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# Comparison of Liquid-Liquid Extraction and Solid Phase Extraction of Mephedrone

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# COMPARISON OF LIQUID-LIQUID EXTRACTION AND SOLID PHASE EXTRACTION OF MEPHEDRONE

by

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ABSTRACT

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Legal high drugs are modified scheduled drugs. As with any new drug, researchers have to conduct studies to gather information about the drug. The problem with obtaining accurate information on new drugs is that by the time information is gathered, drug abusers and *street chemists* have developed new ones. Comparing designer drugs to their illegal counterparts is often helpful in that it can provide a starting point. Mephedrone is a new designer drug that has become a problem over the past few years. Often marketed as *bath salts* and *plant food*, mephedrone has become the knockoff replacement for amphetamines.

This experiment focused on comparing liquid-liquid extraction to solid phase extraction to determine if there was a difference and which was more efficient. Synthetic urine samples were spiked with mephedrone, extracted using both methods, and analyzed with GC-MS. Spiked synthetic urine samples were also analyzed to determine the limit of detection for mephedrone.

A T-ratio test determined that there is less than a 5% chance that LLE and SPE are the same for extracting mephedrone. The *t* ratios for extracting 0.5μg/mL via LLE and SPE were 5.567 and 6.542, which were above the level of significance *t* value 2.086. After determining that the methods were statistically different, the percent recovery of each method was observed. The percent recovery of SPE was higher than that of LLE for

0.5µg/mL, making SPE the better method. For 2.0µg/mL SPE percent recovery was less than LLE, proving LLE to be better in this case.

# DEDICATION

I dedicate my thesis to my grandfathers, who supported every step of my educational career. Although they are not here to witness my accomplishment, I know they are both very proud.

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I would like to recognize the members of my committee for their patience, support, and guidance throughout the process of this thesis project. Dr. Kuppareddi Balamurugan's advice, time, and helping make sure I had everything scheduled. I appreciate Dr. Anthony Bell for the encouragement to continue and different suggestions to help my research. I am very grateful for Dr. Thomas Pittman's dedication to ensure that I completed this project, his wisdom, stories to keep my mind off of family life, and most of all his faith that I could do it.

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# CHAPTER I

# INTRODUCTION

## Designer Drugs

Various drug regulations continue to stir up conflict within a society of drug abusers. As a result, these abusers have found ways to avoid the consequences of law while simultaneously acquiring the drugs they desire. These altered substances, commonly known as *designer drugs*, are formulated to bypass the laws that prohibit their counterparts while mimicking similar effects. Because many designer drugs are not controlled under the Controlled Substances Act, they are referred to as *legal highs*, but are still considered to be drugs of abuse. Most of these drugs are controlled under the Federal Analog Act of Controlled Substances Act due to their being derived from other controlled substances (Drugs of Abuse, 2011).

Due to the lack of availability and purity of common drugs of abuse, clandestine laboratories began producing and selling mephedrone. Mephedrone is a synthetic derivative of cathinone, an active ingredient found in Khat (Drugs of Abuse, 2011). Also known as *plant food* or *bath salts* and often sold online labeled "not for human consumption," mephedrone has become the new drug of choice and interest. The recent spark of mephedrone use appears to have originated primarily in the United Kingdom and eventually began to make headlines in the United States. With increasing popularity and emergency room reports, there have been numerous case studies on mephedrone to determine its effects and toxicity. Although mephedrone has now been scheduled under the Controlled Substances Act as a Schedule I drug, there is still much to be known about this designer drug.

Development of methods for analyzing designer drugs is crucial to the discipline of toxicology. Effective extraction methods, analysis, and toxic research play a huge role in keeping up with the constantly growing and changing drug field. Just as easily as ecstasy and amphetamines were replaced with mephedrone, mephedrone will be replaced with another designer drug. The challenge with developing methodologies for designer drugs is there is usually no previous or current data on the drug. Without established knowledge or reference standards to compare to, analysts seem to fall a step behind clandestine laboratories. Research on methods, such as extractions, is beneficial in that it can cut down on time that is spent on methods and techniques that are not productive.

For the determination of mephedrone toxicity, sample preparations as well as proper extraction methods from biological fluids are an important factor. Extractions have a direct effect on the sensitivity and quantization of drug analysis. An efficient method of extraction should possess high recovery and reproducibility. Extractions are the transfer of an analyte from one miscible phase to another phase and are used to separate the analyte from mediums such as blood, urine, or bile (Harris, 2007). Based around pH and polarity, a drug's $pK_a$ is one of the determining factors for choosing an extraction solvent that the analyte can be partitioned in. The $pK_a$ is the negative logarithm of an acid dissociation constant and describes how an acid ionizes in solution (Harris, 2007). If the $pK_a$ is small, the acid is completely ionized and is a strong acid. A large $pK_a$ correlates to a weak acid, meaning that the acid is partially ionized. The Henderson-Hasselbach equation is used to determine an analyte's pH by expressing the ratio of ionized to unionized ions in relation to its $pK_a$.

Before a drug can be analyzed, it has to be removed from its matrix. Liquid-liquid extractions and solid phase extractions are the most commonly used methods to separate a drug from its matrix. Liquid-liquid extraction (LLE) is the use of two immiscible liquids to extract a drug of interest in a liquid in which it is miscible. Depending on the drug's $pK_a$, a buffer is added to the matrix to convert the drug to its unionized form. Once in its unionized form, the drug can be separated from the matrix with the addition of an extraction solvent that is polar enough to extract the drug of interest. After the solvent is allowed to mix with the sample, the sample is centrifuged to separate the organic solvent from the aqueous layer. The volatile organic extract is removed and allowed to dry, leaving only the drug of interest behind. Now that the drug has been isolated, it can be analyzed.

Solid phase extractions (SPE) use columns with a stationary phase to trap the drug of interest while separating it from its matrix. The same concept with LLE of pH and $pK_a$ drive the separating factors of SPE, except that SPE contains an absorbent solid. The solid phase is made of sorbent silica that has an affinity for the drug of interest, binding it, while allowing unwanted waste materials to pass by. Before separating the drug from its matrix, the columns are conditioned with methanol (MeOH) and water to remove absorbed organic material in the column. The sample containing the drug of interest is poured into the column allowing the drug to adhere to the stationary phase. Columns have different types of systems to help materials pass through the column; there are columns that allow the materials to flow by gravity, or with negative or positive pressure systems. Once the drug is contained in the column, the sample is washed with weak solvents and water to removed unwanted polar solutes. A strong organic solvent is used

to elute the strongly bound drug from the stationary phase. The desired eluent is eluted in its own tube and evaporated to dryness, leaving only the drug.

In some cases, an additional step of derivatization is beneficial before the analysis of the isolated drug. Derivatization is the process of chemically altering an analyte to make it easier to detect or separate (Harris, 2007). Derivatization is used in this study to improve the analyte's chromatographic behavior and assure its identification. Most commonly used derivatizing reagents are bistrimethylsilyltrifluoroacetamide (BSTFA), heptafluorobutyric anhydride (HFBA), pentafluoropropionic anhydride (PFPA), and trifluoroacetic anhydride (TFAA). A study conducted by Cerilliant (2011) determined that TFAA is the best derivatizing reagent to use with designer drugs such as mephedrone.

In studies conducted on mephedrone, both methods have been used, but there has been no specification of which is more conducive for the analysis of mephedrone. The efficiency of either extraction method can be measured via analysis of mephedrone with gas chromatography with mass spectrometric detection (GC-MS). GC-MS assays can provide spectral analysis on mephedrone spiked synthetic urine samples. The spectrum will provide information about the recovery of the extraction and detection limits.

# CHAPTER II

# REVIEW OF LITERATURE

## Liquid-Liquid Extraction

Every step of drug analysis is critical, but preparation of the sample for analysis is the most crucial part to achieving valid results. "Sample preparation is the series of steps required to transform a sample so that it is suitable for analysis" (Harris, 2007, p. 655). Sample preparation can consist of extraction of the desired drug from its matrix, making the sample more concentrated so that it can be detected, or just dissolving the sample. Extraction involves the dissolving of an analyte in a solvent without affecting the drug of interest (Harris, 2007). Analysts attempt to control which direction the analysis can go with each step they take in preparing the sample for analysis.

Liquid-liquid extraction, LLE, is the most commonly used method of extraction because it is direct and blood and urine are the specimens that are frequently tested for the determination of drugs. Because blood and urine are liquids, they are easily "partitioned with an organic solvent without protein precipitation after a pH adjustment of the liquid with a buffer, acid, or base" (Siek, 2010, p. 71). Polarity is the deciding factor behind choosing an organic solvent for extraction of drugs. After deciding on the proper organic solvent to use, a buffer is sometimes added so the drug of interest will be in its non-ionic form and easily partition into the organic solvent of choice. The adjustment of the pH before extraction depends on what class the drug falls in. Drugs are classified into six classes: strong acids, weak acids, neutrals, weak bases, strong bases, and amphoteric bases. When performing chromatographic assays such as HPLC, TLC,

or GC, samples have to be extracted twice in order to have an extract that can be chromatographed (Siek, 2010).

## Solid Phase Extraction

To overcome the disadvantages of LLE, another method of extraction exists, solid phase extraction. Harris (2007) describes solid phase extraction as a process in which a solution is passed through a short column of chromatographic stationary phase to separate the analyte from the matrix and the absorbed analyte on the column is eluted from the column with a solvent. This method of separation is recorded to have existed since the biblical days but has only been of scientific value and used as a technique since the 1970s (Simpson, 2000).

The most important part in solid phase separation is the column. Columns were developed in the 1960s and early '70s by bench toxicologists who developed their own columns for separation (Siek, 2010). These columns contained sodium sulfate to absorb water and shredded filter paper or cotton to retain the desired drug (Siek, 2010). This somewhat successful method involved pouring blood through the column and afterwards pouring a solvent through to recover the drug of interest (Siek, 2010). In October of 1977, the process became more convenient with the development of prepackaged, disposable columns with bonded silica sorbents (Simpson, 2000).

The objective of solid phase extraction is for the analyte to be concentrated, cleaned of unwanted molecules, and/or separated from the matrix (Simpson, 2000). An isolated, cleaned, and concentrated drug is achieved through three steps. The first step is retention, in which the analyte is separated from the matrix and retained by the sorbent particles in the column. After retention of the analyte to the column, the column is

washed with a solvent to remove any interfering compounds that can affect analysis. Finally the analyte is eluted from the column with the passing of a solvent to desorb the analyte and collect it in the solvent (Simpson, 2000).

Developed to overcome the challenges with liquid-liquid extraction, solid phase extraction has its own disadvantages as well. In the case of drugs like benzodiazepines and tricyclics, solid phase extraction uses more solvents, materials, and time. Although this method is more tedious than liquid-liquid extraction, it compensates for that with its reproducibility and minimizing the use of solvents (Siek, 2010). Unlike liquid-liquid extractions that have to use immiscible solvents for the sample, solid phase extraction can use miscible solvents (Simpson, 2000).

## Mephedrone

Mephedrone has become an increasing problem in recent years, along with other designer drugs. These designer drugs are synthetically made to possess similar effects to the drugs from which they are derived while bypassing the laws that legally prohibit their counterparts. Mephedrone (4-methylmethcathinone, 4-MMC) is a psychoactive derivative of cathinone that provides similar results to those of stimulant and hallucinogenic drugs. Cathinone is a naturally occurring alkaloid of the Khat plant, *Catha edulis*, which originates in Northeast Africa and the Arabian Peninsula (National Drug Intelligence Center, 2003). The derivatives of cathinones are the β-keto analogues of the phenethylamine family (Drug Profiles, 2012).

Mephedrone is a ring substituted cathinone that differs from its phenethylamine counterparts "by a keto functional group at the beta carbon" (Sedefov & Gallegos, 2011, p. 27). Systematically named by IUPAC as (RS)-2-methylamino-1-(4-

methylphenyl)propan-1-one, mephedrone's molecular formula, molecular weight, boiling point, and melting point are: $C_{11}H_{15}NO$, 177.242g/mol, 269.51°C, and 66.61°C respectively (Sedefov & Gallegos, 2011).

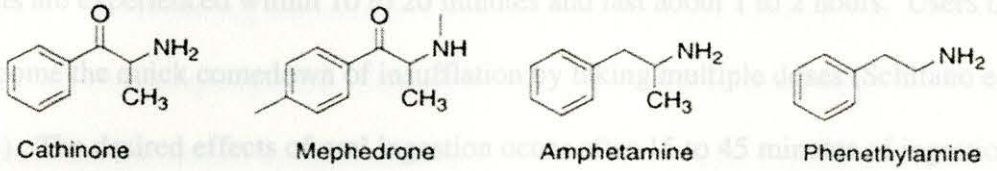


*Figure 1.* Mephedrone and its related structures

Sedefov and Gallegos (2011) report that mephedrone was mentioned as early as 1929 by Saem de Burnaga Sanchez, who described its synthesis, mentioning it as *toluyl-alpha-monomethylaminoethylcetone*. The straightforward synthesis of mephedrone combines the product of brominated 4-methylpropiophenone with methylamine and an acid scavenger. Gaseous or aqueous hydrochloride is then added to provide the hydrochloride salt that will need to be recrystallized. Mephedrone can also be synthesized from the oxidation of 4-methylephedrine with potassium permanganate or potassium dichromate (Sedefov & Gallegos, 2011). Methcathinone is synthesized from the oxidation of ephedrine, which is also a precursor for methamphetamine (Maheux et al., 2010). Mephedrone is an analogue of methcathinone, which "is the β-keto analog of methamphetamine and the *N*-methyl derivative of cathinone" (Maheux et al., 2010, p. 42). Because methcathinone is made from the same precursor of methamphetamine, and mephedrone is the analogue of methcathinone, this can explain why mephedrone has similar central nervous stimulatory effects as those of amphetamines. Mephedrone has recently been scheduled as a schedule I drug in the United States.

Mephedrone, known as *bath salts*, appears as a white/slightly yellow powder or a tablet and is most commonly administered via insufflation, but it can also be administered

orally, smoked, or intravenously (Drugs of Abuse, 2011). When taken orally in its powder form, mephedrone is placed in cigarette paper and swallowed; this is referred to as *bombing* (Prosser & Nelson, 2012). Mephedrone users report that after insufflation, effects are experienced within 10 to 20 minutes and last about 1 to 2 hours. Users try to overcome the quick comedown of insufflation by taking multiple doses (Schifano et al., 2011). The desired effects of oral ingestion occur after 15 to 45 minutes of ingestion and have duration of 2 to 4 hours (Prosser & Nelson, 2012). Because effects last longer with oral ingestion, the need to take more doses to maintain that *high* is less than that of insufflated mephedrone (Schifano et al., 2011). Users report that injection intravenously provides effects within 10 to 15 minutes and tend to last 30 minutes (Prosser & Nelson, 2012).

Mephedrone produces stimulatory and hallucinogenic effects like that of amphetamines and ecstasy, respectively. Although there are no published formal studies on the psychological effects of mephedrone on humans, users do report effects that are "broadly comparable to those reported for better-studied stimulant drugs" (Sedefov & Gallegos, 2011, p. 29). The effects reported by mephedrone users are those of "euphoria, general stimulation, enhanced music appreciation, elevated mood, decreased hostility, improved mental function and mild sexual stimulation" (Sedefov & Gallegos, 2011, p. 29). Other than the desired pleasurable effects, users also experience adverse effects that vary from agitation, depression, paranoia, and panic attacks (Drugs of Abuse, 2011). Schifano et al. (2011) reported user complaints consisting of loss of appetite, nausea, headache, dizziness, anxiety, agitation, elevated blood pressure, chest pain, and difficulty urinating.

Despite mephedrone having become increasingly popular over the recent years, there is still much to be learned of its pharmacology and toxicity. A report from Sedefov and Gallegos (2011) speculate that mephedrone acts as other stimulants by blocking reuptake and stimulating the release of stimulant neurotransmitters such as serotonin, dopamine, and norepinephrine. Support of this speculation is based on its chemical structure and the similar sympathomimetic effects that are seen with mephedrone in comparison to other stimulant drugs such as MDMA and cocaine (Sedefov & Gallegos, 2011). Martínez-Clemente, Escubedo, Pubill, and Camarasa (2012) determined that mephedrone interacts with the transporters of dopamine and serotonin and blocks the uptake of the neurotransmitters. The Encyclopedia of the Human Brain (2002) states that serotonin affects sleep, mood, sexual behavior, and aggressive behavior; dopamine is involved with the reward system and in addiction. Before studies on metabolism were conducted, it was figured that mephedrone is "partly excreted as glucuronides and sulphate conjugates" (Sedefov & Gallegos, 2011, p. 58). Meyer, Wilhelm, Peters, and Maurer (2010) concluded from a study on mephedrone's metabolism in human and rat urine that mephedrone is partly excreted as glucuronides and/or sulfates.

Synthetic cathinones are less potent than their phenethylamine analogues due to the β-keto group increasing the polarity and allowing less chance for passage across the blood-brain barrier (Drug Profiles, 2012). Although less potent and structurally similar to amphetamines, there is still much more to be known about ring substituted cathinones. There have been numerous reports of mephedrone related deaths and admittance to the hospital for poisoning, but there is still no solid conclusion on the toxicity of mephedrone. A case reported by the American College of Medical Toxicology

determined that a male who had ingested 200mg of mephedrone and intramuscularly injected 3.8g suffered from "isolated mephedrone toxicity" (Wood et al., 2010, p. 329).

# CHAPTER III

# METHODOLOGY

## Method Overview

Synthetic urine samples were used for the purposes of this research. Two milliliters of the synthetic urine were placed into 80 16x120 screw cap tubes. Forty tubes of the synthetic urine were spiked with 20µL of mephedrone to make a concentration of 0.5µg/mL; the remaining 40 tubes were spiked with 80µL, making a concentration 2.0µg/mL. Forty samples, which consisted of 20 tubes of 0.5µg/mL mephedrone and 20 tubes of 2.0µg/mL mephedrone, were extracted using liquid-liquid extraction (LLE). The remaining samples were extracted using solid phase extraction (SPE). Each extract was derivatized and injected into the Gas Chromatography-Mass Spectrometer (GC-MS) under specific parameters. Results of the GC-MS analysis were used to compare LLE and SPE to determine which method is more efficient. Spiked samples with concentrations of 0.25µg/mL, 0.125µg/mL, and 0.05µg/mL were analyzed to determine mephedrone's limits of detection for both extractions. Concentrations of 0.5µg/mL and 2.0µg/mL were used as neat standards with which to compare the extracted samples.

### *Preparation of Mephedrone Standard*

A Mephedrone-HCl (Cerilliant, Round Rock, TX) standard with a concentration of 1mg/mL was used to make dilutions of standards with the following steps:

1. 1mL of 1mg/mL mephedrone-HCl was added to a tube with 4mL of methanol (MeOH) and vortexed for approximately 5-10, seconds making a concentration of 200µg/mL.
2. 1mL of 200µg/mL mephedrone was pipetted into another tube with 3mL of MeOH to make 50µg/mL mephedrone.

*Preparation of Neat Mephedrone Standard*

Mephedrone standards of 0.5µg/mL and 2.0µg/mL were used with which to compare analyzed extracts. The neat standards were prepared by the following steps:

1. 2mL of MeOH were pipetted into two separate tubes.
2. 20µL of 50µg/mL standard were pipetted into one tube.
3. 80µL of 50µg/mL standard were pipetted into the other tube.
4. 100µL of 0.1% HCl were added to each tube and vortexed.
5. Each tube was place in a Rapidvap Labconco vacuum pump (Thermo-Fisher, Houston, TX) and evaporated to dryness at 50°C for approximately 1 hour.
6. After drying, the standards were derivatized and dried again.
7. Once dry, 100µL of ethyl acetate were added to the standards and vortexed.
8. The standards were transferred to autosampler vials containing 150µL inserts.
9. Vials were labeled, capped, and placed on the GC-MS for injection.

*Preparation of Synthetic urine Samples*

1. 2mL of synthetic urine were pipetted into 86 16x120 screw cap tubes.
2. 20µL of the 50µg/mL standard were pipetted into 40 tubes containing synthetic urine and vortexed for a concentration of 0.5µg/mL.
3. 80µL of the 50µg/mL standard were pipetted into 40 tubes containing synthetic urine and vortexed for a concentration of 2.0µg/mL.
4. 10µL of the 50µg/mL standard were pipetted into 2 tubes containing synthetic urine and vortexed for a concentration of 0.25µg/mL.
5. 5µL of the 50µg/mL standard were pipetted into 2 tubes containing synthetic urine and vortexed for a concentration of 0.125µg/mL.

6. 2μL of the 50μg/mL standard were pipetted into 2 tubes containing synthetic urine and vortexed for a concentration of 0.05μg/mL.

*Preparation of Sodium Hydroxide Solution*

A 1.0M solution of sodium hydroxide (Fisher Scientific, Houston, TX) was added to the samples extracted via LLE to make the sample basic. The following steps were completed to prepare 100mL of 1.0M sodium hydroxide (NaOH) solution:

1. 4.1g of NaOH were weighed out and placed into a 250mL Erlenmeyer flask.
2. 100mL of Type III water (Millipore, Billerica, MA) were measured with a graduated cylinder and added to the flask containing NaOH.
3. The flask was swirled until NaOH had dissolved.

*Preparation of 0.25M Phosphate Buffer*

A 0.25M phosphate buffer was used to prepare the samples before SPE. The phosphate buffer was prepared with both monobasic ($NaH_2PO_4$) and dibasic ($Na_2HPO_4$) sodium phosphate (Fisher Scientific, Houston, TX) by the following steps:

1. 6.90g of $NaH_2PO_4$ were weighed out and placed in a 200mL Erlenmeyer flask and dissolved to a final volume of 200mL with Type III water.
2. 13.40g of $Na_2HPO_4$ were weighed out and placed in a 200mL Erlenmeyer flask and dissolved to a final volume of 200mL with Type III water.
3. 100mL of the 200mL of $NaH_2PO_4$ were added to a clean beaker and placed on a magnetic hot plate with a stirrer. 5mL of $Na_2HPO_4$ were continuously added to the beaker until a pH of 6.0 was achieved. The pH of the solution was verified with a pH meter (Accumet 25CL, Fisher Scientific, Houston, TX).

*Preparation of 0.1M Glacial Acetic Acid*

A 0.1M solution of Glacial Acetic Acid (Fisher Scientific, Fair Lawn, NJ) was used with SPE for the washing of the sample. The following steps were completed to prepare 100mL of the solution:

1. 100mL of Type III water were measured with a graduated cylinder and poured into a 100mL Erlenmeyer flask.
2. 575µL of 17.4M glacial acetic acid were pipetted into the flask.
3. The flask was swirled for thorough mixing.

*Preparation of N-Butyl Chloride: Ethyl Acetate*

An 80:20 dilution of N-Butyl Chloride: Ethyl Acetate (Fisher Scientific, Fair Lawn, NJ) was used for both LLE and SPE to extract mephedrone. The solution was prepared by the following steps:

1. 400mL of N-Butyl Chloride were measured using a graduated cylinder and poured into a 500mL Erlenmeyer flask.
2. 100mL of ethyl acetate were measured and poured into the flask containing N-Butyl Chloride.
3. The flask was swirled to allow the two to be mixed.

*Preparation of Triethylamine with N-Butyl Chloride: Ethyl Acetate*

Four percent Triethylamine (TEA) (Fisher Scientific, Fair Lawn, NJ) prepared with N-Butyl Chloride: Ethyl Acetate was used with SPE to elute the sample from the column. The solution was prepared by the following steps:

1. 4mL of TEA were pipetted into a 100mL volumetric flask.

2. N-Butyl Chloride: Ethyl Acetate (80:20) was added until the volume was brought to the 100mL mark.
3. The flask was covered and inverted several times to allow adequate mixing.

*Preparation of 0.1% Hydrochloric acid*

Hydrochloric acid (Fisher Scientific, Fair Lawn, NJ) was used for both methods of extraction during evaporation of the sample. Fifty milliliters of 0.1% HCl were prepared as follows:

1. 49.95mL of methanol were measured using a graduated cylinder and poured into a 100mL Erlenmeyer flask.
2. 50µL of 6M HCL were added to the flask.
3. The flask was swirled for thorough mixing.

*Preparation of 10% Hydrochloric acid*

Hydrochloric acid was used to adjust the pH of the samples before SPE. The following steps were completed to prepare 10% HCl:

1. A 100mL volumetric flask was filled halfway with Type III water.
2. 10mL of HCl were added to the flask.
3. Type III water was added until the 100mL mark was reached.

*Liquid-Liquid Extraction of Mephedrone*

Forty-three spiked synthetic urine samples, 20 of 0.5µg/mL, 20 of 2.0µg/mL, 1 of 0.25µg/mL, 1 of 0.125µg/mL, and 1 of 0.05µg/mL were extracted via LLE by the following steps:

1. 100µL of 1.0M NaOH solution were added to the sample and vortexed for approximately 5-10 seconds.

2. 8mL of N-Butyl Chloride: Ethyl Acetate solution (80:20) were added to the sample and capped.
3. The tubes were placed on a platform rotator for 30 minutes and mixed at a slow speed to prevent emulsion.
4. The tubes were then centrifuged on a Sorvall Legend T+ centrifuge (Fisher Scientific, Houston, TX) at 3000 RPM for 10 minutes. After 10 minutes of centrifuging, the tubes were *bumped* to remove the emulsion layer from the sides of the tube and centrifuged for another 10 minutes.
5. The top layer containing N-Butyl Chloride: Ethyl Acetate was transferred to clean 16x120mm tubes.
6. 100µL of 0.1% HCl were added to each sample and vortexed.
7. The samples were evaporated to dryness at 50°C for approximately 1.5 hours.
8. After drying, the samples were derivatized and dried again.
9. Once dry, 100µL of ethyl acetate were added to the samples and vortexed.
10. The samples were transferred to autosampler vials containing 150µL inserts.
11. Vials were labeled, capped, and placed on the GC-MS for injection.

*Solid Phase Extraction of Mephedrone*

Solid phase extraction was performed on "No Vacuum" Gravity GV-65C columns (Biochemical Diagnostics, Inc., Edgewood, NY). Forty-three spiked synthetic urine samples, 20 of 0.5µg/mL, 20 of 2.0µg/mL, 1 of 0.25µg/mL, 1 of 0.125µg/mL, and 1 of 0.05µg/mL were extracted via SPE.

*Sample Preparation*

1. 1mL of 0.25M sodium phosphate buffer (pH = 6) was added to each sample and vortexed.
2. The samples were incubated at room temperature for 20 minutes.
3. 200µL of 10% HCl were added to the samples to adjust the pH to 2.

*Column Conditioning*

1. 43 columns were set up to sit inside 16x120 tubes to allow proper gravity flow.
2. 1mL of MeOH was added to each column to wash the column.
3. After the flowing of MeOH from the column, 1mL of Type III water was added.
4. Extraction of the sample was followed within 20 minutes of column conditioning.

*Sample Extraction*

1. Each sample was poured onto a preconditioned column and allowed to flow through completely.
2. 2mL of 0.1M glacial acetic acid were added and allowed to flow through the column.
3. 3mL of Type III water were added to wash the column.
4. 1mL of MeOH was added to the column.
5. 1mL of Ethyl Acetate was added to the column.
6. Columns were dried at 40°C for 15minutes

*Sample Elution*

1. Each column was placed into clean test tubes for the elution of the sample.
2. 1.5mL of N-Butyl Chloride: Ethyl Acetate (80:20) +4% Triethylamine were added to each column.
3. 100µL of 0.1% HCl were added to each tube and vortexed.
4. Each tube was evaporated to dryness at 50°C for approximately 1 hour.
5. Once dry, the samples were derivatized and dried again.
6. After drying, 100µL of ethyl acetate were added to each tube and vortexed.
7. The samples were transferred to autosampler vials containing 150µL inserts.
8. Vials were labeled, capped, and placed on the GC-MS for injection.

*Derivatization of Dried Samples*

Before extracts were analyzed by GC-MS, the extracts were derivatized with Trifluoroacetic anhydride (TFAA) (Acros Organics, New Jersey, USA) by the following steps:

1. 100µL of TFAA were added to tubes containing dried extract.
2. 500µL of Toluene (Acros Organics, New Jersey, USA) were added to the tubes, capped and vortexed.
3. The tubes were heated with caps on at 70°C for 30 minutes.
4. After heating, the tubes were allowed to cool for approximately 10 minutes.
5. The samples were uncapped and allowed to dry at 70°C for approximately 1.5 hours.

*GC-MS Analysis*

Analysis was performed on a Perkin Elmer Clarus 600 $EI^+$ AutoSystem GC with built-in Autosampler (Shelton, CT).

*GC Parameters*

**Autosampler Method**

Syringe Capacity: 5.0μL

Injection: Auto

Injection Speed: Normal

Viscosity Delay: 0

Pre-Injection Solvent Washes: 2

Post-Injection Solvent Washes: 8

Injection Volume: 2μL

Sample Pumps: 2

Wash/Waste Vial Set: 1

Pre-Injection Sample Washes: 1

**Carrier Parameters**

Carrier Control: He

Capillary column: MS5 30m x .25mm x 250μm phase

Vacuum Compensation: On

Flow Rate: 0.75mL/min

Initial Hold: 999.0 min

**Heated Zones**

Inlet A: CAP

Setpoint: 250°C

**Oven Program**

Cryogenics: Off

Initial Temperature: 100°C

Maximum Temperature: 275°C

Initial Hold: 3.00 min

Equilibration Time: 0.3 min

Ramp: 25°C/min to 275°C, hold for 0.0min

**Timed Events**

Split 1: 0 at -0.20min

Split 2: 50 at 0.30min

*MS Parameters*

The MS analysis was performed on a PerkinElmer Clarus 600 Mass Spectrometer (Shelton, CT).

**Duration: 10.0 minutes**

Solvent Delay Start: 0.0min

Solvent Delay End: 6.80min

Number of Functions: 1

**Function 1: SIR of 3 masses**

Time: 7.00 to 8.50 minutes

Ion Mode: $EI^+$

Scan Time: 0.25 seconds

Inter Scan Delay: 0.005 seconds

| Channel | Mass (Da) | Dwell (s) |
|---|---|---|
| 1 | 91 | 0.050 |
| 2 | 119 | 0.050 |
| 3 | 154 | 0.050 |

# CHAPTER IV

## DATA AND RESULTS

A total of 86 tubes containing 2mL of synthetic urine were spiked with mephedrone and used for this research. Forty of the samples were spiked with 20µL of mephedrone for a concentration of 0.5µg/mL. The other forty were spiked with 80µL of mephedrone for a concentration of 2.0µg/mL. The six remaining synthetic urine samples were spiked with 10, 5, and 2µL of mephedrone to make two concentrations of 0.25, 0.125, and 0.05µg/mL, respectively. An average retention time of 7.69 was seen for the majority of the extractions.

Neat standards of mephedrone were analyzed to compare to the mean area and heights of extracted mephedrone to determine each method's percent recovery. The standards were prepared with 2mL of MeOH in each tube and 20µL and 80µL for concentrations of 0.5µg/mL and 2.0µg/mL, respectively. The samples were dried with 100µL of 0.1% HCl at 50°C. Each standard was derivatized, dried at 70°C for about 1.5 hours, and analyzed by GC/MS using selective ion monitoring of the 91, 119, and 154 ion fragments (see Figure 4). The mean, standard deviation, and standard error for the standard concentrations were calculated using the peak area and height of the 119 ion fragment (Tables 1 and 2). Two standards' chromatograms are displayed in Figures 2 and 3, showing areas and heights.

Table 1

*Mephedrone 119 Ion Area and Height of 0.5µg/mL Standard*

| 0.5µg/mL Sample No. | | 0.5µg/mL Area | 0.5µg/mL Height |
|---|---|---|---|
| 1 | | 33,231 | 1,482,680 |
| 2 | | 26,553 | 1,268,893 |
| 3 | | 21,787 | 996,368 |
| 4 | | 21,798 | 1,025,897 |
| 5 | | 53,249 | 2,929,822 |
| 6 | | 28,639 | 1,508,190 |
| 7 | | 17,722 | 835,784 |
| 8 | | 25,698 | 1,307,290 |
| 9 | | 12,485 | 599,602 |
| 10 | | 18,115 | 833,576 |
| 11 | | 35,276 | 1,324,995 |
| 12 | | 33,109 | 1,434,687 |
| 13 | | 55,969 | 2,755,932 |
| 14 | | 29,986 | 1,431,726 |
| 15 | | 25,500 | 1,168,998 |
| 16 | | 26,372 | 1,266,032 |
| 17 | | 24,704 | 1,227,282 |
| 18 | | 32,633 | 1,682,028 |
| 19 | | 44,487 | 2,451,025 |
| 20 | | 44,310 | 2,423,222 |
| | Mean: | 30,581.150 | 1,497,701.450 |
| | Std. Deviation: | 11481.975 | 647,064.558 |
| | Std. Error: | 1862.622 | 104,967.732 |

Note: Data for Standard 0.5µg/mL area and height of the 119 ion fragment. Results were used to determine percent recovery of LLE and SPE.

Mean $=\frac{\Sigma X}{N}$ Standard Dev. $=\sqrt{\frac{\Sigma(X-\overline{X})^2}{(N-1)}}$ Est. Std. Error $=\frac{SD}{\sqrt{(N_1-1)+(N_2-1)}}$

Table 2

*Mephedrone 119 Ion Area and Height of 2.0µg/mL Standard*

| 2.0µg/mL Sample No. | 2.0µg/mL Area | 2.0µg/mL Height |
|---|---|---|
| 1 | 214,484 | 12,642,950 |
| 2 | 165,478 | 9,734,338 |
| 3 | 136,548 | 7,589,207 |
| 4 | 119,981 | 6,516,962 |
| 5 | 96,626 | 5,493,307 |
| 6 | 150,156 | 8,841,016 |
| 7 | 94,813 | 5,227,638 |
| 8 | 219,481 | 13,192,626 |
| 9 | 150,330 | 9,049,646 |
| 10 | 122,241 | 7,185,256 |
| 11 | 229,914 | 13,892,811 |
| 12 | 100,121 | 5,447,835 |
| 13 | 83,749 | 4,444,461 |
| 14 | 185,437 | 11,349,705 |
| 15 | 91,256 | 5,040,195 |
| 16 | 184,296 | 11,048,184 |
| 17 | 214,065 | 12,976,743 |
| 18 | 109,247 | 6,127,039 |
| 19 | 206,177 | 12,104,322 |
| 20 | 176,544 | 10,598,386 |
| Mean: | 152,547.200 | 8,925,131.350 |
| Std. Deviation: | 48,997.616 | 3,147,659.834 |
| Std. Error: | 7,948.463 | 510,617.852 |

Note: Data for Standard 2.0 µg/mL area and height of the 119 ion fragment. Results were used to determine percent recovery of LLE and SPE.

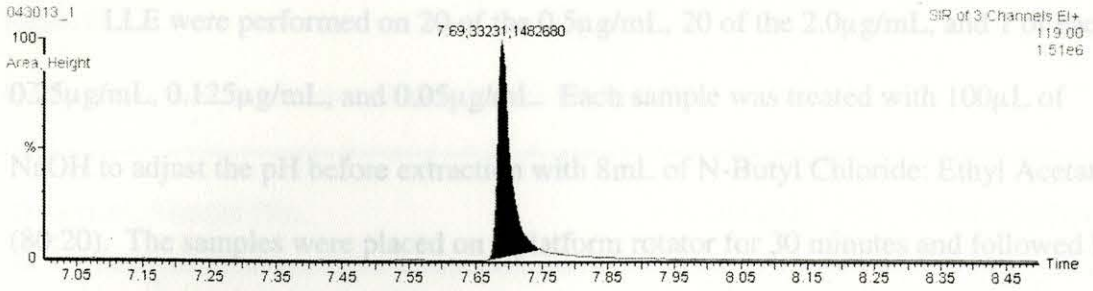


*Figure 2.* Mephedrone chromatogram of neat standard 0.5µg/mL. Mephedrone was eluted from the column at 7.69 minutes. The peak integration shows an area of 33,231 and a height of 1,482,680.

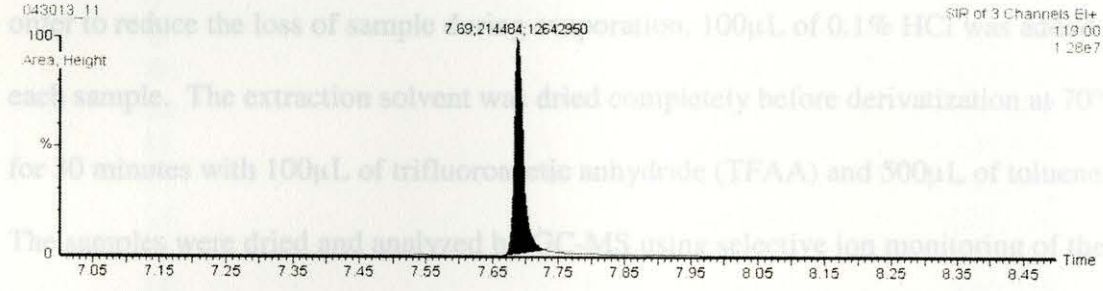


*Figure 3.* Mephedrone chromatogram of neat standard 2.0µg/mL. Mephedrone was eluted from the column at 7.69 minutes. The peak integration shows an area of 214,484 and a height of 12,642,950.

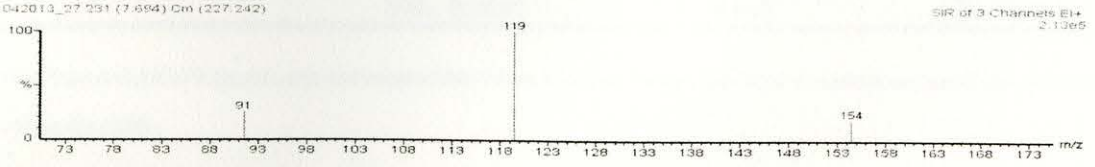


*Figure 4.* TFAA derivatized Mephedrone ion spectra displaying the 119 ion fragment which used for peak integration.

LLE were performed on 20 of the 0.5µg/mL, 20 of the 2.0µg/mL, and 1 of each of 0.25µg/mL, 0.125µg/mL, and 0.05µg/mL. Each sample was treated with 100µL of NaOH to adjust the pH before extraction with 8mL of N-Butyl Chloride: Ethyl Acetate (80:20). The samples were placed on a platform rotator for 30 minutes and followed by centrifugation for a total of 20 minutes. Halfway between centrifugation, the samples were removed and *bumped* to remove the emulsion layer from the walls of the tube. After centrifugation, the extraction solvent was removed and added to clean test tubes. In order to reduce the loss of sample during evaporation, 100µL of 0.1% HCl was added to each sample. The extraction solvent was dried completely before derivatization at 70°C for 30 minutes with 100µL of trifluoroacetic anhydride (TFAA) and 500µL of toluene. The samples were dried and analyzed by GC-MS using selective ion monitoring of the 91, 119, and 154 ion fragments. The mean, standard deviation, and standard error for LLE of 0.5µg/mL and 2.0µg/mL were calculated using the peak area and height of the 119 ion fragment (see Tables 3 and 4).

Table 3

*Mephedrone 119 Ion Area and Height of 0.5µg/mL LLE*

| 0.5µg/mL Sample No. | 119 ion area | 119 ion height |
|---|---|---|
| 1 | 582 | 37,357 |
| 2 | 1,721 | 100,837 |
| 3 | 2,187 | 108,700 |
| 4 | 2,643 | 154,168 |
| 5 | 1,299 | 73,387 |
| 6 | 1,499 | 79,785 |
| 7 | 5,444 | 305,602 |
| 8 | 990 | 48,175 |
| 9 | 2,179 | 113,124 |
| 10 | 1,337 | 72,075 |
| 11 | 2,421 | 137,473 |
| 12 | 2,642 | 141,901 |
| 13 | 4,730 | 263,716 |
| 14 | 3,257 | 180,919 |
| 15 | 2,847 | 160,735 |
| 16 | 2,978 | 175,193 |
| 17 | 3,497 | 194,397 |
| 18 | 2,955 | 172,803 |
| 19 | 2,143 | 124,263 |
| 20 | 5,176 | 287,616 |
| Mean: | 2626.350 | 146611.300 |
| Std. Deviation: | 1324.717 | 74819.919 |
| Std. Error: | 214.898 | 12137.394 |

Note: Data for LLE of 0.5µg/mL area and height of the 119 ion fragment. Results were used to establish any significant difference compared to SPE.

$$\text{Mean} = \frac{\Sigma X}{N} \qquad \text{Standard Dev.} = \sqrt{\frac{\Sigma(X-\bar{X})^2}{(N-1)}} \qquad \text{Std. Error} = \frac{SD}{\sqrt{(N_1-1)+(N_2-1)}}$$

Table 4

*Mephedrone 119 Ion Area and Height of 2.0µg/mL LLE*

| 2.0µg/mL Sample No. | 119 ion area | 119 ion height |
|---|---|---|
| 1 | 5289 | 376,515 |
| 2 | 21,618 | 1,124,926 |
| 3 | 29,385 | 1,577,971 |
| 4 | 34,932 | 1,863,275 |
| 5 | 32,404 | 1,788,215 |
| 6 | 35,050 | 1,903,884 |
| 7 | 83,053 | 4,649,702 |
| 8 | 106,410 | 6,243,251 |
| 9 | 55,559 | 2,993,259 |
| 10 | 150,682 | 8,605,144 |
| 11 | 209,051 | 11,926,742 |
| 12 | 88,895 | 4,806,430 |
| 13 | 84,467 | 4,502,916 |
| 14 | 195,287 | 10,462,803 |
| 15 | 177,427 | 9,915,483 |
| 16 | 225,729 | 12,308,818 |
| 17 | 227,575 | 13,088,955 |
| 18 | 209,289 | 11,666,571 |
| 19 | 169,992 | 9,269,243 |
| 20 | 193,712 | 10,624,808 |
| Mean: | 116790.300 | 6484945.550 |
| Std. Deviation: | 78604.595 | 4400128.071 |
| Std. Error: | 12751.349 | 713795.029 |

Note: Data for LLE of 2.0µg/mL area and height of the 119 ion fragment. Results were used to establish any significant difference compared to SPE.

SPE were performed on the remaining samples: 20 of the 0.5μg/mL, 20 of the 2.0μg/mL, and 1 of each of 0.25μg/mL, 0.125μg/mL, and 0.05μg/mL. One mL of 1.0M sodium phosphate buffer (pH = 6) was added to each sample and incubated at room temperature for 20 minutes. To acidify the sample before extraction, 200μL of 10% HCl was added to each sample. The samples were poured onto columns that were preconditioned with 1mL of MeOH and 1mL of type III water. The samples were washed with 2mL of 0.1M glacial acetic acid, 3mL of type III water, 1mL of MeOH, and 1mL of ethyl acetate, allowing for each solvent to flow through completely. The columns were dried at 40°C for 15 minutes. To elute the samples, 1.5mL of N-Butyl Chloride: Ethyl Acetate + 4% TEA were added to each column. One hundred microliters of 0.1% HCl was added to the eluent and evaporated to dryness at 50°C for 1 hour. The dried samples were derivatized at 70°C for 30 minutes with 100μL of trifluoroacetic anhydride (TFAA) and 500μL of toluene. The samples were dried and analyzed by GC-MS using selective ion monitoring of the 91, 119, and 154 ion fragments. The mean, standard deviation, and standard error for SPE of 0.5μg/mL and 2.0μg/mL were calculated using the peak area and height of the 119 ion fragment (see Tables 5 and 6).

Table 5

*Mephedrone 119 Ion Area and Height of 0.5µg/mL SPE*

| 0.5µg/mL Sample No. | | 119 ion area | 119 ion height |
|---|---|---|---|
| 1 | | 1,967 | 153,399 |
| 2 | | 6,217 | 362,220 |
| 3 | | 3,093 | 183,960 |
| 4 | | 5,563 | 341,736 |
| 5 | | 3,425 | 197,614 |
| 6 | | 3,138 | 186,997 |
| 7 | | 2,985 | 177,646 |
| 8 | | 5,014 | 312,828 |
| 9 | | 4,255 | 266,559 |
| 10 | | 6,391 | 397,219 |
| 11 | | 3,841 | 235,206 |
| 12 | | 6,444 | 411,544 |
| 13 | | 3,981 | 243,242 |
| 14 | | 4,411 | 265,679 |
| 15 | | 2,722 | 159,094 |
| 16 | | 6,165 | 383,412 |
| 17 | | 3,221 | 191,361 |
| 18 | | 5,533 | 340,534 |
| 19 | | 3,797 | 229,480 |
| 20 | | 4,562 | 274,576 |
| | Mean: | 4336.250 | 265715.300 |
| | Std. Deviation: | 1352.750 | 83645.771 |
| | Std. Error: | 219.445 | 13569.136 |

Note: Data for SPE of 0.5µg/mL area and height of the 119 ion fragment. Results were used to establish any significant difference compared to LLE.

Table 6

*Mephedrone 119 Ion Area and Height of 2.0µg/mL SPE*

| 2.0µg/mL Sample No. | | 119 ion area | 119 ion height |
|---|---|---|---|
| 1 | | 17,539 | 966,463 |
| 2 | | 34,427 | 1,958,788 |
| 3 | | 33,617 | 2,035,855 |
| 4 | | 21,767 | 1,310,713 |
| 5 | | 33,940 | 2,040,852 |
| 6 | | 19,630 | 1,153,008 |
| 7 | | 15,088 | 894,503 |
| 8 | | 18,993 | 1,138,131 |
| 9 | | 22,368 | 1,385,448 |
| 10 | | 21,823 | 1,304,416 |
| 11 | | 22,081 | 1,353,607 |
| 12 | | 28,125 | 1,749,587 |
| 13 | | 17,402 | 1,043,270 |
| 14 | | 7,507 | 450,643 |
| 15 | | 28,346 | 1,646,558 |
| 16 | | 25,874 | 1,612,155 |
| 17 | | 25,486 | 1,575,600 |
| 18 | | 31,485 | 1,875,417 |
| 19 | | 27,544 | 1,647,794 |
| 20 | | 28,237 | 1,739,522 |
| | Mean: | 24063.950 | 1444116.500 |
| | Std. Deviation: | 6977.600 | 421209.818 |
| | Std. Error: | 1131.916 | 68329.255 |

Note: Data for SPE of 2.0µg/mL area and height of the 119 ion fragment. Results were used to establish any significant difference compared to LLE.

Concentrations of 0.25µg/mL, 0.125µg/mL, and 0.05µg/mL were extracted via LLE and SPE to determine mephedrone's limit of detection for each method (see Tables 7 and 8).

Table 7

*Mephedrone 119 Ion Area and Height of LLE 0.25µg/mL, 0.125µg/mL, and 0.05µg/mL*

| Concentration of Sample | 119 Ion Area | 119 Ion Height |
|---|---|---|
| 0.25µg/mL | 5,834 | 327,251 |
| 0.125µg/mL | 2,727 | 135,488 |
| 0.05µg/mL | 2,523 | 134,183 |

Note: Data of LLE of 0.25µg/mL, 0.125µg/mL, and 0.05µg/mL to determine mephedrone's limit of detection for LLE.

Table 8

*Mephedrone 119 Ion Area and Height of SPE 0.25µg/mL, 0.125µg/mL, and 0.05µg/mL*

| Concentration of Sample | 119 Ion Area | 119 Ion Height |
|---|---|---|
| 0.25µg/mL | 1,489 | 98,848 |
| 0.125µg/mL | 572 | 38,432 |
| 0.05µg/mL | 161 | 9,897 |

Note: Data of SPE of 0.25µg/mL, 0.125µg/mL, and 0.05µg/mL to determine mephedrone's limit of detection for SPE.

To determine if there was a statistical difference between LLE and SPE, a *t* test was completed at the .05 level of confidence. The standard error of difference for area and height of both concentrations and methods was calculated and used to determine a *t* ratio. Compared to a *t* value of 2.086, the *t* ratio was outside of the 0.5 confidence level, signifying that LLE and SPE are significantly different (see Tables 9 and 10).

Table 9

*Statistical Comparison of LLE and SPE for 0.5µg/mL*

| | Mean | Standard Error | Standard Error of Difference | $t_{(.05)(100)}$ |
|---|---|---|---|---|
| LLE Area | 2626.35 | 214.898 | | |
| SPE Area | 4336.25 | 219.445 | 307.143 | 5.567 |
| LLE Height | 146611.30 | 12137.394 | | |
| SPE Height | 265715.30 | 13569.136 | 18205.433 | 6.542 |

Note: Statistical comparison of 0.5µg/mL LLE and SPE. With a degree of freedom of 19, the *t* value is 2.086. The *t* ratios for 0.5µg/mL are larger than the *t* value, meaning that LLE and SPE are significantly different for this concentration.

$$\text{Std. Error Difference} = \sqrt{(SE_1{}^2 + SE_2{}^2)} \qquad t_{ratio} = \frac{(\bar{X}_1 - \bar{X}_2)}{SED}$$

Table 10

*Statistical Comparison of LLE and SPE for 2.0µg/mL*

| | Mean | Standard Error | Standard Error of Difference | $t_{(.05)(100)}$ |
|---|---|---|---|---|
| LLE Area | 116790.30 | 12751.349 | | |
| SPE Area | 24063.95 | 1131.916 | 12801.490 | 7.243 |
| LLE Height | 6484945.55 | 713795.029 | | |
| SPE Height | 1444116.50 | 68329.255 | 717058.038 | 7.030 |

Note: Statistical comparison of 2.0µg/mL LLE and SPE. With a degree of freedom of 19, the *t* value is 2.086. The *t* ratios for 2.0µg/mL are larger than the *t* value, meaning that LLE and SPE are significantly different for this concentration.

Percent recovery shows the percentage of analyte that is recovered from each method based off of the samples' peak area and height. Peak integration was performed on the 119 ion fragment to provide the samples' area and height. The average of 0.5µg/mL and 2.0µg/mL area and height for both methods were compared to mephedrone's average standard area and height to achieve a percentage (Tables 11 and 12)

Table 11

*Percent Recovery for 0.5µg/mL Mephedrone Extraction*

| | Mean | Percent Recovery |
|---|---|---|
| Standard Area | 30581.15 | |
| LLE Area | 2626.35 | 8.59% |
| SPE Area | 4336.25 | 14.18% |

Table 11 (continued.)

| | Mean | Percent Recovery |
|---|---|---|
| Standard Height | 1497791.45 | |
| LLE Height | 146611.30 | 9.79% |
| SPE Height | 265715.30 | 17.74% |

Note: Percent recovery of 119 ion fragment for LLE and SPE of 0.5µg/mL. SPE has a higher percentage of recovery than LLE; therefore, SPE is a more efficient method for 0.5µg/mL mephedrone.

$$\text{Percent Recovery} = \left(\frac{\text{Extraction } \bar{X}}{\text{Standard } \bar{X}}\right) \times 100$$

Table 12

*Percent Recovery for 2.0µg/mL Mephedrone Extraction*

| | Mean | Percent Recovery |
|---|---|---|
| Standard Area | 152547.20 | |
| LLE Area | 116790.30 | 76.56% |
| SPE Area | 24063.95 | 15.77% |
| Standard Height | 8925131.35 | |
| LLE Height | 6484945.55 | 72.66% |
| SPE Height | 1444116.50 | 16.18% |

Note: Percent recovery of 119 ion fragment for LLE and SPE of 2.0µg/mL. LLE has a higher percentage of recovery than SPE; therefore, LLE is a more efficient method for recovery of 2.0µg/mL mephedrone.

# CHAPTER V

## DISCUSSION

Mephedrone is currently one of the more popular designer drugs of today. Its stimulatory and hallucinogenic effects provide a *high* like that of amphetamines and ecstasy. With its increasing popularity, it is imperative that efficient methods of analysis are employed in an attempt to keep up with the designer world. This study was conducted with goals of confirming that one method of extraction is more efficient and conducive to obtaining knowledge of mephedrone

Liquid-liquid extraction is the oldest and most widely used method of extraction. The use of two immiscible liquids appears to be fairly simple, but can be time consuming and costly due to the large amounts of solvents used. Although not as old in the scientific world as LLE, solid phase extraction involves the use of columns and smaller amounts of solvents.

In this research, the derivatization of mephedrone prior to analysis proved to be a crucial step in detecting the analyte from the extracts. Initially LLE and SPE were conducted on all synthetic urine samples, with no success for SPE. LLE analysis detected mephedrone and displayed peaks that could be integrated. SPE, however, gave distorted chromatograms with no ability to identify mephedrone. Derivatization was applied to help identify mephedrone because of is small molecular weight of 177.242g/mol.

This experiment focused on determining the more efficient method for the extraction of mephedrone from synthetic urine. It was theorized that LLE would be the more efficient method compared to SPE in the recovery of mephedrone. Because

mephedrone is a designer drug, there was concern that the use of columns specified for amphetamine drug testing may not be successful. After numerous trial runs, SPE demonstrated its ability to extract mephedrone just as LLE method. Manufacturer's directions did not specify to dry the columns prior to elution of the sample, but this technique was the determining factor in GC-MS detecting mephedrone from SPE extracts.

Eighty mephedrone spiked synthetic urine samples were extracted; 20 of 0.5µg/mL and 20 of 2.0µg/mL were extracted via LLE and 20 of 0.5µg/mL and 20 of 2.0µg/mL were extracted via SPE. A two tail T-test was completed on the average areas and heights of the analyzed samples. The *t* ratio of area and height of each extraction were compared to a *t* value of 2.086 with a confidence level of .05 and 19 degrees of freedom. The *t* ratios for LLE and SPE of 0.5µg/mL were 5.567 and 6.542 for area and height respectively. For LLE and SPE of 2.0µg/mL, the *t* ratio was calculated to 7.243 for area and 7.030 for height. The *t* ratios for both extractions and concentrations falling under the *t* value signify that LLE and SPE are significantly different for the extraction of mephedrone.

The areas and heights of the analyzed samples were used to determine which method was more efficient in recovering mephedrone from synthetic urine. Standards of 0.5µg/mL and 2.0µg/mL that were not extracted, were derivatized and analyzed 20 times each just as the extracts were. The results of the standards' areas and heights were compared to the average areas and heights of the extracted samples of both methods. The results concluded that for 0.5µg/mL, SPE is more efficient with a recovery of 14.18% and 17.74% for area and height compared to LLE 8.59% and 9.79%. For 2.0µg/mL, LLE

percent recovery is better than that of SPE. LLE percent recovery for area and height are 76.56% and 72.66% while SPE percentages are 15.77% and 16.18%.

Additional samples of 0.25, 0.125, and 0.05µg/mL were extracted via both LLE and SPE to determine mephedrone's limit of detection with analysis of GC-MS. For LLE, a steady decrease in peak area and height is seen with the decreasing concentrations. SPE peak area and height also show an area and height decrease with decreasing concentration. The peak areas and heights are smaller for SPE than LLE. In both cases, mephedrone is still detected at only 0.05µg/mL. For toxicological purposes, there is no need to test lower than this limit.

There is a large variation in the data received, which may be due to inconsistent derivatization of the extract. Capacity limits of SPE *gravity flow* columns may present an issue as well for extracting concentrations beyond 0.5µg/mL. Research would need to be conducted to determine the capacity of this column. The factor of varying results may have been able to be pinpointed had an internal standard been used in this research. In this study, however, there were issues with obtaining an appropriate internal standard for extraction of mephedrone. In the areas of derivatization, column capacity, and use of an internal standard, further research is needed.

This study exhibited that for the extraction of mephedrone spiked synthetic urine, SPE is a better extraction method than LLE in the case of extracting concentrations lower than 2.0µg/mL. If extracting larger concentrations of 2.0µg/mL and beyond, LLE appears to be the better method. Although both methods serve the same purpose of extracting analytes from mediums, they are significantly different. Further studies on this

research would be beneficial to analysts to ensure they are getting the most out of their analysis as well as to the human performance arena of forensic toxicology.

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