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

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ABSTRACT

An automated headspace-solid phase micro extraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS) method has been developed using cannabinoid standard reference materials and actual marijuana plant material samples. Unlike previous methods that would require the sample to be extracted with solvents before analysis, the HS-SPME-GC/MS method required the sample to be sealed in the sample vial and placed on GC/MS autosampler that would carry out the HS-SPME extraction. The HS-SPME extraction parameters were optimized to extract cannabinoids from plant material. Results from the HS-SPME-GC/MS method showed the method to be comparable to the common liquid extraction method. The same cannabinoids can be detected with both methods and in some cases the HS-SPME-GC/MS method could detect more cannabinoids than the liquid extraction.

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. This limitation is compounded by the fact that marijuana has been legalized in 4 states of the United States. There is a risk of legally grown marijuana being taken out of state for illegal reselling, and there is currently no analytical way to differentiate between legally and illegally grown samples. The purpose of this study was to develop a method to link marijuana seizures by their chemical profiles.

A HS-SPME method was developed and linked 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 nondestructive, can extract from complex matrixes, and is sensitive enough to detect trace amounts of target compounds [2,3,4]. HS-SPME has been used to detect illicit drugs in the headspace over urine and blood samples [3], as well as chemically profiling several foodstuffs [4, 5]. Recently Ilias et al successfully extracted cannabinoids from marijuana samples using HS-SPME [6].

In this work, a select amount 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 [6]. 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

Twelve samples, provided by the U. S. Customs and Border Protection Houston Laboratory (Southwest Regional Science Center), were analyzed. A Agilent GC Sampler 120 was used for SPME extraction. SPME extraction was carried out with 23 gauge 100 µm polydimethylsiloxane (PDMS) coated fibers and 20 mL vials with PTFE/silicone septa screw caps. An Agilent 7890B system coupled to dual detectors (5977A Mass selective detector and Flame ionization detector (MSD/FID)) was used for GC/MS analysis. The column used was a Restek Rxi 35Sil-M3 [Length: 15 m, Inner Diameter: 0.25 mm, Film Thickness: 0.25 µm].

Sampling

Plant material was pulverized and sieved according to UNODC recommendations [7]. An amount of marijuana was weighed out and added to a 20 mL vial. The vial was placed in the GC Sampler 120 for HS-SPME extraction and GC/MS analysis.

Liquid extraction was performed using the recommended method from UNODC. Each sample was run in triplicate.

Optimization

HS-SPME parameters such as extraction time, extraction temperature, desorption temperature, and others were varied systematically to find the most efficient method to extract cannabinoids. The optimized extraction was as follows:

The vial was placed in the GC Sampler 120 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. The fiber was then heated to 250°C for 20 minutes to remove any remaining compounds. A blank HS-SPME-GC/MS run was performed to ensure the fiber was clean before next extraction.

RESULTS

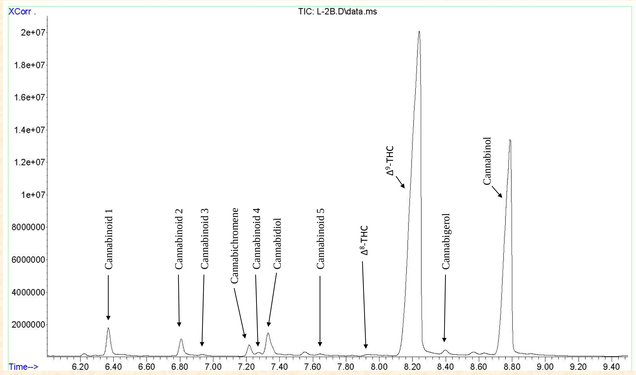

Figure 1: Total Ion Chromatogram (TIC) of cannabinoids extracted from marijuana plant material by HS-SPME

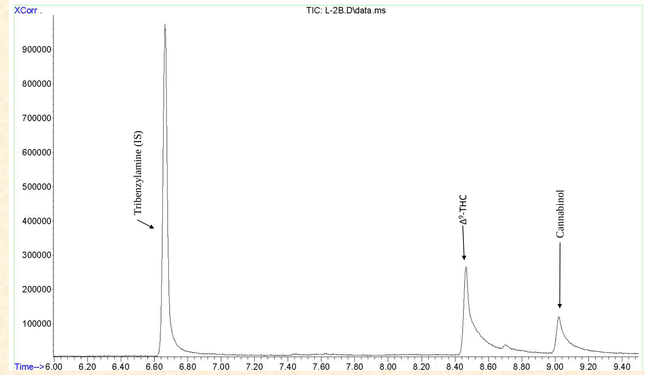

Figure 2: Total Ion Chromatogram (TIC) of cannabinoids extracted from marijuana plant material by liquid extraction

Table 1: Variability in cannabinoids extracted by HS-SPME between triplicate samples for TIC data

	Peak Area										
Triplicate	Cannabinoid 1	Cannabinoid 2	Cannabinoid 3	Cannabichromene	Cannabinoid 4	Cannabidiol	Cannabinoid 5	Δ⁸ - THC	Δ⁹ - THC	Cannabigerol	Cannabinol
A	29691141	23360652	2871976	12716178	5540809	42276649	4047736	6050475	818253995	9971865	333293482
B	26988133	20242317	2808165	12428825	5042275	35684688	4991998	6760505	813358020	9575673	381214822
C	31364104	25592761	3667421	17673357	5459822	44148670	6018951	8516887	955622920	11963418	436860055
mean	29347793	23065243	3115854	14272787	5347635	40703336	5019562	7109289	862411645	10503652	383789453
StDev	2208098	2687427	478735	2948483	267532	4445923	985897	1269659	80760442	1279621	51831268
RSD (%)	8	12	15	21	5	11	20	18	9	12	14

Table 2: Variability in cannabinoids extracted by liquid extraction between triplicate samples for TIC data

	Peak Area		
Triplicate	Tribenzylamine (IS)	Δ⁹ - THC	Cannabinol
A	17964817	7690526	3358442
B	18815483	8174128	3377082
C	18510493	5384943	2216247
mean	18430264	7083199	2983924
StDev	430971	1490477	664893
RSD (%)	2	21	22

Table 3: Variability in cannabinoids extracted by HS-SPME between triplicate samples for FID data

	Peak Area										
Triplicate	Cannabinoid 1	Cannabinoid 2	Cannabinoid 3	Cannabichromene	Cannabinoid 4	Cannabidiol	Cannabinoid 5	Δ⁸ - THC	Δ⁹ - THC	Cannabigerol	Cannabinol
A	3468076	2726990	461813	1957416	512717	4777514	856883	1175527	65354406	1486612	22518887
B	3127208	2238264	424405	2086003	588802	3803310	731923	1090179	64305854	1344221	25030708
C	3640361	2875470	427864	2707945	614843	4628603	740003	1334825	77280111	1788196	29401210
mean	3411882	2613575	438027	2250455	572121	4403142	776270	1200177	68980124	1539676	25650268
StDev	261151	333399	20671	401381	53067	524779	69930	124172	7207094	226694	3482741
RSD (%)	8	13	5	18	9	12	9	10	10	15	14

Table 4: Variability in cannabinoids extracted by liquid extraction between triplicate samples for FID data

	Peak Area		
Triplicate	Tribenzylamine (IS)	Δ⁹ - THC	Cannabinol
A	6453441	94058	3059855
B	6688844	106800	3492036
C	6556898	77847	2578286
mean	6566394	92902	3043392
StDev	117988	14511	457097
RSD (%)	2	16	15

DISCUSSION & CONCLUSIONS

HS-SPME Advantages

- After grinding the plant material, the only preparation needed before putting the sample on the autosampler for extraction and analysis is to weigh it out into vials.
- HS-SPME requires less sample (10 mg) than the liquid extraction (200 mg).
- More cannabinoids are detected with HS-SPME and at greater intensity than liquid extraction.
- Variation between Δ^9-THC, cannabinol, and cannabidiol appear similar. Half of the samples showed less variation with HS-SPME and the other half showed less variation with the liquid extraction.

HS-SPME Limitations

- Carryover of cannabinol, Δ^8-THC, and Δ^9-THC has not been completely eliminated.
- The method is currently limited to qualitative analysis only. When an internal standard was added to the plant material, the signal showed greater variability than the cannabinoids.
- Manufacturer recommendation is to replace the SPME fiber every 100 runs, limiting the number of cases that can be run with one fiber.

Concluding Remarks

Overall the HS-SPME method appears to be comparable to liquid extraction for the identification of marijuana. Continuing efforts will be made to eliminate carryover, to develop the method for quantitative analysis, and to confirm the identity of cannabinoids 1, 2, 3, 4, and 5. Method validation will also be completed.

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