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Application News

Software for Efficient Method Development
Preparative Purification Liquid Chromatograph

Seamless Purification Workflow from Analytical to Preparative Using a Single Quad LC-MS System

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User Benefits

- Scaling-up from analytical column to preparative column and purity/recovery check can be completed in a single system.
- LabSolutions™ MD efficiency optimizes separation conditions by automatically creating analysis schedules.
- Single quadrupole mass spectrometer LCMS-2050 provides *m/z* information for target compounds.

■ Introduction

Preparative LC is utilized in various fields, such as pharmaceuticals, food, and chemical engineering, for purifying target compounds from mixed samples, searching for active ingredients in natural products, and analyzing the structures of impurities and unknown compounds. To achieve high purity and recovery rate for fractionation of target compounds, it is crucial to establish analytical conditions that separate these compounds from other co-existing components. Due to the significant sample and mobile phase consumption associated with preparative LC conditions, the optimization of separation conditions is typically performed on an analytical scale to minimize these consumptions. During this optimization, various HPLC conditions, including gradient profiles, are adjusted to find the optimal separation. This is a time-consuming process for creating each analysis schedule. Also, confirmation of purity and recovery rate after scaling-up is followed by transferring fractions from fraction tubes to an autosampler manually. This article presents an efficient preparative purification workflow (Fig. 1), which includes investigating separation conditions at analytical scale, scaling-up for fractionation, and confirming purity and recovery rate. All of these processes are carried out using single analytical/preparative convertible LC-MS system of Nexera™ Prep.

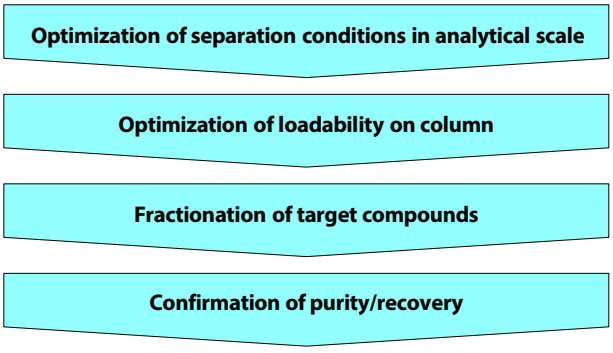

Fig. 1 Workflow of Preparative Purification

■ Overview of LC-MS System

The flow path diagram of analytical/preparative convertible LC-MS system is shown in Fig. 2. The analytical flow path (the upper of Fig. 2) is used to optimize separation conditions, loadability on column, and purity/recovery check, while the preparative flow path (the lower of Fig. 2) is used for the preparative separation of target compounds. The liquid handler (LH-40), which has both analytical and preparative flow paths and can inject fractions from fraction tubes directly into the analytical flow path, allows a complete workflow of preparative purification with this system. In addition, LCMS-2050 provides not only mass information of target compounds when optimizing separation conditions, but also a combination trigger of UV and MS signals during fractionation. Therefore, target compounds can be recovered with high purity. To do so, this system is configured to split the preparative flow path and introduce a portion of the mobile phase into MS with a make-up solvent to achieve both fractionation and MS detection.

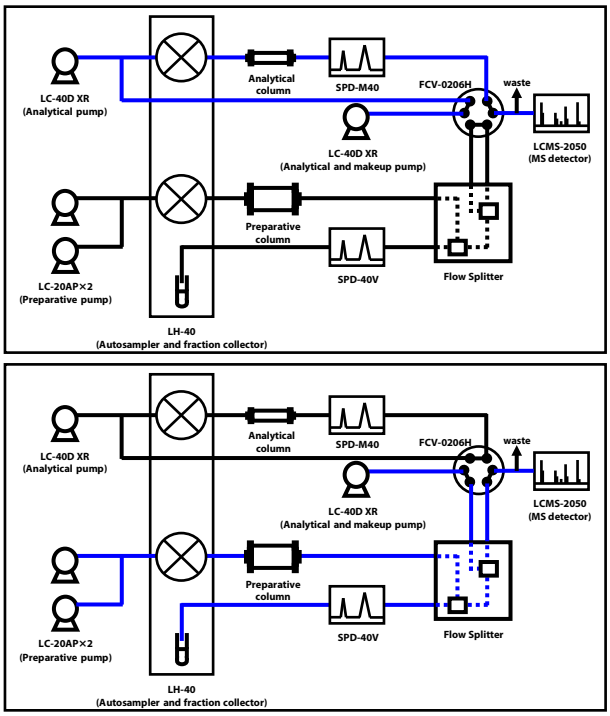

Fig. 2 Analytical Flow Path (Upper), Preparative Flow Path (Lower)
*blue colored flow path is in operation

The following section describes an example of using this analytical/preparative convertible LC-MS system to provide an efficient preparative purification workflow with a mixture of seven pharmaceutical standards (target compound for fractionation : Hydrocortisone) as model compounds.

■ Optimization of Separation Conditions in Analytical Scale

The separation conditions of Hydrocortisone are optimized in analytical scale. LC chromatogram (analytical conditions : Table 1) before optimization is shown in Fig. 3, in which Salicylic acid is eluted very close to Hydrocortisone. Increasing loadability under these conditions may cause further deterioration of the separation of these two compounds and the reduction of the purity at the recovery. Consequently, improvement of the separation is essential. The separation is optimized by varying the gradient conditions in nine different profiles (initial concentration and gradient slope in three different levels each). LabSolutions MD, a dedicated software for supporting method development, was used for automatic analysis schedule generation to improve the efficiency of the optimization (Fig. 4). The obtained chromatograms are shown in Fig. 5. The separation of Hydrocortisone and Salicylic acid was optimized at an initial concentration of 15% and a gradient slope of 20 minutes ((3) in Fig. 5). Then, the next optimization of loadability was conducted based on these conditions. During optimizing separation conditions, mass information (Hydrocortisone : *m/z* 363.3) was simultaneously obtained by LCMS-2050.

Table 1 Analytical Conditions

Mobile Phase	: Pump A : 0.1% formic acid in water
	: Pump B : Acetonitrile
Column	: Shim-pack Scepter™ C18-120
	(150 mm × 4.6 mm I.D., 5 µm)*1
Sample	: (A) Hydrocortisone, (B) Salicylic acid,
	(C) Metoclopramide, (D) Lidocaine,
	(E) Furosemide, (F) Papaverine, (G) Quinidine
Sample Concentration	: 100 mg/L (Hydrocortisone), 10 mg/L (others)
Injection Volume	: 10 µL
LC Conditions	
Time Program	: B Conc. 25%(0 min)→45%(20 min)
	→25%(20.01-25 min)
Column Temp.	: Ambient
Flow rate	: 1 mL/min
Sample loop size	: 500 µL
Syringe size : 500 µL	
Detection (PDA)	: 245 nm (SPD-M40, conventional cell)
MS conditions	
Ionization	: ESI/APCI (DUIS™), positive and negative
Mode	: SCAN (*m/z* 100-500)
Nebulizing Gas Flow	: 2.0 L/min (N2)
Drying Gas Flow	: 5.0 L/min (N2)
Heating Gas Flow	: 7.0 L/min (N2)
DL Temp.	: 200 °C
Desolvation Temp.	: 450 °C
Interface Voltage	: 3.0/-2.0 kV (positive/negative)

*1 P/N : 227-31020-05

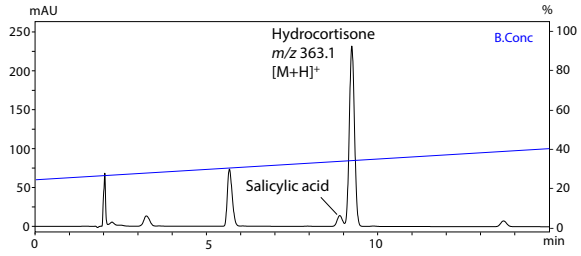

Fig. 3 LC Chromatogram before Optimizing the separation

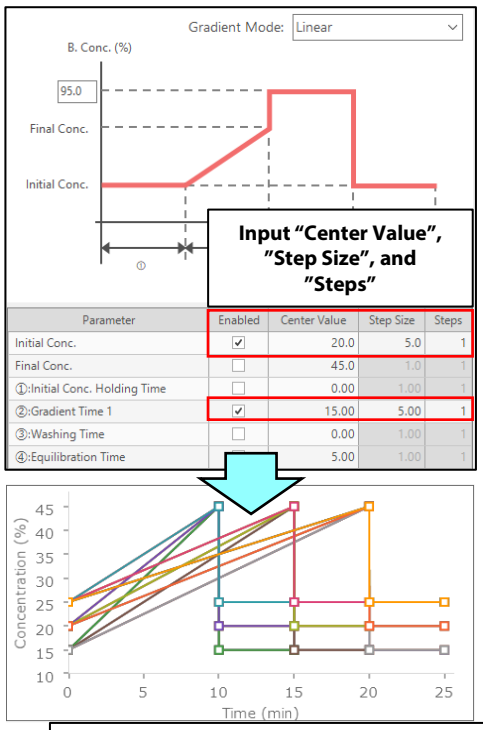

Fig. 4 Automatic Generation of Analysis Schedules by LabSolutions MD

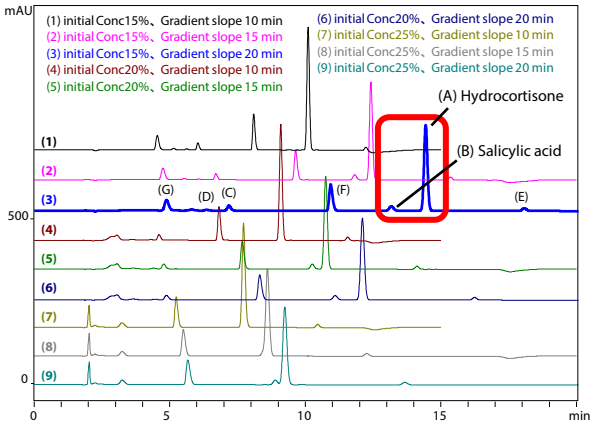

Fig. 5 Result of Optimizing Separation Conditions with LabSolutions MD
*blue colored chromatogram (3) shows the best separation of Hydrocortisone and Salicylic acid

■ Optimization of Loadability on Column

Fig. 6 shows the result of optimization of loadability conducted at injection volumes of 10, 20, 30, 40, 50, and 100 µL using Hydrocortisone (10,000 mg/L) under the optimized conditions at the analytical scale ((3) in Fig. 5). At an injection volume of 100 µL, the separation of Hydrocortisone and Salicylic acid was not sufficient (inside the blue oval in Fig. 6). In addition, a small peak (circled in red in Fig. 6), which seemed to be an impurity, was not clearly separated at the base of the peak. Up to an injection volume of 50 µL, Hydrocortisone is well separated from the neighboring peaks. Consequently, scaling-up was implemented using 50 µL injection volume.

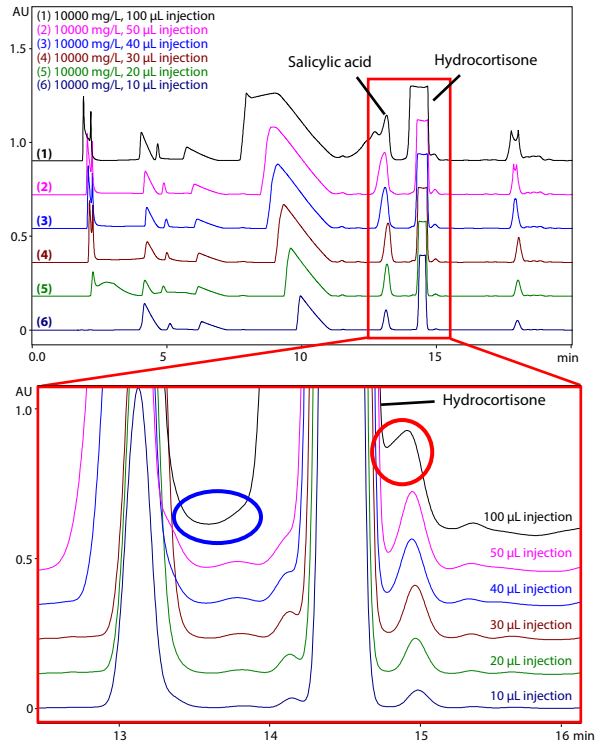

Fig. 6 Result of Optimization of Sample Loading Capacity

■ Fractionation of Target Compounds

Hydrocortisone was fractionated using a UV trigger. The preparative conditions are presented in Table 2 (only the settings differing from those in Table 1 are listed), and the resulting preparative LC chromatogram is shown in Fig. 7 (the blue area represents the fractionation interval). Based on the cross-sectional area ratio (approximately 20-fold) between the preparative column (20 mm I.D.) and the analytical column (4.6 mm I.D.), the flow rate was increased to 20 mL/min (with a constant linear velocity before and after scaling-up), and the injection volume was set at 1 mL. As a result, similar separation profiles were achieved before and after scaling-up, allowing for effective fractionation of Hydrocortisone while maintaining adequate separation from Salicylic acid." When scaling up from analytical to preparative LC, several LC parameters must be recalculated to create a preparative method file. However, this process is often labor-intensive and prone to manual input errors. LabSolutions MD streamlines this workflow by automatically calculating the necessary LC parameters and generating a preparative method file that reflects them (Steps (1) to (4) in Fig. 8). By simply selecting the target system (Fig. 8(1)), entering the column size (Fig. 8(2)) and flow rate (Fig. 8(3)), users can automatically generate a preparative method file, significantly reducing manual work.

Table 2 Preparative Conditions

Column	: Shim-pack Scepter C18-120 (150 mm × 20 mm I.D., 5 µm)*1
Sample Concentration	: 10000 mg/L (Hydrocortisone), 1000 mg/L (others)
Injection Volume	: 1 mL
LC Conditions	
Flow rate (Prep)	: 20 mL/min
Flow rate (Makeup)	: 1.5 mL/min (Methanol)
Sample loop size	: 2 mL
Syringe size	: 5 mL
Detection (UV)	: 245 nm (SPD-40V, preparative cell)
MS conditions	
Desolvation Temp.	: 100 °C

*1 P/N : 227-31102-03

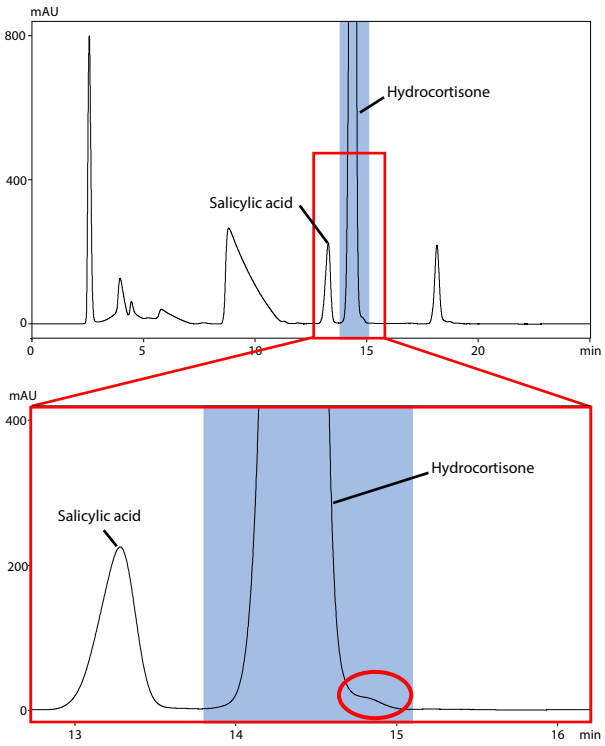

Fig. 7 UV triggered Preparative Chromatogram
*fractionation interval is colored blue

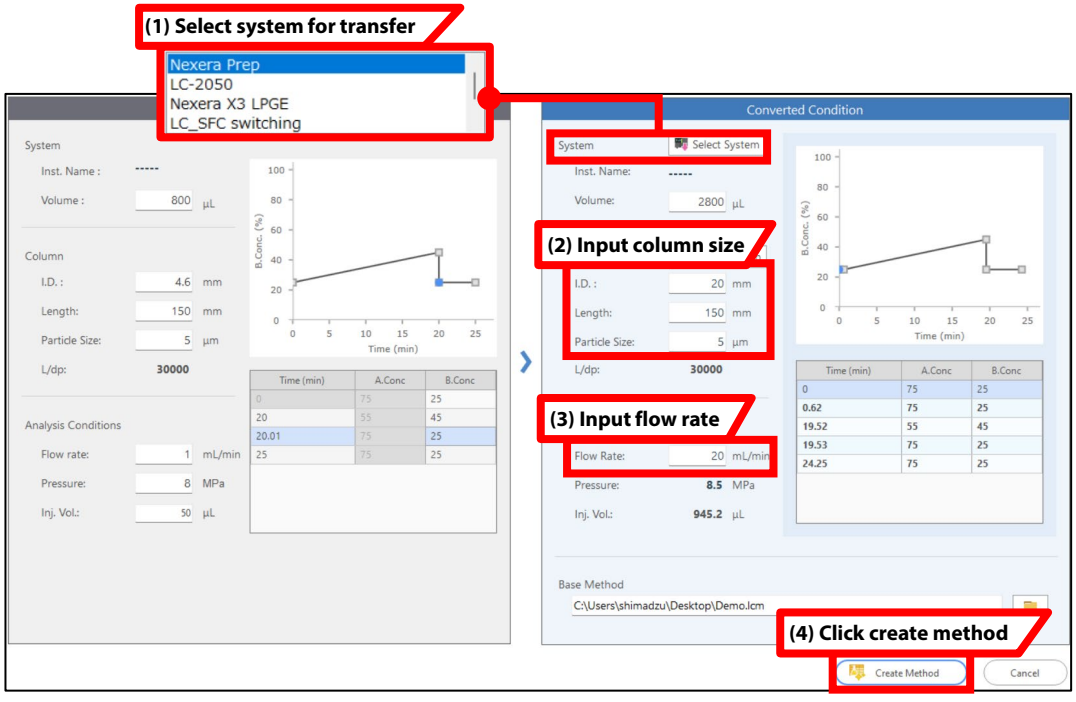

Fig. 8 Automatic Generation of a Preparative Method File by LabSolutions MD

■ Confirmation of Purity/Recovery

Fig. 9 shows the chromatogram obtained when a fractionated Hydrocortisone was re-injected into analytical flow path, as well as the chromatogram of a standard mixture (as a reference for calculating recovery) prepared to be the same concentration as fractionated Hydrocortisone. The purity and recovery rate of Hydrocortisone are shown in Table 3. The purity was more than 99% using area normalization, and the recovery rate was also excellent.

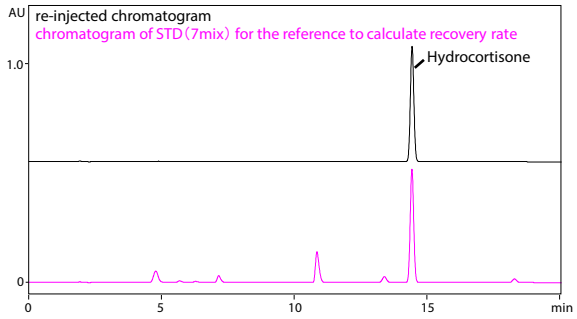

Fig. 9 Re-injected Chromatogram (Upper)
Chromatogram of STD (Lower)

Table 3 Purity and Recovery Rate of Fractionated Hydrocortisone
(n=3, average value)

	Purity (Area %)	Recovery Rate (%)
Hydrocortisone	99.7	101.2

In the lower part of Fig. 7, an enlarged image of the interval where Hydrocortisone was eluted is presented. A very small peak, likely an impurity, is observed at the base of the Hydrocortisone (inside the red oval). The fractionation results demonstrate a purity of 99.7% (Table 3) with UV trigger only. However, the preparative LC-MS system also supports MS triggered fractionation, enabling the separation of target compounds with even higher purity by avoiding impurities, thanks to the high identification performance of the MS. More information on the MS triggered preparative LC-MS system can be found in Application News "01-00651-EN".

■ Conclusion

The analytical/preparative convertible LC-MS system is employed to efficiently complete the preparative purification workflow which consists of the optimization of separation conditions at an analytical scale, scaling-up for fractionation, and confirmation of purity and recovery rate. LabSolutions MD, equipped to automatically generate analysis schedules with various LC parameters, facilitates the efficient optimization of separation conditions. Also, LCMS-2050 provides mass information simultaneously. Moreover, the liquid handler LH-40 allows for the direct injection of obtained fractions into the analytical flow path, ensuring seamless confirmation of purity and recovery rate without transferring fractions from a fraction tubes to an autosampler manually.

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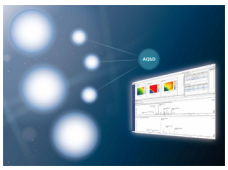

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