Analytical Method for Mirosamycin (Animal and Fishery Products)

1. Analyte

Mirosamycin

2. Instrument

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

3. Reagents

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

Divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (60 mg): Polyethylene tube of 8–9 mm in inside diameter packed with 60 mg of divinylbenzene-*N*-vinylpyrrolidone copolymer, or other cartridge with equal separation characteristics.

Reference standard of mirosamycin: Contains not less than 95% of mirosamycin.

4. Procedure

1) Extraction

i) Muscle, fat, liver, kidney, fish, shellfish, milk and egg

Add 100 mL of acetone to 10.0 g of sample, homogenize, centrifuge for 5 minutes at 3,500 rpm, and collect the supernatant. Add 50 mL of acetone to the residue, homogenize, centrifuge for 5 minutes at 3,500 rpm. Combine the supernatants, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the extract, add 5 mL of 2-propanol, concentrate at below 40°C and remove the solvent. Add 30 mL of *n*-hexane to the residue, and extract with shaking three times with 30 mL of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C and remove the solvent. Dissolve the residue in 10 mL of water/methanol (7:3, v/v).

ii) Honey

Dissolve 10.0 g of sample in 10 mL of water. Add 100 mL of acetone, extract with shaking, centrifuge for 5 minutes at 3,500 rpm, and collect the supernatant. Add 50 mL of acetone to the residue, homogenize, centrifuge for 5 minutes at 3,500 rpm. Combine the supernatants, and add acetone to make exactly 200 mL. Take a 10 mL aliquot of the extract, add 5 mL of 2-propanol, concentrate at below 40°C, and remove the solvent. Add 30 mL of *n*-hexane to the residue, and extract with shaking three times with 30 mL of acetonitrile saturated with *n*-hexane. Combine the extracts, concentrate at below 40°C and remove the solvent. Dissolve the residue in 10 mL of water/methanol (7:3, v/v).

2) Clean-up

Add 5 mL each of methanol and water/methanol (7:3, v/v) to a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge (60 mg) sequentially, and discard the effluent. Transfer the extract obtained in 1) to the cartridge, add 5 mL of water/methanol (7:3, v/v),

and discard the effluent. Elute with 10 mL of acetonitrile, concentrate the eluate below 40°C and remove the solvent. Dissolve the residue in methanol to make exactly 5 mL, and use this solution as the test solution.

5. Calibration curve

Prepare 0.001–0.02 mg/L mirosamycin standard solutions (methanol). Inject 5 μL of each standard solution to LC-MS/MS, and make a calibration curve by peak-height or peak-area method.

6. Quantification

Inject 5 μL of the test solution to LC-MS/MS and calculate the concentration of mirosamycin from the calibration curve made in **5**.

7. Confirmation

Confirm using LC-MS/MS.

8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length and 3.5 μm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from 0.05 vol% formic acid-acetonitrile solution/0.05 vol% formic acid solution (1:4, v/v) to (1:1, v/v) in 15 min.

Ionization mode: ESI (+)

Major monitoring ions (*m/z*): Precursor ion 728, Product ions 158 and 116

Expected retention time: 8 min

9. Limit of quantification

0.01 mg/kg

10. Explanatory note

1) Outline of analytical method

The method consists of extraction of mirosamycin from sample with acetone, defatting by acetonitrile/hexane partitioning, clean-up with a divinylbenzene-*N*-vinylpyrrolidone copolymer cartridge, quantification and confirmation using LC-MS/MS.

2) Notes

i) When the analytical method for mirosamycin using LC-MS/MS was developed, the following monitoring ions were used:

for quantification (*m/z*): precursor ion 728, product ion 158

for confirmation (*m/z*): precursor ion 728, product ion 116

ii) When floating substances appear in the supernatant after centrifuge, filter through a cotton plug.

iii) It has been reported that in the testing of tissues or organs (liver, kidney etc.), especially in fresh state, mirosamycin decreases when samples are left unprocessed after weighing. Thus, it is recommended to start extraction immediately after weighing of the samples. It is also recommended to verify that mirosamycin has not decreased during leaving, if necessary.

11. References

None

12. Type

C