AOAC SMPR 2015.007

Standard Method Performance Requirements℠ (SMPRs) for Withanolide Glycosides and Aglycones of Ashwagandha *(Withania somnifera)*

Intended Use: Reference Method for cGMP Compliance

1 Purpose

AOAC S*tandard Method Performance Requirements*℠ (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 (ERPs) 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* (2012) *Official Methods of Analysis of AOAC INTERNATIONAL*, 19th Ed., AOAC INTERNATIONAL, Rockville, MD, USA.]

2 Applicability

Methods shall quantitatively determine withanolide glycosides and aglycones of interest from biomass, extracts, and Ashwagandha-containing finished products.

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

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 {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.

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

Quantitative method.—Method of analysis which response is the amount of the analyte measured either directly (enumeration in a mass or a volume), or indirectly (color, absorbance, impedance, etc.) in a certain amount of sample.

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 spiked analyte that is recovered when the test sample is analyzed using the entire method.

Table 1. Analytical range and LOQ requirements

Parameter	Total glycosides	Aglycones
Analytical range, ppm	10–250000	10–20000
Limit of quantitation (LOQ), ppm	10	

Table 2. Recovery, repeatability, and recovery for both total glycosides and aglycones

Range, ppm	10–100	>100–1000	1000–10000	>10000
Recovery, %	80–110	90–107	95–105	97–103
Repeatability, %	≤7	≤6	≤4	≤1
Reproducibility, %	≤10	≤9	≤6	≤2

Table 3. Target compound panel[a]

Analyte	Relative retention time
Withanoside IV	0.70
Physagulin D	0.75
27-hydroxywithanone	0.80
Withanoside V	0.89
Withanoside VI	0.89
Withaferin A	0.92
Withastramonolide	0.96
Withanolide A	1.00
Withanone	1.01
Withanolide B	1.14

[a] Withanolide glycosides and aglycones measured with the USP-HPLC method.

5 Method Performance Requirements

See Tables 1 and 2.

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.

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. Available at http://www.eoma.aoac.org/app_f.pdf.

NIST guidance document

Withanoside IV

Withanoside V
Withaferin A
Withanolide A
Withanolide B
Withastramonolide
Withanone

8 Validation Guidance

All target compounds in Table 3 and the following matrixes shall be evaluated: tablets, capsules, liquids, powders, extracts, and plant products.

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. Available at http://www.eoma.aoac.org/app_d.pdf.

Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed. Available at http://www.eoma.aoac.org/app_f.pdf.

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

9 Maximum Time-to-Result

None.

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