Investigative Multi-Step and Quantitative Analysis of Cannabidiol Oil using the Pyroprobe

Application Note
Cannabis

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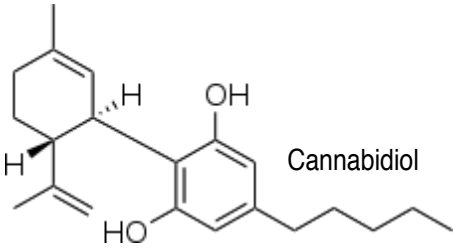
Abstract

This application note demonstrates multi-step analysis of CBD oil, CBD RSDs with a calibration curve.

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 µg 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.

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.

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

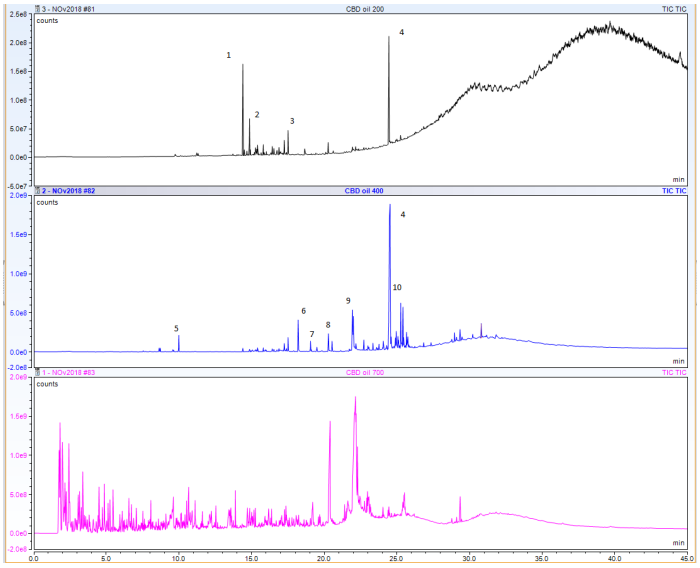
Column:	5% phenyl (30m x 0.25mm)
Carrier:	Helium, 50:1 split
Injector:	320°C
Oven:	40°C for 2 minutes 10°C/min to 300°C hold 15 minutes
Ion Source:	230°C
Mass Range:	35-600amu

RSD Analyses
Trapping Mode
Pyroprobe:
DISC Chamber: 300°C 30min

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, 75:1 split
Injector:	300°C
Oven:	80°C for 10 minutes 10°C/min to 300°C
Ion Source:	230°C
Mass Range:	35-600amu



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).

Table 1. 200°C Library Search Results

Ret.Time (min)	Top Hit
9.74	Linalool
11.23	α-Terpineol
11.32	Decanal
13.71	α-ylangene
14.40	Caryophyllene
14.50	α-Bergamotene
14.64	α-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	α-acorenol
17.18	Pregan-20-one, 2-hydroxy-5,6-epoxy-15-methyl-
17.33	Columbin
17.51	α-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

Table 1. 200°C Library Search Results -cont'd.

Ret.Time (min)	Top Hit
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	β-Selinene
15.36	α-Selinene
15.42	ß-Bisabolene
15.80	α-Bisabolene
15.86	ß-Guaiene
15.95	3,5,11-Eudesmatriene)-
16.42	Caryophyllene oxide
17.00	Aromadendrene oxide-(2)
17.05	Caryophylla-4(12),8(13)-dien-5a-ol
17.11	α-acorenol
17.33	Isoaromadendrene epoxide
17.40	α-Guaiene
17.51	α-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-
19.30	Ethanol, 2-(9-octadecenyloxy)-, (Z)-
19.84	1-Heptatriacotanol
20.29	n-Hexadecanoic acid
20.33	Ethyl iso-allocholate
20.38	13-Heptadecyn-1-ol
20.54	Cannabicyclol
21.49	17-Pentatriacontene
21.73	Phytol
21.79	Gamolenic acid
21.95	(Z)-18-Octadec-9-enolide
22.00	cis-Vaccenic acid
22.18	Octadecanoic acid
22.37	Ethyl iso-allocholate
22.72	Uvaol
23.01	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-
23.06	Z,Z-3,15-Octadecadien-1-ol acetate
23.35	DELTA.8-Tetrahydrocannabinol
23.48	7,8-Epoxylanostan-11-ol, 3-acetoxy-
24.51	Cannabidiol
24.53	Resorcinol, 2-p-mentha-1,8-dien-3-yl-5-pentyl-, (-)-(E)-
25.19	11-Acetoxy-d8-tetrahydrocannabinol
25.26	Dronabinol
25.32	Hydroxy-d 9-tetrahydrocannabinol, 8-a

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

Ret.Time (min)	Top Hit
26.41	7,8-Epoxylanostan-11-ol, 3-acetoxy-
26.59	Ethyl iso-allocholate
26.75	Docosanoic acid, 1,2,3-propanetriyl ester
28.97	Tocopherol
29.34	Astaxanthin
29.44	Rhodopin
30.80	Sitosterol
31.56	Rhodopin

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

After obtaining qualitative data using the multi-step temperature sequence, a CBD calibration curve was developed and RSDs were measured for a quantitative study. First, to decrease the sample's viscosity for easy syringe additions, 1mL CBD oil was diluted to 2mLs with hexane. Three 0.5μL aliquots of this sample were each added to 3 DISC tubes. Each DISC tube was analyzed twice at one of the 3 preset temperatures: 200°C, 300°C, and 400°C. The first run of each temperature was a sample run, and the second run of the same sample was to characterize residual CBD. A setpoint of 300°C for 30 minutes extracted nearly all the CBD, leaving 0.22% for the following 300°C step (Figure 1). So 300°C was chosen to create calibration curve and perform RSD measurements. Five consecutive sample runs yield an Area Count RSD for cannabidiol in CBD oil at 2.18%. Replicate extracted ion chromatograms of m/z 231.2, the mass spectrum's base peak, are shown in Figure 3, and the RSD measurements are found in Table 4.

A CBD calibration curve was created by adding 4, 6, 8, 10, 12, and 14 µL of a 1000μg/mL CBD standard (Restek P/N 34011) to DISC tubes, resulting in 4, 6, 8, 10, 12, and 14 μg of CBD, respectively, in each of the DISC tubes. These masses are equivalent to concentrations of 8, 12, 16, 20, 24, and 28mg/mL from 0.5μL of the tested CBD sample. These tubes were run at 300°C for 30 minutes, and the resulting six-point calibration produced a linear regression >0.99 r2 (Figure 4.) By comparing the average area of the CBD in the replicates in relation to the calibration curve (using the generated calibration line equation and solving for x, concentration), it was determined that the sample has 24mg/mL of CBD. As the sample was diluted in half, the original CBD oil has 48mg/mL of CBD, close to what was claimed by the manufacturer (50mg/mL). As shown here, typical reproducibility and quantitative results experienced with the Pyroprobe can proceed into the analysis of complex samples such as CBD oil.

Performing multi-step thermal analysis on both pharmaceuticals and natural products like CBD oil, can help an analyst find distinct components, providing valuable information for competitive analysis and product development. Additionally, the linearity and RSDs demonstrate that the CDS Pyroprobe is adept at analyzing active substances like CBD in complex sample matricies, making it suitable to use for qualification and regulation purposes.

	Area Counts	
Rep 1	17579813	
Rep 2	16921013	
Rep 3	17600523	
Rep 4	16980330	RSD
Rep 5	16836796	2.18%

Table 4. Area counts of m/z 231.2 for CBD in CBD oil.

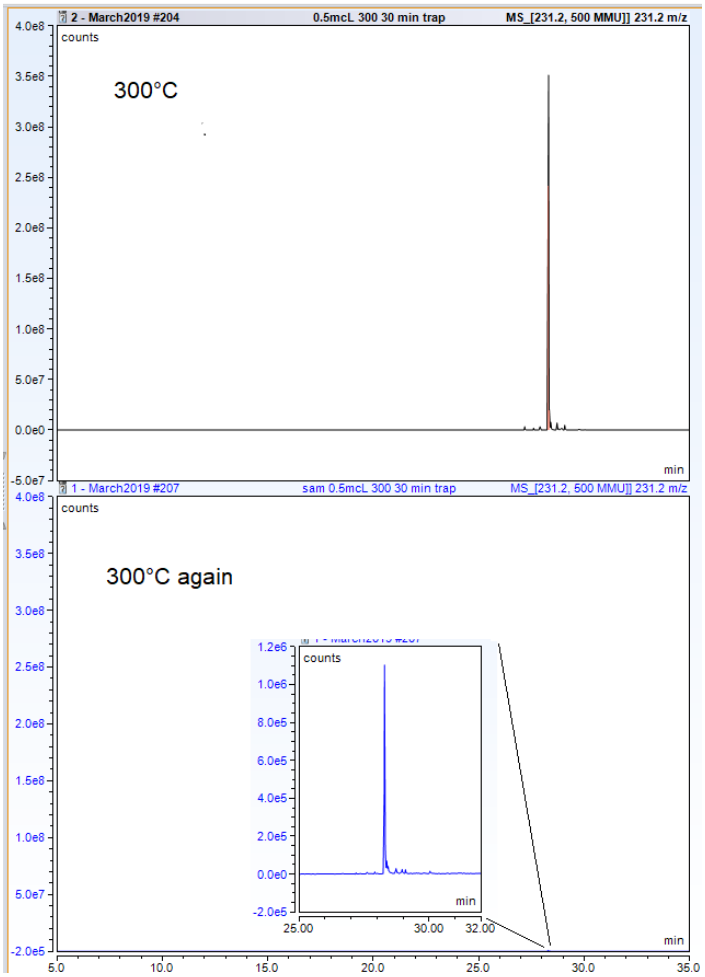


Figure 2. CBD 300°C (top) then 300°C again (bottom), m/z 231.2.

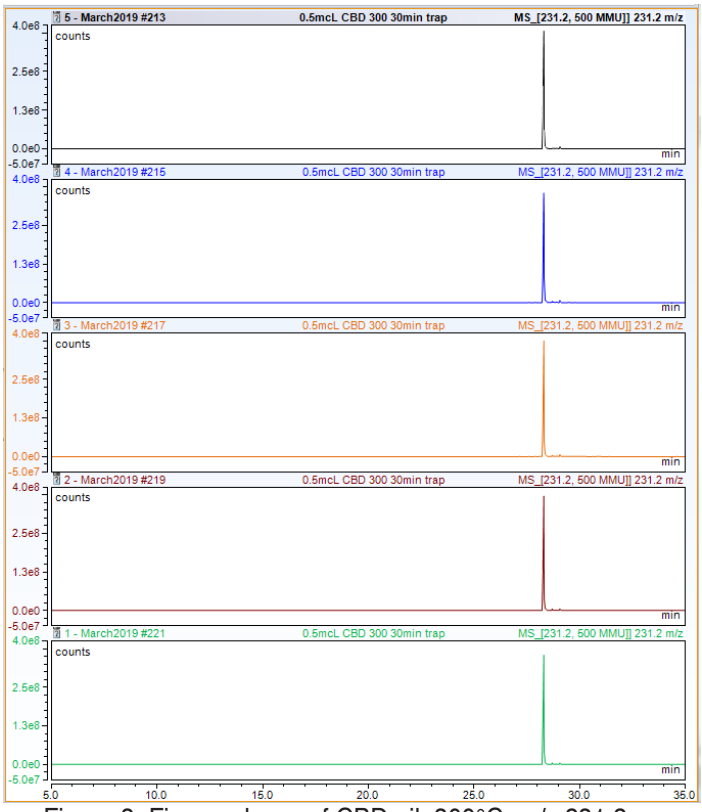


Figure 3. Five analyses of CBD oil, 300°C, m/z 231.2.

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

Quantitative Analyses
Trapping Mode
Pyroprobe:
DISC Chamber: 300°C 30min

Trap Material: Tenax
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, 75:1 split
Injector: 300°C
Oven: 80°C for 10 minutes
10°C/min to 300°C
Ion Source: 230°C
Mass Range: 35-600amu

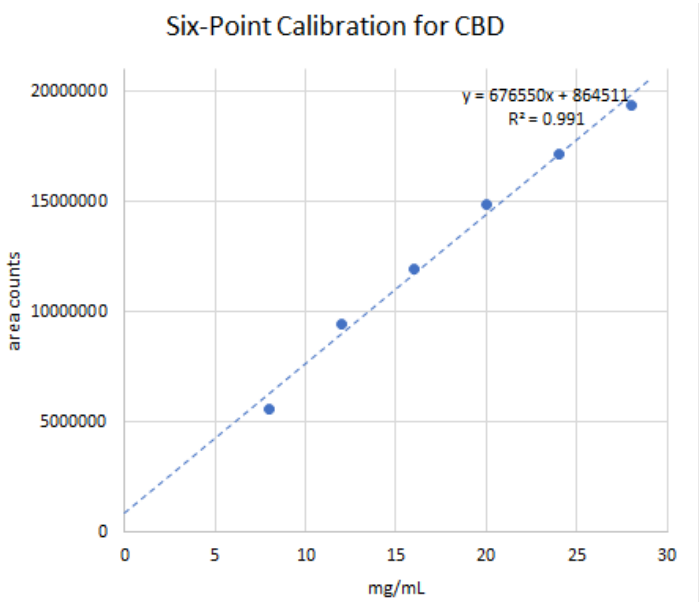


Figure 4. CBD Calibration Plot