# Simultaneous UV and Mass Detection Aids in Rapid Cannabinoid Identification

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## Introduction

The evolution of hemp processing to isolate non-psychoactive cannabinoids has expanded from almost exclusively cannabidiol (CBD) to other, minor cannabinoids – CBG, CBC, CBL, CBCT, CBDV – to name a few.

Identification can be problematic as many cannabinoids possess the same molecular weight limiting the utility of mass detection alone. Fortunately, most cannabinoids absorb UV at different wavelengths but that alone is not enough information of provide an accurate cannabinoid ID, especially if the cannabinoid is contaminated with another compound.

Flash systems outfitted with PDA UV detectors and in-line mass detectors provide individual UV and mass spectra for each eluting cannabinoid which improve their identification.

Compounding the problem of separation and identification is the fact that neither normal-phase nor reversed-phase flash chromatography alone provide complete cannabinoid separations as seen in Figure 1.

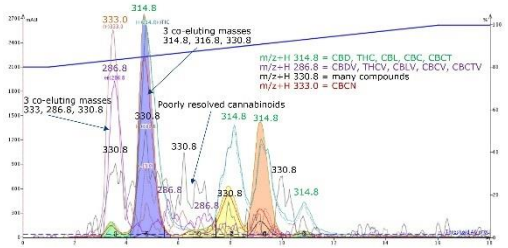


*Figure 1. Reversed-phase flash chromatography of hemp distillate provided a separation of many UV absorbing peaks most which were a mixture of cannabinoids.*

To improve purification success an orthogonal purification strategy was used, first with normal-phase then reversed-phase.

In this poster we show how a single flash system, using an orthogonal purification strategy and both PDA UV and mass detection, helped to separate and identify several cannabinoid compounds.

## Experimental Protocol

### Reagents and Materials

Solvents: methanol, hexanes, methyl t-butyl ether (Reagents, Inc., Charlotte, NC), and in-house deionized water

Sample: hemp distillate

Flash chromatography:

Biotage® Isolera Dalton 2000 system with APCI

10-gram Biotage® Sfär HC column

12-gram Biotage® Sfär C18 flash column

Evaporation:

Biotage® V-10 Touch rapid solvent evaporator

### Normal-phase Purification

Load: 0.5 gram

Solvent A: hexanes

Solvent B: MTBE

Gradient: Multi-step gradient from 0% B to 50% B

Flow rate: 40 mL/min

PDA UV: 200-400 nm

APCI masses: 314.8, 286.8, 330.8, 333.0, monitor TIC

### Results

Though only partially separated, each early eluting UV peak has multiple masses detected, primarily 314.8 and 286.8 corresponding to CBD, THC, CBC, CBL, and CBCT (all 314.8) and CBDV, THCV, CBCV, and CBCTV (all 286.8). The later eluting, higher polarity cannabinoids had detectable m/z+H of 330.8 and 333, Figure 2.

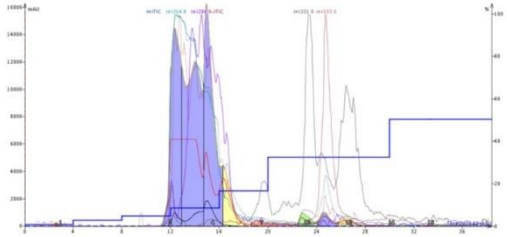

*Figure 2. Hemp distillate normal-phase flash chromatography using both PDA UV and mass detection partially separated many cannabinoids by polarity groups.*

### Reversed-phase Purification

Each of the early eluting normal-phase fractions (2-5) were dried and re-purified by reversed-phase using the same UV wavelengths and targeted m/z.

Solvent A: $H_2O$

Solvent B: MeOH

Gradient: 80%-100% B over 15 CV

Flow rate: 30 mL/min

### Results

Re-purification of the normal-phase fractions showed complete removal of the major high polarity contaminants (m/z+H 330.8 and 333) as well as the separation of the individual cannabinoids and their detected m/z+H, Figure 3.

UV spectrum analysis of each separated peak helped with identification, Table 1.

*Table 1. UV maxima of common cannabinoids*

| Cannabinoid | Wavelength 1 (nm) | Wavelength 2 (nm) | Wavelength 3 (nm) |
|---|---|---|---|
| CBD/CBDV | 207 | shoulder @ 227 | 275 |
| THC/THCV | 209 | shoulder @ 227 | 279 |
| CBC/CBCV | 230 | 283 | |
| CBN | 219 | 283 | |
| CBG | 206 | shoulder @ 231 | 275 |
| CBCT | 212 | shoulder @ 235 | 280 |
| CBDA | 223 | 270 | 308 |

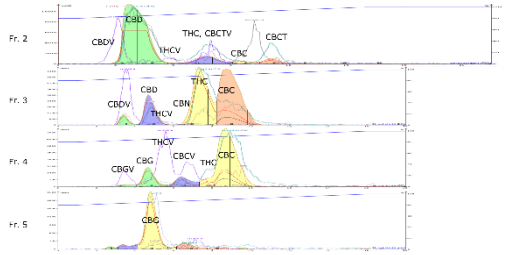


*Figure 3. Reversed-phase purification of normal-phase fractions 2-5 indicated complete removal of the polar contaminants and the separation of many individual cannabinoids.*

This is possible as most cannabinoids do absorb UV light at different wavelengths, such as CBD and CBC, Figure 4.

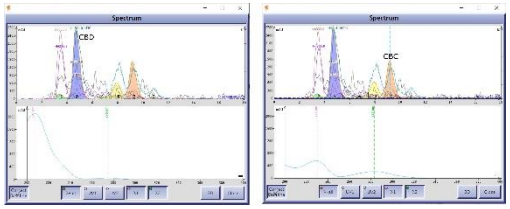


*Figure 4. UV spectra for CBD and CBC captured during reversed-phase flash chromatography displayed very different UV maxima.*

## Conclusions

Orthogonal flash purification combined with in-line UV spectra analysis and mass detection provided an efficient means for the separation, isolation, and identification of many minor cannabinoids in a hemp distillate.

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