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Trinity College

Department of Chemistry

Development of a Rapid SPME/GC-MS Method for the Detection and Quantification of Synthetic Cathinones in Oral Fluid

A Senior Thesis By

David Matthew Correll

Submitted in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in
Neurochemistry

May 2013

Signatures of Thesis Committee:

Dr. Janet Morrison, Advisor: _____

Dr. David Henderson: _____

Dr. William Church: _____

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Abstract

The recent emergence and widespread abuse of new classes of compounds on the designer drug market as “legal” alternatives to scheduled drugs such as Ecstasy has prompted interest in the development of analytical methods for their detection and characterization. Synthetic cathinones, which are structurally similar to amphetamines, are commonly advertised as a “legal highs” or “herbal highs,” and are marketed under names such as “bath salts” and “plant food.” To avoid drug abuse legislation, a “not for human consumption” warning is typically printed on the label. Their ready availability on the Internet and in “head” shops, convenience stores, and even gas stations has spurred the popularity and abuse of these drugs for their euphoric/stimulant effects. The dramatic increase since 2009 in U.S. drug seizures involving cathinones, coupled with a significant rise in calls to poison control centers, emergency room visits, and even deaths due to synthetic cathinone intoxication, have prompted 37 states to ban these substances. In October 2011 the U.S. Drug Enforcement Agency exercised its emergency scheduling authority to temporarily designate three synthetic cathinones as Schedule I substances under the Controlled Substances Act.

The current study explores the development of an analytical method based on headspace and direct immersion solid-phase microextraction (HS-SPME and DI-SPME, respectively) coupled with gas chromatography-mass spectrometry (GC-MS) for the rapid laboratory confirmation of synthetic cathinones in oral fluid. Target analytes included butylone, diethylpropion, flephedrone, mephedrone, methedrone, MDPV, methylone, and naphyrone. Results of method optimization experiments designed to maximize SPME recoveries of cathinones from oral fluid are presented. Parameters investigated include incubation/extraction temperature, sample pH and salting out effects. In-matrix derivatization with ethylchloroformate and 2,2,2-trichloroethylchloroformate was explored. The mass spectrometric fragmentation of the alkylchloroformate derivatives was characterized and selectivity advantages were summarized. An internal standard calibration method was developed using matrix-matched calibrators and deuterated analogs of the target drugs as internal standards. Validation data including limit of detection, limit of quantification, and accuracy of quantification in oral fluid are presented.

Keywords: *cathinone, bath salts, solid-phase microextraction, derivatization, ethylchloroformate, 2,2,2-trichloroethylchloroformate, oral fluid, drug detection*

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This scientific work is lovingly dedicated to my grandmother, Nancy Mann.

Learn from yesterday, live for today, hope for tomorrow. The most important thing is not to stop questioning.

-Albert Einstein

Chapter 1: Introduction

1.1: Background

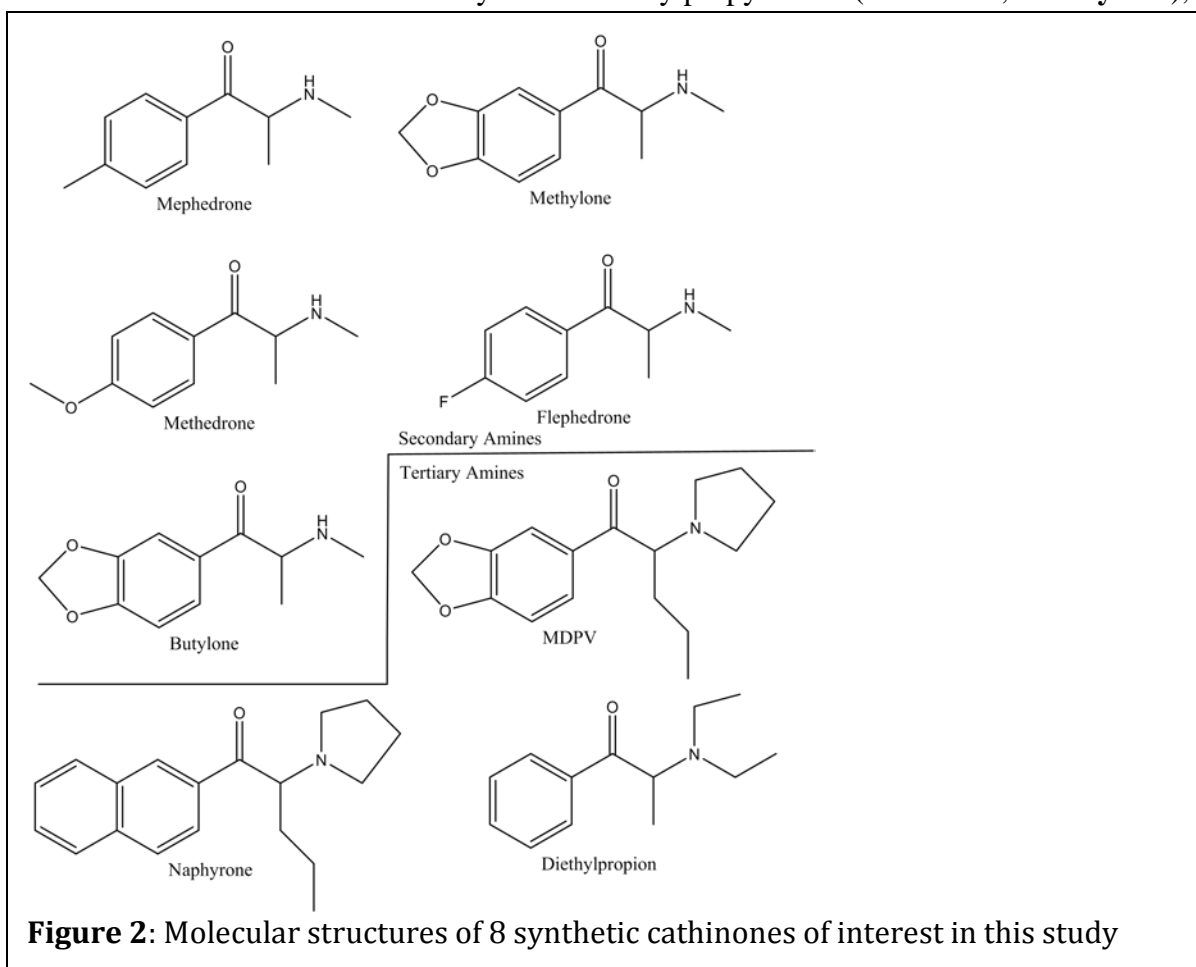
In recent years synthetic cathinones have become an increasingly popular and dangerous class of designer drug, abused by many individuals worldwide due to their ease of acquisition, variable legal status and hallucinogenic/euphoric effects. This most recent designer drug craze, which began in Western Europe in 2007, spread to the United States in 2010 [1]. These synthetic legal intoxicating drugs (SLIDs) are widely available over-the-counter in gas stations, convenience stores, and “head” shops, as well as on the Internet [2]. Synthetic cathinones are



derivatives of cathinone ((S)-2-amino-1-phenyl-1-propanone), a naturally occurring compound found in the leaves of the *Cathu edulis* (khat) plant [3]. As of 1993 cathinone was a scheduled drug in the United States under the Controlled Substance Act. The first appearance of synthetic cathinones dates back to the late 1930’s. The former Soviet Union used methcathinone as an

antidepressant in the 1930’s and an appetite suppressant in the 1950’s [4, 5]. Synthetic analogs of cathinone are commonly advertised as “legal highs” and are typically marketed as “bath salts” or “plant food (**Figure 1**).” In order to avoid regulation as a drug of abuse, a “not for human consumption” warning is typically printed on the label [3]. Popular brand names of these synthetic drugs range from “Ivory Wave,” “White Lighting,” “Meow-meow,” “Blow,” and “Cloud 9” to “Bohemian Bath Salts,” “Columbian Odorizer” and “Serenity” [4]. The major structural difference between synthetic cathinones and amphetamine type drugs is the presence of the beta-keto (bk) moiety on the benzyl side chain (**Figure 2**).

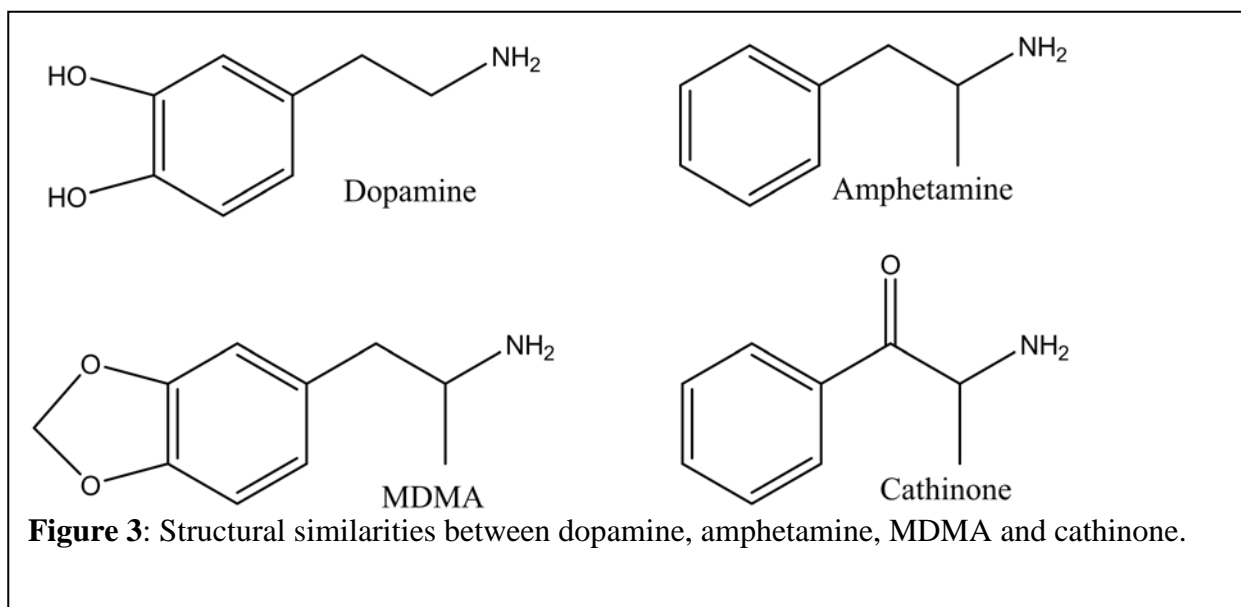
The most prevalent synthetic cathinones that are being marketed as “legal highs” include 3,4-methylenedioxypropylvalerone (**MDPV**), 3,4-methylenedioxy-N-methylcathinone (bk-MDMA, or **methylone**) and 4-methylmethcathinone (4-MMC, or **mephedrone**). These three drugs represent 98% of all synthetic cathinones currently on the market [6]. Other synthetic cathinones of interest are bk-N-methylbenzodioxolylpropylamine (bk-MBDB, or **butylone**), 4-



methoxymethcathinone (bk-PMMA, PMMC, or **methedrone**), naphthylpyrovalerone (NRG-1, or **naphyrone**), 4-fluoromethcathinone (4-FMC, or **flephedrone**), and 2-(diethylamino)-1-phenyl-1-propanone (DI, amfepramone, or **diethylpropion**) [2]. Chemical and structural data for these drugs is provided in **Figure 2**. These eight compounds are the target analytes explored in this study.

1.2: Pharmacology of Synthetic Cathinones

Synthetic cathinones possess similar stimulatory and hallucinogenic properties to those of Schedule I and II stimulants [6]. The extracellular increase of monoamines caused by synthetic cathinones is believed to produce the stimulatory and hallucinogenic effects of these drugs [7]. The common structural feature of both synthetic cathinones and other amphetamine-type stimulants is a phenyl group connected via a two-carbon chain to an amino group, similar to that of dopamine (**Figure 3**) [8]. While the desired effect of synthetic cathinones is a euphoria similar to that of amphetamine or methamphetamine, adverse effects are being increasingly reported by



hospitals and poison control centers [1, 9-10]. Routes of administration include sublingual, intranasal, intravenous, rectal and inhalation [11]. On October 21, 2011, the United States Department of Justice (USDoJ) and the Drug Enforcement Administration (DEA) designated three synthetic cathinones – MDPV, mephedrone and methylone – as temporary Schedule 1 substances under the Federal Analog Act, which controls these substance for up to eighteen months while the DEA and Department of Health and Human Services assess whether these

substances should be permanently controlled [12-13]. Various states have also enacted their own legislation for the scheduling of other synthetic cathinones.

MDPV, an analogue of pyrovalerone, a stimulant used to treat chronic fatigue, is known to be a dopamine (DA) and norepinephrine (NE) reuptake inhibitor [14, 15]. Like MDMA and methamphetamine, MDPV has been experimentally shown to increase extracellular levels of dopamine in mice brains, although with less potency [16]. It has no documented medical uses and produces stimulatory effects similar to those of MDMA [12,14].

Mephedrone also possesses stimulatory properties similar to those of MDMA [17]. While the mechanism of action has not been extensively studied, it is believed that mephedrone acts as a monoamine reuptake inhibitor in addition to stimulating the presynaptic release of monoamines [17]. Mephedrone has been shown experimentally to increase the extracellular concentration of both serotonin and dopamine in rat brains [18,19]. First synthesized in 1929, it was the sixth most popular drug of abuse in Europe only two years after it was classified as a drug of abuse (DOA) in 2007 [17,20].

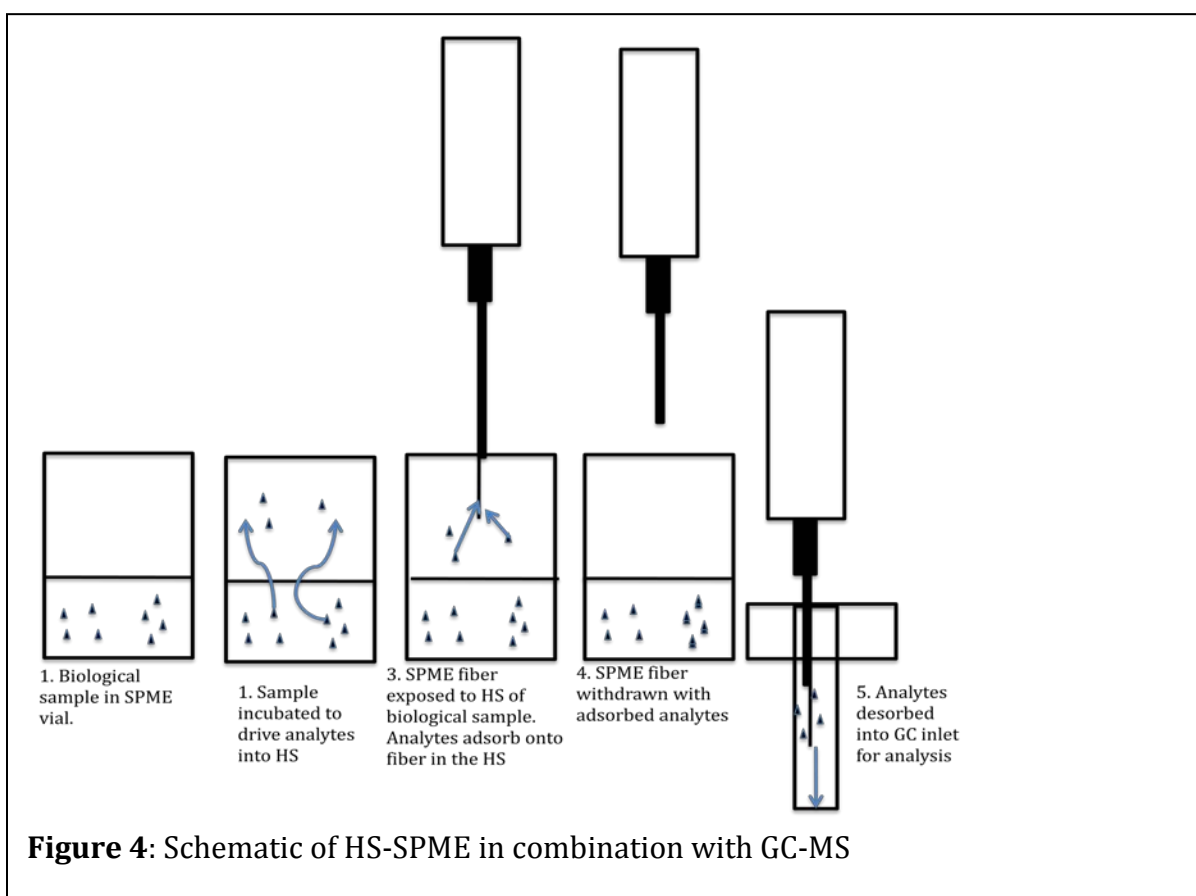
Methylone has no legitimate medical uses, although it was developed during research into anti-depressant and anti-Parkinsonian agents [21]. Methylone initiates an increase in dopamine, norepinephrine and serotonin from cells that express these specific monoamine transporter proteins [22].

Despite their recent emergence, a variety of methods have already been reported for the detection, quantification and characterization of synthetic cathinones using GC-MS [4, 23-34], liquid chromatography-mass spectrometry (LC-MS) [23, 25, 31, 33, 35, 36], high performance liquid chromatography (HPLC) [37], Fourier transform infrared spectroscopy (FTIR) [27, 38], Fourier transform Raman spectroscopy (FT-Raman) [27], nuclear magnetic resonance (NMR)

[24, 26-27, 38-39] and immunoassay (IA) [36, 40]. MDPV and butylone have been known to cause false positive results in amphetamine/Ecstasy [36] and phencyclidine (PCP) [40] IA drug screens, respectively.

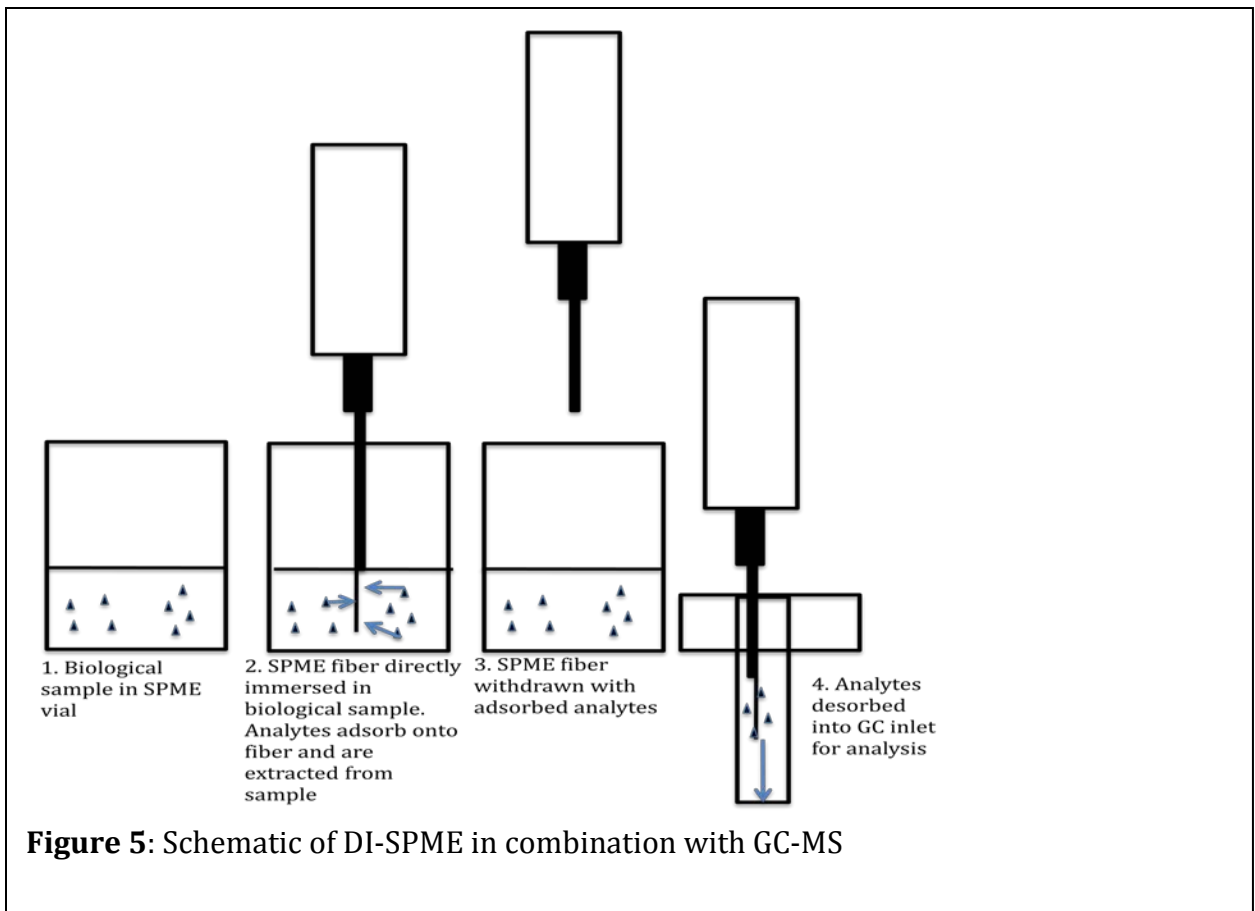
1.3: Solid-Phase Microextraction (SPME)

Solid-phase microextraction (SPME) is a fast, efficient, affordable, sensitive and solvent-free extraction technique that allows for high-throughput and online coupling of sample preparation and separation [41-46]. It also avoids the typical problems (i.e., sample loss, dilution



and/or contamination) associated with other extraction techniques such as liquid-liquid extraction, solid-phase extraction, and purge-and-trap [47]. Most extraction methods require a large amount of technical expertise to obtain accurate and reproducible results and often involve multiple steps, increasing the chance of sample loss [48]. In contrast, SPME is simple to

implement, readily automated and easy to couple with GC or LC. SPME was invented in 1989 by Pawliszyn and colleagues for analysis of water pollutants [49]. Two commonly used modes of SPME are headspace (HS-SPME) and direct immersion (DI-SPME). HS-SPME is well suited for analysis of volatile compounds while DI-SPME is well suited for polar and non-volatile compounds [44]. Both use a fused silica fiber coated with a polymeric organic liquid which acts as the extraction medium. The fiber is encased in a stainless steel sheath to prevent mechanical damage during septum piercing. In HS-SPME (see **Figure 4**) the liquid or solid sample is capped and incubated, driving the analytes of interest into the headspace above the sample. Once a dynamic equilibrium is reached the SPME needle pierces the septum of the cap and the SPME fiber is exposed to the headspace, allowing the gaseous analytes to adsorb onto the fiber phase. After the desired extraction time, the fiber (with adsorbed analytes) is retracted back into its



protective sheath and the needle is removed from the sample vial. If GC-MS is being utilized for extract analysis, the SPME needle subsequently enters the GC injection port and the SPME fiber is exposed. The analytes are then thermally desorbed off the fiber and concentrated at the head of the GC column. Direct immersion (DI) SPME is identical to HS-SPME except the SPME fiber is exposed directly to the liquid sample matrix (**Figure 5**), removing volatility issues that can render HS-SPME ineffective. When employing HS-SPME, sample pretreatment and preheating can increase the volatility of some substances. Pretreatment for amphetamine type drugs often involves adjusting the sample to an alkaline pH (to maintain the free base form of the drug) and addition of salt. Agitation of the sample in both DI and HS-SPME allows for decreased sampling time by decreasing the time for dynamic equilibrium to be achieved. SPME is extremely well suited for the extraction of volatile and semi-volatile compounds from a variety of matrices, making it ideal for biological sample analysis [50]. *On-fiber* as well as *in-matrix* analyte derivatization is also compatible with SPME [51].

1.4: SPME: Methodology and Theory

SPME is a multiphase complex equilibration process, which is completed once a dynamic equilibrium is achieved between the sample matrix and the headspace and the headspace and the fiber coating [52]. The equilibrium conditions are best described by Equation 1, where n is the mass of the analyte extracted by the coating, K_{fs} is a fiber coating–sample matrix distribution constant, K_{hs} is the headspace–solution equilibrium constant, V_f is the fiber coating volume, V_s is the sample volume, V_h is the headspace volume, and c_0 is the initial concentration of a given analyte in the sample [52].

$$\text{Equation 1: } n = \frac{K_{fh}K_{hs}V_fV_s}{K_{fh}K_{hs}V_s + K_{hs}V_h + V_s} c_0$$

Various factors can affect the efficiency and sensitivity of SPME. In order to maximize SPME recoveries, a variety of operational parameters need to be optimized. These parameters include fiber size and nature and thickness of coating; agitation of the sample; sample pH; salting out effects; incubation, extraction and desorption temperatures; and incubation, extraction, and desorption times. Optimization of these parameters is necessary to achieve desirable detection limits and to reduce sampling time. Sample pretreatment is simple and dependent upon the chemical nature of the desired analytes.

1.5: Review of SPME in Analytical Toxicology

The use of SPME in analytical toxicology, reviewed by Pragst [52] and Kataoka [41], has increased dramatically over the past decade. SPME-GC/MS is an ideal method for coupling the extraction, detection and quantification of drugs of abuse in many different biological samples. Merola and colleagues [53] developed a HS-SPME/GC-MS method for the determination and quantification of amphetamines, ketamine, methadone, cocaine, cocaethylene, and THC in hair. Miekisch and colleagues [34] used HS-SPME/GC-MS for the assessment of propofol concentrations in human breath and blood. Hara and colleagues [54] used HS-SPME/GC-MS to determine amphetamine and methamphetamine concentrations in human tissue. Recently, a SPME/GC-MS method for the simultaneous detection of seventeen drugs of abuse and metabolites in hair was developed by Aleksa and colleagues [32].

More relevantly, SPME has been used in combination with GC/MS for the detection of many different types of drugs in oral fluid (OF). OF offers enormous advantages over other biological matrices when attempting to determine if a subject is under the influence of a DOA at the time of testing. While the levels of drugs in OF are often similar to that of plasma, OF can be obtained under direct observation by non-medical personnel, making sample collection easier,

faster and less subject to adulteration [55-56]. Although urine sample collection should be done under direct supervision, subjects are often granted privacy to produce the specimen, allowing for a higher chance of sample adulteration. Furthermore, urinalysis is only useful for establishing recent use, rather than “under the influence” determinations, making it ineffective for roadside testing [55]. A more comprehensive list of the advantages and disadvantages of OF drug monitoring are outlined in **Table 2**.

Table 2: Advantages and Disadvantages of Oral Fluid Testing*

Advantages	Disadvantages
<ul style="list-style-type: none"> • Observed collection • Noninvasive collection • Less stressful collection for cortisol levels • Decreased opportunity for adulteration when compared to urine • Generally represents free plasma levels • Easier to obtain multiple samples than with urine or plasma • Commercial screening assays are available • Oral fluid assays are technically easier than plasma due to the lower content of proteins and lipids • Collection and screening may be done in the home • Risk of infection is lower with oral fluid than plasma 	<ul style="list-style-type: none"> • Limited specimen volume • Low analyte concentrations • Interferences from food and/or beverages • Contamination with oral, smoked, inhaled or insufflation • Lack of standardized testing procedures • If collection devices are used, unknown concentration of analyte • Stimulation of oral fluid changes the pH and drug concentration

*Reproduced from [56]

1.6: Oral Fluid and Drug Analysis

OF consists of saliva, gingival cervicular fluid, buccal and mucosal transudates, cellular debris, bacteria, and residues of ingested products [55]. The salivary glands, which are highly perfusable, allow for the rapid diffusion of DOA in blood to OF, with positive detection of drugs occurring within minutes of use [57].

The most common route in which ionized DOA and their ionized metabolites appear in oral fluid is through passive diffusion from blood [55]. Intranasal administration and inhalation

can also deposit DOA in oral fluid [55]. Passive diffusion is affected by salivary pH, chemical properties of the DOA, concentration of nonionized DOA/metabolites, drug-protein binding, and membrane characteristics [57]. Basic drugs are often found in higher concentrations in plasma compared with OF due to the relative acidity of saliva [57].

1.7: Oral Fluid and SPME for Drug Analysis

Fucci and colleagues [58] developed a SPME (HS and DI) method for the detection of methadone, cocaine, cocaethylene, amphetamine, methamphetamine, MDMA, 3,4-methylenedioxyethylamphetamine (MDEA), N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), cannabidiol, THC and cannabinol in OF. Limits of detection (LOD) and limits of quantification (LOQ) for these analytes ranged from 1-50 ng/mL for DI-SPME and 1-250 ng/mL for HS-SPME [58]. Souza and colleagues [59] developed a DI-SPME method to detect amphetamine, methamphetamine, fenproporex and diethylpropion in OF after in-matrix derivatization with propylchloroformate, which produced detection limits of 0.5-2 ng/mL [58].

1.7: Project Goals

Much of the published literature on the detection, quantification and characterization of synthetic cathinones explores only one or two cathinone analogs at a time. The few studies that have been developed for screening multiple synthetic cathinones have focused on LC-MS separation and detection. At this time no study has used SPME to isolate synthetic cathinones from oral fluid. The goal of the present study was to develop a rapid and reliable method based on SPME/GC-MS for the detection and quantification of eight unique and commonly abused synthetic cathinones (see **Figure 2**) in OF. Because MDMA tablets often contain synthetic

cathinone impurities or are comprised completely of synthetic cathinones [60], MDMA was also included as a target analyte in this study.

Various derivatization schemes with alkylchloroformate reagents were investigated to increase the volatility and improve the chromatographic characteristics of target analytes possessing a secondary amine group. Each analyte and its alkylchloroformate derivative was characterized by electron impact mass spectrometry. HS and DI-SPME were compared for relative efficiency and reproducibility of extraction of the target analytes from OF.

SPME parameters including incubation and extraction temperature and sample pretreatment were optimized. Calibration curves using deuterated internal standards (I.S.) were produced. Analytical figures of merit including limits of detection and quantification (LOD, LOQ) and accuracy were determined. Finally, the optimized method was applied to a commercially available saliva drug test collection device to demonstrate proof of concept.

Chapter 2: Experimental Methods

2.1: Chemicals and Reagents

Certified analytical reference standards for MDPV, methylone, mephedrone, mephedrone- d_3 diethylpropion, butylone, butylone- d_3 , methedrone, naphyrone, flephedrone, amphetamine, methamphetamine, MDMA, MDMA- d_5 , MBDB, MDA, MDEA, PMA and PMMA were purchased from Cerilliant Corporation (Round Rock, TX). All certified standards and working standard solutions were stored at 4°C in clear plastic storage containers. Ethylchloroformate (ECF) and 2,2,2-trichloroethylchloroformate (TCECF) were obtained from Sigma Aldrich (Bellefonte, PA) and stored at 4°C.

2.2: Individual Working Standard and Mixed Calibration Standard Preparation

Stock aqueous solutions (~40 µg/mL) of each analyte were prepared by appropriate dilution of certified reference standards (1.0 mg/mL in methanol) obtained from Cerilliant using an absolute mass method. Mixed aqueous calibration standards with concentrations of 5, 10, 20, 50, 100, 200, 400, 800, 1600, 4800, and 8000 ng/mL were prepared from these stock solutions using a serial dilution method.

2.3: SPME/GC-MS Analysis

Samples were analyzed using a Thermo-Fisher Trace Ultra DSQ II with a TriPlus autosampler equipped with an aluminum heating block which allowed for stable, programmable sample incubation and extraction. SPME vials (10 mL) and septum caps were purchased from Thermo Fisher (Kalamazoo, MI). The SPME fiber was a 24-gauge autosampler-compatible 100 µm polydimethylsiloxane (PDMS) fiber (Supelco Corp, Bellefonte, PA) which was conditioned for 30 minutes at 250°C before use. The GC column was a Restek Rxi-5ms (15 m x 0.25 mm x

0.25 μm) 5% diphenyl/ 95% dimethylpolysiloxane (Restek Corp., Bellefonte, PA). Optimal analyte separation was obtained using the following GC oven temperature program: initial temperature 80°C (3 min hold); ramp 1 at 40°C/min to 140°C; ramp 2 at 8°C/min to 210°C; ramp 3 at 40°C/min to 280°C (3 minute hold). Helium was used as a carrier gas at a flow rate of 1 mL/min. Splitless injection at 250°C was employed with a splitless time of 2.0 minutes. The MS transfer line was maintained at 250°C. The MS was operated in the electron impact ionization mode (70 eV). The ion source was maintained at 200°C. MS data acquisition was initiated at 6 min and was performed in full scan (40-400 m/z) mode for SPME optimization experiments.

2.3.1: Headspace solid-phase microextraction (HS-SPME). Aqueous samples were incubated for 10 minutes at the optimized temperature (see below) in the aluminum block of the autosampler. The PDMS fiber was then introduced to the headspace of the sample and extraction took place for 10 minutes at the optimized temperature. The extracted analytes were then thermally desorbed at 250°C for 6 minutes (optimized in earlier work for MDMA) in the splitless injection mode.

2.3.2: Direct-immersion solid-phase microextraction (DI-SPME). The sample was introduced to the aluminum heating block at 70°C. The 100 μm SPME fiber was then directly immersed into the sample for 10 minutes. In order to insure direct fiber exposure the fiber sampling depth was set to its maximum depth (35 mm) and the samples were diluted with 8.0 mL of water to raise the volume of the sample to an adequate sampling height.

2.4: Sample Pretreatment

HS-SPME samples were pretreated with KOH (1.4 M) to a final sample pH of 10 and saturated with NaCl. DI-SPME samples were pretreated with KOH and diluted with deionized

water to bring the aqueous sample to an appropriate depth for automated sampling. Vials were hermetically sealed with crimp tops.

2.5: HS-SPME Parameter Optimization

Incubation/extraction parameters were optimized by running triplicate analyses of ~320 ng/mL mixed standards at 50, 60, 70, 80, 90 and 100°C and plotting the absolute area of the base peaks of each analyte after derivatization with ECF. A subsequent temperature optimization was run at 80, 90, 100, 110, 120 and 130°C. The optimal temperature for analysis of all samples was determined by averaging the optimal incubation/extraction temperatures determined for each individual analyte.

2.6: Oral Fluid Collection and Drug Fortification

Drug free oral fluid for spike recovery studies and for use as a negative control was collected daily by expectoration from a drug negative user prior to sample preparation.

2.7: SPME with In-Matrix Derivatization

For derivatization studies, 50 µL of derivatizing reagent were added directly to a SPME vial containing 400 µL of the aqueous or oral fluid sample, 400 µL of 1.4 M KOH (pH=10) and saturated with salt (HS-SPME only).

2.8: Preparation and GC-MS Analysis of Derivatized Standards

For retention time determination and mass spectral characterization of derivatized analytes, derivatized standards for liquid injection were prepared in the following manner: 500 µL of an individual stock aqueous standard was spiked into a glass screw top conical test tube, 50 µL of derivatizing reagent (ECF) was added and the tube was vortexed for 1 min. The tube was capped and incubated at 80°C for 15 minutes. Derivatized analytes were then back extracted into 1 mL of ethyl acetate. The ethyl acetate layer was transferred to a GC autosampler vial for

subsequent GC-MS analysis. Prior to injection the GC syringe was prewashed twice in hexane (2 x 5.0 uL), twice in ethyl acetate (2 x 5.0 uL) and twice with the sample (2 x 2.0 uL). The sample (1.0 uL) was injected at 280°C and GC-MS was performed under the operating conditions described above.

2.9: Calibration Curves

HS-SPME calibration standards were prepared by fortifying 400 µL of oral fluid with aqueous mixed analyte solutions to achieve final concentrations of 1, 2, 4, 10, 20, 40, 80, 160 and 320 ng/mL. DI-SPME calibration standards were prepared in the same manner with final concentrations of 20, 40, 80, 160 and 320 ng/mL; these calibrators were subsequently diluted with 8 mL deionized water to bring the volume to an appropriate sampling depth for DI as discussed above. MDMA-*d*₅ (30 ng/mL) was used as an I.S. for DI-SPME while mephedrone-*d*₃ and butylone-*d*₃ (96 ng/mL) were used as I.S. for HS-SPME. Each calibration standard was run under the optimized SPME parameters.

2.10: Accuracy

Accuracy was assessed by analysis of blind high (240 ng/mL) and low (60 ng/mL) fortified samples which were quantified off the calibration curve run that day. Each calibrator and blind sample was fortified with MDMA-*d*₅ internal standard at a concentration of 30 ng/mL.

2.11: Demonstration of Method Applicability to Commercial Saliva Collection Kit

For demonstration of proof-of-concept for the DI-SPME method, analysis was performed using an Intercept oral fluid collection device (Bethlehem, PA). To simulate the saliva collection process, the cotton swab was immersed for 5 minutes in 2 mL of negative control saliva in a conical test tube which had been pre-fortified with the target analytes (16 ng/mL). To accommodate the saliva swab 4 mL of deionized water was added to conical tube. The swab was

then stored in preservative buffer supplied with the commercial kit. The swab and buffer were subsequently transferred to a 10 mL SPME vial and analyzed in the DI-SPME manner described above.

Chapter 3: Results and Discussion

3.1: Cathinone Recovery using HS-SPME

Initial HS-SPME experiments demonstrated that while HS-SPME was a suitable method for the isolation of 3° cathinones from aqueous solution, underivatized 2° cathinones were not recovered (**Figure 6**).

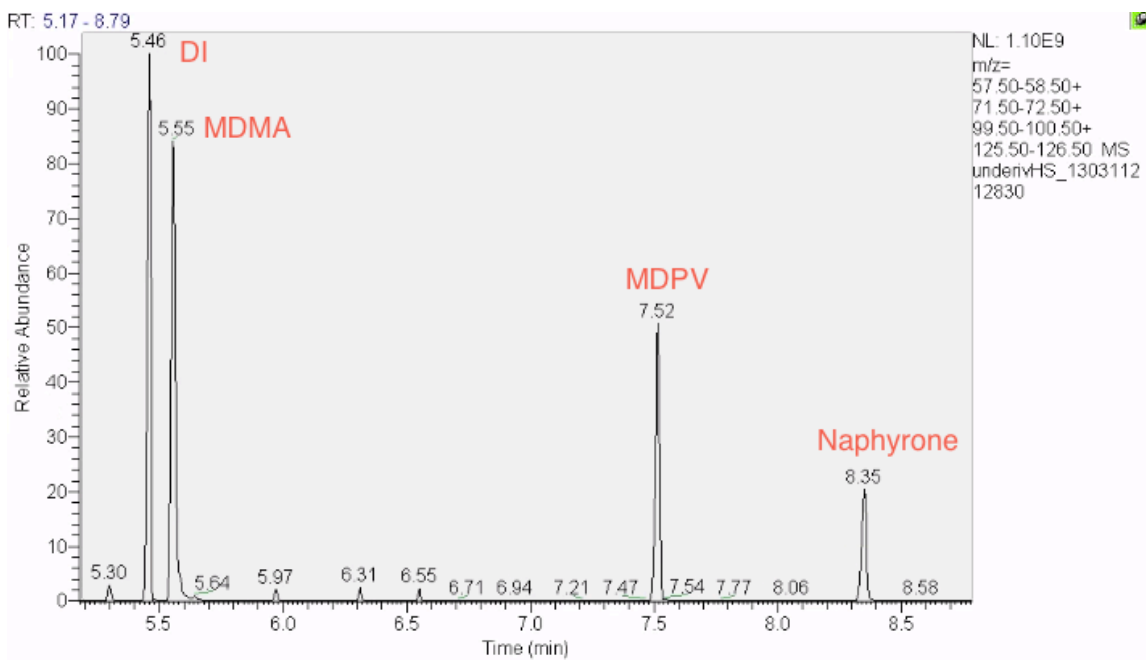


Figure 6: Resulting chromatogram following HS-SPME of an underivatized aqueous standard of mixed cathinones (320 ng/mL), demonstrating the recovery of 3° cathinones and MDMA, while 2° cathinones are absent. SPME conditions: 100 μ m PDMS fiber, 70°C extraction/incubation temp, 10 min extraction time, 6 min desorption time at 250 °C. GC-MS parameters as described in the experimental section.

It is hypothesized that intermolecular hydrogen bonding between neighboring 2° cathinones in solution (**Figure 7**) is sufficiently strong so as to preclude their partitioning into the headspace. In contrast, the 3° cathinones and MDMA (which lacks a β -keto moiety and does not hydrogen bond in a similar manner to 2° cathinones) are sufficiently volatile and efficiently recovered

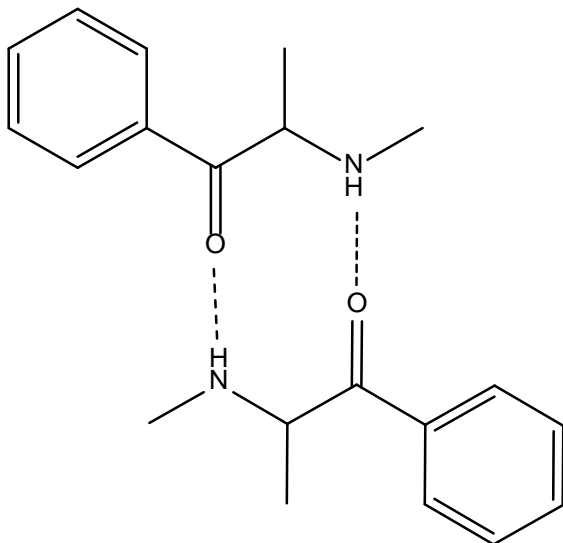


Figure 7: Hypothesized intermolecular bonding schematic which prevented underivatized 2° cathinones from entering the gaseous, making them incompatible with HS-SPME.

using HS-SPME. To overcome this volatility limitation for 2° cathinones, both direct immersion (DI)-SPME and in-matrix derivatization of the 2° amine functional group with alkylchloroformate derivatizing reagents was investigated as a means to eliminate the intermolecular hydrogen bonding and allow the cathinones to enter the HS for SPME extraction.

3.2: Effect of ECF Derivatization on Cathinone Recovery Using HS-SPME

The advantages of ECF derivatization on the recovery of cathinones became immediately apparent, as shown in **Figure 8**. 2° cathinones were easily derivatized within the aqueous matrix (referred to as “in-matrix” derivatization) to their ethylcarbamate analogs using ECF, and were readily extracted from the headspace above incubated oral fluid samples (**Figure 8a**).

Unfortunately, in the presence of the ECF derivatizing reagent the 3° cathinones were either inefficiently extracted (**Figure 8b**) or not extracted at all (**Figure 8c**). While it was initially expected that the 3° cathinones would be unaffected by the ECF reagent, it is now hypothesized that the reagent may in fact react with the tertiary amines at the amine or carbonyl functional

groups to form an unanticipated derivative that is either non-volatile or does not partition into the PDMS fiber film. This hypothesis is discussed in further detail in section 3.7.

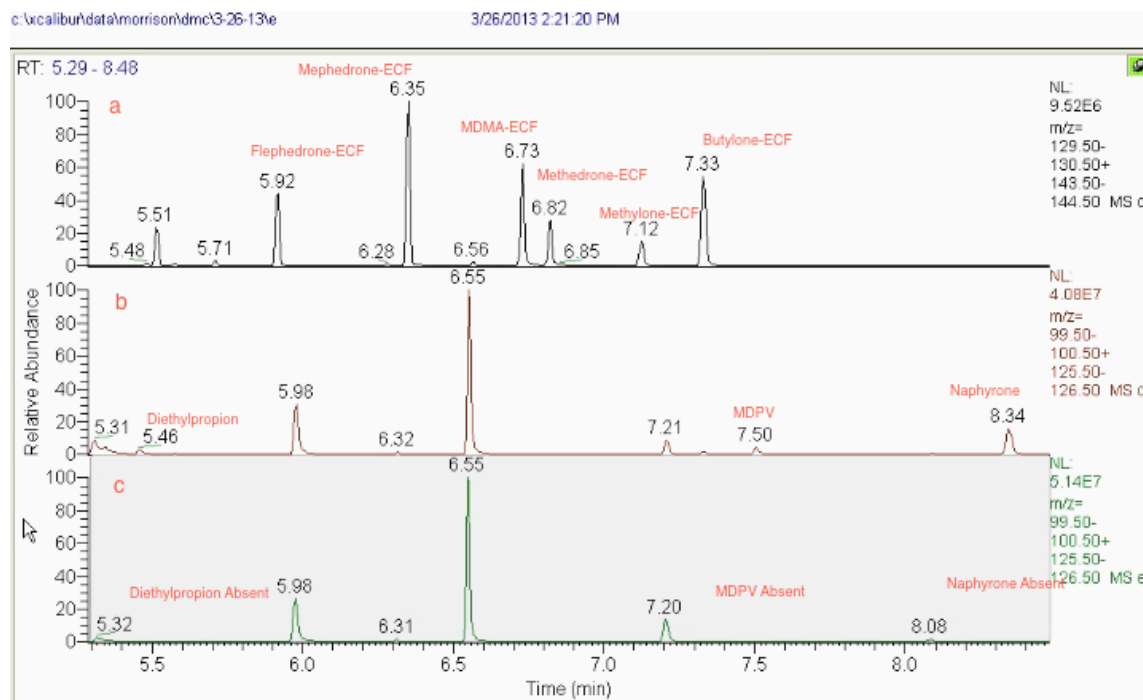


Figure 8: Resulting chromatogram following HS-SPME of ECF-derivatized mixed cathinone standard in oral fluid. Figure 3a shows that 2^o cathinones (320 ng/mL) were successfully extracted from the HS as their ECF derivatives following addition of 50 μ L of the ECF reagent directly to the oral fluid matrix. Figures 3b and 3c show that 3^o cathinones (320 ng/mL and 80 ng/mL, respectively) were inefficiently recovered (3b) or not recovered at all (3c) in the presence of ECF derivatizing reagent. SPME conditions as in Figure 6.

3.3: Effect of ECF Derivatization on GC Peak Shape of Cathinone Analogs

The conversion of the secondary amine functional group to a carbamate by derivatization with ECF allowed for improved GC peak shapes of secondary cathinones (**Figure 9**). While the GC peaks for the underivatized cathinones showed significant tailing (**Figure 9a**), those for ECF-derivatized cathinones exhibit no tailing and are more Gaussian-shaped (**Figure 9b**).

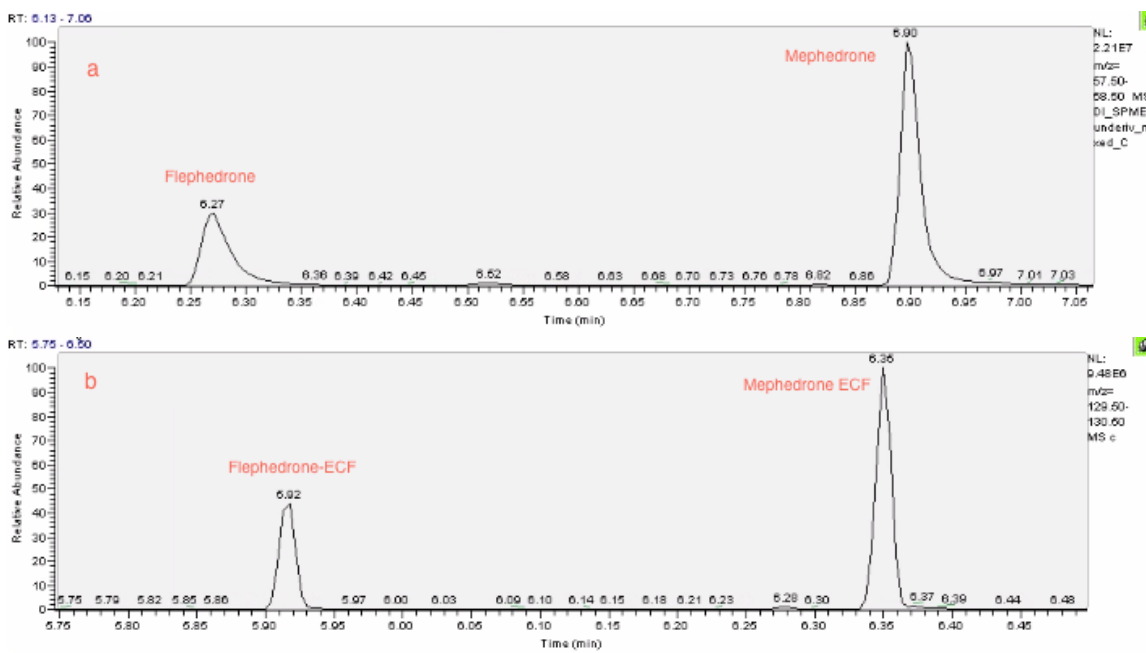


Figure 9: Resulting chromatogram following DI-SPME of underivatized (a) and ECF-derivatized (b) mephedrone and flephedrone from oral fluid, showing the advantages of ECF-derivatization on GC peak shape. SPME conditions as in Figure 7.

3.4: Effect of ECF- and TCECF- Derivatization on MS Selectivity of Cathinone Analogs

The 70 eV EI-mass spectra obtained for underivatized 2^o cathinone analogs, shown in **Figure 10**, illustrate that structurally similar cathinone compounds produce relatively non-selective fragmentation patterns which lack molecular ions and consist mainly of immonium ions at m/z 58 or 72 depending on the particular compound [31, 61]. The lack of selectivity in the mass spectra makes identification of structurally similar analogs extremely challenging in the absence of authentic standards. To address these limitations, derivatization with alkylchloroformates was investigated with the goal of producing analyte derivatives which exhibit more unique fragmentation patterns and consequently provide for more adequate mass

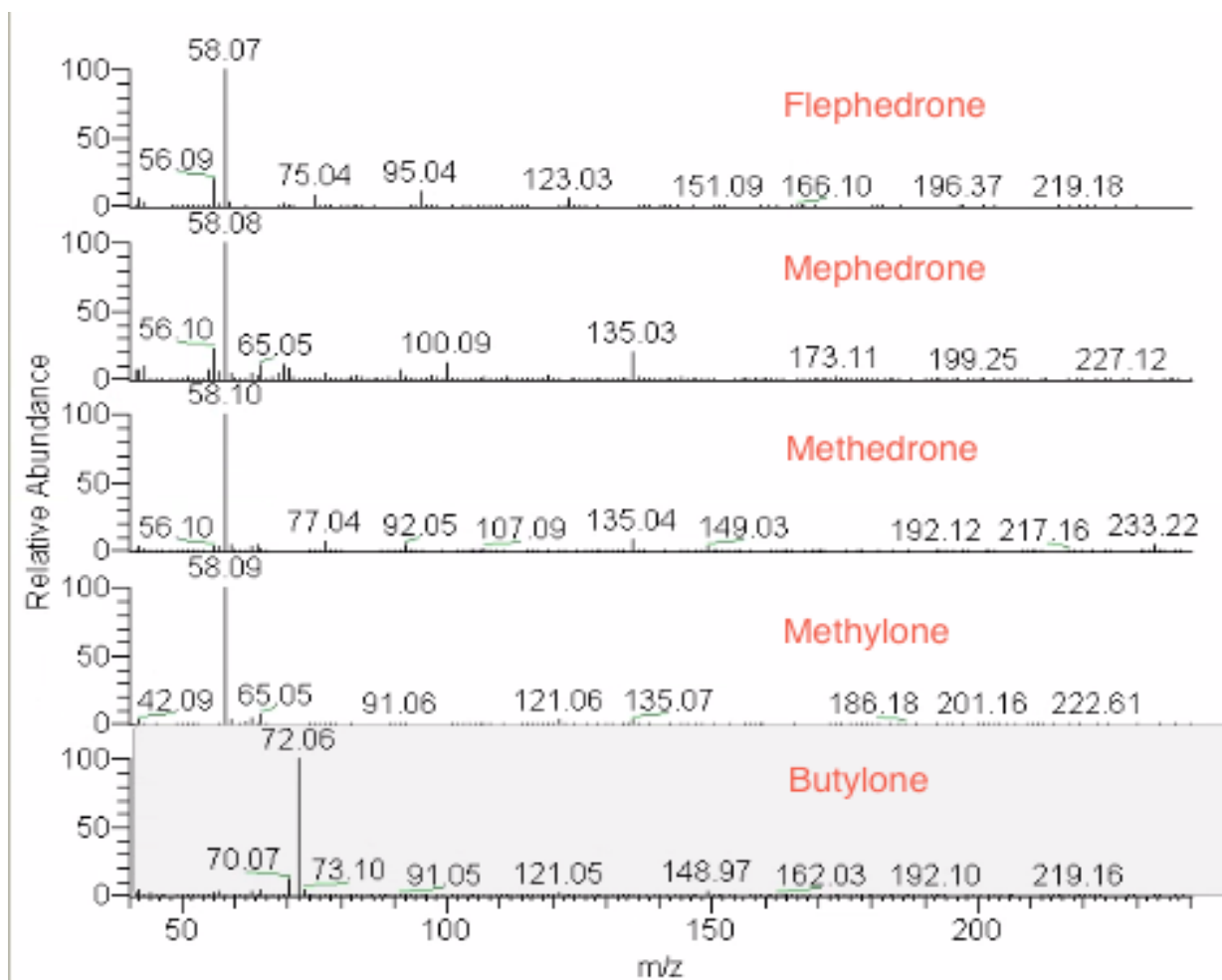


Figure 10: 70 eV MS of underivatized 2° cathinones

spectral discrimination of structurally similar cathinone analogs. Specifically, derivatization with 2,2,2-trichloroethylchloroformate (TCECF) and ethylchloroformate (ECF) was explored. These reactants acylate 1° and 2° amines to produce carbamate derivatives (**Figure 11**). The advantages of ECF derivatization for extraction of 2° cathinones and improved chromatographic performance were described above. Here, the advantages of both ECF- and TCECF-derivatization on MS selectivity are discussed.

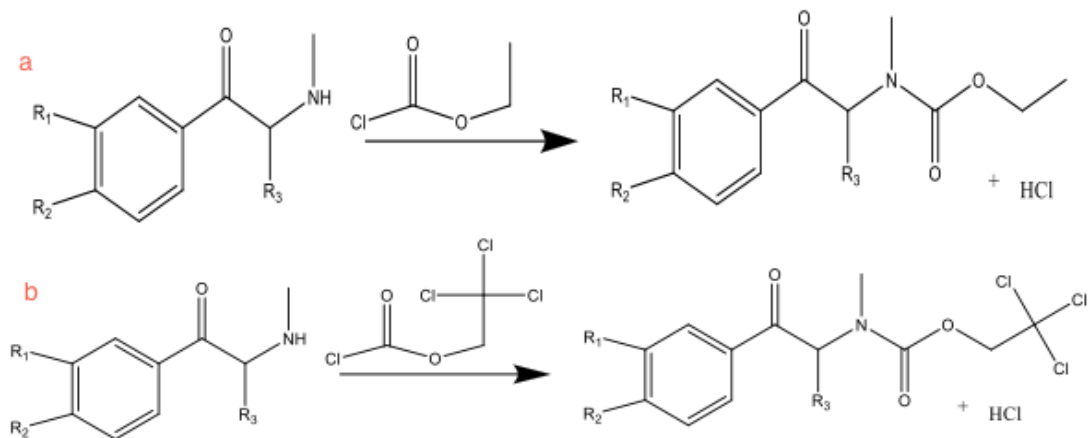


Figure 11: Derivatization reaction of 2^o cathinone with ECF (11a) and TCECF (11b).

Figures 12 and **13** illustrate the improved MS selectivity that derivatization with ECF and TCECF, respectively, provide. Derivatives were prepared by adding 50 μ L of derivatizing reagent to oral fluid fortified with an aqueous cathinone mixed standard (320 ng/mL) and pretreated with KOH (1.4 M) to a pH of 10 and saturated with NaCl(s). The resulting MS data are summarized in **Table 3**. These results demonstrate that derivatization with either ECF or TCECF yields more qualifier ions as well as the molecular ions for the carbamate derivatives, thus facilitating molecular discrimination. Furthermore, the addition of three chlorine atoms to the TCECF-derivatized molecules allowed for unique isotopic peak patterns, providing each molecule with a more easily identifiable fragmentation signature. Frison [28] previously reported the MS advantages of TCECF derivatization on the mass spectral discrimination of mephedrone from methanolic solutions of seized drugs containing mephedrone. At the time of this writing, TCECF-derivatization has not been reported for the analysis of any other cathinone analogs, and the derivatization of cathinone analogs with ECF has never before been reported. Additionally, to our knowledge this work represents the first demonstration of in-matrix alkylchloroformate derivatization of cathinone analogs in oral fluid for subsequent SPME/GC-MS analysis

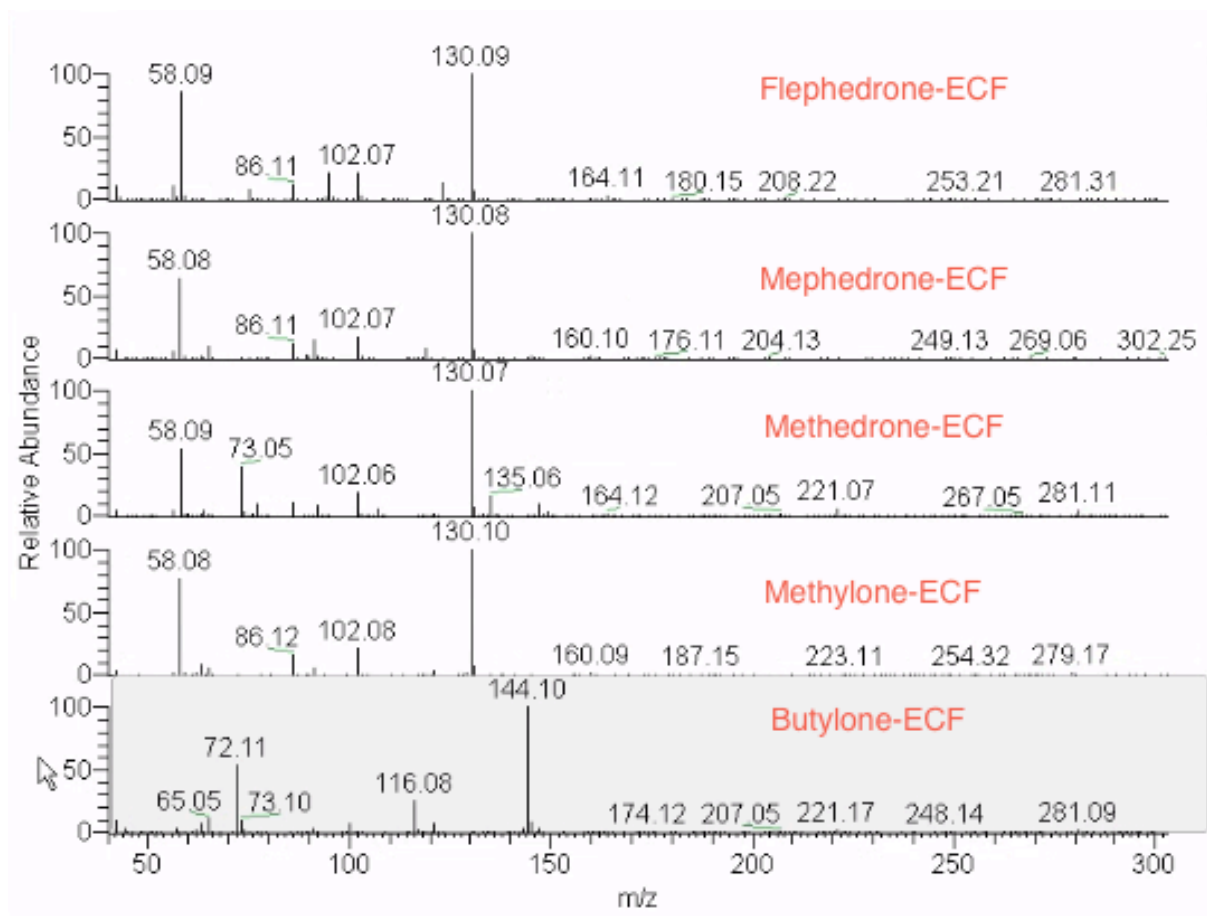


Figure 12: EI mass spectrometric fragmentation (70 eV) of 2° cathinones derivatized with ECF.

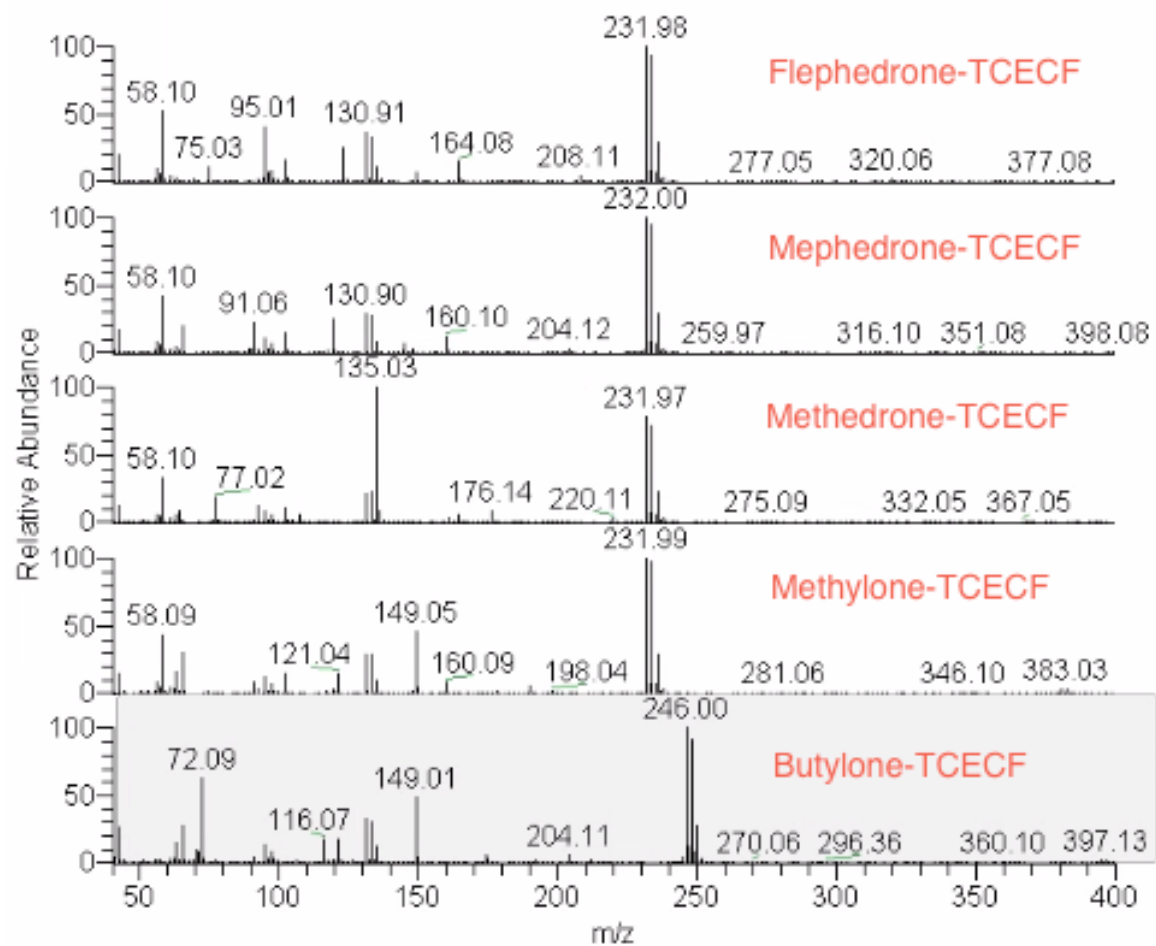


Figure 13: EI mass spectrometric fragmentation (70 eV) of 2° cathinones derivatized with TCECF.

Table 3: Summary of the Mass Spectrometric Fragmentation of Underivatized, ECF Derivatized and TCECF Derivatized Cathinones.*

	Underivatized				ECF-Derivatized				TCECF-Derivatized			
	Molecular Weight (g/mol)	Base Peak (m/z)	Qualifier Ions (m/z)	Molecular Ion	Molecular Weight (g/mol)	Base Peak (m/z)	Qualifier Ions (m/z)	Molecular Ion	Molecular Weight (g/mol)	Base Peak (m/z)	Qualifier Ions (m/z)	Molecular Ion
Flephedrone	181.21	58	75, 95, 123	Not Present	253.21	130	58, 75, 86 , 95, 102 , 123, 164	Present	355.21	232/4/6	58, 95, 102 , 123, 131/3/5 , 149 , 164 , 208	Present
Mephedrone	177.24	58	65, 91, 119	Not Present	249.24	130	58, 65, 86 , 91, 102 , 119, 160	Present	351.24	232/4/6	58, 65, 91, 102 , 119, 131/3/5 , 145 , 160 , 204	Present
Methedrone	193.24	58	77, 92, 135	Present	265.24	130	58, 73 , 77 , 86 , 92, 102 , 107 , 135 , 149	Present	367.24	232/4/6	58, 77, 92, 102 , 131/3/5 , 176 , 220	Present
Methylone	207.23	58	91, 121, 149	Not Present	279.23	130	58, 65, 86 , 91, 102 , 121, 149	Present	381.23	232/4/6	58, 65, 95, 102 , 121, 131/3/5 , 149, 160 , 190	Present
Butylone	221.25	72	91, 121, 135, 149	Not Present	293.25	144	72, 91, 100 , 116 , 121, 149	Present	395.25	246/8/50	65, 72, 95, 116 , 121 , 131/3/5 , 149, 174 , 204	Present

*Qualifier ions that are present in the derivatives but not in the underivatized mass spectra shown in bold

3.5: Proposed Fragmentation of Alkyl Chloroformate-Derivatized Cathinones

Figures 14a and **14b** illustrate the proposed EI fragmentation pathways of TCECF- and ECF-derivatized cathinones, respectively. An in-depth analysis of the fragmentation of these derivatives is given below.

Cleavage *a* for both ECF and TCECF produces a substituted benzyl ion at m/z 91 (mephedrone), m/z 95 (flephedrone) m/z 107 (methedrone), and m/z 121 (methylone, butylone). The loss of CO and acetylene from the tropylium ion (m/z 91) observed in the mephedrone mass spectrum results in a ion at m/z 65 as well [28]. These fragments are useful for the differentiation of R_1 and R_2 groups.

Cleavage *b* for both ECF and TCECF results in the formation of the 4-methylbenzoyl cation at m/z 119 (mephedrone), the 4-fluorobenzoyl cation at m/z 123 (flephedrone), the 4-methoxybenzoyl cation at m/z 135 (methedrone) and the 3,4-methylenedioxybenzoyl cation at m/z 149 (methylone, butylone). These fragments are also useful for differentiating R_1 and R_2 groups.

Cleavage *c* for both ECF and TCECF derivatives results in the well-documented iminium ion, which is observed in straight chain cathinones and phenethylamines [31]. The iminium ion is formed by an α -cleavage process and is highly favorable due to the location

of the C=O bond [31]. The fragments are observed at m/z 58 (mephedrone, methylone, methedrone, flephedrone) and m/z 72 (butylone). The formation of the iminium ion facilitates the production of cleavage *a* [31].

Cleavage *d* after TCECF derivatization results in the formation of isotopic clusters of the $[\text{CHR}_3\text{-NMe-COO-CH}_2\text{-CCl}_3]^+$ cation at m/z 232/4/6 (mephedrone, methylone, methedrone, flephedrone, MDMA) and m/z 246/8/52 (butylone) due to the ethyl R_3 group. These clusters represent the base peak in all of the recorded mass spectra. This cleavage is useful in differentiating R_3 and R_4 groups, but by itself is not very diagnostically useful.

Cleavage *d* after ECF derivatization results in the formation of $[\text{CHR}_3\text{-N(Me)COO-CH}_2\text{-CH}_3]^+$ cation at m/z 130 (mephedrone, methylone, methedrone, flephedrone) and m/z 144 (butylone) and provides diagnostic information on the composition of the R_3 and R_4 groups.

Cleavage *e* after TCECF results in the formation of the $[\text{R}_{1,2}\text{-Ph-CO-CHR}_3\text{-N(Me)(CO)}]^+$ cation at m/z 204 (mephedrone), m/z 208 (flephedrone), m/z 220 (methedrone), m/z 234 (methylone) and m/z 248 (butylone). These fragment ions in methylone and butylone add to the relative abundance of the already present m/z 234 and 248 ions, respectively, which result from cleavage *d*. When used in conjunction with cleavages *a* and *c*, differentiation between R_1 , R_2 , $\text{R}_{3/4}$ groups is possible.

Cleavage *e* after ECF derivatization results in the formation of the $[\text{N(Me)COO-CH}_2\text{-CH}_3]^+$ cation at m/z 102, and provides information about the composition of the R_4 groups. The m/z 102 fragment was present in all of the 2° cathinone derivatives investigated in this study, since they all have the same R_4 group. However, this fragment would be extremely

useful for cathinone compounds that contain unique R₄ groups since it would allow differentiation between R₃ and R₄ by comparing it to cleavage *c*.

Cleavage *f* after TCECF derivatization results in the formation of isotopic clusters of the [CH₂-CCl₃]⁺ ion at 131/3/5 for all derivatives investigated. Since these fragments are present in all derivatives they hold little diagnostic value.

Cleavage *g* after TCECF derivatization results in a low abundance [M - Cl]⁺ at *m/z* 316/8 (mephedrone), *m/z* 320/2 (flephedrone), *m/z* 332/4 (methedrone), *m/z* 346/8 (methylone) and *m/z* 360/2 (butylone). These fragments have similar discriminating value to the fragments from cleavage *e* because they allow for the differentiating of R₁, R₂, R₃ and R₄ groups and exhibit isotopic clustering; however, their low abundance makes these fragments less advantageous.

While TCECF derivatization of 2° cathinones provides slightly more diagnostic ions for MS discrimination, it was discovered that TCECF has limited solubility in aqueous samples, making it an unsuitable reagent for oral fluid applications. ECF, on the other hand, was deemed suitable for derivatization of cathinones in oral fluid due to its aqueous solubility and the increase in the number of diagnostic ions for derivatives (compared to underivatized analytes), which allows for improved mass spectrometric differentiation.

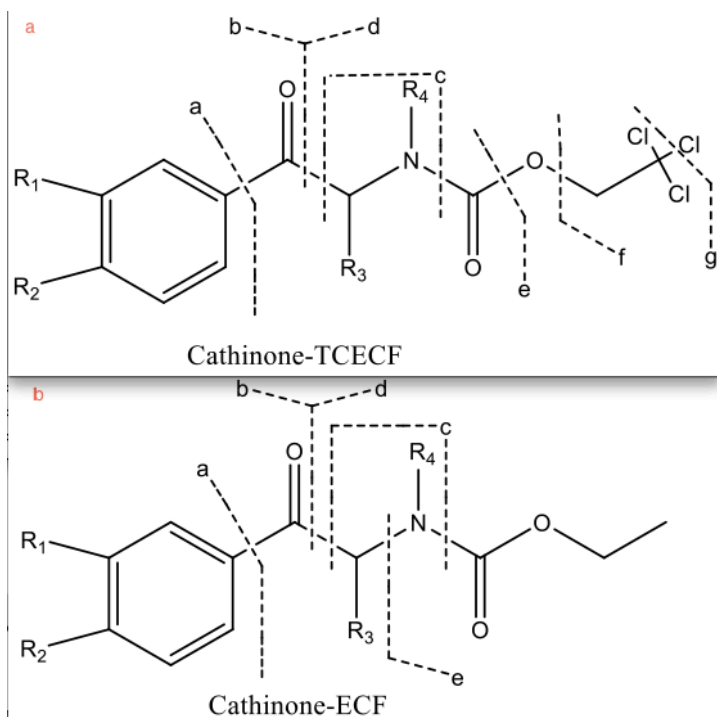


Figure 14: Proposed fragmentation of 2° cathinones after TCECF (a) and ECF (b) derivatization

3.6: Investigation of DI-SPME on Recovery of Underivatized Cathinones

As with HS-SPME experiments, initial DI-SPME experiments demonstrated that while DI-SPME was a suitable method for the isolation of 3° cathinones from aqueous solution, underivatized 2° cathinones were not efficiently recovered (**Figure 15**). Since analyte volatility is not a consideration for DI-SPME, these results suggest that the 2° amines do not partition well into the PDMS fiber film, which caused their lack of extractability. 3° amines and MDMA were able to adequately partition into the PDMS fiber film and were efficiently extracted using DI-SPME.

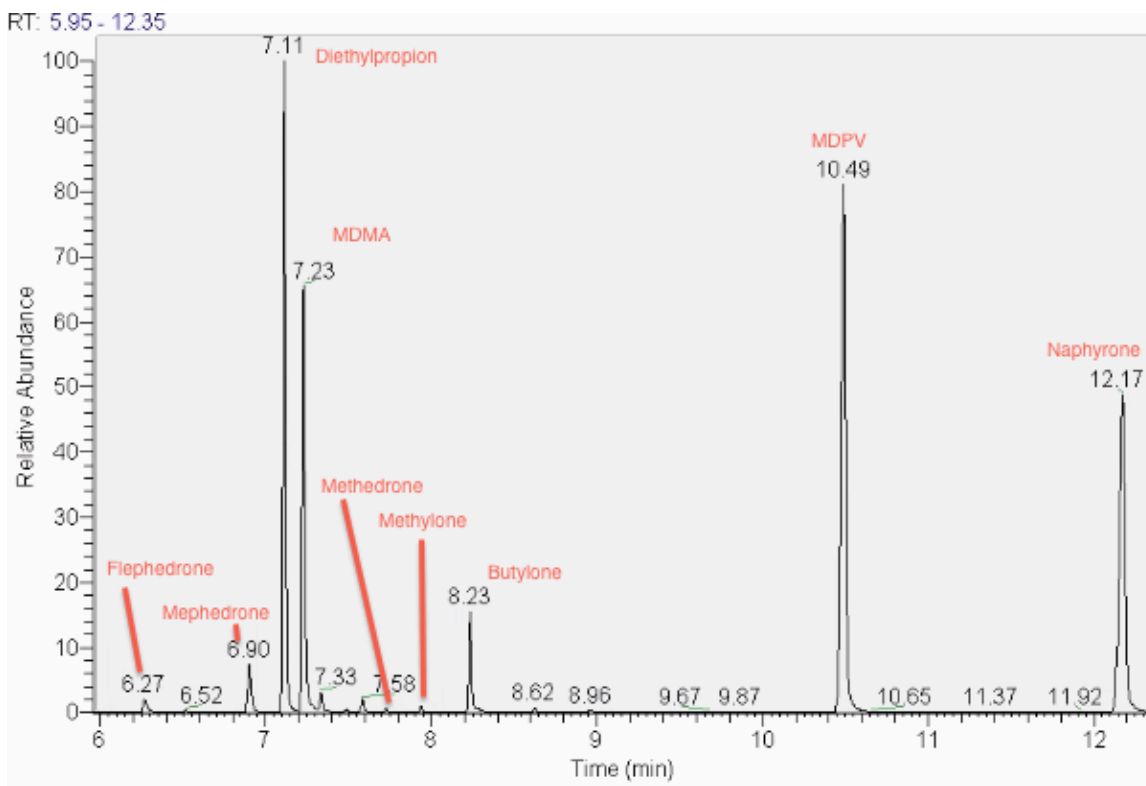


Figure 15: Resulting chromatogram following DI-SPME of an underivatized aqueous mixed cathinone standard (320 ng/mL), demonstrating the recovery of 3^o cathinones and MDMA, while 2^o cathinones are extracted inefficiently. SPME conditions as in Figure 6

3.7: Effect of ECF and TCECF Derivatization on Cathinone Recovery Using DI-SPME

Derivatization with ECF (**Figure 16**) and TCECF (**Figure 17**) drastically improved the DI-SPME recovery of 2^o cathinones from aqueous and oral fluid samples. The improvement in recovery of the 2^o cathinones following derivatization with ECF and TCECF indicates that the alkylchloroformate derivatives are able to partition more efficiently into the PDMS fiber film. Similar to what was observed in the HS-SPME experiments, however, 3^o cathinones were not recovered in the presence of ECF. This unexpected result was hypothesized to occur because of an unanticipated derivatization reaction occurring with the 3^o cathinones. It is postulated that this unexpected derivatization reaction may occur either at the tertiary amine, forming a quaternary

amine salt, or at the carbonyl oxygen. Further theoretical and practical research into this hypothesis is required to adequately determine what is occurring to 3° cathinones in the presence of ECF. All analytes were extracted in the presence of TCECF. While the peaks for MDMA-TCECF and naphyrone co-elute, they can easily be distinguished by searching m/z 232 and 126, the base peaks of MDMA-TCECF and naphyrone, respectively (**Figure 17**).

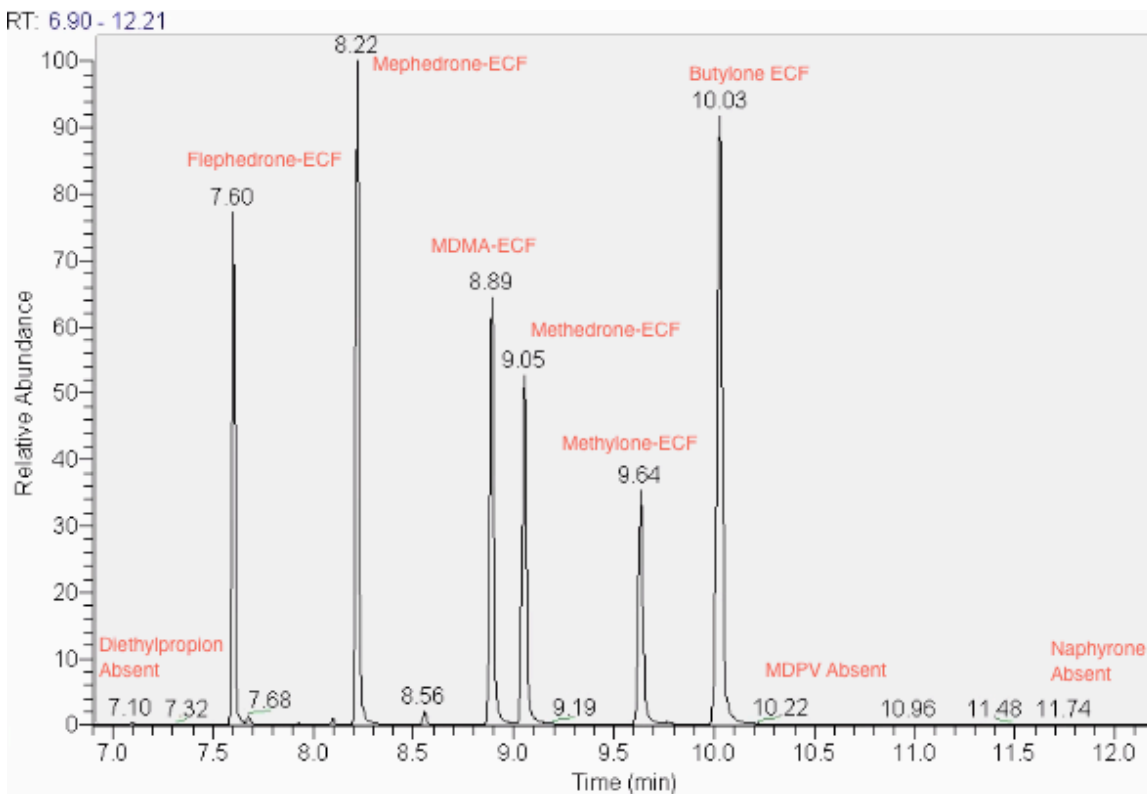


Figure 16: Resulting chromatogram following DI-SPME of an ECF-derivatized mixed aqueous cathinone standard (320 ng/mL), demonstrating the recovery of 2° ECF-derivatized cathinones while showing that 3° cathinones were unable to be extracted. SPME conditions as in **Figure 6**.

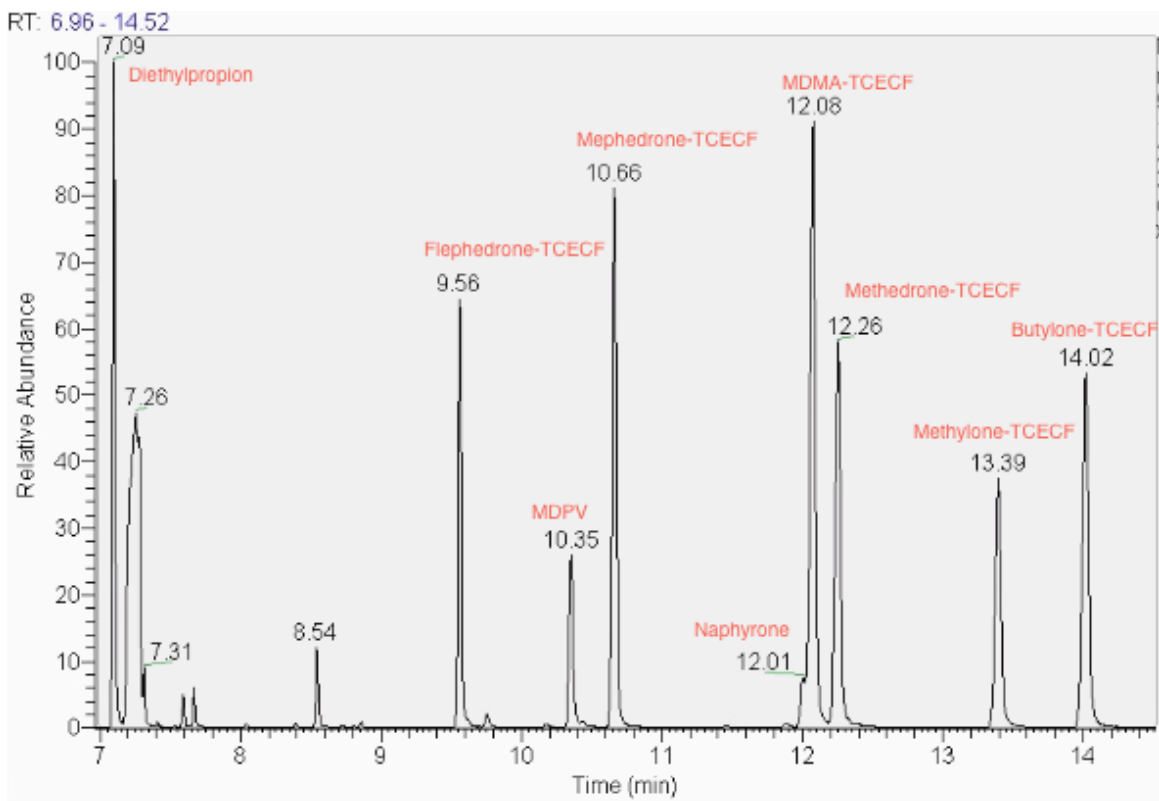


Figure 17: Resulting chromatogram following DI-SPME of a TCECF-derivatized mixed aqueous cathinone standard (320 ng/mL), demonstrating the recovery of all 2° TCECF-derivatized cathinones and 3° cathinones. SPME conditions as in **Figure 6**.

3.8: Comparison of HS and DI-SPME for the Efficient Recovery of Alkylchloroformate Derivatized Cathinones from Aqueous Solutions

Sampling the headspace offers significant advantages over direct immersion sampling. In terms of “cleanliness” of the extract, the headspace contains only volatile and semi-volatile organic compounds, while the oral fluid matrix contains a variety of additional non-volatile endogenous compounds which can potentially interfere with chromatographic analysis or contribute to fiber fouling. The life expectancy of the fiber is enhanced when sampling the headspace because background adsorption and matrix effects are reduced [62]. In this study the life expectancy of the fiber was also prolonged when sampling the headspace rather than the oral fluid matrix itself, since the sample pH was adjusted to a pH of 10 and direct exposure to such a harsh alkaline environment severely reduced fiber lifetime.

The TriPlus autosampler employed in this study was not compatible with automated DI sampling of low sample volumes due to sampling depth limitations. Samples were consequently diluted with 8 mL of deionized H₂O to bring the total volume up to an adequate level for the automated direct immersion of SPME fiber. Ideally, a small-volume insert could be used in the SPME vial to bring the original sample volume up to an adequate sampling height without the need for sample dilution.

The constraints of the autosampler for DI-SPME, combined with the decrease in fiber life experienced with this sampling mode, resulted in HS-SPME being chosen as the optimal SPME sampling mode.

3.9: Optimization of HS-SPME Parameters

The pretreatment of aqueous and oral fluid samples fortified with 2° cathinones and derivatized with ECF was optimized to maximize recovery of derivatized cathinones from samples using HS-SPME. It was determined that pH adjustment to a pH of 10 using 1.4 M KOH and saturation with NaCl(s) allowed for the maximum recovery of ECF-derivatized 2° cathinones (**Figure 18**). The alkaline pH adjustment causes the cathinones to exist in solution in the free base rather than protonated forms, which eliminates ionic interactions that reduce analyte partitioning into the headspace. The addition of salt disrupts the interactions between the analytes and the water molecules in solution, allowing them to more easily partition into the headspace. In the absence of pretreatment, mephedrone and butylone were inefficiently recovered, while flephedrone, methedrone and methylone were not recovered at all. Optimal recoveries for all analytes occurred in the presence of both NaCl and KOH.

To further maximize recoveries of ECF-derivatized cathinones, the extraction/incubation temperature was optimized. The results of temperature optimization experiments are shown in

Figure 19. The extraction/incubation temperature for flephedrone-ECF and mephedrone-ECF was optimized at 90°C, while methedrone-ECF, methylone-ECF and butylone-ECF were most efficiently extracted at 110°C. Consequently, the individual analyte optimum temperatures were averaged and the final optimized temperature was determined to be 102°C.

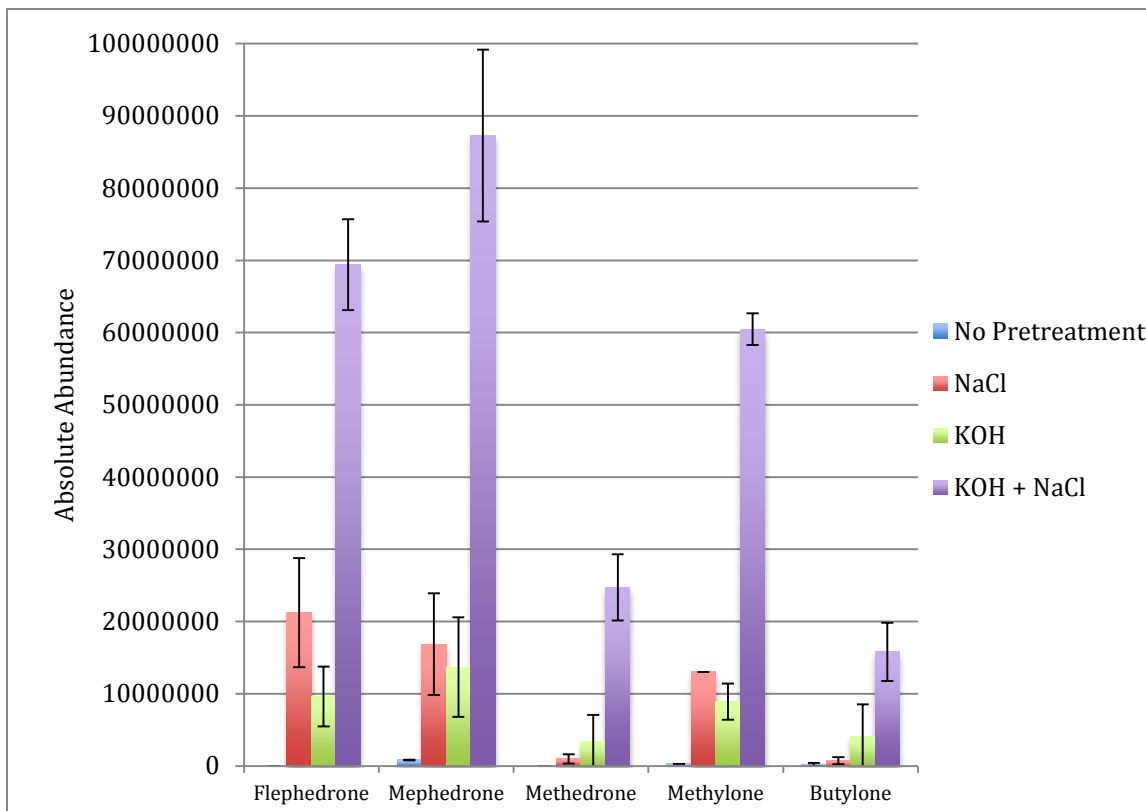


Figure 18: Effect of sample pretreatment on the recoveries of ECF-derivatized 2° cathinones. Samples containing 100 µL of a 320 ng/mL aqueous mixed cathinone standard, 50 µL of ECF and variable sample pretreatment were run in triplicate and the absolute abundances of the base peak for each derivatized analyte were averaged. All ECF-derivatives exhibited maximum recovery in the presence of KOH and NaCl. Error bars represent the standard deviation for three trials.

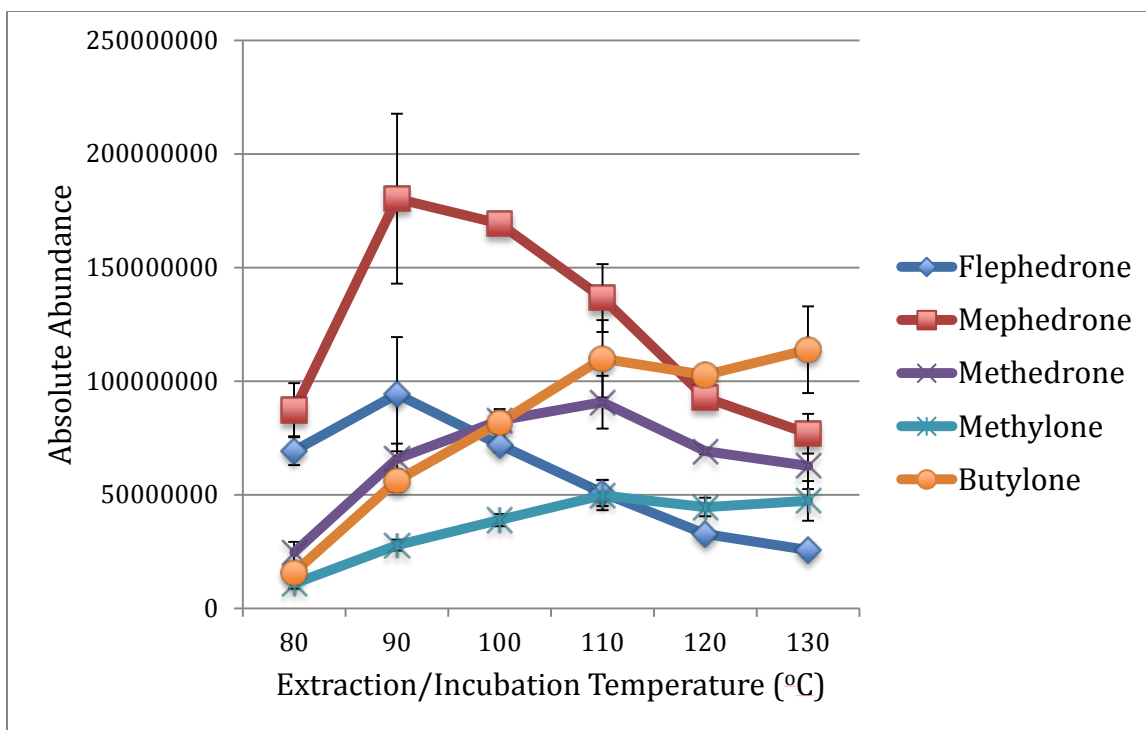


Figure 19: Extraction/incubation temperature optimization for recoveries of ECF-derivatized 2° cathinones. Samples containing 100 μL of a 320 ng/mL aqueous mixed cathinone standard, 50 μL of ECF, 400 μL of 1.4 M KOH and saturated with NaCl(s) were prepared. The optimal temperature was determined to be 102°C, which was the average of the optimum extraction/incubation temperature for each individual analyte. Data represents the average of three extraction trials with error bars representing one standard deviation.

3.10: HS-SPME Calibration of ECF-Derivatized 2^o Cathinones in Oral Fluid

Linear calibration curves for ECF-derivatized cathinones ranging in concentration from 1-320 ng/mL were produced using HS-SPME (**Figure 20**). Because the calibration data in Figure 20 was obtained before extraction/incubation temperature optimization experiments were conducted, the extraction/incubation was set at 80°C for these experiments.

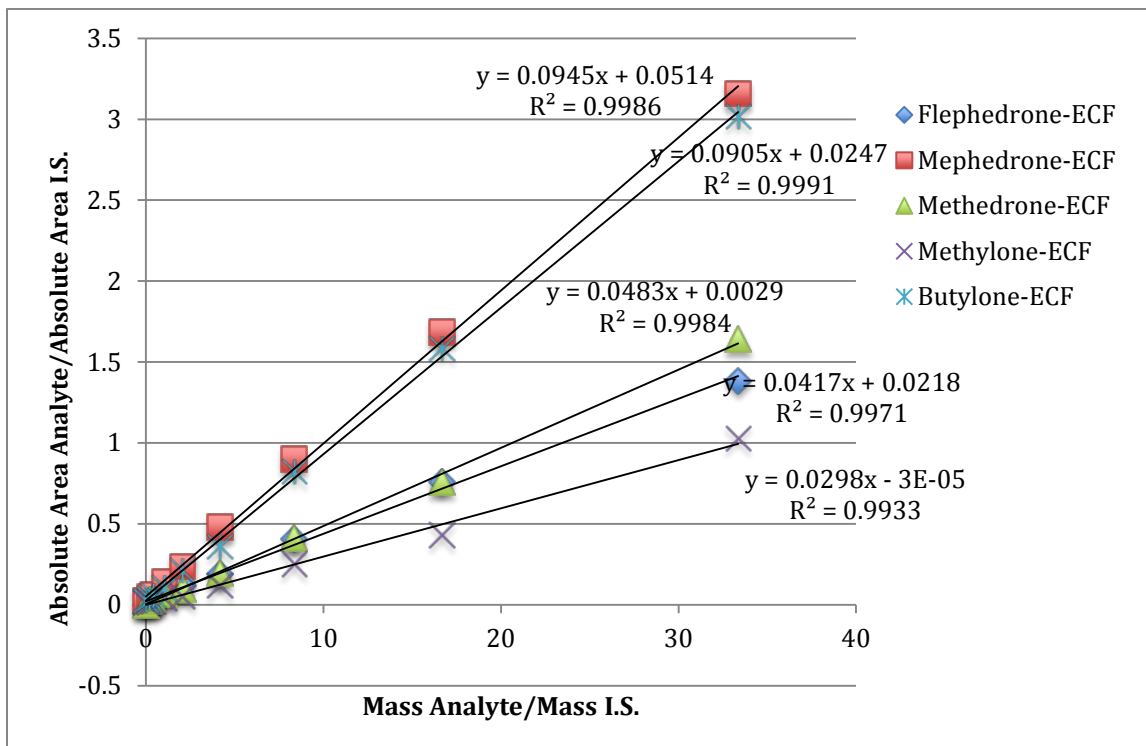


Figure 20: Calibration solutions in simulated oral fluid spanning an MDMA concentration range of 1–320 ng/mL were prepared as described in the Experimental Methods section and subjected to HS-SPME/GC-MS to generate the calibration plot depicted above. All calibration solutions were spiked with mephedrone-*d*₃ and butylone-*d*₃ (96 ng/mL) as internal standards. Mephedrone-*d*₃ was used as an internal standard for flephedrone, mephedrone, methedrone and methylone. Butylone-*d*₃ was used as an internal standard for butylone. All other SPME and GC-MS parameters as described in Experimental Methods section.

3.11: DI-SPME Calibration of ECF-Derivatized 2° Cathinones in Oral Fluid

Linear calibration curves for ECF-derivatized cathinones were produced using DI-SPME from 20-320 ng/mL (**Figure 21**). While HS-SPME was concluded to be a more suitable sampling mode for the reasons described above, the DI calibration data is included here for the sake of completeness.

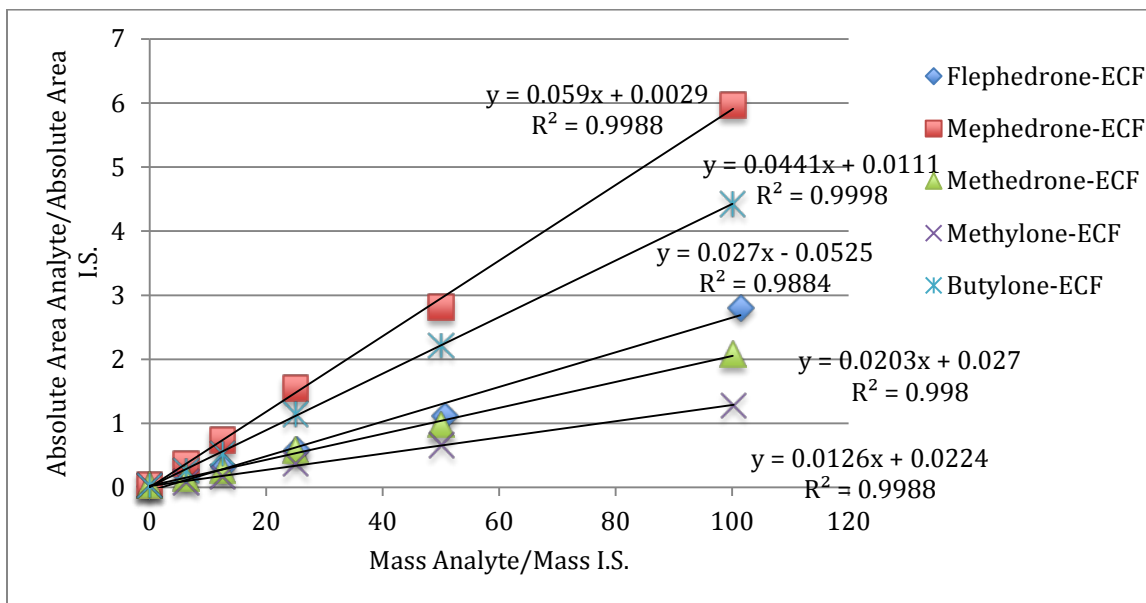


Figure 21: Calibration solutions in simulated oral fluid spanning an analyte concentration range of 20–320 ng/mL were prepared as described in the Experimental Methods section and subjected to DI-SPME/GC-MS to generate the calibration plot depicted above. All calibration solutions were spiked with MDMA_{d-5} as internal standard at 30 ng/mL. All other SPME and GC-MS parameters as described in Experimental Methods section.

3.12: Accuracy of DI-SPME Method

The accuracy of the DI-SPME method was determined by analysis of high (240 ng/mL) and low (60 ng/mL) level spiked blind oral fluid samples and calculating target analyte concentrations off of the appropriate calibration curves generated on the same day as the blind sample analysis. The calculated analyte concentrations were compared to the true concentrations to determine the % error of the quantification. Accuracy data is presented in **Table 4**.

Accuracies ranged between 1-10% with an average error of 5 %.

Table 4: Accuracy Data for Oral Fluid Samples Analyzed by DI-SPME/GC-MS*

	Accuracy (% Error)		Average**
	High (240 ng/mL)	Low (60 ng/mL)	
Flephedrone-ECF	-7.75	10.53	9.14
Mephedrone-ECF	2.68	-1.14	1.91
MDMA-ECF	-8.84	-1.53	5.185
Methedrone-ECF	4.59	2.84	3.715
Methylone-ECF	2.68	-8.19	5.435
Butylone-ECF	6.61	5.66	6.135
Mean Accuracy (all analytes)			5.232

*Results calculated using calibration curve equations from **Figure 21**. MDMA_{d-5} (30 ng/mL) was used as an internal standard.

**Average of absolute values of errors

3.13: Limit of Detection and Limit of Quantitation of DI and HS-SPME Methods

The limit of detection (LOD) is the lowest concentration of an analyte that can be reliably distinguished from a blank. The limit of quantification (LOQ) is the concentration of an analyte at which a positive result can be reported with a high degree of certainty. The LOD (**Equation 2**) and LOQ (**Equation 3**) can be determined from regression statistics using the following equations [63]:

$$\text{Equation 2:} \quad \text{LOD} = \frac{3.3 s_y}{m}$$

$$\text{Equation 3:} \quad \text{LOQ} = \frac{10 s_y}{m}$$

where s_y is the standard deviation associated with y and m is the slope of the regression line. The LOD and LOQ data determined for the HS and DI-SPME methods are shown in **Table 5**. LOD values for ECF-derivatives ranged from 12 to 33 ng/mL for HS-SPME and 6 to 49 ng/mL for DI-SPME. LOQ values ranged from 37 to 99 ng/mL for HS-SPME and 19 to 148 ng/mL for DI-SPME. In general, compared with DI-SPME, HS-SPME produced a lower LOD and LOQ for ECF-derivatives of flephedrone, mephedrone and methedrone while DI-SPME resulted in a lower LOD and LOQ for methylone and butylone.

Table 5: LOD and LOQ Values for HS- and DI-SPME Methods Determined from Linear Regression Statistics

Analyte	HS-SPME		DI-SPME	
	LOD (ng/mL)	LOQ (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
Flephedrone-ECF	20.45	61.99	48.72	147.63
Mephedrone-ECF	14.46	43.85	15.5	46.97
Methedrone-ECF	16.44	49.82	19.96	60.48
Methylone-ECF	32.77	99.39	15.45	46.83
Butylone-ECF	12.11	36.7	6.13	18.57

3.14: Application of Optimized Method to the Qualitative Analysis of 13 Amphetamines and Cathinones in Oral Fluid

To illustrate the wide applicability of the HS-SPME/GC-MS method developed in this work to the extraction, separation and identification of a wide variety of abused drugs, a mixed oral fluid sample fortified with eight commonly abused amphetamines (amphetamine, methamphetamine, PMA, PMMA, MDA, MDEA, MBDB, MDMA) and five 2° cathinones was analyzed. In-matrix ECF derivatization was employed after pretreatment with KOH (1.4 M, pH=10) and NaCl(s). The method was able to successfully extract, separate and detect all 13 drugs (**Figure 22**). While MDEA-ECF and methedrone-ECF were observed to co-elute with the GC temperature program employed, their base peaks and qualifier ions are unique to each molecule, thus allowing their mass spectral resolution.

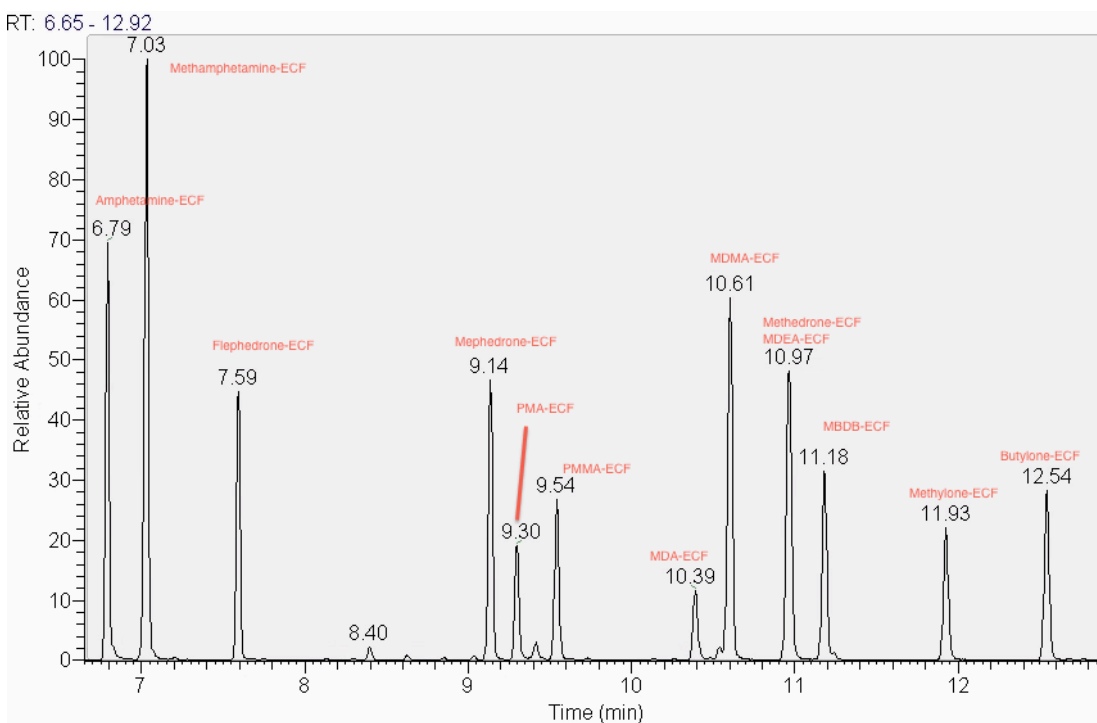


Figure 22: GC after HS-SPME of a mixed cathinone and amphetamine standard solution (320 ng/mL) in oral fluid derivatized with ECF (50 μ L) after pretreatment with KOH (1.4 M) to a pH of 10 and saturated with NaCl(s), demonstrating the applicability of the method to a large variety of abused stimulant drugs of abuse. 100 μ m PDMS fiber, 102°C extraction/incubation temperature, 10 min extraction time, 10 min incubation time, 6 min desorption time, 250°C desorption temperature.

3.15: Application of DI and HS-SPME Methods to Commercial Saliva Collection Kit

The optimized DI-SPME method was applied to a mock OF sample collected using the Intercept oral fluid collection device as described in **Section 2.11**. The resulting GC is shown in **Figure 23**. Although TCECF was sparingly soluble in water, the resulting TCECF-derivatized 2° cathinones could be recovered using DI-SPME. The 3° cathinones were not recovered during this experiment.

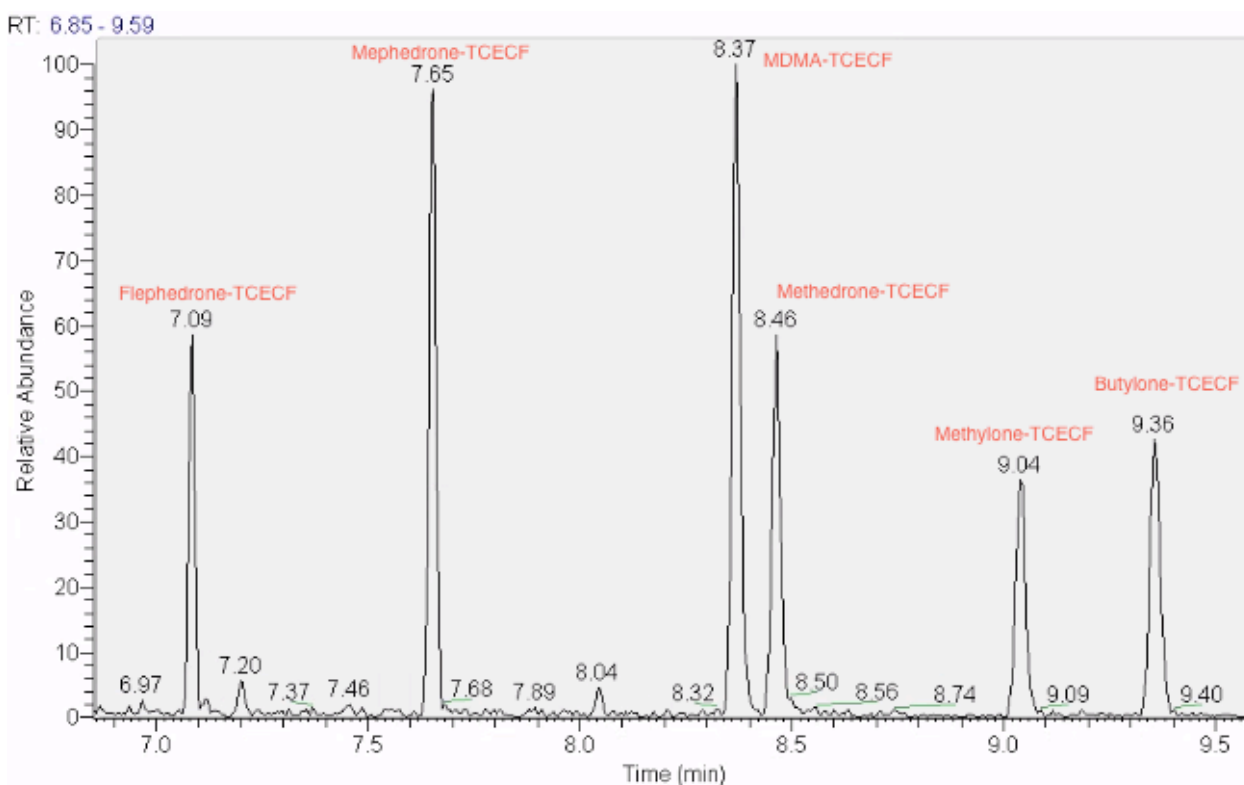


Figure 23: GC after DI-SPME from commercial saliva swab exposed to a 16 ng/mL mixed cathinone solution as described in **Section 2.11**. SPME conditions as in **Figure 6**.

3.16: Further HS-SPME Parameter Optimizations To Consider

The HS-SPME method reported here would benefit from further parameter optimization, including incubation, extraction and desorption times; SPME fiber film thickness; and choice of fiber phase. Optimization of incubation and desorption time could further reduce total sampling time. Optimization of extraction time could further optimize analyte recoveries. Optimization of SPME fiber film thickness and phase might offer improved analyte recoveries, since the analytes or their alkylchloroformate derivatives might more readily partition into a different fiber phase or film thickness.

3.17: Future Work

Future work on this project should include additional HS-SPME parameter optimization (outlined in section 3.14) and further calibration studies to determine intra/interday reproducibility. Additional accuracy and precision studies should take place concurrently. The stability of the cathinone drugs and their alkylchloroformate derivatives in oral fluid should also be studied by re-analyzing prepared samples after allowing them to sit in the autosampler tray for 24 - 48 hours (or other designated time periods), and comparing the quantified results to those obtained when the samples were initially run. Derivatization with other alkylchloroformate reagents should be explored to determine which reagent is most optimal for the recovery of cathinones in oral fluid as well as determining which provides the best MS selectivity. The failure to detect the tertiary amines in the presence of alkylchloroformate derivatizing reagents must be investigated to determine whether these compounds are in fact reacting to form unanticipated products. Finally, a more rigorous evaluation of method applicability to various commercially available oral fluid drug-testing platforms must be undertaken.

Chapter 4: Conclusions

HS-SPME was determined to be a suitable extraction method for the isolation of 2° cathinones derivatized with ECF in oral fluid. ECF derivatives were optimally extracted using a 100 µm PDMS fiber at 102° C after being pretreated with base and salt. Linear calibration curves were produced using both HS and DI-SPME at ranges of 1-320 and 20-320 ng/mL, respectively. Blind sample studies analyzed using DI-SPME/GC-MS demonstrated accuracies of 1-10%. The HS-SPME method produced LODs between 12-33 ng/mL and LOQs between 37-99 ng/mL for the five target 2° cathinones. The DI-SPME method produced LODs between 6-49 ng/mL and LOQs between 19-148 ng/mL for the target analytes. HS-SPME was determined to be a more suitable extraction mode due to increased fiber lifetime, compatibility with the instrument autosampler, and the elimination of background adsorption and matrix effects. Further HS-SPME parameter optimization is required before the method can be considered completely optimized. In particular, further experiments are necessary to understand the derivatization and extraction behavior of the tertiary cathinones, and the method must be improved to isolate these compounds. Additionally, in order to fully validate the method, its application to oral fluid samples collected by various commercially available collection kits will need to be explored in more depth, and, finally, the method must be evaluated and validated on oral fluid samples collected from actual users.

Chapter 5: References

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