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Technical Report

Improved Efficiency of Isomer Preparative Operations by Supercritical Fluid Chromatography with Stacked Injection

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Abstract:

Preparative processes are used in a wide variety of fields, such as for selectively screening seed or lead compounds from newly synthesized compounds, or for structural analysis of impurities in pharmaceuticals or components with specific functional properties in natural substances. Preparative supercritical fluid chromatography (preparative SFC) is widely used in the pharmaceutical industry and many other fields because it can shorten analysis times and simplify post-processing. For analysis involving a limited number of peaks, such as when separating isomers of chiral compounds, stacked injection can improve the efficiency of preparative purification. This report describes an example of using the stacked injection functionality of the Nexera UC Prep preparative supercritical fluid chromatograph system to improve the efficiency of preparative operations.

Keywords: Preparative SFC, stacked injection

1. Shorter Analysis Times Using SFC

Due to the low viscosity and high diffusivity of supercritical carbon dioxide, column back pressure for SFC is low even at high flow rates. That means analysis speeds can be increased without sacrificing column efficiency. Consequently, significantly shorter analysis times can be expected than with HPLC.

A comparison of analysis times using preparative LC versus preparative SFC is shown in Fig. 1, based on an example of separating chiral forms of omeprazole. Using preparative SFC shortened the analysis time to one fourth of the analysis time using preparative LC.

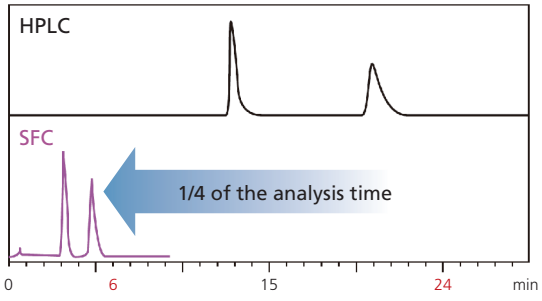

Fig. 1 Chiral Separation of Omeprazole by HPLC vs. SFC (Preparative Scale)

Table 1 Analytical Conditions

Column	: CHIRALPAK IC (250 mm L. × 20 mm I.D., 5 µm)
Mobile phase	: Hexane/Ethanol=70/30 (LC) CO_2/Methanol=75/25 (SFC)
Flow rate	: 20 mL/min (LC) 120mL/min (SFC)
Column temperature	: Room temperature (LC) 40 °C (SFC)
Injection volume	: 500 µL
Detection	: 303 nm
Cell	: High pressure cell for SFC (preparative)
BPR Pressure	: 15 MPa (SFC)

2. Stacked Injection

Stacked injection is a technique that improves the efficiency of preparative operations by successively injecting additional samples and utilizing the time spent waiting for peak elution. Functionality for stacked injection is included in SIL-40 autosampler and FRS-40 sampler and fraction collector. An overview of stacked injection is shown in Fig. 2. The following points require particular care when specifying settings for stacked injection.

- Only the isocratic separation mode can be used.
- Specify an injection interval that results in no overlap between peaks.

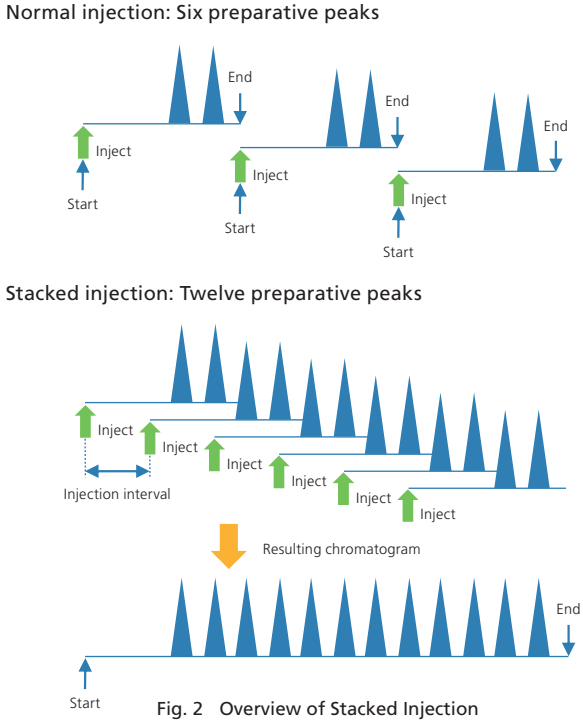

Fig. 2 Overview of Stacked Injection

1 Analytical & Measuring Instruments Division

3. Stacked Injection Settings

Settings for stacked injection can be specified easily in LabSolutions workstation software. The presence of any overlapping peaks can be confirmed easily by specifying [Injection Interval], [Number of Injection], and [Wait Time to Next Pretreat] settings (Fig. 3), and using the single run results (chromatogram) to simulate results for the given injection interval (Fig. 4). To inject samples successively, an appropriate wait time must be specified so that the sample loop can be switched back to the load state (right side of Fig. 5) after the sample is ejected from the sample loop (Fig. 5).

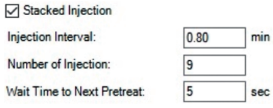

Fig. 3 Stacked Injection Settings (for Example in Fig. 4)

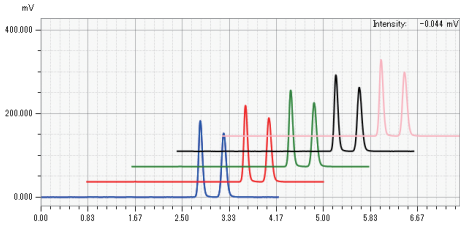

Fig. 4 Simulation of Stacked Injection (LabSolutions)

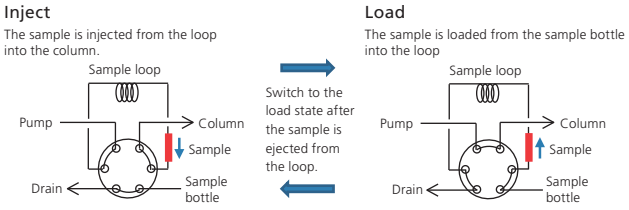

Fig. 5 Sample Valve Actions (FRS-40)

For the data acquisition time, enter a value greater than the analysis time for a single run plus the injection interval multiplied by the number of injections. For example, given 9 injections at an injection interval of 0.8 minutes, enter a setting value that is at least the single-run analysis time plus 7.2 minutes (Fig. 6).

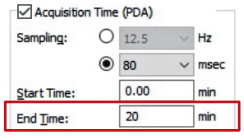

Fig. 6 Data Acquisition Time (SPD-M40)

Stacked injection collects all the peaks for the same compound in the same collection bottle. The same peaks can be collected in the same bottle by returning the fraction valve back to its initial position after each injection cycle. To collect fractions according to a time program, simply enter the time program for a single run analysis, and then the fraction time for subsequent injections is set automatically based on the injection interval (Fig. 7 and 8)

Fig. 7 Reset Time for Stacked Injection

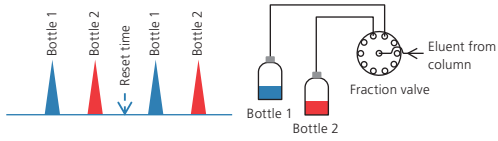

Fig. 8 Relationship between Reset Time and Preparative Intervals

4. Using Stacked Injection for Chiral Separation of Pharmaceuticals

The following describes an example of actually using stacked injection to separate chiral forms of a drug. A 10 mg/mL warfarin solution (in methanol) was used as the sample. Analytical conditions are listed in Table 2, with the resulting chromatograms shown in Fig. 9. The results show that the number of injections during the 10.5 minute analysis time could be increased from three injections using normal injection to nine injections using stacked injection, which tripled the efficiency of preparative operations.

Table 2 Analytical Conditions

Column	: CHIRALPAK IC (250 mm L. × 20 mm I.D., 5 µm)
Modifier	: Methanol
Modifier concentration	: 30 %
Flow rate	: 60 mL/min
Column temperature	: 40 °C
Injection volume	: 200 µL (Loop size: 400 µL)
Detection	: 200 nm
Cell	: High pressure cell for SFC (preparative)
BPR Pressure	: 10 MPa
Injection interval	: 0.80 min
Number of injection	: 9
Wait time	: 5 sec

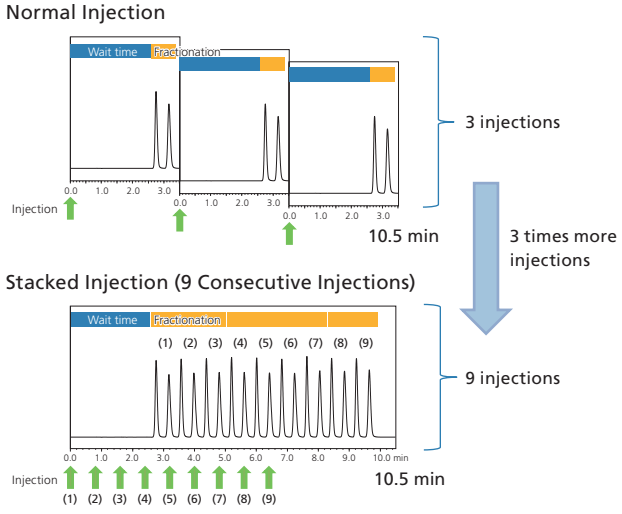

Fig. 9 Warfarin Separation Example

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