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Application News

Software for Efficient Method Development "LabSolutions™ MD"

Automatic Optimization of Gradient Conditions by AI Algorithm on Synthetic Peptide and Impurities

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User Benefits

- The AI algorithm of LabSolutions MD can automatically optimize gradient conditions to greatly reduce labor of LC method development.
- Anyone can optimize gradient conditions, regardless of their experience in chromatography.
- Gradient conditions that meet the resolution criteria for specified peaks are automatically searched (e.g., principal component and related impurities).

■ Introduction

In the typical LC method development, the process begins with "preparation" which includes mobile phase preparation, column installation, and creation of analysis schedules, then the analysis is started. After that, the acquired data is analyzed and "preparation" for the subsequent analysis is carried out, followed by starting the next analysis again. The method development progresses by repeating these processes, but in addition to the significant time required to repeatedly create analysis schedules, expertise in chromatography is necessary to explore optimal conditions based on data analysis. In other words, typical method development requires "human intervention". Therefore, eliminating human involvement and automating such method development processes would be desirable to improve labor efficiency. This article introduces an example of automatic optimization of gradient conditions to meet resolution criteria for synthetic peptide and related impurities using LabSolutions MD (Technical Report C190-E309), a dedicated software for supporting method development.

■ Target Compounds and Analytical Conditions

A target peptide and three related impurities with different sequences were used as a model sample of a synthetic peptide (Table 1). Full length peptide (FLP : beta-Melanotropin), deletion sequences of p.A1_K3del and p.A1_D5del as shorter length of products, and Met(O2) (methionine in FLP is oxidized to methionine sulfone) were prepared as a sample mixture. The analytical conditions are shown in Table 2. In this article, the resolution criteria were set for FLP, and LabSolutions MD automatically explored the gradient conditions to meet the criteria.

Table 1 Target Compounds

Name	Sequence
FLP	AEKKDEGPYRMEHFRWGSPPKD
p.A1_K3del	KDEGPYRMEHFRWGSPPKD
p.A1_D5del	EGPYRMEHFRWGSPPKD
Met(O2)	AEKKDEGPYR{Met(O2)}EHFRWGSPPKD

Note : Met(O2) = methionine sulfone

Table 2 Analytical Conditions

LC (Nexera™ X3)	
Mobile phase	
Pump A : 0.1% TFA (Trifluoroacetic acid) in water	
Pump B : Acetonitrile	
Analytical conditions	
B Conc.	: 5%(0 min)→60%(X*1 ~X+2 min) →5%(X+2~X+7 min)
Column Temp.	: 80 °C
Flow rate	: 0.6 mL/min
Injection Vol.	: 2 µL
Detection	: 220 nm (SPD-M40, STD cell)
Column : Shim-pack Scepter™ C8-120 (100 mm × 3.0 mmI.D., 1.9 µm)*2	
Criteria of automatic optimization of gradient conditions	
Resolution	: > 2.0 (FLP)
MS (LCMS-2050)	
Ionization	: ESI/APCI (DUIS™), positive mode
Mode	: SCAN (*m/z* 300-2000)
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.	: 450 °C
Interface voltage	: +1.0 kV

*1 : X = 3, 4, 5, 6, 7 (5 patterns)

*2 P/N : 227-31034-03 (Shimadzu GLC product number)

■ Automatic Optimization of Gradient Conditions

Fig. 1 shows the workflow of automatic optimization of gradient conditions using LabSolutions MD. This software has a unique AI algorithm to automatically explore gradient conditions that satisfy resolution criteria by alternately repeating "improvement of gradient conditions (condition search)" and "analysis under improved conditions (correction analysis) by AI. For the criteria, "resolution" and "elution time of the last peak" can be set. The peaks to be optimized can be easily specified by simply entering the molecular weight of the target compound, as LabSolutions MD can calculate molecular weight by deconvolution of MS spectrum (Fig. 2). FLP (molecular weight : 2660) was specified as a target peak to optimize the separation from related impurities, with a minimum resolution criteria set at 2.0 (Fig. 2).

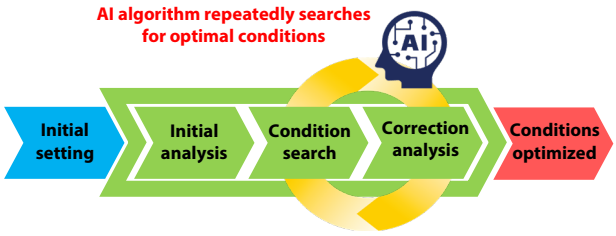

Fig. 1 Workflow for Automatic Optimization of Gradient Conditions by LabSolutions MD

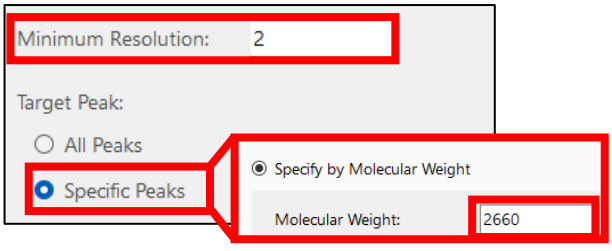

Fig. 2 Criteria Setting for Automatic Optimization

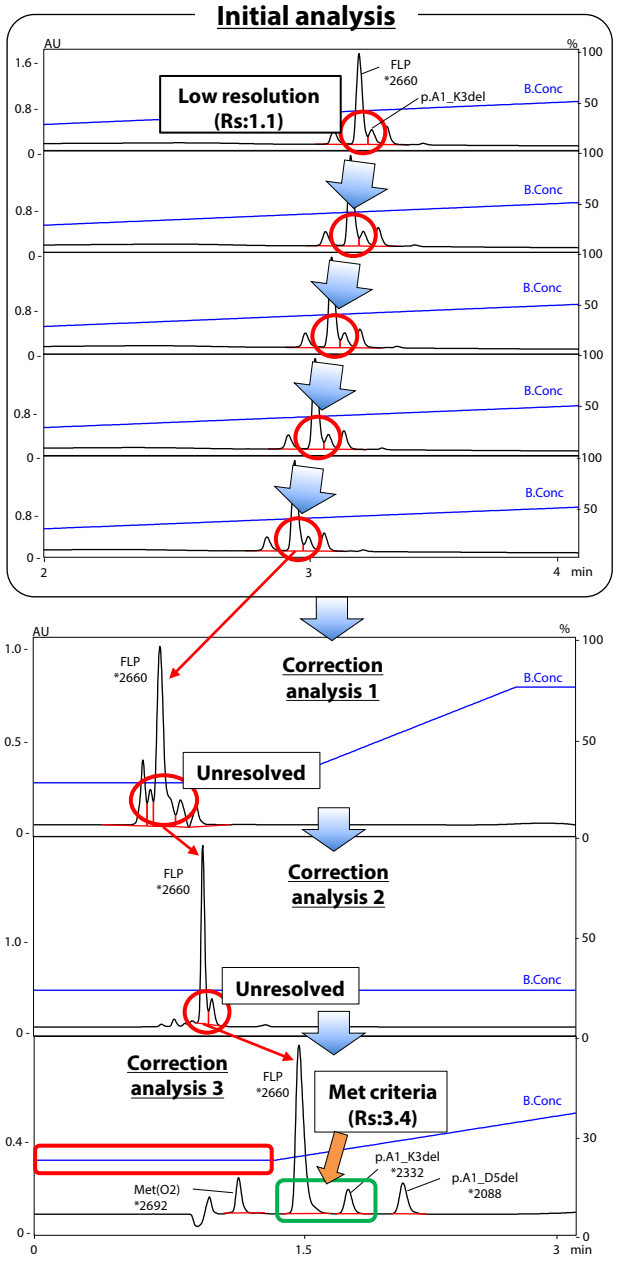

Fig. 3 Result of Automatic Optimization of Gradient Conditions
(blue line shows gradient conditions)
* : molecular weights estimated by deconvolution

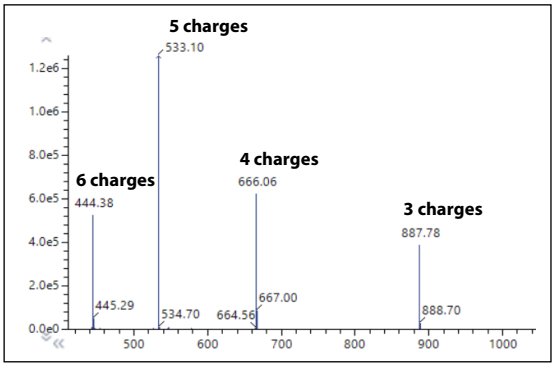

Fig. 4 MS Spectrum of FLP

The results of automatic optimization of gradient conditions and MS spectrum of FLP are shown in Fig. 3 and Fig. 4, respectively. Multiply-charged ions with charges from 3 to 6 were detected for FLP, and the molecular weight (2660) was calculated by deconvolution. In the initial analyses of the five gradient profiles (Table 1), FLP and related impurity (p.A1_K3del) were not sufficiently separated (red circles in Fig. 3 initial analyses). After repeated corrections by AI algorithm (3 times), the gradient conditions that met the criteria were automatically searched (correction analysis 3). In correction analysis 3, the resolution criteria (resolution > 2.0) was achieved (green box) by applying an isocratic elution (red box). By optimizing with LCMS-2050, molecular weight information for each compound can also be obtained, allowing for reliable optimization without missing changes in peak elution order.

■ Conclusion

Automatic optimization of gradient conditions using AI algorithm of LabSolutions MD was applied to synthetic peptide and related impurities. As a result, gradient conditions that met the criteria (resolution for FLP > 2.0) were successfully explored. This result indicates that significant labor saving in method development can be expected by LabSolutions MD. This article introduces an automatic optimization of gradient conditions in method development while LabSolutions MD also supports a series of workflow of method development, including screening of mobile phases and columns. For details, please refer to the application news, "Efficient Method Development for Synthetic Peptide and Related Impurities (01-00780)".

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