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Method of Test for Pesticide Residues in Livestock and Poultry Products - Test of Chlormequat, Cyromazine, Diquat and Paraquat

1. Scope

This method is applicable to the determination of 4 pesticides including chlormequat, cyromazine, diquat, and paraquat in muscle, eggs, viscera, and milk of livestock and poultry products.

2. Method

After extraction and purification, 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: SIELC Obelisc R, 5 μm, 2.1 mm i.d. × 15 cm, or an equivalent product.

2.1.2. Grinder.

2.1.3. Blender.

2.1.4. Vortex mixer.

2.1.5. High speed dispersing device: SPEX SamplePrep 2010 GenoGrinder® , >1000 rpm, or an other mechanical shaker.

2.1.6. Centrifuge: centrifugal force ≥ 5000 ×g, temperature control < 15°C.

2.2. Chemicals

Formic acid, reagent grade;
Ammonium formate, reagent grade;
Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Octadecylsilane (C18), analytical grade;
Deionized water, resistivity ≥ 18 MΩ・cm (at 25°C);
Chlormequat, cyromazine, diquat, and paraquat, reference standards.

2.3. Apparatus

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

2.3.2. Volumetric flask: 10 mL, PP.

2.3.3. Ultrafiltration filter tube: 15 mL, 10 kDa molecular weight cutoff suitable for centrifuges, PP.

2.3.4. Membrane filter: 0.22 μm, PTFE.

2.4. Reagents

2.4.1. Methanol containing 1% formic acid

Dilute 5 mL of formic acid with methanol to 500 mL.

2.4.2. 50% methanol containing 0.5% formic acid

Dilute 500 mL of methanol containing 1% formic acid with deionized water to 1000 mL.

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 3.16 g of ammonium formate with deionized water to 1000 mL. Adjust pH to 3.0 with formic acid, and filter with a membrane filter.

2.5.2. Solvent B

Acetonitrile.

2.6. Standard solution preparation

Transfer about 10 mg of chlormequat, cyromazine, diquat, and paraquat reference standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute to volume with methanol containing 1% formic acid 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 methanol containing 1% formic acid to 0.5 μg/mL as the standard solution.

2.7. Sample solution preparation

2.7.1. Muscle and eggs:

Transfer about 2 g of the homogenized muscle sample accurately weighed; remove eggs' shell, and transfer about 2 g of the mixed egg white and yolk sample accurately weighed into a 50-mL centrifuge tube. Add appropriate amount of deionized water to adjust the water content to 2 mL (add 0.4 mL for muscle, and 0.5 mL for eggs). Add 10 mL of 50% methanol containing 0.5% formic acid, shake at 1000 rpm by the high speed dispersing device for 5 min, and ultrasonicate at 80°C for 15 min. After cooling to room temperature, shake at 1000 rpm by the high speed dispersing device for 1 min. Freeze at -20°C for at least 90 min, take out, and immediately centrifuge at 5000 ×g for 5 min. Transfer 2 mL (a) of the supernatant to a 15-mL centrifuge tube containing 2 mL (b) of acetonitrile and 100 mg of C18. Shake at 1000 rpm by the high speed dispersing device for 1 min, and centrifuge at 3000 ×g for 5 min. Filter with a membrane filter, and dilute

0.8 mL (c) of the filtrate with 50% methanol containing 0.5% formic acid to 1 mL (d), and mix well as the sample solution.

2.7.2. Milk and viscera:

Transfer about 2 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube. Add appropriate amount of deionized water to adjusted the water content to 2 mL (add 0.3 mL for milk, 0.6 mL for liver, and 0.4 mL for kidney). Add 10 mL of 50% methanol containing 0.5% formic acid solution, shake at 1000 rpm by the high speed dispersing device for 5 min, and ultrasonicate at 80°C for 15 min. After cooling to room temperature, shake at 1000 rpm by the high speed dispersing device for 1 min. Freeze at -20°C for at least 90 min, take out, and immediately centrifuge at 5000 ×g for 5 min. Transfer 2 mL (a) of the supernatant to a 15-mL centrifuge tube containing 2 mL (b) acetonitrile and 100 mg of C18, shake at 1000 rpm by the high speed dispersing device for 1 min, and centrifuge at 3000 ×g for 5 min. Transfer 3 mL of the supernatant into an ultrafiltration filter tube, and centrifuge at 5000 ×g for 5 min. Dilute 0.8 mL (c) of the filtrate with 50% methanol containing 0.5% formic acid to 1 mL (d), and mix well as the sample solution.

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 0.8 mL (c) of the supernatant, add 1-100 μL of the standard solution, dilute with 50% methanol containing 0.5% formic acid to 1 mL (d), 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 (0.0005 - 0.05 μg/mL).

LC-MS/MS operating conditions(note):

Column: SIELC Obelisc R, 5 μm, 2.1 mm × 15 cm.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	A (%)	B (%)
0.0 → 1.0	20 → 40	80 → 60
1.0 → 4.0	40 → 80	60 → 20
4.0 → 6.0	80 → 80	20 → 20

6.0 → 6.5	80 → 20	20 → 80
6.5 → 10.0	20 → 20	80 → 80

Flow rate: 0.4 mL/min.

Injection volume: 2 μL.

Ionization mode: ESI+.

Capillary voltage: 3.5 kV.

Ion source temperature：150°C.

Desolvation temperature：450°C.

Cone gas flow：10 L/hr.

Desolvation flow：900 L/hr.

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

Note: 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 2 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 each pesticide based on the retention time and the relative ion intensities(note). Calculate the amount of each pesticide in the sample by the following formula:

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

Where,

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

V: the volume of the water content adjusted and 50% methanol containing 0.5% formic acid for sample extraction (12 mL)

M: the weight of the sample (g)

F: the dilution factor (2.5), (a+b)×d/(a×c)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions. Maximum permitted tolerances for relative ion intensities by LC-MS/MS are as follows:

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

Remark

1. Limits of quantitation (LOQs) are listed in the attached table.
2. Further validation should be performed when interfering compounds appear in samples.

Reference

1. Taiwan Food and Drug Administration. 2019. Method of test for pesticide residues in foods - test of paraquat, a herbicide (TFDAP0014.00). Published on August 5, 2019.
2. Anastassiades, M., Kolberg, D. I., Eichhorn, E., Wachtler, A. K., Benkenstein, A., Zechmann, S., Mack, D., Wildgrube, C., Barth, A., Sigalov, I., Görlich, S., Dörk, D. and Cerchia, G. 2021. Quick method for the analysis of numerous highly polar pesticides in food involving extraction with acidified methanol and LC-MS/MS measurement. I. Food of plant origin (QuPPe-PO-Method) - Version 11.1. EURL-SRM.
3. Anastassiades, M., Wachtler, A. K., Kolberg, D. I., Eichhorn, E., Benkenstein, A., Zechmann, S., Mack, D., Barth, A., Wildgrube, C., Sigalov, I., Görlich, S., Dörk, D. and Cerchia, G. 2019. Quick method for the analysis of numerous highly polar pesticides in food involving extraction with acidified methanol and LC-MS/MS measurement. II. Food of animal origin (QuPPe AO Method) - Version 3.2. EURL-SRM.

Reference chromatogram

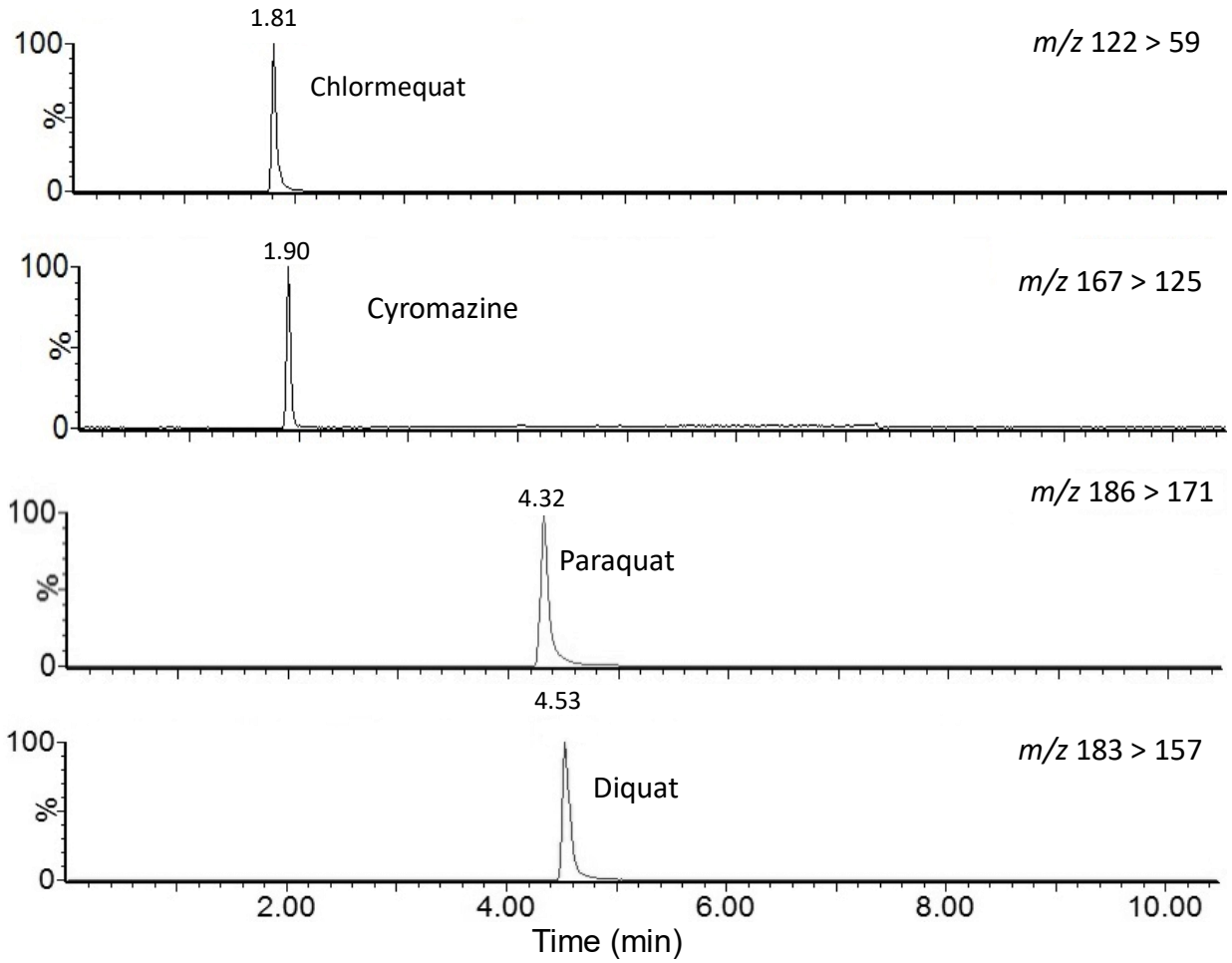

Figure. MRM chromatograms of 4 pesticide standards analyzed by LC-MS/MS.

Table. MRM parameters and LOQs of chlormequat, cyromazine, diquat and paraquat

No.	Analyte	Quantitative ion pair			Qualititative 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	Eggs	Milk
1	Chlormequat	122 > 59	37	20	124 > 65	32	18	0.05	0.05	0.05	0.05
2	Cyromazine	167 > 125	30	18	167 > 108	30	20	0.01	0.05	0.01	0.01
3	Diquat	183 > 157	50	18	184 > 156 184 > 106	20 20	20 17	0.01	0.01	0.01	0.01
4	Paraquat	186 > 171	10	15	186 > 155	40	36	0.01	0.01	0.01	0.01