Comprehensive 2D-LC Analysis of Tea (*Camellia sinensis*) with the Agilent 1290 Infinity II 2D-LC Solution

Quantification of Purine Alkaloids and Catechins in Green and Black Tea

Application Note

Food Testing and Agriculture

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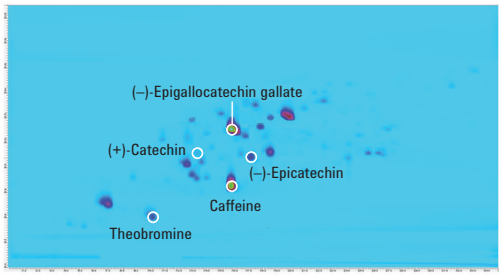
Abstract

Tea is one of the most widely consumed beverages in the world, and is produced from the tea plant *Camellia sinensis*. Depending on the processing methods of the leaves of *Camellia sinensis* after harvesting, green, oolong, or black tea is obtained. This Application Note shows the comprehensive 2D-LC analysis of purine alkaloids and catechins in green and black tea using the Agilent 1290 Infinity II 2D-LC solution. The precision of retention time and peak volume is determined, and the purine alkaloids caffeine and theobromine as well as the catechins catechin, epicatechin, and epigallocatechin gallate contained in green and black tea are quantified.



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Introduction

Tea, produced from the tea plant *Camellia sinensis*, is one of the most widely consumed beverages worldwide[1-5]. Comprising diverse polyphenols, purine alkaloids, polysaccharides, amino acids, vitamins, lipids, and volatiles, tea is characterized by a highly complex composition[3]. The consumption of tea is associated with a range of health benefits, which is in part attributed to the antioxidant activity of polyphenols contained in tea[2-6].

Depending on the processing methods of the leaves of *Camellia sinensis* after harvesting, three forms of tea are obtained; green, oolong, and black tea. Generally, after harvesting, the leaves are rolled, which leads to disruption of the cellular compartmentation and brings phenolic compounds into contact with the enzyme polyphenol oxidase. In the production of green tea, the rolled leaves are steamed or dried immediately to inactivate the enzyme and minimize oxidation. To produce black tea, the rolled leaves undergo oxidation (referred to as fermentation) before drying. Oolong tea is produced similarly to black tea while deploying a shorter fermentation period[1-4].

The predominant polyphenols contained in green tea are catechins (flavan-3-ols) such as catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate[1-4,6]. Epigallocatechin gallate is the most abundant catechin present in green tea[2,3]. In the production of black tea, the monomeric catechins undergo oxidative polymerization to form the condensation products theaflavins and their polymers thearubigins[1,3,4].

Due to the complex composition of tea and the structural similarity of green tea phenolics, complete separation of the phenolic compounds contained in tea cannot be achieved using conventional one-dimensional liquid chromatography (1D-LC)[3]. Using comprehensive two-dimensional liquid chromatography (comprehensive 2D-LC), the separation power can be greatly increased[3,7].

This Application Note shows the comprehensive 2D-LC analysis of green and black tea using the Agilent 1290 Infinity II 2D-LC solution. Quantification of the purine alkaloids caffeine and theobromine as well as of the tea catechins catechin, epicatechin, and epigallocatechin gallate (Figure 1) enables a comparison of green and black tea.

Experimental

Equipment

The Agilent 1290 Infinity II 2D-LC solution was comprised of the following modules:

- Agilent 1290 Infinity II High-Speed Pumps (2 × G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B) with cooler
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with 2-position/4-port-duo valve (2D-LC valve head, G4236A) equipped with two 60-µL loops
- Agilent 1290 Infinity II Diode Array Detector (G7117B) with 10-mm Max-Light cartridge cell (G4212-60008)

Software

- Agilent OpenLAB CDS ChemStation Edition Software, version C.01.07 [27] with 1290 Infinity 2D-LC acquisition software, version A.01.02 SP1
- GC Image LCxLC-HRMS Edition Software, version 2.5b0 for 2D-LC data analysis from GC Image LLC., Lincoln, NE, USA

Columns

First dimension

Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 3.5 µm (p/n 959793-902)

Second dimension

Agilent Poroshell 120 Bonus-RP, 3.0 × 50 mm, 2.7 µm (p/n 699968-301)

Chemicals

Caffeine, theobromine, theophylline, (+)-catechin, (–)-epicatechin, and (–)-epigallocatechin gallate were purchased from Sigma-Aldrich (Steinheim, Germany). All solvents were LC grade. Acetonitrile, methanol, and acetone were purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Acetic acid and trifluoroacetic acid were purchased from Sigma-Aldrich (Steinheim, Germany).

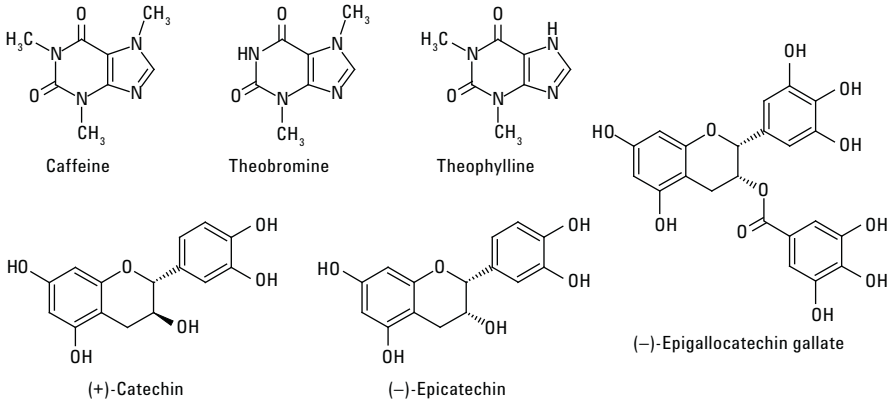

Figure 1. Structures of purine alkaloids and catechins.

Standards

Stock solutions with concentrations of 1 mg/mL of caffeine, theobromine, theophylline, (+)-catechin, (–)-epicatechin, and (–)-epigallocatechin gallate were prepared by dissolution in acetonitrile/water/acetic acid (20/80/1, v/v/v). Standard solutions in the concentration range of 2 to 100 µg/mL were obtained by dilution of the stock solutions with acetonitrile/water/acetic acid (20/80/1, v/v/v).

Samples and sample preparation

Ten different samples of green tea and black tea were obtained from a German retail market. Sample preparation was carried out using a modification of the method described by Kalili[3]. Approximately 2 g of finely ground tea were accurately weighed and extracted three times with 15 mL of acetone/water (70/30, v/v). The suspension was centrifuged at 5,000 rpm for 5 minutes after every extraction, and the resulting supernatants were combined and made up to 50 mL. A 100-µL aliquot of the combined supernatant was evaporated to dryness using a SpeedVac, and redissolved in 1 mL of acetonitrile/water/acetic acid (20/80/1, v/v/v). The resulting solution was filtered using a 1-mL plastic syringe with Captiva Premium Syringe Filters Regenerated Cellulose, 15 mm, 0.45 µm (p/n 5190-5109) before injection into the LC system.

2D-LC Method

Parameter	Value
First dimension pump	
Solvent A	Water + 0.05 % trifluoroacetic acid
Solvent B	Methanol + 0.05 % trifluoroacetic acid
Flow rate	0.1 mL/min
Gradient	5 %B at 0 minutes 60 %B at 30 minutes 95 %B at 32 minutes
Stop time	40 minutes
Post time	10 minutes
Second dimension pump	
Solvent A	Water + 0.05 % trifluoroacetic acid
Solvent B	Acetonitrile + 0.05 % trifluoroacetic acid
Flow rate	2.5 mL/min
Gradient and gradient modulation	5 %B at 0.00 minutes to 22 %B at 35 minutes to 95 %B at 35.1 minutes 20 %B at 0.25 minutes to 57 %B at 35 minutes to 95 %B at 35.1 minutes
^{2}D Gradient stop time	0.25 minutes
Modulation time	0.35 minutes
Multisampler	
Injection volume	5 µL for standard solutions, 2 µL for tea extracts
Sample temperature	6 °C
Needle wash	3 seconds in methanol/water (50/50, v/v)
Multicolumn thermostat	
First-dimension column	30 °C at right side
Second-dimension column	50 °C at left side
2-position/4-port-duo valve	
The 2-position/4-port-duo valve was switched automatically after each second dimension modulation cycle of 21 seconds. The loops were used in a cocurrent manner (filling and elution of the loops in the same flow direction).	
Diode array detector	
Wavelength	280 nm/4 nm, reference 395 nm/10 nm
Data rate	80 Hz
Stop time	35 minutes

Results and Discussion

Deploying reversed-phase LC in the first and second dimension, a comprehensive 2D-LC method for the analysis of purine alkaloids and catechins in green and black tea was developed. Figure 2 shows the separation of a mixture of the purine alkaloids caffeine, theobromine and theophylline as well as the catechins catechin, epicatechin, and epigallocatechin gallate. It can be seen that only the two-dimensional setup enables a complete separation of the purine alkaloids and catechins. In the first-dimension separation, a coelution of caffeine and epigallocatechin gallate is observed, which is resolved in the second-dimension separation. Deploying only the second-dimension separation, catechin and epicatechin would coelute.

The precision of retention times and peak volumes was determined by multiple injection (n = 10) of the mixture of purine alkaloids and catechins (10 µg/mL each). The results are shown in Table 1. For the first-dimension separation, the retention time precision cannot be calculated as fractions of 0.35 minutes (corresponding to the modulation time) are transferred to the second dimension separation. In the second dimension, the retention time precision is always below 2.5 %, and the peak volume precision is always below 1 %.

To enable quantification of purine alkaloids and catechins in green and black tea, calibration was performed in the concentration range from 2 to 100 µg/mL. Excellent linearity was achieved for all purine alkaloids and catechins. Figure 3 exemplarily shows the calibration curve for epicatechin, and Table 2 summarizes the coefficients of linearity obtained.

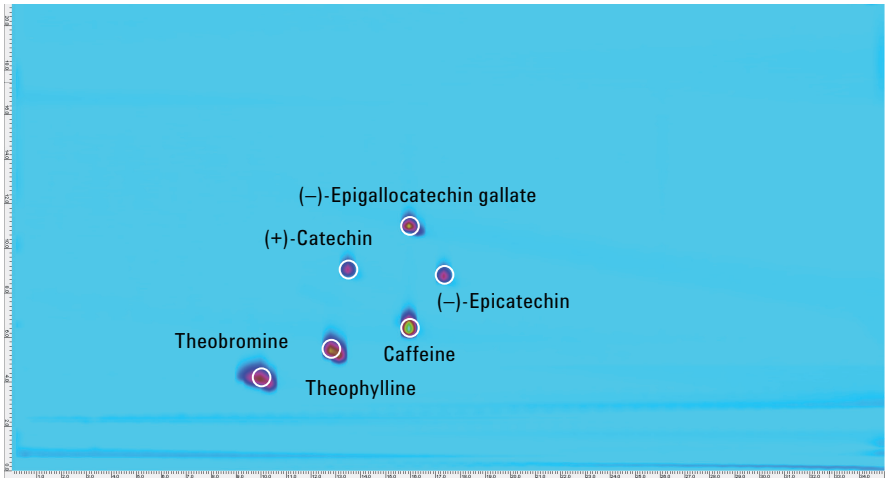

Figure 2. Comprehensive 2D-LC separation of purine alkaloids and catechins (10 µg/mL each).

Table 1. Precision of retention times and peak volumes (n = 10).

Substance	Second dimension Retention time (s)	RT RSD (%)	Peak volume (arbitrary units)	Peak volume RSD (%)
Caffeine	6.44	1.19	567,766	0.32
Theobromine	4.20	1.59	543,889	0.22
Theophylline	5.42	2.27	529,877	0.17
(–)-Epigallocatechin gallate	11.04	0.70	283,588	0.84
(–)-Epicatechin	8.77	0.78	140,757	0.32
(+)-Catechin	9.07	0.87	136,431	0.45

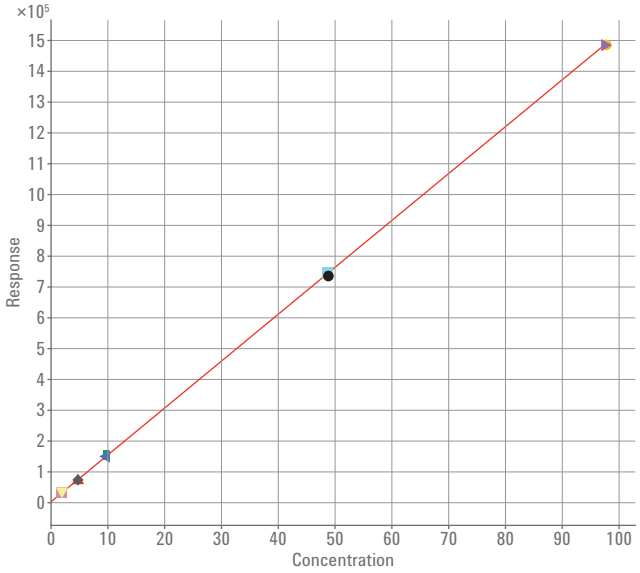

Figure 3. Calibration curve for epicatechin from 2 to 100 µg/mL.

Table 2. Coefficients of linearity (R^2) for calibration in the range from 2 to 100 µg/mL.

Substance	R^2
Caffeine	0.99998
Theobromine	0.99993
Theophylline	0.99998
(–)-Epigallocatechin gallate	0.99984
(–)-Epicatechin	0.99996
(+)-Catechin	0.99998

Ten different samples of green and black tea were analyzed, and purine alkaloids and catechins were quantified. Theophylline could not be detected in any of the analyzed tea samples. Figure 4 shows the chromatograms from the 2D-LC analysis of one green and one black tea sample. In green tea, the epigallocatechin gallate peak shows higher intensity compared to black tea, whereas in black tea, peaks detected at higher first-dimension retention time (30 to 32 minutes) might originate from theaflavins and thearubigins.

Table 3 and Figure 5 show the quantification results for purine alkaloids and catechins in green and black tea. As expected, the green tea samples generally contain higher amounts of the catechins epigallocatechin gallate and epicatechin than the black tea samples.

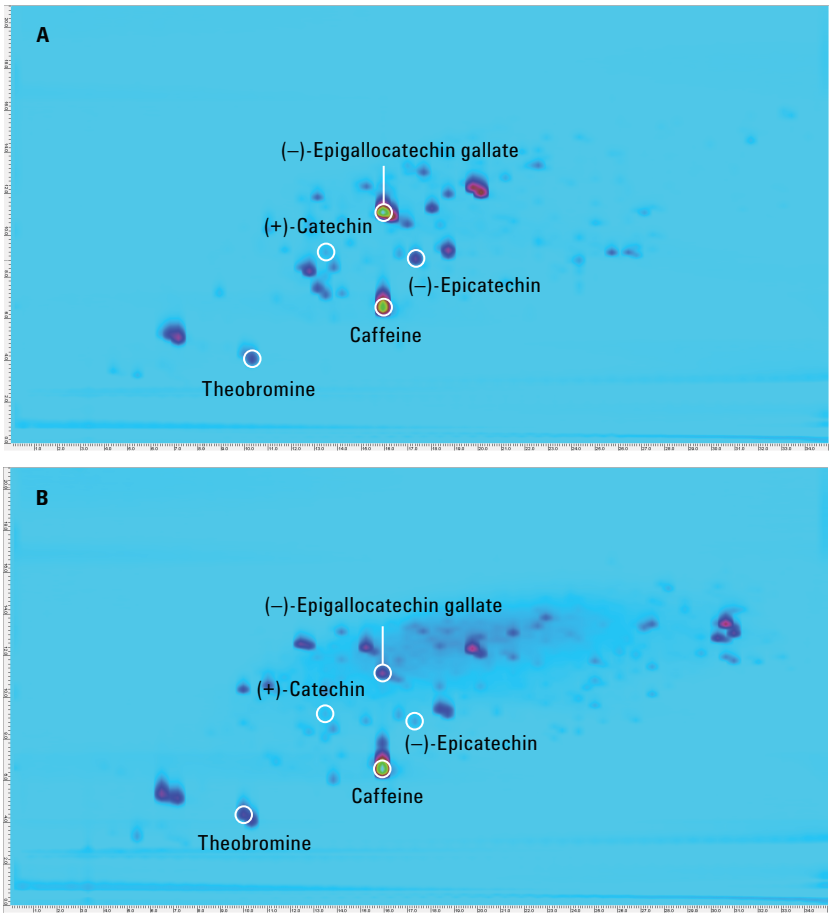

Figure 4. Comprehensive 2D-LC analysis of green tea (A) and black tea (B).

Table 3. Quantification of purine alkaloids and catechins in green and black tea.

Tea	Caffeine (mg/g)	Theobromine (mg/g)	(+)-Catechin (mg/g)	(–)-Epicatechin (mg/g)	(–)-Epigallocatechin gallate (mg/g)
Green tea	15.0	0.5	1.1	6.6	42.0
	26.5	1.5	1.5	7.4	69.0
	18.8	0.4	0.9	9.5	49.3
	17.2	0.5	1.0	6.4	47.2
Black tea	23.2	1.2	0.9	2.6	15.0
	24.1	1.3	2.0	2.3	6.4
	22.6	1.2	1.7	4.0	29.3
	33.7	2.6	1.8	2.4	11.7
	25.9	1.7	2.0	3.7	29.9
	24.2	1.2	2.0	5.4	42.1

Conclusions

The Agilent 1290 Infinity II 2D-LC solution with reversed-phase LC in the first and second dimension enables the analysis and quantification of purine alkaloids and catechins in green and black tea. For the purine alkaloids and catechins analyzed, second-dimension retention time precision was below 2.5 % RSD, and peak volume precision was below 1 % RSD. Excellent linearity was obtained in the concentration range from 2 to 100 µg/mL. As expected, the analyzed green tea samples contained higher amounts of the catechins epigallocatechin gallate and epicatechin than the analyzed black tea samples.

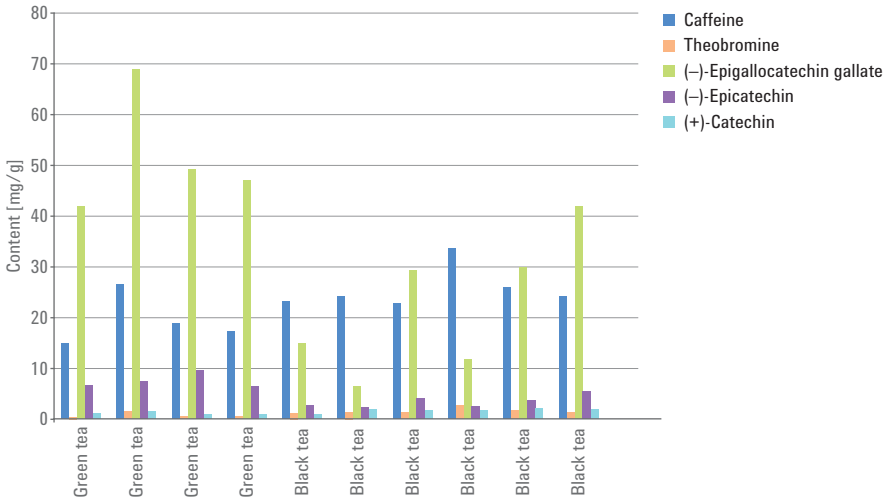

Figure 5. Quantification of purine alkaloids and catechins in green and black tea.

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Published in the USA, August 1, 2015
5991-6059EN

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