Selective and Sensitive Analysis of 20 Category I and II Residual Solvents for Cannabis Testing

Application using Headspace-GCMS

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1. Introduction

In the cannabis industry, many different solvents are used in the extraction process, each one classified based on the level of toxicity. Also, sample preparation is difficult with cannabis matrices due to their lack of solubility, and the limitation of available non-interferent diluent solvents. The ideal way for testing is direct analysis of the sample in a headspace vial without any sample preparation involved. However, this can be challenging if high sample amounts are required. Furthermore, other method development challenges exist, such as co-elution of several residual solvent analytes or mass-to-charge interferences from the environment. Finding an acceptable balance between proper separation for unequivocal identification and quantitation, and reasonable analysis throughput time can be complicated. The work described herein demonstrates the viability of headspace-GCMS with SIM mode for the analysis of twenty Category I and II residual solvents, including three soluble gases, which may be present in cannabis products.

2. Experimental Methods

A 6 point calibration curve was created from CPI Category I and II standards. An aliquot of 150 µL was placed on a 20 mL headspace vial and capped. Butyl Acetate was used as diluent solvent. Concentration ranges and calculated sample amounts are listed on Table 1. Quality Control standards were prepared by weighting ~250 mg of methyl cellulose (substituted matrix), and spiked with residual solvents standards to obtain a Level 5 concentration in the QC samples.

Table 1. Calculated Amount of Residual Solvent Calibration Curve Standards

Calibration Curve Standards	Concentration (µg/mL)	Volume in vial (mL)	Amount (µg)
Category I Residual Solvent			
Calibrator Level 1	0.781	0.150	0.117
Calibrator Level 2	1.563	0.150	0.234
Calibrator Level 3	3.125	0.150	0.469
Calibrator Level 4	6.25	0.150	0.938
Calibrator Level 5	12.5	0.150	1.875
Calibrator Level 6	25.0	0.150	3.75
Category II Residual Solvent			
Calibrator Level 1	312.5	0.150	46.875
Calibrator Level 2	625	0.150	93.75
Calibrator Level 3	1250	0.150	187.5
Calibrator Level 4	2500	0.150	375
Calibrator Level 5	5000	0.150	750
Calibrator Level 6	10000	0.150	1500

3. Analytical Conditions

Table 2. System Configuration and Instrument Parameters

Headspace	**HS-20 Loop Model**
Operation Mode	Static headspace with loop
Sample	150uL sample volume 20-mL headspace vial
Equilibration	15:00 minutes at 120°C
Sample Loop	0.2-mL Loop Vial pressure 350kP; Pressurizing Time-1.50 min Loop load time-0.20 min, equilibration 0.20 min Injection time 0.20min
Sample Line Temperature	150°C
Transfer Line Temperature	150°C
Gas Chromatograph	**GC-2010 Plus or 2030 NX**
Injection	Split injection from HS-20, with 50:1 split ratio
Column	Rxi-624 Sil MS 30.0m x 0.25 mm x 1.40 um Helium carrier gas Constant linear velocity, 39.9cm/sec Column Flow 1.24mL/min Purge flow 0.0mL/min
Oven Program	30°C, hold 3.0 min 10°C /min to 140°C, hold 0.0 min 45°C /min to 200°C , hold 1.0 min Total GC run time 16.33 min Total GC cycle time: 25.00 mins
Detector	**GCMS-QP2010 SE or 2020 NX**
Operation Mode	Selected Ion Monitoring Mode (SIM)
Ion Source	200°C, EI mode, 70eV
Solvent Cut Time	0.1 min
MS Interface	300°C

Figure 1. Shimadzu GCMS-QP2020 NX with HS-20 autosampler

4. Results

Figure 2. Left Panel - Calibration Curves for Category I Residual Solvents required by the state of California (CA), with representative SIM chromatograms for highest calibrator level for each solvent. Right Panel - Calibration Curves for Category II Residual Solvents required by the state of CA, with representative SIM chromatograms for highest calibrator level for each solvent.

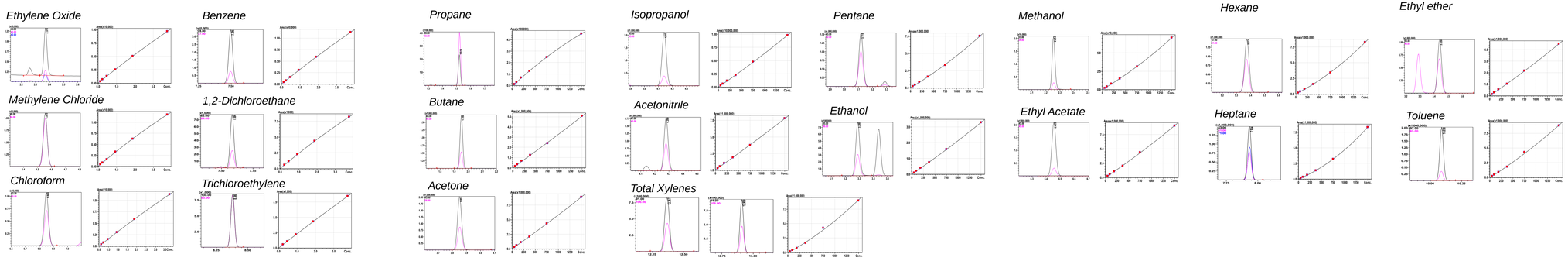

Figure 3. TIC chromatogram of 20 Residual Solvent standards (required in CA).

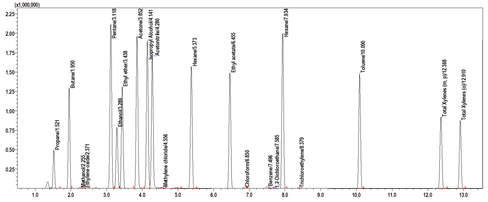

Figure 4. Zoomed-in TIC chromatogram of 20 Residual Solvent standards to show smaller solvent peaks.

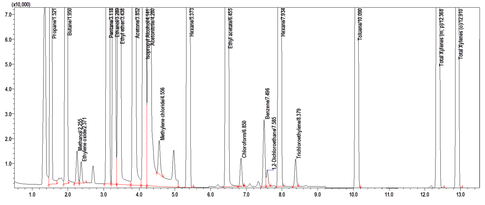

Table 3. Accuracy % and R^2 results for calibration curves.

Residual Solvents Calibration Curve Standards	Accuracy % (Criteria: 80-120% for Standards) Lowest Level in Cal Curve (ug)	Result (%)	Highest Level in Cal Curve (ug)	Result (%)	R^2 (Criteria: ≥ 0.99)
Propane	46.875	97.46	1500	100.40	0.9991
Butane	46.875	91.54	1500	100.61	0.9989
Methanol	46.875	92.07	1500	100.24	0.9996
Ethylene Oxide	0.117	93.56	3.75	100.15	0.9996
Pentane	46.875	91.19	1500	100.43	0.9993
Ethanol	46.875	94.71	1500	100.03	0.9997
Ethyl Ether	46.875	96.21	1500	99.95	0.9997
Acetone	46.875	94.54	1500	100.03	0.9996
Isopropanol	46.875	96.44	1500	99.85	0.9996
Acetonitrile	46.875	89.69	1500	100.47	0.9990
Methylene Chloride	0.117	99.04	3.75	100.07	0.9998
Hexane	46.875	91.67	1500	100.27	0.9996
Ethyl Acetate	46.875	97.59	1500	99.73	0.9994
Chloroform	0.117	100.12	3.75	100.00	1.0000
Benzene	0.117	99.58	3.75	99.99	0.9999
1,2-Dichloroethane	0.117	98.64	3.75	99.98	1.0000
Heptane	46.875	94.04	1500	100.10	0.9999
Trichloroethylene	0.117	100.67	3.75	99.93	0.9997
Toluene	46.875	104.01	1500	99.32	0.9984
Total Xylenes (m, p, and o)	46.875	109.97	1500	99.02	0.9964

Table 4. Accuracy % results for spiked QC samples.

Residual Solvents Quality Control Standards	Accuracy % (Criteria: 70-130% for spiked QC's) ICV (level 5; %)	CCV (level 5, %)	LCS (level 5; %)	LRS (level 5; %)
Propane	109.27	106.68	105.23	101.03
Butane	109.68	106.53	105.83	101.22
Methanol	109.11	105.78	88.00	83.33
Ethylene Oxide	106.61	101.09	94.18	87.46
Pentane	107.83	104.79	104.84	100.93
Ethanol	107.19	104.95	88.56	83.77
Ethyl Ether	104.80	101.74	100.72	97.26
Acetone	104.63	103.22	97.54	93.28
Isopropanol	104.34	102.80	92.49	88.08
Acetonitrile	108.30	107.54	93.25	88.30
Methylene Chloride	102.65	99.70	94.46	90.08
Hexane	106.91	104.14	106.12	102.74
Ethyl Acetate	100.91	100.51	100.27	96.56
Chloroform	100.92	99.77	96.68	93.66
Benzene	100.25	99.27	98.73	95.10
1,2-Dichloroethane	98.80	97.66	96.95	92.56
Heptane	102.68	101.70	108.45	105.40
Trichloroethylene	101.75	99.05	100.95	97.05
Toluene	95.80	96.28	99.92	96.64
Total Xylenes (m, p, and o)	91.29	92.71	98.08	95.36

5. Conclusions

- In this poster, a GCMS method using Selected Ion Monitoring (SIM) with headspace injection was developed for the analysis of cannabis concentrates. This includes the identification and use of appropriate standard mixes and diluent solvents for simple preparation of calibration curves, as well as method optimization to eliminate or minimize co-elution and ambient interferences for maximum sensitivity and accuracy in quantitation, all without compromising run time. Moreover, method parameters and hardware configuration changes were performed to accommodate high sample size requirements for compliant testing. These were conducted to avoid analytical column overloading and MS detector saturation, while directly testing sample without preparation steps.
- Results showed good coefficient of determination and accuracy % for calibrator standards. The curve fit type used is Quadratic with a 1/C weighting regression. All QC samples (Initial Calibration Verification, Continuing Calibration Verification, Laboratory Control Sample, and Laboratory Replicate Sample) showed good accuracy %.

6. References

- "Analysis of Residual Solvents – Class 1, Class 2A, Class 2B in Pharmaceuticals using Headspace-GC/MS", Shimadzu's application note.
- "A Fast, Simple FET Headspace GC-FID Technique for Determining Residual Solvents in Cannabis Concentrates", Restek's application note.
- "Protocol for Quantitative Determination of Residual Solvents in Cannabis Concentrates", Restek's protocol.
- Current BCC Cannabis Regulations (January 2019), https://www.bcc.ca.gov/law_regs/cannabis_order_of_adoption.pdf