Multi-residue Method I for Veterinary Drugs by HPLC
(Animal and Fishery Products)

1. Analytes

See Table 7.

2. Instruments

High performance liquid chromatograph-photodiode array detector (HPLC-DAD)

High performance liquid chromatograph-fluorometric detector (HPLC-FL)

Liquid chromatograph-mass spectrometer (LC-MS)

3. Reagents

Use the reagents listed in Section 3 of the General Rules, except the following.

Acetonitrile: Prepared for high-performance liquid chromatography.

Water: Prepared for high-performance liquid chromatography.

Reference standards of veterinary drugs: Reference standards of known purities for each veterinary drug.

4. Procedure

Weigh 5.00 g of sample, add 30 mL of acetonitrile, 20 mL of *n*-hexane saturated with acetonitrile and 10 g of anhydrous sodium sulfate, homogenize, centrifuge at 3,000 rpm for 5 minutes, and take the organic layer. Remove an acetonitrile layer from the obtained organic layer, add the resultant *n*-hexane layer to the centrifuged residue, add 20 mL of acetonitrile, shake vigorously, and centrifuge at 3,000 rpm for 5 minutes. Discard the *n*-hexane layer, combine the resulting acetonitrile layers, add 10 mL of *n*-propanol, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 1.0 mL of acetonitrile/water (4:6, v/v), add 0.5 mL of hexane saturated with acetonitrile on the acetonitrile/water layer, centrifuge at 3,000 rpm for 5 minutes, and use the acetonitrile-water layer as the test solution.

5. Calibration curve

Prepare standard solutions (methanol) of each veterinary drug, and prepare mixed standard solutions (acetonitrile/water (4:6, v/v)) of several concentrations. Inject 10 µL of each standard solution to HPLC, and make calibration curves by peak-height or peak-area method.

6. Quantification

Inject 10 µL of the test solution to HPLC, and calculate the concentration of each veterinary drug from the calibration curves made in **5**.

7. Confirmation

Confirm using LC-MS or LC-MS/MS.

8. Measurement conditions

Detector: See Table 7.

Column: Octadecylsilanized silica gel, 3.0 mm in inside diameter, 150 mm in length and 3 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.05% trifluoroacetic acid solution (1:99, v/v) to (1:0, v/v) in 35 min. and hold for 5 min. When LC-MS with ESI negative is used, use 0.1% formic acid instead of 0.05% trifluoroacetic acid solution.

Detecting conditions: See Table 7.

9. Limits of quantification

See Table 7.

10. Explanatory notes

1) Outline of analytical method

The method consists of extraction of veterinary drugs from sample with acetonitrile, removal with *n*-hexane for fat and fat-soluble matrix components, removal with anhydrous sodium sulfate for water and water-soluble matrix components, and quantification and confirmation using HPLC-DAD, HPLC-FL or LC-MS.

2) Notes

i) Table 7 list the analytes for which this method is applicable in the order they appear in the Japanese syllabary. Note that the maximum residue limits (MRLs) defined for some agricultural chemicals include not only the parent compounds, but also their metabolites or other transformation products, which are inapplicable to this method.

ii) This method does not ensure simultaneous analysis of all of the analytes listed in Table 7. In advance, confirm that degradation or interference does not occur as the result of interaction between the target analytes.

iii) Because some veterinary drugs easily cause the air oxidation and the photolysis, all procedures should be performed under shading promptly.

iv) If a reference standard is difficult to dissolve in methanol, dissolve it in a small amount of *N*, *N*-dimethylformamide and then dilute with methanol.

v) Concentration and complete removal of the solvent should be performed under a gentle stream of nitrogen.

vi) Depending on the sensitivity of the LC-MS or LC-MS/MS, it may be necessary to dilute the test solution with HPLC mobile phase.

vii) When steady trueness and precision cannot be obtained by the absolute calibration curve method, it might be able to correct it by the internal standard method using stable isotope or the standard addition method.

viii) Because the limit of quantification differs depending on the instrument used, the concentration rate of the test solution, and the injection volume, it may be necessary to

optimize the conditions.

11. References

1) Mitsunori Murayama, et al., Food Hygiene and Safety Science, 32, 155-160, 1991

2) Standard Methods of Analysis in Food Safety Regulation (for Veterinary Drugs and Feed Additives) edited by MHLW, Japan Food Hygiene Association, 26-43, 2003.

12. Type

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Table 7. Multi-residue Method I for Veterinary Drugs by HPLC (Animal and Fishery Products)

Veterinary drugs	Analytes	Monitoring wavelength (nm)	Monitoring ions (m/z)	Limit of quantification (mg/kg)
Aklomide	Aklomide		199	0.5
Azaperone	Azaperone		328	0.01
2-Acetylamino-5-nitrothiazole	2-Acetylamino-5-nitrothiazole		186	0.01
Altrenogest	Altrenogest		311	0.003
Albendazole	5-(Propylsulfinyl)-1H-benzimidazol-2-amine	300	240	0.01
Allethrin	Allethrin		303	0.01
Amprolium	Amprolium	245	243	0.01
Ethopabate	Ethopabate		238	0.01
Eprinomectin	Eprinomectin B1a		914	0.03
Emamectin benzoate	Emamectin B1a		886	0.003
Erythromycin	Erythromycin		716	0.01
Enrofloxacin	Enrofloxacin	280	360	0.01
	Ciprofloxacin	280	332	0.01
Oxibendazole	Oxibendazole		250	0.01
Oxolinic acid	Oxolinic acid	260	262	0.01
Ofloxacin	Ofloxacin		362	0.01
Orbifloxacin	Orbifloxacin		396	0.01
Ormetoprim	Ormetoprim	230	275	0.02
Oleandomycin	Oleandomycin		688	0.01
Xylazine	Xylazine		221	0.001
Crostebol	Crostebol		365	0.01
Clopidol	Clopidol	230	192	0.01
Chlorhexidine	Chlorhexidine		506	0.01
Chlormadinone	Chlormadinone		405	0.01
Ketoprofen	Ketoprofen		255	0.005
Sarafloxacin	Sarafloxacin		386	0.01
Diaveridine	Diaveridine		261	0.02
Diclazuril	Diclazuril	275	382	0.01
Dicyclanil	Dicyclanil		191	0.01
Diflubenzuron	Diflubenzuron		311	0.03
Difloxacin	Difloxacin		400	0.01
Josamycin	Josamycin		829	0.01
Sulfaethoxypyridazine	Sulfaethoxypyridazine		295	0.01
Sulfaquinoxaline	Sulfaquinoxaline	270	301	0.01
Sulfaguanidine	Sulfaguanidine		215	0.01

Sulfachlorpyridazine	Sulfachlorpyridazine		285	0.01
Sulfadiazine	Sulfadiazine		251	0.01
Sulfadimidine	Sulfadimidine	270	279	0.01
Sulfadimethoxine	Sulfadimethoxine	275	311	0.01
Sulfacetamide	Sulfacetamide		215	0.01
Sulfathiazole	Sulfathiazole		256	0.01
Sulfadoxine	Sulfadoxine		311	0.01
Sulfanitran	Sulfanitran		336	0.01
Sulfapyridine	Sulfapyridine		250	0.01
Sulfabenzamide	Sulfabenzamide		277	0.01
Sulfamethoxazole	Sulfamethoxazole		254	0.01
Sulfamethoxypyridazine	Sulfamethoxypyridazine		281	0.01
Sulfamerazine	Sulfamerazine	270	265	0.01
Sulfamonomethoxine	Sulfamonomethoxine	275	281	0.01
Cefoperazone	Cefoperazone		646	0.01
Cefuroxime	Cefuroxime		364	0.01
Tylosin	Tylosin		916	0.01
Danofloxacin	Danofloxacin		358	0.01
Thiabendazole	Thiabendazole	300	202	0.01
	5-Hydroxythiabendazole	300	218	0.01
Tiamulin	Tiamulin		494	0.05
Thiamphenicol	Thiamphenicol	225	-354	0.01
Tilmicosin	Tilmicosin	235	870	0.05(muscle, fat, organ) 0.01 (milk)
Dexamethasone	Dexamethasone		393	0.01
Temephos	Temephos		467	0.05
Trichlorfon	Trichlorfon		258	0.1
Tripelennamine	Tripelennamine		256	0.002-0.02
Trimethoprim	Trimethoprim	230	291	0.02
Tolfenamic acid	Tolfenamic acid		261	0.005
Trenbolone acetate	α- Trenbolone (liver)	340	271	0.002
	β- Trenbolone (muscle)	340	271	0.002
Nafcillin	Nafcillin		447	0.01
Nalidixic acid	Nalidixic acid	260	233	0.01
Nitarsone	Nitarsone		276	0.003
Nitroxynil	Nitroxynil		291	0.05

Valnemulin	Valnemulin		565	0.01
Halofuginone	Halofuginone	245	416	0.01
Nicarbazin	*N*,*N*′-Bis(4-nitrophenyl) urea	350	303	0.02
Hydrocortisone	Hydrocortisone		405	0.01
Pyrantel tartrate	Pyrantel tartrate		207	0.01
Pyrimethamine	Pyrimethamine	230	249	0.02
Famphur	Famphur		326	0.02
Phenoxymethylpenicillin	Phenoxymethylpenicillin		383	0.002
Fenobucarb	Fenobucarb		208	0.01
Butylhydroxyanisol	Butylhydroxyanisol		195	0.01
Prifinium	Prifinium		306	0.002
Flunixin	Flunixin		297	0.005
Flubendazole	Flubendazole	315	314	0.01
Flumequine	Flumequine		262	0.01
Prednisolone	Prednisolone		361	0.002
Brotizolam	Brotizolam		395	0.0005
Bromacil	Bromacil		207	0.005
Florfenicol	Florfenicol		356	0.01
Benzocaine	Benzocaine		166	0.005
Mafoprazine	Mafoprazine		402	0.005
Marbofloxacin	Marbofloxacin		363	0.01
Miloxacin	Miloxacin		264	0.01
Mecillinum	Mecillinum		259	0.01
Methylprednisolone	Methylprednisolone		375	0.01
Mebendazole	Mebendazole		296	0.01
Meloxicam	Meloxicam		352	0.005
Menbutone	Menbutone		241	0.001
Monensin	Monensin		679	0.001
Morantel	Morantel		221	0.01
Lasalocid	Lasalocid		613	0.005
Rifaximin	Rifaximin		786	0.01
Lincomycin	Lincomycin		407	0.05
Levamisole	Levamisole	220	205	0.01
Robenidine	Robenidine		334	0.01
Warfarin	Warfarin		309	0.001

・The compounds are listed in the order of the Japanese syllabary.

・The monitoring wavelengths represent the wavelength measured by a high performance liquid chromatograph equipped with an ultraviolet spectrophotometric detector or a photodiode array detector.

・A high performance liquid chromatograph with a fluorometric detector (ex 300 nm, and em

370 nm) can also be used to measure the level of

5-propylsulphonyl-1H-benzimidazole-2-amine and thiabendazole.

- Ions are monitored with an ESI positive mode (+) and an ESI negative mode (-) in LC-MS measurement.