Highly Efficient Analytical Cannabinoid Determination using an Automated Dispersive Pipette Extraction Method

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Introduction

Cannabis testing facilities and forensic labs alike require rapid, robust analytical methods to keep pace with increasing consumer demand and use according to local laws. Current cannabis sample preparation workflows can be highly labor-intensive, which contributes to backlogs and risks of human error and variability. On the other hand, simple dilute-and-shoot methods that bypass sample preparation to save time incur a high risk of skewed results from interfering compounds contained in the sample as well as fouling or damage to sensitive analytical instrumentation.

Solid phase extraction (SPE), where analytes of interest in a given sample are bound to an affinity-based solid sorbent, washed to remove interferents, then eluted, is conducive to the chemical complexity of cannabis sample matrices, including plant matter, extracts, and edibles. SPE also produces clean extracts without problematic interferents, however, the active labor time and dedicated equipment required limits sample throughput.

A novel SPE technology from DPX Technologies, known as dispersive solid phase extraction (dSPE) enables hands-free sample processing in a high throughput manner when used in an automated workflow. The technology uses a freely moving resin sorbent contained between lower frit and upper porous barriers in a specialized pipette tip. As sample containing an analyte(s) of interest is aspirated into the tip, it interacts with and binds to the sorbent for which it has an affinity (Figure 1A), while a baffle system provides turbulence to ensure thorough mixing. The analyte-bound resin is washed to remove interferents (Figure 1B), and the purified analyte is then eluted into an assay plate (Figure 1C) for downstream analysis.

Here, we demonstrate an automated dSPE sample preparation workflow (see *Workflow* at end) on the Microlab NIMBUS96 liquid handling workstation. The Microlab NIMBUS96 uses air displacement pipetting for high precision and accuracy when aspirating and dispensing volumes from 1–1,000 µL, while Compressed O-Ring Expansion (CO-RE®) Technology creates an air-tight seal between dSPE tips or other disposable pipetting tips and the pipetting channel mandrels to maximize performance. Up to 96 samples may be processed in under 15 minutes to maximize throughput with little to no sample variability. The flexible and open deck layout (see *Deck Layout* at end) allows easy loading of a variety of sample vessels and peripheral device integration, while barcode reading eliminates the risk of sample mishandling and manual documentation errors. Cannabinoid levels from nine cannabis plant samples are determined using the automated sample preparation workflow followed by LC-MS/MS analysis. We show that the automated method is rapid and robust while enabling hands-free efficiencies.

Benefits-Based Highlights

- Increase sample throughput while decreasing sample extraction time.
- Reduce or eliminate sample preparation complexity and risks of human error and variability.

Materials and Methods

Nine dried cannabis samples obtained from the Beaufort County Sheriff's Office in South Carolina and the South Carolina Law Enforcement Division were ground and weighed, and 200 mg of each sample was combined in microtubes with 2 mL of acetonitrile (P/N A955, Fisher Chemical, Waltham, MA) using sonication for 30 minutes to extract the cannabinoid compounds. After incubation, sample solutions were transferred from the microtubes to a 96-well plate and loaded on the Microlab NIMBUS96 deck.

On the Microlab NIMBUS96, 200 µL of the sample solutions (containing 10 µL plant extract, 20 µL internal standard, 50 µL methanol, and 120 µL distilled water) were aspirated into 300 µL dSPE tips (P/N DPX170052, DPX Technologies, Columbia, SC) containing 5 mg of Reverse Phase sorbent to allow analyte binding. The tips were then automatically washed with 300 µL 50% Optima LC/MS grade methanol (P/N A456, Fisher Chemical, Waltham, MA) in water to remove the matrix and unwanted compounds. Analyte was then eluted by aspirating 300 µL acetonitrile and dispensing the fluid containing pure analyte into an assay plate. The automated process was also performed on standards in a mix of tetrahydrocannabivarin (THCV), cannabigerol (CBG), cannabidiol (CBD), cannabidivarin (CBDV), cannabigerolic acid (CBGA), cannabinol (CBN), delta 9 tetrahydrocannabinol (Δ9 THC), cannabichromene (CBC), tetrahydrocannabinolic acid (THCA-A) (P/N 220-91239-20, Shimadzu Scientific Instruments, Columbia, MD), and the internal standard, delta 9 tetrahydrocannabinol D3 (P/N T-003, Cerilliant, Round Rock, TX). Purified samples and standards were diluted before injecting 2 µL of each into the LC-MS/MS.

LC-MS/MS 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. 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 set as 5000 V electrospray voltage, 2 psi auxiliary gas pressure, 35 psi sheath gas pressure, 330ºC vaporizer temperature, and 400ºC capillary temperature.

Results and Discussion

Cannabinoid levels from the nine cannabis samples obtained from law enforcement and processed using the automated dSPE tip-based method are shown in Table 1. Positive CBG levels ranged from 0.05–0.22%, CBD 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 3.5–16%.

Recovery of cannabinoids were also analyzed using the standards mix. As seen in Figure 2, the percent recovery of cannabinoids from spiked samples/standards ranged from 75.9–98.0%.

Conclusion

Pipette tip-based dispersive solid phase extraction, using dSPE tips from DPX Technologies, is a time- and resource-saving alternative to traditional solid phase extraction techniques during cannabis analyses. Automating the workflow on the Hamilton Microlab NIMBUS96 liquid handling workstation increases hands-free sample throughput. Together as a single system, the automated dSPE method is rapid and effective when used to determine cannabis potency.

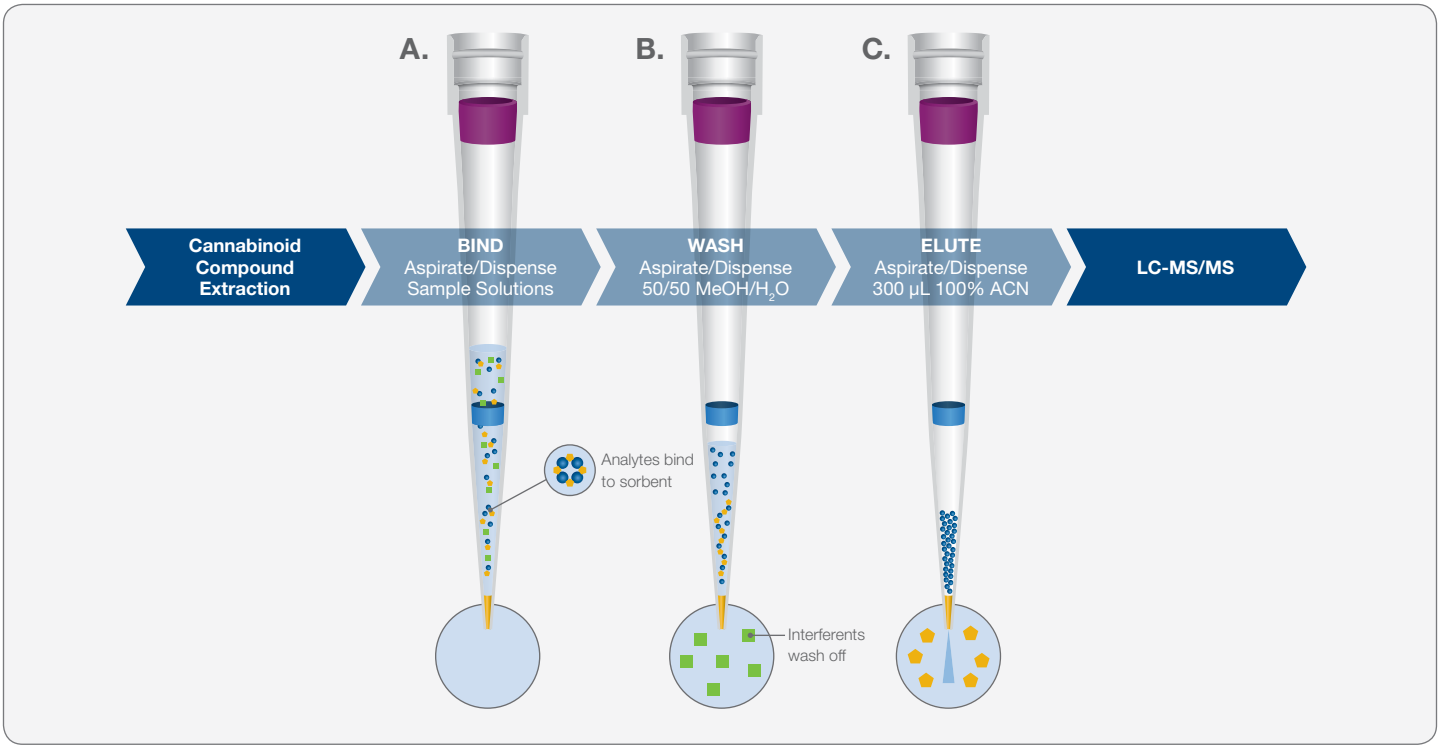


Figure 1. Schematic representation of the dispersive pipette extraction procedure.

(A) Sample is aspirated into automation-ready dSPE tips, where the analytes of interest are captured on the sorbent contained within. (B) Wash steps remove interfering compounds while the analyte remains bound to the sorbent. (C) Pure analyte is then eluted for downstream analysis.

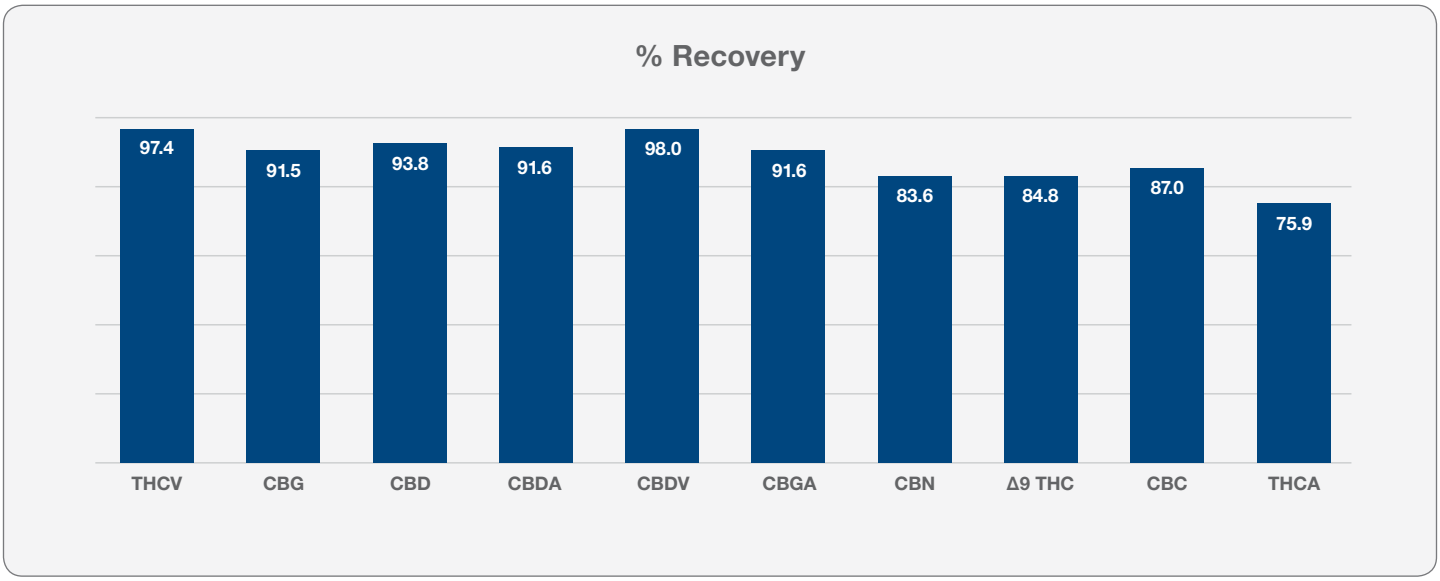
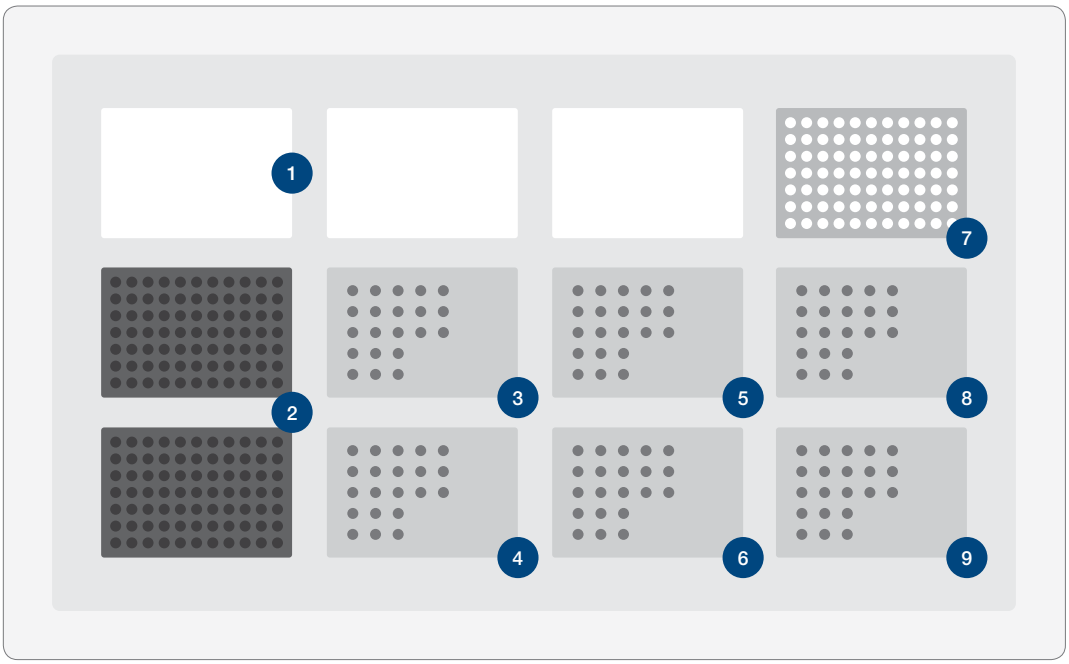


Figure 2. Cannabinoid percent recovery using the automated dispersive pipette extraction method.

Table 1: Monitored Cannabinoid Levels, Expressed in Percentage, of Nine Seized Cannabis Samples

Sample	THCV	CBG	CBD	CBDA	CBDV	CBGA	CBN	Δ9 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

Microlab NIMBUS96 Deck Layout with DPX dSPE Tips for Use in Cannabis-Based Extractions



1. ACN
2. dSPE Tips
3. 96-Well Sample Plate–Sample 1
4. 96-Well Sample Plate–Sample 2
5. 96-Well Sample Plate–Wash 1
6. 96-Well Sample Plate–Wash 2
7. 300 µL Tips
8. 96-Well Sample Plate–Elution 1
9. 96-Well Sample Plate–Elution 2

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