Application Note: 433

Identification of Cannabinoids in Baked Goods by UHPLC/MS

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Key Words

- Accela™ UHPLC
- Hypersil GOLD™ PFP
- MSQ Plus™
- Δ^9-tetrahydro-cannabinol (THC)
- Forensic Analysis

Goal

Positively identify trace amounts of cannabinoids in a complex food matrix quickly, with minimal sample preparation and no chemical derivatization.

Introduction

Marijuana is the most common illegal drug in the United States, and each year U.S. law enforcement agencies seize more than two million pounds of marijuana in various forms. Seized evidence submitted to forensic laboratories is screened for marijuana by microscopic inspection and simple chemical tests such as the Duquenois-Levine test. Presumptive positive results are confirmed by using gas chromatography-mass spectrometry (GC/MS) to positively identify cannabinoids including Δ^9-tetrahydrocannabinol (THC, the main psychoactive component), cannabinol (the main degradation product of THC) and cannabidiol. This traditional approach works fairly well for leaf marijuana, hashish, hash oil and residue collected from smoking paraphernalia.

GC/MS is less useful for confirming the presence of marijuana in complex food matrices such as baked goods. Simple sample preparation procedures using methanol or methylene chloride coextract many small molecules found in baked goods that can coelute with the target cannabinoids. Cholesterol, fatty acids, and caffeine can contaminate the gas chromatograph, forcing the analyst to clean the instrument and rerun all subsequent samples. More extensive sample preparation methods are time-consuming and often require greater amounts of the controlled substance than are present in the evidence.

An alternative method to positively identify marijuana cannabinoids in complex food matrices is to use ultra high performance liquid chromatography with mass spectrometry detection (UHPLC/MS). UHPLC/MS offers a threefold benefit compared to GC/MS; simpler sample preparation, no derivatization, and less instrument clean up time. This application note demonstrates how a working forensic laboratory uses UHPLC/MS to analyze baked goods for three cannabinoids of forensic importance. The cannabinoids are extracted, separated within 8 minutes on a Hypersil GOLD PFP 1.9 µm, 100 x 2.1 mm column and detected by a fast scanning single quadrupole mass spectrometer.

Experimental Conditions

1. Standard and Sample Preparation

The standard compounds THC, cannabidiol and cannabinol were purchased from Alltech (State College, PA, USA) and used as received. These compounds were mixed and diluted to about 10 ppm with methanol to prepare a stock standard solution.

Brownie and cookie samples were obtained from evidence archived after adjudication. Two (2) mL methanol was added to 25 mg of baked-good material. This mixture was vortexed for 30 seconds, allowed to settle for 2 min, and the supernatant was filtered through a cotton-plugged Pasteur pipette. The filtrate was centrifuged at 12,000 rpm for 90 seconds, and filtered again. The second filtrate was diluted 50 fold with methanol prior to analysis.

2. Chromatographic Conditions

Chromatographic analyses were performed using the Accela UHPLC system (Thermo Fisher Scientific, San Jose, CA). The chromatographic conditions were as follows:

Column:	Hypersil GOLD PFP (perfluorinated phenyl) 1.9 µm, 100 x 2.1 mm			
Flow Rate:	1 mL/min			
Mobile Phase:	A: Water with 0.06 % acetic acid B: Acetonitrile (ACN) with 0.06% acetic acid C: Methanol with 0.06% acetic acid			
Gradient:	T (min)	A%	B%	C%
	0.00	95.0	0.0	5.0
	1.00	60.0	32.5	7.5
	2.00	50.0	40.0	10.0
	5.00	45.0	45.0	10.0
	6.00	25.0	60.0	15.0
	6.50	5.0	0.0	95.0
	7.50	5.0	0.0	95.0
	7.51	95.0	0.0	5.0
	8.00	95.0	0.0	5.0
Column Temperature:	45 °C			
Injection:	2 µL partial loop injection, 25 µL loop size Syringe Speed: 8 µL/sec Flush Speed: 100 µL/sec Flush Volume: 400 µL Wash Volume: 100 µL Flush/Wash Source: Bottle with methanol			

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3. Mass Spectrometer Conditions

MS analysis was carried out on a MSQ Plus single quadrupole LC/MS detector with Xcalibur 2.05 (Thermo Fisher Scientific, San Jose, CA). The MS conditions were as follows:

Ionization:	Electrospray (ESI)
Polarity:	Positive
Probe Temperature:	500 °C
Cone Voltage:	90 V
Scan Mode:	Full scan with mass range of 50-500 *m/z*
ESI Voltage:	3.5 kV
Scan Time:	0.2 s

Results

The cannabinoid standards elute with good resolution at 4.1 min (cannabidiol), 5.1 min (THC) and 5.4 min (cannabinol). The cannabinoids were detected by using full scans (50-500 *m/z*) of the single quadrupole mass spectrometer, and the extracted ion chromatograms from *m/z* 310.5-311.5 + 314.5-315.5 are displayed in Figure 1A. Molecular ions of each compound (*m/z* 315 for cannabidiol and THC and *m/z* 311 for cannabinol) are observed (Figure 2A-C).

The brownie sample, which was taken from an adjudicated case and was known to contain THC, tested positive for THC (Figure 1B, 2D), demonstrating that the sample preparation required for this LC/MS method is simpler, faster and requires less sample than the GC/MS method employed for the original casework.

After ten years in the forensic laboratory's training vault, cannabinoids in the cookie sample had degraded significantly, but by increasing the sample injection from 2 µL to 10 µL, THC was detected with good signal-to-noise (Figure 1C, 2E).

Solvent blanks were analyzed after each sample run, with no apparent carryover from one run to the next (results not shown).

Conclusion

Cannabinoids in baked goods can be identified using UHPLC/MS with minimal sample preparation. The preparation time (10 min) and run time (8 min) make this a very efficient analytical method.

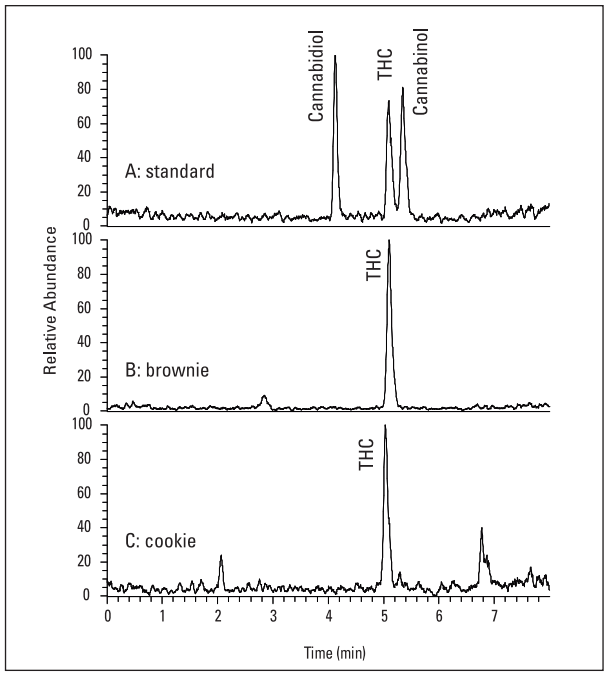

Figure 1: Extracted ion chromatograms (*m/z*, 310.5-311.5, 314.5-315.5) of cannabinoid standards (A) and extracts from brownie (B) and cookie (C) by UHPLC/MS

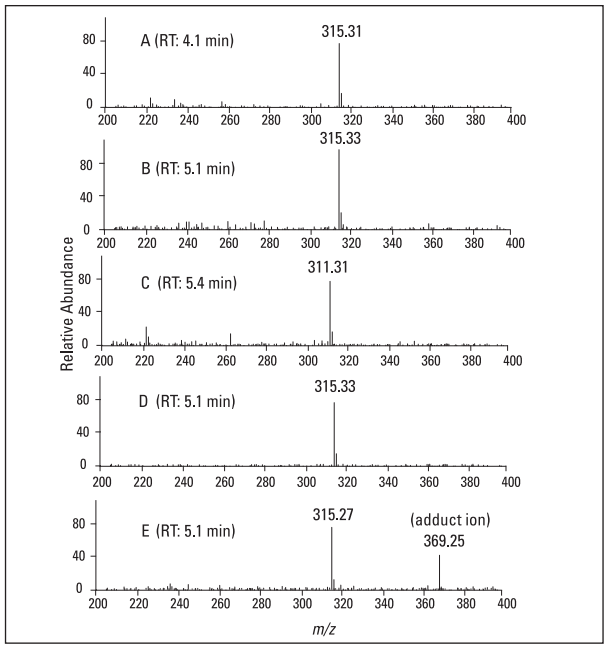

Figure 2: MS spectra of cannabinoid standards, cannabidiol (A), THC (B), cannabinol (C), eluted at 4.1 min, 5.1 min and 5.4 min respectively, and extracts from brownie (D) and cookie (E), eluted at 5.1 min

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