AOAC SMPR® 2015.014

Standard Method Performance Requirements for Determination of Catechins, Methyl Xanthines, Theaflavins, and Theanine in Tea (*Camellia sinensis*) Dietary Ingredients and Supplements

Intended Use: Quality Control, Routine Testing, and Dispute Resolution

1 Purpose

AOAC *Standard Method Performance Requirements* (SMPRs®) describe the minimum recommended performance characteristics to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by AOAC stakeholder panels composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for method being considered for *Performance Tested Methods*SM or AOAC *Official Methods of Analysis*, and can be used as acceptance criteria for verification at user laboratories [Refer to Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA.]

2 Applicability

Quantitative determination of catechins, methyl xanthenes, theaflavins, and theanine in tea dietary ingredients and supplements.

3 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable. It is acceptable to have a different analytical method for each class of analytes.

4 Definitions

Catechins.—Catechin, epicatechin, epigallocatechin, catechin gallate, epicatechin gallate, epigallocatechin gallate, and

Table 1. Catechins

Common name	IUPAC nomenclature	CAS No.
Catechin	(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol	154-23-4
Epicatechin	(2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol	490-46-0
Epigallocatechin	(2*R*,3*R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol	970-74-1
Catechin gallate	[(2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2*H*-chromen-3-yl] 3,4,5-trihydroxybenzoate	130405-40-2
Epicatechin gallate	(2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-5,7-dihydroxy-2H-1-benzopyran-3-yl-ester-3,4,5-trihydroxy-benzoic acid	1257-08-5
Epigallocatechin	(−)-*cis*-3,3′,4′,5,5′,7-Hexahydroxyflavane, (−)-*cis*-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1(2*H*)-benzopyran-3,5,7-triol	970-74-1
Gallate	3,4,5-Trihydroxybenzoic acid	149-91-7
Gallocatechin	(2*S*,3*R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1(2*H*)-benzopyran-3,5,7-triol	3371-27-5

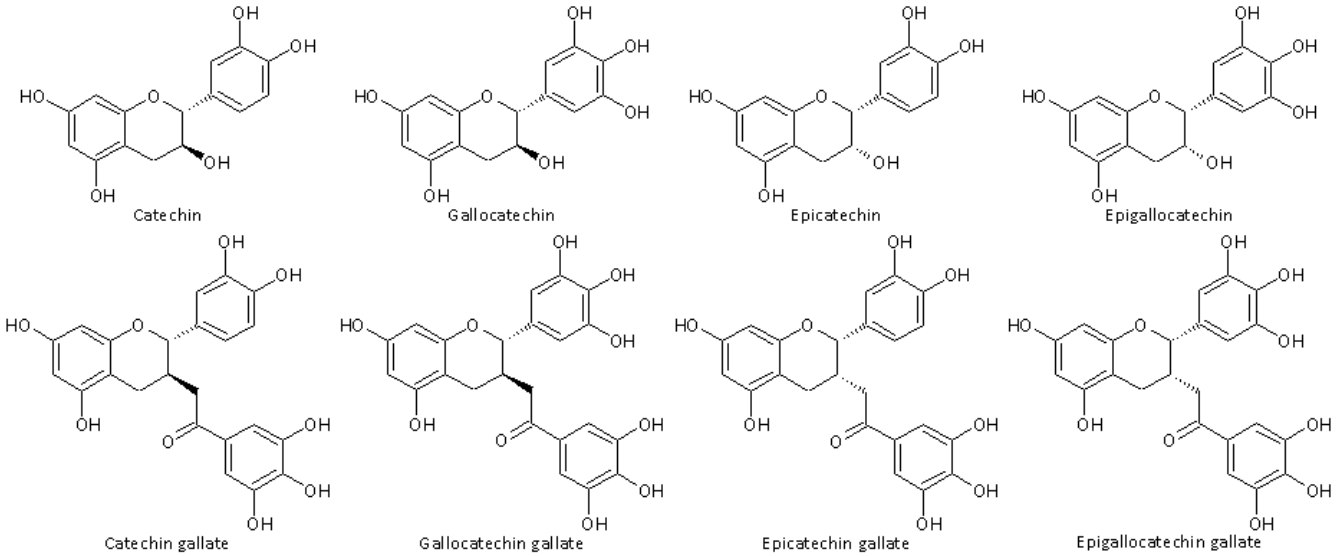

Figure 1. Chemical structure of catechin and derivatives.

Table 2. Methyl xanthenes		
Common name	IUPAC nomenclature	CAS No.
Caffeine	1,3,7-trimethylpurine-2,6-dione	58-08-2
Theobromine	3,7-dimethyl-1H-purine-2,6-dione	83-67-0
Theophylline	1,3-dimethyl-7H-purine-2,6-dione	58-55-9

gallocatechin. Refer to Table 1 for IUPAC nomenclature and CAS registry numbers. *See* Figure 1 for chemical structures.

Methyl xanthines.—Caffeine, theobromine, and theophylline. Refer to Table 2 for IUPAC nomenclature and CAS registry numbers. *See* Figure 2 for chemical structures.

Theaflavins.—Theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3-3'-digallate. Refer to Table 3 for IUPAC nomenclature and CAS registry numbers. *See* Figure 3 for chemical structures.

Theanine.—*N*-ethyl-L-glutamine, (2*S*)-2-ammonio-5-(ethyl- amino)-5-oxopentanoate. CAS registry number: 3081-61-6. *See* Figure 4 for chemical structure.

Dietary ingredients.—Vitamin, mineral, herb, or other botanical; an amino acid; a dietary substance for use by man to supplement the diet by increasing total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the above dietary ingredients {United States Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]}.

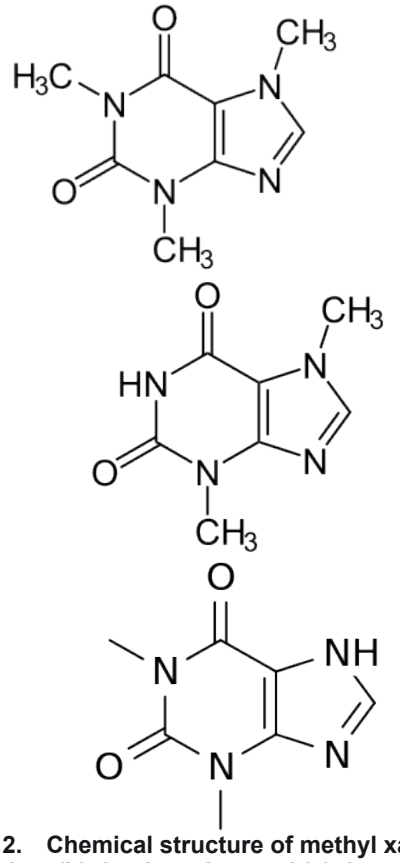

Figure 2. Chemical structure of methyl xanthines: (a) caffeine, (b) theobromine, and (c) theophylline.

Dietary supplements.—Product intended for ingestion that contains a "dietary ingredient" intended to add further nutritional value to (supplement) the diet. Dietary supplements may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders.

Limit of quantitation (LOQ).—Minimum analyte concentration for which quantitative results may be obtained with 95% confidence.

Repeatability.—Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator, and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r), or % repeatability relative standard deviation ($\%RSD_r$).

Reproducibility.—Standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD_R) or % reproducibility relative standard deviation ($\%RSD_R$).

Recovery.—Fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method.

5 Method Performance Requirements

See Tables 4 and 5.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples, and check standards at the lowest point and midrange point of the analytical range, and a protocol to demonstrate suitability.

7 Reference Material(s)

ISO Guide 34:2009 *General requirements for the competence of reference material producers*

SRM 3254 *Camellia sinensis* (Green Tea) Leaves

SRM 3255 *Camellia sinensis* (Green Tea) Extract

SRM 3256 Green Tea-Containing Solid Oral Dosage Form

SRM 3257 Catechil Calibration Materials

8 Validation Guidance

All matrixes listed in Table 6 must be evaluated for LOQ, repeatability, and recovery for First Action *Official Methods of Analysis*^{SM} approval.

Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, *Official Methods of Analysis of AOAC INTERNATIONAL* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA. Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix K: Guidelines for Dietary Supplements and Botanicals, *Official Methods of Analysis of AOAC INTERNATIONAL* (current edition), AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_k.pdf). Also at: *J. AOAC Int*. **95**, 268(2012); DOI: 10.5740/jaoacint.11-447

9 Maximum Time-to-Result

No maximum time to result.

Approved by AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: September 25, 2015. Effective Date: September 25, 2015.

Table 3. Theaflavins		
Common name	IUPAC nomenclature	CAS No.
Theaflavin	3,4,5-Trihydroxy-1,8-bis[(2*R*,3*R*)-3,5,7-trihydroxy-3,4-dihydro-2*H*-chromen-2-yl]-6*H*-benzo[7]annulen-6-one	4670-05-7
Theaflavin-3-gallate	[(2*R*,3*R*)-5,7-dihydroxy-2-[3,4,5-trihydroxy-6-oxo-8-[(2*R*,3*R*)-3,5,7-trihydroxychroman-2-yl]benzo[7]annulen-1-yl]chroman-3-yl] 3,4,5-trihydroxybenzoate	30462-34-1
Theaflavin-3'-gallate	[(2*R*,3*R*)-5,7-dihydroxy-2-[3,4,5-trihydroxy-6-oxo-8-[(2*R*,3*R*)-3,5,7-trihydroxychroman-2-yl]benzo[7]annulen-1-yl]chroman-3-yl] 3,4,5-trihydroxybenzoate	28543-07-9
Theaflavin-3-3'-digallate	[1-[(2*R*,3*R*)-3,5-dihydroxy-7-(3,4,5-trihydroxybenzoyl) oxychroman-2-yl]-3,5-dihydroxy-6-oxo-8-[(3*R*)-3,5,7-trihydroxychroman-2-yl]benzo[7]annulen-4-yl] 3,4,5-trihydroxybenzoate	33377-72-9

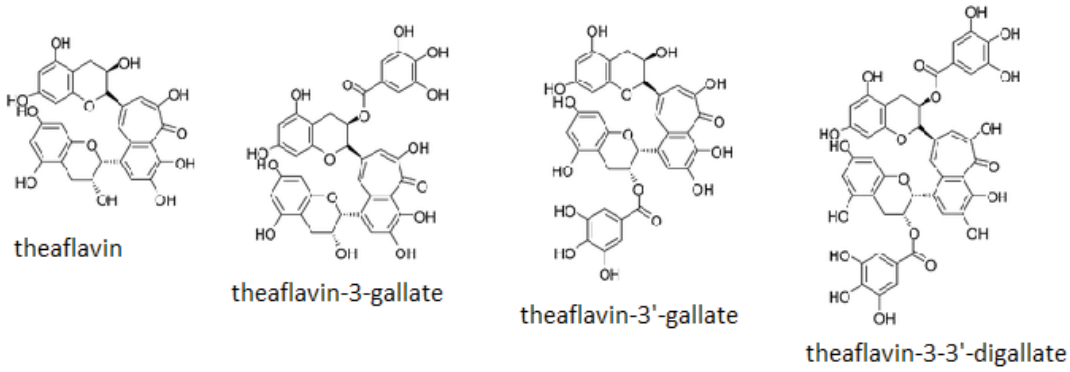

Figure 3. Chemical structure of theaflavins.

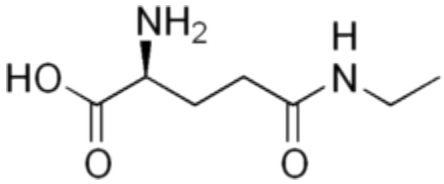

Figure 4. Chemical structure of theanine.

Table 4. Analytical ranges and LOQs				
Component	Catechins	Methyl xanthenes	Theaflavins	Theanine
Analytical range, ppm	10–500 000	10–500 000	10–100 000	10–100 000
LOQ, ppm	≤5			

Table 5. Method performance requirements by range					
Range, ppm	10–50	51–500	–4000	4001–20 000	>20 000
Recovery, %	80–110	90–107	95–105	95–105	95–105
RSD_r, %	≤7	≤5	≤5	≤5	≤5
RSD_R, %	≤10	≤8	≤8	≤8	≤8

Table 6. Matrixes
Tablets
Capsules
Softgels
Gelcaps
Gummies
Chewables
Liquids
Powders