

Standard Method Performance Requirements (SMPRs®) for Determination of Kavalactones and/or Flavokavains from Kava (*Piper methysticum*)

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*SM or AOAC *Official Methods of Analysis*SM, 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 the six major kavalactones (desmethoxyyangonin, dihydrokavain, yangonin, kavain, dihydromethysticin, and methysticin) and/or flavokavains A, B, and C (see Table 1 for more detailed information on analytes and Figure 1 for molecular structures) derived from the underground portions of kava (*Piper methysticum*) in plant material, dietary ingredients, and dietary supplements as listed Table 2. Methods will be accepted that identify and quantify either flavokavains and/or kavalactones.

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, gelscaps, 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.

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).

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 kavalactone and flavokavains materials, and Table 6 for sources of plant materials.

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.aoc.org/app_f.pdf.

8 Validation Guidance

All target analytes and all matrices listed in Table 1 claimed by the method submitter shall be evaluated. Data from different matrixes may be pooled together to determine overall analytical range, LOQ, recovery, RSD_r, and RSD_R. However, all target analytes and claimed matrixes must be represented in the complete evaluation.

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.aoc.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.aoc.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. Information on kavalactones and flavokavainines of interest

No.	Common name	IUPAC name	CAS No. (alternative)	UNII code	InChI key	PubChem
1	Kavain ((R)-(+)-kavain)	(6R)-5,6-Dihydro-4-methoxy-6-[(1E)-2-phenylethenyl]-2H-pyran-2-one	500-64-1	W1E506373M	XEAQIWGXBCYFX-GUOLPTJISA-N	5281565
2	d,l-Kavain	5,6-Dihydro-4-methoxy-6-[(1E)-2-phenylethenyl]-2H-pyran-2-one	3155-48-4 (1635-33-2)	5L1NI60TGB	XEAQIWGXBCYFX-BQYQJAHWSA-N	5369129
3	Dihydrokavain ((+)-(S)-dihydrokavain, marindirin)	(6S)-5,6-dihydro-4-methoxy-6-(2-phenylethyl)-2H-pyran-2-one	587-63-3	NW8ZGW9XRZ	VOOYTQRREPYRIW-LBPRGKRZSA-N	10220256
4	Methysticin ((+)-methysticin, kavahin)	(6R)-6-[(1E)-2-(1,3-Benzodioxol-5-yl)ethenyl]-5,6-dihydro-4-methoxy-2H-pyran-2-one	495-85-2	M832AIJ6HX	GTEXBOVBADJOQH-FWEMWIAWSA-N	5281567
5	Dihydromethysticin ((S)-(+)-dihydromethysticin)	(6S)-6-[2-(1,3-Benzodioxol-5-yl)ethyl]-5,6-dihydro-4-methoxy-2H-pyran-2-one	19902-91-1	FZ66MQ73GS	RSIWXFIBHXYNFM-NSHDSACASA-N	88308
6	Yangonin	4-Methoxy-6-[(1E)-2-(4-methoxyphenyl)ethenyl]-2H-pyran-2-one	500-62-9	R970U49V3C	XLHIYUYCSMZCCC-VMPITWQZSA-N	5281575
7	Desmethoxyyangonin (5,6-dehydrokawain)	4-Methoxy-6-[(1E)-2-phenylethenyl]-2H-pyran-2-one	15345-89-8 (1952-41-6)	F2MBQ8QRUN	DKKJNZYHGRUXBS-BQYQJAHWSA-N	5273621
8	Flavokavain A (flavokawain A, 4-methoxyflavokawain B)	(2E)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one	37951-13-6 (3420-72-2)		CGIBCVDFTMPT-RMKNXTCFSA-N	5355469
9	Flavokavain B (flavokawain B, persicochalcone)	(2E)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-phenyl-2-propen-1-one	1775-97-9		QKQLSQLKXBHUSO-CMDGGGBGSA-N	5356121
10	Flavokavain C (flavokawain C, 4-hydroxyflavokawain B)	(2E)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one	37308-75-1 (56798-34-6)		UXUFMIJZNYXWDX-VMPITWQZSA-N	6293081

Table 2. Examples of plant material, dietary supplements, and dietary ingredients
Dried plant material
Liquid extracts (including tinctures)
Soft extracts
Dry extracts
Tablets
Capsules (including softgels)

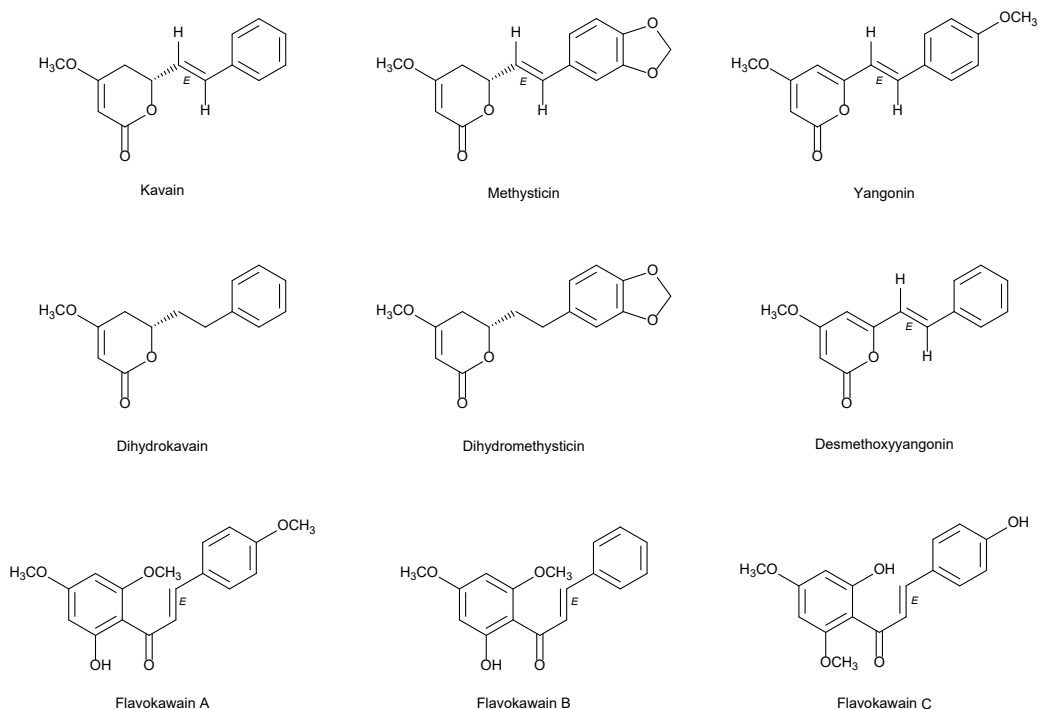


Figure 1. Molecular structure of kavalactones and flavokavains of interest.

Table 3. Analytical range and LOQ based on matrix		
Parameter	Kavalactones ^a	Flavokavains ^a
Analytical range, mg/g ^b	5–750	0.1–25
Limit of quantitation, mg/g	≤5	≤0.1
^a Reported as individual constituents.		
^b Range may be narrower depending on the analytical matrix.		

Table 4. Method performance requirements as a function of range			
Parameter	Acceptance criteria kavalactones ^a	Acceptance criteria lower range flavokavains ^a (0.1–5 mg/g)	Acceptance criteria upper range flavokavains ^a (>5–25mg/g)
Recovery, %	90–110	90–110	
RSD _P , %	≤7.5	≤15	≤7.5
RSD _R , %	≤10	≤20	≤10
^a Reported as individual constituents.			

Table 5. Sources of kavalactone and flavokavain materials

No.	Compound	PhytoLab	Extrasynthese (Alkemist)	Sigma	AvaChem Scientific	AK Scientific Inc.	ACC Corp.	Ceriliant
1	Kavain ((R)-(+)-kavain)				1801	8163AH	API0003191	
2	d,l-Kavain	89239	6550	5790585				PHY89239
3	Dihydrokavain ((+)-S)-dihydrokavain, marindinin)	89185		41866				PHY89185
4	Methysticin ((+)-methysticin, kavahin)	89250		80488				PHY89186
5	Dihydromethysticin ((S)-(+)-dihydromethysticin)	89186		52007				PHY89186
6	Yangonin	89293	4989	75575				PHY89293
7	Desmethoxyyangonin (5,6-dehydrokavain)	89184		51773				
8	Flavokavain A (flavokawain A, 4-methoxyflavokawain B)	83762	1043					
9	Flavokavain B (flavokawain B, persicochalcone)	83763	1045					
10	Flavokavain C (flavokawain C, 4-hydroxyflavokawain B)	83854	1042					

Table 6. Sources of reference plant materials

No.	Botanical reference material	UNII code	USP	Extrasynthese (Alkemist)	American Herbal Pharmacopoeia	Botanical Liaisons, Inc.
1	Rhizome and root			B0061		
2	<i>Piper methysticum</i> root	BOW48C81XP			http://www.herbal-ahp.org/documents/BRM-CRS%20List/AHP-BRM%20List%20Order%208.4.17.pdf	
3	Plant	3P306S300W				http://www.botanicalliaisons.com/materials.html
4	Powdered kava extract		1355709			