Application Note

Cannabis & Hemp Testing

Agilent

Trusted Answers

Simple and Accurate Quantification of THC and CBD in Cannabis-Infused Chocolate Edibles using Agilent Captiva EMR—Lipid Removal and the Agilent 1260 Infinity II LC System

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Abstract

Accurate measurement of Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) in samples with high fat content like chocolate, brownies, and cookies is an important testing requirement to meet the evolving regulatory landscape for cannabis, including for product labeling, safety and for forensic purposes in jurisdictions where edibles are permitted under law. Chocolate is a complex matrix, rich in protein, fat, and cocoa, making it particularly challenging to analyze. This application note will demonstrate a simple and optimized procedure to grind chocolate, extract cannabinoids, and to quantify them by LC/UV. Emphasis is put on simple sample preparation and minimized cost per sample. Data show accuracy, precision, and linear response over multiple days for the target analytes. Using patented Agilent Captiva EMR—Lipid filtration technology, the cleanest samples were produced. The method provided superior robustness, enhanced sensitivity, and accuracy for potency testing in cannabis-infused milk chocolate, dark chocolate, and white chocolate.

Key advantages

- Fast sample preparation resulting in increased sample throughput
- Optimized sample cleanup with simple, yet effective, filtration step
- Accurate and precise THC and CBD quantitation
- Reduced HPLC maintenance and increased lab productivity

Introduction

There is an increased demand to test cannabinoids in edibles in order to meet established or evolving regulatory requirements. These regulatory requirements vary greatly depending on country and state. Each food type has specific challenges related to their unique physical consistency but also because of their different ingredient's impact on analytical instrumentation. There is therefore a need for more robust and reliable procedures to quantify cannabinoids such as Δ9-THC and CBD in foods such as chocolate, brownies, cookies, candies, and beverages.[1,2,3] Accuracy of such quantification procedures is paramount for legal considerations, for safety reasons, and to insure adequate labeling of commercially available products. Vandrey *et al.* (2015) found that only 17% of edible products were truthfully labeled, while 23% were under-labeled and 60% over-labeled with respect to Δ9-THC concentrations.[4]

Why is chocolate challenging?

Potency testing is routinely performed by HPLC with UV detectors, and in some instances by LC/MS or LC/MS/MS. Independent of the analytical platform, sample preparation is key to accurate determination of cannabinoids. If food matrix is not sufficiently removed from samples prior to injection, backpressure may increase on the HPLC system, which may result in extra maintenance and instrument downtime. Chromatographic columns may also get plugged more rapidly and, most importantly, the accuracy of THC and CBD quantitation may shift with time.

One of the most challenging edibles to analyze is chocolate because it is sticky, rich in fats (30 to 40%), sugars (60%), proteins (10%), and flavonoids.[5] This high level of matrix interferences drastically reduces accuracy and precision for potency testing using LC/UV, as documented in the literature.[6] More specifically, fats represent 30 to 40% of dry weight in chocolate and their presence has a direct impact on cannabinoid's solubility in solvents compatible with HPLC analysis. If fats are not adequately removed prior to injection, THC and CBD may bind to those lipids and precipitate. As a result, the inadequate and insufficient removal of lipids from chocolate samples causes inaccuracies in cannabinoid quantification.[6] Fats also increase maintenance of HPLC systems as they can easily clog reversed-phase LC columns, requiring longer LC gradients in order to extensively rinse the columns with organic solvents. That extra run time decreases lab productivity and requires extra mobile phase, increasing environmental and waste-handling costs.

Another contributor to poor quantification of cannabinoids in chocolate is the presence of cocoa that is rich in flavonoids like catechin, epicatechin, and gallocatechin. These flavonoids can represent up to 8% of dry weight in cocoa powder and could interact through noncovalent interactions with planar aromatic cannabinoids like cannabinol (CBN) and so reduce UV signal on the HPLC.[6] It is therefore critical to remove interferences in order to have accurate potency testing in cannabis-infused chocolate.

Sample processing and homogenization

Different techniques can be used to process chocolate prior to cannabinoid extraction in solvent. Some of those techniques are labor-intensive and require various lab equipment. Food samples can be ground mechanically using a commercial blender or they can be crushed using a mortar and pestle. Either approach is not ideal for chocolate, as it is sticky in nature and can partially melt in the process. Alternatively, chocolate can be melted, or it can be frozen prior to solvent extraction. When frozen, chocolate is easier to grind, and low temperature hinders any sample degradation. When sample processing is not optimized, lengthy sonication is often required to ensure full extraction of cannabinoids from the chocolate. For these techniques, sample throughput can be challenging, as blenders, mortars, and sonication devices can usually process a small number of samples per hour and need to be cleaned for each sample.

Extraction techniques to recover cannabinoids from edibles

Once chocolate is finely ground, different extraction techniques can be used to capture THC and CBD while removing as many interferences as possible. Quick, easy, cheap, effective, rugged, and safe (QuEChERS) is a technique widely used to process food samples.[7] It is a two-step procedure that first involves an extraction step based on water partitioning using salts, and a second step based on a dispersive SPE (dSPE) approach to remove more matrix components. However, for chocolate and other fat-rich edibles such as brownies and cookies, QuEChERS is not a good choice, as both cannabinoids and fats are hydrophobic; therefore, no fat is removed after the water-partitioning step. Unfortunately, water will also capture interferences from cocoa compared to an aprotic solvent like acetonitrile, as demonstrated in this application note. Moreover, QuEChERS removes polar interferences like sugars and salts, but those are typically not retained in reversed-phase chromatography, and will not interfere with cannabinoids, which are well retained on a C18 column. Additionally, no dispersive used in the second QuEChERS step has enough specificity and capacity to completely capture the amount of lipids present in chocolate. QuEChERS dispersives kits can have 150 to 400 mg of C18 to potentially capture lipids, which is largely insufficient

for the fattest edibles. Dispersives can also unfortunately capture cannabinoids. For these reasons, QuEChERS is not a good choice for potency testing in lipid-rich edibles like chocolate, brownies, and cookies, even if this technique is advisable for other matrices like hard candies and gummies.

As an alternative to QuEChERS, chocolate samples can be extracted in solvents and put in a freezer to precipitate lipids. This temperature-induced lipid precipitation, also called winterization, is time- and temperature-dependent. Typically, samples will need to be put at –20 °C for a few hours for winterization to show some efficiency. However, in some instances, the fat content will require extremely low temperatures (–80 °C) and overnight timeframes for winterization to work. Winterization also reduces solubility of cannabinoids, potentially resulting in significant losses in recoveries, sensitivity, and accuracy when testing potency.

Agilent Enhanced Matrix Removal-Lipid (EMR—Lipid) is a unique sorbent that selectively removes lipids in complex matrices and challenging high-fat samples using both size exclusion and chemical bonding, while leaving target analytes in solution.[8, 9] In the case of cannabis edibles, this means EMR will remove a maximum amount of lipids without capturing cannabinoids. The Captiva EMR—Lipid filtration comprises EMR sorbent packed between two frits in a cartridge format. The addition of 20% water to chocolate extracted in acetonitrile optimally precipitates proteins and prepares samples for easy filtration on Captiva EMR—Lipid cartridges. In one easy step, both proteins and lipids will be captured without losing any cannabinoids. For the first time, this specificity of the EMR technology towards lipids enables removal of almost 100% of fats without losing THC and CBD in the process.[10]

To summarize, potency testing in chocolate is challenging for laboratories because it is laborious to process, especially when you have many samples. The specific challenge with chocolate can be attributed to the presence of fat and cocoa matrix. Fat directly interferes with detection of cannabinoids in addition to causing maintenance issues with analytical columns and systems. Flavonoids from cocoa are also a documented source of interference. QuEChERS extraction is not optimal for cannabinoids in chocolate because they carry significantly dirtier matrix that interferes with detection and requires more instrument maintenance. Solvent extraction followed by winterization is also not optimal for potency testing in fatty edibles because it is time consuming and provides lower UV signal for cannabinoids, negatively impacting accuracy. The improved procedure developed here simplifies sample preparation to process large numbers of chocolate samples at the lowest cost possible and, more importantly, with the highest accuracy and precision currently possible.

Experimental

Materials and reagents

- 50 mL polypropylene (PP) centrifuge tubes (VWR part number 89039-660)
- 15 mL polypropylene (PP) centrifuge tubes (VWR part number 89039-668)
- Agilent ceramic homogenizers (part number 5982-9313)
- Agilent InfinityLab ultrapure LC/MS acetonitrile (part number 5191-4496)
- Agilent InfinityLab ultrapure LC/MS water (part number 5191-4498)
- Agilent InfinityLab ultrapure LC/MS methanol (part number 5191-4497)
- Agilent Captiva EMR—Lipid 3 mL (part number 5190-1003)
- Agilent vials with screw caps (part number 5182-0553)
- Agilent formic acid (part number G2453-85060)
- THC certified reference material, 1.0 mg/mL in methanol
- CBD certified reference material, 1.0 mg/mL in methanol

Since the development of this application, Agilent has introduced cannabinoid certified reference materials. These can be used in place of the standards used here.

- Cannabinoid Mix A - CBD, CBN, and Δ^9-THC at 1.0 mg/mL each (part number 5190-9430)
- Cannabinoid Mix B - THCA, CBDA, and CBG at 1.0 mg/mL each (part number 5190-9429)
- Cannabinoid Mix C - CBDV, CBGA, and CBC at 1.0 mg/mL each (part number 5190-9428)
- Cannabinoid Mix D - THCV and Δ^8-THC at 1.0 mg/mL each (part number 5190-9427)

Lab equipment

- Agilent positive pressure manifold (PPM) 48 processor (part number 5191-4101) (optional, for increased throughput)
- Agilent 3 mL cartridge rack (part number 5191-4103)
- Waste rack for Agilent PPM-48 (part number 5191-4112)
- Automated mechanical homogenizer (Geno/Grinder 1600 MiniG from SPEX SamplePrep or the equivalent)
- Centrifuge 5804 R from Eppendorf with 50 mL tube adaptor
- Scissors
- Analytical balance
- Mini vortexer

Instrument conditions

HPLC conditions

In this work, we implemented the Agilent standard potency analytical method described below.[11]

Parameter	Value
LC Modules	Agilent 1260 Infinity II Prime pump (G7104C)
	Agilent 1260 Infinity II vialsampler (G7129C) with tray cooling option
	Agilent InfinityLab integrated column compartment (G7130A)
	Agilent 1260 Infinity II Diode Array Detector WR
MS	Agilent 6545 LC/Q-TOF
Run Time	9.5 min
Post-time	1.5 min
Analytical Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Guard Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 5 mm, 2.7 µm
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.05% formic acid in methanol
Injection Volume	5 µL
Multisampler Temperature	20 °C
Column Temperature	50 °C
Detection	UV at 230 nm for all quantitative results
Flow	1 mL/min
Gradient	**Time (min) / %A / %B** 0 / 40 / 60 1 / 40 / 60 7 / 23 / 77 8.2 / 5 / 95
Needle Wash	3 sec in flush port with 25:25:50 isopropanol:acetonitrile:methantol

MS conditions

Parameter	Value
MS	Agilent 6545 LC/Q-TOF*
Acquisition Mode	TOF scan, 40 spectra/sec, *m/z* range 100 to 1,700
Source	Agilent Jet Stream ESI
Drying Gas Flow	12 L/min
Sheath Gas Temperature	350 °C
Nebulizer Pressure	40 psi
Drying Gas Temperature	350 °C
Sheath Gas Flow	11 L/min
Polarity	Positive
Capillary Voltage	3,500 V
Nozzle Voltage	1,000 V
Fragmentor	135 V

* Time-of-flight (TOF) mass spectrometry was used as a qualitative tool in this study to evaluate the matrix charge resulting from different sample preparation procedures.

Sample processing and cannabinoids extraction

Cannabis-infused chocolate samples

1. Using an analytical balance, weigh the piece of infused chocolate associated with the label claim (bar, square, bite, etc.), and record the value.
2. Reduce the chocolate piece to fine bits or flakes with scissors (Figure 1); transfer 1 ±0.005 g in a 50 mL PP centrifuge tube.
3. Add two ceramic homogenizers in the tube to ensure complete and faster homogenization; cap.
4. Put tube in –20 °C freezer for 20 minutes.
5. Add 10 mL of cold acetonitrile containing 2% formic acid (previously put in –20 °C freezer).
6. Place the tube on an automated mechanical homogenizer for aggressive vertical shaking (1,500 rpm) for 5 minutes. Chocolate should be completely dissolved and homogeneous.
7. Centrifuge the tube at 3,600 to 5,000 rpm for 5 minutes at room temperature (20 °C).
8. Transfer 2 mL of the supernatant into a 15 mL PP centrifuge tube.
9. Add 500 µL of nanopure (Type 1) water, cap, and briefly vortex.
10. Place 3 mL cartridge rack (part number 5191-4101) on top of waste rack (part number 5191-4112).
11. Place a 3 mL Captiva EMR—Lipid tube in the cartridge rack, and a clean 15 mL PP centrifuge tube directly under the EMR tube in the waste rack.
12. Pour all contents (2.5 mL) from Step 8 into the 3 mL Captiva EMR—Lipid tube. This will flow by gravity.
13. After complete elution of the initial 2.5 mL portion, pour an additional 1.5 mL portion of (4:1 acetonitrile:nanopure water) solution to the Captiva EMR—Lipid tube, also flowing by gravity.
14. Do not forget to vortex collection tube, transfer to vial, and cap.

Note: Alternatively to gravity flow and to make the EMR cleanup 4x faster, place the 2 racks above with the Captiva EMR—Lipid tubes in an Agilent PPM-48 processor (part number 5191-4101) at a pressure of 1 psi, and control flow rate to a maximum of 1 drop every 3 to 5 seconds (see comment in Lab equipment section).

Note: Nonhomogenous baked goods and chocolates containing nuts and fruits need to be fully homogenized before extraction in order to get representative sampling and to obtain a totally homogeneous acetonitrile extract. To do so, the entirety of these samples needs to be processed in a blender/cryomill or an automated mechanical homogenizer prior to weighing at Step 2 in the procedure above.

Noninfused chocolate samples for matrix-matched calibrators

Following the procedure described in the previous section, make sure to prepare enough chocolate matrix (noninfused milk, dark or white), required for matrix-matched calibrators, by loading a minimum of two 15 mL PP centrifuge tubes with 2 mL aliquots of supernatant at Step 8, then treat each tube as recommended in Step 9 and beyond. In the end, the two eluates must be combined and vortexed in a single 15 mL PP centrifuge tube. Table 1 shows the serial dilutions used to prepare the calibrators.

Table 1. Preparation of matrix-matched calibrators using a serial dilution approach.

Calibrator Level	Concentration (µg/mL)	Serial Dilution Prepared With
6	100	100 µL of CBD standard + 100 µL of THC standard + 800 µL of chocolate matrix
5	50	500 µL of calibrator 6 + 500 µL of chocolate matrix
4	10	200 µL of calibrator 5 + 800 µL of chocolate matrix
3	5	500 µL of calibrator 4 + 500 µL of chocolate matrix
2	1	200 µL of calibrator 3 + 800 µL of chocolate matrix
1	0.5	500 µL of calibrator 2 + 500 µL of chocolate matrix
0	0	1,000 µL of chocolate matrix

* Following this preparation, the final volume of calibrators 2, 4, and 6 will be 500 µL. Make sure to adjust the settings of the autosampler to accommodate this volume.

Results and discussion

Several conditions were tested in order to have optimal sample processing and extraction conditions. Parameters of success included cleanliness determined by gravimetric analysis and LC/Q-TOF. Analyte recovery was determined by LC/UV. Stability of the analytes in solution was tested over time in addition to accuracy and precision on a range of concentrations between 0.5 to 100 µg/mL, equivalent to 10 to 2,000 µg CBD and THC per g/chocolate.

Sample processing

For sample processing, blenders and cryomills were not tested as they only can process one sample at the time and require to be cleaned between samples. Melting chocolate was also not used in order to avoid warm temperatures that could affect analyte stability. It was therefore simpler to finely chop chocolate with scissors (Figure 1) and to put those samples in disposable polypropylene tubes with two ceramic homogenizers for complete homogenization. Different shaking times were tested in order to optimize cannabinoid extraction. Note that it was easier to handle chocolate samples when they were just out of storage at 4 °C. It was also observed that freezing weighed samples at –20 °C in PP tubes with ceramic homogenizers for 20 minutes was beneficial to avoid chocolate sticking to the tube wall during mechanical vertical shaking.

Figure 1. Milk chocolate chopped with scissors prior to weighing.

Extraction solvent

Several extraction solvents were tested: acetonitrile, methanol, 80/20 acetonitrile/ethyl acetate, and 40/60 ethanol/water. Although the extraction of CBD and THC has been shown to be slightly more efficient using methanol compared to acetonitrile (https://blog.restek.com/medical-marijuana-solvent-extraction-efficiency-%e2%80%93-potency-determinations-with-gc-fid/), methanol was found to produce "dirtier" chocolate extracts (with more undesired matrix content) when analyzed by LC/Q-TOF in MS scan mode, which is considered to be a nonspecific yet very sensitive way to evaluate sample cleanliness. In short, a Q-TOF total ion chromatogram (TIC) obtained from a "dirty" sample will have a more intense signal (more peaks, higher noise level) compared to a "clean" sample. As observed in Figure 2, the red trace (methanol) shows higher background noise and a few additional peaks compared to the black trace (acetonitrile). Over time, injecting dirtier samples will result in additional system maintenance, increasing costs, and reduced profitability of testing laboratories. A 2% formic acid was added to acetonitrile in order to improve protein precipitation and reduce the binding of THC and CBD to chocolate protein. The stability of CBD and THC in these gentle acidic conditions was confirmed for over a week.

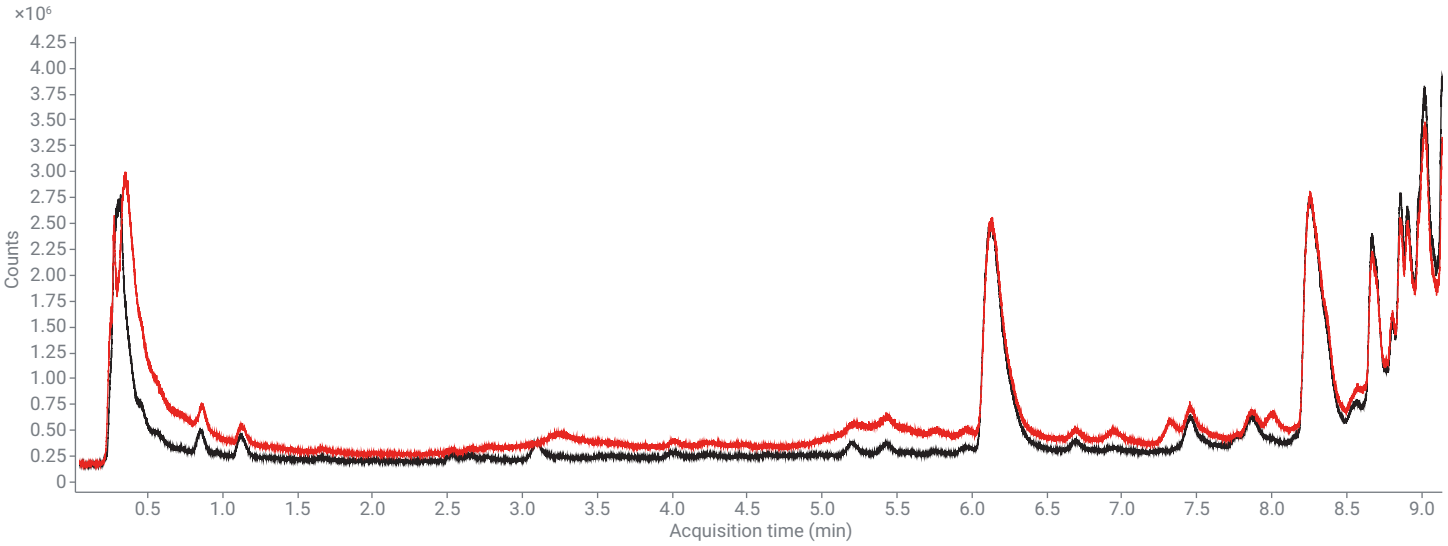

Figure 2. LC/Q-TOF TIC comparison of methanol (red trace) and acetonitrile (black trace) white chocolate extracts fortified with CBD and THC.

Extract cleanup

An increase in system backpressure, likely caused by lipid accumulation and ultimately clogging of the reversed-phase column, has been reported by multiple cannabis testing labs when analyzing chocolate-based products. This suggests that these samples require thorough lipid removal following cannabinoid extraction. Different cleanup procedures on a THC-infused commercial milk chocolate sample were compared, including filtration, using QuEChERS extraction salts and dispersives, winterization (lipid precipitation at –80 °C), and filtration on Captiva EMR—Lipid. Results were compared by both gravimetric analysis of the resulting extracts as well as LC/Q-TOF analysis. As shown in Figure 3, filtration on Captiva EMR—Lipid and winterization gave an identical average residual weight, while simple acetonitrile extraction without further treatment yielded the highest residual weight, followed by QuEChERS treatment without dispersive. Adding a fat-specific dispersive to QuEChERS extraction did help to remove some chocolate matrix components and reduced the residual weight, but not quite to the extent of Captiva-EMR—Lipid and winterization (Figure 4).

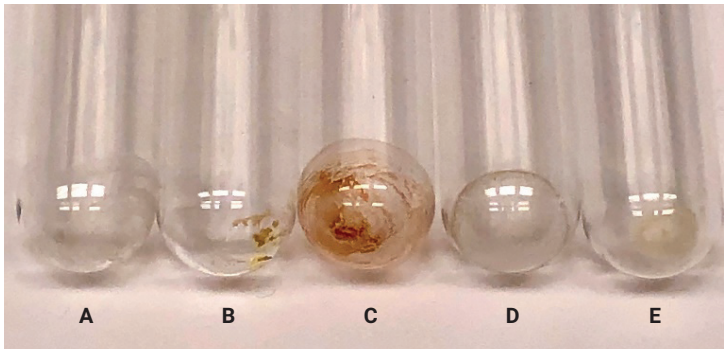

Figure 3. Sample cleanliness comparison after sample preparation and dry-down (average weight (n = 3) of residuals, in mg, for each treatment between parentheses).
(A) Acetonitrile extraction + Captiva EMR—Lipid cleanup (0.4 mg).
(B) Acetonitrile extraction (no treatment) (30.3 mg).
(C) QuEChERS extraction (26.7 mg).
(D) QuEChERS extraction + dispersive for fatty samples (8.3 mg).
(E) Acetonitrile extraction + cold stabilization (winterization procedure) (0.4 mg).

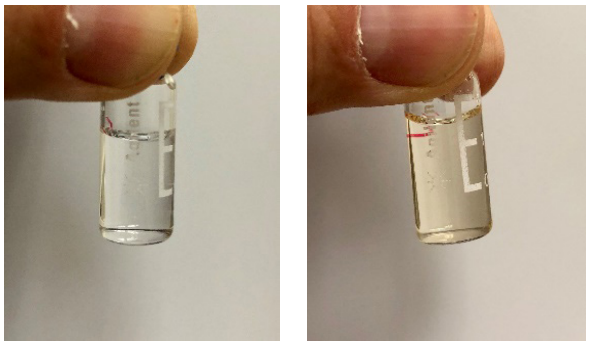

Figure 4. Visual comparison between milk chocolate extracted with Agilent Captiva EMR—Lipid (left) and QuEChERS (right).

In addition to gravimetric analysis, the extracts from the various sample treatments were also compared by LC/Q-TOF for further cleanliness assessment. Looking at the resulting TIC profiles, the Captiva EMR—Lipid extracts showed a significantly lower baseline compared to other cleanup techniques (Figures 5A and 5B). This extra cleanliness was especially noticeable between 4.5 and 6 minutes, and after 8.5 minutes. Moreover, the LC/UV analysis of the various cleanup techniques showed a significantly higher signal for THC when using a Captiva EMR—Lipid cleanup compared to other cleanup methodologies (Figure 6). This increase in UV signal can be attributed to superior lipid removal using Captiva EMR—Lipid filtration. Cannabinoids are fat soluble, and as such, lipids can interfere with the UV detection of cannabinoids when not effectively removed.[6] QuEChERS dispersives do not have enough specificity and capacity to fully capture fats in baked goods and chocolates. QuEChERS dispersives can only remove lipids from samples using C18, which is not selective and can also capture cannabinoids. Lipid precipitation at cold temperature or winterization does remove a fair amount of lipids, but can also coprecipitate a significant quantity of cannabinoids, as shown in Figures 5A and 5B.

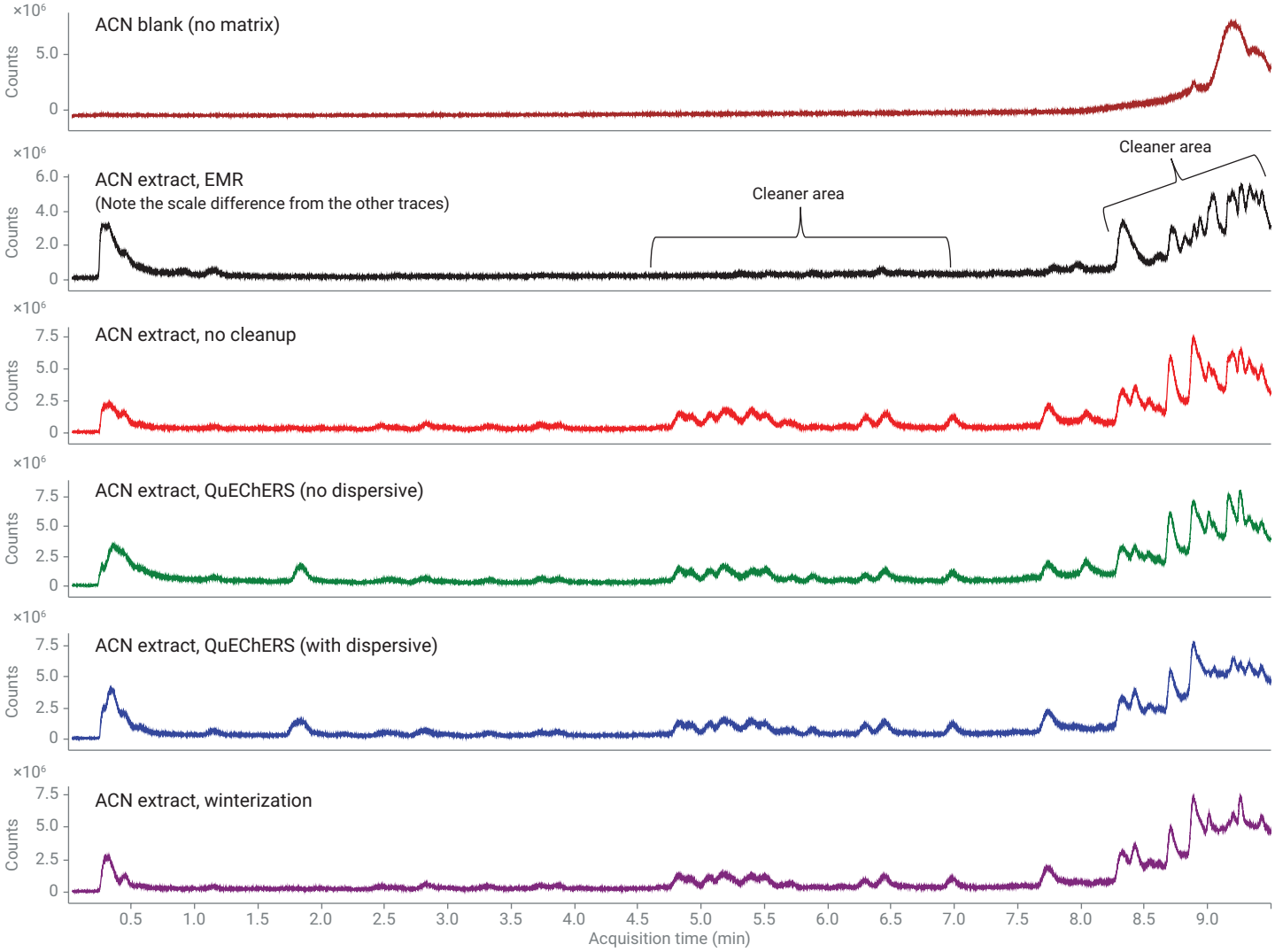

Figure 5A. LC/Q-TOF TIC comparison.

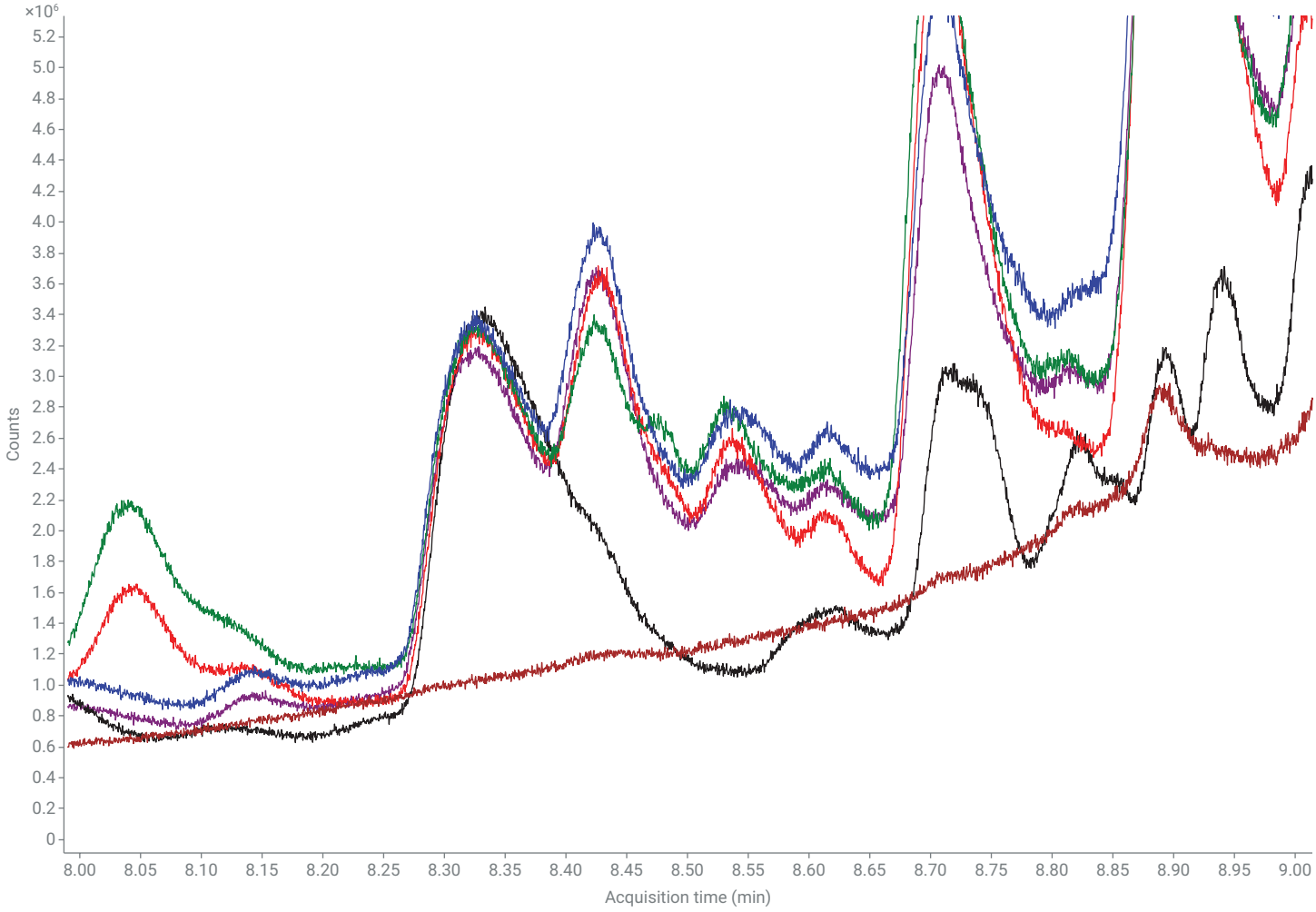

Figure 5B. Zoomed-in region between minute 8 and 9. THC elutes at 8.32 minutes.

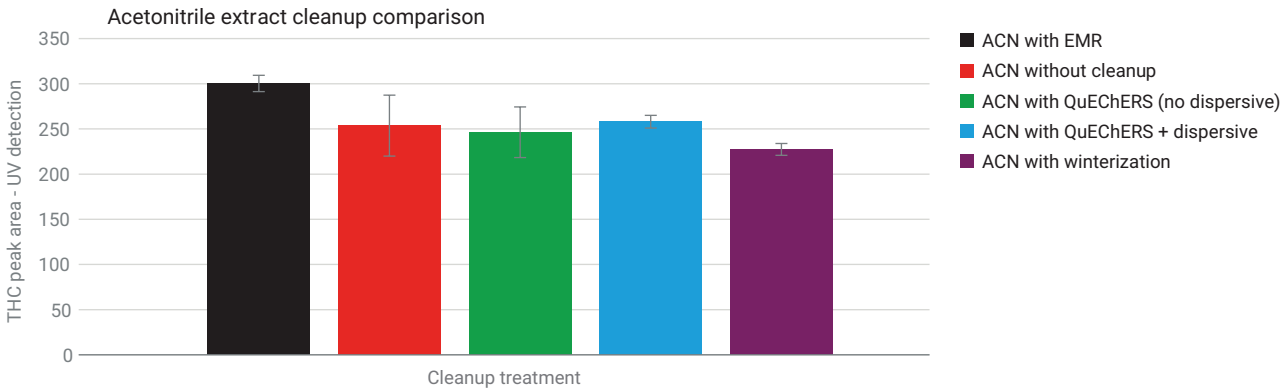

Figure 6. Comparison of the THC peak area (UV signal) from multiple cleanup treatments of an acetonitrile extract of infused milk chocolate (three samples were prepared per treatment, error bars show standard deviation).

Method performance characteristics

The optimized methodology (extraction of CBD and THC with acidified acetonitrile combined with extract filtering on Captiva EMR—Lipid) was tested for robustness, accuracy, and precision over 4 different days for milk chocolate, and over 2 different days for each white chocolate and dark chocolate (Tables 2, 3, and 4). Matrix-matched standard curves were prepared with six points in triplicate injections at concentrations ranging from 0.5 to 100 µg/mL for each cannabinoid (Table 5 and Figure 7). The stability of these calibration curves was demonstrated over a period of 7 days (Table 6). Noninfused chocolate samples were spiked before and after extraction/filtration to establish recoveries for the two analytes (Table 7). Finally, commercially available THC-infused milk chocolate was tested to validate accuracy of the quantification procedure (Table 8).

Intraday accuracy and interday accuracy and precision

Table 2. Milk chocolate.

Calibrator 1 (0.5 ug/mL CBD, 0.5 ug/mL THC)	CBD				THC			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Cal 1 - First Preparation	114.1	106.6	103	117.4	96.3	97.8	96.7	109.8
Cal 1 - Second Preparation	105.3	97.8	123.5	110.1	103.4	93.5	100.2	105.9
Cal 1 - Third Preparation	106.1	101.4	108.1	108.6	97.6	101.9	92.1	106.2
Intra-Day Average Accuracy (n=3)	108.5	101.9	111.5	112.0	99.1	97.7	96.3	107.3
Inter-Day Average Accuracy (n=12)	108.5				100.1			
Inter-Day Standard Deviation (n=12)	7.1				5.4			
Inter-Day Precision (%RSD, n=12)	6.5				5.4			

Table 3. Dark chocolate.

Calibrator 1 (0.5 ug/mL CBD, 0.5 ug/mL THC)	CBD		THC	
	Day 1	Day 2	Day 1	Day 2
Cal 1 - First Preparation	105.9	112.6	109.0	96.9
Cal 1 - Second Preparation	107.0	106.9	106.8	96.5
Cal 1 - Third Preparation	103.8	93.7	106.4	97.4
Intra-Day Average Accuracy (n=3)	105.6	104.4	107.4	96.9
Inter-Day Average Accuracy (n=6)	105.0		102.2	
Inter-Day Standard Deviation (n=6)	6.2		5.8	
Inter-Day Precision (%RSD, n=6)	6.0		5.7	

Table 4. White chocolate.

Calibrator 1 (0.5 ug/mL CBD, 0.5 ug/mL THC)	CBD		THC	
	Day 1	Day 2	Day 1	Day 2
Cal 1 - First Preparation	98.4	114	103.7	107.2
Cal 1 - Second Preparation	111.9	107.8	103.5	103
Cal 1 - Third Preparation	106.8	101.4	107.8	102.6
Intra-Day Average Accuracy (n=3)	105.7	107.7	105.0	104.3
Inter-Day Average Accuracy (n=6)	106.7		104.6	
Inter-Day Standard Deviation (n=6)	6.0		2.3	
Inter-Day Precision (%RSD, n=6)	5.6		2.2	

Calibration curves and linearity range

Table 5. Calibration curve average fit (R^2) for milk, dark, and white chocolate.

Name	Range (ug/mL)	Number of Calibrators	Curve Type	Weight	Average Fit, Milk Chocolate (R^2, n=4)	Average Fit, Dark Chocolate (R^2, n=2)	Average Fit, White Chocolate (R^2, n=2)
CBD	0.5 to 100	6	Linear	1/x	0.99962	0.99988	0.99984
THC	0.5 to 100	6	Linear	1/x	0.99983	0.99984	0.99987

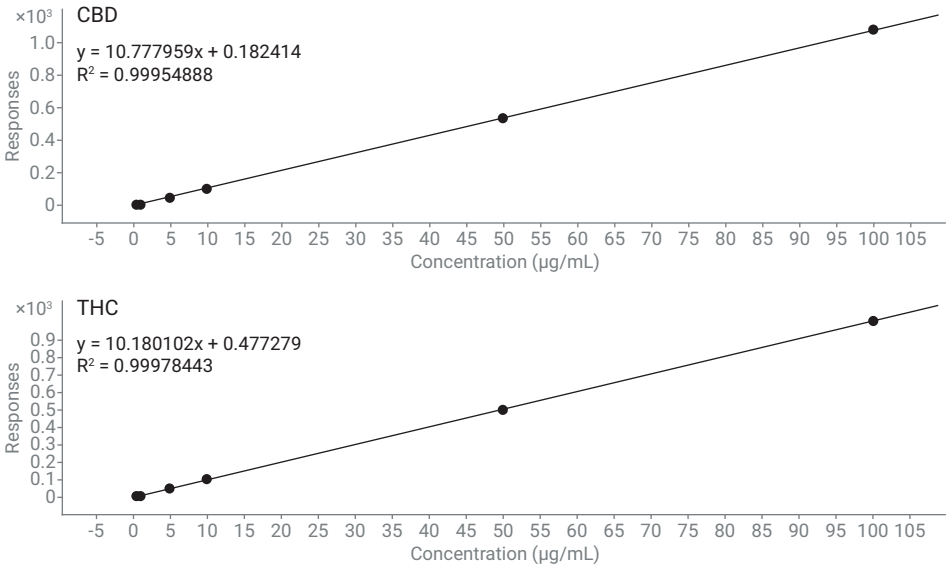

Figure 7. Typical matrix-matched calibration curves for CBD and THC, respectively.

Calibrator stability in milk chocolate

Table 6. Seven-day stability study of CBD and THC calibrators. The calibrators were stored in an HPLC vialsampler at 20 °C.

Calibrator Level	Concentration (µg/mL)	CBD		THC	
		UV Peak Area on Day 1	UV Peak Area on Day 7	UV Peak Area on Day 1	UV Peak Area on Day 7
6	100	1025	1142	946	1060
5	50	504	582	471	537
4	10	92	109	94	103
3	5	46	54	45	49
2	1	9	11	8	9
1	0.5	4	5	4	5

Recovery study

Table 7. Recovery efficiency and matrix effect for CBD and THC in milk, dark, and white chocolate (where % recovery efficiency = (pre-extraction spike/post-extraction spike) × 100, and % matrix effect = (post-extraction spike/solvent spike) × 100.

	CBD			THC		
	Milk	Dark	White	Milk	Dark	White
Pre-Extraction Matrix Spike Average Peak Area (n = 3)	292.9	294.9	298.1	257	268.8	261.2
Post-Extraction Matrix Spike Average Peak Area (n = 3)	274.4	275.9	275.7	246.3	245	250.4
% Recovery Efficiency (n = 3)	106.7	106.9	108.1	104.3	109.7	104.3
Post-Extraction Matrix Spike Average Peak Area (n = 3)	274.4	275.9	275.7	246.3	245	250.4
Solvent (no matrix) Spike Average Peak Area (n = 3)	277.2			255.9		
% Matrix Effect (n = 3)	99	99.5	99.4	96.3	95.7	97.9

Commercial sample analysis

Calculation to convert in-vial concentration to finished product concentration (using the protocol above – if using different dilutions, calculations will need to be modified accordingly)

A) Weight (µg) of THC/CBD in starting material: in-vial concentration (µg/mL) × 4/2.5 × 2.5/2 × 10 mL/1 g chocolate

B) Starting material concentration: weight of THC/CBD (µg THC/CBD) × 1 (mg THC/CBD)/1,000 (µg THC/CBD) × weight of chocolate sample (g chocolate)

Table 8. Milk chocolate. Commercially available THC-infused milk chocolate was tested to validate accuracy of the quantification procedure.

	THC			
	Day 1	Day 2	Day 3	Day 4
Average Calculated In-vial Concentration (n = 3, ug/mL)	16.9	15.9	15.4	16.3
Bar Weight (g)	31.4	31.4	31.4	31.4
Average Calculated In-Bar Concentration (n = 3, mg THC/bar)	10.6	9.9	9.6	10.2
Label Claim (mg THC/ bar)	10	10	10	10
Accuracy %	105.6	99.4	96.2	102.3

Conclusion

Potency testing in chocolate and baked products, such as brownies and cookies, is challenging because of high matrix complexity and lipid content. Removing lipids prior to analysis by LC/UV is critical in order to achieve robust and accurate quantification of cannabinoids given their affinity for fat. The procedure developed here enabled the highest lipid removal compared to other common preparation techniques for high-lipid content, resulting in a higher LC/UV signal for THC and CBD. The short LC gradient used reduces solvent consumption, and with cleaner samples, the analytical column is less likely to suffer from lipid accumulation. These optimized method parameters provide increased system uptime, lab productivity, and profitability. Implementation of potency testing in fatty edibles using this approach is therefore simple, accurate, and reliable, in addition to providing increased lab productivity.

Disclaimer

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state and country law.

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