AOAC SMPR® 2025.002

Standard Method Performance Requirements (SMPRs®) for Detection of Benzodiazepines, Fentanyl, Medetomidine, Nitazenes, and Xylazine in Selected Drug Product Solids, Liquids, and Residues

Intended Use: Rapid Screening of Known or Suspected Drug Products in Non-Laboratory Environments

Purpose

What: AOAC *Standard Method Performance Requirements* (SMPRs®) are voluntary consensus standards developed in accordance with the AOAC policy, "AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents." SMPRs describe a scientific community's recommended minimum method performance characteristics and analytical requirements for a specific method-related intended use.

Who: Drafted by AOAC working groups, SMPRs are adopted by AOAC by a consensus of stakeholders affiliated with its integrated science programs and projects, which are composed of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.

Use: AOAC SMPRs are used in AOAC core science programs as a resource for AOAC method experts, including expert review panels, in the evaluation of validation study data for methods submitted to the AOAC *Official Methods of Analysis*SM and AOAC *Performance Tested Methods*SM programs. AOAC SMPRs also may be used to provide acceptance criteria for verification of methods and serve as a resource to guide method development and optimization.

1 Applicability

Detection of drug analytes (*see* Table 1) in matrices to include bulk solids, liquids, and residues (*see* Table 2), excluding plant material, beverages, and biological fluids by lateral flow immunoassay or aptamer test strips. Methods may be targeted to detect individual or multiple analytes in one or more matrices. The method scope (test strip instructions for use) shall specifically define the individual analyte(s) being tested, evaluated matrices, intended users, and sample preparation procedures. These SMPRs can be used as a basis for validation of methods for drugs and drug classes beyond those specifically defined in the document, but compound-specific cross-reactivity requirements, specified in Table 3a, would need to be established for these drug analytes.

Table 1. Test strip target analytes

Test strip target	Drug compound	CAS No.
Benzodiazepines	Alprazolam	28981-97-7
Fentanyl	Fentanyl • HCl	1443-54-5
Medetomidine	*dl*-Medetomidine • HCl	86347-15-1
Nitazene	Isotonitazene • $C_6H_8O_7$	NA
Xylazine	Xylazine • HCl	23076-35-9

Table 2. Target matrices

Matrix category	Representative matrices
Bulk solids	Powder, crystalline powder, tablet, capsule
Liquids	Liquids (excluding beverages and biological fluids), softgels/gelcaps
Residues	Residue from used paraphernalia or packaging, combusted or smoked material (excluding plant material)

2 Analytical Technique

Immunoassays and aptamer-based tests in lateral flow or test strip formats.

3 Definitions

Aptamer-based test.—Test that uses short, single-stranded nucleic acid sequences to identify and measure certain substances (1).

Cross-reactivity.—Reaction of test strip to material other than target analyte(s) (2; *see* Table 3a). *Note*: In the context of this document, cross-reactivity includes all reactions to compounds other than the defined target compound. In some instances, these reactions may be considered favorable because the cross-reacting compound belongs to the same drug class as the target analyte or may be considered unfavorable because the compound is not related to the compound(s) of interest. For example, the target compound for a benzodiazepine test strip may be alprazolam, but cross-reactivity with bromazolam is a desired reaction and cross-reactivity with xylazine is not.

Immunoassay.—Test that uses binding of antibodies to antigens to identify and measure certain substances (3).

Laboratory probability of detection (LPOD).—Composite POD pooled across laboratories (4). 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 (5).

Limit of detection (LOD).—Lowest concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level (6).

Powders.—Fine, dry particles that may vary in morphology, size, and density; dosage form that is a solid or a mixture of solids in a finely divided state intended for internal or external use (7).

Probability of detection (POD).—Proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent (6).

Tablet.—Oral solid dosage forms containing drug substance(s) with or without excipients (7).

Target compound.—Chosen analyte used for characterizing test strips (*see* Table 1).

4 Method Performance Requirements

See Table 4.

5 System Suitability Tests and/or Analytical Quality Control

Positive and negative controls shall be embedded in assays as appropriate (*see* Table 5). Manufacturers must provide written justification if controls are not appropriate for an assay.

6 Reference Material(s)

Use of reference materials for each analyte is required for testing. Reference materials should be obtained as powders, as the

Table 3. Potential cross-reactants

(a) Potential cross-reactants (including compounds that may interfere with the analyte detection) to be tested for all test strips

Drug compound	CAS No.
3,4-MDA • HCl	6292-91-7
3,4-MDMA • HCl	64057-70-1
5-APB • HCl	286834-80-8
Acetaminophen	103-90-2
Alprazolam[a]	28981-97-7
dl-Amphetamine • HCl	2706-50-5
Aspirin	50-78-2
Benzocaine	94-09-7
Brorphine • HCl	2707204-49-5
Buprenorphine • HCl	53152-21-9
Bupropion • HCl	31677-93-7
Buspirone • HCl	33386-08-2
Caffeine	58-08-2
dl-Cathinone • HCl	16735-19-6
Citric acid	77-92-9
Cocaine • HCl	53-21-4
Codeine • H_3PO_4 [½H_2O]	41444-62-6
Creatine • H_2O	6020-87-7
Dextromethorphan • HBr [H_2O]	6700-34-1
Dimethylsulfone ($DMSO_2$)	67-71-0
N,N-Dimethyltryptamine (DMT) • $C_4H_6O_4$	2853570-32-6
Diphenhydramine • HCl	147-24-0
Eutylone • HCl	17764-18-0
Fentanyl • HCl	1443-54-5
D-Fructose	57-48-7
Gabapentin	60142-96-3
Guaifenesin	93-14-1
Heroin • HCl	1502-95-0
Hydroxyzine • 2HCl	2192-20-3
dl-Ibuprofen	15687-27-1
Imipramine • HCl	113-52-0
myo-Inositol	87-89-8
Isotonitazene • $C_6H_8O_7$[a]	NA
Ketamine • HCl	1867-66-9
D-Lactose • H_2O	64044-51-5
Levamisole • HCl	16595-80-5
Lidocaine • HCl	73-78-9
Lisdexamfetamine • 2CH_3SO_3H	608137-33-3
D-Mannitol	69-65-8
dl-Medetomidine • HCl	86347-15-1

Table 3. (*continued*)

Drug compound	CAS No.
Melatonin[a]	73-31-4
Metamizole (sodium salt)	68-89-3
dl-Methadone • HCl	1095-90-5
dl-Methamphetamine • HCl	300-42-5
Methcathinone • HCl	49656-78-2
p-Methyl AP-237 • HCl	2749048-12-0
dl-threo-Methylphenidate • HCl	23655-65-4
Naloxone • HCl	357-08-4
Naproxen • Na	26159-34-2
dl-Nicotine	22083-74-5
Oxycodone • HCl	124-90-3
Papaverine • HCl	58-74-2
Pentedrone • HCl	879669-95-1
Pentobarbital	76-74-4
Phenacetin[a]	62-44-2
Phencyclidine (PCP) • HCl	956-90-1
dl-Phenylephrine • HCl	61-76-7
Polyethylene glycol–400 (PEG-400)	25322-68-3
Primidone[a]	125-33-7
Procaine • HCl	51-05-8
Psilocin • ½$C_4H_4O_4$	2769752-98-7
Pseudoephedrine • HCl	345-78-8
α-Pyrrolidinoisohexanophenone (α-PiHP) • HCl	2705245-60-7
Quinine	130-95-0
Sildenafil • $C_6H_8O_7$	171599-83-0
Sodium bicarbonate	144-55-8
d-Sorbitol	50-70-4
Tetracaine • HCl	136-47-0
cis-Tramadol • HCl	36282-47-0
Trazodone • HCl	25332-39-2
Tryptamine • HCl	343-94-2
Vitamin C (*l*-ascorbic acid)	50-81-7
Xylazine • HCl	23076-35-9
Zolpidem	82626-48-0

(b) Additional compounds required to be tested for benzodiazepine test strips[a]

Bromazepam[a]	1812-30-2
Bromazolam[a]	71368-80-4
Chlordiazepoxide[a]	58-25-3
Clobazam[a]	22316-47-8
Clonazepam[a]	1622-61-3
Clorazepate • 2K[a]	57109-90-7

Table 3. (*continued*)

Drug compound	CAS No.
Desalkylflurazepam[a]	2886-65-9
Desalkylgidazepam[a]	2894-61-3
Diazepam[a]	439-14-5
Estazolam[a]	29975-16-4
Ethylbromazolam[a]	105470-75-5
Etizolam[a]	40054-69-1
Flualprazolam[a]	28910-91-0
Flubromazepam[a]	2647-50-9
Flubromazolam[a]	612526-40-6
Flunitrazepam[a]	1622-62-4
Fluoxetine • HCl	56296-78-7
Flurazepam[a]	17617-23-1
Loprazolam • CH_4O_3S[a]	70111-54-5
Lorazepam[a]	846-49-1
Lormetazepam[a]	848-75-9
Medazepam[a]	2898-12-6
Midazolam[a]	59467-70-8
Nitrazepam[a]	146-22-5
Nordiazepam[a]	1088-11-5
Oxazepam[a]	604-75-1
Phenazolam[a]	87213-50-1
Prazepam[a]	2955-38-6
Temazepam[a]	846-50-4
Triazolam[a]	28911-01-5
(c) Additional compounds required to be tested for fentanyl test strips	
4-ANPP[a]	21409-26-7
Acetyl fentanyl • HCl	117332-89-5
Carfentanil[a]	59708-52-0
Furanyl fentanyl • HCl	101365-56-4
Norfentanyl[a]	1609-66-1
Methoxyacetyl fentanyl • HCl	101365-54-2
o-Methylfentanyl • HCl	1443-53-4
p-Chlorofentanyl • HCl	117994-27-1
p-Fluorofentanyl • HCl	117332-92-0
p-Fluoroisobutyryl fentanyl • HCl (FIBF)	2309383-06-8
Phenethyl-4-ANPP • HCl	2751624-03-8
Remifentanil • HCl	132539-07-2
Sufentanil • $C_6H_8O_7$	60561-17-3

Table 3. (*continued*)

Drug compound	CAS No.
(d) Additional compounds required to be tested for medetomidine test strips	
Clonidine • HCl	4205-91-8
Detomidine • HCl	90038-01-0
Dexmedetomidine • HCl	145108-58-3
Etomidate	33125-97-2
Guanfacine • HCl	29110-48-3
Levomedetomidine • HCl	190000-46-5
Lofexidine • HCl	21498-08-8
dl-Metomidate • HCl	35944-74-2
Romifidine • HCl	65896-14-2
Tizanidine • HCl	64461-82-1
(e) Additional compounds required to be tested for nitazene test strips[a]	
5-Amino isotonitazene	NA
5-Methyl etodesnitazene • $C_6H_8O_7$	NA
N-Desethyl isotonitazene • HCl	NA
Ethylene nitazene • $C_6H_8O_7$	NA
Ethyleneoxynitazene • $C_6H_8O_7$	NA
Etodesnitazene • $C_6H_8O_7$	NA
Etonitazene • $C_6H_8O_7$	NA
Fluetonitazene • $C_6H_8O_7$	NA
Metonitazene • $C_6H_8O_7$	14680-51-4
N-Desethyl metonitazene • HCl	NA
Nitazene • $C_6H_8O_7$	NA
N-Piperidinyl etonitazene • $C_6H_8O_7$	NA
N-Piperidinyl metonitazene • $C_6H_8O_7$	NA
N-Pyrrolidino isotonitazene • $C_6H_8O_7$	NA
N-Pyrrolidino metonitazene • $C_6H_8O_7$	NA
Protodesnitazene • $C_6H_8O_7$	NA
Protonitazene • HCl	119276-01-6
(f) Additional compounds required to be tested for xylazine test strips	
Chlorpromazine • HCl	69-09-0
Clonidine • HCl	4205-91-8
Detomidine • HCl	90038-01-0
Lofexidine • HCl	21498-08-8
Romifidine • HCl	65896-14-2
Thioridazine • HCl	130-61-0
Tizanidine • HCl	64461-82-1

[a] Analyte not soluble at 2 mg/mL; needs to be analyzed at maximum dissolvable concentration.

Table 4. Method performance requirements

Type of study	Parameter	Requirement	Target test concentration	Minimum acceptable result, %
Single-laboratory	POD at low concentration	Minimum of 33 positive replicates[a] per matrix type, spiked at or below low-level target test concentration	*See* Table 6	90% POD with 95% confidence
	POD at high concentration	Minimum of five replicates[a] per matrix type, spiked at high-level target test concentration	Pure target analyte in aqueous solution (no matrix)	100% positive result
Multi-laboratory	POD in blank matrix	Minimum of five replicates[a] per matrix type	Blank matrix	100% negative result
	LPOD	Use AOAC OMA "Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods"	*See* Table 6	≥85
			Pure target analyte in aqueous solution (no matrix)	≥95
	$LPOD_{(0)}$		Blank matrix	≤5

[a] Replicates are meant to be aliquots of same solution tested using individual test strips.

Table 5. Assay controls

Type	Description	Requirement	Result[a]
Positive	Designed to demonstrate appropriate test response; positive controls should be included at 10x LOD and should monitor performance of entire assay	Single use per sample (or sample set) run	*Success*: Positive test response at tested concentration *Failure:* Negative or inconclusive test response
Negative	Designed to demonstrate that assay itself does not produce positive test response in the absence of any target analytes and any potential interference from matrix	Single use per sample (or sample set) run	*Success:* Negative test response *Failure*: Positive or inconclusive test response

[a] If test strip control line fails to develop or solution does not fully wick up the strip, result will not be considered a negative, but it must be documented. Additional test strip can be used in place of faulty strip in this instance.

Table 6. Sample preparation for high and low concentrations for POD experiments for different matrix categories

Matrix category	Bulk solids	Liquids	Residues
Amount of solvent, mL	5	1	1
Maximum amount of material	10 mg	0.1 mL	100 µg
Maximum (high) concentration (assuming pure analyte substance)	2 mg/mL or maximum solubility, whichever is lower	2 mg/mL or maximum solubility, whichever is lower	0.1 mg/mL or maximum solubility, whichever is lower
Low concentration for POD	2x LOD or 0.002 mg/mL (2000 ng/mL), whichever is greater prepared in the presence of matrix compounds to total sample size of 2 mg/mL	2x LOD or 0.002 mg/mL (2000 ng/mL), whichever is greater prepared in the presence of matrix compounds to total sample size of 2 mg/mL	2x LOD or 0.0001 mg/mL (100 ng/mL), whichever is greater prepared in the presence of matrix compounds to total sample size of 0.1 mg/mL
Matrix compound	Mannitol	PEG-400	Mannitol
	Sodium bicarbonate	Glycerin	Sodium bicarbonate
	Caffeine	MCT oil	Caffeine
	Lactose	Benzyl alcohol	Lactose

Table 7. Environmental factors

Environmental condition	Test strips to study	Criteria
Elevated storage temperature	All	3/3 Positive results obtained
Decreased storage temperature	All	
Low pH solution	All	
High pH solution	All	
Mechanical manipulation	Test strips not housed in rigid cassette	
Elevated humidity	Test strips not stored in sealed packaging	
Water hardness	Test strips not accompanied with pre-portioned water packets	
Lighting	All	

salt forms, whenever possible. Reference materials should have purity of ≥95%.

7 Validation Guidance

Methods may detect individual or multiple drug analyte compounds. All validation studies must include use of reference materials and assay controls (*see* Table 5). Method validation study designs must include LOD, cross-reactivity, and environmental factors studies. Matrix compounds listed in Table 6 are to be used individually as the matrix for the validation study. Sample preparation for each type of matrix should be assessed using the criteria in Table 6. Information on LOD, cross-reactivity, environmental factors, and field use studies are as follows, however, review accompanying standard method validation protocol for details on all required studies and data collection requirements to support this document:

LOD.—Establishing LOD requires establishment of preliminary and final LODs. LOD should be determined for target analyte in aqueous solution. This process is adapted from the U.S. Food and Drug Administration's (FDA) Template for Developers of Molecular Diagnostic Tests (https://www.fda.gov/media/135900/download).

Cross-reactivity study.—Using Table 3, cross-reactivity should be tested at maximum solubility or 2 mg/mL in water, whichever is lower, if validating solid and/or liquid matrices. Cross-reactivity should be tested at maximum solubility or 0.1 mg/mL in water, whichever is lower, if validating residue matrix. Conduct triplicate analyses for each compound. If matrix compound cross-reacts on test strip in cross-reactivity study (produces positive result), it should not be included as a matrix in this study.

Environmental factors.—Each relevant factor listed in Table 7 should be assessed in triplicate at 2x LOD. If test strip does not produce positive result for target analyte, repeat analysis with increasing concentration of target analyte until 3/3 positive results are obtained. If control band is not observed in all initial target analyte and negative control tests, additional testing does not need to be conducted.

Field use study.—The field use study should assess how a minimum of 15 individuals with no experience using lateral flow tests for the analysis of drugs can perform critical tasks required to use the test strips. Users should encompass a variety of ages, levels of education, and socioeconomic backgrounds.

8 Maximum Time to Result

Five minutes from sample introduction to result.

9 References

(1) Ni, X., Castanares, M., Mukherjee, A., & Lupold, S.E. (2011) *Curr. Med. Chem*. **18**(27), 4206–4214. doi: 10.2174/092986711797189600

(2) Majdinasab, M., Badea, M., & Marty J.L. (2022) *Pharmaceuticals* **15**(1), 90. https://doi.org/10.3390/ph15010090

(3) National Cancer Institute (2025) *NCI Dictionary of Cancer Terms: Immunoassay*, U.S. National Institutes of Health, https://www.cancer.gov/publications/dictionaries/cancer-terms/def/immunoassay (accessed 3/13/2025)

(4) Wehling, P., LaBudde, R.A., Brunelle, S.L., & Nelson, M.T. (2011) *J. AOAC Int*. **94**, 335–347

(5) *Official Methods of Analysis of AOAC INTERNATIONAL* (2023) "Appendix M: Guidance on Food Allergen Immunoassay Validation," G.W. Latimer, Jr. (Ed), 22nd Ed., Oxford University Press, New York, NY, USA. https://doi.org/10.1093/9780197610145.005.013 (accessed June 13, 2025)

(6) ISO Standard No. 5725-1:2023 (2023) Accuracy (trueness and precision) of measurement methods and results—Part 1: General principles and definitions, International Organization for Standardization. https://www.iso.org/standard/80974.html

(7) USP Nomenclature Guidelines, G01.11-03, Effective Date 3/20/2020. https://www.usp.org/sites/default/files/usp/document/usp-nomenclature-guidelines.pdf

Additional References

Official Methods of Analysis of AOAC INTERNATIONAL (2023) "Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces," G.W. Latimer, Jr. (Ed), 22nd Ed., Oxford University Press, New York, NY, USA. https://doi.org/10.1093/9780197610145.005.010

Official Methods of Analysis of AOAC INTERNATIONAL (2023) "Appendix N: ISPAM Guidelines for Validation of Qualitative Binary Chemistry Methods," G.W. Latimer, Jr. (Ed), 22nd Ed., Oxford University Press, New York, NY, USA. https://doi.org/10.1093/9780197610145.005.014

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (2023) *Bacteriological Analytical*

Manual, Chapter 5. https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

United States Pharmacopeia 40–National Formulary 35 (USP 40–NF 35) (2017) <61> "Microbiological examination of nonsterile products: Microbial enumeration tests," U.S. Pharmacopeia Convention, Rockville, MD, USA

United States Pharmacopeia 40–National Formulary 35 (USP 40–NF 35) (2017) <62> "Microbiological examination of nonsterile products: Tests for specified microorganisms," U.S. Pharmacopeia Convention, Rockville, MD, USA

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