

Standard Method Performance Requirements for Authentication of Selected *Vaccinium* species (Anthocyanins) in Dietary Ingredients and Dietary Supplements

Intended Use: Reference Method for Dispute Resolution or Routine Use

1 Purpose

AOAC *Standard Method Performance Requirements*SM (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 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* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA.]

2 Applicability

Authentication of selected *Vaccinium* species in dietary ingredients and dietary supplements containing a single *Vaccinium* species using anthocyanin profiles.

3 Analytical Technique

Any analytical technique(s) that measures the analytes of interest and meets the following method performance requirements is/are acceptable.

4 Definitions

Anthocyanins.—Flavanoid phenolic compounds based on an oxonium ion ring structure (commonly pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin), with one of the following substitutions at the 3, 5, or 7 site: simple sugars such as glucoside, arabinoside, or galactoside; oligosaccharides such as

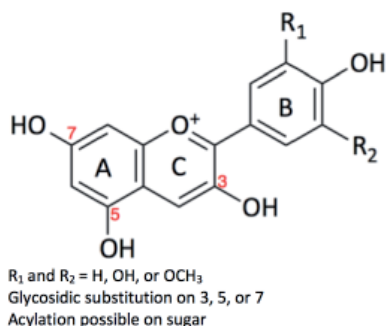


Figure 1. Basic chemical structure of anthocyanin.

Table 1. Method performance requirements

Type of study	Minimum acceptable results
Selectivity study	100% correct identification of all the authenticity panel (Annex I) materials in the presence or absence of exclusivity (Annex II) materials ^a
^a 100% correct analyses are expected. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.	

rutinoside, sambubioside, etc.; or acylation on sugar with coumaric acid, acetic acid, etc. (See Figure 1.)

Identification.—Identification is the characterization of the substance being analyzed, including its chemical, mineral, or biological classification, as applicable. In many investigations the identity of the analyte is assumed and the correctness of the assumption is merely confirmed.

Authenticity panel.—Authenticated materials to be used in the validation study for identity.

Authentication validation study.—A study to verify that a candidate method can correctly authenticate *Vaccinium* species in materials specified in the authenticity panel.

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, gelcaps, liquids, or powders.

Selected *Vaccinium* species.—*Vaccinium* species identified by the AOAC Anthocyanins Working Group that are the focus of methods for authentication by anthocyanin profile. See Annex I.

5 Method Performance Requirements

See Table 1.

6 System Suitability Tests and/or Analytical Quality Control

Methods will include a protocol to demonstrate resolution sensitivity and repeatability.

7 Reference Material(s)

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* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoc.org/app_f.pdf).

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

8 Validation Guidance

Method developers must submit validation data for all of the species listed in Annex I and Annex II in combination with the forms in Annex III for First Action *Official Methods*SM consideration.

Appendix K: *Guidelines for Dietary Supplements and Botanicals, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoc.org/app_k.pdf)

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_d.pdf)

9 Maximum Time-to-Result

No maximum time to result.

Approved by AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: September 5, 2014. Effective Date: October 16, 2014.

ANNEX I

Selected *Vaccinium* species/Authenticity Panel

Lowbush Blueberry (*Vaccinium angustifolium* Aiton)
Northern Highbush Blueberry (*Vaccinium corymbosum* L.)
Southern Highbush Blueberry (*Vaccinium corymbosum* L. x
Vaccinium darrowii Camp)
American Cranberry (*Vaccinium macrocarpon* Aiton)
Bilberry (*Vaccinium myrtillus* L.)
European Cranberry (*Vaccinium oxycoccos* L.)
Rabbiteye Blueberry (*Vaccinium virgatum* Aiton)
Lingonberry (*Vaccinium vitis-idaea* L.)

ANNEX II Exclusivity Panel

Selected Non-*Vaccinium* Fruit

Chinese mulberry (*Morus* L.)
Red raspberry (*Rubus idaeus* L. and hybrids)
Grape (*Vitis vinifera* L., *Vitis labrusca* L. and hybrids)
Elderberry (*Sambucus nigra* L. and *Sambucus canadensis* L.)
Blackberry (*Rubus* spp.)
Black raspberry (*Rubus occidentalis* L. and *Rubus leucodermis*
Douglas ex Torr. & A. Gray)

Strawberry (*Fragaria* L.)
Açaí (*Euterpe oleracea* Mart.)
Black currant (*Ribes nigrum* L.)
Red currant (*Ribes rubrum* L.)
Pomegranate (*Punica granatum* L.)
Sweet cherry (*Prunus avium* L.)
Sour cherry (*Prunus cerasus* L.)
Aronia/chokeberry [*Aronia melanocarpa* (Michx.) Elliott]

Selected Nonfruit Sources

Blue, red, purple potato (*Solanum tuberosum* L.)
Purple sweet potato [*Ipomoea batatas* (L.) Lam.]
Purple corn/maize (*Zea mays* L.)
Black carrot (*Daucus carota* L.)
Beet (*Beta vulgaris* L. and *Beta procumbens* C. Sm.)
Black soybean [*Glycine max* (L.) Merr.]
Black bean (*Phaseolus vulgaris* L.)
Black rice (*Oryza sativa* L.)
Carmine, cochineal, E120 (*Dactylopius coccus* Costa)

Selected Artificial Coloring

Allura Red AC (FD&C Red No. 40, E129)
Amaranth (former FD&C Red No. 2, E123)
Carmoisine (E122)
Ponceau 4R (E124)

ANNEX III Forms

Dry product (dry plant part, dry juices, etc.)
Dry extracts
Liquid extracts
Juice concentrates (marketed as dietary supplements)
Capsules
Softgels
Tablets