AOAC SMPR 2014.012

Standard Method Performance Requirements for Screening Method for Phosphodiesterase Type 5 (PDE5) Inhibitors in Dietary Ingredients and Supplements

Intended Use: Routine Surveillance of Dietary Ingredients and Products

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

Qualitative assay for phosphodiesterase type 5 (PDE5) inhibitors in dietary ingredients and supplements.

3 Analytical Technique

Any analytical technique(s) that detects 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.

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 detection (POD).—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. [Appendix H: *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA (http://www.eoma.aoac.org/app_h.pdf)]

Laboratory probability of detection (LPOD).—The POD value obtained from combining all valid collaborator data sets for a method for a given matrix at a given analyte level or concentration [Appendix H: *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, Maryland, USA (http://www.eoma.aoac.org/app_h.pdf)].

Qualitative assay.—A method of analysis with two possible outcomes.

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*.

Selectivity study.—A study designed to demonstrate that a candidate method does not detect nontarget compounds, and at the same time, demonstrate a candidate method's ability to detect the different types of PDE5 inhibitors (as a minimum the target panel provided in Annex I).

5 Method Performance Requirements

See Tables 1 and 2.

6 System Suitability Tests and/or Analytical Quality Control

The controls listed in Annex III shall be embedded in assays as appropriate. Interference controls should 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 claimed matrices shall be evaluated (see Annex IV for matrices relevant to the detection of PDE5 inhibitors.) Minimum matrices for validation study shall include at least one raw ingredient, such as Epimedium herbal extract and/or powder, and at least one finished product, such as capsules (both the content and the capsule shell).

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)

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)

9 Maximum Time-to-Result

1 hour.

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

Table 1. Matrix-dependent criteria

Type of study	Parameter	Parameter requirements	Target test concn	Minimum acceptable results
Single laboratory validation	POD at low concn	Minimum of 33 replicates per matrix type, spiked at or below the designated low level target test concentration	100 ppm	90% POD[a]
	POD 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 per matrix type[b]
	POD at 0 concn	Minimum of five replicates per matrix type	0 ppm	
Multi-laboratory validation	LPOD[c]	Use Appendix N: *ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods*	Low concn	≥0.85[a]
			10× low concn	≥0.95[a]
	$LPOD_{(0)}$[c]		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.

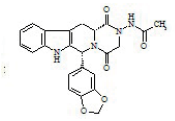
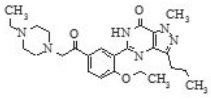
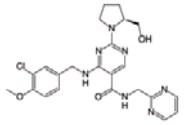
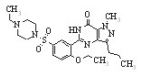
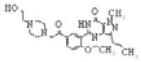
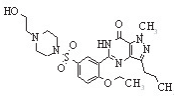
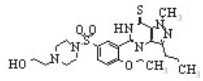
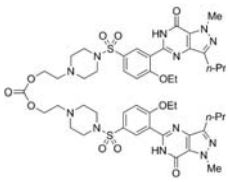
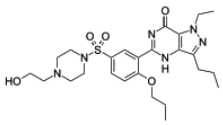
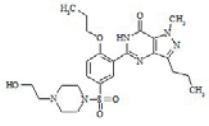
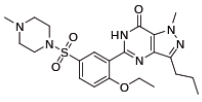
c LPOD = Laboratory probability of detection. The POD value obtained from combining all valid collaborator data sets for a method for a given matrix at a given analyte level or concentration [Appendix H: *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods, Official Methods of Analysis of AOAC INTERNATIONAL* (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, Maryland, USA]. LPOD and $LPOD_{(0)}$ are not required for single-laboratory validations.

Table 2. Selectivity study

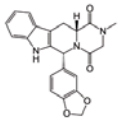
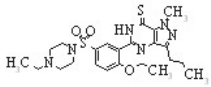
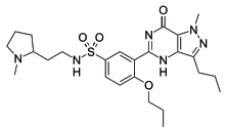
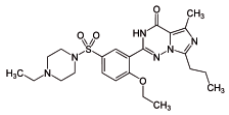
Type of study	Parameter	Parameter requirements	Final test concn	Minimum acceptable results
Single laboratory validation	Target	Test each target compound listed in Annex I at the final test concentration	Low concn	100% positive results[a]
	Nontarget	Test each nontarget panel compound at the final test concentration or at the highest expected matrix concentration in the case of naturally occurring matrix components. A list of potential nontarget compounds for immunoassays is provided in Annex II.	10× low concn	≥95% negative results

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.

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
Nontarget Panel

A suitable nontarget panel shall be selected based on the analytical technique.

For nonbioassays: It is expected that an appropriate nontarget panel will be developed for the selectivity study in Table 2 of the method performance requirements.

For a bioassay based on PDE5 inhibitors: Natural components/matrices with PDE5 inhibition activity shall be evaluated, such as extracts/powders of *Tribulus terrestris, Cnidium monieri, Morinda officinalis, Cuscuta chinensis,* and *Epimedium koreanum.*

For immunoassay, structurally similar compounds should be tested, such as the following examples of compounds that are subcomponents of PDE5 inhibitors, which are provided for illustration purposes:

2-Pyrimidinecarboxamide, 5-pyrimidinecarboxamide, benzenesulfonyl, piperazin, piperazinyl, piperazinylphenolate, 3,6,7,12,12a-hexahydro-2-methyl-pyrazino [1′,2′:1,6] pyrido[3,4-b]indole-1,4-dione, benzenesulfonamide

ANNEX III
Controls

Control	Description	Implementation	Acceptance criteria
Positive control	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 Control	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 Control	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

ANNEX IV
Matrixes

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