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Application Note

An Easy Approach for the Analysis of Active Cannabis Compounds in Edible Food Products: Gummy Bears and Brownies

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Introduction

Potency testing in marijuana-infused edibles can be a challenging analysis for many laboratories due to the complexity of the involved matrices. To ensure correct labeling and proper dosing, accurate testing of these commodities is extremely important. HPLC is the preferred technique for determination of potency, and in this application it was used in conjunction with a simple and fast extraction procedure for the determination of cannabinoids in two edible commodities; gummy bear candy and chocolate brownies. Analysis was performed using UV detection, thus all cannabinoids had to be chromatographically resolved. A biphenyl stationary phase was chosen for this purpose.

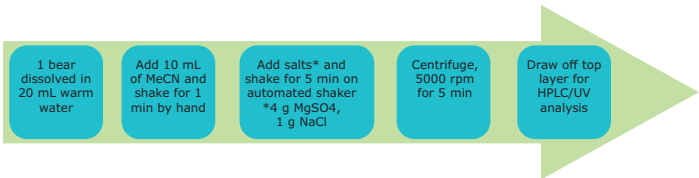
Experimental

Cannabinoid standards from Cerilliant were used. These were available as 1 mg/mL solutions in methanol or acetonitrile. The concentration of cannabinoids allowed the spiking of both gummy extract and brownies at about 40 ppm with all compounds.

Sample Preparation

- The procedure used to extract gummy bear candies is shown in **Figure 1**. Cannabinoid spikes were added to the candy/water solution prior to extraction.
- The procedure used to extract brownie (chocolate with frosting) is shown in **Figure 2.** Cannabinoid spikes were added directly to the brownie and allowed to equilibrate for 30 minutes prior to extraction.

Figure 1. Extraction procedure used for gummy bears



HPLC Analysis

A calibration curve was constructed in acetonitrile bracketing the expected concentration of 10 ug/mL in the final extracts. The following calibration points were included: 2 ug/mL, 5 ug/mL, 10 ug/mL , 20 ug/mL and 25 ug/mL. The HPLC run conditions are listed in **Table 1**.

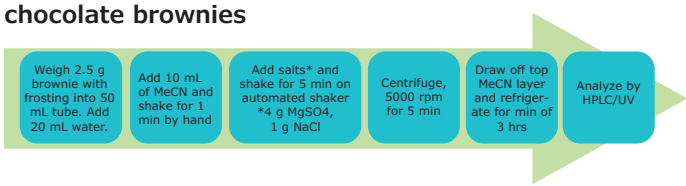
Table 1. HPLC Conditions

instrument:	Agilent 1200-with UV detector.
column:	Ascentis® Express Biphenyl, 10 cm x 2.1 mm, 2.7 µm
mobile phase:	(A) 0.1% TFA in water (B) 0.1% TFA in acetonitrile
gradient:	start at 47% B, to 50% B in 13 minutes, to 100% B in 0.1 min, 100% B for 3 minutes, to 47% B in 0.1 min, at 47% B for 2.5 minutes
flow rate:	0.70 mL/min
column temp.:	35 °C
detector:	UV, 220 nm and 280 nm
injection:	5 µL
pressure :	340 bar

Results

- The color from the yellow and green gummy bear candies remained in the water layer after extraction. Color from the red candy was only partially extracted into the acetonitrile. Thus there were no interferences with detection of the cannabinoids at 220 nm from these candies. The color from the orange candy extracted into the acetonitrile, producing an interfering peak that co-eluted with CBDVA, requiring this peak to be quantitated at 280 nm.
- No cleanup was required for the gummy bear extract. The brownie extracts contained co-extracted fats, which were precipitated out of the samples using refrigeration.

Figure 2. Extraction procedure used for frosted chocolate brownies



- Typical chromatograms obtained for the gummy bears and brownies are shown in **Figures 3 & 4**.
- Recoveries of the cannabinoids from gummy bear candies averaged > 90%, with RSDs < 10%. Recoveries from brownies averaged >85% for most compounds, with RSDs < 5% (**Figure 5**).
- Method ruggedness was evaluated with multiple injections of a brownie extract, preceded and followed by a 10 ug/mL standard. Peak retention times were stable, indicating the column was being sufficiently washed to prevent buildup of matrix components. Peak areas for the standard declined slightly (4%) from before and after the test.

Figure 3. HP LC/UV analysis of cannabinoids in gummy candy, 220 nm

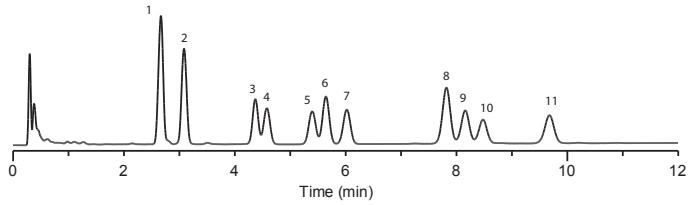


Figure 4. HPLC/UV analysis of cannabinoids in brownie, 220 nm

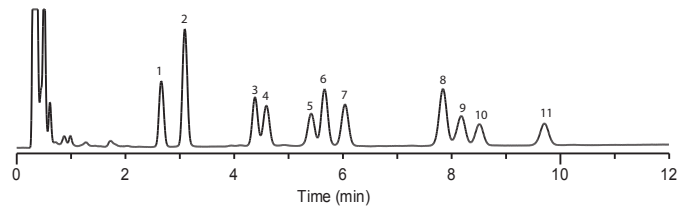
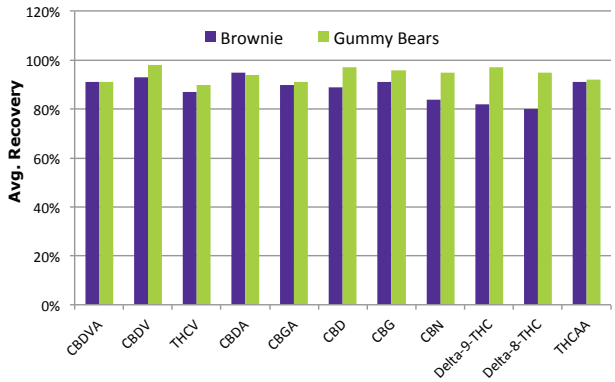


Figure 5. Avg. recoveries of cannabinoids from edibles; spiked at 40-45 ppm.

Peak List for Figures 3 and 4			
1	CBDVA	7	CBG
2	CBDV	8	CBN
3	THCV	9	Delta-9-THC
4	CBDA	10	Delta-8-THC
5	CBGA	11	THCAA
6	CBD		

Conclusions

A method was presented for analysis of cannabinoids in both brownies and gummy bears. The extraction procedure involved a salting out step into acetonitrile, and did not require intensive cleanup. Good recoveries and reproducibility were obtained for both matrices. Using UV detection required chromatographic separation of all eleven cannabinoids tested. This was achieved with a run time of 13 minutes using a biphenyl stationary phase and acetonitrile gradient.



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Cannabidiolic acid solution 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material	**45-C-144**
Cannabidivarin solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	**45-C-140**
Cannabidivarinic Acid (CBDVA) solution 1.0 mg/mL in acetonitrile, certified reference material, ampule of 1 mL	**45-C-152**
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Cannabigerolic acid solution 1.0 mg/mL in acetonitrile, ampule of 1 mL, certified reference material	**45-C-142**
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(−)-trans-Δ9-THC solution 1.0 mg/mL in methanol, ampule of 1 mL, certified reference material	**45-T-005**

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