Published, OCT 13, 2023
TFDAP0027.00

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

1. Scope

This method is applicable for the determination of metaldehyde 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), metaldehyde 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: 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. Centrifuge: centrifugal force > 3000 ×g, temperature control <15℃.

2.1.6. Vortex-Mixer.

2.2. Chemicals

Glacial acetic acid, reagent grade;
Formic acid, reagent grade;
Acetonitrile, HPLC grade;
Methanol, HPLC grade;
Sodium acetate anhydrous, 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℃);
Metaldehyde, reference standard;
Triphenylphosphate (TPP), 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 and 1 g of sodium acetate anhydrous.

2.3.6. Clean-up centrifuge tube I(note 2): 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).

2.3.7. Clean-up centrifuge tube II(note 2): 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).

2.3.8. Clean-up centrifuge tube III(note 2): 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).

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. 1% Acetic acid in acetonitrile

Mix 10 mL of glacial acetic acid and 990 mL of acetonitrile.

2.5. Mobile phase

2.5.1. Solvent A

Take 1 mL of formic acid, dilute with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Take 1 mL of formic acid, dilute with acetonitrile to 1000 mL, and filter with a membrane filter.

2.6. Internal standard solution preparation

Transfer about 50 mg of triphenylphosphate internal standard accurately weighed into a 50-mL volumetric flask, dissolve and dilute to volume with methanol as the internal standard stock solution. Store under freezing 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 for 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 matrix-matched calibration curve preparation in section 2.9.

2.7. Standard solution preparation

Transfer about 25 mg of metaldehyde reference standard accurately weighed to a 25-mL volumetric flask, dissolve and dilute to volume with methanol as the standard stock solution. Store under freezing in the dark. When to use, dilute with methanol to 1 μg/mL as the standard solution.

2.8. Sample solution preparation

2.8.1. 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, and add 10 mL of 1% acetic acid in acetonitrile and 10 μL of 50 μg/mL internal standard solution after freezing. 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 1 min. Centrifuge at 3000 ×g for 1 min at 15℃, 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℃. Take 1 mL of the supernatant, and filter with a membrane filter as the sample solution.

2.8.2. 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, and add 10 mL of 1% acetic acid in acetonitrile 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 1 min. Centrifuge at 3000 ×g for 1 min at 15℃, 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℃. Take 1 mL of the supernatant, and filter with a membrane filter as the sample solution.

2.8.3. 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 of pre-cooled deionized water, stand for 20 min, and add 10 mL of 1% acetic acid in acetonitrile 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 1 min. Centrifuge at 3000 ×g for 1 min at 15℃, 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℃. Take 1 mL of the supernatant, and filter with a membrane filter 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 2-200 μL of 1 μg/mL the standard solution, 10 μL of 5 μg/mL the internal standard solution 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 metaldehyde by the ratios of the peak area to that of the internal standard vs. the added concentrations in the range of 0.002-0.2 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℃.
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 → 9.0	30 → 1	70 → 99
9.0 → 10.0	1 → 1	99 → 99
10.0 → 10.5	1 → 99	99 → 1
10.5 → 12.0	99 → 99	1 → 1

Flow rate: 0.35 mL/min.
Injection volume: 5 μL.
Interface voltage: 5 kV.
Ionization mode: ESI^+.
Interface temperature: 250℃.
Nebulizing gas flow: 3.0 L/min.
Heating gas flow: 15.0 L/min
DL temperature: 200℃.
Heat block temperature: 350℃.
Drying gas flow: 5 L/min.
Detection mode: multiple reaction monitoring (MRM). Detection ion pair, Q1/Q3 Pre Bias and collision voltage 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, and operate according to the conditions described in section 2.9. Identify metaldehyde based on the retention time and the relative ion intensities(note 4). Calculate the amount of metaldehyde in the sample using the following formula:

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

Where,

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

V: the volume of 1% acetic acid in acetonitrile for sample extraction (10 mL)

M: the weight of the sample (g)

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

Remark

1. Limits of quantification (LOQs) for metaldehyde are listed in the attached table.
2. Since the internal standard, triphenylphosphate (TPP), used in the procedure cannot represent the physicochemical property of metaldehyde, it is not necessary to use it in the formula to calculate the amount of metaldehyde in the sample. It can serve as quality control to confirm the procedure.
3. Further validation should be performed when interfering compounds appear in samples.

Reference

Lin, S. K., Huang, C. H., Shyu, T. H., Chuang, W. C. and Wang, S. Y. 2022. Research on development of testing methods for pesticide residues in agricultural, poultry and livestock products. Commissioned Research Report of Taiwan Food and Drug Administration.

Reference chromatogram

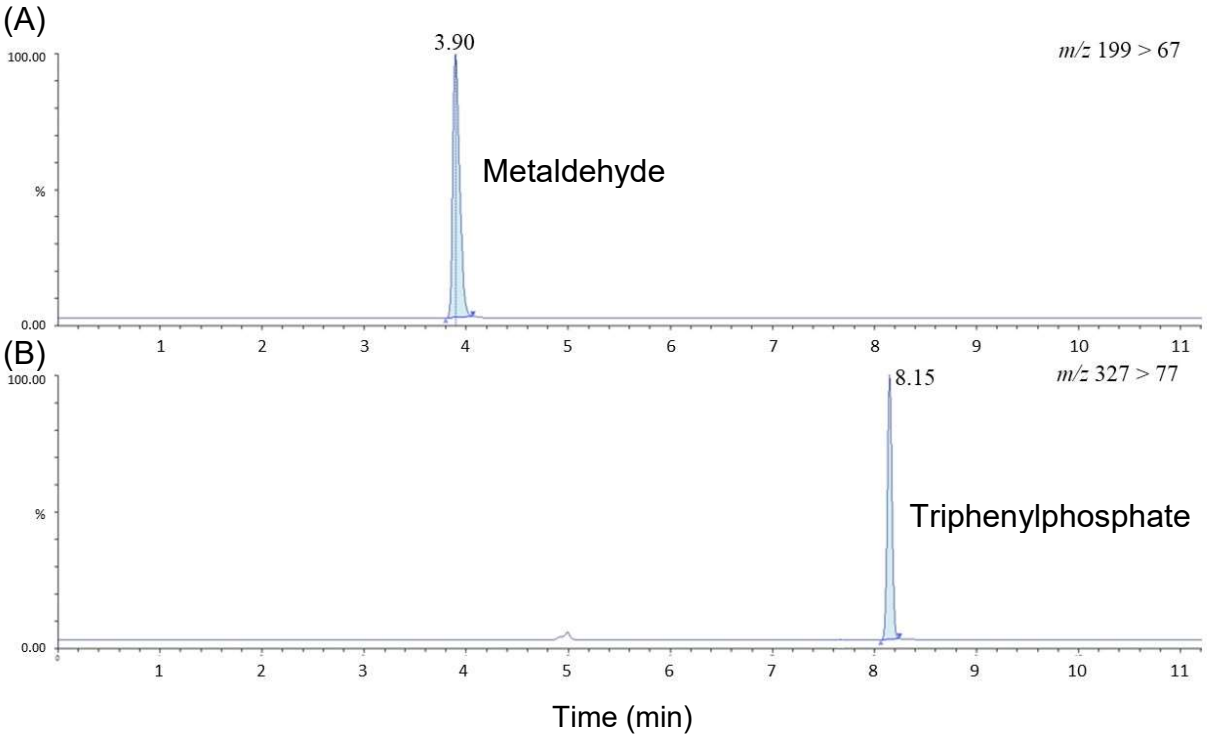

Figure. MRM chromatograms of metaldehyde standard (A) and the internal standard (TPP) (B) analyzed by LC-MS/MS.

Table. MRM parameters and LOQs of metaldehyde (positive ion mode)

Analyte	Ion pair			LOQ (ppm)		
	Precursor ion (*m/z*) > product ion (*m/z*)	Q1/Q3 Pre Bias (V)	Collision voltage (V)	Type I[a]	Type II[b]	Type III[c]
Metaldehyde	199 > 67*	10/11	13	0.01	0.02	0.1
	194 > 62	23/20	6			
	194 >106	21/10	6			
Triphenylphosphate (I.S.)	327 > 77*	26/13	45	—	—	—

[a]Applicable for fresh fruits, vegetables, spice plants and other herbs with high water content.

[b]Applicable for crops and dried beans with high wax, fat and sugar content.

[c]Applicable for dried tea, fruits, vegetables, spice plants and other herbs with high pigment content.

* The quantitative ion, and a qualitative ion can be selected based on the matrix condition.