# It's time for LC/MS to Replace HPLC for Routine Cannabinoid Testing

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The most common analytical technique for the quantitative determination of cannabinoids in cannabis plants and other cannabinoid products, is HPLC with UV detection. However, given the complexity of the matrix in these products, interferences and co-eluting chemicals can often bias the results when using nonselective UV detectors. We compared HPLC-UV with electrospray LC/MS to illustrate the power and need of mass spectrometry for the selective and quantitative determination of 16 cannabinoids including Δ9-THC, Δ8-THC, THCA, CBD, and CBDA in food-grade hemp seed oil.

## Experimental

### Methods

Three HPLC-UV and LC/MS methods were developed that demonstrated baseline resolution for the commercially available cannabinoids. Simultaneous SIM/SCAN data were acquired with the LC/MS method, and full spectral (3D) data were acquired with the HPLC-UV method. A standard curve was created in hemp seed oil over the concentration range of 0.05 µg /mL through 50.00 µg /mL for both methods. A Hemp standard consisting of 500 µg/mL of CBD and 1.5 µg /mL of Δ9-THC was diluted 10:1 and analyzed. Limits of detection (LOD), limits of quantitation (LOQ), linearity, selectivity, accuracy, and precision were determined for both methodologies. Ten samples of commercially available hemp products were purchased and analyzed six times each to determine selectivity and precision. The samples were diluted at 1:10 and 1:100 ratios with a 60/40 Ethanol and Water mixture.



## Experimental

### Method 1

Column: Agilent Poroshell EC-C18, 3.0 x 150mm, 2.7um
Flow Rate: 0.5 mL/min
Mobile Phase: A) 0.1% Formic Acid in H2O
B) 100% ACN
C) 100% MeOH
D) 10 mM Ammonium Formate in $H_2O$

Gradient:

| Time | %A | %B | %C | %D |
|------|-----|-----|-----|-----|
| 0.00 | 29 | 70 | 0 | 1 |
| 3.20 | 29 | 70 | 0 | 1 |
| 7.20 | 12 | 0 | 87 | 1 |
| 10.00 | 0 | 0 | 95 | 5 |

Column Temperature: 30 °C
Post Time: 5 minutes
UV: 228 nm

### Method 2

Column: Agilent Poroshell EC-C18, 3.0 x 150mm, 2.7um
Guard Column: Poroshell 120 EC-C18, 3.0 x 5mm, 2.7 µm
Flow Rate: 0.8 mL/min
Mobile Phase: A) 0.1% 5mM Ammonium Formate in Water
B) 0.1% Formic Acid in Acetonitrile

Gradient:

| Time | %A | %B |
|------|-----|-----|
| 0.00 | 25 | 75 |
| 10.00 | 10 | 90 |
| 11.00 | 10 | 90 |

Column Temperature: 30 °C
Stop Time: 11 minutes
Post Time: 3.5 minutes
Injection Volume: 5 uL
Autosampler Temperature: Ambient
Peak Width >0.0063 min, 40 Hz
UV: 230 nm

### Method 3

Column: Agilent ZORBAX Bonus RP 3.0 × 150 mm, 1.8 µm
Flow Rate: 0.5 mL/min
Mobile Phase: A) Water
B) Methanol
C) 0.1 % Formic Acid + 2.2 mL of 5mM Ammonium Formate in Water

Gradient:

| Time | %A | %B | %C |
|------|-----|-----|-----|
| 0.00 | 23 | 72 | 5 |
| 12.50 | 0 | 95 | 5 |

Column Temperature: 50 °C
Stop Time: 12.5 minutes
Post Time: 6.5 minutes
Injection Volume: 0.25 µL
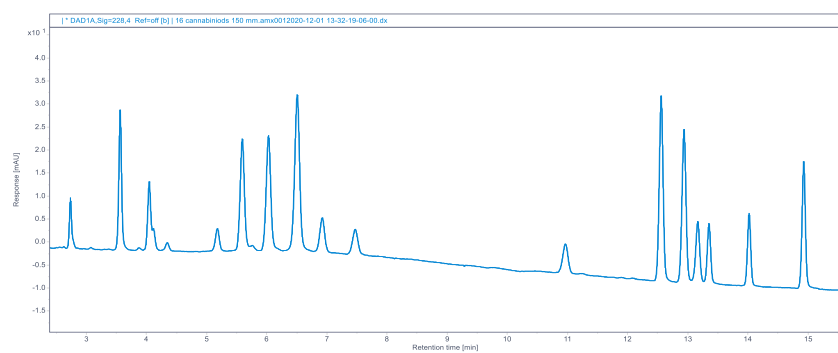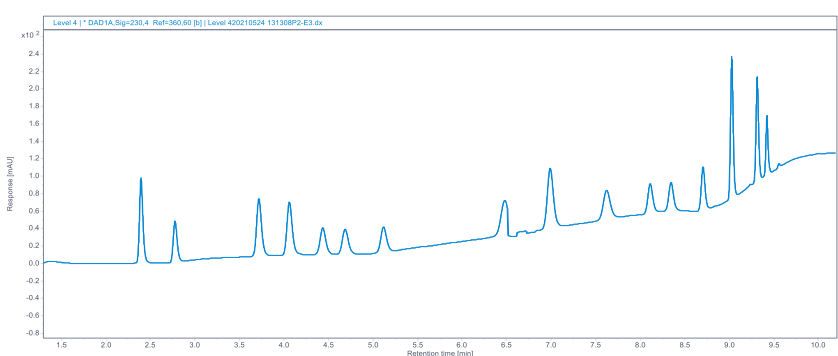Autosampler Temperature: 25 °C
UV: 230 nm
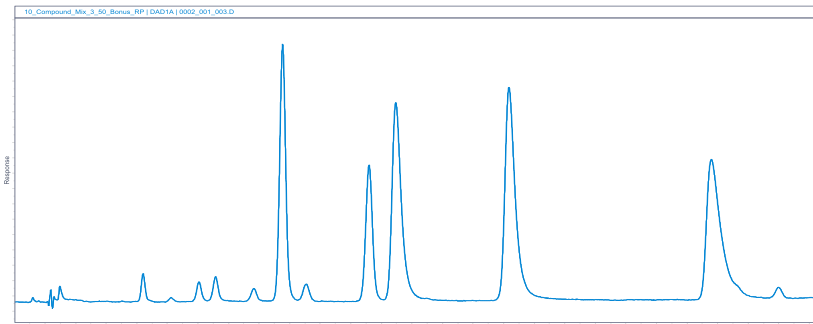Overall run time: 20.0 minutes (including re-equilibration)

## UV Chromatograms
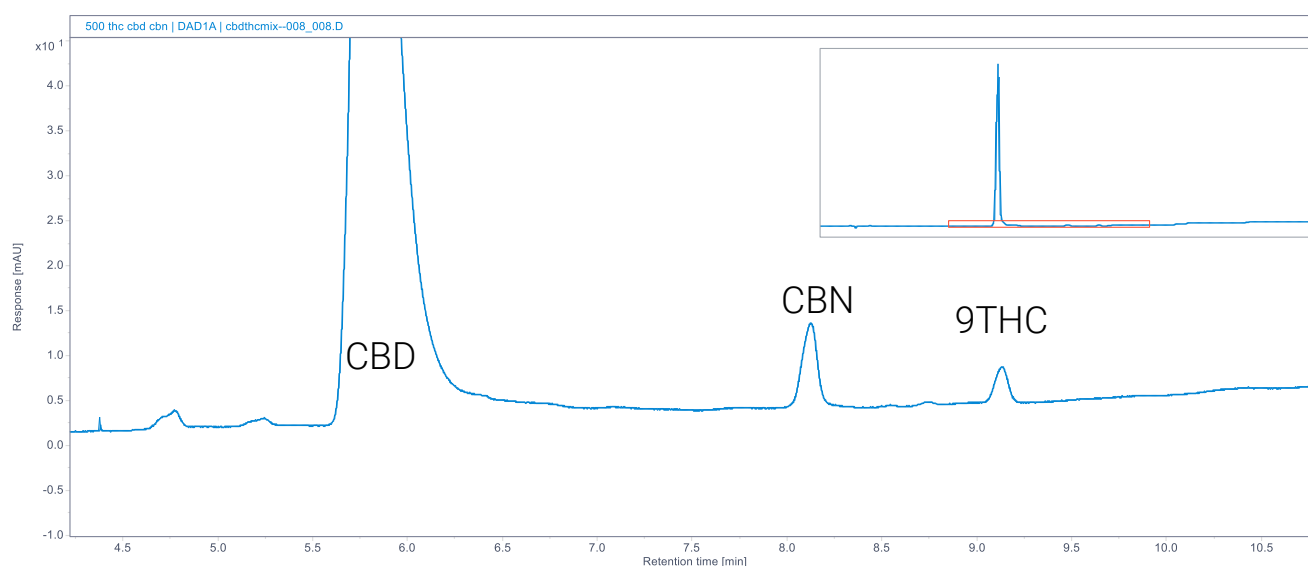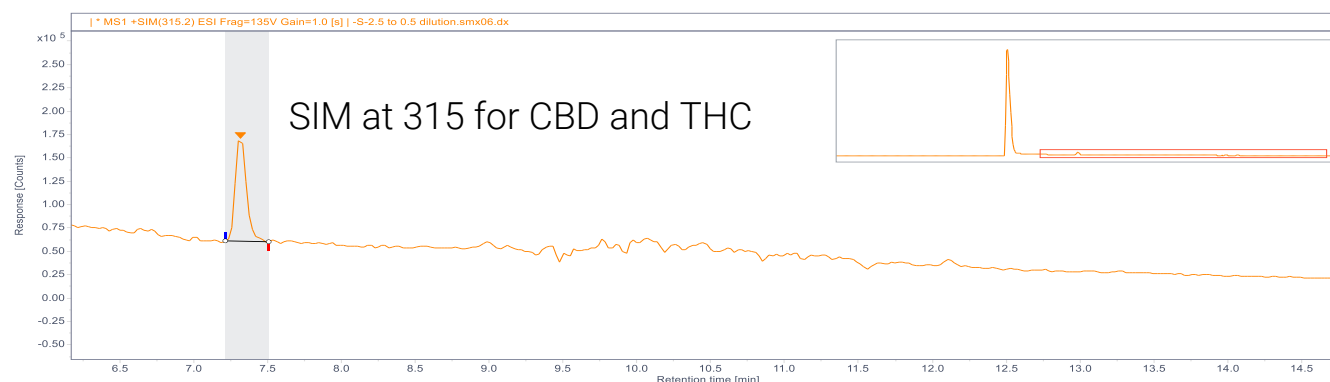### Method 1



### Method 2



### Method 3



## UV Chromatograms compared to LC/MS TIC for Hemp Standard
Data with *Method 2*



Hemp standard CBD concentration is 500 µg/mL which is typical for many of the Pet CBD oils. The THC concentration is 1.5 µg/mL which is less than 0.3% (wt/wt) of the CBD oil note the $10^1$ of the UV axis compared to $10^6$ of the Y axis of the SIM at 315.2



SIM at 315 for CBD and THC

## Hemp oil results with both UV and LC MS data

The following is the results of Delta 9 - THC concentrations in 7 Samples of commercial Pet Hemp oil run with all three methods.

| Sample | Δ9-THC UV 1 | Δ9-THC MS 1 | Δ9-THC UV 2 | Δ9-THC MS 2 | Δ9-THC UV 3 | Δ9 THC MS 3 |
|---|---|---|---|---|---|---|
| 1 | 620 µg/ml | 712 µg/ml | 650 µg/ml | 740 µg/ml | 600 µg/ml | 690 µg/ml |
| 2 | Not detected | 5.5 µg/ml | Not detected | 5.5 µg/ml | Not detected | 5.5 µg/ml |
| 3 | Not detected | 13.5 µg/ml | Not detected | 15.4 µg/ml | Not detected | 12 µg/ml |
| 4 | Not detected | 4.1 µg/ml | Not detected | 3.6 µg/ml | Not detected | 2.8 µg/ml |
| 5 | Not detected | 14.3 µg/ml | Not detected | 15.1 µg/ml | Not detected | 13.9 µg/ml |
| 6 | 158 µg/ml | 165µg/ml | 148 µg/ml | 167 µg/ml | 141 µg/ml | 163 µg/ml |
| 7 | 45 µg/ml | 58 µg/ml | 50 µg/ml | 70 µg/ml | Not detected | 66 µg/ml |
| Hemp oil | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected |
| Hemp Std | 1.4 µg/ml | 1.5 µg/ml | 1.6 µg/ml | 1.5 µg/ml | 1.5 µg/ml | 1.5 µg/ml |

1:10 dilutions calculated for this table. Average of 6 replicates

## Method 1 Results for Sample 2
### (1:100 dilution)                                    (1:10 dilution)



Delta 9 - THC is not found in the DAD on several samples. This is an example of 1:100 and a 1:10 dilution. These values are easily above our lowest calibration curve in a standard. However, matrix plays a role in these samples. Delta 9 - THC was found in samples by LC/MS. In calculation, this made the sample give greater than 0.3% Delta 9 - THC by weight.

## Method 3 Results for Sample 5



## Conclusions

Based on the defined analytical metrics, the LC/MS method demonstrated better overall performance, sensitivity, and selectivity compared to the HPLC-UV method. Matrix interferences at low level cannabinoid concentrations in samples affected the HPLC-UV spectral confirmation and purity. Most importantly, interfering, and co-eluting matrix compounds can be readily distinguished from the target analytes using the specificity of electrospray quasi-molecular ions. However, it is still imperative to chromatographically resolve isobaric compounds such as Δ9-THC, Δ8-THC, and CBD.

## References

Quantitation of Phytocannabinoid Oils Using the Agilent Infinity II 1260 Prime/infinityLab LC/MSD iQ LC/MS System

Agilent Application Brief:  5991-8210EN.pdf (agilent.com)

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