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Application News

LCMS™-8050 Liquid Chromatograph Mass Spectrometer

Simultaneous Analysis of Catechins and Theaflavins in Tea Using Triple Quadrupole LC/MS/MS

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User Benefits

- Eight catechins and four theaflavins in tea can be analyzed simultaneously.
- Complicated sample preparation is not necessary, as preparation requires only filtration with a membrane filter followed by dilution.
- Multivariate analysis can be performed easily with Traverse MS™ software.

■ Introduction

Tea, which can be purchased easily and contains a large amount of polyphenol, has attracted attention as a functional food with expected effects such as anticancer activity, prevention of dementia, and antiviral action. Catechins are compounds which are particularly contained in green tea leaves and include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). By heating treatment, these compounds transform into catechin (C), gallocatechin (GC), catechin gallate (CG), and gallocatechin gallate (GCG). In the fermentation process, catechins also react with enzymes contained in tea leaves to form four types of theaflavins, depending on combination of catechins. These are theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B), and theaflavin-3,3'-digallate (TF3). This article introduces an example of a simultaneous analysis of 8 catechins and 4 theaflavins in several types of commercially-available PET-bottled tea beverages using a triple quadrupole mass spectrometer LCMS-8050 with Shim-pack™ XR-ODS III column using a simple sample pretreatment procedure. In addition, the results of green teas were conducted multivariate analysis.

■ Simultaneous Analysis of 8 Catechins and 4 Theaflavins

Table 1 shows the analytical conditions, and Table 2 shows the MRM transitions of the compounds. Fig. 1 shows the chromatogram obtained by a simultaneous analysis of a standard mixture solution of the 12 compounds, and Fig. 2 shows the structural formulas of the catechins and theaflavins. In terms of catechins, the various epimers were eluted continuously and satisfactory separation was achieved. For the theaflavins, TF2A and TF2B which are structural isomers having the same mass can also be separated, and good peak shapes were obtained.

Table 1 Analytical Conditions

[HPLC conditions] (Nexera™ X2)	
Column	: Shim-pack XR-ODSIII (150 mm L. × 2.0 mm I.D., 2.2 µm)
Mobile phase	: A: Water containing 0.1 % formic acid/ Tetrahydrofuran=95/5 (v/v) B: Acetonitrile
Time program	: B.CONC. 3 % (0 min) → 35 % (15-17 min) → 3 % (17.01-20 min) Using the front cut valve, introduced into the MS only from 0.7 to 16 min.
Flow rate	: 0.25 mL/min
Injection volume	: 10 µL
Column temp.	: 50 ˚C
[MS conditions] (LCMS-8050)	
Ionization	: ESI (Positive mode)
Mode	: MRM
Nebulizing gas flow	: 2.5 L/min
Drying gas flow	: 10 L/min
Heating gas flow	: 10 L/min
DL temp.	: 250 ˚C
Block heater temp.	: 400 ˚C
Interface temp.	: 300 ˚C
Probe position	: +1 mm

Table 2 MRM Conditions

Compound	MRM Transition(*m/z*)	Collision energy(V)
GC	307.05>139.00	-13.0
	307.05>151.00	-10.0
	307.05>163.00	-21.0
EGC	307.05>139.00	-16.0
	307.05>151.00	-10.0
	307.05>163.05	-22.0
C	291.10>139.00	-14.0
	291.10>123.00	-15.0
	291.10>161.00	-18.0
EC	291.10>139.05	-14.0
	291.10>123.00	-14.0
	291.10>165.00	-12.0
EGCG	459.10>139.10	-21.0
	459.10>289.05	-10.0
	459.10>151.05	-14.0
GCG	459.10>139.10	-25.0
	459.10>289.05	-11.0
	459.10>151.10	-13.0
ECG	443.05>139.10	-25.0
	443.05>123.05	-19.0
	443.05>273.10	-9.0
CG	443.05>139.10	-27.0
	443.05>123.05	-23.0
	443.05>273.00	-10.0
TF1	565.10>139.05	-37.0
	565.10>427.00	-17.0
	565.10>276.95	-10.0
TF2A	717.10>139.15	-37.0
	717.10>277.05	-17.0
	717.10>150.95	-43.0
TF2B	717.10>579.05	-17.0
	717.10>139.10	-40.0
	717.10>276.95	-14.0
TF3	869.10>138.95	-48.0
	869.10>276.95	-17.0
	869.10>333.25	-18.0

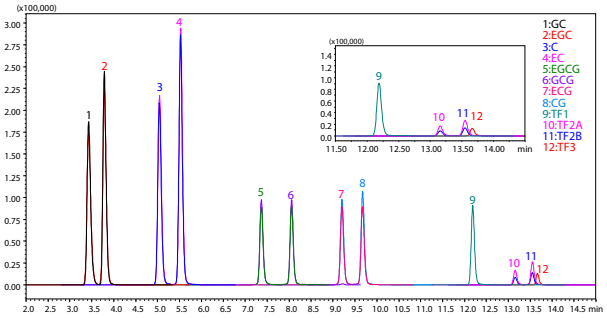

Fig. 1 MRM Chromatogram of Standard Mixture Solution of 12 Compounds

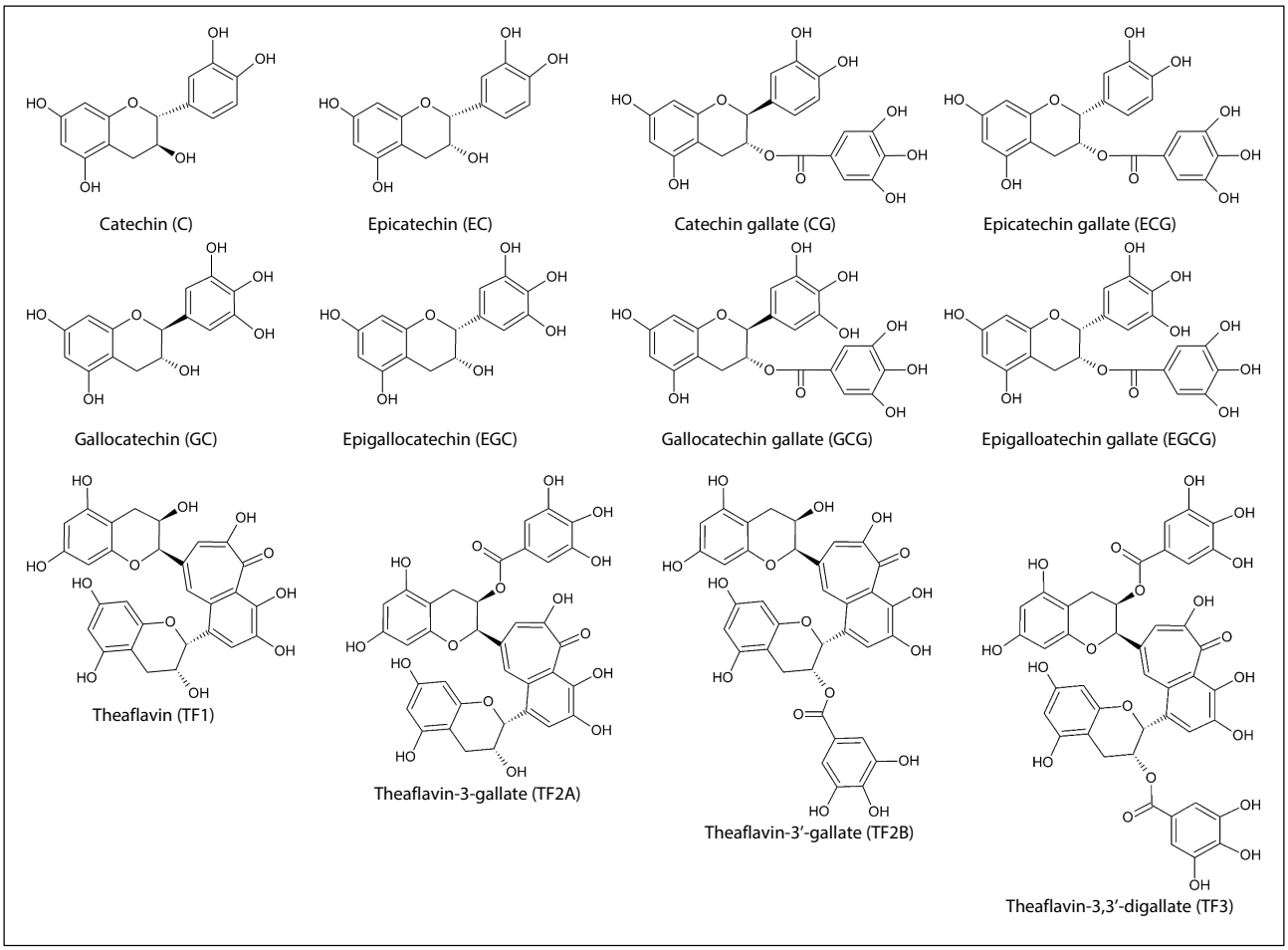

Fig. 2 Structural Formulas of 8 Catechins and 4 Theaflavins

■ Qualitative Analysis of Various Types of Tea

Nine types of teas including black tea, oolong tea and green tea were filtrated through a 0.22 µm hydrophilic of cellulose-ester membrane filter and then were diluted 200 times for the catechin analysis and 10 times for the theaflavin analysis. TORAST™-H Glass Vial (Shimadzu GLC Ltd.) was utilized for the analysis.

Fig. 3 to 5 show the MRM chromatograms of the 10x diluted black tea, oolong tea and green tea (represented by green tea K), respectively. Although peaks of theaflavins were not detected in the green teas, peaks were confirmed for the black tea and oolong tea. It is suggested that the judge whether a tea is a green tea or other teas can be determined by the presence of peaks for theaflavins.

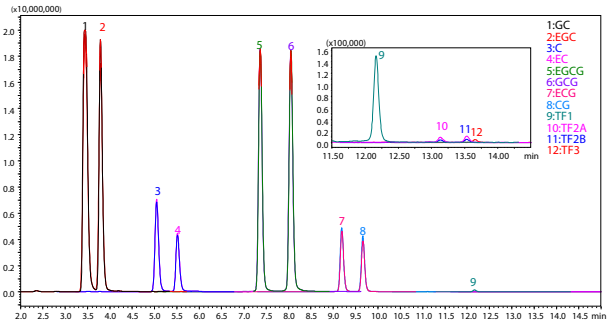

Fig. 4 MRM Chromatogram of Oolong Tea (Diluted 10x)

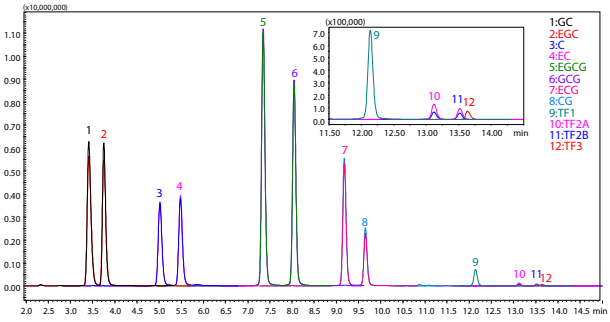

Fig. 3 MRM Chromatogram of Black Tea (Diluted 10x)

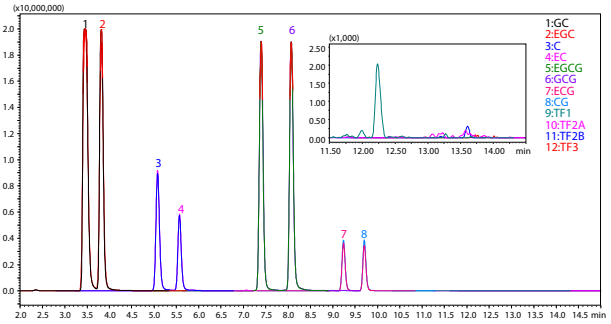

Fig. 5 MRM Chromatogram of Green Tea K (Diluted 10x)

■ Quantitative Analysis of Various Types of Tea

Table 3 shows the results of a quantitative analysis for 11 teas by a standard addition method. The teas were diluted 200 times for the catechin analysis and 10 times for the theaflavin analysis. Fig. 6 shows the analysis workflow, and Fig.7 and Table 4 show the calibration curves and coefficient of determination (r^2) for each compound in the black tea as a representative example.

It is known that commercial tea beverages generally contain non-epimer gallate type catechins (GCG and CG). These are considered to transform from EGCG and ECG by epimerization during heat treatment in the manufacturing process. In this experiment, it was established that all 11 teas contained non-epimer catechins. As in the qualitative analysis results, the content of the theaflavins in the green teas was negligible, but all four theaflavins were detected in the black tea and the oolong tea. Theaflavins are formed from two types of catechins which get oxidized and polymerized by the polyphenol oxidase in tea leaves. Their content increases as fermentation proceeds. Likewise, these results also showed that the black tea, which is a fermented tea, contained a higher concentration of theaflavins than the oolong tea, which is a semi-fermented product.

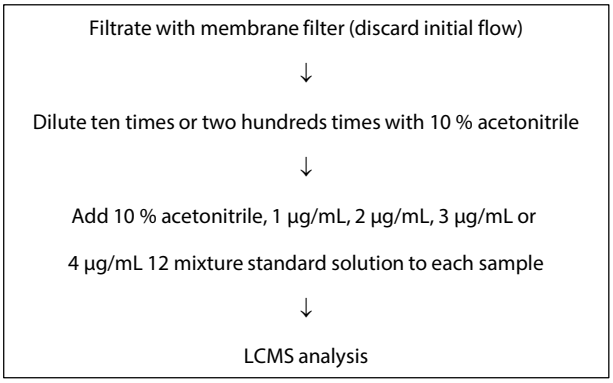

Fig. 6 Workflow of Analysis

Table 4 Coefficients of Determination of Calibration Curves for Compounds Added to Black Tea
(Catechins :tea diluted 200 times, Theaflavins : tea diluted 10 times)

Compound	Coefficient of determination (r^2)
GC	0.9995
EGC	0.9993
C	0.9990
EC	0.9995
EGCG	0.9978
GCG	0.9970
ECG	0.9994
CG	0.9984
TF1	0.9996
TF2A	0.9995
TF2B	0.9996
TF3	0.9999

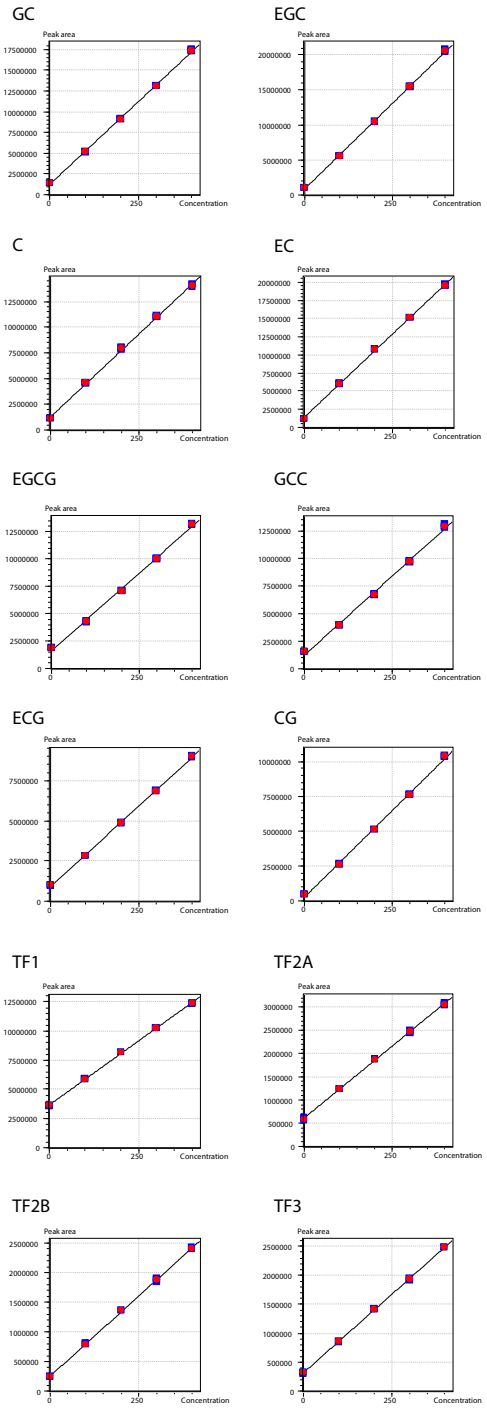

Fig. 7 Calibration Curves of Compounds Added to Black Tea
(Catechins :tea diluted 200 times, Theaflavins : tea diluted 10 times)

Table 3 Quantitative Analysis Results of Tea Samples (Unit: µg/mL)

	Black tea	Oolong tea	Green tea C	Green tea D	Green tea E	Green tea F	Green tea G	Green tea H	Green tea I	Green tea J	Green tea K
GC	6.170	32.699	101.001	63.913	66.084	85.903	48.791	69.243	74.618	82.820	44.205
EGC	3.339	11.042	37.162	21.345	24.399	32.488	17.042	23.611	24.828	28.054	15.939
C	7.605	13.447	36.209	24.163	22.066	27.251	23.640	22.126	24.974	28.772	17.951
EC	6.115	7.072	14.645	8.663	9.607	12.943	9.306	8.552	9.969	10.778	8.378
EGCG	11.456	22.780	41.257	15.062	36.465	23.276	33.937	16.639	19.786	22.252	29.937
GCG	8.629	30.820	59.899	22.020	51.511	32.272	42.991	23.008	25.486	35.025	36.531
ECG	8.951	7.320	9.472	2.368	8.192	4.732	7.993	4.376	3.442	4.592	6.248
CG	2.151	3.828	9.123	2.227	7.492	3.334	6.659	4.114	2.759	4.512	4.788
TF1	1.679	0.342	0.071	N.D.	N.D.	0.041	0.076	N.D.	N.D.	0.060	N.D.
TF2A	0.989	0.045	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TF2B	0.468	0.022	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TF3	0.581	0.017	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. : <0.012 µg/mL

■ Repeatability

Three analytical samples of green tea K were prepared and analyzed to investigate diurnal variation. A similar analysis was also carried out over a 3-day period to investigate day-to-day variation. Table 5 shows the results of these analyses. The relative standard deviation (RSD) of all compounds was within 10%, indicating that this analytical method has excellent repeatability for both daily and day-to-day analysis.

Table 5 Repeatability Results (n=3)

	Diurnal repeatability Relative standard deviation (RSD) (%)	Day-to-day repeatability Relative standard deviation (RSD) (%)
GC	1.56	2.21
EGC	1.11	1.52
C	1.65	2.09
EC	3.43	4.33
EGCG	1.37	1.93
GCG	0.30	1.73
ECG	3.95	2.35
CG	9.22	3.2
TF1	N.D.	N.D.
TF2A	N.D.	N.D.
TF2B	N.D.	N.D.
TF3	N.D.	N.D.

■ Multivariate Analysis of Green Teas

A principal component analysis (PCA) of the 9 green tea samples measured in this experiment was carried out with Traverse MS software (*Reifycs* Inc.) using the peak areas of the 8 catechins. For the data, the lower limit of the calibration curve for the standard addition method was used.

Fig. 8 shows the score plot and loading plot. Two clusters were identified from the score plot. The loading plot indicated that cluster X had large contents of free catechin compounds (C, EC, GC, EGC), while the contents of the gallate-type catechin compounds (CG, ECG, GCG, EGCG) tended to be small. The opposite tendencies were found in cluster Y. The products in cluster X include a high-quality refined green tea (*gyokuro*) and powdered tea (*maccha*) and emphasize a smooth tea. Since these two types of tea leaves (*gyokuro* and *maccha*) are shaded from light during growth, they are thought to have higher contents of amino acids than general middle-grade green tea (*sencha*), and a lower content of catechins results in sweetness and an umami flavor. The astringency of tea depends on ECG and EGCG and the sweetness of the aftertaste derives from EC and EGC, which suggests that tea leaves produced by the covering method were used in order to increase sweetness while suppressing astringency. Thus, it is possible to evaluate samples objectively and at a single glance not only by sensory evaluation but also by multivariate analysis.

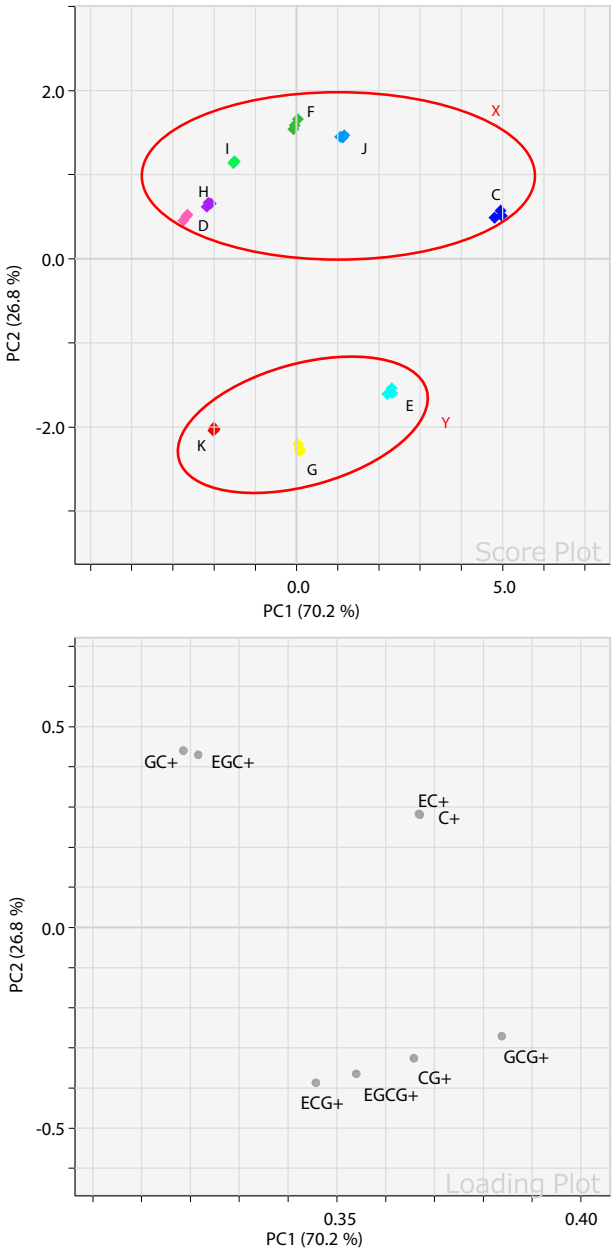

Fig. 8 PCA Results

■ Conclusion

This experiment demonstrated that 8 catechins and 4 theaflavins in tea can be analyzed simultaneously using a triple quadrupole mass spectrometer LCMS-8050 with a general ODS column. This analytical method enables quantitative analysis of 12 compounds contained in tea with easy pretreatment procedure. A simple multivariate analysis can also be conducted with the Traverse MS software.

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