Quantitative LC-UV Method for CBD in Topicals with Simplified Extraction of Lotions, Balms, and Creams

By Jamie York, PhD

Abstract
A universal extraction method has been developed for the LC-UV analysis of cannabidiol (CBD) in infused lotions, balms, and creams. The method was evaluated using fortified samples with percent recoveries ranging from 90.2–108%. Linearity was evaluated from 2–300 ppm and returned an R^2 value of 0.9997. The sample preparation methods developed here produce clean extracts and reliable results. The LC-UV analysis was conducted using a Raptor ARC-18 (150 mm x 4.6 mm ID x 2.7 µm) column with a Raptor ARC-18 EXP guard column cartridge. Isocratic mobile phase conditions were used for a total run time of five minutes per sample.

Introduction

Cannabidiol is a nonpsychoactive cannabinoid found in cannabis plants and cannabis-related products. Currently, CBD is not an FDA scheduled drug in the United States, and it is sold in a number of different topical products, including lotions, balms, and creams. Developing extraction and analytical methods for these matrices can be very difficult because they are composed of a diverse array of ingredients, which makes it challenging to develop universal procedures that work for all samples. In the LC-UV method for CBD in topicals developed here, one sample preparation and method of analysis was successfully used to quantify CBD in all three sample types. The product of this sample preparation method is a clean extract that will ensure instrument uptime and maximize column lifetime while providing fast and reliable quantitation of CBD in these complex matrix samples.

Experimental

A lotion blank and CBD-containing lotion, balm, or cream samples were weighed (0.5 grams) into a 50 mL centrifuge tube (cat.# 25846). The sample was distributed in as thin a layer as possible inside the centrifuge tube to avoid forming large flocculation particles upon the addition of solvent. Solvent was added to the centrifuge tube (5 mL of 50:50 methyl *tert*-butyl ether:methanol) and vortexed for ~90 seconds. To help facilitate the extraction of CBD, samples were put on a shaker table for 5 minutes. Centrifugation was then performed for 5 minutes at 3000 rpm at 4 °C. The supernatant was transferred (50 µL) to a vial, and the solvent was dried down with a stream of nitrogen. Each sample was reconstituted with 25:75 water:acetonitrile (1 mL) and vortexed. A 300 µL aliquot was transferred to a 0.2 µm PTFE Thompson filter vial (cat.# 25893) and filtered prior to LC-UV analysis.

All samples were analyzed in triplicate by LC-UV at a wavelength of 228 nm. The oven was set to 30 °C and equipped with a Raptor ARC-18 150 mm x 4.6 mm ID x 2.7 µm column (cat.# 9314A65) paired with a Raptor ARC-18 5 x 4.6 mm ID x 2.7 µm EXP guard column (cat.# 9314A0250). The flow rate was set to 1.5 mL/min, and 5 µL of sample was injected. Mobile phase A consisted of 5 mM ammonium formate with 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. The samples were analyzed under isocratic conditions at 75% B.

To evaluate this LC-UV method for CBD in topicals, recovery experiments were prepared in triplicate and performed by fortifying CBD (cat.# 34011) into samples to achieve a final concentration of 6 ppm. Calibration standards were prepared at 2, 3, 5, 10, 20, 50, 100, 200, and 300 ppm and analyzed in triplicate. Quality control standards were prepared at 8, 80, and 280 ppm and analyzed six times each.

A comparison of label claims to experimental values was made by calculating the native concentrations of CBD in lotion, balm, and cream containers based on the measured concentrations in each sample. This value was compared to the advertised amount of CBD by calculating percent difference (Equation 1). Percent recovery experiments were performed by fortifying a set of samples prior to sample preparation and another set of samples post-sample preparation at a concentration of 6 ppm. These values were then used to calculate percent recovery to determine if any analyte was lost during the sample preparation procedure (Equation 2).

$$\text{Equation 1:}\quad \textbf{\textit{Percent Difference}} = abs\,\frac{label\ value - experimental\ value}{label\ value} \times 100$$

$$\text{Equation 2:}\quad \textbf{\textit{Percent Recovery}} = \frac{Pre\text{-}Sample\ Prep\ Fortification}{Post\text{-}Sample\ Prep\ Fortification} \times 100$$

Results and Discussion

Initial solubility experiments were performed to determine which extraction solvent could best dissolve the majority of samples. Ultimately, the extraction solvent chosen was not able to fully dissolve most samples but was able to quantitatively extract CBD from the matrix. The LC-UV method for CBD in topicals developed here allows for multiple CBD-infused samples (lotions, balms, creams) to be analyzed by the same sample preparation procedure and method of analysis, saving time and allowing for higher throughput. The LOD for these experiments was determined to be 2 ppm. Experimentally determined CBD per bottle, precision (%RSD), percent difference, and percent recovery are displayed in Tables I and II, respectively. A typical calibration curve is presented in Figure 1 and representative chromatograms for each sample matrix are shown in Figure 2.

Table I: Advertised label value of CBD, experimentally calculated concentration of CBD, calculated precision, and percent difference (using Equation 1) compiled for each sample.

Sample	CBD Label Value per Bottle (mg)	Experimental CBD per Bottle (mg)	Precision (%RSD)	% Difference (Experimental vs. Label)
1. Hemp-infused lotion	348	343	0.84	1.42
2. CBD-infused lotion	600	617	1.43	2.90
3. CBD-infused cream	150	151	3.08	0.69
4. CBD-infused balm	100	88.6	2.94	11.4
5. Hemp-infused balm	82.6	82.3	2.96	0.39
6. CBD-infused lotion	250	269	2.02	7.58
7. CBD-infused cream	100	90.5	9.51	9.55
8. CBD-infused lotion	100	97.1	5.02	2.91
9. CBD- infused balm	70.0	55.8	4.46	20.3

Table II: Percent recovery values of fortified samples calculated by using Equation 2.

Sample	% Recovery
1. Hemp-infused lotion	106
2. CBD-infused lotion	108
3. CBD-infused cream	96.8
4. CBD-infused balm	90.2
5. Hemp-infused balm	99.2
6. CBD-infused lotion	102
7. CBD-infused cream	98.8
8. CBD-infused lotion	99.0
9. CBD-infused balm	90.7
10. Blank lotion (no CBD)	101

Figure 1: Calibration Curve

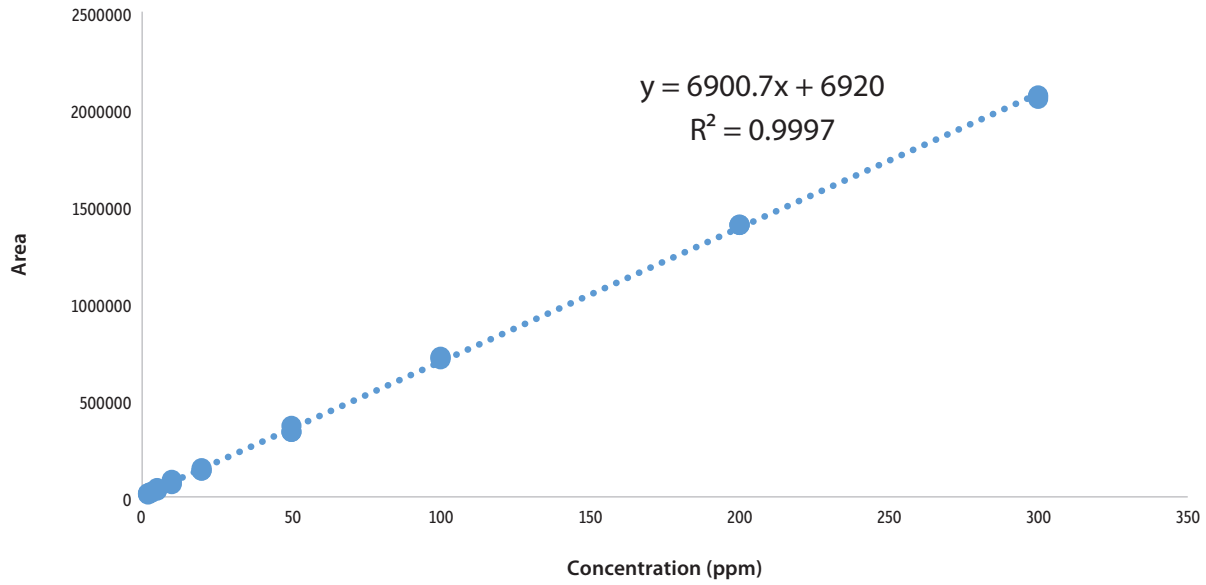
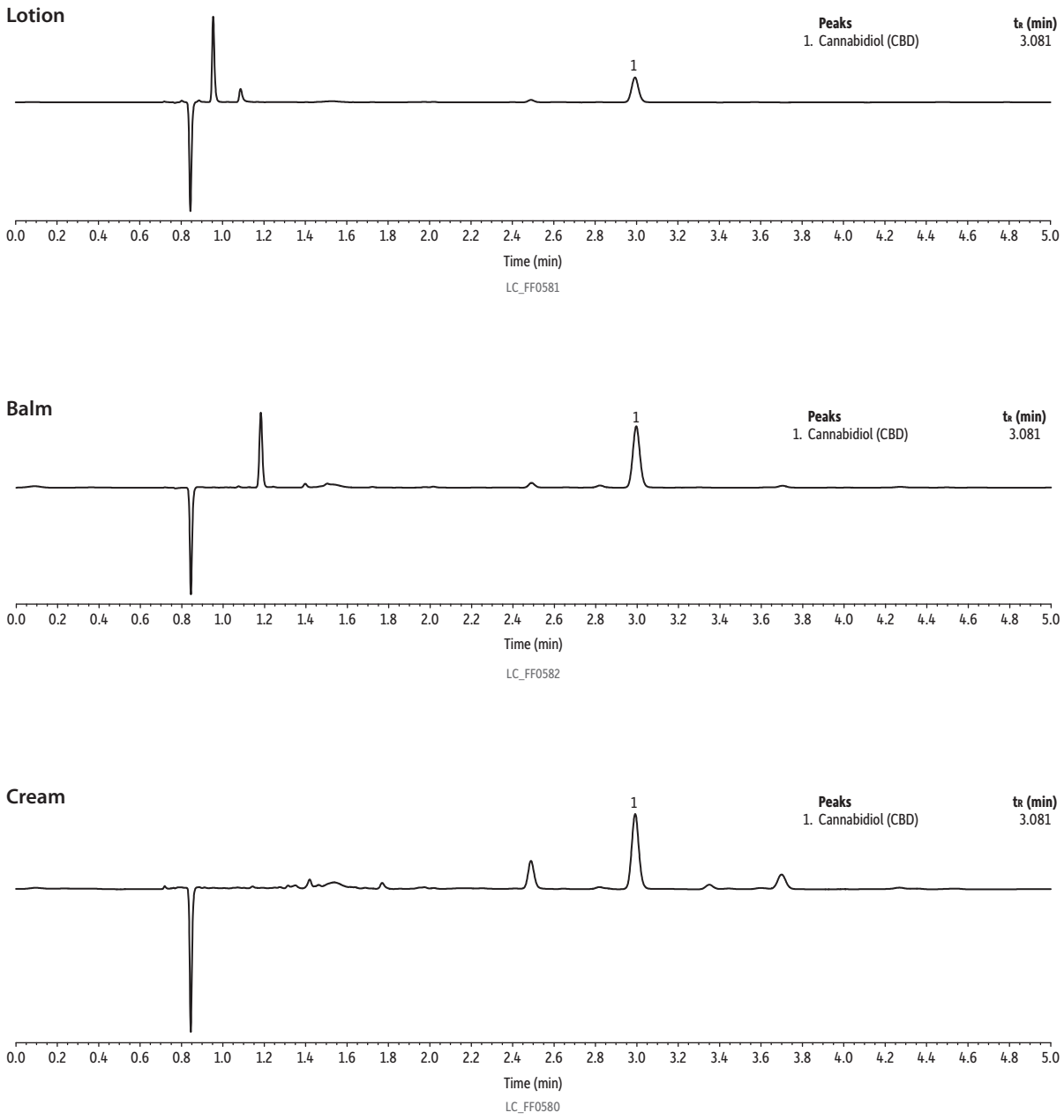


Figure 2: Example chromatograms for this LC-UV method for CBD in topicals.



Conclusion

The LC-UV method for CBD in topicals that is presented here allows for a fast and straightforward sample preparation and subsequent detection of CBD in lotions, balms, and creams. The total run time for the analysis is five minutes per sample. The applicability of this methodology for multiple sample types provides labs with an opportunity for higher sample throughput by applying a single method to a wide variety of CBD-infused lotions, balms, and creams.

Raptor ARC-18 LC Column (USP L1)

- Ideal for high-throughput LC-MS/MS applications with minimal sample preparation.
- Well-balanced retention profile for better detection and integration of large, multiclass analyte lists.
- Sterically protected to endure low-pH mobile phases without sacrificing retention or peak quality.
- Part of Restek's Raptor LC column line featuring 1.8, 2.7, and 5 µm SPP core-shell silica.

Designed and intended specifically for use on LC-MS/MS systems, the Raptor ARC-18 column offers a well-balanced retention profile without the drawbacks of using an ordinary C18 in the harsh, acidic mobile phases needed for mass spectrometry (MS). Even after extended use in these low-pH (≤ 2.0) conditions, the sterically protected ARC-18 offers consistent retention, peak shape, and response for charged bases, neutral acids, small polar compounds, and more. For the rapid analysis of large, multiclass assays by LC-MS/MS, the acid-resistant Raptor ARC-18 truly is ahead of the curve.

ID	Length	qty.	cat.#
2.7 µm Particles			
4.6 mm	150 mm	ea.	9314A65

Raptor EXP Guard Column Cartridge and Direct Connect Holder

- Free-Turn architecture lets you change cartridges by hand without breaking inlet/outlet fluid connections—no tools needed.
- Patented titanium hybrid ferrules can be installed repeatedly without compromising high-pressure seal.
- Auto-adjusting design provides ZDV (zero dead volume) connection to any 10-32 female port.
- Pair with EXP hand-tight fitting (cat.# 25937–25938) for tool-free installation.

To help protect your investment and further extend the life of our already-rugged LC columns, Restek offers the patent-pending guard column hardware developed by Optimize Technologies. A Restek LC guard cartridge in an EXP direct connect holder is the ultimate in column protection, especially when using dilute-and-shoot or other minimal sample preparation techniques.

Raptor EXP Guard Column Cartridge

- Guard column cartridges require EXP direct connect holder (cat.# 25808).

Description	Particle Size	Size	qty.	cat.#
Raptor ARC-18 EXP Guard Column Cartridge	2.7 µm	5 x 4.6 mm	3-pk.	9314A0250

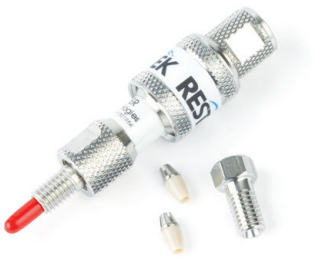
Maximum cartridge pressure: 1,034 bar/15,000 psi* (UHPLC), 600 bar/8,700 psi (2.7 µm); 400 bar/5,800 psi (5 µm)
* For maximum lifetime, recommended maximum pressure for UHPLC particles is 830 bar/12,000 psi.
Intellectual Property: optimizetech.com/patents

EXP Direct Connect Holder

- EXP direct connect holder requires separate guard column cartridges; available from Restek in 2.1, 3.0, and 4.6 mm.

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808

Intellectual Property: optimizetech.com/patents



25808

Maximum holder pressure: 20,000 psi (1,400 bar)

25846

Empty Centrifuge Tubes, Polypropylene

Description	qty.	cat.#
Empty 50 mL Centrifuge Tube, Polypropylene w/Blue Cap	50-pk.	25846

25893

Simply squeeze particulates and contaminants out of your sample!

Thomson SINGLE StEP Standard Filter Vials

- Recommended for samples containing less than 10% solid particulates.
- Easy-to-use vials offer fast sample filtration and require only a squeeze of your fingers.
- Minimize sample loss by eliminating multiple transfers.
- Color-coded caps allow easy identification of 0.2 µm or 0.45 µm membranes in PVDF, PTFE, PES, or nylon.
- Preslit PTFE/silicone caps help eliminate broken autosampler needles and cored septa.
- Rugged polypropylene vial houses insert with 450 µL loading capacity and low dead volume (120 µL).
- Fit most standard 12 x 32 mm autosamplers, including UHPLC instruments.

Description	Color	Porosity	qty.	cat.#
PTFE (polytetrafluoroethylene)				
Thomson SINGLE StEP Standard Filter Vial	green preslit cap	0.2 µm	100-pk.	25893

Patent No. 7,790,117

Cannabidiol (CBD) Standard

Excluded from U.S. DEA Controlled Substances Act (CSA) regulatory controls—no customer permits or licensing required to purchase within the U.S.

Description	CAS #	Conc. in Solvent	CRM?	DEA Status	Canadian Test Kit Registration	Min Shelf Life on Ship Date	Max Shelf Life on Ship Date	Shipping Conditions	Storage Temp.	Type	qty.	cat.#
Cannabidiol (CBD)	13956-29-1	1,000 µg/mL in P&T methanol, 1 mL/ampul	Yes	Not Controlled	T.K.# 71-047	6 months	24 months	On Ice	10 °C or colder	Cannabinoids	ea.	34011

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