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Method of Test for Pesticide Residues in Livestock and Poultry Products - Test of 2,4-D and Fenbutatin Oxide

1. Scope

This method is applicable for the determination of 2,4-D and fenbutatin oxide in muscle, viscera, fat, eggs, and milk of poultry and livestock products.

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. × 100 mm, 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 P 2010 GenoGrinder®, >1000 rpm, or an equivalent product.

2.1.5. Centrifuge: centrifugal force > 4000 ×g, temperature control < 15°C.

2.1.6. Nitrogen evaporator.

2.1.7. Vortex mixer.

2.2. Chemicals

Acetonitrile, HPLC grade;
Methanol, HPLC grade;
Formic acid, trace grade;
Sodium citrate, trace grade;
Sodium dihydrogen citrate, trace grade;
Sodium chloride, trace grade;
Magnesium sulfate anhydrous, trace grade;

Ammonium acetate, trace grade;
Primary secondary amine (PSA), AR grade;
Octadecylsilane end-capped (C18 EC), AR grade;
Graphitized carbon black (GCB), AR grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
2,4-D and fenbutatin oxide, reference standards;
Triphenylphosphate, internal standard.

2.3. Apparatus

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

2.3.2. Membrane filter: 0.22-μm, PTFE.

2.3.3. Volumetric flask: 25 mL and 50 mL, amber.

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

2.3.5. Extraction powder(note 2): containing 4 g of magnesium sulfate anhydrous, 1 g of sodium chloride, 1 g of sodium citrate, and 0.5 g of sodium dihydrogen citrate

2.3.6. Clean-up centrifuge tube(note 2): containing 125 mg of PSA, 750 mg of magnesium sulfate anhydrous, 250 mg of C18 EC and 45 mg of GCB, 5 mL.

Note 1: Ceramic homogenizer can be used depending on the viscosity of the sample.

Note 2: Commercial extraction/clean-up kit can be used as needed.

2.4. Reagents

2.4.1. Acetonitrile: methanol (4:1, v/v)

Mix acetonitrile and methanol at the ratio of 4: 1 (v/v).

2.4.2. 1% Formic acid in acetonitrile: methanol (4:1, v/v).

Mix 10 mL of formic acid and 990 mL of acetonitrile: methanol (4:1, v/v).

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 0.4 g of ammonium acetate with deionized water to 1000 mL. Add 1 mL of formic acid, and filter with a membrane filter.

2.5.2. Solvent B

Dissolve and dilute 0.4 g of ammonium acetate with methanol to 1000 mL, and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer about 50 mg of triphenylphosphate internal standards accurately weighed into a 50-mL volumetric flask, dissolve and dilute with methanol to volume as the internal standard stock solution. Store at -18°C in the dark.

2.6.1. Dilute appropriate volume of the internal standard stock solution with methanol to 50 μg/mL as the internal standard solution used for the sample solution preparation in section 2.8.

2.6.2. Dilute appropriate volume of the internal standard stock solution with methanol to 5 μg/mL as the internal standard solution for the preparation of matrix-matched calibration curve in section 2.9.

2.7. Standard solution preparation

Transfer about 10 mg of 2,4-D and fenbutatin oxide reference standards accurately weighed to each 10-mL volumetric flask, dissolve and dilute to volume with acetonitrile as the standard stock solutions. Store at -18°C in the dark. When to use, mix appropriate volume of each standard stock solution, and dilute with acetonitrile to 1 μg/mL as the standard solutions.

2.8. Sample solution preparation

Transfer about 10 g of the homogenized muscle, visceral, or milk sample accurately weighed; remove eggs' shell and transfer about 10 g of mixed egg white and yolk sample accurately weighed; transfer about 2 g of homogenized fat sample accurately weighed into a 50-mL centrifuge tube. Add 10 mL of 1% formic acid in acetonitrile: methanol (4:1, v/v) and 10 μL of 50 μg/mL internal standard solution. Add 1 granule of a 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 3 min. Centrifuge at 4000 ×g for 5 min at 15°C, and transfer 5 mL of the supernatant (avoiding the fat layer) to a clean-up centrifuge tube. Shake at 1000 rpm by the high speed dispersing device or shake vigorously by hands for 1 min, and centrifuge at 4000 × g for 5 min at 15°C. Take 1 mL of the supernatant, and evaporate to near dryness by gently flushing with a stream of nitrogen. Dissolve the residue with 1 mL of methanol, mix well, and filter with a membrane filter. Take the filtrate as the sample solution.

2.9. Matrix-matched calibration curve

Take a blank sample without adding the internal standard, and follow the procedure described in section 2.8 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 appropriate volume of methanol, 5-200 μL of the standard solution (for fat matrix, add 2-100μL) and 10 μL of 5 μg/mL the internal standard solution 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 ratios of the peak area of each pesticide to that of the internal standard vs. the added concentrations (0.002-0.1 μg/mL for fat matrix, and 0.005-0.2 μg/mL for other matrices).

LC-MS/MS operating conditions(note 3)

Column: CORTECS UPLC, C18, 1.6 μm, 2.1 mm i.d. × 100 mm.

Guard column: CORTECS UPLC, C18, 1.6 μm, 2.1mm i.d. × 5 mm.

Mobile phase: 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 → 10.0	30 → 1	70 → 99
10.0 → 13.0	1 → 1	99 → 99
13.0 → 13.5	1 → 99	99 → 1
13.5 → 15.0	99 → 99	1 → 1

Flow rate: 0.3 mL/min.

Injection volume: 5 µL.

Capillary voltage: ESI^+, 3.5 kV; ESI^-, 1.6 kV.

Ion source temperature: 150°C.

Desolvation temperature: 450°C.

Cone gas flow: 30 L/hr.

Desolvation flow rate: 900 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are shown in the attached table.

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

2.10. Identification and quantification

Accurately inject 5 µL of the sample solution and the matrix-matched standard solutions into LC-MS/MS separately. Operate according to the conditions in section 2.9. Identify each pesticide based on the retention time and the relative ion intensities(note 4). Calculate the amount of each pesticide in the sample by the following formula:

$$\text{The amount of each pesticide in the sample (ppm)} = \frac{C \times V}{M}$$

Where,

C: the concentration of each pesticide in the sample solution calculated by the matrix-matched calibration curve (µg/mL)

V: the volume of 1% formic acid in acetonitrile: methanol

(4:1, v/v) for sample extraction (10 mL)

M: the weight of the sample (g)

Note 4: Relative ion intensities are calculated by peak areas of quantitative ions divided by peak areas of qualitative ions (≤100%). Maximum permitted tolerances of relative ion intensities are as follows:

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

Remark

1. Limit of quantification (LOQ) for each pesticide is listed in the attached table.
2. Because the added internal standard, triphenylphosphate (TPP), used in the procedure may not represent the physicochemical properties of all items of pesticides, it is optional for applying it in the formula to calculate the amount of pesticides in the sample. The TPP is recommended to serve as a quality control factor to confirm the procedure.
3. Further validation should be performed when interfering compounds are found in the samples.

Reference

European Committee for Standardization. 2018. Foods of plant origin– Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE– Modular QuEChERS-method. NF EN 15662:2018 (English version).

Reference chromatogram

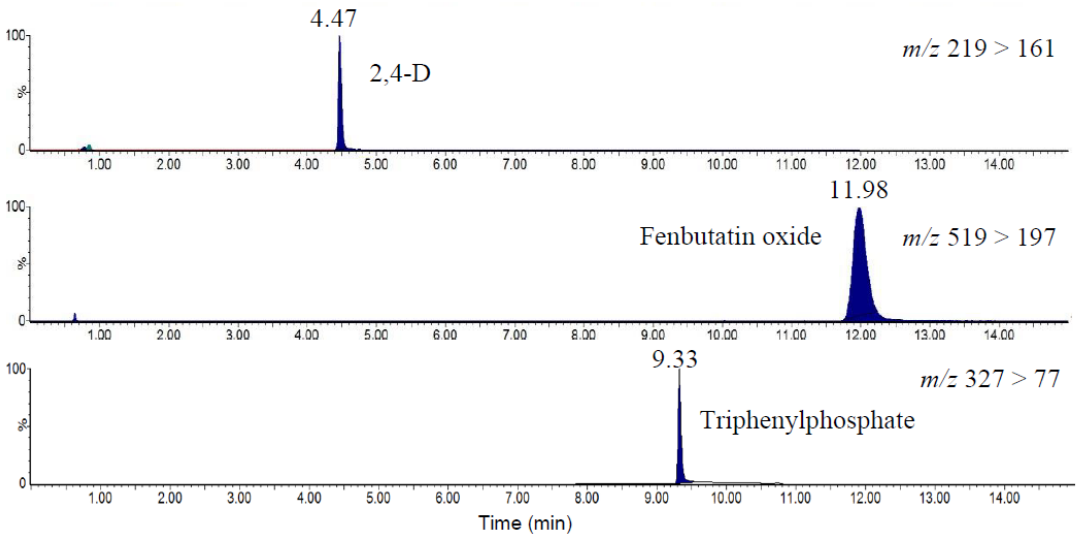

Figure. MRM chromatograms of 2 pesticide standards and the internal standard (TPP) analyzed by LC-MS/MS.

Table. MRM parameters and LOQs of 2,4-D, fenbutatin oxide and the internal standard

No	Analyte	Ionization mode	Quantitative ion pair			Qualitative ion pair			LOQ (ppm)				
			Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)	Muscle	Viscera	Fat	Eggs	Milk
1	2,4-D	ESI^-	219 > 161	23	15	221 > 125	23	15	0.01	0.01	0.01	0.01	0.01
2	Fenbutatin oxide	ESI^+	519 > 197	44	54	519 > 351	44	32	0.01	0.01	0.01	0.01	0.01
I.S.	Triphenylphosphate	ESI^+	327 > 77	40	35	—	—	—	—	—	—	—	—