

Analysis of Δ-8-THC, Δ-9-THC, and Their Metabolites in Whole Blood by LC-MS/MS

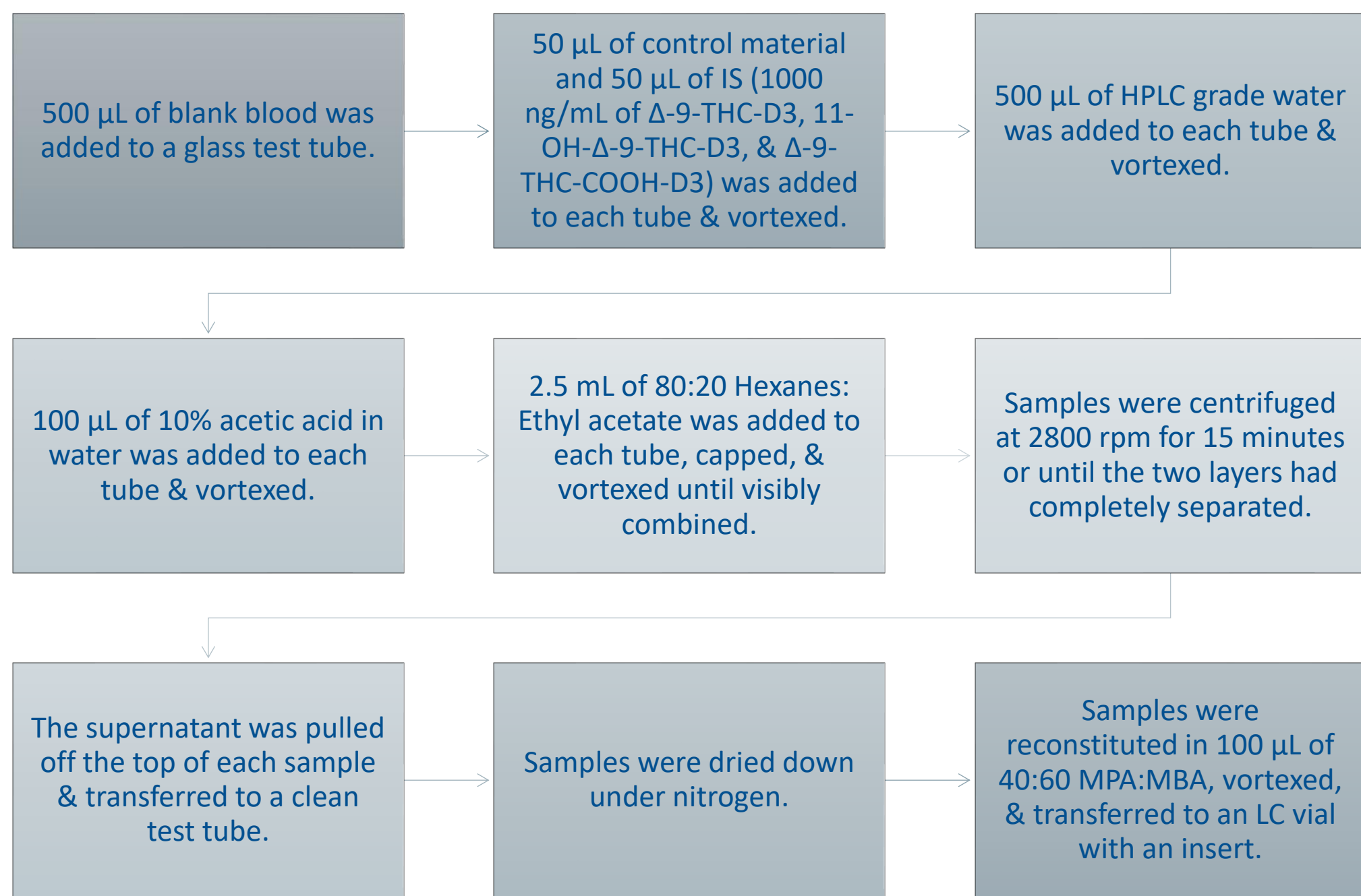
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Introduction

The testing of whole blood samples for tetrahydrocannabinol (Δ-9-THC) consumption is routine and has been around for many decades. Δ-9-THC is metabolized into 11-Hydroxy-Δ9-tetrahydrocannabinol (11-OH-Δ-9-THC) and further into 11-nor-9-carboxy-Δ-9-THC (Δ-9-THC-COOH). It is important to test for the parent and both metabolites to properly monitor for THC usage.¹ As more isomers of Δ-9-THC become available on the market, testing becomes more complicated and novel methods are needed to achieve isomeric resolution. One such isomer, Δ-8-THC, is currently not federally regulated in the United States and is readily available for purchase in many states. This compound forms its own hydroxylated and carboxylated metabolites, (11-OH-Δ-8-THC and Δ-8-THC-COOH), that must be resolved from their isomeric metabolites. The resolution of these metabolites is key in reporting accurate results. Poor resolution, especially when one isomer is in much greater abundance than the other, can result in invalid data. In this study, an LC-MS/MS method was developed to adequately resolve the parent, hydroxy, and carboxy isomer compounds in whole blood.

Materials and Methods

Liquid-Liquid Extraction Procedure



Instrument Parameters

The method was developed on Shimadzu Nexera X2 LC coupled to a SCIEX 4500 MS/MS. The following parameters were optimized for ideal isomer separation.

HPLC Column

The method utilized a Raptor FluoroPhenyl 100 x 3.0 mm, 2.7 µm column with a Raptor FluoroPhenyl EXP guard column cartridge 5 x 3.0 mm, 2.7 µm. The column oven temperature was 40°C.

Mobile Phases

The best separation was achieved for this method using water and methanol as mobile phases, both modified with 0.1% formic acid (v/v).

Chromatography Gradient

The following chromatography gradient was used to achieve separation of all isomers. The flow rate was 0.8 mL/min.

Table 1: Optimized LC Pump Program

Time (min)	%A	%B
0.00	36	64
6.50	36	64
6.60	32	68
13.00	32	68
13.10	0	100
14.00	0	100
14.10	36	64
16.00	36	64

MS Parameters

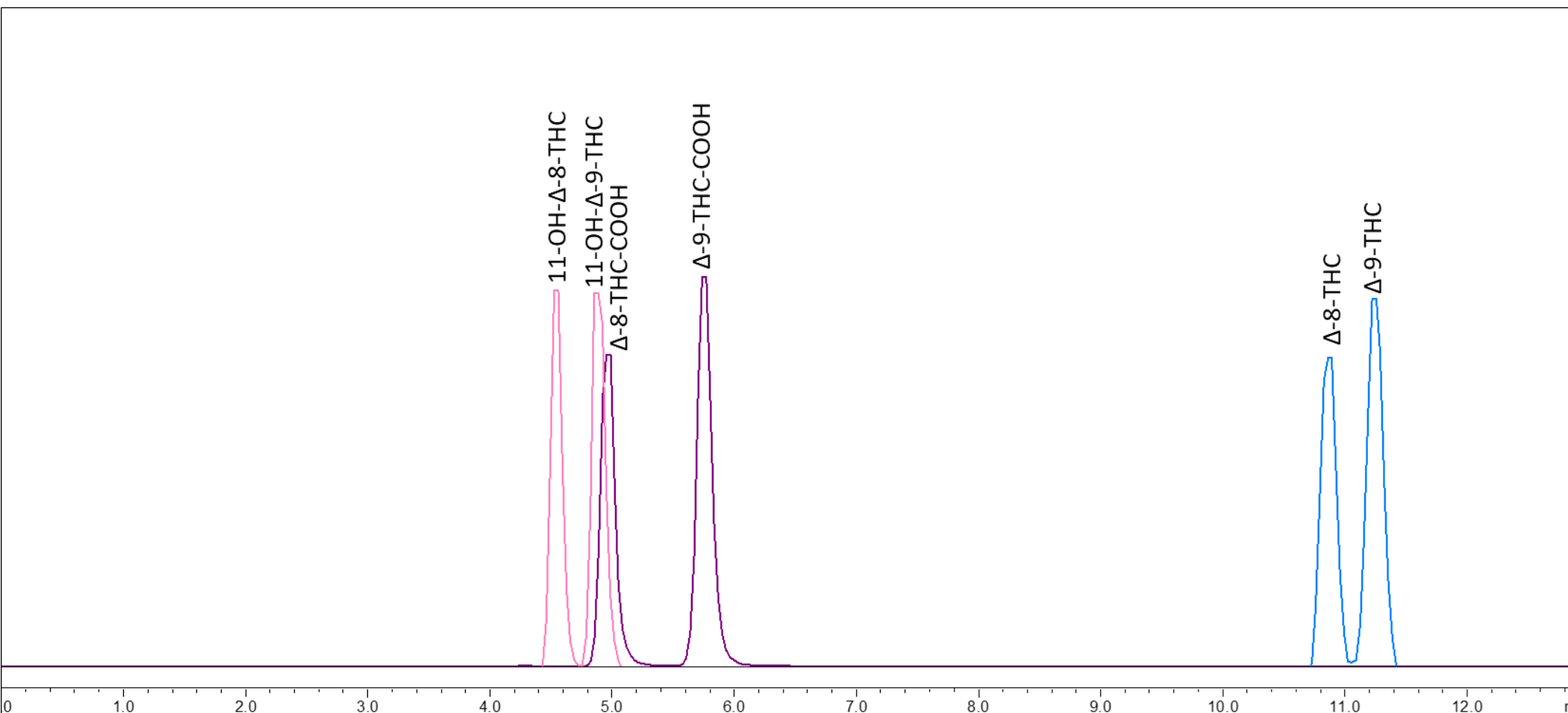
Δ-8/9-THC and 11-OH-Δ-8/9-THC were collected in ESI+ mode, while Δ-8/9-THC-COOH were collected in ESI- mode.

Results

Chromatographic Performance

The developed method successfully resolved all three pairs of isomers in a 13-minute gradient (16-minutes total analysis time). Deuterated internal standards eluted at the same retention time as corresponding nondeuterated analytes.

Figure 1: Separation of Δ-8/9-THC, Hydroxy, and Carboxy Isomers in Whole Blood



Linearity

Linearity was demonstrated using a 1/x weighted linear regression. All analytes showed acceptable R² values (≥0.99). The calibration range was made up of 6 calibrators.

Table 2: Calibration Ranges and Example R² Values

Analyte	Calibration Range (ng/mL)	R ²
11-OH-Δ8-THC	0.5 – 100	0.9932
11-OH-Δ9-THC	0.5 – 100	0.9984
Δ8-THC-COOH	2.5 – 500	0.9956
Δ9-THC-COOH	2.5 – 500	0.9964
Δ8-THC	0.5 – 100	0.9976
Δ9-THC	0.5 – 100	0.9950

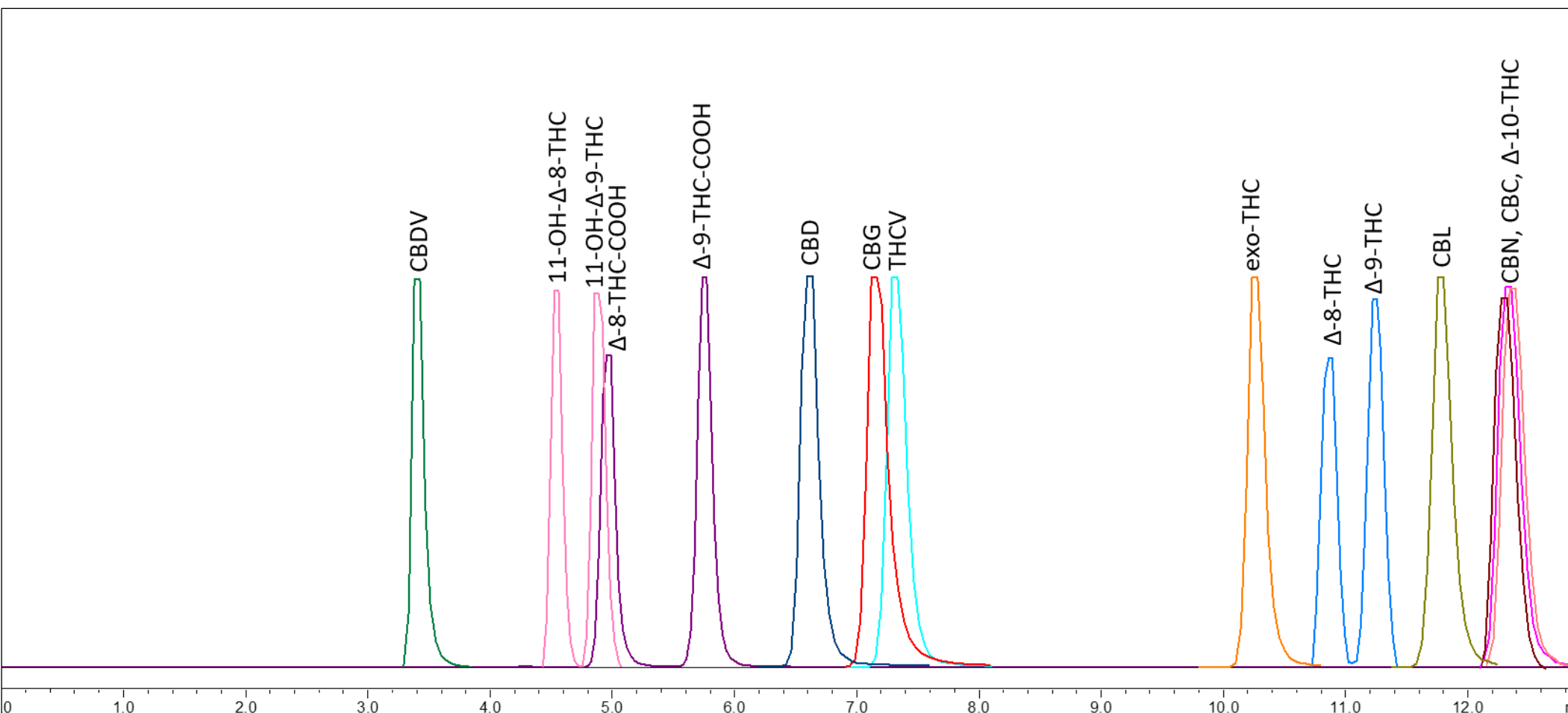
Precision and Accuracy

Precision and accuracy were assessed at four different concentrations (LLOQ, Low QC, Medium QC, High QC) and evaluated within a day and as an average of three days (N=9). The average method accuracy ranged from 86.2-98.8% for all levels. Interday method precision ranged from 1.56-9.90% for all levels. This method demonstrates acceptable precision and accuracy.

Cross-Analyte Interferences

Interferences were tested with nine commonly encountered and/or structurally similar cannabinoids including cannabidivarin (CBDV), cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabivarin (THCV), exo-tetrahydrocannabinol (exo-THC), cannabicyclol (CBL), cannabinol (CBN), 9(S)-Δ^{6a,10a}-THC (Δ10-THC), and cannabichromene (CBC). MRMs for these analytes were added to the acquisition method to identify any potential interferences. All nine cannabinoids were well resolved from the analytes of interest and no cross-analyte interferences were observed.

Figure 2: Separation of Δ-8/9-THC Isomers and Isomer Metabolites from CBDV, CBD, CBG, THCV, exo-THC, CBL, Δ10-THC, CBN, and CBC



Discussion

In this study, a method was developed to analyze the parent compounds, hydroxy, and carboxy metabolites in a whole blood matrix. Samples were prepared with a straightforward liquid-liquid extraction procedure. This method was demonstrated to be fast, rugged, and sensitive enough to meet reporting requirements and is suitable for clinical and toxicology labs that are interested to implement accurate reporting of intoxication at time of incident and/or differentiating consumption of Δ-8-THC and Δ-9-THC.

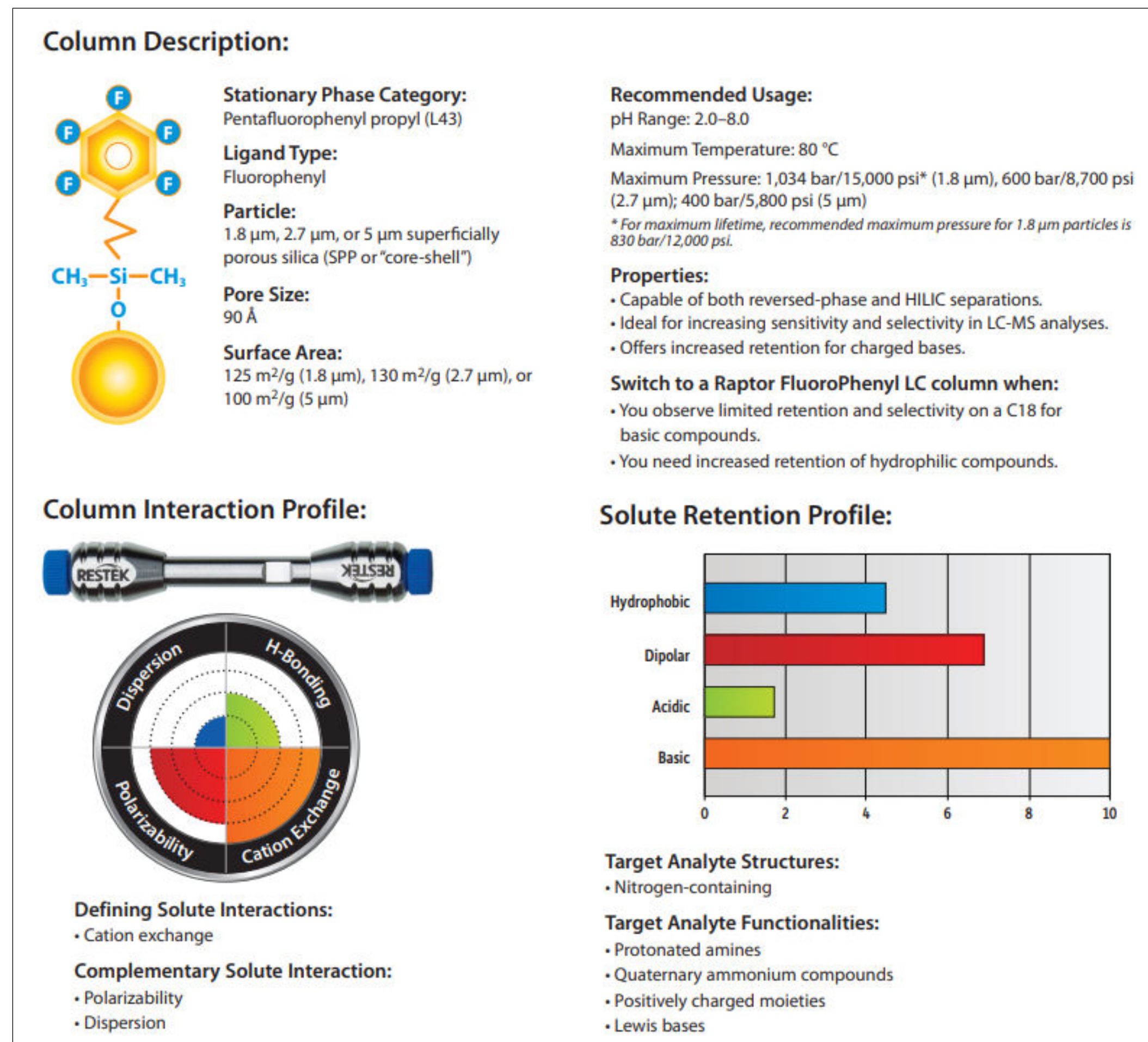
Liquid-Liquid Extraction Procedure

The developed workflow utilized a liquid-liquid extraction (LLE) procedure adapted from Tiscione, Nicholas B et al. (2016).² While LLE is a common sample preparation technique for the analysis of cannabinoids in whole blood, this procedure is advantageous as it uses a smaller sample volume than is typical (500 µL vs. ≥ 1 mL), as well as less extraction solvents (5 mL vs ≥ 10 mL). This allows for the analyst to converse sample volume for further testing as well as reducing extraction costs while still maintaining accuracy and robustness. Internal standard recoveries were consistent for all analytes.

Raptor FluoroPhenyl Column

The Raptor FluoroPhenyl column was an ideal choice for this analysis. While C18 phase columns may show some selectivity for the three isomer pairs, full resolution of the isomers is needed for accurate quantitation. To achieve this resolution on a C18 phase would likely result in a lengthy analytical runtime. The FluoroPhenyl ligand shows selectivity for all three isomer pairs, allowing for adequate separation of the analytes in a reasonable analytical runtime.

Figure 3: FluoroPhenyl Retention Properties



References

- Karschner, E. L.; Swortwood-Gates, M. J.; Huestis, M. A. Identifying and Quantifying Cannabinoids in Biological Matrices in the Medical and Legal Cannabis Era. Clinical Chemistry 66:7 (2020) 888-914.
- Tiscione NB, Miller R, Shan X, Sprague J, Yeatman DT. An Efficient, Robust Method for the Determination of Cannabinoids in Whole Blood by LC-MS-MS. J Anal Toxicol. 2016;40(8):639-648. doi:10.1093/jat/bkw063

Disclosure: I have (or a member of my immediate family has) a financial relationship with a company as defined in the AACC policy on potential bias or conflict of interest