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3 **Method Name: Determination of Catechins, Methyl Xanthines, Theaflavins, and**
4 **Theanines in Tea Dietary Ingredients And Supplements**

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6 **Purpose:** AOAC SMPR's describe the minimum recommended performance characteristics to be
7 used during the evaluation of a method. The evaluation may be an on-site verification, a single-
8 laboratory validation, or a multi-site collaborative study. SMPRs are written and adopted by
9 AOAC Stakeholder Panels composed of representatives from the industry, regulatory
10 organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC
11 SMPRs are used by AOAC Expert Review Panels in their evaluation of validation study data for
12 method being considered for *Performance Tested Methods* or *AOAC Official Methods of*
13 *Analysis*, and can be used as acceptance criteria for verification at user laboratories.¹

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15 **Approved by:** Stakeholder Panel on Dietary Supplements (SPDS)

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17 **Intended Use:** Quality control, routine testing and dispute resolution.

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19 **1. Applicability:**

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

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23 **2. Analytical Technique:**

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

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28 **3. Definitions:**

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30 **Catechins**

31 Catechin, epicatechin, epigallocatechin, catechin gallate, epicatechin gallate,
32 epigallocatechin gallate, and galocatechin. Refer to table 3 for IUAC nomenclature and CAS
33 registry numbers. See figure 1 for chemical structures.

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35 **Dietary Ingredients**

36 A vitamin; a mineral; an herb or other botanical; an amino acid; a dietary substance for use
37 by man to supplement the diet by increasing total dietary intake; or a concentrate,
38 metabolite, constituent, extract, or combination of any of the above dietary ingredients.²

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40 **Dietary supplements**

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

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¹ Refer to [Appendix F: Guidelines for Standard Method Performance Requirements](#) in the 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis (2012).

² United States Federal Food Drug and Cosmetic Act §201(ff) [U.S.C. 321 (ff)]

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Limit of Quantitation (LOQ):

The minimum analyte concentration for which quantitative results may be obtained with 95% confidence.

Methyl xanthines

Caffeine; theobromine; and theophylline. Refer to table 4 for IUPAC nomenclature and CAS registry numbers. See figure 2 for chemical structures.

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:

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R) or %reproducibility relative standard deviation (% RSD_R).

Recovery:

The fraction or percentage of the analyte that is recovered when the test sample is analyzed using the entire method.

Theaflavins

Theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3-3'-digallate. Refer to table 6 for IUPAC nomenclature and CAS registry numbers. See figure 3 for chemical structures.

Theanine

N-ethyl-L-glutamine; (2S)-2-ammonio-5-(ethylamino)-5-oxopentanoate. CAS registry number: 3081-61-6. See figure 4 for chemical structure.

4. Method Performance Requirements:

Table 1: 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			

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Table 2: Method Performance Requirements by Range

Ranges (ppm)	10 – 50	51 - 500	501– 4,000	4,001 – 20,000	>20,000
Recovery (%)	80 -110	90-107	95-105	97-103	98-102
RSD_r (%)	≤ 7	5	4	2	2
RSD_R (%)	≤ 10	8	6	3	3

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- 85 **5. System suitability tests and/or analytical quality control**
86 Suitable methods will include blank check samples, and check standards at the lowest point and
87 midrange point of the analytical range, and a protocol to demonstrate suitability.
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- 89 **6. Reference Material(s)**
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91 ISO Guide 34:2009 General requirements for the competence of reference material
92 producers.
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94 SRM 3254 *Camellia sinensis* (Green Tea) Leaves
95 SRM 3255 *Camellia sinensis* (Green Tea) Extract
96 SRM 3256 Green Tea-Containing Solid Oral Dosage Form
97 SRM 3257 Catechil Calibration Materials
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99 Other compounds?
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- 101 **7. Validation Guidance:**
102 All matrices listed in Table 3 must be evaluated for LOQ, repeatability, and recovery for First
103 Action *Official Methods of Analysis* approval.
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105 [Appendix D](http://www.eoma.aoc.org/app_d.pdf): Guidelines for Collaborative Study Procedures To Validate Characteristics of a
106 Method of Analysis; 19th Edition of the AOAC INTERNATIONAL Official Methods of Analysis
107 (2012). Available at: http://www.eoma.aoc.org/app_d.pdf
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109 [Appendix K](http://www.eoma.aoc.org/app_k.pdf): Guidelines for Dietary Supplements and Botanicals 19th Edition of the AOAC
110 INTERNATIONAL Official Methods of Analysis (2012). Also at: . AOAC Int. 95, 268(2012); DOI:
111 10.5740/jaoacint.11-447 and available at: http://www.eoma.aoc.org/app_k.pdf
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- 114 **8. Maximum Time-To-Result:** No maximum time to result.
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116 **Table 3: Matrices**

117 Tablets

118 Capsules

119 Softgels

120 Gelcaps

121 Gummies

122 Chewables

123 Liquids

124 Powders

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126 **Table 4: Catechins**

Common name	IUPAC Nomenclature	CAS No.
Catechin	(2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	154-23-4?
epicatechin	(2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	490-46-0
epigallocatechin	(2R,3R)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	970-74-1
catechin gallate	[(2R,3R)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2H-chromen-3-yl] 3,4,5-trihydroxybenzoate	130405-40-2
epicatechin gallate	(2R,3R)-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	(-)- <i>cis</i> -3,3',4',5,5',7-Hexahydroxyflavane, (-)- <i>cis</i> -2-(3,4,5-Trihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol	970-74-1
Gallate	3,4,5-Trihydroxybenzoic acid	149-91-7
gallo catechin	(2S,3R)-2-(3,4,5-Trihydroxyphenyl)-3,4-dihydro-1(2H)-benzopyran-3,5,7-triol	3371-27-5

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128 **Table 5: 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

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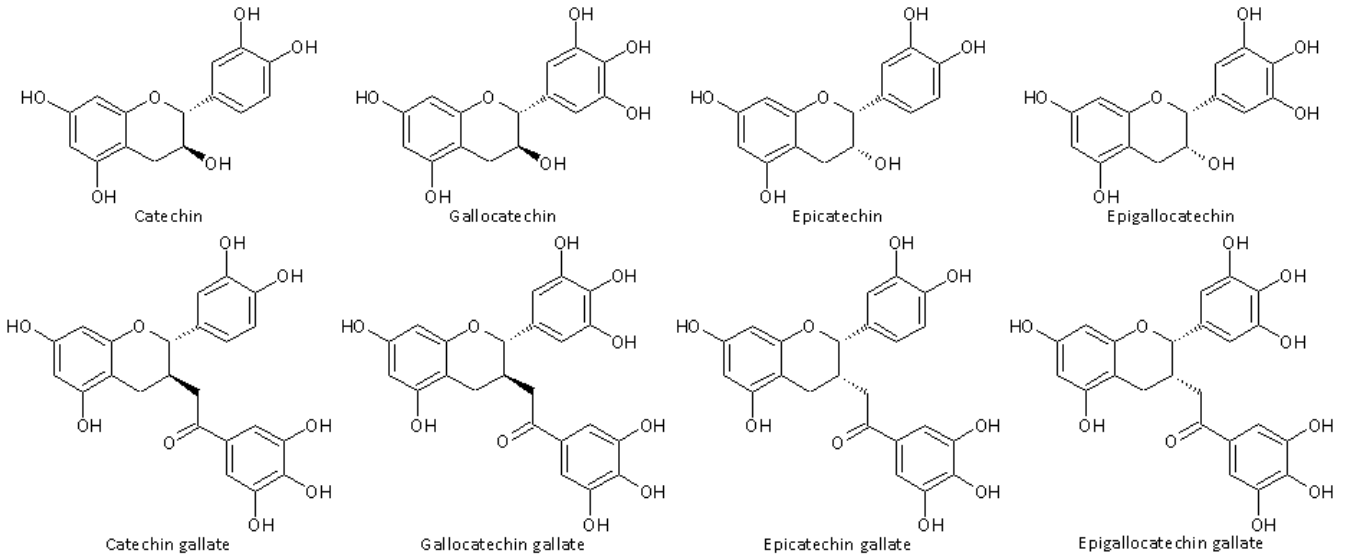
131 **Table 6: Theaflavins**

Common name	IUPAC Nomenclature	CAS No.
theaflavin	3,4,5-Trihydroxy-1,8-bis[(2R,3R)-3,5,7-trihydroxy-3,4-dihydro-2H-chromen-2-yl]-6H-benzo[7]annulen-6-one	4670-05-7
theaflavin-3-gallate	[(2R,3R)-5,7-dihydroxy-2-[3,4,5-trihydroxy-6-oxo-8-[(2R,3R)-3,5,7-trihydroxychroman-2-yl]benzo[7]annulen-1-yl]chroman-3-yl] 3,4,5-trihydroxybenzoate	30462-34-1
theaflavin-3'-gallate	[(2R,3R)-5,7-dihydroxy-2-[3,4,5-trihydroxy-6-oxo-8-[(2R,3R)-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-[(2R,3R)-3,5-Dihydroxy-7-(3,4,5-trihydroxybenzoyl)oxochroman-2-yl]-3,5-dihydroxy-6-oxo-8-[(3R)-3,5,7-trihydroxychroman-2-yl]benzo[7]annulen-4-yl] 3,4,5-trihydroxybenzoate	33377-72-9

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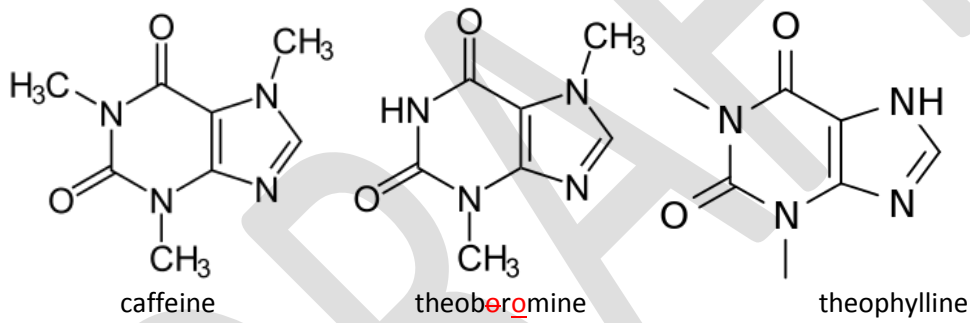
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Figure 1: Chemical structure for catechin and derivatives



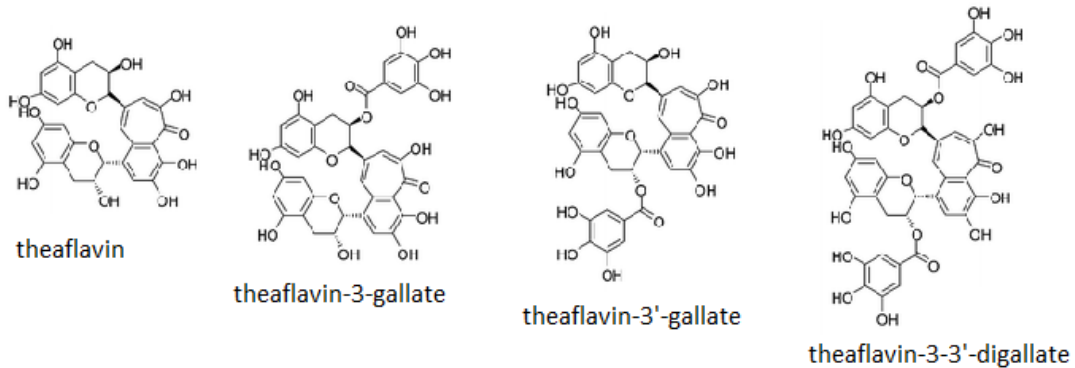
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Figure 2: Chemical structure of methyl xanthines



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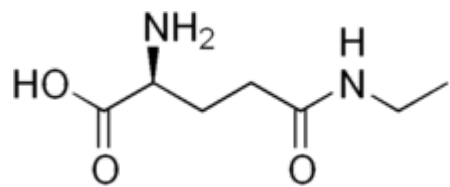
Figure 3: Chemical structures for theaflavins



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147 **Figure 4: Chemical structure of theanine**

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