

Application News Software for Efficient Method Development "LabSolutions™ MD"

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

Shinichi Fujisaki

## **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 <sup>TM</sup> X3)				
Mobile phase				
Pump A : 0.1% TFA (Trifluoroacetic acid) in water				
Pump B : Acetonitrile				
Analytical conditions				
B Conc.	: 5%(0 min)→60%(X <sup>*1</sup> ~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 $ imes$ 3.0 mml.D., 1.9 $\mu$ m)*2		

Criteria of automatic optimization of gradient conditions			
Resolution	: > 2.0 (FLP)		

MS (LCMS-2050)		
lonization	: ESI/APCI (DUIS™), positive mode	
Mode	: SCAN ( <i>m/z</i> 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	

\*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 Al 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 Al. 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).

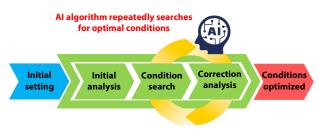
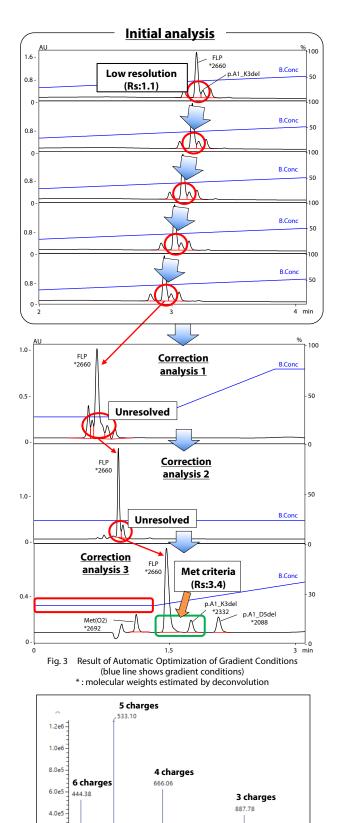


Fig. 1 Workflow for Automatic Optimization of Gradient Conditions by LabSolutions MD

Minimum Resolution:	2		
Target Peak:			
○ All Peaks			
<ul> <li>Specific Peaks</li> </ul>	Specify by Molecular Weight		
	Molecular Weight	: 2	2660

Fig. 2 Criteria Setting for Automatic Optimization



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)".

LabSolutions, Nexera, DUIS and Shim-pack Scepter are trademarks of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.

1000

888.70

900

800



Shimadzu Corporation

www.shimadzu.com/an/

45.29

500

534.70

664.5

Fig. 4 MS Spectrum of FLP

600

2.0e5

0.0e0

01-00814-EN First Edition: Nov. 2024

For Research Use Only. Not for use in diagnostic procedures. This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. See <a href="http://www.shimadzu.com/about/trademarks/index.html">http://www.shimadzu.com/about/trademarks/index.html</a> for details. Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not

they are used with trademark symbol "TM" or "®". Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.