

## **Analytical Method for Tiadinil (Agricultural Products)**

### **1. Analytes**

Tiadinil

4-Methyl-1,2,3-thiadiazole-5-carboxylic acid (hereinafter referred to as "carboxylic acid compound")

4-Hydroxymethyl-1,2,3-thiadiazole-5-carboxylic acid (hereinafter referred to as "hydroxymethyl carboxylic acid compound")

### **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 tiadinil: Contains not less than 98% of tiadinil.

Reference standard of carboxylic acid compound: Contains not less than 95% of carboxylic acid compound.

Reference standard of hydroxymethyl carboxylic acid compound: Contains not less than 95% of hydroxymethyl carboxylic acid compound.

Strongly basic anion-exchange resin cartridge (500 mg): Polyethylene tube of 12-13 mm in inside diameter packed with 500 mg of strongly basic anion-exchange resin, or other cartridge with equal separation characteristics.

0.05% aqueous ammonia: Mix ammonia water (28%) and water with the ratio of 1:559.

Acetonitrile/10 mmol/L ammonium acetate solution (17:3, v/v) (pH 5.0): Weigh 0.77 g of ammonium acetate, dissolve in about 950 mL of water, adjust the pH to 5.0 by adding 5 vol% acetic acid, add water to make 1 L, and mix 150 mL of the solution with 850 mL of acetonitrile.

### **4. Procedure**

#### 1) Extraction

For grains, weigh 10.0 g of sample, and for tea leaves, weigh 5.00 g of sample, add 20 mL of 0.1 mol/L hydrochloric acid, and let stand for 30 minutes. For fruits and vegetables, weigh 20.0 g of sample and add 20 mL of 0.1 mol/L hydrochloric acid. Add 80 mL of acetonitrile, homogenize, and filter with suction. Add 50 mL of acetonitrile/water (4:1, v/v) to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates, and add acetonitrile/water (4:1, v/v) to make exactly 200 mL. Take a 4 mL aliquot of the solution for grains, an 8 mL for tea leaves, and a 2 mL for fruits and vegetables. Add 4 mL of acetonitrile/0.05 mol/L hydrochloric acid (4:1, v/v) for grains, and add 6 mL for fruits and vegetables. Concentrate the solution to about 2 mL at below 40°C, and add 4 mL of 0.05%

aqueous ammonia to the concentrated solution.

## 2) Clean-up

Add 5 mL each of methanol and water to an octadecylsilylanized silica gel cartridge (1,000 mg) sequentially, and discard the effluents. Transfer the solution obtained in 1) to the cartridge, elute with 5 mL of water/methanol (4:1, v/v), collect the total eluate, and use this eluate as fraction of carboxylic acid compound and hydroxymethyl carboxylic acid compound (Eluate I). Then, elute with 10 mL of water/methanol (1:4, v/v), collect the eluate, and use this eluate as the tiadinil fraction (Eluate II).

### i) Test solution of tiadinil

Concentrate Eluate II at below 40°C, and remove the solvent. Add 1 mL of methanol to the residue, dissolve, and mix with 9 mL of ethyl acetate.

Add 5 mL of ethyl acetate to an alumina (neutral) cartridge (1,710 mg), and discard the effluent. Transfer the above solution to the cartridge, elute with 10 mL of ethyl acetate/methanol (9:1, v/v), collect the total eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in water/methanol (3:7, v/v) to make exactly 20 mL, and use this solution as the test solution.

### ii) Test solution of carboxylic acid compound and hydroxymethyl carboxylic acid compound

Add 5 mL each of methanol and water to a strongly basic anion-exchange resin cartridge (500 mg) sequentially, and discard the effluents. Transfer Eluate I to the cartridge, and then add 5 mL of methanol, and discard the effluents. Elute with 10 mL of 0.01 mol/L hydrochloric acid-methanol solution, collect the eluate, concentrate at below 40°C, and remove the solvent. Dissolve the residue in acetonitrile/10 mmol/L ammonium acetate solution (17:3, v/v) (pH 5.0) to make exactly 20 mL, and use this solution as the test solution.

## 5. Calibration curve

Dissolve reference standard of tiadinil in acetonitrile, and use the solution as stock standard solution. Prepare tiadinil standard solutions of several concentrations by dilution of the stock standard solution with water/methanol (3:7, 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 tiadinil gives the test solution of 0.0001 mg/L in concentration.

Dissolve reference standards of carboxylic acid compound and hydroxymethyl carboxylic acid compound in acetonitrile separately, and use the solutions as stock standard solutions. Prepare mixed standard solutions of carboxylic acid compound and hydroxymethyl carboxylic acid compound of several concentrations by mixing stock standard solutions and dilution with acetonitrile/10 mmol/L ammonium acetate solution (17:3, v/v) (pH 5.0). 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 0.02 mg/kg of

carboxylic acid compound or hydroxymethyl carboxylic acid compound gives the test solution of 0.0002 mg/L in concentration (calculated as tiadinil).

## 6. Quantification

Inject the test solution to LC-MS/MS, and calculate the concentration of tiadinil, carboxylic acid compound and hydroxymethyl carboxylic acid compound from the calibration curves made in 5. Use the following equation to calculate the concentration of tiadinil including carboxylic acid compound and hydroxymethyl carboxylic acid compound.

Concentration (ppm) of tiadinil (including those of carboxylic acid compound and hydroxymethyl carboxylic acid compound)

$$= A + B \times 1.857 + C \times 1.672$$

A: Concentration (ppm) of tiadinil

B: Concentration (ppm) of carboxylic acid compound

C: Concentration (ppm) of hydroxymethyl carboxylic acid compound

## 7. Confirmation

Confirm using LC-MS/MS.

## 8. Measurement conditions

Example

### i) Tiadinil

Column: Octadecylsilanized silica gel, 2.1 mm in inside diameter, 150 mm in length and 3  $\mu\text{m}$  in particle diameter

Column temperature: 40°C

Mobile phase: 0.1 vol% formic acid/methanol (3:7, v/v)

Ionization mode: ESI (-)

Major monitoring ions ( $m/z$ ): Precursor ion 266, product ion 238, 71

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

Expected retention time: 7 min

### ii) Carboxylic acid compound and hydroxymethyl carboxylic acid compound

Column: Triazole-bonded 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: Acetonitrile/10 mmol/L ammonium acetate solution (17:3, v/v) (pH 5.0)

Ionization mode: ESI (-)

Major monitoring ions ( $m/z$ ):

Carboxylic acid compound: precursor ion 143, product ion 71

Hydroxymethyl carboxylic acid compound: precursor ion 159, product ion 87

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

Expected retention time: Carboxylic acid compound 13 min, hydroxymethyl carboxylic acid compound 11 min

## 9. Limits of quantification

Tiadinil: 0.01 mg/kg

Carboxylic acid compound: 0.02 mg/kg (calculated as tiadinil)

Hydroxymethyl carboxylic acid compound: 0.02 mg/kg (calculated as tiadinil)

## 10. Explanatory note

### 1) Outline of analytical method

The method consists of extraction of tiadinil, carboxylic acid compound and hydroxymethyl carboxylic acid compound from sample with acetonitrile under acidic condition (hydrochloric acid), fractionation to fraction of tiadinil and fraction of carboxylic acid compound and hydroxymethyl carboxylic acid compound using an octadecylsilanized silica gel cartridge, clean-up, and quantification and confirmation using LC-MS/MS. For fraction of tiadinil, clean-up with an alumina (neutral) cartridge, and for fraction of carboxylic acid compound and hydroxymethyl carboxylic acid compound, clean-up with a strongly basic anion-exchange resin cartridge. Tiadinil, carboxylic acid compound and hydroxymethyl carboxylic acid compound are quantified individually. For calculation of the concentration of tiadinil including carboxylic acid compound and hydroxymethyl carboxylic acid compound, each concentration of carboxylic acid compound and hydroxymethyl carboxylic acid compound is converted to the concentration of tiadinil by multiplying by the conversion factor, and the sum of the concentration of tiadinil, carboxylic acid compound and hydroxymethyl carboxylic acid compound is regarded as the analytical result of tiadinil.

### 2) Notes

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

for quantification ( $m/z$ ): precursor ion 266, product ion 71

for confirmation ( $m/z$ ): precursor ion 266, product ion 238

For carboxylic acid compound and hydroxymethyl carboxylic acid compound, appropriate monitoring ions for confirmation could not be selected under the measurement conditions of this method.

ii) For agricultural products, which is applied a uniform limit of 0.01 mg/kg, it is preferable that carboxylic acid compound and hydroxymethyl carboxylic acid compound are analyzed at limits of quantification of 0.01 mg/kg (calculated as tiadinil).

iii) In procedure 4 1), 4 mL of 0.05% aqueous ammonia is added to change the concentrated solution from neutral to weak basic. Confirm the pH of the sample, using pH test paper for example, and adjust the additive amount if necessary.

iv) In clean-up using an alumina (neutral) cartridge, the residue after clean-up with the octadecylsilanized silica gel cartridge is difficult to dissolve in ethyl acetate/methanol (9:1, v/v). Therefore, dissolve the residue in 1 mL of methanol first, then add 9 mL of ethyl acetate and mix. For some samples, the residue is also difficult to dissolve in methanol. In this case, ultrasonication is recommended.

v) In LC-MS/MS analysis for agricultural products containing high amounts of interfering

components, it is recommended that the LC column is washed with a mobile phase thoroughly after analytes have been eluted to decrease the effect of carry-over of interfering components.

#### **11. References**

None

#### **12. Type**

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