



Recycling Preparative HPLC  
LaboACE LC-5060

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## Recycling by SEC Column 1 Purification of Peptides

**Keyword:**

Purification of peptides, SEC Column, Size Exclusion Chromatography

### Introduction

In preparative HPLC, the column length is one of the key factors to get better separation. However, there is a limit in length due to restriction on the pressure the column can endure.

Recycling preparative HPLC is the solution to the problem. By cycling the sample solution back to the same column repeatedly, it causes the same effect as a longer column is used. Further, no solvent is consumed during the cycles. So it is the ideal way to efficiently increase separation (resolution) ability.

Also, combined use of SEC column, which separates compounds by their size, has gained great popularity among synthetic organic chemists since it can considerably save labor and time for method development work as long as the sample is dissolved in some solvent.

Here is an example of recycling preparative HPLC using aqueous SEC column.

### Experiment and Results

Sample: Mixture of peptides having estimated molecular weights of 2851 and 2648  
Reversed phase columns using gradient gave inadequate separation. So we tried recycling preparative HPLC using aqueous SEC column.

Instrument : LC-9101 (Detector : UV (220 nm))  
Column : JAIGEL-GS310 in series  
Mobile phase : 0.01 % Trifluoroacetic acid in Water  
Flow rate : 5 mL/min

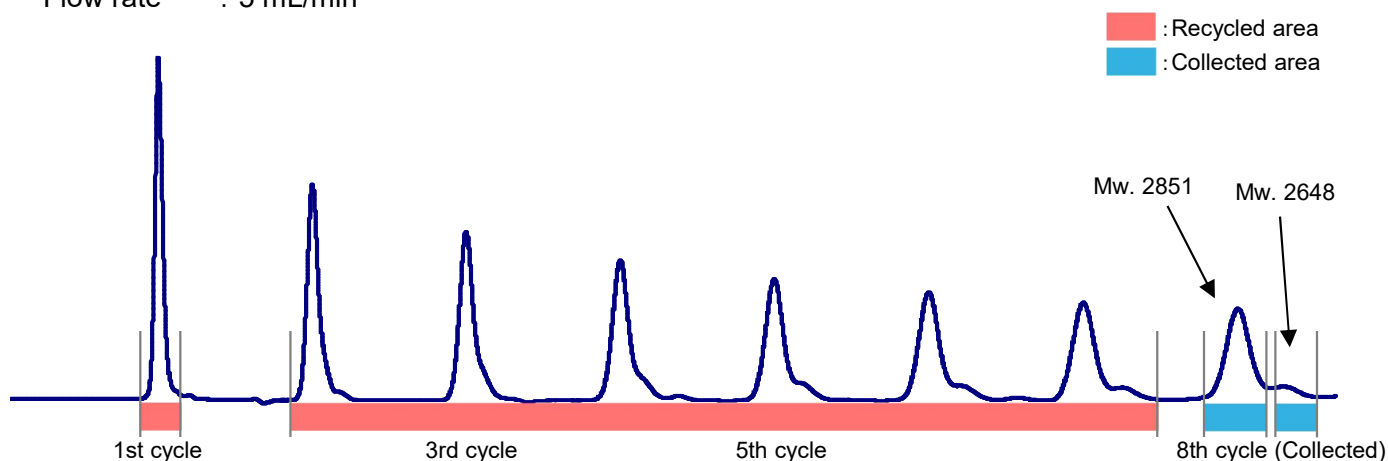


Fig. 1

### Conclusion

The two peptides were separated at the 8th cycle.  
The result suggests that recycling preparative HPLC using aqueous SEC column is very effective for peptides and nucleic acids that are often inseparable by reversed phase HPLC.