C190-E250

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Technical Report

Evaluating the Performance of the LotusStream Gas-Liquid Separator for Preparative Supercritical Fluid Chromatography

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Abstract:

Preparative supercritical fluid chromatography (SFC) is one of the most common technologies in preparative purification. Unlike preparative liquid chromatography, preparative SFC is prone to low recovery rates because the liquid splatters if it is not appropriately separated from the CO_2 during recovery. This report provides an evaluation of recovery rates, carryover, and contamination using the newly developed LotusStream™ gas-liquid separator in a Nexera UC Prep preparative supercritical fluid chromatograph system.

Keywords: Preparative SFC, gas-liquid separator

1. Shimadzu's Unique Gas-Liquid Separation Technology

When CO_2 transitions from the supercritical fluid state to the gas state during preparative SFC, its volume immediately expands by about 500 times, which can cause the eluate from the column to splatter, a factor leading to decreased recovery rates during preparative SFC.

The newly developed LotusStream gas-liquid separator (patented) uses multiple flow channels to limit the flowrate without increasing the tubing diameter. As a result, the CO_2 is discharged externally, and the liquid travels along the column and then drips directly below, so the eluate does not splatter. Fig. 1 shows an illustration of the LotusStream separator.

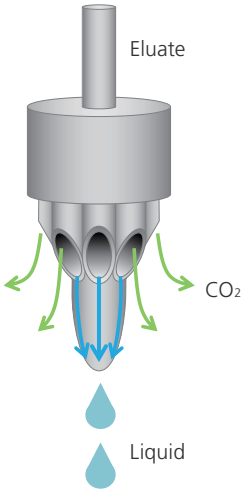

Fig. 1 Illustration of the LotusStream Separator

Fig. 2 shows gas-liquid separation using/not using the LotusStream separator. (Refer to Table 1 for the test parameters.) Without the LotusStream separator, CO_2 expansion causes the liquid to splatter, which makes it difficult to recover the liquid appropriately. In contrast, Fig. 2 shows that with the LotusStream separator, the liquid is separated appropriately from the CO_2, enabling its recovery.

The remainder of this report provides an example of a performance evaluation of the LotusStream separator.

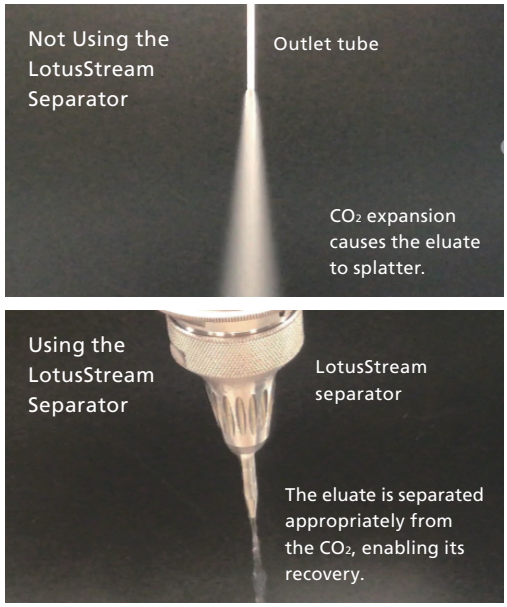

Fig. 2 Gas-Liquid Separation Using and Not Using the LotusStream Separator

Table 1 LotusStream Separator Test Parameters

Modifier	: Methanol
Modifier concentration	: 20 %
Modifier flow rate	: 100 mL/min

1 Analytical & Measuring Instruments Division

2. Improving Recovery Rates with the LotusStream Separator

The recovery rate with the LotusStream separator was evaluated using a Nexera UC Prep stacked fraction system. The stacked fraction system is intended for chiral preparative separation and other large-volume preparative separation. The FRS-40, which is used as the fraction collector, collects fractions in each bottle using a valve switching method. The system is shown in Fig. 3 and the flow channel diagram is shown in Fig. 4.

Caffeine, linalool (a volatile compound), and hydrocortisone (a compound with low solubility) were used as samples. An overview of the evaluation procedures is shown in Fig. 5, and the details are indicated below.

(1) Inject 1 mL of the sample solution according to the specified SFC parameters (refer to Table 2), and recover the eluate from the peak elution intervals into the recovery bottles. Repeat the injection three times, with peak elution from each injection recovered in the same bottle.
(2) Transfer the recovered liquid to a 100 mL volumetric flask. Rinse the recovery bottle about three times with methanol, and transfer the rinse solution to the flask as well. Then fill up the flask to 100 mL.
(3) After filling up the flask, reinject 1 mL of the recovered liquid using the SFC parameters. Then check the peak area values. Repeat steps (1) to (3) three times to obtain N = 3 values.
(4) Configuring LC parameters (refer to Table 3) as a reference, inject 1 mL of the sample solution via flow injection, and recover the eluate. Repeat the injection three times, with the eluate from each injection recovered in the same bottle.
(5) Fill up the flask of recovered liquid to 100 mL, as described in step (2).
(6) After filling up the flask, reinject 1 mL of the recovered liquid using the SFC parameters. Then check the peak area values.
(7) Calculate the recovery rate according to the following formula.
Recovery rate (%)
= Peak area from step (3)/Peak area from step (6) × 100

Fig. 3 Nexera UC Prep Stacked Fraction System

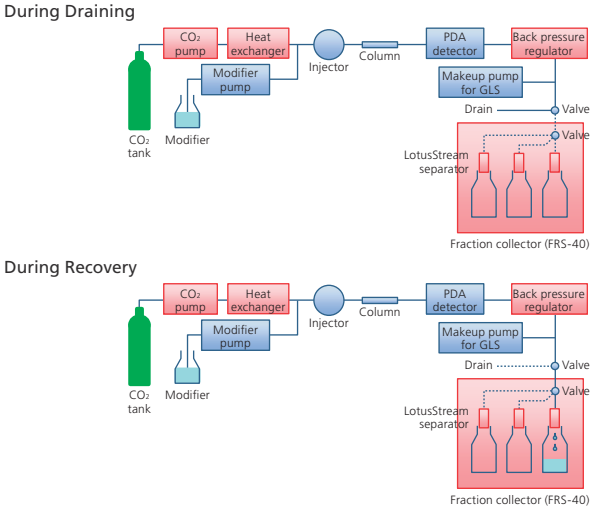

Fig. 4 Flow Channel Diagram for the Nexera UC Prep Stacked Fraction System

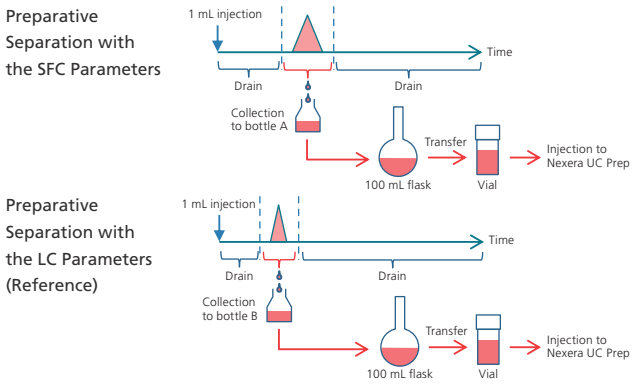

Fig. 5 Overview of the Recovery Rate Evaluation Procedures

Table 2 Analytical Conditions (SFC Parameters)

System	:	Nexera UC Prep Stacked fraction system
Column	:	Silica column (250 mm L. × 20 mm I.D.)
Modifier	:	Methanol
Modifier concentration	:	10 %
Flow rate	:	125 mL/min
Makeup	:	Methanol
Makeup flow rate	:	10 mL/min
Column temperature	:	40 °C
Injection volume	:	1000 µL (Loop size: 2000 µL)
Detection	:	272 nm (Caffeine) 205 nm (Linalool) 238 nm (Hydrocortisone)
Cell	:	High pressure cell for SFC (preparative)
BPR pressure	:	10 MPa
BPR temperature	:	50 °C
Sample	:	Caffeine 10 mg/mL Linalool 1 % Hydrocortisone 10 mg/mL

Table 3 Analytical Conditions (LC Parameters)

System	:	Nexera UC Prep Stacked fraction system
Column	:	No column (Flow injection)
Modifier	:	Methanol
Modifier concentration	:	100 %
Flow rate	:	20 mL/min
Column temperature	:	40 °C
Injection volume	:	1000 µL (Loop size: 2000 µL)
Detection	:	272 nm (Caffeine) 205 nm (Linalool) 238 nm (Hydrocortisone)
Cell	:	High pressure cell for SFC (preparative)
Sample	:	Caffeine 10 mg/mL Linalool 1 % Hydrocortisone 10 mg/mL

The recovery rate results are shown in Table 4. It was confirmed that consistently good results are obtained for all three compounds despite their different properties.

Table 4 Recovery Rate Results

Compound	Peak Area (Reference)	Peak Area (1st Recovery)	Peak Area (2nd Recovery)
Caffeine	309936	303480	300797
Linalool	207554	204007	206255
Hydrocortisone	334839	322162	323639

Compound	Peak Area (3rd Recovery)	SFC Recovery Rate (Mean Value)	Recovery Rate %RSD
Caffeine	301443	97.4 %	0.46 %
Linalool	205288	98.9 %	0.55 %
Hydrocortisone	323464	96.5 %	0.32 %

3. Checking for Contamination of Adjacent Test Tubes

The Nexera UC Prep multi-fraction system was used to evaluate whether or not there was any contamination in adjacent test tubes. The multi-fraction system is used for preparative separation of multiple peaks arising from impurities, natural ingredients, or other substances. It uses an FRC-40SF fraction collector, which collects fractions in each test tube using a mobile arm. The system is shown in Fig. 6 and the flow channel diagram is shown in Fig. 7.

Linalool was used as the sample. An overview of the evaluation procedures is shown in Fig. 8, and the details are indicated below.

(1) Inject 1 mL of the sample solution using the parameters in Table 5, and recover eluate from the peak elution intervals into the recovery containers (60 mL test tubes with an 18 mm diameter).
(2) Transfer the recovered liquid to a 50 mL volumetric flask. Rinse the test tube about three times with methanol, and transfer the rinse solution to the flask as well. Then fill up the flask to 50 mL.
(3) After filling up the flask, dilute the recovered solution by 2000 times with methanol. Then using the reinjection parameters (Table 6), check the peak area values.
(4) Remove the adjacent test tube after recovery in step (1), rinse it with a small amount of methanol, transfer the liquid to a 50 mL volumetric flask, and fill it up to 50 mL.
(5) Using the reinjection parameters, check the peak area values for the liquid prepared in step (4).
(6) Calculate the contamination level according to the following formula.
Contamination (%)
= Peak area from step (5)/Peak area from step (3)/2000 × 100

Fig. 6 Nexera UC Prep Multi-Fraction System

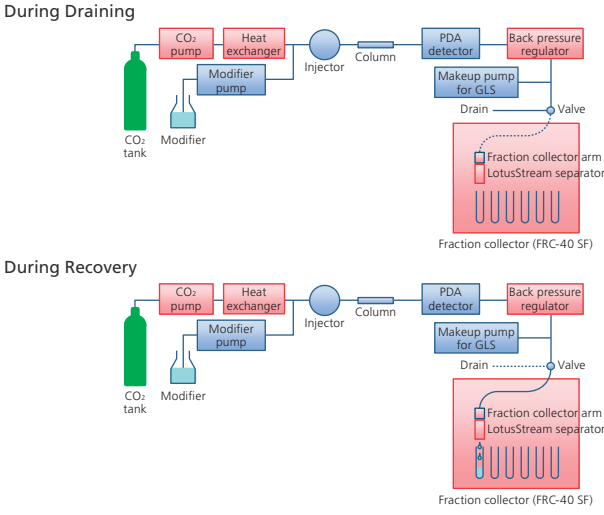

Fig. 7 Flow Channel Diagram for the Nexera UC Prep Multi-Fraction System

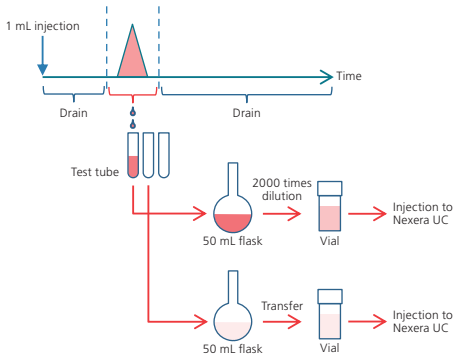

Fig. 8 Overview of the Procedures for Evaluating the Degree of Contamination of Adjacent Test Tubes

Table 5 Analytical Conditions

System	: Nexera UC Prep Multi-fraction system
Column	: Silica column (250 mm L. × 20 mm I.D.)
Modifier	: Methanol
Modifier concentration	: 10 %
Modifier flow rate	: 60 mL/min
Makeup	: Methanol
Makeup flow rate	: 15 mL/min
Column temperature	: 40 °C
Injection volume	: 1000 µL (Loop size: 2000 µL)
Detection	: 205 nm
Cell	: High pressure cell for SFC (preparative)
BPR pressure	: 10 MPa
BPR temperature	: 50 °C
Sample	: Linalool 1 %

Table 6 Analytical Conditions (Reinjection)

System	: Nexera UC
Column	: Silica column (250 mm L. × 4.6 mm I.D.)
Modifier	: Methanol
Modifier concentration	: 20 %
Flow rate	: 3 mL/min
Column temperature	: 40 °C
Injection volume	: 20 µL
Detection	: 205 nm
BPR pressure	: 10 MPa
BPR temperature	: 50 °C

The evaluation results are shown in Table 7. No peaks were detected in the reinjected solution, which confirms that the concentration was less than the detection limit (i.e., a peak area of less than 2667). Consequently, it was confirmed that contamination of the adjacent test tube was 0.006 % or less, even during the preparative separation of volatile compounds.

Table 7 Contamination Results

Compound	Peak Area (Reference Diluted by 2000 Times)	Peak Area (Recovered Liquid)
Linalool	22491	N.D.

Compound	Peak Area for Detection Limit	Contamination
Linalool	2667	0.006 % max.

4. Evaluating Carryover by the LotusStream Separator

Carryover by the LotusStream separator was evaluated using a Nexera UC Prep stacked fraction system. Whenever a sample is changed, residual substances are flushed out by rinsing the flow channels and the LotusStream separator. Accordingly, a check for carryover was performed after rinsing.

Hydrocortisone, a compound with low solubility, was used as the sample. The evaluation procedures are described below.

(1) Inject 1 mL of the sample solution using the parameters in Table 8, and recover the eluate from the peak elution intervals into the recovery bottles.
(2) Transfer the recovered liquid to a 50 mL volumetric flask. Rinse the recovery bottle about three times with methanol, and transfer the rinse solution to the flask as well. Then fill up the flask to 50 mL.
(3) After filling up the flask, dilute the recovered solution by 1000 times with methanol. Then using the reinjection parameters (Table 9), check the peak area values.
(4) Prepare a separate recovery bottle, and flow solution through the LotusStream separator for two minutes. (Use the LotusStream separator and the flow channel rinsing process for this step, configured as shown in the middle illustration in Fig. 9.)
(5) When the rinsing process is finished, install a separate recovery bottle, and recover solution for the same amount of recovery time as in step (1). (Refer to the lower illustration in Fig. 9.)
(6) Then fill up the flask of recovered liquid to 50 mL, as described in step (2).
(7) After filling up the flask, check the peak area values using the reinjection parameters.
(8) Calculate the carryover according to the following formula.
Carryover (%)
= Peak area from step (7)/Peak area from step (3)/1000 × 100

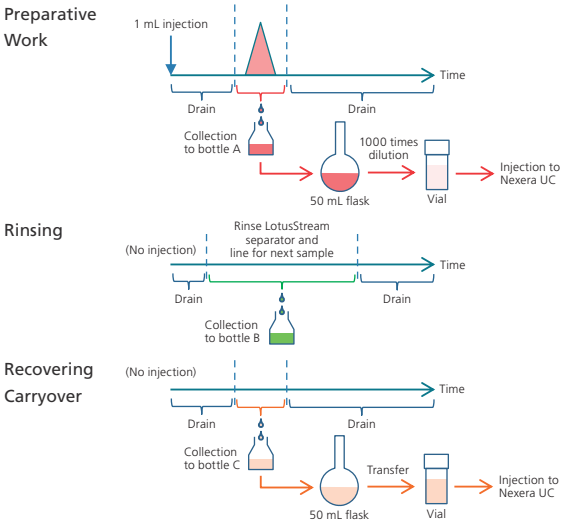

Fig. 9 Overview of the Carryover Evaluation Procedures

Table 8 Analytical Conditions (Preparative Separation)

System	: Nexera UC Prep Stacked fraction system
Column	: Silica column (250 mm L. × 20 mm I.D.)
Modifier	: Methanol
Modifier concentration	: 10 %
Flow rate	: 125 mL/min
Makeup	: Methanol
Makeup flow rate	: 10 mL/min
Column temperature	: 40 °C
Injection volume	: 1000 µL (Loop size: 2000 µL)
Detection	: 238 nm
Cell	: High pressure cell for SFC (preparative)
BPR pressure	: 10 MPa
BPR temperature	: 50 °C
Sample	: Hydrocortisone 30 mg/mL

Table 9 Analytical Conditions (Reinjection)

System	: Nexera UC + LCMS-2020
Column	: Silica column (250 mm L. × 4.6 mm I.D.)
Modifier	: Methanol
Modifier concentration	: 20 %
Flow rate	: 3 mL/min
Column temperature	: 40 °C
Injection volume	: 20 µL
Detection	: ESI Positive, m/z 364
BPR pressure	: 10 MPa
BPR temperature	: 50 °C

The evaluation results are shown in Table 10. The results confirmed that after only two minutes of rinsing, carryover by the LotusStream separator was a very low 0.024 %, even for compounds prone to precipitation.

Table 10 Carryover Results

Compound	Peak Area (Reference Diluted by 1000 Times)	Peak Area (Recovered Liquid)	Carryover
Hydrocortisone	7261078	1749519	0.024 %

First Edition: February, 2020

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3655-01017-ODPIT