Application Note

Cannabis & Hemp Testing

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Quantification of THC and CBD in Gummies and Hard Candies

Authors

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Abstract

Accurate measurement of Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) in edibles with a high sugar content such as gummies and hard candies is an important testing requirement to ensure product labeling and safety. This application note demonstrates a simple procedure to grind candies efficiently and extract and quantify cannabinoids by liquid chromatography coupled to UV detection (LC/UV).

Key advantages

- Process more samples per hour
- Optimized extraction procedure for better accuracy and precision
- Works for a variety of candies including gelatin, pectin, and corn starch-based gummies

Introduction

There is an increased demand to test cannabinoids in edibles to meet established or evolving regulatory requirements that vary greatly depending on country and state. Each food type has specific challenges related to their unique physical consistency but also because how their different ingredients impact analytical instrumentation uptime. 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, topicals, 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. A 2015 study 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]

Potency analysis of gummies and hard candies is challenging

Gummies are very sticky and are hard to grind mechanically. Current procedures often call for the use of cryo-milling, which is a very effective way to grind food samples at low temperatures. Cryo-milling devices can unfortunately process only a small number of samples per hour, reducing sample throughput. Moreover, even after cryo-milling, gummy samples can turn into gels at room temperature, regaining their sticky nature.

The second challenge with gummies and hard candies is their inability to fully dissolve in common solvents like methanol. Temperature increase has a positive impact on the ability to dissolve high-sugar candies, but the addition of water to those solvents enables a complete and faster melting at room temperature.

The third challenge associated with gummies is the variability of their chemical nature. Most gummies are made of gelatin, but some vegan gummies use pectin or corn starch to achieve the desired consistency. As a result, not all gummies will dissolve in solvents in the same way, potentially causing variability and resulting in a general lack of method robustness.

Finally, the external surface of gummies is often coated with sugar, coconut oil, palm kernel oil, carnauba wax, palm oil, or beeswax. It is therefore advisable to only use the middle or inside of gummies to get optimal accuracy and reproducibility when testing for cannabinoids. Grinding and extracting gummies with their outside coating will generate potency results artificially lower than actual values. Furthermore, because cannabinoids such as THC and CBD are fat-soluble, oils and waxes used for gummy coating can interfere with their detection and cause significant analytical challenges.[5,6]

This application note provides a methodology to increase lab productivity in a context of gummy potency testing, by avoiding time-consuming grinding procedures. An optimized extraction procedure using a combination of water, solvent, and high pH will provide wide applicability to high-sugar candies, including for hard candies and gummies made of gelatin, pectin, and corn starch.

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.

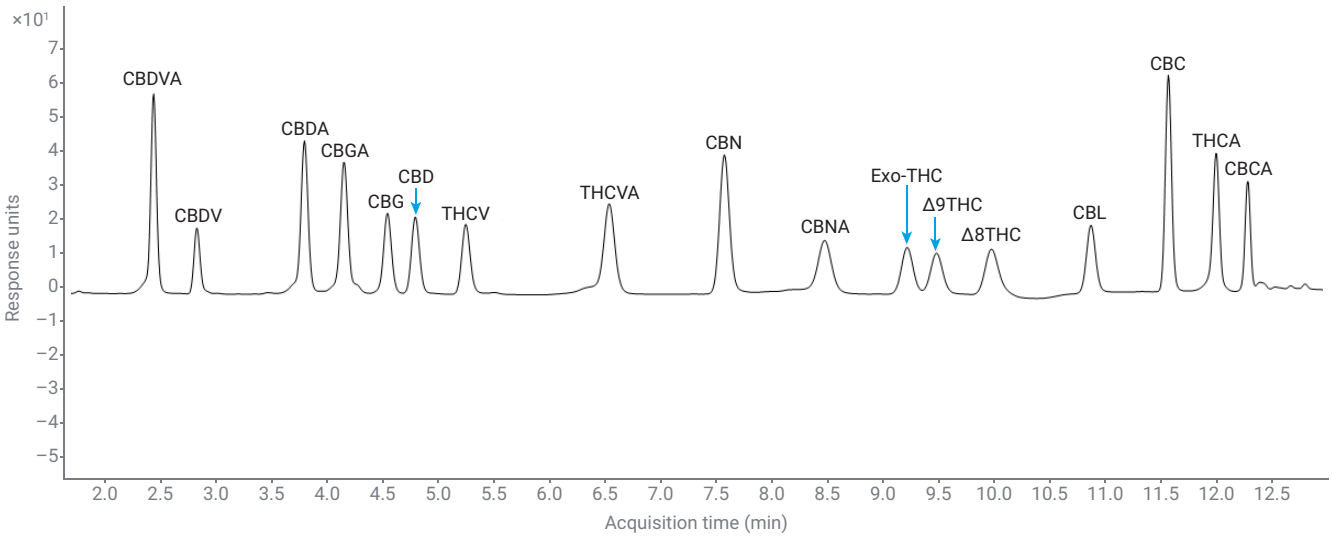


Figure 1. Separation of 17 cannabinoids using a 13-minute gradient.

MS conditions

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.

Materials and reagents

- 50 mL polypropylene (PP) centrifuge tubes (part number 5610-2049)
- Agilent disposable ceramic homogenizers (part number 5982-9313)
- Agilent InfinityLab Ultrapure LC/MS acetonitrile (part number 5191-4496)
- Acetonitrile containing 2% ammonia: to make 100 mL, add 2 mL of concentrated ammonium hydroxide solution (28.0 to 30.0% in water) to 98 mL of acetonitrile.
- Agilent InfinityLab Ultrapure LC/MS water (part number 5191-4498)
- Agilent EU QuEChERS extraction kit (part number 5982-6650 or 5982-7650)
- Agilent PTFE 0.2 µm syringe filter (part number 5190-5082)
- Agilent Captiva disposable syringes (part number 9301-6476)
- 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

Lab equipment

- Automated mechanical homogenizer (Geno/Grinder 1600 MiniG from SPEX SamplePrep or the equivalent)
- Centrifuge 5804 R from Eppendorf with 50 mL tube adaptor (or equivalent)
- Scissors
- Stainless-steel lab spatula
- Analytical balance
- Mini vortexer

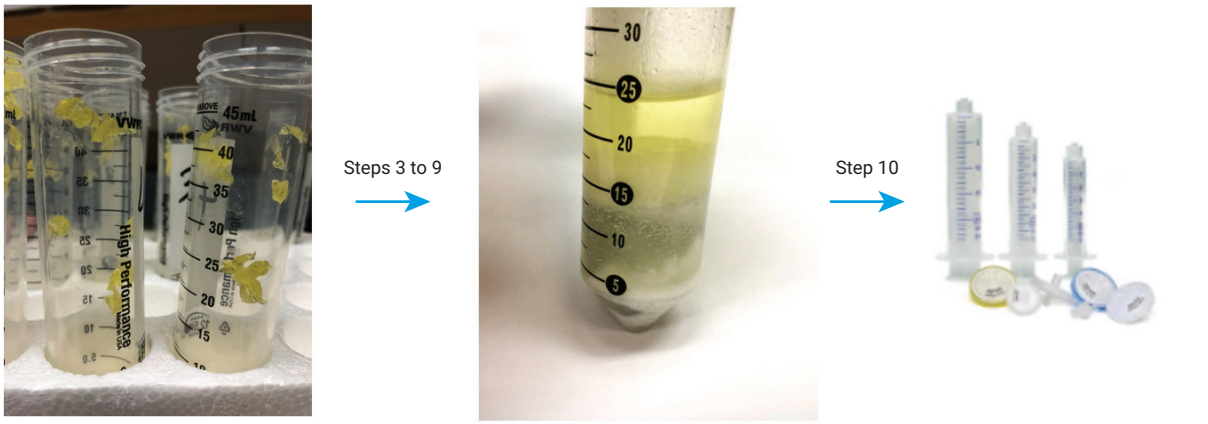
Sample processing and cannabinoid extraction

1. Weigh the whole gummy precisely and record weight for later calculations. Then take 1 ±0.005 g from the middle of an infused gummy (cut into four pieces and use middle to avoid sugar sanding and waxy/oily coating) and chop very finely with scissors. If the gummy is too small and the coating cannot be excluded when sampling 1 g, use a smaller sample weight. Put at the bottom and sides of a 50 mL PP conical-bottom tube using a stainless-steel spatula. Hard candies need to be mechanically crushed to powder beforehand, then take 1 ±0.005 g and put into a 50 mL PP conical-bottom tube.
2. Add two disposable ceramic homogenizers in the tube to ensure complete and faster homogenization.
3. Add 10 mL of ultrapure water at room temperature, and cap.
4. Place the tube on an automated mechanical homogenizer for aggressive vertical shaking (1,500 rpm) for 3 minutes (gummies) or less (crushed hard candies).
5. Add 10 mL of acetonitrile containing 2% ammonia hydroxide prepared on the same day and cap. (To make 100 mL, add 2 mL of concentrated ammonium hydroxide solution (28.0 to 30.0% in water) to 98 mL of acetonitrile.)
6. Place the tube on an automated mechanical homogenizer for aggressive vertical shaking (1,500 rpm) for 5 minutes. Candies should be completely dissolved and homogeneous.
7. Add the contents of an Agilent EU QuEChERS extraction kit. Immediately shake for 10 seconds manually to avoid clumping. Open cap to degas, then tighten cap again.
8. Place the tube on an automated mechanical homogenizer for aggressive vertical shaking (1,500 rpm) for 1 minute.
9. Centrifuge the tube at 3,600 rpm minimum for 5 minutes at room temperature (20 °C).
10. Filter 2 mL of acetonitrile supernatant with Agilent PTFE filters, put in vials, and cap. The final dilution factor is 10x.

Notes:

- Alternatively, instead of using room-temperature water at step 3, it is possible to put the 50 mL tube containing 1.00 g of candy and 10.0 mL of water in a sonication bath at 60 °C for approximately 10 minutes. Then add two ceramic homogenizers. This combination of heat and sonication will better melt the candies and shorten shaking times at step 4 and step 6.
- It is not recommended to use whole gummies for potency testing because they are coated with coconut oil, palm kernel oil, carnauba wax, palm oil, or beeswax. These fatty additives will compromise accuracy and

QuEChERS procedure to melt gummies without Cryo-Milling



1.00g of gummies chopped with scissors before water addition (two disposable Ceramic Homoginizers required)

Upper acetronitrile layer after QuEChERS Extraction at high pH

Filtration on Agilent 0.2 µm PTFE

Figure 2. Pictures representing the 10-step procedure for potency testing in gummies.

precision both by LC/UV and LC/MS/MS. If testing the entire/whole gummy is absolutely required, the procedure above can be used on gummies of 4.00 g or less with the following modifications: Take the entire gummy, chop finely with scissors, add 10.0 mL of water, and sonicate for approximately 10 minutes in a heated bath at 60 °C. Add two ceramic homogenizers and perform steps 4 to 9 above inclusively. Then, instead of filtering with PTFE syringe filter, take 2 mL of the top acetonitrile layer generated at step 9, mix with 500 µL of water and process on a Captiva EMR—Lipid filter (part number 5190-1003) as described in the chocolate and baked goods application note.[6] This lipids removal step will increase signal for cannabinoids and improve method robustness on whole gummies. Calculate the new dilution factor accordingly.

– Using the unbuffered Agilent Original QuEChERS salts (part number 5982-5550) at step 7 will help to maintain a high pH and will therefore better neutralize basic analytes, improving their partitioning from the water layer to the acetonitrile layer. This different QuEChERS extraction kit will have no significant impact on recoveries of THC and CBD, but will help if other analytes need to be extracted and quantified in addition to cannabinoids.

Noninfused gummy/candy samples for matrix-matched calibrators

Use the procedure described in the previous section to prepare noninfused gummy/candy matrix for matrix-matched calibrators. 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
7	200	200 µL of CBD standard + 200 µL of THC standard + 600 µL of gummy/candy matrix
6	100	500 µL of calibrator 7 + 500 µL of gummy/candy matrix
5	50	500 µL of calibrator 6 + 500 µL of gummy/candy matrix
4	10	200 µL of calibrator 5 + 800 µL of gummy/candy matrix
3	5	500 µL of calibrator 4 + 500 µL of gummy/candy matrix
2	1	200 µL of calibrator 3 + 800 µL of gummy/candy matrix
1	0.5	500 µL of calibrator 2 + 500 µL of gummy/candy matrix
0	0	1,000 µL of gummy/candy matrix

* Following this preparation, the final volume of calibrator levels 2, 4, 6, and 7 will be 500 µL. Please make sure to adjust the settings of the autosampler to accommodate this volume.

Results and discussion

Several conditions were tested to achieve optimal sample processing and extraction conditions. Parameters of success 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 200 µg/mL of each cannabinoid, corresponding to 0.005 to 2 mg of each cannabinoid per gram of infused candy.

Sample processing and extraction of cannabinoids

As described above, high-sugar edibles like candies can be laborious to process by cryo-milling and they do not dissolve well in solvents like methanol and acetonitrile. Water is however much more efficient to get candies to melt at room temperature. For these reasons, and because candies have a relatively low fat content, QuEChERS is a good technique to prepare more samples per hour to test for cannabinoids and other drugs.

QuEChERS is an extraction technique widely used for food testing.[8] QuEChERS requires high water content, which is the case for candy when 10.0 mL of water is added. The addition of two disposable ceramic homogenizers is important to speed the extraction process and to ensure full dissolution of candies. Then an equal amount of acetonitrile containing 2% ammonia is added to complete the extraction of cannabinoids and to increase solubility of cannabinoids. Different pH and solvent conditions were tested; no significant difference was observed by LC/Q-TOF when comparing the cleanliness of the various extracts, but 2% ammonia consistently provided higher UV signals for various gummy types extracted with this procedure (Figures 3 and 4). It is the first time this positive impact of high pH is reported for gummies. The hypothesis is that alkalinity melts them more efficiently, especially pectin-based gummies that require acidity to stay hard. For that reason, the effect of pH was not investigated on crushed hard candies as they melt more quickly and consistently in water. Acetonitrile provides similar solubility for cannabinoids compared to methanol but provides cleaner extracts because it is an aprotic solvent. Figure 2 in the Agilent application note 5994-2873EN documents extra cleanliness of acetonitrile compared to methanol.[6] In addition, higher water temperature and sonication positively impacts candy homogenization and can reduce shaking times. It was observed that the use of a sonication bath at 60 °C can reduce the shaking time required at steps 4 and 6 of the procedure described above.

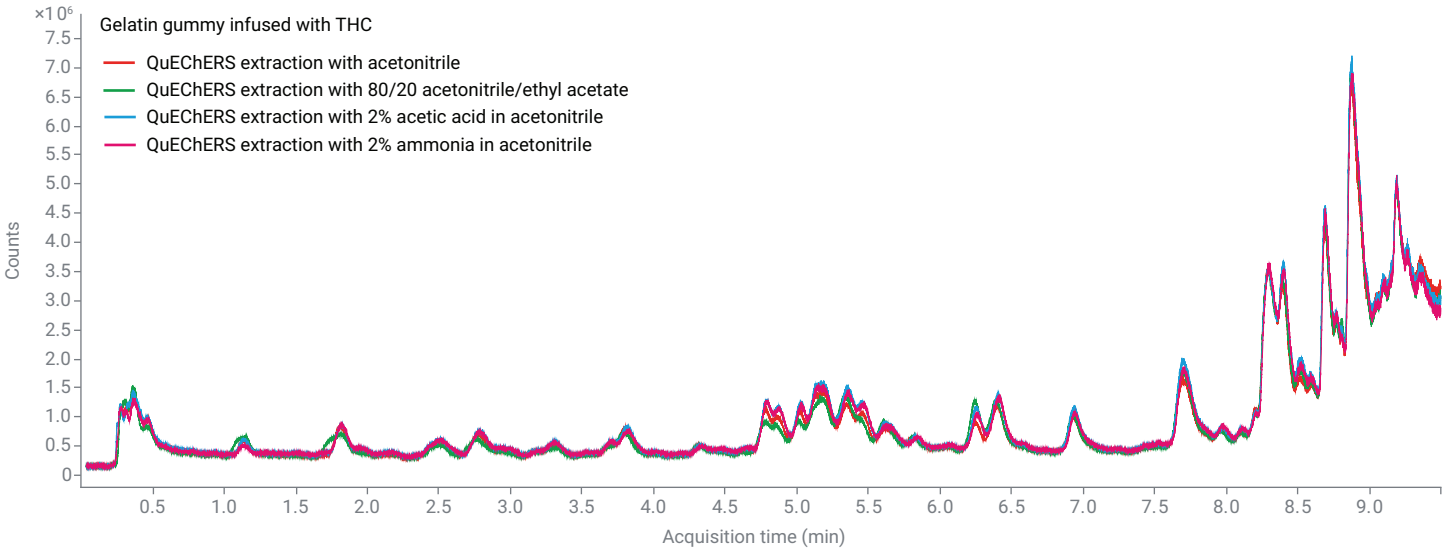


Figure 3. LC/Q-TOF TIC comparison of various solvent and pH conditions for a QuEChERS extract from a gelatin gummy infused with THC (QuEChERS extraction with acetonitrile: red trace, QuEChERS extraction with 80/20 acetonitrile/ethyl acetate: green trace, QuEChERS extraction with 2% acetic acid in acetonitrile: blue trace, QuEChERS extraction with 2% ammonia in acetonitrile: pink trace).

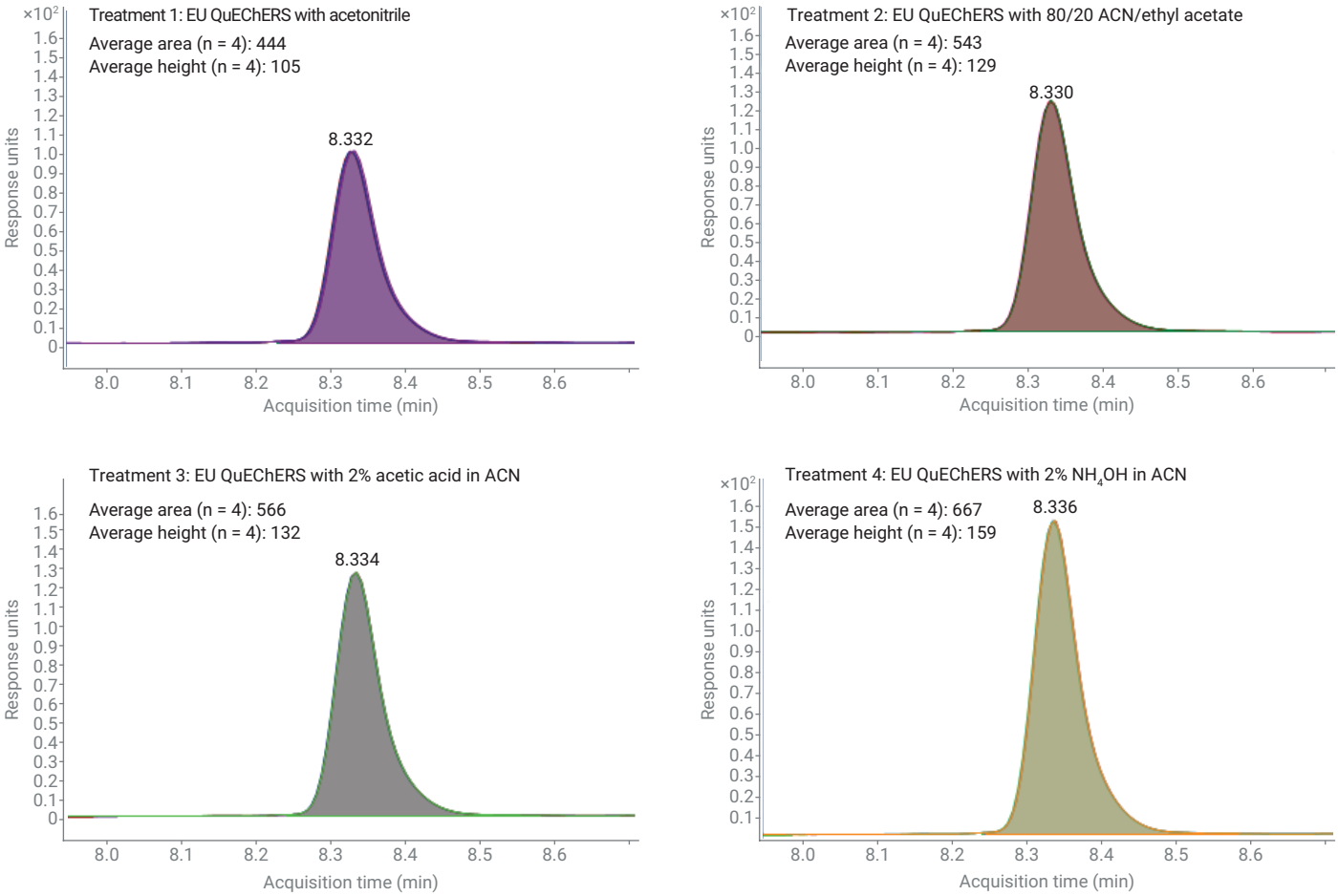


Figure 4. LC/UV comparison of various solvent and pH conditions for a QuEChERS extract from a gelatin gummy infused with THC. THC average peak height and peak area were obtained from four technical replicates.

The extraction partitioning step of QuEChERS separates the water from acetonitrile after addition of extraction salts and centrifugation. As a result, polar interferences such as sugars and starch were removed in the water layer, generating a cleaner acetonitrile layer at the top. Starch can become viscous in the presence of organic solvents, so removing it in the water partitioning step of QuEChERS prevents pressure issues during chromatography. The use of QuEChERS dispersives to further cleanup the acetonitrile layer was investigated and did not provide any benefit. Filtration was however required prior to injection. Several filter types were tested. Regenerated cellulose and PTFE provided equivalent UV signal and repeatability, but PTFE was chosen because of its superior stability at high pH.

Method performance characteristics

Although the applicability of the procedure described here (alkaline QuEChERS extraction of CBD and THC followed by PTFE filtration) was assessed with multiple gummy and candy matrices, gelatin gummies and pectin gummies were chosen to test method performance. Parameters including accuracy and precision (Table 2) were monitored over 2 days. Matrix-matched standard curves were prepared with seven levels in triplicate injections at concentrations ranging from 0.5 to 200 µg/mL for each cannabinoid (Table 3). The gelatin gummies were spiked before and after extraction-filtration to establish recoveries of CBD and THC (Table 4). Finally, commercially available gelatin gummies from a reputable manufacturer and infused with CBD were tested to validate accuracy of the quantification procedure (Table 5).

Table 3. Calibration curve average fit (R^2) and linearity range.

Name	Range (µg/mL)	Number of Calibrators	Curve Type	Weight	Average Fit	
					Gelatin Gummy (R^2, n = 2)	Pectin Gummy (R^2, n = 2)
CBD	0.5 to 200	7	Linear	1/x	0.99983	0.99987
THC	0.5 to 200	7	Linear	1/x	0.99984	0.99984

Table 4. Recovery study in gelatin gummy (where % recovery efficiency = (pre-extraction spike/post-extraction spike) * 100).

	CBD	THC
Pre-Extraction Matrix Spike Average Peak Area (n = 3)	452.1	423.3
Post-Extraction Matrix Spike Average Peak Area (n = 3)	490.0	451.8
Recovery Efficiency % (n = 3)	92.3	93.7

Table 5. Commercial sample analysis: Accuracy against label claim.

Parameter	Value (µg/mL)	
Sample 1	124.72	
Sample 2	125.71	
Sample 3	124.59	
Average	125.01	
	CBD per Gram of Gummy (mg)	CBD per Gummy (mg)
Experimental Value	1.25	5.38
Theoretical Value	—	5
% Accuracy		107.51

Table 2. Intraday accuracy and interday accuracy and precision.

Calibrator 1 (0.5 µg/mL CBD, 0.5 µg/mL THC)	Gelatin				Pectin			
	CBD		THC		CBD		THC	
	Day 1	Day 2	Day1	Day 2	Day 1	Day 2	Day1	Day 2
Calibrator 1: First Preparation	101.6	101.6	103.3	110.7	98.0	100.0	100.2	101.8
Calibrator 1: Second Preparation	101.7	105.2	102.7	105.3	100.6	95.9	104.6	102.1
Calibrator 1: Third Preparation	108.8	110.2	102.9	106.4	101.2	98.7	102.8	104.0
Intraday Average Accuracy (n = 3)	104.0	105.7	103.0	107.5	99.9	98.2	102.5	102.6
Interday Average Accuracy (n = 6)	104.9		105.2		99.1		102.6	
Interday Standard Deviation (n = 6)	3.9		3.1		2.0		1.6	
Interday Precision (%RSD, n = 6)	3.7		2.9		2.0		1.6	

Commercial sample analysis: Calculations

Calculations to convert in-vial concentration to (A) amount of cannabinoid (mg) per gram of gummy/candy or (B) amount of cannabinoid (mg) in entire gummy/candy:

* Using the protocol above—if using different dilutions, calculations will need to be modified accordingly.

** As mentioned earlier in this application note, cannabinoids may or may not be evenly distributed throughout a gummy. The choice of reporting units for potency in gummies must reflect the manufacturing process of the product that is tested.

A) **Weight of cannabinoid per gram of gummy:**
in-vial concentration (µg/mL) × 10 mL × 1
(mg cannabinoid)/1,000 (µg cannabinoid)/weight of gummy piece (g)

B) **Weight of cannabinoid in the entire gummy:**
Weight (mg) of THC/CBD per gram of gummy × weight of entire gummy (g)

Example:

An entire gummy weighs 4.30 g. A 1.000 g portion of this gummy was processed as described earlier, and was found to contain 125.01 µg/mL of CBD.

A) **To calculate the weight of CBD per gram of gummy:**
125.01 µg/mL × 10 mL × (1 mg/1,000 µg)/1.000 g = 1.25 mg CBD per gram of gummy

B) **To calculate the weight of CBD in the entire gummy, assuming an even distribution of CBD:**
1.25 mg/g × 4.30 g = 5.38 mg CBD

Conclusion

Potency testing in candies and gummies is challenging because they are sticky, hard to process, and they do not dissolve well in organic solvents. In addition, the coating of gummies is sanded or waxed, which can reduce method accuracy and precision if that exterior part of the candies is sampled for testing. The procedure developed here effectively extracted cannabinoids in gelatin, pectin, and starch gummies without the use of a cryo-milling device, saving time and increasing lab productivity. Extraction conditions were optimized for pH, water content, and the best solvent for fast and complete dissolution of gummies. The LC/UV method provided quantification of THC and CBD with great accuracy and precision.

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