Separating Cannabinoid Stereoisomers Utilizing LabSolutions MD with an Analytical Quality by Design (AQbD) Approach

Ciara N. Pitman, Lena Jackson, Kodai Imaeda, Evgenia Barannikova, Nivesh K. Mittal
Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia MD 21046, USA
Email: cnpitman@shimadzu.com

Best Poster Award Participant

Introduction

The cannabis market has been rapidly growing with the list of identified cannabinoids constantly increasing. Analytical methods for detecting over 25 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 stereoisomers 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 predicted 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 stereoisomers.

Methods

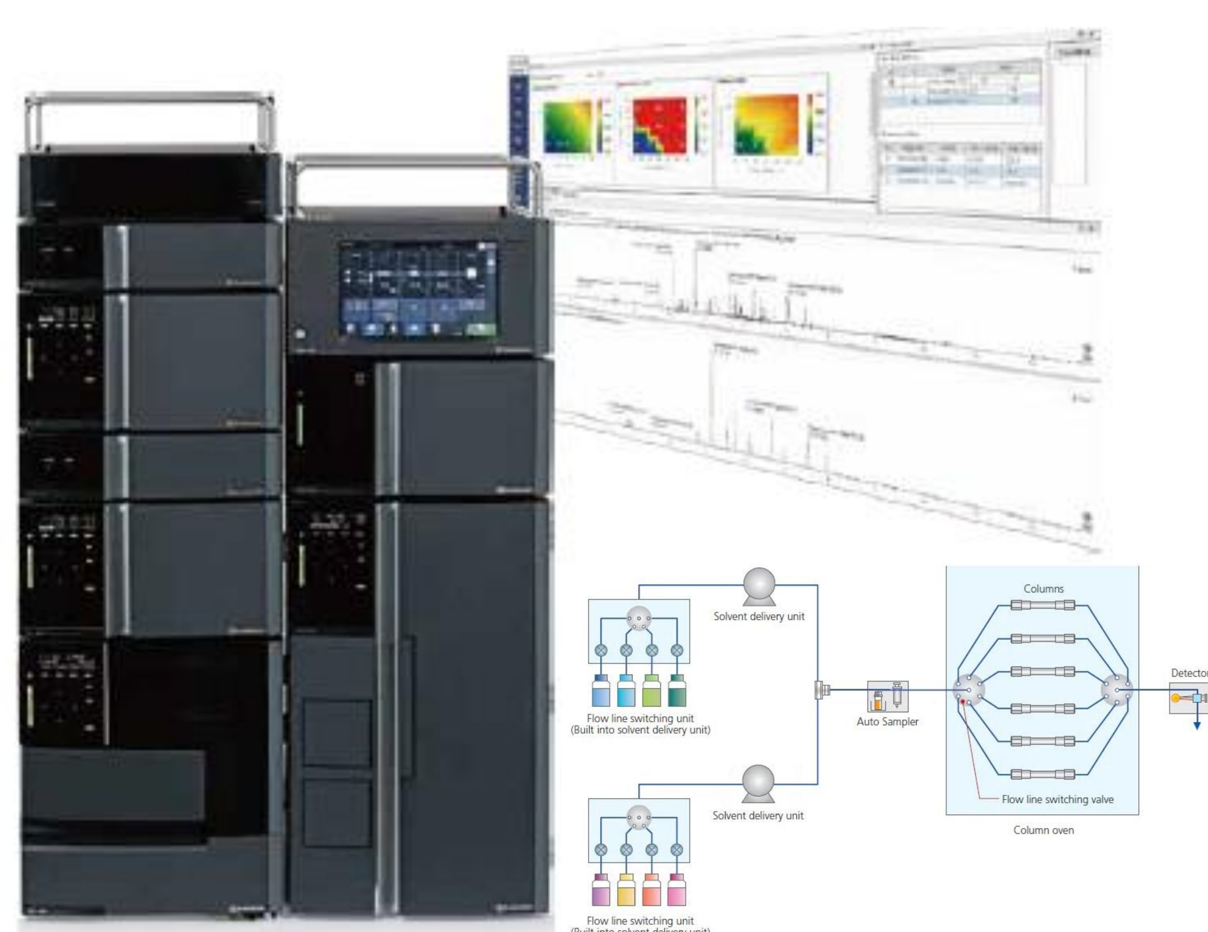

Figure 1. Shimadzu's Nexera X3 Method Scouting System with LabSolutions Method Development (MD) software.

- Six Daicel chiralpak columns with varying stationary phase chemistries were used
- Shimadzu's Method Scouting system and LabSolutions MD were utilized to separate cannabinoids
- Different mixtures of cannabinoids were analyzed

Table 1. Base method used for LabSolutions MD

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

Method Optimization of Stereoisomers

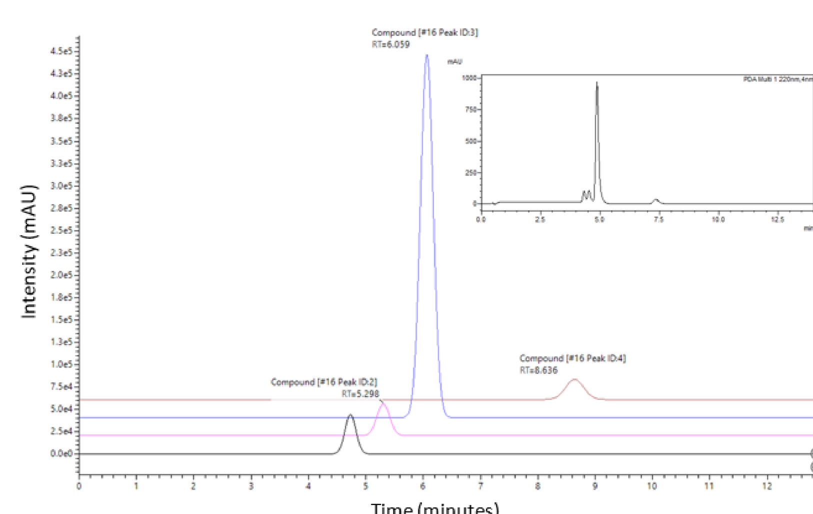

Figure 2. Four stereoisomers were separated using the IG-U chiralpak column. Elution order of the isomers are 9(R)- $\Delta^{6a,10a}$-THC, and 9(S)- $\Delta^{6a,10a}$-THC, (6aR, 9R)-Δ^{10}-THC, (6aR,9S)-Δ^{10}-THC, respectively. The predicted optimal chromatogram from the optimization step is shown above. The resulting chromatogram after optimization is shown as the inset. Optimal conditions were determined to be 50:50 mobile phase ratio, 0.8mL/min flow rate, and 35°C oven temperature.

Method Optimization and Robustness of 25 Cannabinoids

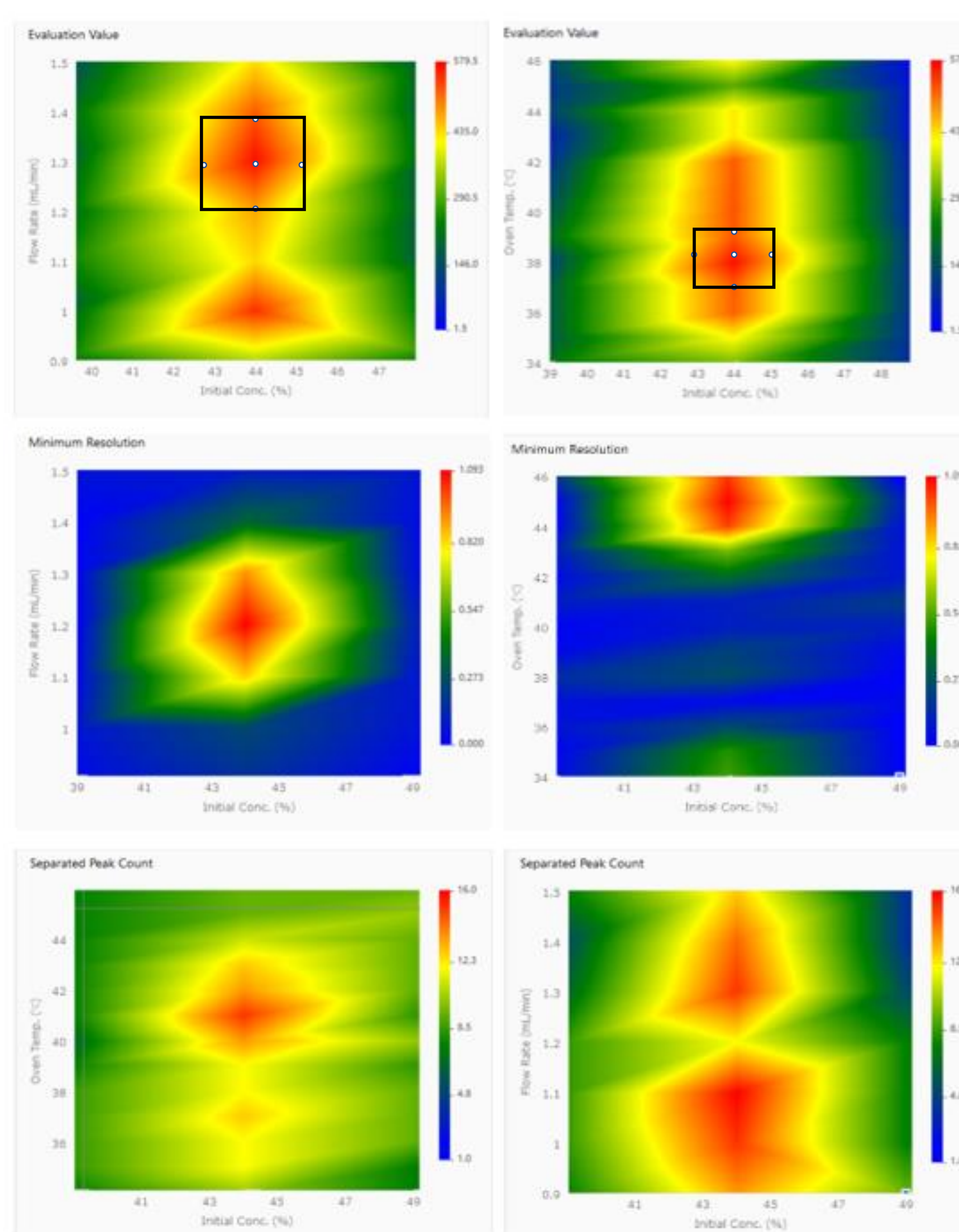

Figure 3. During the optimization phase, the optimal conditions are selected using statistical analysis. Heat maps for the evaluation value, minimum resolution, and separated peak count are displayed above. The optimal point indicates that a mobile phase ratio of 56A:44B, 1.3mL/min flow rate, and 38°C oven temperature.

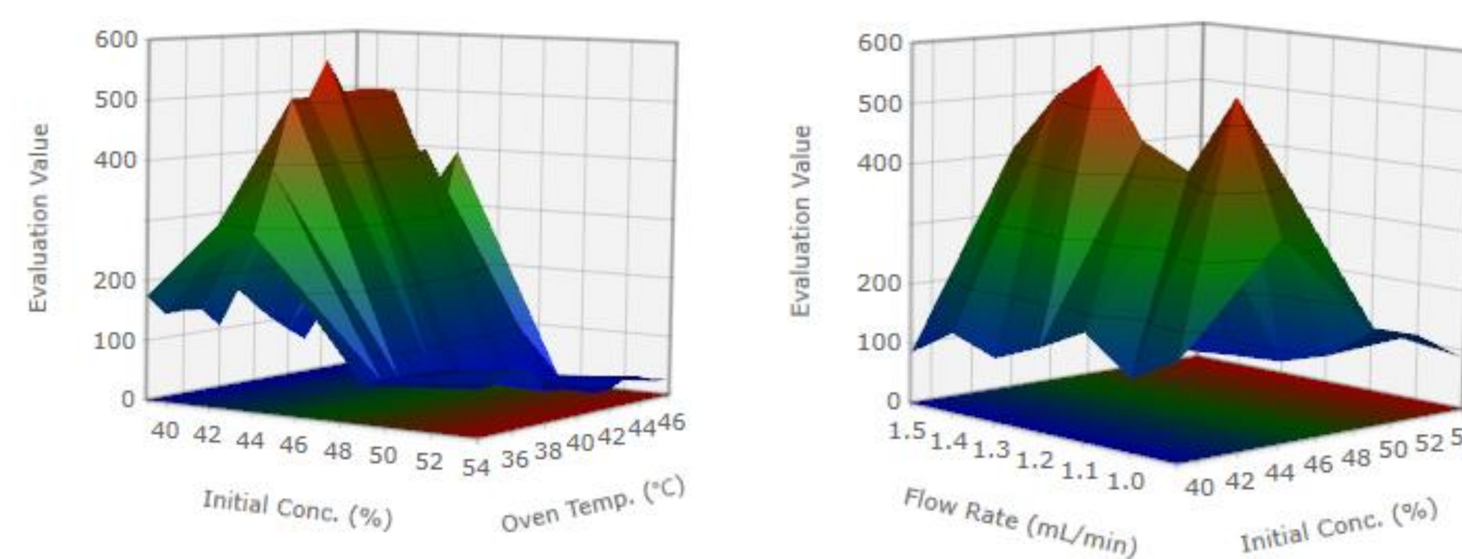

Figure 4. In addition to heat maps, 3D modeling can be utilized to determine the optimal method conditions. At a concentration of 44B, it was determined that the optimal oven temperature was 38°C and the optimal flow rate was 1.3mL/min.

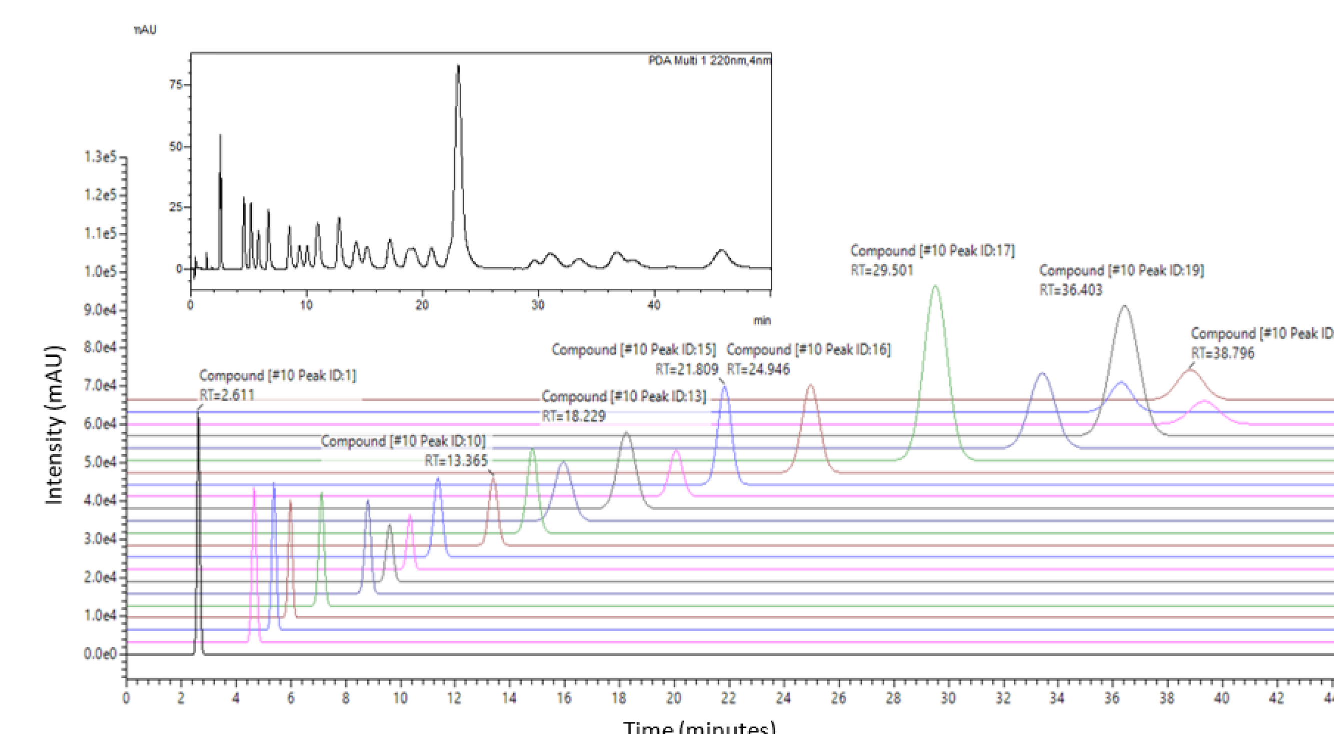

Figure 5. At the proposed optimal conditions, a predicted chromatogram was generated to display the peak elution at the set conditions. The inset is the resulting chromatogram using the optimal conditions. The elution order is CBDVA, CBDV, CBDA, CBCO, CBG, CBGA, CBCV, CBD, THCV, THCA, CBN, CBNA, CBL/D9-THC, 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.

- Parameters tested in the robustness stage were determined by the predicted optimal conditions
- The center point and the conditions indicated in the box were tested to evaluate the method robustness
- ANOVA was utilized to assess statistical deviations in the proposed optimal range

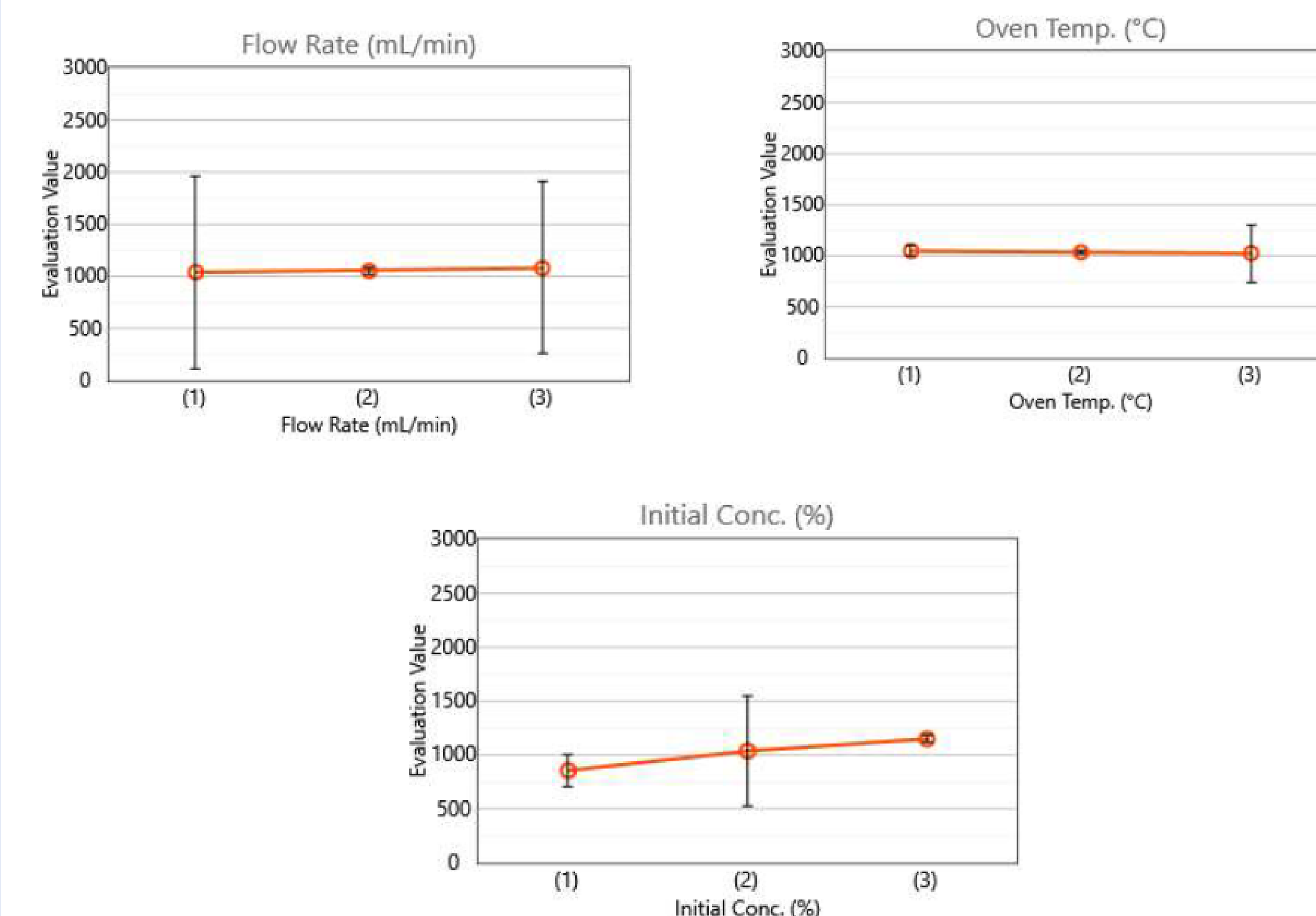

Figure 6. Using the ANOVA feature in LabSolutions MD the method robustness was determined. There results were not significantly different and therefore the optimal method is robust.

Conclusion

In conjunction with Method Scouting, LabSolutions MD is powerful tool for method development and optimization. It alleviates the monotonous task of testing, analyzing, and comparing all the individual runs. LabSolutions MD can be used for both simple and complex samples. Heat mapping and 3D modeling assisted in the identification of the most robust analytical condition for both 4 and 25 cannabinoid mixtures.

Future Directions

- Exploration of gradients to reduce run times
- Increase resolution with the use of in-line columns

Acknowledgments:
Dr. Weston Umstead and Daicel Corporation
Dr. Eberhardt Kuhn