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Technical Report

Optimization of Analytical Conditions, and Scale-up from Analytical to Preparative Scale Using Supercritical Fluid Chromatography

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Abstract:

High-purity fractionation requires proper separation between peaks, and in order to achieve this, both an exhaustive investigation of conditions and the optimization of analytical conditions (method scouting) are important. Particularly with supercritical fluid chromatography (SFC), retention behavior changes substantially depending on the stationary phase, so it is useful to investigate conditions with a variety of different columns. Normally, optimal conditions are verified at the analytical scale by method scouting, and are then scaled up to the preparative scale by increasing flow rate and injection volume in proportion to the column cross-sectional area. This report describes an investigation of conditions on a Nexera UC (analytical scale) system, after which the method obtained was scaled up for a Nexera UC Prep (preparative scale) system.

Keywords: preparative supercritical fluid chromatography, scale-up, method scouting

1. Method Scouting at the Analytical Scale

When fractionating compounds by preparative supercritical fluid chromatography (SFC), improving the purity requires a search for the optimal conditions. However, as method scouting at the preparative scale consumes large amounts of sample and modifier solvent, methods are normally first developed at the analytical scale.

This report examines the separation of a mixed solution of five compounds (linalool, ibuprofen, ketoprofen, caffeine, and theophylline). First, a Nexera UC chiral screening system was used to perform method scouting and to search for analytical conditions that produce good separation. Shim-pack UC-series analytical columns designed for SFC were used in this case.

Method scouting was performed using Method Scouting Solution (Fig. 1) dedicated software. Method Scouting Solution automatically generates a batch table, and simply executing this batch table enables even first-time users to perform method scouting for SFC analysis with ease. When multiple modifiers and columns are used, Method Scouting Solution can switch automatically between the modifiers and columns, so method scouting can be continued through the day and night.

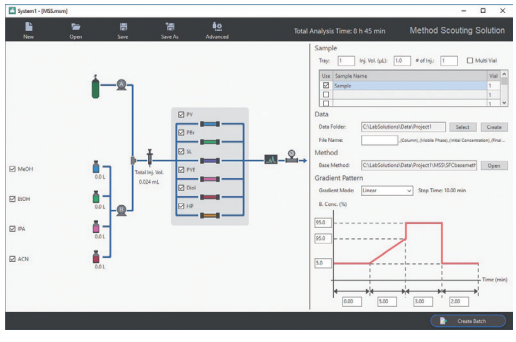

Fig. 1 Method Scouting Solution Ver. 2

When Method Scouting Solution is used, the fixed parameters not investigated (flow rate, wavelength, column temperature, etc.) are specified in advance in LabSolutions; the standard software (base method). In contrast, the parameters changed when investigating conditions (column, modifier type, modifier concentration, injection volume, gradient profile, etc.) are configured in the Method Scouting Solution software. The six columns used for method scouting are shown in Table 1, and the analytical conditions used in method scouting are shown in Table 2.

Table 1 Columns Used for Method Scouting

Column name	Functional group
Shim-pack UC-Diol II	Diol group
Shim-pack UC-Sil II	–
Shim-pack UC-HyP	3-Hydroxyphenyl group
Shim-pack UC-Py	Pyridinyl group
Shim-pack UC-PBr	Pentabromobenzyl group
Shim-pack UC-PyE	Pyrenyl ethyl group

Table 2 Analytical Conditions

System	: Nexera UC (Analytical scale)
Column	: Shim-pack UC-SIL II Shim-pack UC-HyP Shim-pack UC-PBr Shim-pack UC-PYE Shim-pack UC-PY Shim-pack UC-Diol II (250 mm L. × 4.6 mm I.D., 5 µm)
Modifier	: Methanol
Modifier concentration	: 20 %
Flow rate	: 3.0 mL/min
Column temperature	: 40 °C
Injection volume	: 2 µL
Detection	: 225 nm
Cell	: High pressure cell for SFC (analytical)
BPR	: 10 MPa
Sample	: Linalool, Ibuprofen, Ketoprofen, Caffeine, Theophylline (500, 50, 20, 5, 5 mg/mL in methanol, respectively)

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2. Comparing Scouting Results

After method scouting, the data browser can be used to determine which chromatograms show good separation by displaying multiple chromatograms alongside one another for visual comparison.

Fig. 2 shows the chromatograms obtained by method scouting, displayed together in the data browser. From a comparison of the separation in each chromatogram, it is evident that good separation of all sample constituents was achieved using Shim-pack UC-PBr.

If scouting produces a large number of chromatograms that cannot easily be compared visually, Multi Data Report function can score the degree of separation achieved in each chromatogram, and rank the chromatograms by this score. For a description of how to compare chromatograms using Multi Data Report, please refer to the technical report entitled "Improving Efficiency in the Preparation of Test Reports for Chemistry, Manufacturing, and Control (CMC) Using Multi Data Report" (C191-E046).

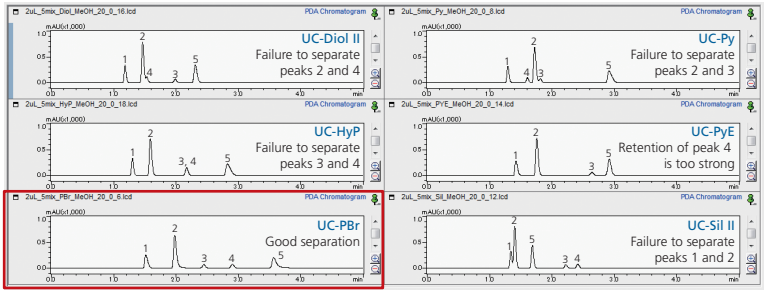

Fig. 2 Method Scouting Results (Data Browser)

3. Investigation of Loading (Analysis Scale)

Next, loading was investigated using the optimal conditions obtained by method scouting. To avoid saturation of peak intensities, the cell was changed from an analytical cell to a preparative cell. The issue of whether increasing the injection volume caused peak collapse, failed separation, and so on was investigated. The analytical conditions used are shown in Table 3, and the chromatogram obtained is shown in Fig. 3. Increasing the injection volume from the 2 µL used to investigate analytical conditions to 20 µL did not cause a major collapse in peak shape, and it was confirmed that separation of each peak was maintained in comparison with Fig. 2.

Table 3 Analytical Conditions

System	: Nexera UC (Analytical scale)
Column	: Shim-pack UC PBr (250 mm L. × 4.6 mm I.D., 5 µm)
Modifier	: Methanol
Modifier concentration	: 20 %
Flow rate	: 3.0 mL/min
Column temperature	: 40 °C
Injection volume	: 20 µL
Detection	: 225 nm
Cell	: High pressure cell for SFC (preparative)
BPR	: 10 MPa

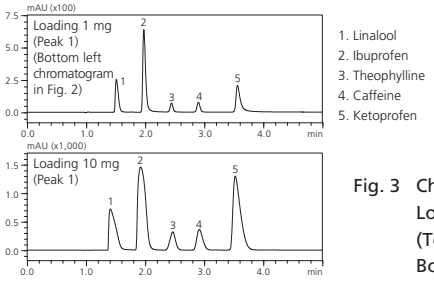

Fig. 3 Chromatogram Obtained during Loading Investigation (Nexera UC) (Top: Before loading increase. 2 µL injected, Bottom: After loading increase. 20 µL injected)

4. Scale-up

Scale-up is normally performed after conditions are investigated at an analytical scale. A preparative-sized column is used, and flow rate and injection volume are changed accordingly. Essentially, equivalent separation can be achieved at the preparative scale by increasing flow rate and injection volume in proportion to the column cross-sectional area. For example, scaling up from a 4.6 mm I.D. column to a 20 mm I.D. column is an approximate 18.9-fold increase in column cross-sectional area. For this report, an 18.9-fold scale-up of flow rate and a 25-fold scale-up of injection volume were attempted. The analytical conditions used are shown in Table 4, and the chromatogram obtained is shown in Fig. 4. Results confirmed that using a preparative-scale column with the same stationary phase produced an equivalent separation to that obtained in Fig. 3.

Table 4 Analytical Conditions

System	: Nexera UC Prep (Preparative scale)
Column	: Shim-pack UC-PBr (250 mm L. × 20 mm I.D., 5 µm)
Modifier	: Methanol
Modifier concentration	: 20 %
Flow rate	: 56.7 mL/min
Column temperature	: 40 °C
Injection volume	: 500 µL
Detection	: 225 nm
Cell	: High pressure cell for SFC (preparative)
BPR	: 10 MPa

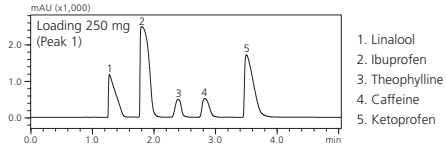

Fig. 4 Chromatogram of 500 uL Injection (Nexera UC Prep)

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