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Method development for the determination of phosphine residues in foods

Shu-Wei Lin, Yu-Ting Liu, Ya-Chun Chou*, Yu-Ching Hung, Shu-Han Chang, Ya-Min Kao, Su-Hsiang Tseng, Der-Yuan Wang

Division of Research and Analysis, Taiwan Food and Drug Administration, Ministry of Health and Welfare, No.161-2, Kunyang St, Nangang District, Taipei City 11561, Taiwan, ROC

Abstract

Phosphine (PH_3) is a fumigant used for pest control of stored products and foods. In Taiwan, PH_3 has the maximum residue level (MRL) in 14 foods, including dried fruits, vegetables, spices, nuts, crops, roots, and tuber vegetables. In this study, gas chromatography/mass spectrometry coupled with a headspace sampler (HS-GC/MS) was used to determine the PH_3 content in foods. The stability of the PH_3 standard was evaluated either directly using the gas standard or indirectly using zinc phosphide (Zn_3P_2) with an acid. The optimal conditions for the headspace agitator were determined to be an incubation temperature of 65 °C, rotation speed of 750 rpm, and shaking time of 20 min. The PH_3 residue in the sample was sufficiently released when 15 mL of 5% sulfuric acid was added. The PH_3 gas standard was applicable for quantification because the correlation coefficients of the standard and matrix-matched calibration curves were greater than 0.99, and the coefficient of variation of the repeatability test was less than 20%. The signal-to-noise ratio was greater than 3 when PH_3 was fortified into the testing matrix at 5 ng/g; therefore, the limit of quantification of PH_3 was determined to be 0.005 ppm. Because of the significant matrix effects and difficulty in obtaining representative matrices, the developed method referred to the EU Reference Laboratory-single residue method to estimate the PH_3 content in the sample using a standard curve and the PH_3 content was accurately quantified using the standard addition method. The proposed method was used to analyze 44 real samples obtained from markets and food factories, including various food commodities. Among these samples, three showed detectable PH_3 residues, with concentrations ranging from 0.006 to 0.066 ppm, which complies with the MRLs in Taiwan. This study successfully utilized HS-GC/MS with a gaseous PH_3 reference standard to develop a facile sample preparation method for detecting PH_3 in foods.

Keywords: Fumigant, GC/MS, Headspace sampler, Phosphine

1. Introduction

Methyl bromide, formerly an extensively utilized fumigant, is subject to restrictions under the Montreal Protocol on Substances that Deplete the Ozone Layer because of its potential to deteriorated the ozone layer. Currently, it is exempt from use in a few quarantine and pest control applications [1]. Methyl bromide has since been replaced by phosphine (PH_3), which is equally effective for agricultural pest control. PH_3 is a fumigant used globally for the pest control of both stored products and processed foods. It has been authorized for application in various food storage systems and is commonly used in cereals, dried fruits, spices, nuts, pulses, and oil seeds. Despite the rapid diffusion and high volatility of PH_3, its residues persist in foodstuffs after fumigation, even when applied at the recommended rates [2]. The Taiwan Food and Drug Administration (TFDA) has established the maximum residue limit (MRL) for PH_3 in 14 types of food. The MRL for PH_3 residues originates from the use of aluminum, magnesium, and zinc phosphides or the direct use of gaseous PH_3 [3]. In particular, the PH_3 must be used as follows: According to the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ), PH_3 can only be used as a fumigant in designated warehouses, cargo containers, rice barrels, tents, and BAPHIQ-approved facilities. Metal phosphides are stable crystals formed under dry conditions. Upon

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* Corresponding author.
E-mail addresses: cyc0516@fda.gov.tw, dreamy0516@gmail.com (Y.-C. Chou).

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exposure to atmospheric moisture, aluminum, magnesium, and zinc phosphides readily react with water, gradually releasing gaseous PH_3 that disperses within the warehouse and exterminates pests [4]. This procedure must be performed in accordance with the "Operating guidelines for phosphine fumigants," and the warehouse must be properly ventilated thereafter [5].

PH_3 is a colorless and highly toxic flammable gas with a molecular weight of 33.99 g/mol, boiling point of −87.8 °C, and specific gravity of 1.185 (at 20 °C). At an ambient concentration of 0.3 ppm, PH_3 has a fishy or garlicky odor. In addition, it may cause poisoning in humans upon skin or eye contact, inhalation, or consumption. The 4-h LC_{50} for PH_3 in rats was determined to be 11 ppm, with inhalation being the primary route of exposure. PH_3 inhalation may cause drowsiness, lethargy, poor coordination, central inhibition, headaches, vertigo, asthma, breathing difficulties, and may be lethal in severe cases [6]. PH_3 is a neuroactive insecticide that enters the body of insects via their spiracles. It affects the physiological mechanisms of insects according the following mechanisms: In terms of neural and behavioral aspects, PH_3 binds to acetylcholinesterase in the synapses of the insect nervous system, inhibiting the hydrolysis of neurotransmitters, such as acetylcholine. In the cytoplasm, PH_3 binds to glycerophosphate dehydrogenase, preventing the conversion of dihydroxyacetone phosphate to glycerol-3-phosphate, which facilitates lipid metabolism. In the mitochondria, PH_3 binds to cytochrome oxidase, disrupting the electron transport chain, and thereby impeding the energy supply within the insect body [7]. Because humans and animals are also susceptible to these effects, PH_3 should be handled carefully [8].

To monitor the MRLs and improper application of PH_3, an appropriate testing method for PH_3 is required. Currently, the most commonly used techniques for determining PH_3 residues in foodstuffs include confirmatory colorimetric methods and headspace gas chromatography [9]. The colorimetric method involves the combination of orthophosphate and $(NH_4)2MoO_4$ under acidic conditions to form $(NH_4)3PO_4 \cdot 12MoO_3$, which is a yellow precipitate that can be filtered and quantified by gravimetric analysis. However, when the phosphate content is below 30 μg/mL, a color reaction is initiated by adding hydrazine sulfate ($N_2H_6SO_4$). The sample preparation procedure for the colorimetric method is complicated and time-intensive (approximately 9 h) [9].

In this study, gas chromatography/mass spectrometry coupled with a headspace sampler (HS-GC/MS) is used to determine the PH_3 content in foods. The sample preparation procedure is based on a previous study [10–12]. In addition, this study focuses on optimizing the headspace agitator parameters, including the incubation temperature, rotation speed, and shaking time. Furthermore, this study evaluates the stability of a PH_3 standard for the first time, either directly from a gas standard or indirectly from zinc phosphide (Zn_3P_2) with an acid. Rice, oats, dried corn, fresh onions, raisin, dried cabbage, almond, Sichuan pepper, chili powder, sesame, and cocoa are used as representative food matrices for testing. This PH_3-testing method is developed for border and post-market surveillance to ensure food safety in Taiwan.

2. Materials and methods

2.1. Reagents and chemicals

Steel cylinders (116 L) containing 50 ppm (*v/v*) PH_3 in nitrogen were obtained from PortaGas (Pasadena, CA, USA). To convert the volume to weight, each liter of analytical standard PH_3 gas was assumed to be 1.39 g (1 atm, 25 °C). Thus, 50 ppm (v/v) PH_3 was assumed to be equal to 69.5 ng/mL of PH_3. Analytical-grade propylene glycol, toluene, benzene, isopropanol, 1,3-dimethyl-2-imidazolidinone, and zinc phosphide (Zn_3P_2, 87.047%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Analytical grade silica gel was purchased from the SiliCycle Company (Quebec, Canada). Ultrapure water (18.2 MΩ cm^{-1}) was deionized using a Milli-Q SP Advantage A10 system (Millipore, Bedford, MA, USA). Reagent-grade sulfuric acid (98%) was obtained from J. T. Baker (Phillipsburg, NJ, USA). The deionized water used in the experiments had a specific resistance of greater than 18 MΩ cm at 25 °C.

2.2. Equipment and materials

Glass headspace vials (20 mL, P/N 5188-2753) and magnetic steel screw caps with polytetrafluoroethylene (PTFE)/silicone septa (P/N 5188-2759) were purchased from Agilent Technologies (Santa Clara, CA, USA). Polyvinyl fluoride gas sampling bags (1 L) with polypropylene connectors and switching valves were obtained from SKC (Blandford Forum, Dorset, UK). Gastight SampleLock syringes of various sizes (50 μL, 100 μL, 500 μL, 1 mL, and 2.5 mL) were purchased from Hamilton® (Reno, NV, USA). A stainless steel pressure-reducing valve (flow rate = 0.5 L/min) was obtained from PortaGas (Pasadena, CA, USA). PTFE tubing, with both ends connected to Tygon tubing, was obtained from Hsing Sheng (New Taipei City, Taiwan). A Blixer (3.7 L, P/N GETPP3100)

suitable for operation at 3000 rpm was obtained from Robot Coupe® (Paris, France).

2.3. Sampling and sample preparation

Rice, oat, dried corn, fresh onion, raisin, dried cabbage, almond, Sichuan pepper, chili powder, black pepper, millet, adlay, sesame, barley, quinoa, taro, jujube, soy bean, cocoa bean, and coffee bean samples were collected from supermarkets, hypermarkets, traditional markets, organic food vendors, and food factories in Taiwan and stored in freezers before use. Based on the sample preparation method developed by the EU Reference Laboratory-single residue method (EURL-SRM) [10], the optimal extraction process was as follows: Each test sample was initially homogenized using the Robot Coupe® Blixer (Paris, France) with dry ice cooling. After complete sublimation of the dry ice, 1 g of the homogenized sample powder was accurately weighed in a headspace vial. Subsequently, deionized water (7.5 mL) was added and the mixture was vortexed thoroughly until the particles were completely dispersed. Next, 10% sulfuric acid (7.5 mL) was added to obtain a final sulfuric acid concentration of 5%. Finally, all vials were immediately sealed for analysis.

2.4. Headspace GC/MS analysis

A PAL RTC 120 headspace sampler (HS) with a three-dimensional (XYZ) robotic autosampler capable of a shanking speed of $\leq$ 750 rpm and temperature control of $\geq$ 65 °C was purchased from Industriestrasse (Zwingen, Switzerland). Table 1 lists the HS specifications. A gas chromatography/mass spectrometer (GC/MS) equipped with an Agilent Intuvo 9000 GC and a 7000D mass selective detector (MSD) was used with electron ionization (EI) as the ion source. The MSD was operated in the selected ion monitoring (SIM) mode with an EI energy of 70 eV and detected ions at *m/z* values of 34, 33, and 31. The QQQ quantitative analysis software obtained from Agilent Technologies was used. An HP-PLOT/Q + PT capillary column (20 μm, 0.32 mm × 30 m) and liner (P/N 5190-4047, ultra inert, straight, 1 mm) were also obtained from Agilent Technologies. Table 2 summarizes the operating conditions used for the GC–MS analyses.

Table 1. Analytical conditions for the headspace sampler.

Parameter	Condition
Incubation temperature	65 °C
Incubation time	20 min
Shaking speed	750 rpm
Shaking interval	60 s followed by a 90 s break
Syringe temperature	70 °C

2.5. Calibration curve

The gas cylinder containing 50 ppm (*v/v*) PH_3 in nitrogen was used as the reference standard. The cylinder was connected to a stainless-steel flow control valve, which was connected to a gas-sampling bag using the PTFE tubing. PH_3 was added to the gas sampling bag at 0.5 L/min for 2 min. Fresh standards were prepared before use. A standard curve was established by adding 15 mL of the 5% sulfuric acid to the headspace vial and tightly screwing the cap. Subsequently, 29–1450 μL of headspace air was removed from the headspace vials using gastight syringes, and then 29–1450 μL of the PH_3 standard gas (69.5 ng/mL) in the gas sampling bag was injected into the headspace vials to prepare the calibration curve standards with a linear range from 2 to 100 ng. Matrix-matched calibration was performed using the blank samples prepared as described in Section 2.3. Similarly,

Table 2. Analytical conditions for the gas chromatograph/mass spectrometer.

Parameter	Condition			
GC column	HP-PLOT/Q + PT, 30 m × 0.32 mm × 20 μm			
Flow gas	Helium			
Gradient program		Rate (°C/min)	Value (°C)	Hold time (min)
	Initial	–	35	3
	Ramp 1	35	70	0
	Ramp 2	60	250	3
Flow rate	2.2 mL/min			
Injection volume	0.5 mL			
Injection temperature	130 °C			
Inject mode	Split 1:1			
Interface temperature	240 °C (MSD transfer line temperature)			
Ionization temperature	230 °C			
Ionization mode	EI, 70 eV			
Detection mode	SIM, detection ions are 34, 33 and 31 *m/z*			

29–1450 μL of headspace air was removed from the headspace vials using gastight syringes, and then 29–1450 μL of the PH_3 standard gas (69.5 ng/mL) in the gas sampling bag was injected into the headspace vials containing the blank samples. The matrix-matched calibration for PH_3 ranged from 2 to 100 ng. Finally, 1 mL of gas was injected into the GC/MS system for analysis under the conditions described in Section 2.4. Matrix-matched calibration and standard curves (0.002–0.100 μg) were established by plotting the peak area of PH_3 and its corresponding concentration.

2.6. Identification and quantification of phosphine

PH_3 was identified based on retention time and relative ion intensities. When the signal intensity of the positive samples exceeded the lowest concentration value (0.002 μg/vial) on the standard curve, the PH_3 content has been estimated. For accurate quantification, the samples were reanalyzed using the standard addition method, which was performed as follows: Between zero and three times the headspace gas volume, which is equivalent to the estimated amount of PH_3 in the sample in the headspace vials prepared using gas-tight syringes, was removed. Subsequently, an equal volume of the PH_3 standard gas (69.5 ng/mL) in the gas sampling bag was injected into each headspace vials containing the samples. A linear regression curve ($y = mx + n$) was constructed by plotting the PH_3 peak areas against the added concentration. Subsequently, the amount of PH_3 in the samples is determined as follows: The amount of PH_3 in the sample (ppm) = C/M, where C is the concentration of PH_3 in the sample solution calculated from n/m (μg) and M is the weight of the sample (g).

2.7. Repeatability test and matrix effect

Repeatability tests were conducted using sesame (blank) and PH_3-fumigated brown sesame (1 g) according to established methods. The mean, coefficient of variation (CV), and relative percent difference (RPD) of the quantitative results were calculated. The matrix effect was calculated using the slopes of the standard and matrix-matched calibration curves: (formula)

$$\text{Matrix effect}\,(\%) = \frac{\text{matrix-matched calibration curve slope - standard curve slope}}{\text{standard curve slope}} \times 100$$

3. Results and discussion

3.1. Selection of reference standards

In previous studies, either a zinc phosphide (Zn_3P_2) standard solution or gaseous PH_3 were commonly used as the reference standard for testing [9–12]. In this study, deionized water, propylene glycol, toluene, benzene, isopropanol, 1,3-dimethyl-2-imidazolidinone, and silica gel were used to prepare the phosphide standard solutions [12–14]. With the exception of silica gel, a magnetic stirrer was needed to prepare the standard solutions. The standard solutions prepared using propylene glycol demonstrated superior stability compared to the other solvents and dispersion media, with a CV of 18.5% at a concentration of 20 ng/vial (n = 5). During the repeated analyses, the standard solutions prepared using the other solvents exhibited excessively high CV values and the linear correlation coefficient (r) values of the standard curves were less than 0.99 (Table 3). This may be attributed to the insolubility of zinc phosphide in various solvents. Unfortunately, the stability of the phosphide standard solutions was inadequate for dissolving or suspending zinc phosphide in various solvents and dispersion media. Therefore, a gaseous PH_3 standard was tested using the EURL-SRM. The equivalent volume of air in the headspace vial was removed according to the EURL-SRM before the PH_3 gas standard was added [10,11]. A stability assessment of the PH_3 gas standard

Table 3. Stability testing of the zinc phosphide standard solution in various solvents and dispersion media.

Solvents and dispersing media	Peak area (n = 5)	
	Average	CV (%)
Deionized water	23129	69.6
Propylene glycol	12797	18.5
Toluene	4410	35.8
Benzene	10337	132.1
Isopropanol	21655	72.5
1,3-Dimethyl-2-Imidazolidinone	18015	33.7
Silica gel	87209	32.4

revealed excellent repeatability and reproducibility with a CV of 5.13% at 3 ng/vial. Therefore, for subsequent method development, gaseous PH_3 was selected as the reference standard.

3.2. Development of a sample preparation procedure

In this study, the concentrations and added volumes of sulfuric acid were evaluated according to a study based on the EURL-SRM [10]. As shown in Fig. 1, at a sulfuric acid concentration of 0%, PH_3 was not released from the test samples. When the sulfuric acid concentration was increased to 5%, PH_3 was released. However, sulfuric acid is a strong oxidant at a concentration of 10%, which causes PH_3 to be over-oxidized, resulting in a decrease in the analytical signal. To accommodate the selected headspace autosampler, 20 mL headspace vials were used. Generally, at a fixed sample weight (g), PH_3 becomes concentrated with an increasing volume of sulfuric acid because of the smaller headspace in the vial, resulting in an enhanced analytical signal and improved peak separation of PH_3 from the noise signals. Consequently, 15 mL of 5% sulfuric acid solution was used in this study. Notably, cocoa contains hydrophobic substances, such as lipids (54.7%) and crude fibers (2.1%). Therefore, the direct addition of homogenized cocoa powder to a 5% sulfuric acid solution may cause the formation of a hydrophobic film at the gas–liquid interface. In this case, the cocoa bean powder could not be adequately mixed with the sulfuric acid solution, leading to an incomplete release of PH_3 from the test sample during the reaction in the headspace autosampler. Therefore, in this study, a cocoa test sample was prepared by mixing the cocoa bean

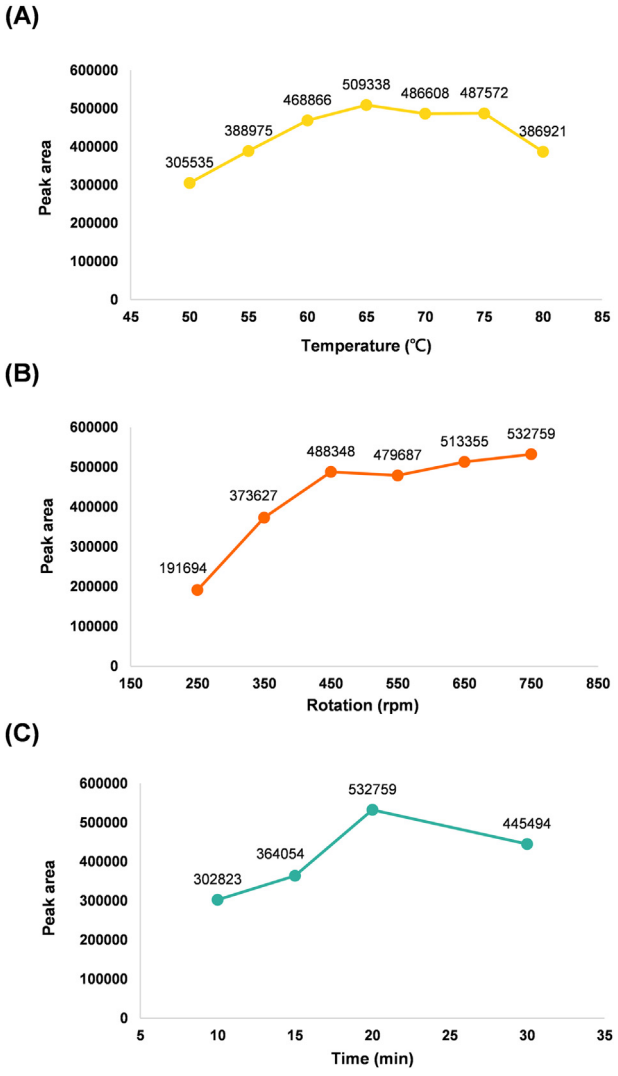

Fig. 2. Testing of the incubation temperature (A), rotation speed (B), and shaking time (C) of the headspace sampler.

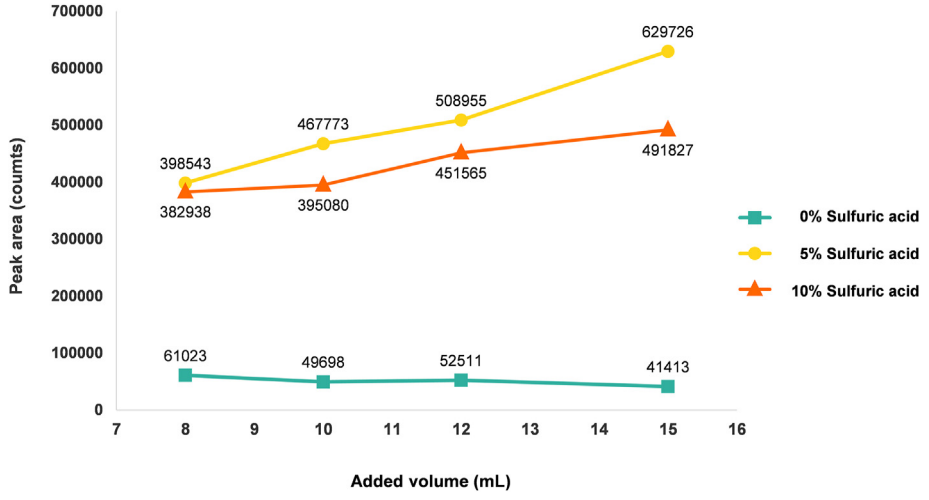

Fig. 1. The pretreatment process for phosphine analysis using different concentrations and volumes of sulfuric acid.

powder with deionized water (7.5 mL), vortexing until the particles were dispersed, adding 7.5 mL of 10% sulfuric acid, and quickly sealing the vial for analysis.

3.3. Headspace GC/MS parameter optimization

Fig. 2 shows the results of the evaluation of the incubation temperature, rotation speed, and shaking time of the headspace agitator. At a temperature of 65 °C, the largest amount of PH_3 was released. However, when the temperature exceeded 65 °C or decreased to room temperature, the analytical signal of PH_3 weakened, presumably because of an inadequate reaction or overoxidation of PH_3. The largest amount of PH_3 was released after shaking for 20 min. However, at a shaking time of 30 min, the analytical PH_3 signal was considerably weakened. At high rotation speeds, the rate of PH_3 release increased. According to the analytical method developed by the EURL, test samples must be analyzed within 4 h of the addition of the sulfuric acid solution to prevent PH_3 degradation [10,11]. In this study, real samples of black sesame and brown samples were simultaneously added to a sulfuric acid solution for analysis. The PH_3 peak area decreased over time, from acid addition to the analysis. The peak area of PH_3 decreased from approximately 49.4%–40.8% when the analysis was conducted 4 h after acid addition (Fig. 3).

3.4. Matrix effect and limit of quantitation

This study selected representative matrices for evaluating the matrix effect according to the "Standards for pesticide residue limits in foods" [3]. The selected matrices included rice (rice MRL: 0.1 ppm), oat (cereals and crops MRL: 0.1 ppm), dried corn (corn MRL: 0.1 ppm), white sesame, black sesame, fresh onion (bulb vegetables MRL: 0.1 ppm), dried cabbage (vegetables (dry) MRL: 0.01 ppm), raisin (fruits (dry) MRL: 0.01 ppm), Sichuan pepper (spices MRL: 0.01 ppm), chili powder (spices MRL: 0.01 ppm), cocoa bean (cocoa bean MRL: 0.01 ppm), and almond (tree nuts MRL: 0.01 ppm). The slopes of the standard- and matrix-matched calibration curves were used to evaluate the matrix effects. Both the standard and matrix-matched calibration curves of the 12 matrices exhibited a good linearity with correlation coefficient (r) values of >0.99, in accordance with the validation specifications of the TFDA [15]. The matrix effect of white rice was 5.22%, whereas those of the other matrices ranged from −20.12% to −83.50%, indicating their suppression effects (Table 4). Due to these suppression effects, the calibration curves of 0.002–0.1 μg/vial is suitable for screening purposes. When 0.005 ng/vial PH_3 was added to the aforementioned matrices, the analytical signals were easily distinguished from the noise signals. Therefore, the limit of quantification (LOQ) of PH_3 for the developed method was determined to be 0.005 ppm, which achieves the requirements for

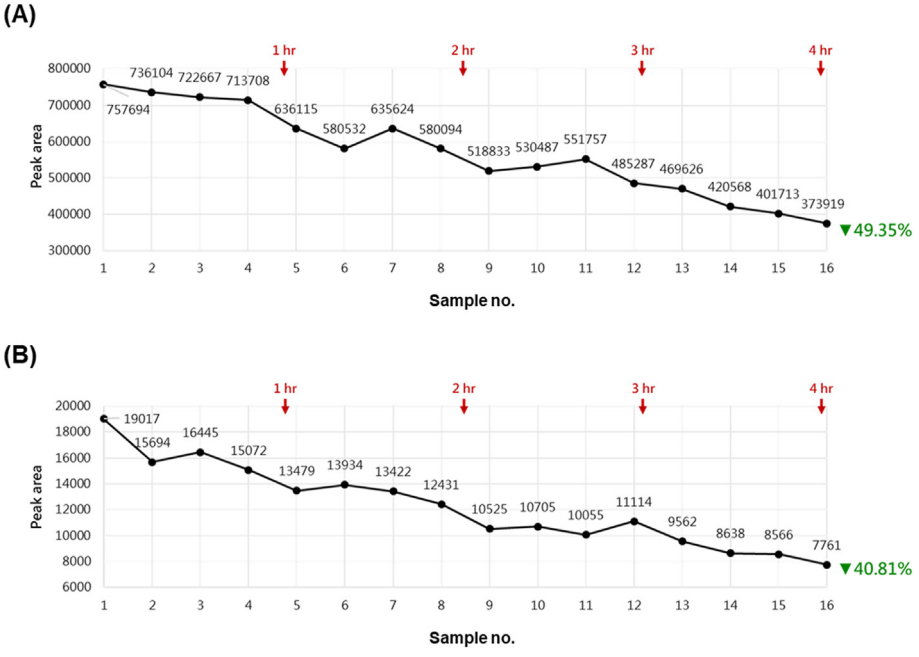

Fig. 3. Variations in the phosphine content in black sesame (A) and brown sesame (B) after sulfuric acid treatment for 4 h.

Table 4. Linear correlation coefficients and matrix effects of phosphine in 12 types of food matrices.

Matrix	r	Matrix effect (%)
Standard curve	0.9929	–
Rice	0.9986	5.22
Oat	0.9996	−20.12
Dried corn	0.9949	−21.42
Almond	0.9965	−26.18
White sesame	0.9985	−30.51
Fresh onion	0.9985	−39.37
Black sesame	0.9994	−44.17
Raisin	0.9979	−49.16
Dried cabbage	0.9976	−53.04
Chili powder	0.9989	−65.04
Sichuan pepper	0.9985	−75.13
Cocoa	0.9941	−83.50

regulatory compliance (the MRLs for PH_3 in foodstuffs range from 0.01 to 0.1 mg kg^{-1} in Taiwan) [3].

3.5. Method validation

Because PH_3 is a naturally gaseous compound, common recovery experiments are unsuitable [16]. Therefore, in this study, a validation test was conducted using brown sesame, which is a real sample containing PH_3. Both the matrix-matched calibration curve and standard addition methods were used to evaluate the accuracy and precision of the proposed method. In the matrix-matched calibration curve method, the black sesame was used as a representative matrix to establish a matrix-matched calibration curve. The intraday and interday quantification results for brown sesame were 7.6 and 6.9 ng/g, respectively, both with a CV of 17.0%. In the standard addition method, a standard curve was used to estimate the PH_3 content of the test samples at a concentration of 2.5 ng/g. Subsequently, the standard addition method was used to determine the PH_3 content. The intraday and interday quantification results for brown sesame were 3.6 and 4.3 ng/g, respectively, with a mean of 4.0 ng/g and an RPD of 17.2%. Therefore, the two methods yielded slightly different quantification results. Considering the wide variety, complexity, considerable matrix effects, and challenges in selecting representative food matrices, we adopted the approach referenced by the EURL for PH_3 quantification [10]. A standard curve was constructed to estimate the PH_3 content of the test samples, followed by precise quantification using the standard addition method.

3.6. Analysis of real samples

To determine the PH_3 content using the proposed method, 44 samples of rice, barley, cereals, cocoa beans, dried beans, spice herbs, root vegetables, and dried fruits were collected from various locations in Taiwan, including department stores, supermarkets, convenience stores, and food factories. Among the 44 samples, three contained detectable PH_3 residues. The PH_3 concentrations in rice and barley ranged from 0.006 to 0.066 ppm (Table 5). Notably,

Table 5. Determination of the phosphine contents in commercially available products.

Commodity	Country of origin	No. Samples	No. Positive Samples	MRL category	MRLs (ppm)	Result (ppm)
Rice	Taiwan	15	1	Rice	0.1	0.066
Black rice	Unspecified (import)	1	1	Rice	0.1	0.006
Barley	Unspecified	1	1	Barley	0.1	0.014
Millet	USA	1	0	Cereals	0.1	N. D.
Adlay	Laos	1	0	Cereals	0.1	N. D.
Quinoa	Unspecified	1	0	Cereals	0.1	N. D.
Taro	Unspecified	1	0	Root vegetables	0.1	N. D.
Cocoa bean	Taiwan	8	0	Cocoa beans	0.01	N. D.
Cocoa bean	Vietnam	1	0	Cocoa beans	0.01	N. D.
Cocoa bean	Unspecified	1	0	Cocoa beans	0.01	N. D.
Black pepper	India	1	0	Spice herbs	0.01	N. D.
Black pepper	Belgium	1	0	Spice herbs	0.01	N. D.
Black pepper	Spain	1	0	Spice herbs	0.01	N. D.
Sichuan pepper	Unspecified (import)	1	0	Spice herbs	0.01	N. D.
Jujube	China	1	0	Dried fruits	0.01	N. D.
Sesame	Taiwan	2	0	Dried beans	–	N. D.
Sesame	Japan	1	0	Dried beans	–	N. D.
Soy bean	Australia	1	0	Dried beans	–	N. D.
Soy bean	Unspecified (import)	1	0	Dried beans	–	N. D.
Black bean	China	1	0	Dried beans	–	N. D.
Coffee bean	Guatemala	1	0	–	–	N. D.
Coffee bean	Africa	1	0	–	–	N. D.

N. D. = Not Detected.

all the detected residue levels in rice and barley complied with the MRLs in Taiwan (0.1 ppm). To ensure food safety, continued surveillance programs for monitoring PH_3 residues in foodstuffs are essential.

4. Conclusion

This study referred to the EURL-SRM and utilized headspace GC–MS with a gaseous PH_3 reference standard to develop a facile sample preparation method for detecting PH_3 in food samples. The test samples were homogenized by dry ice cooling, followed by the addition of 15 mL of 5% sulfuric acid after the sublimation of the dry ice. In this study, the optimal conditions for the headspace agitator were determined to ensure the complete release of PH_3 from the samples. The LOQ of this method was determined to be 0.005 ppm. Additionally, the samples were analyzed immediately after the addition of sulfuric acid to prevent PH_3 degradation. To quantify PH_3, the concentration in the food sample was first estimated using a standard curve, followed by precise quantification using the standard addition method. The proposed method was published by the TFDA, and can serve as a reference for regular border inspections and post-market surveillance to safeguard consumer health.

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Conflict of interest

The authors declare no conflict of interest.

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