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

1. Scope

This method is applicable for the determination of phosphine in dried fruits, vegetables, spice plants, nuts, crops, dried beans, cocoa beans, root and tuber vegetables.

2. Method

After pretreatment, phosphine is determined by gas chromatography/mass spectrometry (GC/MS) with headspace sampler (HS).

2.1. Equipment

2.1.1. Gas chromatograph/mass spectrometer.

2.1.1.1. Ion source: electron ionization, EI.

2.1.1.2. Column: HP-PLOT/Q+PT capillary column, 20 μm, 0.32 mm × 30 m, or an equivalent product.

2.1.1.3. Liner: Agilent P/N 5190-4047, ultra inert, straight, 1 mm, or an equivalent product.

2.1.2. Headspace sampler: XYZ robotic autosampler, shanking speed ≤ 750 rpm, temperature control ≥ 65℃.

2.1.3. Grinder.

2.1.4. Vortex mixer.

2.2. Chemicals

Sulfuric acid (98%), reagent grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);

Phosphine, 50 ppm (v/v) in nitrogen, reference standard gas cylinder.

2.3. Apparatus

2.3.1. Headspace vial: 20 mL, glass, with pre-assembled magnetic steel screw cap and PTFE/silicone septa.

2.3.2. Gas sampling bag: 1 L, polyvinyl fluoride (PVF), with PP valve.

2.3.3. Gas tight syringe: Hamilton® SampleLock syringe, 50 μL, 100 μL, 500 μL, 1 mL and 2.5 mL, or an equivalent product.

2.3.4. Stainless steel pressure reducing valve: flow rate 0.5 L/min.

2.3.5. Tubing: PTFE tubing, both ends are connected with Tygon tubing.

2.4. Reagents

2.4.1. 10% Sulfuric acid

Take 10 mL of sulfuric acid, slowly add to 75 mL of deionized water,

and dilute with deionized water to 100 mL.

2.4.2. 5% Sulfuric acid

Take 5 mL of sulfuric acid, slowly add to 80 mL of deionized water, and dilute with deionized water to 100 mL.

2.5. Standard gas preparation

Connect the stainless steel pressure reducing valve to the 50 ppm (v/v)(note1) phosphine reference standard gas cylinder, then use the tubing to connect the gas cylinder and the gas sampling bag, and turn on the stainless steel pressure reducing valve to a flow rate of 0.5 L/min for 2 min to prepare the standard gas. Prepare freshly before use.

Note 1: At 1 atm and 25℃, 1 L of phosphine gas has a mass of 1.39 g, so 50 ppm (v/v) of phosphine reference standard gas is equivalent to 69.5 ng/mL of phosphine standard gas.

2.6. Sample solution preparation

Homogenize the sample using dry ice. After the dry ice is completely sublimated, transfer about 1 g of the sample accurately weighed into a headspace vial, add 7.5 mL of deionized water, vortex-mix well to until particles are dispersed completely, add 7.5 mL of 10% sulfuric acid, and immediately close the cap as the sample solution.

2.7. Standard curve preparation

Add 15 mL of 5% sulfuric acid to each headspace vial, and close the cap. Remove 29-1450 µL of headspace air from the headspace vials using gas tight syringes, and then inject 29-1450 µL of the phosphine standard gas (69.5 ng/mL) from the gas sampling bag into the headspace vials as the standard solutions. Operate HS-GC/MS according to the following conditions. Establish the standard curve of phosphine by the peak areas of phosphine vs. the added concentrations (0.002-0.1 µg).

Headspace sampler operating conditions(note 2):

Incubation temperature: 65℃.

Incubation time: 20 min.

Shaking speed: 750 rpm.

Shaking interval: 60 sec followed by a 90 sec rest.

Syringe temperature: 70℃.

Injection volume: 0.5 mL.

GC/MS operating conditions[note 2]:

Column: HP-PLOT/Q+PT capillary column, 20 μm, 0.32 mm × 30 m.

Column temperature:

Initial temperature: 35℃, 3 min;

Temperature rising rate: 35℃/min;

Middle temperature: 70℃, 0 min ;

Temperature rising rate: 60℃/min;

Final temperature: 250℃, 3 min.

Carrier gas and flow rate: helium, 2.2 mL/min.

Injector temperature: 130℃.

Injection mode: split, 1:1.

Interface temperature: 240℃.

Ion source: electron ionization, 70 eV.

Ion source temperature: 230℃.

Detection mode: selected ion monitoring (SIM). Detection ions are shown as follows:

Analyte	Detection ion (*m/z*)
Phosphine	34*
	33
	31

*Quantitative ion.

Note 2: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.8. Identification

Place the headspace vials with the sample solution and the standard solutions on the headspace sampler, and operate according to the conditions described in section 2.7. Identify phosphine based on the retention time and the relative ion intensities[note 3].

Note 3: 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	± 10
> 20～50	± 15
> 10～20	± 20
≤ 10	± 50

2.9. Quantification

The standard addition method is used to quantify the amount of phosphine in the sample which is estimated by the standard curve. The sample which was found to contain phosphine from section 2.8 was prepared as the sample solution described in section 2.6(note 4). Remove 0-3 times of the volume of headspace gas which is equivalent to the estimated amount of phosphine in the sample from the headspace vials of the sample by gas tight syringes, then inject equal volume of the phosphine standard gas (69.5 ng/mL) from the gas sampling bag into each headspace vial of the sample separately, and operate according to the conditions described in section 2.7. Establish a linear regression curve (y = mx + n) by the peak areas of phosphine vs. the added concentrations. Calculate the amount of phosphine in the sample by the following formula:

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

Where,

C: the concentration of phosphine in the sample solution calculated from n/m (μg)

M: the weight of the sample (g)

Note 4: The weight of the sample for analyzing can be moderately reduced based on the estimated concentration of phosphine in the sample by the standard curve in section 2.8 to ensure that the measured value of phosphine in the sample is in the linear range.

Remark

1. Limit of quantification (LOQ) for phosphine is 0.005 ppm.
2. Further validation should be performed when interfering compounds are found in the samples.

Reference

1. European Union Reference Laboratory for Residues of Pesticides. 2014. Analysis of the fumigant phosphine applying headspace-GC-MSD. Methods for Individual SRM-Compounds (SRM-18).
2. Amrein, T. M., Ringier, L., Amstein, N., Clerc, L., Bernauer, S., Baumgartner, T., Roux, B., Stebler, T. and Niederer, M. 2014. Determination of phosphine in plant materials: method optimization and validation in interlaboratory comparison tests. J. Agric. Food Chem. 62: 2049-2055.
3. Perz, R., Benkenstein, A., Köbler H., Scherbaum, E., Köhl, D., Barth A. and Anastassiades, M. 2014. Analysis of phosphine in dried foodstuffs via headspace-GC-MSD. CVUA Stuttgart 02(May): 1-17.
4. European Union Reference Laboratory for Residues of Pesticides. 2017. Workflow to perform quantification by standard addition procedure. Single Residue Methods.

Reference chromatogram

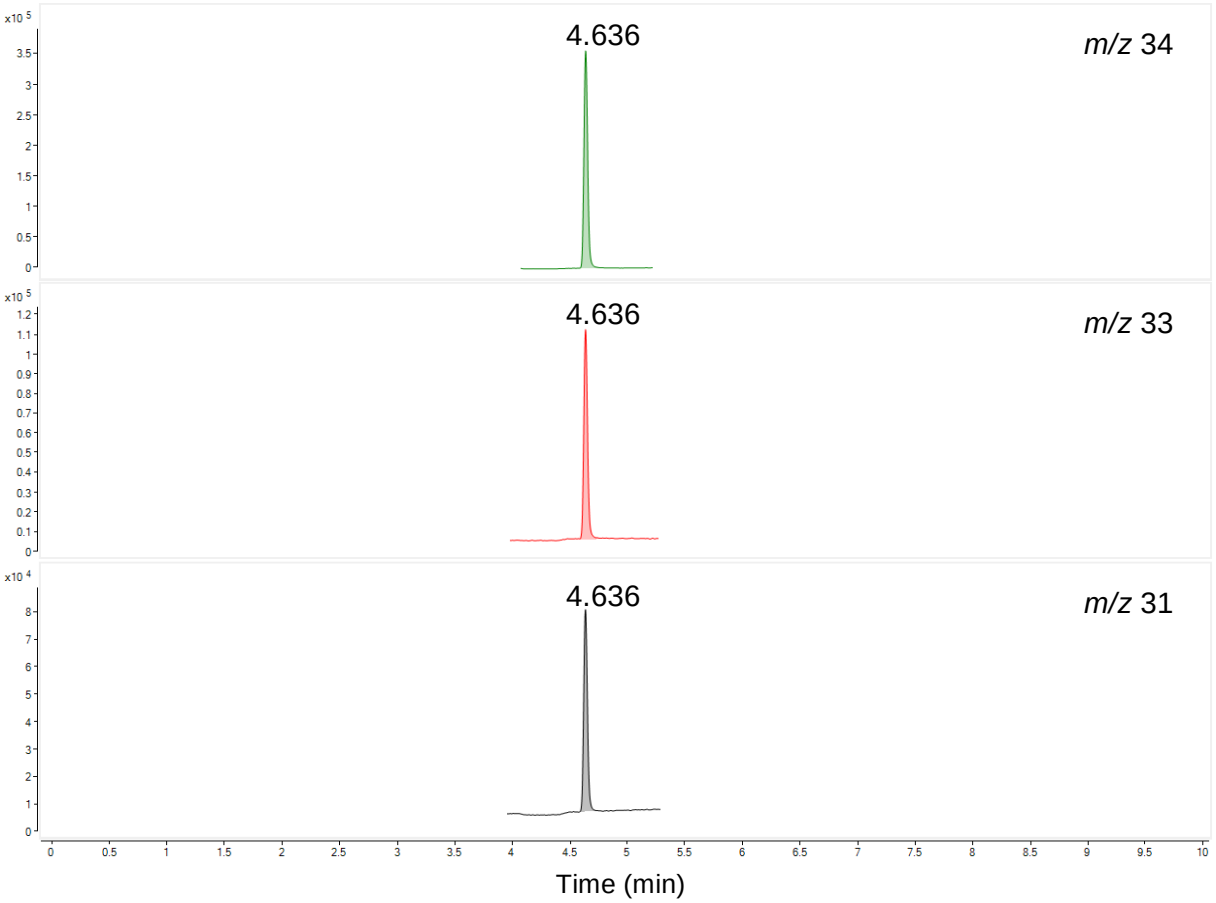

Figure. SIM chromatograms of phosphine standard gas analyzed by GC/MS.