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.

INTRODUCTION

SPME is a prevalent extraction technique that was first introduced by Arthur and Pawliszyn in which compounds undergo a process of adsorption and desorption. 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.

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. 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 delta-9-tetrahydrocannabinol (δ9-THC), cannabidiol (CBD), cannabigerol (CBG), cannabinoids (CBD), cannabigerol (CBG), cannabinol (CBN), and tetrahydrocannabinovarin (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 derivatization reagent was purchased from Cayman Chemical Company (Ann Arbor, MI).
- A polyethylene tethered trifluoroacetic acid (MSTFA) was obtained from Sigma-Aldrich (St. Louis, MO).
- A SPME device for an auto-needle with a replaceable 100-μL 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) derivatized products were placed in separate 20 mL headspace vials.
- After drying 4 mL solvent, 1, 2.5, 7.5, 12.5, 15, 20, and 25 µL of MSTFA was added to the inserts inside headspace vials.
- The vials were sealed with a silicone septum and magnetic cap.

Sample Preparation

- For HHS-SPME extraction, 4 mL of 100 ng/mL solutions of common cannabinoids and their acidic forms were placed in separate 20 mL headspace vials and dried.
- After drying, 4 mL MSTFA was added in situs 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.

GC-MS Method

- An Agilent model 7890 Series gas chromatograph in combination with a FID and PS-5 Auto-Sampler and an Agilent 5975C mass selective detector.

HS-SPME Method

- Each sample vial was spiked for 2 min at 150ºC in the agitate of the auto-sampler (200 rpm, agitate on time 0:02 seconds).
- 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

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 detected as low as 0.4 µg in the vial including: CBD, CBG, CBN, ∆8-THC, ∆9-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 CBN had the most increase in abundance, with their abundance of their peaks increased by 101x, 10.9x, and 7.5x post derivatization.

Fig 1. Mass spectra of ∆9-THC-TMS.

DISCUSSION & CONCLUSIONS

The acidic forms of the cannabinoids were not detectable due to their thermally labile nature.

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