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Application News

Semi-Preparative Supercritical Fluid Chromatograph – Nexera™ UC Prep

Highly Effective Purification of Drug Compounds

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User Benefits

- Preparative SFC provides significant reduction of fraction drying time comparing with conventional preparative HPLC
- Preparative SFC provides notable reduction of organic solvent consumption comparing with conventional preparative HPLC
- The stacked injection function provides consecutive purification and reduces run time

■ Introduction

High throughput purification is required in various fields. Preparative LC is widely used for purification of compounds., but it takes a long time. Additionally, high consumption of organic solvents leads to ineffective running cost. Here, we introduce a high throughput and low running cost preparative SFC purification.

■ Purification by Preparative LC system

Reverse phase mode is one of the most popular separation modes that is widely used in analytical HPLC and preparative HPLC. HPLC with reverse phase mode has high resolution and is suitable for purification of various compounds. On the other hand, water is often used as mobile phase in reverse phase mode and can cause extensive drying time. Table 1 and Fig. 1 show a practical example for ketoprofen and indomethacin of preparative LC purification.

Table 1. Preparative LC Conditions

Column	: Shim-pack™ Scepter C18-120 *1 (50 mm× 20 mm I.D., 5 µm)
Mobile phase	: A: Water (containing 0.1 % (v:v) formic acid) B: Acetonitrile
Flow rate	: 20 mL/min
Time program	: B conc. 10 %(0-1 min)→ 90 % (7-9 min) →10 %(9.01-10 min)
Column temp.	: Ambient temperature
Injection vol.	: 500 µL (10 mg/mL for each compound in acetonitrile)
Vial	: 10 mL screw vial *2
Detection	: 250 nm (PDA)

*1 P/N: 227-31102-01
*2 P/N: 220-97331-09

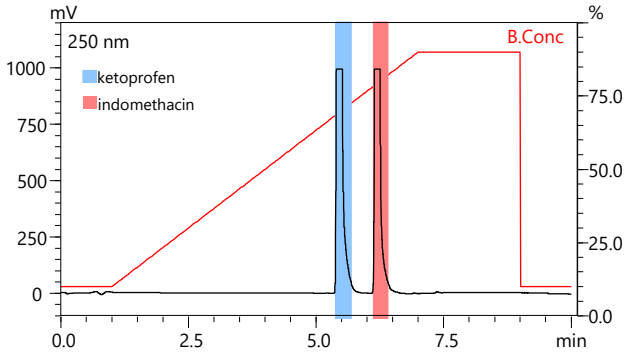

Fig. 1 Preparative LC chromatogram of drug compounds

■ Features of Preparative SFC

Supercritical Fluid Chromatography (SFC) is one of the chromatography using supercritical fluid (i.e., supercritical carbon dioxide) as mobile phase. The low cost of carbon dioxide (purity: 99.9 %) can significantly trim the total running cost of preparative SFC. Moreover, fraction drying time is shorter than conventional preparative LC because carbon dioxide naturally evaporates under atmospheric pressure.

■ Retention time modulation by gradient mode in Preparative SFC

In SFC analysis, organic solvent , called as "modifier" is used to modulate the retention time and/or selectivity. By continuously changing the amount of modifier through gradient mode, retention of compounds can be modulated. Table 2 and Fig. 2 show preparative SFC conditions and preparative SFC chromatogram of drug compounds that are the same compounds analyzed by conventional preparative LC in figure 1. A good separation was achieved on Shim-pack UC Diol column.

Table 2. Preparative SFC conditions (gradient mode)

Column	: Shim-pack UC Diol II *3 (250 mm× 20 mmI.D., 5 m)
Mobile phase	: CO_2 B: Methanol
Flow rate	: 60 mL/min
Time program	: B conc. 0 %(0-1 min)→ 40 % (7-9 min) →0 %(9.01-10 min)
Column temp.	: 40℃
Injection vol.	: 500 µL (10 mg/mL for each compound in *n*-Heptane/2-propanol = 2:1, v:v)
Vial	: 10 mL screw vial
BPR setting	: 10 MPa
Detection	: 250 nm (PDA)

*3 P/N: 227-32606-04

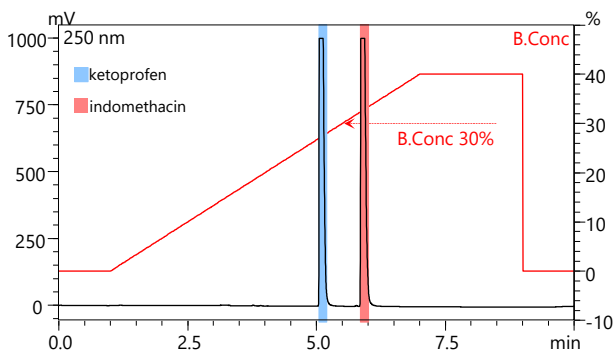

Fig. 2 Preparative SFC chromatogram of drug compounds (Gradient mode)

■ Improved efficiency of purification with stacked injection mode

"stacked injection" is a technique to improve the efficiency of preparative operations by consecutively injecting a sample in a queue and utilizing the buffer/waiting time for peak elution (Technical Report C190-E247). An isocratic program was developed for stacked injection mode based on results of gradient mode. Table 3 and Fig. 3 show Preparative SFC conditions and chromatogram by isocratic mode separation and with stacked injections. Repeated injections of for ketoprofen and indomethacin (n=5) were set and shown in Fig. 4 and Fig. 5.

Table 3. Preparative SFC conditions by (Isocratic mode)

Column	: Shim-pack UC Diol II (250 mm× 20 mmI.D., 5 µm)
Mobile phase	: A: CO_2 B: Methanol
Flow rate	: 60 mL/min
Time program	: B conc. 30 %(0-4 min)
Column temp.	: 40 ℃
Injection vol.	: 500 µL (10 mg/mL of each compound in *n*-Heptane/ 2-propanol = 2:1)
Vial	: 10 mL screw vial
BPR setting	: 10 MPa
Detection	: 250 nm (PDA)

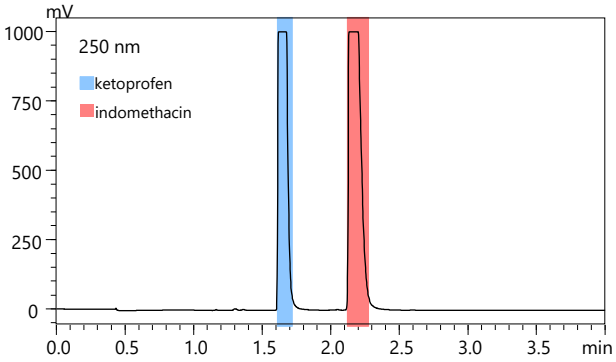

Fig. 3 Preparative SFC chromatogram (Isocratic mode)

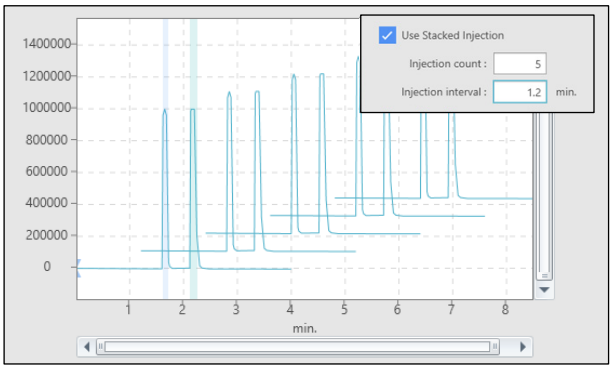

Fig. 4 Setting in Prep Solution *4 for stacked injection mode

*4 Prep Solution: Dedicated software for Nexera UC Prep

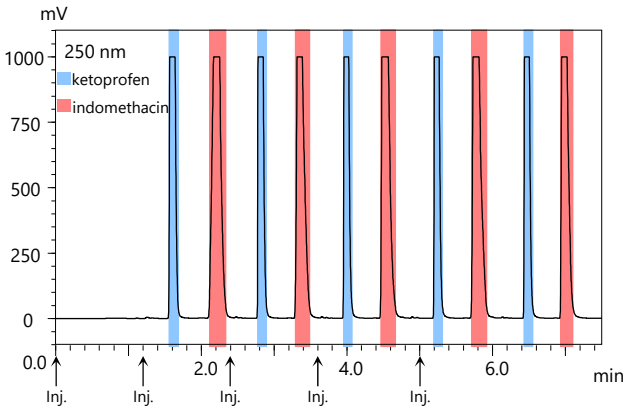

Fig 5. Preparative SFC chromatogram "stacked Injection mode"

■ Comparison of fraction drying time by Preparative LC and Preparative SFC

Comparison of mobile phase consumption and drying time of the collected fraction from Preparative LC and Preparative SFC is shown in Table 4 and Fig. 6. Preparative LC utilized a massive amount of water and organic solvents. By Preparative SFC, significant reduction of running cost was achieved due to the usage of carbon dioxide instead of them. Moreover, cost reduction is supported by shorter preparative time from stacked injection.

Fig. 6 shows the comparison of the fraction drying time collected by Preparative LC and Preparative SFC. Preparative SFC significantly shortened the drying time by 20 times or greater.

Table 4 Comparison of organic solvents consumption

	Mobile Phase	Volume	Mobile Phase	Volume	Total time
Preparative HPLC	Water	630 mL	Acetonitrile	470 mL	55 min
Preparative SFC	CO_2	315 mL	Methanol	135 mL	7 min

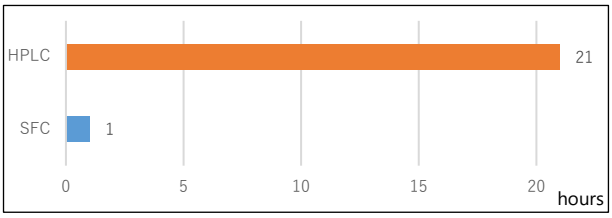

Fig. 6 Comparison of solvent drying time

■ Conclusion

This article introduced purification process of ketoprofen and indomethacin by Preparative SFC as an example of practical drug compounds purification. Preparative SFC provides superior advantages compared to Preparative LC in terms of process time length, drying time and running cost. Nexera UC Prep is expected to be a game changer for preparative workflow.

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