AOAC SMPR 2015.008

Standard Method Performance Requirements[SM] for Alkaloids of *Mitragyna speciosa*

Intended Use: Reference Method for GMP Compliance and Surveillance

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 (ERPs) 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, Rockville, MD, USA.]

2 Applicability

Methods must be able to quantitate mitragynine, 7-hydroxymitragynine, and separate other relevant indole alkaloids of *Mitragyna speciosa*, in a broad range of matrices, including plant material, extracts, and finished products. Methods should be able to quantitate the analytes for which appropriate standards are available and account for interfering compounds.

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)].

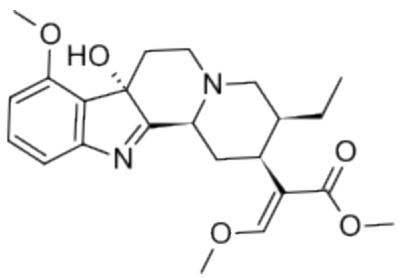

Figure 1. Molecular structure of 7-OH mitragynine.

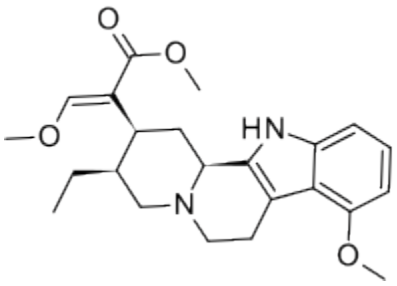

Figure 2. Molecular structure of mitragynine.

Dietary supplements.—Products intended for ingestion, containing at least one "dietary ingredient," and not represented as a conventional food or as a sole item of a meal or the diet. They are products intended to add (supplement) further nutritional value to the diet. Dietary supplements may be found in many forms, such as tablets, capsules, softgels, gelcaps, liquids, or powders.

7-Hydroxymitragynine.—(α*E*,2*S*,3*S*,7a*S*,12b*S*)-3-Ethyl-1,2,3,4,6,7,7a,12b-octahydro-7a-hydroxy-8-methoxy-α-(methoxymethylene)indolo[2,3-a]quinolizine-2-acetic acid methyl ester (CAS No. 174418-82-7). *See* Figure 1 for molecular structure.

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

Mitragynine.—(*E*)-2-[(2*S*,3*S*)-3-ethyl-8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[3,2-h]quinolizin-2-yl]-3-methoxyprop-2-enoic acid methyl ester (CAS No. 4098-40-2). *See* Figure 2 for molecular structure.

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.

5 Method Performance Requirements

See Table 1.

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.

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

Table 1. Method performance requirements

Type of study	Parameter		Minimum acceptance criteria	
			7-OH mitragynine	Mitragynine
Single-laboratory validation	Analytical range		0.01–0.5%	0.1–15%
	Limit of quantitation (LOQ)		≤0.005% (≤50 ppm)	≤0.05% (≤500 ppm)
	Limit of detection (LOD)		15 ppm	150 ppm
	Recovery	0.01–0.1%	90–110%	—
		>0.1–0.5%	95–105%	95–105%
		>0.5–15%	—	95–105%
	Repeatability (RSD_r)	0.01–0.1%	≤5	—
		>0.1–0.5%	≤4	≤4
		>0.5–15%		≤3
Multi-laboratory validation	Reproducibility (RSD_R)	0.01–0.1%	≤8	—
		>0.1–0.5%	≤6	≤6
		>0.5–15%		≤4

Cerilliant or equivalent
H-099 7-Hydroxymitragynine, 100 µg/mL
H-109 7-Hydroxymitragynine-D_3, 100 µg/mL
M-152 Mitragynine, 100 µg/mL
M-182 Mitragynine-D_3, 100 µg/mL

8 Validation Guidance

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.

Approved by AOAC Stakeholder Panel on Dietary Supplements (SPDS). Final Version Date: June 15, 2015. Effective Date: March 19, 2015.