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Document Title:	**Automated Derivatization and Identification of Controlled Substances via Total Vaporization Solid Phase Microextraction (TV-SPME) and Gas Chromatography/Mass Spectrometry (GC/MS)**
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Document Number:	**252919**
Date Received:	**May 2019**
Award Number:	**2015-DN-BX-K058**

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FINAL SUMMARY OVERVIEW

Project Title: Automated Derivatization and Identification of Controlled Substances via Total Vaporization Solid Phase Microextraction (TV-SPME) and Gas Chromatography/Mass Spectrometry (GC/MS)

Grant Period: 01/01/2016 - 11/31/2018

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PROJECT SUMMARY AND PURPOSE

Overall, this project sought to improve the analysis of thermally unstable drugs by gas chromatography/mass spectrometry through a combination of derivatization and a novel total vaporization technique (Total Vaporization – Solid Phase Microextraction or TV-SPME). The primary aims of this project were to:

1) Establish derivatization protocols for several controlled substances that are problematic in gas chromatography/mass spectrometry (GC/MS)
2) Establish Total Vaporization Solid Phase Microextraction (TV-SPME) methods for the same analytes "as is" as well as achieving automated on-fiber derivatization
3) Demonstrate the utility of the TV-SPME methods for seized drugs and biological samples

Our overall hypothesis was that TV-SPME will offer greater sensitivity than traditional liquid injection for controlled substances (either "as is" or derivatized). In addition, TV-SPME was easily adapted to include either a pre-extraction or a post-extraction on-fiber derivatization step for thermally labile species. This means that samples were prepared quickly and simply. Derivatization agents were introduced automatically by a SPME autosampler via exposure to the vapors of the derivatization agent.

PROJECT DESIGN AND METHODS

The project was divided into four distinct phases:

PHASE 1: Identify appropriate derivatization agents using off-line derivatization and liquid injection

PHASE 2: Adapt and optimize the off-line/liquid injection methods to TV-SPME

PHASE 3: Evaluate the traditional and TV-SPME methodologies for identifying controlled substances in seized drug exhibits

PHASE 4: Evaluate the traditional and TV-SPME methodologies for identifying controlled substances and/or their metabolites in biological matrices

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FINDINGS

TRADITIONAL DERIVATIZATION AND LIQUID INJECTION ANALYSIS

Basic and zwitterionic drugs can be amongst the most difficult to analyze via GC/MS due to their thermal instability and non-ideal behavior resulting in broad, asymmetric peaks. In the first phases of this project, several drugs and derivatization agents were evaluated, as summarized below:

Table 1. A summary of traditional liquid injection results. + indicates the formation of a single chromatographic peak which could be unambiguously identified. – indicates no relevant peak was formed. 0 indicates multiple peaks were formed.

Drug	Underivatized	TFAA	BSTFA	DMF-DMA
Amphetamine	+	+	0	+
2C-I	+	+	0	+
Gabapentin	+	+	-	+
Lorazepam	+	-	+	-
Vigabatrin	-	-	+	+
Pregabalin	-	-	0	0
Clorazepate	-	-	+	-

Amphetamine, 2C-I, gabapentin, and lorazepam were successfully analyzed by GC/MS without modification. However, the number of theoretical plates achieved was dramatically increased by derivatization and peak symmetry was greatly improved. Direct GC/MS analysis of vigabatrin, pregabalin and clorazepate yielded negative results, as no peaks were formed.

Among the drugs of interest, amphetamine and 2C-I were readily derivatized with TFAA. The derivatives produced more intense and narrower chromatographic peaks than their underivatized forms. Gabapentin was also successfully derivatized with TFAA, but the resulting chromatographic peak was smaller in magnitude than that of the underivatized drug. Lorazepam, vigabatrin, pregabalin, and clorazepate were not successfully derivatized by TFAA – lorazepam and vigabatrin produced no chromatographic peaks, while pregabalin and clorazepate both produced multiple peaks, none of which were the target derivatives.

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Derivatization with BSTFA + 1% TMCS produced single chromatographic peaks for lorazepam, vigabatrin, and clorazepate. The derivatization was incomplete for amphetamine and wholly unsuccessful for gabapentin, producing no derivative. BSTFA derivatization yielded multiple derivatives with one, two, or even three trimethylsilyl (TMS) groups for 2C-I and pregabalin.

Potential future work could include methyl-N-t-butyldimethylsilyltrifluoroacetamide (MTBSTFA) and other silylation reagents that replace active hydrogens with larger t-butyldimethylsilyl rather than TMS groups. These derivatives generally take longer to form, but are more stable. Additionally, the t-butyldimethylsilyl group is more sterically hindering than the TMS group, so t-butyldimethylsilyl reagents will likely form only one derivative with primary amines. It is therefore possible that reactions with BSTFA that were unsuitable due to multiple products or for which no derivative was detected here may produce useful results when reacted with larger silylation reagents such as MTBSTFA.

Lastly, DMF-DMA proved an effective new method for the derivatization of the primary amines amphetamine, 2C-I, gabapentin, and vigabatrin. The primary amine hydrogens in amphetamine and 2C-I were replaced with a DMAM group. Gabapentin and vigabatrin, containing both a primary amine and a carboxyl group, underwent the addition of a methyl group and a DMAM group. Derivatization with DMF-DMA did not yield favorable results for lorazepam, pregabalin, or clorazepate. As previously discussed, the derivative for pregabalin was formed, but there were several other peaks present in the chromatogram that could not be identified. The chromatograms for both lorazepam and clorazepate showed multiple peaks, none of which could be attributed to the target derivatives.

DEVELOPMENT OF TV-SPME ON-FIBER DERIVATIZATION OF CONTROLLED SUBSTANCES

TV-SPME proved to be an effective technique for analyzing controlled substances both with and without on-fiber derivatization. A summary of the results is shown in the table below:

Table 2. Summary of results for liquid injection and TV-SPME methods. + indicates the formation of a single chromatographic peak which could be unambiguously identified. 0 indicates that multiple peaks formed, and – indicates that no peak formed.

Drug	Liquid Injection	TV-SPME
Amphetamine	+	+

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Amphetamine + TFAA	+	+
Methamphetamine	+	+
Methamphetamine + TFAA	+	+
Ephedrine	+	+
Ephedrine + TFAA	+	0
2C-I	+	+
2C-I + TFAA	+	+
25I-NBOMe	+	+
25I-NBOMe + TFAA	+	+
25I-NBOH	-	0
25I-NBOH + TFAA	-	0
Psilocin	+	+
Psilocin + BSTFA + 1% TMCS	+	+
Psilocybin	+	-
Psilocybin + BSTFA + 1% TMCS	-	-
GHB	-	-
GHB + BSTFA + 1% TMCS	+	+
Gabapentin	+	+
Gabapentin + BSTFA + 1% TMCS	-	-
Gabapentin + DMF-DMA	+	0
Lorazepam	+	+
Lorazepam + BSTFA + 1% TMCS	+	-
Lorazepam + DMF-DMA	-	-
Vigabatrin	-	-
Vigabatrin + BSTFA + 1% TMCS	+	-
Vigabatrin + DMF-DMA	+	-
Pregabalin	-	+
Pregabalin + BSTFA + 1% TMCS	0	-
Pregabalin + DMF-DMA	0	-
Clorazepate	-	-

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Clorazepate + BSTFA + 1% TMCS	+	-
Clorazepate + DMF-DMA	-	-

Amphetamine, 2C-I, 25I-NBOMe, psilocin, gabapentin, lorazepam, and pregabalin were all identified by TV-SPME without derivatization. Methamphetamine and 25I-NBOH were also identified without derivatization, but chromatographic performance was unsatisfactory. To our knowledge, this is the first report of identification of 25I-NBOH by GC/MS.

Derivatization with TFAA was chosen for the amine and hydroxylamine drugs. TFAA derivatization was effective for amphetamine, methamphetamine, 2C-I, and 25I-NBOMe, and successful but incomplete for 25I-NBOH. On-fiber derivatization with TFAA proved ineffective for psilocin and psilocybin, producing no derivatives for either.

Derivatization with BSTFA + 1% TMCS was chosen for the zwitterionic drugs as well as GHB, psilocin, and psilocybin. DMF-DMA derivatization was attempted for the zwitterionic drugs. On-fiber derivatization was unsuccessful for all the zwitterionic drugs chosen, with either BSTFA + 1% TMCS or DMF-DMA. On-fiber derivatization of gabapentin with DMF-DMA was incomplete, with most of the drug remaining in the underivatized state. The other zwitterions produced no derivative with either derivatization agent.

Psilocybin produced no derivative using on-fiber derivatization with BSTFA + 1% TMCS. On-fiber derivatization of psilocin produced a psilocin-TMS derivative which dominated the chromatogram despite a small chromatographic peak for underivatized psilocin still present. GHB proved an excellent candidate for on-fiber derivatization with BSTFA + 1% TMCS. As expected, GHB could not be detected by GC/MS in the underivatized form. On-fiber derivatization, however, produced a single chromatographic peak for GHB di-TMS with strong signal.

In a separate study, each drug was analyzed “as is” with extraction temperatures ranging from 30°C to 200°C to determine the optimum temperature. Figures 1-3 illustrate the effect of extraction temperature on analyte signal for those drugs that were detected in the underivatized form by TV-SPME. The optimum extraction temperature was then chosen as the starting extraction temperature for on-fiber derivatization.

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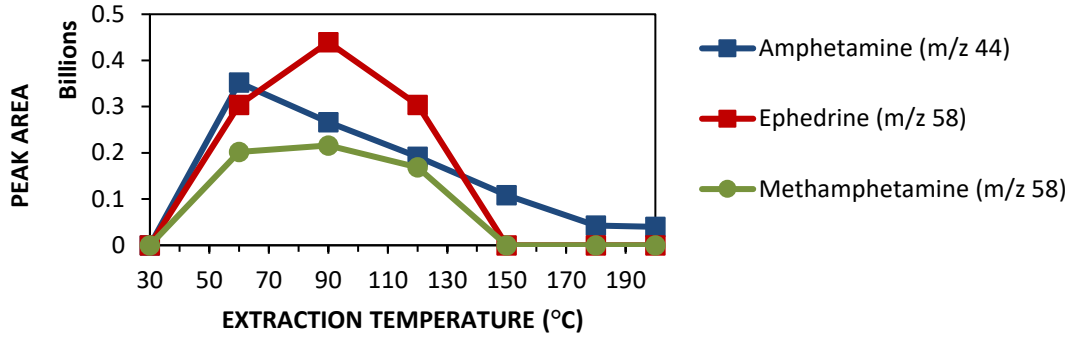

Figure 1. Graph of peak area vs extraction temperature for underivatized phenethylamines. Peak area was calculated using the extracted ion profile (EIP) for the ion indicated.

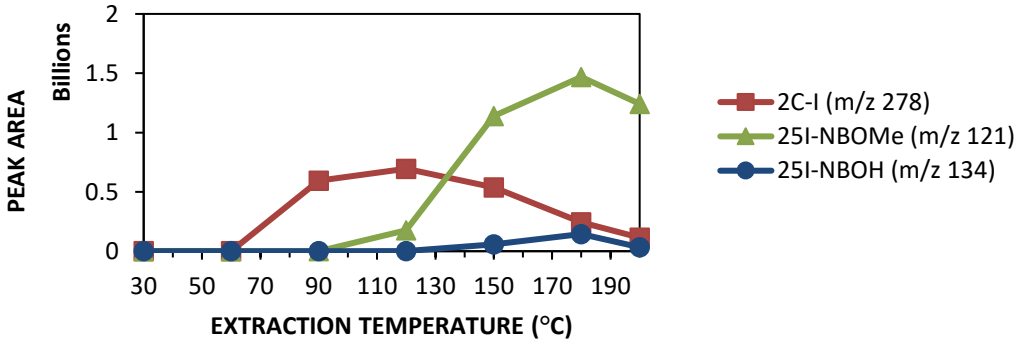

Figure 2. Graph of peak area vs extraction temperature for underivatized designer drugs. Peak area was calculated using the extracted ion profile (EIP) for the ion indicated.

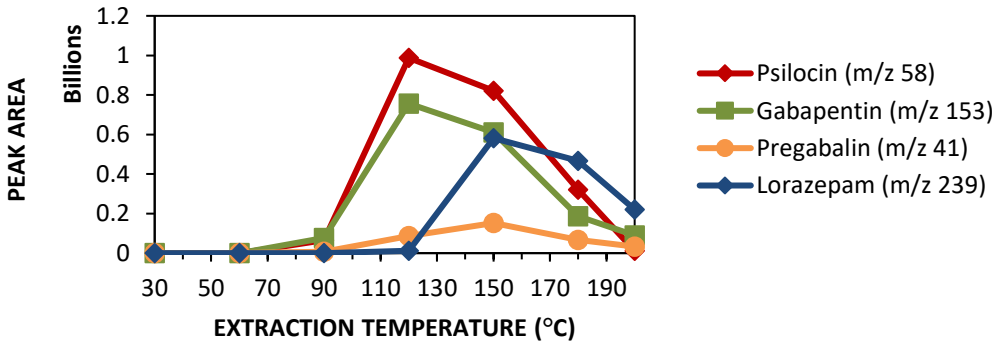

Figure 3. Graph of peak area vs extraction temperature for underivatized zwitterions. Peak area was calculated using the extracted ion profile (EIP) for the ion indicated.

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The results were promising for all drug classes that were analyzed successfully by on-fiber derivatization as solutions. This discovery greatly improves the utility of the technique, as controlled substances are most often encountered in their solid forms in forensic science laboratories. The application of this technique to beverage samples and solid drug powders is of most interest, as these applications represent a significant decrease in sample preparation.

While not ideal for all analytes, TV-SPME with on-fiber derivatization could be a powerful technique for amine and hydroxylamine controlled substances as well as GHB. The technique could increase analyst efficiency by reducing sample preparation time for these types of analytes. The method is particularly well-suited to the analysis of GHB since the drug cannot be analyzed by GC/MS in its native state.

REALISTIC SAMPLES AND SOLID DRUG DERIVATIZATION

On-fiber derivatization has been proven to be an effective and useful tool for analysis of realistic seized drug samples, particularly for amines and GHB. On-fiber derivatization was unsuccessful for hallucinogenic mushrooms in this study, however, and requires further research. TV-SPME with on-fiber derivatization using TFAA was able to identify all three components of a simulated impure sample of methamphetamine, detecting methamphetamine and ephedrine as the TFA derivatives and caffeine underivatized (see Figure 4 below):

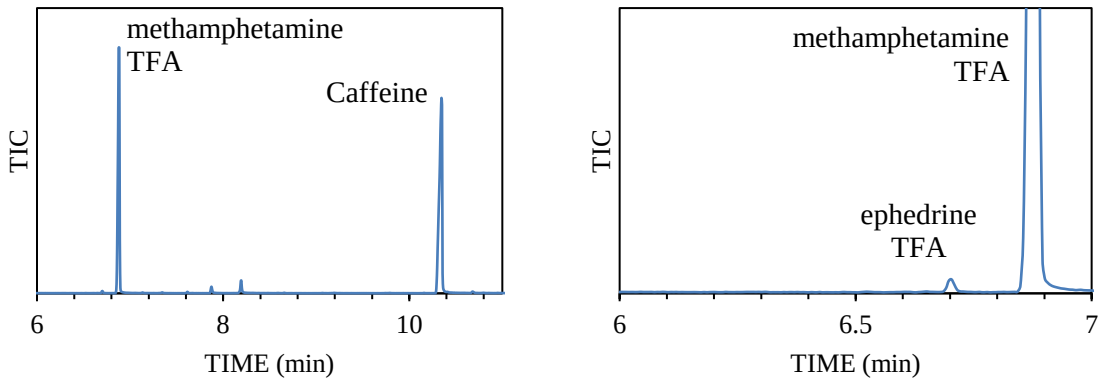

Figure 4. "street meth" chromatogram showing the methamphetamine derivative and caffeine (left) and zoomed in to show the ephedrine derivative (right).

This method was applied to the identification of GHB in mixed drinks, where it excelled. Despite the presence of protic solvents, on-fiber derivatization with BSTFA + 1% TMCS resulted in unambiguous identification of GHB in samples of water, Coke®, rum, and a 2:1 mixture of rum and Coke®.

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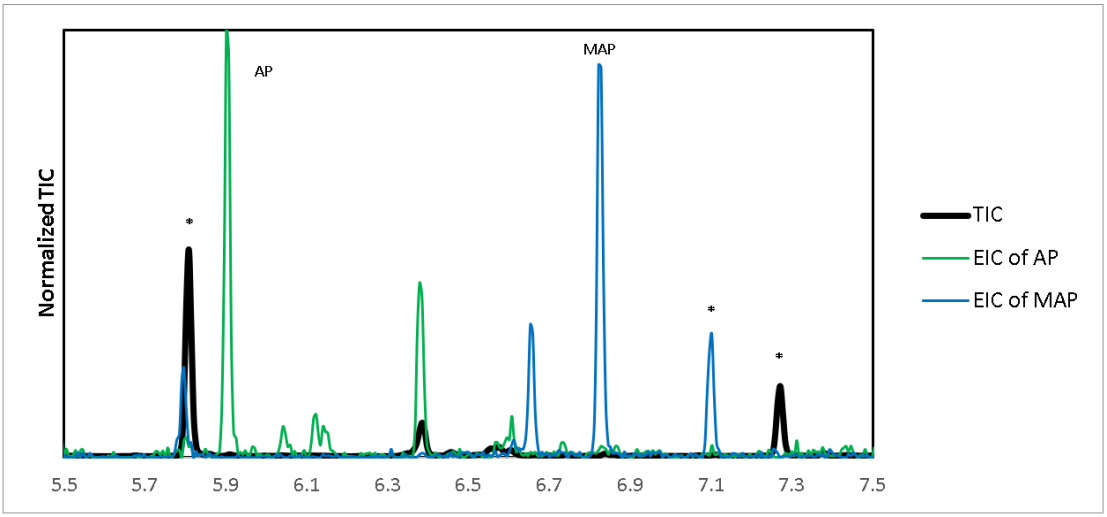
Solid samples of amphetamine, ephedrine and methamphetamine produced single chromatographic peaks for their derivatives when analyzed as solid powders. Underivatized solid GHB did not produce any peaks, whereas on-fiber derviatization produced a single peak from GHB-di-TMS. The derivatization of 2C-I was promising, however, the chromatogram showed not only 2C-I TFA, but also a side product corresponding to the 2C-I TFA derivative after losing its iodine atom. Analysis of underivatized ephedrine and methamphetamine powders did not produce any chromatographic peaks. This indicates that analysis of a powder with neither solvent nor derivatization agent will be difficult to impossible – particularly as the drugs in question are present as their salt forms. However, analysis of solid drugs in their free base form (e.g., "freebase" and/or "crack" cocaine) should be possible.

Lastly, TV-SPME was able to detect and identify amphetamine and methamphetamine in urine. For example, the figure below is a normalized total ion chromatogram for methamphetamine (MAP) and amphetamine (AP) in urine (black), as well as the extracted ion chromatogram for AP at 140 m/z (green), and the extracted ion chromatogram for MAP at 154 m/z (blue).



*Siloxane

The results for realistic samples were the most promising for drugs analyzed by on-fiber derivatization as solutions. However, identifying controlled substances in their solid form has great potential in forensic science laboratories. This far, pure standards of phenylethylamines and GHB have been analyzed successfully. Overall, the application of this technique to beverage samples and solid drug powders represents a significant decrease in sample preparation.

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IMPLICATIONS FOR CRIMINAL JUSTICE POLICY AND PRACTICE IN THE UNITED STATES

Controlled substances units in forensic science laboratories are put under significant pressure to analyze samples using rigorous methods that offer high throughput and cost-effectiveness. In addition, it is the nature of the field of drug chemistry that new chemical compounds appear in exhibits and forensic chemists must react to this by developing instrumental methods with high specificity. The main results of this work are a set of optimized derivatization methods that can be used in liquid injection or TV-SPME. The TV-SPfsME approach offers the possibility of automated sampling and derivatization for a wide variety of thermally labile compounds as well as successful analysis of compounds that require no derivatization.

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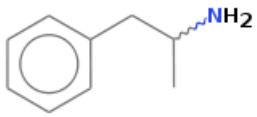
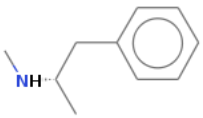
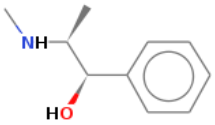
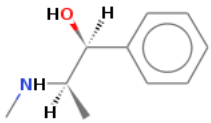
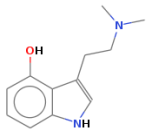
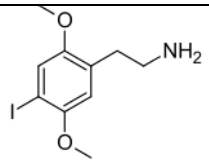
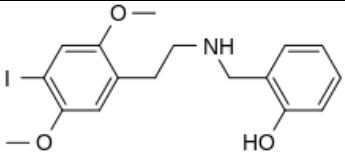
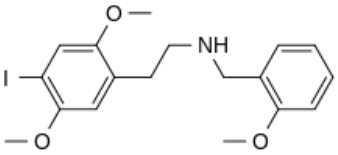
APPENDIX

A summary of the analytes and their structures are summarized below:

COMPOUND CLASS	DRUG	STRUCTURE	MW
Amines / Hydroxylamines	Amphetamine		135
	Methamphetamine		149
	Ephedrine		165
	Pseudoephedrine		165
	Psilocyn		204
	2C-I		307
	25I-NBOH		413
	25I-NBOMe		427

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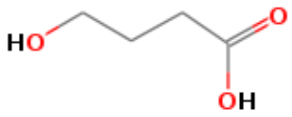
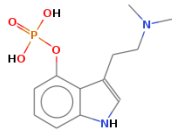
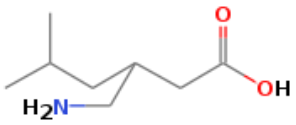
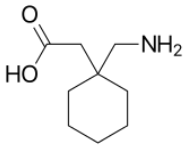
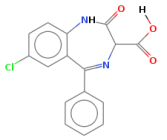
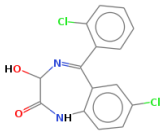
Carboxylic / Phosphonic Acids	GHB		104
	Psilocybin		284
Zwitterions (amine + carboxylic acid)	Pregabalin		159
	Gabapentin		171
	Chlorazepate		315
	Lorazepam		322

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