

## Application News

Software for Efficient Method Development "LabSolutions™MD"  
Preparative Purification Liquid Chromatograph "Nexera™Prep / LH-40 / LCMS-2050"

# Efficient Preparative Purification Workflow of Synthetic Peptide Using Analytical/Preparative Switching LC-MS System

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### User Benefits

- ◆ A multi-step seamless preparative purification workflow of synthetic peptides can be performed within a single LC-MS system setup.
- ◆ LabSolutions MD can provide easy investigation for optimal separation conditions for target compounds.
- ◆ Target compounds can be purified from crude samples with high purity and high recovery based on the excellent identification capability of LCMS-2050.

### Introduction

In the 2000s, biopharmaceuticals such as antibody drugs have emerged but there are many challenges to overcome because of their manufacturing process using genetic technology. Consequently, middle molecule drugs are paid attention, peptide therapeutics, one of middle molecule drugs which have advantages of being manufactured at low cost, easily taken into cells because of their small molecular weight, and prevented from degradation by adopting a specific three-dimensional structure when they are taken into human body. Since such peptides are produced by chemical synthesis like small molecule drugs, it is essential to purify, fractionate, and confirm the purity of the final synthesized product. In this article, we present a case study of multi-step seamless preparative purification workflow (optimizing separation conditions, scaling-up, fractionation, and confirming purity/recovery) of a peptide using preparative purification liquid chromatograph Nexera Prep (Fig. 1) based on Application News [01-00650-EN](#) and [01-00651-EN](#).



Fig.1 System setup of Nexera™ Prep

### Overview of analytical/preparative switching LC-MS

In this article, an analytical/preparative switching LC-MS system equipped with both analytical and preparative flow paths was used. The analytical flow path was used to evaluate separation conditions, loadability, and purity/recovery, while the preparative flow path was used for fractionation of a target peptide. LCMS-2050 provides not only mass information of target compounds when optimizing separation conditions but can also be effectively used for sample collection (MS triggered fraction collection). Therefore, target compounds can be recovered with high purity. Refer to Application News [01-00650-EN](#) for detailed information.

### Optimization of separation conditions in analytical scale

Separation conditions for a crude synthetic sample containing the target synthetic peptide (parathormone (1-34): PTH) were investigated in an analytical scale. Fig. 2① shows a UV chromatogram (analytical conditions: Table 1) of the sample before separation conditions were optimized. Under these conditions, the separation between PTH and co-existing impurities was not sufficient, and increased loading amount would deteriorate the separation from impurities furthermore, so improving the separation was essential to recover PTH with high purity.

LabSolutions MD, which enables comprehensive investigation for HPLC separation conditions using different parameter settings (twenty-five patterns of gradient profiles and five patterns of combination of initial and final concentrations of organic solvent) was employed for this study.

The best result for the separation of PTH (blue arrow) and impurities in the synthesized crude sample were obtained under conditions of 25% initial concentration and 35% final concentration of organic solvent (Fig. 2 ③).

Table 1 Analytical conditions

Mobile Phase	: Pump A : 0.1% TFA in water Pump B : 0.1% TFA in acetonitrile
Column	: Shim-pack Scepter™ C18-120 (150 mm × 4.6 mm I.D., 5 μm)*1
Sample Concentration	: 2 mg/mL in N-methylpyrrolidone
Injection Volume	: 10 μL
<b>LC Conditions</b>	
Time program (%B)	: B Conc. X%(0 min)→Y%(10 min) →90%(10.01-15 min)→X%(15.01-20 min) X : 10, 15, 20, 25, 30 Y : 30, 35, 40, 45, 50
Column Temp.	: Ambient
Flow rate	: 1 mL/min
Sample loop size	: 500 μL
Syringe size	: 500 μL
Detection (PDA)	: 220 nm (SPD-M40, conventional cell)
<b>MS Conditions</b>	
Ionization	: ESI/APCI (DUIS™), positive mode SCAN (m/z 500-2000)
Nebulizing gas Flow	: 2.0 L/min
Drying gas Flow	: 5.0 L/min
Heating gas Flow	: 7.0 L/min
DL Temp.	: 200 °C
Desolvation Temp.	: 250 °C
Interface Voltage	: 0.5 kV

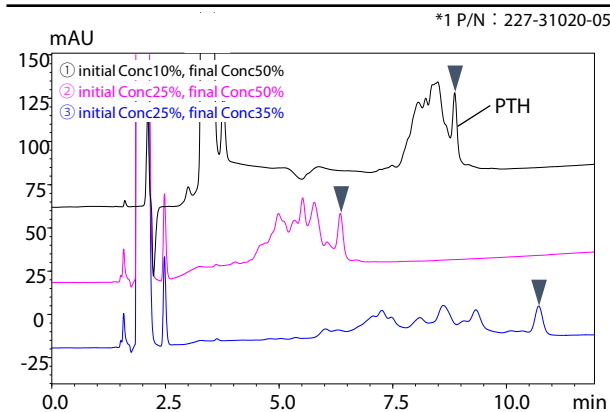


Fig. 2 Result of optimization for separation conditions

### Evaluation of loading capacity

Under the conditions optimized in analytical scale (③ in Fig. 2), the loading amount was investigated at injection volumes of 5, 10, 20, and 50 μL using a synthetic sample (10 mg/mL) (Fig. 3). Since the separation of PTH did not deteriorate as the injection volume was increased, it was decided to scale up and perform preparative analysis with 50 μL of injection volume.

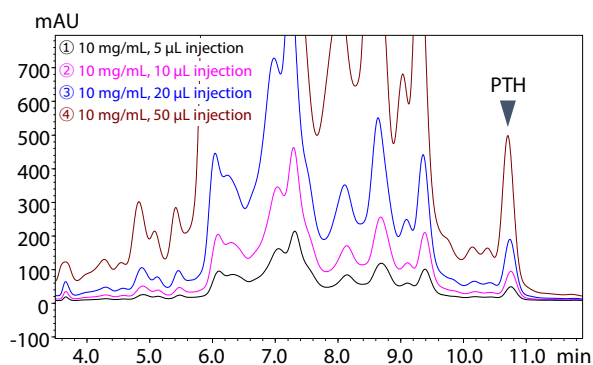


Fig. 3 Result of evaluation for loading amount

## ■ Fractionation of PTH

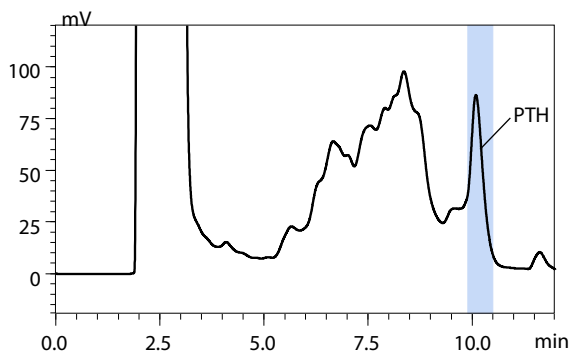
PTH was fractionated using UV and MS triggers. The preparative conditions are shown in Table 2 (only parameters different from Table 1 are listed), and the resulting LC chromatogram is shown in Fig. 4 (blue area is the fractionated interval). Based on the ratio of the cross-section area (approximately 20-fold) of the preparative column (20 mm i.d.) to the analytical column (4.6 mm i.d.), the flow rate was scaled up to 20 mL/min (linear velocity was constant before and after scaling-up) and the injection volume to 1 mL.

Similar separation patterns were obtained before and after scaling-up, and separation from impurities was maintained; the combination use of MS and UV triggers resulted in highly selective PTH fractionation.

Table 2 Analytical condition

Column	: Shim-pack Scepter C18-120 (150 mm × 20 mm I.D., 5 µm)*1
Sample Concentration	: 10 mg/mL in N-methylpyrrolidone
Injection Volume	: 1000 µL
<b>LC Conditions</b>	
Flow rate (Prep)	: 20 mL/min
Flow rate	: 1.5 mL/min
(Makeup for MS)	: (0.1% propionic acid in water/methanol = 90/10)
Sample loop size	: 2 mL
Syringe size	: 5 mL
Detection (PDA)	: 220 nm (SPD-40V, preparative cell)

\*1 P/N : 227-31102-03

Fig. 4 Preparative chromatogram with UV+MS triggers  
\*Blue band indicates fractionated interval

## ■ Confirming purity of collected fraction

Fig. 5 shows the chromatogram obtained by reinjecting the fractionated PTH into the analytical path and the chromatogram of a synthetic sample before fractionation, which was prepared to have the same theoretical concentration as that of collected PTH fraction. Comparison of the chromatograms indicates that the target PTH was successfully purified.

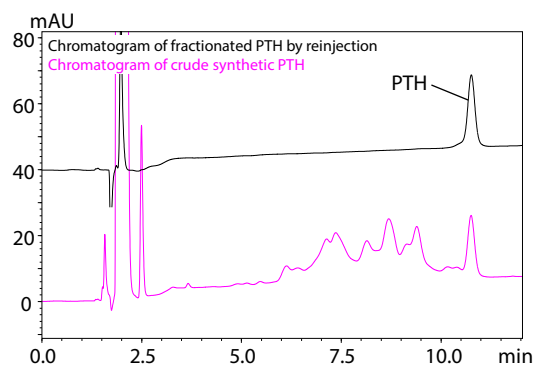


Fig 5 Purity confirmation of fractionated PTH

## ■ Evaluation of purity and recovery using standard PTH

The preparative performance (purity and recovery) of this system setup was evaluated using a standard solution of angiotensin I. Fig. 6 shows the chromatogram obtained when the fractionated angiotensin I was reinjected into the analytical path and the chromatogram of the standard solution prepared to have the same theoretical concentration as that of the collected angiotensin I fraction. The purity and recovery are shown in Table 3. The purity was 100% in terms of area-normalization, and the recovery calculated from the comparison of peak areas was 97.9%, indicating that the reliable fractionation was able to be performed.

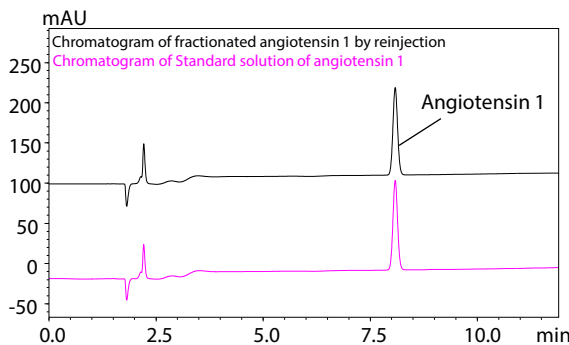


Table 3 Purity and recovery of fractionated angiotensin I

	Purity (area%)	Recovery (%)
Angiotensin I	100.0	97.9

## ■ Conclusion

The seamless preparative purification workflow can be executed using analytical/preparative switching LC-MS system. In addition, LabSolutions MD can automatically create an analytical batch schedule in which various HPLC parameter settings are examined, resulting in efficient optimization of separation conditions.

Furthermore, LCMS-2050 enables highly selective fractionation of target compounds based on MS trigger. The analytical/preparative switching LC-MS used in this article has both analytical and preparative flow paths, enabling an efficient preparative purification workflow in the preparative purification of peptide including its synthesis confirmation process.

### <Related Applications>

1. Seamless Purification Workflow from Analytical to Preparative in Single LC-MS System, [01-00650-EN](#)
2. High Purity Preparative Purification Enabled by UV/MS Trigger on LC-MS System, [01-00651-EN](#)

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## Related Products

Some products may be updated to newer models.



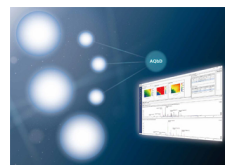
### > Nexera Prep

Preparative Purification Liquid Chromatograph



### > LCMS-2050

High-Performance Liquid Chromatograph Mass Spectro...



### > Method

### Development System

Automatic Optimization of Gradient Conditions with...



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