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

1. Analytes

See Table.

2. Instruments

Liquid chromatograph-mass spectrometer (LC-MS)

Liquid chromatograph-tandem mass spectrometer (LC-MS/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.

Methanol: Prepared for high-performance liquid chromatography.

Divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (60 mg): Polyethylene tube of 12-13 mm in inside diameter packed with 60 mg of divinylbenzene-*N*-vinylpyrrolidone copolymer, or other cartridge with equal separation characteristics.

Reference standard of each veterinary drug: Veterinary drugs which clearly show its purity.

4. Procedure

1) Extraction

Weigh 5.00 g of sample. Add 100 mL of acetonitrile/methanol/0.2% metaphosphoric acid (1:1:3, v/v/v) to the sample, homogenize, and filter through a filter paper, covered with a 2-3 -cm-thick layer of diatomaceous earth, with suction into a vacuum rotary evaporator flask. Add 20 mL of acetonitrile/methanol/0.2% metaphosphoric acid (1:1:3, v/v/v) to the residue on the filter paper, treat as described above, combine the filtrate and concentrate to about 20 mL at below 40°C.

2) Clean-up

Add 5 mL each of methanol and water to a 60 mg divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge sequentially and discard the effluents. Transfer the solution obtained in **1)** to the cartridge, add 5 mL of water, and discard the effluent. Elute with 5 mL of methanol, combine the eluates in the vacuum rotary evaporator flask, and remove methanol at below 40°C. Add 1.0 mL of acetonitrile/water (1:9, v/v) to the residue, and use this solution as the test solution.

5. Calibration curve

Prepare standard solutions (methanol) of each veterinary drug, and prepare several solutions (acetonitrile/water (1:9, v/v)) with appropriate concentration range. Inject 5 µL of each standard solution to LC-MS or LC-MS/MS, and make calibration curves by peak-height or peak-area method.

6. Quantification

Inject 5 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.

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 100 mm in length and 3 µm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/0.005 vol% formic acid/water (1:1:18, v/v/v) to (16:1:3, v/v/v) in 15 min and hold for 5 min.

Detecting conditions: See Table.

9. Limit of quantification

See Table.

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of each veterinary drug from sample with acetonitrile/methanol/0.2% metaphosphoric acid, clean-up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, and quantification and confirmation using LC-MS or LC-MS/MS.

2) Notes

i) Table shows analytes which are applicable to this method in the order of the Japanese syllabary. Veterinary drugs could include chemicals like metabolites which are inapplicable to this method.

ii) This method does not ensure all simultaneous analysis using analytes listed in Table. In advance, confirm the interaction by the intended combination of analytes does not interfere decomposition and measurement.

iii) Some veterinary drugs are easy to be oxidized in air and to be photodegraded. All procedure should be performed avoiding direct sunlight.

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

v) Depending on sensitivity of LC-MS or LC-MS/MS, dilute the test solution with mobile phase of HPLC.

vi) Even if certain accuracy and precision are not obtained by absolute calibration curve method, internal standard method using stable isotope and standard addition method may correct the accuracy and the precision.

vii) Limit of quantification differs by the instrument to use, the concentration rate and the injection volume of the test solution. Consider optimum condition depending on the situation.

11. References

None

12. Type

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

Veterinary Drugs	Analytes	Monitoring ions (m/z)	Limit of quantification (mg/kg)
Albendazole	5-propylsulfonyl-1h-benzimidazole-2-amine	+240→133	0.01
Ethopabate	Ethopabate	+260→260	0.01
Oxytetracycline/Chlortetracycline/Tetracycline	Oxytetracycline	+461→426	0.02
	Chlortetracycline	+479→462	0.03
	Tetracycline	+445→410	0.02
Oxibendazole	Oxibendazole	+250→218	0.01
Ormetoprim	Ormetoprim	+275→123	0.02
Clopidol	Clopidol	+192→101	0.01
Spiramycin	Spiramycin I	+422→174	0.05
	Neospiramycin I	+350→174	0.05
Sulfaquinoxaline	Sulfaquinoxaline	+301→156	0.01
Sulfachlorpyridazine	Sulfachlorpyridazine	+285→156	0.01
Sulfadiazine	Sulfadiazine	+251→156	0.01
Sulfadimidine	Sulfadimidine	+279→186	0.01
Sulfadimethoxine	Sulfadimethoxine	+311→156	0.01
Sulfathiazole	Sulfathiazole	+256→156	0.01
Sulfadoxine	Sulfadoxine	+311→156	0.01
Sulfanitran	Sulfanitran	-334→136	0.01
Sulfapyridine	Sulfapyridine	+250→156	0.01
Sulfabenzamide	Sulfabenzamide	+277→156	0.01
Sulfamethoxazole	Sulfamethoxazole	+254→92	0.01
Sulfamethoxypyridazine	Sulfamethoxypyridazine	+281→156	0.01
Sulfamerazine	Sulfamerazine	+265→156	0.01
Sulfamonomethoxine	Sulfamonomethoxine	+281→156	0.01
Zeranol	Zeranol	-321→321	0.01
Thiamphenicol	Thiamphenicol	-354→185	0.01
Trimethoprim	Trimethoprim	+291→123	0.02
Trenbolone Acetate	α- Trenbolone (liver)	+271→253	0.002
	β-Trenbolone (Muscle)	+271→253	0.002
Nicarbazin	*N,N'*- Bis(4-Nitrophenyl)urea	-301→137	0.02
Flubendazole	Flubendazole	+314→282	0.01
Levamisole	Levamisole	+205→178	0.01

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

・The figures in "Monitoring ions" shows [precursor ion → product ion] in LC-MS/MS measurement, and the code before the figures means ionization mode in ESI (-) or ESI (+) measurement.