

Quantitative Analysis of Residual Solvents in Hemp Oil by Full Evaporation Headspace Gas Chromatography/Mass Spectrometry

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KEYWORDS

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ABSTRACT

The number of cannabis containing products, such as extracts, tinctures, edibles, waxes and oils, available in the United States has increased significantly due to changes in state law and the 2018 Farm Bill. Cannabis concentrates are legally manufactured for both medicinal and recreational use and are quickly becoming more commonly used than the Cannabis sativa flower. The concentrates containing cannabinoids and terpenes are typically extracted from plant material using a solvent such as supercritical CO₂, butane, propane, other hydrocarbons, water or alcohol. Residual solvents and solvent impurities in the extracted material can be a cause for concern. There is a need for an analytical methodology to determine the amount of solvent present in these concentrates to ensure consumer safety and to perform quality

control. This study describes the use of the GERSTEL MultiPurpose Sampler (MPS robotic) with automated headspace option for the analysis of residual solvents in hemp oil samples by full evaporation technique headspace gas chromatography/mass spectrometry (FET-HS-GC/MS)[1]. The technique is fast, accurate and precise and enables quantitation of the analytes of interests (solvents, etc.) within the allowable concentration limits for the State of California.

INTRODUCTION

Currently, there are no federal regulations in the United States on the allowable concentration of residual solvents present in cannabis or cannabis concentrates. The limits for each residual solvent are defined by the individual state in which the cannabis is grown. The allowable concentration of residual solvents and corresponding action levels in cannabis and cannabis products for the State of California as defined by the Bureau of Cannabis Control [2] are shown in Table 1.

Table 1. State of California proposed action levels for residual solvents in cannabis.

Compound	CA Action Levels (ppm)
Propane	1000
Butane	1000
Methanol	600
Ethylene oxide	1
Pentane	1000
Ethanol	1000
Ethyl ether	1000
Acetone	1000
Isopropanol	1000
Acetonitrile	80
Dichloromethane	1
Hexane	60
Ethyl Acetate	1000
Chloroform	1
Benzene	1
1,2-Dichloroethane	1
Heptane	1000
Trichloroethylene	1
Toluene	180
Xylenes total	430

The GERSTEL MultiPurpose Sampler (MPS robotic) in combination with the headspace option provides the user with a multitude of analytical tools for chemical analysis. This study describes the use of the GERSTEL MPS robotic for full evaporation headspace GC/MS used for quantitative analysis of residual solvents in hemp oil. This technique may be applied to cannabinoid containing products for evaluation of consumer safety, as many of these residual solvents and their corresponding byproducts may be toxic. The automation provided by the GERSTEL Maestro software enables this headspace technique to be fast, simple and accurate. This use of the MSD in single ion monitoring (SIM) mode enables quantification of residual solvent analytes at very low levels.

EXPERIMENTAL

Instrumentation.

Agilent 7890 GC / 5977B MSD, GERSTEL MPS robotic with Headspace Option and Cooled Injection System CIS 4 (PTV).

Analysis conditions

MPS: 100°C incubation temperature
110°C HS syringe temperature
CIS: SPME liner
split 3:1 (Res. solv. cat. I and II)
split 10:1 (Res. solv. cat. III, IV and V)
250°C isothermal
Pneumatics: He, constant flow, 1 mL/min
Column: 30 m CP-Select 624 CB (Agilent)
 $d_i = 0.25$ mm $d_f = 1.40$ μ m
Oven: 30°C (3 min); 15°C/min; 260°C (5 min)

Sample Preparation. Cold-pressed hemp oil was purchased at a local store. 20 mg of hemp oil was directly weighed into a 10 mL crimp-cap vial. A ten microliter aliquot of the standard used was spiked into the 10 mL crimp cap vial containing the hemp oil and capped. Five-point calibration curves were generated with each level prepared in triplicate. Precision was obtained from n=3 replicates at the median concentration level of each calibration curve. California residual solvent standards were obtained from AccuStandard:

Category I: 1,2-Dichloroethane, benzene, chloroform, dichloromethane, trichloroethene

Category II: Ethylene oxide

Category III: Acetone, acetonitrile, ethanol, ethyl acetate, diethyl ether, isopropanol, methanol

Category IV: n-Heptane, n-hexane, n-pentane, toluene, o-xylene, p-xylene, m-xylene

Category V: n-Butane, n-propane

Sample Introduction. The 10 mL vials were placed in a VT15 tray on the MPS Robotic sampler. The samples were incubated at 100°C for 15 minutes with 500 RPM orbital agitation. One mL of sample headspace was introduced into the CIS 4 (250°C) in split mode with a split ratio of 3:1 (Residual solvent standards I and II) or 10:1 (Residual solvent standards III, IV and V). The headspace syringe was purged for 120 seconds between samples.

RESULTS AND DISCUSSION

Table 2 lists the linearity and precision for all analytes included in this study. Excellent linearity and precision were observed for all compounds in this study, with an average percent relative standard deviation (% RSD) of 2.56 % and an average correlation coefficient (r^2) value of 0.995.

Table 2. Linearity and precision of residual solvents in hemp oil.

Compound	Correlation Coefficient	Precision n=3
Propane	0.9374	7.66
Butane	0.9972	1.81
Methanol	0.9998	5.77
Ethylene oxide	0.9992	1.52
Pentane	0.9998	1.23
Ethanol	0.9996	2.32
Ethyl ether	0.9983	2.72
Acetone	0.9994	1.09
Isopropanol	0.9999	2.18
Acetonitrile	0.9999	0.89
Dichloromethane	0.9971	0.40
Hexane	1.0000	1.12
Ethyl acetate	0.9997	1.99
Chloroform	0.9938	0.90
Benzene	0.9992	2.77
1,2-Dichloroethane	0.9984	2.19
Heptane	0.9982	1.55
Trichloroethylene	0.9982	2.51
Toluene	0.9991	2.79
Xylenes total	0.9971	2.65

Two methods were developed for the quantification of these categories of compounds, one for a concentration range of 0.005 – 1 ppm and one for a concentration range of 1 – 1000 ppm. For residual solvent category I and II compounds with action levels at 1 ppm, a single ion monitoring (SIM) mode method was developed for detection. This method employed a 3:1 split at the inlet to reduce the interference with air. Table 3 shows the reporting limit (ppm) for this method. The method reporting limit corresponds to the lowest concentration of standard that was detected, quantified and incorporated into the calibration curve for linearity.

Table 3. Action levels and method reporting limits for residual solvents category I and II.

Compound	Ions* [m/z]	CA Action Level [ppm]	Reporting Limit [ppm]
Ethylene Oxide	29 , 44	1	0.500
Dichloromethane	49 , 85	1	0.005
Chloroform	83 , 85	1	0.005
Benzene	78 , 77	1	0.005
1,2-Dichloroethane	62 , 64	1	0.005
Trichloroethylene	130 , 132	1	0.025

*Plot ions used in SIM mode are shown in bold.

A representative calibration curve for the category I and II residual solvents is shown in Figure 1, which shows the calibration curve for benzene. Excellent linearity is observed.

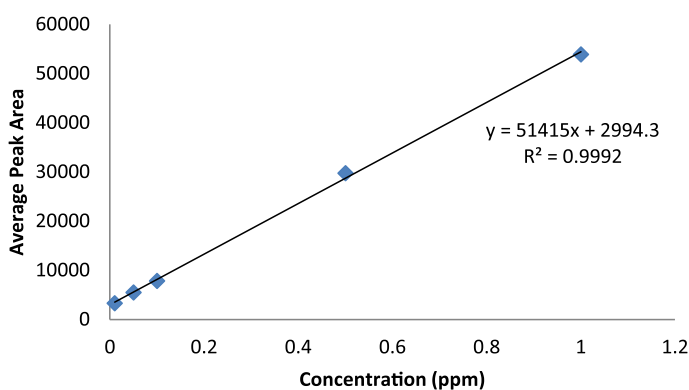


Figure 1. Calibration curve for 0.005 - 1 ppm benzene calibration standards in hemp oil.

Figure 2 shows a representative extracted ion chromatogram of a 1 ppm residual solvent category I standard in hemp oil. The plot ion for each compound was extracted and overlaid. All compounds in the residual solvent category I were identified and labeled in the figure.

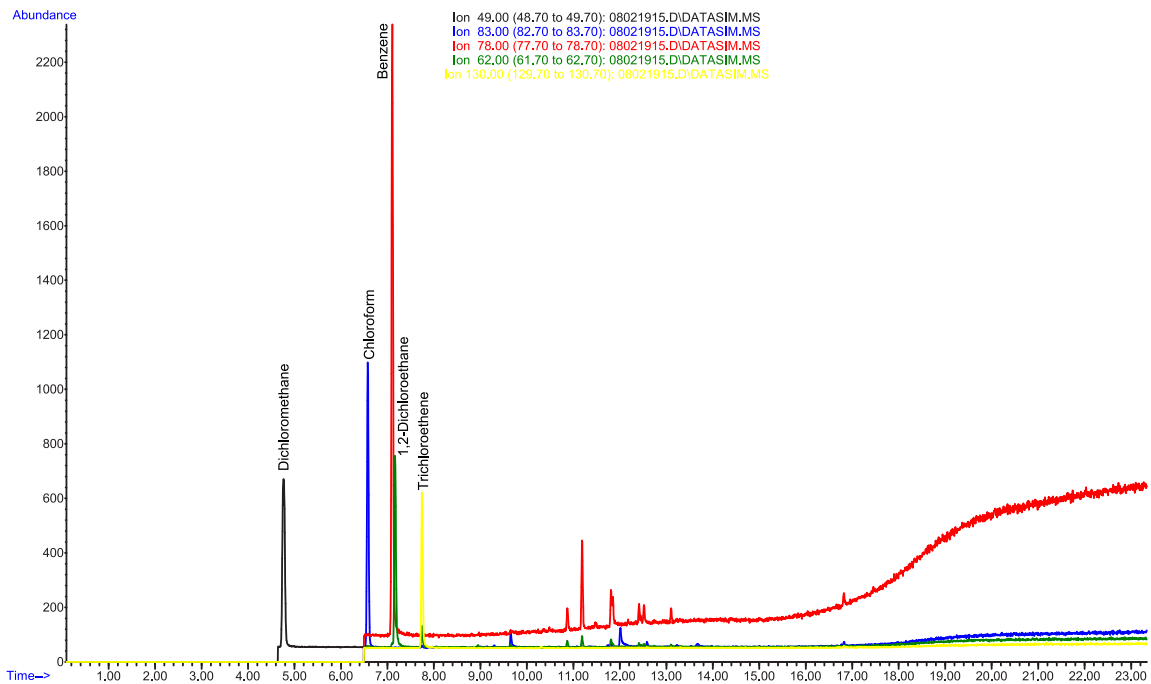


Figure 2. Representative extracted ion chromatogram of FET-HS-GC/MS analysis of 1 ppm residual solvents category I in hemp oil.

Table 4 shows the method reporting limit for residual solvent category III, IV and V compounds. For these compounds, the California action levels were 60 - 1000 ppm. Because of the relatively high action levels, the lowest concentration standard incorporated into the calibration curve was 1 ppm. This method utilized a 10 to 1 split at the inlet and full scan mode for detection in the MSD. The m/z ions, including the plot (shown in bold) and one or two additional ions used for identification of each compound, are listed in Table 3. Lower detection limits are achievable by decreasing the split ratio at the inlet and performing detection in the MSD in SIM mode, as shown for the residual solvent category I and II analytes.

Table 4. Action levels and method reporting limits for residual solvent category III, IV and V compounds.

Compound	Ions* [m/z]	Precision n=3	CA Action Level [ppm]	Reporting Limit [ppm]
Propane	29 , 44	7.66	1000	50.000
Butane	29, 43 , 58	1.81	1000	5.000
Methanol	29, 31	5.77	600	1.000
Pentane	42, 43 , 72	1.23	1000	1.000
Ethanol	31 , 45, 46	2.32	1000	1.000
Ethyl ether	31, 59 , 74	2.72	1000	1.000
Acetone	43 , 58	1.09	1000	1.000
Isopropanol	43, 45 , 59	2.18	1000	1.000
Acetonitrile	39, 40, 41	0.89	80	1.000
Hexane	43, 57 , 86	1.12	60	1.000
Ethyl acetate	43 , 61, 88	1.99	1000	1.000
Heptane	43 , 57, 100	1.55	1000	1.000
Toluene	91 , 92, 93	2.79	180	1.000
Xylenes total	91 , 106	2.65	430	1.000

*Extracted ions used for quantification are shown in bold.

A representative calibration curve for the residual solvent category III, IV and V compounds is shown below. Figure 3 shows the calibration curve for hexane from the category IV residual solvent standards. Excellent linearity is observed.

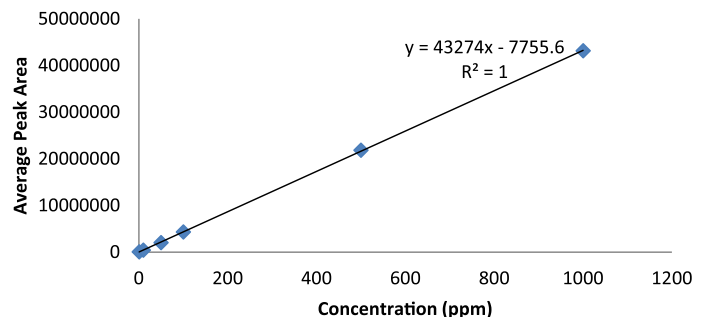


Figure 3. Calibration curve for 1- 1000 ppm hexane calibration standards in hemp oil.

Figure 4 shows a representative total ion chromatogram of 1000 ppm residual solvent category IV standard in hemp oil. All compounds in the residual solvent category IV standard were identified and labeled in the figure. Good chromatographic separation is achieved.

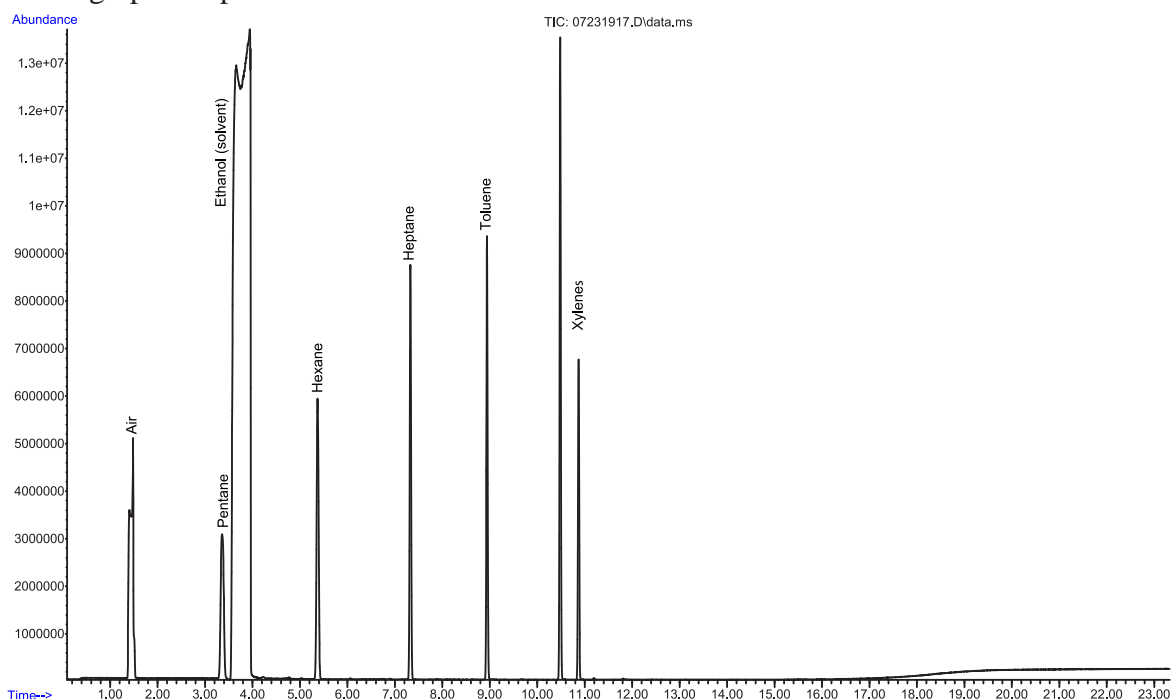


Figure 4. Chromatogram of full evaporation headspace GC/MS analysis of 1000 ppm residual solvent category IV in hemp oil.

As demonstrated in Tables 3 and 4, excellent linearity is observed for all compounds with an average correlation coefficient of 0.995. The FET-HS-GC/MS technique is very precise, with an average relative standard deviation of 2.56 % for all compounds. Low detection levels were demonstrated using the SIM mode for the category I and II residual solvent compounds. If necessary, the SIM mode method can be applied to the residual solvent category III, IV and V compounds to achieve lower detection limits. Method reporting limits three orders of magnitude below the action levels were observed for the category III, IV and V residual solvent compounds.

CONCLUSION

This study has demonstrated the efficacy of full evaporation technique headspace GC/MS analysis for the quantification of residual solvents in hemp oil and other cannabis concentrates. The method requires only minimal sample preparation, and automation using GERSTEL Maestro software allows this technique to be simple to perform, fast and accurate. The use of the MSD in SIM mode allows the operator to achieve low levels of detection for analytes with action levels at 1 ppm.

REFERENCES

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