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Application News

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Separation of Styrene Oligomers Using Recycle Fractionation System

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User Benefits

- Recycle fractionation enables high-purity prep purification of compounds that are difficult to separate.
- GPC analysis and recycle fractionation can be performed within a single HPLC setup.
- Automated recycle fractionation using the time program function originally equipped in LabSolutions™ and batch analysis function.

■ Introduction

Prep LC is widely used across pharmaceutical, food, and chemical industries for purifying target compounds from mixtures, identifying active ingredients in natural products, and structural analysis of impurities and unknown compounds.

It is important to separate target compounds from co-existing compounds for the collection with high purity. However, it is difficult in some cases to separate target compounds due to limitations of optimizing separation conditions such as column selection. This article introduces the principles and workflow of prep purification using the recycle separation method—a technique for improving separation—using the example separation of styrene oligomers.

■ Recycle fractionation system

When target compounds are not separated sufficiently during HPLC prep purification, separation conditions such as mobile phase composition are typically changed. However, in non-aqueous size exclusion chromatography (GPC), the eluent cannot be changed, and there is almost no way for improving separation by changing conditions other than the column.

A simple method to enhance column separation performance without changing the packing material is to increase the column length. However, this approach has the drawbacks of "increased column back pressure" and "Expensive cost for additional columns". Preparing multiple columns is not practical considering expensive prep GPC column price.

In such cases, the "recycle separation method" is employed. This method achieves the same effect as extending length of the column by making sample repeatedly pass through a single column. Here, prep purification using the recycle separation method is referred to as "recycle preparative chromatography."

Fig. 1 shows the flow diagram of a recycle fractionation system. the waste/collection flow path connected to the fraction collector and the recycle flow path connected to the pump inlet can be selected by switching the position of the recycle valve. Selective re-introduction of the elution band containing the target compounds from the column outlet into the column inlet improves the separation of the target compounds.

Furthermore, eluent consumption is reduced, since the eluent is recycled in the recycling flow path.

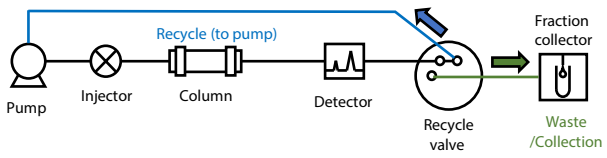

Fig. 1 Typical flow path diagram of recycle fractionation setup

■ Expansion of Nexera series to recycle fractionation system

Nexera lite analytical HPLC system or Nexera GPC analytical GPC system can be expanded into a recycle fractionation system. Taking Nexera GPC system as an example, adding (1) a recycle kit, (2) a recycle valve, and (3) a fraction collector to the standard configuration enables not only GPC analysis but also recycle fractionation. Using a single instrument for both analysis and recycle fractionation maximizes equipment utilization. Fig. 2 shows an example configuration of recycle fractionation system based on Nexera GPC.

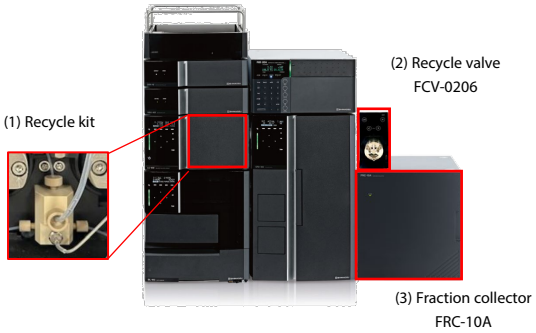

Fig. 2 System setup for recycle fractionation

■ Workflow of recycle fractionation

In recycle fractionation, the following workflow is typically used to obtain target compounds at high purity:

(1) Identification of target peak groups: Perform trial analysis under non-recycle conditions preliminary to identify peak groups containing the target compounds.

(2) Confirmation of separation and peak shape during recycling: Conduct trial analysis with recycling to understand the separation and peak shape of the target components during recycling.

(3) Manual recycle fractionation: Only specific peak groups can be selectively recycled by manual operation of the recycle valve while monitoring the chromatogram. This allows removal of impurities other than the target compounds from the recycling flow path.

(4) Automated recycle fractionation using time programs: Automatic recycle fractionation of complicated samples can be performed by using the time program function originally equipped in LabSolutions. This significantly reduces workload when a single recycle fractionation is not sufficient to recover desired fraction amount of target compounds or when recycle fractionation is performed routinely.

■ Separation of Styrene Oligomers

A commercially available polystyrene molecular weight marker (MW = 580, styrene oligomer, Figure 3 (left)) was used as a simulated sample. This styrene oligomer contains multiple compounds that have different number of repeating monomer units. In this article, a fractionation of a specific molecule from the styrene oligomer following the workflow described above is introduced.

Shodex GPC K-2002, suitable for the molecular weight of the target component, was used as the prep GPC column. The chromatogram obtained without recycling conditions for target compound confirmation is shown in Fig. 3 (right), and the analytical conditions are listed in Table 1.

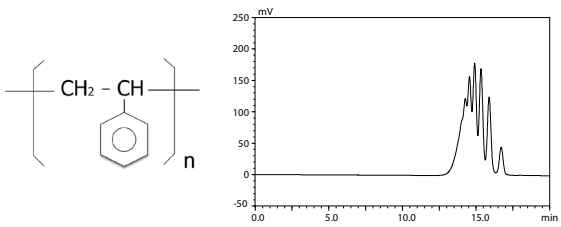

Fig. 3 (Left) Structural formula of polystyrene,
(Right) Chromatogram of styrene oligomer

Table 1 Analytical conditions

System	:	Nexera GPC + LC-40D recycle kit
Column	:	Shodex GPC K-2002 (300 mm × 20 mm I.D.)
Flow rate	:	3.0 mL/min
Mobile phase	:	Chloroform
Column temp.	:	Room temperature
Injection vol.	:	100 µL
Vial	:	TORAST for LC 1.5 mL, Glass
Detection	:	UV (254 nm, conventional cell)

In recycling fractionation, if all peaks—including those other than the target compounds—are recycled, the target peaks may coelute with other lapped peaks in the previous cycle.

Therefore, it is appropriate to remove all peaks except the target peaks intentionally during the initial elution. Consequently, after confirming the elution retention times of the target peaks to be fractionated, the timing for selecting the recycle valve position of either "waste/collection" or "recycle" must be determined. This ensures that only the target component peak and its nearby contaminant peaks are recycled, making the separation achieved by recycling visually apparent on the chromatogram.

An overview diagram of the recycle fractionation is shown in Fig. 4. At the timing when the target peak elutes during the initial elution, the recycle valve is switched to the "recycle" side. Subsequently, when good separation of the target peak from the nearby contaminant peaks is confirmed through recycling, the recycle valve is switched to the "waste/collection" side to remove contaminant peaks and collect the target peak.

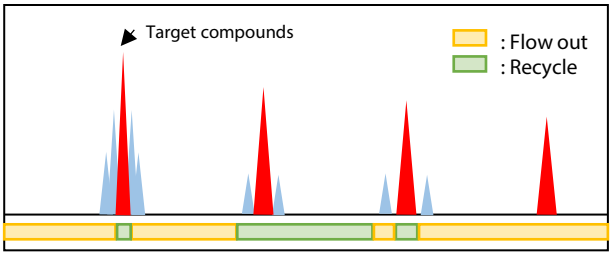

Fig. 4 Overview diagram of recycle fractionation

■ Automated operation using time program

Automatic recycle fractionation can be performed using the time program function equipped in LabSolutions. Here, a chromatogram was obtained by recycling all peaks including those other than the target compound as a preliminary trial. Then the recycle valve time program was determined based on this chromatogram. The red colored portion in Fig. 5 (Top) represents the target compound.

During recycling fractionation (Fig. 5 (Bottom)), unwanted peaks on both sides of target compound were roughly removed in the first elution. The second elution and the third elution were performed to improv the separation against the remained unwanted peaks. The separated neighboring peaks were removed in the fourth elution, and the appropriate interval around the peak top of the target compound was fractionated. The time program for this operation is shown in Table 2.

Combination with the analytical batch in LabSolutions provides fully automated repeated recycle fractionation involving sample injection, recycling, and collection.

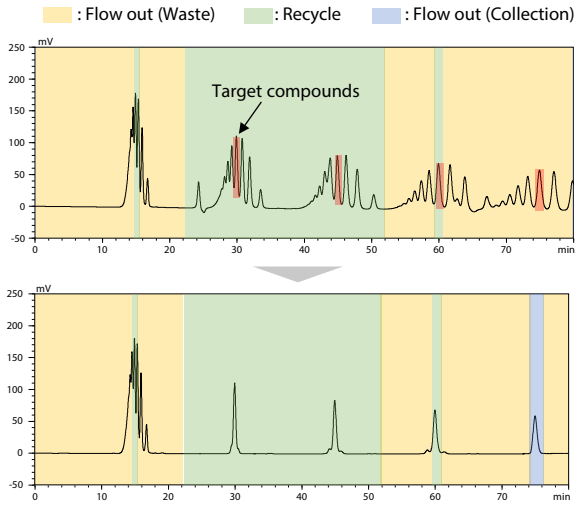

Fig. 5 (Top) Chromatogram of recycling all peaks,
(Bottom) Chromatogram of recycling following valve switching time program

Table 2 Time program for recycle valve

	Time	Unit	Command	Value	Recycle valve position
1	0.01	Controller	Event	0	Waste/Collection
2	14.70	Controller	Event	1	Recycle
3	15.10	Controller	Event	0	Waste/Collection
4	20.00	Controller	Event	1	Recycle
5	52.00	Controller	Event	0	Waste/Collection
6	59.40	Controller	Event	1	Recycle
7	60.00	Controller	Event	0	Waste/Collection
8	80.00	Controller	Stop		

■ Conclusion

This article introduced the principles and workflow of recycle fractionation using the separation of styrene oligomers as a simulated sample. Utilizing recycle fractionation improves the separation of compounds that are difficult to separate using a single column, resulting in high purity purification. Furthermore, switching use of a single HPLC setup for both analysis and recycle fractionation depending on the purpose can be performed by adding a recycle fractionation unit to ordinary HPLC and GPC systems, resulting in maximized instrument utilization. Additionally, recycle fractionation can be automated using the time program function and batch analysis function of LabSolutions.

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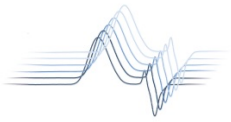

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