Original: Japanese
Provisional translation

Multi-residue Method I for Veterinary Drugs by LC/MS (Animal and Fishery Products)

1. Analytes

Refer to Table.

2. Application

Animal and fishery products

3. Instrument

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

4. Reagents

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

Reference standard of each veterinary drug: Veterinary drugs, which clearly show their purity. (When individual testing methods for each veterinary drug designate purities of reference standards, follow the direction. If not, desirably use reference standards with a purity of not less than 95%.)

5. Procedure

1) Extraction

Weigh 10.0 g of sample. For honey, weigh 10.0 g of sample, and then dissolve the sample in 10 mL of water. Add 50 mL of acetonitrile saturated with *n*-hexane, 50 mL of *n*-hexane and 1 mL of acetic acid to the sample, homogenize, add 20 g of anhydrous sodium sulfate, and homogenize again. Centrifuge at 3,000 rpm for 5 minutes, discard the *n*-hexane layer, and collect the acetonitrile layer. Add 50 mL of acetonitrile to the residue, homogenize, and centrifuge as described above. Collect the acetonitrile layer, combine the previously obtained acetonitrile layer, and add acetonitrile to make exactly 100 mL. Take a 5 mL aliquot of the solution accurately, concentrate at below 40℃, and remove the solvent. Dissolve the residue in 1 mL of 0.1 vol% formic acid/methanol (1:4, v/v).

2) Clean-up

Add 5 mL each of methanol and 0.1 vol% formic acid/methanol (1:4, v/v) to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially and discard the effluents. Transfer the solution obtained in **1)** to the cartridge, add 15 mL of 0.1 vol% formic acid/methanol (1:4, v/v), and collect the total eluate including the transferred solutions. Concentrate the eluate at below 40℃ and remove the solvent. Dissolve the residue in acetonitrile/0.1 vol% formic acid (1:3, v/v) to make exactly 1 mL, and use this solution as the test solution.

6. Calibration curve

Dissolve the reference standard of each veterinary drug in the appropriate solvent respectively, and prepare stock standard solutions. Mix these stock standard solutions appropriately, and prepare solutions, acetonitrile/0.1 vol% formic acid (1:3, v/v) of several concentrations. Inject each standard solution to LC-MS/MS respectively and make a calibration curve by the peak-height or peak-area method.

7. Quantification

Inject the test solution to LC-MS/MS and calculate the concentration of each veterinary drug from the calibration curves made in 6.

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

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: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Mobile phase A: 0.1 vol% formic acid

Mobile phase B: 0.1 vol% formic acid-acetonitrile solution

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	99	1
5.0	99	1
35.0	0	100
40.0	0	100

Ionization mode: ESI (+) and ESI (-)

Major monitoring ions (*m/z*): Refer to Table.

Injection volume: 5 μL

Expected retention time: Refer to Table.

10. Limit of quantification

Refer to Table.

11. Explanatory note

1) Outline of analytical method

The method consists of extraction of each veterinary drug from sample with acetonitrile in the presence of *n*-hexane and anhydrous sodium sulfate under an acidic condition with acetic acid, clean-up with an octadecylsilanized silica gel cartridge, and quantification

and confirmation using LC-MS/MS.

2) Notes

i) Table shows analytes which are applicable to this method in the order of the Japanese syllabary. Veterinary drugs may include chemicals like metabolites which are inapplicable to this method. Isomers having different retention times are listed separately in “Analytes”.

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

iii) Some analytes listed in the Table reduce with time under analysis operation, therefore all procedure should be performed promptly.

iv) Use reference standards of veterinary drugs in the highest purity, if possible.

v) If the suspended solids are found in the acetonitrile extract after fixing the volume, centrifuge, and the supernatant can be used next measurement.

vi) Concentration and complete removal of the solvent should be performed gently in the nitrogen stream.

vii) Before using cartridges for clean-up, perform a pretest on the elution of each veterinary drug and confirm the elution position under the use condition.

viii) Dilution of test solution, matrix-matched calibration or standard addition may be required to obtain accurate measurement results.

ix) Because the limit of quantification varies depending on the instrument used and measurement conditions, it may be necessary to optimize the conditions.

x) Depending on the sensitivity of LC-MS/MS, it may be necessary to dilute the test solution with acetonitrile/0.1 vol% formic acid solution (1:3, v/v).

xi) Food items used to develop the analytical method: Cattle muscle, chicken muscle, cattle fat, cattle liver, milk, chicken eggs, honey, eel, salmon, corbicula

12. References

None

13. Type

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Ministry of Health, Labour and Welfare

Table. Multi-residue Method I for Veterinary Drugs by LC/MS (Animal and Fishery Products)

Veterinary Drugs	Analytes[1)]	RRT[2)]	Major monitoring ions (*m/z*)[3)]						Limit of quantification (mg/kg)[4)]
2-Acetylamino-5-nitrothiazole	2-Acetylamino-5-nitrothiazole	0.68	-186 → 139	-186 → 96					0.01
Azaperone	Azaperol	0.55	+330 → 312	+330 → 121	+330 → 109	+330 → 78			0.01
Albendazole	5-propylsulfonyl-1h-benzimidazole-2-amine (Albendazole Metabolite I)	0.52	+240 → 198	+240 → 133	+240 → 91				0.01
Ethopabate	Ethopabate	0.75	+238 → 206	+238 → 136	+238 → 80				0.01
Oxibendazole	Oxibendazole	0.68	+250 → 218	+250 → 176	+250 → 80				0.01
Orbifloxacin	Orbifloxacin	0.60	+396 → 352	+396 → 295	+396 → 267	+396 → 226			0.01
Ormetoprim	Ormetoprim	0.57	+275 → 259	+275 → 123	+275 → 81				0.01
Carazolol	Carazolol	0.68	+299 → 222	+299 → 194	+299 → 116				0.001
Xylazine	Xylazine	0.61	+221 → 164	+221 → 90					0.01
Clopidol	Clopidol	0.51	+194 → 101	+192 → 101	+192 → 87				0.01
Ketoprofen	Ketoprofen	0.91	+255 → 209	+255 → 194	+255 → 105	+255 → 77			0.01
Diaverdine	Diaverdine	0.52	+261 → 245	+261 → 123	+261 → 107	+261 → 81			0.01
Dicyclanil	Dicyclanil	0.43	+191 → 163	+191 → 150	+191 → 109	+191 → 92	+191 → 41		0.01
Dinitolmide	Dinitolmide	0.67	-224 → 181	-224 → 77	-224 → 42				0.01
Diflubenzuron	Diflubenzuron	1.04	+311 → 158	+311 → 141					0.01
Difloxacin	Difloxacin	0.63	+400 → 356	+400 → 306	+400 → 299	+400 → 256			0.01
Josamycin	Josamycin	0.84	+828 → 174	+828 → 109					0.01
Sulfatroxazole	Sulfatroxazole	0.69	+268 → 156	+268 → 108	+268 → 92				0.01
Sulfanitran	Sulfanitran	0.84	+336 → 156	+336 → 134	+336 → 65	+334 → 136	+334 → 133		0.01
Sulfamerazine	Sulfamerazine	0.57	+265 → 156	+265 → 108	+265 → 92				0.01
Thiabendazole	Thiabendazole	0.53	+202 → 175	+202 → 131	+202 → 104				0.01*
Tiamulin	Tiamulin	0.80	+495 → 91	+495 → 73	+494 → 192	+494 → 119			0.01
Thiamphenicol	Thiamphenicol	0.57	-354 → 290	-354 → 185					0.01
Trichlorfon	Trichlorfon	0.61	+257 → 127	+257 → 109	+257 → 79				0.004
Trimethoprim	Trimethoprim	0.54	+291 → 261	+291 → 230	+291 → 123				0.01*
Nicarbazin	*N,N'*- Bis(4-Nitrophenyl)urea	0.99	-301 → 137	-301 → 107					0.01
Nalidixic Acid	Nalidixic Acid	0.82	+233 → 215	+233 → 187	+233 → 159	+233 → 104			0.01
Nitroxinil	Nitroxinil	0.87	-289 → 162	-289 → 127					0.01
Valnemulin	Valnemulin	0.81	+565 → 263	+565 → 164					0.01
Halofuginone	Halofuginone	0.66	+416 → 120	+416 → 100					0.01
Pyrantel	Pyrantel-1	0.52	+207 → 150	+207 → 136	+207 → 109	+207 → 97			0.01
	Pyrantel-2	0.57	+207 → 150	+207 → 136	+207 → 109	+207 → 97			0.01
Pyrimethamine	Pyrimethamine	0.67	+249 → 233	+249 → 198	+249 → 177	+249 → 128			0.01
Famphur	Famphur	0.98	+326 → 281	+326 → 217	+326 → 109	+326 → 93			0.01*
Phenoxymethylpenicillin	Phenoxymethylpenicillin	0.82	+351 → 229	+351 → 160	+351 → 137	+351 → 114	+349 → 208	+349 → 93	0.01
Praziquantel	Praziquantel	0.92	+313 → 203	+313 → 174	+313 → 83				0.01
Prifinium	Prifinium	0.82	+307 → 87	+307 → 86	+306 → 91	+306 → 86			0.01
Flunixin	Flunixin	0.94	+297 → 279	+297 → 264	+297 → 109				0.01
Flubendazole	Flubendazole	0.81	+314 → 282	+314 → 123	+314 → 95				0.01
Flumequine	Flumequine	0.83	+262 → 244	+262 → 202	+262 → 126				0.01
Brotizolam	Brotizolam	0.89	+395 → 316	+395 → 314	+393 → 314	+393 → 279			0.0005
Bromacil	Bromacil	0.77	+261 → 205	+261 → 188					0.01
Florfenicol	Florfenicol	0.69	-356 → 336	-356 → 185					0.01
Mafoprazine	Mafoprazine	0.68	+402 → 193	+402 → 122	+402 → 70				0.01
Methylprednisolone	Methylprednisolone	0.80	+375 → 339	+375 → 185	+375 → 161	+375 → 135			0.01
Mebendazole	Mebendazole	0.78	+296 → 264	+296 → 131	+296 → 105	+296 → 77			0.01
Meloxicam	Meloxicam	0.94	+352 → 141	+352 → 115	+352 → 73				0.01
Menbutone	Menbutone	0.89	+259 → 241	+259 → 185	+259 → 159	+259 → 114			0.01
Levamisole	Levamisole	0.51	+205 → 178	+205 → 91					0.01

1) The analytes are listed in the order of the Japanese syllabary. Veterinary Drugs may include chemicals which are inapplicable to this method. Isomers having different retention time are listed separately in “Analytes”.

2) Relative retention time (RRT) is the relative value for retention time (22-27 minutes) of Isoxaflutole, and shows the average of values which obtained from laboratories.

3) The figures in "Major monitoring ions" shows [precursor ion → product ion] in LC-MS/MS measurement, and the code before the figures (+ or -) means ionization mode (ESI (+) or ESI (-)) in ESI measurement. Each ion is listed in descending order.

4) We described 0.01 mg/kg (or the minimum additive concentration) as the limit of quantification when S/N ratio at the peak of an analyte in at least one sample in recovery test which conducted with fortification 0.01 ppm (or minimum fortification level) was not less than 10. When recovery test with additive concentration 0.01 ppm was not existed, we conducted recovery test using matrix-containing standard solution, and when the S/N ratio at peak of an analyte which corresponded to 0.01 ppm in sample got value which is not less than 10 at least in one sample, we assumed the limit of quantification was 0.01 mg/kg and described with "*".