

HIGHLIGHTS: 96 samples < 15 minutes **PRODUCT:** RP dSPE Tips

INTRODUCTION

The need for fast robust methods of evaluating cannabinoid content continues to rise with the increase in cannabis use. The chemical complexity of cannabis plant material and subsequent extracts may benefit from solid phase extraction prior to LC-MS/MS analysis. Current procedures either have very laborious sample preparation methods or lack them altogether. An analytical procedure for the determination of 9 cannabinoids in plant material was developed using dispersive pipette extraction on a Hamilton Microlab® NIMBUS96® platform followed by LC-MS/MS analysis. Dispersive Pipette Extraction requires minimal sorbent resulting in reduced matrix effects and reasonable levels of THC to avoid saturating the detector. The automated method can process up to 96 samples in under 15 minutes thereby minimizing within-run sample variability and maximizing throughput. The versatility of the sample preparation method could allow for its use with a variety of matrices including many edible forms of cannabis for both qualitative and quantitative analysis.

MATERIALS AND METHODS

Reagents and Standards

All standards were obtained from Shimadzu in a mix of tetrahydrocannabivarin (THCV), cannabigerol (CBG), cannabidiol (CBD), cannabidivarin (CBDV), cannabigerolic acid (CBGA), cannabinol (CBN), delta 9 tetrahydrocannabinol (d9 THC), cannabichromene (CBC), and tetrahydrocannabinolic acid (THCA-A). Internal standard, delta 9 tetrahydrocannabinol D3, was obtained from Cerilliant (Round Rock, TX). dSPE tips with 5 mg of Reverse Phase (RP) sorbent were purchased from DPX Technologies, LLC (Columbia, SC).

Instrumental Analysis

Analyses were performed using a Thermo TSQ Vantage™ triple quadrupole mass spectrometer (Milwaukee, WI) coupled to an Agilent 1260 Series HPLC (Agilent Technologies, Santa Clara, CA) equipped with an Agilent Poroshell EC-C18 column (3.0 × 50 mm, 2.7 µm) with column temperature held at 50°C and



Nimbus96 with dSPE Tips

a 2 µL sample injection volume. The mobile phase was composed of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient started at 20% B, ramped to 95% B at 2 minutes where it remained until 4.1 minutes and was then re-equilibrated to 20% B for a total run time of 5 minutes. The column flow rate was 0.65 mL/min. Mass spectrometer parameters were: electrospray voltage, 5000V; auxiliary gas pressure, 2 psi; sheath gas pressure, 35 psi; vaporizer temperature was 330 °C, and capillary temperature was 400 °C.

Sample Preparation

Cannabis samples were obtained from local law enforcement agencies. The dried cannabis plant material was ground and weighed into 200 mg samples. An aliquot of 2 mL of acetonitrile (ACN) was added and the solution was sonicated for 30 minutes.

The Dispersive Pipette Extraction method and deck layout is depicted in Figure 1A and 1B respectively. The analyte partitions into the sorbent from mixing the sample with the sorbent through aspiration and dispensing steps. The matrix is efficiently removed from the sorbent by washing with 50% methanol in water. The elution step uses just 300 µL of ACN, which is subsequently diluted and injected into the LC-MS/MS system. The extraction procedure was performed in less than 5 minutes.

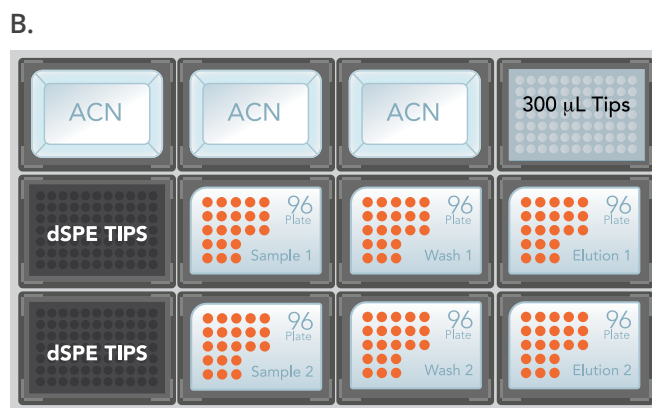
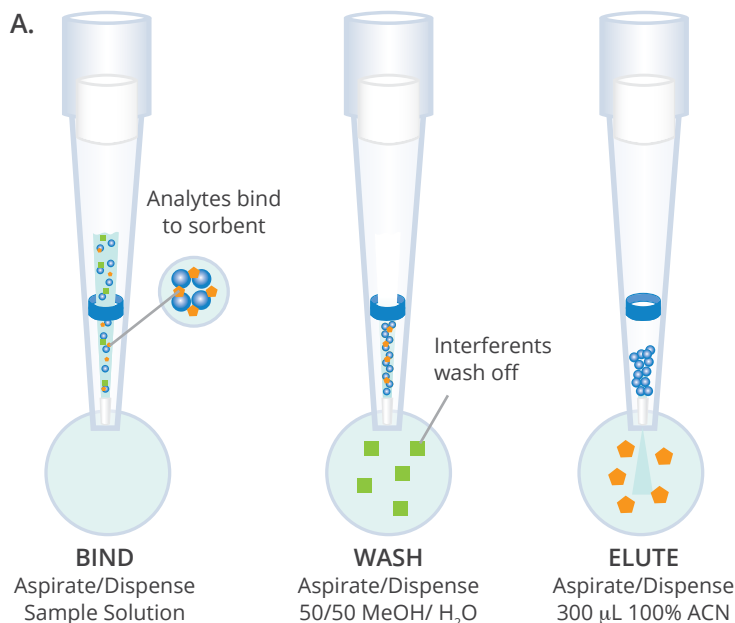


Figure 1
A. Schematic of Dispersive Pipette Extraction procedure.
B. Deck layout for cannabinoid extraction.

RESULTS AND DISCUSSION

The levels of each cannabinoid present in the nine forensic case samples are shown in Table 1. Positive CBG levels ranged from 0.05-0.22%, CBD at only 0.01%, CBDA 0.03-0.08%, CBDV 0.01-0.06%, CBGA 0.09-0.96%, CBN 0.12-1.6%, delta 9 THC 1.9-7.7%, CBC 0.02-0.20%, and THCA-A 3.5 to 16%.

CONCLUSIONS

The method described herein is a rapid, effective and automated sample preparation for the determination of cannabis potency. This method was successfully employed to determine the potency of 9 seized cannabis samples.

ACKNOWLEDGMENTS

This work would not have been possible without the help of the South Carolina Law Enforcement Division and the Beaufort County Crime Lab.

Table 1. The levels of monitored cannabinoids in the 9 seized cannabis samples.

Sample	THCV	CBG	CBD	CBDA	CBDV	CBGA	CBN	d9 THC	CBC	THCA
1	NF	0.05	NF	0.06	0.01	0.25	0.12	2.4	NF	12
2	NF	0.05	0.01	0.07	0.03	0.14	0.27	7.3	0.12	11
3	NF	0.20	0.01	0.05	0.03	0.68	0.13	4.0	0.20	7.6
4	NF	0.08	NF	0.06	0.02	0.27	0.19	4.1	0.13	10
5	NF	0.22	NF	0.03	0.02	0.36	0.74	1.9	0.05	3.5
6	NF	0.07	0.01	0.04	0.01	0.09	1.6	2.3	0.12	4.9
7	NF	0.14	NF	0.05	0.02	0.65	0.18	2.8	0.02	9.7
8	NF	0.16	0.01	0.08	0.06	0.35	0.20	7.7	0.11	13
9	NF	0.16	NF	0.08	0.03	0.96	0.15	3.4	0.08	16

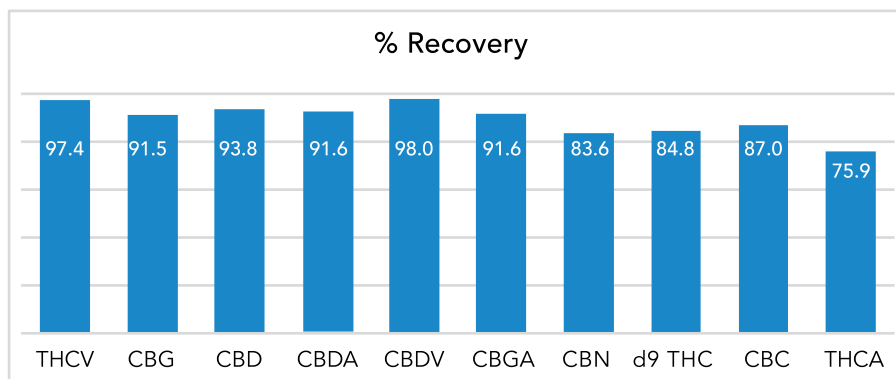


Figure 4. The percent recovery of each cannabinoid with Dispersive Pipette Extraction.

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