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Quantification of THC and CBD in Beverages Containing Nano-Emulsions

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Abstract

Accurate measurement of Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) in cannabis-infused beverages is a pivotal testing requirement to ensure regulatory compliance including product labeling and product safety. This application note demonstrates a simple and robust procedure to extract cannabinoids in the presence of emulsifying agents, and to quantify those cannabinoids by liquid chromatography coupled to UV detection (LC/UV). The method provides excellent quantitation accuracy and precision for a great variety of beverages, including iced tea, beer, soda water, and carbonated fruit drinks.

Key advantages

– Removal of emulsifiers and carrier oils that can potentially lead to column clogging and erratic testing results

– Optimized extraction procedure for better accuracy and precision

– Reliable and robust LC/UV detection applicable to a great variety of beverages

Introduction

There is a demand for a better, more accurate and reproducible methodology to test cannabinoid potency in edibles in order to meet regulatory requirements, which vary greatly depending on country and/or state. Each food type has specific challenges due to its consistency and its excipients, all directly impacting results, uptime, and maintenance of analytical instrumentation. There is 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. In a peer-reviewed study, Vandrey, *et al.* 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 potency testing in cannabis-infused beverages challenging?

Cannabinoids are at much lower concentrations in beverages compared to other edibles like baked goods and candies. Therefore, extraction and detection methods designed for beverages need to be optimized to reach lower detection limits.

Cannabinoids have relatively high Log P values, making them more fat-soluble than water-soluble. This chemical feature makes molecules such as THC and CBD hard to dissolve and stabilize in water-based drinks. To do so, manufacturers of cannabis-infused drinks must use carrier oils and emulsifiers such as glycerin, ethyl alcohol, Tween 80, modified palm oil, and lecithin. These additives pose several challenges to analytical labs trying to perform potency testing in these drinks with accuracy and robustness.

The first issue with high concentrations of additives such as oils and emulsifiers in extracts is the gradual contamination of the LC flow path, potentially leading to a steady increase of instrument backpressure over multiple injections, and eventually to column clogging. That buildup in analytical columns is often irreversible and increases the frequency of guard column and analytical column changes, leading to higher operating costs. Buildups in analytical columns also cause several chromatography problems including disruption in peak shape, lower signal-to-noise ratios, and poor reproducibility. All these technical issues increase labor and consumable costs.

Secondly, fatty additives and emulsifiers encapsulate cannabinoids and can potentially interfere with their extraction from beverages, ultimately impacting LC/UV potency results. Dawson, *et al*. have described in part this lipid interference problem in chocolate.[5,6] If using liquid chromatography coupled to mass spectrometry detection (LC/MS), in addition to the buildup and interferences challenges described above, the surfactants used in beverages can cause ion suppression. Polysorbate 80 (Tween 80) and phospholipids are known ion suppressors in electrospray ionization (ESI) and they can cause significant quantification inaccuracies in beverages, even after dilution.

This application note provides a methodology to increase lab productivity by quicky and selectively removing problematic emulsifiers from cannabis-infused beverage extracts, leading to more accurate potency results and increased instrument uptime.

Experimental

HPLC conditions

Parameter	Value
LC Modules	− Agilent 1260 Infinity II Flexible pump (G7104C) − Agilent 1260 Infinity II vialsampler (G7129C) with tray cooling option − Agilent integrated column compartment (G7130A) − Agilent 1260 Infinity II DAD (G7115A)
Run Time	13 min
Post-Time	3 min
Analytical Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 150 mm, 2.7 µm
Guard Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 5 mm, 2.7 µm
Mobile Phase A	5 mM Ammonium formate + 0.1 % formic acid in acetonitrile/water (70/30)
Mobile Phase B	0.1% Formic acid in methanol
Injection Volume	5 µL
Multisampler Temperature	20 °C
Column Temperature	30 °C
Detection	UV at 230 nm for all quantitative results
Flow	0.8 mL/min
Gradient	Time (min) %A %B 0 99 1 4 99 1 4.5 75 25 8.5 75 25 10.5 25 75 11 0 100 13 0 100
Needle Wash	3 seconds in flush port with 25/25/50 isopropanol/acetonitrile/methanol

*   Although this application note shows quantitative results for CBD and THC only, the previously mentioned HPLC conditions can resolve the 17 cannabinoids shown in Figure 1.

MS conditions—Agilent 6545 LC/Q-TOF

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.

Parameter	Value
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

Materials and reagents

–   50 mL polypropylene (PP) centrifuge tubes (part number 5610-2049)

–   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 10 g Original QuEChERS extraction kit (part numbers 5982-5550, 5982-6550 or 5982-7550)

–   15 mL polypropylene (PP) centrifuge tubes (part number 5610-2039)

–   Agilent Captiva EMR—Lipid 3 mL (part number 5190-1003)

–   Agilent vials with screw caps (part number 5182-0553)

–   Agilent cannabidiol (CBD) certified reference material, 1.0 mg/mL (part number 5191-3924)

–   Agilent Δ9-THC certified reference material, 1.0 mg/mL (part number 5191-3929)

–   More Agilent standards for potency testing:

| Part Number	Product Description	Concentration
5191-3928	Cannabichromene (CBC)	1 mg/mL
5191-3930	Cannabidiolic Acid (CBDA)	1 mg/mL
5191-3920	Cannabidivarin (CBDV)	1 mg/mL
5191-3923	Cannabigerol (CBG)	1 mg/mL
5191-3927	Cannabigerol Acid (CBGA)	1 mg/mL
5190-9430	Cannabinoid Mix A - CBO, CBN, delta9-THC	multiple
5190-9429	Cannabinoid Mix 8 - CBG, THCA, CBOA	multiple
5190-9428	Cannabinoid MIX C - CBC, CBGA, CBDV	multiple
5190-9427	Cannabinoid Mix D - THCV, delta8-THC	multiple
5191-3926	Cannabinol (CBN)	1 mg/mL
5191-3922	delta8-Tetrahydrocanoabinol (deltas-THC)	1 mg/mL
5191-3925	delta9-Tetrahydrocannabinolic acid (THCA)	1 mg/mL
5191-3921	Tetrahydrocannabivann (THCV)	1 mg/mL
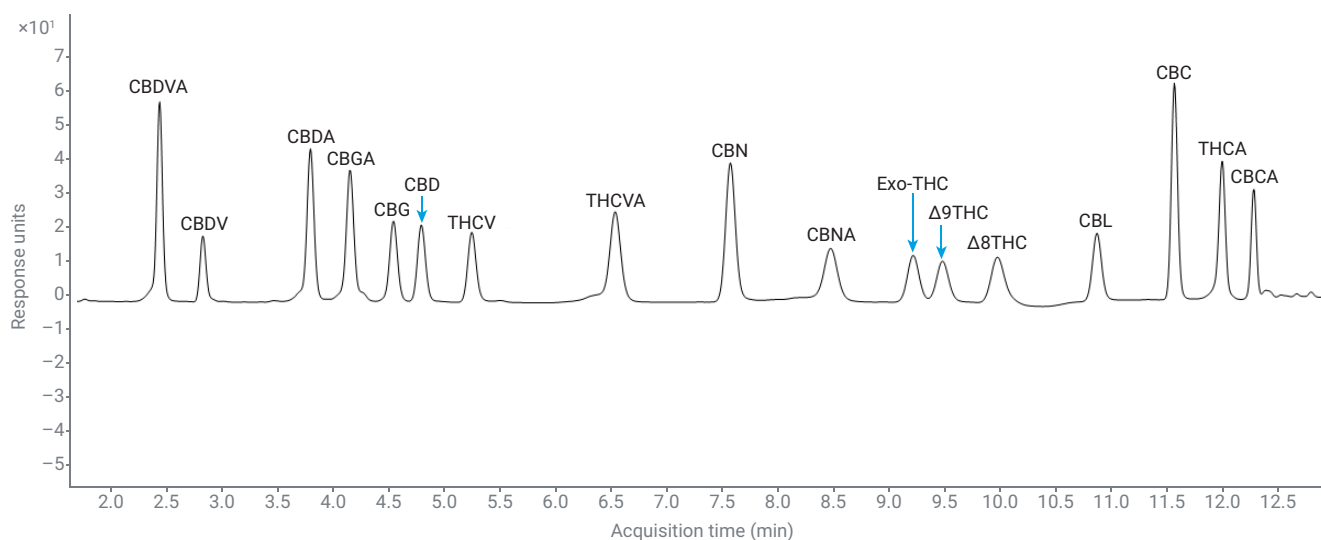
Figure 1. Separation of 17 cannabinoids using a 13-minute gradient.

Lab equipment

- Sonicator with a temperature-controlled bath
- Mechanical homogenizer (Geno/Grinder 1600 MiniG from SPEX SamplePrep or equivalent)
- Centrifuge 5804 R from Eppendorf with 50 mL tube adaptor, or equivalent
- Agilent positive pressure manifold (PPM) 48 processor (part number 5191-4101). This is optional, for increased throughput.
- Agilent 3 mL cartridge rack (part number 5191-4103)
- Waste rack for Agilent PPM-48 (part number 51914112)
- Mini vortexer

Sample processing and cannabinoid extraction

1. Take a bottle of infused beverage. Warm the can/bottle with warm tap water for 10 minutes. Shake for 10 seconds before opening.

2. Sonicate at approximately 50 °C for 20 minutes to degas and to dislodge cannabinoids from emulsifiers.

3. Put 10.0 mL of sonicated, cannabis-infused beverage into a 50 mL conical-bottom centrifuge tube. Add 10.0 mL of acetonitrile and shake aggressively for 3 minutes on a mechanical homogenizer (1,500 rpm).

4. Add Agilent Original extraction salts (part number 5982-6550), immediately shake by hand for 10 seconds, and open the cap to degas. This will prevent leaks and help to avoid salt agglomeration/clumping.

5. Shake for 1 minute aggressively on a mechanical homogenizer (1,500 rpm).

6. Centrifuge the tube at 3,000 to 5,000 rpm for 5 minutes at room temperature.

7. Transfer 2 mL of supernatant to a 15 mL conical-bottom centrifuge tube. Add 500 µL of water, and mix by inversion.

8. Place a 3 mL cartridge rack (part number 5191-4103) on top of a waste rack (part number 5191-4112).

9. 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.

10. Pour all of the contents (2.5 mL) from step 7 into the 3 mL Captiva EMR—Lipid tube. This will flow by gravity.

11. After complete elution of the initial 2.5 mL portion, pour an additional 1.5 mL of 80/20, acetonitrile/water into the Captiva EMR—Lipid tube, also flowing by gravity.

12. Vortex the 15 mL collection tube and aliquot in vials (part number 5182-0553) prior to injection. The final dilution factor is 2.

   Note: Do not forget to vortex the collection tube.

Notes: As an alternative to using a mechanical shaker for steps 3 and 5 above, add two disposable ceramic homogenizers (part number 5982-9313) and shake by hand vigorously with an up and down motion. This alternative procedure was not tested.

Including 2% ammonium hydroxide (200 µL of ammonium hydroxide solution, 28.0 to 30.0% + 9.8 mL acetonitrile) in step 3 can potentially help with recoveries of polar analytes with high pKa values. The high pH can neutralize basic analytes and increase their partitioning from the water layer to the acetonitrile layer during QuEChERS extraction. This alternative procedure was not tested, but it can be used if you need to test other analytes in addition to cannabinoids.

As an alternative to gravity flow elution, make the EMR cleanup 4x faster by placing the two racks with the Captiva EMR—Lipid tubes in step 8 in an Agilent PPM-48 processor (part number 5191-4101) at a pressure of 1 psi, and control the flow rate to a maximum of one drop every 3 to 5 seconds (see comment in Lab equipment section).

Noninfused beverage samples for matrix-matched calibrators

Following the procedure described in the previous section, make sure to prepare enough noninfused beverage matrix required for matrix-matched calibrators by loading two 15 mL PP centrifuge tubes with 2 mL aliquots of supernatant at step 7, then treat each tube as recommended in step 8 and beyond. In the end, the two eluates must be combined and vortexed in a single 15 mL PP centrifuge tube. That combined fraction is the beverage matrix. 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)	Prepared With
6	100	100 µL of CBD standard + 100 µL of THC standard + 800 µL of beverage matrix
5	50	500 µL of calibrator 6 + 500 µL of beverage matrix
4	10	200 µL of calibrator 5 + 800 µL of beverage matrix
3	5	500 µL of calibrator 4 + 500 µL of beverage matrix
2	1	200 µL of calibrator 3 + 800 µL of beverage matrix
1	0.5	500 µL of calibrator 2 + 500 µL of beverage matrix
0	0	1,000 µL of beverage matrix

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

Results and discussion

Several parameters were tested to achieve optimal sample processing and extraction conditions. Evaluation criteria included reproducibility and analyte recovery determined by LC/UV, as well as sample cleanliness determined by LC/Q-TOF total ion chromatogram (TIC) analysis. Accuracy and precision were tested on a range of in-vial concentrations from 0.5 to 100 µg/mL, corresponding to 1 to 200 µg/mL CBD and THC in infused beverages.

Sample processing

The stability of beverages containing cannabinoids is an important consideration. First, emulsions containing THC and CBD can be unstable. They can degrade during pasteurization and manufacturing. These emulsions also tend to precipitate during storage over time. Secondly, oxidation must be controlled to avoid transformation of THC during storage, for example. Finally, emulsions need to be compatible with liner material in cans and with the plastic or glass used in bottles.

It is therefore recommended to test beverages for potency in the first days after reception to mitigate any of the stability concerns described above. In the procedure described here, beverage cans and bottles were placed in a sink with warm tap water before testing to reduce any possible interaction cannabinoids of interest may have with their containers. Higher temperature potentially increases analyte solubility and may reduce unwanted binding to bottles and cans. Hydrophobic polymers are often sprayed in the inside of aluminum cans and can potentially bind THC and CBD. Similarly, plastic bottles are often made with polyethylene terephthalate (PET), which can interact with THC and CBD. Glass bottles are hydrophilic and less unwanted interactions with cannabinoids emulsions are expected.

Sonication at 50 °C facilitates the degassing of carbonated drinks and may help to disrupt and brake micelles formed by carrier oils and emulsifiers in cannabinoid nano-emulsions. In addition, the presence of acetonitrile in the QuEChERS extraction further helps to disrupt nano-emulsions for better solubility and extraction efficiency of cannabinoids.

Extraction of cannabinoids

QuEChERS is an extraction technique widely used for food testing and is a great fit for beverages.[7] QuEChERS requires high water content, which is the case for beverages. An equal amount of acetonitrile, in this case 10 mL, is added to the sonicated beverage for extraction. Note that acetonitrile provides equivalent solubility for cannabinoids compared to methanol but provides cleaner extracts because it is an aprotic solvent. Figure 2 in

Agilent application note 5994-2873EN documents the enhanced cleanliness of acetonitrile compared to methanol.[6] The extraction partitioning step of QuEChERS separates water from acetonitrile after addition of extraction salts and centrifugation. As a result, polar interferences are removed in the water layer, generating a cleaner acetonitrile layer at the top. However, that top acetonitrile layer still has a significant amount of oil, fatty acids, and other emulsifying agents that need to be removed to avoid the issues with detection, chromatography, and accuracy of potency testing described earlier.

Extract cleanup

Because of the aqueous nature of the cannabis-infused beverages and the relatively low levels of cannabinoid they contain, it would be tempting to inject them directly into the HPLC system with very little or no treatment at all. However, the presence of oils and emulsifiers in beverages suggests that they require targeted cleanup in order to perform accurate potency testing on them. Therefore, different cleanup procedures were tested on several commercially available THC- and CBD-infused beverages, including beer, iced tea, soda water, and carbonated fruit drinks. All currently reported cleanup techniques were compared, including filtration on various syringe filters, using several combinations of QuEChERS extraction salts with dispersives, and Captiva EMR—Lipid filtration. Resulting samples were compared by LC/UV and LC/Q-TOF analysis. The information generated provided clear conclusions about sample cleanliness and method accuracy.

As shown in Figure 2, QuEChERS extraction combined with a dispersive cleanup or followed by Captiva EMR—Lipid filtration seemed to be the most efficient at removing undesired matrix. When looking at the resulting UV peak area of the various treatments, the combined QuEChERS Captiva EMR—Lipid filtration yielded a significantly larger peak compared to any other treatment (Table 2). 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.[5,6] QuEChERS dispersives do not have enough specificity and capacity to fully capture oils and fatty emulsifiers in cannabinoid-infused beverages. Regenerated cellulose and PTFE filters are considered hydrophobic membranes and can interact with some nonpolar interferences. However, they will also partially capture cannabinoids as demonstrated in Table 2, which will negatively impact accuracy and reproducibility in potency testing.
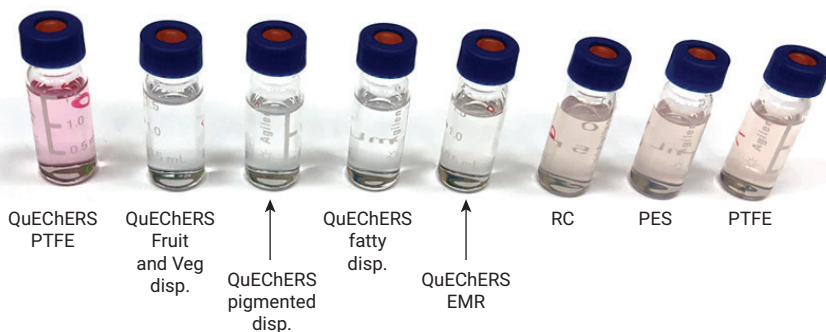
Figure 2. Visual comparison between various treatments of a carbonated fruit beverage infused with a THC nano-emulsion. From left to right: (1) QuEChERS extraction followed by PTFE filtration; (2) QuEChERS followed by a fruits and vegetables dispersive (PSA, MgSO$_4$) followed by PTFE filtration; (3) QuEChERS extraction followed by a dispersive cleanup for pigmented fruits and vegetables (PSA, GCB, and MgSO$_4$) followed by PTFE filtration; (4) QuEChERS extraction followed by a dispersive cleanup for fatty samples (PSA, C18EC, MgSO$_4$) followed by PTFE filtration; (5) QuEChERS extraction followed by Captiva EMR—Lipid filtration; (6) 4 mm, 0.2 μm regenerated cellulose (RC) filtration only; (7) 4 mm, 0.2 μm PES filtration only; and (8) 4 mm, 0.2 μm PTFE filtration only.

Table 2. LC/UV peak area for THC following various treatments of a carbonated cannabis fruit beverage infused with a THC nano-emulsion.

Sample	THC UV Peak Area
PTFE Filtration	353.37
QuEChERS + PTFE Filtration	363.58
QuEChERS + Pigment Dispersive + PTFE Filtration	370.89
QuEChERS + EMR	419.62

In addition to LC/UV 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 (Figure 3). This extra cleanliness was especially noticeable after 6 minutes (note that the HPLC gradient was increased to 15 minutes total to monitor all eluting compounds). QuEChERS dispersives can only remove lipids from samples using C18, which is not selective and can also capture cannabinoids. As a result, a lower UV signal for THC is reported in Table 2 for samples treated with QuEChERS dispersives. Note that lipid precipitation at cold temperatures or winterization has been reported for baked goods and chocolate. That technique does remove a reasonable amount of lipids but can also co-precipitate a significant quantity of cannabinoids, as demonstrated on work done on chocolate.[6]

Method performance characteristics

Although the fit of the procedure described here (QuEChERS extraction of CBD and THC followed by Captiva EMR—Lipid filtration) was assessed with multiple beverage matrices, a carbonated fruit beverage infused with a THC nano-emulsion was chosen to test the method performance. Parameters including accuracy and precision (Table 3) were monitored over several days. 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 4). The stability of these calibration curves was demonstrated over a period of 6 days (Table 5). A carbonated fruit beverage noninfused with cannabinoids but containing a nano-emulsion was spiked before and after extraction-filtration to establish recoveries of CBD and THC (Table 6). Finally, a commercially available beverage infused with a THC nano-emulsion was tested to validate accuracy of the quantification procedure.
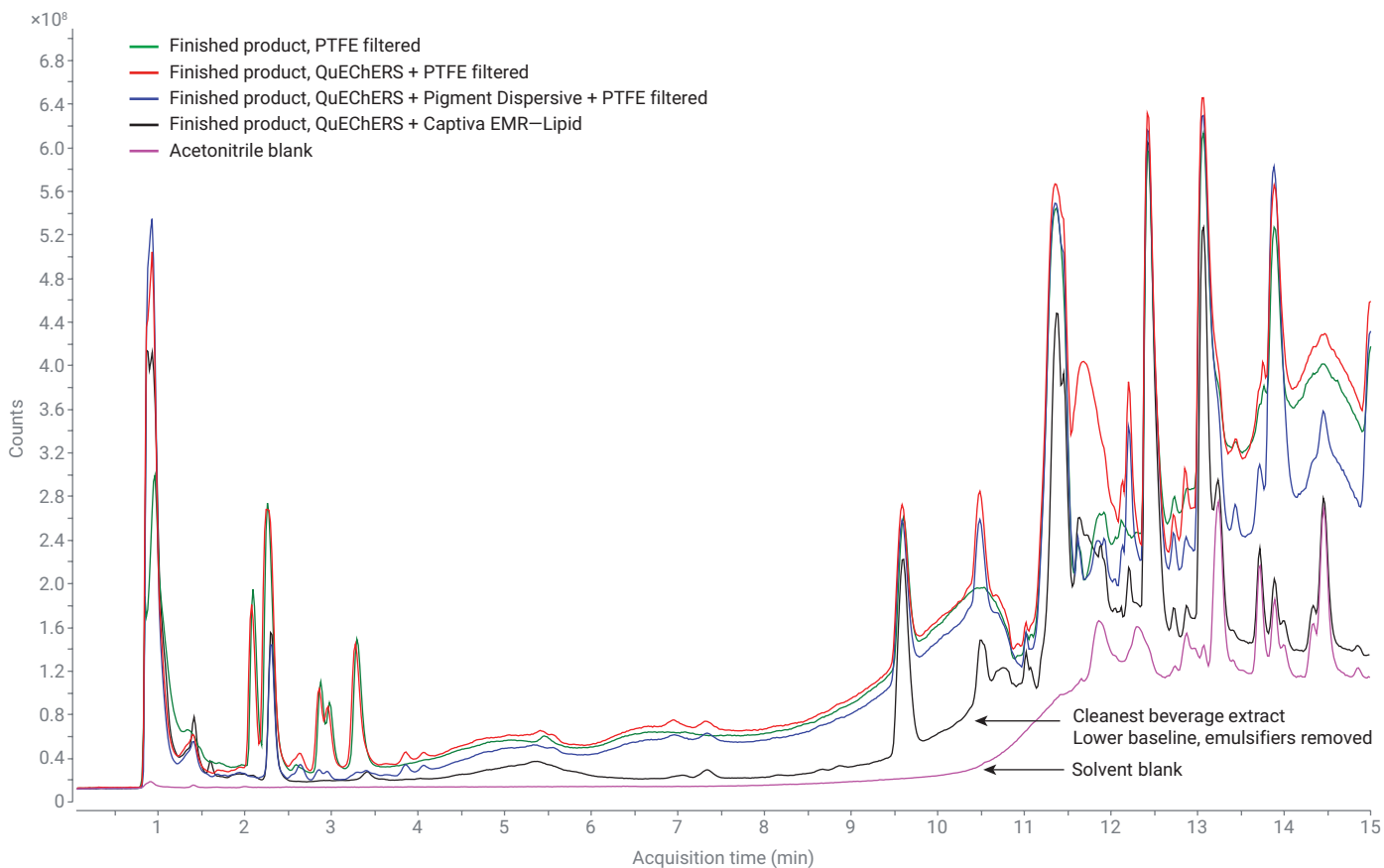
Table 3. Intraday accuracy and interday accuracy and precision.

Calibrator 1	CBD			THC		
(0.5 μg/mL CBD, 0.5 μg/mL THC)	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Calibrator 1: First Preparation	100.9	103.3	103.4	99.8	101.9	103.1
Calibrator 1: Second Preparation	103	102.4	103.4	101.7	103	104.5
Calibrator 1: Third Preparation	102	101.1	104.9	102.3	102.4	104
Intraday Average Accuracy (n = 3)	102.0	102.3	103.9	101.3	102.4	103.9
Interday Average Accuracy (n = 6)	102.7			102.5		
Interday Standard Deviation (n = 6)	1.3			1.4		
Interday Precision (%RSD, n = 6)	1.2			1.3		

Figure 3. LC/Q-TOF TIC comparison of various cleanup treatments of an acetonitrile extract from a carbonated fruit beverage infused with a THC nano-emulsion (acetonitrile blank: pink trace; Agilent QuEChERS extraction + EMR filtration: black trace; beverage filtered on PTFE: green trace; QuEChERS extraction (no dispersive) followed by PTFE filtration: red trace; QuEChERS extraction followed by pigmented dispersive (PSA, GCB, $MgSO_4$) and PTFE filtered: blue trace).

Table 4. Calibration curve average fit (R^2) and linearity range.

Name	Range (µg/mL)	Number of Calibrators	Curve Type	Weight	Average Fit, XMG (R^2, n = 3)
CBD	0.5 to 100	6	Linear	1/x	0.99994
THC	0.5 to 100	6	Linear	1/x	0.99995

Table 5. Calibrator stability (stored in HPLC autosampler at 20 °C).

Calibrator Level	Concentration (µg/mL)	CBD Peak Area on Day 1	CBD Peak Area on Day 6	THC Peak Area on Day 1	THC Peak Area on Day 6
1	0.5	9	9	7	7
2	1	18	19	15	16
3	5	93	93	81	82
4	10	188	191	165	169
5	50	948	961	832	851
6	100	1926	1968	1693	1741

Table 6. Recovery study (where % recovery efficiency = (pre-extraction spike/post-extraction spike) × 100.

	CBD	THC
Pre-Extraction Matrix Spike Average Peak Area (n = 3)	274.6	249.1
Post-Extraction Matrix Spike Average Peak Area (n = 3)	243.2	223.3
Recovery Efficiency % (n = 3)	112.9	111.5

Commercial sample analysis

Calculations to convert in-vial concentration to A) concentration of cannabinoid (mg/mL) in finished product, or B) weight of cannabinoid (mg) in finished product (using the protocol above – if using different dilutions, calculations will need to be modified accordingly).

A) Concentration (mg/mL) of THC/CBD in finished product: in-vial concentration (µg/mL) × (4 mL/2.5 mL) × (2.5 mL/2 mL) × (1 mg/1,000 µg)

B) Weight (mg) of cannabinoid in finished product: cannabinoid concentration in finished product (mg/mL) × volume of beverage in container (mL)

Example:

After being processed as described earlier, an extract from a 355 mL can of cannabis-infused beverage is found to contain 3.148 µg/mL of THC.

A) Concentration of THC in the beverage:
3.148 µg/mL × (4 mL/2.5 mL) × (2.5 mL/2 mL) × (1 mg/1,000 µg) = 0.00630 mg THC/mL

B) Weight of THC in the can:
(0.00630 mg/mL) × 355 mL = 2.24 mg THC

Conclusion

Potency testing on beverages infused with cannabinoids can be challenging because of their relatively low concentration and because nano-emulsions can cause several analytical challenges that will negatively impact accuracy and lab productivity. QuEChERS extraction followed by filtration on Agilent Captiva EMR—Lipid provides a quick and powerful cleanup before LC/UV and LC/MS/MS analysis. Results generated with this more robust procedure demonstrated superior cleanliness, accuracy, and precision for potency testing on a wide variety of beverages.

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.

Acknowledgments

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