Cannabidiol Oil Analysis with the Pyroprobe

Application Note
Pharmaceutical

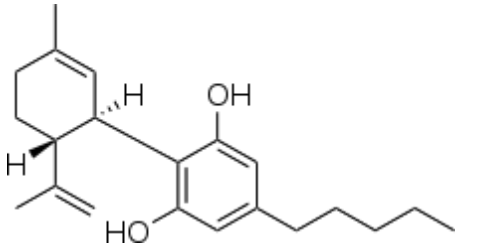
Author:

Karen Sam

Abstract
This application note demonstrates multi-step analysis of CBD oil with RSDs of cannabidiol

With recent legislation paving the way for entertainment usage of marijuana, and the promise of cannabidiol (CBD) for treating a variety of ailments, CBD oil, derived from the cannabis plant, has a growing interest in nutraceutical and pharmaceutical industries. Similar in structure to psychoactive δ9-Tetrahydrocannabinol (THC) found in marijuana, this non-intoxicating extract is being credited with helping treat many medical issues. Diluted with hemp oil prior to use, CBD oil is a complex natural product, containing many volatile and non-volatile constituents. Analytical testing using the Pyroprobe can clarify ingredients in natural materials such as CBD oil by separating ingredients based on their volatility, then pyrolyzing the non-volatile portion, like the oil itself.

About 500 micrograms of CBD oil purchased from a therapeutic hemp company, was placed into a Drop-In-Sample-Chamber (DISC) tube and run using a multi-step sequence on a CDS 6000 Series Pyroprobe interfaced to a GC/MS. The Pyroprobe was programmed to heat first to 200°C, and then to 400°C to analyze semi-volatiles in the oil; then finally to 700°C to qualify pyrolysis products. Before each of these heating steps, the Pyroprobe waits for the GC to become ready, then starts the GC as the heating begins.



Cannabidiol

Chromatograms of all 3 multi-step runs are shown in Figure 1. At 200°C, some of the oil starts to vaporize, exhibiting as an unresolved mixture at the end of the chromatogram. Along with this, active compounds in the oil, like the sesquiterpene α-Bisabolol (with anti-irritant, anti-inflammatory, and anti-microbial properties), α-Caryophyllene (often found in aromatic plants), and the cannabinoid, CBD (active ingredient) vaporize. Then at 400°C, remaining CBD continues to vaporize, along with some fatty acids, alcohols like olivetol and phytol (a diterpene alcohol), and additional cannabinoids, including THC, whose amount must be small enough to pass regulations. Finally, at 700°C, the remainder of the oil pyrolyzes, breaking down the remaining triglycerides of the oils into long chain alkenes, alkanes, alkynes, aldehydes, and alcohols. A more detailed list of peak search results are found in Tables 1-3.

The worth of quantitative results is contingent on the reproducibility of the approach. This relies on instrument precision, as well as issues like consistent sample preparation and homogeneity. A series of 20 firings of a Pyroprobe filament at 1100°C produces an average measured temperature of 1100.15°C with a relative standard deviation of only 0.04%, establishing that Pyroprobe instruments perform with the highest precision. As previously observed, CBD oil contains a wide array of compounds. So fortunately, reproducibility seen with the Pyroprobe proceeds into complex samples such as CBD oil. As CBD's boiling point is between 160-180°C, a preliminary setpoint of 200°C for 20 minutes was used to vaporize cannabidiol while minimizing interference from other constituents in

Experimental Parameters
Samples were pyrolyzed in a DISC tube, using a CDS Model 6200 Pyroprobe.

Multi-step Analyses
Direct-Py Mode
Pyroprobe:
DISC Chamber: 200, 400, 700°C 30s

Interface: 300°C
Transfer Line: 325°C
Valve Oven: 300°C

GC/MS
Column: 5% phenyl (30m x 0.25mm)
Carrier: Helium, 50:1 split
Injector: 320°C
Oven: 40°C for 2 minutes
10°C/min to 300°C
hold 15 minutes
Ion Source: 230°C
Mass Range: 35-600amu

RSD Analyses
Trapping Mode
Pyroprobe:
DISC Chamber: 200°C 20min

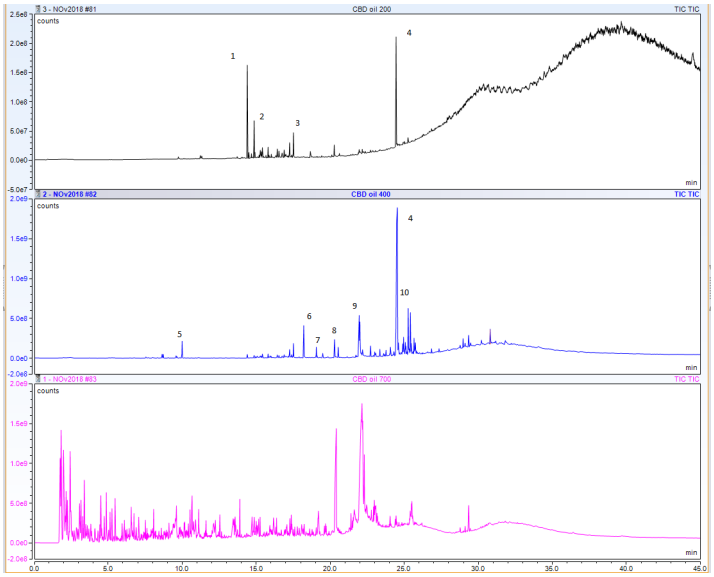
Trap Rest: 50°C
Trap Final: 300°C 10min

Interface: 300°C
Transfer Line: 300°C
Valve Oven: 300°C

GC/MS
Column: 5% phenyl (30m x 0.25mm)
Carrier: Helium 1.25mL/min, 16:1 split
Injector: 300°C
Oven: 80°C for 10 minutes
10°C/min to 300°C
Ion Source: 230°C
Mass Range: 35-600amu

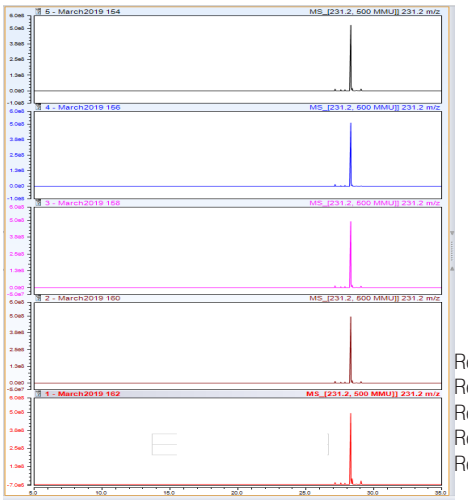
the oil for RSD trials. One half a microliter of the CBD in hemp oil diluted in half with hexane and extracted to a 200°C setpoint produced an RSD for identified cannabidiol at around 3% (Figure 2).

Performing multi-step thermal analysis on both pharmaceuticals and natural products which make up pharmaceuticals, can help an analyst find distinct components, providing valuable information for competitive analysis and product development. Additionally, reliable results for thermal extraction of active and illegal ingredients like CBD and THC respectively can be achieved.



Peak #	Identification		
1	Caryophyllene	6	Olivetol
2	Humulene	7	Phytol
3	a-Bisabolol	8	n-Hexadecanoic acid
4	Cannabidiol	9	Linoleic acid
5	1,3,8-p-Menthatriene	10	δ1-Tetrahydrocannabinol

Figure 1. CBD in hemp oil, 200°C (top), 400°C, and 700°C (bottom).



	Area Counts	
Rep 1	24649718	
Rep 2	23491890	
Rep 3	24151015	
Rep 4	24630748	RSD
Rep 5	25671887	3.26%

Figure 2. Five analyses of CBD oil, 200°C, m/z 231.2.

Table 1: 200°C Library Search Results

Ret.Time (min)	Top Hit
9.74	Linalool
11.23	a-Terpineol
11.32	Decanal
13.71	a-ylangene
14.40	Caryophyllene
14.50	a-Bergamotene
14.64	a-ylangene
14.68	cis-ß-Farnesene
14.86	Humulene
14.92	Alloaromadendrene
15.05	ß-copaene
15.20	Patchoulene
15.28	g-Selinene
15.66	Patchoulene
15.80	a-Bisabolene
15.86	ß-Guaiene
15.95	Columbin
16.02	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester
16.08	N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester)
16.42	Caryophyllene oxide
16.54	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro-a,a,3,8-tetramethyl-
16.69	Ethyl iso-allocholate
16.89	ß-Guaiene
17.00	a-acorenol
17.18	Pregan-20-one, 2-hydroxy-5,6-epoxy-15-methyl-
17.33	Columbin
17.51	a-Bisabolol
17.82	5,8,11,14-Eicosatetraynoic acid
18.66	Phenanthrene
20.27	n-Hexadecanoic acid
21.39	Ethyl iso-allocholate
22.00	7,8-Epoxylanostan-11-ol, 3-acetoxy-
22.26	Astaxanthin
22.88	Arenobufagin
24.45	Cannabidiol
24.51	7,8-Epoxylanostan-11-ol, 3-acetoxy-
24.58	Cinobufotalin
32.21	Lycoxanthin

Table 2: 400°C Library Search Results

Ret.Time (min)	Top Hit
5.02	Hexane, 2,4-dimethyl-
5.25	2-Octene
6.48	2-Methyl-1-hepten-3-yne
6.78	Heptanal
7.54	2-Heptenal, (Z)-
7.72	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-
8.05	Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans-(-)-
8.64	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-
8.72	D-Limonene
9.59	Cyclohexene, 1-methyl-4-(1-methylethylidene)-
9.65	Benzene, 1-methyl-4-(1-methylethenyl)-
9.85	13-Heptadecyn-1-ol
10.00	p-Mentha-1,5,8-triene
10.18	Cyclopentene,1-hexyl-
11.06	6-Dodecene, (Z)-
14.40	Caryophyllene
14.96	5-Hexadecyne
15.06	9,17-Octadecadienal, (Z)-
15.28	b-Selinene
15.36	a-Selinene
15.42	ß-Bisabolene
15.80	a-Bisabolene
15.86	ß-Guaiene
15.95	3,5,11-Eudesmatriene)-
16.42	Caryophyllene oxide
17.00	Aromadendrene oxide-(2)

Table 2: 400°C Library Search Results -cont'd.

Ret.Time (min)	Top Hit
17.05	Caryophylla-4(12),8(13)-dien-5a-ol
17.11	a-acorenol
17.33	Isoaromadendrene epoxide
17.40	ß-Guaiene
17.51	a-Bisabolol
17.90	1-Hexadecanol, 2-methyl-
18.21	1,3-Benzenediol, 5-pentyl-
18.46	Z,Z-3,15-Octadecadien-1-ol acetate
18.61	Pregan-20-one, 2-hydroxy-5,6-epoxy-15-methyl-
19.00	1-Heptatriacotanol
19.06	Neophytadiene
19.11	2-Pentadecanone, 6,10,14-trimethyl-

Table 3: 700°C Library Search Results

Ret.Time (min)	Top Hit
1.71	Carbon dioxide
2.12	1,3-Pentadiene
2.24	Bicyclo[2.1.0]pentane
2.44	1-Hexene
2.71	(Z),(Z)-2,4-Hexadiene
2.85	1,4-Cyclohexadiene
2.97	(Z),(Z)-2,4-Hexadiene
3.07	Benzene
3.16	1,3-Cyclopentadiene, 1-methyl-
3.24	trans-1,4-Hexadiene
3.29	Bicyclo[3.1.0]hexane
3.39	1-Heptene
3.94	1-Methylcyclohexa-2,4-diene
4.31	3,4-Heptadiene
4.88	1-Octene
5.03	Hexane, 2,4-dimethyl-
5.26	4-Octene, (E)-
5.38	Cyclopentene, 3-propyl-
5.70	Tricyclo[3.2.1.0(1,5)]octane
5.89	1,4-Heptadiene, 3-methyl-
5.94	2,4-Octadiene
6.04	E,Z-4-Ethylidenecyclohexene
6.07	2,4-Octadiene
6.10	Ethylbenzene
6.19	E,Z-4-Ethylidenecyclohexene
6.56	2-Nonene
6.65	3-Nonene, (E)-
6.74	Cyclooctene, (Z)-
7.07	1,3-Nonadiene, (E)-
8.07	1-Decene
9.38	3-Heptenoic acid
9.61	1-Undecene
13.89	1-Hexadecanol
20.37	n-Hexadecanoic acid
21.95	(Z)-18-Octadec-9-enolide
22.19	9,12-Octadecadienoic acid (Z,Z)-
24.40	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester

FOR MORE INFORMATION CONCERNING THIS APPLICATION, WE RECOMMEND THE FOLLOWING READINGS:

CDS Application Note #184a Pyrolysis GC-MS of Pharmaceutical Packaging

CDS Application Note #105a Cracking Products of Oleic Acid and Olive Oil

Sam, K. American Lab Multistep Thermal Characterization of Liquid-Filled Capsules and Medication Packaging Using GC/MS. Am. Lab, April 2018.