A Comparison of Potency Extraction Methods on the EDGE

Abstract

With the continued growth of the cannabis industry, and the further progression of the legislation around it, accurate methods for the determination of the cannabinoids in cannabis plant material and products are increasingly needed. The cannabinoid content in cannabis and its products is of interest because it defines the legality of the material. In addition to the legal aspect, accurately measuring the cannabinoid content can also help guide consumers who are pursuing cannabis for recreational versus medicinal purposes. A major hindrance in the development of potency methods is the inconsistent approaches to how the material is stored and extracted, which can alter the cannabinoid profile in the material.

The EDGE®, an automated extraction system, was utilized to extract cannabinoids from cannabis material. Several methods for potency were compared to observe extraction-dependent changes in the cannabinoid profile of a high-THC cannabis plant, and the resulting extraction data for the cannabinoids were compared to a hand method validated by Convergence Laboratories. The EDGE's extractions produced excellent recoveries for the typical cannabinoids measured, meeting or exceeding the results of the validated manual method. Thus, the EDGE is an excellent option for laboratories seeking to automate their potency extraction workflow.

Introduction

With the increasing prevalence of the legalization of cannabis, including industrial hemp, the need for extraction methods that accurately measure the cannabinoids in cannabis and its products continues to grow. Potency methods are of interest because they can determine the legality of the material, as some regulations require industrial hemp to contain less than 0.3% of Δ9-tetrahydrocannabinol (D9-THC) and tetrahydrocannabinolic acid (THCA) combined on a dry weight basis. Furthermore, the determination of the cannabinoid levels gauge whether materials can be used medicinally or recreationally, affecting the price of the dried plant material and its products. The five major compounds of interest in cannabis are D9-THC, THCA, cannabinol (CBN), cannabidiol (CBD), and cannabidiolic acid (CBDA). However, there are many other cannabinoids of interest.

Cannabis and its various products have proven to be challenging matrices. A major difficulty in potency measurements is that the storage and drying conditions of the plant, and how the cannabis material is extracted, can change the cannabinoid profile. Heat and sun exposure can cause the interchange between cannabinoids. For example, if exposed to heat or light, THCA and CBDA can be decarboxylated to D9-THC and CBD, respectively, and D9-THC can be oxidized into CBN with oxygen and light exposure.[1] Thus, the extraction conditions, particularly temperature, should be considered with care when examining cannabinoid data. Furthermore, cannabinoid extractions have typically been done using the "hand method." This technique is often used to extract analytes from plants and produce.

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The hand method is an adaptation of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method, which utilizes buffered salts and dispersive clean-up materials to extract pesticides from food. It is a highly manual method that requires multiple sample transfers and generates waste. Thus, ideal extraction techniques for cannabis would work around these difficulties found with making potency measurements but would be less manual and tedious than QuEChERS extractions.

The EDGE is an automated pressurized solvent extraction system from CEM Corporation. The EDGE utilizes pressure and heat in a sequential system to quickly extract analytes of interest from a variety of matrices, including cannabis. The EDGE uses a two-piece open sample vessel called a Q-Cup® to hold samples for extraction. During an extraction, solvent is added to the sample in the Q-Cup, and the sample and solvent are pressurized and heated to the selected temperature for a programmed time period. When the extraction is finished, the extract passes through the bottom of the Q-Cup through the Q-Disc® filter and moves through the fluidic pathway of the system, including a cooling coil, to be dispensed into the collection vial. The final extraction volume is at room temperature, filtered, and ready for analysis. Although the EDGE does not require the use of sorbents or clean-up material, the Q-Cup technology offered by the EDGE allows for the incorporation of these materials, if needed.

In the context of cannabis extractions, the EDGE has several advantages over other extraction techniques. The EDGE extraction is temperature-controlled and quick, with most methods lasting less than 10 minutes. It generates less waste, does not require the use of clean-up salts but allows their incorporation if desired, and removes human error, making extractions more consistent. In conjunction with Convergence Laboratories and Restek, the EDGE was used to extract cannabis for its cannabinoid content. To address the issue of how extraction conditions can change the cannabinoid footprint, a panel of methods utilizing different temperatures and extraction volumes was screened. The results for the extractions were compared to a hand method validated by Convergence Laboratories. The EDGE was found to extract cannabinoids from cannabis with high recoveries. Thus, the EDGE is an excellent automated solution for the extraction of cannabinoids.

Materials and Methods

Potency Extractions

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.

Methods

The Q-Cups were manually cleaned with HPLC-grade methanol and dried using Kimwipes. The Q-Cups were assembled with the S1 stack of Q-Discs. The S1 stack of Q-Discs is a sandwich of three filters including a glass fiber filter surrounded by two cellulose filters. Its filtering capacity is 0.35-0.5 µm. A 0.5 g cannabis sample was weighed directly into the Q-Cup after its assembly. The EDGE rack was loaded with the Q-Cups and polypropylene conical collection tubes. The samples were extracted using the methods indicated in **Table 1** (page 4). Cycles were collected separately. Duplicate samples were run for each method, with the exception of the 30 °C and 90 °C exhaustive methods, which were run in triplicate.

For comparison, a validated hand method was used to extract the cannabinoids from the cannabis samples. A 0.5 g cannabis sample was weighed into a conical tube, 20 mL of HPLC-grade methanol was added, and the samples were shaken for 20 minutes on a shaker table. Subsequently, the samples were centrifuged, and an aliquot was pipetted off the top for further dilutions with water prior to chromatographic analysis.

Analysis

All EDGE extracts were brought to exactly 20 mL and vortexed. 100 µL of the sample was diluted in 900 µL of methanol, vortexed, and then filtered using a 0.45 µm Thompson syringe filter. The samples were subjected to UV-Vis analysis using validated retention times for each analyte. The cannabinoids were separated 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 injection volume was 5 µL. The mobile phases consisted of 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 for a total of 10 minutes. The wavelength monitored for detection was 228 nm. The separation of the cannabinoids at this wavelength is shown in **Figure 1** (page 4). To calculate the cannabinoid content, a 7-point calibration curve (0.5, 1, 5, 12.5, 25, 50, and 100 mg/L) was used for each cannabinoid, and the total cannabinoid content was related to the sample mass extracted. If the EDGE method was more than one cycle, the cannabinoid content was totaled across all cycles for a final cannabinoid content measurement.

Results

Initially, only the D9-THC and THCA levels were examined. The results for the hand method validated by Convergence Laboratories was regarded as the reference values for the levels of D9-THC, THCA, and total THC. The hand method's results were considered 100% recovery.

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The highest temperature assessed on the EDGE was 90 °C using an exhaustive method of three cycles. It is known that at 90 °C, conversion between cannabinoids can occur. The EDGE method at 90 °C yielded total THC levels of 223.69 mg/g of sample as compared to 207.12 mg of total THC/g of sample determined by the hand method (shown in **Table 2**, page 5). This results in 108.00% recovery of the total THC by the EDGE (shown in **Table 3**, page 5). However, the level of D9-THC alone for the EDGE was greater than double that of the hand method, 23.93 mg D9-THC/g of sample for the EDGE compared to 10.13 mg/g of sample for the hand method (shown in **Table 2**). This results in a recovery of 236.23% for this compound alone (shown in **Table 3**). Thus, extracting cannabis samples at 90 °C on the EDGE causes a shift from THCA to D9-THC, which is unfavorable. Because of this, two lower temperatures, 30 °C and 45 °C, were examined.

An exhaustive method of three cycles, a two-cycle method without a rinse cycle, and a one-cycle method with a rinse were all examined at 30 °C (all methods defined in **Table 1**). For 45 °C, a two-cycle method without a rinse cycle and a one-cycle method with a rinse were also tested (all methods defined in **Table 1**). Recoveries for the total THC for each of these methods are shown in **Table 3**. The exhaustive method at 30 °C yielded a lower recovery for D9-THC compared to the other methods. When comparing the one-cycle and two-cycle methods for each temperature, the one-cycle-rinse methods had higher recoveries for each temperature compared to the two-cycle methods. The one-cycle method also had the advantage of being shorter overall because, although the total extraction time for each method is 5 minutes, dividing the total time between two cycles increases the total method time, due to the purging time of the initial extract used by the EDGE. Furthermore, when comparing the one-cycle methods with a rinse for 30 °C and 45 °C, the recoveries were essentially the same. When considering which of these methods would be the most advantageous, the method time length was considered. Between samples, the EDGE cools down the temperature of the programmed method. Cooling to 30 °C takes a greater amount of time than cooling to 45 °C; thus, the 45 °C one-cycle method with a rinse was selected for further study.

The 45 °C method was used to obtain the full cannabinoid profile and was compared to the profile obtained by the validated hand method. The results are shown in **Table 4** (page 5).

The recoveries of all compounds measured, with the exception of CBD and CBN, as extracted by the EDGE, were in the range of 86% to 115% compared to the hand method. The level of CBD found with both methods was found to be negligible, and the EDGE was able to extract CBN, while the hand method was not. Thus, the EDGE was able to extract cannabinoids as efficiently, if not better, than the hand method employed by Convergence Laboratories.

Conclusion

The potency profile of cannabis material is important to its legality, purpose of use, and sale value. When determining the potency profile of cannabis, the extraction conditions can drastically alter the cannabinoid content. Thus, the extraction conditions of the material should be selected with great care. In this work, the EDGE, an automated extraction system, was used to extract cannabinoids from cannabis material.

A panel of methods at 30 °C, 45 °C, and 90 °C were employed to optimize the extraction of the cannabinoids without causing drastic changes to the cannabinoid fingerprint. A one-cycle method at 45 °C was found to yield the highest recoveries in the shortest amount of time without the requirement of additional clean-up, and the determined recoveries were as good as, if not better than, the hand method used by our partnering laboratory. The EDGE provides an automated system that can quickly extract cannabinoids in a temperature-controlled manner without the use of clean-up materials and is an ideal solution for cannabis laboratories seeking to automate their workflow.

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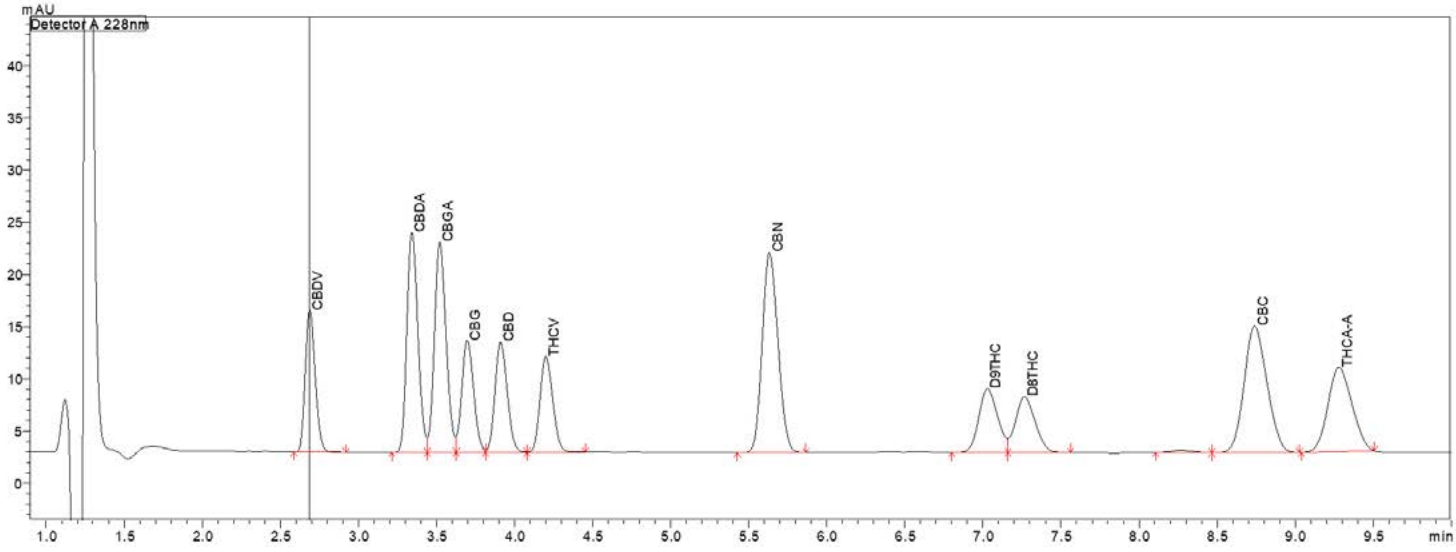

Figure 1. The Separation of the Cannabinoids Measured at 228 nm

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
	Top Add (mL)	20	10	15	10	15	20
	Bottom Add (mL)	0	0	0	0	0	0
	Rinse (mL)	0	0	5	0	5	0
	Temperature (°C)	30	30	30	45	45	90
	Hold Time (mm:ss)	05:00	02:30	05:00	02:30	05:00	05:00
Cycle 2	Solvent	HPLC-Grade MeOH	HPLC-Grade MeOH	---	HPLC-Grade MeOH	---	HPLC-Grade MeOH
	Top Add (mL)	20	10	---	10	---	20
	Bottom Add (mL)	0	0	---	0	---	0
	Rinse (mL)	0	0	---	0	---	0
	Temperature (°C)	30	30	---	45	---	90
	Hold Time (mm:ss)	03:00	02:30	--:--	02:30	--:--	03:00
Cycle 3	Solvent	HPLC-Grade MeOH	---	---	---	---	HPLC-Grade MeOH
	Top Add (mL)	20	---	---	---	---	20
	Bottom Add (mL)	0	---	---	---	---	0
	Rinse (mL)	0	---	---	---	---	0
	Temperature (°C)	30	---	---	---	---	90
	Hold Time (mm:ss)	03:00	--:--	--:--	--:--	--:--	03:00
Wash 1	Solvent	HPLC-Grade MeOH	IPA	IPA	IPA	IPA	HPLC-Grade MeOH
	Volume (mL)	20	20	20	20	20	20
	Temperature (°C)	30	80	80	80	80	30
	Hold Time (mm:ss)	03:00	00:05	00:05	00:05	00:05	03:00
Wash 2	Solvent	---	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH	HPLC-Grade MeOH	---
	Volume (mL)	---	10	10	10	10	---
	Temperature (°C)	---	---	---	---	---	---
	Hold Time (mm:ss)	--:--	--:--	--:--	--:--	--:--	--:--

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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	0.00	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

References

[1] Dussy, F. E.; Hamberg, C.; Luginbühl, M.; Schwerzmann, T.; Briellmann, T. A. Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. *Forensic Sci Int.* **2005**, *149*(*1*), 3-10.

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