Total Vaporization of Derivatization Reagent for In Situ Headspace Derivatization Solid Phase Microextraction

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ABSTRACT

Solid phase micro extraction (SPME) is a solvent-free extraction technique in which analytes are adsorbed on a polymeric coated fiber, and subsequently desorbed into an instrument for analysis. Many derivatization SPME techniques have been reported including, but not limited to, doping fiber and in vial derivatization post SPME. For this project, we combined total vaporization technique (TVT) and in vial derivatization with SPME in one single step. The approach consists of samples dried in a 20 mL headspace vial and an insert which is placed in the same vial with derivatization reagent. Total vaporization of the derivatization reagent then occurs in combination with heated headspace SPME. Our approach reduced contaminations during derivatization, and also provides the possibility of detecting less volatile compounds.

INTRODUCTION

SPME is a prevalent extraction technique that was first introduced by Arthur and Pawliszyn[1] in which compounds undergo a process of adsorption and desorption[2]. For headspace SPME, other techniques such as TVT and heated headspace SPME (HHS-SPME) have been used to assist headspace extraction of analytes. These techniques enhance the extraction of non-volatiles, polar analytes, and analytes with a high boiling point in liquid or solid samples.

An effective means of reducing equilibration time is to decrease the sample volume. TVT is a method wherein a minimal amount of sample (under 15µL) is added to the sample vial and heated to an adequate temperature in order to vaporize the entire sample inside the vial, but not burst the vial, thereby allowing for greater sensitivity and selectivity[3]. Another effective way to reduce equilibration time is to use increased temperature programming[3]. In HHS-SPME, analytes are separated from the headspace of a dried solid at temperatures between 100-200°C, so that the SPME fiber does not come into direct contact with the original medium[4].

The combination of derivatization with SPME can often enhance the SPME extraction and the later separation of extracts by gas chromatography (GC) or liquid chromatography (LC). Derivatization can optimize volatility and the partition coefficients between the fiber and the headspace[5]. For this project, we combined TVT, heated headspace, and in vial derivatization with SPME in one single step for application to the detection of common cannabinoids, such as delta9-Tetrahydrocannabinol (THC), cannabidiol (CBD), cannnabigerol (CBG), cannabinol (CBN), and tetrahydrocannabivarin (THCV) and cannabichromene (CBC) .

MATERIALS AND METHODS

Reagents and Materials

- Common cannabinoids and their acidic forms were purchased from Cerilliant (Austin, TX) and from Restek (Oklahoma City, OK) as standard methanolic solutions.
- The phytocannabinoid mixture 1 was purchased from Cayman Chemical Company(Ann Arbor, MI).
- *N*-methyl-*N*-trimethylsilyl-trifluoroacetamine (MSFTA) was obtained from Sigma Aldrich (St. Louis, MO).
- A SPME device for an auto-sampler with a replaceable 100-µm polydimethylsiloxane (PDMS) fiber was obtained from Supelco (St. Louis, MO).

Derivatization

- In order to achieve optimal yield of the derivatized product, varying amounts of derivatization reagent were analyzed.
- For HHS-SPME extraction, 400 ng (4 µL of 100 µg/mL) delta9-tetrahydrocannabinol were placed in separate 20 mL headspace vials.
- After drying 4µL solvent, 1, 2.5, 5, 7.5, 12.5, 15, 20, and 25 µL of MSTFA was added to the inserts inside headspace vials. The vials were then sealed with a silicone septum and magnetic cap.

Sample Preparation

- For HHS-SPME extraction, 4 µL of 100 µg/ml solutions of common cannabinoid solutions and phytocannabinoid mixture 1 were placed in separate 20 mL headspace vials and dried.
- After drying, 5 µL MSFTA was added to an insert inside the headspace vial. Separate headspace vials containing each cannabinoid were also prepared without derivatization reagent.
- An aqueous internal standard, delta9-tetrahydrocannabinol-d3, was added in 2 µL aliquots to every 20 mL headspace vial. The vials were sealed with a silicone septum and a magnetic cap.

GC-MS Method

- Agilent model 7890 Series gas chromatograph in combination with a CTC Combi PAL Auto sampler and an Agilent 5975C mass selective detector.
- Substances were separation on a fused silica capillary column (Rxi®-35 Sil MS, 15m x 0.25mm i.d., film thickness 0.25 µm)
- Temperature program: 170ºC hold for 1 min.; 15ºC/min up to 228ºC, hold for 3 min.; 10ºC/min. up to 250ºC, hold for 0 min.; 5ºC/min up to 270ºC. SPME injected using split mode (20:1); initial flow rate 1.2 mL/min.

HS-SPME Method

- Each sample vial was agitated for 2 min. at 150ºC in the agitator of the auto-sampler (250 rpm, agitator on time 0:02 min, agitator off time 0:10 min).
- For absorption, the needle of the SPME assembly containing the fiber was inserted through the septum of the vial, and the fiber was exposed to the headspace in the vial for 60 second.
- In the final step, the SPME fiber with the adsorbed derivatized compound was placed into the injection port of the GC/MS for 30 seconds to finalize desorption.

RESULTS

Phytocannabinoid	Retention Time (min)	Base Peak	Molecular Ion Peak	Qualifier Ions
CBC	8.306	231	314	174, 231
CBD	8.497	231	314	231, 246
CBG	10.234	193	316	193, 231, 316
CBN	10.625	295	310	238, 295
Δ8-THC	9.53	231	314	231, 314
Δ9-THC	9.868	299	314	231, 271, 299
THCV	7.396	271	286	203, 343, 271, 286
CBC-TMS	6.063	303	386	303,371
CBD-diTMS	4.982	390	458	73, 301, 337, 390
CBG-diTMS	6.158	337	460	337, 391
CBN-TMS	7.844	367	382	367
Δ8-THC-TMS	6.449	303	386	303, 330,386
Δ9-THC-TMS	6.73	371	386	303, 343, 371, 386
THCV-TMS	5.206	343	358	275, 315, 343, 358

Table 1. Base peak, molecular ion peak, and qualifier ions for the derivatized and underivatized versions from laboratory standards of phytocannabinoids.

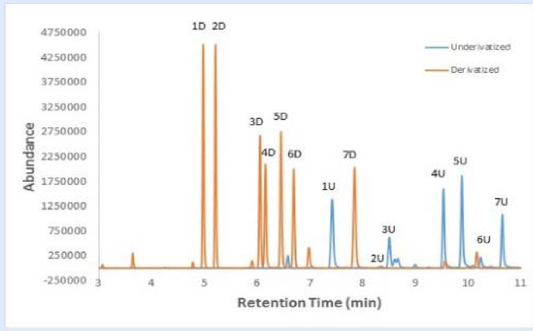

Fig 2. Compilation Chromatogram of derivatized and underivatized phytocannabinoids: 1D) CBD-diTMS, 2D) THCV-TMS, 3D) CBC-TMS, 4D) CBG-TMS, 5D) Δ8-THC-TMS, 6D) Δ9-THC-TMS, 7D) CBN-TMS, 1U) THCV, 2U) CBC, 3U) CBD, 4U) Δ8-THC, 5U) Δ9-THC, 6U) CBG, and 7U) CBN.

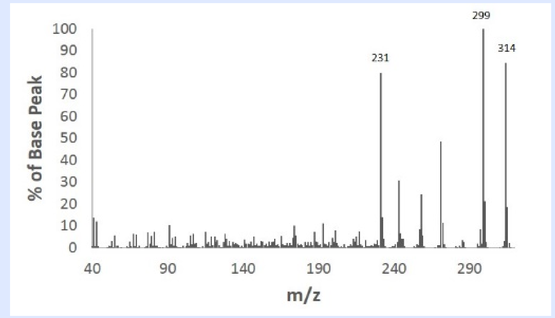

Fig 1. Mass spectra of Δ9-THC.

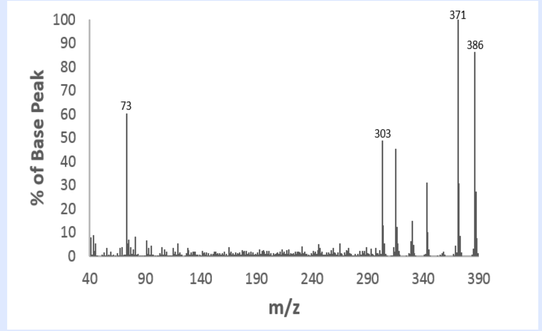

Fig 3. Mass spectra of Δ9-THC-TMS.

DISCUSSION &CONCLUSIONS

The optimal amount of derivatization reagent for in situ derivatization of phytocannabinoids in a 20 mL headspace vial was found to be 5 µL.

All seven of the phytocannabinoids and their tri-methyl silyl (TMS) derivatized products were detectable as low as 0.4µg in the vial including: CBC, CBD, CBG, CBN, Δ8-THC, Δ9-THC, and THCV. The acidic forms of the cannabinoids were not detectable due to their thermally labile nature.

After derivatization, chromatographically, the peaks were narrower, less peak tailing was observed along with baseline separation, as well as, increased abundance. CBC, CBG, and CBD had the most increase in abundance, with their abundance of their peaks increased by 101x, 10.9x, and 7.5x post derivatization.

Δ9-THC-TMS had a regression equation from the calibration curve of y = 2E+07x - 1E+07, and an R^2 value of 0.976, indicating this in situ headspace derivatization approach could be adopted for quantitative analysis.

ACKNOWLEDGEMENTS

This work was supported by Award #2014-R2-CX-K005, National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the author(s) and do not necessarily reflect those of the Department of Justice.

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