

## Analytical Method for Fipronil (Animal Products)

### 1. Analyte

Fipronil

(±)-5-amino-1-(2,6-dichloro- $\alpha,\alpha,\alpha$ -trifluoro-*p*-tolyl)-4-trifluoromethylsulphonylpyrazole-3-carbonitrile (hereinafter referred to as metabolite B)

### 2. Applicable food

Animal products

### 3. Instrument

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

### 4. Reagents

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

Alumina (neutral) cartridge (1,000 mg): A polyethylene tube of 12–13mm in inside diameter packed with alumina (neutral) 1,000 mg or a cartridge equivalent to the specified one in separation capability.

10 mmol/L ammonium formate solution (pH 9): weigh 0.63 g of ammonium formate, dissolve it in approximate 950 mL of water. Add ammonia water to adjust pH to pH 9. Add water to make exactly 1 L.

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

Reference standard of metabolite B: Contains not less than 98% of metabolite B.

### 5. Procedure

#### 1) Extraction

Add 50 mL of acetonitrile saturated with *n*-hexane, 50mL of *n*-hexane and 1 mL of acetic acid to 10.0 g of sample, homogenize. Add 20 g of anhydrous sodium sulphate thereto, homogenize. Centrifuge at 3,000 rpm for 5 minutes, discard the *n*-hexane layer and collect an acetonitrile layer. Add 50 mL of acetonitrile to the residue, homogenize, and centrifuge as describe above. Collect an acetonitrile layer again, combine the previously obtained acetonitrile layer, and add another portion of acetonitrile to make exactly 100 mL. Take an exact 5 mL aliquot of the solution, concentrate it at below 40°C, and remove the solvent. Dissolve the residue in 2 mL of *n*-hexane..

#### 2) Clean-up

Add 5 mL of *n*-hexane to an alumina (neutral) cartridge (1,000 mg), and discard the effluent. Transfer the solution obtained in 1) to the cartridge, add 5 mL of *n*-hexane, discard effluent. Elute with 15 mL of ethanol/*n*-hexane (1:19, v/v). Collect the eluate and concentrate it below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/10 mmol/L ammonium formate solution (pH 9) (4:1, v/v) to make exactly 1 mL, and use this

solution as the test solution.

## 6. Calibration curve

Dissolved the reference standards of fipronil and metabolite B respectively in acetonitrile to prepare standard stock solutions. Mix them, prepare solutions (acetonitrile/10 mmol/L ammonium formate solution (pH 9) (4:1, v/v)) of several concentrations. Inject each standard solution to 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 0.001 mg/kg of fipronil (including metabolite B) gives the test solution of 0.0005 mg/L (as fipronil) in concentration.

## 7. Quantification

Inject the test solution to LC-MS/MS and calculate the concentration of fipronil and metabolite B from the calibration curve made in 6. Use the following equation to calculate the sum of concentrations of fipronil and metabolite B converted to fipronil.

Concentration of fipronil (including metabolite B) (ppm) =  $A + B * 0.9647$

A: Concentration of fipronil (ppm)

B: Concentration of metabolite B (ppm)

## 8. Confirmation

Confirm using LC-MS/MS.

## 9. Measurement conditions

Example

Column: Octadecylsilanized silica gel, 3 mm in inside diameter, 150 mm in length, 3  $\mu$ m in particle diameter.

Column temperature: 40°C

Mobile phase: acetonitrile/10 mmol/L ammonium formate solution (pH 9) (4:1, v/v)

Ionization mode: ESI (-)

Major monitoring ion (*m/z*)

Fipronil: Precursor ion 435, product ion 330

Precursor ion 437, product ion 332, 330

Metabolite B: Precursor ion 451, product ion 282

Precursor ion 453, product ion 284, 282

Injection volume: 5  $\mu$ L

Expected retention time

Fipronil: 3 minutes

Metabolite B: 3 minutes

## 10. Limit of quantification

0.001 mg/kg (the concentration of metabolite B is calculated as fipronil.)

## 11. Explanatory note

### 1) Outline of analytical method

The method consists of extraction of fipronil and metabolite B with acetonitrile under an acidic condition and in presence of *n*-hexane and anhydrous sodium sulphate, clean-up with an alumina (neutral) cartridge, quantification and confirmation using LC-MS/MS.

Notes

### i) When the analytical method for fipronil and metabolite B using LC-MS/MS was developed, the following monitoring ions were used:

#### - Fipronil

for quantification (*m/z*): precursor ion 435, product ion 330

for confirmation (*m/z*): precursor ion 437, product ion 332

#### - Metabolite B

for quantification (*m/z*): precursor ion 451, product ion 282

for confirmation (*m/z*): precursor ion 453, product ion 284

### ii) Food items considered when the analytical method was developed:

Cattle muscle, beef fat, beef liver, milk, chicken muscle and chicken egg

## 12. References

None

## 13. Type

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