# Profiling hemp oils using liquid chromatography-time of flight mass spectrometry

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## Introduction

*Cannabis sativa* is typically associated with psychotropic marijuana however, it is also the taxonomic classification of hemp. Hemp has been used as fibrous material for more than 10.000 years [1] and is used as a raw material for many industrial products including paper, plastics, and biofuels [2]. *C. sativa* is highly complex in the number and type of endogenous chemotypes synthesized in the various plant organs. These include at least 66 cannabinoids, and diverse terpenoids, phenols, flavonoids, alkaloids, phytosterols, glycosides, and fatty acids [3, 4]. The distribution of these chemicals can vary widely across genetic sub-strains and are further influenced by growing conditions. Understanding these distributions requires sophisticated analytical and data analysis techniques. Herein, we demonstrate liquid chromatography-time of flight mass spectrometry (LC-TOF) for the quantification of cannabinoids in oils derived from hemp and how LC-TOF can be used for discovery in cannabinoid-related investigations.

## Experimental

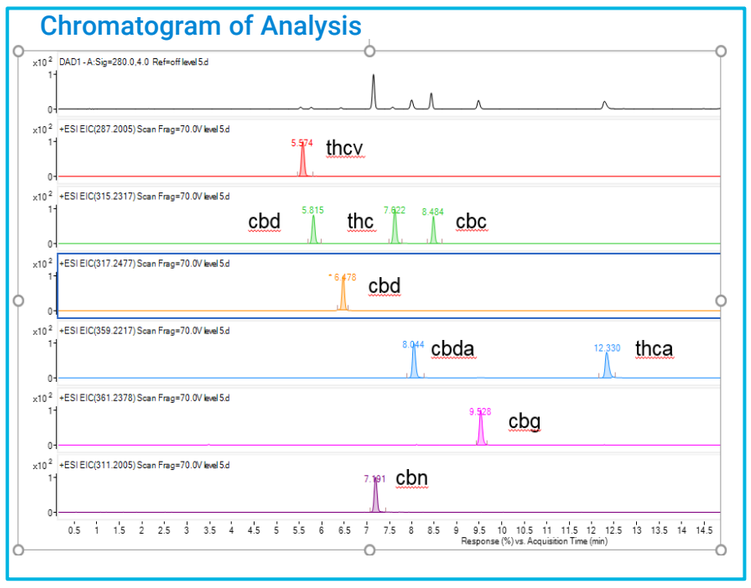
An Agilent 1290 Infinity II combined with an Agilent 6230B LC-TOF (Agilent Technologies, Santa Clara, CA USA) system was used for this investigation. Targeted analytes were $\Delta^9$-THC, tetrahydrocannabivarin (THCV), tetrahydrocannabinolic acid (THCA), cannabinol (CBN), cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), cannabidiolic acid (CDBA), cannabigernol (CBG), and cannabigerolic acid (CBGA).

The analytical system included an Agilent 1290 Infinity II UHPLC series quaternary pump, multisampler with wash, and multi-column thermostat equipped with an Agilent ZORBAX Bonus RP. 2.1 × 50 mm, 1.8 µm column held isothermally at 50 °C. The mobile phases were: Channel A – water, channel B – methanol, and channel C – 0.1 % formic acid plus 0.22 % 5 mM ammonium formate$_{(aq)}$. All reagents were high purity, HPLC-grade. The mobile phase gradient was 72% B and 5% C at time zero, increasing linearly to 95% B and 5% C at 12.5 minutes with a constant flow rate of 0.5 mL/min. The mass spectrometer was an Agilent 6230B time of flight instrument operated in electrospray positive mode over a mass range of 100 m/z to 1700 m/z at a 1.0 Hz acquisition rate. The drying gas flow was 12 L/min, the drying gas temperature was 350 °C with a capillary voltage of 4000 V, and fragmentor voltage of 175 V.

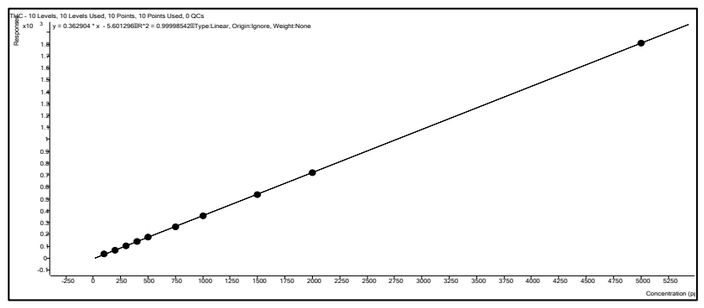
Calibrators containing a mixture of cannabinoids noted above were prepared over a range of 100 µg/mL to 5,000 µg/mL for method development, and 10 µg/mL to 1,000 µg/mL for the analysis of the unknown samples.

Seven samples of commercially available hemp oil products were purchased. Samples were prepared from 100-microliter aliquots of each commercially purchased sample diluted 100-fold with dichloromethane, followed by a 10-fold dilution with methanol.

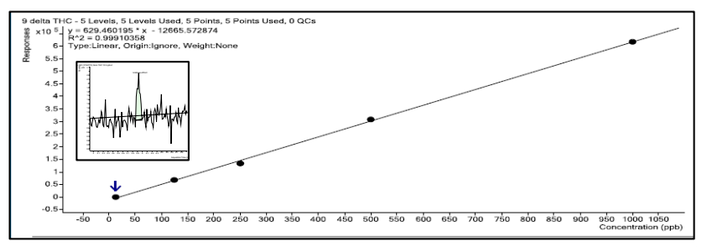
## Results and Discussion




### Quantitation of THC at high and low levels

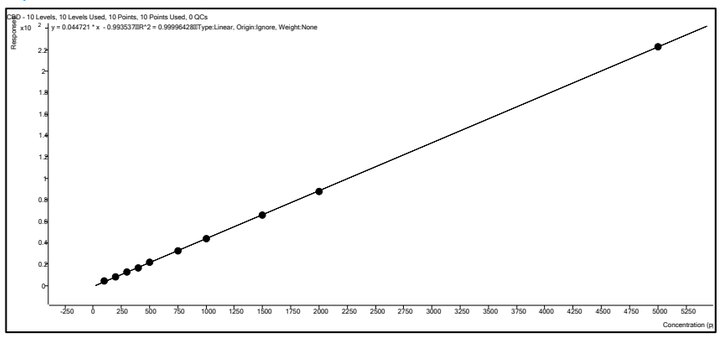


| Sample | | | | THC Method | THC Results | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Name | Data File | Type | Level | Exp. Conc. | RT | Resp. | Calc. Conc. | Final Conc. | Accuracy |
| 9 peak mix | level 1.d | Cal | 1 | 100.0000 | 8.408 | 35 | 110.7183 | 110.7183 | 110.7 |
| 9 peak mix | level 2.d | Cal | 2 | 200.0000 | 8.422 | 68 | 203.0966 | 203.0966 | 101.5 |
| 9 peak mix | level 3.d | Cal | 3 | 300.0000 | 8.427 | 103 | 299.8599 | 299.8599 | 100.0 |
| 9 peak mix | level 4.d | Cal | 4 | 400.0000 | 8.427 | 138 | 396.4041 | 396.4041 | 99.1 |
| 9 peak mix | level 5.d | Cal | 5 | 500.0000 | 8.435 | 177 | 503.9937 | 503.9937 | 100.8 |
| 9 peak mix | level 6.d | Cal | 6 | 750.0000 | 8.446 | 266 | 747.4100 | 747.4100 | 99.7 |
| 9 peak mix | level 7.d | Cal | 7 | 1000.0000 | 8.437 | 356 | 996.0243 | 996.0243 | 99.6 |
| 9 peak mix | level 8.d | Cal | 8 | 1500.0000 | 8.438 | 536 | 1491.6483 | 1491.64... | 99.4 |
| 9 peak mix | level 9.d | Cal | 9 | 2000.0000 | 8.445 | 719 | 1995.9952 | 1995.99... | 99.8 |
| 9 peak mix | level 10.d | Cal | 10 | 5000.0000 | 8.436 | 1811 | 5004.8495 | 5004.84... | 100.1 |



| Data File | Type | Level | Acq. Date-Time | Exp. Conc. | RT | Resp. | Calc. Conc. | Final Conc. |
|---|---|---|---|---|---|---|---|---|
| THC 12.5 ppb.d | Cal | 1 | 8/9/2016 3:20 AM | 12.5000 | 5.925 | 4703 | 17.9468 | 17.9468 |
| THC 125 ppb.d | Cal | 2 | 8/9/2016 3:40 AM | 125.0000 | 5.910 | 68067 | 118.1281 | 118.1281 |
| THC 1000 ppb... | Cal | 5 | 8/9/2016 4:42 AM | 1000.0000 | 5.922 | 626243 | 1000.6274 | 1000.62... |
| THC 500 ppb.d | Cal | 4 | 8/9/2016 4:21 AM | 500.0000 | 5.920 | 316156 | 510.3682 | 510.3682 |
| THC 250 ppb.d | Cal | 3 | 8/9/2016 4:01 AM | 250.0000 | 5.923 | 138773 | 229.9177 | 229.9177 |

### Quantitation of CBD



| Sample | | | CBD Method | CBD Results | | | | |
|---|---|---|---|---|---|---|---|---|
| Data File | Type | Level | Exp. Conc. | RT | Resp. | Calc. Conc. | Final Conc. | Accuracy |
| level 1.d | Cal | 1 | 100.0000 | 7.549 | 4 | 114.6761 | 114.6761 | 114.7 |
| level 2.d | Cal | 2 | 200.0000 | 7.566 | 8 | 203.0707 | 203.0707 | 101.5 |
| level 3.d | Cal | 3 | 300.0000 | 7.567 | 12 | 299.8328 | 299.8328 | 99.9 |
| level 4.d | Cal | 4 | 400.0000 | 7.568 | 17 | 393.2657 | 393.2657 | 98.3 |
| level 5.d | Cal | 5 | 500.0000 | 7.576 | 21 | 502.1863 | 502.1863 | 100.4 |
| level 6.d | Cal | 6 | 750.0000 | 7.585 | 32 | 745.9138 | 745.9138 | 99.5 |
| level 7.d | Cal | 7 | 1000.0000 | 7.578 | 44 | 1006.1116 | 1006.11... | 100.6 |
| level 8.d | Cal | 8 | 1500.0000 | 7.581 | 66 | 1493.4677 | 1493.46... | 99.6 |
| level 9.d | Cal | 9 | 2000.0000 | 7.587 | 88 | 1983.6854 | 1983.68... | 99.2 |
| level 10.d | Cal | 10 | 5000.0000 | 7.577 | 223 | 5007.7899 | 5007.78... | 100.2 |

### Linearity and replicates of CBD

| Name | Data File | Type | Level | Acq. Date-Time | Dil. | Pos. | RT | Final Conc. | Accuracy | Resp. |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 | 25 ppm replicates 01.d | Sample | | 9/5/2016 21:22 | 1 | P3-A1 | 5.444 | 22.4003 | | 3436709 |
| 25 | 25 ppm replicates 02.d | Sample | | 9/5/2016 21:42 | 1 | P3-A2 | 5.443 | 21.5165 | | 3295961 |
| 25 | 25 ppm replicates 03.d | Sample | | 9/5/2016 22:03 | 1 | P3-A3 | 5.41 | 23.7939 | | 3658652 |
| 25 | 25 ppm replicates 04.d | Sample | | 9/5/2016 22:23 | 1 | P3-A4 | 5.406 | 23.9299 | | 3680308 |
| 25 | 25 ppm replicates 05.d | Sample | | 9/5/2016 22:44 | 1 | P3-A5 | 5.373 | 23.818 | | 3662489 |
| 25 | 25 ppm replicates 06.d | Sample | | 9/5/2016 23:04 | 1 | P3-B1 | 5.377 | 23.6223 | | 3631311 |
| 25 | 25 ppm replicates 07.d | Sample | | 9/5/2016 23:25 | 1 | P3-B2 | 5.395 | 23.3784 | | 3592482 |
| 25 | 25 ppm replicates 08.d | Sample | | 9/5/2016 23:45 | 1 | P3-B3 | 5.408 | 23.6481 | | 3635420 |
| 25 | 25 ppm replicates 09.d | Sample | | 9/6/2016 0:06 | 1 | P3-B4 | 5.406 | 23.5704 | | 3623045 |
| 25 | 25 ppm replicates 10.d | Sample | | 9/6/2016 0:26 | 1 | P3-B5 | 5.405 | 23.5852 | | 3625404 |
| 25 | 25 ppm replicates 11.d | Sample | | 9/6/2016 0:47 | 1 | P3-C1 | 5.397 | 23.0039 | | 3532828 |
| 25 | 25 ppm replicates 12.d | Sample | | 9/6/2016 1:07 | 1 | P3-C2 | 5.38 | 22.651 | | 3476628 |
| 25 | 25 ppm replicates 13.d | Sample | | 9/6/2016 1:28 | 1 | P3-C3 | 5.376 | 22.8665 | | 3510951 |
| 25 | 25 ppm replicates 14.d | Sample | | 9/6/2016 1:48 | 1 | P3-C4 | 5.409 | 23.0619 | | 3542068 |
| 25 | 25 ppm replicates 15.d | Sample | | 9/6/2016 2:09 | 1 | P3-C5 | 5.392 | 22.8092 | | 3501832 |
| 25 | 25 ppm replicates 16.d | Sample | | 9/6/2016 2:29 | 1 | P3-D1 | 5.362 | 22.9727 | | 3527863 |
| 25 | 25 ppm replicates 17.d | Sample | | 9/6/2016 2:50 | 1 | P3-D2 | 5.363 | 22.9097 | | 3517831 |
| 25 | 25 ppm replicates 18.d | Sample | | 9/6/2016 3:10 | 1 | P3-D3 | 5.363 | 22.9547 | | 3524993 |
| 25 | 25 ppm replicates 19.d | Sample | | 9/6/2016 3:31 | 1 | P3-D4 | 5.362 | 22.6298 | | 3473258 |
| 25 | 25 ppm replicates 20.d | Sample | | 9/6/2016 3:51 | 1 | P3-D5 | 5.375 | 23.1343 | | 3553608 |
| 2 ppm | 2 ppm.d | Cal | 1 | 9/5/2016 17:56 | 1 | P1-A1 | 5.47 | 2.2898 | 114.5 | 234015 |
| 5 ppm | 5 ppm.d | Cal | 2 | 9/5/2016 18:17 | 1 | P1-A2 | 5.459 | 4.896 | 97.9 | 649066 |
| 15 ppm | 15 ppm.d | Cal | 3 | 9/5/2016 18:37 | 1 | P1-A3 | 5.474 | 14.6092 | 97.4 | 2195935 |
| 25 ppm | 25 ppm.d | Cal | 4 | 9/5/2016 18:58 | 1 | P1-A4 | 5.459 | 25.0264 | 100.1 | 3854923 |
| 40 ppm | 40 ppm.d | Cal | 5 | 9/5/2016 19:18 | 1 | P1-A5 | 5.458 | 40.2786 | 100.7 | 6283917 |
| 60 ppm | 60 ppm.d | Cal | 6 | 9/5/2016 19:39 | 1 | P1-A6 | 5.44 | 59.9 | 99.8 | 9408715 |

### Linearity and replicates of other cannabinoids

| Sample | | THCV Results | | CBC Results | | CBG Results | | CBN Results | | CBD Results | | CBDA Results | | THC Results | | CBGA Results | | Δ9-THCA Results | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Data File | Level | RT | Final Conc. | RT | Final Conc. | RT | Final Conc. | RT | Final Conc. | RT | Final Conc. | RT | Final Conc. | RT | Final Conc. | RT | Final Conc. | RT | Final Conc. |
| level 1.d | 1 | 5.553 | 45.8677 | 5.793 | 55.6911 | 6.448 | 68.1147 | 7.177 | 23.53 | 7.600 | 40.8614 | 8.031 | 88.0906 | 8.462 | 90.4250 | 9.507 | 68.1370 | 12.342 | 82.6832 |
| level 2.d | 2 | 5.566 | 170.7265 | 5.807 | 169.0788 | 6.462 | 173.2007 | 7.191 | 160.9... | 7.614 | 167.5536 | 8.037 | 179.4669 | 8.468 | 185.4560 | 9.520 | 169.9906 | 12.339 | 168.6954 |
| level 3.d | 3 | 5.570 | 297.8734 | 5.810 | 293.2463 | 6.465 | 283.8862 | 7.191 | 290.3... | 7.618 | 291.1512 | 8.040 | 285.5188 | 8.471 | 290.0830 | 9.524 | 283.3952 | 12.326 | 268.4161 |
| level 4.d | 4 | 5.577 | 404.9664 | 5.817 | 413.9471 | 6.464 | 394.0739 | 7.185 | 424.3... | 7.608 | 413.8514 | 8.039 | 404.8989 | 8.470 | 399.6527 | 9.523 | 410.5962 | 12.333 | 390.3144 |
| level 5.d | 5 | 5.578 | 549.5927 | 5.815 | 545.4278 | 6.479 | 540.0666 | 7.191 | 561.2... | 7.622 | 546.4598 | 8.044 | 526.6426 | 8.484 | 518.9580 | 9.528 | 536.6802 | 12.330 | 531.5719 |
| level 6.d | 6 | 5.588 | 807.6517 | 5.828 | 801.2261 | 6.483 | 796.0720 | 7.208 | 823.9... | 7.636 | 828.8500 | 8.058 | 780.3652 | 8.496 | 773.4770 | 9.542 | 789.1688 | 12.327 | 808.5492 |
| level 7.d | 7 | 5.580 | 1048.84... | 5.812 | 1035.8498 | 6.475 | 1065.6733 | 7.197 | 1076... | 7.628 | 1048.3231 | 8.042 | 1018.2177 | 8.490 | 1005.6598 | 9.534 | 1058.43... | 12.320 | 1082.08... |
| level 8.d | 8 | 5.592 | 1486.93... | 5.824 | 1480.3461 | 6.487 | 1480.8181 | 7.202 | 1474... | 7.630 | 1487.0882 | 8.046 | 1489.8548 | 8.485 | 1530.9807 | 9.521 | 1483.92... | 12.298 | 1507.72... |
| level 9.d | 9 | 5.582 | 1922.17... | 5.823 | 1943.7187 | 6.486 | 1938.0747 | 7.208 | 1893... | 7.638 | 1905.3750 | 8.051 | 1970.6716 | 8.495 | 1947.7245 | 9.537 | 1934.30... | 12.297 | 1886.90... |

The $r^2$ coefficients for the LC–TOF-MS data were at least 0.999 for all compounds over both the 100–5000 µg/mL and the 10–1000 µg/mL ranges.

## Results and Discussion

Since the non-psychoactive cannabinoid concentration in the samples is very high compared to THC levels, and the laboratory was not limited by sample amounts, LOQ and limit of detection (LOD) may not be as important for these compounds. However, the unknown samples were purported to contain very low levels of THC and this assertion should be confirmed. Therefore, we also evaluated the LC–TOF-MS data down to 12.5 ng/mL for THC LOD and LOQ experiments. A limit of detection of 1.0ng/mL could be achieved using a signal-to-noise ratio (S/N) of 3:1 as the criterion, and an LOQ of 3.0 ng/mL (S/N ≥ 10:1) for THC was determined.

| Sample ID | Product Description | amount from label or spike | Amount detected |
|---|---|---|---|
| A1 | Hemp oil | 250 mg/ml | 298 |
| A2 | Hemp oil | 250 mg/ml | 272 |
| B | Cold pressed hemp oil | None | None |
| C | Hemp oil | 500 mg/mg | 564 |
| D | Hemp oil | 500 mg/ml | 539 |
| E | Hemp Oil unflavored | 100 mg/ml | 164 |
| F | Hemp Oil flavored | 100 mg/ml | 186 |
| G | Spike into sample B | 1 mg/ml | 0.96 |

It is typical in the industry to set the label as a target minimum concentration value. In order to prove accuracy of our method, we tested cold pressed Hemp oil which is not cannabinoid containing. After confirmation of no cannabinoids we spiked pure sample of CBD oil into the CBD oil, we showed about 96 percent recovery.

## Conclusions

Hemp oil extracts contain 100's of chemical compounds representing a multitude of chemical classes. LC-TOF was used to evaluate the advantages of linear dynamic range, linearity, specificity, and the ability to profile and quantify cannabinoids in unknown hemp oil samples. This method achieves excellent chromatographic separation and LOD for target cannabinoids and offers the ability of discovery-based analysis.

## References

1. Tourangeau, W. (2015) Re-defining Environmental Harms: Green Criminology and the State of Canada's Hemp Industry. *Canadian Journal of Criminology and Criminal Justice*, 57(4):528-554.
2. Keller, N. M. (2013). The legalization of industrial hemp and what it could mean for Indiana's biofuel industry. *Indiana International & Comparative Law Review*, 23(3):555.
3. Huestis, M. A. (2007). Human Cannabinoid Pharmacokinetics. *Chemical Biodiversity*. 4(8): 1770–1804.
4. Brenneisen, R. (2007). Chemistry and analysis of phytocannabinoids and other cannabis constituents. *Forensic Science and Medicine: Marijuana and the Cannabinoids*. Edited by: M. A. ElSohly © Humana Press Inc., Totowa, New Jersey.

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