AOAC SMPR 2014.011

Standard Method Performance Requirements for Determination of Phosphodiesterase Type 5 (PDE5) Inhibitors in Dietary Ingredients and 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 method 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

Quantitative method for phosphodiesterase type 5 (PDE5) in dietary ingredients and supplements for use in testing laboratories by trained technicians.

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. {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.

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

PDE5 inhibitors.—For the purposes of this SMPR: PDE5 inhibitors are defined as avanafil, lodenafil carbonate, mirodenafil, sildenafill, tadalafil, udenafil, or vardenafil; or any of their analogs. Refer to the *Supplemental List of Known PDE5 Inhibitors*.

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 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. Method performance requirements

Type of study	Parameter	Minimum acceptable criteria
Single-laboratory validation	Analytical range	50–500 000 ppm
	Limit of quantitation (LOQ)	≤50 ppm
	Repeatability (RSD_r)	≤20%
	Recovery	70 to 120% of mean spiked recovery over the analytical range
Multi-laboratory validation	Reproducibility (RSD_R)	≤30%

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., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_f.pdf)

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

8 Validation Guidance

All target compounds in Annex I and ideally in all matrices in Annex II shall be evaluated.

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)

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.aoac.org/app_f.pdf)

Appendix K: *Guidelines for Dietary Supplements and Botanicals*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2012)

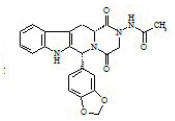
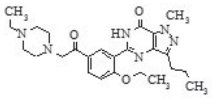
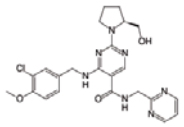
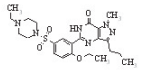
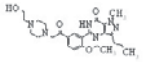
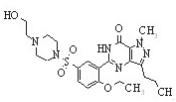
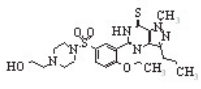
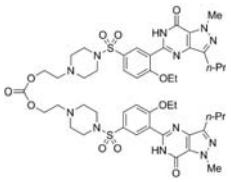
19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_k.pdf)

8 Maximum Time-to-Result

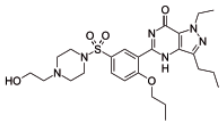
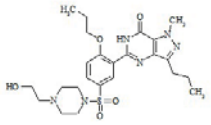
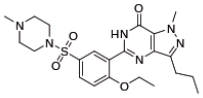
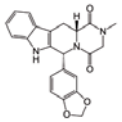
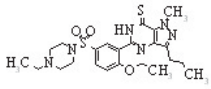
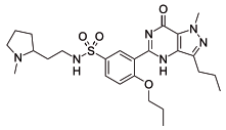
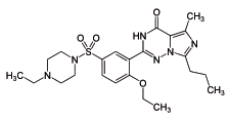
No maximum time.

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

ANNEX I
Target Compound Panel

Analyte	CAS No.	Formula	Structure
Acetaminotadalafil	1446144-71-3	$C_{23}H_{20}N_4O_5$	
Acetildenafil	831217-01-7	$C_{25}H_{34}N_6O_3$	
Avanafil (sold under the brand names *Stendra* and *Spedra*)	330784-47-9	$C_{23}H_{26}ClN_7O_3$	
Homosildenafil	642928-07-2	$C_{23}H_{32}N_6O_4S$	
Hydroxyacetildenafil	147676-56-0	$C_{25}H_{34}N_6O_4$	
Hydroxyhomosildenafil	139755-85-4	$C_{23}H_{32}N_6O_5S$	
Hydroxythiohomosildenafil	479073-82-0	$C_{23}H_{32}N_6O_4S_2$	
Lodenafil carbonate (sold under the brand name *Helleva* in Brazil)	398507-55-6	$C_{43}H_{54}N_{12}O_9S_2$	

ANNEX I
Target Compound Panel (*continued*)

Analyte	CAS No.	Formula	Structure
Mirodenafil (sold under the trade name of *Mvix*.)	862189-95-5	$C_{26}H_{37}N_5O_5S$	
Propoxyphenyl hydroxyhomosildenafil	139755-87-6	$C_{24}H_{34}N_6O_5S$	
Sildenafil (sold under the brand names *Viagra* and *Revatio*, and other various brand names)	139755-83-2	$C_{22}H_{30}N_6O_4S$	
Tadalafil (sold under the brand names *Cialis* and *Adcirca*)	171596-29-5	$C_{22}H_{19}N_3O_4$	
Thiohomosildenafil	479073-80-8	$C_{23}H_{32}N_6O_3S_2$	
Udenafil (sold under the brand name *Zydena*)	268203-93-6	$C_{25}H_{36}N_6O_4S$	
Vardenafil (sold under the brand names *Levitra*, *Staxyn*, and *Vivanza*)	224785-90-4	$C_{23}H_{32}N_6O_4S$	

ANNEX II
Matrixes

Tablets
Capsules (both the content and the capsule shells)
Softgels
Gelcaps
Liquids
Powders
Extracts