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Application News

No. L559

High Performance Liquid Chromatography

High Efficiency in Workflow from Preparative HPLC to Analytical HPLC by Nexera™ Prep System

Preparative LC is widely used as a technique for separation and purification of a target compound from a mixture. Identification of the target compound peak and confirmation of fraction purity are extremely time-consuming, and automation of these processes can be expected to shorten the total working time for purification process. This article introduces a high throughput analysis conducted for simple fractionation using MS triggers and purity confirmation using an analytical/preparative LC-MS system, which is one configuration of the Nexera Prep brand.

K. Nakajima

Outline of Analytical/Preparative LC-MS System

The liquid handler (LH-40) used in this system has parallel preparative and analytical flow paths, enabling seamless reanalysis of the collected fractions. Fig. 1 and Fig. 2 show the flow line of the analytical/preparative LC-MS system used in this experiment. Fig. 1 shows the flow path when the system is used as preparative LC-MS. In this flow path, the target compounds injected from the LH-40 is separated by the preparative column, and part of the fraction is introduced into the LCMS™-2020. When the LCMS-2020 identifies peaks originating from the target compounds, an solenoid valve mounted on the tip of the LH-40 nozzle opens and the target compounds are collected respectively in the fraction collector.

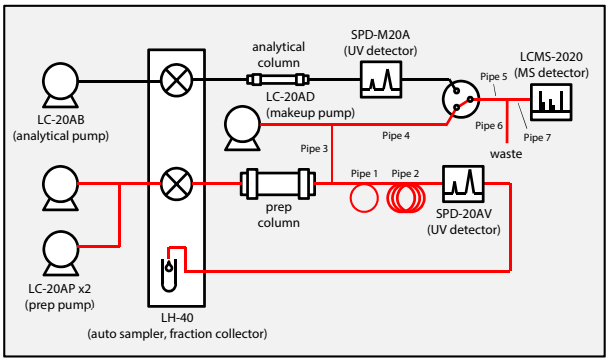

Fig. 1 Flow Diagram of Analytical/Preparative LC-MS During Fractionation

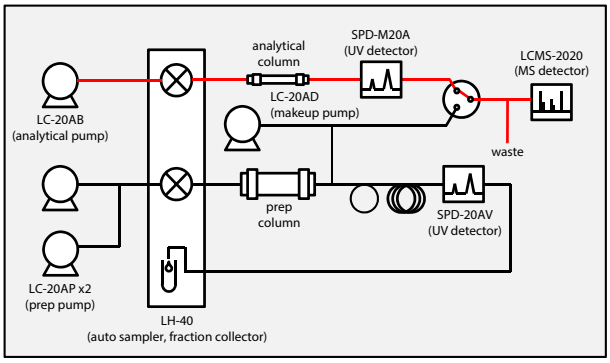

Fig. 2 Flow Diagram of Analytical/Preparative LC-MS During Analysis

Table 1 Configuration of Splitter Piping Used for LC-MS Trigger Fractionation

Pipe No.	Pipe 1	Pipe 2	Pipe 3	Pipe 4	Pipe 5	Pipe 6	Pipe 7
Material	PEEK	PTFE	PEEK	PEEK	PEEK	PEEK	PEEK
Diameter (mm)	0.25	1.0	0.025	0.25	0.13	0.13	0.13
Length (mm)	150	6000	40	1000	900	60	480

Fig. 2 is the flow diagram when the system is used as an analytical LC-MS. The LH-40 has a function that reinjects the fraction collected into the analytical flow path. In this system, gradient analysis is supported using a Shimadzu LC-20AB gradient solvent delivery unit, in which two pumps are incorporated in one compact module as solvent delivery pumps for analytical LC-MS, and introduction of a high pressure flow change-over valve in the downstream part of the analytical flow path allows to utilize the same LCMS-2020 in analytical LC-MS.

Both the preparative and analytical functions provided by this system can be controlled by the Shimadzu workstation LabSolutions™. All processes from switching of preparative LC-MS and analytical LC-MS during batch analysis, fractionation of the target compounds, and purity confirmation are conducted automatically in one system, realizing high efficiency in the entire workflow from fractionation to reanalysis.

Fractionation of Drugs by Preparative LC-MS

Purification was conducted by this preparative LC-MS system using a standard mixture consisting of 2 drugs, ketoprofen and indomethacin, as model compounds. Table 2 shows the fractionation conditions. A Shim-pack Scepter™ C18 with a particle diameter of 5 µm was used as the preparative column. MS detection was carried out in the high-speed positive/negative ionization switching scan mode (*m/z* range: 50 to 1,000). MS-triggered fractionation is conducted based on the XIC (extracted ion chromatogram) of ion species originating from the target compound. The XIC is drawn automatically by setting the molecular weight of the target compound. Fig. 3 shows the chromatograms obtained by preparative LC-MS. Two types of fractions, A and B, were detected by both the UV detector and the MS, and were successfully collected.

Table 2 Fractionation Conditions (Preparative LC-MS Flow Line)

Prep conditions	
Column	: Shim-pack Scepter C18 (75 mm L. × 30 mm I.D., 5 µm)
Mobile phase	: A: water (containing 1%(wt/v) formic acid) B: acetonitrile (containing 1%(wt/v) formic acid)
Flow rate	: 40 mL/min
Makeup	: 1.5 mL/min (methanol)
Time program	: B conc. 10% (0 min) → 90% (6-8 min)
Column temp.	: Ambient
Injection vol.	: 1000 µL (containing 10 mg/mL for each compound)
Detection	: UV 250 nm (prep cell) MS (Posi. Nega. Scan *m/z* 50 - 1000)

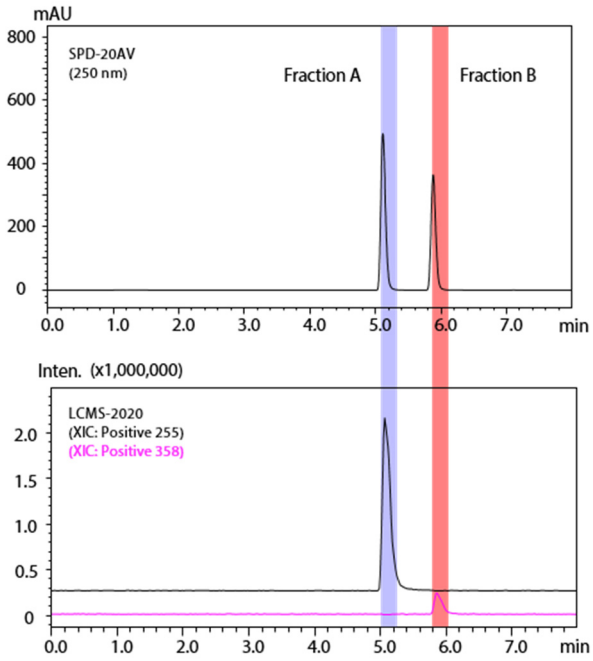

Fig. 3 Preparative Chromatograms of 2 Drugs (Top: UV Detector Chromatogram, Bottom: LC-MS Chromatogram)

Purity Confirmation of Fractions by Analytical LC-MS

The obtained fractions A and B were analyzed by the analytical flow path (Fig. 2) of this system, and a purity confirmation test was conducted. Table 3 shows the analytical conditions. A large number of fractions are obtained in one fractionation in preparative LC, and high throughput analysis is required to confirm their purity. Here, a Shim-pack Scepter C18 with a particle size of 3 µm was used as the analytical column, aiming at a high separation capacity and good peak shapes in high throughput analysis.

Fig. 4 and Fig. 5 show the obtained chromatograms for ketoprofen and indomethacin, respectively. Purity of 99% or more was obtained for both ketoprofen and indomethacin in peak area normalization (UV chromatogram) (Table 4), and the analysis times for both compounds were within 3 min.

Table 3 Analytical Conditions (Analytical LC-MS Flow Path)

Analytical conditions	
Column	: Shim-pack Scepter C18 (50 mm L. × 4.6 mm I.D., 3 µm)
Mobile phase	: A: water (containing 1%(wt/v) formic acid) B: acetonitrile (containing 1%(wt/v) formic acid)
Flow rate	: 2.5 mL/min
Time program	: B conc. 10% (0 min) → 90% (2-3 min)
Column temp.	: Ambient
Injection vol.	: 1 µL
Detection	: UV 250 nm (prep cell) MS (Posi. Nega. Scan *m/z* 50 - 1000)

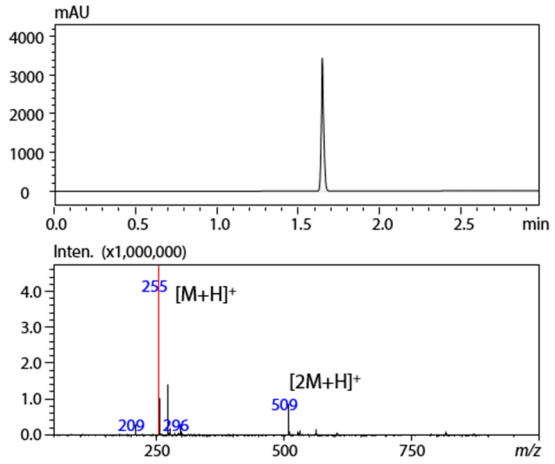

Fig. 4 Reanalysis Results of Fraction A (Ketoprofen) (Top: UV Detector, Bottom: MS Spectrum of Detected Peak)

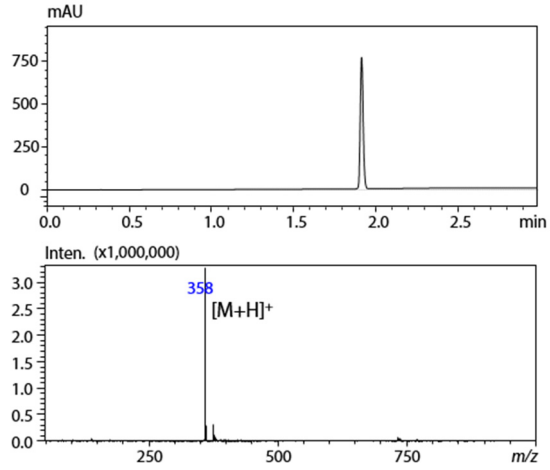

Fig. 5 Reanalysis Results of Fraction B (Indomethacin) (Top: UV Detector, Bottom: MS Spectrum of Detected Peak)

Table 4 Purity of Target Compounds Contained in Fractions (Area Normalization, UV 250 nm)

	Area %
Ketoprofen*	99.8
Indomethacin	99.9

* Because the peak intensity was near the saturation point, the area normalization value is a reference value.

Conclusion

This article introduced a convertible system setup of preparative and analytical LC-MS using LH-40 liquid handler and its application. Use of the reinjection function of the LH-40 allowed to carry out all the processes from purification of the target compounds by preparative LC-MS to purity confirmation of the acquired fractions and acquisition of the MS spectrum in a single series batch analysis, significantly improving the efficiency in the conventional preparative LC workflow.

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