Poster Reprint

**ASMS 2023**
**Poster number WP 675**

# Fast LC/MS/MS Analytical Method for the Separation of Isobaric Isomers of Delta 8, 9 and 10 THC and Carboxy THC metabolites

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## Introduction

The main goal of this work was to provide chromatographic resolution with baseline separation between delta-8-tetrahydrocannabinol (Δ8-THC), delta-9-tetrahydrocannabinol (Δ9-THC), and delta-10-tetrahydrocannabinol (Δ10-THC) as well as two metabolites, 11-nor-9-carboxy-Δ8-THC (Δ8-THCA) and 11-nor-9-carboxy-Δ9-THC (Δ9-THCA), utilizing liquid chromatography/mass spectrometer triple quadrupole (LC/MS TQ) technology. The THC isobars are structurally almost identical, with the only difference in the position of the delta double bond, the same also applies to the THCA isobars (Figure 1). The mass spectrometer can not distinguish between compounds with the same molecular formula and structure. Therefore, chromatography needs to be used to provide good enough separation for proper identification and quantitation. During method development, four HPLC columns packed with different packing stationary phases (phenyl-hexyl, stable bond AQ, charged surface CS-C18, end capped EC-C18) were compared. The Poroshell EC-C18 and CS-C18 were almost even, however, Poroshell CS-C18 with 2.7 µm particles was chosen as it gave slightly better separation. In addition, various mobile phases (acetonitrile, methanol, isopropanol) and additives (ammonium formate, ammonium acetate, ammonium fluoride, acetic acid and formic acid) were evaluated. It was concluded that water/methanol with ammonium formate and formic acid provided best chromatographic results. The chromatographic method and mass spectrometer conditions are provided in tables 1-4.

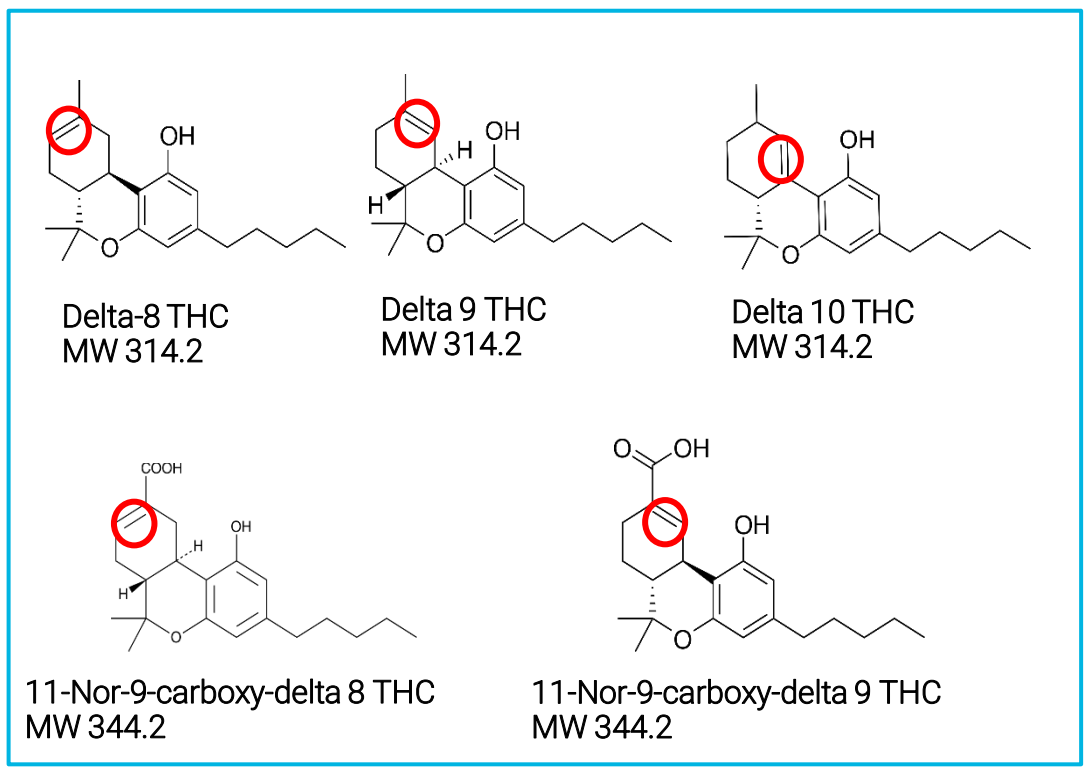


Figure1. Analyte Structures

## Experimental

### Sample Prep

Drug standards were made from a working stock solution. The calibration curve was made by serial dilutions from 1 ng/mL to 1000 ng/mL Samples were injected onto an analytical column column and analyzed in positive mode via LC-MS/MS.

### LC-MS/MS Analytical Method

The LC-MS/MS system consisted of a 1290 binary pump, a thermostatted autosampler, a temperature-controlled column compartment, and a 6495C triple quadrupole mass spectrometer. Separation conditions are given in Tables 1 and 2.

| | |
|---|---|
| Column | Poroshell EC-C18 2.1x100 mm 2.7 µm |
| Injection | 20 µL |
| Solvent A | Water + 5mM ammonium formate |
| Solvent B | Methanol |
| Needle Wash | 50:20:20:10 IPA:MeOH:ACN:H2O |
| Sampler Temp. | 5 °C |
| Column Temp. | 55 °C |

Table 1. UHPLC Parameters

| | | |
|---|---|---|
| Flow Rate | 0.300 mL/min | |
| Gradient | Time<br>0.00<br>5.00<br>6.50<br>8.48<br>8.50 | %B<br>75<br>82<br>98<br>98<br>75 |
| Stop Time | 8.5 min | |
| Post Time | 1.5 min | |

Table 2. Pump Parameters

Detection of all analytes was undertaken in multiple reaction monitoring (MRM) mode. MS source conditions for the mass spectrometer are shown in Table 3. Data was acquired and analyzed using MassHunter software suite version 12.

## Experimental

| | | | |
|---|---|---|---|
| Gas Temperature | 300 °C | Sheath Gas Flow | 12 L/min |
| Gas Flow | 12 L/min | Capillary Voltage | 4000 V |
| Nebulizer Pressure | 50 psi | Nozzle Voltage | 1500 V |
| Sheath Gas Temperature | 350 °C | Polarity | Positive |

Table 3. Parameters of Agilent's 6495C with JetStream ESI Source.

| Name | Formula | ISTD? | Precursor *m/z* | Product *m/z* | Dwell (ms) | Fragmentor (V) | CE (V) |
|---|---|---|---|---|---|---|---|
| Delta 10 THC | CH21H30O2 | No | 315.2 | 123.0/193.0 | 20 | 100 | 36/24 |
| Delta 9 THCA | CH21H28O4 | No | 345.2 | 193.0/299.1 | 20 | 105 | 20/12 |
| Delta 9 THC | CH21H30O2 | No | 315.2 | 123.0/193.0 | 20 | 100 | 36/24 |
| Delta 8 THCA | CH21H28OH | No | 345.2 | 193.0/299.1 | 20 | 105 | 20/12 |
| Delta 8 THC | CH21H30O2 | No | 315.2 | 123.0/193.0 | 20 | 100 | 36/24 |

Table 4. MRM Transitions and Parameters

## Results and Discussion

### Chromatography: Column Selection

Four columns with different packing materials (charged surface CS-C18, end capped EC-C18 phenyl-hexyl P-H and stable bond SB-AQ) were tested for baseline separation between isobaric compound isomers. As shown in Figure 2, the best separation was obtained by the CS-C18 column and for final analysis 2.1x100 mm with 2.7 µm particles was chosen.

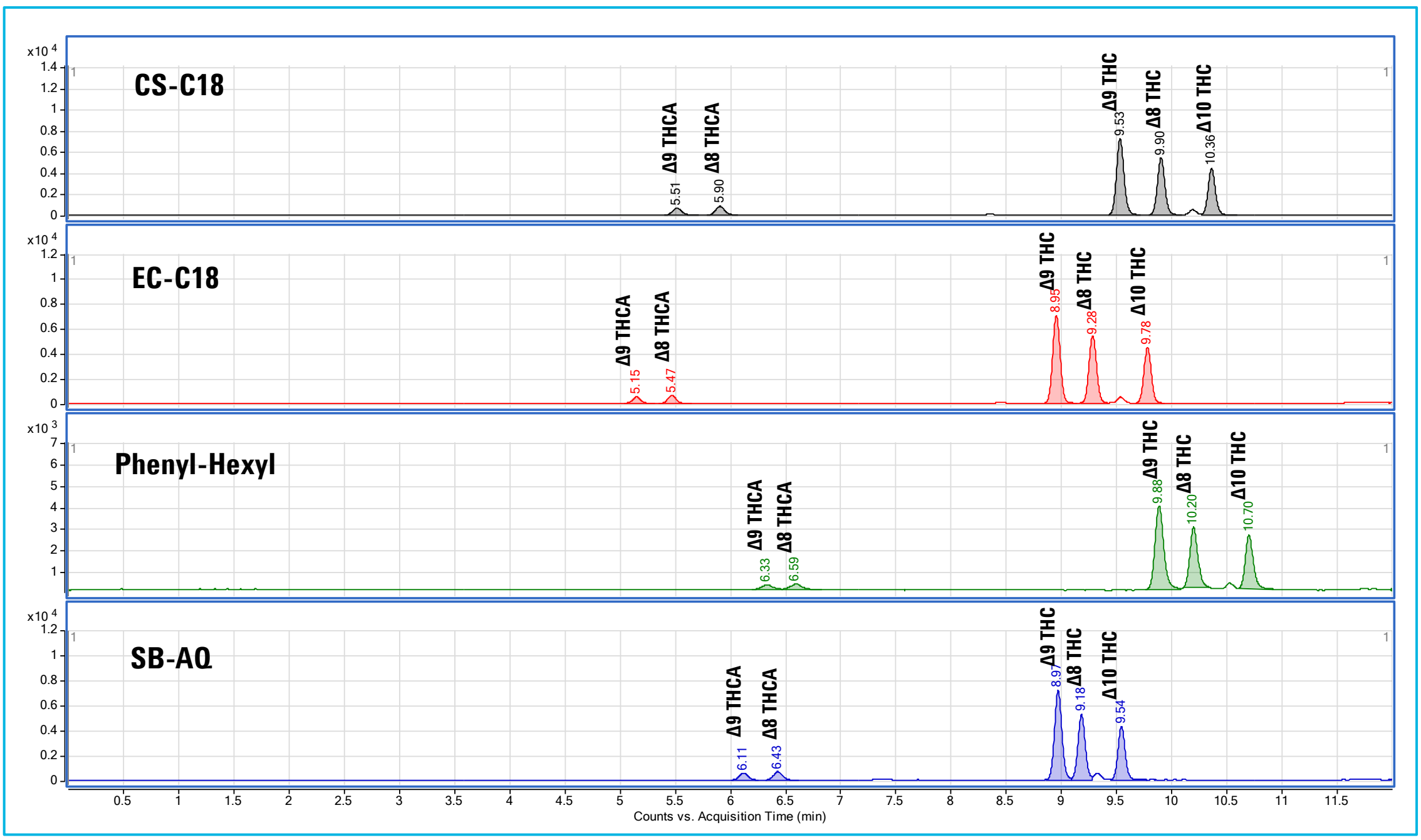


Figure 2. Column Comparison

## Results and Discussion

### Chromatography: MRM vs dMRM

In order to obtain accurate quantitation, it was critical to get baseline separation between isobars. Therefore, the analysis time was extended from a typical 5 minutes to 10 minutes. With regular multi reaction monitoring (MRM) analysis, all isobars were labeled with the same color and there were matrix peaks observed (Figure 3A). When using Dynamic MRM (dMRM), all isobars were labeled with different colors and no matrix peaks were observed (Figure 3B ).

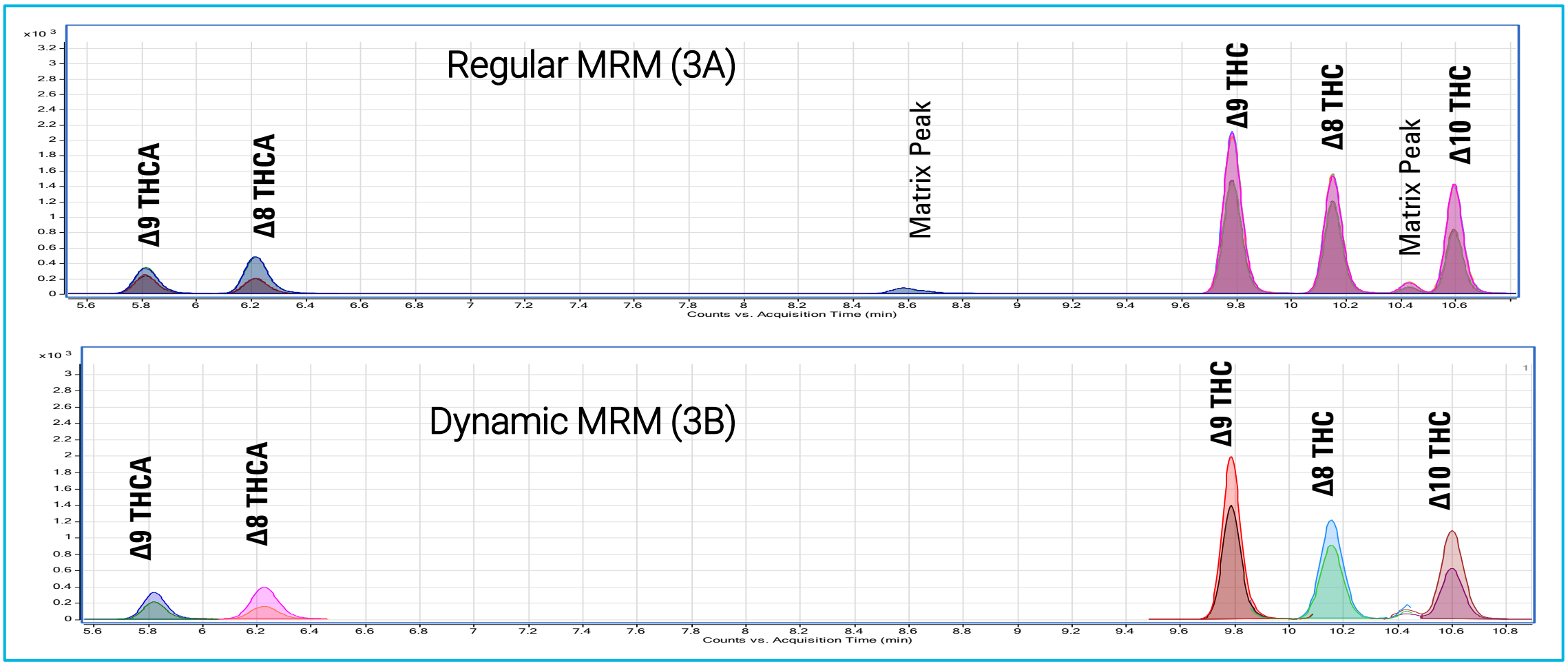


Figure 3. Regular MRM vs Dynamic MRM

### Quantitation: Calibration Curve

The calibration range was tested from 1 ng/mL to 1000 ng/mL for all compounds and showed excellent linearity with correlation coefficient, R2 of 0.998 or better. Example calibration curves are shown below (Figure 4).

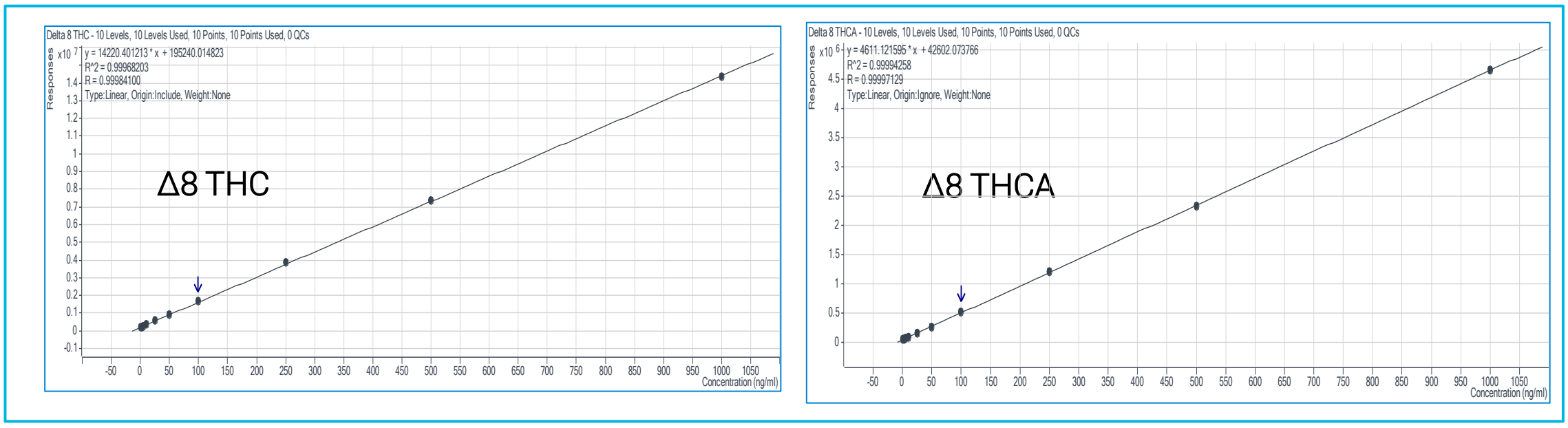


Figure 4. Example Calibration Curves

## Conclusions

Satisfactory chromatographic separation between delta 8, 9 and 10 THC and delta 8 and 9 THCA metabolites was achieved with the CS-C18 UHPLC column. Quantitation of results showed a linear response over the entire concentration range with an excellent correlation of variation. This method can be used in a variety of applications by any analytical lab that has access to the LC/MS TQ instrumentation.

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DE07292755


Published in USA, May 31,2023

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