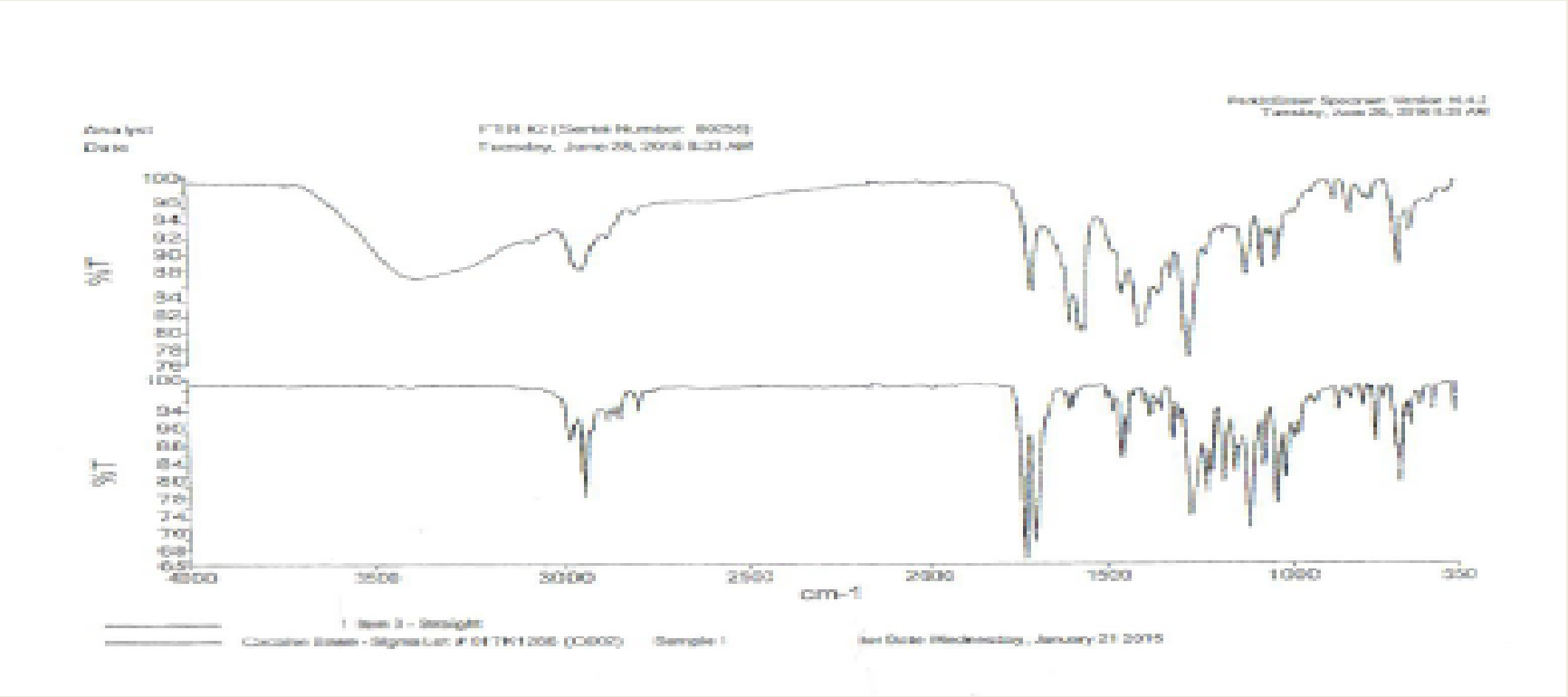


Presumptive Tests: IR Spectroscopy Examples:[25]



Presumptive Tests: IR Spectroscopy Example from Cocaine Case:

Top IR Spectrum: Evidence Sample
Bottom IR Spectrum: Cocaine Base Standard

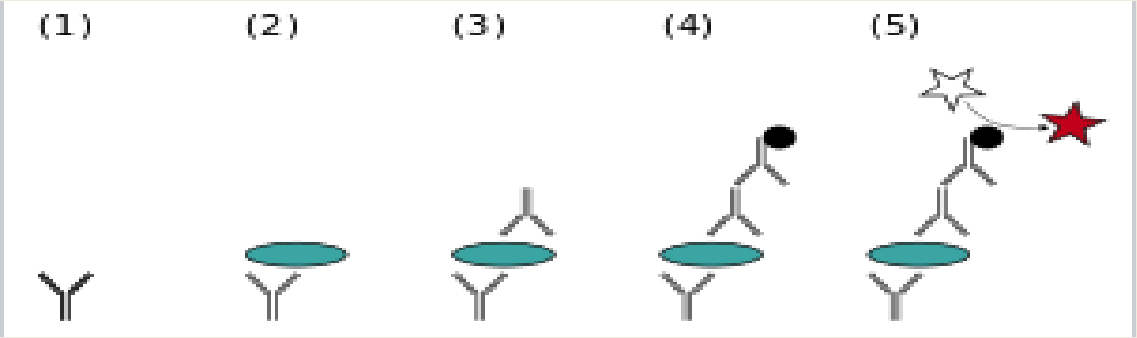


Presumptive Tests: Immunoassays for Bodily Fluids:[1]

Immunoassays: How They Work:

- Immunoassay involves the binding of an antibody that is selective for the drug or drug group of interest (antigen) and a label that will be part of the antibody-antigen complex that can be detected using some means (such as fluorescence or color).
- Antigen-antibody binding is based on a typical immune system response in which antibodies in biological tissue bind to antigens in order to neutralize or remove them.
- Various opioids and cocaine can be detected rapidly and somewhat effectively using the "old-style" immunoassay technology. There are problems with specificity regarding the original types of immunoassays, and there have been many instances of false positives due to similarity in drug structures or metabolites.
- Newer immunoassay techniques such as ELISA (Enzyme-Linked ImmunoSorbant Assay) methods can be more selective and sensitive than the original immunoassays.
- Immunoassays are most often employed to detect drug usage after the fact, such as in urine drug screens.
- Immunoassay testing is fast and relatively inexpensive.
- Two examples of ELISAs in current use by forensic labs are provided in the next slide.

Presumptive Tests: ELISA Example 1:[26]



Example of one type of a sandwich ELISA method:

(1) Plastic plate is coated with a capture antibody (= "Y"; think: "Velcro" glued to the bottom of a plastic plate); the plate is washed to remove anything that is free;

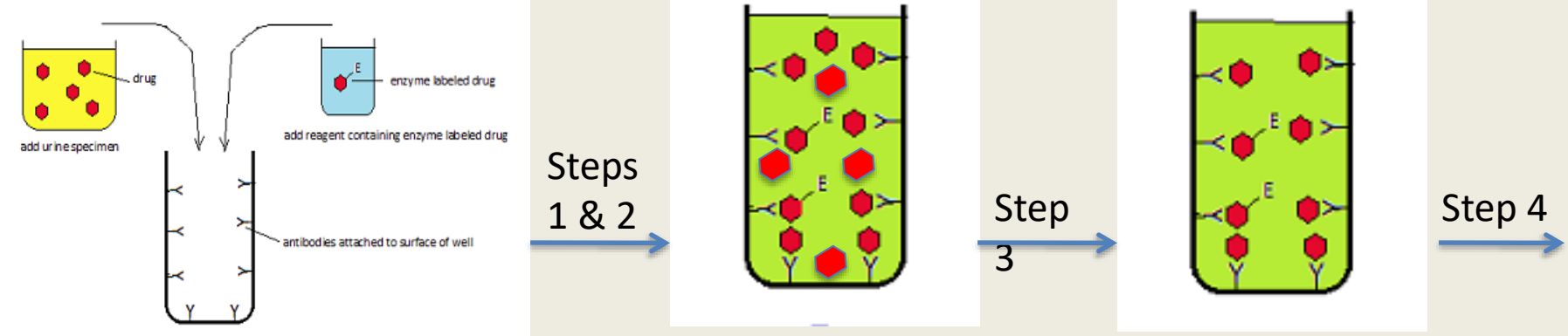
(2) Sample is added; any antigen (drug = ⬬) present binds to capture antibody (= Velcro partner piece); the plate is washed to remove anything that is free;

(3) Detecting antibody is added (= " ⅄ "); binds to antigen (drug); binds as new Velcro; again, the plate is washed to remove anything in solution, not adhering to complex;

(4) Enzyme-linked secondary antibody (= " ⅄● "); is added; binds detecting antibody; sticks as a new type of Velcro only to the antibody stuck to the drug; plate washed;

(5) Substrate is added (= " ☆ "); is converted by enzyme to detectable form (e.g., color change); the more drug present, the more color that can be produced.

Presumptive Tests: ELISA Example 2:[27]



Example of a second type of an ELISA method: Competition Assay:

(1) A well in a plastic plate is coated with a capture antibody (= "Y"; think: "Velcro" glued to the bottom of a plastic plate); the plate is washed to remove any free aBody.

(2) A solution of a test sample (= drug = ⬢) plus a solution of an enzyme-linked drug moiety reference sample (= enzyme-linked drug = ⬢-E) are added to the well; the mix is allowed time to equilibrate; any antigen (= drug) present competes with the special enzyme-linked drug moiety reference to bind with the capture antibody on the sides of the well (both = Velcro partner pieces);

(3) The reaction well is drained to remove anything that isn't bound to the sides of the well and it is washed to remove any traces of the two original solutions;

(4) Two different chemicals are added that form a color only if an enzyme is present; if less drug, the more enzyme is present, the more reaction and the more color forms.

Presumptive Tests: False Positives & Negatives:

Table of Known Immunoassay False Positives for Commonly-Detected Drugs:[29]

Medication	AMP/MET	BAR	BZO	THC	LSD	MTD	OPI	PCP	TCA
Amitriptyline					X				
Bupropion	X				X				
Buspirone					X				
Carbamazepine									X
Cyclobenzaprine									X
Dextromethorphan							X	X	
Diltiazem					X				
Diphenhydramine						X		X	
Doxylamine						X	X	X	
Fentanyl					X				
Fluoxetine	X				X				
Ibuprofen		X		X				X	
Labetalol	X				X				
Lamotrigine								X	
Metformin	X								

AMP/Met = Amphetamine/Methamphetamine; BAR = Barbiturates; BZO = Benzodiazepines; LSD = Lysergic Acid Diethylamide; MTD = Methadone; OPI = Opiates; PCP = Phencyclidine; TCA = Tricyclic antidepressant; THC= Cannabinoid

Presumptive Tests: False Positives & Negatives:

Table of Known Immunoassay False Positives for Commonly-Detected Drugs:[29]

Medication	AMP/MET	BAR	BZO	THC	LSD	MTD	OPI	PCP	TCA
Methylphenidate	X				X				
Metoclopramide					X				
Naproxen		X		X					
Prochlorperazine					X				
Promethazine	X								
Pseudoephedrine	X								
Quetiapine						X			X
Quinolones[a]							X		
Ranitidine	X								
Risperidone					X				
Sertraline			X		X				
Tramadol								X	
Trazodone	X				X				
Venlafaxine								X	
Verapamil					X	X			

AMP/Met = Amphetamine/Methamphetamine; BAR = Barbiturates; BZO = Benzodiazepines; LSD = Lysergic Acid Diethylamide; MTD = Methadone; OPI = Opiates; PCP = Phencyclidine; TCA = Tricyclic antidepressant; THC= Cannabinoid

Presumptive Tests: False Positives & Negatives:

Questions and Answers:

???

Summary:

My intention has been to describe the basics about various analytical tools and techniques used by forensic analysts when determining if drugs or alcohol are present.

I hope you have a better understanding of these issues now.

Please remember that there are many types of data that need to be presented in order to prove to a level of scientific certainty that drugs or alcohol are present in a person's body. It takes much less evidence, however, to make a presumptive guess about the presence or absence of drugs or alcohol.

In many cases, questions need to be asked about subtle assumptions and data differences in the evidence; without a detailed knowledge an Expert has about chemistry and toxicology, these questions might not be asked at the appropriate times; this matters.

Drug and alcohol chemistry topics can be complicated, but an understanding of these issues doesn't have to be impossible. I would recommend finding an Expert who can help you to understand the details of the science in each of your cases.

We can provide expertise in chemistry and toxicology issues.

Our First Hour of Work is Free!

Thank You for Your Time and Attention!

Thank you!!!

Edward G. Brown, Ph.D.

Expert Chemistry Services

92 Cornerstone Drive #198

Cary, NC 27519

919-607-4626

Ed.Brown@ExpertChemistryServices.Com

Presumptive Tests: False Positives & Negatives:

Sources of False Positive Results & False Negative with Presumptive Tests:

Because presumptive tests only measure the chemical reactivities of samples, rather than provide direct measurements about the actual molecules present in a sample, other chemicals, impurities or additives can interfere with the testing. These can give misleading results or may lead to mis-assumptions on the part of the analyst.

- Color/Spot tests:
- With over 100 million known chemicals reported in the chemistry literature so far, it is unlikely that a specific color will only form with one specific drug in a color test (e.g., assuming that only cocaine forms a blue color from an evidence sample treated with cobalt thiocyanate would be unlikely to be true). If one assumes that each test only gives one specific color for one specific drug substance, this assumption will lead to a possible false-positive if further testing is not done.
- A sample which contains two substances that turn different colors can lead to a combining of colors to give a new color (e.g., a yellow colored material in a solution which also contains a blue color may produce a green-colored solution). This can lead to mis-assumptions about the type of drug present in the sample.

Presumptive Tests: False Positives & Negatives:

Sources of False Positive Results & False Negatives with Presumptive Tests:

- Color/Spot tests, continued:
- With some spot tests, if the analyte sample is prepared in too dilute a form, the test will give a false-negative.
- The presence of some types of impurities in a sample may inhibit the formation of a color in a color test even though a drug is present in the evidence sample; or the presence of the impurity may create its own color with the reagent that will overwhelm the color from the drug present. Ths type of interference can lead to false negatives or mis-interpretations of the results.
- Color tests cannot differentiate between D- and L- isomers of chemical compounds such as between D-methamphetamine (illegal) and L-methamphetamine (legal).
- D- and L-isomers of chemical compounds such as D-methamphetamine (illegal) and L-methamphetamine (legal), are related to one another on the molecular scale in the same way that a person's hands are related to one another: they are mirror images of one-another. They are not identical. This property of having a mirror image is related to a property called chirality. The body uses isomers in different ways: D-meth is a stimulant while L-meth (Levmetamfetamine) is a decongestant.

Presumptive Tests: False Positives & Negatives:

Sources of False Positive & False Negative Results with Presumptive Tests:

- Microcrystalline tests:
- As for color tests, it is unlikely that a specific crystal pattern will only form with just one specific drug when treated with a reagent. That is, it is unlikely that all other chemical types out of one hundred million known chemicals will be excluded from forming a similar crystal pattern in a microcrystalline test. If one assumes that each microcrystaline test only gives one specific crystal pattern for exactly one specific drug substance, this assumption will lead to a possible false-positive if further testing is not performed.
- The enantiomeric D- and L-isomeric forms of drugs will not be differentiated between when using microcrystalline tests. However many can differentiate between a pure D-isomer (or a pure L-isomer) and a 50:50 (racemic) mixture of D- and L-isomers of a drug or other chemical substance.
- Most microcrystalline tests which employ an acid solution will not be able to differentiate between the acid and free-base form of drugs such as for powder cocaine (salt form) and freebase cocaine. Further testing will be required to differentiate between the two forms.

Presumptive Tests: False Positives & Negatives:

Sources of False Positive & False Negative Results with Presumptive Tests:

- Microcrystalline tests, continued:
- The presence of impurities such as cutting agents in evidence samples can distort the shapes of crystal patterns that drugs form with the reagents; this can lead to possible false negatives if incorrect assumptions are made about the distorted crystal shapes.[18,19,28]
- Sometimes, if impurities or cutting agents are present in large amounts in an evidence sample, it has been noted that some reagents will not react to form crystals with the drugs in that sample,[19] leading to a possible false negative result.
- The reagents for some microcrystalline tests have a shelf life and usually need to be replaced one or more times per year. If an analyst uses an expired reagent, the microcrystalline test may have extra impurities present and not form crystals with an evidence sample that contains a drug substance, leading to possible false negatives.

Presumptive Tests: False Positives & Negatives:

Sources of False Positive & False Negative Results with Presumptive Tests:

- UV Spectroscopic Analyses:
- Some chemicals have very high absorbances of UV light per milligram at certain wavelengths, while others have fairly low absorbances of UV light per milligram. This means that, occasionally, the presence of small amounts of impurities which have very high absorbances of UV light in an evidence sample can add to the lower amounts of UV absorbances due to the major components in a sample and skew the UV spectrum obtained from the analysis. This can lead to irregularities in the shape of the UV spectrum obtained and lead to false assumptions about what drug may or may not be present in a sample.
- The enantiomeric D- and L-isomeric forms of drugs will not be differentiated when using UV spectrophotometry.
- UV spectrophotometric analysis of samples can give slightly different results for the UV spectra from the same chemical if the pH of the solution that the chemical is dissolved in for the UV analysis is acidic, basic or neutral. These UV spectral changes must be kept in mind when analyzing the spectra from evidence samples.

Presumptive Tests: False Positives & Negatives:

Sources of False Positive & False Negative Results with Presumptive Tests:

- IR Spectroscopic Analyses:
- In the case of some seized drugs, the complexity of the matrices (# of cutting agents) and the low percentage of drug in the evidence sample compared to the amount of matrix present makes qualitative tests especially prone to produce false negatives.
- The enantiomeric D- and L-isomeric forms of drugs will not be differentiated when using IR spectroscopy. In the case of methamphetamine, the L-isomer is legal to buy, sell and possess and one can purchase products made with this isomer at drug stores over-the-counter. An analysis by IR or FTIR will not prove that the illegal isomer is present, only that some type of methamphetamine is present.
- IR spectroscopic analysis of samples can give different results for the IR spectra from the same chemical when in different salt forms, in other words, if the pH of the chemical matrix is either acidic or basic. These IR spectral changes must be kept in mind when analyzing the spectra from evidence samples.
- If an evidence sample contains many different chemicals, such that many of the absorbances are broadened by the overlapping of peaks and troughs from the impurities, it may be possible to mistake the peak pattern for that of a drug.

Presumptive Tests: False Positives & Negatives:

Sources of False Positive & False Negative Results with Presumptive Tests:

- Immunoassay tests:
- Immunoassays involve the use of antibodies raised to antigens. The process of raising antibodies to antigens is not perfectly selective, and therefore, the assays are not perfectly selective in their drug binding properties. False-positives can occur due to the structural similarities of the drugs used as antigens to other chemicals when other non-drug chemicals are bound to the antibodies; when this happens, the tests show positives due to this "mistaken" binding to structurally-similar chemicals.
- Immunoassays involve the use of antibodies that are developed to bind chemicals having a certain molecular shape, i.e., to molecules having particular distances between various functional groups in a molecular structure. There are many different chemicals with similar shapes that can cross-react with antibodies to a particular drug; these cross-reactions lead to false positives in drug screening assays.
- A certain level of drug is necessary to be present in solution for detection to occur using the immunoassay technique. If a sample such as urine, for example, is diluted to bring a drug concentration to below the required level for detection, it is possible to produce false-negatives.

Presumptive Tests: False Positives & Negatives:

Sources of False Positive & False Negative Results with Presumptive Tests:

- Immunoassay tests[29]:
- Usually, immunoassays are used to detect the presence of drugs in bodily fluids such as urine and saliva. Drugs, however, are metabolized over time and decrease in concentration in the blood and tissues over time. This lower concentration in the body over time is reflected in lower and lower concentrations of drugs and metabolites in the bodily fluids as time proceeds. If removal of a sample of a bodily fluid is not done soon enough after a drug has been used, one runs the risk of an immunoassay giving a false-negative for prior drug use.
- Samples of bodily fluids can be adulterated using a number of commercial products with the active ingredients of: peroxide (peroxidase), glutaraldehyde, sodium or potassium nitrite, and pyridinium chlorochromate, among others. These chemicals have the potential to interact with immunoassay chemistry and the enzymatic processes used for drug detection and can lead to false-negatives.
- Some immunoassays infer the presence of drugs in a sample by detection of the drugs' metabolites instead of the actual drugs. If certain members within a family of drug compounds are not metabolized by the body in the same way that most of the others in the family are, these different metabolites will not be detectable by the assay.

References:

1. See also: "*An overview of forensic drug testing methods and their suitability for harm reduction point-of-care services*", L. Harper, J. Powell and E. M. Pijl, *Harm Reduct J.* 2017; 14: 52., published online Jul 31, 2017 at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5537996/ : Accessed 6/14/2020.
2. For a publication detailing the recommendations for "Best Practices" from the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) for the development of analytical techniques, protocols and policies applied to drug analysis, see: "*Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations*", Version 8.0, SWGDRUG 2019-June-13, U.S. Department of Justice, Drug Enforcement Administration, at: http://swgdrug.org/Documents/SWGDRUG%20Recommendations%20Version%208_FINAL_ForPosting_092919.pdf : Accessed 6/14/2020.
3. For further information, see: "*Color Test Reagents/Kits for Preliminary Identification of Drugs of Abuse NIJ Standard–0604.01*", July 2000, The Law Enforcement and Corrections Standards and Testing Program, National Institute of Justice at: https://www.ncjrs.gov/pdffiles1/nij/183258.pdf : Accessed 6/14/2020.
4. For cobalt thiocyanate test, see: https://en.wikipedia.org/wiki/Cobalt(II)_thiocyanate : Accessed 6/14/2020.

References:

5. For Dille–Koppanyi reagent, see: https://en.wikipedia.org/wiki/Dille–Koppanyi_reagent : Accessed 6/14/2020.
6. For Duquenois–Levine reagent, see: https://en.wikipedia.org/wiki/Duquenois–Levine_reagent#/media/File:Duquenois_levine_step2.jpg : Accessed 6/14/2020.
7. For Mandelin reagent, see: https://en.wikipedia.org/wiki/Mandelin_reagent : Accessed 6/14/2020.
8. For Marquis reagent, see: https://en.wikipedia.org/wiki/Marquis_reagent : Accessed 6/14/2020.
9. For nitric acid test, see: "*Validation of twelve chemical spot tests for the detection of drugs of abuse*", C. L. O'Neal, D. J. Crouch, A. A. Fatah, *Forensic Science International,* Volume 109, Issue 3, 2000, pp. 189-201 : Abstract accessed: 6/14/2020.
10. For para-dimethylaminobenzaldehyde reagent, see: https://en.wikipedia.org/wiki/Para-Dimethylaminobenzaldehyde : Accessed 6/14/2020.

References:

11. For ferric chloride test, see: https://en.wikipedia.org/wiki/Ferric_chloride_test : Accessed 6/14/2020.
12. For Froehde test, see: https://en.wikipedia.org/wiki/Froehde_reagent : Accessed 6/14/2020.
13. For Mecke reagent, see: https://en.wikipedia.org/wiki/Mecke_reagent : Accessed 6/14/2020.
14. For Zwikker reagent, see: https://en.wikipedia.org/wiki/Zwikker_reagent : Accessed 6/14/2020.
15. For Simon's (nitroprusside) reagent, see: https://en.wikipedia.org/wiki/Simon%27s_reagent : Accessed 6/14/2020.
16. Photo for Duquenois-Levine reagent test on THC sample, see: http://www.coloradogreenlab.com/blog/archives/07-2016 : Accessed 6/14/2020.
17. "*Microcrystalline tests in forensic drug analysis*", M. P. Elie, L. E. Elie. In: Meyers RA, editor. *Encyclopedia of analytical chemistry*. Hoboken: Wiley; 2009. pp. 471–481 : Accessed 6/14/2020.

References:

18. *"Microcrystal Analysis of Cocaine Hydrochloride and Added Adulterants"* H. C. Nelson, E. A. Gardner, D. Matteo, *J. Forensic Sci*, 2011 56(3):736-40. Photo: see also gold chloride microcrystal test with cocaine hydrochloride: https://www.semanticscholar.org/paper/Microcrystal-analysis-of-cocaine-hydrochloride-and-Nelson-Gardner/1b25d497ae08c1bc19dba96eef2e8b9dc373e94c : Accessed 6/14/2020.

19. *"A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals, Compendium"*, K. M. Brinsko, D. Golemis, M. B. King, G. J. Laughlin, S. B. Sparenga, November 2016, final report to: National Institute of Justice, Office of Justice Programs, U.S. Department of Justice; Published online, see p.28:https://www.ncjrs.gov/pdffiles1/nij/grants/249854.pdf : Accessed 6/14/2020.

20. Ibid, See p.6: Accessed 6/14/2020.

21. Ibid, See p.63: Accessed 6/14/2020.

22. *"A Comparative Study of UV-Spectrophotometry and First-Order Derivative UV-Spectrophotometry Methods for the Estimation of Diazepam in Presence of Tween-20 and Propylene Glycol"*, D. G. Dastidar, B. Sa, *AAPS Pharm Sci Tech*, 2009 Dec; 10(4): 1396.

References:

22. Published online Nov 20, 2009 at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2799609/ : Accessed 6/14/2020.

23. For UV spectra of codeine phosphate and lidocaine: See: *"Ultraviolet-Visible Reference Spectra"*, at: https://www.pmda.go.jp/files/000203136.pdf : Accessed 6/14/2020.

24. For IR spectra of acetaminophen and cocaine hydrochloride, see *"Infrared Reference Spectra"*, at: https://www.pmda.go.jp/files/000152482.pdf : Accessed 6/14/2020.

25. For IR spectra of heroin base and heroin hydrochloride, see Scientific Working Group for the Analysis of Seized Drugs Monographs website, at: http://www.swgdrug.org/monographs.htm and the link to the heroin page at: http://www.swgdrug.org/Monographs/HEROIN.pdf : Accessed 6/14/2020.

26. For figure of ELISA method 1, see: https://en.wikipedia.org/wiki/ELISA : Accessed 6/14/2020.

27. For figure of ELISA method 2, see:https://www.labce.com/spg2056292_immunoassay_techniques_used_in_drug_test_screening.aspx : Accessed 6/14/2020.

References:

28. For an example of distortion of microcrystals from impurities, see: https://issuu.com/sciencejournal.inquiro/docs/inquiro_magazine_volume_3/80 : Accessed 6/14/2020.

29. For immunoassay false-positive and false-negative data, see: “Urine Drug Screening: Minimizing False-Positives and False-Negatives to Optimize Patient Care”, A. Schwebach, J. Ball, US Pharm. 2016;41(8):26-30 at: https://www.uspharmacist.com/article/urine-drug-screening-minimizing-false-positives-and-false-negatives-to-optimize-patient-care and references therein : Accessed 6/14/2020.

30. For a good general summary of information of IR spectroscopy, see: https://www.ru.nl/systemschemistry/equipment/optical-spectroscopy/infrared/ : Accessed 6/14/2020 and https://en.wikipedia.org/wiki/Infrared_spectroscopy accessed 6/16/2020.

References:

31. For a drawing of this GCMS cartoon and video of GCMS in use, see: https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.youtube.com%2Fwatch%3Fv%3DzjXBwbGG0e8&psig=AOvVaw3XX8nh5rAfYlIf7MX7inh7&ust=1592542863551000&source=images&cd=vfe&ved=0CAIQjRxqFwoTCNDqus_KiuoCFQAAAAAdAAAAABAD : Accessed 6/16/2020.

32. GC chromatogram taken from: *"Three Peak Shapes You Really Don't Want to See in Your Gas Chromatogram"*, T. Taylor, The Column, Volume 14, Issue 3, Mar 01, 2018, pg 25–31 Accessed on 6/17/2020 from:http://files.alfresco.mjh.group/alfresco_images/pharma//2018/03/13/e559a519-f215-4c78-9a5a-f6a833107cc6/figure%202%20L.png .

33. For an overview of LCMS techniques, see: https://en.wikipedia.org/wiki/Liquid_chromatography–mass_spectrometry : Accessed 6/14/2020.

34. See: https://ifs.harriscountytx.gov/eDiscovery/eDiscovery/Forensic%20Toxicology/Standard%20Operating%20Procedures/Technical%20procedures/GCMS/TOX.GC.3617%20Ethanol%20Quant%20using%20Agilent%20GC%20Headspace/TOX.GC.3617%20Rev%2008,%20Effective%202013-12-20%20to%202014-01-31.pdf : 6/17/20.