Automated High Throughput Potency Testing of Cannabis Samples using GC-MS in a Forensic Laboratory

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Abstract #B138

Introduction

Many traditional cannabis identification methods used in crime laboratories cannot accurately quantify total tetrahydrocannabinol (THC) in accordance with federal and state regulations; or they do so with increased time, labor, and risks of instrument damage. An automated INTip™ solid phase extraction method uses Dispersive Pipette XTRaction (DPX) technology and an automated liquid handler to enable fast, hands-free selective isolation of THC and precursors such as tetrahydrocannabinolic acid A (THCA-A). The isolated compounds are then precisely quantified using gas chromatography mass spectrometry (GC-MS) systems, which are already used in most crime laboratories.

The automated workflow eliminates user variability and bias while empowering analysts to reduce repetitive manual pipetting time and effort so that they may focus on high value tasks such as data analysis. It also reduces the frequency of instrument maintenance compared to dilute-and-shoot methods. Using this automated method, crime labs can legally and accurately distinguish between marijuana and industrial hemp.

Materials and Methods

Previously confirmed marijuana samples from adjudicated cases originally processed by the Richland County Sheriff's Department, hemp samples from a hemp farmer and a smoke shop, and internal standards were used. Tubes each containing 100 mg cannabis plant sample and 10 mL methanol are sonicated for 15 minutes then transferred to the Microlab NIMBUS96 personal pipetting workstation deck (Figure 1A) previously set up with all necessary reagents and consumables. The Microlab® NIMBUS96 is configured with a 96-channel multi-pipetting head, choice of two deck styles and air displacement pipetting volumes from 1 µL to 1 mL. We used Microlab NIMBUS96 with 24 channels (every other position of the 96 head), to pipette all reagents and facilitate the DPX workflow.

The Microlab NIMBUS96 picks up 1 mL XTR tips (P/N DPX170096, Figure 1B) and conditions them by aspirating and dispensing in a 50:50 MeOH:H_20 wash buffer, then aspirates and dispenses the samples several times to facilitate complete mixing. In the XTR tip, the sample contacts a freely moving styrene divinyl benzene sorbent contained between an upper porous barrier and a lower frit barrier. A disperser contained within the tip disrupts the liquid sample flow and encourages turbulent mixing when binding any THC and THCA-A analyte in the sample to the sorbent.

After binding, the Microlab NIMBUS96 washes the tips once to remove interferents, then uses 100% acetonitrile in triplicate aspirate/dispense cycles to elute analytes into GC vials. An Agilent 7890A GC coupled to a 5975C UL mass selective detector and 7693 automatic liquid sampler (Santa Clara, CA) is used with a 1 µL injection volume and a 20:1 split at 20 mL/min along with a J&W CP8944VF-5ms column, 18.8 psi pressure, and 1 mL/min flow rate. The GC oven temperature begins at 250 ˚C and ramps at 20 C˚/min to 310 ˚C, with a 5-minute total run time. The method used a 1.27-minute holdup time, 1.75-minute solvent delay, 0.35 electron multiplier (EM) voltage gain factor/resulting EM voltage of 1447. We set the MS source to maintain at 230 ˚C and the MS Quad to hold at 150 ˚C. We monitor tribenzylamine at a m/z of 91 with an expected retention time of 2.157, and Delta-9 THC at a m/z of 299 with an expected retention time of 2.634. We perform GC-MS analysis in full scan mode to utilize in-house library matching and maintain the ability to identify other potential adulterants in the plant material.

Automated INTip DPX Workflow

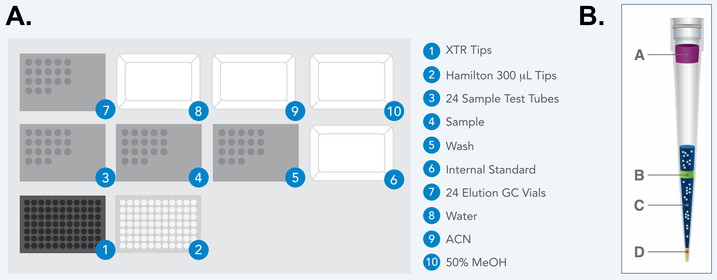

Figure 1. A. Hamilton Microlab NIMBUS96 deck layout used for the DPX method. B. XTR tip schematic, showing (A) upper barrier, (B) disperser, (C) sorbent, and (D) lower frit barrier.

Method Verification

Automated extraction allows simultaneous processing of up to twenty-four samples from tube post-sonication through extraction and elution into GC vials in less than eleven minutes with no human involvement. To verify this method to semi-quantitatively report THC percentage in plant material, we performed the following evaluations:

REPRODUCIBILITY, LINEARITY, SENSITIVITY

Inter/intra-day precision and accuracy were determined by extracting three standard concentrations (30, 100, and 500 µg/mL) on four different days with three injections of each standard on each day. Average precision values were below 3% CV and average accuracy values were above 98% (Table 1). We determined linearity and sensitivity by extracting and injecting 10, 30, 50, 100, 300, 500, and 1000 µg/mL twice. Linearity was determined by averaging the R^2 values, which gave 0.998. Further, sensitivity was determined by evaluating the signal-to-noise ratio at the lower calibrators from the linearity study as well as the strength of the match from the library. The limit of quantitation was determined to be 0.3%. However, the laboratory only reports above 1% THC or proceeds with further testing using a different method.

Concentration	30 µg/mL	100 µg/mL	500 µg/mL
Average Intra-Day Precision	1.7%	2.9%	2.4%
Average Interday Precision	0.5%	0.6%	0.7%
Accuracy	98.7%	99.1%	99.0%

Table 1. Precision and accuracy sample results at three concentrations.

RECOVERY

THC recovery using the DPX method was 93% at 30 µg/mL and 78% at 500 µg/mL. Similarly, THCA-A recovery was 100% at 30 µg/mL and 74% at 500 µg/mL. Data is shown in Figure 2.

THCA-A DECARBOXYLATION

We injected THCA-A controls and THC controls at the same concentration and calculated the percent difference of the area counts. Decarboxylation of THCA-A to THC in the GC injection port showed a 90% conversion rate, represented by the chromatogram in Figure 3.

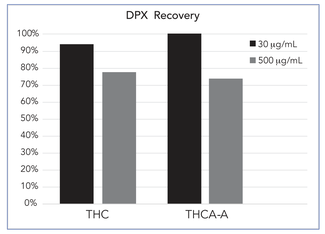

Figure 2. *THC and THCA-A recovery using XTR tips with Reverse Phase sorbent for extraction at 30 µg/mL and 500 µg/mL.*

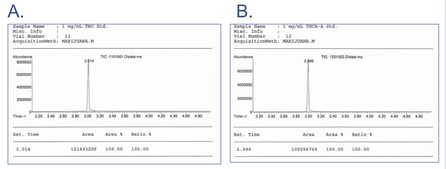

Figure 3. *Chromatograms representing the decarboxylation of THCA-A to THC in the GC injection port. A. 1 mg/mL THC. B. 1 mg/mL THCA.*

CROSS-CONTAMINATION

Using the Microlab NIMBUS96, a checkerboard pattern was made with 12 blank solvent samples and 12 non-probative positive THC extracts. All samples were processed using the automated DPX method with RP-XTR tips. Blank samples were determined to have undetectable THC levels.

STABILITY

Seventeen samples were extracted and injected over the course of several days. Approximately 20% THC loss was found after 2 days and approximately 37% loss after 4 days. As the stability study showed significant THC loss at 48 hours, probative casework will be analyzed within 48 hours or will have to be re-processed.

SAMPLING ROBUSTNESS

Sampling robustness was evaluated using three different nonprobative cases with two replicates at each sampling weight (20, 50, 100 mg). The average percent coefficient of variation (%CV) of the percent THC across all sampling weights were 12.2, 17.5, and 13.8% for the three cases tested.

Blind Study

Sixteen samples evaluated in a blind study (proficiency, hemp, and non-probative case samples) were all accurately identified as greater than or less than 1% THC, as shown in Table 2. Four samples were < 1% and 12 samples were > 1% THC. The hemp samples were the only samples that also had cannabidiol present. Chromatograms from two case samples are shown in Figure 4. Tribenzylamine (TBA) internal standard was consistent for both samples. CBD was present in high concentration in sample 1 (black), and not present in sample 2 (grey). THC was present in much higher concentration in sample 2 than sample 1. In all, sample 1 had a 7.4% THC content and sample 2 had a 1% THC content. While the CBD content of sample 2 suggests a hemp plant variety, the %THC content was still greater than the legal limit in South Carolina.

Sample	Expected	Concentration	Reported Value
Proficiency 1.1	Positive	3.83%	>1.0%
Proficiency 1.2	Negative	0.00%	<1.0%
Proficiency 1.3	Positive	6.55%	>1.0%
Proficiency 2.1	Positive	3.57%	>1.0%
Proficiency 2.2	Positive	5.30%	>1.0%
Proficiency 2.3	Negative	0.24%	<1.0%
Hemp 1	Negative	0.53%	<1.0%
Hemp 2	Negative	0.58%	<1.0%
Non-Probative 1	Positive	7.37%	>1%
Non-Probative 2	Positive	6.21%	>1%
Non-Probative 3	Positive	3.93%	>1%
Non-Probative 4	Positive	6.53%	>1%
Non-Probative 5	Positive	2.54%	>1%
Non-Probative 6	Positive	3.20%	>1%
Non-Probative 7	Positive	4.51%	>1%
Non-Probative 8	Positive	1.84%	>1%

Table 2. Sample results from a blind study of proficiency, hemp, and non-probative case samples.

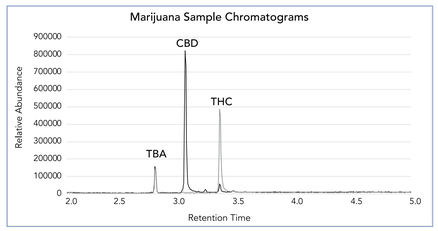

Figure 4. *Overlay of two different marijuana case samples, represented by black and grey lines.*

Conclusions

The automated DPX method using XTR tips was verified for several parameters showing its utility for discriminating marijuana from hemp-based material through detection of % total THC. The Microlab NIMBUS96 and DPX tips provided the desired hands-off procedure that allowed robust processing of plant extracts to clean and prepare samples for GC-MS analysis.

Acknowledgements

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