

HPLC method scouting system using ultra high performance liquid chromatography coupled to single quadrupole mass spectrometer

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Introduction

Traditionally, the process of LC/MS method development has been a time consuming as well as labor intensive task. Optimization of separation conditions that also maximize mass spectrometry sensitivity requires a methodical approach that can involve multiple experiments involving column switching and mobile phase screening.

In this study a UHPLC based method scouting system was used to dramatically shorten total experiment time compared to conventional approaches. This enabled

multiple separations based on the combination of different mobile phase and multiple columns allowing comprehensive investigation of analytical conditions. Mass spectrometry sensitivity, also influenced by mobile phase pH buffer, can be optimized via this approach. Method scouting was applied to the simultaneous analysis of geno-toxic impurities, several amines and acidic compounds using UHPLC coupled to a single quadrupole LC/MS instrument.

Overview of the Nexera Method Scouting System

- Capable of searching conditions based on a maximum of 6 columns and 16 mobile phases
- Can be used with basically all current UHPLC columns (100 MPa valve pressure resistance)
- Easily configured scouting conditions enabled through proprietary software
- Automated control of entire analysis from system checks to scouting, and then shut down

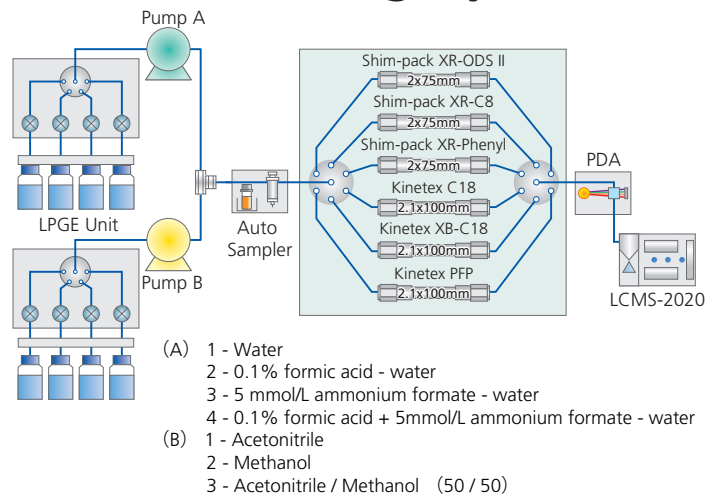


Fig. 1 Schematic representation and features of the Nexera Method Scouting System.

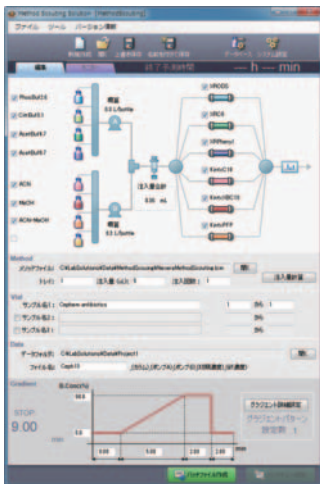


Fig. 2 Main screen of the Method Scouting Solution.

- **Easy Operation**
Mobile phases and columns can be selected in the same window. Integrated user interface allows simple operation.
- **Seamless Connection**
Software links with LabSolutions Ver. 5.42 or later versions.
- **Improved Workflow**
Batch analysis files are automatically created.

Analysis by the Nexera Method Scouting System

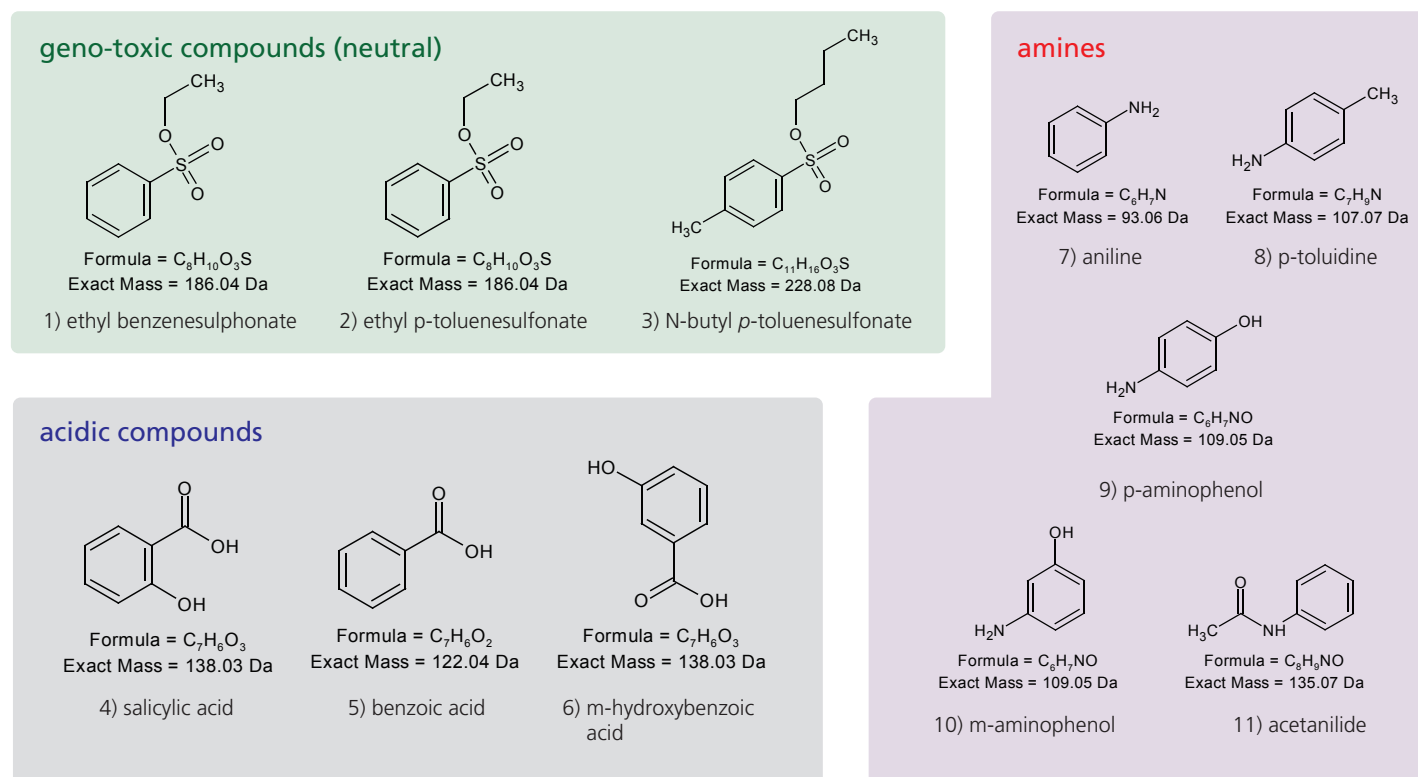
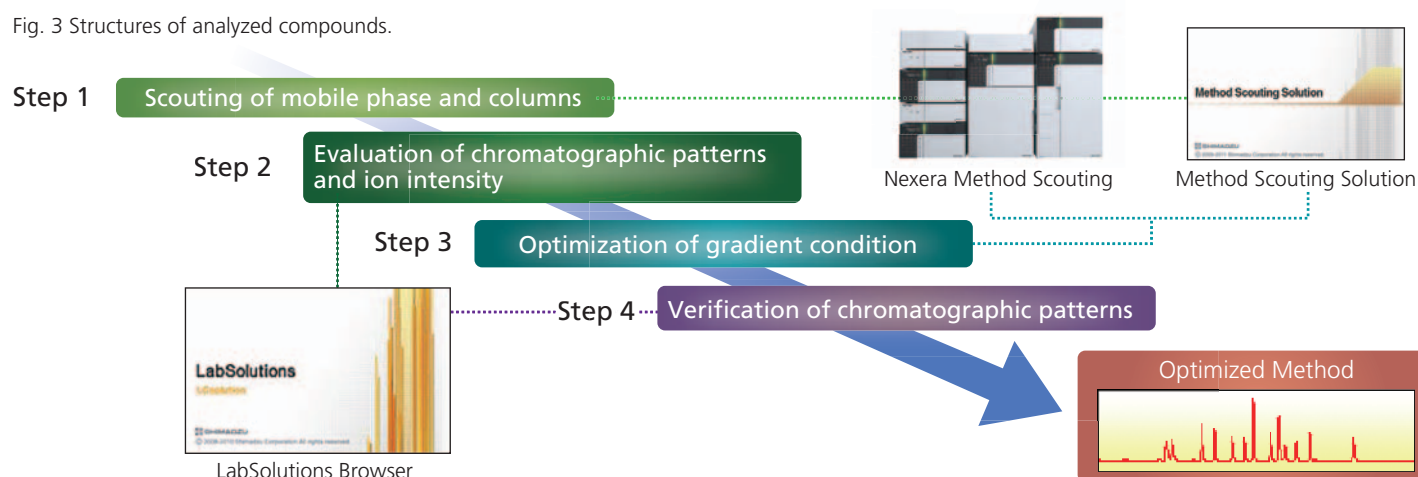


Fig. 3 Structures of analyzed compounds.



Scouting of mobile phases and columns (Step 1)

The purpose of this step is to find out for the best combination of mobile phase and column using a typical gradient condition (Table 1). In these experiments we used 12 combinations of mobile phases and 6 different columns (Fig. 1).

Table 1 Analytical conditions of Step 1.

Binary gradient	: 8 conc. 5% (0 min) → 95% (5-7 min) → 5% (7.01-12 min)
Flow Rate	: 0.3 mL/min
Injection vol.	: 2 µL (Mixture of each 20 ppm of ingredients)
Column Temp.	: 40°C
Ionization	: ESI (Positive and Negative)
Detection	: SIM ([M+H] ⁺ , [M+NH ₄] ⁺ , [M-H] ⁻ , [M+HCOO] ⁻) of each compound

Evaluation of chromatographic patterns and ion intensity (Step 2)

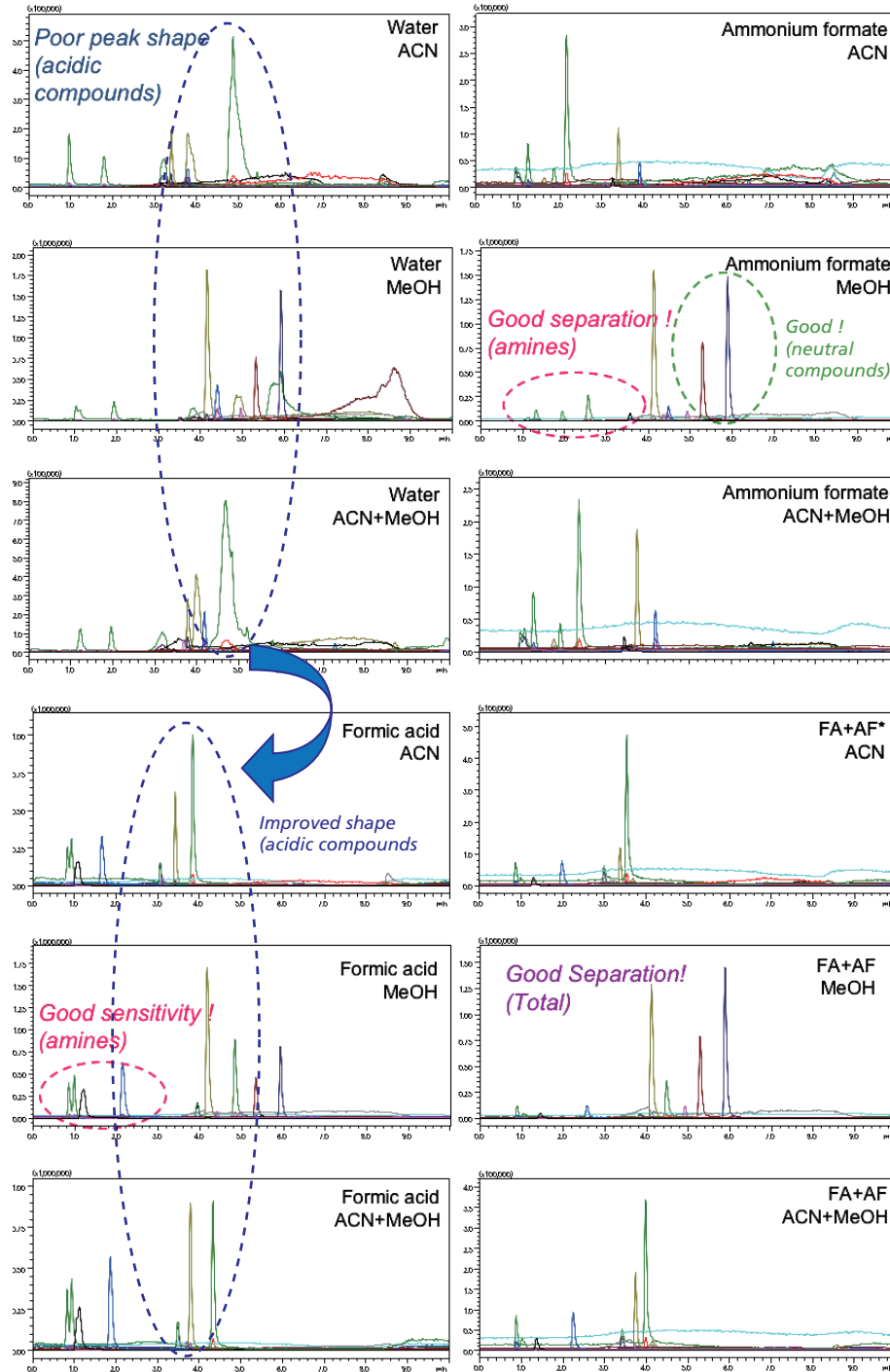


Fig. 5 Representative chromatograms under selected mobile phase conditions using Kinetex C18 column. * FA and AF indicate formic acid and ammonium formate.

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Optimization of gradient condition (Step 3 and 4)

For improved separation and sensitivity for amines, we optimized the gradient condition using method scouting system.

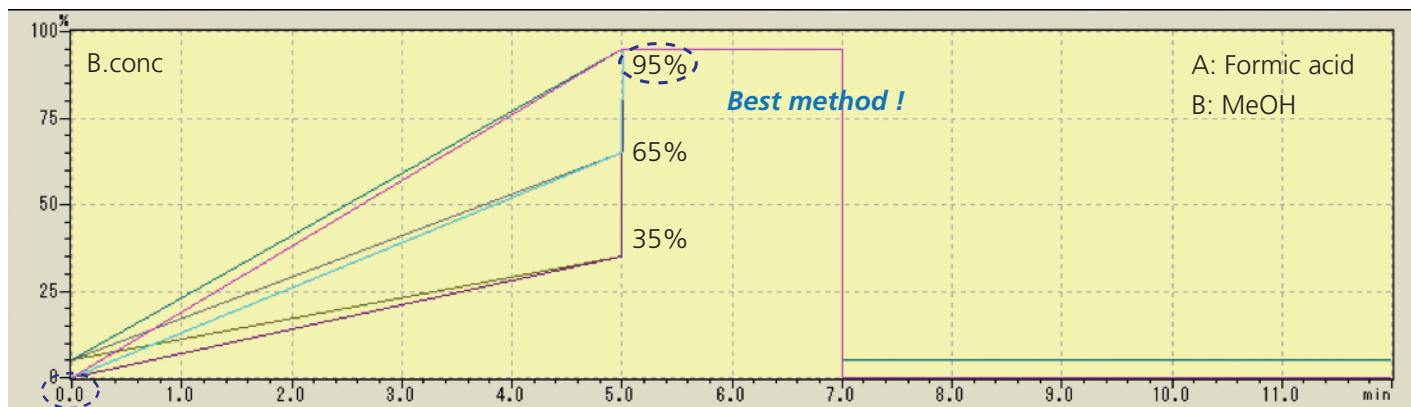


Fig. 6 Optimization of gradient conditions for separation of amines

Optimized method

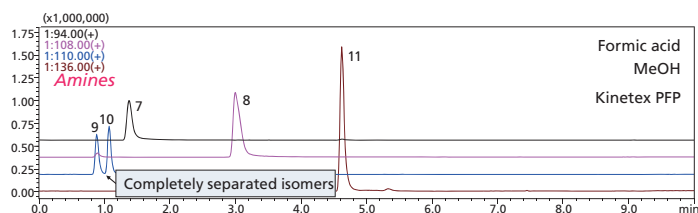
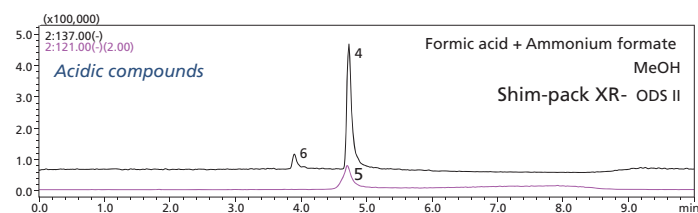
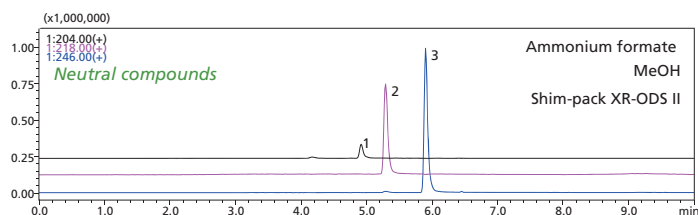


Fig. 7 Optimized method for each group.

Conclusion

- Method Scouting Solution enabled optimization of the analytical method separating compounds of differing properties in a single batch.
- The most suitable method for a single compound class could be chosen, alternatively a generic method could also be selected allowing separation of all compounds.
- Through method optimization LC/MS sensitivity was enhanced significantly.
- Seamless integration of software provided improved speed and efficiency in method optimization processes.



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