AOAC SMPR 2014.010

Standard Method Performance Requirements for Identification 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

Identification of phosphodiesterase type 5 (PDE5) inhibitors (as listed in Annex I) in dietary ingredients and supplements.

3 Analytical Technique

Any analytical technique(s) that identifies 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.

Identification method.—A method that is capable of identifying the PDE5 inhibitors listed in Annex I and providing at least class identification of other PDE5 inhibitors based on their structural similarity to the compounds listed in Annex I. A *Supplemental List of Known PDE5 Inhibitors* provides an overview of currently known PDE5 inhibitors, for which analytical standards are available in the majority of cases. The identification should be done using technique-specific, generally acceptable criteria, such as those given in the European Commission Decision 2002/657/EC.

Interference control.—A control designed to confirm that a test matrix does not interfere with the assay's ability to detect target compounds.

Probability of identification (POI).—The proportion of positive analytical outcomes for an identification method for a given matrix at a given analyte level or concentration.

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*.

5 Method Performance Requirements

See Table 1.

Table 1. Method performance requirements

Type of study	Study	Parameter	Parameter requirements	Target test concn	Minimum acceptable results
Single-laboratory validation	Matrix study	POI at low concn	Minimum of 33 replicates representing all target compounds in Annex I and ideally all matrix types listed in Annex II, spiked at or below the designated low level target test concentration	100 ppm	90% POI[a] of the pooled data for all target compounds and matrixes
		POI at high concn	Minimum of five replicates per matrix type spiked at 10× the designated low level target test concentration	10× low concn	100% correct analyses are expected[b]
		POI at 0 concn	Minimum of five replicates per matrix type	0 ppm	
Multi-laboratory validation	Matrix study[c]	LPOI	Use Appendix N: *ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods*	Low concn	≥0.85[a]
				10× low concn	≥0.95[a]
		$LPOI_{(0)}$		0 ppm	≤0.05[a]

[a] 95% confidence interval.
[b] 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.
[c] Multi-laboratory validation matrix study (LPOI and $LPOI_{(0)}$) are not required for First Action *Official Methods of Analysis* approval.

6 System Suitability Tests and/or Analytical Quality Control

The controls listed in Annex III shall be embedded in assays as appropriate. Interference controls shall be used for method verification for each new matrix.

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 matrixes 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 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). Also at: *J. AOAC Int.* (2012) **95**, 268; DOI: 10.5740/jaoacint.11-447.

Appendix N: *ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_n.pdf)

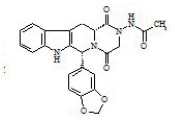
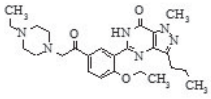
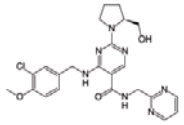
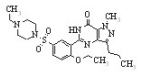
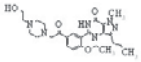
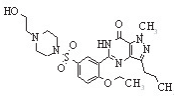
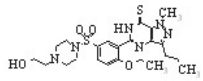
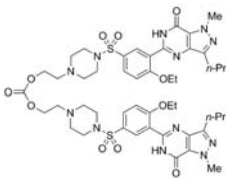
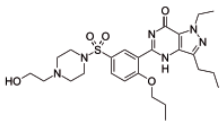
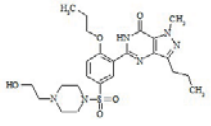
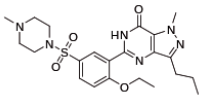
European Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:221:0008:0036:EN:PDF)

9 Maximum Time-to-Result

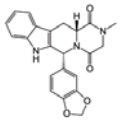
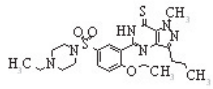
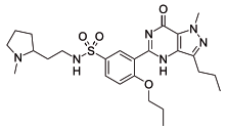
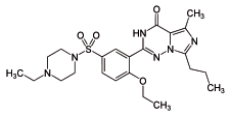
No maximum time.

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	$C_{43}H_{54}N_{12}O_9S_2$	
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	$C_{22}H_{30}N_6O_4S$	

ANNEX I
Target Compound Panel (*continued*)

Analyte	CAS No.	Formula	Structure
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

ANNEX III
Controls

Control	Description	Implementation	Acceptance criteria
Positive	Designed to demonstrate an appropriate test response. This positive control should be included at a low but easily detectable concentration, and should monitor the performance of the entire assay. The purpose of using a low concentration of positive control is to avoid contamination of the test sample and/or instrument.	Single use per sample (or sample set) run	Success: Control detected at expected levels Failure: Control not detected or at levels below expected
Negative	Designed to demonstrate that the assay itself does not produce a positive detection in the absence of target compounds. The purpose of this control is to rule out contamination in the assay or test.	Single use per sample (or sample set) run	Success: No detections made Failure: Detections made
Interference	Designed to specifically address the impact of a sample or sample matrix on the assay's ability to detect target compounds	Single use per sample run	Success: Control detected at expected levels Failure: Control not detected or at levels below expected