Published, Nov 13, 2024
TFDAP0032.00

Method of Test for Pesticide Residues in Foods - Test of Diafenthiuron

1. Scope

This method is applicable for the determination of diafenthiuron and its metabolites, diafenthiuron-urea and diafenthiuron methaneimide-amide, in fruits and vegetables, crops, dried beans, tea, spice plants and other herbs.

2. Method

After preparation of the sample solution by the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe), pesticides are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: electrospray ionization, ESI.

2.1.1.2. Column: CORTECS UPLC C18, 1.6 μm, 2.1 mm i.d. × 10 cm, or an equivalent product.

2.1.1.3. Guard column: CORTECS UPLC C18, 1.6 μm, 2.1 mm i.d. × 5 mm, or an equivalent product.

2.1.2. Blender.

2.1.3. Grinder.

2.1.4. High speed dispersing device: SPEX SamplePrep 2010 GenoGrinder®, >1000 rpm, or another mechanical shaker.

2.1.5. Vortex mixer.

2.1.6. Centrifuge: centrifugal force ≥ 3000 ×g, temperature control ≤ 15°C.

2.2. Chemicals

Formic acid, reagent grade;
Ammonium acetate, reagent grade;
β-Carotene Type I, reagent grade;
Acetonitrile, HPLC grade;
Methanol, HPLC grade;
Dichloromethane, HPLC grade;
Sodium chloride, AR grade;
Magnesium sulfate anhydrous, AR grade;
Primary secondary amine (PSA), AR grade;
Octadecysilane, end-capped (C18 EC), AR grade;

Graphitized carbon black (GCB), AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Diafenthiuron, diafenthiuron-urea and diafenthiuron methaneimide-amide, reference standards.

2.3. Apparatus

2.3.1. Centrifuge tube: 15 mL and 50 mL, PP.

2.3.2. Volumetric flask: 25 mL, amber.

2.3.3. Ceramic homogenizer(note 1): Bond Elut QuEChERS P/N 5982-9313, or an equivalent product.

2.3.4. Extraction powder(note 1): Containing 4 g of magnesium sulfate anhydrous and 1 g of sodium chloride.

2.3.5. Clean-up centrifuge tube I(note 1): containing 300 mg of PSA and 900 mg of magnesium sulfate anhydrous, 6 mL, used for type I samples (fresh fruits, vegetables, spice plants and other herbs with high water content).

2.3.6. Clean-up centrifuge tube II(note 1): containing 300 mg of PSA, 300 mg of C18 EC and 900 mg of magnesium sulfate anhydrous, 6 mL, used for type II samples (crops and dried beans with high wax, fat and sugar content).

2.3.7. Clean-up centrifuge tube III(note 1): containing 450 mg of PSA, 900 mg of magnesium sulfate anhydrous, 300 mg of C18 EC and 50 mg of GCB, 6 mL, used for type III samples (dried tea, fruits, vegetables, spice plants and other herbs with high pigment content).

2.3.8. Membrane filter: 0.2 μm, PTFE.

Note 1: Commercial extraction/clean-up kit can be used as needed.

2.4. 1 mg/mL β-carotene in dichloromethane

Dissolve and dilute 20 mg of β-carotene with dichloromethane to 20 mL.

2.5. Mobile phase

2.5.1. Solvent A

Dissolve 0.39 g of ammonium acetate with deionized water to 1000 mL, add 1 mL of formic acid, mix well, and filter with a membrane filter.

2.5.2. Solvent B

Dissolve 0.39 g of ammonium acetate with methanol to 1000 mL, and

filter with a membrane filter.

2.6. Standard solution preparation

Transfer about 25 mg of diafenthiuron, diafenthiuron-urea and diafenthiuron methaneimide-amide reference standard accurately weighed to each 25-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the standard stock solutions. Store under freezing in the dark. When to use, dilute appropriate amount of the standard stock solutions with acetonitrile to 1 μg/mL separately as the standard solutions.

2.7. Sample solution preparation(note 2)

2.7.1. Type I samples (fresh fruits, vegetables, spice plants and other herbs with high water content)

Transfer about 10 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube, add 1 mL of 1 mg/mL β-carotene in dichloromethane, shake at 1000 rpm by the high speed dispersing device for 1 min, add 10 mL of acetonitrile, and shake vigorously several times immediately. Add 1 granule of the ceramic homogenizer and the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of salt, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min. Centrifuge at 3000 ×g for 1 min at 15°C, and transfer 6 mL of the supernatant to a clean-up centrifuge tube I. Shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 3000 ×g for 2 min at 15°C. Take 1 mL of the supernatant, and filter with a membrane filter as the sample solution.

2.7.2. Type II samples (crops and dried beans with high wax, fat and sugar content)

Transfer about 5 g of the powdered sample accurately weighed into a 50-mL centrifuge tube, add 10 mL of pre-cooled deionized water, stand for 20 min, add 10 mL of acetonitrile, and shake vigorously several times immediately. Add 1 granule of the ceramic homogenizer and the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of

salt, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min. Centrifuge at 3000 ×g for 1 min at 15°C, and transfer 6 mL of the supernatant to a clean-up centrifuge tube II. Shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 3000 ×g for 2 min at 15°C. Take 1 mL of the supernatant, and filter with a membrane filter as the sample solution.

2.7.3. Type III samples (dried tea, fruits, vegetables, spice plants and other herbs with high pigment content)

Transfer about 2 g of the powdered sample accurately weighed into a 50-mL centrifuge tube, add 10 mL acetonitrile, and shake vigorously several times immediately. Add 1 granule of the ceramic homogenizer and the extraction powder, cap the centrifuge tube, shake vigorously several times by hands to prevent coagulation of salt, and then shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min. Centrifuge at 3000 ×g for 1 min at 15°C, and transfer 6 mL of the supernatant to a clean-up centrifuge tube III. Shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 3000 ×g for 2 min at 15°C. Take 1 mL of the supernatant, and filter with a membrane filter as the sample solution.

Note 2: The entire process needs to be protected from light.

2.8. Matrix-matched calibration curve

Take a blank sample, and follow the procedure described in section 2.7 to obtain the supernatant after the clean-up procedure. Take several 1 mL of the supernatant, and evaporate to near dryness by gently flushing with a stream of nitrogen. Separately add 5-200 μL of 1 μg/mL the standard solutions and acetonitrile to achieve a final volume of 1 mL, and mix well as the matrix-matched standard solutions. Operate LC-MS/MS according to the following conditions. Establish the matrix-matched calibration curve of each pesticide by the peak areas of each pesticide vs. the added concentrations in the range of 0.005-0.2 μg/mL.

LC-MS/MS operating conditions (note 3):

Column: CORTECS UPLC C18, 1.6 μm, 2.1 mm i.d. × 10 cm.

Guard column: CORTECS UPLC C18, 1.6 μm, 2.1 mm i.d. × 5 mm.
Column temperature: 40°C.
Mobile phase: a gradient program of solvent A and solvent B is as follows:

Time (min)	A (%)	B (%)
0.0 → 2.0	99 → 50	1 → 50
2.0 → 8.0	50 → 30	50 → 70
8.0 → 15.0	30 → 1	70 → 99
15.0 → 18.0	1 → 1	99 → 99
18.0 → 18.5	1 → 99	99 → 1
18.5 → 20.5	99 → 99	1 → 1

Flow rate: 0.35 mL/min.
Injection volume: 3 μL.
Interface voltage: 1 kV.
Ionization mode: ESI^+.
Interface temperature: 250°C.
Nebulizing gas flow: 3 L/min.
Heating gas flow: 15 L/min
Desolvation line temperature: 200°C.
Heat block temperature: 350°C.
Drying gas flow: 5 L/min.
Detection mode: multiple reaction monitoring (MRM). Detection ion pair, Q1/Q3 Pre Bias and collision voltage are shown as follows:

	Ion pair	Q1/Q3	Collision
	Precursor ion (*m/z*) > product ion (*m/z*)	Pre Bias (V)	voltage (V)
Diafenthiuron	385 > 329*	19/20	20
	385 > 278	19/20	20
Diafenthiuron-urea	369 > 229*	14/24	27
	369 > 271	13/28	22
	369 > 313	19/22	17
Diafenthiuron methaneimide-amide	353 > 297*	10/14	22
	353 > 280	10/13	27

*Quantitative ion, and a qualitative ion can be selected based on

the matrix condition.

Note 3: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.9. Identification and quantification

Accurately inject 3 μL of the sample solution and the matrix-matched standard solutions into LC-MS/MS separately, and operate according to the conditions described in section 2.8. Identify diafenthiuron based on the retention time and the relative ion intensities(note 4). Calculate the amount of diafenthiuron(note 5) in the sample using the following formula:

$$\text{The amount of diafenthiuron in the sample (ppm)} = \frac{\sum[C \times V \times F]}{M}$$

Where,

C: the concentration of diafenthiuron or its metabolites in the sample solution calculated by the matrix-matched calibration curve (μg/mL)

V: the volume of acetonitrile for sample extraction (10 mL)

M: the weight of the sample (g)

F: the conversion factor

Diafenthiuron: 1.00

Diafenthiuron-urea: 1.04

Diafenthiuron methaneimide-amide:1.09

Note 4: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions. Maximum permitted tolerances of relative ion intensities are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20～50	± 25
> 10～20	± 30
≤ 10	± 50

Note 5: The amount of diafenthiuron in the sample is the sum of residues of diafenthiuron, diafenthiuron-urea and diafenthiuron methaneimide-amide, calculated as diafenthiuron.

Remark

1. Limits of quantification (LOQs) for diafenthiuron, diafenthiuron-urea and

diafenthiuron methaneimide-amide are 0.01 ppm in Type I samples, 0.02 ppm in Type II samples, and 0.05 ppm in Type III samples.

2. Further validation should be performed when interfering compounds appear in samples.

Reference

Anastassiades, M. and Lehotay, S. J. 2003. Fast and easy multiresidue method employing acetonitrile extraction/Partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. J. AOAC Int. 86: 412-431.

Reference chromatogram

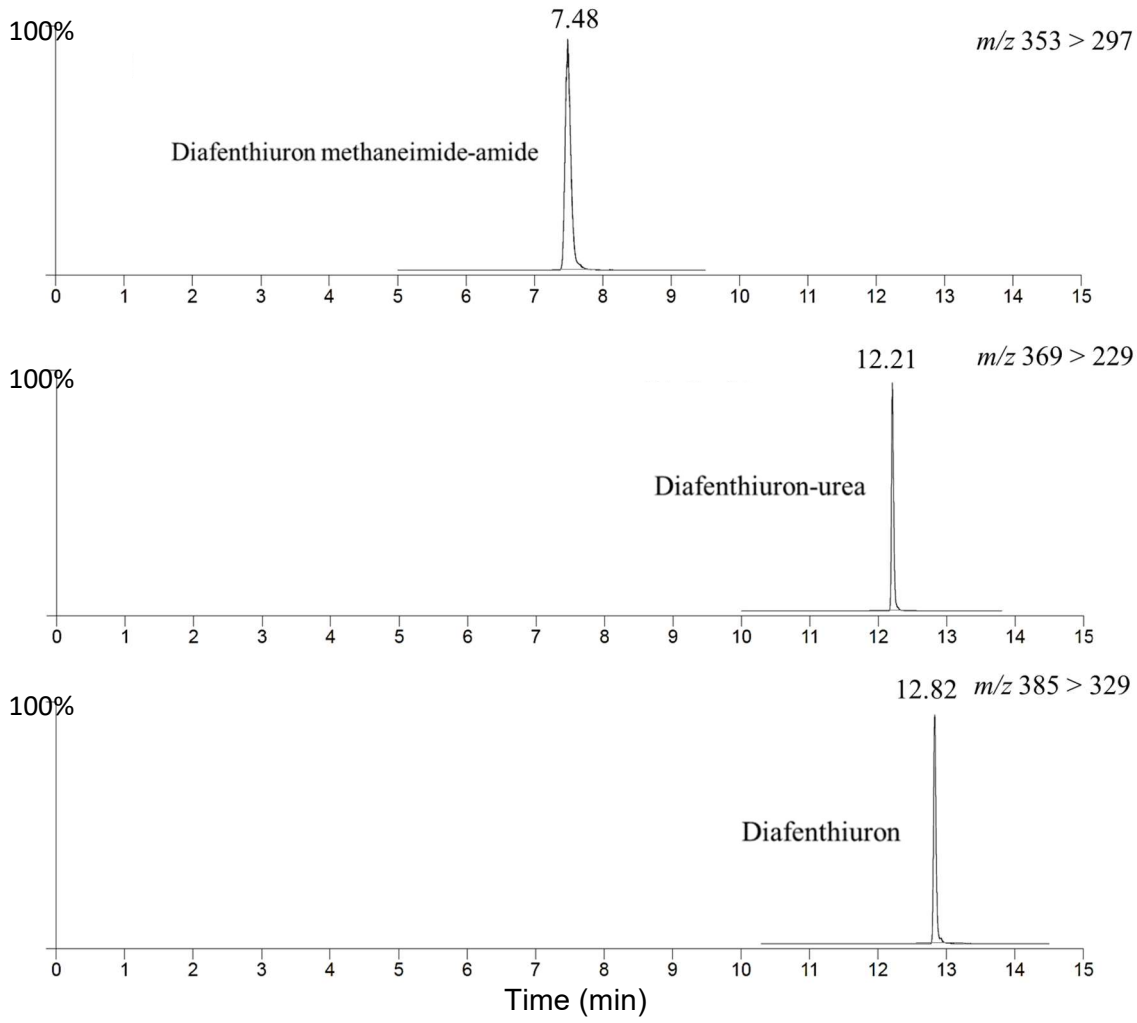

Figure. MRM chromatograms of diafenthiuron, diafenthiuron-urea and diafenthiuron methaneimide-amide standards analyzed by LC-MS/MS.