Evaluating Cannabinoids and Terpenes in Challenging Matrices Using High-Temperature Headspace-Gas Chromatography-Mass Spectrometry

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OVERVIEW

OBJECTIVE

- To address the need for a simple method for extracting and quantitating cannabinoids from difficult sample matrices (*e.g.,* chocolates and gummies)

METHODS

- Chocolates with and without cannabidiol (CBD) were produced for method development
- Extractions were assessed using:
  1) high-temperature headspace sampling (HS-GC-MS)
  2) heated ultrasonic extraction into MeOH (uHPLC-MS)

DATA SUMMARY

- GC-based methods are viable, with fit-to-purpose performance, but are of limited use in jurisdictions were acidic cannabinoids must be quantitated separately from neutrals
- Heated ultrasonic extraction with HPLC-MS/DAD is an easy approach that could be extended to gummies and is applicable to all cannabinoids

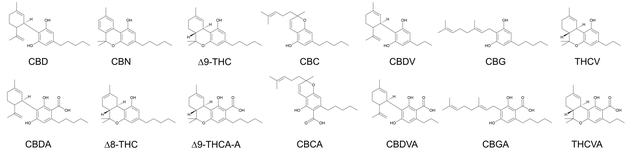


Figure 1. Structures of common cannabinoids.

METHODS

CHOCOLATES

Coconut oil (30 g)
Cocoa powder (7.5 g)
Corn Syrup (20 g)
→ 0.5 g CBD → homogenize → 8200 ppm CBD

HIGH-TEMPERATURE HEADSPACE GC-MS

TriPlus 500 Parameters	Values
Incubation time/temp	15 min/200 °C
Vial shaking/pressure	fast/100 kPa
Loop volume/temp	1.00 mL/220 °C
Loop pressure	50 kPa
Loop equilibration	0.50 min
Injection time	0.50 min

ISQ™ 7000 Single Quadrupole MS
TRACE™ 1300 GC
TriPlus 500 Headspace Autosampler

Time (min)	Rate (°C/min)	Target (°C)	Hold (min)
0.0	0	60	0.5
4.9	50.0	130	3.0
6.9	5.0	140	0.0
20.0	22.0	325	4.0

Column: ZB-5MSplus, 30m x 0.25 mm, x 0.25 µm
Carrier Gas (He): 1.5 mL/min (100:1 split)
Scan type: EI full scan (35 – 400 *m/z*)
ISQ Transfer Line/Source: 300/300 °C

UHPLC-ORBITRAP MASS SPECTROMETRY

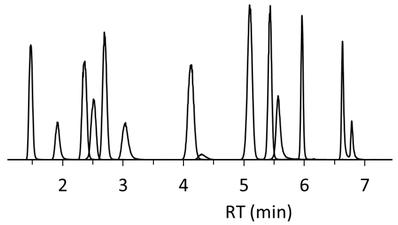
HESI-II Parameters	Values
Sheath/aux gas flow-rate	40/15
Sweep gas flow-rate	1
Spray voltage	3.50 kV
Capillary temp	320 °C
S-lens RF level	50.0
Aux gas heater temp	350 °C

Vanquish Flex UHPLC System
Q Exactive™ Orbitrap™ MS

Time (min)	% B
0.0	80
4.0	80
6.5	100
8.5	100
8.6	80

Column: Kinetex® C18 2.6 µm, 100 x 3.0 mm
Mobile phase A: 0.05% formic acid
Mobile phase B: 0.05% formic acid, MeOH
Flow-Rate: 750 µL/min (10.5 min total run time)
Ionization: +ve electrospray (HESI-II)

Q Exactive™ Parameters	Full-MS	dd-MS²
Resolution	70,000	17,500
AGC target	1e6	2e5/4e3
Maximum IT (ms)	250	50
Scan Range (m/z)	200 – 400	2.0
Loop Count	-	5
NCE	-	17.5, 35.0, 52.5
Dynamic Exclusion (s)	-	2.0



1: CBDV
2: CBDVA
3: CBG
4: CBD
5: THCV
6: CBDA
7: CBGA
8: CBN
9: Δ⁹-THC
10: Δ⁸-THC
11: THCVA
12: CBC
13: THCA
14: CBCA

Figure 2. Sample UHPLC-Orbitrap data for a 1 ppm solvent standard.

SAMPLE PREPARATION

HS-GC-MS

- 0.5g chocolate
- 5 mL glycerol

Heated ultrasonic

- 0.5g chocolate
- 10mL MeOH
- 50 °C with sonication and manual mixing
- 3,000 x g for 5min

RESULTS

HIGH-TEMPERATURE HEADSPACE

- Developed and validated high-temperature HS-GC-MS method for terpenes → used same HS parameters to evaluate cannabinoids in chocolate

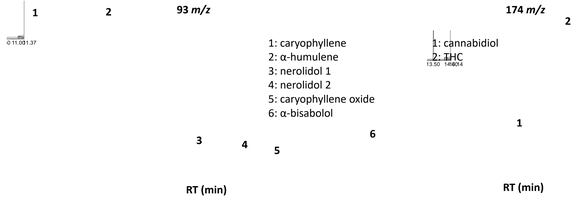


Figure 3. Extracted ion chromatograms demonstrating the detection of terpenes and cannabinoids from the same HS injection of cannabis plant material.

ADVANTAGES

88% recovery of CBD from chocolate
minimal sample preparation
terpenes in same injection as cannabinoids
sensitivity is fit-to-purpose

DRAWBACKS

no information on acidic cannabinoids
sensitivity may preclude cost-effective calibration
specialized instrumentation needed

HEATED ULTRASONIC EXTRACTION

- Melting the chocolate during extraction and winterizing the resulting methanolic extract to reduce the presence of co-extracted waxes
- Evaluated the impact of winterization time/temperature and ultrasonication time

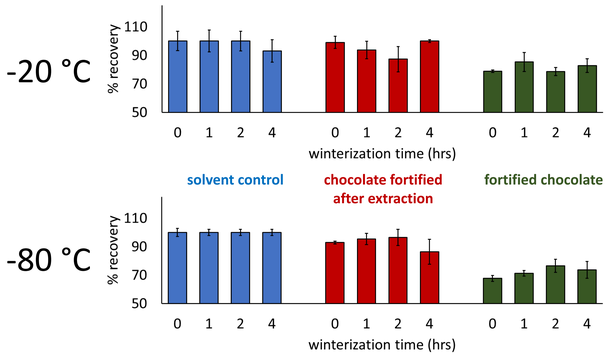


Figure 6. Effect of winterization time and temperature on the recovery of CBD from chocolates extracted for 40 min via heated (50 °C) ultrasonication. All data are shown ±1 SEM (*n* = 3/condition).

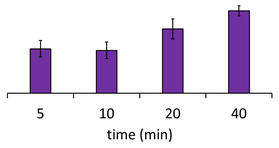
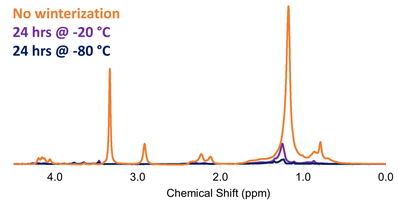


Figure 4. Relative recoveries of CBD from chocolates following ultrasonic-assisted extraction into MeOH @ 50 °C (*n* = 3/condition). Data are shown ±1 SEM.



- ^{1}H-NMR (60 MHz) demonstrated a decrease in the triglycerides that are co-extracted into MeOH following winterization
- -80 °C provides the most benefit, but -20 °C also effective

Figure 5. ^{1}H-NMR of residue in methanolic extracts before and after winterization (-20/-80 °C).

- No statistical differences between 0, 1, 2, 4 hrs winterization for a given temperature
- No evidence of ion suppression or recovery issues for solvent standard or chocolate extracts fortified after all sample processing

ADVANTAGES

removal of waxes that may prematurely foul analytical hardware
Increased throughput relative to GC-MS
leverages existing infrastructure in most labs

DRAWBACKS

longer sample preparation time
not possible to get information on terpenes in the same injection

CONCLUSIONS AND FUTURE WORKS

- CBD was extracted from fortified chocolates using both a high-temperature HS-GC-MS method and a heated ultrasonic UHPLC-Orbitrap method
- HS-GC-MS allowed for high recovery of CBD with low sample preparation, but will not allow for the analysis of acidic cannabinoids due to thermal decomposition in the GC inlet
- Heated ultrasonic UHPLC-Orbitrap was used to obtain high recoveries of CBD, in conjunction with winterization that removed co-extracted lipids (expected to improve UHPLC column life-time)
- In future:
  - Improve recoveries for heated ultrasonic extraction
  - Determine optimal winterization temperature
  - Validate an ultrasonic extraction method for submission to AOAC International

ACKNOWLEDGEMENTS

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