Quantitation of Major Cannabinoids Found in Seized Marijuana Using Automated Headspace Solid-Phase Microextraction Coupled with Gas Chromatography/Mass Spectrometry

Anastasia Brown[1], BS*; James Sweet[2], PhD; Jorn Chi-Chung Yu[1], PhD, D-ABC

[1]Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340
[2]U.S. Customs and Border Protection, Laboratories and Scientific Services Directorate, Houston Laboratory (Southwest Regional Science Center)

U.S. Customs and Border Protection

ABSTRACT

There are over 60 natural cannabinoids found in the plant material of *Cannabis sativa*, commonly known as marijuana. The primary psychoactive component is Δ^9-tetrahydrocannabinol (Δ^9-THC). Other important components for forensic purposes in states with legalized marijuana include cannabinol (CBN) and cannabidiol (CBD). Current analytical methods for the detection of cannabinoids include solvent extractions followed by gas or liquid chromatography. An optimal automated headspace solid phase micro extraction gas chromatography mass spectrometry (HS-SPME-GC/MS) method has been developed using anthracene as an internal standard for seized marijuana samples. Results from the optimized HS-SPME-GC/MS method show that the same major cannabinoids can be detected with both traditional liquid extraction and HS-SPME methods. The HS-SPME-GC/MS method can potentially offer a new, nearly nondestructive marijuana sample preparation method for quantitation purposes.

INTRODUCTION

The analysis of marijuana is currently limited to identification and determination of potency. Unlike other drugs, such as ecstasy [1], there is currently no effective way to confidently link different seizures by common origin. The purpose of this study was to develop a method to rapidly quantitate cannabinoids to aid in linking seized samples through headspace chemical profiling.

A HS-SPME method was developed and coupled with GC/MS to analyze the cannabinoid profile of seized marijuana samples. HS-SPME is advantageous over traditional liquid extraction because it may not require solvents, is nearly nondestructive, can extract from complex matrixes, and is sensitive enough to detect trace amounts of target compounds [2,3,4].

In this work, 10mg of marijuana was placed in a headspace vial. A polydimethylsiloxane (PDMS) coated SPME fiber was used as it has been shown to be the most efficient at extracting cannabinoids [5]. Mepivacaine and anthracene were tested as potential internal standards due to the high cost of deuterated Δ^9-THC. The new method was compared to the liquid extraction method recommended by the United Nations Office on Drugs and Crime (UNODC).

MATERIALS AND METHODS

Materials

Twenty-five samples were analyzed. An automated sampler was used for SPME extraction. SPME extraction was carried out with 23 gauge 100µm polydimethylsiloxane (PDMS) coated fibers and 20mL vials with PTFE/silicone septa screw caps. A GC system coupled to dual flame ionization (FID) and mass selective (MS) detectors was used for analysis. A 15 meter 35MS fused silica mid-polar column was used.

Sample Extraction

HS-SPME

10mg of marijuana was analyzed. 25µg of internal standard was added and dried off under an airstream. The vial was placed in the sampler and heated to 150°C for 5 minutes with agitation. A SPME fiber was inserted into the vial for 5 minutes. The fiber was then exposed to the GC inlet at 250°C for 30 seconds.

Liquid Extraction

Liquid extraction was performed using the recommended method from UNODC.

Method

GC-MS/FID

Carrier gas flow: 1.2mL/min, initial oven temperature: 170°C held 1 minute, 1st ramp: 15°C/min to 250°C, 2nd ramp: 5°C/min to 270°C, 1.4 minute hold at 270°C, Detectors: MS scanned 40-450amu; FID at 250°C

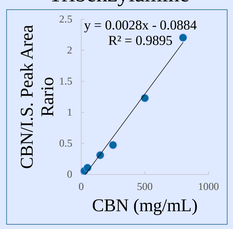
RESULTS

Internal Standard Testing – HS-SPME-GC/MS
% Relative Standard Deviation (N=3)

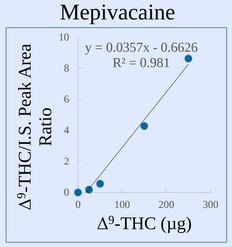
Tribenzylamine				Mepivacaine				Anthracene			
I.S.	THC	CBN	CBD	I.S.	THC	CBN	CBD	I.S.	THC	CBN	CBD
50	14	16	11	124	17	18	22	9	9	5	7

Comparison of Calibration Curves with Different Internal Standards

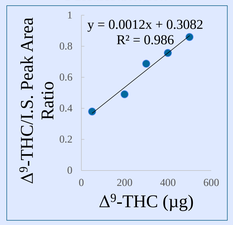
Tribenzylamine



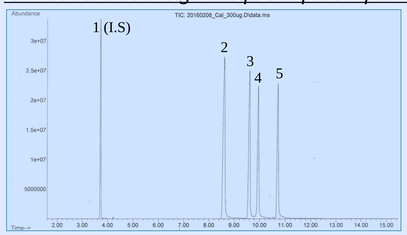
Mepivacaine



Anthracene



Total Ion Chromatogram of Certified Reference Standards (HS-SPME-GC/MS)



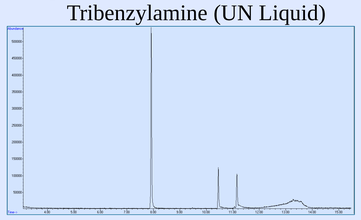
1. Anthracene (25µg)
2. Cannabidiol (100µg)
3. Δ^8-Tetrahydrocannabinol (100µg)
4. Δ^9-Tetrahydrocannabinol (100µg)
5. Cannabinol (100µg)

RESULTS

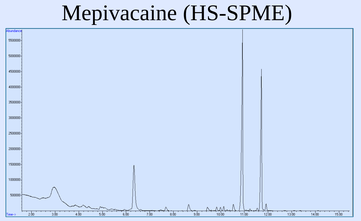
Comparison of Total Ion Chromatograms for Seized Marijuana Samples with Different Internal Standards

Sample 65

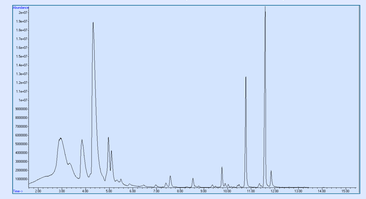
Tribenzylamine (UN Liquid)



Mepivacaine (HS-SPME)

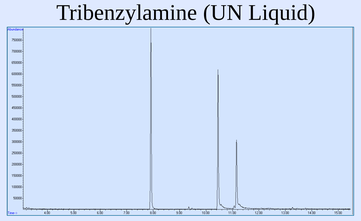


Anthracene (HS-SPME)

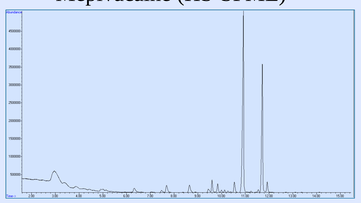


Sample 91

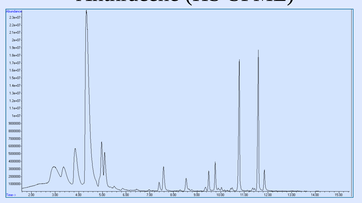
Tribenzylamine (UN Liquid)



Mepivacaine (HS-SPME)



Anthracene (HS-SPME)



Sample 65

Method	% Δ^9-THC	% CBN	% CBD
UN Liquid	1.13	1.06	N/A
Mepivacaine HS-SPME	0.81	0.65	0.22
Anthracene HS-SPME	OCR	OCR	OCR

Sample 91

Method	% Δ^9-THC	% CBN	% CBD
UN Liquid	3.19	1.84	N/A
Mepivacaine HS-SPME	7.07	4.77	0.64
Anthracene HS-SPME	OCR	OCR	OCR

DISCUSSION & CONCLUSIONS

Deuterated Δ^9-THC was originally tested as the desired internal standard (IS). However, it was abandoned because it is not cost-effective for this application. Tribenzylamine (TBA), mepivacaine, and anthracene were tested as potential candidates. TBA was tested due to its use as an IS in the UNDOC method, but it showed large deviations in peak area when tested in multiple samples of one marijuana specimen. Mepivacaine was evaluated due to its low cost and ease of availability. It also showed large deviations in peak area when tested in multiple samples of one marijuana specimen. Anthracene was chosen due to it being readily available and similar to another commonly used IS for liquid marijuana extraction, phenanthrene. Anthracene shows a much smaller deviation in peak area when tested in multiple samples of one marijuana specimen. When used to quantitate Δ^9-THC, CBN, and CBD, the calibration curve showed a R^2 value of 0.986. The quantitation of cannabinoids in real marijuana samples fall outside calibrator range (OCR) due to matrix effects during HS-SPME because the calibration curve was constructed with reference cannabinoid materials that lack the other matrices present in marijuana. Future work will be to determine the matrix effect on cannabinoid during HS-SPME, the inter-variability of anthracene in differing marijuana specimens, and improve the selectivity of SPME fiber for cannabinoids. At the present time, in order to quantify cannabinoids in marijuana by HS-SPME-GC/MS, preparation of calibrators using standard marijuana reference material is recommended.

REFERENCES

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