A comparison of cannabinoid extraction methods using an automated extraction system

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Abstract

As the cannabis industry continues to expand because of its legalisation, highly accurate methods to assess the cannabinoid profile in cannabis products are increasingly needed. In this work, an automated extraction system, the EDGE by CEM, was used to extract the cannabinoids from cannabis plant. Several methods were tested to assess their performance as well as observe any extraction-dependent changes in the cannabinoid profile. The results were compared to a highly-validated hand method performed by Convergence Laboratories. The automated extraction system results produced acceptable recoveries for the cannabinoids measured.

Introduction

As cannabis becomes legalised in various parts of the US, the need for extraction methods that accurately measure the cannabinoid content in cannabis plant and products continues to grow. It is critical that these methods be developed because if the material is legal depends on the potency profile of cannabis and its products. According to the Agriculture Improvement Act of 2018, hemp is defined as 'plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis' [1]. Also, the cannabinoid profile is important to the intended purpose of the material, thus also affecting its price. The 5 most commonly measured cannabinoids of interest are D9-THC, THCA, cannabinol (CBN), cannabidiol (CBD), and cannabidiolic acid (CBDA), but there are many others.

Cannabis and its products, such as edibles, are challenging matrices to extract and analyse. Depending on how plant material is stored and dried, changes to the cannabinoid profile of the material can occur. Sun exposure and heat can cause cannabinoids to interchange. With heat or light exposure, THCA and CBDA can be decarboxylated to D9-THC and CBD, respectively, and with oxygen or light exposure, D9-THC can be oxidised into CBN [2]. Thus, it is important to consider the storage and extraction conditions of the sample when examining potency data. Also, in the past, cannabis has been extracted using manual methods, such as the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method [3]. This method was originally developed for pesticide extractions from food and requires multiple sample transfers and generates waste.

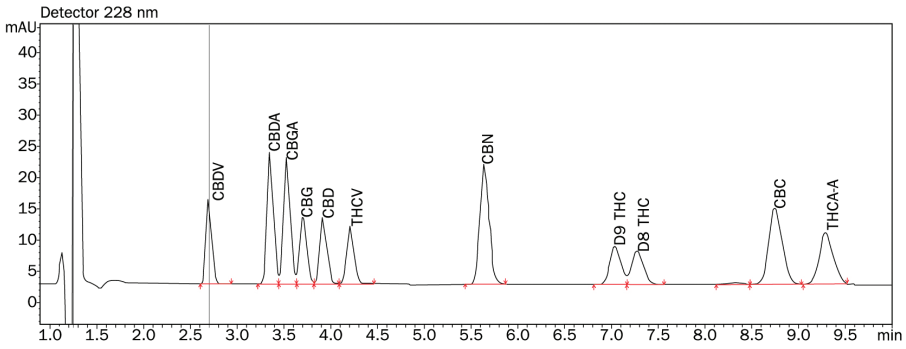

Figure 1. The separation of the cannabinoids measured at 228 nm.

In this work, in partnership with Convergence Laboratories and Restek, the EDGE, an automated pressurised solvent extraction system by CEM (Matthews, NC), was used to extract cannabinoids from dried cannabis plant using a panel of methods using different volumes of solvent and different temperatures. The system uses applied heat and pressure to extract analytes from samples contained in its Q-Cup, an open sample vessel. The results for the extractions were compared to a manual method developed by Convergence Laboratories. The automated system was found to extract cannabinoids from cannabis with high recoveries.

Experimental

Materials

HPLC-grade methanol, HPLC-grade water, HPLC-grade acetonitrile, formic acid, and isopropanol (IPA) were purchased from Sigma-Aldrich. The high-THC cannabis material extracted was obtained by Convergence Laboratories. Other materials were provided by CEM.

Automated Extraction Methods

Before the extractions, the Q-Cups were

rinsed with HPLC-grade methanol and dried with Kimwipes. The S1 stack of Q-Discs was inserted into each Q-Cup. The S1 stack of Q-Discs is a glass fiber filter surrounded by two cellulose filters, creating a filter with a filtering capacity of 0.35-0.5 µm. Samples of cannabis weighing 0.5 g were weighed directly into each Q-Cup. The samples were placed, along with polypropylene conical tubes, into a rack. Samples were then extracted using the methods indicated in Table 1. Each cycle was collected by the automated extraction system in separate tubes. Duplicate samples were extracted for each automated extraction system method, except for the 30°C and 90°C exhaustive methods, which were ran in triplicate.

Hand Method

A validated hand method developed by Convergence Laboratories was used to extract the cannabinoids from cannabis planet material. 5 g cannabis samples were placed into a conical tube. Then, 20 mL of HPLC-grade methanol were added to each sample. The samples were shaken for 20 minutes on a shaker table. Finally, the samples were placed in a centrifuge. After being spun down, an aliquot was removed to dilute with water prior to chromatographic analysis.

Analysis

All the extracts were brought to up to volume to exactly 20 mL and vortexed. The samples were diluted 10-fold in methanol and filtered using a 0.45 µm Thompson

Table 1. Methods used on the EDGE for extraction.

Method Name	30°C Exhaustive	30°C 10 mL 2X	30°C Rinse	45°C 10 mL 2X	45°C Rinse	90°C Exhaustive
Cycle 1 Solvent	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH
Cycle 1 Top Add (mL)	20	10	15	10	15	20
Cycle 1 Bottom Add (mL)	0	0	0	0	0	0
Cycle 1 Rinse (mL)	0	0	5	0	5	0
Cycle 1 Hold Time (min)	5	2.5	5	2.5	5	5
Cycle 1 Temperature (°C)	30	30	30	45	45	90
Cycle 2 Solvent	HPLC-Grade MeOH	HPLC-Grade MeOH	---	HPLC-Grade MeOH	---	HPLC-Grade MeOH
Cycle 2 Top Add (mL)	20	10	---	10	---	20
Cycle 2 Bottom Add (mL)	0	0	---	0	---	0
Cycle 2 Rinse (mL)	0	0	---	0	---	0
Cycle 2 Hold Time	3	2.5	---	2.5	---	3
Cycle 2 Temperature (°C)	30	30	---	45	---	90
Cycle 3 Solvent	HPLC-Grade MeOH	---	---	---	---	HPLC-Grade MeOH
Cycle 3 Top Add (mL)	20	---	---	---	---	20
Cycle 3 Bottom Add (mL)	0	---	---	---	---	0
Cycle 3 Rinse (mL)	0	---	---	---	---	0
Cycle 3 Hold Time	3	---	---	---	---	3
Cycle 3 Temperature (°C)	30	---	---	---	---	90
Wash 1 Solvent	HPLC-Grade MeOH	IPA	IPA	IPA	IPA	HPLC-Grade MeOH
Wash 1 Volume (mL)	20	20	20	20	20	20
Wash 1 Hold Time	3 min	5 s	5 s	5 s	5 s	3 min
Wash 1 Temperature (°C)	30	80	80	80	80	30
Wash 2 Solvent	---	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH	---
Wash 2 Volume (mL)	---	10	10	10	10	---
Wash 2 Hold Time	---	---	---	---	---	---
Wash 2 Temperature (°C)	---	---	---	---	---	---

Table 2. Total THC values for the methods of extraction.

Method Name	30°C Exhaustive	30°C 10 mL 2X	30°C Rinse	45°C 10 mL 2X	45°C Rinse	90°C Exhaustive	Hand method
D9-THC (mg/g sample)	8.52	10.12	10.33	9.93	10.39	23.93	10.13
THCA (mg/g sample)	249.79	227.25	226.17	218.06	223.12	227.78	224.62
Total THC (mg/g sample)	227.58	209.41	208.68	201.16	206.07	223.69	207.12

Table 3. Total THC recoveries for the methods of extraction used on the EDGE compared to the hand method.

Method Name	30°C Exhaustive	30°C 10 mL 2X	30°C Rinse	45°C 10 mL 2X	45°C Rinse	90°C Exhaustive
D9-THC	84.07%	99.90%	101.97%	97.98%	102.57%	236.23%
THCA	111.21%	101.17%	100.69%	97.08%	99.33%	101.41%
Total THC	109.88%	101.11%	100.75%	97.12%	99.49%	108.00%

Table 4. Cannabinoid values for the EDGE method '45°C Rinse' compared to the hand method.

Analyte	45°C Rinse (mg/g sample)	Hand method (mg/g sample)	Recovery of 45°C Rinse Method (%)
CBDV	0.06	0.07	85.71%
CBDA	0.74	0.83	89.16%
CBGA	8.74	8.52	102.52%
CBG	1.75	1.53	114.75%
CBD	0.00	0.00	0.00%
THCV	0.10	0.11	95.24%
CBN	0.26	ND	0.00%
D9-THC	10.39	10.13	102.62%
D8-THC	0.52	0.55	94.55%
CBC	0.48	0.46	104.40%
THC-A	223.12	224.62	99.33%

syringe filter. The samples were then analysed using HPLC/UV-Vis using validated retention times for each analyte. 5 µL of diluted sample was injected on a Restek Raptor ARC-18 column (150 x 4.66 mm, 2.7 µm) with a Restek Raptor ARC-18 guard column (5 x 4.6 mm, 2.7 µm). The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The flow rate was 1.25 mL/min, and the isocratic flow used for separation was 25% A. The method time was 10 minutes. The absorbance was monitored at 228 nm. A chromatogram showing the separation of the cannabinoids is shown in Figure 1. A 7-point calibration curve (0.5, 1, 5, 12.5, 25, 50, and 100 mg/L) was used to quantify each cannabinoid. Cycles were quantified separately, and to determine recovery, the cannabinoid content was added across all cycles.

Results and Discussion

For the preliminary analysis, only the total THC levels were determined, as these values are the greatest priority for these extractions. The reference values used for the levels of D9-THC and THCA were obtained using a validated hand method developed by Convergence Laboratories. These values were considered to be 100% recovery of the tested cannabinoids.

It has been found that at 90°C, cannabinoids can interconvert. Thus, the highest temperature used on the automated extraction system was 90°C. Utilising an exhaustive automated extraction system method with three cycles, the extraction produced total levels of THC of 223.69 mg/g of sample, as compared to the hand method, which produced 207.12 mg/g (Table 2), resulting in a recovery of 108.00% (Table 3). When examining the individual levels of D9-THC, the level of D9-THC for the automated extraction system extraction, 23.93 mg/g of sample, was greater than double the level found by the hand method, 10.13 mg/g of sample. Therefore, because of this shift in the cannabinoid profile, it is inappropriate to extract cannabinoids at 90°C, and cooler temperatures should be examined.

Three methods at 30°C, another exhaustive method with three cycles, a method with two cycles without rinsing, and a method containing one cycle with a rinse, were tested (all methods described in Table 1). Two methods at 45°C, a two cycle method without a rinse and a one cycle method with a rinse, were also assessed. For each method, the total THC content was determined, and the recoveries for each method is documented in Table 3. The recovery for the exhaustive method that used 30°C was lower compared to the other methods. When the one cycle method and the two cycle method were compared for each temperature, the one cycle method with a rinse had higher recoveries than the two cycle method. One cycle methods are favourable on the automated extraction system because they have the overall advantage of being shorter compared to a two cycle method with the same total extraction time. This is true because each cycle requires a purging time and a pressurising period before moving to a subsequent cycle. Also, the one cycle methods with a rinse at 30°C and 45°C produced nearly identical results. In a similar vein to comparing one cycle methods to two cycle methods with the same extraction times, when hot washes are used in a program, hotter methods are faster on the automated extraction system. This is true because between samples, the automated extraction system cools from the wash temperature to the extraction temperature. Thus, it takes a greater amount of time for the automated extraction system to reach 30°C compared to 45°C. Because of this, the 45°C one cycle method was selected for further study.

The method was used to determine the full cannabinoid profile, and the profile was compared to data obtained using the hand method validated by Convergence Laboratories. The final data are shown in Table 4. The automated extraction system extracted all compounds measured, except CBD and CBN, with recoveries in the range 86% to 115%. Both methods found no CBD present. The hand method did not extract CBN, while the automated extraction system extract did contain CBN. The automated extraction system extracted the cannabinoids with similar or better recoveries compared to the validated hand method used by Convergence Laboratories.

Conclusion

The cannabinoid profile of cannabis and its products is of interest for a variety of reasons, including the legal state of the material, the purpose of the material, and its market value. Storage and extraction conditions can easily alter the cannabinoid content. Thus, when determining the cannabinoid measurements, the extraction temperature should be considered. In this article, the automated extraction system extracted cannabinoids from cannabis material using a variety of methods at 30°C, 45°C, and 90°C to assess temperature-related changes in the cannabinoid profile and determine what method would be most comparable to a validated hand method. A method of one cycle at 45°C for the system produced cannabinoid recoveries comparable or better than the validated hand method in the shortest sample run time. Thus, the EDGE automated extraction system manufactured by CEM is an option for cannabis laboratories interested in automating their cannabinoid extractions.

Acknowledgements

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