RESTEK Pure Chromatography

The Study of Three HPLC Column Chemistries for Optimal Separation of THC Isomers

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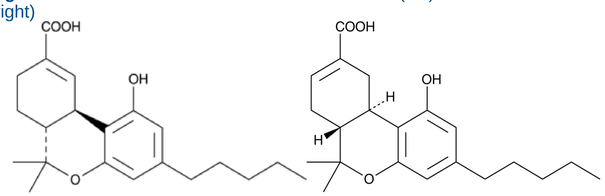
Introduction

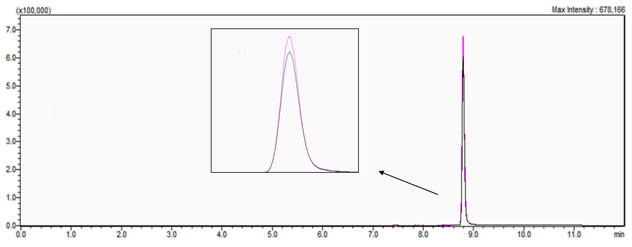
The emergence of Δ9-tetrahydrocannabinol (Δ9-THC) isomers, particularly Δ8-tetrahydrocannabinol (Δ8-THC), have created analytical challenges as they are often not easily resolved by traditional chromatographic methodologies. When consumed, Δ9-THC forms the metabolite 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol (Δ9-THC-COOH). Similarly, Δ8-THC is metabolized to 11-Nor-9-carboxy-Δ8-tetrahydrocannabinol (Δ8-THC-COOH). Traditional methods for separating Δ8-THC and Δ9-THC do not adequately resolve these metabolites, resulting in quantitation issues and the inability to determine an accurate value for one or both isomers. This issue is especially prevalent in urine samples, where these metabolites may be detected at high concentrations.

Figure 1: Chemical structures of Δ9-THC-COOH (left) and Δ8-THC-COOH (right)



Typical LC-MS/MS drug screening or quantitative assays may not be able to detect that an isomer is present in a sample. Restek's "Big Pain" assay utilizes a Raptor Biphenyl column to analyze 231 compounds, including Δ9-THC-COOH. When a sample containing Δ8-THC-COOH and Δ9-THC-COOH was run on this method, no separation of the isomers is observed (Figure 2). An alternative chromatography method would be necessary to detect and quantify the isomers. The objective of this study was to evaluate the capability of different HPLC column chemistries to separate Δ8-THC-COOH and Δ9-THC-COOH.

Figure 2: Unresolved Δ8-THCCOOH and Δ9-THCCOOH isomers when run using Restek's "Big Pain" drug screening assay



Materials and Methods

HPLC Columns

The following HPLC columns were used to develop methods to separate Δ8-THC-COOH and Δ9-THC-COOH:

Table 1: HPLC Columns used for method development

Column	Dimensions	Particle Size
Raptor FluoroPhenyl	100 x 2.1 mm	2.7 µm
Raptor C18	100 x 2.1 mm	2.7 µm
Raptor Biphenyl	100 x 2.1 mm	2.7 µm

Mobile Phases

The following mobile phase compositions were used for all method development:

Table 2: Mobile phase system used for method development

Mobile Phase A	Mobile Phase B
0.1% formic acid in water	0.1% formic acid in methanol

Instrument Parameters

A Shimadzu LCMS-8045 was used for all analysis. The ionization mode used was ESI (positive). The column temperature was 30°C.

Results

Optimized Chromatography Methods

Chromatography methods were optimized to provide as much separation as possible in the shortest run time without exceeding the recommended pressure limit of the columns (8700 psi).

Figure 3: Separation of Δ8-THC-COOH (left peak) and Δ9-THC-COOH (right peak) on the Raptor FluoroPhenyl column

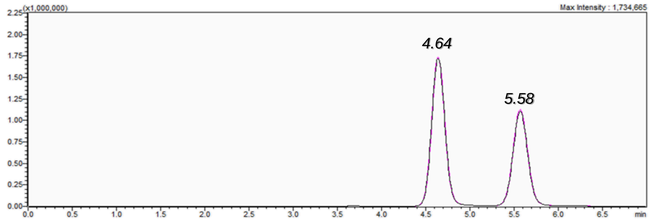


Figure 4: Separation of Δ8-THC-COOH (left peak) and Δ9-THC-COOH (right peak) on the Raptor C18 column

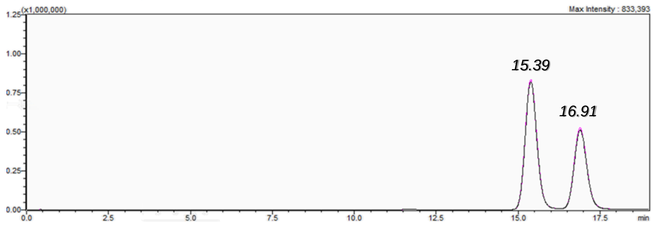


Figure 5: Attempt to separate Δ8-THC-COOH and Δ9-THC-COOH on the Raptor Biphenyl column

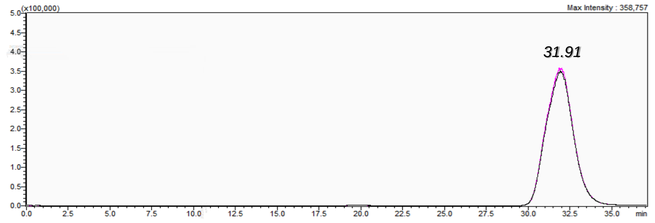


Table 3: Optimized LC Pump Program for Raptor FluoroPhenyl

Time (min)	Flow Rate (mL/min)	%A	%B
0.0	0.6	44	56
7.0	0.6	44	56

Table 4: Optimized LC Pump Program for Raptor C18

Time (min)	Flow Rate (mL/min)	%A	%B
0.0	0.6	37	63
19.0	0.6	37	63

Table 5: Optimized LC Pump Program for Raptor Biphenyl

Time (min)	Flow Rate (mL/min)	%A	%B
0.0	0.6	41	59
37.0	0.6	41	59

Interferences at High Isomer Concentration Ratios

Of the three column chemistries tested, the FluoroPhenyl column provided the most optimized separation of the isomers. Samples were prepared at concentrations of 1000 ng/mL Δ8-THC-COOH:10 ng/mL Δ9-THC-COOH and 1000 ng/mL Δ9-THC-COOH:10 ng/mL Δ8-THC-COOH and analyzed using the developed method. Even at extreme isomer ratios, the peaks were completely resolved and did not interfere with one another.

Figure 6: Analysis of sample containing 1000 ng/mL Δ8-THC-COOH:10 ng/mL Δ9-THC-COOH using developed method

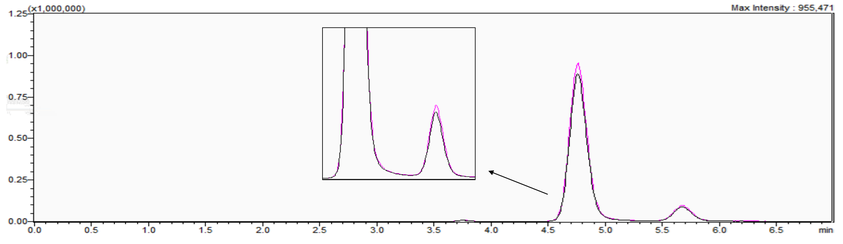
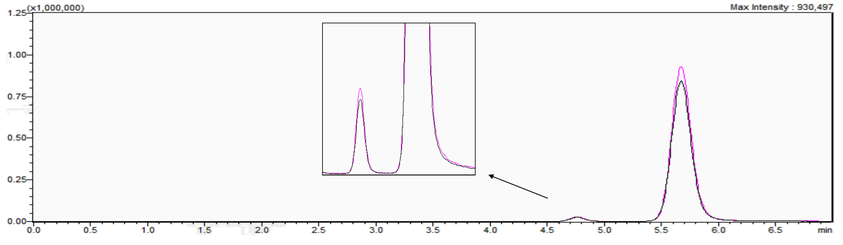


Figure 7: Analysis of sample containing 1000 ng/mL Δ9-THC-COOH:10 ng/mL Δ8-THC-COOH using developed method

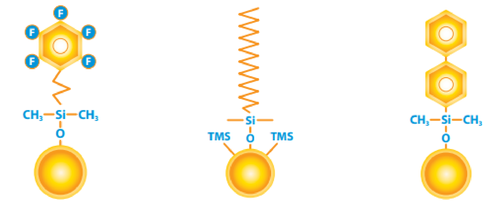


Discussion

Comparing Column Chemistries

Three column chemistries were evaluated for the separation of Δ8-THC-COOH and Δ9-THC-COOH. Raptor Biphenyl provided no separation of the isomers under the conditions tested. Raptor C18 did provide some separate of the isomers but did not adequately resolve them and resulted in a long run time. Raptor FluoroPhenyl provided excellent separation of the analytes in a run time of 7 minutes.

Figure 8: FluoroPhenyl stationary phase (left), C18 stationary phase (middle), and Biphenyl stationary phase (right)



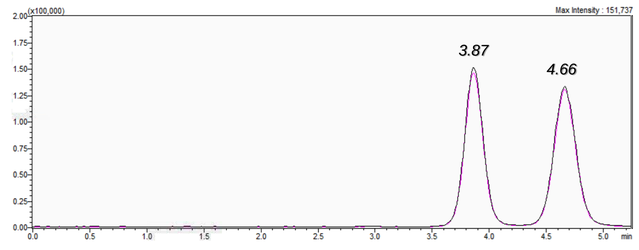
Optimal Separation of Δ8/9-THC-COOH on the Raptor FluoroPhenyl

The Raptor FluoroPhenyl proved the obvious choice for separation of these isomers. The optimized method is quick and reproducible, providing complete resolution of Δ8-THC-COOH and Δ9-THC-COOH that was not achievable with traditional HPLC column chemistries. The high degree of separation was demonstrated by running samples with extreme ratios of the isomers, which showed that the peaks did not interfere with one another even at high concentrations.

Further Optimization

The developed method was adapted to a Raptor FluoroPhenyl 50 x 2.1, 2.7 µm column in an effort to reduce the run time. Complete resolution of Δ8/9-THC-COOH was achieved in a method run time of 5.25 minutes using these column dimensions. This demonstrates that the FluoroPhenyl phase provides multiple effective solutions for separating the isomers.

Figure 9: Separation of Δ8-THC-COOH (left peak) and Δ9-THC-COOH (right peak) on the Raptor FluoroPhenyl 50 x 2.1, 2.7 µm column resulting in reduced run time



Conclusions

The Raptor FluoroPhenyl column provided a solution for the difficult separation of Δ8-THC-COOH and Δ9-THC-COOH. In this case, the FluoroPhenyl phase allowed for increased separation of the isomers in comparison to phases like C18 and Biphenyl. The resolution was adequate to eliminate any interference between the peaks even at high concentrations. The developed method is quick, efficient, reproducible, and can be amendable to different column dimensions to meet any laboratory's analytical needs.

References

Lupo, S. "The Big Pain": Development of Pain-Free Methods for Analyzing 231 Multiclass Drugs and Metabolites by LC-MS/MS. https://www.restek.com/en/technical-literature-library/articles/the-big-pain-development-of-pain-free-methods-for-analyzing-231-multiclass-drugs-and-metabolites-by-LC-MSMS (accessed Feb 28, 2023).

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