Detection of Phytocannabinoids from Buccal Swabs Using One Vial Headspace Vaporization Derivatization Coupled with SPME-GC/MS

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INTRODUCTION

Marijuana is classified as a Schedule I controlled substance at the federal level despite its legalization in medical and recreational uses in multiple states. However, with the increase in the recreational uses of marijuana, it was reported as the most prevalent illicit drug in drug-related accidents and/or in motor vehicle incidents associated with driving under the influence of drugs (DUID).[1]

The choices of biological matrices to collect and analyze in DUID cases are important to determine impairment of the driver at the time of driving. Blood and urine specimens are the most common biological matrices collected for toxicological tests. Although the testing of blood specimen has a strong indication of impairment due to the presence of the metabolite, delta-9-tetrahydrocannabinnol (Δ9-THC), the sampling process is invasive.[2] As for urine samples, the major metabolite, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH), does not always indicate impairment and the sampling process is subjected to adulteration issues.[3] Other than that, both types of evidence are required to undergo lengthy sample preparation to extract the analytes and remove interferences before instrumental analysis.

In the past decade, oral fluid has been suggested to be an alternative matrix for forensic analysis due to its ease and lower cost of sample collection. When oral fluid is collected by buccal swaps, it has lower chance to be subjected to adulteration when compared to other matrices, such as urine. Most importantly, the drug concentration in oral fluid is found to have a stronger correlation to that in plasma than in urine and thus maybe a better indicator of recent use of marijuana.[3-5]

Due to the relatively simple matrix of oral fluid, extraction of targeted drugs using heated headspace solid phase micro-extraction (HHS-SPME) is a promising alternative to bypass the lengthy and labor intensive steps in the conventional sample preparation. This project explored the application of HHS-SPME-GC/MS coupled with in-vial derivatization to facilitate automated extraction and detection of phytocannabinoids from buccal swabs.

RESULTS & DISCUSSION

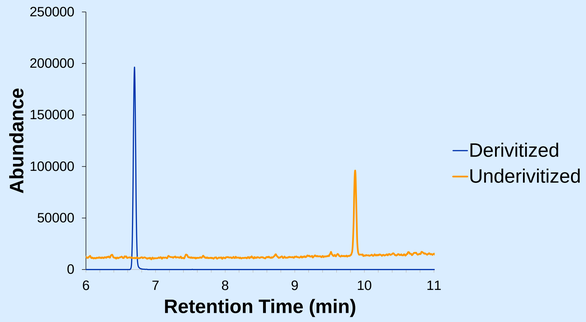

Figure 1: Overlap of total ion chromatograms for underivitized (orange) and derivitized (blue) Δ9-THC using the HHS-SPME-GC/MS technique. Spiking level was 0.4 ug of Δ9-THC and 5mg of swab materials were sampled for HHS-SPME-GC/MS.

- Seven phytocannabinoids (CBC, CBD, CBG, CBN, Δ8-THC, Δ9-THC, and THCV) and their derivitized products can be detected using the in vial derivatization with our optimal HHS-SPME-GC/MS condition. CBDA, CBGA, and THCA were not detectable with our method. These phytocannabinoids might be thermally unstable.
- Optimal amount of derivitization agent (MSTFA) was found to be 5 µL in a 20mL headspace vial for 04 ug of Δ9-THC in the same vial.
- Derivitization improved sensitivity, resolution, peak shape, and abundance of the phytocannabinoids.
- Push off buccal swab has the least background noise among the five tested swabs, and thus may provide better limit of detection (LOD) for Δ9-THC.

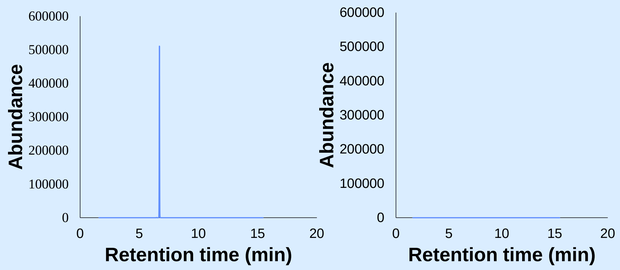

Figure 2: Extract ion chromatograms (m/e 317, 386) showing derivitized Δ9-THC (left) from buccal swab using HHS-SPME-GC/MS and blank buccal swab (right). Spiking level was 0.4 ug of Δ9-THC and 5mg of swab materials were sampled for HHS-SPME-GC/MS

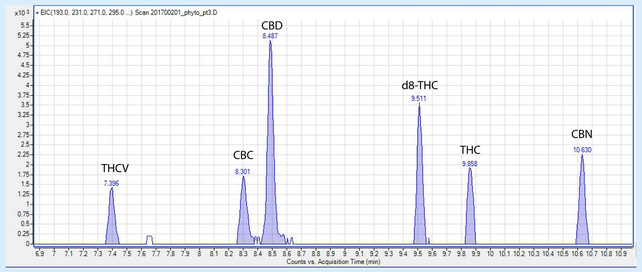

Figure 4: Summed ion chromatogram (m/z 193, 213, 271, 295, 314) showing the detection of phytocannabinoids from s spiked buccal swab using HHS-SPME-GC/MS. 0.2 ug of standard mixture of phytocannabinoid was spiked onto a buccal swab, and 5mg of air dried swab materials were sampled for HHS-SPME-GC/MS

MATERIALS AND METHODS

Interference Studies from Buccal Swabs

Background interference from the direct analysis of buccal swabs were determined by testing five different buccal swabs: cotton push off swabs, cotton breakoff swabs, regular buccal swabs, CEP swabs, and Omni swabs. Each swab was spiked with 0.4 µg of Δ9-THC and approximately 5 mg of the swabs were transferred to individual headspace vials and sealed for HHS-SPME-GC/MS analysis.

Sample Preparation

To determine whether HHS-SPME-GC/MS can be applied to extract and detect phytocannabinoids, 4 µL of 100 µg/mL of common phytocannabinoid standards (Δ9-THC, cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), cannabigerol (CBG), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), tetrahydrocannabivarin (THCV), tetrahydrocannabinolic acid (THCA), Δ8-THC, and a mixture of the above standards) were added to separate headspace vials, let dry and analylzed with and without derivitization.

To determine whether HHS-SPME-GC/MS can be applied to extract and detect phytocannabinoids directly from buccal swabs, 0.2 – 10 µg of Δ9-THC standard was transferred on separate cotton push-off swabs, let dry overnight, and approximately 5 mg of the swabs were transferred to individual headspace vials and subjected to analysis with and without derivitization.

CONCLUSIONS

- The combined step of in vial derivatization with HS-SPME is cost-efficient. The process requires minimal reagents and preparation time, and can be easily automated.
- This method is promising for the detection of phytocannabinoids from buccal swab.
- Application of this method provides a non-invasive sample collection alternative for road-side and workplace drug testing for residual phytocannabinoids in oral cavity.
- This novel methodology has a potential to be applied to detect other illicit substances in different biological matrices.

MATERIALS AND METHODS

Derivitization Optimization

To achieve optimal yield of derivitized products, various amounts of derivitization agent, *N*-methyl-*N*-trimethylsilyl-trifluoroacetamine (MSFTA), were evaluated. Four µL of 100 µg/mL Δ9-THC standard solution were added in separate headspace GC vials and were allowed to dry. Glass inserts containing different amounts of MSTFA (1, 2.5, 5, 7.5, 12.5, 15, 20, and 25 µL) were placed in the prepared headspace vials, sealed, and subjected to HHS-SPME-GC/MS analysis.

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ACKNOWLEDGEMENTS

This project was partly supported by Award No. 2014-R2-CX-K005 awarded by the National Institute of Justice. The opinions, findings, and conclusions or recommendations expressed in this report are those of the author and do not necessarily reflect those of the Department of Justice.