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Sensitive Determination of Catechins in Tea by HPLC

INTRODUCTION

Consumption of tea has become increasingly popular in North America and tea is currently one of the most consumed non-alcoholic drinks worldwide.[1-3] Studies have shown that tea (*Camellia sinensis*) provides several health benefits, such as reduction of cholesterol and obesity, and protection against cardiovascular disease and cancer. Catechins are powerful antioxidants found in tea that are thought to provide several of these health benefits. Figure 1 shows the structures of the most abundant catechins found in tea.

The composition of catechins in commercial teas varies based on the species, season, horticultural conditions, and most importantly, the degree of oxidation during the manufacturing process.[4] There are four major varieties of teas: white, green, oolong, and black. Although all teas are derived from the same *Camellia sinensis* plant, the processing methods for each tea are different. For example, white tea is naturally dried using either sun drying or steaming methods before being minimally processed to prevent oxidation. These processing methods protect the tea flavor and preserve the high catechin concentrations.

Green tea represents about 20% of the total tea production. It is primarily popular in Japan and parts of China, but its popularity is growing in other parts of the world due to its wide availability and reported health benefits. The production process for green tea is similar to white tea, and therefore, it also contains a relatively high concentration of catechins.

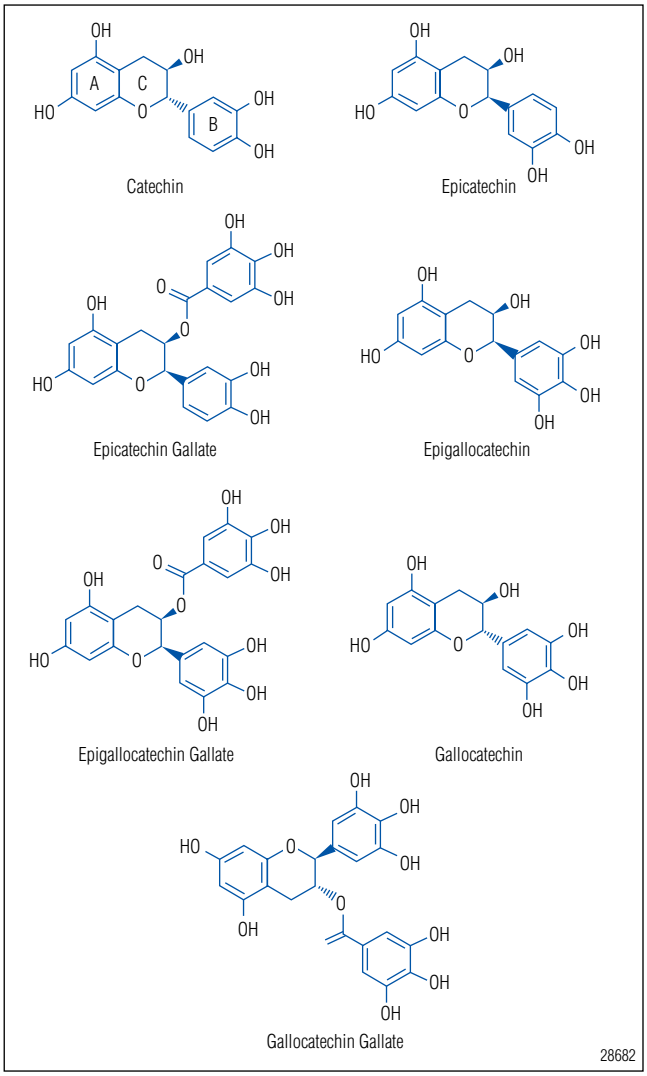

Figure 1. Structures of catechins in Camellia sinensis.

Black tea represents approximately 78% of the global tea production and is the most common type of tea in the United States and Europe.[5] It is made by completely oxidizing the harvested leaves for several hours before drying. Oxidation imparts a dark coloration to the tea and also triples the caffeine content.[5] Due to variability in the composition of tea catechins and their potential health benefits, it is critical to establish a simple and reliable analytical method for the determination of these compounds in different tea products.

This work describes a sensitive, fast, and accurate high-performance liquid chromatography (HPLC) method to determine catechins in tea. The most abundant catechins in tea products include catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), gallocatechin gallate (GCG), and epigallocatechin gallate (EGCG). The method uses a high-resolution silica-based 2.2 µm Acclaim® C18 RSLC column and a wavelength of 280 nm to separate, detect, and quantify catechins in white, green, black, and a blended white and green tea.

In addition, this work evaluated two standard reference materials (SRMs) provided by the National Institute of Standards and Technology (NIST) as part of a collaborative study. The control material (SRM 3255) was a spray-dried green tea extract and the sample (SRM 3256) contained ground and homogenized green tea tablets.

The method demonstrates good sensitivity, enabling the detection of a wide variety of catechins with concentrations ranging from 2.46 mg/g for catechin to 80.8 mg/g for EGCG, and has a total run time of less than 20 min. The reported limits of detection (LODs) using the method range from 0.20 µg/mL for EG and catechin to 1.17 µg/mL for GC, and limits of quanititation (LOQs) range from 0.59 µg/mL for EC to 3.56 for GC. The method described here is ideal for simple, sensitive, accurate, rapid, and routine analysis of catechins in different tea products.

EXPERIMENTAL

Dionex UltiMate® 3000 Rapid Separation LC System

SRD-3600 Solvent Rack with 6 degasser channels (P/N 5035.9230) and Eluent Organizer, including pressure regulator and 2 L glass bottles for each pump (eluents were maintained under helium or nitrogen headspace from 5–8 psi)

DGP 3600RS Pump (P/N 5040.0066)

WPS-3000TRS Well Plate Sampler (P/N 5840.0020)

Sample Loop, 25 µL (P/N 6820.2415)

TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)

DAD-3000RS Photodiode Array Detector (P/N 5082.9920)

Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL volume, 7 mm path length (P/N 6080.0300)

CONSUMABLES

Acclaim 120 C18, 2.2 µm, RSLC column, 2.1 × 150 mm (P/N 059130)

Centrifuge equipped with a ten-place, aluminum fixed-angle rotor (Beckman Spinchron R, GS-6R Series, Beckman Coulter P/N 358702 or equivalent)

Viper™ fingertight fitting system, SST Flex. –Cap., i.d. × L, 0.13 × 250 mm (P/N 6040.2325)

Viper fingertight fitting system, SST Flex. –Cap., i.d. × L, 0.13 × 350 mm (P/N 6040.2335)

Viper fingertight fitting system, SST Flex. –Cap., i.d. × L, 0.18 × 450 mm (P/N 6040.2365)

Static mixer, mixing volume: 350 µL (P/N 6040.0040)

Glass injection vials with caps and septa, 1.5 mL (P/N 055427)

Borosilicate glass scintillation vials with closures attached, 20 mL (VWR P/N 66022-129)

REAGENTS AND STANDARDS

Reagent grade water, Type I, 18 MΩ-cm resistance or better, filtered through a 0.2 µm filter immediately before use (referred to here as deionized [DI] water)

Acetonitrile, HPLC grade (Honeywell P/N AH015-4)

Trifluoroacetic acid (TFA), 98% pure (Pierce P/N 208901)

Epigallocatechin (Chromadex P/N ASB-00005145-010)

Epicatechin gallate (Chromadex P/N ASB-00005135-010)

Catechin (Chromadex P/N ASB-00003310-010)

Epicatechin (Chromadex P/N ASB-00005125-010)

Epigallocatechin gallate (Chromadex P/N ASB-00005150-010)

Gallocatechin (Sigma P/N G6657)

Gallocatechin gallate (Sigma P/N G6782)

SAMPLES

NIST SRM 3255: *Camellia sinensis* extract used as a control material

NIST SRM 3256: Green tea-containing tablets

White tea

White tea blended with green tea

Green tea brand A

Green tea brand B

Black tea

CONDITIONS

Column:	Acclaim 120 C18, 2.2 µm (2.1 × 150 mm)
Flow Rate:	0.450 mL/min
Inj. Volume:	1.0 µL
Tray Temp.:	4 ºC
Detection:	Absorbance, UV, 280 nm
Column Temp.:	25 ºC
Eluents:	A: 0.1% TFA, 5% acetonitrile B: 0.1% TFA in acetonitrile
System Backpressure:	~6025–6200 psi during the gradient

Gradient Conditions:

Time (min)	*A %*	*B %*
0.0	100.0	0.0
1.2	100.0	0.0
15.5	71.5	28.5
17.0	71.5	28.5
17.0	100.0	0.0
25.0	100.0	0.0

PREPARATION OF SOLUTIONS AND REAGENTS

Trifluoroacetic Acid (0.1%) in Acetonitrile (5%)

Transfer 100 mL of acetonitrile into a glass 2 L volumetric flask containing approximately 1700 mL of DI water. Add 2 mL of TFA to the volumetric flask. Bring to volume using DI water and invert flask several times to mix.

Trifluoroacetic Acid (0.1%) in Acetonitrile

Transfer 900 mL of acetonitrile into a glass 1 L volumetric flask, then add 1 mL of TFA to the flask. Bring to volume using acetonitrile and invert flask several times to mix.

Formic Acid (0.05%) in 70% Methanol (Extraction Solvent)

Transfer 700 mL of methanol into a glass 1 L volumetric flask and add 500 µL of formic acid. Bring to volume using DI water and invert several times to mix.

Standard Concentrates (1 mg/mL)

Catechin standards of EGC, ECG, catechin, EC, EGCG, GC, and GCG were prepared by accurately weighing 1–2 mg of solid and adding 1–2 mL of 0.05% formic acid in 70% acetonitrile to make a stock solution of 1.0 mg/mL for each individual catechin. The stocks were prepared in 1.5 mL glass vials, vortexed to mix, and stored at -40 °C until needed. All standard concentrates can be stored for up to six months at -40 °C when protected from light.

Working Standards and Standards for Method Linearity

To prepare working standards, use a calibrated pipette to deliver the appropriate volume of the 1 mg/mL stock standard into a glass vial containing the appropriate volume of 0.05% formic acid in 70% acetonitrile. To prepare mixed catechin standards, combine appropriate volumes of the individual stock catechin standards in a glass vial containing the appropriate volume of 0.05% formic acid in 70% methanol. Diluted intermediate standards are stable for 3 months at -40 °C and working and mixed standards are stable for 4 weeks at 2–4 °C.

SAMPLE PREPARATION

Two SRMs used in this study—control material (SRM 3255) and sample (SRM 3256)—were provided by NIST as part of a collaborative study. All commercial tea samples were purchased locally.

NIST Control Material

Prepare the NIST control material by weighing 20 mg of solid, then adding 7 mL of the extraction solvent. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Collect the supernatant in a glass vial and add another 7 mL of the solvent to the pellet. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min at 4 ºC. Add the supernatant to the first 7 mL to make a total volume of 14 mL. Filter the samples using 0.2 µm cellulose acetate sterile syringe filters, and dilute 1:5 in the extraction solvent prior to analysis.

Commercial Tea Samples and NIST SRM 3256

Prepare all samples by weighing 60 mg of solid and adding 7 mL of the extraction solvent. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Collect the supernatant in a glass vial and add another 7 mL of the solvent to the pellet. Vortex the mixture, sonicate for 90 min, and centrifuge at 5000 RPM for 10 min. Add the supernatant to the first 7 mL to make a total volume of 14 mL. Filter the samples using 0.2 µm cellulose acetate sterile syringe filters and dilute 1:5 or 1:20 in the extraction solvent prior to analysis, depending on the sample type.

RESULTS AND DISCUSSION

Separation of Catechin Standards

The initial investigation for the separation of catechins used a 2.2 µm RSLC Acclaim 120 C18 column in the 2.1 × 150 mm format. This column format was chosen to increase sample throughput and reduce sample and eluent consumption. Shorter column formats were also evaluated, but the 2.1 × 150 mm format was chosen because this column provided the best resolution of the target compounds.

Figure 2 shows a chromatogram of a mixed standard containing the predominant catechins in tea. In addition, free gallic acid and moderate amounts of caffeine are naturally present in tea; and therefore were included in the mixed standard. The retention times of gallic acid, GC, EGC, caffeine, catechin, EC, EGCG, GCG, and ECG are 2.15, 4.60, 7.00, 7.25, 7.40, 8.70, 8.90, 9.30, and 10.7 min, respectively. All catechins are well resolved and the total analysis time is less than 20 min.

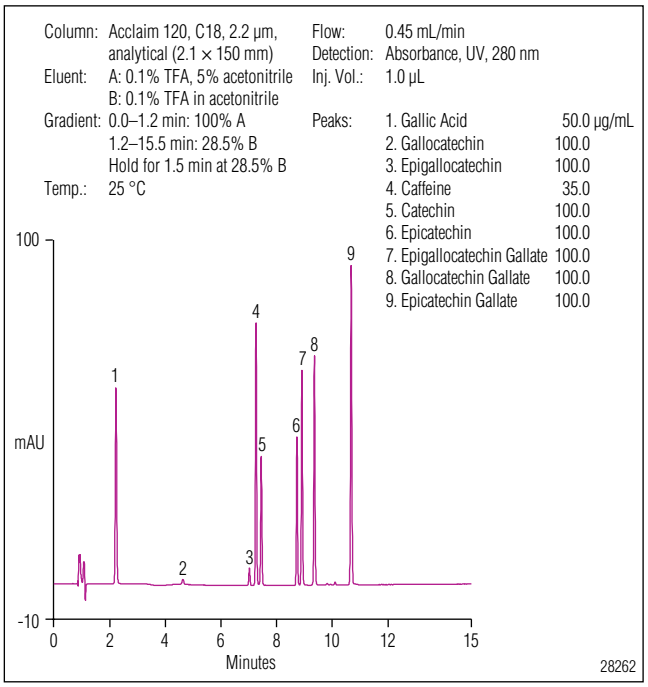

Figure 2. Separation of a mixed catechin standard on the Acclaim C18 RSLC column.

Preliminary Sample Analysis

Prior to analyzing commercial tea samples, a NIST sample and control were evaluated for their catechin profiles using the method described here. The sample and control were prepared as described in the Sample Preparation section. The chromatography demonstrated that all peaks were resolved, suggesting that the method can be used for further system suitability studies.

System Suitability

The linearity, LODs, and LOQs were evaluated to determine the suitability of the method for this analysis. To determine the appropriate calibration ranges for the target compounds, each sample was analyzed and compared to a mixed catechin standard. EGCG, catechin, GCG, GC, EGC, ECG, and EC exhibited a linear peak area response in the ranges summarized in Table 1.

Table 1. Data for Linearity, LOD, and LOQ of Catechins

Analyte	Range (µg/mL)	Coeffic. of Determin. (r^2)	LOD (µg/mL)	LOQ (µg/mL)	RSD	
					Ret. Time* (n=30)	Peak Area* (n=30)
Gallocatechin	3.56–75	0.9993	1.17	3.56	0.11	1.17
Epigallocatechin	1.8–50	0.9993	0.59	1.80	0.18	1.45
Catechin	0.78–50	0.9992	0.20	0.78	0.13	1.19
Epicatechin	0.59–50	0.9999	0.20	0.59	0.06	1.51
Epigallocatechin Gallate	1.17–150	0.9994	0.39	1.17	0.04	1.00
Gallocatechin Gallate	1.2–10	0.9998	0.39	1.20	0.02	1.37
Epicatechin gallate	1.56–50	0.9995	0.39	1.56	0.02	1.30

*EGC, Catechin, EGCG, EC, GCG, and ECG at concentrations of 15, 1, 30, 1, 2, and 3 µg/mL, respectively, were used for precision studies

The LODs for the catechins were determined based on the concentration of the analyte that provides a peak height of 3× the measured noise (S/N = 3). The LOQs were determined as the concentration of the analyte that provides a peak height of 10× the measured noise (S/N = 10). The LODs ranged from 0.20 µg/mL for EC to 1.17 µg/mL for GC, and the LOQs ranged from 0.59 µg/mL for EC to 3.56 µg/mL for GC. Retention time precisions of the standards were excellent, with RSDs ranging from 0.02% for ECG to 0.18% for EGC. This demonstrated good precision of the gradient delivered by the DPG 3600RS pump. Peak area precision ranged from 1.00% for EGCG to 1.51% for EC. Peak height precision ranged from 0.25% for EGC to 1.70% for GC over 30 runs.

Sample Analysis

Catechin concentrations were determined in a NIST control and sample prior to the analysis of commercial teas. The samples and control were prepared as described in the Sample Preparation section. Table 2 summarizes the catechin concentrations in the NIST controls and samples with a comparison to the certified values.

The concentrations for all the catechins in the control were consistent with the certified NIST values. EGCG is the catechin present at the highest concentration, contributing to 60% of the total catechin content based on the determination presented here, and 58% based on the NIST certified value.

Table 2. Determination of Catechins in a 1:5 Diluted NIST Control and Reference Sample

Analyte	NIST Control (mg/g)	NIST Control Certified Value (mg/g)	NIST Sample (mg/g)	NIST Sample Certified Value (mg/g)
Gallocatechin	22.8 ± 1	24 ± 1	7.84	7.60
Epigallocat-echin	84.7 ± 1	88 ± 3	29.6	30.7
Catechin	9.70 ± 0.5	9.8 ± 0.4	2.46	2.60
Epicatechin	47.3 ± 1	46 ± 2	11.9	12.0
Epigallocat-echin Gallate	427.3 ± 12	417 ± 16	80.8	71.1
Gallocatechin Gallate	40.9 ± 1	38 ± 3	4.46	4.60
Epicatechin Gallate	76.8 ± 2	94 ± 5	17.4	17.1
Total Catechins	709.6 ± 12.5	716.8 ± 27.4	154.5	145.7

The total catechin content calculated using the method described here was determined to be 709.6 mg/g, compared to the NIST certified value of 716.8 ± 27 mg/g. This agreement of the control results with the NIST values confirms that the method is accurate for the determination of catechins.

A NIST reference sample was also evaluated using this method. The individual determined catechin concentrations for the NIST sample ranged from 2.46 mg/g for catechin to 80.8 mg/g for EGCG, compared to certified values of 2.60 mg/g for catechin to 71.7 mg/g for EGCG. The total catechin content is 154.5 mg/g, compared to the certified value of 145.7 mg/g, which is within 6% of the certified value.

Several different brands of teas were evaluated for their catechin content. The samples investigated in this study included two different types of green tea, white tea, a blend of white tea with green tea, and black tea. White tea is minimally processed, so it is expected to be very high in catechins. Figure 3 shows the separation of catechins in white tea. Concentrations ranged from 2.73 mg/g for EC to 42.6 mg/g for EGCG. The total catechin content in this sample was 98.5 mg/g.

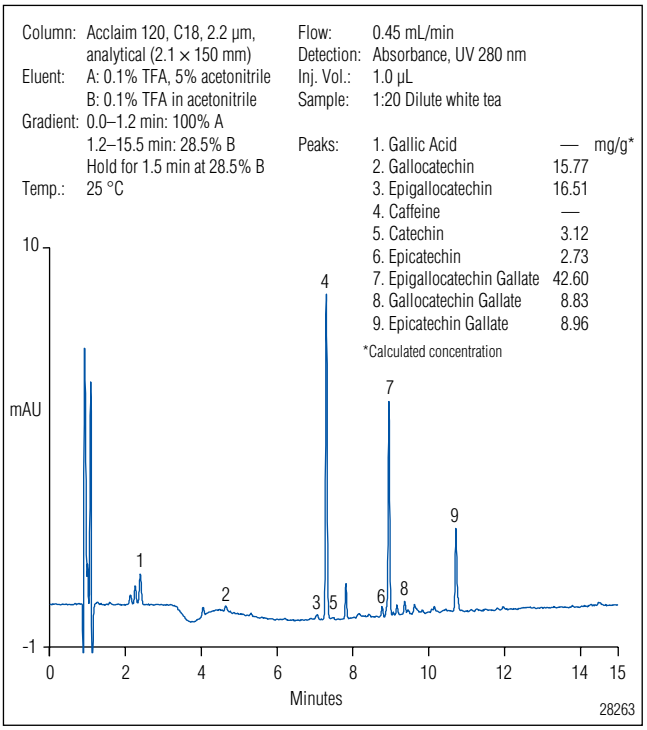

Figure 3. Separation of catechins in a 1:20 diluted sample of white tea.

Figure 4 shows the separation of catechins present in the two different commercially available green teas. The catechin concentrations ranged from 3.45 mg/g for catechin to 64.0 mg/g for EGCG in brand A green tea. In brand B, the concentrations ranged from 3.57 mg/g for catechin to 60.6 mg/g for EGCG. The health benefits of consuming green tea are attributed to the high concentrations of catechins, which account for approximately 30% of the dry weight of green tea leaves.

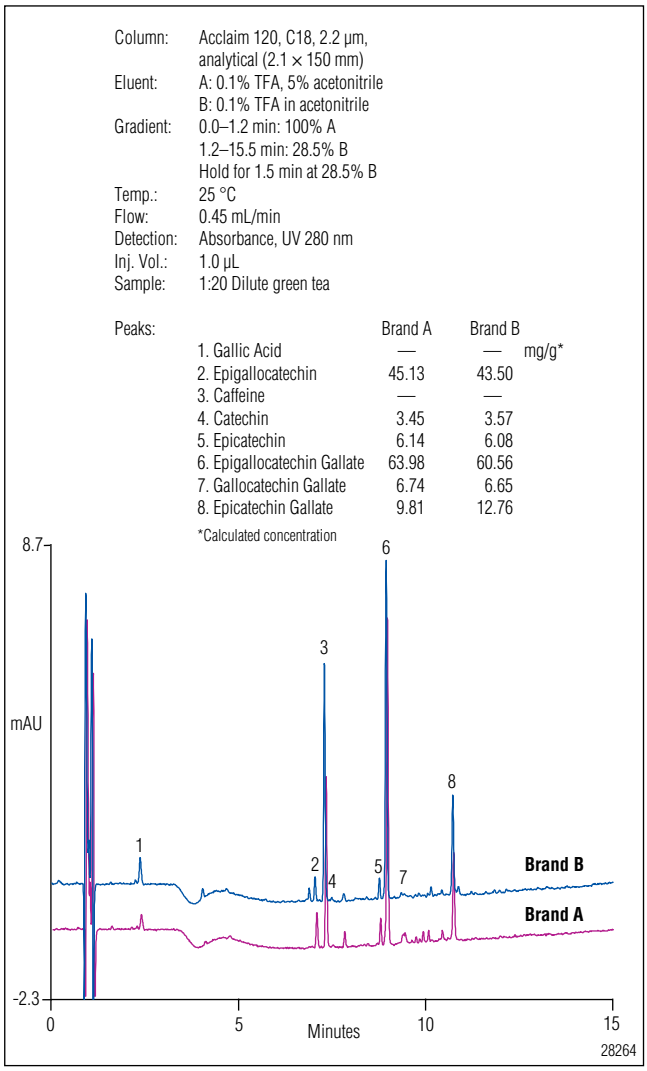

Figure 4. Comparison of catechins in a two different brands of green tea (diluted 1:20).

As shown in Figure 4, the most abundant catechin is EGCG, which is about 50% of the total catechin content. One cup of green tea may contain 100–200 mg of EGCG. The concentrations of individual catechins were determined to be similar for both brands of the green tea, with the exception of ECG. Brand A green tea had 9.81 mg/g and brand B had 12.8 mg/g of ECG. The total catechin content were determined to be similar for both green tea samples with the total concentrations determined at 135.3 mg/g and 133.1 mg/g for brands A and B, respectively. The data shows that the total catechin concentration in the white tea is unexpectedly lower than the total catechin concentrations in the green tea products that were evaluated in this study. However, additional white tea samples were not analyzed to determine if this was representative of white tea products.

The majority of tea produced in the world is black tea, but it is also reported to contain the lowest concentration of catechins due its additional processing. In this study, the content in black tea ranged from 2.25 mg/g for EC to 27.8 mg/g for EGC (Figure 5). Unlike the other teas studied, the EGC concentration in black tea is higher than the EGCG content. The total catechin content is 63.3 mg/g, which is nearly 50% less than the total catechins in the green tea products analyzed.

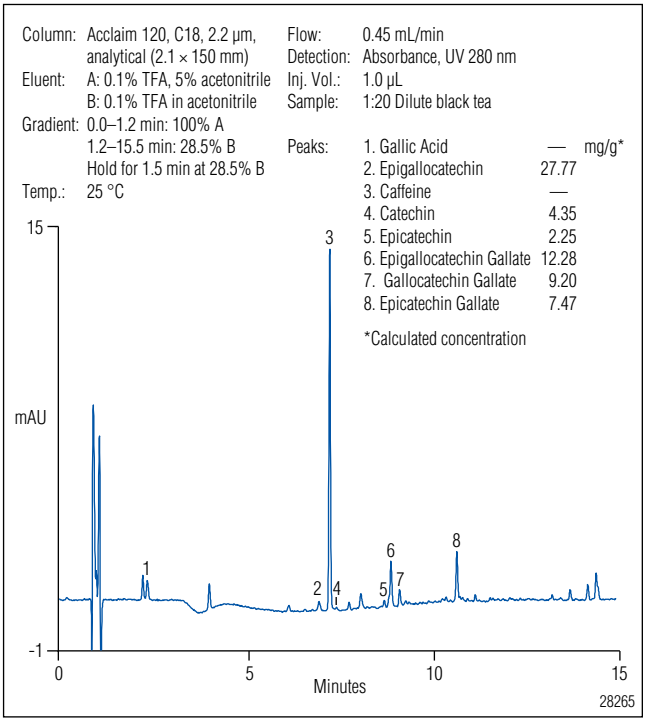

Figure 5. Separation of catechins in a 1:20 diluted sample of black tea.

Table 3. Intraday and Between-Day Precision						
Sample	Analyte	Amount (mg/g)	Intraday Precision RSD (n=3)			Between-day Precision
			Retention Time	Peak Area	Peak Height	Peak Area (n=9 over 3 days)
White Tea	GC	15.8	0.02	0.56	0.90	1.30
	EGC	16.5	0.03	1.04	0.98	2.13
	Catechin	3.12	0.06	0.78	0.89	1.24
	EC	2.73	0.02	1.18	0.83	1.51
	EGCG	42.6	0.02	0.91	0.35	1.74
	GCG	8.83	0.02	0.54	0.85	1.83
	ECG	8.96	0.02	1.06	0.54	2.03
White/Green Tea Blend	EGC	26.7	0.10	1.16	0.50	1.44
	Catechin	2.63	0.14	1.27	1.58	1.49
	EC	2.88	0.08	1.63	1.35	2.15
	EGCG	30.7	0.06	1.17	0.90	1.66
	GCG	4.40	0.02	1.40	1.18	1.62
	ECG	7.44	0.02	0.93	0.74	1.88
Black Tea	EGC	27.8	0.05	1.00	1.39	1.15
	Catechin	4.35	0.09	0.61	0.51	1.08
	EC	2.25	0.03	0.47	0.70	1.07
	EGCG	12.3	0.02	1.33	1.65	1.42
	GCG	9.20	0.05	1.76	1.57	1.97
	ECG	7.47	0.03	1.66	1.37	1.72
Green Tea Brand A	EGC	45.1	0.12	1.10	0.57	1.71
	Catechin	3.45	0.09	1.16	0.87	1.93
	EC	6.14	0.04	1.34	0.98	1.72
	EGCG	64.0	0.02	1.30	1.00	1.91
	GCG	6.74	0.36	1.35	1.09	1.52
	ECG	9.81	0.01	0.33	1.37	1.04
Green Tea Brand B	EGC	43.5	0.01	0.43	0.48	1.92
	Catechin	3.57	0.01	1.49	1.69	1.50
	EC	6.08	0.01	0.25	0.41	1.81
	EGCG	60.6	0.01	0.45	0.36	1.93
	GCG	6.65	0.01	0.66	0.27	1.14
	ECG	12.8	0.01	0.92	0.50	1.08

Sample Precision and Accuracy

Five different samples of teas were analyzed over three days to evaluate the method precision. Representative data from each of the teas are summarized in Table 3. For all the samples analyzed in this study, the intraday retention time RSDs ranged from 0.01% for several catechins to 0.36% for GCG. Intraday peak area RSDs ranged from 0.25% for EC to 1.76% for GCG. The between-day peak area RSDs ranged from 1.04% for ECG to 2.15% for EC.

The accuracy of the method was confirmed by determining catechin concentrations in the NIST control and comparing against the NIST certified values. The NIST certified values for the control and the samples were in agreement with the values reported in this study. Recovery studies were performed on all five tea samples by spiking known amounts of the seven catechins to determine method accuracy.

Table 4. Recovery of Catechins in Different Commercial Tea Products

Sample	Analyte	Amount Spiked µg/mL	% Recovery
White Tea	GC	4.0	103.5
	EGC	4.0	92.1
	Catechin	1.0	99.8
	EC	1.0	112.0
	EGCG	10.0	93.3
	GCG	3.0	105.1
	ECG	3.0	84.2
White/Green Tea Blend	GC	1.0	93.4
	EGC	6.5	91.3
	Catechin	1.0	94.3
	EC	1.0	96.4
	EGCG	10.0	99.1
	GCG	1.0	97.6
	ECG	2.0	93.6
Black Tea	GC	1.0	94.0
	EGC	7.0	93.3
	Catechin	1.0	96.8
	EC	0.5	90.2
	EGCG	3.0	101.0
	GCG	2.0	102.3
	ECG	2.0	102.0
Green Tea Brand A	GC	1.0	85.4
	EGC	15.0	99.9
	Catechin	1.0	100.5
	EC	1.5	90.9
	EGCG	15.0	99.9
	GCG	2.0	95.5
	ECG	3.0	96.5
Green Tea Brand B	GC	1.0	84.2
	EGC	15.0	94.4
	Catechin	1.0	89.8
	EC	2.0	95.1
	EGCG	15.0	96.8
	GCG	2.0	93.8
	ECG	3.0	91.7

Table 4 summarizes the amounts spiked and the calculated recoveries. Recoveries ranged from 84.2% for ECG to 112% for EC.

CONCLUSION

This work describes a simple, sensitive, rapid, and accurate method to separate and quantify catechins in different commercially available teas with a simple solvent extraction. The method uses a high-resolution silica-based Acclaim RSLC C18 column and absorbance at a wavelength of 280 nm to separate and detect catechins in less than 20 min. Catechin concentrations in NIST controls and samples were determined using the described method, and the values reported were in agreement with certified NIST values. The catechin concentrations varied over a wide range in the samples, from 427.3 mg/g of EGCG in the NIST control to 2.25 mg/g of EC in black tea. The method described here is ideal for routine screening and quantification of catechins in different tea products.

PRECAUTIONS

Trifluoroacetic acid is corrosive, causes burns, and is harmful if swallowed, inhaled, or absorbed through skin. This material is extremely destructive to the upper respiratory tract, eyes, and skin. In case of contact, immediately flush eyes or skin with plenty of water for at least 15 min while removing contaminated clothing and shoes. Wash clothing before reuse. If inhaled, quickly move the exposed person to a source of fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. If the product is swallowed, do not induce vomiting. Give large quantities of water. Never give anything by mouth to an unconscious person. In all cases, call a physician immediately. Please read the material safety data sheets (MSDS) prior to handling and contact a licensed waste disposal organization to ensure all disposals are in accordance with existing federal, state, and local environmental regulations.

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SUPPLIERS

Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO 63103, U.S.A. Tel: 800-521-8956. www.sigmaaldrich.com

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