AOAC SMPR® 2018.006

Standard Method Performance Requirements (SMPRs®) for Determination of Select Flavonoids from Skullcap

Intended Use: Quality Assurance and Compliance to Current Good Manufacturing Practices

1 Purpose

AOAC 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 the 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 methods being considered for *Performance Tested Methods*℠ 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* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA.]

2 Applicability

Identification and quantitation of baicalin from skullcap in plant material, dietary ingredients and dietary supplements (*see* Table 1). Determination of chrysin, baicalein, wogonin, wogonoside, scutellarein and scutellarin is also desirable. *See* Table 2 for additional information on analytes and Figure 1 for molecular structures.

3 Analytical Technique

Any analytical technique that meets the following method performance requirements is acceptable.

4 Definitions

Analytical range.—Includes all steps of the analytical procedure including sample preparation and further dilutions.

Dietary ingredient.—A vitamin; a mineral; an 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)]}

Dietary supplement.—A 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).—The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

Plant materials.—Fresh, dried, or cut plant materials.

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

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 standard deviation (SD_R); or % reproducibility relative standard deviation ($\%RSD_R$).

Skullcap.—For the purposes of this SMPR, the term skullcap refers to the species *Scutellaria lateriflora* and S*cutellaria baicalensis*.

5 Method Performance Requirements

See Tables 3 and 4.

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. A control sample must be included.

7 Reference Material(s)

See Table 5 for sources of flavonoids reference materials. *See* Table 6 for sources of *Scutellaria* species.

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements*, 20th Ed. of the *Official Methods of Analysis of AOAC INTERNATIONAL* (2016). Available at: http://www.eoma.aoac.org/app_f.pdf

8 Validation Guidance

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, 20th Ed. of the *Official Methods of Analysis of AOAC INTERNATIONAL* (2016). Available at: http://www.eoma.aoac.org/app_d.pdf

Appendix K: *Guidelines for Dietary Supplements and Botanicals,* 20th Ed. of the *Official Methods of Analysis of AOAC INTERNATIONAL* (2016). Also at: *J. AOAC Int*. **95**, 268(2012); DOI: 10.5740/jaoacint.11-447 and available at: http://www.eoma.aoac.org/app_k.pdf

9 Maximum Time-to-Determination

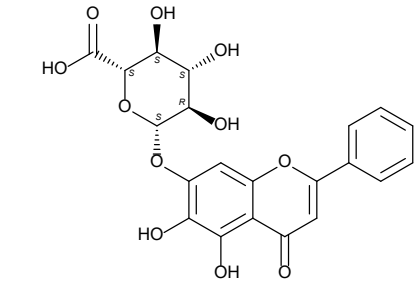
No maximum time.

Approved by the AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: March 16, 2018.

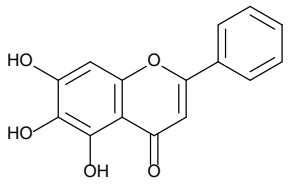
Table 1. Examples of plant material, dietary supplements, and dietary ingredients
Dried plant material
Liquid extracts (including tinctures)
Dry extracts
Tablets
Capsules
Multi-botanical finished product

Table 2. Additional information on select flavanoids

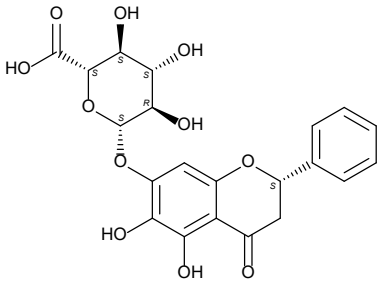
Compound	Name	CAS No.	UNII	InChI key	PubChem
Baicalein (noroxylin)	5,6,7-Trihydroxy-2-phenyl-4*H*-1-benzopyran-4-one	491-67-8	49QAH60606	FXNFHKRTJBSTCS-UHFFFAOYSA-N	5281605
Baicalin (baicalein-7-*O*-glucuronide)	5,6-Dihydroxy-4-oxo-2-phenyl-4*H*-1-benzopyran-7-yl β-D-glucopyranosiduronic acid	21967-41-9	347Q89U4M5	IKIIZLYTISPENI-ZFORQUDYSA-N	64982
Chrysin (5,7-dihydroxyflavone)	5,7-Dihydroxy-2-phenyl-4*H*-1-benzopyran-4-one	480-40-0	3CN01F5ZJ5	RTIXKCRFFJGDFG-UHFFFAOYSA-N	5281607
Dihydrobaicalin (5,6,7-trihydroxyflavanone 7-*O*-*β*-*D*-glucuronide)	(2*S*)-3,4-Dihydro-5,6-dihydroxy-4-oxo-2-phenyl-2*H*-1-benzopyran-7-yl β-D-glucopyranosiduronic acid	56226-98-3	NA	UVNUGBQJLDGZKE-XDZPIWCFSA-N	14135325
Ikonnikoside I	5,6-Dihydroxy-2-(2-hydroxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl β-D-glucopyranosiduronic acid	119152-49-7	NA	ARCCSELFQKSKDR-ZFORQUDYSA-N	10183148
Lateriflorein (5,6,7-trihydroxy-2'-methoxyflavone)	5,6,7-Trihydroxy-2-(2-methoxyphenyl)-4*H*-1-benzopyran-4-one	1329-06-2	NA	OHWUYMZEFLQFQB-UHFFFAOYSA-N	10913608
Lateriflorin (lateriflorein 7-O-β-D-glucuronide)	5,6-Dihydroxy-2-(2-methoxyphenyl)-4-oxo-4H-1-benzopyran-7-yl	521066-22-8	NA	NA	NA
Oroxylin A 7-*O*-β-D-glucuronide (6-methoxybaicalein 7-*O*-β-D-glucuronide)	5-Hydroxy-6-methoxy-4-oxo-2-phenyl-4*H*-1-benzopyran-7-yl β-D-glucopyranosiduronic acid	36948-76-2	O84RM2NAEQ	QXIPXNZUEQYPLZ-QSUZLTIMSA-N	14655552
Scutellarein (6-hydroxyapigenin)	5,6,7-Trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one	529-53-3	P460GTI853	JVXZRQGOGOXCEC-UHFFFAOYSA-N	5281697
Scutellarin (scutellarein 7-*O*-β-D-glucuronide)	5,6-Dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-1-benzopyran-7-yl β-D-glucopyranosiduronic acid	27740-01-8 (1329-06-2)	16IGP0ML9A	DJSISFGPUUYILV-ZFORQUDYSA-N	185617
Teucrin A	(3*S*,5*S*,5'a*S*,7'*S*,8'*S*,8'a*R*)- 5-(3-furanyl)-3',4,5,5',5'a,7',8',8'a-octahydro-8'-hydroxy-7'-methyl-spiro[furan-3(2*H*),6'-[6*H*]naphtho[1,8-*bc*]furan]-2,2'(4'*H*)-dione	12798-51-5	NA	AONLJCCUYGGOSW-PJERILTQSA-N	159529
Teucrioside	(*E*)-4-[3-(3,4-dihydroxyphenyl)-2-propenoate]-2-(3,4-dihydroxyphenyl)ethyl *O*-α-L-lyxopyranosyl-(1→2)-*O*-6-deoxy-α-L-mannopyranosyl-(1→3)-β-D-glucopyranoside	115872-98-5	NA	UDHCHDJLZGYDDM-JPCQEIDESA-N	14034194
Verbascoside (acteoside, kusaginin, russetinol, stereospermin)	(*E*)-4-[3-(3,4-Dihydroxyphenyl)-2-propenoate]-2-(3,4-dihydroxyphenyl)ethyl *O*-α-rhamnopyranosyl(1→3)-4-*O*-caffeoyl-β-D glucopyranoside	61276-17-3	3TGX09BD5B	FBSKJMQYURKNSU-ZLSOWSIRSA-N	5281800
Wogonin	5,7-Dihydroxy-8-methoxy-2-phenyl-4*H*-1-benzopyran-4-one	632-85-9	POK93PO28W	XLTFNNCXVBYBSX-UHFFFAOYSA-N	5281703
Wogonoside (oroxinidin, glychionide B, wogonin 7-O-β-D-glucuronide)	5-Hydroxy-8-methoxy-4-oxo-2-phenyl-4*H*-1-benzopyran-7-yl β-D-glucopyranosiduronic acid	51059-44-0	ETX4944Z3R	LNOHXHDWGCMVCO-NTKSAMNMSA-N	3084961



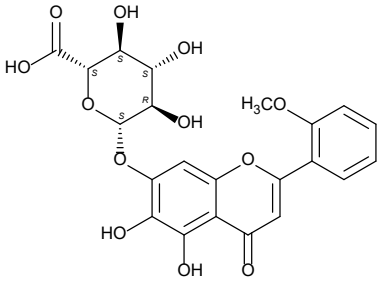
Baicalin



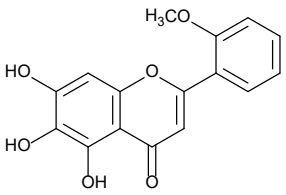
Baicalein



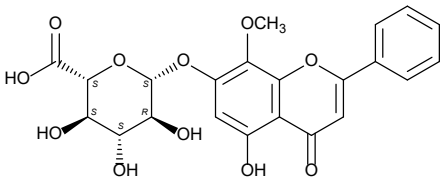
Dihydrobaicalin



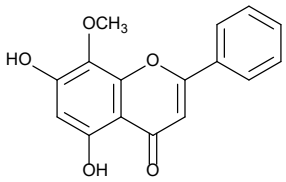
Lateriflorin



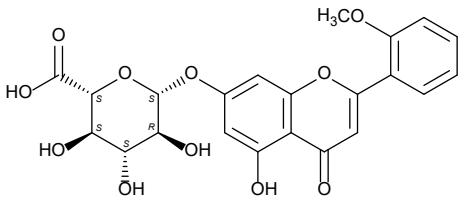
Lateriflorein



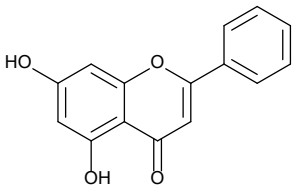
Wogonoside



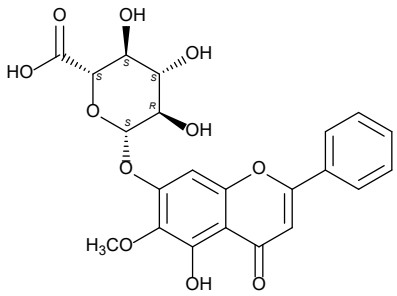
Wogonin



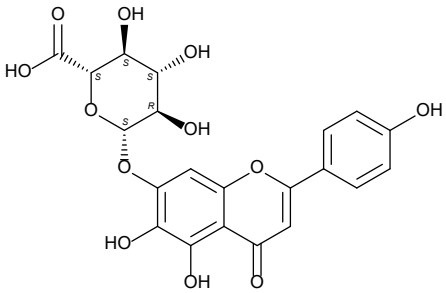
2'-Methoxy-chrysin 7-*O*-Glucoronide



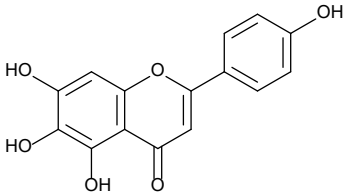
Chrysin



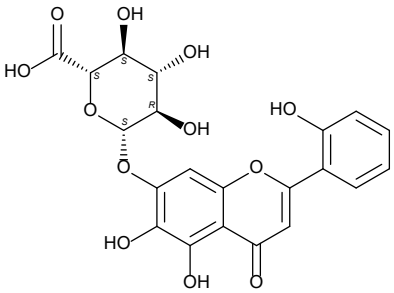
Oroxylin A 7-*O*-Glucoronide



Scutellarin



Scutellarein



Ikonnikoside I

Figure 1. Molecular structures of selected flavanoids.

Table 3. Analytical range and LOQ	
Parameter	Minimum requirement
Analytical range, % (w/w)	1–50
Limit of quantitation, % (w/w)	1

Table 4. Method performance requirements	
Parameter	Minimum requirement
Recovery, %	92–105
RSD_r, %	≤5
RSD_R, %	≤8

Table 5. Sources of selected flavanoids					
Compound	Phytolab	Extrasynthese	Sigma	EP	USP
Baicalein (noroxylin)	89577	1171	92081		1048357
Baicalin (baicalein-7-*O*-glucuronide)	89322	1280 S	94121	Y0001273	1048368
Chrysin (5,7-dihydroxyflavone)	80382	1362 S	95082	NA	NA
Dihydrobaicalin (5,6,7-trihydroxyflavanone 7-*O*-*β*-*D*-glucuronide)	NA	NA	NA	NA	NA
Ikonnikoside I	NA	NA	NA	NA	NA
Lateriflorein (5,6,7-trihydroxy-2'-methoxyflavone)	NA	NA	NA	NA	NA
Lateriflorin (lateriflorein 7-O-β-D-glucuronide)	NA	NA	NA	NA	NA
Oroxylin A 7-*O*-β-D-glucuronide (6-methoxybaicalein 7-*O*-β-D-glucuronide)	NA	NA	NA	NA	NA
Scutellarein (6-hydroxyapigenin)	83283	1334 S	S0327	NA	NA
Scutellarin (scutellarein 7-*O*-β-D-glucuronide)	89788	NA	73577	NA	NA
Teucrin A	89285	NA	NA	NA	NA
Teucrioside	NA	NA	NA	NA	NA
Verbascoside (acteoside, kusaginin, russetinol, stereospermin)	89289	4994 S	v4015	NA	1711455
Wogonin	89825	1304 S	681670	NA	NA
Wogonoside (oroxinidin, glychionide B, wogonin 7-O-β-D-glucuronide)	82684	NA	SMB00081	NA	NA

Table 6. Sources of *Scutellaria* species
Skullcap aerial parts: American Herbal Pharmacopoeia, Product Code 524
Scutellaria baicalensis, root: American Herbal Pharmacopoeia, Product Code 563474
Scutellaria lateriflora, herb: Alkemist Labs, Product Code 942495
Scutellaria lateriflora, herb: American Herbal Pharmacopoeia, Product Code 533097