

## Analytical Method for Propoxycarbazone (Agricultural Products)

### 1. Analytes

Propoxycarbazone

Methyl 2-[(4,5-Dihydro-3-(2-hydroxypropoxy)-4-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)carbonyl]amino)sulfonyl]benzoate (hereinafter referred to as metabolite A)

### 2. Application

Grains

### 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 propoxycarbazone sodium salt: Contains not less than 98% of propoxycarbazone sodium salt.

Reference standard of metabolite A: Contains not less than 97% of metabolite A.

### 5. Procedure

#### 1) Extraction

Add 20 mL of water to 10.0 g of sample and let stand for 30 min. Add 100 mL of methanol, homogenize, and filter with suction. Add 50 mL of methanol to the residue on the filter paper, homogenize, and filter as described above. Combine the resulting filtrates and add methanol to make exactly 200 mL. Take exactly a 20 mL aliquot of the solution, concentrate at below 40°C, and remove the solvent. Dissolve the residue in 5 mL of acetonitrile.

#### 2) Clean-up

Inject 5 mL each of water and acetonitrile into an ethylenediamine-*N*-propylsilanized silica gel cartridge (500 mg) sequentially and discard each effluent. Transfer the solution obtained in 1) to the cartridge, add 5 mL of acetonitrile, and discard the effluent. Then, add 10 mL of acetonitrile and water (7:3) mixed solution, collect the eluate, add a mixed solution of acetonitrile and water (7:3) to make exactly 10 mL, and use this solution as the test solution.

### 6. Calibration curve

Dissolve the reference standard of propoxycarbazone sodium salt in methanol to make 200 mg/L (equivalent to propoxycarbazone), and use the solution as a stock standard solution. Likewise, dissolve the reference standard of metabolite A in methanol to make 200 mg/L, and use the solution as a stock standard solution. Mix each stock standard solution appropriately, dilute with a mixed solution of acetonitrile and water (7:3) appropriately, and prepare standard solutions of several concentrations. Inject each 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 concentration of propoxycarbazone in the test solution corresponding to 0.01 mg/kg (The concentration of metabolite A is calculated as propoxycarbazone.) in the sample is 0.001 mg/L (The concentration of metabolite A is calculated as propoxycarbazone.).

## 7. Quantification

Inject the test solution into LC-MS/MS and calculate the concentration of propoxycarbazone and metabolite A from the calibration curve prepared in 6. Use the following equation to calculate the concentration of propoxycarbazone including metabolite A.

$$\text{Concentration (ppm) of propoxycarbazone (including metabolite A)} = A + B \times 0.9614$$

A: Concentration (ppm) of propoxycarbazone

B: Concentration (ppm) of metabolite A

## 8. Confirmation

Confirm using LC-MS/MS.

## 9. Measurement conditions

(Example)

Column: Octadecylsilanized silica gel, 2.1 mm inside diameter, 150 mm in length and 5 µm in particle diameter

Column temperature: 40°C

Mobile phase: Linear gradient from a mixed solution of acetonitrile and 0.1 vol% formic acid (3:7) to (7:3) in 10 min and hold for 5 min at (9:1).

Ionization mode: ESI (–)

Major monitoring ions (*m/z*)

Propoxycarbazone: Precursor ion 397, product ions 156, 113

Metabolite A: Precursor ion 413, product ions 172, 113

Injection volume: 4 µL

Expected retention time

Propoxycarbazone: 9 min

Metabolite A: 5 min

## 10. Limit of quantification

0.01 mg/kg for each analyte (The concentration of metabolite A is calculated as propoxycarbazone.)

## 11. Explanatory note

### 1) Outline of analytical method

The method consists of extracting propoxycarbazone and metabolite A from sample with methanol, cleaning up with an ethylenediamine-*N*-propylsilanized silica gel cartridge and quantifying and confirming using LC-MS/MS.

### 2) Notes

- i) When analytical methods for propoxycarbazone and metabolite A using LC-MS/MS

were developed, the following monitoring ions were used:

Propoxycarbazone

for quantitative ions ( $m/z$ ): precursor ion 397, product ion 156

for qualitative ions ( $m/z$ ): precursor ion 397, product ion 113

Metabolite A

for quantitative ions ( $m/z$ ): precursor ion 413, product ion 172

for qualitative ions ( $m/z$ ): precursor ion 413, product ion 113

- ii) Since propoxycarbazone is hard to dissolve in acetonitrile, care should be taken when preparing standard stock solutions depending on the concentration.
- iii) Food items used to develop the analytical method: wheat.

## 12. Reference

None

## 13. Type

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