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Technical Report

Optimizing Preparative Workflow with the LH-40 Liquid Handler

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Abstract:

Preparative LC is a separation technique for isolating target compounds from a mixture. It makes use of the excellent separation performance of HPLC to recover target compounds at high purity levels. Preparative LC is used in pharmaceuticals, chemistry, and many other fields to recover target compounds from synthesized products at high purity levels, and to selectively recover functional constituents from natural products. Such applications of preparative LC require a preliminary investigation and optimization of the separation and loading conditions, and a way of streamlining this step is needed.

The Shimadzu Nexera™ Prep preparative purification LC system supports and optimizes the entire preparative workflow Including the preliminary method development phase. This article describes the use of the LH-40 liquid handler and LabSolutions™ control software to streamline the preliminary investigation of conditions, scale-up to the preparative scale, optimize the preparative parameters, and perform purity check. The article also explains how this system can be expanded for the successive preparative separation of large numbers of samples.

Keywords: Nexera Prep, multi-liquid handler, preparative LC, LH-40

1. Streamlining Preparative LC

Preparative LC is a technique for isolating target compounds from a mixture. It makes use of the excellent separation performance of HPLC to recover target compounds at high purity levels. Given the purpose of preparative LC, there is a need for ways not only to (1) maximize recovery yields and purity levels, but also to (2) streamline the entire preparative workflow from sample injection to purity checks.

This article describes the use of the LH-40 liquid handler, which combines the functions of a fraction collector and autosampler, and LabSolutions control software to seamlessly implement the entire preparative workflow, including the preliminary investigation of conditions, scale-up to preparative scale, optimization of preparative parameters, and purity checks. It also describes the features of the LH-40 liquid handler and LabSolutions software responsible for streamlining this preparative work.

2. Investigating Conditions at the Analytical Scale

An important initial step in preparative LC is identifying the amount of target compound to be fractionated. This amount can be estimated based on the concentration and amount of target compound in the sample, and is subsequently used in selecting the preparative system and column. Large amounts of target compound require a column with a large internal diameter and increased loading volumes.

Preparative LC consumes large volumes of mobile phase and sample, so separation conditions are typically investigated in advance using analytical scale. The LH-40 adds an analytical LC flow path into the preparative LC system (fig. 1). The possibility to inject up to 400 µL into the analytical flow path allows to perform accurate investigations on loading volumes.

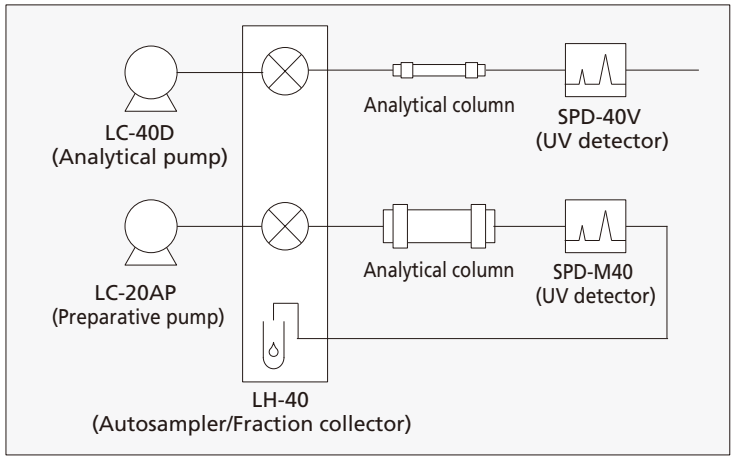

Fig. 1 Example of a Flow Path Diagram for a Combined Analytical/Preparative System

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3. Optimizing the Preparative Conditions

After investigation at the analytical scale, conditions are scaled up for preparative applications.

Identical separation is achieved in the preparative column by using the same column packing, while the mobile phase flowrate and loading volume are increased in proportion to the increase in column cross-sectional area. Fig. 2 shows an example scale-up based on the loading volume determined at the analytical scale. Increasing the internal diameter of the column from 4.6 mm to 20 mm increased the cross-sectional area by approx. 18.9 times, so the preparative scale flowrate was set to 19 mL/min or 19 times the analytical flowrate. After the scale up the chromatogram resulted the same.

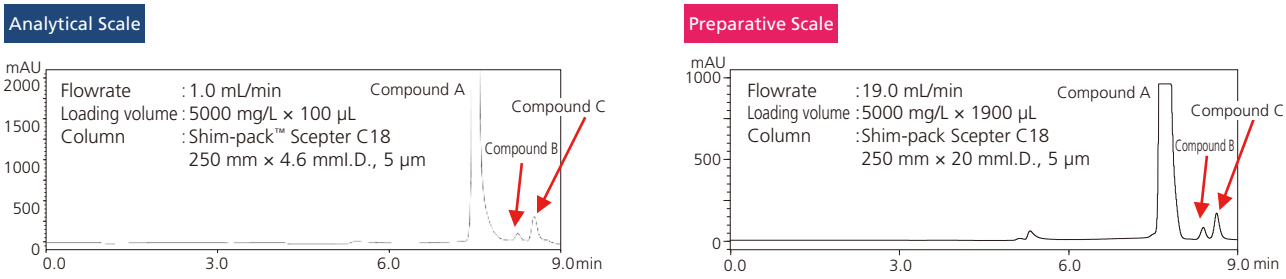


Fig. 2 Example Scale-up from Analytical to Preparative Scale

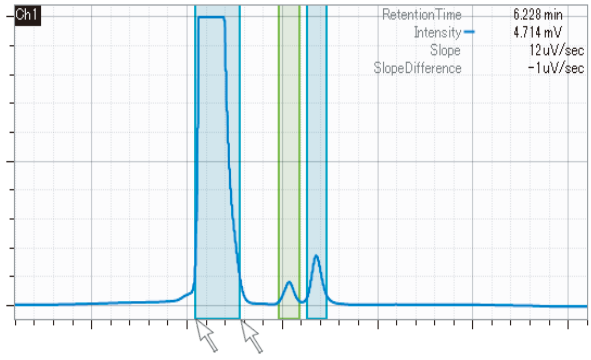
The next step is to configure the parameters for fractionation. Fractionation can be implemented using automatic fraction collection, time-based fraction collection, or manual fraction collection.

Automatic fraction collection involves setting a signal slope and intensity threshold for peak detection, and collecting fractions based on peak recognition. This method allows to collect fraction independently from their retention time variation.

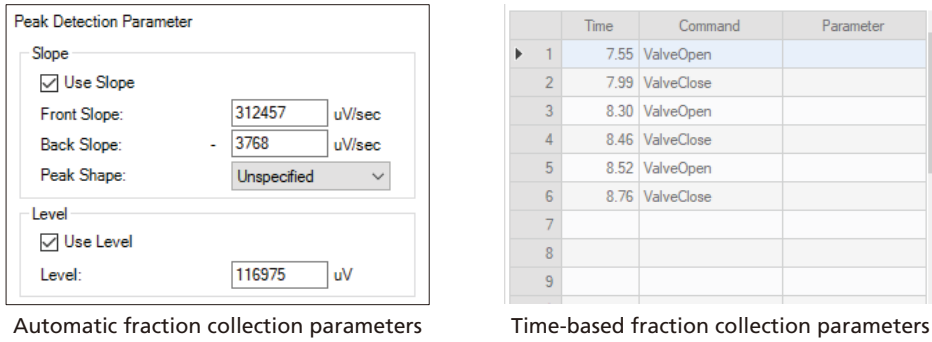
Time-based fraction collection involves setting elution time ranges in advance, and collecting fractions based on these intervals. This method is useful for collecting fractions during a predetermined period when performing preparative separation repeatedly.

Manual fraction collection involves manually controlling when to collect fractions. With the LabSolutions software, the operator can monitor the chromatogram from the software interface and collect fractions simply with a single click.

The fraction collection function in LabSolutions allows an easy setting of all collection parameters. While referring to the acquired chromatogram, the operator simply selects the peak range to collect (as indicated by the blue and green regions in Fig. 3), and LabSolutions automatically calculates the signal slope and threshold intensity to trigger automatic fraction collection. Alternatively, fraction collection can be set based on the elution times for specified peak ranges.



Selection of desired peak range by clicking on the chromatogram



Automatic fraction collection parameters

Time-based fraction collection parameters

Fig. 3 Automatic Configuration of Fraction Collection Parameters

4. Checking the Purity

Collected fractions are analyzed at the analytical scale to check their purity. The LC-40 enables the direct analysis of fractions without need of any sample transfer. Fig. 4 shows a chromatogram before fractionation and the chromatograms obtained from analysis of compound A, B, and C fractions after fractionation. The chromatograms show that each fraction contains no other compounds and consists of a high-purity fraction of the respective target compounds.

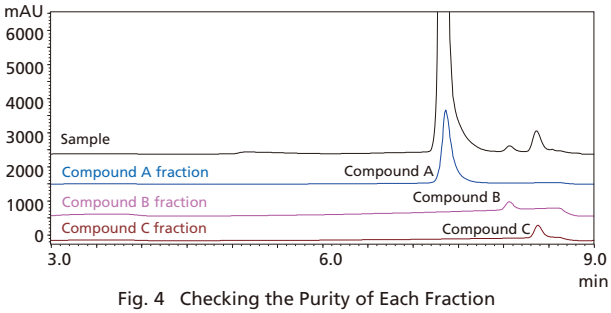


Fig. 4 Checking the Purity of Each Fraction

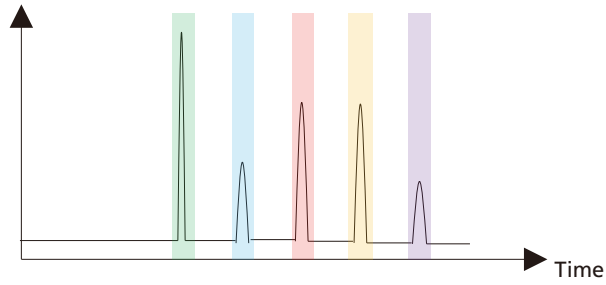
5. Multiple Analysis of Large Numbers of Samples

Up to six LH-40 liquid handlers can be coupled together (Fig. 5) for even greater workflow efficiency. When coupled together, all LH-40 liquid handlers are capable of injecting samples, collecting fractions, and checking the purity of the collected fractions. Coupling multiple LH-40 liquid handlers together enables successive preparative separation and purity checks for large numbers of samples.

As an example, 2 liquid handlers can hold up to 432 18-mm test tubes (Fig. 6). Assuming 5 fractions are collected from each sample, this configuration allows up to 72 samples to sequentially fractionated, followed by injection of the fraction collected from each sample into a total of 360 test tubes, and completion of the entire workflow from sample injection to purity checks with a single system.

Fig. 5 Multi-Liquid Handler System (Two Coupled LH-40 Units)

Example of a Chromatogram Showing Preparative Separation of 5 Compounds from One Sample



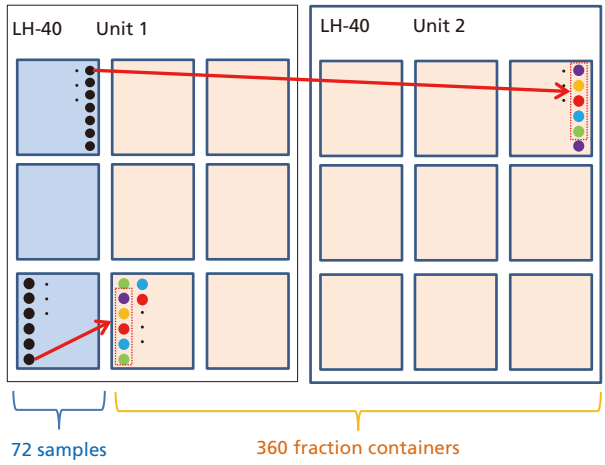


Fig. 6 Example of a Multi-Liquid Handler System

6. Conclusions

- The combination of the LH-40 liquid handler and LabSolutions control software enables streamlined, single-system implementation of the entire preparative workflow, from a preliminary investigation of preparative conditions to optimization of preparative parameters and purity checks.

- Up to six LH-40 liquid handlers can be coupled together and used in tandem to inject samples, collect fractions, and check fraction purity levels for collected fractions. Coupling LH-40 units together enables successive preparative separation and purity checks for large numbers of samples.

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