

## Analytical Method for Fenhexamid (Animal and Fishery Products)

### 1. Analyte

Fenhexamid

### 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.

Reference standard of fenhexamid: Contains not less than 98% of fenhexamid.

### 4. Procedure

#### 1) Extraction

##### i) Muscle, liver, kidney, milk, egg, fish, shellfish, and honey

Add 30 mL of 1.5 mol/L phosphoric acid to 10.0 g of sample and mix well. Add 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take a 4 mL aliquot of the extract, and add 16 mL of 1 vol% formic acid.

##### ii) Fat

Add 30 mL of 1.5 mol/L phosphoric acid to 5.00 g of sample and mix well. Add 100 mL of acetone, homogenize, and filter with suction. Add 50 mL of acetone to the residue on the filter paper, homogenize, and filter with suction. Combine the resulting filtrates, and add acetone to make exactly 200 mL. Take an 8 mL aliquot of the extract, and add 20 mL of 1 vol% formic acid.

#### 2) Clean-up

Add 5 mL each of acetonitrile and water to an octadecylsilanized silica gel cartridge (1,000 mg) sequentially, and discard the effluent. Add 5 mL each of acetonitrile and water to a graphitized carbon black cartridge (500 mg) sequentially, and discard the effluent. Transfer the extract obtained in 1) to the octadecylsilanized silica gel cartridge, add 10 mL of 1 vol% formic acid-acetonitrile/water (3:7, v/v), and discard the effluent. Connect the graphitized carbon black cartridge at the bottom of the octadecylsilanized silica gel cartridge, add 10 mL of 1 vol% formic acid-acetonitrile/water (7:3, v/v), and discard the effluent. Remove the octadecylsilanized silica gel cartridge, elute with 30 mL of 1 vol% formic acid-acetonitrile from the graphitized carbon black cartridge. Concentrate the eluate at below 40°C and remove the solvent. Dissolve the residue in acetonitrile/water (1:1, v/v) to make exactly 2 mL, and use this solution as the test solution.

## 5. Calibration curve

Prepare fenhexamid standard solutions of several concentrations (acetonitrile/water (1:1, v/v)). Inject each standard solution to LC-MS/MS, and make a calibration curve by peak-height or peak-area method. When the test solution is prepared following the above procedure, the sample containing 0.01 mg/kg of fenhexamid gives the test solution of 0.001 mg/L in concentration.

## 6. Quantification

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

## 7. Confirmation

Confirm using LC-MS/MS.

## 8. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 2.0 mm in inside diameter, 150 mm in length and 5  $\mu\text{m}$  in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from acetonitrile/2 mmol/L ammonium acetate solution (3:7, v/v) to (9:1) in 10 min and hold for 5 min.

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

Major monitoring ions ( $m/z$ ):

ESI(-): Precursor ion 300, product ion 264

ESI(+): Precursor ion 302, product ion 97

Injection volume: 5  $\mu\text{L}$

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 fenhexamid from sample with acetone in acidic condition, clean-up with an octadecylsilanized silica gel cartridge and a graphitized carbon black cartridge, quantification and confirmation using LC-MS/MS.

2) Notes

i) Fenhexamid should be extracted at pH lower than 3.

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

for quantification ( $m/z$ ): ESI (-): precursor ion 300, product ion 264

for confirmation ( $m/z$ ): ESI (+): precursor ion 302, product ion 97

Examples of other ions are shown below:

ESI(-): Precursor ion 300, product ion 249

**11. References**

None

**12. Type**

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