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Detection of phytocannabinoids from buccal swabs by headspace solid phase microextraction – gas chromatography/mass spectrometry

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Headspace solid phase microextraction (HS-SPME), which is a solvent-free extraction technique, was configured with gas chromatography/mass spectrometry (GC/MS) to detect phytocannabinoids from buccal swabs. The HS-SPME extraction procedure, *i.e.* extraction time, extraction temperature, thermal desorption parameters as well as headspace derivatization, were evaluated to extract major phytocannabinoids from the headspace of air-dried buccal swab samples. Sub micrograms of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) spiked onto buccal swabs could be extracted and detected by the HS-SPME-GC/MS approach. The analytical system can be readily automated without the use of solvent extraction. No interference peaks for phytocannabinoids were found in the total ion chromatograms obtained from the tested buccal swabs using cotton as the substrate. Interference background can also be minimized by using selected ion monitoring. This analytical approach potentially could be adopted to detect marijuana smokers by the identification of residual phytocannabinoids from oral cavities for forensic applications.

Introduction

While marijuana is the most widely available and commonly used illicit drug, and remains illegal under the federal law in the United States, some state legislation has passed marijuana programs to regulate the cultivation, possession, and use of marijuana within their respective states.[1] As of the end of 2017, 29 states, including Washington, D.C., have legislation permitting the use of marijuana for medical purposes. Some states have legalized marijuana for recreational use. With such changes in legislation, marijuana and its related products sales increased. For example, Colorado sold $1.3 billion worth of marijuana in 2016.[2] According to the World Drug Report 2015, the most recent data predicted an increase in the prevalence of marijuana, or cannabis, use in the United States, because of ongoing changes in state legislation.[3] The legalization of medical or recreational use of marijuana in some of the states has raised concerns of situations of driving under the influence of smoking marijuana.[4] Because of this potential increase of marijuana use in the community, the detection of recent smoking or using marijuana from an individual is becoming an important forensic task in law enforcement.

Marijuana is the dried plant material of *Cannabis*. Although more than 60 phytocannabinoids have been identified from marijuana, Δ^9-tetrahydrocannabinol (THC) is known as the primary psychoactive chemical and cannabidiol (CBD) has been promoted as the medical component.[5] Other than phytocannabinoids, several classes of compounds, including limonene, pinene (in α- and β-form), β-myrcene, and over 100 others could be found from marijuana.[6,7] The most common way of using marijuana is smoking, particularly the flowering tops which contain the highest concentration of THC in the plant.[8] The plant material can also be baked in food products such as brownies or cakes. Pressed resins and oils are extracts of marijuana that can be ingested. Marijuana is used, whether licitly or illicitly, on a global scale.[9]

Marijuana is also the most prevalent illicit controlled substance reported in motor vehicle accidents.[10] Experimental studies show that severe intoxication due to consumption of marijuana takes its toll on a variety of cognitive and motor skills that are pertinent to driving, including response time, attention, information processing, perceptual motor performance, and tracking behaviour.[11] The detection of the use of marijuana often requires collection of specimen from the person in question and perform toxicological analysis of the specimen.[12] Most of the toxicological methods offer high sensitivity and accuracy for the detection of THC, and its metabolites from blood,[13] urine,[14] as well as oral fluids.[15] However, the positive detection of THC or metabolites cannot eliminate "second hand" exposure to marijuana smoke.[16] In blood, besides THC, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) is the major metabolite found from marijuana smokers.[17] THC-

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COOH is also the metabolite that is present in urine from marijuana users. The presence of THC-COOH in blood or urine samples may not always indicate impairment because the detection window is in days.[18]

Headspace solid phase microextraction (HS-SPME) is a solvent-free extraction technique used to isolate analytes of interest from the headspace of a sample. SPME fibers are commercially available in a variety of compositions of polymeric components immobilized on a fused silica or metal core. Common fiber compositions are polydimethylsiloxane (PDMS) and polyacrylate (PA). A variety of SPME fiber types are becoming available from the commercial source to accommodate various purpose for extraction. SPME fibers are chemically inert and can undergo high temperatures without interfering with extraction and analysis.[19] The major advantages of HS-SPME are that it is simple, solvent-free, nearly non-destructive, fast, capable of automation, and sensitive enough to extract sub microgram levels of analyte.[20,21] High sensitivity headspace extraction was also demonstrated by combining headspace single-drop microextraction (SDME) and SPME.[22]

In this research, HS-SPME-GC/MS was adopted and optimized to detect major phytocannabinoids from the headspace of air-dried buccal swabs. The aim of this work was to develop a novel analytical method to identify marijuana smokers by the identification of residual phytocannabinoids from their oral cavities.

Experimental

Materials and methods

Individual CBD, Δ^9-THC, cannabinol (CBN), standards were purchased from Cerilliant (Round Rock, TX). Certified reference material containing 10 phytocannabinoids (THC, Δ^8-THC, tetrahydrocannabinolic acid (THCA), cannabinol (CBN), CBD, cannabidiolic acid (CBDA), cannabichromene (CBC), cannabigerol (CBG), cannabigerolic acid (CBGA) and tetrahydrocannabivarin (THCV)) mixture was obtained from Cayman Chemical (Ann Arbor, MI) for the purpose of optimizing chromatography conditions and identifying phytocannabinoids by the MS detector. Polydimethylsiloxane (PDMS) 23 gauge 100 μm was selected for HS-SPME and was purchased form Supelco (Bellefonte, PA). The fiber was conditioned *via* thermal desorption for 30 min as per manufacturer instructions. The GC/MS was performed on an Agilent 7890 GC System with a 5975C MS Detector. Data acquisition and analysis was performed using standard software supplied by the manufacturer (Agilent Chemstation C00.01). Summed ion chromatogram (SIC) was generated with Agilent MassHunter Qualitative Analysis software (Version B.06.00). SPME methods were fully automated, and controlled by the Agilent PAL software. A fused silica capillary column (Rxi-35 Sil MS, 15 m × 0.25 mm × 0.25 μm) was used as the separation column with helium gas (ultrapure, 99.999%) as carrier gas. The GC oven programming for the optimal separation of major phytocannabinoids was as the following: initial temperature was set at 170 °C for 1 min, then ramped to 228 °C at 15 °C min^{-1} and hold for 3 min, then to 250 °C at 10 °C min^{-1}, lastly to 270 °C at 5 °C min^{-1}.

For HS-SPME, each sample vial was incubated for 5 min at 150 °C in the agitator of the auto-sampler. 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. After HS-SPME, the SPME fiber was placed into the injection port of the GC/MS for 30 seconds for sample injection.

To optimize HS-SPME-GC/MS conditions using reference phytocannabinoids, 4 μL of 100 μg mL^{-1} solutions of standard phytocannabinoid mixture were placed in 20 mL headspace vials. The solvent of the standard was dried under gentle air stream. This procedure placed 0.4 μg of each phytocannabinoids in the vial for the subsequent HS-SPME-GC/MS method development.

Headspace derivatization

In order to evaluate headspace derivatization during HS-SPME, 1, 2.5, 5, 7.5, 12.5, 15, 20, and 25 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), purchased from Sigma-Aldrich (St. Louis, MO), was added to the GC inserts placed inside the 20 mL headspace vials. Derivatization of THC was tested at 0.4 μg level in the vial, which was prepared by drying 4 μL of 100 ng μL^{-1} aliquots of THC in the vial before HS-SPME.

Buccal swab sample preparation and swab substrate evaluation

To first develop a HS-SPME condition for the extraction of phytocannabinoids from dried buccal swabs, THC was selected for the optimization of the extraction condition. THC levels ranging from 0.1 to 25 μg were transferred onto separate buccal swabs (Fitzco, Spring Park, Minnesota) and dried overnight. After drying, approximately 5 mg of the swab sample was placed into a 20 mL headspace vial for HS-SPME-GC/MS method development and optimization. The initial HS-SPME-GC/MS condition was adopted from Ilias *et al.*[23] The performance of the optimal HS-SPME-GC/MS was evaluated with buccal swabs spiked with 0.2, 0.4, and 10 μg of phytocannabinoid mixture for the separation and detection of all phytocannabinoids. Cotton push off swabs, cotton break off swabs, regular buccal swabs, CEP swabs, and Omni swabs were purchased from Fitzco and matrix effect from their headspace interferences were evaluated.

Results and discussion

HS-SPME method evaluation

Swab sampling technique is one of the most versatile sampling technique in forensic evidence collection, including biological sample and trace evidence. For biological sample collection, air drying swabs is a common practice to prevent the growth of fungi in the sample that might interfere with subsequent biological tests. The use of HS-SPME to extract headspace chemicals from solid samples has been demonstrated by several researchers. In this work, the idea was to adopt HS-SPME approach for the headspace analysis of air dried buccal swab. Residual phytocannabinoids collected from the oral cavity of a marijuana smoker could be extracted and detected by HS-

SPME-GC/MS approach. In this way, the buccal swab not only could be used for chemical analysis, but also for DNA analysis. As shown in Fig. 1, 7-phytocannabinoids (THC, Δ^8-THC, CBN, CBD, CBC, CBG, and CBV) could be readily extracted and detected by HS-SPME-GC/MS from the headspace of a buccal swab spiked with 0.4 μg certified reference material in a 20 mL vial. Note that THCA, CBDA, and CBGA could not be detected by this HS-SPME-GC/MS approach.

In vial headspace derivatization

Because THCA, CBDA, and CBGA could not be detected by this HS-SPME-GC/MS approach, headspace derivatization was evaluated during HS-SPME step. From a study performed by Liu and Huang,[24] urine samples containing amphetamines were placed in a headspace vial, with a fritted insert containing derivatization reagent, exposed to a PDMS fiber, and heated to 100 °C for adsorption and derivatization during HS-SPME process. Several studies have been performed using one vial derivatization, also known as in tube/in vial/*in situ* derivatization, including but not limited to chlorophenols in water,[25] acidic pesticides,[26] methylmercury in water,[27] anti-inflammatory drugs in water,[28] phenolic acids and flavonoids,[29] GHB in bio fluids,[30] amphetamines and ecstasy from water,[31] methamphetamine,[32] primary aromatic amines[33] and cannabinoids in urine.[34] In this work, 5 μL of derivatization reagent was chosen as the suitable amount of derivatization in a 20 mL sample vial, because this was minimal sample volume yielding a reduction in equilibrium time.[35] As shown in Fig. 2, the reaction between the phytocannabinoids and MSTFA produced favorable chromatograms. It demonstrated the presence of MSTFA in sample headspace during HS-SPME successfully derivatized THC, CBD. CBN, CBC, CBG, Δ^8-THC, and THCV. Their tri-methyl silyl (TMS) derivatized products were detected by in vial derivatization HS-SPME-GC/MS. In vial derivatization improved the peak shape of Δ^9-THC on a buccal swab. The abundance of Δ^9-THC increased post derivatization. THCA, CBDA, and CBGA still could not be detected by the GC/MS approach due to their thermally labile nature. In order to detect THCA, CBDA and CBGA, the derivatization step may be performed before HS-SPME step.

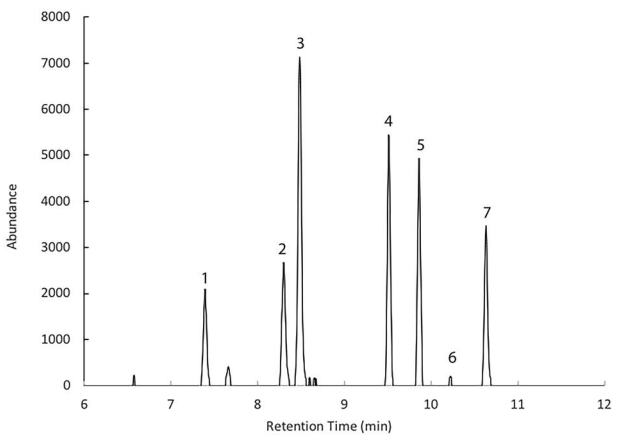

Fig. 1 Summed ion chromatogram (SIC) of *m*/*z* 193, 231, 271, 295, 299, 314 from an air dried, phytocannabinoid-spiked buccal swab sample obtained by HS-SPME-GC/MS. (1) THCV (*m*/*z* 271), (2) CBC (*m*/*z* 231), (3) CBD (*m*/*z* 231), (4) Δ^8-THC (*m*/*z* 231, 314), (5) Δ^9-THC (*m*/*z* 231, 299, 314), (6) CBG (*m*/*z* 193, 231), and (7) CBN (*m*/*z* 295). 0.4 μg of standard phytocannabinoids were spiked onto the sample.

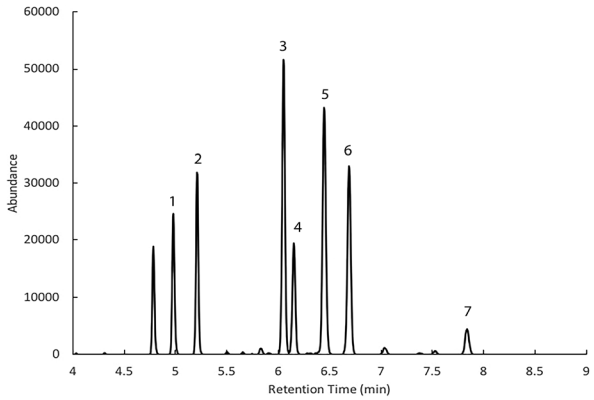

Fig. 2 Summed ion chromatogram (SIC) of *m*/*z* 303, 310, 337, 343, 374, 389 from an air dried, phytocannabinoid-spiked buccal swab sample obtained by in vial derivatization HS-SPME-GC/MS. (1) THCV–TMS (*m*/*z* 343), (2) CBC–TMS (*m*/*z* 303), (3) CBD–TMS (*m*/*z* 303), (4) Δ^8-THC–TMS (303), (5) Δ^9-THC–TMS (*m*/*z* 374), (6) CBG–TMS (337), and (7) CBN–TMS (310). 0.4 μg of standard phytocannabinoids were spiked onto the sample. 15 μL MSTFA was added to a GC insert and placed inside the 20 mL headspace sample vials.

Interference study with different swab substrates

When assessing the swab substrates for the present study, minimal background noise was the most predominant determining factor. As shown in Fig. 3, the CEP swab and foam swab had the highest background with the highest abundances exceeding 25 million abundance count in a typical TIC chromatogram. The Omni and buccal swab with a wooden handle had their highest abundances between 10–17 million in a typical TIC chromatogram, while the push off buccal swab's highest abundance was approximately 14 000. Therefore, the push-off buccal swab was chosen because it had the least amount of background noise compared to all the swabs tested. Alternatively, single ion monitoring (SIM) mode can be set up to remove interference background (swab substrate) from the chromatogram.

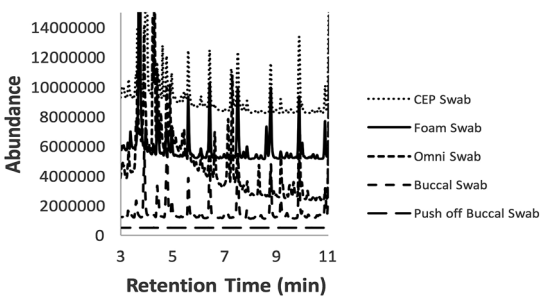

Fig. 3 Headspace background profiles obtained from different swab substrates by HS-SPME-GC/MS.

Conclusions

In a study performed by Moore *et al.*,[36] 17 μg L^{-1} THC and CBN were detected in oral fluid after 3 hours of exposure to cannabis smoke in Dutch coffee shops, but THC-COOH was not present. Therefore, it was suggested that the metabolite could be used as a marker for distinguishing between active and passive exposure to marijuana. However, it was also suggested that typical instrumentation used to target this metabolite was not sensitive enough due to minimal concentrations in oral fluid. Cryogenic focusing, two-dimensional gas chromatography, and negative ion chemical ionization detection were viable alternatives for the detection of metabolites in oral fluids. Furthermore, to differentiate between residual THC and contamination, the use of CBN or CBD as a marker for analysis has also been suggested.[37] Consequently, detection of multiple phytocannabinoids may be used to confirm active smoking.[38] In this work, the detection of phytocannabinoids from buccal swabs was studied for qualitative purposes. For quantitative measurement of each phytocannabinoids using HS-SPME-GC/MS, proper internal standards combining with SIM mode would be suggested. Under our experimental condition, CBC, CBD, CBN, Δ^8-THC, Δ^9-THC, THCV and their TMS derivatized products were less interfered by matrix effect between spiking level of 0.2–10 μg from 5 mg of buccal swabs. The limit of detection (LOD) of our method is below 0.2 μg/5 mg of buccal swab samples. Different swab substrates may result in different LOD. Derivatization would be recommended when better LOD is required. Sample stability studies for each phytocannabinoids under different storage condition should be investigated in the future.

The investigated HS-SPME-GC/MS method is simple, sensitive, and nearly non-destructive to evidence collected and preserved by swabbing technique. HS-SPME-GC/MS is also applicable to a wide variety of forensic uses in both trace evidence and drug chemistry. It may be particularly helpful in detecting trace amounts of contaminants or additives. In the scenarios of controlled substance collection and crime scene investigation, by swabbing suspected surface or paraphernalia, an analyst can expect to yield results which are reliable and unadulterated by the sampling substrate. Further testing of different swabbing materials (*i.e.* nylon flock) would be beneficial to determining the various benefits and limitations of this method in trace residual drug sampling. Moreover, micro scale liquid phase headspace extraction technique[39,40] can be used for comparison. With proper selection of internal standards and operating the mass detector under SIM mode, the HS-SPME-GC/MS could also be adopted for quantitative analysis of residual phytocannabinoids from samples.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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