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Automatic Optimization of Gradient Conditions Using AI Algorithm for LC Method Development with Functional Foods

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1. Introduction

In the LC method development, the process begins with "preparation" which includes mobile phase preparation, column installation, and creation of analysis schedules, then 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 poster employs a fifteen-standard mixed solution of catechins, theaflavins, and gallic acid, which are functional components in tea leaves. The AI algorithm equipped with LabSolutions™ MD, a dedicated software for supporting method development, was utilized for the automatic optimization of gradient conditions. Furthermore, the optimized method was applied to several tea leaves and comparisons were made among different tea species.

2. Analytical conditions

Analytical conditions and target compounds are shown in Table 1. Ten catechins, including Epigallocatechin gallate, Epigallocatechin, Epicatechin gallate, and Epicatechin (mainly present in tea leaves), along with four theaflavins and gallic acid (a total of fifteen compounds) were subjected to LC analysis. First, the gradient conditions of a mixed standard solution were automatically optimized by LabSolutions MD. Then, the optimized gradient conditions were applied to the analysis of non-fermented green tea and fermented black tea.

Table 1 Analytical Conditions and Target Compounds

System : Nexera™ X3

Sample : Catechin, Theaflavin and Gallic acid (15 compounds)

C1) Gallocatechin	C8) Epicatechin gallate
C2) Epigallocatechin	C9) Catechin gallate
C3) Catechin	C10) Epicatechin 3-(3''-O-methyl) gallate
C4) Epicatechin	T1) Theaflavin
C5) Epigallocatechin gallate	T2) Theaflavin 3-gallate
C6) Gallocatechin gallate	T3) Theaflavin 3'-gallate
C7) Epigallocatechin 3- (3''-O-methyl)gallate	T4) Theaflavin 3,3'-digallate
	G1) Gallic acid

Mobile phase A	: 0.2% phosphoric acid in water
Mobile phase B	: Acetonitrile
B Conc.	: 15%(0 min)→45%(X*1 min)→15%(X~X+5 min) *1 : X = 6, 8, 10, 12, 14 (5 patterns)
Column Temp.	: 55 °C
Flow rate	: 0.6 mL/min
Injection Vol.	: 5.0 µL
Detection	: 242 nm (SPD-M40, UHPLC cell)

Column : Shim-pack™ GISS C18 (100 mm × 3.0 mmI.D., 1.9 µm)*2
*2 P/N : 227-30049-02 (Shimadzu GLC product number)

Parameters for automatic optimization of gradient conditions
Criteria of minimum resolution : 1.5
Gradient mode for optimization : Linear

3. Software

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 profile that meets resolution criteria by alternately repeating "improvement of gradient prifile (condition search)" and "analysis under improved conditions (correction analysis).

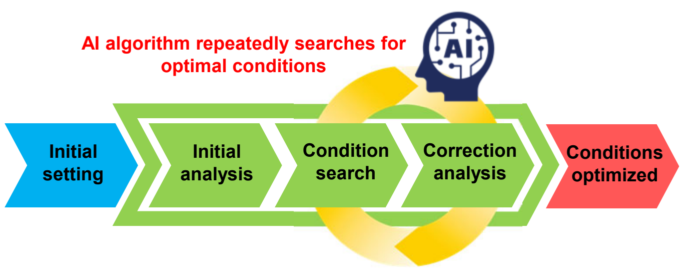

Fig. 1 Workflow of Automatic Optimization of Gradient Conditions by LabSolutions MD

4. Results and discussion

For a mixture of catechins, theaflavins, and gallic acid (a total of fifteen compounds), gradient conditions were automatically searched with a minimum resolution criteria of 1.5 (Fig. 2). The result of initial analysis shows that the resolution between peaks C4/C5, and between peaks T3/T4 were not sufficient (shown in a red circle at the top of Fig. 2).

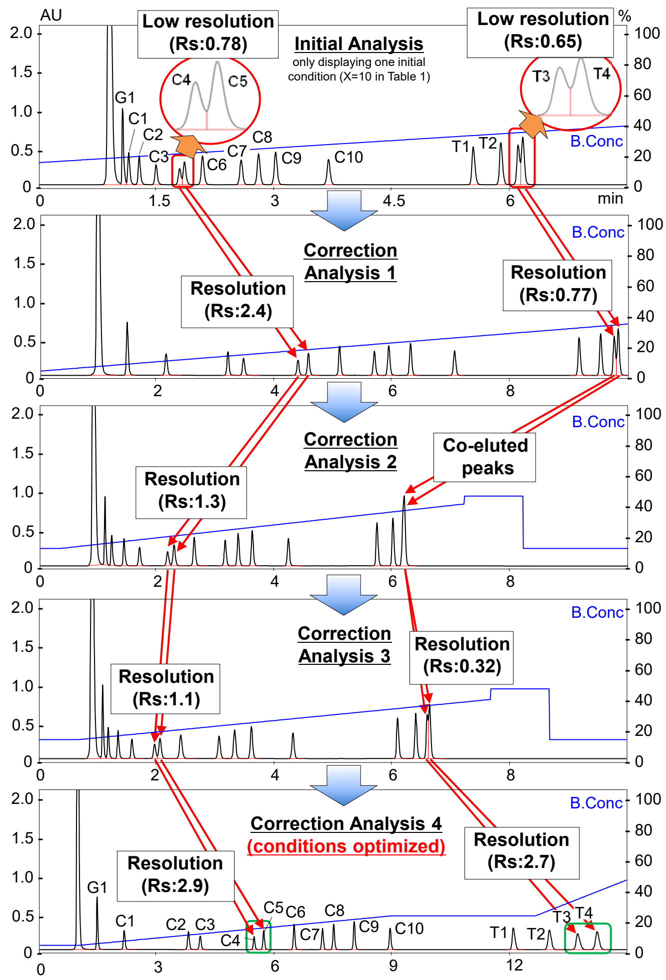

Fig. 2 Automatic Optimization of Gradient Conditions (blue lines show gradient curves)

However, by using AI algorithm to repeatedly perform correction analyses, the gradient profile that met the criteria (minimum resolution of 1.5) was finally discovered (shown in a green box at the bottom of Fig. 2). In this case, T3 and T4 were successfully separated by applying an isocratic elution after 9 minutes. Then, the optimized method was applied to the quantitative analysis of tea-leaves. The chromatograms of representative tealeaves are shown in Fig. 3. Green tea D contained more catechins, including the four major catechins, than those in black tea A. Notably, in green tea D, two methylated catechins were detected, which have garnered attention for their anti-allergic effects and ability to reduce hay fever. On the other hand, the four types of theaflavins were detected in black tea A. Although both green tea D and black tea A were "Benifuki" species, the comparison between the two suggests that catechins were converted to theaflavins during fermentation.

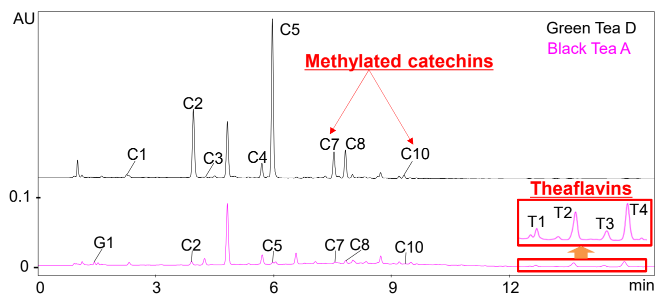

Fig. 3 Chromatograms of typical tealeaves under optimized conditions

5. Conclusion

Using a model sample of a mixture of fifteen standards solution of catechins, theaflavins, and gallic acid, the AI algorithm of LabSolutions MD was employed for automatic optimization of gradient profile. As a result, the gradient profile that met the criteria (minimum resolution of 1.5) were automatically searched, which provided significant labor savings. Furthermore, the optimized method was applied to tealeaf analysis to compare the quantity of functional components among different tea species. This method is expected to facilitate various scientific discussions on catechins and theaflavins.