

A Dynamic Calibration Approach for Determining Catechins and Gallic Acid in Green Tea Using LC-ESI/MS

Mary Bedner and David L. Duewer
National Institute of Standards and Technology
Analytical Chemistry Division
Gaithersburg, MD 20899

* Corresponding author contact information: email: mary.bedner@nist.gov; Voice: 301-975-4142; FAX: 301-977-0685

Abstract

Catechins and gallic acid are antioxidant constituents of *Camellia sinensis*, or green tea. Liquid chromatography with both ultraviolet (UV) absorbance and electrospray ionization mass spectrometric (MS) detection was used to determine catechins and gallic acid in three green tea matrix materials that are commonly used as dietary supplements. The results from both detection modes were evaluated with fourteen quantitation models, all of which were based on the analyte response relative to an internal standard. Half of the models were static, where quantitation was achieved with calibration factors that were constant over an analysis set. The other half were dynamic, with calibration factors calculated from interpolated response factor data at each time a sample was injected to correct for potential variations in analyte response over time. For all analytes, the relatively non-selective UV responses were found to be very stable over time and independent of the calibrant concentration; comparable results with low variability were obtained regardless of the quantitation model used. Conversely, the highly-selective MS responses were found to vary both with time and as a function of the calibrant concentration. A dynamic quantitation model based on polynomial data-fitting was used to reduce the variability in the quantitative results using the MS data.

Keywords: catechins, gallic acid, green tea, mass spectrometry, liquid chromatography, dynamic calibration, dietary supplements, ultraviolet absorbance detection

Copyright Notice

Official contribution of the National Institute of Standards and Technology; not subject to copyright in the United States.

INTRODUCTION

Dietary supplements containing green tea consist at least partially of dried leaves or extracts of the plant *Camellia sinensis*. Green tea dietary supplements have gained popularity because of the antioxidant properties of many constituents including catechins and gallic acid (GA). While epigallocatechin 3-gallate (EGCG) receives the most public attention, there have been at least twelve catechins identified in green tea.¹ Most measurements of the catechin content in tea focus primarily on the seven major catechins, which include catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), gallocatechin 3-gallate (GCG), EGCG, and epicatechin 3-gallate (ECG) (structures in Figure 1). While there are several published studies in which individual catechins and other bioactive constituents have been measured in green tea dietary supplements, the measured values were rarely in agreement with the values indicated on the product labels.^{2;3} This demonstrates a need for both reliable methods and standards for catechins in green tea dietary supplements. The National Institute of Standards and Technology (NIST) is working in collaboration with the National Institutes of Health, Office of Dietary Supplements and the Food and Drug Administration, Center for Food Safety and Applied Nutrition and Center for Drug Evaluation and Research to develop dietary supplement Standard Reference Materials (SRMs). Unprocessed, extracted, and/or processed forms of each material are prepared that represent different analytical challenges. NIST has developed three SRMs that represent different matrices commonly used for green tea dietary supplements: SRM 3254 *Camellia sinensis* Leaves, SRM 3255 *Camellia sinensis* Extract, and SRM 3256 Green Tea-Containing Solid Oral Dosage Form. The goal of the present investigation was to develop a procedure for accurately determining the seven major catechins and gallic acid in each of these SRMs.

Catechins are commonly measured in green tea using liquid chromatographic methods with ultraviolet absorbance (UV)³⁻¹² and/or mass spectrometric (MS) detection,^{1;13-17} but electrochemical detection has also been used for green tea matrices^{18;19} and human urine.²⁰ Quantitation of catechins in green tea is most frequently performed with absorbance detection and calibration with external standards^{3-7;10-12}, but there is one report of UV detection and calibration with an internal standard approach.⁸ In that study, catechins and gallic acid were determined in green tea matrix materials using catechol as an internal standard,⁸ even though it is not a structural analogue of the analytes.

Catechins have been determined in biological samples^{21;22} and cosmetics²³ using MS detection, but reports of quantitation of catechins in green tea using MS detection are more limited.¹³⁻¹⁷ In one investigation, Pelillo *et al.* compared quantitation of catechins in a green tea extract using both UV and electrospray ionization (ESI)-MS detection and calibration with external standards.¹³ They compared catechin calibration curves obtained for freshly prepared standards to those obtained when the standards were a week old, and the UV responses were found to be stable while the MS responses had changed considerably. They concluded that MS detection lacks both precision and accuracy for catechin determinations and that frequent calibration is necessary. In another study, Shen *et al.* utilized LC-ESI/MS with the isoflavone glycitein as an internal standard for quantitation of the catechins C, EC, ECG, and EGCG in the plant catechu.²⁴ Glycitein is structurally related to the catechins, but it is less polar and had a significantly longer retention time than the catechin analytes. Linear calibration curves were obtained for the four catechins, but the measurement relative standard deviation was reported to be as high as 9.4 %. These studies indicate there are issues with MS detection that may limit the development of a

quantitative method with low variability for catechins. A quantitative method for catechins is desired because MS detection provides increased sensitivity and selectivity over UV detection.

We sought to address the measurement need for a reliable quantitative LC-MS approach for determining catechins in green tea matrix materials. In our experience, the absolute peak areas in MS detection can decrease as much as 40% over the course of a set of measurements, making calibration with external standards unreliable. We therefore used an internal standard to compensate for potential variations with the sample preparation and instrumental analysis. To develop a quantitative MS method, stable-isotope labeled compounds for each analyte are the preferred choice for use as internal standards. In comparison to unlabeled compounds, stable-isotope labeled internal standards can provide improved measurement precision for LC-MS measurements, which has been demonstrated for LC-MS measurements of alkaloids in SRMs containing ephedra and bitter orange.^{25;26} In those measurements, ephedrine-d3 and synephrine-d3 were used as internal standards for all alkaloid analytes, and lower measurement variability was obtained for ephedrine and synephrine. Stable-isotope labeled standards that coelute with the corresponding unlabeled analyte can compensate for variations in ionization efficiency in MS, but they are less effective when used as internal standards for other (separated) analytes. For the catechins, stable-isotope labeled standards were not readily available. Proxiphylline was selected as the internal standard even though it is not structurally related to the catechins or gallic acid (Figure 1), but it is a structural analogue of the xanthine components of green tea including caffeine, theobromine, and theophylline. The use of a general, non-labeled internal standard has already been demonstrated for catechins;^{8;24} it also permitted quantitative determinations with both UV and MS detection for the same samples, calibrants, and LC analyses. As validation for

our method with the non-labeled internal standard, we compared quantitation of catechins in the three green tea dietary supplement SRMs using several different calibration models for both UV and ESI-MS detection. While the UV responses were found to be stable, variable responses were observed for the catechins and gallic acid with MS detection. To address changing analyte responses, we developed novel quantitation models that were dynamic, indicating that calibration varied with time. The results from the dynamic models were compared to those from more traditional static, or non-time dependent, calibration approaches. The data from the different calibration models were evaluated both graphically and statistically, and the best models for quantitation of catechins and gallic acid in the green tea dietary supplement SRMs using each detection mode were identified.

EXPERIMENTAL SECTION*

Materials. Proxiphylline (7-(β -Hydroxypropyl)theophylline) was obtained from Sigma (St. Louis, MO) and was used as the internal standard, from here “IS.” Gallic acid was obtained from MP Biomedicals (Solon, OH). Standards for (-)-gallocatechin, (-)-epigallocatechin, (-)-catechin, (-)-epicatechin, (-)-epigallocatechin 3-gallate, (-)-gallocatechin 3-gallate, and (-)-epicatechin 3-gallate were obtained from Blaze Science Industries (Lawndale, CA). Formic acid was reagent grade, and water, methanol, and acetonitrile were HPLC-grade. The Green tea SRMs were obtained from the Standard Reference Materials Program (NIST, Gaithersburg, MD). SRM 3254 *Camellia sinensis* Leaves consists of ground and sieved green tea leaves, and SRM 3255 *Camellia sinensis* Extract consists of a dried powder from a solvent extract of green tea

* Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

leaves and was designed to have high catechin content. SRM 3256, Green Tea-Containing Solid Oral Dosage Form, consists of four commercial green tea dietary supplements (tablets and caplets) that were individually ground and sieved, then blended together. The three SRMs were packaged in nitrogen-flushed 4 mil polyethylene bags, which were then placed in nitrogen-flushed aluminized plastic bags containing two silica gel packets. The individual SRM packets were irradiated (absorbed dose 7.9 kGy to 9.5 kGy) to prevent mold growth.

Chromatographic conditions. An Agilent Technologies (Palo Alto, CA, USA) 1100 series liquid chromatograph with both a UV absorbance detector and an SL series MS detector (in series) was used to determine all analytes. The injection volume was 2 μ L of each calibration solution and sample. The analytical column, a Zorbax Eclipse XDB-C18 column from Agilent that was 25 cm x 4.6 mm with 5 μ m particles, was selected based on a literature separation of catechin standards.²⁷ Security Guard C18, 4 mm x 3.0 mm guard cartridges (Phenomenex, Torrance, CA) were used and replaced after every 24 injections of green tea matrix samples. The mobile phase was delivered at a flow rate of 1.0 mL/min and consisted of two solvents where A was 0.1 % formic acid in water and B was 0.1 % formic acid in acetonitrile (volume fractions). The composition of the mobile phase was increased linearly from 93% A, 7% solvent B to 83% A, 17% B in 55 min, followed by a linear increase to 20% A, 80% B from 55 to 60 min. The mobile phase was returned to the initial conditions in 1 min, followed with a post-run time of 10 min to ensure equilibration. The total elapsed time required for a given chromatographic analysis was thus 70 min.

Catechins have absorption maximums at both 210 nm and 280 nm, but 280 nm was selected to achieve better selectivity and to avoid baseline shifts from absorbance of the mobile phase during the gradient. MS detection was achieved using electrospray ionization (ESI) in positive polarity. Selected ion monitoring (SIM) was used for quantitation at m/z 171 for gallic acid (GA), m/z 239 for proxiphylline (IS), m/z 291 for catechin (C) and epicatechin (EC), m/z 307 for gallocatechin (GC) and epigallocatechin (EGC), m/z 443 for epicatechin gallate (ECG), and m/z 459 for gallocatechin gallate (GCG) and epigallocatechin gallate (EGCG). ESI/MS detection conditions included: fragmentor voltage, 130 V; drying gas flow, 12 L/min; drying gas temperature, 350 °C; nebulizer pressure, 379 kPa (55 psi); capillary voltage, 3500 V.

Calibration. All solutions were gravimetrically prepared in reduced lighting using a solvent composition of 30 % methanol and 0.05 % formic acid in water (volume fractions), which was also used as the extraction solvent for the green tea samples. An acidified solvent was used to prevent degradation of the catechins, which is accelerated at room temperature conditions and neutral pH.²⁸ The concentrations of the analytes were varied to generate calibration curves that covered the ranges expected for all green tea SRM samples, and the SRM sample sizes and extraction volumes were chosen to minimize the covered ranges. Four independent stock calibration solutions were prepared that contained weighed amounts of C, EC, GC, EGC, GCG, ECG, and GA in 20 mL weighed solution. The mass fraction ranges in the stock solutions were as follows: C, 12.41 µg/g to 41.34 µg/g; EC, 94.67 µg/g to 177.5 µg/g; GC, 23.68 µg/g to 104.8 µg/g; EGC, 200.6 µg/g to 387.0 µg/g; GCG, 10.83 µg/g to 108.0 µg/g; ECG, 192.2 µg/g to 294.4 µg/g; GA, 7.895 µg/g to 170.2 µg/g. Four independent stock calibration solutions were prepared that contained weighed amounts of EGCG in 10 mL weighed solution for a concentration range

of 812.2 µg/g to 1.485 mg/g. A solution of proxyphylline was gravimetrically prepared to be 0.64283 mg/g and was used as the IS. Working calibration solutions were prepared by weighing and mixing equal volumes of each of the stock calibration solutions and the IS solution to yield levels “A”, “B”, “C”, and “D” (in order of increasing concentration) for each analyte. All solutions were stored at 4 °C when not in use.

SRM Sample Preparation. Duplicate test portions were sampled from each of six units of each SRM, resulting in 12 independent measurements ($n=12$) of the mass fractions of each analyte. All sample preparation was performed in reduced lighting to minimize instability of the catechins. The following approximate sample sizes were used for each of the SRMs: SRM 3254, 90 mg; SRM 3255, 20 mg; SRM 3256, 60 mg. Approximately 5 mL (5 g) of internal standard was used for each SRM 3254 and SRM 3256 sample, and approximately 7 mL (7 g) was used for each SRM 3255 sample. To prepare samples, the appropriate amount of SRM and internal standard solution were accurately weighed into a 15 mL plastic tube with a screw cap. For SRM 3254 and SRM 3256 samples, the tubes were vortex-mixed for 10 sec then ultrasonically extracted for 90 min. The extractions were performed at room temperature to minimize analyte epimerization that can occur when hot solvents are used.¹⁸ The tubes were centrifuged at 6000 rpm for 10 min. The supernatant was transferred to another 15 mL plastic tube and stored in the refrigerator; 5 mL fresh solvent not containing the IS was then added to the original sample tube. The extraction process was repeated, based on a study by Perva-Uzunalić *et al* that recommended a multi-step extraction procedure for catechins.²⁹ The supernatant was combined with the first and vortex-mixed for 10 sec. For SRM 3255 samples, the tubes were vortex-mixed for 10 sec then sonicated for 5 min until the material was completely dissolved. An additional 7

mL solvent not containing the IS was added to the tube and the samples were vortex-mixed for 10 sec. For all SRM samples, a portion of the extract was transferred to a 5 mL plastic syringe and syringe-filtered with a 25 mm, 0.45 µm pore size, cellulose acetate filter with a GD/X prefilter into an LC vial for analysis. The sample vials were placed on the autosampler tray, which was maintained at 7 °C to preserve the samples and calibrants.

LC Analysis. The chromatographic analyses of the samples and calibrants for each SRM were analyzed as part of a single set, with each set requiring approximately three days for completion. The long time for each set was a result of the large number of injections and the 70 min chromatographic analysis time. The samples and calibrants were alternated throughout the analysis order so that the responses for the calibrants could be monitored over the entire set. For each analysis, the time of injection was noted and the UV and MS peak areas for each analyte and the internal standard were manually integrated. When a calibrant was analyzed, a relative response factor (RRF) was calculated for both UV and MS detection. The RRF was calculated as:

$$\text{Relative Response Factor (RRF)} = \frac{\text{Mass Analyte} \times \text{Area IS}}{\text{Mass IS} \times \text{Area Analyte}}$$

RESULTS AND DISCUSSION

MS SIM and UV chromatograms for a sample of SRM 3254, green tea leaves, are presented in Figure 2. Proxiphylline (IS) elutes in the middle of the eight target analytes, which minimizes the potential ionization differences between the internal standard and any given analyte during the gradient separation. The MS chromatograms (Figure 2, A-D) exhibit well-separated peaks with excellent signal-to-noise. MS detection is very selective and sensitive for the catechins and

gallic acid; the chromatograms in Figure 2 are representative of the MS chromatograms for all three GTDS SRMs. Conversely, the UV chromatogram (Figure 2, E) is less selective and contains many peaks in addition to the analytes and internal standard. The gradient conditions had been optimized to obtain the best separation of the catechins from matrix peaks, but baseline resolution was not possible for some of the analytes. The UV chromatograms for both SRM 3255 and SRM 3256 were similar to Figure 2E, but the chromatograms for the SRM 3255 purified extract material samples had slightly fewer matrix peaks.

Descriptions of Quantitation models. While the subsequent data discussion applies to all analytes in this study, for brevity epicatechin will be used to represent all catechins from this point forward. Graphical representations of calibrant RRF data for epicatechin and gallic acid obtained with both UV and MS detection are presented in Figure 3. Figure 3 presents the RRF value (normalized to the mean of all the RRF values in a given measurement set) over time for each of the four calibrant levels, designated A, B, C, and D. For UV detection, the RRF values are independent of calibrant level and consistent across the measurement set for both epicatechin and gallic acid. Conversely, the RRF values exhibit significant level- and time-dependence with MS detection, and gallic acid is the most variable of all analytes investigated. The consistent UV RRF values confirm that the analytes and the internal standard were stable in the calibration solutions. The variability of the RRF values with time and level (A, B, C, D) for the MS responses indicate that there are ionization differences attributable to both instrument fluctuations with time and to the concentrations of the analytes injected.

To address the significant differences between the UV and MS results, modeling of the calibration behavior was employed. The calibrants and sample data for each SRM measurement set and each detection method were analyzed with 14 different quantitation models, which are described in Table 1. Half of the models were static (“Stat”) or non-time dependent, and the other half were dynamic (“Dyn”), indicating that the calibration varied with time. For the dynamic models, quantitation was achieved using calibrant data that was interpolated at each time a green tea SRM sample was analyzed. Six of the models were based on calibrant RRF values (“RRF”) and did not emphasize differences between calibration levels. Figure 3 graphically represents four of the RRF-based quantitation models (black and gray-shaded lines) including one static model (StatRRF, All) and three dynamic models (DynRRF, All; DynRRF, Close; DynRRF, Closest); although all six models could have been depicted, four were selected as examples to illustrate the fundamental differences between the RRF-based models. For UV detection the four depicted quantitation models mostly overlap, but with MS detection the models are distinct, especially for gallic acid. The other eight models were calibration-based (“Cal”) and utilized linear or quadratic data fitting with either forced-zero or empirical intercepts. The four static calibration-based models were straightforward approaches based on both linear and quadratic fits of the average RRF for each calibrant level. The four dynamic quantitation models utilized a straight-line interpolation of the RRF data for each calibrant level (A-D) with time, depicted as the color-coordinated lines in Figure 3. The individual lines for each calibrant level were then interpolated at each time when a SRM sample was analyzed, resulting in four time-corrected calibration points. These points were then fit to different calibration curves based on linear and quadratic models (zero and non-zero intercepts). Each SRM set was comprised of duplicate injections of 12 SRM samples, resulting in 24 total time-corrected calibration curves

for each of the four dynamic quantitation models. The analyte concentration in the sample was determined using the time-corrected calibration curve corresponding with the time of injection.

Choice of Quantitation Model. The mass fractions of each of the analytes in the three SRMs were calculated using all 14 quantitation models for both detection modes. Figure 4 depicts the calculated mass fractions over time for epicatechin and gallic acid in SRM 3256 and SRM 3254, respectively, as determined with both UV and MS detection. Each point in the graph represents the mass fraction value (MF; normalized to the mean MF from all quantitation models) calculated for the sample that was injected at the indicated time. The mass fraction data are displayed for four quantitation models representing the basic model types of StatRRF, DynRRF, StatCal, and DynCal, but the choice of data-fit was different for the two analytes as indicated in the figure legend. The mass fraction values for both epicatechin and gallic acid are overlapping for the four depicted quantitation models for UV detection. There is no trending of the UV results for either analyte, but the mass fraction of gallic acid in SRM 3254 exhibits more variability due to the very low levels in the leaf material. For MS detection, the mass fraction results for gallic acid are significantly different with the four depicted quantitation models, but the RRF-based methods (depicted in blue) are the least divergent from zero on the normalized scale. The epicatechin mass fraction values obtained with MS detection exhibit significant upward trending with time for the static models (lines with solid markers), but the trending is minimized with the two dynamic quantitation models (open markers).

To estimate the average measurement variability associated with the different types of quantitation models, the percent relative standard deviations (% RSD) of the mass fraction data

for the seven catechins were pooled for each of the fourteen quantitation models, each detection mode, and each SRM. These pooled % RSDs were in turn pooled for the seven static, seven dynamic, six RRF, and eight Cal (level-dependent) models. Comparing the results for the catechins for each SRM, the lowest variability with MS detection was obtained with the dynamic models (% RSD = 3.1 to 4.7) while the highest variability was obtained with the static models (% RSD = 4.9 to 6.1). For gallic acid, the RSDs were pooled for the seven static, seven dynamic, six RRF, and eight “Cal” models; the variability with MS detection was lowest with the RRF models (% RSD = 2.6 to 5.0) and highest with the Cal models (% RSD = 3.2 to 6.5). Thus to minimize measurement variability with MS detection, the catechins should be quantitated with a dynamic model while gallic acid should be quantitated with a RRF model. For UV detection, the pooled variabilities were the same for all model types for both catechins and gallic acid.

Selection of the best quantitation model required consideration of many parameters. For UV detection, the RRF values for the calibrants were the same regardless of injection time or calibrant concentration (Figure 3). Also, the mass fractions for the SRM sample data were remarkably similar, regardless of the analyte or the quantitation model used (Figure 4). The pooled RSDs were also the same regardless of model type. Since all quantitation models are equally valid for the UV data, the simple static linear quantitation model with a zero intercept (model “StatCal, bX”) was selected for quantitation of all analytes.

Based on the pooled % RSDs for the different model types, the catechins had the lowest measurement variability for MS detection with the dynamic quantitation models. Also, since the RRF data for the catechins revealed both time- and level-dependence (exemplified for

epicatechin in Figure 3), the best model choice must be both dynamic and calibration-based, or DynCal. The selection of the best DynCal model for quantitation of the catechins utilized the following observations: the intercept is probably zero, which was demonstrated for the same calibrants with UV detection; the curve that best describes the data is quadratic, due to the slight curvature of the calibration lines (data not shown) and the RRF data (Figure 3). The time-dependent, zero-intercept quadratic model, “DynCal, $bX+cX^2$ ”, was therefore selected for quantitation of the catechins for the MS data.

For gallic acid, the lowest measurement variability for MS detection was obtained with the RRF quantitation models. Gallic acid also exhibited RRF values that varied significantly with level (A-D) and with time for MS detection (Figure 3), indicating the best model choice for quantitation is both RRF-based and dynamic, or DynRRF. The greater level-dependence for gallic acid is attributable to the larger concentration range covered for that analyte; calibrant D was ~ 21 times higher than calibrant A, whereas for the catechins the largest difference in calibrant concentration was a factor of 10 (for GCG). A wide concentration range for gallic acid calibrants was required to cover the concentration differences expected for the SRM samples. However, the gallic acid concentration in calibrant A was designed to match the concentration in the samples for both SRM 3254 and SRM 3256, and the concentration in calibrant D was designed to match the concentration in the samples for SRM 3255 (the concentrations for calibrant B and C were designed to fill the calibration range between the high and low levels). Since the SRM sample data for gallic acid were centered on single calibrants, model “DynRRF, closest” was selected for quantitation of gallic acid with the MS data.

Quantitative Results. A summary of the results for the catechins and gallic acid in the green tea SRMs obtained with UV and MS detection and the quantitation models just described is presented in Table 2, which includes the average, standard deviation (SD), and % RSD of the results (duplicate injections of $n=12$ samples for each SRM); the results are not corrected for the purities of the reference standards. On average, the measurement % RSDs are lower for UV detection than for MS detection. However, all results exhibit low measurement variability for both detection methods and all SRMs, with % RSDs $\leq 6\%$ for all analytes except gallocatechin gallate, which has % RSDs ranging from 3.3 to 7.5 %. It is not understood why the results for GCG exhibit higher variability, but it might be due to micro-heterogeneity of this analyte in the green tea materials. A mathematical comparison of the MS and UV results are also included in Table 2, which lists the difference (Dif) in the mean results obtained with each method, the uncertainty (u) in the difference, as well as the ratio Dif/u ($|\text{Dif}/u|$) that are greater than 2 indicate the difference in results for the two methods is greater than the random measurement variability within each method. When the MS and UV results are compared for SRM 3255, there are no differences in the mean mass fractions for any of the analytes ($|\text{Dif}/u| < 2$). This is attributed to SRM 3255 containing higher analyte levels and peak areas, so any potential matrix peaks and associated interferences are less significant with this material. When the MS and UV results are compared for SRM 3254 and SRM 3256, the mean values for catechin, epicatechin, and epigallocatechin are all higher by the UV method ($|\text{Dif}/u| > 2$). In addition, the mean value for gallocatechin gallate is higher by the UV method in SRM 3254 ($|\text{Dif}/u| = 5.7$). These results suggest that the peaks for these analytes in the UV chromatogram contain matrix inferences that are biasing the results, and/or that the lack of

baseline resolution from matrix components has introduced systematic errors when the peak areas were determined.

CONCLUSIONS

Catechins can be determined in green tea with either UV absorbance or MS detection and an internal standard approach to quantitation. Response factors for the catechins obtained with UV detection are stable, but vary with time and level when MS detection is used with the non-labeled internal standard proxyphylline. However, measurement variability can be minimized and reliable quantitation of the catechins obtained when a dynamic calibration approach is used with MS detection. The use of a dynamic calibration approach for the MS results is somewhat difficult and time-consuming but potentially yields more reliable results for the catechins when compared to UV detection, which may suffer bias from interferences for certain green tea matrices. To the best of our knowledge, this is the first study that compared static and dynamic quantitation models for the determination of catechins in green tea using both MS and UV detection, and the first reported use of a dynamic calibration approach for quantitation of the MS results.

ACKNOWLEDGEMENTS

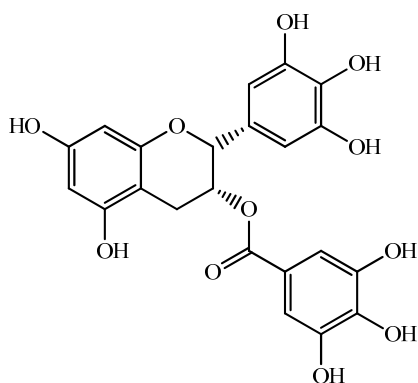
The authors wish to thank Michael Tims for his recommendation of theophylline for the internal standard and Rachel Lieberman for preparing most of the structures in Figure 1.

Reference List

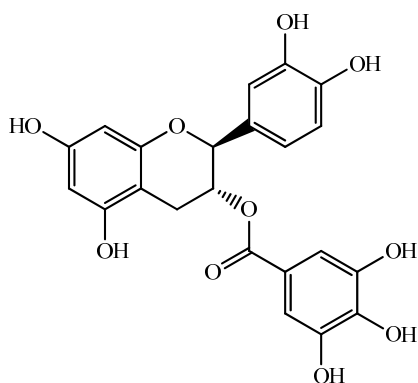
1. Zeeb, D. J.; Nelson, B. C.; Albert, K.; Dalluge, J. J. *Analytical Chemistry* **2000**, *72*, 5020-26.
2. Weiss, D. J.; Austria, E. J.; Anderton, C. R.; Hompesch, R.; Jander, A. *Journal of Chromatography A* **2006**, *1117*, 103-08.
3. Seeram, N. P.; Henning, S. M.; Niu, Y. T.; Lee, R.; Scheuller, H. S.; Heber, D. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 1599-603.
4. Friedman, M.; Levin, C. E.; Choi, S. H.; Kozukue, E.; Kozukue, N. *Journal of Food Science* **2006**, *71*, C328-C337.
5. Del Rio, D.; Stewart, A. J.; Mullen, W.; Burns, J.; Lean, M. E. J.; Brighenti, F.; Crozier, A. *Journal of Agricultural and Food Chemistry* **2004**, *52*, 2807-15.
6. Peng, L.; Song, X. H.; Shi, X. G.; Li, J. X.; Ye, C. X. *Journal of Food Composition and Analysis* **2008**, *21*, 559-63.
7. Hu, B.; Wang, L.; Zhou, B.; Zhang, X.; Sun, Y.; Ye, H.; Zhao, L. Y.; Hu, Q. H.; Wang, G. X.; Zeng, X. X. *Journal of Chromatography A* **2009**, *1216*, 3223-31.
8. Mizukami, Y.; Sawai, Y.; Yamaguchi, Y. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 4957-64.
9. Wang, H.; Provan, G. J.; Helliwell, K. *Food Chemistry* **2003**, *81*, 307-12.
10. Nishitani, E.; Sagesaka, Y. M. *Journal of Food Composition and Analysis* **2004**, *17*, 675-85.
11. Wang, D.; Lu, J.; Miao, A.; Xie, Z.; Yang, D. *Journal of Food Composition and Analysis* **2008**, *21*, 361-69.
12. Goto, T.; Yoshida, Y.; Kiso, M.; Nagashima, H. *Journal of Chromatography A* **1996**, *749*, 295-99.
13. Pelillo, M.; Bonoli, M.; Biguzzi, B.; Bendini, A.; Gallina Toschi, T.; Lercker, G. *Food Chemistry* **2004**, *87*, 465-70.
14. Pelillo, M.; Biguzzi, B.; Gallina Toschi, T.; Vanzini, M.; Lercker, G. *Food Chemistry* **2002**, *78*, 369-74.
15. Wu, J. C.; Xie, W.; Pawliszyn, J. *Analyst* **2000**, *125*, 2216-22.

16. Sultana, T.; Stecher, G.; Mayer, R.; Trojer, L.; Qureshi, M. N.; Abel, G.; Popp, M.; Bonn, G. K. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 3444-53.
17. Pongsuwan, W.; Bamba, T.; Harada, K.; Yonetani, T.; Kobayashi, A.; Furusaki, E. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 10705-08.
18. Sano, M.; Tabata, M.; Suzuki, M.; Degawa, M.; Miyase, T.; Maeda-Yamamoto, M. *Analyst* **2001**, *126*, 816-20.
19. Long, H.; Zhu, Y.; Huang, T.; Coury, L. A.; Kissinger, P. T. *Journal of Liquid Chromatography and Related Technologies* **2001**, *24*, 1105-14.
20. Yang, B.; Arai, K.; Kusu, F. *Analytical Biochemistry* **2000**, *283*, 77-82.
21. Mata-Bilbao, M. D. L.; Andres-Lacueva, C.; Roura, E.; Jauregui, O.; Torre, C.; Lamuela-Raventos, R. M. *Journal of Agricultural and Food Chemistry* **2007**, *55*, 8857-63.
22. Masukawa, Y.; Matsui, Y.; Shimizu, N.; Kondou, N.; Endou, H.; Kuzukawa, M.; Hase, T. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **2006**, *834*, 26-34.
23. Frauen, M.; Rode, T.; Rapp, C.; Steinhart, H. *Chromatographia* **2002**, *55*, 43-48.
24. Shen, D.; Wu, Q.; Wang, M.; Yang, Y.; Lavoie, E. J.; Simon, J. E. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 3219-24.
25. Sander, L. C.; Putzbach, K.; Nelson, B. C.; Rimmer, C. A.; Bedner, M.; Thomas, J. B.; Porter, B.; Wood, L. J.; Schantz, M. M.; Murphy, K. E.; Sharpless, K. E.; Wise, S. A.; Yen, J. H.; Siitonen, P. H.; NguyenPho, A.; Roman, M. C.; Betz, J. M. *Analytical and Bioanalytical Chemistry* **2008**, *391*, 2023-34.
26. Sander, L. C.; Sharpless, K. E.; Satterfield, M. B.; Ihara, T.; Phinney, K. W.; Yen, J. H.; Wise, S. A.; Gay, M. L.; Lam, J. W.; McCooeye, M.; Gardner, G.; Fraser, C.; Sturgeon, R.; Roman, M. C. *Analytical Chemistry* **2005**, *77*, 3101-12.
27. Dalluge, J. J.; Nelson, B. C.; Thomas, J. B.; Sander, L. C. *Journal of Chromatography A* **1998**, *793*, 265-74.
28. Zhu, Q. Y.; Zhang, A.; Tsang, D.; Huang, Y.; Chen, Z.-Y. *Journal of Agricultural and Food Chemistry* **1997**, *45*, 4624-28.
29. Perva-Uzunalic, A.; Skerget, M.; Knez, Z.; Weinreich, B.; Otto, F.; Gruner, S. *Food Chemistry* **2006**, *96*, 597-605.

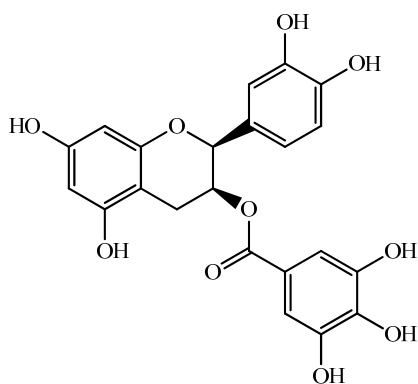
Figure 1. Structures of catechins measured in this investigation, gallic acid, and proxiphylline (IS).



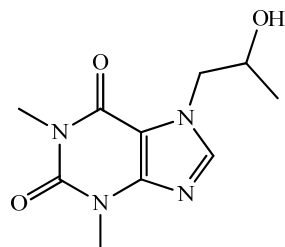
(-)-Epigallocatechin 3-gallate (EGCG)



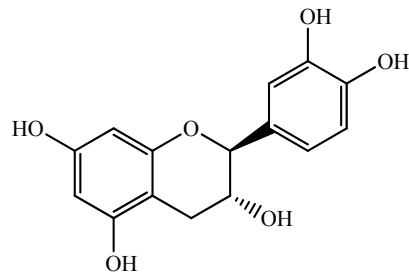
(-)-Gallocatechin 3-gallate (GCG)



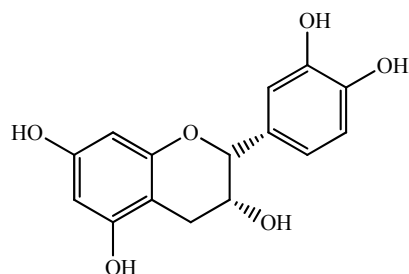
(-)-Epicatechin 3-gallate (ECG)



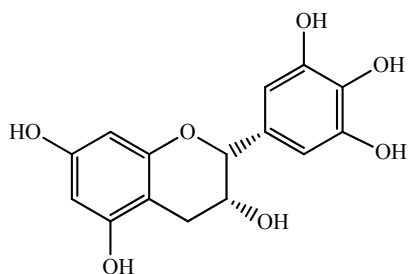
Proxiphylline (IS)



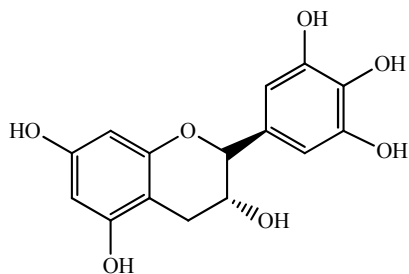
(-)-Catechin (C)



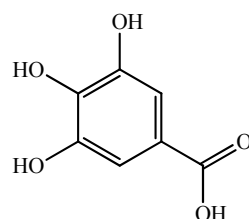
(-)-Epicatechin (EC)



(-)-Epigallocatechin (EGC)



(-)-Gallocatechin (GC)



Gallic Acid (GA)

Figure 2. Chromatograms of a sample of SRM 3254, green tea leaves. A: MS detection at m/z 171 and m/z 473; B: MS detection at m/z 239; C: MS detection at m/z 307; D: MS detection at m/z 291 and m/z 459; E: UV detection at 280 nm (plotted off-scale to emphasize the low-level analytes and matrix peaks).

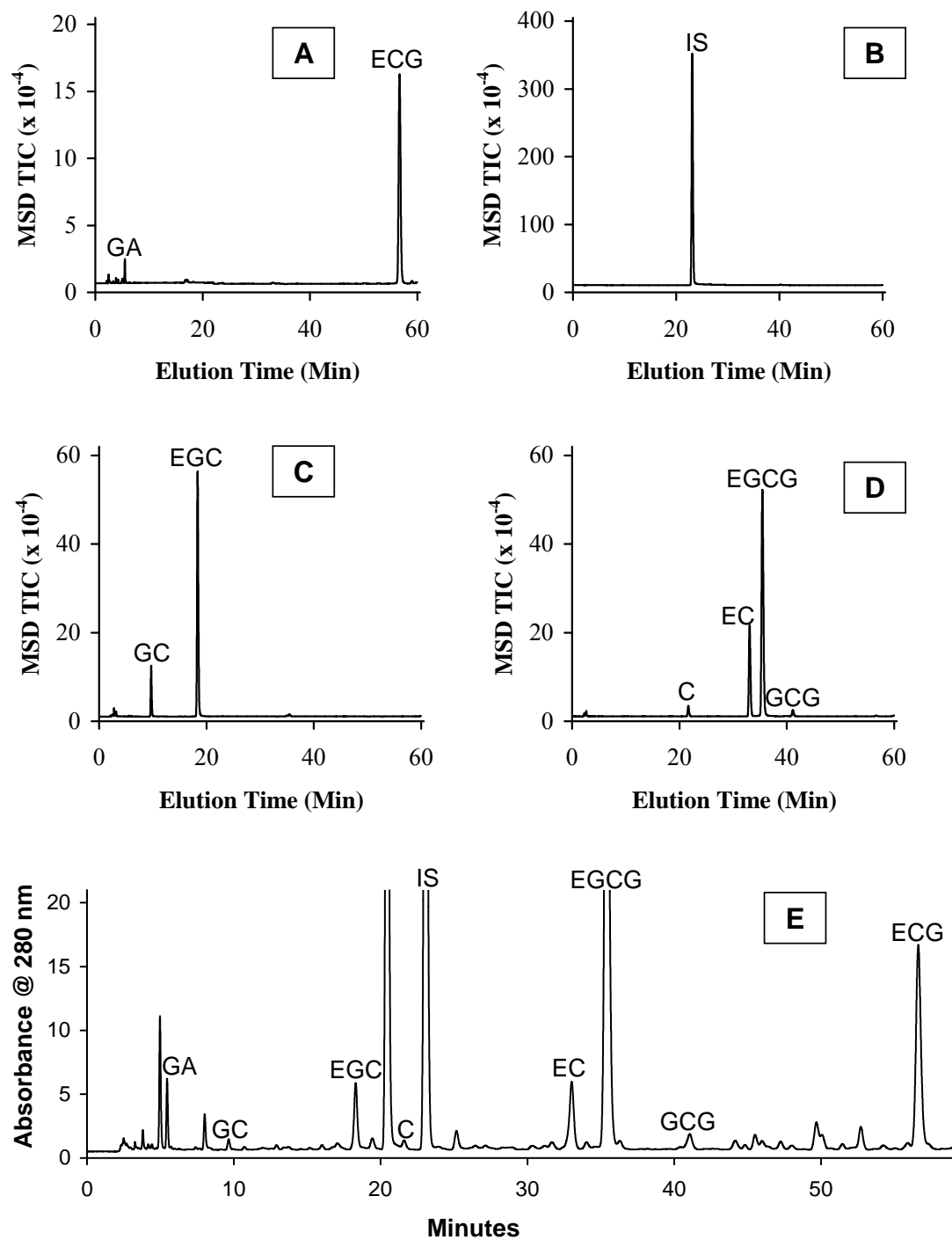
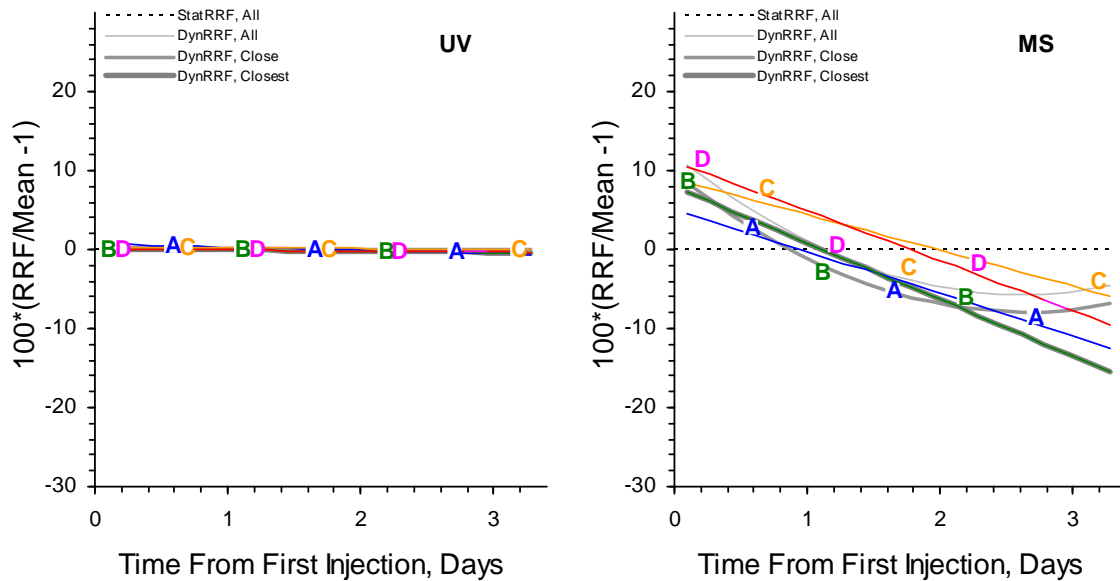


Figure 3. Normalized RRF data for epicatechin and gallic acid calibrants A, B, C, and D obtained with UV and MS detection. The color-coordinated lines are linear fits of the RRF data for each calibrant level (A-D) with time. The gray lines represent four of the RRF quantitation models, where “Stat” and “Dyn” refer to static, and dynamic, respectively: StatRRF, All; DynRRF, All; DynRRF, Close; DynRRF, Closest. Note: the dark grey lines for the DynRRF, Closest model overlap one of the colored lines in each of the MS plots.

Epicatechin calibrants: SRM 3256 measurement set



Gallic acid calibrants: SRM 3254 measurement set

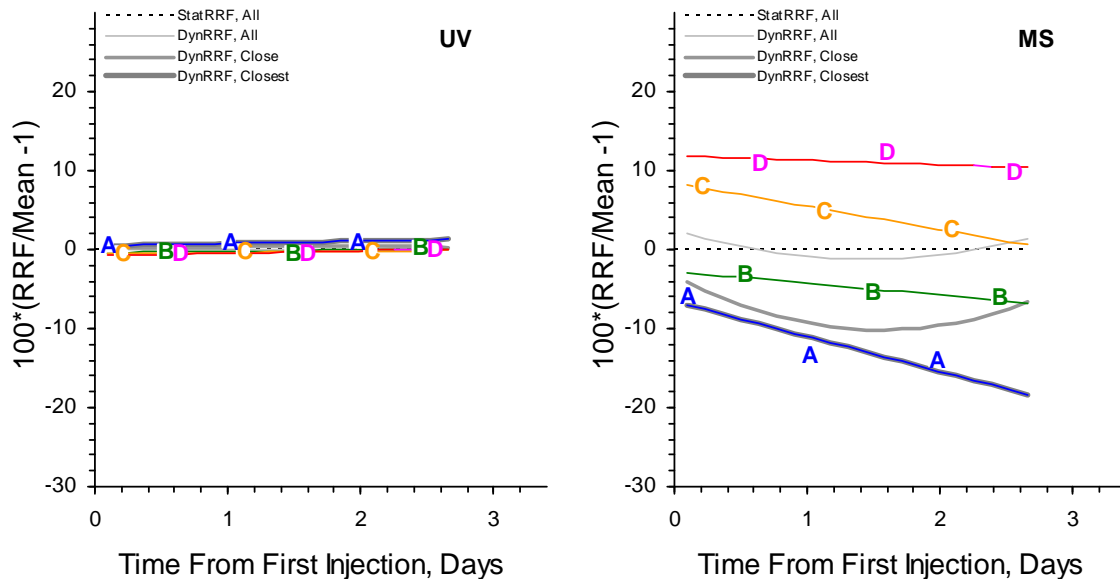


Figure 4. Normalized mass fraction (MF) data for epicatechin in SRM 3256 (green tea oral form) and gallic acid in SRM 3254 (green tea leaves). The individual points represent the normalized MF for each analyzed sample as a function of injection order. The mass fraction data were calculated with four quantitation models as indicated in the figure legends.

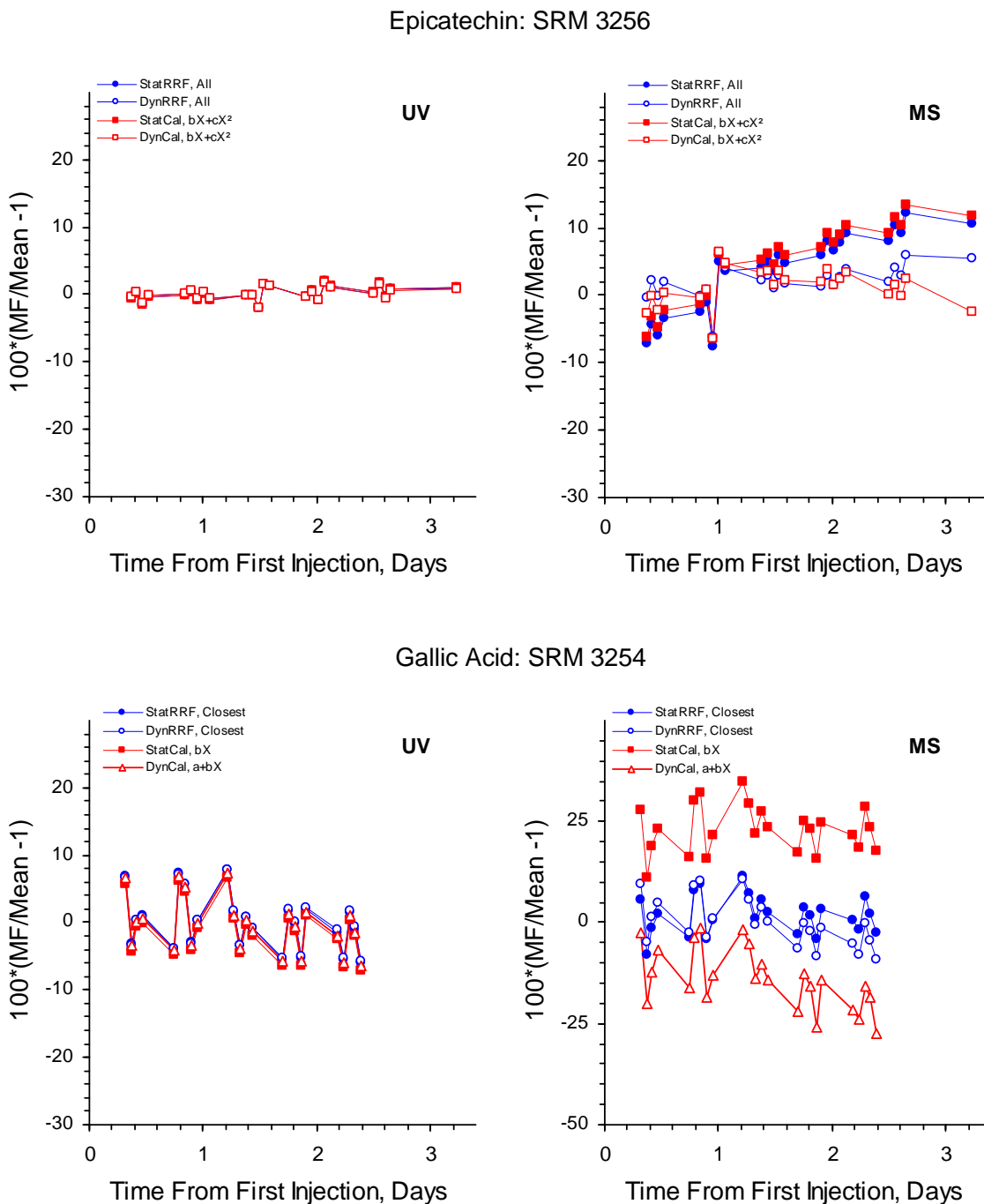


Table 1. Summary of the fourteen quantitation models used to determine the catechins and gallic acid in the green tea SRMs.

Model ID	Model Description
StatRRF, All	Average RRF of all calibrant levels
StatRRF, Close	Average RRF of the two calibrant levels closest to the level in the sample
StatRRF, Closest	Average RRF of the calibrant level closest to the level in the sample
DynRRF, All	Least squares quadratic fit of all calibrant RRF data to time; RRF was interpolated at each time a sample was injected
DynRRF, Close	Least squares quadratic fit of RRF data to time for the two calibrant levels closest to the level in the sample; RRF was interpolated at each time a sample was injected
DynRRF, Closest	Linear least squares fit of RRF data to time for the calibrant level closest to the level in the sample; RRF was interpolated at each time a sample was injected
StatCal, bx	Linear least squares fit of the average RRF values calculated for each calibrant level; forced zero-intercept
StatCal, a+bx	Linear least squares fit of the average RRF values for each calibrant level
StatCal, bx+cx ²	Least squares quadratic fit of the average RRF values for each calibrant level; forced zero-intercept
StatCal, a+bx+cx ²	Least squares quadratic fit of the average RRF values for each calibrant level
DynCal, bx	Linear fit of interpolated RRF values for each calibrant level at each time a sample was injected, forced zero intercept
DynCal, a+bx	Linear fit of interpolated RRF values for each calibrant level at each time a sample was injected
DynCal, bx+cx ²	Quadratic fit of interpolated RRF values for each calibrant level at each time a sample was injected, forced zero intercept
DynCal, a+bx+cx ²	Quadratic fit of interpolated RRF values for each calibrant level at each time a sample was injected

Table 2. Average mass fraction (reported in mg/g), standard deviation (SD), and percent relative standard deviation (% RSD) of catechins and gallic acid in each SRM for both UV and MS detection of each SRM (duplicate injections of $n=12$ samples for each analyte). A comparison of the UV and MS results is also presented. Data are not corrected for the purities of the reference standards.

SRM 3255: Extract		MS			UV			UV - MS			
Analyte	Model	Mean	SD	% RSD	Model	Mean	SD	% RSD	Dif	u	Dif/u
C	DynCal, bX+cX ²	9.62	0.41	4.2	StatCal, bX	9.92	0.30	3.0	0.29	0.51	0.6
EC	DynCal, bX+cX ²	45.87	1.61	3.5	StatCal, bX	45.10	1.32	2.9	-0.77	2.08	-0.4
ECG	DynCal, bX+cX ²	95.07	5.70	6.0	StatCal, bX	93.10	4.78	5.1	-1.97	7.43	-0.3
EGC	DynCal, bX+cX ²	87.79	2.79	3.2	StatCal, bX	88.01	2.39	2.7	0.22	3.67	0.1
EGCG	DynCal, bX+cX ²	410.52	14.75	3.6	StatCal, bX	423.72	16.39	3.9	13.20	22.05	0.6
GA	DynRRF, Closest	3.48	0.15	4.3	StatCal, bX	3.42	0.09	2.6	-0.06	0.17	-0.3
GC	DynCal, bX+cX ²	24.38	1.24	5.1	StatCal, bX	23.33	0.63	2.7	-1.04	1.39	-0.8
GCG	DynCal, bX+cX ²	37.96	2.83	7.5	StatCal, bX	38.13	2.28	6.0	0.17	3.63	0.0

SRM 3256: Oral Form		MS			UV			UV - MS			
Analyte	Model	Mean	SD	% RSD	Model	Mean	SD	% RSD	Dif	u	Dif/u
C	DynCal, bX+cX ²	2.35	0.05	2.2	StatCal, bX	2.91	0.05	1.6	0.55	0.07	7.8
EC	DynCal, bX+cX ²	9.57	0.26	2.8	StatCal, bX	10.80	0.11	1.0	1.23	0.28	4.3
ECG	DynCal, bX+cX ²	16.37	0.86	5.2	StatCal, bX	17.33	0.46	2.7	0.97	0.97	1.0
EGC	DynCal, bX+cX ²	29.14	0.78	2.7	StatCal, bX	33.52	0.48	1.4	4.38	0.92	4.8
EGCG	DynCal, bX+cX ²	75.02	2.39	3.2	StatCal, bX	73.61	1.38	1.9	-1.41	2.76	-0.5
GA	DynRRF, Closest	14.58	0.34	2.3	StatCal, bX	14.05	0.25	1.8	-0.53	0.42	-1.2
GC	DynCal, bX+cX ²	8.01	0.18	2.3	StatCal, bX	7.86	0.20	2.6	-0.15	0.28	-0.5
GCG	DynCal, bX+cX ²	5.50	0.19	3.5	StatCal, bX	5.46	0.19	3.5	-0.04	0.27	-0.1

SRM 3254: Leaves		MS			UV			UV - MS			
Analyte	Model	Mean	SD	% RSD	Model	Mean	SD	% RSD	Dif	u	Dif/u
C	DynCal, bX+cX ²	0.73	0.03	4.1	StatCal, bX	1.08	0.02	1.5	0.35	0.03	10.2
EC	DynCal, bX+cX ²	7.21	0.16	2.3	StatCal, bX	8.99	0.13	1.5	1.78	0.21	8.4
ECG	DynCal, bX+cX ²	11.34	0.47	4.2	StatCal, bX	12.42	0.27	2.2	1.07	0.55	2.0
EGC	DynCal, bX+cX ²	25.72	0.84	3.3	StatCal, bX	29.42	0.89	3.0	3.70	1.23	3.0
EGCG	DynCal, bX+cX ²	51.28	1.62	3.2	StatCal, bX	50.38	0.88	1.7	-0.90	1.84	-0.5
GA	DynRRF, Closest	0.88	0.05	6.0	StatCal, bX	0.83	0.03	4.1	-0.05	0.06	-0.7
GC	DynCal, bX+cX ²	3.05	0.08	2.6	StatCal, bX	2.93	0.13	4.6	-0.12	0.15	-0.8
GCG	DynCal, bX+cX ²	0.82	0.06	7.2	StatCal, bX	1.22	0.04	3.4	0.40	0.07	5.7