Efficient SFC Method Optimization for Benzoic Acid Derivatives using LabSolutions MD

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1. Introduction

As the prediction of separation in SFC analysis is not trivial, software that supports method development during both the screening and optimization phases is an important tool. This example demonstrates that Shimadzu LabSolutions MD (method development) software can predict optimal separation conditions after creating a design space from acquired data.

The tested sample was a synthetic mixture of eight benzoic acid derivatives. This mixture was chosen because it includes neutral, basic, and acidic compounds, as well as isomers, making it a challenging test for separation techniques and peak tracking.

In preparation for this experiment, different columns and modifiers were screened, and a column was selected for the experiment discussed here.

2. Target Compounds

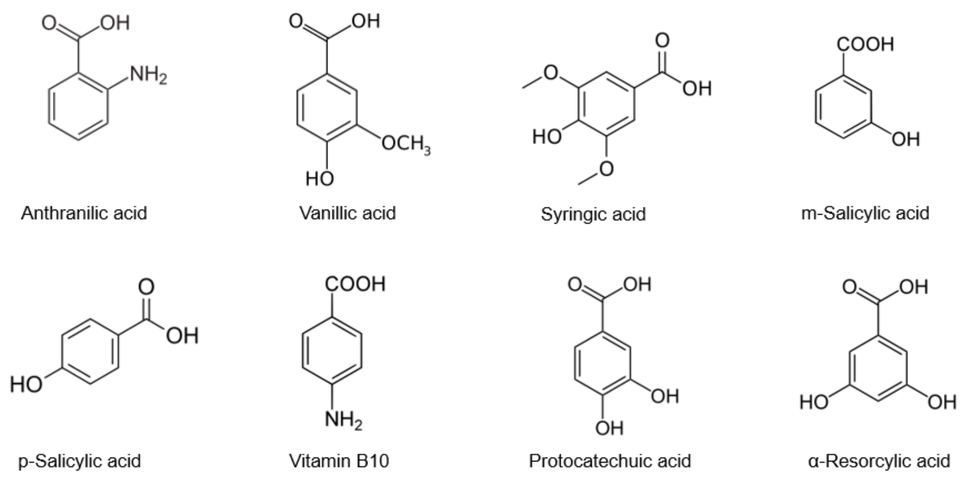

Figure 1: Benzoic acid derivatives included in the sample

3. Method

The method was created by optimization of: (i) the composition of modifier, (ii) the oven temperature and (iii) the gradient time.

Table 1: Analytical conditions

SFC System	**Nexera™ UC**
Column	Shim-pack™ UC-Diol, 150 mm x 4.6 mm I.D., 3 µm
Mobile phase A	CO_2
Mobile phase B (composition modified by solvent blending)	B1) Isopropanol B2) Water B3) Isopropanol + 0.1 % Formic acid B4) Methanol + 0.1 % Formic acid
Gradient	5 - 40 %B in 5 min, 10 min, 15 min
Composition %B	B2: 4 %; B3: 0, 96, 48 %; B4: 96, 0, 48 %
Flow rate	2.5 mL/min
Column temp.	35 °C, 40 °C, 45 °C
Injection volume	10 µL
Detection	PDA Max Plot 220 – 320 nmm
MS System	**LCMS-2050**
Ionization	ESI/APCI (DUISTM), positive mode
Mode	Scan
Nebulizing gas	2.0 L/min
Drying gas	5.0 L/min
Heating gas	7.0 L/min
DL temperature	350 °C
Desolvation temperature	200 °C
Interface voltage	3 kV

Using the LabSolutions MD workflow, it is not necessary to manually create methods and batch tables. The procedure is automatically carried out by the software.

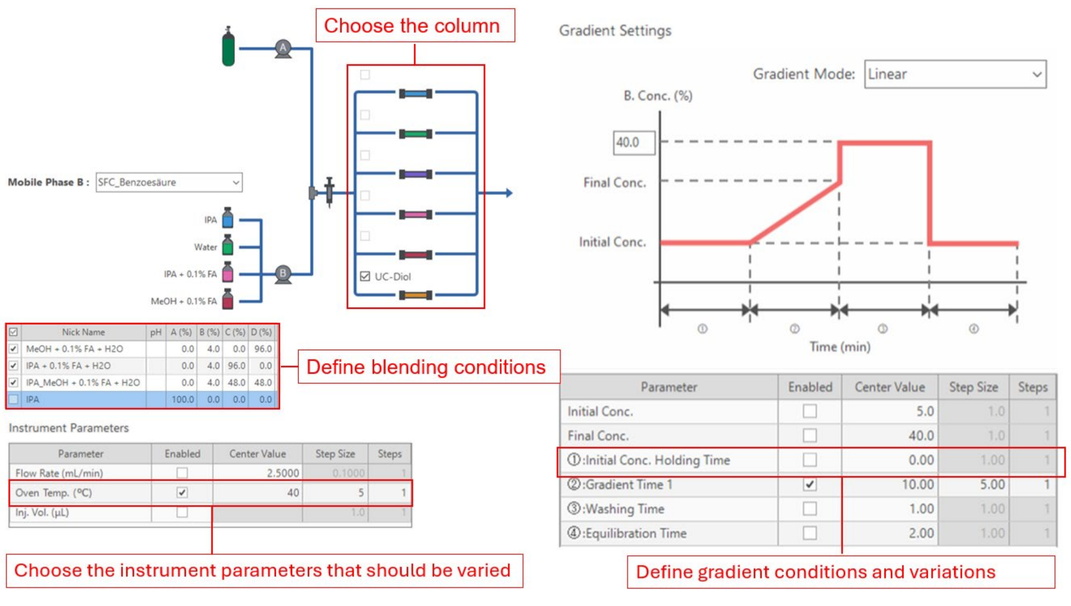

Figure 2: LabSolutions MD workflow

4. Results

The resulting data was reviewed in the software. Using the automatic peak tracking function, all peaks were identified by comparison of their UV and MS spectral data across the obtained chromatograms.

After successfully identifying the peaks, the design space for the varied parameters (modifier, oven temperature, and gradient time) was created and verified using statistical data generated by the software. The response criteria selected were the minimal resolution and the retention time of the last peak, in order to find the best compromise between run time and peak resolution.

Additionally, the software could simulate expected chromatograms across the entire design space.

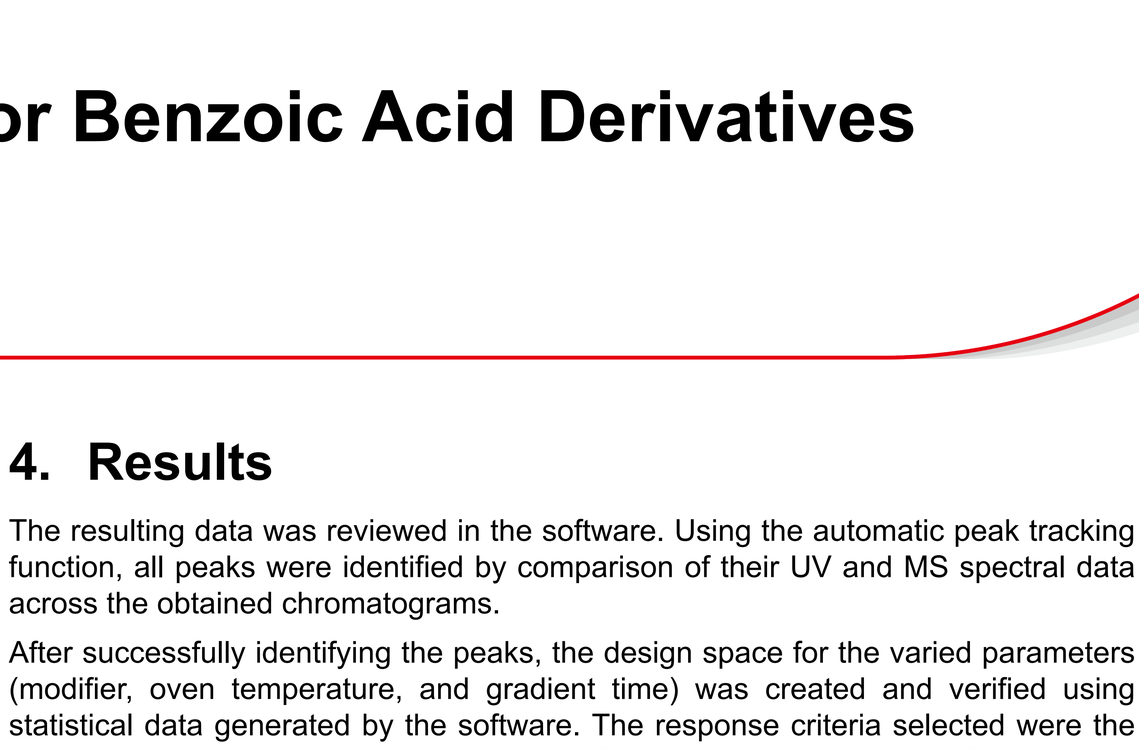

Figure 3: 2D counter plot

The area in the design space where all response criteria were met was clearly visible in the 2D contour plot (see Figure 3). Using the automatic search for optimal conditions, the combination of method parameters expected to provide the optimum and most robust separation was identified.

To verify, a confirmation batch was created using the automated function of the software. The chromatogram obtained from the confirmation batch aligned with the predicted chromatogram, with a maximum retention time difference of 2 %.

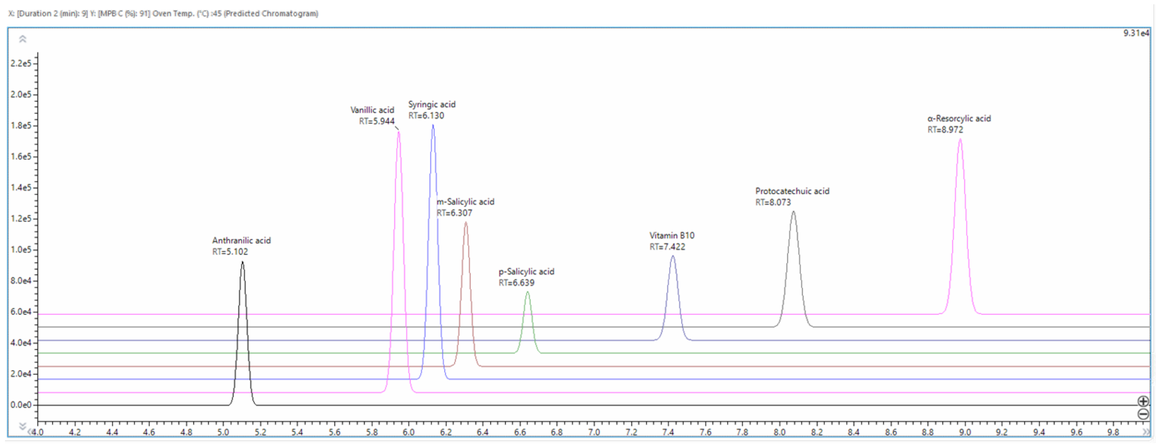

Figure 4: Predicted chromatogram at optimized conditions

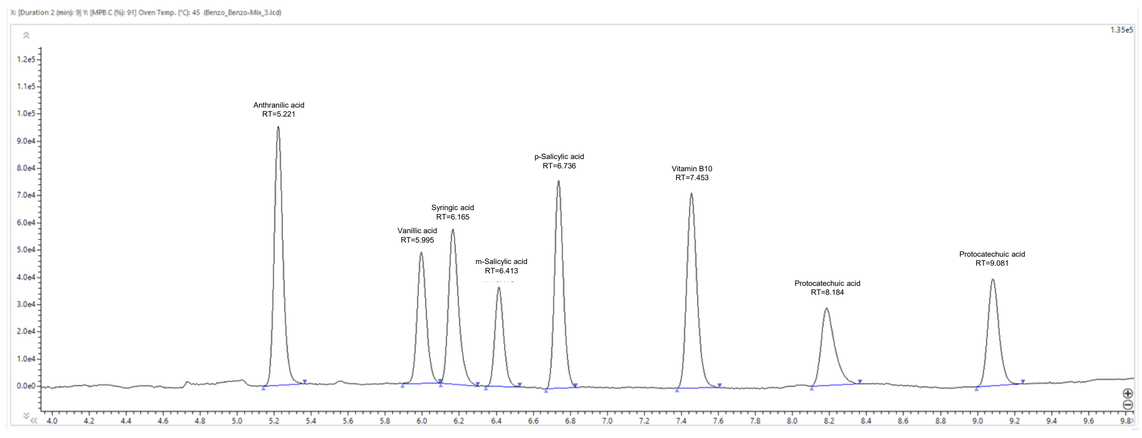

Figure 5: Measured chromatogram at optimized conditions

5. Conclusion

This study highlighted the value of method development software, such as LabSolutions MD, in optimizing SFC methods. Features like automatic peak tracking and design space analysis made it possible to efficiently identify optimal separation conditions for a complex mixture of benzoic acid derivatives.

The successful verification of a model chromatogram by comparison with an actual analytical run demonstrated the software's ability to accurately predict SFC separations. This marks a significant advancement in eliminating the need for the often complex "trial-and-error" process and providing a more user-friendly and reliable solution.