Development of Microcrystal Tests

for Butylone, Ethylone, and Methylone Detection

BY

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THESIS

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LIST OF ABBREVIATIONS

3-OH-4-MeO-MC	3-Hydroxy-3-methoxymethcathinone
3'OH-MDMC	3'-hydroxy-methylenedioxymethcathinone
4-OH-3-MeO-MC	4-hydroxy-3-methoxymethcathinone
ATR-IR	Attenuated Total Reflection-Infrared Spectroscopy
FTIR	Fourier Transform-Infrared Spectrometer/Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
GHB	Gamma-Hydroxybutyric Acid
LC-MS	Liquid Chromatography-Mass Spectrometry
LSD	Lysergic acid diethylamide
MDC	3,4-methylenedioxycathinone
MDMA	3,4-Methylenedioxymethamphetamine
MS-MS	Tandem Mass Spectrometer
PLM	Polarized Light Microscope/Microscopy
PPP	Periods on a Printed Page
RI	Refractive Index/Indices
RTL	Redwood Toxicology Laboratory
TOF-MS	Time of Flight-Mass Spectrometer
UV	Ultra Violet

SUMMARY

Microcrystal tests are a microchemistry technique developed in the 1800's. Recently, they have been used more frequently in forensic science to help identify drugs of abuse, such as amphetamine and cocaine. Microcrystal tests are advantageous due to relatively short analysis time, small sample quantity needed, and little to no sample preparation. Although many U.S. forensic laboratories have turned to instrumental techniques, such as gas chromatography-mass spectrometry, microcrystal tests are still used in laboratories in the U.S. and around the world for drug identification. While microcrystal tests have been developed for many common drugs of abuse, newer drugs of abuse do not have validated tests, among which are the synthetic cathinones. Therefore, the purpose of this thesis is to develop microcrystal tests that can detect butylone, ethylone, and methylone, through optical characteristics of precipitated crystals. Synthetic cathinones, street name "Bath Salts", are newer drugs of abuse that are man-made compounds similar to cathinone. Cathinone is a naturally occurring compound found in khat (*Catha edulis*) leaves. Since cathinone, and subsequently synthetic cathinones, is a stimulant, increased euphoria, alertness, sexual arousal, sociability, concentration, and motivation can occur upon administration. Since 2010, synthetic cathinones have become more prominent on the illegal drug market; signifying, validated microcrystal tests could be beneficial. Microcrystal tests were successfully developed to detect butylone, ethylone, and methylone individually and in the presence of adulterants. Each test utilized either picrolonic or picric acid as the reagent. All three drugs were identified by precipitation of rosettes. Methylone formed rosettes with both reagents while butylone and ethylone precipitated crystals with either picrolonic and picric acid respectively. All four crystals are distinguishable either by reagent used or optical properties. Attenuated total reflectance-infrared spectroscopy was also performed on each drug and butylone

and methylone crystals precipitated from picrolonic acid, for reference purposes. These tests are unique since no other drug is known to produce the same crystals and optical characteristics with these reagents; thus, they are good options for use in forensic science.

I. INTRODUCTION

A. Background

1. Microcrystal Tests

Microcrystal tests are used to identify a compound within an unknown sample. This is extremely useful for forensic samples because all samples are unknowns to be analyzed. When analyzing a compound, a preliminary test is used to exclude multiple drugs so only a select few are left as possibilities. A widely used preliminary test is a color test. A reagent is added to the sample and a change in color will categorize the drug present: amphetamine, opiate, cocaine, etc. However, since color tests are presumptive, the drug is analyzed a second time with a confirmatory test. A presumptive test can give indications of a drug which may be present. This is because presumptive tests will react with a class of drugs, meaning that there is cross reactivity with many other compounds. For example, cocaine will turn blue with the cobalt thiocyanate test; however, lidocaine, procaine, and other compounds will give the same result.

Recently, microcrystal tests have been included as a presumptive test for drug analysis after being ignored for instrumental methods. At the highest form of microcrystal test application, they can be used to conclusively identify a drug sample (Fulton, 1969). Presently, instrumental analysis is prioritized over microcrystal tests. Therefore, usually, microcrystal tests are solely used as a presumptive technique.

There are several different applications when using a microcrystal test, but the overarching technique entails a specific reagent added to a specific compound, creating unique and identifiable crystals. The two main application methods are direct and hanging drop. When using the direct method, the reagent is added right to the sample and

can be stirred. Stirring can affect crystal precipitation, so it is important to note when stirring is required for crystal formation. For hanging drop, the compound of interest must become volatile when added to a reagent, usually sodium hydroxide. The gaseous compound will rise up and meet a reagent drop hanging underneath a coverslip. This technique forms crystals in the reagent droplet the same as the direct method; however, contaminants are not included in crystal formation (Fulton, 1969). An example of what the hanging drop technique looks like is shown in figure 1.

Figure 1. Hanging drop method. From bottom to top: glass slide, glass ring, reagent drop with sample, coverslip (illustrated by author).

Microcrystal tests are used to identify minute amounts of individual chemical substances, requiring a microscope to be used when performing the test and analyze the results. While the ability to analyze minute quantities is an added bonus for this technique, it is not the main purpose. Instead, the primary objective is to produce a chemical reaction, placing microcrystal tests in the overarching microchemistry technique category. Microchemistry also does not always utilize a microscope; thus, small quantities are a result of pairing a microscope with microcrystal tests. Also, microcrystal tests are not an instrumental identification, but rather a chemical identification (Fulton, 1969). Instrumental techniques require a machine, such as a gas chromatography-mass

spectrometer (GC-MS). Instrumental techniques are predominantly used for conclusive identification. This means that an analyst can definitively identify a sample. Chemical identification is most often used for presumptive testing. It requires a chemical reaction to occur, such as the example previously given—cocaine turns blue with the cobalt thiocyanate test. Microcrystal tests, therefore, are chemical tests. A reagent is added to a sample and a reaction occurs, but depending on what other tests are being utilized it can be used as a presumptive or conclusive method. When used with an instrumental technique, microcrystal tests are often used for presumptive screening. When used in conjunction with a second microcrystal tests, it is both presumptive and conclusive (Fulton, 1969).

The polarizing microscope is used to aid in observing and distinguishing the many crystals formed. In the 1800's, when microcrystal tests were first used, they were primarily for the identification of alkaloids. Alkaloids are heavy and complex molecules, which easily precipitate with certain reagents. These are still relevant today because there are alkaloid drugs, such as cocaine and codeine, and many other drugs have similar precipitation properties as alkaloids. Acidic compounds, such as barbiturates, can form precipitates with a halogen reagent; for instance, iodine in iodide solution. Microcrystal tests, nevertheless, are not just limited to those types of compounds previously mentioned. If a reagent and compound produce a crystalline product it is considered a microcrystal test (Fulton, 1969).

The reason microcrystal tests are easy to perform and do not require substantial training is because they focus on "matching identification" instead of "structural identification". The difference between the two is exactly how they are named.

"Structural identification" involves identifying a compound based on atoms, radicals, and their relation to each other. "Matching identification" identifies a substance as a known chemical compound. While the two sound similar, the difference can be observed in morphine's history. Morphine's structure was not identified and proven for over a hundred years after the substance was known; however, it was identified in toxicology, drug analysis, and law enforcement chemistry through matching identification. While the substance was recognized and analyzed, the exact chemical composition was not determined for over a hundred years. "Matching identification" is often used in toxicology where pure samples are difficult to obtain. Many times, the compound of interest is in a body fluid matrix that contains extra compounds that can be difficult to separate. This also applies to drugs because most drugs are a combination of the desired drug and various other compounds. For example, caffeine is often found with cocaine because it is cheaper, but still provides stimulating effects (Fulton, 1969).

Finally, since the crystals formed via microcrystal tests are through molecular-addition, combining the reagent and drug, the original product can be obtained after the analysis has been completed. In other words, the microcrystal test can be reversed and be considered a non-destructive method. Addition reactions result in a drug and reagent complex; for microcrystal tests the precipitated crystal is the complex. When addition reactions occur, the drug and reagent molecules form weak bonds that can easily be broken by adding a solvent to the crystals. The crystal will dissolve, and the drug molecules will be in solution. This is useful because it allows the drug sample to be analyzed a second time in cases where seized samples are not sufficient to perform confirmatory tests. Research has already been done that supports gamma-hydroxybutyric

acid (GHB) and methadone can be identified with GC-MS and liquid chromatography-mass spectrometry (LC-MS) after microcrystal analysis was reversed (Fulton, 1969; Elie et al., 2011).

Although there is no known error rate for microcrystal tests, between 1985-1993, seventeen drug proficiency tests and results were evaluated for errors. Methodology ranged from standard GC-MS and infrared spectroscopy (IR) for identification to microcrystal tests and ultra-violet spectroscopy for classification. The samples were a mixture of drugs that could easily be distinguished through common techniques, but the results of the evaluation showed many mistakes were made when instrumental techniques were used. Unexpectedly, when multiple microcrystal tests were used to analyze a sample there were no errors. Of the 2,237 scorable tests, there were 63 total errors, resulting in 2.8% error rate. With even distribution, five errors out of the 148 participants who only used microcrystal tests for analysis would be expected. This was not the case. Instead, many of the errors occurred when GC-MS or IR or a combination of the two were used for identification. When a microcrystal test was used in conjunction with an instrumental test, the microcrystal test results were correct, however, the analyst chose to rely on the instrumental techniques and interpretation for analysis. Errors with instrumental techniques were not caused because the method was unable to identify the compound, but because the analyst missed key information when analyzing the results (Nichols, 1997).

Microcrystal tests have not been used in forensic laboratories as often because microscopy units have dwindled. Although microcrystal tests are used in identifying drugs, extensive microscopy knowledge is needed for one to testify in court and to be

recognized as an expert witness, despite the analysis requiring little microscopy understanding. This poses a challenge in forensic science because microscopy and drug chemistry are considered two different units; therefore, drug chemists are not trained in microscopy, and vice versa. Microcrystal tests are different in that they do not fall in just one discipline. They require both drug and microscopy knowledge. Before a microcrystal test can be performed, the microscope must be properly set up. This requires optimizing the microscope through Köhler Illumination, which is not well known outside of microscopy. It also requires knowledge of how a polarized light microscope (PLM) works and how each different analysis can be used to help identify a crystal. So, while the analysis itself is simple, the preparation and interpretation requires considerable knowledge.

2. Polarized Light Microscopy

Microcrystal tests are performed on a microscopic scale; thus, a microscope is needed when analyzing crystals. Crude forms of microscopes have been around since the late 1500's, although lenses were used as early as Egyptian times to magnify items. For chemical and petrographic analysis, a specific microscope was developed to ease analysis. These microscopes were equipped with a polarizer, analyzer, compensator, Bertrand lens, and a rotating stage. This type of microscope is now referred to as a polarized light microscope (Allen, 1940). An illustration of a polarized light microscope is shown in appendix A (McCrone et al., 2014, appendix 2; used with permission).

A polarized light microscope has a polarizer underneath the stage that transmits filtered light traveling in one direction of vibration. There is also a second filter, called an analyzer (which is removable below the eyepieces) that only allows light traveling

perpendicular to the polarizer, to be transmitted. (McCrone et al., 2014). How a polarizer and analyzer works is shown in figure 2.

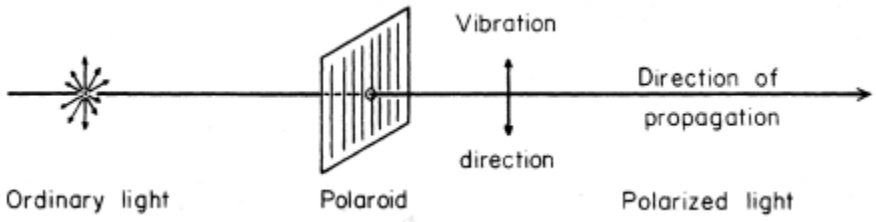

Figure 2. Illustrates how a polaroid allows only one vibrational direction through. Regular light has 360 degrees of vibrational rotation, but when a polaroid filters light, only one vibrational direction is transmitted (McCrone et al., 2014, pg.135; used with permission).

These two filters help more information to be known about samples such as whether the sample is isotropic or anisotropic. An isotropic sample would look completely black when viewing it through the microscope with both the polarizer and analyzer because it only has one refractive index (RI). Refractive index is a ratio of light's velocity in a vacuum to its velocity in a medium. Thus, if a compound is dense or has a high atomic number, the RI will be higher because the velocity of light in that medium will be much slower compared to the velocity in a vacuum. All compounds have at least one RI, but if the compound has more than one refractive index it will be viewable with both the polarizer and analyzer in place. When a compound has multiple refractive indices light can travel through the substance in multiple directions. In a sense, it is like having multiple exits. An isotropic substance has one path light can exit through

and if that one exit is blocked it will not be viewed. If, however, the sample has multiple indices, three is the maximum, thus multiple exits, light can find a path through the substance so that it can be seen through the microscope even with both polarizer and analyzer inserted (McCrone et al., 2014).

A compound's refractive indices can be determined by adding liquids of a known RI to the sample until the sample becomes translucent. If the sample has only one RI, it will completely transparent in the liquid of the same refractive index. If there are multiple RI, then the sample will only disappear in specific orientations. For the purpose of this research, the crystals have two identifiable refractive indices so the crystals would disappear either in the horizontal or vertical orientation. The sign of elongation can be determined by comparing the RI. If the refractive index of the elongated portion of the crystal is higher than the refractive index of the width of the crystal then the sign of elongation is positive and vice versa (McCrone et al., 2014).

Polarized light microscopes also have a first order red compensator. It consists of a layer of selenite or quartz at the correct thickness to usually produce about 530 nm of retardation. A first order red compensator can be used to determine the sign of elongation in conjunction with the Michel-Lévy Chart, if RI liquids cannot be used. The Michel-Lévy Chart, shown in appendix B, relates thickness, retardation, and birefringence for substances that are transparent, colorless or lightly-colored. Retardation is the difference in distance between the fast and slow vibration directions of a crystal. As a sample's thickness and/or birefringence increases, so does retardation. Birefringence is the numerical difference between a compound's highest and lowest RI. The compensator has a high and low refractive index perpendicular to each other. If a

crystal's high and low RI are oriented in the same direction as the compensator's, a higher order color is viewed and the sign of elongation is positive. If oriented oppositely, then a lower order color is viewed and the sign of elongation is negative. Color order is determined by the Michel-Lévy Chart.

Optic sign, which is another optical property, independent of sign of elongation, can be determined by conoscopy. However, conoscopy is only available if crystal orientation allows. When the Bertrand lens is inserted, an interference figure is visible that is specific to a uniaxial or biaxial crystal. A uniaxial crystal is a crystal with two RI while a biaxial crystal has three. Uniaxial crystals will form an interference pattern of a cross. A biaxial crystal will also form a cross, but when rotated 45°, a pair of hyperbolic isogyres will appear, instead of the cross. Optic sign is determined by inserting the compensator while viewing the interference figure. Figure 3 illustrates how the interference figure and compensator are used to determine optic sign. This method was not used because the crystals were not suitable. The crystal must be large enough and oriented appropriately for the interference figure to be present. The crystals precipitated in this research were not suitable because the needles of the rosettes were too small and closely packed together (McCrone et al., 2014).

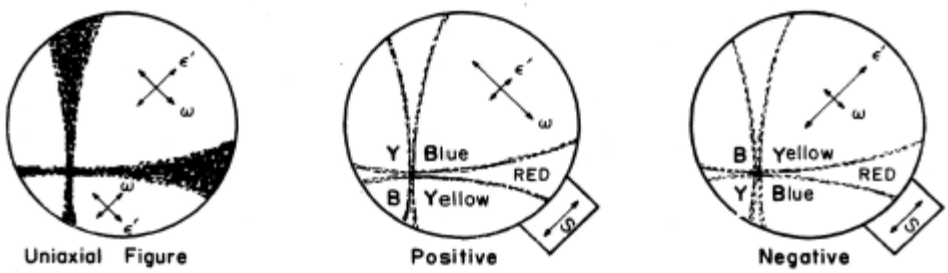

Figure 3A

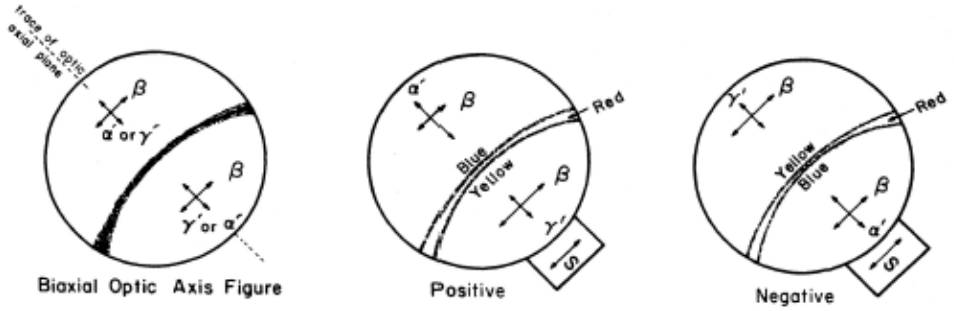

Figure 3B

Figure 3. Illustrates how optic sign can be determined via conoscopy for 3A uniaxial crystal will have a positive optic sign if the bottom left quadrant and upper right quadrant are blue, 3B biaxial crystal will have a positive optic sign if the outer portion of the semi-circle is blue (McCrone et al., 2014, pg 153, 155; used with permission).

Like all microscopes, a polarized light microscope works best when all components are optimized to each other. This is accomplished using Köhler illumination. As the name suggests, this method was published by August Köhler, in 1893. Köhler illumination results in even illumination of the field of view by optimizing the field diaphragm, substage iris, and light intensity. The field diaphragm controls the size of the illuminated field of view and the substage iris controls the angle at which light is transmitted through the sample. Together, by optimizing those three components, the microscopist has better control over contrast, resolution, and depth of field. When Köhler illumination is set up correctly, two images can be observed: one is observed

orthoscopically (no Bertrand lens) and the other conoscopically (with Bertrand lens). To achieve an image observed orthoscopically, the field diaphragm, specimen, and ocular focal plane are focused and centered. To observe an image conoscopically, the lamp filament must be focused and centered in the substage condenser aperture diaphragm plane and the objective back focal plane (McCrone et al., 2014; Gill, 2013). Other than Köhler Illumination, diffuse illumination can be used instead and requires less set-up. Diffuse illumination requires no lamp filament adjustment because a piece of ground glass is placed between the lamp filament and microscope condenser. The illumination is still high quality, but there is less intensity (McCrone et al., 2014)

3. Attenuated Total Reflectance-Infrared Spectroscopy

Attenuated total reflectance-infrared spectroscopy, referred to as ATR-IR, is a form of infrared spectroscopy that analyzes the chemical composition of a compound. It differs from Fourier transform-infrared spectroscopy (FTIR) in that it utilizes a microscope, internal reflection rather than transmission, and there is little sample preparation. Transmission has the infrared beam pass directly through the sample while internal reflection creates an evanescent wave when ATR-IR is performed. The evanescent wave interacts with the sample and loses intensity due to the sample absorbing energy. Figure 4 illustrates an evanescent wave. ATR-IR also requires a crystal so the infrared beam is not released before the detector detects it. Usually these crystals are made out of diamond because it is more robust and durable, but it can be made from germanium, silicon, or zinc selenide. These materials are used for the crystal because the crystal must have a higher refractive index than the sample so internal reflectance occurs. If it does not have a higher refractive index, then the light will be

transmitted and it will no longer be ATR-IR. The light is transmitted by hitting the crystal at a specific angle that allows it to be bounced throughout the crystal.

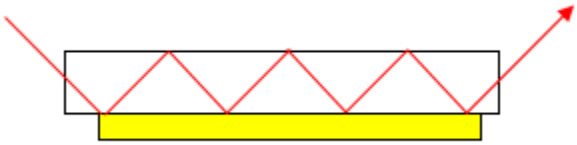

Figure 4. Illustrates an evanescent wave during ATR-IR. The infrared beam passes through the crystal, shown in white, and continuously interacts with the sample, shown in yellow. As it interacts with the sample, the beam loses intensity before it enters the detector.

A low-e microscope slide is used when collecting ATR-IR data so there is no interfering absorptions from 400 to 4000 cm^{-1}. The slide is coated with two layers of silver for infrared reflectance techniques. The microscope slide is also thicker than standard slides and can therefore withstand more pressure when performing this technique (Kevley Technologies, 2010). This is important because for the sample to absorb some of the infrared light, producing a spectrum, the crystal and sample must be touching. If they are not, the sample will not be able to absorb a small amount of the evanescent wave.

Infrared spectroscopy provides chemical information of a compound. It vibrates molecules to generate a unique spectrum specific to the molecular structure of a compound (Perkin Elmer, 2005; Tasumi, 2015). This is possible because each functional group is made up of different bonds and atoms that will absorb varying energy

wavelengths. The energy absorbed by the sample is contained in the infrared region. For example, a carboxylic group will have distinct vibrational bands at about 1700 cm^{-1} wavelength and 3200 cm^{-1}.

4. Synthetic Cathinones

Synthetic cathinones are man-made compounds similar to cathinone. Cathinone, chemical structure shown in figure 5, is a naturally occurring stimulant drug found in the plant khat (*Catha edulis*) and is an amphetamine analogue.

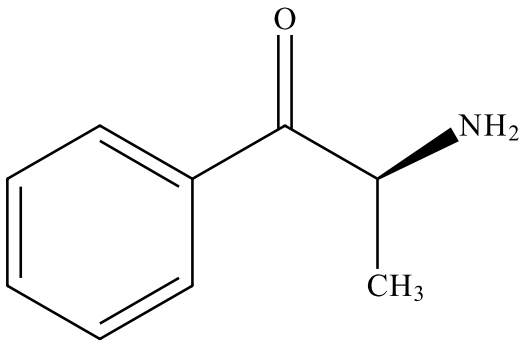

Figure 5. Chemical structure of cathinone (illustrated by author).

While many synthetic cathinones have adverse effects, bupropion and diethylpropion, both cathinone derivatives, have therapeutic properties. Bupropion is an antidepressant for major and seasonal depression. Recently, it has been hypothesized bupropion could be used to treat induced dependence and craving of synthetic cathinones. However, there has been no research to date to support the hypothesis (Cappola and Mondola, 2012). Diethylpropion is an appetite suppressant used to treat obesity, but is rarely prescribed

due to abuse and dependence (Coppola and Mondola, 2012). The U.S. Poison Control Centers started receiving "Bath-Salt" related reports in 2010. In the first four months of 2011, the U.S. Poison Control Centers received 2,237 reports related to "Bath-Salts". Based on the 2011 NDIC report, an increase in synthetic cathinone distribution and abuse was expected. In 2010, state and local forensic laboratories analyzed 594 more synthetic cathinone samples compared to 34 reports in 2009 (National Drug Intelligence, 2011).

Khat is indigenous to East Africa and the Arabian Peninsula. Chewing khat leaves dates back many centuries and is still prevalent in Somalia, Yemen, Kenya, and Ethiopia, but the khat shrub was not catalogued until the 18th century by Peter Forskål. In 1928, methcathinone was synthesized in Germany and mephedrone the following year. As of now, there are about 30 known synthetic cathinones. Like many drugs, methcathinone was first marketed as an antidepressant in the Soviet Union; this is similar to the use of cocaine for toothache in the late 1800s (Capriola, 2013). About twenty years later, methcathinone started to be abused in the Soviet Union. Methcathinone abuse did not start in the U.S. until 1991, and subsequently, methcathinone became a Schedule I drug. Finally, in 2009, there were global concerns about cathinone as a "legal high," resulting in the U.K. scheduling cathinone derivatives as Class B substances (Kelly, 2011). Class B is the middle drug class in the U.K.'s four tier system: Class A, B, C and temporary class. Drugs placed in Class A are considered to be the most dangerous and receive the harshest penalties (Legislation.gov.UK; Prosser and Nelson, 2012). The U.S. has five schedule drug classes, so in comparison, the U.K. has classified synthetic cathinones in a class comparable to the U.S. Schedule III class, while the U.S. has classified synthetic cathinones as Schedule I, comparable to the U.K.'s Class B. In the

U.S., the legal status of synthetic cathinones is continuously evolving as new compounds are introduced to evade existing scheduling. This is a problem for forensic science because timely development of tests to accurately identify new derivatives is difficult due to new synthetic cathinones being constantly manufactured. The manufacturer can easily add another functional group to the chemical structure, which can potentially alter a method's analysis. An example can be found with synthetic cannabinoids. Redwood Toxicology Laboratory (RTL) developed a test to detect synthetic cannabinoids JWH-018 and JWH-073 metabolites in urine; however, in response, manufacturers synthesized JWH-250, JWH-019, JWH-081, and CP47 497-C8 and started using them in various products (Redwood Toxicology Laboratory, Inc.).

Since 2004, Asia, Israel, the E.U., and U.S. have reported synthetic cathinone abuse, which could be fueled by decreased purity and availability of other stimulant drugs of abuse, such as 3,4-methylenedioxymethamphetamine (MDMA) and cocaine. According to law-enforcement data, the People's Republic of China, Pakistan, and India are frequent suppliers of synthetic cathinones. They are sold on the internet, "head shops," gas stations, convenience stores, and skateboard shops. To circumvent law enforcement, they are often labeled as bath salts, plant food/fertilizer, vacuum freshener, pond cleaner, and insect repellant or have "not for human consumption" written on packaging. It is often sold as powder or tablets and previously oral ingestion and nasal insufflation were the most common forms of use, but more recently injection has become more common (Capriola, 2013; Prosser and Nelson, 2012).

With all of the various cathinone derivatives, research has not been done for each individual drug. Instead, a few synthetic cathinones have been studied and those results

act as a basis for the remaining synthetic cathinones. Since cathinone is a stimulant, it affects dopamine, serotonin, and norepinephrine similarly to cocaine, amphetamine, and MDMA. Dopamine is responsible for euphoric and pleasurable feelings and drugs that increase dopamine can be more addicting. Dopamine can also cause delusions and paranoia. Increased serotonin causes hallucinations; that is why delusions are connected with lysergic acid diethylamide (LSD). Norepinephrine increases heart rate and constricts blood vessels. Collectively, all three neurotransmitters increase euphoria, alertness, sexual arousal, sociability, concentration, and motivation. Together, dopamine, serotonin, and norepinephrine also induce psychological or physiological changes, which is why they are used in religious ceremonies. There is also an increase in empathy.

Cathinones, including synthetic cathinones, increase dopamine, serotonin, and norepinephrine levels by inhibiting reuptake. When a neurotransmitter is released, synapses reabsorb the excess. This means neurotransmitters will be taken back into synapses, rather than being transferred to the cell. Cathinones inhibit neurotransmitter reuptake by increasing the acidity of vesicle sacks. Vesicle sacks carry neurotransmitters and release them. Compared to the cytoplasm, vesicle sacks are marginally more acidic. This difference prevents neurotransmitters from being released into the cell too early. However, amphetamine and other similar drugs, decrease the pH difference, resulting in neurotransmitters being released into the cytoplasm before the vesicle has fused with the cell membrane. This also means the reuptake of neurotransmitters will not occur because the vesicle sack does not have time to fuse to the cell membrane (McGraw, 2012). Cathinones also inhibit monoamine oxidase enzymes A and B. Both metabolize excess monoamines. Enzyme A metabolizes serotonin and norepinephrine while enzyme B

metabolizes dopamine. These three side effects significantly increase dopamine, serotonin, and norepinephrine levels by increasing output and decreasing reuptake and metabolism. Butylone, ethylone, and methylone all have these side effects, but butylone and methylone also compete with dopamine and serotonin substrates during reuptake. This makes it even more difficult for synapses to reuptake dopamine and serotonin (Simmler et al., 2013).

All drugs that are administered orally follow the enterohepatic recirculation model when processed by the body: it will be absorbed in the gut, some will be transferred into the blood stream, excess will move to the liver, bile, then back to the gut where it gets reabsorbed. However, not all of the drug will move from liver to bile, instead some will travel to the kidneys and be excreted out through urine. Pharmacokinetically, cathinone concentration in plasma reaches its peak level at about 1.5 h after capsule ingestion. Within 10-20 minutes of insufflation, mephedrone's effects will start and last 1-2 h, but taken orally will peak at 20-40 minutes and last 2-4 h. Once ingested, Phase I metabolism begins for all cathinone derivatives. Phase I prepares compounds for Phase II reactions through various reactions: reduction, hydrolysis, hydration, isomerization, to name a few. In Phase II, drugs will undergo more reactions, such as glycosidation, sulfation, or methylation. An example of metabolism looks like this: 3,4-methylenedioxy ring-substituted cathinones (i.e. methylone) become metabolized mainly by demethylation, but also by *N*-dealkylation, *O*-methylation, and reduction of β-keto moiety. Most cathinone derivatives are eliminated from the body as metabolites in urine, either in free form or conjugates, via Phase II metabolism. Generally, metabolism occurs

in the liver, kidneys, intestines, or blood. Research has suggested that the liver, kidney, and perhaps other organs help in eliminating synthetic cathinones (Kelly, 2011).

While extensive pharmacokinetic research has not been performed for ethylone and butylone specifically, methylone's pharmacokinetics have been studied in rats. Since ethylone, butylone, and methylone are all cathinone β analogues, ethylone and butylone's pharmacokinetics are thought to be similar to methylone. When methylone was administered orally, the peak level was reached within 30-60 minutes and was undetectable at 24 hours. Bioavailability, the amount of drug absorbed by a system compared to the amount ingested, was between 78-89%. Four metabolites, 3,4-methylenedioxycathinone (MDC), 4-hydroxy-3-methoxymethcathinone (4-OH-3-MeO-MC), 3-Hydroxy-3-methoxymethcathinone (3-OH-3-MeO-MC), and 3'-hydroxy-methylenedioxymethcathinone (3'OH-MDMC), were consistently detected in rats at 60, 120, and 180 minute time intervals (Ellefsen et al., 2016). Methylone also displays a flip-flop phenomenon that takes place during metabolism. This phenomenon occurs when drug absorption rate is much slower than its elimination rate; thus, increasing the half-life. Half-life is the amount of time it takes for half of a drug dosage taken, to be eliminated. Flip-flop occurs with extravascular dosing (Lopéz-Arnan et al., 2013 Yáñez et al., 2011).

B. Purpose of the Study

The purpose of this study is to develop a method to identify butylone, ethylone, and methylone; chemical structures are shown in figure 6.

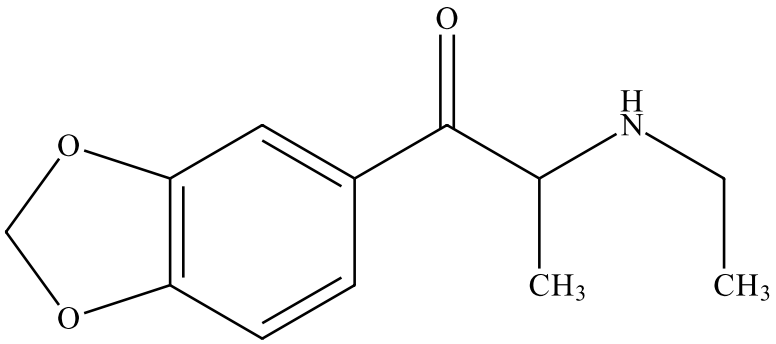

Figure 6A

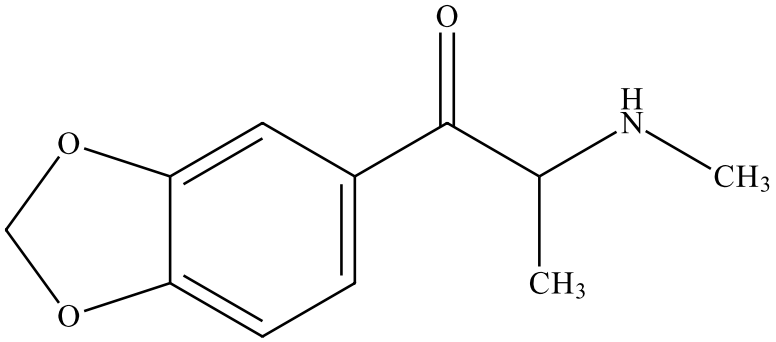

Figure 6B

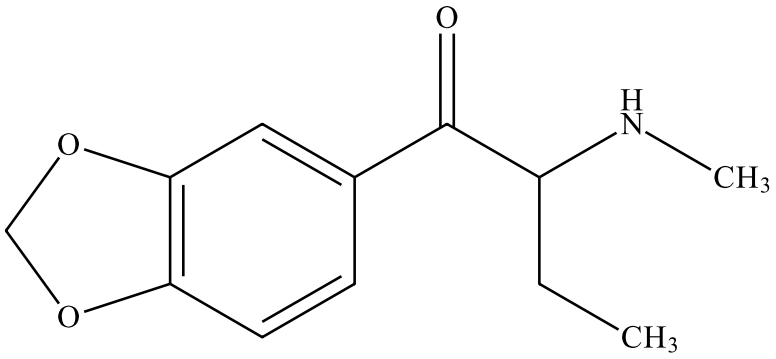

Figure 6C

Figure 6. Chemical structures of 6A ethylone, 6B methylone, 6C butylone (structures illustrated by author).

This is important because rapid synthetic cathinone development from the illegal drug market has made it difficult for forensic science to stay up to date. While illegal drugs can easily be synthesized and distributed, developing and accepting a new analytical method takes more time in the scientific field. Also, traditional methods for analyzing drugs, such as GC-MS, are not as useful in differentiating synthetic cathinones. Studies

have shown that methcathinone and derivatives are thermally unstable; thus, will undergo thermal degradation when analyzed with GC-MS. While degradation can be minimized: lowering injection temperatures, decreasing time in the inlet, and eliminating active sites during chromatographic analysis, separation may not be resolved enough to be useful in forensic analysis. For forensic science, retention time and mass spectrum are used to identify a compound; therefore, fully resolved compounds, peaks that are completely separated on a chromatogram, are required when performing a forensic analysis. Also, because separation is not fully resolved, mass spectral data will be a combination of the two or more coeluting compounds. This will result in a mass spectrum containing data from multiple compounds and make it more difficult to compare and identify cathinones with GC-MS (Kerrigan et al., 2016). While there are some GC-MS methods for distinguishing synthetic cathinones, they are usually in a blood, urine, or saliva mixture. Meaning, the methods are more useful for toxicology analysis, not drug analysis. There has also been success using HPLC, but again the drug is in a matrix and often the detector used with HPLC is not the traditional MS. Detectors such as ultra violet (UV), tandem mass spectrometer (MS-MS) and time of flight-mass spectrometer (TOF-MS) are used in research, but are not commonly found in a forensic laboratory. Both GC-MS and HPLC have been used in chiral separation, but each drug is tested individually and the analyst already knows what the compound is (NMS Laboratory). Methods do not consider how to narrow down the possible synthetic cathinones present. Analysis of synthetic cathinones is possible with microcrystal tests. Microcrystal tests require little sample, are quick, efficient, and will still work when adulterants are present. Also, even if

adulterants are present, the hanging drop method is a simple solution to separate the compound of interest from the adulterants.

A method to identify the three drugs was developed using microcrystal tests. Crystal formation and optic properties were used to further differentiate the drugs. Attenuated total reflectance-infrared spectroscopy was used to additionally identify the drugs and precipitated crystals.

C. Related Literature

1. *A Modern Compendium of Microcrystal tests* is a compilation of microcrystal tests for commonly found drugs in forensic analysis. It was compiled by McCrone Research Institute and is used by state forensic laboratories to assist in identification of unknown compound

II. Materials and Methods

A. Samples

All three synthetic compounds were procured from Cayman Chemical in their hydrochloride salt form. Lot numbers are 0463055-6 for Methylone, 0444658-36 for Butylone, and 0470794-8 for Ethylone.

Acetaminophen, procaine HCl, starch (potato), and palmitic acid were procured from Sigma-Aldrich. Lot numbers are 088120040, S42504-365, 33H0566 respectively. Lactose anhydrous is from USP (Sheffield), lot number 07839.

Picrolonic acid is from Santa Cruz Biotechnology, lot number F2812. Picric acid was obtained from McCrone Research Institute.

B. Microcrystal Tests

A Leica EZ4 compound stereomicroscope was used to aid in transferring drugs onto a slide. A tungsten needle was used to transfer the drugs. An Olympus BX51-P microscope was used to view the crystals and a SPOT Insight Firewire camera was used to take photomicrographs.

There are two ways to sharpen tungsten needles: chemical and electrolytic. Chemically, tungsten needles are made by clipping a tungsten wire into about two-inch-long pieces. Each piece is then sharpened to a fine point using sodium nitrite. Solid sodium nitrite is adhered onto a piece of cork. Afterwards, using a controlled flame, the two-inch-long tungsten wire is drawn through the sodium nitrite as it softens from the flame. This is continued until the wire is sharpened. For more control over the shape and fineness of the needle, the electrolytic method is preferred (Bowen).

While compiling the microcrystal test compendium, McCrone Research Institute developed an easy method to measure the amount of sample used. The unit of measurement is a period on a printed page (PPP). One PPP is equal to a sample filling the area of a period at Times New Roman 10 point font (Figure 7). The word document is either viewed at 100% or printed out. The resulting period is the measurement used to determine sample amount. 1 PPP is approximately 0.1 mg. This measurement is used by McCrone Research and can be found in their compendium. PPP was used to determine the tested limit of detection and the tested upper limit of detection. It was also used so consistent amounts were used each time.

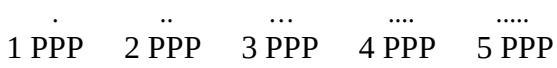

Figure 7. PPP measurement system used to measure samples.

Each drug was tested with multiple reagents to determine the most suitable reagent. The list of reagents was compiled by McCrone Research Institute from various sources. The reagents at the top are ones most commonly found in a forensic laboratory with the likelihood decreasing down the list. More tests were accomplished with butylone and methylone because the procured samples were larger for those drugs than ethylone. To narrow down the reagents, precipitated crystals had to be abundant, form when adulterants were added, and reproducible. A standard of precipitated crystals for each drug was taken each day tests were performed with that drug, over thirty times each.

Since adulterants are commonly found in forensic samples, common adulterants for synthetic cathinones were tested with each microcrystal test. Most samples are composed of 1 part drug to 10 parts adulterant(s). Therefore, 10 PPP of each adulterant was added separately to 1 PPP of each drug. If the same crystals did not form, then the adulterant concentration was lowered to 5 PPP adulterant to 1 PPP drug. A combination of all six adulterants, totaling 10 PPP, was tested with each drug to ensure crystals still formed when adulterants were combined. Microcrystal tests with adulterants were performed more than three times.

1. Direct Method

There are two microcrystal test methods: direct and hanging drop. The direct method can then be split into two different methods. The reagent can either be added directly to a dry sample or the drug can be dissolved in a solution and then the reagent added. Both of these methods can also be performed with or without a coverslip. When a coverslip is used, the reagent is placed on the coverslip and then placed onto the sample. When no coverslip is used, the reagent can be stirred once added to the sample. All samples were tested with 5 μL of reagent without a coverslip or stirring the solution. For an aqueous solution, 5 μL of water was added to the sample and then 5 μL of the reagent was added the aqueous solution with or without a coverslip, but with no stirring. The aqueous (adding 5 μL of water) and dry methods with and without a coverslip were tested for all of the reagents listed in tables I-III, appendix C, with butylone, ethylone, and methylone. A list of all the reagent recipes is shown in appendix D (Fulton, 1969). Adulterants were tested with each drug in various mixtures to ensure identifiable crystals would still form. The adulterants were starch, lactose, caffeine, palmitic acid, procaine

HCl, acetaminophen, butylone, ethylone, and methylone. A blank of each reagent was taken to ensure the reagent was not contaminated and precipitated reagent crystals were not mistaken for sample crystals. A positive control of each drug was taken first. 1 PPP was used first and if crystals formed with 1 PPP of the drug, then that was determined as the tested limit of detection. The tested upper limit of detection was 5 PPP and was tested to ensure crystals still formed at higher concentrations.

2. Hanging Drop Method

The hanging drop method is used to separate a drug from adulterants. Many forensic drug samples are mixed with adulterants because the adulterants are cheaper and sometimes they give a bonus effect. However, when using a microcrystal test for analysis, those adulterants can hinder crystal growth so it is important to be able to separate the drug from adulterants. This is possible by using the hanging drop method. A small glass ring is placed around the sample and then a small drop of 10% concentrated sodium hydroxide is added to the sample. Immediately afterwards, a glass coverslip, with a 5 μL of reagent placed on it, is inverted onto the glass ring. The sodium hydroxide volatilizes the sample, forcing it into a gaseous state. As the sample rises, it comes into contact with the reagent and crystals will form within the reagent droplet. This method will only work if the drug can be volatilized.

C. Polarized Light Microscopy

An Olympus BX51-P microscope was used to view crystals and determine optical properties. The microscope had a first order red compensator (530 nm) to assist in determining sign of elongation. Liquids of known RI from Cargille Labs were used to identify RI.

1. Köhler Illumination

The Olympus microscope was set up with Köhler Illumination. This was accomplished by focusing and centering the field diaphragm and aligning the center of rotation to the middle of the field of view for each objective lens.

2. Calibration of the Micrometer Ocular

To determine the size range of each precipitated crystal, the micrometer ocular was calibrated. The micrometer ocular is an eyepiece containing an uncalibrated scale. The eyepiece is used as either the right or left eyepiece, depending on which eye is dominant. Once inserted, the ocular scale was brought into focus by rotating the focusing top lens. A microscope slide containing a known calibrated scale was then placed on the microscope stage. Usually, the scale is equivalent to 1000 μm split into one hundred divisions, each division equivalent to 10 μm. The scale on the microscope slide was then brought into focus. Using Köhler illumination, both the microscope slide and ocular scale were simultaneously in focus. The two scales were aligned so they overlay each other. From there, the furthest left and right portion where each scale aligned was determined. The difference between the furthest right and left was subtracted for each individual scale. The microscope slide scale was then divided by the ocular scale; thus, calibrating the ocular scale. For example, when calibrating the ocular scale for the 10x objective, the microscope slide scale and ocular scale align at three and thirteen divisions respectively and at eighty-eight and ninety-seven respectively, then the lower number is subtracted from the higher number for each scale. In this example, three is subtracted from eighty-eight to equal eighty-five divisions for the microscope slide and thirteen is subtracted from ninety-seven to equal eighty-four for the ocular scale. The stage scale is

then multiplied by the number of microns per each division, if 10 μm per division, then multiplied by ten. The product is divided by the ocular scale to equal 10.12 μm per ocular scale division. Equation 2.1 represents this example in a mathematical format.

$$\frac{SS \times 10}{OS} = microns\ per\ ocular\ stage\ division \quad (2.1)$$

where SS is the stage scale divisions and OS is the ocular stage divisions. This process must be repeated for each objective lens and if different ocular scales are used (McCrone et al., 2014, appendix 6).

3. Sign of Elongation

To determine sign of elongation using a compensator, the crystal must be anisotropic. Next, the slow direction of the compensator was determined. It is usually marked with a γ, n_g, or Z to indicate the slow direction. The crystal was oriented so the crystal's length is diagonal from bottom left to top right. This positioned the refractive index parallel to the length of an elongated crystal, n1, parallel with the compensator's slow direction of vibration and the refractive index perpendicular to the length, n2, parallel with the compensator's fast direction of vibration. An example can be seen in figure 8. Once the crystal is aligned, the polars are crossed and the crystal's retardation is judged using the Michel-Lévy chart, appendix B. Then the compensator is inserted. The sample has a positive sign of elongation if 530 nm are added, but negative if it is subtracted. For instance, nylon has an average retardation of 730 nm. When a first order red compensator is added, its retardation is about 1260 nm. Thus, it has a positive sign of elongation.

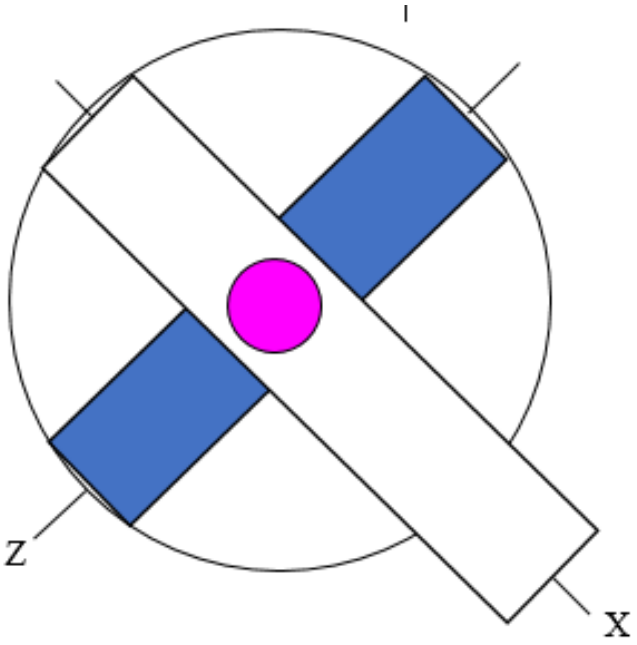

Figure 8. Shows the parallel alignment of the compensator's slow vibrational direction with a crystal's elongated RI (n1). Where the blue represents the crystal, pink is the first order red compensator, X indicates the compensator's fast vibrational direction, and Z the slow vibrational direction (illustrated by the author).

4. Refractive Index

To determine the exact refractive indices of a sample, Cargile liquids of known and standardized refractive indices were used. Refractive index was determined by using the Becke line test. The Becke line test allows a microscopist to determine which medium has a higher refractive index: the mounting medium, in this case the Cargille liquid, or the sample. The Becke line is the orange halo around the sample. Before the Becke line test was be performed, the microscope was properly set up. An orange filter was placed on top of the field lens so that only one wavelength was transmitted through the microscope. An orange filter was used because the Becke line will only be visible at the sodium D line wavelength, 589.3 nm, when used with those Cargille liquids.

Unfiltered light will not show the Becke line because it is an average of light's wavelengths. If the Cargille liquids used required a sodium C or F line to view the Becke line, then a red or blue filter would have been used respectively. The liquids were used at room temperature so the liquid's RI were that which was labeled. Otherwise, the new RI needs to be calculated if the temperature is not about twenty-five degrees centigrade. As the temperature increases the RI of the liquids will decrease. Next, the condenser aperture was closed so resolution and depth of field were increased. By increasing both resolution and depth of field, it is much easier to see which medium the Becke line enters. Finally, liquids of known refractive index were used as a point of measurement. Once the microscope was set up a sample was prepared.

Since Cargille liquid was used as the mounting medium, the crystals had to be dried. There can be no other liquid combined with the sample, except the Cargille liquid. Therefore, the sample was either air dried or the reagent was wicked away with a Kimwipe®. If the reagent could not be fully removed, then the refractive indices were not determined. Also, if the crystals were too small or thin to accurately see the Becke line, the refractive indices were not determined. After the sample was completely dried, a coverslip was placed over the sample and Cargille liquid of known refractive index was added to the edge of the coverslip. The liquid then migrated underneath the coverslip until the entire area was filled. The sample was placed on the stage and focused. The focus was then raised, meaning the stage was lowered. The Becke line test was performed until the needles of the rosettes were transparent in the liquid, indicating the RI of that particular orientation, or until it was determined the RI was greater than 1.700. 1.700 was the cut off because most RI sets in a laboratory do not exceed that RI.

D. Attenuated Total Reflectance Infrared Spectroscopy

An Olympus BX60 microscope with a Smiths IlluminatIR II and a SensIR ATR objective lens was used to collect ATR-IR data. The crystals were placed on a MirrIR slide.

ATR-IR utilizes a diamond on the end of an objective lens to gather infrared spectra. This method was used because both reagents are acidic and would erode other materials. Also, diamond is more robust than other materials. Before the instrument was turned on, liquid nitrogen was poured into the IR detector to keep it cool (ThermoScientific, 2013). This helps increase scan speed, sensitivity, and spatial resolution. The crystal, diamond in this case, was cleaned with an absolute ethanol dampened Kimwipe®. An infrared background was collected first to eliminate noise collected during runs. This was collected before each test. The sample was then placed on the stage and the ATR objective was then lowered until crystal was directly in contact with the sample. This caused the crystals to become flattened and lose their form. However, this is acceptable because then the entire area in contact with the ATR-IR crystal was from the precipitated crystal or the drug's hydrochloride salt form. A spectrum of the sample was then collected and the process was repeated three times.

III. Results and Discussion

A. Microcrystal Tests

For butylone, ethylone, and methylone, the hanging drop method did not work because they are too heavy to be volatilized; therefore, the hanging drop method is not included Tables I-III, appendix C, showing the results of how each drug reacted with each reagent. Although several reagents precipitated crystals when added to a sample, when adulterants were added, many times the crystals would not form unless the adulterants were at a lower concentration (usually 5 or 1 PPP). For that reason, picrolonic and picric acid were the final reagents used to identify butylone, ethylone, and methylone because precipitated crystals are abundant with and without adulterants, the results are instantaneous, and only two reagents are required to identify three drugs.

Each drug formed rosettes when added to picrolonic or picric acid. Tables IV-VI, appendix E, summarize the crystals formed, various limitations, and optical properties if applicable. When more than one drug was tested together, there were no hybrid crystals. Instead, each drug formed its own crystals as if the other drug was not present. If methylone was added to a butylone sample and 5 μL of reagent was added, each crystal associated with either drug formed. The same occurred with the other combinations. This is important because it means even if there is a mixture of these three drugs, their individual crystals will still precipitate and can be identified. Of the adulterants, only caffeine precipitated needle crystals, shown in figure 9, in both picric and picrolonic acid. All six adulterants were tested individually with each drug and with each combination of butylone, ethylone, and methylone and all six adulterants were combined and tested with each drug. When all six adulterants were combined, to be tested with a synthetic

cathinone, they were analyzed as a 10:1 concentration of adulterants to drug. Thus, there was a little more than 1 PPP of each adulterant when executing this test. Tables VII-IX, appendix E, summarize the ratio of adulterant(s) to drug that still allowed crystals to precipitate. The highest ratio was 10 PPP of adulterant(s) to 1 PPP of a drug.

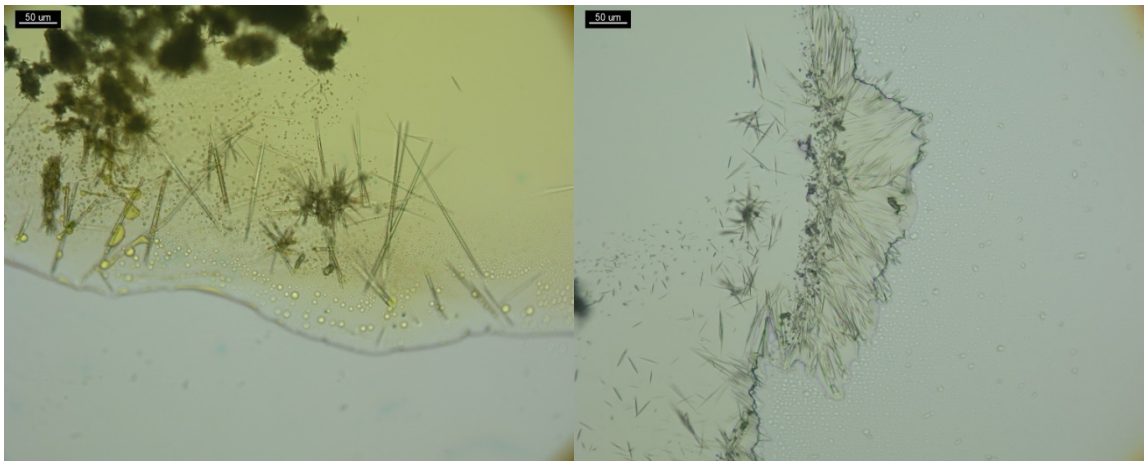

Figure 9A Figure 9B

Figure 9. Photomicrographs at 200x magnification of caffeine needle crystals precipitated in 9A picric acid, 9B picrolonic acid.

1. <u>Picrolonic Acid</u>

Both butylone and methylone formed rosette crystals immediately when 5 µL of picrolonic acid was added to 1 PPP of each. Butylone can be identified separately from methylone based on the rosettes formed and optical properties. Butylone forms one kind of rosette with varying sizes, shown in figure 10, while methylone forms two distinct types of rosettes, shown in figure 11. Methylone's smaller rosettes are more condensed

and the rosette's needles are more sparse. The smaller rosettes also have a yellow-brown color while the larger rosettes have a more transparent color. Butylone formed one type of rosette that are yellow-brown in color and the needles are very abundant. Butylone and methylone formed crystals with a (+) sign of elongation. When tested with adulterants, both drugs formed the same unique crystals when 5 µL of reagent was added.

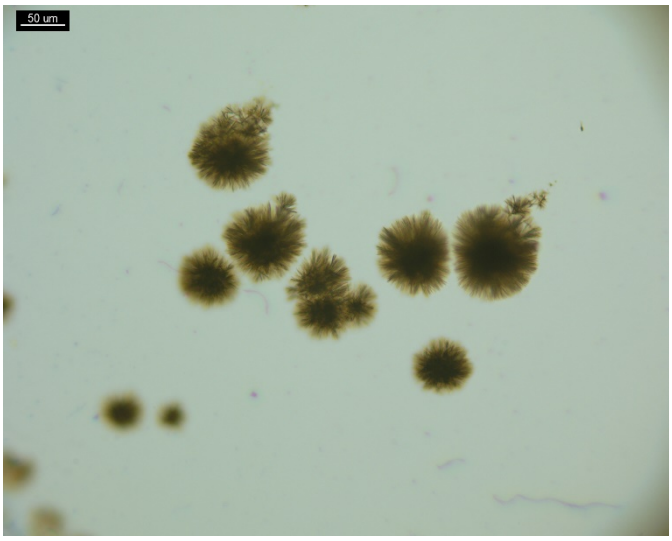

Figure 10. Photomicrograph of 1 PPP butylone and 5 µL of picrolonic acid at 200x magnification in polarized light. Depicts precipitated crystals that are yellow-brown and the needles are straight with little to no side branching.

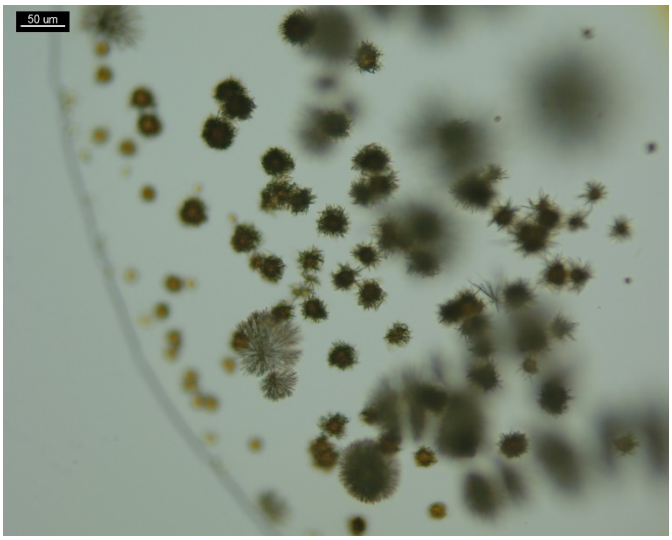

Figure 11. Photomicrograph of 1 PPP methylone in 5 µL of picrolonic acid taken at 200x magnification. Polarized light displays two distinct crystals. One crystal is smaller and yellow-brown while the larger crystals are translucent.

2. Picric Acid

Picric acid formed rosettes immediately when added to ethylone and methylone. It also formed crystals when added to butylone, but the results were inconsistent. Therefore, picric acid was not used as a reagent for butylone. Ethylone's needles start to branch off almost immediately from the rosette center, as shown in figure 12. Methylone's needles do not branch off until closer to the middle of the needle, as shown in figure 13. Adulterants were added and crystal habit remained the same for each drug.

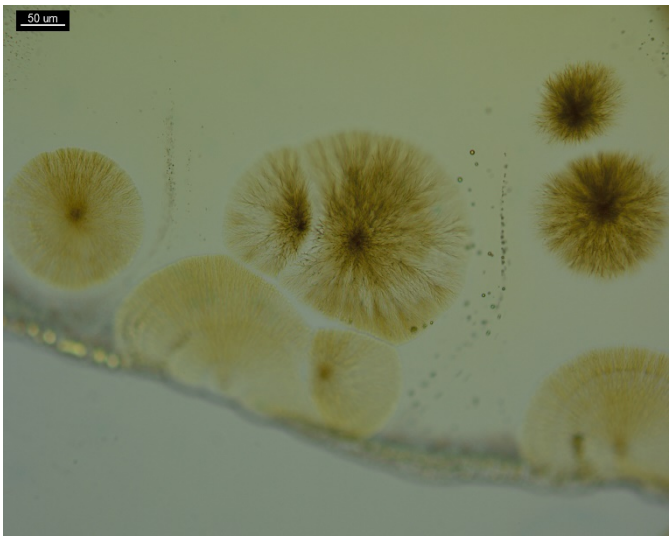

Figure 12. Photomicrograph of 1 PPP ethylone in 5 μL of picric acid at 200x magnification. Polarized light depicts large yellow rosettes that have very slight branching

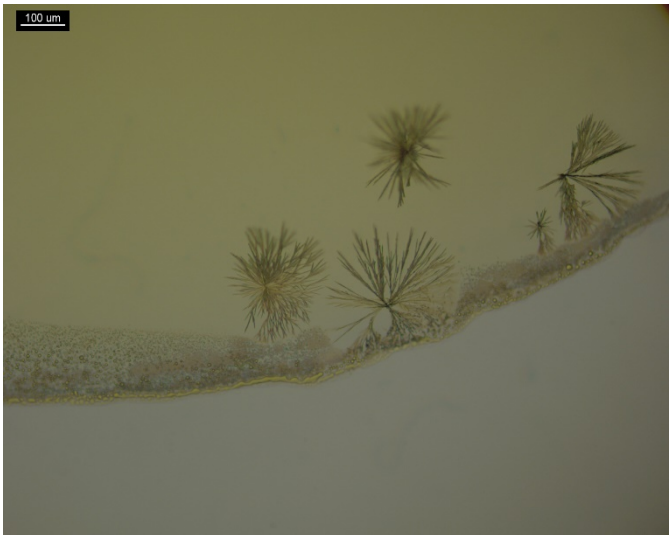

Figure 13. Photomicrograph of 1 PPP methylone in 5 μL of picric acid. Polarized light at 100x magnification illustrates rosettes that are feathery and branch closer to the tips.

B. Optical Properties of Microcrystals

1. Sign of Elongation

Ethylone and methylone crystals precipitated in picric acid have a negative sign of elongation, shown in figure 14 and 15.

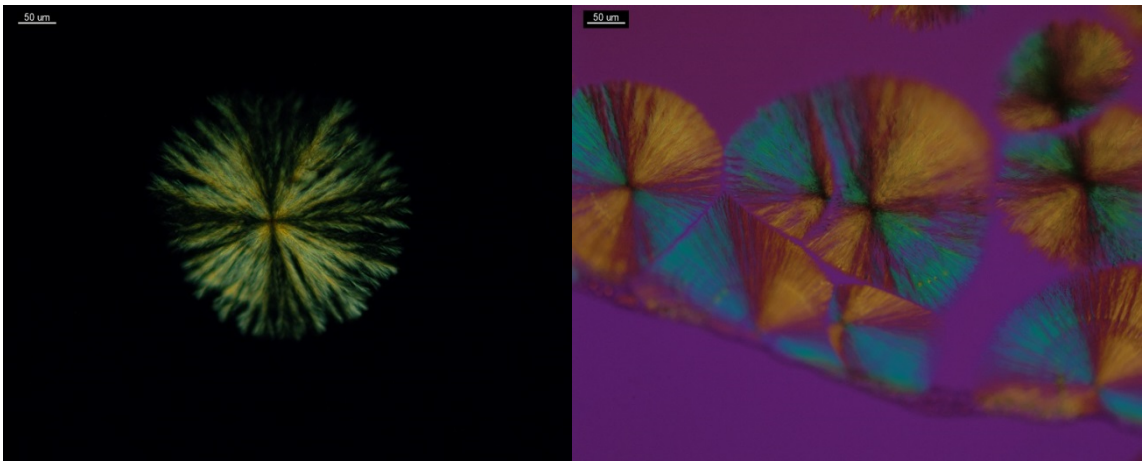

Figure 14A Figure 14B

Figure 14. Photomicrographs of 1 PPP ethylone in 5 µL of picric acid at 200x magnification 14A crossed polars depict the rosette needles have a first order grey color, 14B crossed polars with 1st order red compensator, indicating (-) sign of elongation because the rosette needles now have a yellow color due to 530 nm being subtracted

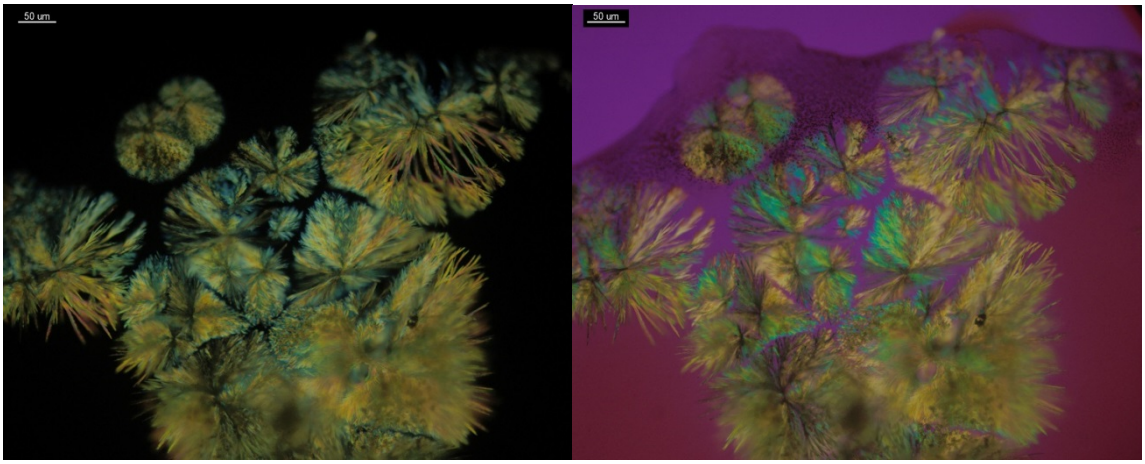

Figure 15A Figure 15B

Figure 15. Photomicrographs of 1 PPP methylone in 5 μL of picric acid at 200x magnification 15A crossed polars illustrates the rosette needles have a first order grey color. This color is only apparent at the tips of the needles because the other parts are overlapping with several needles, 15B crossed polars with 1st order red compensator, indicating (-) sign of elongation because the color changes to a first order yellow, which indicates that 530 nm is subtracted.

Butylone precipitated crystals in picrolonic acid have a positive sign of elongation. This is noted by the first order grey, grey along the needle's tips, changing to blue when the first order red compensator is inserted and is shown in figure 16. Methylone's two crystals precipitated in picrolonic acid have a different sign of elongation. The smaller rosettes have a positive and the larger rosettes have a negative sign of elongation. Figures 17 and 18 correspondingly depict the sign of elongation for the smaller and larger crystals.

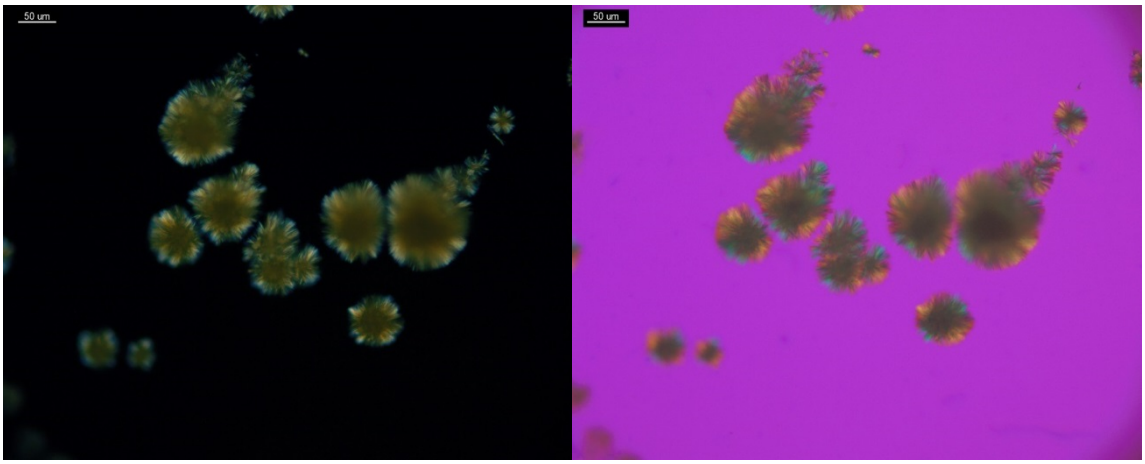

Figure 16A Figure 16B

Figure 16. Photomicrographs of 1 PPP butylone and 5 μL of picrolonic acid at 200x magnification 16A depicts crystals in crossed polars, showing the needles are grey, thus, in the first order on the Michel-Lévy chart, 16B crossed polars with 1st order red compensator, indicating (+) sign of elongation because the needles oriented from bottom left to upper right are blue, which is achieved when 530 nm are added to the grey on the Michel-Lévy chart.

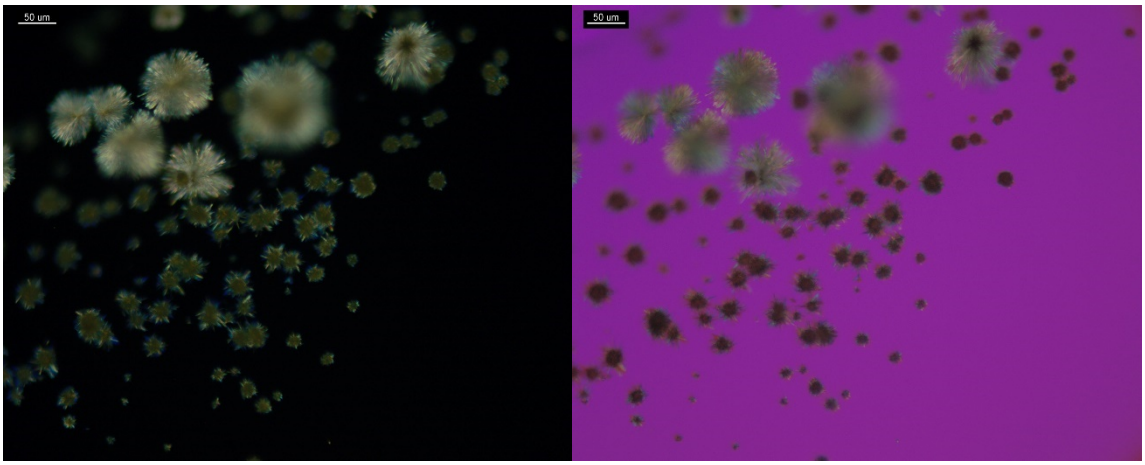

Figure 17A Figure 17B

Figure 17. Photomicrographs of 1 PPP methylone in 5 μL of picrolonic acid taken at 200x magnification 17A crossed polars display the smaller crystals have first order grey on the Michel-Lévy chart, 17B crossed polars with 1st order red compensator indicate (+) sign of elongation for the smaller crystals because the needles are now blue due to the addition of 530 nm.

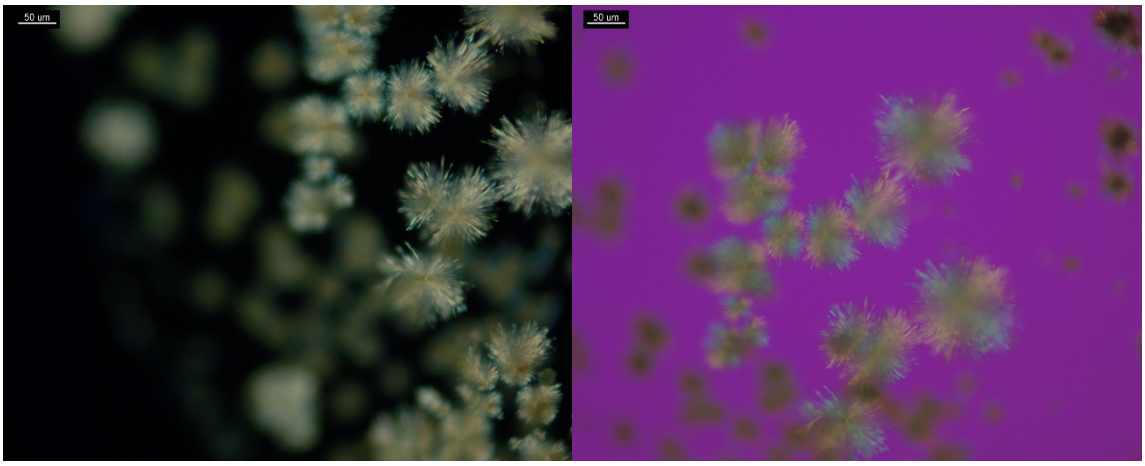

Figure 18A Figure 18B

Figure 18. Photomicrographs of 1 PPP methylone in 5 μL of picrolonic acid taken at 200x magnification 18A crossed polars display the larger crystals have first order grey on the Michel-Lévy chart, 18B crossed polars with 1st order red compensator indicate (-) sign of elongation for the larger crystals because the needles are now yellow due to the subtraction of 530 nm.

Sign of elongation can be used to differentiate two crystals. Butylone and methylone can easily be distinguished from each other because butylone crystals have a positive sign of elongation while methylone's larger rosettes have a positive sign of elongation, but the smaller rosettes have a negative sign of elongation. This is unusual because the optical properties of a crystal cannot change. Instead, this indicates the precipitated methylone rosettes are biaxial crystals with two different orientations. The larger rosettes are oriented with a lower RI along the length of the needles while the smaller rosettes are oriented with a higher RI along the length of the needles. This uncommon occurrence will aid in detecting methylone. Comparing the ethylone and methylone crystals precipitated in picric acid, the ethylone crystals are more orange when

the first order red compensator is inserted. If an analyst is uncomfortable identifying ethylone or methylone with only picric acid, the sample can be tested a second time using picrolonic acid. Methylone forms two different rosettes when picrolonic acid is added, but ethylone will not precipitate crystals.

2. **Refractive Index**

Refractive index is a method commonly combined with microcrystal tests. Of the four crystals formed, only one, methylone in picrolonic acid, could be examined to determine its refractive indices. The other three crystals have needles with a diameter which are too small to accurately perform the Becke line test or the reagent, picric acid, is not easily dried or wicked away from the crystals. Methylone has two refractive indices: $n1 = 1.532$ and $n2 > 1.700$. Figure 19 is a photomicrograph of methylone in 1.532 RI liquid. The horizontal needles appear transparent because the RI of the needles are the same as the RI medium. A photomicrograph of methylone in 1.700 RI was not taken because the n2 RI is greater than 1.700.

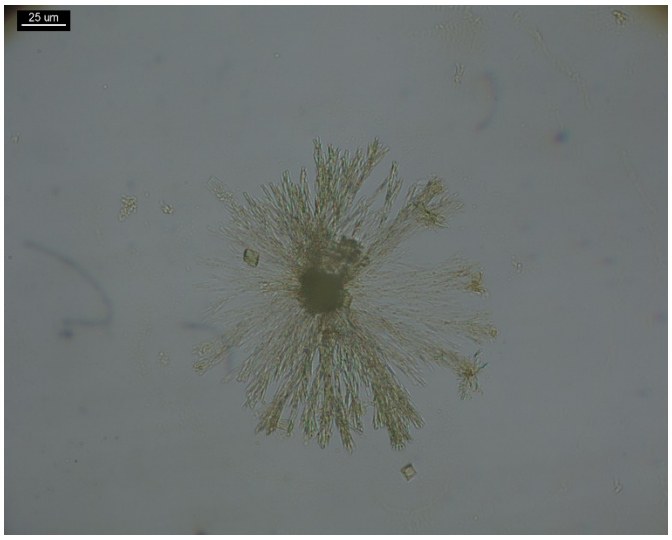

Figure 19. Photomicrograph of methylone crystal precipitated in picrolonic acid mounted in 1.532 RI liquid. The horizontal needles are transparent because the RI of the medium and crystal are the same. However, the outline of the needles is still slightly visible because the edges are not perfectly straight.

C. Attenuated Total Reflectance Infrared Spectroscopy

The spectrum of each crystal does not match the spectrum of its associated drug. This occurs because the crystal no longer has the same composition of the drug. Instead, the crystal is a mixture of drug and reagent. Figures 20-22 are spectra of each drug: butylone, ethylone, and methylone. Figures 23 and 24 are spectra of butylone and methylone crystals in picrolonic acid. Crystals precipitated with picric acid were not taken because picric acid did not dry and was not easily wicked away. Changes between the drug and precipitated crystals can be seen in the fingerprint region, where carbon bonds are indicated, and in 2200 – 3000 cm^{-1} range.

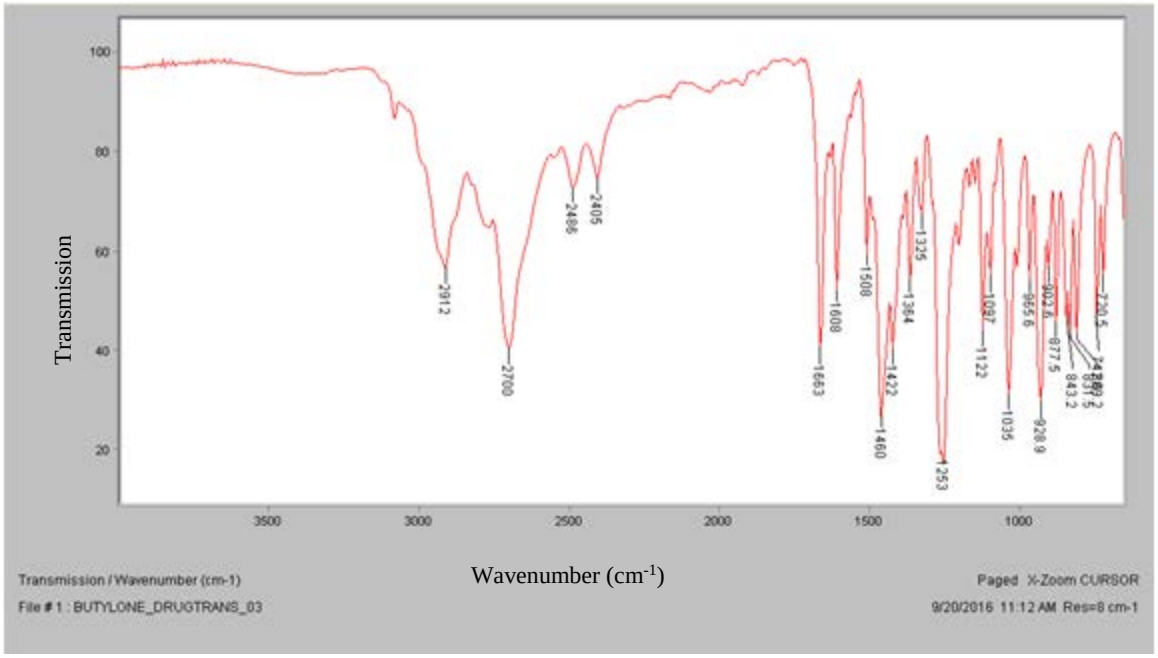

Figure 20. ATR-IR spectrum of butylone hydrochloride salt.

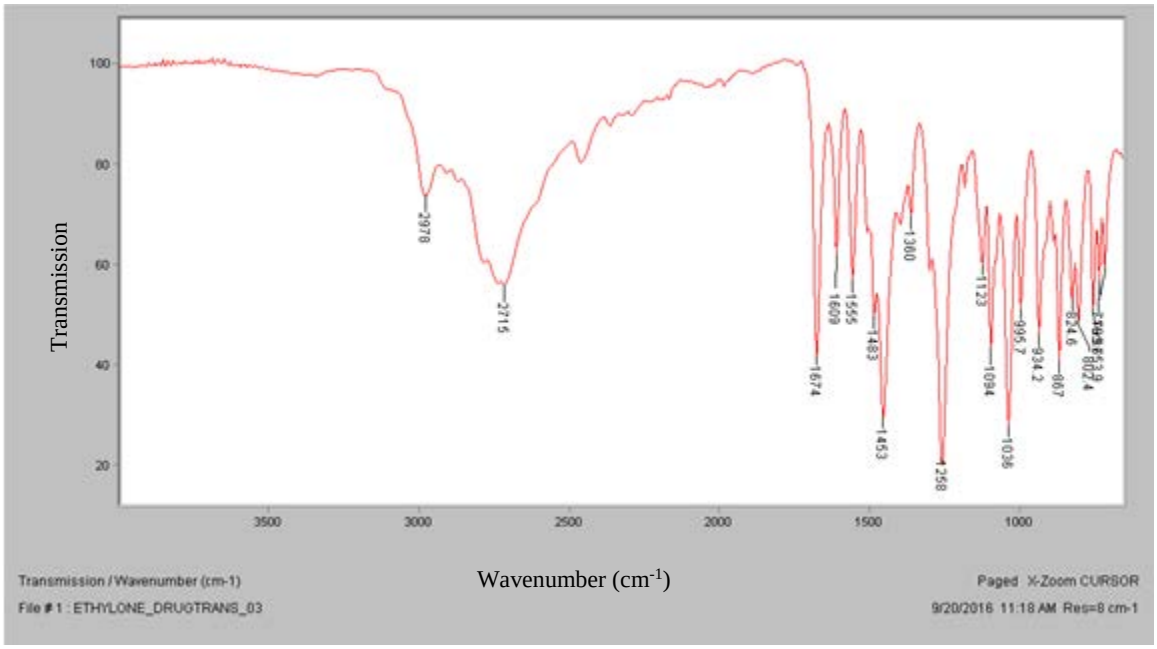

Figure 21. ATR-IR spectrum of ethylone hydrochloride salt.

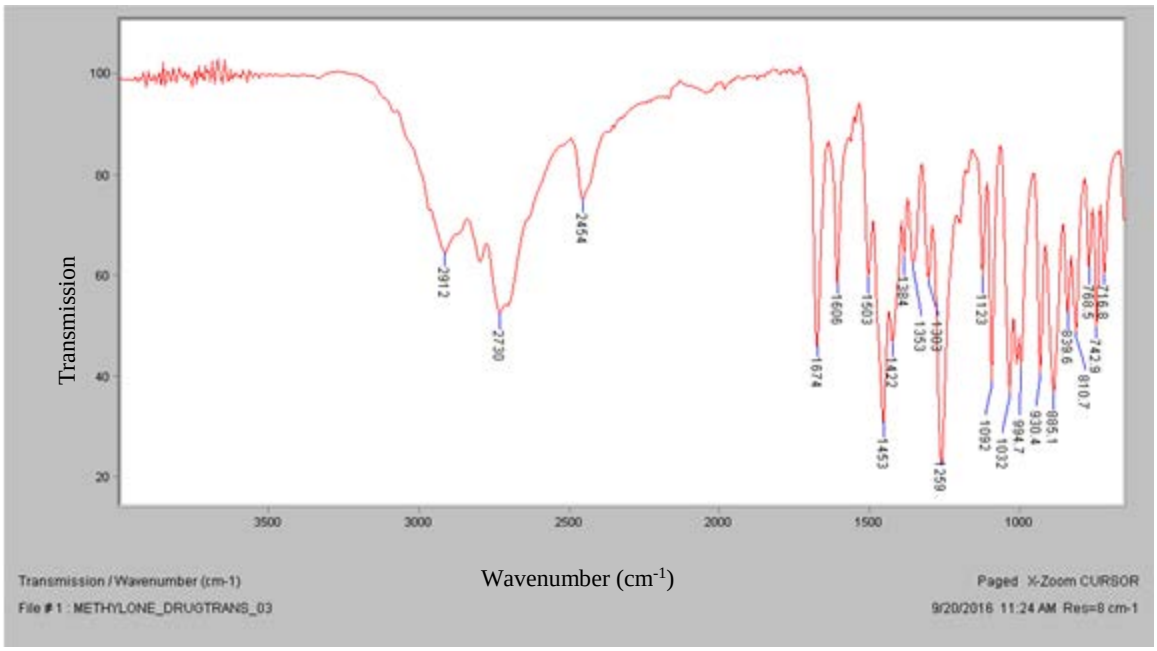

Figure 22. ATR-IR spectrum of methylone hydrochloride salt.

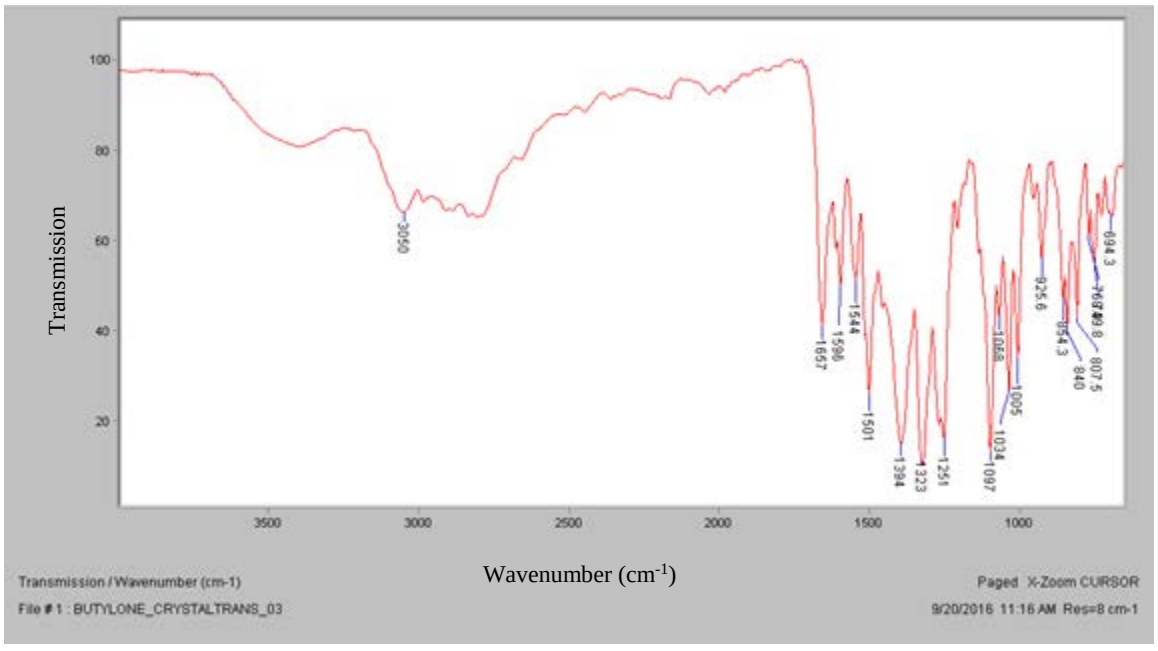

Figure 23. ATR-IR spectrum of butylone picrolonic acid precipitate. Changes occur in the fingerprint region and between 2200 – 3000 cm^{-1} when compared to Figure 20.

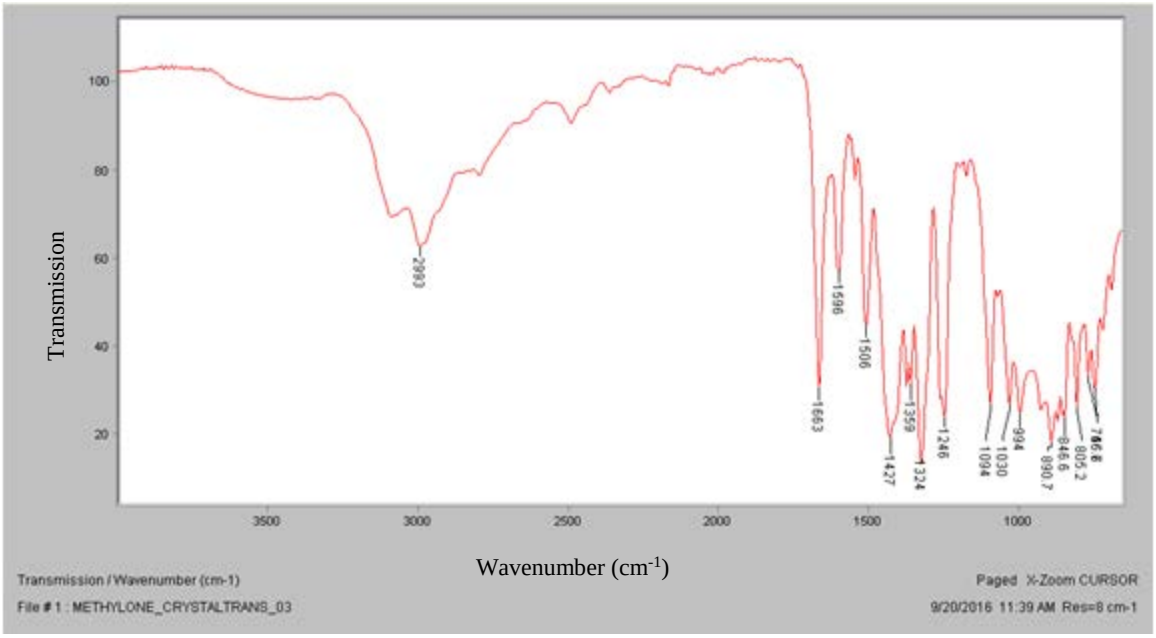

Figure 24. ATR-IR spectrum of methylone picrolonic acid precipitate. Changes occur in the fingerprint region and between 2200 – 3000 cm^{-1} when compared to Figure 22.

IV. Conclusion

There are many synthetic cathinones on the illegal drug market and new ones are developed every year. Due to the increasing volume, it is difficult for acceptable methods to be validated before new synthetic cathinones replace the old ones. The amount of time required to perform an analysis is also important. However, if microcrystal tests are used to presumptively identify unknown compounds, it will be easier and more expedient to analyze unknown samples. Microcrystal tests require small amounts of sample and even that amount can, in theory, be recovered. Crystals can precipitate in as little as five seconds, with minimal sample preparation required. When multiple microcrystal tests are used together there is little to no error rate when compared to instrumental techniques. When morphology, optical properties, and ATR-IR spectra are compared, drug identification is obtainable. Butylone, ethylone, and methylone formed unique crystals that can be identified when observing crystal morphology and optical properties.

V. FUTURE WORK

While a method to identify three synthetic cathinones has been developed, there are still many more synthetic cathinones that do not have identification methods. Future work should be focused on developing methods that can help identify the remaining and any newly developed synthetic cathinones. New synthetic cathinones are developed constantly to circumvent law enforcement, so it is important that new methods are developed accordingly. Also, to further enhance identification, a second microcrystal test should be developed for butylone and ethylone. This will further decrease the likelihood of misidentifying a sample.

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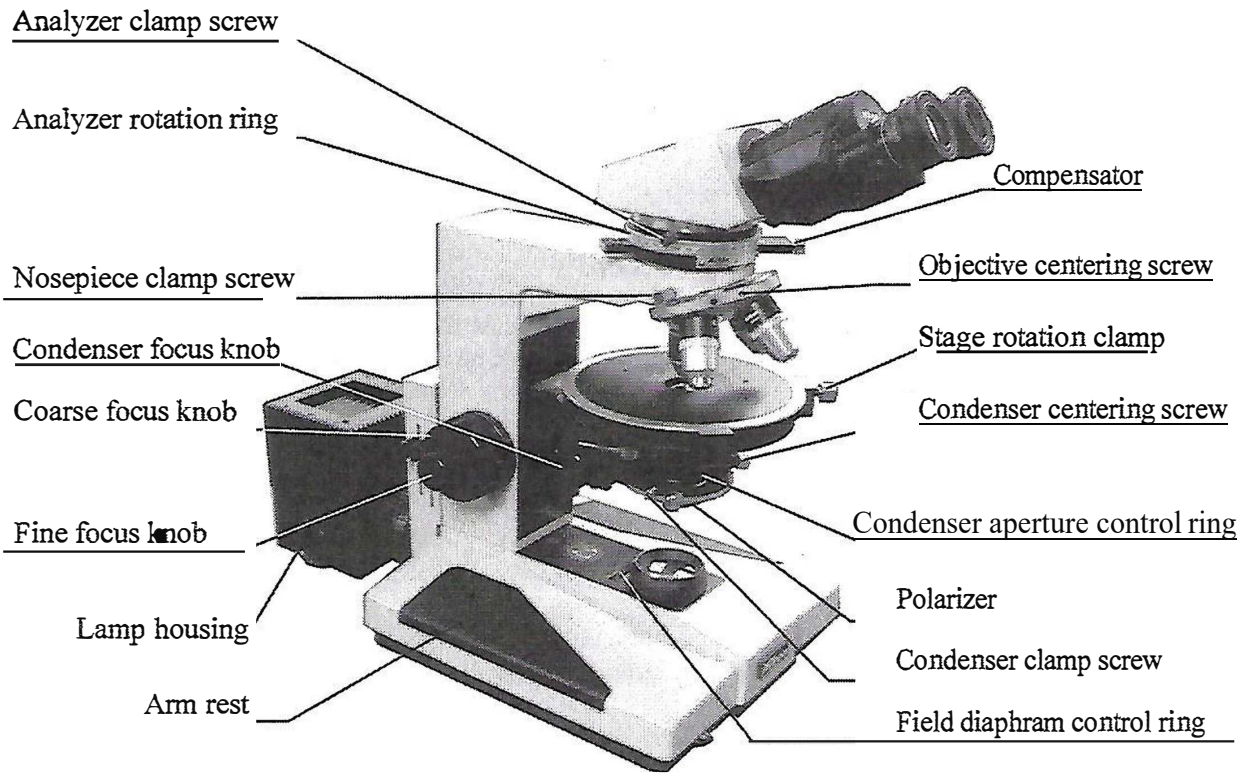
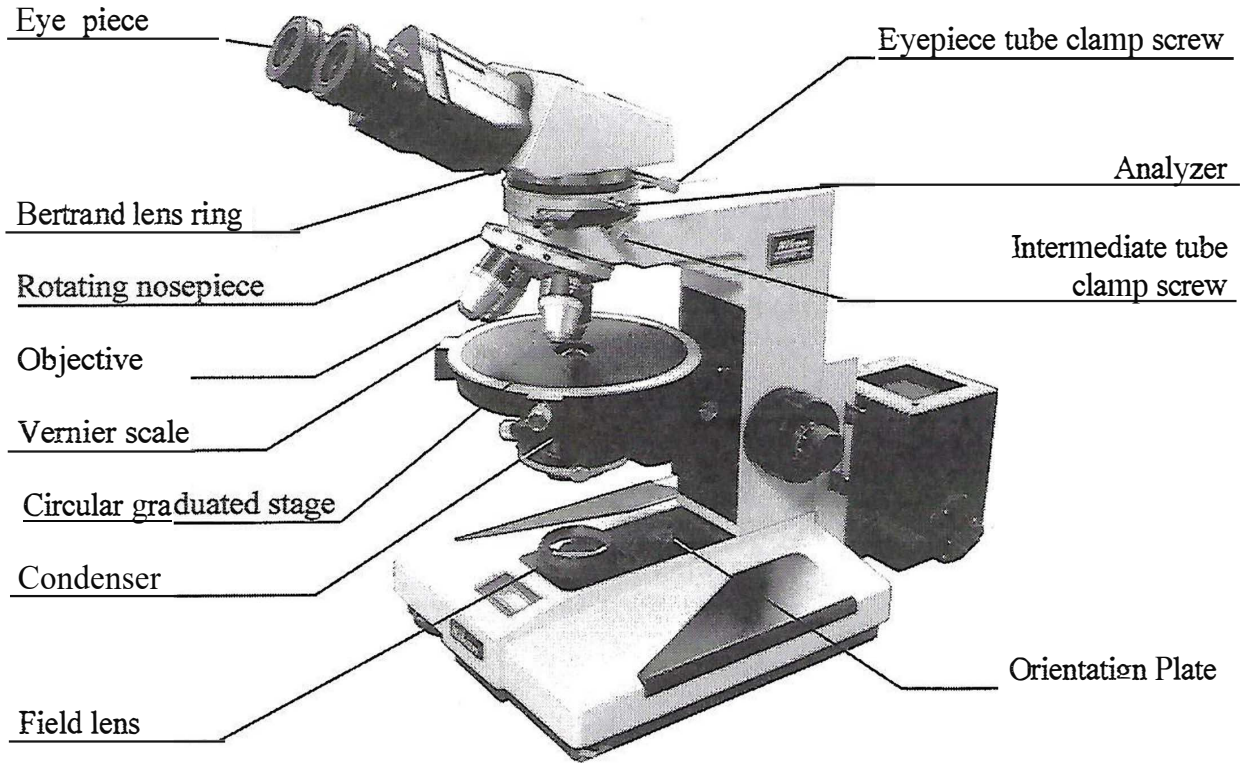
APPENDIX A

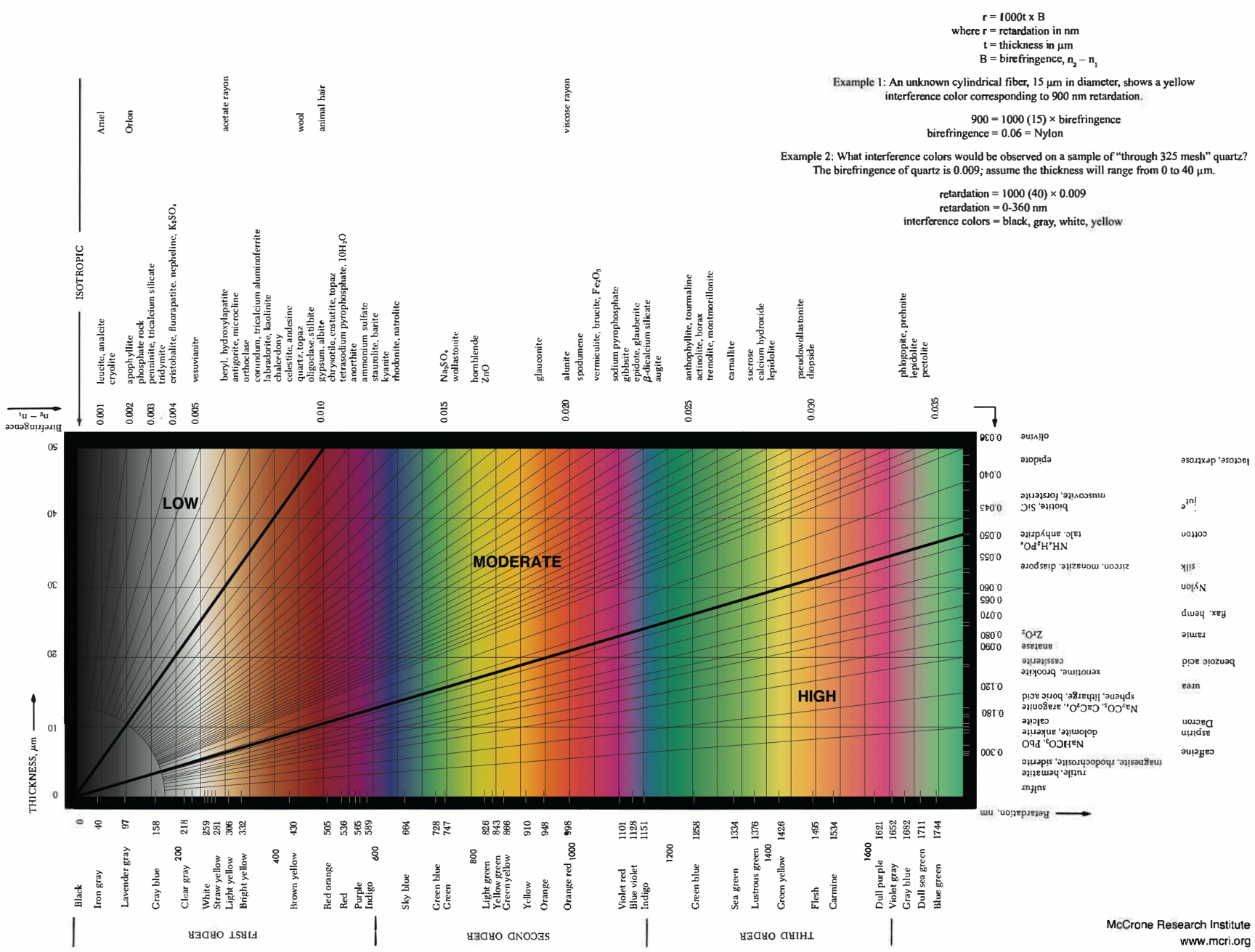
NIKON OPTIPHOT-POL





APPENDIX B

Michel-Lévy Chart



APPENDIX C

TABLE I
RESULTS OF REAGENTS TESTED WITH BUTYLONE

Reagent	Test Drop: Crystal formation?				Crystal(s)
	Dry		Aqueous		
	Coverslip	Without	Coverslip	Without	
H_3BiI_6-(aqueous)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_3BiI_6 in (1+7) H_2SO_4	☐ Y ☒ N	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	Large red clusters
I-(2.75)KI	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
I-(50)KI	☐ Y ☒ N	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	Plates and pyramids
$HAuBr_4$ in (2+3) H_2SO_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuBr_4$ in conc. HCl	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuCl_4$ (aqueous)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuCl_4$ in (1+3) HCl	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Picric acid (sat'd aqueous)	☐ Y ☒ N	☒ Y ☐ N	☐ Y ☒ N	☒ Y ☐ N	Dry: Clusters Aq: Fans and tuffs
K_2CdI_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
PbI_2-KOAc solution	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_2PtCl_6 (aqueous)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_2PtCl_6 in (1+3) H_3PO_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_2PtBr_6 in (2+3) H_2SO_4	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	Clusters and plates
Platinum cyanide reagent	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuBr_4$ in H_3PO_4-5HOAc (50 mL)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuBr_4$ in $2H_3PO_4$-1HBr	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	

APPENDIX C (continued)

$HAuBr_4$ in HOAc-3(2+3)H_2SO_4 (40 mL)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuCl_4$ in (1+1) H_2SO_4 (20 or 60 mL)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
I-HI-7H_3PO_4, cg	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Picric acid in HOAc-$Mg(OAc)_2$ solution	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
PbI_2-KOAc in HOAc-$Mg(OAc)_2$ solution	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Iodine-KI	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_2PtI_6 (HCl)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Phosphoritungstic acid	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Bromine-HBr	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
K_2HgI_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HgCl_2$	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_2PdCl_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Picrolonic acid	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	Rosettes
$KMnO_4$	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
CrO_3	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
K_2CrO_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
KOH	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Na_2CO_3	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$K_4Fe(CN)_6$ in dilute hydrochloric acid	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	

Thiocyanate (NH_4SCN or KSCN)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	

TABLE II

RESULTS OF REAGENTS TESTED WITH ETHYLONE

Reagent	Test Drop: Crystal formation?				Crystal(s)
	Dry		Aqueous		
	Coverslip	Without	Coverslip	Without	
H_3BiI_6-(aqueous)	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
H_3BiI_6 in (1+7) H_2SO_4	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
I-(2.75)KI	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
I-(50)KI	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuBr_4$ in (2+3) H_2SO_4	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuBr_4$ in conc. HCl	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuCl_4$ (aqueous)	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuCl_4$ in (1+3) HCl	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
Picric acid (sat'd aqueous)	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	
K_2CdI_4	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
PbI_2-KOAc solution	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
H_2PtCl_6 (aqueous)	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
H_2PtCl_6 in (1+3) H_3PO_4	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
H_2PtBr_6 in (2+3) H_2SO_4	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
Platinum cyanide reagent	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	

APPENDIX C (continued)

$HAuBr_4$ in H_3PO_4-5HOAc (50 mL)	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuBr_4$ in $2H_3PO_4$-1HBr, cg	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuBr_4$ in HOAc-3(2+3)H_2SO_4 (40 mL)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuCl_4$ in (1+2) H_3PO_4 (20 mL)	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
$HAuCl_4$ in (1+1) H_2SO_4 (20 or 60 mL)	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
I-HI-7H_3PO_4, cg	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	
Picric acid in HOAc-$Mg(OAc)_2$ solution	☒ Y ☐ N	☒ Y ☐ N	☐ Y ☐ N	☐ Y ☐ N	Rosettes
PbI_2-KOAc in HOAc-$Mg(OAc)_2$ solution	☐ Y ☐ N	☐ Y ☒ N	☐ Y ☐ N	☐ Y ☐ N	

TABLE III

RESULTS OF REAGENTS TESTED WITH METHYLONE

Reagent	Test Drop: Crystal formation?				Crystal(s)
	Dry		Aqueous		
	Coverslip	Without	Coverslip	Without	
H_3BiI_6-(aqueous)	☐ Y ☒ N	☒ Y ☐ N	☐ Y ☒ N	☒ Y ☐ N	Burs and rosettes
H_3BiI_6 in (1+7) H_2SO_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
I-(2.75)KI	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
I-(50)KI	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuBr_4$ in (2+3) H_2SO_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HauBr_4$ in conc. HCl	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HauCl_4$ (aqueous)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HauCl_4$ in (1+3) HCl	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	

APPENEDIX C (continued)

Picric acid (saturatedd aqueous)	☒ Y ☒ N	☒ Y ☐ N	☐ Y ☒ N	☒ Y ☐ N	Dry: Numerous rosettes Aq: Rosettes, but fewer
K_2CdI_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
PbI_2-KOAc solution	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_2PtCl_6 (aqueous)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☒ Y ☐ N	Rods and plates
H_2PtCl_6 in (1+3) H_3PO_4	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	☒ Y ☐ N	Dry: Florets Aq: Rods and plates
H_2PtBr_6 in (2+3) H_2SO_4	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	☒ Y ☐ N	Dry: Clusters and plates Aq: Clusters
Platinum cyanide reagent	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuBr_4$ in H_3PO_4-5HOAc (50 mL)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuBr_4$ in $2H_3PO_4$-1HBr, cg	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuBr_4$ in HOAc-3(2+3)H_2SO_4 (40 mL	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuCl_4$ in (1+2) H_3PO_4 (20 mL)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HAuCl_4$ in (1+1) H_2SO_4 (20 or 60 mL)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
I-HI-7H_3PO_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Picric acid in HOAc-$Mg(OAc)_2$ solution	☒ Y ☐ N	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	Cg: Rosettes and dendrites Without: Rosettes
PbI_2-KOAc in HOAc-$Mg(OAc)_2$ solution	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
H_2PtI_6 (HCl)	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Phosphoritungstic acid	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	

APPENDIX C (continued)

Bromine-HBr	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
K_2HgI_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$HgCl_2$	☐ Y ☒ N	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	Burrs and fans
H_2PdCl_4	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	Burs and clusters
Picrolonic acid	☒ Y ☐ N	☒ Y ☐ N	☐ Y ☒ N	☐ Y ☒ N	Cg: Dendrites Without: Rosettes
$KMnO_4$	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
CrO_3	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
K_2CrO_4	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
KOH or NaOH	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Na_2CO_3 or NH_4OH	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
$K_4Fe(CN)_6$ in dilute HCl	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	
Ammonium Thiocyanate (NH_4SCN)	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	☐ Y ☒ N	

APPENDIX D

RECIPES FOR MICROCRYSTAL TEST REAGENTS

1. 5% mercuric chloride ($HgCl_2$)

   Add 1 g of mercury (II) chloride to 20 mL of water.

2. 5% sodium hydroxide (NaOH)

   Add 5 g of sodium hydroxicd to water to make 100 mL.

3. 10% ammonium thiocyanate

   Add 10 g of ammonium thiocyanate to water to make 100 mL.

4. Bismuth (III) iodide (H_3BiI_6) (aqueous)

   Mix 1.25 g of potassium iodide, 0.05 g of sodium hypophosphite, 4.5 mL of water, and 0.5 mL of concentrated bismuth (III) nitrate solution together.

5. Bismuth (III) iodide (H_3BiI_6) in (1+7) sulfuric acid

   Mix 1.25 g of potassium iodide, 2 mL of water, 2.5 mL of (1+3) sulfuric acid, 0.5 mL of concentrated bismuth (III) nitrate solution, and 0.1 g of sodium hypophosphite.

6. Bromine-hydrobromic acid

   Add one part 5% sodium bromate to one part (1+1) hydrobromic acid.

7. Bromoauric acid ($HAuBr_4$) in acetic acid—3(2+3) sulfuric acid (40 mL total)

   Dilute three parts by volume of bromoauric acid in (2+3) sulfuric acid, with one part glacial acetic acid.

8. Bromoauric acid ($HAuBr_4$) in conc. Hydrochloric acid

   Add 1.3 g of bromoauric acid to 30 mL of concentrated hydrochloric acid.

APPENDIX D (continued)

9. Bromoauric acid ($HAuBr_4$) in phosphoric acid-acetic acid

   Combine 1 g of gold (III) chloride trihydrate, 1.5 mL of 40% concentrated hydrobromic acid, 8 mL of phosphoric acid, and 40 to 40.5 mL of acetic acid.

10. Bromoauric acid ($HAuBr_4$) in phosphoric acid-hydrobromide acid

11. Bromoauric acid ($HAuBr_4$) in (2+3) sulfuric acid

    Add 1.3 g of bromoauric acid to (2+3) sulfuric acid. To make (2+3) sulfuric acid, combine two part of concentrated sulfuric acid (e.g. 12 mL) with three parts of water (e.g. 18 mL). Then add 60 mL of concentrated phosphoric acid.

12. Chlorauric acid ($HAuCl_4$) (aqueous)

    Add 1 g of gold (III) chloride trihydrate to 60 mL of water.

13. Chlorauric acid ($HAuCl_4$) in (1+3) hydrochloric acid

    Add 1 g of gold (III) chloride trihydrate to 60 mL of (1+3) hydrochloric acid. To make (1+3) hydrochloric acid, mix one part hydrochloric acid (e.g. 5 mL) to three parts water (e.g. 15 mL).

14. Chlorauric acid ($HAuCl_4$) in (1+2) phosphoric acid (20 mL)

    Dissolve 1 g of gold (III) chloride trihydrate crystals in 20 mL of (1+2) phosphoric acid. To make (1+2) phosphoric acid, combine one part phosphoric acid (e.g. 5 mL) with two parts water (e.g. 10 mL)

15. Chlorauric acid ($HAuCl_4$) in (1+1) sulfuric acid (20 or 60 mL total)

    Dissolved 1 g of gold (III) chloride trihydrate crystals in 20 or 60 mL of (1+1) sulfuric acid. To make (1+1) sulfuric acid, combine one part sulfuric acid (e.g. 10 mL) with one part water (e.g. 10 mL).

APPENDIX D (continued)

16. Chloroplatinic acid (H_2PtCl_6) (aqueous)

    Dissolve 1 g of chloroplatinic acid hexahydrate in 20 mL of water.

17. Chloroplatinic acid (H_2PtCl_6) in (1+3) phosphoric acid

    Dissolved 1 g of chloroplatinic acid hexahydrate in 20 mL of (1+3) phosphoric acid. To make (1+3) phosphoric acid, mix one part phosphoric acid (e.g. 5 mL) to three parts water (e.g. 15 mL).

18. Chromium trioxide (CrO_3)

    Add 5 g of chromium trioxide to 100 mL of water.

19. Iodine-Hydroiodic-phosphoric acid

    Dissolve 0.08 g of iodine in 0.5 mL of 57% hydroiodic acid, then add 3.5 mL of 85-88% concentrated phosphoric acid

20. Iodine-potassium iodide

    Dissolve 1 g of iodine and 1 g of potassium iodide in water then dilute to 100 mL.

21. Iodine-potassium iodide (I-(2.75)KI)

    Dissolve 1 g of iodine and 2.75 g of potassium iodide in water then dilute to 100 mL.

22. Iodine-potassium iodide (I-(50)KI)

    Dissolve 1 g of iodine and 50 g of potassium iodide in water then dilute to 100 mL.

APPENDIX D (continued)

23. Iodo-platinic acid (H_2PtI_6) (HCl)

    Add 0.625 g of sodium iodide to 0.5 mL of hydrochloric acid and 2 mL of (1+20) chloroplatinic acid solution. To make (1+20) chloroplatinic acid solution, add one part chloroplatinic acid (e.g. 1 mL) to twenty parts water (e.g. 20 mL).

24. Lead (II) iodide-potassium acetate (PbI_2-KOAc) solution

    Dissolve 4 g of lead acetate trihydrate and 20 g of potassium acetate in 100 mL of water.

25. Lead (II) iodide-potassium acetate in acetic acid-magnesium acetate solution

    Add 0.24 g of Lead (II) acetate trihydrate and 0.27 g of potassium iodide to 1 mL of acetic acid and 5 mL of magnesium acetate solution (40g/100 mL). Once mixed, slowly add potassium acetate until the lead iodide dissolves.

26. Palladous chloric acid (H_2PdCl_4) in (1+2) phosphoric acid

    Add 1 g of palladous chloride ($PdCl_2 \cdot 2H_2O$) to 0.8 mL of (1+2) phosphoric acid. To make (1+2) phosphoric acid, combine one part phosphoric acid (e.g. 5 mL) to two parts water (e.g. 10 mL).

27. Phosphoritungstic acid

    Add 10 g of commercial phosphotungstic acid to 100 mL of water.

28. Picrolonic Acid (saturated aqueous solution)

    Saturated picrolonic acid in water

29. Picric Acid in acetic acid-magnesium acetate

    Dissolve 0.03 g picric acid in 1 mL glacial acetic acid and 5 mL magnesium acetate solution (40 g/100mL)

30. Platinic bromide (H_2PtBr_6) in (2+3) sulfuric acid

    To convert chloroplatinic hexahydrate crystals to platinic bromide, add 1 g of chloroplatinic hexahydrate crystals to 1.7 mL of 40% concentrated hydrobromic acid. Dilute resulting product to 20 mL with (2+3) sulfuric acid. To make (2+3) sulfuric acid, mix two parts sulfuric acid (e.g. 12 mL) to three parts water (e.g. 18 mL).

31. Platinum cyanide

    Dissolve 1 g of chloroplatinic acid hexahydrate crystals in 18 mL of water and add 1.5 g sodium cyanide.

32. Potassium chromate (K_2CrO_4) (aqueous)

    Add 10 g of potassium chromate to water to make 100 mL.

33. Potassium hexacyanoferrate (II) ($K_4Fe(CN)_6$) in dilute HCl

    Add 5 g of potassium hexacyanoferrate (II) trihydrate to 50 mL of (1+11) hydrochloric acid. To make (1+11) hydrochloric acid, combine one part hydrochloric acid (e.g. 5 mL) to eleven part water (e.g. 55 mL).

34. Potassium Iodocadmate (K_2CdI_4)

    Add 5 g of cadmium iodide and 4.5 g of potassium iodide to 100 mL of water.

35. Potassium mercuric iodide (II) (K_2HgI_4)

    Dissolve 2 g of potassium iodide in 100 mL of water and saturate with mercury (II) iodide—usually about 3 g required.

APPENDIX D (continued)

36. Potassium permanganate ($KMnO_4$)

Add 2 g of potassium permanganate to 5 drops of concentrated phosphoric acid.

Dilute up to 100 mL

37. Sodium carbonate (Na_2CO_3) (aqueous)

5 g of sodium carbonate ($Na_2CO_3 \cdot H_2O$) to water to make 100 mL

APPENDIX E

TABLE IV

METHYLONE CRYSTALS PRECIPITATED IN PRICROLONIC AND PICRIC ACID

Reagent	Picrolonic Acid	Picric Acid
Crystal Morphology	Rosettes	
Color	Smaller: Yellow brown Larger: Translucent	Translucent, tinge of yellow due to reagent
Time Lapsed	Immediately	
Limit of Detection	1 PPP	
Upper Limit of Detection	5 PPP	
Size	Smaller: 20 – 30 μm Larger: ~50 μm	20 – 200 μm
Sign of Elongation	Smaller: (+) Larger: (-)	(-) sign of elongation
Refractive Indices	n1 = 1.532 n2 > 1.700	N/A

TABLE V

ETHYLONE CRYSTALS PRECIPITATED IN PICRIC ACID

Crystal Morphology	Rosettes
Color	Yellow
Time Lapsed	Immediately
Limit of Detection	1 PPP
Upper Limit of Detection	5 PPP
Size	50 – 200 μm
Sign of Elongation	(-) sign of elongation
Refractive Indices	N/A

APPENDIX E (continued)

TABLE VI
BUTYLONE CRYSTALS PRECIPITATED IN PICROLONIC ACID

Crystal Morphology	Rosettes
Color	Yellow Brown
Time Lapsed	Immediately
Limit of Detection	1 PPP
Upper Limit of Detection	5 PPP
Size	20 – 100 μm
Sign of Elongation	(+) sign of elongation
Refractive Indices	N/A

TABLE VII
METHYLONE:ADULTERANT RATIO IN PICROLONIC AND PICRIC ACID

Reagent	Picrolonic Acid	Picric Acid
Starch (Potato)	1:10	1:10
Lactose	1:10	1:10
Cafeine	1:10	1:10
Palmitic Acid	1:10	1:10
Procaine HCL	1:10	1:10
Acetaminophen	1:10	1:10
All Adulterants	1:10	1:10

APPENDIX E (continued)

TABLE VIII

ETHYLONE:ADULTERANT RATIO IN PICRIC ACID

Starch (Potato)	1:10
Lactose	1:10
Cafeine	1:5
Palmitic Acid	1:10
Procaine HCL	1:5
Acetaminophen	1:10
All Adulterants	1:10

TABLE IX

BUTYLONE:ADULTERANT RATIO IN PICROLONIC ACID

Starch (Potato)	1:10
Lactose	1:10
Cafeine	1:10
Palmitic Acid	1:10
Procaine HCL	1:10
Acetaminophen	1:10
All Adulterants	1:10

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