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Examination of the sugar analysis using HPLC method scouting system coupled to single quadrupole mass spectrometer

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1. Introduction

Optimization of peak separation and sensitivity is important for decision of LC/MS analytical conditions. However, the evaluation of them has been tedious and time-consuming operation. The HPLC method scouting system coupled to single quadrupole mass spectrometer used in this study can dramatically shorten total run time compared with the conventional system, because this system can make enormous combinatorial analysis methods and run batch program automatically. In this study, we developed the optimized method for the simultaneous analysis of seventeen kinds of sugars based on the result of evaluation for columns, mobile phases and gradient programs using this system.

2. Overview of the Nexera Method Scouting System

- Capable of searching conditions based on a maximum of six columns and sixteen mobile phases
- Can be used with basically all current UHPLC columns (100 MPa valve pressure resistance)
- Easily configured scouting conditions enabled through proprietary software (Fig. 1)
- Automated control of entire analysis from system checks to scouting, and then shut down

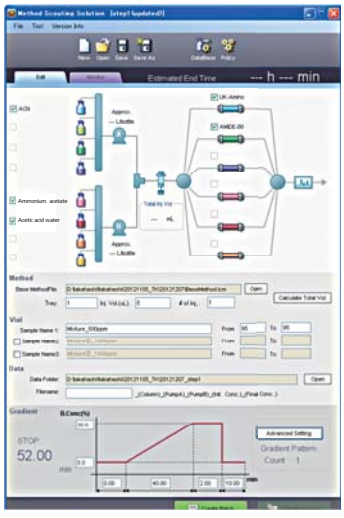

Fig. 1 Main screen of the Method Scouting Solution

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. 5.53 SP3 or later versions.
- *Improved Workflow*
  Batch analysis files are automatically created.

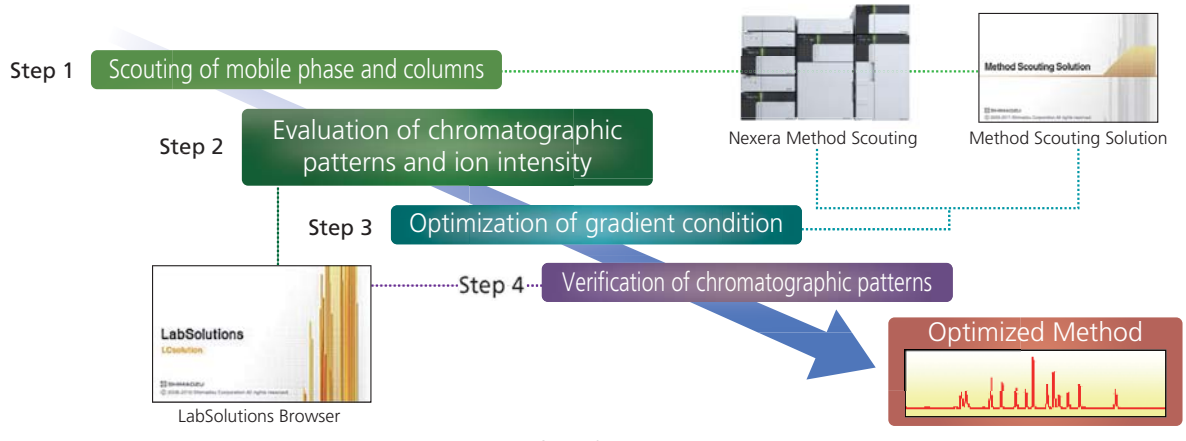

Fig. 2 Work-flow of the method scouting

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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 2 combinations of mobile phases and 2 different columns (Fig. 3).

Table 1 Analytical conditions of Step 1

Binary gradient	: B conc. 5% (0 min)
	→ 30% (40-42 min)
	→ 5% (42.01-52 min)
Flow Rate	: 1.0 mL/min
Injection vol.	: 5 µL
Column Temp.	: 55 deg. C
Ionization	: ESI (Negative)
Detection	: SCAN (range : *m/z* 100-500)

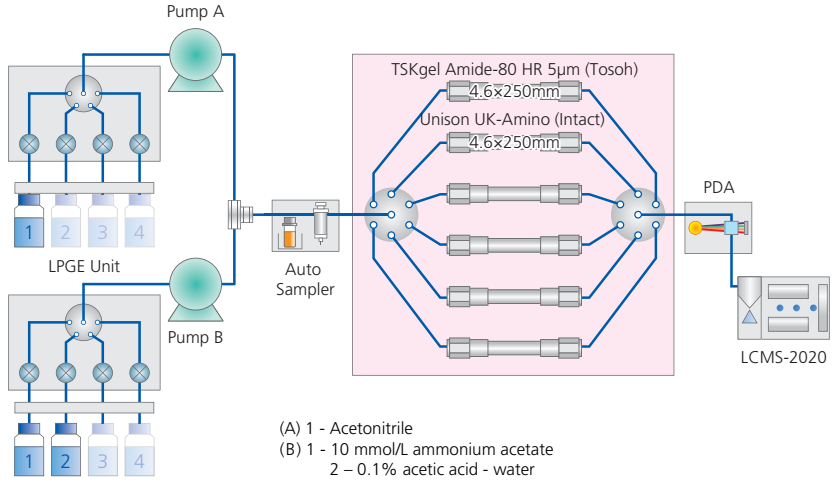

Fig. 3 Schematic representation and features of the Nexera Method Scouting System

Analysis by the Nexera Method Scouting System

We targeted seventeen sugars and analyzed them simultaneously. (Fig. 4)

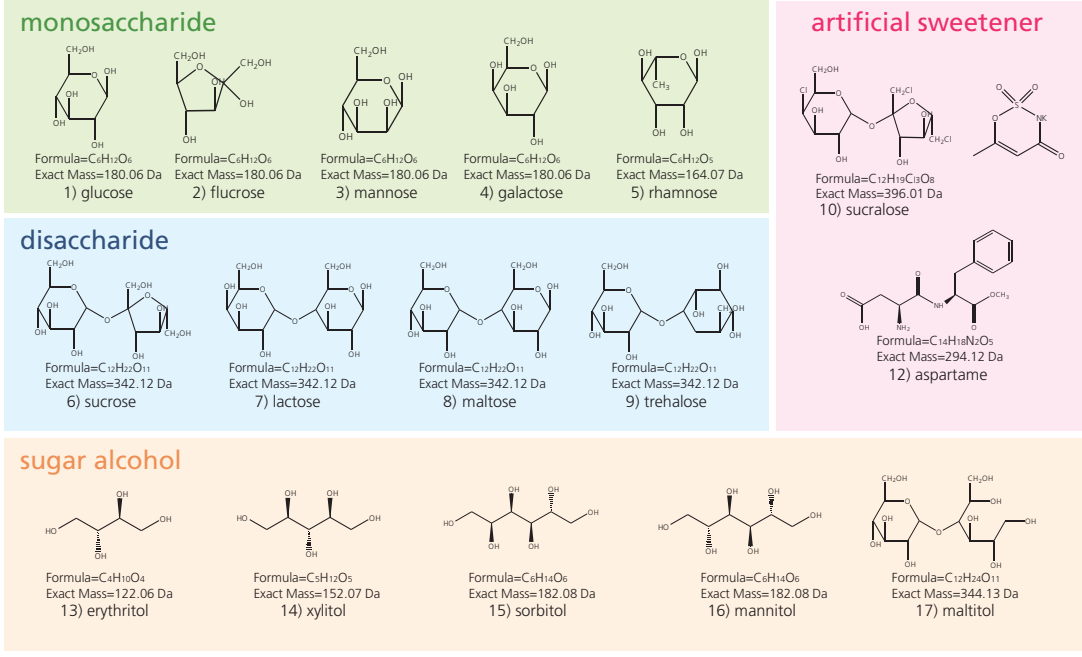

Fig. 4 Structures of analyzed compounds

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Evaluation of chromatographic patterns and ion intensity (Step 2)

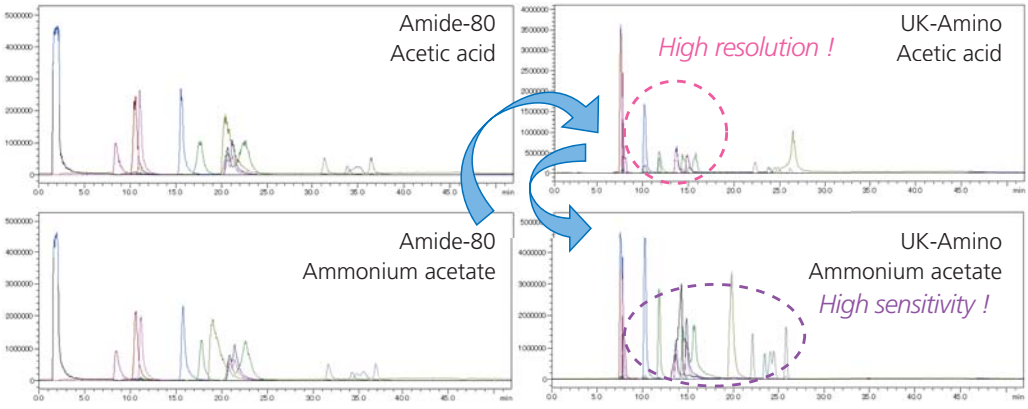

Fig. 5 Typical chromatograms in selected mobile phases and column conditions

Optimization of gradient condition (Step 3 and 4)

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

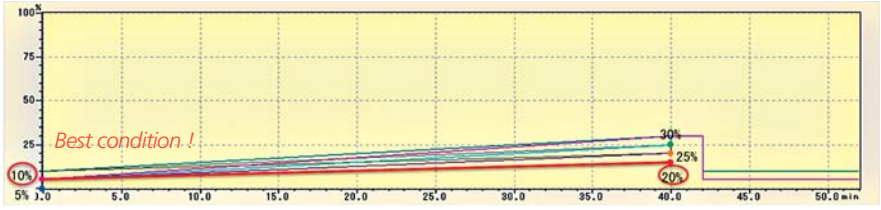

Fig. 6 Optimization of gradient conditions for separation of sugars

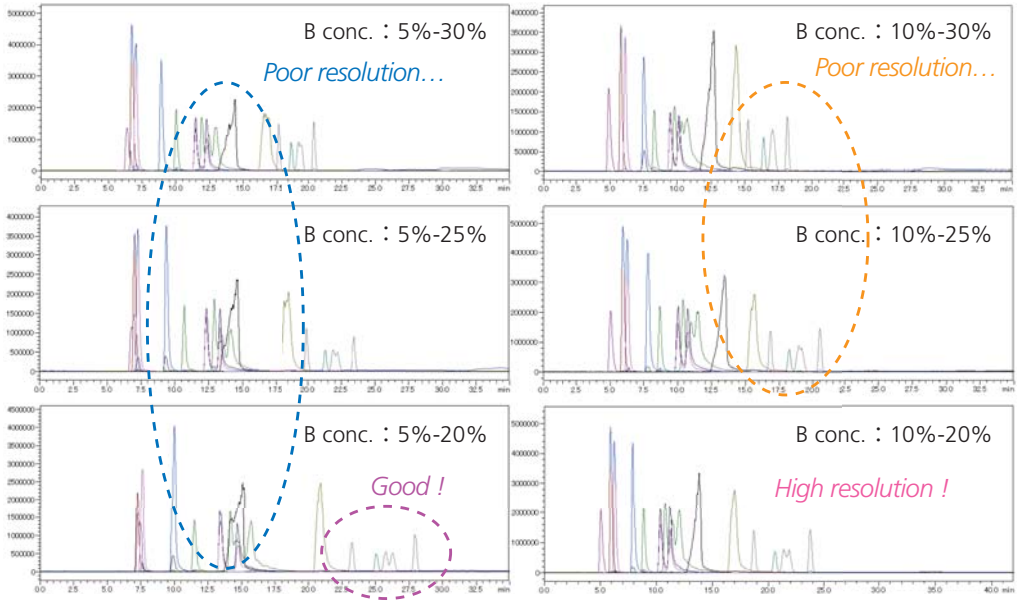

Fig. 7 Typical chromatograms in selected gradient conditions using ammonium acetate and Amide-80 column

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Optimized method

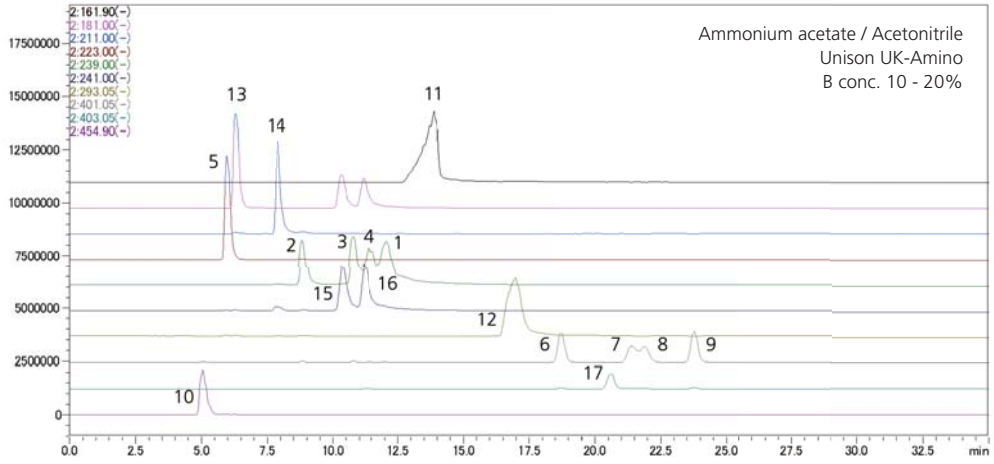

Fig. 8 Optimized method for seventeen sugars

Calibration curves

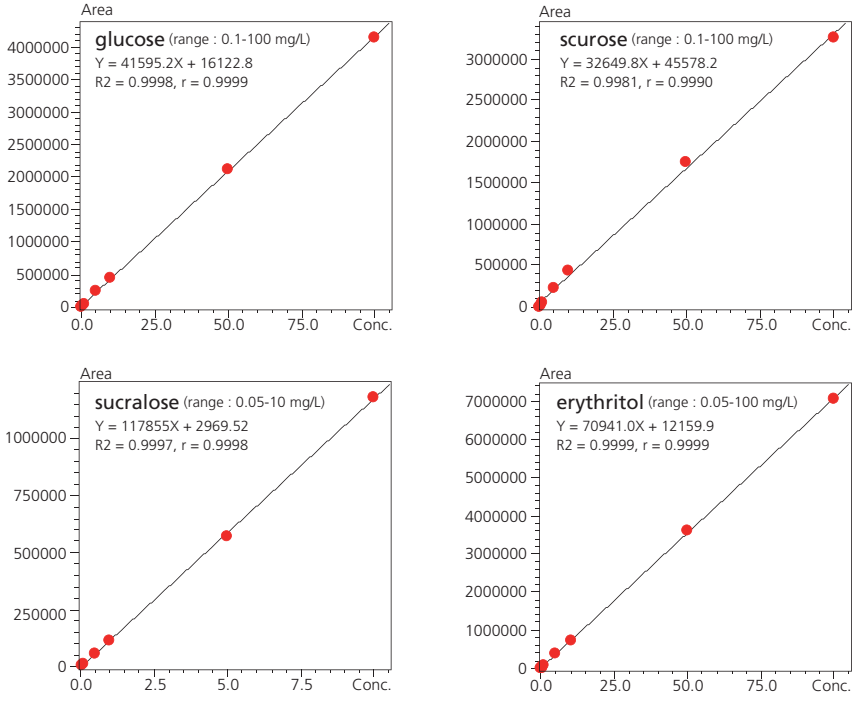

Fig. 9 Calibration curves of each sugar using optimized method

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3. Conclusion

- Method Scouting Solution, dedicated software for controlling method scouting system, 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 development processes.
- Using an optimized method file, high quantifiability was provided.

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