Gas Chromatography−Mass Spectrometry Determination of Phosphine Residues in Stored Products and Processed Foods

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A gas chromatography−mass spectrometry (GC-MS) method was used for the quantitative confirmation of phosphine residues in stored products and processed foods. An established extraction technique was utilized for the preparation of headspace samples, which were analyzed by GC-MS and gas chromatography−nitrogen−phosphorus detection (GC-NPD). Wheat, oats, maize, white rice, brown rice, cornflakes, tortilla cornchips, groundnuts, and raisins were validated, showing excellent agreement between detectors when spiked at levels equivalent to 0.001 and 0.01 mg/kg phosphine and for samples containing incurred residues. The GC-MS method was reproducible and accurate when compared to the GC-NPD method and allowed five samples to be quantified in a working day. Subambient GC-MS oven temperatures were most suitable for phosphine residues ranging from 0.001 to 0.005 mg/kg, and a GC oven temperature of 100 °C was appropriate for residues >0.005 mg/kg. The method was sufficiently robust to be evaluated for other similar commodities as the need arises.

Keywords: *Phosphine; headspace analysis; gas chromatography−mass spectrometry (GC-MS); stored products; processed foods*

INTRODUCTION

Phosphine is used worldwide to disinfest stored products and processed foods. Phosphine is approved for use in many food storage situations and is typically applied to cereals, nuts, dried fruit, pulses, oilseeds, and dried animal products. Although phosphine diffuses rapidly and is extremely volatile, residues persist in food commodities (Anonymous, 1998) following fumigation using recommended application rates. The U.K. maximum residue level (Anonymous, 1994) permitted in cereals is 0.1 mg/kg, and a CODEX guideline level of 0.01 mg/kg exists for dried fruits, tree nuts, groundnuts, bread, cooked cereal products, and cooked cocoa products.

Detection methods for determining phosphine residues in foodstuffs utilize colorimetry (Rangaswamy, 1984), electrochemical conductivity detection (Carlson and Thompson, 1998), nitrogen−phosphorus detection (Scudamore and Goodship, 1986), and flame photometric detection (Nowicki, 1978). These methods of detection are robust and sensitive and can quantify phosphine residues as low as 0.001 mg/kg. Such methods are selective, but the possibility of obtaining false positives exists. Mass spectrometric detection can provide unequivocal quantitative confirmation of phosphine residues. A few published methods have used mass spectrometry to determine phosphine, but none describe the determination of phosphine residues in food commodities. Mass spectrometry was used to detect phosphine (Halmann and Klein, 1964), and GC-MS was used to determine phosphine as an impurity in other gases such as silane (de Saint Etienne and Mettes, 1989).

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This paper describes a determinative GC-MS procedure based on an established vacuum distillation extraction method (Scudamore and Goodship, 1986) using manual headspace sampling. A subambient GC column oven temperature was required to meet a quantitation limit of 0.001 mg/kg, which is required for surveillance data provided for the Working Party on Pesticide Residues. A comparison between GC-MS and GC-NPD was carried out with spiked commodities, and samples containing incurred phosphine residues, to evaluate the performance of the GC-MS method.

EXPERIMENTAL PROCEDURES

Reagents. A cylinder of phosphine in nitrogen (0.934 μg/mL) was purchased from BOC Gases (Guildford, U.K.).

Caution: Phosphine is highly toxic.

Preparation of Standard and Calibration Curves. A calibrated, nominally 1 L, round-bottom flask containing a Teflon-coated spin-stirrer was sealed with a Teflon-coated silicone septum in a Quickfit adapter. A steady flow of gas was set up through a nylon capillary tube ($^1/_8$ in.) from a calibrated cylinder (Scudamore and Goodship, 1986), containing phosphine in nitrogen (0.934 μg/mL). Gas (1 mL) was slowly withdrawn from the capillary tube using a gastight syringe fitted with a Teflon-tipped plunger. Two aliquots were discarded, and the third 1 mL aliquot was injected through the Teflon-coated silicone septum into the 1 L flask, the contents of which were stirred for 20 min until homogenization. Fresh standards were prepared daily.

Calibration curves were prepared by injecting 10−100 μL of the phosphine standard onto the GC-NPD and 20−500 μL onto the GC-MS. The syringe needle was cleaned with a wire following each injection. Each detector was calibrated with five levels of standard using duplicate injections for the GC-NPD and a single injection for the GC-MS. Duplicate injections of samples and spiked samples were bracketed by complete sets of calibration standards. Phosphine recoveries and residue levels were calculated from the average of the bracketed standards.

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Extraction and Spiking of Samples. A subsample (40 g) of the whole unprocessed commodity was vacuum distilled with dilute sulfuric acid (100 mL, 10% v/v) and the resulting sample vapor collected in a calibrated, nominally 1 L, collection flask (Scudamore and Goodship, 1986). The previously described procedure was modified by sealing ground glass joints using Teflon sleeves and replacing glass taps with Rotaflo Teflon taps. The contents of the collection flask were homogenized with a Teflon-coated magnetic spin-stirrer for 20 min.

Samples shown to be free of phosphine residues were used for spiked recovery checks. The extraction apparatus was spiked, prior to the addition of dilute sulfuric acid, with 44 or 440 μL of phosphine gas in nitrogen (0.934 μg/mL), equivalent to about 0.001 and 0.01 mg/kg of phosphine residue in a sample. Two replicates of each spike level were extracted, and two replicate injections were made of each spike level.

Gas Chromatography. Gastight syringes with Teflon-tipped plungers were used to sample vapor for determination by GC-MS (500 μL) and GC-NPD (100 μL). Two replicates of each spike level were extracted and analyzed in duplicate by both detection systems. The syringe needle was cleaned with a wire following each injection.

Analyses using GC-NPD were carried out on a Pye Unicam PU4550 gas chromatograph (Cambridge, U.K.). Manual injections were made into a flash vaporization glass liner at 175 °C. The detector was at 250 °C. A 30 m × 530 μm i.d. J&W GS-Q capillary column was used with helium carrier gas at a constant flow of ~11 mL/min. The oven was used isothermally at 50 °C. A signal-to-noise ratio of 10 was obtained for samples (40 g) containing a residue of 0.001 mg/kg. The detector response was found to be linear over the range of 3−83 pg of phosphine.

Analyses using GC-MS were carried out on a Hewlett-Packard 5890 series II gas chromatograph fitted with a Hewlett-Packard 5972 mass selective detector (Palo Alto, CA). Manual injections were made into a flash vaporization glass liner (4 mm i.d.) at 150 °C in split mode with a split flow of 4 mL/min, and the detector transfer line was at 300 °C. A 30 m × 250 μm i.d. J&W GS-Q capillary column was used with helium carrier gas at a constant flow of ~0.9 mL/min set by electronic pressure control. A length (30 cm × 250 μm i.d.) of J&W DB-624 column was connected to the end of the GS-Q column using a press-fit connector and passed through the heated transfer line to the MS. The initial oven temperature was held at −20 °C for 1 min and programmed to 100 °C at 50 °C/min, held for 1.7 min, and then raised from 100 to 150 °C at 50 °C/min, and held for 14 min. The MS was used with an electron ionization at 70 eV in selective ion monitoring (SIM) mode. Following a 3 min solvent delay, three ions were collected during a dwell time of 140 ms: *m*/*z* 31 (30), 33 (30), and 34 (100). Ion *m*/*z* 32 was not collected because of interference from oxygen. A signal-to-noise ratio of 5 was obtained for samples (40 g) containing a residue of 0.001 mg/kg using *m*/*z* 34. The detector response was found to be linear over the range of 15−410 pg of phosphine.

RESULTS AND DISCUSSION

Development of the GC Method for Mass Spectrometric Detection. Porous layer open tubular (PLOT) columns containing GS-Q were found to exhibit the necessary selectivity to resolve phosphine from air using GC oven temperatures above ambient. A quantitation limit of 0.001 mg/kg phosphine was possible for NPD analysis using a GS-Q column and was desirable for MS analysis. Although PLOT columns tend to cause peak broadening, effectively lowering the signal-to-noise ratio for a given amount of analyte, the MS was less sensitive than the NPD and it was only possible to determine phosphine residues to 0.005 mg/kg using a column oven temperature of 100 °C. Subambient column oven temperatures offer the potential to focus analyte bandwidth, giving narrower peaks and increasing signal-to-noise ratios. GC oven temperatures of −30, −20, and −10 °C were evaluated. An initial GC oven temperature of −30 °C gave an extrapolated limit of detection (LOD) for a signal-to-noise ratio equal to 4 of 0.0006 mg/kg, −20 °C gave an extrapolated LOD of 0.0007 mg/kg, whereas −10 °C gave an extrapolated LOD of 0.0009 mg/kg phosphine. An initial column oven temperature of −20 °C was selected to minimize the GC oven cycle time, reduce cryogenic nitrogen consumption, and allow for day-to-day variation in the MS response to ensure the quantitation limit was possible. Random peaks, which were narrow for their retention times, were present in many chromatograms, but the ions did not correspond with those of phosphine. It is likely they were particles from the porous polymer of the GS-Q column. The incorporation of a length of DB-624 column in the heated transfer line prior to the MS minimized this phenomenum. Injections were made in the split mode with a minimum split flow of 4 mL/min and were suitable for injection sizes between 20 and 500 μL.

Table 1. Relative Measurement of Phosphine Spike Levels Determined by GC-MS and GC-NPD

commodity	spike level (mg/kg)	relative measurement by GC-MS/GC-NPD
wheat	0.001	0.82, 0.99
	0.010	1.01, 1.11
oats	0.001	1.12, 1.28
	0.010	1.22, 1.02
maize	0.001	0.95, 1.06
	0.011	1.01, 0.95
white rice	0.001	1.28, 0.89
	0.010	0.97, 0.90
brown rice	0.001	1.06, 0.82
	0.010	1.04, 0.92
cornflakes	0.001	0.93, 0.91
	0.010	0.80, 0.81
tortilla cornchips	0.001	0.91, 0.89
	0.010	0.99, 0.90
groundnuts	0.001	0.97, 0.86
	0.010	0.85, 0.77
raisins	0.001	1.01, 1.05
	0.010	0.94, 0.92

Method Validation. Ideally, the accuracy of the GC-MS method would be assessed using certified reference materials. Unfortunately, these do not exist for such a volatile analyte as phosphine, and therefore comparison of GC-MS and GC-NPD determinations from spiked samples and determinations of incurred phosphine residues from a variety of field samples were used. Each sample extract was analyzed using both GC-MS and GC-NPD. This gave a direct comparison of detector performance. Any errors associated with subsampling or sample preparation did not affect the comparison as the contents of the calibrated 1 L collection flask were homogeneous. Samples were spiked at levels equivalent to the quantitation limit of 0.001 mg/kg and 10 times this level.

Table 1 shows the relative phosphine spike levels determined using GC-MS and GC-NPD. A ratio of 1.00 represents a perfect agreement between detector systems. Only four results were outside the range of 0.80−1.20, which shows good agreement between detectors for each commodity and both spike levels. The largest differences between detector systems were with two of the spiked oats subsamples, but the other two subsamples gave good agreement at each spike level. Ratios of 0.77 and 1.28 were observed for groundnuts spiked at the high level and white rice spiked at the low level, respectively. These may be random errors as these data

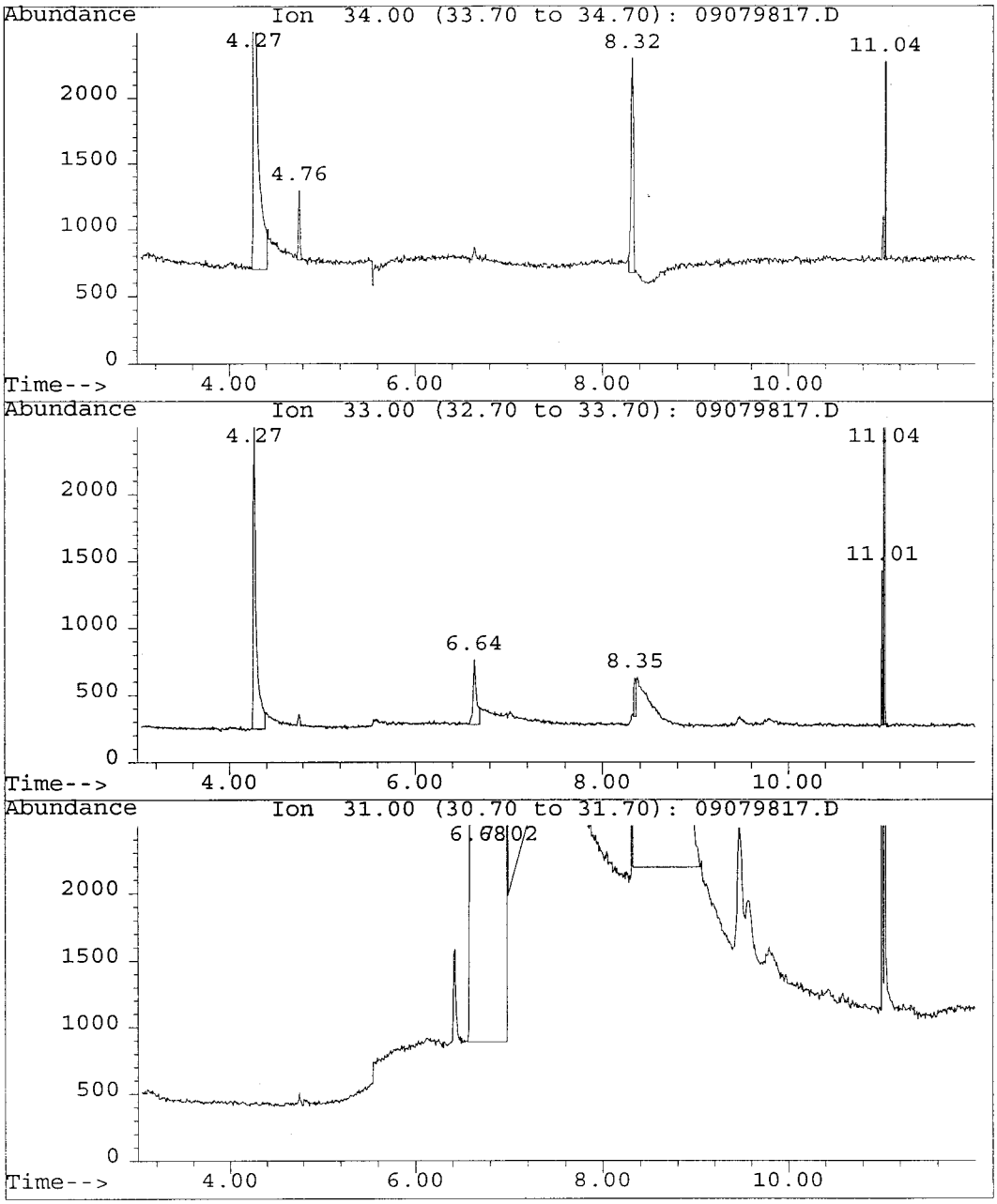

Figure 1. GC-MS ion chromatograms of a brown rice control sample using a subambient GC oven temperature.

do not suggest any systematic difference between detectors. The performance of the GC-MS using the subambient GC oven temperature was satisfactory under the conditions used and gave the necessary sensitivity to meet the required quantitation limit. Figure 1 shows ion chromatograms of a brown rice control sample, and Figure 2 shows ion chromatograms of brown rice spiked with phosphine (retention time = 3.97 min) equivalent to a residue level of 0.01 mg/kg.

Wheat, oats, maize, white rice, brown rice, cornflakes, tortilla cornchips, groundnuts, and raisins previously screened using the GC-NPD method and found not to contain phosphine residues were analyzed using the GC-MS. No peaks were found for any of these commodities at a retention time corresponding to phosphine for any of the three ions collected. Random narrow peaks (retention times = 11.01 and 11.04 min, Figure 1) were probably particles from the GS-Q column and contained all three ions.

The injection repeatability was assessed by analyzing eight aliquots of phosphine standards of 15.7 and 393 pg, as well as a spiked tortilla chip sample with a nominal residue level of 0.001 mg/kg and a spiked pistachio nut sample with a nominal residue level of 0.01 mg/kg. Relative standard deviations (RSD) of 13.7 and 3.4%, respectively, were obtained for the phosphine standards and of 5.6 and 3.7%, respectively, for the spiked samples. The RSD obtained for the spiked samples were excellent and showed there were no precision problems due to these commodities.

Analysis of Incurred Residues. Wheat, maize, brown rice, pistachio nuts, and barley known to contain incurred residues of phosphine were extracted and analyzed using the GC-MS and GC-NPD (Table 2). Residue levels ranged from 0.001 to 0.093 mg/kg determined using GC-MS and from 0.001 to 0.094 mg/kg determined using GC-NPD. A difference of 20% was noted for the high wheat residue (0.067 and 0.084 mg/

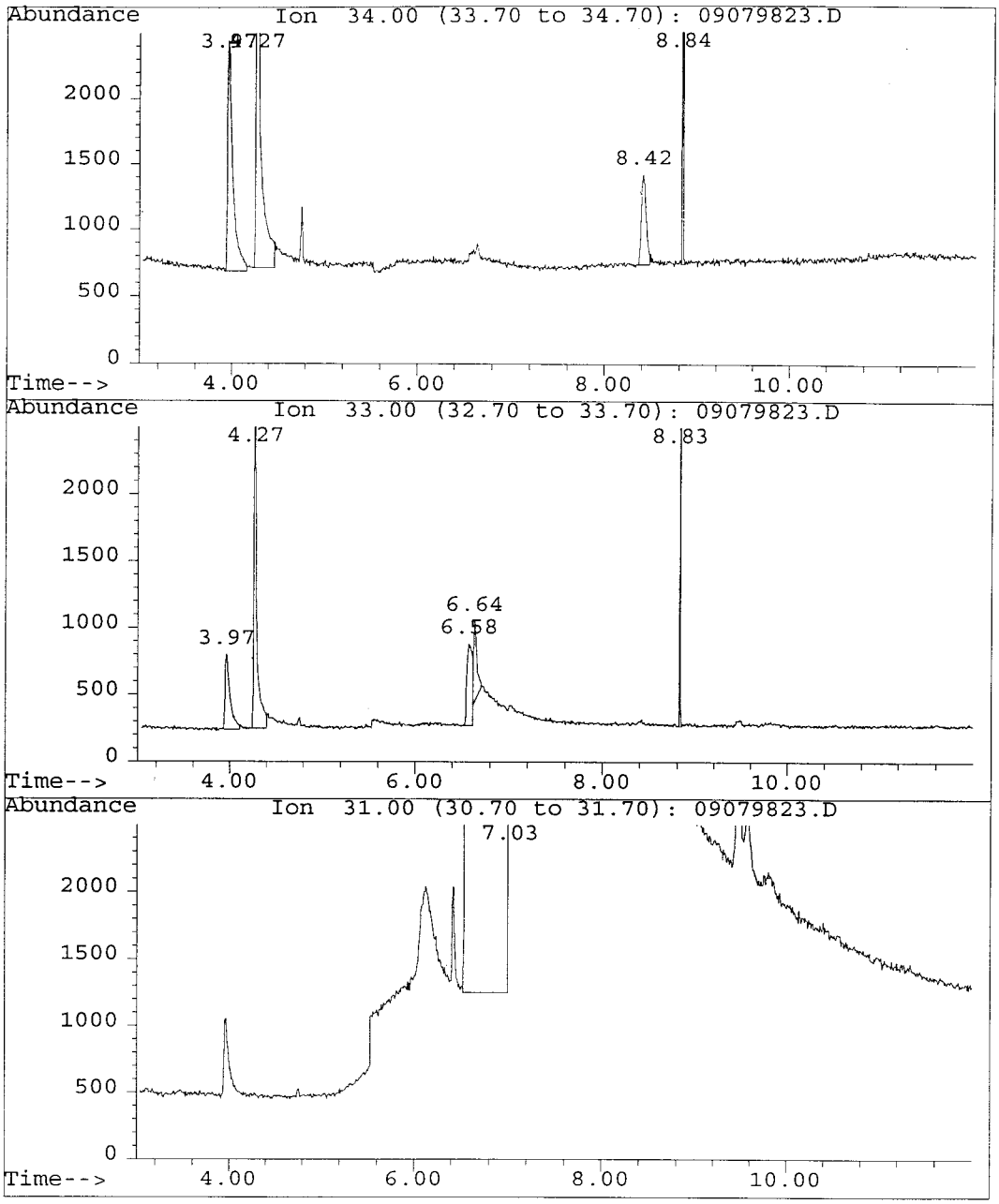

Figure 2. GC-MS ion chromatograms of brown rice spiked with phosphine (3.97 min) equivalent to a residue level 0.01 mg/kg using a subambient GC oven temperature.

Table 2. Incurred Phosphine Residues Determined by GC-MS and GC-NPD

commodity	GC-MS (mg/kg)	GC-NPD (mg/kg)
wheat	0.005	0.006
	0.067*	0.084
maize	0.019*	0.017
	0.093*	0.094
brown rice	0.045*	0.051
pistachio nuts	0.004	0.004
barley	0.001	0.001

* A 100 μL injection was used for GC-MS.

kg), which was likely to be a random error and not a function of the injection volume used (100 μL) for the GC-MS analysis, as other high-level residues such as maize used the same volume and gave excellent agreement with the GC-NPD. Excellent agreement between detectors was obtained for maize, brown rice, pistachio nuts, and barley, particularly for residues in the 0.001−0.02 mg/kg range.

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