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Application News

No. HPLC-046

High Performance Liquid Chromatography

Cannabinoid Isomer Separation Utilizing LabSolutions MD with an Analytical Quality by Design (AQbD) Approach and i-PDeA Technology

■ Background

The cannabis market has been rapidly growing with the list of identified cannabinoids constantly increasing. Analytical methods for detecting cannabinoids by means of HPLC (High Pressure Liquid Chromatography) analysis have been explored and currently there are methods to separate 21 cannabinoids in a single run. Expanding the list of cannabinoids is possible; however, isomers challenge the current methods and, therefore, further method optimization is needed.

Shimadzu's new analytical method development software, LabSolutions MD (Method Development), alleviates the tedious task of testing, analyzing, and comparing all the individual runs. LabSolutions MD uses Analytical Quality by Design (AQbD) concepts to determine the optimal method for cannabinoid separation. Experimental design during the entire method development process, identification of the most robust analytical conditions, and prediction of chromatograms provide the user with the power to automate the development of robust analytical methods.

This software can be an asset to many fields and will be used in this study to efficiently separate cannabis isomers. In addition to LabSolutions MD, Intelligent Peak Deconvolution Analysis (i-PDeA) technology can be utilized to deconvolute remaining co-eluting peaks after optimization and can be a powerful technique to use in conjunction with MD.

Analytical Quality by Design (AQbD) Principles of LabSolutions MD

Per AQbD principles, ICH recommends that the user first charts out an analytical target profile. The analytical target profile provides scientifically rational explanations of why a particular mobile phase, stationary phase, detector, etc. must be used for analyzing a molecule of interest.

Once the profile is set, we start the screening phase. The screening phase will determine the best combination of column and mobile phase for use in the method optimization phase. During the optimization phase, method conditions such as column temperature, gradient changes, and flow rate are tested to determine the optimal running conditions. After the optimal running conditions are determined, the robustness of the method is tested.

i-PDeA Technology

i-PDeA technology can be utilized to identify coeluting peaks or impurities with a photo diode array (PDA) detector. i-PDeA uses Multivariate Curve Resolutions-Alternating Least Squares (MCR-ALS) deconvolution to detect co-eluting peaks and impurities. This technique is used either when there are co-eluting peaks or suspicion of impurities present in the sample.

■ Method and Instrumentation

Six Daicel chiralpak columns (IA-U, IB-U, IC-U, ID-U, IG-U, IH-U: 3.0mmx100mmx1.6µm) with varying stationary phase chemistries were used with Shimadzu's Method Scouting system and LabSolutions MD to separate several cannabinoids. Mixtures of four, fifteen, and twenty-five cannabinoids were tested using LabSolutions MD. Standards were diluted in methanol to prepare a 25µg/mL mix standard for analysis. The mobile phase was 0.085% phosphoric acid in water and 0.085% phosphoric acid in acetonitrile. i-PDeA technology was performed to deconvolute a mixture of four coeluting cannabinoids.

Nexera Method Scouting System

Item	Description
HPLC System	Shimadzu's Method Scouting System (Nexera X3)
Detection	221nm (SPD-M40)
Mobile Phase A	0.085% Phosphoric Acid in Water
Mobile Phase B	0.085% Phosphoric Acid in Acetonitrile
Columns	1. Chiralpak IA-U (3.0mmx100mmI.D., 1.6µm) 2. Chiralpak IB-U (3.0mmx100mmI.D., 1.6µm) 3. Chiralpak IC-U (3.0mmx100mmI.D., 1.6µm) 4. Chiralpak ID-U (3.0mmx100mmI.D., 1.6µm) 5. Chiralpak IG-U (3.0mmx100mmI.D., 1.6µm) 6. Chiralpak IH-U (3.0mmx100mmI.D., 1.6µm)
Injection Volume	5uL
Column Temp.	40°C
Flow Rate	1.0 mL/min

■ Results

Four Compound Screening with i-PDeA deconvolution

(9R)-Δ6a,10a-THC, (9S)-Δ6a,10a-THC, (6aR,9S)-Δ10-THC, and (6aR,9R)-Δ10-THC isomers have gained popularity in cannabinoid research but are difficult to separate. Chiral columns were screened to separate the four isomers from themselves and other pre-separated cannabinoids. Utilizing the full factorial screening function of LabSolutions MD, six columns and 2 solvents (1 organic and 1 aqueous) were tested to determine the best column and mobile phase pairing for separating cannabinoids. The mobile phases were determined in the analytical target profile based on previous research conducted on cannabinoid separation (See HPLC-039).

The mixture of four isomers were tested first to determine the most effective column in separating the isomers. The columns with separation of all four isomers were IA-U and IG-U, with IG-U having the best separation and faster retention time (~8 minutes). Figure 1 displays an example of the IH-U column that was not able to separate the four isomers. The four isomers can be detected by utilizing i-PDeA peak deconvolution technology. The 3D mapping shows that there are multiple peaks under the chromatographic peak that was detected. In the inset of Figure 1, the deconvolution of the shouldering chromatographic peak is shown with the four isomers detected. This technology can be applied to all the other columns that were unsuccessful in separating all four isomers.

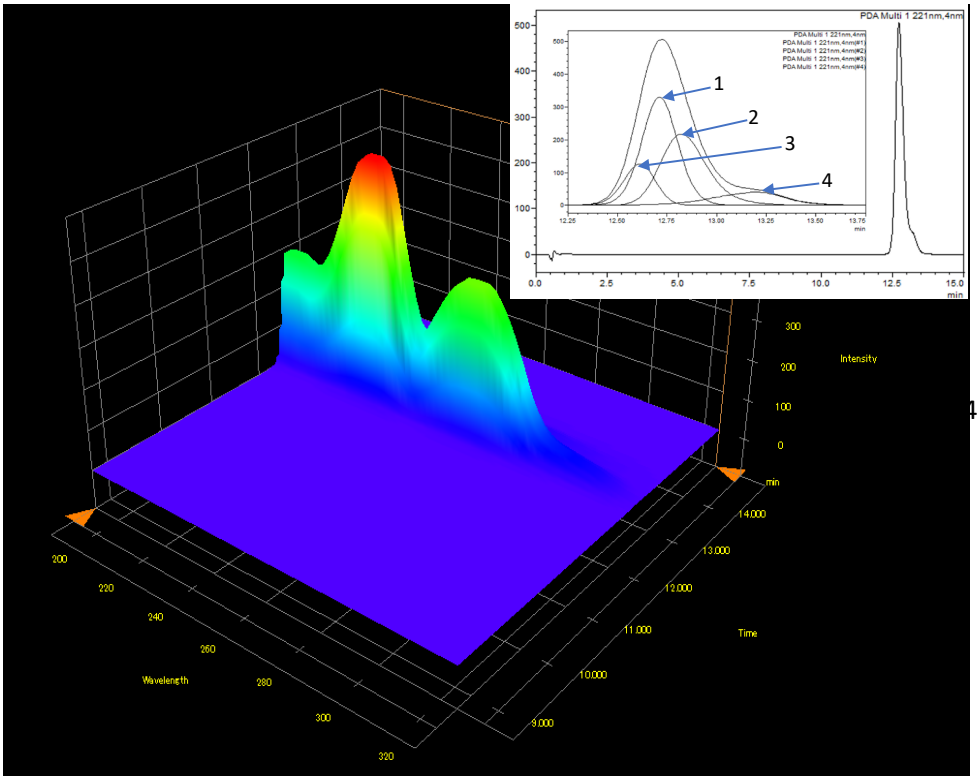

Figure 1: i-PDeA deconvolution of the IH-U column separation of the (9R)-Δ6a,10a-THC, (9S)-Δ6a,10a-THC, (6aR,9S)-Δ10-THC, and (6aR,9R)-Δ10-THC isomers. The 3D mapping shows the presence on multiple peaks within the one chromatographic peak. The inset displays the deconvolution of the shouldering chromatographic peak into four separate peaks (the four target isomers in the mixture).

Addition of Four Isomer Mix to Existing Eleven and Twenty-one Cannabinoid Mis using MD

Like the analysis of four isomers, the screening phase of MD was used to determine which of the chiral columns was the most efficient at separating all the cannabinoids. This process was completed with mixtures of fifteen and twenty-five cannabinoids. The results of the screening phase determined that the highest evaluation value was attributed to the IG-U column and therefore was selected to be used in the optimization phase of MD.

Mobile phase concentration, flow rate, and column oven temperature were tested in the optimization phase using a central composite DOE (Design of Experiment). In the post-run section of LabSolutions MD, modeling features can be used to select the optimal running conditions. The first feature is the predictive chromatograms, which will give a visual representation of where the peaks will elute under specified conditions.

Figure 2A and 3A display the predictive chromatogram of the optimal running conditions for the fifteen and twenty-five cannabinoid mixes and the inset is the real-time chromatogram received when those conditions were executed.

Optimal running conditions for the fifteen-cannabinoid mixture were determined to be a 30-minute isocratic run at a concentration of 46%B (0.085% phosphoric acid in acetonitrile), a flow rate of 1.1mL/min and an oven temperature of 45℃. The twenty-five-cannabinoid mixture had optimal running conditions at a concentration of 44%B (0.085% phosphoric acid in acetonitrile), a flow rate of 1.3mL/min, and an oven temperature of 38℃ The elution order of the cannabinoids is CBDVA, CBDV, CBDA, CBCO, CBG, CBGA, CBCV, CBD, THCV, THCA, CBN, CBNA, CBL/D9-THC, 9(R)- $\Delta^{6a,10a}$-THC, 9(S)- $\Delta^{6a,10a}$-THC, (6aR,9R)- Δ^{10a}-THC, CBDP, CBC, (6aR,9R)- Δ^{10A}-THC, THCA, CBT, CBCA.

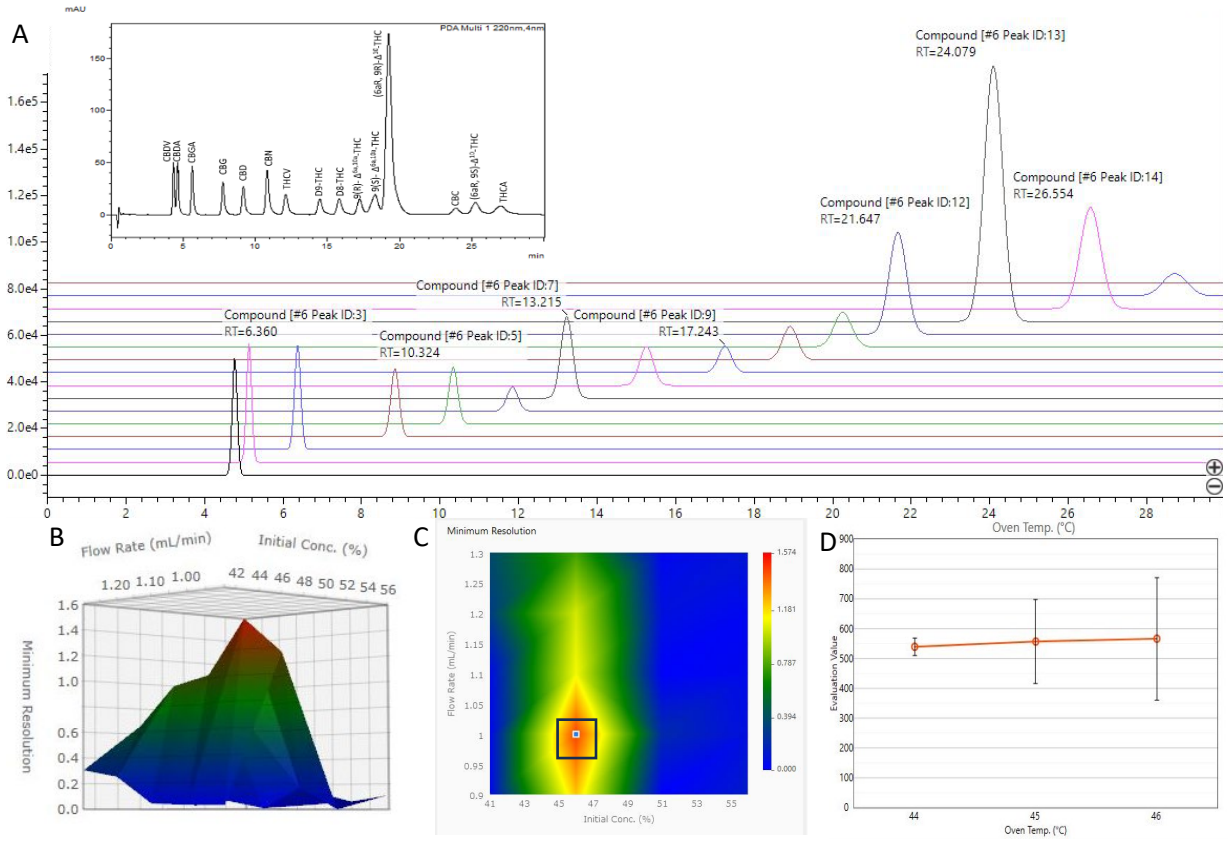

Figure 2: Predictive chromatogram (A), 3D modeling (B), heat map (C), and robustness plot (D) of the fifteen-cannabinoid mixture.

In addition to predictive chromatograms, modeling such as heat maps and 3D modeling can help select the optimal running conditions. Figures 2 and 3 display the 3D models and heat maps for the cannabinoids. The 3D models display the highest evaluation values with peaks at the optimal temperature, concentration, and flow rate. Heat maps (2C & 3C) display the optimal points and the areas of robustness. The red areas of the heat map are the more optimal conditions whereas the blue and green sections are the least optimal. The boxes indicate the area of robustness and where the conditions for optimal separation fall within. This box can be used as a reference in the robustness testing phase as well.

Testing the robustness of the optimal conditions is the last phase of the method scouting process. In the robustness phase, the optimal conditions are tested by making small but deliberate changes to the method parameters (such as 0.1 mL / min flow rate change or 1°C column temperature change etc.). ANOVA is then utilized to generate statical data for the running conditions to determine how robust the optimized method is. Examples of the robustness plots are found in Figure 2D and 3D.

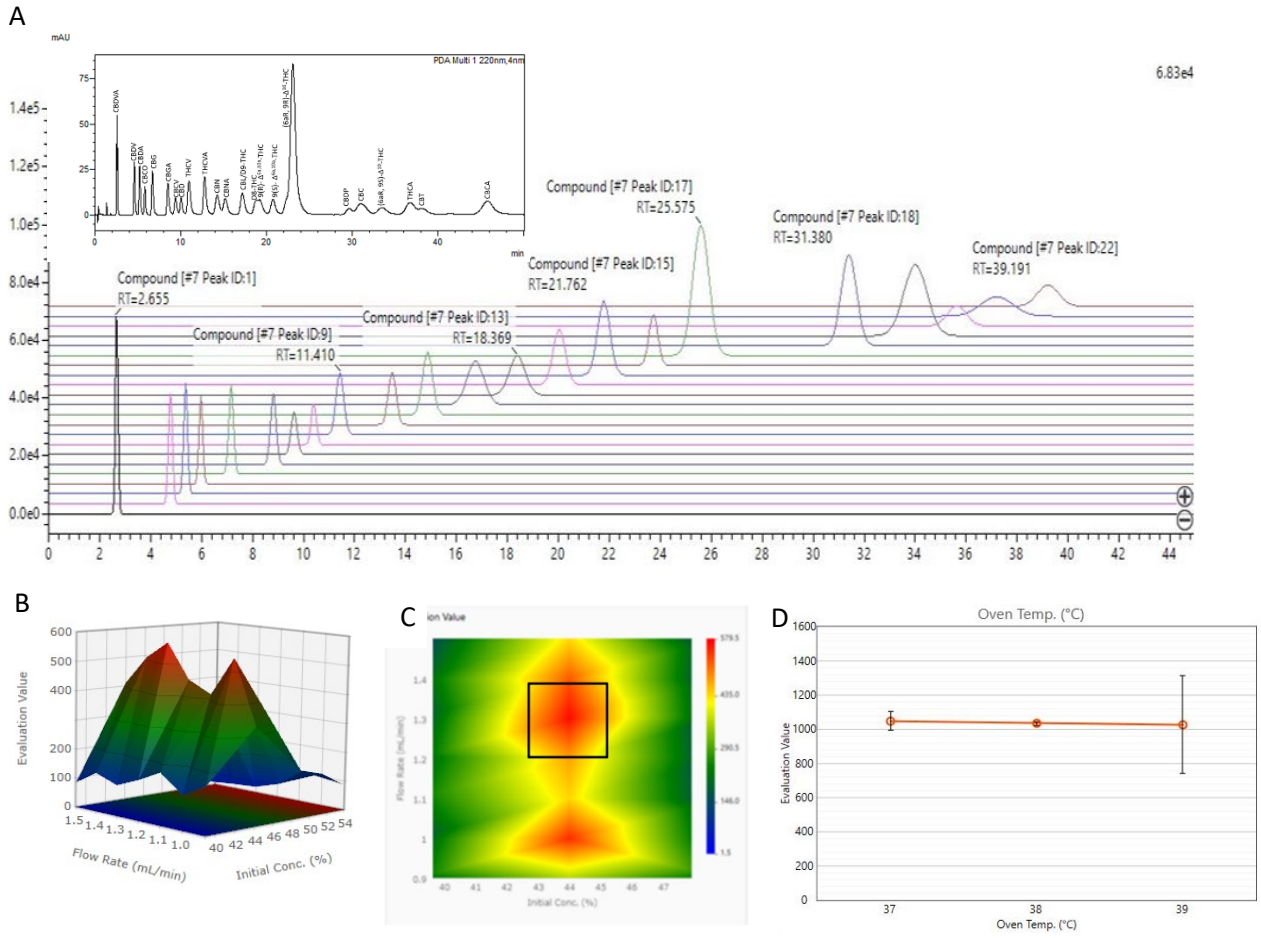

Figure 3: Predictive chromatogram (A), 3D modeling (B), heat map (C), and robustness plot (D) of the twenty-five-cannabinoid mixture.

■ Conclusion

Cannabinoid separation can be difficult with the presence of coeluting isomers. This method was used to determine optimal running conditions for a four, fifteen, and twenty-five cannabinoid mixture. Utilizing method development technologies can assist with identifying and separating difficult cannabinoid mixtures. LabSolutions MD can be utilized to screen and optimize parameters to efficiently produce the most optimal running conditions for complex mixtures.

In addition to MD, i-PDeA deconvolution technology can be a powerful technique to use in conjunction with MD modeling to determine the presence of co-eluting isomers or peak impurities. It could also be advantageous with cannabis samples that may experience terpene interference. These techniques give users the tools to confidently identify and separate complex sample mixtures.

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