

Analytical Method for Emamectin benzoate (Animal and Fishery Products)

1. Analytes

Emamectin B1a

8,9-*Z*-emamectin B1a

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 emamectin B1a benzoate: Contains not less than 92% of emamectin B1a benzoate.

Reference standard of 8,9-*Z*-emamectin B1a benzoate: Contains not less than 80% of 8,9-*Z*-emamectin B1a benzoate.

5. Procedure

1) Extraction

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

Add 50 mL of acetone to 10.0 g of sample, homogenize, and filter with suction. Add 25 mL of acetone to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add acetone to make exactly 100 mL. Take a 10 mL aliquot of the solution accurately, and concentrate to about 1 mL at below 40°C. Add 10 mL of 20 w/v% sodium chloride and 1 mL of ammonia solution to the solution above, and extract by shaking twice with 10 mL each of ethyl acetate. Combine the extracts, concentrate at below 40°C, and remove the solvent. Add 10 mL of *n*-hexane to the residue, and extract by shaking twice with 10 mL each 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 acetonitrile/water (1:1, v/v) and 0.5 mL of ammonia solution.

ii) Honey

Dissolve 10.0 g of the sample in 5 mL of water. Add 50 mL of acetone, homogenize, and filter with suction. Dissolve the residue on the filter paper in 5 mL of water, add 25 mL of acetone, homogenize, and filter as described above. Combine the resulting

filtrates, and add acetone to make exactly 100 mL. Take a 10 mL aliquot of the solution accurately, and concentrate to about 1 mL at below 40°C. Add 10 mL of 20 w/v% sodium chloride and 1 mL of ammonia solution to the solution above, and extract by shaking twice with 10 mL each of ethyl acetate. Combine the extract, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 10 mL of acetonitrile/water (1:1, v/v) and 0.5 mL of ammonia solution.

2) Clean-up

Add 10 mL each of acetonitrile and water to a styrene-divinylbenzene copolymer mini-column (500 mg) sequentially and discard the effluents. Transfer the solution obtained in “1)” to the column, add 10 mL of acetonitrile/water (1:1, v/v), and discard the effluent. Then, add 5 mL of 2 vol% formic acid-acetonitrile solution, collect the eluate, add water to make exactly 10 mL, and use this solution as the test solution.

6. Calibration curve

Prepare standard solutions by dissolving emamectin B1a benzoate and 8,9-*Z*-emamectin B1a benzoate in acetone respectively. Prepare several diluted solutions at appropriate concentration range using acetonitrile containing 1 vol% formic acid/water (1:1, v/v), inject each standard solution into LC-MS/MS, and make calibration curves by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.0005 mg/kg of each analyte (calculated as emamectin benzoate) gives the test solution of 0.00005 mg/L (calculated as emamectin benzoate) in concentration.

7. Quantification

Inject the test solution into LC-MS/MS, and calculate the concentration of emamectin B1a and 8,9-*Z*-emamectin B1a from the calibration curve made in 6, and use the following equation to calculate the sum of concentrations of emamectin benzoate.

$$\text{Concentration of emamectin benzoate (ppm)} = A \times 1.138 + B \times 1.138$$

A: Concentration of emamectin B1a (ppm)

B: Concentration of 8,9-*Z*-emamectin B1a (ppm)

8. Confirmation

Confirm using LC-MS/MS.

9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel (2.1 mm in inside diameter, 100 mm in length and 3.5 μm in particle diameter)

Column temperature: 40°C

Mobile phase: Initially 0.02 vol% formic acid/0.02 vol% formic acid-acetonitrile (1:1, v/v) for 0.5 minutes, followed by a linear gradient from (1:1, v/v) to (1:9, v/v) in 9.5 0.5 0.5 minutes, and hold at (1:9, v/v) for 5 minutes.

Ionization mode: ESI (+)

Major monitoring ions (m/z): Emamectin B1a: precursor ion 887, product ions 158, 82
8,9-*Z*-emamectin B1a: precursor ion 887, product ions 158,
82

Injection volume: 5 μ L

Expected retention time: Emamectin B1a: 5 minutes

8,9-*Z*-emamectin B1a: 6 minutes

10. Limit of quantification

0.0005 mg/kg for each analyte (calculated as emamectin benzoate).

11. Explanatory note

1) Outline of analytical method

The method consists of extracting emamectin B1a and 8,9-*Z*-emamectin B1a from sample with acetone, transferring into ethyl acetate under basic condition of ammonia solution, defatting by acetonitrile/hexane partitioning (omit for honey), clean-up with a styrene-divinylbenzene copolymer mini column, and quantification and confirmation using LC-MS/MS. In the method, emamectin B1a and 8,9-*Z*-emamectin B1a are quantified respectively, converted to the concentration of emamectin benzoate by multiplying by a conversion factor, and the sum of the concentrations of emamectin B1a and 8,9-*Z*-emamectin B1a is regarded as the analytical result of emamectin benzoate.

2) Notes

i) When the analytical method for emamectin B1a and 8,9-*Z*-emamectin B1a using LC-MS/MS was developed, the following monitoring ions were used:

Emamectin B1a

for quantitative ions (m/z): precursor ion 887, product ions 158

for qualitative ions (m/z): precursor ion 887, product ion 82

8,9-*Z*-Emamectin B1a

for quantitative ions (m/z): precursor ion 887, product ion 158

for qualitative ions (m/z): precursor ion 887, product ion 82

ii) Emamectin B1a and 8,9-*Z*-emamectin B1a may adhere to glass. Therefore, do not use scratched or deteriorated glass instruments.

iii) At the time of the development of the analytical method, the reference standard for emamectin B1a benzoate was only available with not less than 92% purity. Therefore, "Reference standard of emamectin B1a benzoate: Contains not less than 92% of emamectin B1a benzoate." is mentioned in 4. However, if available, it is desirable to use the reference standard with a purity of 95% or higher. Also, the reference standard for 8,9-*Z*-emamectin B1a was only available with not less than 80% purity. Therefore, "Reference standard of 8,9-*Z*-emamectin B1a benzoate: Contains not less

than 80% of 8,9-*Z*-emamectin B1a benzoate." is mentioned in 4. However, if available, it is desirable to use the reference standard with a purity of 95% or higher.

iv) Food items used to develop the analytical method: cattle muscle, cattle fat, cattle liver, milk, egg, buckwheat honey, eel, Corbicula

12. References

None

13. Type

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