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Method of Test for Pesticide Residues in Foods-Test of Paraquat, a Herbicide

1. Scope

This method is applicable for the determination of paraquat in fruits, vegetables, crops, dried beans, tea, spice plants and other herbs.

2. Method

After extraction, paraquat is 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: CAPCELL PAK ST, 2.0 μm, 2.0 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. Ultrasonicator: temperature control ≥ 80°C.

2.1.6. High speed dispersing device: SPEX SamplePrep 2010 GenoGrinder®, > 1000 rpm, or other mechanical shakers.

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

2.1.8. Nitrogen evaporator.

2.2. Chemicals

Formic acid, reagent grade;
Ammonium acetate, reagent grade;
Methanol, HPLC grade;
Acetonitrile, HPLC grade;
Deionized water, resistivity ≥ 18 MΩ·cm (at 25°C);
Paraquat dichloride, reference standard.

2.3. Apparatus

2.3.1. Centrifuge tube: 50 mL, PP.

2.3.2. Volumetric flask: 1 mL and 10 mL, PP.

2.3.3. Sample vial: 1 mL and 10 mL, PP.

2.3.4. Membrane filter: 0.22 μm, PTFE.

2.4. Reagents

2.4.1. 50% methanol containing 0.5% formic acid

Dilute 5 mL of formic acid and 495 mL of deionized water with methanol to 1000 mL.

2.4.2. Methanol containing 1% formic acid

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

2.5. Mobile phase

2.5.1. Solvent A

Dissolve and dilute 0.04 g of ammonium acetate with deionized water to 500 mL. Add 0.5 mL of formic acid, mix well, and filter with a membrane filter.

2.5.2. Solvent B

Acetonitrile.

2.6. Standard solution preparation

Transfer equivalent to about 10 mg of paraquat reference standard accurately weighed to a 10-mL volumetric flask, dissolve and dilute to volume with methanol containing 1% formic acid as the standard stock solution. Store in a plastic vial under freezing in the dark. When to use, mix appropriate volume of the standard stock solution, and dilute with 50% methanol containing 0.5% formic acid to 1 μg/mL as the standard solution.

2.7. Sample solution preparation

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

Transfer about 4 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube. Add 6 mL of deionized water, and stand for 10 min. Add 10 mL of methanol containing 1% formic acid, shake at 1000 rpm by the high speed dispersing device for 5 min, and ultrasonicate at 80°C for 30 min. After cooling, shake at 1000 rpm by the high speed dispersing device for 1 min, centrifuge at 4500 ×g for 30 min at 15°C, and filter the supernatant with a membrane filter. Take the filtrate as the sample solution.

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

Transfer about 2 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube. Add 10 mL of deionized water, and stand for 10 min. Add 10 mL of methanol containing 1% formic acid, shake at

1000 rpm by the high speed dispersing device for 5 min, and ultrasonicate at 80°C for 30 min. After cooling, shake at 1000 rpm by the high speed dispersing device for 1 min, centrifuge at 4500 ×g for 30 min at 15°C, and filter the supernatant with a membrane filter. Take the filtrate 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 1 g of the homogenized sample accurately weighed into a 50-mL centrifuge tube. Add 10 mL of deionized water, and stand for 10 min. Add 10 mL of methanol containing 1% formic acid, shake at 1000 rpm by the high speed dispersing device for 5 min, and ultrasonicate at 80°C for 30 min. After cooling, shake at 1000 rpm by the high speed dispersing device for 1 min, centrifuge at 4500 ×g for 30 min at 15°C, and filter the supernatant with a membrane filter. Take the filtrate 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. Take several 1 mL of the supernatant, and evaporate to near dryness by gently flushing with a stream of nitrogen at 45°C. Separately add 2-200 μL of the standard solution, dilute with 50% methanol containing 0.5% formic acid to 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 paraquat by the peak areas vs. the added concentrations in the range of 0.002-0.2 μg/mL.

LC-MS/MS operating conditions(note)

Column: CAPCELL PAK ST, 2.0 μm, 2.0 mm i.d. × 15 cm.

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

Time (min)	A (%)	B (%)
0.0 → 5.0	30 → 90	70 → 10
5.0 → 5.1	90 → 90	10 → 10
5.1 → 7.0	90 → 30	10 → 70
10.0 → 13.0	30 → 30	70 → 70

Flow rate: 0.4 mL/min.

Injection volume: 5 μL.

Capillary voltage: 3 kV.

Ionization mode: ESI^+.

Ion source temperature: 150°C.

Desolvation temperature: 500°C.

Cone gas flow: 150 L/hr.

Desolvation flow rate: 1000 L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage and collision energy are shown as follows:

Analyte	Ion pair Precursor ion (*m/z*) > product ion (*m/z*)	Cone voltage (V)	Collision energy (eV)
Paraquat	171 > 77*	40	35
	171 > 155	40	35

*The quantitative ion pair.

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

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

Where,

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

V: the water content of the sample, and the volume of deionized water and methanol containing 1% formic acid to extract the sample (20 mL)

M: the weight of the sample (g)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative 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 paraquat is 0.01 ppm in type I samples, 0.02 ppm in rice,0.1 ppm in type II samples (except for rice), and 0.05 ppm in type III samples.
2. Further validation should be performed when interfering compounds are found in the samples.

Reference

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. 2019. 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 10.1. EURL SRM.

Reference chromatogram

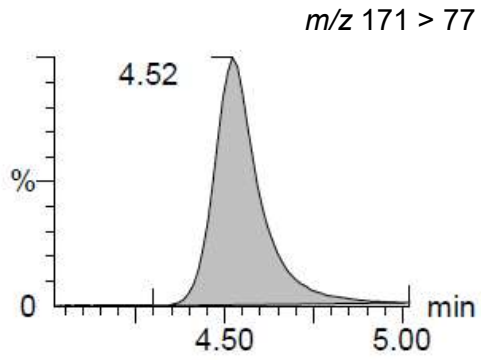

Figure. MRM chromatograms of paraquat standard analyzed by LC-MS/MS.