Application Note

Cannabis Testing

Agilent

Trusted Answers

Novel Residual Solvents Analysis of Cannabinoid Products with the Agilent Headspace-GC/MS System

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Abstract

Residual solvent analysis (RSA) of cannabinoid products made available for medicinal or recreation use programs is required to ensure the safety of the products before retail distribution. Testing of residual solvents in pharmaceutical products has been performed for decades and is well defined in method USP <467>. However, cannabinoid products differ greatly from pharmaceuticals, especially in the varied nature of the products themselves (oils, tinctures, concentrates, edibles, etc.), and the methodological approach to analysis must reflect these differences. Therefore, it must be stated as a matter of fact that RSA of cannabinoid products is not akin to USP <467>. This study defined a unique analytical approach specific to cannabinoid products for the analysis of residual solvents. Our work identified that sample preparation is critical for success and determined the conditions to optimize accuracy and precision. We further defined novel static headspace-GC/MS parameters to establish a methodological robustness that meets the needs of high-throughput cannabis laboratories. Intra-day and inter-day limits of detection (LOD) as defined by method detection limits (MDL), limits of quantitation (LOQ), accuracy and precision, and range and linearity were determined for the target analytes. Our results demonstrated that this analytical procedure is robust, repeatable, and exceeds regulatory requirements as defined by the California Bureau of Cannabis Control (BCC).

Introduction

A robust analytical method for residual solvents in cannabis extracts is necessary for the emerging markets of medicinal and recreational cannabis where approved by local legislation. RSA is typically performed in the pharmaceutical industry using headspace GC with flame ionization detection (FID) or headspace GC/mass spectrometry (HS-GC/MS). Like USP <467>, this analysis requires HS-GC/MS systems to identify and verify that residual solvents are not present in the products in quantitative concentrations that exceed regulated limits. However, unlike the pharmaceutical industry, the hemp and cannabis industries are using different solvents to extract cannabinoids from cannabis and hemp plant material. In our work, it was quickly realized that: 1) USP <467> cannot be used for the analysis of residual solvents in the myriad cannabinoid products; 2) sample preparation is critical – one cannot simply weigh a product into an auto-sampler vial and cap it for analysis; and 3) the use of selected ion monitoring (SIM) facilitated the linear dynamic range needed to measure multiple target analytes over a concentration range of 0.15 to 20 ppm for Category I solvents and 20 to 6,000 ppm for Category II solvents as defined by California regulations. Another critical observation was, by using the Agilent Technologies backflush technique, the method run-time was greatly reduced, fitting the needs of high-throughput laboratories that have moved to liquid injection gas phase methodologies for the analysis of terpenes.

Materials and methods

Hardware and software

An Agilent 7697A Headspace sampler configured with a 0.5 mL sample loop was plumbed to the Intuvo 9000 gas chromatograph (G3950A) configured with a Capillary Flow Technology Mid-Column Backflush Flow Chip (option #881), a MultiMode Inlet (MMI), and Guard Chip (G4587-60665). Please note, a split/split-less inlet (S/SL) can be used as an alternative (the Guard Chip part number for the S/SL is G4587-60565). The Intuvo 9000 system included the XLSI weldment (G3969A) for side mount of the 7697A transfer line and liquid injection capabilities through the same inlet. A 4 mm Ultra Inert, low pressure drop, glass wool split liner (5190-2295) and two DB-Select 624 Ultra Inert columns (30 m × 0.25 mm id × 1.4 µm film thickness, 122-0334UI-INT) were used for all analyses. The GC system was connected to a 5977B Mass Selective Detector (MSD) with an Extractor EI Ion Source (G7077BA) and a 9 mm Extractor Lens. Data were collected using Agilent MassHunter Workstation – Acquisition B.10 SR1 GC/MS software. All data analyses were performed using MassHunter Workstation – Quantitative Analysis B.10.1 GC/MS software. Tables 1 to 4 define the system parameters.

Table 1. Agilent 7697A headspace autosampler parameters.

Parameter	Value
Oven Temp	80 °C
Loop Temperature	80 °C
Transfer Line	140 °C
Vial Equilibration Time	12 minutes
Injection Volume	0.5 mL (loop)
Injection Time	1.00 minute
GC Cycle Time	20.00 minutes
Vial Shake	Level 2, 25 shakes/min
Fill Mode	Default
Fill Pressure	15 psi
Loop Fill	Default
Extraction Mode	Single
Post Injection Purge	200 mL/min for 3 minutes
Acceptable Leak Check	0.2 mL/min

Table 2. Agilent Intuvo 9000 GC parameters.

Parameter	Value
Inlet Temperature (MMI or S/SL)	200 °C
Guard Chip Temperature	200 °C
XLSI Temperature	200 °C
Flow Rate Column 1	1.4 mL/min
Flow Rate Column 2	1.6 mL/min
Initial Oven Temperature	35 °C
Initial Hold Time	1 minute
Ramp Rate 1	15 °C/min
Final Temperature	180 °C
Hold Time	0 minutes
Ramp Rate 2	30 °C/min
Final Temperature 2	240 °C
Final Hold	0.333 minutes
Total Run Time	13.0 minutes
Post Run Backflush	1.9155 minutes
Split Ratio	150:1

Table 3. Agilent 5977B MS parameters.

Parameter	Value
Solvent Delay	3 minutes
Acquisition Mode	SIM
EM Setting mode Gain	10
Source Temperature	250 °C
Quadrupole Temperature	200 °C
Trace Ion Detection	On
MS Tuning	Autotune
Tune File	Atune.u
Number of SIM Groups	17
Run Time (if MS Only)	10 minutes

Table 4. MS SIM parameters.

SIM Group	Mass, Dwell Groups
Group 1 Group ID: Propane Gain: 10	
Resolution: 1	
Group Start Time: 3.8	
Number of Ions: 5 Ions	
Dwell In Group: (Mass Dwell)	(29.00 40) (31.00 40) (41.00 40) (43.00 40) (44.00 40)
Group 2 Group ID: Butane Gain: 10	
Resolution: 1	
Group Start Time: 4.6	
Number of Ions : 3 Ions	
Dwell In Group: (Mass Dwell)	(41.00 60) (43.00 60) (58.00 60)
Group 3 Group ID: Methanol Gain: 20	
Resolution: 1	
Group Start Time: 4.9	
Number of Ions: 3 Ions	
Dwell In Group: (Mass Dwell)	(29.00 60) (31.00 60) (32.00 60)
Group 4 Group ID: Ethylene oxide Gain: 20	
Resolution: 1	
Group Start Time : 5.30	
Number of Ions : 2 Ions	
Dwell In Group :(Mass Dwell)	(29.00 90) (44.00 90)
Group 5 Group ID: *n*-Pentane + ethanol Gain: 15	
Resolution: 1	
Group Start Time: 5.65	
Number of Ions: 6 Ions	
Dwell In Group: (Mass Dwell)	(31.00 30) (43.00 30) (45.00 30) (46.00 30) (57.00 30) (72.00 30)
Group 6 Group ID: Ethyl ether Gain: 10	
Resolution: 1	
Group Start Time: 5.92	
Number of Ions: Ions	
Dwell In Group: (Mass Dwell)	(45.00 60) (59.00 60) (74.00 60)
Group 7 Group ID: Acetone + IPA Gain: 10	
Resolution: 1	
Group Start Time: 6.2	
Number of Ions: 4 Ions	
Dwell In Group: (Mass Dwell)	(43.00 50) (45.00 50) (58.00 50) (59.00 50)
Group 8 Group ID: Acetonitrile Gain: 15	
Resolution: 1	
Group Start Time: 6.55	
Number of Ions: 3 Ions	
Dwell In Group: (Mass Dwell)	(40.00 60) (41.00 60) (78.00 60)
Group 9 Group ID: Methylene chloride Gain: 10	
Resolution: 1	
Group Start Time: 6.75	
Number of Ions: 3 Ions	
Dwell In Group: (Mass Dwell)	(49.00 60) (84.00 60) (86.00 60)
Group 10 Group ID: *n*-Hexane Gain: 10	
Resolution: 1	
Group Start Time: 7.2	
Number of Ions: 3 Ions	
Dwell In Group: (Mass Dwell)	(43.00 60) (57.00 60) (86.00 60)
Group 11 Group ID: Ethyl acetate Gain: 10	
Resolution: 1	
Group Start Time: 7.8	
Number of Ions: 3 Ions	
Dwell In Group: (Mass Dwell)	(43.00 60) (61.00 60) (88.00 60)

SIM Group	Mass, Dwell Groups
Group 12 Group ID: Chloroform Gain: 10	
Resolution: 1	
Group Start Time: 8.15	
Number of Ions: 3 Ions	
Dwell In Group: (Mass Dwell)	(83.00 60) (85.00 60) (87.00 60)
Group 13 Group ID: Benzene - 1, 2-DCA, *n*-Heptane Gain: 10	
Resolution: 0	
Group Start Time: 8.70	
Number of Ions: 7 Ions	
Dwell In Group: (Mass Dwell)	(43.00 25) (51.00 25) (57.00 25) (62.00 25) (64.00 25) (78.00 25) (100 25)
Group 14 Group ID: Trichloroethene Gain: 10	
Resolution: 1	
Group Start Time: 9.30	
Number of Ions: 3 Ions	
Dwell In Group: (Mass Dwell)	(95.00 60) (130.00 60) (132.00 60)
Group 15 Group ID: Trifluorotoluene (ISTD) Gain: 10	
Resolution: 0	
Group Start Time: 9.50	
Number of Ions: 2 Ions	
Dwell In Group: (Mass Dwell)	(127 90) (146 90)
Group 16 Group ID: Toluene Gain: 5.0	
Resolution: 1	
Group Start Time: 10.25	
Number of Ions: 2 Ions	
Dwell In Group: (Mass Dwell)	(91.00 90) (92.00 90)
Group 17 Group ID: *m* + *p*-Xylene Gain: 10	
Resolution: 1	
Group Start Time: 11.5	
Number of Ions: 2 Ions	
Dwell In Group: (Mass Dwell)	(91.00 90) (106.00 90)
Group 18 Group ID: *o*-Xylene Gain: 10	
Resolution: 1	
Group Start Time: 12.35	
Number of Ions: 2 Ions	
Dwell In Group: (Mass Dwell)	(91.00 90) (106.00 90)

Chemicals

Unless otherwise stated, use high-purity grade chemicals listed below:

- N,N-Dimethylacetamide (DMA)
- Sodium chloride (reagent grade)
- α,α,α-Trifluorotoluene (TFT)
- Organic-free water
- Food-grade hemp oil, cannabis extract or equivalent, free of residual solvents
- Residual Solvent standards

Table 5. Class 1 Standards – CPI International #Z G34-115300-03.

Standard	Concentration (µg/mL)
Ethylene oxide	100
Methylene chloride	100
Benzene	100
1,2-Dichloroethane	100
Chloroform	100
Trichloroethylene	100

Table 6. Class 2 Standards – CPI International #Z G34-115301-02.

Standard	Concentration (µg/mL)
Propane	10,000
Butane or Isobutane	10,000
n-Pentane	10,000
Acetone	10,000
Acetonitrile	10,000
Hexane	10,000
Ethyl acetate	10,000
Heptane	10,000
Methanol	10,000
Diethyl ether	10,000
Ethanol	10,000
Isopropanol (2-Propanol)	10,000
Toluene	10,000
m+*p*-Xylene	5,000
o-Xylene	5,000

Data collection

A total of three independent datasets were collected over multiple days. Each dataset was comprised of quintuplicate injections of solvent blanks, matrix blanks, and eight levels of calibrators ranging from 20 ppm through approximately 6,000 ppm for Category II solvents, and 0.15 ppm through approximately 20 ppm for Category I solvents. Each sample, calibrator, etc. contained TFT as an internal standard (ISTD). A separate MDL study was performed, which entailed collecting three individual datasets of eight replicate injections at the low calibrator concentrations for each compound.

Statistics

- Average = $\Sigma x_i/n$
- Standard deviation, $s = \left[\frac{\Sigma(x - \bar{x})^2}{n - 1}\right]^{1/2}$
- MDL (LOD) = (s) × (student t-value, n – 1 df, 99% confidence)
- MDL (LOD) test = calculated MDL <spike level <10 × calculated MDL
- LOQ = 10 × (s)
- Percent accuracy = [(spiked concentration – calculated average concentration/spiked concentration)] × 100
- Precision, (%RSD) = [(s)/Average] × 100

Preparation of α,α,α-trifluorotoluene internal standard (TFT ISTD).

1. Dilute 27 µL of neat TFT in 100 mL of dimethylacetamide (DMA) for a concentration of 321 µg/mL.
2. Aliquot 20 mL of solution in Step 1 and dilute to 1,000 mL with DMA for a final ISTD concentration of 6.42 µg/mL. In 5 mL of this solution there is 32.1 µg/g TFT. Prepare all samples using this solution.

Preparation of DMA-TFT ISTD sample matrix

Carefully weigh 2.5 g ±0.10 g of extract or product into a 20 mL scintillation vial and dissolve in 10 mL of DMA containing TFT ISTD as described above. Accurately record the weight of sample transferred to the vial. In this study, food-grade hempseed oil was used as the matrix for all calibrators and matrix blanks. Alternative blank matrices can also be used if they do not contain any of the target solvents. Our choice of hemp seed oil as the blank matrix fits this criterion. Transfer the solution to a 50 mL volumetric flask. Rinse the vial with another 10 mL of DMA-TFT ISTD and transfer it to the 50 mL volumetric containing the initial solution. Repeat this step with a third 10 mL aliquot of DMA-TFT ISTD and transfer to the same 50 mL volumetric. Dilute to volume with DMA-TFT ISTD and mix thoroughly. Ensure that all the material dissolves before removing an aliquot for analysis. Five milliliters of this preparation represents 0.25 g of the sample. Example: For 0.25 g sample prepared as described above, the resulting ISTD concentration is 32.1 µg/0.25 g = 128.4 µg/g. Additional dilutions of this initial preparation can be made, if necessary, before analysis if the results for any target compound are outside of the existing calibration range.

Preparation of saturated saline solution

In a 1 L volumetric flask, 900 mL of organic free water and 360 g of NaCl was added. The mixture was gently shaken, and organic-free water was added to the 1 L mark. A stir bar was placed in the flask and the flask was placed on a stir plate at ambient temperature. The mixture was stirred for 30 minutes. The solution was allowed to settle and the contents were decanted into a 1 L glass bottle.

Sample, calibrator/QC preparation

Add 5 mL DMA-TFT ISTD solution, 1.0 mL of saturated saline, and 0.20 mL of each calibration standard, QC, etc., to a 20 mL headspace vial. If gas standards are used, it is recommended to add the calibration standards, QCs etc., through the sealed vial.

Serial dilutions of calibration standards

Depending on the number of standards, the volume necessary may change. Calibration level 8 is prepared by adding the correct aliquots from each mixture. For example, if the volume for level 8 is 1.0 mL, then 0.5 mL is taken from level 8 and added to 0.5 mL of DMA in a second vial to produce 1.0 mL of level 7. Repeating this step again using level 7 will produce level 6, and so on. Table 7 provides the starting concentrations and the serial dilution procedures for the target analytes defined by BCC.[2]

Agilent consumables

- Sample loop 0.5 mL (G4556-80105)
- Two DB Select 624 UI columns (30 m × 0.25 mm, 1.4 µm for Intuvo (122-0334UI-INT)
- XLSI connector (G3520-20210)
- 9 mm GC/MS extractor lens (G3870-20449)
- Intuvo compression bolts (G4581-60260)
- Intuvo MMI Guard Chips (G4587-60665)
- Polyimide gaskets (5190-9072)
- Ultra Inert, low pressure drop, glass wool split liner (5190-2295)

Ancillary equipment

- 20 mm vial crimper and 20 mm vial decapper
- 10, 25, 50, and 100 mL class "A" volumetric flasks
- 10, 25, 100, 250, and 1,000 µL gas-tight syringes
- 15 and 20 mm vial caps configured with MinInert valves
- 1, 2, and 3 mL clear graduated micro reaction vials
- 1.0 and 5.0 mL glass disposable serological pipets
- Manual pump pipettor
- Re-pipette bottle top organic solvent dispenser with 1 to 10 mL capacity
- Analytical balance capable of weighing to 0.001 g
- Laboratory freezer for storing chemical standards
- 20 mm borosilicate glass headspace vials with crimp caps
- Disposable 20 mL liquid scintillation vials or equivalent
- 4 mL amber glass vials with screw caps for storing standards

Results and discussion

At the outset of this study, it was quickly determined that traditional USP <467> was not appropriate. Most cannabinoid products are not single active pharmaceutical ingredient (API) pills, injectables, or presented in other common pharmaceutical formats. Cannabinoid products can be edibles, teas, gummies, oils, tinctures, concentrates, shatter, etc. Moreover, cannabinoid products quite commonly have myriad chemicals beyond that of the cannabinoids. These may include natural or added levels of terpenes, polyphenols, fatty acids, steroids,

Table 7. Preparation of calibration levels using serial dilution.

Analytes	Conc.	Mix Conc.	Volume Taken	Sample High Calibrator Level Preparation			Cal_07 ppm	Cal_06 ppm	Cal_05 ppm	Cal_04 ppm	Cal_03 ppm	Cal_02 ppm	Cal_01 ppm
Z-G34-115301-02	(µg/mL)	(µg/mL)	(mL)	Amt. (µg)	Wt. (g)	Cal_08 ppm							
Acetone	10120	7590	0.2	1518.0	0.25	6072.0	3036.0	1518.0	759.0	379.5	189.8	94.9	47.4
Acetonitrile	10060	7545	0.2	1509.0	0.25	6036.0	3018.0	1509.0	754.5	377.3	188.6	94.3	47.2
n-Butane	9999	7499	0.2	1499.9	0.25	5999.4	2999.7	1499.9	749.9	375.0	187.5	93.7	46.9
Ethanol	10050	7538	0.2	1507.5	0.25	6030.0	3015.0	1507.5	753.8	376.9	188.4	94.2	47.1
Ethyl acetate	10110	7583	0.2	1516.5	0.25	6066.0	3033.0	1516.5	758.3	379.1	189.6	94.8	47.4
Diethyl ether	10020	7515	0.2	1503.0	0.25	6012.0	3006.0	1503.0	751.5	375.8	187.9	93.9	47.0
n-Heptane	10050	7538	0.2	1507.5	0.25	6030.0	3015.0	1507.5	753.8	376.9	188.4	94.2	47.1
2-Propanol	10110	7583	0.2	1516.5	0.25	6066.0	3033.0	1516.5	758.3	379.1	189.6	94.8	47.4
Methanol	10020	7515	0.2	1503.0	0.25	6012.0	3006.0	1503.0	751.5	375.8	187.9	93.9	47.0
n-Hexane	10090	7568	0.2	1513.5	0.25	6054.0	3027.0	1513.5	756.8	378.4	189.2	94.6	47.3
N-Pentane	10030	7523	0.2	1504.5	0.25	6018.0	3009.0	1504.5	752.3	376.1	188.1	94.0	47.0
Propane	10000	7500	0.2	1500.0	0.25	6000.0	3000.0	1500.0	750.0	375.0	187.5	93.8	46.9
Toluene	10070	7553	0.2	1510.5	0.25	6042.0	3021.0	1510.5	755.3	377.6	188.8	94.4	47.2
m+p-Xylene	5050	3788	0.2	757.5	0.25	3030.0	1515.0	757.5	378.8	189.4	94.7	47.3	23.7
o-Xylene	5050	3788	0.2	757.5	0.25	3030.0	1515.0	757.5	378.8	189.4	94.7	47.3	23.7
Analytes	Conc.	Mix Conc.	Volume Taken	Sample High Calibrator Level Preparation			Cal_07 ppm	Cal_06 ppm	Cal_05 ppm	Cal_04 ppm	Cal_03 ppm	Cal_02 ppm	Cal_01 ppm
Z-G34-115300-03	(µg/mL)	(µg/mL)	(mL)	Amt. (µg)	Wt. (g)	Cal_08 ppm							
1,2-Dichloroethane	100.6	23.5	0.2	4.7	0.25	18.78	9.39	4.69	2.35	1.17	0.59	0.29	0.15
Benzene	100.7	23.5	0.2	4.7	0.25	18.80	9.40	4.70	2.35	1.17	0.59	0.29	0.15
Chloroform	100.3	23.4	0.2	4.7	0.25	18.72	9.36	4.68	2.34	1.17	0.59	0.29	0.15
Ethylene oxide	100.5	23.5	0.2	4.7	0.25	18.76	9.38	4.69	2.35	1.17	0.59	0.29	0.15
Trichloroethene	100.3	23.4	0.2	4.7	0.25	18.72	9.36	4.68	2.34	1.17	0.59	0.29	0.15
Methylene chloride	100.8	23.5	0.2	4.7	0.25	18.82	9.41	4.70	2.35	1.18	0.59	0.29	0.15

alkanes, alcohols, diglycerides, and triglycerides just to name a few. This complexity requires a wholistic approach to residual solvent testing specific to cannabinoid products. The methodology begins with sample preparation, leverages unique analytical tools such as Agilent Capillary Flow Technologies to enable backflush, and uses column dimensions and chemistries to facilitate fast, high-resolution chromatography, as shown in Figures 1 and 2.

The primary goal of the method development was to ensure robustness and repeatability. To this end, the experimental design was broken into two objectives. For each target analyte:

- Determine intra-day and inter-day method detection limits (MDL) which defined the limit of detection (LOD)
- Determine intra-day and inter-day accuracy, precision, working range, and linearity

LOD and LOQ determinations

To meet BCC regulations, it is imperative that the LOD is empirically determined on each instrument in each laboratory for the defined Category I solvents. Any Category I solvent detected in an unknown sample is actionable and must be reported as a failure of the product in the Certificate of Analysis (CoA). To determine the LOD for all targets in the BCC list, we collected data in three independent studies of eight replicate injections over three days at low calibrator levels prepared in hempseed oil matrix. The intra-day and inter-day LODs were calculated statistically with a T-statistic of 2.998 for n – 1 degrees of freedom at the 99% confidence level. The calculated LOD was further subjected to a MDL (LOD) test to ensure that the proper calibrator concentrations were used for the determination. A value of TRUE indicates a passing result. BCC has defined LOQ action limits for all Category II solvents. LOQs for each analyte were determined statistically from this dataset using (10 × standard deviation) for both the intra-day and inter-day data. Intra-day and inter-day precision were also determined in this dataset as %RSD. Table 8 illustrates these results. For day 1 of the intra-day results, variability was observed for several targets. The cause of this variability was determined to be the result of an exothermic reaction initiated by the addition of saturated saline to DMA. Therefore, before addition of the sample, the exotherm must be allowed to cool for approximately 60 minutes. This new procedure was used for Days 2 and 3.

Table 8. LOD as defined by MDL, LOQ, and precision as defined by %RSD.

Compound Information			Intra-Day (Day 1, n = 8)			Intra-Day (Day 2, n = 8)			Intra-Day (Day 3, n = 8)			Inter-Day (n = 24)			
Name	Category	RT	MDL	LOQ	Conc. %RSD	MDL	LOQ	Conc. %RSD	MDL	LOQ	Conc. %RSD	Average MDL	MDL Test	Average LOQ	Average Conc. %RSD
n-Propane	II	4.116	32.35	107.90	6.70	33.79	112.70	6.80	49.77	166.01	7.80	38.63	TRUE	128.87	7.10
n-Butane	II	4.727	34.69	115.73	6.20	17.72	59.10	3.00	34.93	116.52	5.30	29.12	TRUE	97.12	4.83
Methanol	II	5.003	59.58	198.74	7.50	16.82	56.12	1.80	27.87	92.97	3.00	34.76	TRUE	115.94	4.10
Ethylene Oxide	I	5.207	0.19	0.65	14.20	0.29	0.98	13.20	0.12	0.42	5.80	0.20	TRUE	0.68	11.07
n-Pentane	II	5.821	47.30	157.78	6.50	17.72	59.10	2.60	35.23	117.51	4.90	33.42	TRUE	111.46	4.67
Ethanol	II	5.839	68.99	230.12	8.60	20.19	67.33	2.40	17.21	57.41	2.00	35.46	TRUE	118.29	4.33
Ethyl Ether	II	6.024	52.84	176.27	6.70	16.31	54.41	2.00	27.07	90.29	3.30	32.08	TRUE	106.99	4.00
Acetone	II	6.341	47.69	159.09	5.80	20.29	67.69	2.40	30.44	101.53	3.60	32.81	TRUE	109.44	3.93
Isopropyl Alcohol	II	6.415	65.44	218.30	8.00	18.79	62.66	2.20	14.76	49.24	1.70	33.00	TRUE	110.07	3.97
Acetonitrile	II	6.631	46.47	154.99	5.50	21.68	72.31	2.30	34.21	114.13	3.50	34.12	TRUE	113.81	3.77
Methylene Chloride	I	6.832	0.30	0.99	12.90	0.19	0.62	2.30	0.23	0.77	2.80	0.24	TRUE	0.79	6.00
n-Hexane	II	7.330	59.39	198.11	7.10	37.05	123.59	4.20	47.71	159.16	5.20	48.05	TRUE	160.29	5.50
Ethyl Acetate	II	7.970	44.31	147.79	5.40	19.75	65.86	2.30	21.06	70.26	2.40	28.37	TRUE	94.64	3.37
Chloroform	I	8.313	0.11	0.37	4.60	0.10	0.33	3.50	0.26	0.87	9.30	0.16	TRUE	0.52	5.80
Benzene	I	8.858	0.19	0.63	10.10	0.11	0.37	3.90	0.21	0.72	7.00	0.17	TRUE	0.57	7.00
1,2-Dichloroethane	I	8.870	0.16	0.55	7.40	0.47	1.57	18.50	0.33	1.09	13.30	0.32	TRUE	1.07	13.07
n-Heptane	II	8.987	56.69	189.10	7.60	48.22	160.84	6.80	59.48	198.41	8.00	54.80	TRUE	182.78	7.47
Trichloroethene	I	9.408	0.23	0.78	10.40	0.20	0.68	8.70	0.17	0.56	7.30	0.20	TRUE	0.67	8.80
Toluene	II	10.596	13.22	44.10	1.50	24.61	82.07	2.70	17.01	56.74	1.90	18.28	TRUE	60.97	2.03
m+p-Xylene	II	12.107	77.38	258.10	18.30	14.18	47.29	3.30	8.99	29.98	2.10	33.51	TRUE	111.79	7.90
o-Xylene	II	12.481	115.20	384.27	27.60	15.41	51.40	3.60	9.47	31.60	2.20	46.69	TRUE	155.75	11.13

Accuracy and precision

To determine the LOD for all targets in the BCC list, we collected three independent batches of eight calibrator levels prepared in hempseed oil matrix. Each batch was collected over the course of three days and designated P2, P3, and P4, respectively. Each calibrator level in each batch was injected five times. The intra- and inter-day accuracy was determined. Percent accuracy acceptability criteria were defined as an average percent accuracy greater than 80% and less than 120%. The intra-day batch precision was determined as %RSD.

As shown in Table 9, the toluene accuracy in batch P2 was extremely low (26%), causing it to fall outside the acceptable range of 80% <% accuracy <120%. A Grubb's test determined that although 26% is furthest from 83% and 87%, it was not a significant outlier (P >0.01) with a critical value of Z = 1.15. Therefore, that data point was maintained in the dataset. As noted above, this variability was determined to be the result of an exothermic reaction.

If we remove batch P2 due to the variability from the exotherm observed only after preparing the batch, Table 10 illustrates excellent accuracy and precision. The accuracy range was 85% for toluene and 110 for *n*-hexane. The %RSD was <5 for most of the target solvents. Only *n*-butane (5.95), *n*-pentane (5.95), and methylene chloride (12.6) exceeded a %RSD >5.

Range and linearity

Using intra-day batch results, the range and linearity of the compounds were determined and are shown in Table 10. Category I solvents that must be reported for any concentration greater than the LOD cover a range two orders of magnitude lower than the Category II solvents. These data were further used to determine the curve type,

Table 9. Intra-day and inter-day accuracy and precision.

Sample Information			Intra-Day Average % Accuracy (n = 5)			Inter-Day % Accuracy (n = 15)			
Name	LOQ	Calibrator Level	Ave % P2	Ave % P3	Ave % P4	80 <Average <120 (n = 15)	SD (n = 15)	SE (n = 15)	%RSD (n = 15)
n-Propane	128.87	2	74	94	92	86.67	11.02	6.36	12.71
n-Butane	97.12	2	81	99	91	90.33	9.02	5.21	9.98
Methanol	115.94	2	101	101	103	101.67	1.15	0.67	1.14
Ethylene Oxide	0.68	3	89	103	106	99.33	9.07	5.24	9.13
n-Pentane	111.46	2	107	99	91	99.00	8.00	4.62	8.08
Ethanol	118.29	2	123	103	102	109.33	11.85	6.84	10.83
Ethyl Ether	106.99	2	81	101	95	92.33	10.26	5.93	11.12
Acetone	109.44	2	87	100	99	95.33	7.23	4.18	7.59
Isopropyl Alcohol	110.07	2	97	101	100	99.33	2.08	1.20	2.10
Acetonitirile	113.81	2	89	98	103	96.67	7.09	4.10	7.34
Methylene Chloride	0.79	3	110	92	110	104.00	10.39	6.00	9.99
n-Hexane	160.29	3	81	110	109	100.00	16.46	9.50	16.46
Ethyl Acetate	94.64	2	70	99	98	89.00	16.46	9.50	18.50
Chloroform	0.52	3	95	100	97	97.33	2.52	1.45	2.59
Benzene	0.57	3	96	104	100	100.00	4.00	2.31	4.00
1,2-Dichloroethane	1.07	4	101	101	99	100.33	1.15	0.67	1.15
n-Heptane	182.78	3	71	109	109	96.33	21.94	12.67	22.77
Trichloroethene	0.67	3	87	95	90	90.67	4.04	2.33	4.46
Toluene	60.97	1	26	83	87	65.33	34.12	19.70	52.23
m+p-Xylene	111.79	2	86	98	96	93.33	6.43	3.71	6.89
o-Xylene	155.75	4	106	104	104	104.67	1.15	0.67	1.10

Table 10. Intra-day and inter-day accuracy and precision with batch P2 removed.

Sample Information			Intra-Day Average % Accuracy (n = 5)		Inter-Day % Accuracy (n = 15)			
Name	LOQ	Calibrator Level	Ave % P3	Ave % P4	80 <Average <120 (n = 15)	SD (n = 15)	SE (n = 15)	%RSD (n = 15)
n-Propane	128.87	2	94	92	93	1.41	1.00	1.52
n-Butane	97.12	2	99	91	95	5.66	4.00	5.95
Methanol	115.94	2	101	103	102	1.41	1.00	1.39
Ethylene Oxide	0.68	3	103	106	105	2.12	1.50	2.03
n-Pentane	111.46	2	99	91	95	5.66	4.00	5.95
Ethanol	118.29	2	103	102	103	0.71	0.50	0.69
Ethyl Ether	106.99	2	101	95	98	4.24	3.00	4.33
Acetone	109.44	2	100	99	100	0.71	0.50	0.71
Isopropyl Alcohol	110.07	2	101	100	101	0.71	0.50	0.70
Acetonitirile	113.81	2	98	103	101	3.54	2.50	3.52
Methylene Chloride	0.79	3	92	110	101	12.73	9.00	12.60
n-Hexane	160.29	3	110	109	110	0.71	0.50	0.65
Ethyl Acetate	94.64	2	99	98	99	0.71	0.50	0.72
Chloroform	0.52	3	100	97	99	2.12	1.50	2.15
Benzene	0.57	3	104	100	102	2.83	2.00	2.77
1,2-Dichloroethane	1.07	4	101	99	100	1.41	1.00	1.41
n-Heptane	182.78	3	109	109	109	0.00	0.00	0.00
Trichloroethene	0.67	3	95	90	93	3.54	2.50	3.82
Toluene	60.97	1	83	87	85	2.83	2.00	3.33
m+p-Xylene	111.79	2	98	96	97	1.41	1.00	1.46
o-Xylene	155.75	4	104	104	104	0.00	0.00	0.00

Table 11. Intra-day range, linearity and regression statistics.

Name	Range (ppm)	Number of Calibrators	Curve Type	Weighting	Regression	Linearity (R^2)
n-Propane	47 to 6000	8	Linear	1/x	y = 0.0288x – 1.116	0.9915
n-Butane	47 to 6000	8	Linear	1/x	y = 0.00942 – 0.195	0.9972
Methanol	47 to 6000	8	Linear	1/x	y = 0.00221 + 0.130	0.9996
Ethylene Oxide	0.29 to 19.00	7	Linear	1/x	y = 0.0144 – 7.436E-5	0.9981
n-Pentane	47 to 6000	8	Linear	1/x	y = 0.0108 + 0.0123	0.9992
Ethanol	47 to 6000	8	Linear	1/x	y = 0.00231 + 0.0419	0.9993
Ethyl Ether	47 to 6000	8	Linear	1/x	y = 0.0154 + 0.112	0.9994
Acetone	47 to 6000	8	Linear	1/x	y = 0.0102 + 0.109	0.9992
Isopropyl Alcohol	47 to 6000	8	Linear	1/x	y = 0.00313 + 0.0481	0.9999
Acetonitirile	47 to 6000	8	Linear	1/x	y = 0.00369 + 0.181	0.9991
Methylene Chloride	0.29 to 19.00	7	Linear	1/x	y = 0.00571 + 0.0166	0.9990
n-Hexane	94 to 6000	7	Linear	1/x	y = 0.0312 + 2.533	0.9999
Ethyl Acetate	47 to 6000	8	Linear	1/x	y = 0.0103 + 0.162	0.9990
Chloroform	0.29 to 19.00	7	Linear	1/x	y = 0.00566 + 8.268E-4	0.9990
Benzene	0.29 to 19.00	7	Linear	1/x	y = 0.182 + 0.00469	0.9995
1,2-Dichloroethane	0.58 to 19.00	6	Linear	1/x	y = 0.00394 + 3.089E-4	0.9973
n-Heptane	94 to 6000	7	Linear	1/x	y = 0.00420 + 0.152	0.9980
Trichloroethene	0.29 to 19.00	7	Linear	1/x	y = 0.00524 + 8.324E-4	0.9999
Toluene	47 to 6000	8	Linear	1/x	y = 0.00565 + 0.0846	0.9988
m+p-Xylene	47 to 3000	8	Linear	1/x	y = 0.00299 + 0.0209	0.9994
o-Xylene	94 to 3000	6	Linear	1/x	y = 0.00216 + 0.0310	0.9998

weighting, and regression statistics for each analyte. In all cases, the curves were linear, with six or more calibration levels, and coefficients of determination (R^2) >0.99. Figure 1 is the SIM TIC chromatogram for the target solvents at the highest calibration level. It shows the extreme concentration difference between the Category I solvents, which must be reported for any detection greater the LOD, and the Category II solvents with limits of quantitation defined in the BCC regulations. Figure 2 zooms in on the Category I solvents in the chromatogram. Figures 3 through 5 are example calibration curves for two Category I solvents, ethylene oxide and 1,2-dichloroethane, and one Category II solvent, *o*-xylene.

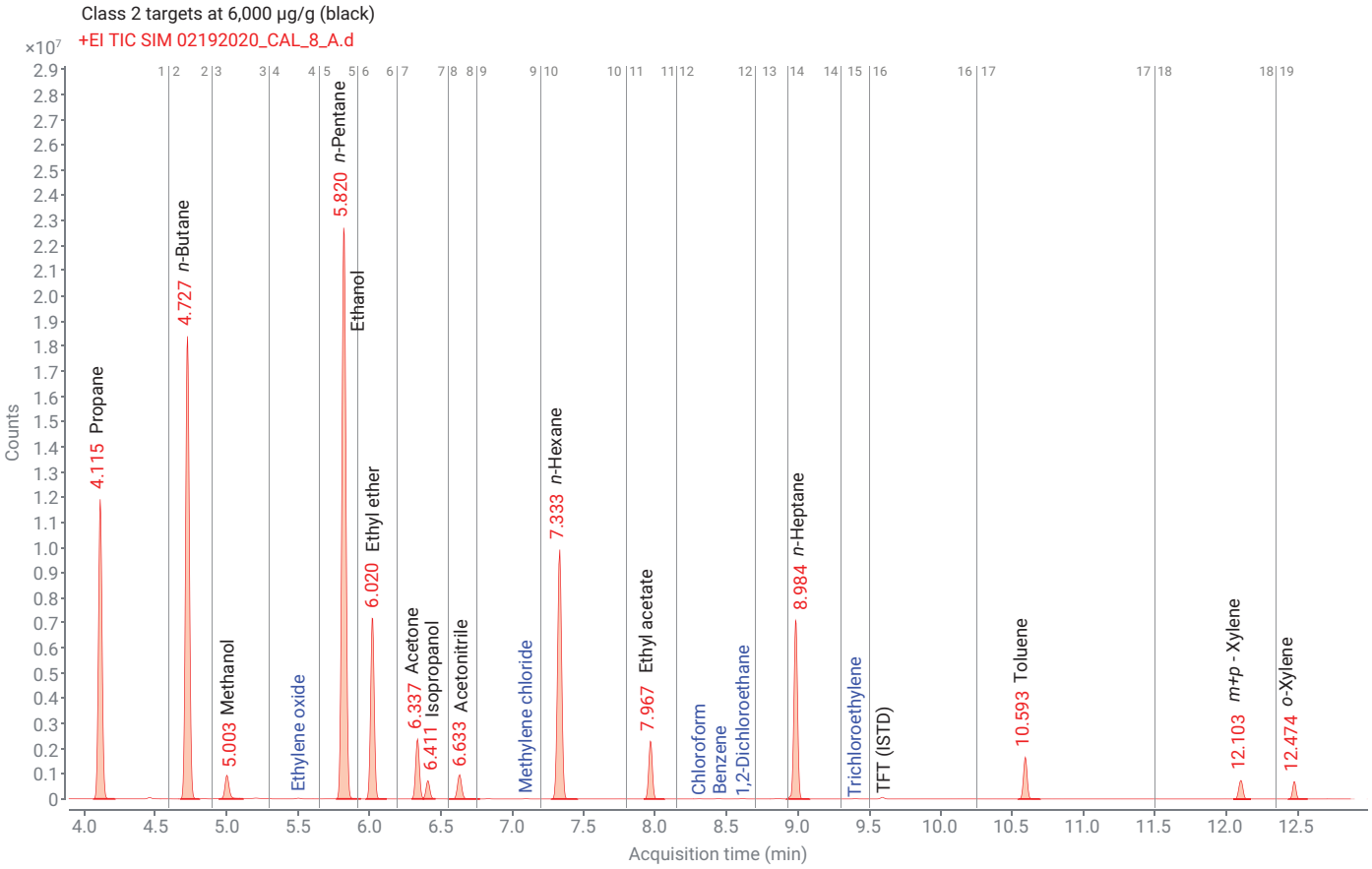

Figure 1. SIM TIC chromatogram of the Category I and II solvents defined by the CA BCC. The red trace are the Category II solvents at the high calibrator level of 6000 ppm. The blue trace is the Category I solvents at the high calibration level of 19 ppm.

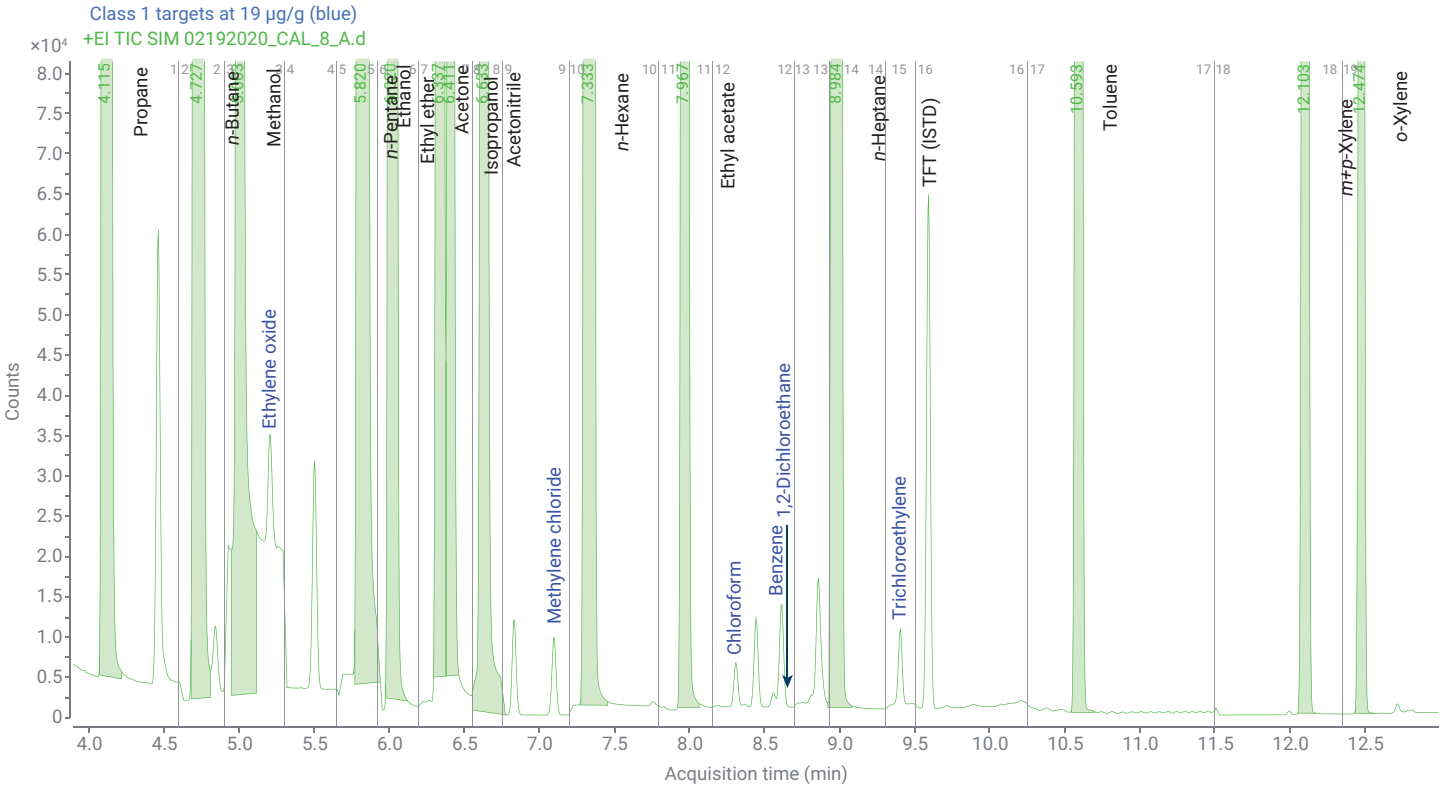

Figure 2. Zoomed SIM TIC illustrating the Category I solvents at the high calibration level of 19 ppm.

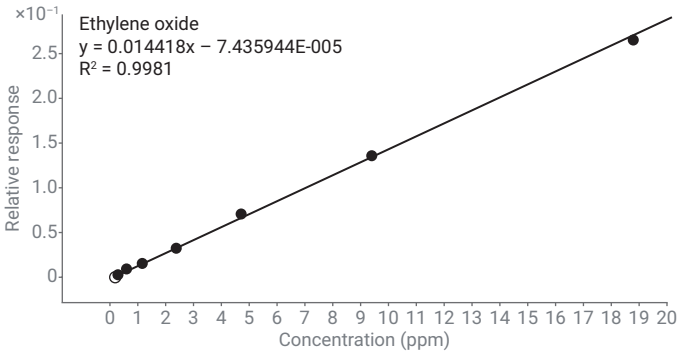

Figure 3. Ethylene oxide calibration curve.

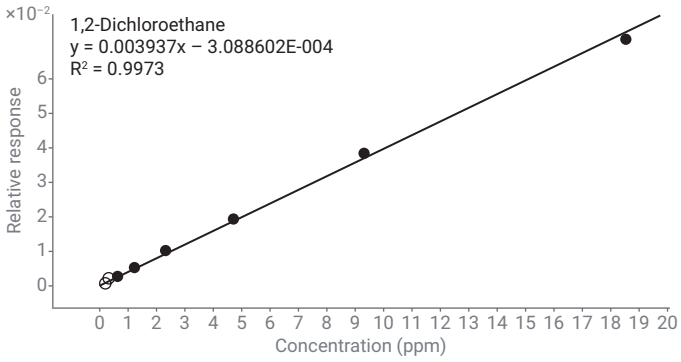

Figure 4. 1,2-Dichlorethane calibration curve.

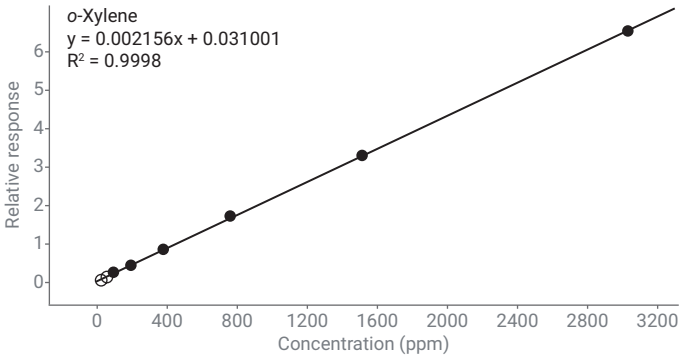

Figure 5. o-Xylene calibration curve.

Calculation to convert µg/mL of residual solvent in matrix to ppm

Using the empirically determined linear regression curve for each analyte, the concentration of the analyte in the sample was determined in µg/mL. This value was then plugged into Equation 1 to determine the amount of analyte in the original sample in µg/g (ppm).

Best practices

The exothermal reaction of DMA and saline solution caused variability and loss of target analytes when preparing samples for analysis. We allowed the vial to cool to ambient temperature before analysis. Another observation was that ethylene oxide degrades in the presence of water at HS oven temperatures above 80 °C. A total loss of ethylene oxide was observed in the presence of water when the HS oven was set above 100 °C.

Commercially available mixed solvent standards manufactured for the testing of cannabinoid products limit the options for performing LOD measurements. Commercial mixtures are manufactured to address regulatory "limits" and do not reflect empirical LODs. In this work, the Category I concentrations were very low compared to the Category II concentrations, which were >300 times higher in the mix. Therefore, analyzing both Categories I and II solvents simultaneously for LOD experiments is not recommended. Separate experiments must be performed to determine method parameters. However, with regulatory LOQ action limits at 5,000 ppm for Category II solvents, establishing Category I limits of detection for Category II solvents is not necessary.

Amount compound (ppm) = [Concentration (µg/mL) × dilution (mL)] / sample weight (g)

Equation 1.

Conclusion

This work developed and verified method parameters and outcomes for the analysis of residual solvents in cannabinoid products using the Agilent 7697A Intuvo 9000/5977B headspace-GC/MS system. This novel method used a unique sample preparation procedure and Agilent Capillary Flow Technology to backflush terpenes which begin to elute after *o*-xylene (the last target solvent in the BCC list) under these experimental conditions. The unique advantages observed for the Intuvo GC were ferrule-free, rapid, and easy column changes, efficient power consumption, reduced heat output, and stable retention times for SIM segments as there is no need for column trimming due to the Intuvo Guard Chip protection. Accuracy, precision, range, linearity, limits of detection (defined as MDL), and limits of quantitation were determined through intra- and inter-day studies. The RSA method presented here was designed specifically for the analysis of cannabinoid products, and is unlike any other published methodology.

The LOD (MDL) determinations in this study are specific to the instrument and laboratory conditions. It is imperative that each laboratory perform a similar study to determine the empirical LOD that accounts for perturbations and bias inherent to each independent laboratory. This, however, is required for any testing performed in any cannabis testing laboratories.

This application note, along with additional information, and ready-to-run acquisition and quantitation methods, are available as eMethod G5280#010, Residual solvent test using Headspace sample introduction with the Intuvo/5977 GC/MS system.

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