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Application News

Software for Efficient Method Development "LabSolutions™ MD"

Automatic Optimization of Gradient Conditions by AI Algorithm

- Application to LC Method Development for Simultaneous Analysis of Functional Components in Foods -

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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.
- Comparison and evaluation of functional components, such as catechins and theaflavins, in tea leaves can be performed among different tea species.

■ 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 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 employs a fifteen-standard mixed solution of catechins, theaflavins, and gallic acid, which are functional components in tea leaves. The AI algorithm (See Technical Report C190-E309) 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.

■ Analytical Conditions and Target Compounds

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 (ascorbic acid and EDTA-2Na were added as antioxidants at 1.76 g/L and 1.00 g/L, respectively) were automatically optimized by LabSolutions MD. Then, the optimized gradient conditions were applied to the analyses of four species of non-fermented green tea and two species of fermented black tea (pretreatment procedure: Fig. 1).

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	
C5) Epigallocatechin gallate	T1) Theaflavin
C6) Gallocatechin gallate	T2) Theaflavin 3-gallate
C7) Epigallocatechin 3-(3''-O-methyl)gallate	T3) Theaflavin 3'-gallate
	T4) Theaflavin 3,3'-digallate
	G1) Gallic acid

Mobile phase:
Pump A : 0.2% phosphoric acid in water
Pump B : Acetonitrile

Column : Shim-pack™ GISS C18 (100 mm × 3.0 mmI.D., 1.9 µm)*1
*1 P/N: 227-30049-02

Analytical conditions	:
B Conc.	: 15%(0 min)→45%(X*2 min) →15%(X~X+5 min)
	*2 : X = 6, 8, 10, 12, 14 (5 patterns)
Column Temp.	: 55 °C
Flow rate	: 0.6 mL/min
Injection Vol.	: 5.0 µL
Detection	: 242/272 nm (SPD-M40, UHPLC cell)
Parameters for automatic optimization of gradient conditions :	
Criteria of minimum resolution	: 1.5
Gradient mode for optimization	: Linear

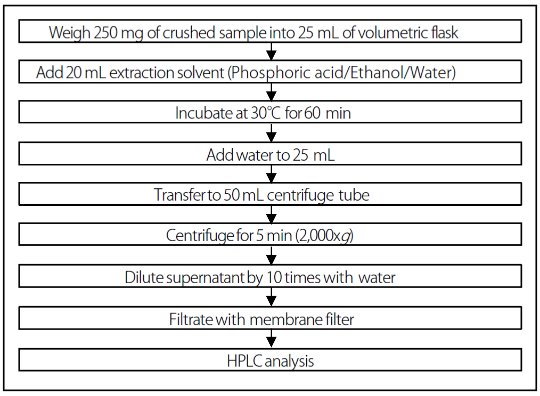

Fig. 1 Pretreatment Procedures for Tea Leaf

■ Automatic Optimization of Gradient Conditions

Fig. 2 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 by AI (condition search)" and "analysis under improved conditions (correction analysis). For a mixture of catechins, theaflavins, and gallic acid (a total of fifteen compounds), gradient conditions were automatically searched with a minimum separation criterion of 1.5 (Fig. 3).

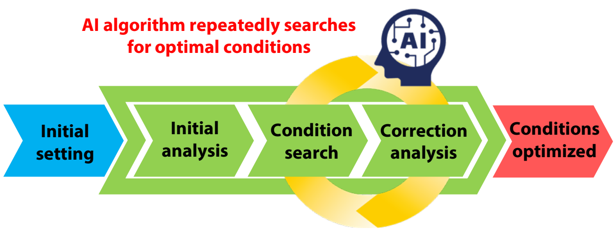

Fig. 2 Workflow for Automatic Optimization of Gradient Conditions by LabSolutions MD

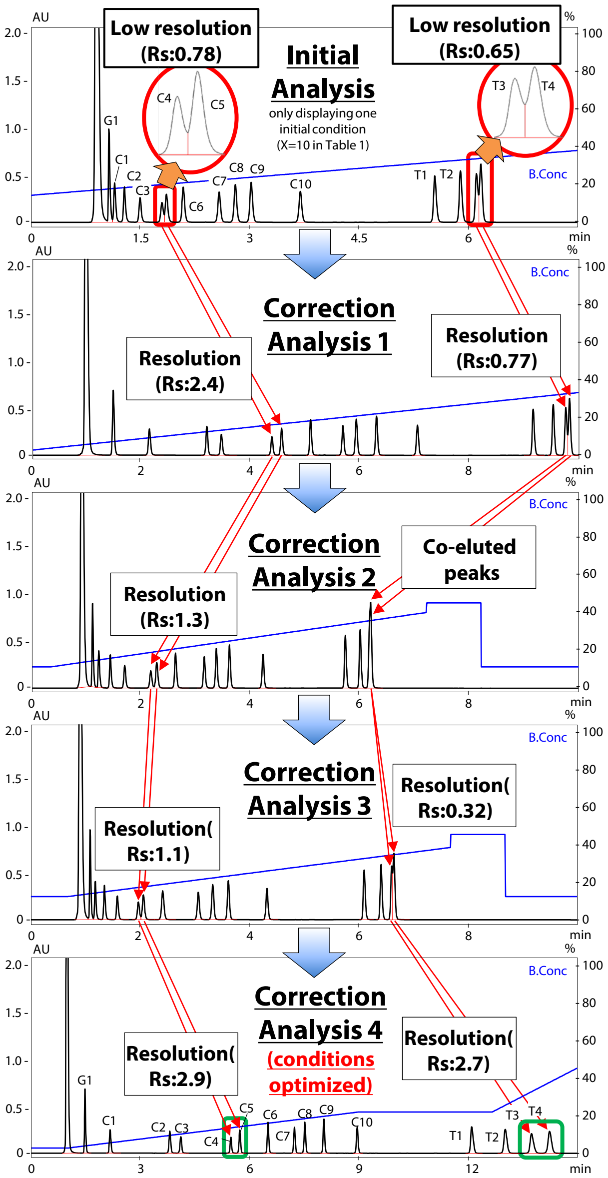

Fig. 3 Automatic Optimization of Gradient Conditions
(blue lines show gradient curves)

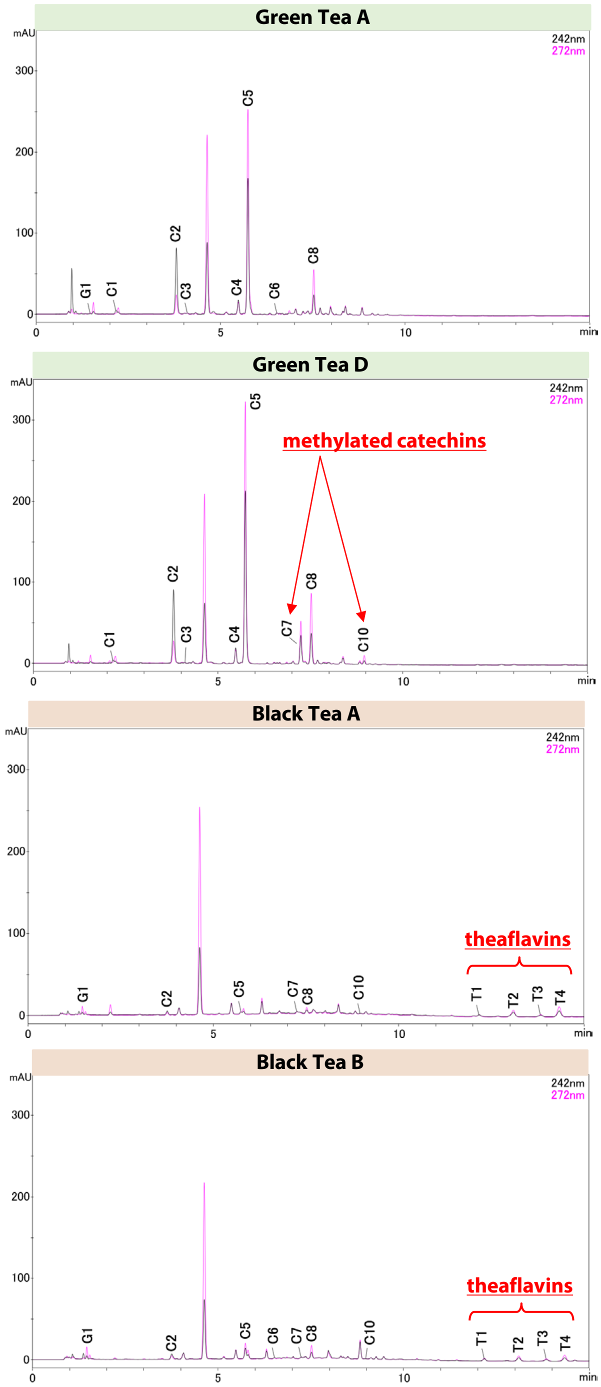

Fig. 4 Chromatograms of Representative Tea Leaf Extracts

The result of initial analysis shows that the resolution between peaks C4/C5, and between peaks T3/T4 was not sufficient (shown in a red box at the top of Fig. 3). However, by using AI algorithm to repeatedly perform correction analyses, the gradient condition that satisfies the criterion (minimum resolution of 1.5) was finally discovered (shown in a green box at the bottom of Fig. 3). In this case, T3 and T4 were successfully separated by applying an isocratic elution after 9 minutes.

■ Application to Tea Leaves

The optimized method was applied to the quantitative analysis of extracts from six different species of tea leaves. The chromatograms of representative tea leaf extracts are shown in Fig. 4 and the graphs comparing the quantity of fifteen compounds are shown in Fig. 5. In addition, Table 2 lists the ranges of calibration curve, coefficients of determination, quantity (green tea A and black tea A), and repeatability (green tea A and black tea A) for the targeted fifteen compounds. Green teas A to D contained more catechins, including the four major catechins, than those in black tea, with the highest concentration of the functional component in all four green teas being Epigallocatechin gallate, known for its potential to inhibit elevated blood glucose. 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, four types of theaflavins were detected in black teas A and B. 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.

■ Conclusion

Using a model sample of a mixture of fifteen standards solution of catechins, theaflavins, and gallic acid, all of which are functional components, the AI algorithm of LabSolutions MD was employed for automatic optimization of gradient conditions. As a result, the gradient conditions that satisfied the criteria (minimum resolution of 1.5) were automatically searched, resulting in significant labor savings. Furthermore, the optimized method was applied to tea leaf 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.

<Acknowledgment>

The tea leaves used in this study were provided by the National Agriculture and Food Research Organization. We also thank Dr. Mari Yamamoto of the National Agriculture and Food Research Organization for her invaluable advice. We would like to express our sincere gratitude.

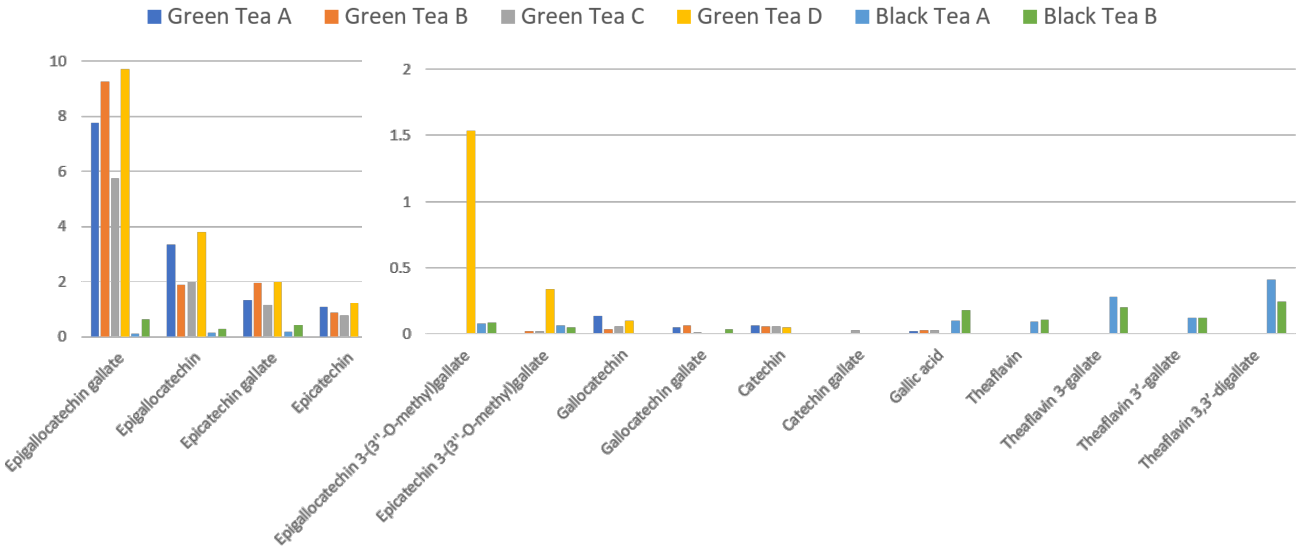

Fig. 5 Catechins and Theaflavins in Tea Leaves (g/100 g)

Table 2 Calibration Range, Coefficient of Determination, Quantity, and Repeatability (%RSD)

				Quantity (g/100 g)		%RSD (n=6)	
Compound		Calib. Range (mg/L)	Coefficient of Determination(r^2)	Green Tea A	Black Tea A	Green Tea A	Black Tea A
C5	Epigallocatechin gallate	1~100	>0.9999	7.74	N.D.*	0.09	-
C2	Epigallocatechin	1~100	>0.9998	3.35	0.16	0.73	0.26
C8	Epicatechin gallate	1~100	>0.9999	1.32	0.18	0.14	0.60
C4	Epicatechin	1~100	>0.9999	1.09	N.D.	0.31	-
C7	Epigallocatechin 3-(3''-O-methyl)gallate	1~100	>0.9999	N.D.	N.D.*	-	-
C10	Epicatechin 3-(3''-O-methyl)gallate	1~100	>0.9999	N.D.	N.D.*	-	-
C1	Gallocatechin	1~100	>0.9998	0.14	N.D.	0.72	-
C6	Gallocatechin gallate	1~100	>0.9999	N.D.*	N.D.	-	-
C3	Catechin	1~100	>0.9999	N.D.*	N.D.	-	-
C9	Catechin gallate	1~100	>0.9999	N.D.	N.D.	-	-
G1	Gallic acid	1~100	>0.9999	N.D.*	0.10	-	1.17
T1	Theaflavin	1~100	>0.9999	N.D.	0.10	-	0.57
T2	Theaflavin 3-gallate	1~100	>0.9999	N.D.	0.28	-	0.57
T3	Theaflavin 3'-gallate	1~100	>0.9999	N.D.	0.12	-	1.53
T4	Theaflavin 3,3'-digallate	1~100	>0.9999	N.D.	0.41	-	0.86

* Less than 0.1 g/100 g

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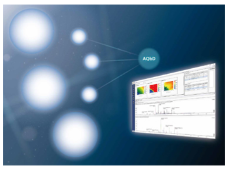

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