

## Standard Method Performance Requirements (SMPRs®) for Determination of Phenolic Compounds in Dietary Supplements and Dietary Ingredients Containing Echinacea

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

### 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 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*<sup>SM</sup> or *AOAC Official Methods of Analysis*<sup>SM</sup>, and can be used as acceptance criteria for verification at user laboratories.

### 2 Applicability

Quantitative determination of individual phenolic compounds, including caffeoyl, chlorogenic acid, cichoric acid, cynarin, and echinacoside (Table 1 and Figure 1) in *Echinacea angustifolia* DC., *E. pallida* (Nutt.) Nutt., and *E. purpurea* (L.) Moench (Table 2) in raw materials, and dietary ingredients and dietary supplement products listed in Table 3.

### 3 Analytical Technique

Any analytical technique that meets the method performance requirements in this SMPR is acceptable.

### 4 Definitions

**Dietary ingredients.**—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 supplements.**—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, gels, liquids, or powders.

***Echinacea angustifolia*.**—See USP 40 Dietary Supplements, Echinacea 6923; or *American Herbal Pharmacopoeia*<sup>®</sup> (AHP) Monograph: *Echinacea angustifolia* root.

***Echinacea pallida*.**—See USP 40 Dietary Supplements, Echinacea 6931; or AHP Monograph: *Echinacea pallida* root.

***Echinacea purpurea*.**—USP 40 Dietary Supplements, Echinacea 6937; or AHP Monograph: *Echinacea purpurea* aerial and AHP Monograph: *Echinacea purpurea* root.

**Limit of quantitation (LOQ).**—Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

**Raw materials.**—Fresh, dried, or cut plant material.

**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<sub>r</sub>); or % repeatability relative standard deviation (%RSD<sub>r</sub>).

**Reproducibility.**—The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility standard deviation (SD<sub>R</sub>); or % reproducibility relative standard deviation (%RSD<sub>R</sub>).

### 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. A control sample must be included.

### 7 Reference Material(s)

See Tables 2 and 6 for a list and sources of reference and testing materials.

Refer to Annex F: *Development and Use of In-House Reference Materials* in Appendix F: *Guidelines for Standard Method Performance Requirements, Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA ([http://www.eoma.aoc.org/app\\_f.pdf](http://www.eoma.aoc.org/app_f.pdf))

### 8 Validation Guidance

All target analytes and examples of *claimed* matrixes listed in Tables 1–3 shall be considered. Data for at least one analyte per *claimed* matrix/plant is acceptable provided that all analytes and *claimed* matrices/plants are represented in the complete evaluation.

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA ([http://www.eoma.aoc.org/app\\_d.pdf](http://www.eoma.aoc.org/app_d.pdf))

Appendix K: *Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis of AOAC INTERNATIONAL* (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA ([http://www.eoma.aoc.org/app\\_k.pdf](http://www.eoma.aoc.org/app_k.pdf)). Also at: *J. AOAC Int.* **95**, 268(2016); DOI: 10.5740/jaoacint.11-447

### 9 Maximum Time-to-Determination

No maximum time.

Approved by the AOAC Stakeholder Panel on Dietary Supplements (SPDS) on September 22, 2017. Final Version Date: September 22, 2017.

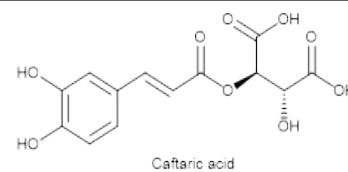
**Table 1. Polyphenols**

No.	Reference standard	CAS No.	Alternative CAS No.	Formula	FDA UNII No.	InChI Key	PubChem ID	MW, g/mol
1	Caftaric acid	67879-58-7		C <sub>13</sub> H <sub>12</sub> O <sub>9</sub>	WCV7W3174L	SWGKAHCIOQPKFW-JTNORFRNSA-N	6440397	312.230
2	Chicoric (cichoric) acid	70831-56-0	6537-80-0	C <sub>22</sub> H <sub>18</sub> O <sub>12</sub>	S4YY3V8YHD	YDDGKXBLOXEEMN-IABMMNSOSA-N	5281764	474.374
3	Chlorogenic acid	327-97-9		C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	318ADP12RI	CWVRJTMFETXNAD-JUHZACGLSA-N	1794427	354.311
4	Echinacoside	82854-37-3		C <sub>35</sub> H <sub>46</sub> O <sub>20</sub>	I04O1DT48T	FSBUXLDOLNLABB-ISAKITKMSA-N	5281771	786.733
5	1,3-Dicaffeoylquinic acid (cynarin)	212891-05-9	30964-13-7	C <sub>26</sub> H <sub>24</sub> O <sub>12</sub>	85D81U9JAV	YDDUMTOHNYZQPO-RVXRWRUFUSA-N	5281769	516.455
6	Powdered <i>Echinacea angustifolia</i> extract	84696-11-7						
7	Powdered <i>Echinacea pallida</i> extract	97281-15-7						
8	Powdered <i>Echinacea purpurea</i> extract	90028-20-9						

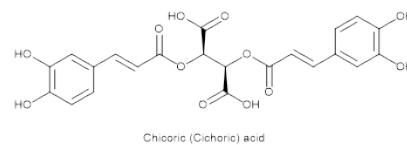
Polyphenol

Molecular structure

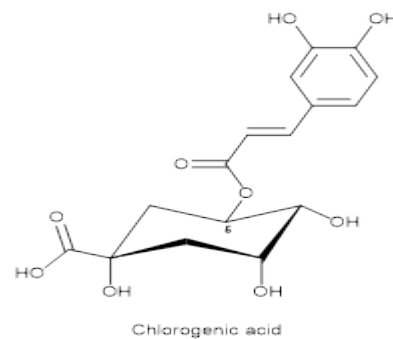
Caftaric acid



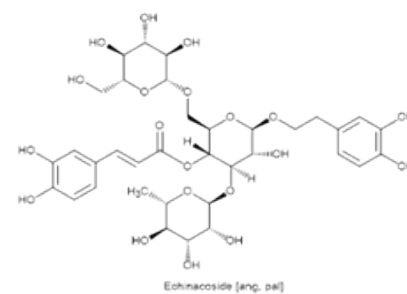
Chicoric (cichoric) acid



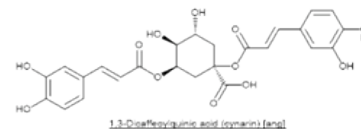
Chlorogenic acid



Echinacoside



1,3-Dicaffeoylquinic acid (cynarin)



**Figure 1. Molecular structures.**

**Table 2. Echinacea**

Echinacea	CAS No.	Form	Source (ID No.)
<i>Echinacea angustifolia</i>	84696-11-7	Powdered extract	USP (1231706)
		Root	Alkemist Labs
		Herb	Alkemist Labs
<i>Echinacea pallida</i>	97281-15-7	Powdered extract	USP (1231717)
		Root	Alkemist Labs
<i>Echinacea purpurea</i>	90028-20-9	Powdered extract	USP (1231728)
		Aerial parts	Alkemist Labs
		Root	Alkemist Labs
		Seed	Alkemist Labs

**Table 3. Examples of dietary ingredient and dietary supplement matrixes**

Ingredients
Extracts (including encapsulated)
Herb powders
Juice
Supplements
Powders
Tablets
Gummies
Liquids
Capsules
Softgel capsules
Tinctures
Gelcaps
Chewables
Juice

**Table 4. LOQ and analytical range**

Parameter	
Analytical range, %	0.01 to 5
LOQ, %	≤0.01

**Table 5. Method performance requirements as a function of range**

Parameter	Range, %	
	0.01–1	>1
Recovery, %	90–107	95–105
RSD <sub>r</sub> , %	≤6	≤3
RSD <sub>R</sub> , %	≤8	≤4

**Table 6. Sources of phenolic compound reference materials**

Polyphenol	CAS No. (alternative)	Source	Product No.
Caftaric acid	67879-58-7	Chromadex	ASB-00003028
		PhytoLab	89170
		Sigma	15029
		USP	1086039
Chicoric (cichoric) acid	70831-56-0 (6537-80-0)	Chromadex	ASB-00003640
		Extrasynthese	4987S
		PhytoLab	89177
		Sigma	C7243
		USP	1105315
Chlorogenic acid	327-97-9	Acro	109240000
		Alfa Asean	J60457
		Chromadex	ASB-00003450
		Extrasynthese	4991S
		LKT Labs	C2943
Echinacoside	82854-37-3	MP Biomedicals	0215061801
		PhytoLabs	89175
		Sigma	C3878
		USP	1115545
		Chromadex	ASB-00005020
1,3-Dicaffeoylquinic acid (cynarin)	212891-05-9 (30964-13-7)	Extrasynthese	4988S
		LKT Labs	E0929
		PhytoLab	89188
		Sigma	01710580
		USP	1231750
1,3-Dicaffeoylquinic acid (cynarin)	212891-05-9 (30964-13-7)	Chromadex	ASB-00003990
		Extrasynthese	4995S
		PhytoLab	89179
		Sigma	91801
		USP	