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Determination of Catechins and Caffeine in *Camillia sinensis* Raw Materials, Extracts, and Dietary Supplements by HPLC-UV: Single-Laboratory Validation

Mark C. Roman,
Tampa Bay Analytical Research, 13160 56th Ct, Suite 606, Clearwater, FL 33760

Jana Hildreth, and
Blaze Science Industries, 4547 West 171st St, Lawndale, CA 90260

Silvia Bannister
Hightower Pharmaceutical Services Corp., 5035 Barrowe Dr, Tampa, FL 33624

Abstract

A rapid method has been developed to quantify seven catechins and caffeine in green tea (*Camillia sinensis)* raw material and powdered extract, and dietary supplements containing green tea extract. The method utilizes RP HPLC with a phenyl-based stationary phase and gradient elution. Detection is by UV absorbance. The total run time, including column re-equilibration, is 13 min. Single-laboratory validation (SLV) has been performed on the method to determine the repeatability, accuracy, selectivity, LOD, LOQ, ruggedness, and linearity for (+)-catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, (−)-gallocatechin gallate, (−)-epigallocatechin gallate, and (+)-gallocatechin, as well as caffeine. Repeatability precision and recovery results met AOAC guidelines for SLV studies for all catechins and caffeine down to a level of approximately 20 mg/g. Finished products containing high concentrations of minerals require the use of EDTA to prevent decomposition of the catechins.

Green tea is derived from the leaves of *Camellia sinensis*, and produced by drying and steaming the leaves (1). Unlike black tea, it is not fermented. Green tea contains a significant amount of catechins, of which at least seven have been identified: (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-gallocatechin gallate (GCG), (−)-epigallocatechin gallate (EGCG), and (+)-gallocatechin (GC; 2; Figure 1). Of these, EGCG is generally the most abundant. These catechins are potent antioxidants. When green tea is fermented (oxidized) to make black tea, the catechins polymerize to form theaflavins and thearubigins.

In addition to the catechins, green tea and products derived from it may contain methylxanthines (caffeine, theophylline, and theobromine), of which caffeine is the most significant, with approximately 0.8–3.4% in leaf material on a dry weight basis (2). The presence and amount of methylxanthines in green tea extracts is dependent upon the specific extraction and processing procedures.

Corresponding author's: mroman@tampabayanalytical.com.

There presently are a large number of dietary supplements containing green tea in the U.S. marketplace, mostly in products marketed for weight loss. These products usually contain an extract of green tea that may or may not contain caffeine. The extracts can be present in the supplement alone, but they are often combined with other ingredients, including vitamins; other botanicals, such as ginseng, bitter orange, white willow bark, and guarana; and ingredients like caffeine, coenzyme Q10, and others.

Green tea has been investigated for use in preventing and treating a variety of cancers, such as breast and skin cancers (3, 4). It has also been studied for prevention of cardiovascular disease by lowering cholesterol levels (5), aiding in weight loss (6), protecting skin from sun damage (7), and improving cognitive function (8). Regular consumption of either green or black tea also may help reduce the risk of age-related degenerative brain disorders such as Alzheimer's disease (9).

Currently, there are no AOAC *Official Methods of Analysis* for the determination of catechins in green tea raw material, extracts, and/or dietary supplements. As a result, the National Institutes of Health, Office of Dietary Supplements, submitted a Request for Proposals to develop, optimize, and validate an analytical method for the determination of EGCG and related catechins (EC, ECG, EGC, GC, and GCG) in tea [*C. sinensis (*L.) Kuntze] raw materials and finished products for use by the greater dietary supplement community.

A number of papers have been published for the analysis of catechins in green tea materials (e.g., 10–22). These papers generally do not report data on their performance with finished products, have long run times (>30 min), demonstrate incomplete resolution of the catechins, and/or lack sufficient validation data to evaluate whether or not the methods are performing as expected.

A method capable of separating all seven catechins of interest, as well as caffeine in green tea raw materials (ground botanical and extracts) and dietary supplement products that contain green tea extract was developed and validated. The method uses aqueous extraction followed by RP-HPLC with UV detection. A phenyl stationary phase was found to provide optimum resolution between the analytes, and use of 3 μm particle size and short column length (100 mm) enabled rapid analysis (10 min). Detection was achieved at 278 nm. The accuracy, repeatability, linearity, range, selectivity, and ruggedness of the method were demonstrated.

Experimental

Materials

Powdered, lyophilized *C. sinensis* leaves, standard reference material (SRM 3254), *C. sinensis* extract (SRM 3255), and powdered solid oral dosage form containing *C. sinensis* extract (SRM 3256) was obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD). A commercially available multivitamin/mineral supplement tablet product was used as the negative control.

Apparatus

a. *HPLC system*.—Dionex Summit (Dionex Corp., Sunnyvale, CA) or Ultimate 3000 RSLC systems with quaternary (low-pressure mixing) gradient pumps, autosampler, temperature-controlled column compartment, and photodiode array (PDA; Dionex Summit system) or variable wavelength UV (Dionex Ultimate) detector. Systems were controlled and data collected and analyzed by Dionex Chromeleon software (version 6.8). The system was operated under the following conditions: mobile phase flow rate, 0.96 mL/min; column temperature, 35°C; injection volume, 30 μL; and detection, 278 nm.

b. *HPLC column*.—Ascentis Phenyl, 3.0 × 100 mm, 3 μm particle size (Supelco, Bellefonte, PA).

c. *Analytical balance*.—AT261 (Mettler Toledo, Columbus, OH), ±0.01 mg readability.

d. *Wrist action shaker*.—Model 75 (Burrell, Pittsburgh, PA).

e. *Ultrasonic bath*.—DSD150A2QS (Kwun Wah Intl Ltd, Wanchai, Hong Kong).

f. *Benchtop centrifuge*.—Quest Diagnostics Vanguard V6500 (Hamilton Bell Co., Montvale, NJ), 1000–1300 g.

g. *Mobile phase filtration apparatus*.—Equipped with a 0.2 μm nylon membrane filter (Sigma-Aldrich, St. Louis, MO).

h. *Laboratory micromill*.—A11 Basic (IKA Works Inc., Wilmington, NC).

Reagents

a. *Reference standards*.—(*Note*: All purities were obtained from the supplier certificate of analysis and were determined by chromatographic purity, water content, and residual solvent content. No independent confirmation of the purity was performed).

1. *GC*.—Blaze Science Industries (BSI, Lawndale, CA) Lot 01062009, 97.2% purity.
2. *EGC*.—BSI Lot 01052009, 96.0% purity.
3. *C*.—BSI Lot 01062009, 98.3% purity.
4. *EC*.—BSI Lot 01052009, 98.0% purity.
5. *EGCG*.—BSI Lot 01052009, 96.3% purity.
6. *GCG*.—BSI Lot 01052009, 97.9% purity.
7. *ECG*.—BSI Lot 01052009, 97.8% purity.
8. *Caffeine*.—Sigma Lot 105K0089, 99.7% purity.

All standards were stored in a desiccator at room temperature until use.

b. *Solvents*.—Acetonitrile (Spectrum, New Brunswick, CT), methanol (Pharmco, Brookfield, CT), and water (in-house), HPLC grade.

c. *Phosphoric acid, 85%*.—American Chemical Society (ACS) reagent grade (EMD).

d. *Acetic acid*.—Glacial (Sigma-Aldrich).

e. *Potassium hydroxide, 85%*.—ACS reagent grade (Sigma-Aldrich).

f. *EDTA disodium salt*.—Reagent grade (Reagents, Inc., Charlotte, NC).

g. *1 N KOH in water*.—Dissolve 5.6 g KOH in 100 mL water and allow to equilibrate to room temperature.

h. *Diluent 1*.—Dissolve 5 g EDTA disodium salt in 500 mL containing approximately three drops 1 M KOH solution. Add 500 mL methanol and 10 mL acetic acid, and stir until mixed and equilibrated to ambient temperature.

i. *Diluent 2*.—Dissolve 200 mg EDTA disodium salt in 2 L water with stirring. Add 2.0 mL 85% phosphoric acid and mix well.

j. *Mobile phase A (50 + 950 + 1, v/v/v acetonitrile–water–H_3PO_4 + 0.1 g/L EDTA)*.—Add 50 mL acetonitrile and 100 mg EDTA disodium salt to 950 mL water. Stir until all of the EDTA is dissolved and add 1 mL phosphoric acid, 85%. Filter through a 0.2 μm nylon membrane filter.

k. *Mobile phase B (350 + 650 + 1, v/v/v acetonitrile–water–H_3PO_4 + 0.1 g/L EDTA)*.—Add 350 mL acetonitrile and 100 mg EDTA disodium salt to 650 mL water. Stir until all of the EDTA is dissolved and add 1 mL of phosphoric acid, 85%. Filter through a 0.2 μm nylon membrane filter.

Preparation of Test Solutions

a. *Stock standard solution*.—Accurately weigh about 10 mg each of (+)-C, (−)-EC, (−)-ECG, (−)-EGC, (−)-GCG, (−)-EGCG, (+)-GC, and caffeine reference standards and transfer into a 100 mL volumetric flask. Add approximately 2 mL methanol and 70 mL Diluent 2 to the flask and sonicate for 15 min. Allow to cool to room temperature; then dilute to volume with Diluent 2. This solution contains about 100 μg/mL each catechin and caffeine.

b. *Instrument calibration solutions*.—Prepare serial dilutions of the stock standard solution in water at concentrations of about 1, 5, 10, 20, and 50 μg/mL each compound.

c. *Botanical raw materials*.—Accurately weigh about 150 mg powdered *C. sinensis* leaf material and transfer into a 100 mL volumetric flask. Add approximately 60 mL Diluent 2 and sonicate the slurry for 15 min with occasional shaking. Allow the solution to cool to room temperature and dilute to volume with Diluent 2. Mix the resulting material well and centrifuge a 15 mL portion for 10 min. Transfer an aliquot of the supernatant solution into an HPLC autosampler vial for analysis.

d. *Powdered extracts*.—Accurately weigh about 400 mg powdered *C. sinensis* extract and transfer into a 100 mL volumetric flask. Add 60 mL Diluent 2 and sonicate the

slurry for about 15 min. After cooling to room temperature, dilute the mixture to volume with Diluent 2 and mix well. Pipet 5.0 mL of this solution into a 50 mL volumetric flask and dilute to volume with Diluent 2. Mix the resulting solution well and centrifuge a 15 mL portion for 10 min. Transfer an aliquot of the supernatant solution into an HPLC autosampler vial for analysis.

e. *Dietary supplement capsules*.—Empty the contents of 20 whole capsules and mix the fill material well. Weigh about 1000 mg capsule fill material into a 100 mL volumetric flask. Add 60 mL Diluent 1 and sonicate the slurry for about 15 min, then shake the slurry on a mechanical shaker for 10 min. After cooling to room temperature, dilute the mixture to volume with Diluent 1 and mix well. Pipet 5.0 mL this solution into a 50 mL volumetric flask and dilute to volume with Diluent 2. Mix the resulting solution well and centrifuge a 15 mL portion for 10 min. Transfer an aliquot of the supernatant solution into an HPLC autosampler vial for analysis.

f. *Dietary supplement tablets*.—Grind 20 tablets in a laboratory micromill to a fine powder so that it passes through a 60 mesh screen. Weigh about 1000 mg powdered tablet material into a 100 mL volumetric flask. Add 60 mL Diluent 1 and sonicate the slurry for about 15 min; then shake the slurry on a mechanical shaker for 10 min. After cooling to room temperature, dilute the mixture to volume with Diluent 1 and mix well. Pipet 5.0 mL this solution into a 50 mL volumetric flask and dilute to volume with Diluent 2. Mix the resulting solution well and centrifuge a 15 mL portion for 10 min. Transfer an aliquot of the supernatant solution into an HPLC autosampler vial for analysis.

Determination

a. *Mobile phase gradient program*.—Elute the analytes with the following linear gradient program of mobile phases A and B: 0 min, 100% mobile phase A to 100% mobile phase B at 9 min. The column should be re-equilibrated at the starting mobile phase conditions for at least 4 min after each injection.

b. *System suitability tests*.—Make duplicate injections of the stock standard solution and each calibration standard. The correlation coefficient of the calibration line for each catechin and caffeine must be >0.999. The RSD of the calibration curve should be no more than 3.0% for each compound. The resolution between catechin and caffeine in the first stock standard solution injection must be ≥2.0. The resolution between caffeine and EC in the first stock standard solution injection must be no less than 2.0. The resolution between EGCG and GCG in the first stock standard solution injection must be no less than 1.0. The tailing factor, calculated at 5% peak height, must be no more than 1.2 for ECG in the first stock standard solution chromatogram.

c. *Injection*.—Make single injections of each standard and test solution. After every 20 sample injections, and after all of the sample injections are completed, make a single injection of each standard solution.

d. *Retention times*.—The approximate retention times for each analyte are presented in Figure 2.

e. *Chromatograms*.—Representative standard and sample chromatograms are presented in Figures 2–5.

Calculations

a. *Concentration of standards in stock standard solution*.— The concentration (C) of each standard in the stock standard solution, in μg/mL, is calculated using the following equation:

$$C = \frac{w}{100} \times 1000$$

where *w* = mass of the standard in mg, 100 = dilution volume in mL, and 1000 = conversion factor from mg to μg.

b. *Percentage (w/w) in leaf material*.—The percentage of each catechin and caffeine in powdered leaf material and extract samples is calculated using the following equation:

$$\frac{A_i - b_i}{m_i} \times \frac{100}{W} \times \frac{100\%}{1000}$$

where A_i = peak area of compound "*i*" in the sample chromatogram, b_i = y-intercept of calibration curve for compound "*i*," m_i = slope of calibration curve for compound "*i*," 100 = sample volume in mL, *W* = mass of sample in mg, 100% = conversion to % (w/w), and 1000 = conversion from μg to mg.

c. *Percentage (w/w) in extract*.—The percentage of each catechin and caffeine in powdered leaf material and extract samples is calculated using the following equation:

$$\frac{A_i - b_i}{m_i} \times \frac{100}{W} \times \frac{25}{1000} \times 100\%$$

where A_i = peak area of compound "*i*" in the sample chromatogram, b_i = y-intercept of calibration curve for compound "*i*," m_i = slope of calibration curve for compound "*i*," 100 = sample volume in mL, *W* = mass of sample in mg, 25 = dilution factor, 1000 = conversion from μg to mg, and 100% = conversion to % (w/w).

d. *Mg/capsule/tablet*.—The mg of each catechin and caffeine/capsule or tablet in dietary supplements is calculated using the following equation:

$$\frac{A_i - b_i}{m_i} \times \frac{100}{W} \times 25\frac{DW}{1000}$$

where A_i = peak area of compound "*i*" in the sample chromatogram, b_i = y-intercept of calibration curve for compound "*i*," m_i = slope of calibration curve for compound "*i*," 100 = sample volume in mL, *W* = mass of sample in mg, 25 = dilution factor, *DW* = average dosage weight in mg, and 1000 = conversion from μg to mg.

Validation Design

Linearity

The stock standard solution and each calibration dilution were each injected at the beginning of each chromatographic injection sequence, after every 20 sample injections, and at the end of each sequence. A five-point standard curve was generated for each analyte, and the slope, y-intercept, correlation coefficient, and RSD of the standard curve were calculated for each analyte on each day.

Accuracy

a. *C. sinensis powdered leaf material*.—Because of limitations in spike recovery results with botanical raw materials, a portion of the NIST *C. sinensis* powdered leaf material used in the study was exhaustively extracted using Soxhlet extraction with methanol to obtain a reference value with which to compare the proposed sample extraction procedure using sonication. The results obtained using the sonication extraction technique were compared to these reference values.

b. *Spike recovery of dietary supplement finished products.*—Spike recovery experiments were performed using the NIST *C. sinensis* extract. About 1000 mg of the composited negative control was transferred into each of 10 separate 100 mL volumetric flasks. About 100 mg NIST SRM 3255 was weighed and transferred into three of the flasks. Into another three flasks, about 250 mg NIST SRM 3255 was accurately weighed and transferred, and into another three, about 50 mg NIST SRM 3255. The tenth flask was used as a negative control. All samples were prepared and analyzed according to the method on 3 separate days, for a total of nine determinations at each spiking level. New calibration solutions were prepared on each day.

Repeatability

Five replicates of each of the three materials (powdered leaf material, powdered extract, and solid oral dosage form) were prepared on each of 3 days, for a total of 15 replicate preparations of each material. New calibration solutions were prepared on each day. The within-day, between-day, and total repeatability were calculated. The HorRat value (23) for each material was also calculated.

Ruggedness

Analyses were performed on two different HPLC systems (Dionex Summit and Dionex Ultimate 3000 RSLC). In addition, two different lots of HPLC columns were used. A Youden ruggedness study (24) examining the seven variables presented in Table 1 was performed.

Selectivity

Catechin and caffeine retention times were verified by injection of the individual reference standards. Selectivity of the method was confirmed the PDA detector, and by injecting the dietary supplement matrix blanks.

Results and Discussion

The optimized chromatographic system was able to achieve baseline resolution of all seven catechins and caffeine in fewer than 10 min. The short column length, small particle size, and high linear flow rate of the mobile phase enabled a short re-equilibration time between injections. Total turnaround time from injection to injection was 13 min.

Catechins are reactive with metal ions, unstable in basic solution, and can bind to various materials. For these reasons, analysis of catechins in complex matrixes, such as dietary supplements, can be challenging. The multivitamin/multimineral negative control used for the spike recovery studies was chosen to challenge the candidate method and provide a "worst-case" scenario for catechin analysis due to the presence of high concentrations of nutritional elements, such as calcium, magnesium, iron, and zinc, as well as trace levels of other minerals that could react with the catechins, and the inclusion of numerous water and fat soluble vitamins that could act as potential interferents. The addition of EDTA as a chelating agent and acetic and phosphoric acids to the diluents helped stabilize the catechins in solution and prevent decomposition. A small amount of EDTA in the mobile phases also helped prevent decomposition/binding with trace ions in the chromatographic system. Centrifugation of the test solutions as opposed to filtration prevented binding of the catechins to filter membranes.

Selectivity

The selectivity of the method was demonstrated by injecting each of the reference standards to show resolution among all of them, and injecting the negative control dietary supplement to show that there were no interfering peaks above the LOQ of the method. PDA detector analysis was used to ensure the peak purity of the seven catechins and caffeine in representative sample solutions. Typical standard and sample chromatograms are presented in Figures 2–5. The negative control chromatogram is presented in Figure 6.

Linearity

The calibration solutions contained catechins and caffeine and concentrations ranging from approximately 1 to 100 μg/mL. A five-point calibration curve was generated at the beginning and end of each day of analysis. Linear regression was used to calculate the slope and y-intercept of the standard curve for each analyte. For those analytes present in green tea samples in the lowest concentration, C and GCG, unit-weighted regression (1/X) was used. Nonweighted linear regression was used for all other analytes. The correlation coefficient and RSD of each standard curve for each day was determined. The data showed standard curves were linear from a concentration of about 1 to about 100 μg/mL for each analyte. Table 2 summarizes the linearity data.

Accuracy

C. sinensis powdered leaf material—The results for each catechin and caffeine for the *C. sinensis* powdered leaf material obtained using exhaustive Soxhlet extraction were used as reference values. Recovery was calculated from the average results obtained for the precision study for this material. Individual analyte recovery values ranged from 91.0 to 109%, with a total catechin recovery of 97.4%, and were within acceptable limits for an AOAC single-laboratory validation (SLV) study (25). Table 3 presents the recovery data of catechins and caffeine from the *C. sinensis* powdered leaf material.

Dietary supplements—Spike recovery studies were used to determine the recovery of the catechins and caffeine from a complex dietary supplement matrix. A multivitamin/multimineral tablet product was selected as a negative control (matrix blank). The NIST SRM 3255 *C. sinensis* powdered extract was used for the spiking studies due to the limited amount of individual reference standards available. Recoveries were calculated from the weight fraction of the analyte in the extract found compared to the reference value obtained from the precision study on the extract. Table 4 presents the recovery data of the catechins and caffeine from the dietary supplement matrix. Recoveries of the catechins decreased with decreasing concentration in the negative control. It is believed that even with the use of a high concentration of EDTA in the extraction solvent, the high mineral content of the negative control still caused some decomposition of the catechins. The negative control contained approximately 150 mg/g calcium, 40 mg/g magnesium, 1.2 mg/g iron, and 1 mg/g zinc; it was also fortified with other elements in lesser amount. Nevertheless, the recoveries were still in the range of 78.7–105% at the lowest spike concentration, corresponding to a mass fractions of 1.74 to 19.9 mg/g of analyte in the sample (extract + negative control). At the middle spike level, corresponding to analyte mass fractions of 3.22–38 mg/g in the sample, recoveries were an acceptable 90.5–116%. At the high spike level, corresponding to analyte mass fractions of 10.1–119 mg/g in the sample, recoveries were 93.2–121%.

Repeatability

The method exhibited good repeatability for all three matrixes. Tables 5–7 present a summary of the repeatability results. GC and C typically exhibited the worst precision in terms of the HorRat values; these analytes had the poorest sensitivity, and were present in relatively low concentrations in the samples. Other catechins and caffeine typically had HorRat values between 0.2 and 1.3, with the exception of GCG in the solid oral dosage form, which had a HorRat >2. The repeatability RSD for GCG in this sample was 11%; however, the total catechin RSD was still acceptable because GCG is a minor component constituting <5% of the total catechin amount. Total catechin content yielded HorRat values <1.

Ruggedness

The Youden ruggedness trial was conducted using the NIST SRM 3255 green tea extract. The results of the Youden ruggedness study are presented in Table 8, and the factors plotted in Figure 6. Catechin was not included in the ruggedness study because of the low concentration in the sample and poor precision at this level.

Based on the results of the Youden ruggedness trial, the most significant factor affecting the results was detection wavelength. The largest effect was on GC, followed by EGC.

The precision studies were conducted on two different HPLC instruments: a Dionex Summit with a PDA detector and a Dionex Ultimate 3000 RSLC (ultra-performance LC-type) with multiwavelength detector. Although the RSLC instrument yielded higher efficiency separations and better sensitivity, particularly for the early-eluting GC and EGC peaks, there was no statistical differences in the quantitative results obtained by the two instruments. The chromatograms presented in Figures 2–5 were obtained on the conventional Summit HPLC system. Two different lots of the HPLC column were also used during the precision studies, with no statistically meaningful differences in efficiency, resolution, or retention.

Conclusions

A method was developed and optimized, and an SLV study performed for the determination of EGCG and the related C, EC, ECG, EGC, GCG in tea [*C. sinensis* (L.) Kuntze] raw materials and finished products. The method is rapid (13 min from injection to injection) and able to achieve baseline resolution of all major catechins and caffeine. Spike recovery and exhaustive extraction studies showed good accuracy for the determination of these compounds using the proposed method. Repeatability studies showed good precision for most individual catechins, total catechins, and caffeine in all three materials, with the exception of GCG in the solid oral dosage form. The higher-than-expected RSD should be noted if the method is used for quantification of GCG in solid oral dosage forms; however, the RSD of the total catechin content is not significantly affected.

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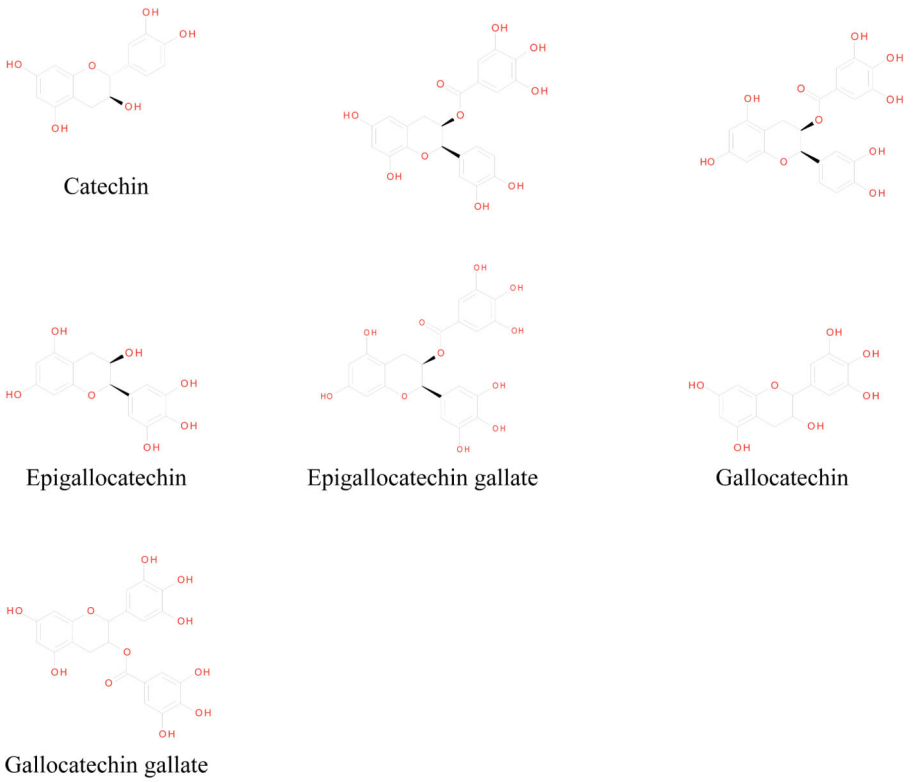

Figure 1.
Structures of catechins.

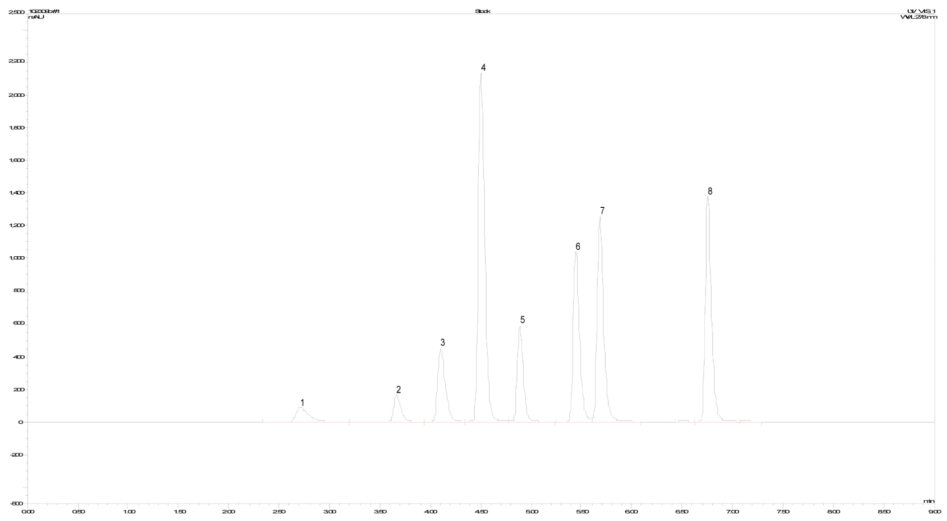

Figure 2.

Stock standard solution chromatogram. Peak assignments and approximate retention times: (1) GC (2.7 min), (2) EGC (3.7 min), (3) C (4.1 min), (4) caffeine (4.5 min), (5) EC (4.9 min), (6) EGCG (5.5 min), (7) GCG (5.7 min), and (8) ECG (6.8 min).

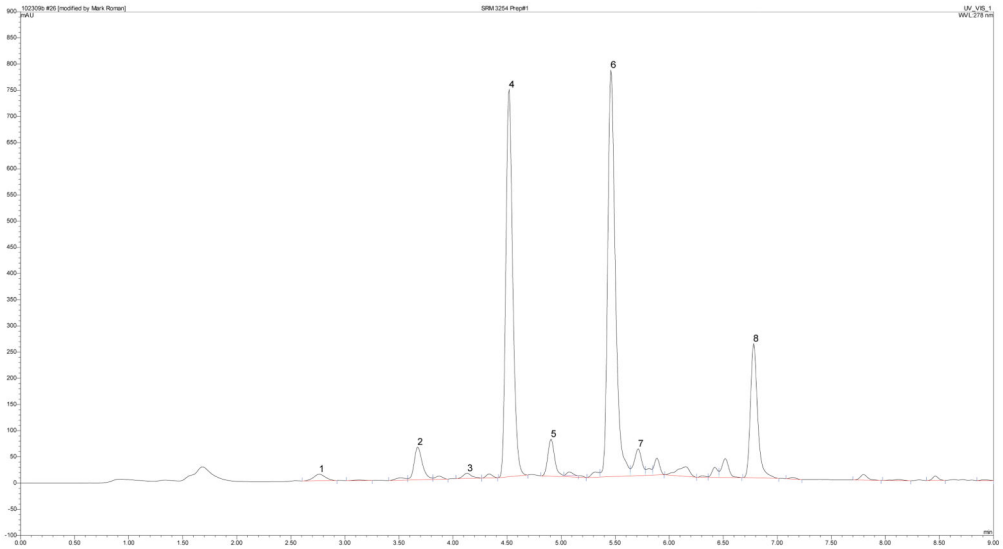

Figure 3.
NIST SRM 3254 *C. sinensis* powdered leaf chromatogram; peaks identified as in Figure 2.

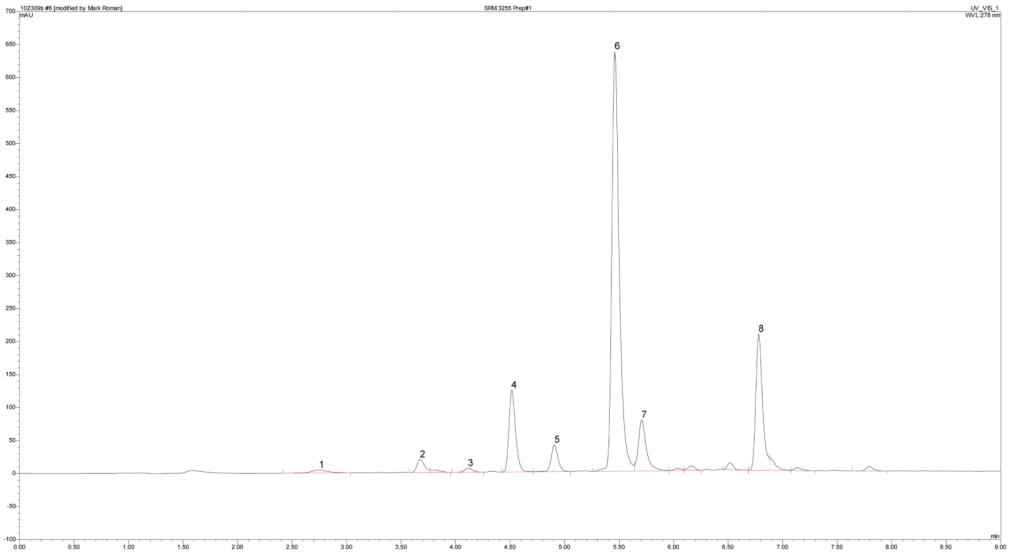

Figure 4.
NIST SRM 3255 *C. sinensis* powdered extract chromatogram; peaks identified as in Figure 2.

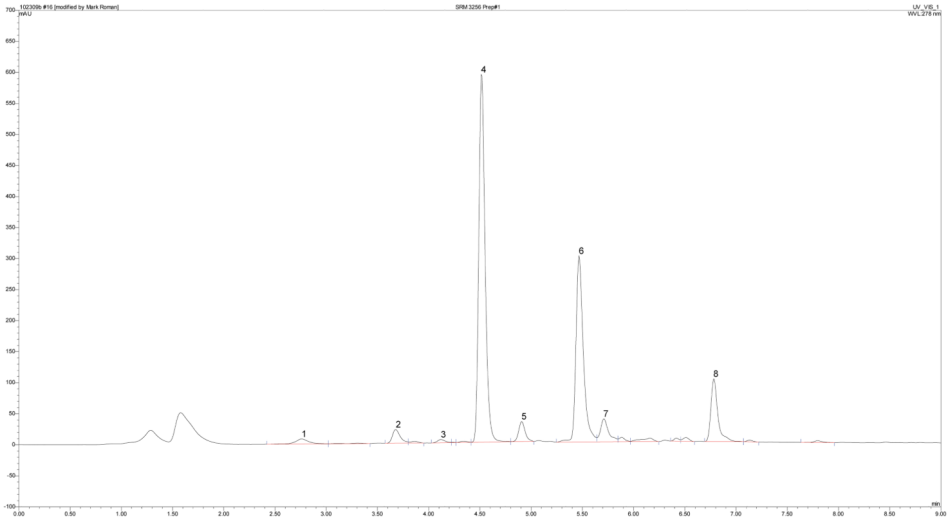

Figure 5.
NIST SRM3256 solid oral dosage form containing green tea chromatogram; peaks identified as in Figure 2.

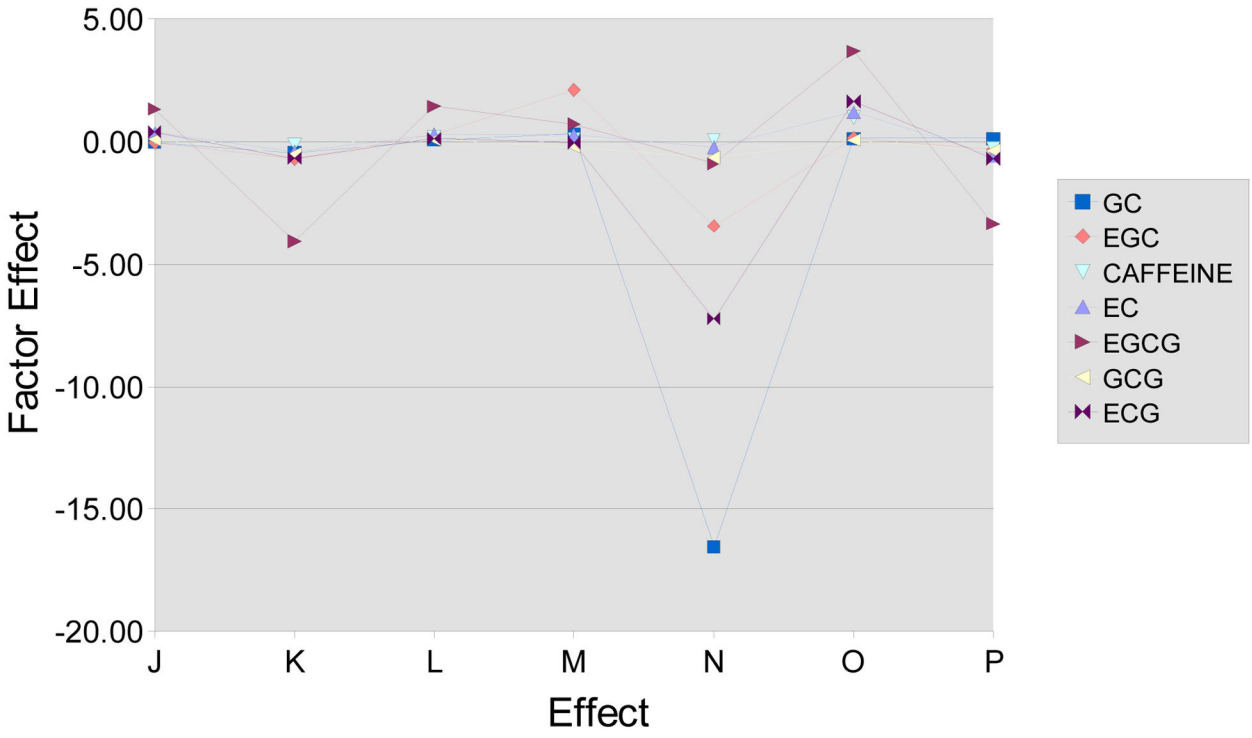

Figure 6.

Youden ruggedness trial plot: J = sonication time, K = sample weight, L = H_3PO_4 concentration in diluents, M = EDTA concentration in diuents, N = detection wavelength, O = injection volume, and P = light exposure.

Table 1

Ruggedness testing—factors examined

Factor	High value	Low value
Sonication time, min	A = 15	a = 10
Sample weight, mg	B = 400	b = 300
H_3PO_4 concn in diluents, %	C = 0.1	c = 0.05
EDTA concn in diluents, mg/mL	D = 0.1	d = 0.05
Detection wavelength, nm	E = 282	e = 274
Injection volume, μL	F = 50	f = 25
Light exposure, min	G = 30	g = 0

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Table 2

Linearity data

	Day 1		Day 2		Day 3	
	r^{2a}	RSD, %	r^2	RSD, %	r^2	RSD, %
GC	0.99958	2.60	0.99997	0.66	0.99975	1.98
EGC	0.99960	2.52	0.99998	0.58	0.99994	0.97
C	0.99969	2.22	0.99999	0.91	0.99991	2.99
Caffeine	0.99942	2.97	0.99997	0.74	0.99992	1.25
EC	0.99970	2.18	0.99999	0.46	0.99989	1.34
EGCG	0.99964	2.42	0.99999	0.29	0.99991	1.21
GCG	0.99964	2.42	0.99999	0.30	0.99987	1.46
ECG	0.99977	1.92	0.99997	0.71	0.99939	3.07

[a] r2 = Coefficient of determination.

Table 3

NIST *C. sinensis* powdered leaf material recovery

Compound	Soxhlet extraction, mg/g	Sonication, μg/g	Recovery, %
GC	7.32	8.01	109
EGC	24.5	25.5	104
C	1.26	1.31	104
Caffeine	22.9	23.1	101
EC	8.7	8.38	96.3
EGCG	55.0	51.6	93.8
GCG	2.91	3.06	105
ECG	14.4	13.1	91
Total	114	111	97.4

Table 4

Recovery of catechins and caffeine from dietary supplement negative control

Spike	Compound	Actual concn, mg/g[a]	Total mass fraction, mg/g[b]	Amt found, mg/g[a]	Recovery, %	RSD, %[c]
Low	GC	35.4	1.69	A[d]	NA[e]	NA
	EGC	73.9	3.52	80.6	105	4.39
	C	8.14	0.39	A	NA	NA
	Caffeine	36.7	1.74	28.9	78.7	4.85
	EC	46.4	2.21	37.0	79.7	3.91
	EGCG	418	19.9	364	87.1	0.72
	GCG	46.8	2.23	46.0	98.3	3.46
	ECG	105	5.00	92.8	88.4	1.16
Middle	GC	35.4	3.22	32.4	91.5	3.15
	EGC	73.9	6.72	85.7	116	2.85
	C	8.14	0.74	A	NA	NA
	Caffeine	36.7	3.34	33.3	90.7	1.89
	EC	46.4	4.22	42.0	90.5	1.82
	EGCG	418	38.0	383	91.6	1.73
	GCG	46.8	4.25	47.7	102	2.74
	ECG	105	9.55	97.7	93.0	2.04
High	GC	35.4	10.1	33.0	93.2	0.90
	EGC	73.9	21.1	89.5	121	1.21
	C	8.14	2.33	A	NA	NA
	Caffeine	36.7	10.5	37.0	101	0.86
	EC	46.4	13.3	46.0	99.1	0.84
	EGCG	418	119.0	400	95.7	1.35
	GCG	46.8	13.4	46.6	99.6	4.38
	ECG	105	30.0	102	97.1	0.77

[a] Mass fraction of the extract.

[b] Mass fraction of the extract + negative control.

[c] RSD estimated from triplicate determinations.

[d] A = Below LOQ of method.

[e] NA = Not applicable.

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Table 5

NIST SRM 3254 *C. sinensis* powdered leaf material repeatability results

Material	n^a	Result, mg/g	SD, mg/g	RSD, %	PRSD, %^{b}	HorRat
GC	15	8.01	1.2	14.9	4.14	3.6
EGC	15	25.5	0.64	2.51	3.47	0.72
C	15	1.31	0.14	10.8	5.43	1.98
Caffeine	15	23.1	0.31	1.35	3.53	0.38
EC	15	8.38	0.43	5.16	4.11	1.26
EGCG	15	51.6	1.12	2.17	3.12	0.69
GCG	15	3.06	0.18	5.72	4.78	1.2
ECG	15	13.1	0.74	5.66	3.84	1.47
Total catechins	15	111	2.1	1.9	2.79	0.68

[a] n = Total number of samples tested.

[b] PRSD = Predicted RSD.

Table 6

NIST SRM 3255 *C. sinensis* powdered extract repeatability results

Material	n^a	Result, mg/g	SD, mg/g	RSD, %	PRSD, %^{b}	HorRat
GC	15	35.4	1.55	4.37	3.31	1.32
EGC	14	73.9	0.72	0.98	2.96	0.33
C	15	8.14	0.71	8.66	4.13	2.1
Caffeine	14	36.7	0.33	0.89	3.29	0.27
EC	14	46.4	1.26	2.71	3.17	0.85
EGCG	14	418	11.6	2.76	2.25	1.21
GCG	15	46.8	1.19	2.54	3.17	0.8
ECG	14	105	1.63	1.55	2.81	0.55
Total catechins	14	731	8.18	1.12	2.1	0.53

[a] n = Total number of samples tested.

[b] PRSD = Predicted RSD.

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Table 7

NIST SRM 3256 solid oral dosage form repeatability results

Material	n^a	Result, mg/g	SD, mg/g	RSD, %	PRSD, %^{b}	HorRat
GC	15	20	1.11	5.57	3.6	1.55
EGC	15	33.8	1.09	3.21	3.33	0.96
C	15	2.6	0.23	8.94	4.9	1.82
Caffeine	15	68.6	2.42	3.52	2.99	1.18
EC	15	13.7	0.51	3.71	3.82	0.97
EGCG	15	75.8	2.24	2.96	2.95	1
GCG	15	8.02	0.88	11	4.14	2.66
ECG	15	19.1	0.71	3.71	3.63	1.02
Total catechins	15	171	3.39	1.98	2.61	0.76

[a] n = Total number of samples tested.

[b] PRSD = Predicted RSD.

Table 8

Results of Youden ruggedness trial

Effect	J[a]	K[b]	L[c]	M[d]	N[e]	O[f]	P[g]
GC	−0.05	−0.47	0.05	0.29	−16.55	0.11	0.11
EGC	−0.07	−0.73	0.25	2.10	−3.46	0.14	−0.35
Caffeine	0.10	−0.14	0.16	0.11	0.03	0.97	−0.29
EC	0.34	−0.43	0.26	0.27	−0.24	1.19	−0.57
EGCG	1.32	−4.09	1.42	0.70	−0.92	3.67	−3.36
GCG	0.07	−0.53	0.06	−0.23	−0.68	0.05	−0.39
ECG	0.38	−0.71	0.11	−0.06	−7.25	1.63	−0.72

[a] J = Sonication time.

[b] K = Sample weight.

[c] L = H_3PO_4 concentration in diluent.

[d] M = EDTA concentration in diluent.

[e] N = Detection wavelength.

[f] O = Injection volume.

[g] P = Light exposure.