A Novel Analysis of Solvent Residual in Cannabis Oil Using Dynamic Headspace by PAL System

Application Note

Cannabis

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Abstract

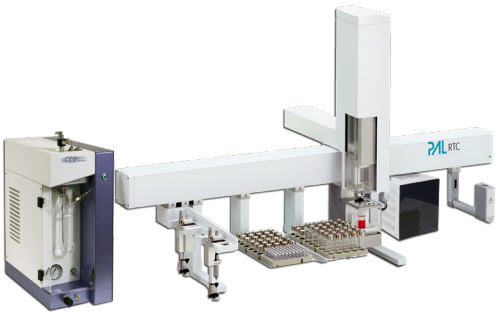
This application note adopted the Full Evaporation Technique (FET) to analyze the solvent residual, based on California Residual Solvents Category, in cannabis oil by a CDS Analytical 7000C concentrator equipped with a dynamic headspace (DHS) module. This setup was mounted on a PAL RTC rail and connected to a GC/MS for compounds separation and detection.

Introduction

In the pharmaceutical manufacturing process, residual solvents may remain in the final product besides the active pharmaceutical ingredients (APIs). These residual solvents are not beneficial to the therapeutic treatment and are regulated by USP 467 method. In the USP 467 method, the dynamic headspace technique was used as the Gas Chromatography (GC) sample introduction method. Three procedures are followed as (1) Identification and limit test, (2) Confirmatory test and (3) Quantitative test. In the USP 467 method, the calibration standard and samples could be insoluble in water, and need to be diluted into a heavier, water-miscible solvent, such as N,N-Dimethylacetamide (DMA). These samples are further prepared by adding an aliquot of the diluted sample to water in a headspace vial for GC analysis. The process involves wet chemistry and may complicate the results with solvent effect.

On the other hand, more states in U.S. have legalized marijuana products for therapeutic and entertainment purposes. However, there are currently no federal regulations to define the allowable concentration limits residual solvent, rather than certain pioneering state-level regulations. For example, Table 1 shows the list of proposed residual solvents and action levels for cannabis products in the State of California. Based on the max allowed concentration limit, these solvents are categorized in two classes as California Residual Solvents Category I and II.

From previous applications, the Full Evaporation Technique (FET) is proven to be an exhaustive way to transfer analytes from the sample to the GC through the headspace, which eliminates the balance time and uses minimized sample amount. In this application note, a dynamic headspace system is setup to explore its capability to analyze the residual solvents in cannabis oil by FET.



Experimental Setup

DHS Module:

Vial Station:	180 °C
Valve Oven:	230 °C
Transfer Line:	250 °C

GC/MS:

Column:	Supelco SPB-624, 30m,0.25mmx1.4μm
Carrier Gas:	Helium 1.24mL/min
GC Oven:	30°C, 3 min 10°C/min to 140 °C 45 °C/min to 200 °C 2min
MSD:	SIM

7000C Concentrator:

Valve Oven:	250 °C
Transfer Line:	250 °C
Vial Volume:	10 mL
Purge Flow:	Helium 40 mL/min 10 min
Dry Purge:	Off
Desorb:	265 °C 6 min
Bake:	285 °C 6 min
Wet Trap:	Bypassed
Analytical Trap:	Type X

Table 1: California proposed residual solvents and action limits in cannabis products

Compound	CAS	CA Residual Solvents Category	Action Limit (ppm)
1,2-dichloroethane	107-06-2	I	1
benzene	71-43-2	I	1
chloroform	67-66-3	I	1
ethylene oxide	75-21-8	I	1
methylene chloride	75-09-2	I	1
trichloroethylene	79-01-6	I	1
acetone	67-64-1	II	1000
acetonitrile	75-05-8	II	80
butane	106-97-8	II	1000
ethanol	64-17-5	II	1000
ethyl acetate	141-78-6	II	1000
ethyl ether	60-29-7	II	1000
heptane	142-82-5	II	1000
isopropyl alcohol	67-63-0	II	1000
methanol	67-56-1	II	600
hexane	110-54-3	II	60
pentane	109-66-0	II	1000
propane	74-98-6	II	1000
toluene	108-88-3	II	180
xylenes (total)	1330-20-7	II	430

A CDS 7000C concentrator and a DHS dynamic headspace module were setup on a CTC RTC rail as the automated sampling platform. This system is controlled by Pal Sample Control (PSC) software with two individual plug-ins for the 7000C concentrator and the DHS module.

To quantify the solvent residual, the first step was to calibrate the DHS and GC/MS with the calibration standard. The calibration standards were purchased from CPI International as CA Residual Solvents Category I (Z-G34-115300-03) in 100 mg/L concentration and CA Residual Solvents Category II (Z-G34-115301-02) in 10,000 mg/L concentration. Triacetin, which was the same solvent in the calibration standard, was used to mix and dilute the two standards into two separate calibration mix solutions (Calibration Mix 1 and Calibration Mix 2). The final concentrations in the Calibration Mix 1 were 8 ppm for each compound in the CA Residual Solvents Category I list and 80 ppm for each compound in the CA Residual Solvents Category II list respectively. The Calibration Mix 2 had a higher 64 ppm and 640 ppm concentration.

By following FET method, a series of aliquots of calibration mix were injected to headspace vials for dynamic headspace analysis. Table 2 shows the calibration mix volume and resulting mass of each target solvent residual,

Five replicates of Calibration Point 3 were tested to evaluate the precision of the method. A 1 μL aliquot of Calibration Mix 2 solution was spiked on to 150 mg methyl cellulose in a headspace vial to test the recoveries of all the compounds. A 10 μL 5% CBD hemp oil was added to a headspace vial as an unknown sample to quantify the residual solvents.

Table 2: Calibration points

Calibration Point	Calibration Standard	Addition Volume (µL)	Individual CA Residual Solvents Category I Mass (ng)	Individual CA Residual Solvents Category II Mass (ng)
1	Calibration Mix 1	1	8	80
2		2	16	160
3		4	32	320
4	Calibration Mix 2	1	64	640
5		2	128	1280
6		4	256	2560

Results

Figure 1 showed an example chromatogram obtained in SIM mode in the Calibration Point 6. The calibration mix contains 256 ng of each compound in CA Residual Solvents Category I list and 2560 ng of each compound in CA Residual Solvents Category II list.

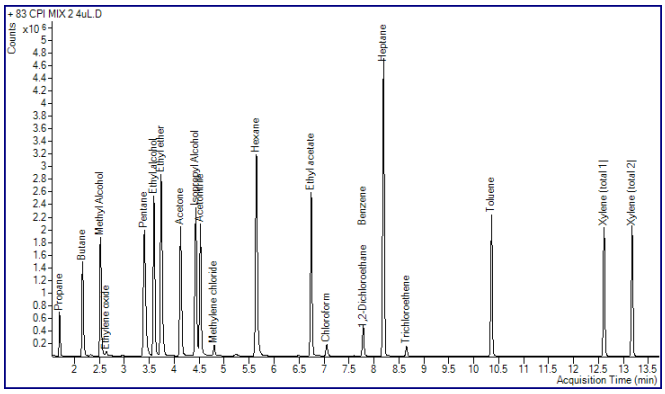


Figure 1: Chromatogram of the Calibration Mix II

Figure 2 and Figure 3 depicted the calibration curve for both CA Residual Solvents Category I compounds and CA Residual Solvents Category II compounds. Most of the R^2 were greater than 0.999.

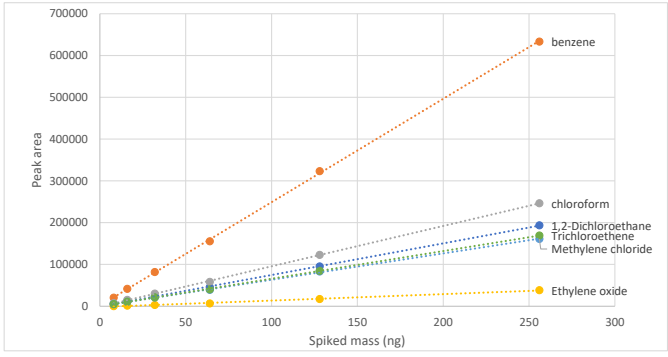


Figure 2: CA Residual Solvents Category I compounds calibration curve

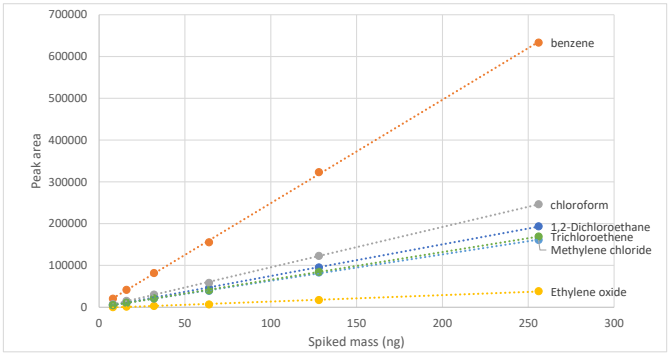


Figure 3: CA Residual Solvents Category II compounds calibration curve

Besides the calibration linearity, Table 3 summarized the retention time, ions in the SIM mode, reproducibility and recovery results. The RSDs for of all the compounds were under 3.5%, the recovery rates, except for methanol, ranged from 88.6% to 106.1%. These data validated the system in the quantification study. The high recovery rate of methanol was due to the high background level in the methyl cellulose.

Table 3: System calibration and validation data

Compound Name	RT (min)	Ions (m/z)	RSD, n=5 (%)	R^2	Recoveries (%)
Propane	1.65	29, 39	3.13	0.9984	93.1
Butane	2.12	43, 41	2.39	0.9987	92.4
Methyl alcohol	2.47	31, 33	3.00	0.9988	719.8
Ethylene oxide	2.64	44, 42	2.83	0.9964	88.6
Pentane	3.35	57, 42	2.18	0.9992	92.3
Ethyl alcohol	3.55	45, 46	1.79	0.9993	98.1
Ethyl ether	3.69	59, 45	2.67	0.9992	93.6
Acetone	4.08	58, 43	2.10	0.9995	97.8
Isopropyl alcohol	4.39	45, 43	1.73	0.9997	95.1
Acetonitrile	4.53	41, 40	2.01	0.9997	99.6
Methylene chloride	4.76	84, 49	1.81	0.9996	97.3
Hexane	5.62	57, 41	2.22	0.9987	93.3
Ethyl acetate	6.72	43, 45	1.97	0.9997	101.0
chloroform	7.03	83, 85	1.57	0.9998	97.0
Benzene	7.76	78, 77	1.74	0.9998	98.7
1,2-Dichloroethane	7.77	62, 64	1.85	0.9998	102.9
Heptane	8.18	71, 57	1.50	0.9995	89.1
Trichloroethene	8.64	130, 95	1.62	0.9998	100.9
Toluene	10.34	92, 65	2.19	0.9998	106.1
Xylene (total)	12.62	91, 106	1.71	0.9993	105.4

After the calibration and validation of the system, a commercially purchased 5% CBD hemp oil from NuLeaf Naturals was tested against the calibration curve to quantify solvent residual. Figure 4 showed the chromatogram and Table 4 listed all the solvent residual over 1 ppm. From the testing result, this CBD oil failed the CA regulation due to the fact that the concentration of methylene chloride exceeded the action limit.

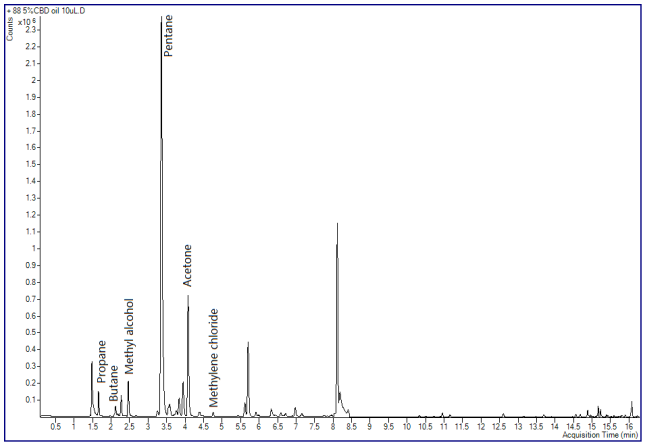


Figure 4: Chromatogram of 5% CBD oil sample

Table 4: Detected solvent residual

Compound Name	Detected in 5% CBD oil (ppm)	Action Limit (ppm)
Propane	46.0	1000
Butane	8.3	1000
Methyl alcohol	22.8	600
Pentane	311.9	1000
Acetone	86.9	1000
Methylene chloride	3.6	1

Conclusions

The dynamic headspace system, including the CDS 7000C concentrator and DHS module coupled to an automated a PAL System, demonstrated a fast and accurate turnkey solution to study the residual solvents in the cannabis oil. Besides this application, the FET is also proven to be capable in analyzing compounds in challenging matrices.