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Application News

Preparative Purification Liquid Chromatograph
Software for Efficient Method Development
Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer

Investigation of Separation Conditions and Prep Purification of GLP-1 Receptor Agonist Semaglutide

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User Benefits

- AI algorithm equipped in LabSolutions™ MD automatically optimizes gradient profile and automatically searches for conditions that meet the specified separation criteria for desired peaks (such as the main component and related impurities).
- Seamless method transfer from analytical scale to semi-prep scale can be performed.
- Purity of collected fraction can be simply performed using MALDI.

■ Introduction

In typical HPLC method development, "preparation" steps such as setting up mobile phase and column and creating analytical batch are executed before starting "analysis." Subsequently, the obtained data undergoes "data processing" followed by repeating "preparation" for the next analysis and another "analysis" was started. This repeated process drives method development. HPLC analysts are required not only huge time to repeatedly create analytical batches but also expertise in chromatography during optimization of analytical conditions based on the results of he obtained data. In other words, conventional method development requires human intervention. Consequently, labor savings through the automation of the entire method development processes are highly anticipated. In this article, the gradient profile that meet separation criteria for semaglutide, a GLP-1 receptor agonist, and its impurities were automatically searched using the analytical method development support software LabSolutions MD (Technical Report C190-E309). Subsequently, the analytical conditions were scaled up to semi-prep scale and fractionation was performed. Furthermore, the purity of the collected samples was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-8030).

■ GLP-1 receptor agonist

In recent years, GLP-1 receptor agonists (GLP-1 agonists), a drug for type 2 diabetes, have been paid significant attention. These drugs are used not only for diabetes treatment but also as anti-obesity medications.

The human body contains two types of peptide hormones that control blood glucose levels: GLP-1 (31 amino acids) and GIP (39 amino acids). These hormones promote insulin secretion from the pancreas to suppress blood glucose level by activating their respective receptors. However, these hormones are prone to enzymatic degradation in human body. Therefore, some amino acid in the sequence is chemically modified when used as pharmaceuticals. Such pharmaceuticals are called "GLP-1 analogs" because they are modified analogues of GLP-1 with modified amino acids. Furthermore, GLP-1 analogs exhibit similar effects to GLP-1 in human body after administration and possess the efficacy to activate GLP-1 receptors, hence they are also called "GLP-1 receptor agonists."

In this article, a mixture containing semaglutide, (Fig. 1) one of "GLP-1 analogs," was used as a simulated sample to investigate LC separation conditions and perform prep purification.

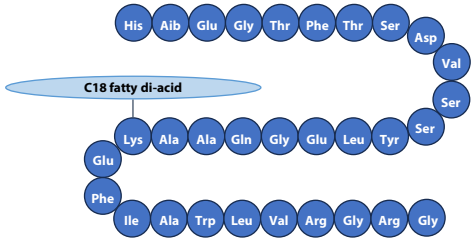

Fig. 1 Chemical structure of semaglutide

■ Optimization of gradient profile

Fig. 2 shows the workflow of automatic optimization for gradient profile using LabSolutions MD, which contains original AI algorithm searching for gradient profile to meet specified criteria. It reaches optimal profile by alternately repeating "gradient profile improvement by AI (condition search)" and "analysis under improved conditions (correction analysis)". "Resolution for desired peak" and "Elution time of the final peak" can be set as criteria. Since LabSolutions MD can calculate molecular weight through mass spectrum deconvolution, peaks can be easily identified simply by inputting the molecular weight of the target compound for optimization (Fig. 3). In this investigation, semaglutide (Average mass: 4113) was designated as the target peak to be separated from nearby-eluted analogues with at least resolution 2.0.

Furthermore, the gradient profile employed during automatic optimization can be specified. When an ordinary linear gradient profile fails to achieve the resolution criterion of 2.0, appropriate separation conditions can be found by trying multi-linear gradient (multi-step gradients) or stepwise gradient. Consequently, advanced separation can be achieved by specific gradient profile, which is difficult to be created with conventional manual analysis methods, facilitating.

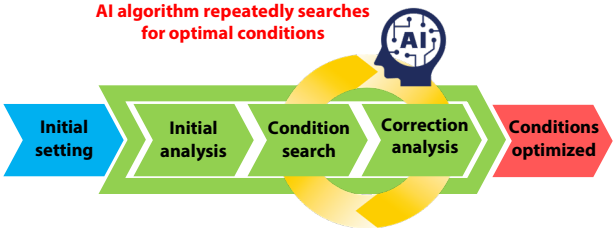

Fig. 2 Workflow of gradient profile optimization using LabSolutions MD

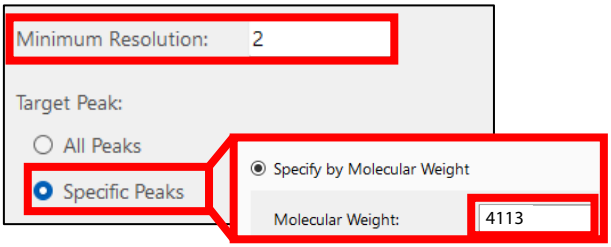

Fig. 3 Criteria settings for automatic optimization

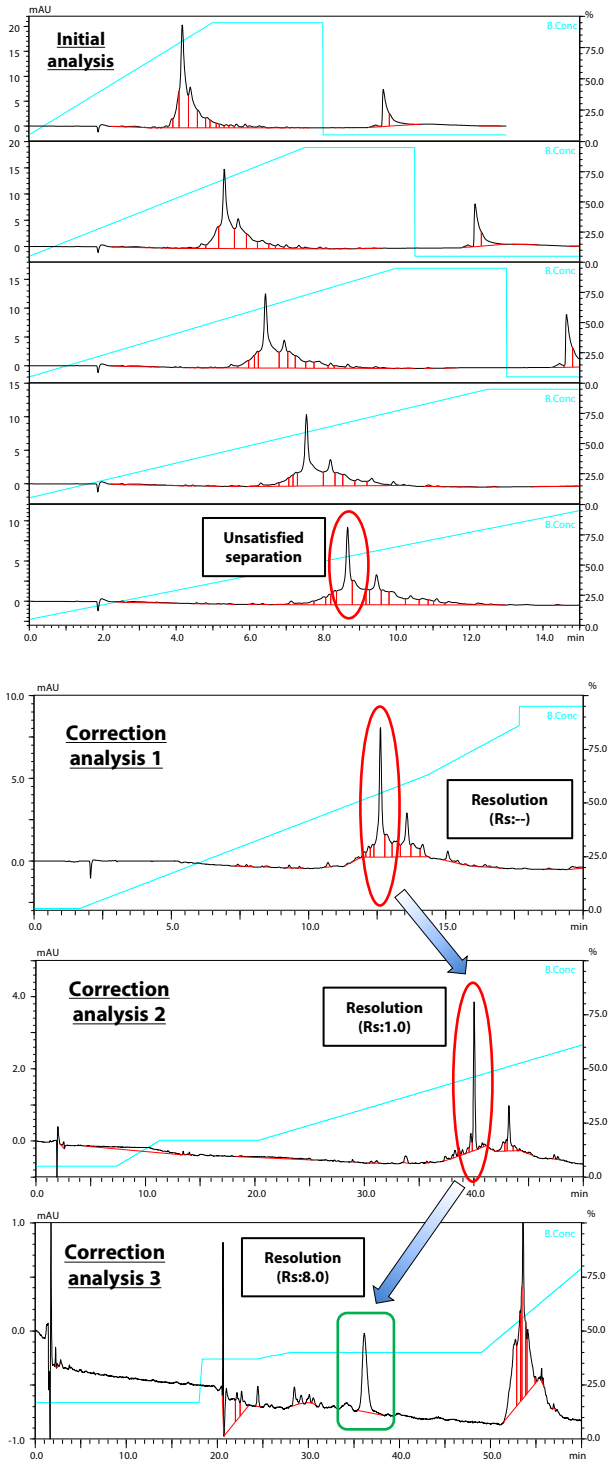

Fig. 4 Result of automatic optimization of gradient profile
(Blue line in each chromatogram shows employed gradient profile)

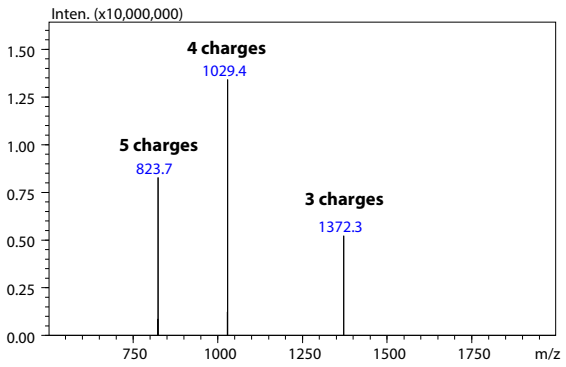

Fig. 5 Mass spectrum of semaglutide

Table 1 Analytical conditions

Instrument	: Nexera XR
Mobile Phase	: Pump A : 0.1% formic acid in water
	: Pump B : 0.1% formic acid in acetonitrile
Column	: Shim-pack Scepter™ C18-120
	(150 mm × 4.6 mm I.D., 5 µm)
Sample	: Unpurified Semaglutide
Sample Concentration	: 1 mg/mL
Injection Volume	: 5 µL
LC conditions	
Time Program	: 5% (0 min) → 95% (X*1 ~X+3 min)
	→ 5% (X+3~X+8 min)
Column Temp.	: 25 °C
Flow rate	: 1.0 mL/min
Detection (PDA)	: 280 nm (SPD-M40, conventional cell)
Criteria of automatic optimization of gradient conditions	
Resolution	: >2.0 (Semaglutide)
MS conditions	
Ionization	: ESI/APCI (DUIS™), positive
Mode	: SCAN (*m/z* 200-2000)
Nebulizing Gas Flow	: 2.0 L/min (N_2)
Drying Gas Flow	: 5.0 L/min (N_2)
Heating Gas Flow	: 7.0 L/min (N_2)
DL Temp.	: 200 °C
Desolvation Temp.	: 450 °C
Interface Voltage	: 1.0 kV

*1 X = 5, 7.5, 10, 12.5, 15 (5 patterns)

Fig. 4 shows the results of the automatic searching for gradient profile, and Fig. 5 shows the mass spectrum of semaglutide. For semaglutide, multivalent ions with charges from three to five were detected, and the molecular weight (4113) was calculated via deconvolution. As a result, semaglutide was recognized as the target peak for separation optimization, and gradient profile that met the criteria was automatically searched.

In the initial analysis results, the separation of semaglutide and impurities was insufficient across five gradient profiles (Table 1) (red circles in Fig. 4). Subsequently, after three times repeated correction analyses provided by AI algorithm, the gradient profile that met the criteria was finally automatically found (correction analysis 3). Corrective analysis 3 created a multi-step gradient with an isocratic interval, achieving the separation criterion (resolution: 2.0) (green box).

Using the LCMS-2050 enables peak tracking based on molecular weight information. This allowed more precise separation studies owning to reliable tracking of the target peak for optimization even when multiple peaks were detected on the UV chromatogram.

■ Performing prep purification

The flow rate was scaled up to 20 mL/min (with linear velocity kept constant before and after scale-up) based on the cross-sectional area ratio (approximately 20-fold) between the preparative column (inner diameter 20 mm) and the analytical column (inner diameter 4.6 mm). The UV chromatogram obtained after prep scale-up is shown in Fig. 6 (the blue interval indicates the fractionation area).

In scaling up process from analytical LC to preparative LC, there are concerns on complicated effort involved in LC-parameter calculations and transcription errors from calculated results into method file. To address these challenges, LabSolutions MD can automatically calculate various LC parameters for scaling up and create a method file reflecting these values (steps (1) to (4) in Fig. 7).

Simply select the target system (Fig. 7 (1)), input the column size (Fig. 7 (2)), and flow rate (Fig. 7 (3)), and the prep method file is automatically.

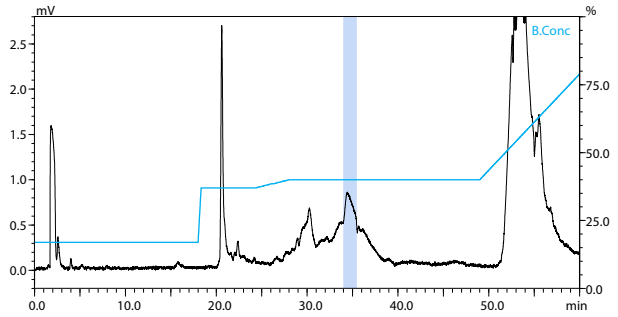

Fig. 6 Chromatogram for prep fractionation of semaglutide
Blue slice shows fractionation interval

Table 2 Analytical conditions

Instrument	: Nexera Prep
Column	: Shim-pack Scepter C18-120 (150 mm × 20 mm I.D., 5 µm)
Sample Concentration	: 5 mg/mL
Injection Volume	: 1 mL
LC conditions	
Flow rate (Prep)	: 20 mL/min
Flow rate (Makeup)	: 1.5 mL/min (Methanol)
Detection (UV)	: 280 nm (SPD-40, preparative cell)
MS conditions	
Desolvation Temp.	: 100 °C

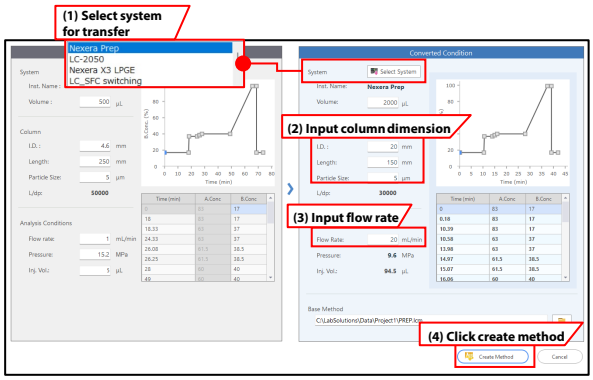

Fig. 7 Creation of prep method file using LabSolutions MD

■ Purity confirmation for collected fraction using MALDI

The purity of the collected fraction was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-8030). MALDI enables mass spectrometry with simple pretreatment. Required measurement time per sample is only a few seconds, providing rapid confirmation of the purities of multiple fractions (Fig. 8).

Fig. 8 Benchtop MALDI-TOF MS MALDI-8030

Table 3 shows the analytical conditions for MALDI-MS, and Fig. 9 and Fig. 10 show the mass spectra of unpurified semaglutide and the collected fraction. In unpurified semaglutide analysis, ionization was suppressed due to the presence of co-eluting impurities, resulting in unreliable data acquisition. Although peaks were detected by increasing the laser power, but they showed different m/z values from $[M+H]^+$ of semaglutide (Fig. 9). In contrast, the mass spectrum of the fraction after collection showed no suppression of ionization, yielding a good mass spectrum of semaglutide $[M+H]^+$ (Fig. 10). Furthermore, no peaks originated from impurities were detected, confirming that semaglutide was fractionated at a high degree of purity.

Table 3 Measuring conditions of MALDI-MS

Instrument	: MALDI-8030 Benchtop MALDI-TOF MS system
Matrix	: 5 mg/mL α-Cyano-4-hydroxycinnamic acid (CHCA) 1% Methylenediphonic acid
Polarity	: Positive-ion linear mode
Pulsed extraction	: *m/z* 4100

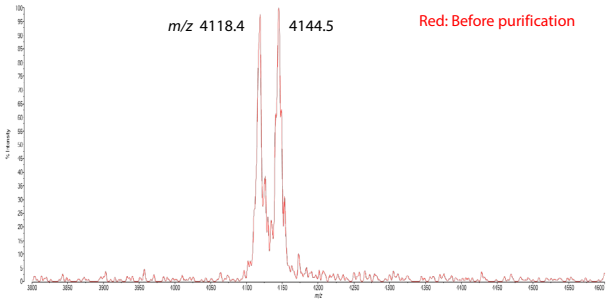

Fig. 9 Mass spectrum of unpurified sample

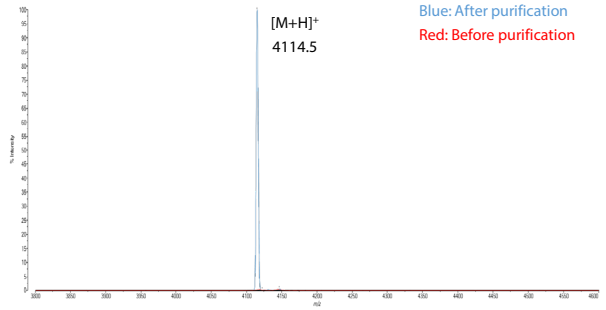

Fig. 10 Mass spectrum of fractionated sample

■ Conclusion

This article introduced a comprehensive automated prep purification workflow targeting semaglutide, GLP-1 receptor agonist.

Using LabSolutions MD enables automatic searching for gradient profile in which semaglutide peak meets resolution criteria.

Furthermore, the method-transfer function enables straightforward scale-up from analytical scale to preparative scale.

Additionally, MALDI streamlined confirming the purities of collected fractions by rapid mass spectrometry and simple operation.

These features enable rapid method development, which improves operational efficiency in pharmaceutical development and manufacturing.

01-01079-EN First Edition: Nov. 2025

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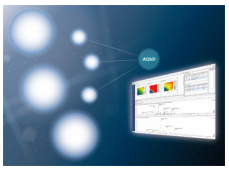

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