AOAC SMPR® 2018.010

Standard Method Performance Requirements (SMPRs®) for Screening and Identification Method for Regulated Veterinary Drug Residues in Food

Intended Use: Routine Surveillance for GMP Compliance

1 Purpose

AOAC 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 the 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.

2 Applicability

A method or a suite of methods that can screen for and identify regulated veterinary drug residues with established maximum residue limits (MRLs) in bovine milk, muscle, and fat; chicken muscle, skin with adhering fat, and eggs; and fish. Table 1 is provided as guidance on veterinary drug residue/matrix combinations and associated MRLs. Additional matrices may be added as appendices to this SMPR in the future. A single method is not required to cover all drug/matrix combinations, but method developers should strive to include as many relevant drug residues as possible for each matrix claimed. Method developers may choose to claim one or more matrices.

3 Analytical Technique

Liquid chromatography-tandem mass spectrometry (LC-MS/MS), using low- or high-resolution MS.

4 Definitions

Maximum residue limit (MRL).—The maximum allowable concentration of a drug residue in a particular matrix. Also known as "tolerance" in the United States. MRL varies by matrix and by country or regulatory agency. For the purposes of this SMPR, the lowest MRL currently in effect amongst European Union (EU), Codex, Canada, China, and U.S. regulations will be used as "the MRL." In cases in which an MRL applies to the sum of metabolites or sum of drugs, that MRL was chosen over MRLs for a single metabolite or marker residue. If no MRL is provided in Table 1 (shaded cells), then the drug is either prohibited in that matrix by one or more regulatory agencies or an MRL is not required.

Probability of detection (POD).—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* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA (http://www.eoma.aoac.org/app_h.pdf)]

5 Method Performance Requirements

See Table 2.

6 System Suitability Tests and/or Analytical Quality Control

Suitable methods will include blank check samples and check standards at 0.5x MRL prepared in matrix. Method developers will provide information on how cutoffs are determined.

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*, 21st Ed. of the *Official Methods of Analysis of AOAC INTERNATIONAL* (2019). Available at http://www.eoma.aoac.org/app_f.pdf

8 Validation Guidance

Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, 21st Ed. of the *Official Methods of Analysis of AOAC INTERNATIONAL* (2019). Available at http://www.eoma.aoac.org/app_d.pdf

Appendix F: *Guidelines for Standard Method Performance Requirements*, 21st Ed. of the *Official Methods of Analysis of AOAC INTERNATIONAL* (2019). Available at http://www.eoma.aoac.org/app_f.pdf

Method developers may prepare cocktails of multiple drug residues. Method developers are cautioned that some drug residues may have additive or masking effects when combined and should be prepared to demonstrate that these concerns have been addressed with their submitted materials/data. Method developers should consider the stability of drug residues in cocktails and be prepared to demonstrate that these concerns have been addressed in their data submission package.

Performance criteria in Table 2 are for single-laboratory validation. Method developers should contact AOAC for developing a collaborative study design.

Method developers must provide the precursor ion and at least two standard reference material (SRM) transitions with ion ratios and retention parameters for each veterinary drug.

9 Maximum Time-to-Result

None

Table 1. Veterinary drugs residues in bovine milk, bovine muscle, bovine fat, chicken muscle, chicken skin/fat, fish, and eggs[a]

Compound	Regulated marker	Lowest global MRL, µg/kg						
		Liquid bovine milk	Raw bovine muscle	Raw bovine fat	Raw chicken muscle	Raw chicken skin/fat	Raw fish	Raw whole chicken egg
Abamectin (B1a)		[b]						
Acetylisovaleryltylosin					40	40		
Albendazole	Albendazole sulfone	100	100	100				
Albendazole	Albendazole sulfoxide							
Albendazole	Albendazole 2-aminosulfone							
Albendazole Oxide	Albendazole oxide							
Albendazole Oxide	Albendazole sulfone							
Albendazole Oxide	Albendazole 2-aminosulfone							
Amitraz	Sum of metabolites containing 2,4-DMA moiety	10		200				
Amoxicillin		4	10	10	10	10	50	
Ampicillin		4	10	10	10	10	50	
Amprolium			500	2000	500	500		4000
Apramycin			50	50	50	50		
Avilamycin					50	50		
Bacitracin (A, B, C)	Dichloroisoeverninic acid	100	500	500	500	500		500
Baquiloprim		30						
Betamethasone		0.3	0.75	0.75				
Bicozamycin			200	50	50	50		
Buquinolate			100		100	100		200
Cabergoline		0.1						
Carazolol		1	5	5				
Carprofen	Carprofen		500	1000			12	
Carprofen	Carprofen glucuronide							
Cefacetril		125						
Cefalexin		100	200	200	200	200		
Cefalonium		20						
Cefapirin	Cefapirin	60	50	50				
Cefapirin	Deacetylcefapirin							
Cefazolin		50						

Table 1. (*continued*)

Compound	Regulated marker	Lowest global MRL, µg/kg						
		Liquid bovine milk	Raw bovine muscle	Raw bovine fat	Raw chicken muscle	Raw chicken skin/fat	Raw fish	Raw whole chicken egg
Cefoperazone		50						
Cefquinome		20	50	50				
Ceftiofur	Ceftiofur	100	1000	2000				
Ceftiofur	Desfuroylceftiofur							
Cefuroxime			20	20				
Chloramine-T	Para-toluenesulfonamide						900	
Chlormadinone		2.5		4				
Chlortetracycline	Chlortetracycline[c]	100	200	200	200	200	200	400
Chlortetracycline	4-epi-chlrotetracycline[c]							
Clavulanic acid		200	100	100				
Clenbuterol		0.05	0.1	0.2				
Clopidol		20	200	200	5000	5000		
Clorsulon		16	35					
Closantel		45	1000	3000				
Cloxacillin		10	10	10	300	300	300	300
Colistin		50	150	150	150	150	150	
Cyfluthrin		20	10	50				
Cyhalothrin		30	20	400				
Cypermethrin	Cypermethrin	20	20	200			50	
Cypermethrin	Alpha-Cypermethrin							
Cyromazine		30	70	100	100	50		300
Danofloxacin				100	200	100	100	
Decoquinate		20	1000	2000	1000	2000		
Deltamethrin			10	50	30	500	10	30
Dexamethasone		0.3	0.75	1	1	1		
Diaveridine					50	50		
Diazinon		20	20	700				
Dichlorvos				50		50		
Diclazuril		50	50		500	1000		50
Diclofenac		0.1	5	1				

Table 1. (continued)

Compound	Regulated marker	Lowest global MRL, µg/kg						
		Liquid bovine milk	Raw bovine muscle	Raw bovine fat	Raw chicken muscle	Raw chicken skin/fat	Raw fish	Raw whole chicken egg
Dicloxacillin		30	300	300	300	300	300	
Difloxacin			400	100	300	400	300	
Dihydrostreptomycin[d]	Sum of dihydrostreptomycin + streptomycin	200	600	600	600	600		
Diminazene		150	500				500	
Doramectin (B1a)		15	10	150				
Doxycycline	Doxycycline	100	100	300	100	300	10	
Emamectin (B1a)							100	
Enramycin					30	30		
Enrofloxacin	Ciprofloxacin	100	100	100	100	100		
Enrofloxacin	Enrofloxacin						100	
Eprinomectin	Eprinomectin (B1a)	12	50	150				
Erythromycin		100	100	100	100	100	200	25
Ethopabate					500	40		
Eugenol							50	
Famphur			100	100				
Febantel	Oxfendazole sulphone		50	50				
Fenbendazole/Oxfendazole	Oxfendazole sulphone	10						
Fenbendazole/Oxfendazole	Fenbendazole							
Fenbendazole	Fenbendazole Sulfoxide							
Fenvalerate		40	25	250				
Flavophospholipol								20
Florfenicol	Florfenicol	10	200	10	30	30		
Florfenicol	Florfenicol-amine		200	200	100	200	1000	
Fluazuron			200	7000				
Flubendazole	Flubendazole		20	20	50	50		
Flubendazole	2-amino 1H-benzimidazol-5-yl-(4-fluorophenyl)methanone							400
Flugestone acetate		1						
Flumequine		50	200	300	400	250	500	

Table 1. (continued)

Compound	Regulated marker	Lowest global MRL, µg/kg						
		Liquid bovine milk	Raw bovine muscle	Raw bovine fat	Raw chicken muscle	Raw chicken skin/fat	Raw fish	Raw whole chicken egg
Flumethrin		30	10	150				
Flunixin	Flunixin		20	30				
Flunixin	5-hydroxyflunixin	2						
Fluralaner							10	1300
Fluvalinate								
Gamithromycin			20	20				
Gentamicin(s)	Sum of C1, C1a, C2, C2a	100	50	50	100	100	50	
Halofuginone			10	20	10	20		
Haloxon			100	100		40		
Hexaflumuron							500	
Hydrocortisone		10						
Imidocarb		50	300	50				
Isoeugenol							6000	
Isometamidium		100	100			100		
Ivermectin	Ivermectin B1a		10	40				
Josamycin					40	40		
Kanamycin A		150	100	100	100	100		
Ketoprofen		50	250					
Kitasamycin					200	200	50	200
Lasalocid A			50	100	20	100		50
Levamisole			10	10	10	10		
Lincomycin		150	100	50	100	50	100	50
Lufénurone							1350	
Maduramicin					100	100		
Marbofloxacin		75	150	50		75		
Mebendazole			20	20				
Melengestrol acetate			1	5				
Meloxicam		15	20					
Metamizol	4-Methylaminoantipyrin	50	100	100				
Methylprednisolone		2	10	10				

Table 1. (*continued*)

Compound	Regulated marker	Lowest global MRL, µg/kg						
		Liquid bovine milk	Raw bovine muscle	Raw bovine fat	Raw chicken muscle	Raw chicken skin/fat	Raw fish	Raw whole chicken egg
Metoserpate					20	20		
Monensin			2	10	10	50		
Morentel	N-methyl-1,3-propanediamine	50	100	100				
Moxidectin		40	20	500				
Nafcillin		30	300	300				
Narasin			15	50	15	50		
Neomycin	Neomycin B	150	500	500	500	500	500	500
Nequinate					100	100		
Netobimin	Albendazole oxide	100			100	100		
Netobimin	Albendazole sulfone							
Netobimin	Albendazole 2-aminosulfone							
Nicarbazin					200	200		
Nitroxinil			400	200				
Norgestomet		0.12	0.2	0.2				
Nosiheptide								
Novobiocin		50	1000	1000	1000	1000		
Oleandomycin		50	150	150	150	150		
Orbifloxacin		20	20	20	20	20		
Ormetoprim					100	100	100	
Oxacillin		30	300	300	300	300	300	
Oxibendazole		50	100	100				
Oxolinic acid			100	50	100	50	50	
Oxyclozanide		10	20	20				
Oxytetracycline	Oxytetracycline[c]	100	200	200	200	200	200	400
Oxytetracycline	4-epi-oxytetracycline[c]							
Paromomycin			500		500		500	
Penethamate	Penicillin G		50	50		50		
Penicillin G (Benzylpenicillin)	Penicillin G		50	50	50	50	50	

Table 1. (*continued*)

Compound	Regulated marker	Liquid bovine milk	Raw bovine muscle	Raw bovine fat	Raw chicken muscle	Raw chicken skin/fat	Raw fish	Raw whole chicken egg
					Lowest global MRL, µg/kg			
Penicillin V (Phenoxymethylpenicillin)					25	25		25
Permethrin		50	50	500				
Phoxim			50	400	25	550		60
Piperazine		50	50	50	100	100		2000
Piperonyl butoxide		50	100	50	500			1000
Pirlimicyn		100	100	100				
Prednisolone		6	4	4	0.7	0.7		0.7
Pyrantel	Pyrantel		500	500				
Pyrantel	N-methyl-1,3-propanediamine							
Pyrimethamine					50	50		
Ractopamine		10	10	10				
Rafoxanide			30	30				
Rifaximin		60						
Robenidine					100	200		100
Roxarsone (Arsanilic acid)	Arsenic				500	500		500
Salinomycin			50	50	100	100		20
Sarafloxacin					10	10	10	
Semduramicin					50	500		
Spectinomycin		200	250	500	100	100	300	
Spiramycin	Spiramycin	200	200	300	200	300	200	
Spiramycin	Neo-Spiramycin							
Streptomycin[d]	Sum of dihydrostreptomycin + streptomycin	200	600	600	600	600		
Sulfonamide	Sum of all substances belonging to the sulfonamide group	100	100	100	100	100	100	
Teflubenzuron							300	
Tetracycline[c]	Tetracycline[c]	100	100	100	200	200	200	400
Tetracycline	4-epi-tetracycline[c]							

Table 1. *(continued)*

Compound	Regulated marker	Liquid bovine milk	Raw bovine muscle	Raw bovine fat	Raw chicken muscle	Raw chicken skin/fat	Raw fish	Raw whole chicken egg
				Lowest global MRL, µg/kg				
Thiabendazole	Thiabendazole	50	100	100				
Thiabendazole	5-hydroxy-thiabendazole							
Thiamphenicol		50	50	50	50	50	50	
Tiamulin	Tiamulin				100	100		1000
Tiamulin	8-alpha-hydroxymutilin							
Tildipirosine			400	1000				
Tilmicosin		50	50	50	75	75	50	
Tolfenamic acid		50	50	50				
Toltrazuril	Toltrazuril sulfone		100	150	100	200		
Trenbolone	Beta-trenbolone		2					
Tricaine methanesulfonate							10	
Trichlorfon		50	50	50			10	
Triclabendazole	Ketotriclabendazole	10	200	100				
Trimethoprim		50	50	50	50	50	50	
Tripelennamine			200	200				
Tulathromycin A		20	100	100				
Tylosin		50	100	100	100	100	100	200
Tylvalosin	Tylvalosin					50		200
Tylvalosin	3-O-acetyltylosin							
Virginiamycin (M1)					100	400		
Zeranol			2					
Zilpaterol			2					
Zoalene (Dinitolmide)	Zoalene		3000		3000	2000		
Zoalene (Dinitolmide)	3-amino-5-nitro-o-toluamide							

a   Method developers should verify the definition of the marker residue.

b   Shaded cells = If no MRL is provided, then the drug is either prohibited in that matrix by one or more regulatory agencies or an MRL is not required.

c   MRL applies to the sum of chlortetracycline, oxytetracyclin, tetracycline, and their epimers.

d   MRL applies to the sum of dihydrostreptomycin and streptomycin.

Table 2. Method performance requirements

Residue concn in matrix	N	Acceptance criterion
0 (Blank)	30	≤10% POD with 95% confidence
0.5x MRL	30 per drug[a]	≥90% POD with 95% confidence[b]

[a] Tested as drug cocktail(s).

[b] All incorrect results must be investigated for determination of concentration at which POD_{90} with 95% confidence is achieved.