



National Institute of Standards & Technology

# Certificate of Analysis

Standard Reference Material<sup>®</sup> 3257

## Catechin Calibration Solutions

This Standard Reference Material (SRM) consists of three separate solutions containing catechins. Solution 1 contains (+)-catechin, (–)-gallicocatechin, and (–)-gallicocatechin 3-gallate; Solution 2 contains (–)-epicatechin, (–)-epigallocatechin, and (–)-epicatechin 3-gallate; Solution 3 contains (–)-epigallocatechin 3-gallate. The solutions were prepared in a mixture of 30 % methanol, 70 % water, and 0.05 % formic acid (volume fractions). Formic acid was added to stabilize the catechins. SRM 3257 is intended primarily for use in calibration of instruments and techniques used for the determination of these analytes. A unit of SRM 3257 consists of twelve 2 mL ampoules, four vials each of Solution 1, Solution 2, and Solution 3. Each ampoule contains approximately 1.2 mL of solution.

The development of SRM 3257 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health (NIH), Office of Dietary Supplements (ODS).

**Certified Values:** The certified values for the catechins in Table 1 are based on the results from the gravimetric preparation of the solutions and from the analytical results determined using liquid chromatography with ultraviolet absorbance detection (LC-UV). A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Values are provided in mass fraction units and, for user convenience, mass concentrations [2]. The mass concentration was calculated from the mass fraction values using the density of the solvent, which was determined at NIST using the Lang-Levy pipette method [3]. An allowance for the change in density over the range 20 °C to 25 °C is included in the uncertainty. The measurand is the value for each catechin listed in Table 1. Metrological traceability is to the SI derived units of mass fraction (expressed as micrograms per gram) and mass concentration (expressed as micrograms per milliliter).

**Expiration of Certification:** The certification of **SRM 3257** is valid, within the measurement uncertainty specified, until **15 April 2021**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Handling, Storage, and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

**Maintenance of SRM Certification:** NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements leading to certification was under the direction of K.E. Sharpless and L.C. Sander of the NIST Chemical Sciences Division.

Preparation and analytical measurements were performed by M. Bedner of the NIST Chemical Sciences Division.

Statistical consultation was provided by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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Certificate Issue Date: 14 January 2016  
*Certificate Revision History on Last Page*

Steven J. Choquette, Acting Director  
Office of Reference Materials

## INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

**Handling:** These solutions contain methanol and a small amount of formic acid, which are hazardous substances; therefore, care should be exercised during handling and use. Use proper methods for disposal of waste. The solutions are contained in tip-sealed borosilicate glass ampoules with prescored stems. Therefore, all appropriate safety precautions, including use of gloves during handling, should be taken.

**Storage:** Sealed ampoules, as received, should be stored in the dark at temperatures at or below  $-20^{\circ}\text{C}$ . The certified values do not apply to contents of previously opened and stored ampoules, as the stability of analytes has not been investigated.

**Use:** The contents of the ampoule should be mixed thoroughly prior to opening. Test portions for use should be withdrawn immediately after opening the ampoules and should be processed without delay for the certified concentrations to be valid within the stated uncertainty. Depending on the sensitivity of the analytical method being used, SRM 3257 may be used as-is or diluted. The certified concentration values listed in Table 1 apply only to aliquots removed at  $20^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ . The stability of catechins in opened ampoules has not been investigated, but catechins are known to be less stable at pH values above 4 [4].

## PREPARATION AND ANALYSIS<sup>(1)</sup>

The solutions were prepared gravimetrically at NIST from a solution of 30 % methanol, 70 % water, and 0.05 % formic acid (volume fractions) and primary standards for the catechins obtained from Blaze Science Industries (Lawndale, CA). After preparation, the solutions were mixed for 60 min to 120 min and then stored at  $-20^{\circ}\text{C}$  overnight. The following morning the solutions were removed from the freezer, stirred for an additional 20 min to 70 min, chilled on ice, and aliquoted into 2 mL amber glass ampoules that had been purged with argon prior to addition of the solution. The ampoules were then flame-sealed. The masses of the standards and the total masses of the solutions were used to calculate the gravimetric concentrations. The concentrations were adjusted for the purity estimates of the primary standards, which were determined using LC-UV and Karl Fischer titration.

Aliquots from 10 ampoules, selected using a stratified random sampling scheme, were analyzed using LC-UV and a  $\text{C}_{18}$  column. Three independent calibration solutions were gravimetrically prepared with catechin concentrations that represent the levels in each of the three SRM 3257 solutions and were used as external standards for quantitation. Representative chromatograms and the separation conditions are presented in Figure 1.

**Certified Value Assignment:** Certified values are unweighted means of concentrations determined by gravimetric preparation and chromatographic measurements. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it incorporates Type B uncertainty components related to purity determination, components related to density (for concentration results), and expresses both the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide and its Supplement 1 [5–7]. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  is the combined uncertainty, and  $k$  is a coverage factor corresponding to approximately 95 % confidence for each analyte [5]. For all measurands,  $k = 2$ . The mass concentrations (micrograms per milliliter) were obtained by multiplying the certified values in mass fraction units by the density of the solvent at  $22^{\circ}\text{C}$  ( $0.9596\text{ g/mL}$ ). These concentrations are for use in the temperature range of  $20^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ .

Table 1. Certified Values for Catechins in SRM 3257

	Mass Fraction (μg/g)			Mass Concentration (μg/mL)		
<b>Solution 1</b>						
(+)-catechin	23.54	±	0.53	22.58	±	0.52
(-)-gallocatechin	62.7	±	1.8	60.2	±	1.8
(-)-gallocatechin 3-gallate	76.6	±	1.7	73.5	±	1.7
<b>Solution 2</b>						
(-)-epicatechin	93.9	±	2.1	90.1	±	2.0
(-)-epigallocatechin	232.9	±	6.6	223.5	±	6.4
(-)-epicatechin 3-gallate	203.9	±	4.6	195.6	±	4.5
<b>Solution 3</b>						
(-)-epigallocatechin 3-gallate	549	±	12	527	±	12

<sup>(1)</sup> Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

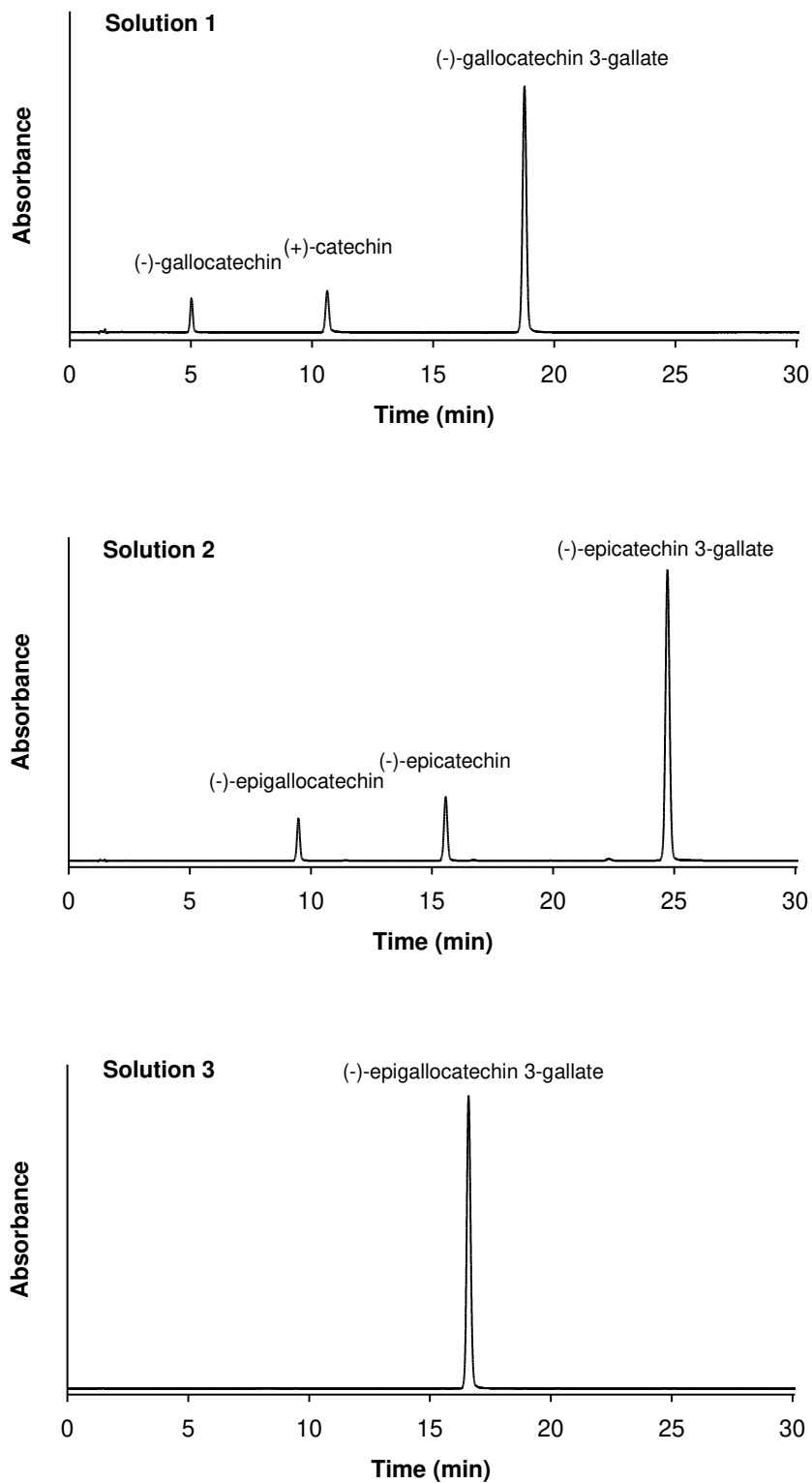


Figure 1. LC-UV chromatograms of SRM 3257 Solution 1, Solution 2, and Solution 3. A Zorbax SB column (Agilent Technologies, Wilmington, DE) with dimensions of 250 mm  $\times$  4.6 mm ID and containing 5  $\mu$ m diameter particles was used with a gradient mobile phase of water and acetonitrile, each with 0.05 % formic acid. Absorbance was measured at 280 nm.

## REFERENCES

- [1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at <http://www.nist.gov/srm/publications.cfm> (accessed Jan 2016).
- [2] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at <http://www.nist.gov/pml/pubs/index.cfm/> (accessed Jan 2016).
- [3] Sneigoski, L.T., Moody, J.R.; *Determination of Serum and Blood Densities*; Anal. Chem., Vol. 51, pp. 1577–1578 (1979).
- [4] Zhu, Q.Y., Zhang, A., Tsang, D., Huang, D., Chen, Z.-Y.; *Stability of Green Tea Catechins*; J. Agric. Food Chem., Vol. 45, pp. 4624–4628 (1997).
- [5] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at [http://www.bipm.org/utis/common/documents/jcg/JCGM\\_100\\_2008\\_E.pdf](http://www.bipm.org/utis/common/documents/jcg/JCGM_100_2008_E.pdf) (accessed Jan 2016); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at <http://www.nist.gov/pml/pubs/index.cfm> (accessed Jan 2016).
- [6] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at [http://www.bipm.org/utis/common/documents/jcg/JCGM\\_101\\_2008\\_E.pdf](http://www.bipm.org/utis/common/documents/jcg/JCGM_101_2008_E.pdf) (accessed Jan 2016).
- [7] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall, London, UK (1993).

**Certificate Revision History:** 14 January 2016 (Change of expiration date; editorial changes); 14 September 2011 (Original certificate date).

*Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*