Evaluation of headspace Solid Phase Micro-extraction method for analysis of phosphine residues in wheat

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Abstract

This new method utilizes headspace-solid-phase micro extraction (HS-SPME) for pre-concentration of PH_3. Phosphine was determined with gas chromatography/pulsed flame photometric detector (PFPD). Spiked samples were used for calculation of phosphine residue in grain. Four types of fibres (100µm-PDMS, 85µm-CAR/PDMS, 75µm-CAR/PDMS and 65µm-PDMS/DVB) were tested. The bipolar fibres (CAR/PDMS and PDMS/DVB) can extract PH_3, but the non-polar fibre (PDMS) did not. Larger size fibres extracted PH_3 more efficiently than the smaller size fibres (e.g., 85 µm > 75 µm > 65 µm). The 85µm CAR/PDMS fibre was used to optimize the different parameters that affect the SPME extraction efficiency of PH_3. In the validation study, 50 grams of wheat in a 250 mL glass flask and capped with an open-top screw cap and PTFE/Silicon septa were spiked at 0.02 ng PH_3/g of wheat. The flask was then heated to 45°C in an oil bath for 45 min, after which time the 85 µm CAR/PDMS fibre was exposed for 20 min and then exposed in the heated injection port of a GC/PFPD and desorbed for 2 min. Under conditions of the validation study, the limit of detection (LOD) or level of quantification (LOQ) was in the range of 0.005–0.01 ng PH_3/g of wheat.

Keywords: Fumigant, Phosphine, Residue, SPME, HS-SPME

1. Introduction

The world grain industry relies heavily on chemicals for grain treatment. Currently, phosphine (PH_3) is the only fumigant available to treat bulk grains and oil seeds (more than 85% grains are treated/re-treated with PH_3) in each of the linkages from on-farm storage to central storage (Collins et al., 2000; Collins et al., 2002; Ren and Mahon, 2007). However, a restrictive Codex Maximum Residue Limit (MRL) of 0.1 mg/kg, and lower limits set by some purchasers, are challenging the use of PH_3 for treatment of grain. Phosphine residues can remain in the grain for several months. Multi-fumigation with PH_3 can also cause accumulation of residues in the grain exceeding the MRLs. It is also important to note that levels of PH_3 residues in grain, producing low aerial concentrations of PH_3, can also facilitate resistance. Therefore, it is necessary to understand PH_3 residues in bulk grain to establish better procedures for multi-fumigation. This will guide industry in the conduct of good PH_3 fumigation practice to minimise PH_3 residue in fumigated grain. These include application methods and choice of the right periods of exposure and airing.

Typical procedures for analysis of PH_3 residues include: 1) removal from the commodity matrix by either purge and trap techniques or by solvent extraction (Miyahara and Saito, 1994), and 2) and then analysis by gas chromatography (GC) or gas chromatography-mass spectra (GC-MS). However, purge and trap methods are not suitable for highly volatile fumigants such as PH_3 as they are unable to trap all fumigant (Ren and Desmarchelier, 1998). Solvent extraction has the problem of solvent interference and is time-consuming. None of the above methods are suitable for analysis of PH_3 residues in grains, particularly at very low levels. Microwave irradiation is being increasingly used in the digestion of samples (Ren, 2001; Desmarchelier et al., 1998), and, in recent work (Chemfate 2004), excellent recoveries and precision have been obtained from microwave extractions of PH_3 from wheat and hay (Ren and Mahon, 2007). However, care is required in selecting the appropriate power setting and the safety implications of heating sealed flasks in microwave ovens is of concern, and water vapour generated from microwave irradiation can significantly interfere with GC results (Ren and Mahon, 2007).

Solid-phase microextraction (SPME) in combination with head space (SH-SPME) analysis by gas chromatography is a convenient alternative method for volatiles. Solid-phase micro extraction is a simple, sensitive, and solvent-free technique that has become popular in a wide range of applications (Penalver et al., 2001). It has been used to study pollutant gases and volatile degradation products (Lattuai-Derieux, et al., 2004). For example residues of methyl isothiocyante (MITC) were analysed by HS-SPME with the 85 µm Polyacrylate (PA) fibre (Ren et al., 2008a). Using the HS-SPME method, MITC residues from wheat can be successfully analysed at levels below 0.1 ng/g, compared with purge and trap methods where the limit of detection is 10 ng/g (Ren et al., 2008a,b). This paper describes a new method for analysis of PH_3 residues in wheat which utilises headspace-solid-phase micro extraction (HS-SPME). It includes selection of the SPME fibre and factors that affect the extraction efficacy of PH_3.

2. Materials and methods

2.1. Reagents and apparatus

One litre Erlenmeyer flasks (Bibby Sterilin, Staffordshire, Cat. No. FE 1 L/3) were used for fumigation of wheat and preparation of standards. The measured volume of each Erlenmeyer flask and inlet system was calculated from the weight of water required to fill the container and was used for calculations. Bottles of 250 mL (Alltech Cat. No. 9535) were used for the microwave "extraction". Each bottle was fitted with a Mininert valve equipped with septa (Alltech Cat. No. 95326). A 100 µL air tight syringe with valve (SGE, Melbourne, Australia; Cat. No 005279) was used for GOW-MAC gas density balance injection and transfer of PH_3 from source to fumigation chambers and flasks.

The SPME fibres used were coated with 85 µm Carboxen/Polydimethylsiloxane (CAR/PDMS) fibre (Sigma-Aldrich Australia, Cat. 57334-U), 75 µm Carboxen/Polydimethylsiloxane (CAR/PDMS) fibre (Sigma-Aldrich Australia, Cat. 57344-U), 65µm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fibre (Sigma-Aldrich Australia, Cat. 57310-U) and 100 µm Polydimethylsiloxane (PDMS) fibre (Sigma-Aldrich Australia, Cat. 57301), respectively. All fibres were conditioned at 270°C for 1 h prior to use in accordance with manufacturers' recommendations.

Phosphine (85.0% PH_3 and 15.0% air and CO_2) was laboratory prepared by the FAO method (FAO, 1975). The purity of PH_3 was determined using a GOW-MAC gas density balance (GOW-MAC Instrument Co., Madison, N.J.) after separation of the gases on a 1 m × 5 mm i.d. Porapak Q 100/120 mesh (Alltech Associates, Cat. No. 2702) column at 105°C with a carrier flow (N_2) of 150 mL/min. The reference gas used was tetrafluoroethane (> 99.9%), which was supplied by ACTROL Ltd, Australia.

Phosphine (PH_3) was determined on a Varian CP-3800 (Varian Instruments, Sunnyvale, CA), equipped with a pulsed flame photometric detector (PFPD) with phosphorus filter. Separation was achieved on a 30 m × 0.53 mm ID, AT-Q column (Alltech Associates, Cat. No. 0810025, Part No. 13939) at 125°C and carrier flow (N_2) of 5.0 mL/min at 5.0 psi. Injector and detector temperatures were 200°C. Injection volumes of gases were 40 µL. A minimum interval of 5 min was kept between injections, in order to elute interfering chemicals.

2.2. Wheat sample and fumigation of wheat

Wheat used was Australian standard white wheat, 10.9% moisture content, w/w wet basis. The moisture content of the wheat was measured by oven drying at 105°C for 2 hours (ISO 712 International Standard 1998). In order to ensure the moisture result is precise, 500 kg wheat sample was quartered equally using a Boerner grain divider. One quarter sample (125 g) was quartered equally again and then one quarter sample of about 30 g was collected and ground with a laboratory sealed metal grinder for measurement of moisture content. Four replicates samples were dried at 105°C in mechanical convection oven (DK 62 American Scientific Products Columbus, OH, USA) and the loss of weight was used to calculate the moisture content of the sample.

Wheat samples (860 g) were fumigated in an Erlenmeyer flask (1 L) equipped with a lid fitted a septum injection system for 7 days at concentrations of 0.5 mg (PH_3)/L. After 7 days exposure, the flask was opened and aired for 7 days in a fume hood to obtain samples containing residual fumigant.

2.3. Preparation of diluted PH_3 gas and spiking standards

Diluted PH_3 gases were prepared by first removing the same volume of air as the known volume (0.5 mL) of concentrated PH_3 to be injected into an Erlenmeyer flask (1 L) containing six glass beads (2-3 mm OD). For validation study, the limit of detection (LOD) or level of quantification (LOQ) was investigated using spiked standards. Spiked wheat samples at and 0.02 and 0.5 ng PH_3/g of wheat were prepared by adding appropriate volumes of PH_3 into sealed flasks (250 mL) containing wheat (50 g) 3 min before oil bath heating. Triplicate samples were used and each sample was injected duplicates (*n=6*). The dosages and required volumes for PH_3 concentrations, calibrated to the current laboratory temperature and pressure, were calculated from Eq. 1

$$\mathbf{V_f} = (1 - \frac{T}{273})\,(\frac{1.7 \times 10^4 \times C \times V}{P \times M \times N}) \quad \text{Eq. 1}$$

Where: ***V*** is volume of fumigation container (L); ***P*** is pressure (mm Hg); ***T*** is temperature (°C); ***C*** is the intended concentration of methyl bromide (mg/L); $\mathbf{V_f}$ is dosage volume of fumigant (mL); ***M*** is molecule weight of fumigant; ***N*** is purity of gas (%).

2.4. Selection of fibre and HS-SPME extraction time

Four different types of fibres (100 µm-PDMS, 85 µm-CAR/PDMS, 75 µm-CAR/PDMS and 65 µm-PDMS/DVB) were tested at 20 min extraction time. Diluted PH_3 gas was injected into a 250 mL flask fitted with a sample port to obtain 0.3, 0.1, 0.05 and 0.01 ppm of PH_3, respectively. Headspace samplings were carried out on all four types of fibres. The needle was carefully inserted into the headspace of the flask and the fibre exposed into the headspace for 20 min. At the end of the defined extraction time, the fibre was withdrawn from the headspace into the needle. The fibre holder was removed from the extraction flask and inserted into the injection port. The fibre was extended into a GC-PFPD inlet where sample components were desorbed at 200°C for 5 min to clean it between extractions.

For evaluation of HS-SPME extraction time on the efficacy of PH_3 extraction, the SPME equilibration time was determined by exposing the fibres (85 µm-CAR/PDMS, 75 µm-CAR/PDMS *and* 65 µm-PDMS/DVB) to 0.3 ppm of PH_3 for 1, 5, 10, 15, 20 and 30 min, respectively. Finally the fibre was retracted and then exposed in the heated injection port (200°C) of a GC-PFPD and desorbed for 2 min. Results are the mean of duplicate samples and injections for each sample (*n=4*).

2.5. Evaluation of GC Injector temperature for desorption of PH_3 from fibre (85µm CAR/PDMS)

Efficient thermal desorption of the analysis in a GC injection port is dependent on the type of fibres and on the GC injector temperature. The 85 µm CAR/PDMS fibre was used to optimize the injector temperature. It was inserted into the headspace of the flask containing 0.1 ppm of PH_3 and exposed for 20 min. The fibre was carefully injected into the GC at different injector temperatures (100,150, 200 and 250°C) and desorbed for 5 min. Results are the mean of duplicate samples and injections for each sample (*n=4*).

2.6. Evaluation of wheat treatment and HS-SPME extraction temperature for extraction efficacy of PH_3

A fumigated wheat sample of 50 g in a 250 mL flask fitted with a sample port was immersed in a heated oil bath at 30°C for 30 min, after which time the fibre was inserted into the headspace of the flask and exposed for 20 min. Equivalent flasks were heated a 35, 40, 45 and 50°C. The fibre was injected (200°C) into a GC-PFPD and desorbed for 5 min. Spiked wheat samples were treated in exactly the same manner. Levels of PH_3 residue were determined against spiked standards. The peak areas were calibrated periodically using a fortified standard, and the data presented are the mean of duplicate samples and injections for each sample (*n=4*).

2.7. Statistical analysis

The GC readings of fumigant concentration were averaged respectively. The average variation of GC readings and PH_3 concentration and standard deviations (SD) between the duplicate treatments and injections were analysed with Microsoft Excel.

3. Result and discussion

3.1. Effect of fibre types and HS-SPME extraction time on extraction efficacy of PH_3

The amounts of PH_3 absorbed by each of four different SPME fibres are shown in Figure 1 for each of four PH_3 concentrations. These four fibres showed different efficiency and selectivity in extracting PH_3 in the headspace. The bipolar fibres (CAR/PDMS and PDMS/DVB) which dispersed solid adsorbents in polymer extracted PH_3, but the nonpolar fibres (PDMS) extracted no PH_3. The larger size of polymer fibre (e.g., 85 µm) extracted PH_3 more efficiently than the smaller size of polymer fibre (e.g., 75 µm and 65 µm).

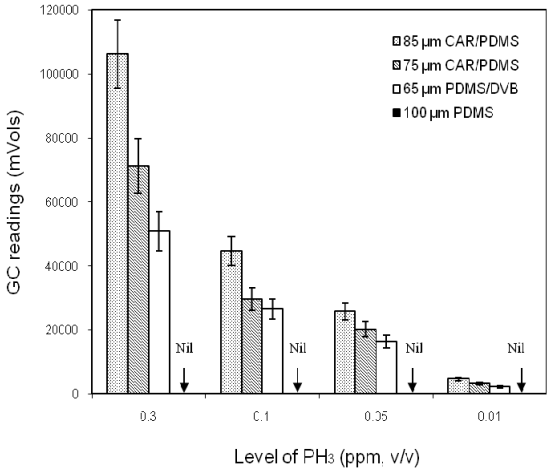

Figure1 Fibre types (100 µm PDMS, 85 µm CAR/PDMS, 75 µm CAR/PDMS and 65 µm PDMS/DVB) affect the extraction efficacy of PH_3 after 20 minutes extraction.

The effect of time of extraction of PH_3 is shown in Figure 2. The responses of GC peak areas progressively increased with increasing the extraction time, but no increase in the response occurred after 20 min. That is, the amount of PH_3 absorbed by the tested fibres increased over a period of 20 min and then attained equilibrium.

After 30 min extraction, the amount of absorption was 85 µm CAR/PDMS > 75 µm CAR/PDMS > 65 µm PDMS/DVB. The 85 µm CAR/PDMS fibre was used in this study to optimize the different parameters that affect the SPME extraction efficiency of PH_3.

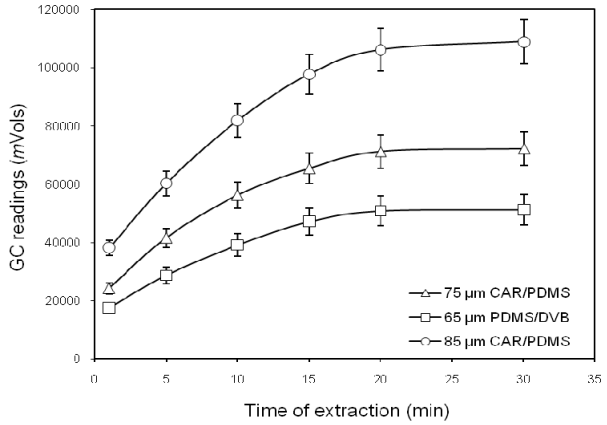

Figure 2 Time of extraction with different type of fibres (85 µm CAR/PDMS, 75 µm CAR/PDMS and 65 µm PDMS/DVB) affects the extraction efficacy of PH_3 (0.3 ppm) at 1, 5, 10, 15, 20 and 30 min.

3.2. Effect of GC Injector temperature for desorption of PH_3 from fibre (85 µm CAR/PDMS)

The effects of GC injector or desorption temperature were evaluated by varying the temperature from 100 to 250ºC. The injector temperature profile obtained is shown in Figure 3. The amount of desorbed PH_3 increased with increasing the injector temperature and then reached a maximum at 200ºC. The GC used had split/splitless capillary injectors. It was suitable for direct introduction of the fibre into the injection port. In this study, the CG injector operated at 200ºC of optimal desorption temperature and did not cause peak broadening and tailing (Fig. 4).

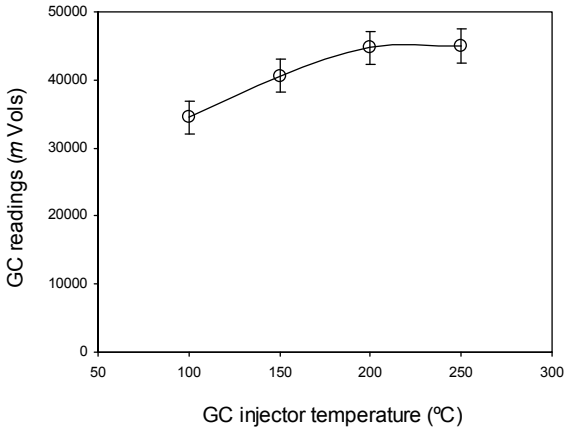

Figure 3 The GC injector temperature affects desorption of PH_3 from fibre (85 µm CAR/PDMS) at 0.1 ppm of PH_3 after 20 minutes extraction.

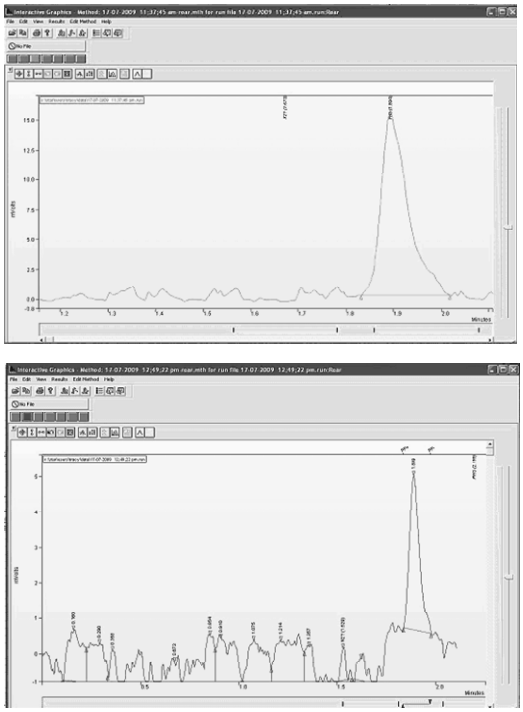

Figure 4 GC spectra with fibre (85 um CAR/PDMS) after 20 min extraction at levels of PH_3 at 0.5 ng/g (A) and 0.02 ng/g (B).

3.3. Effect of wheat treatment and HS-SPME extraction temperature on extraction efficacy of PH_3

The effects of temperature for treatment of wheat sample and extraction efficacy were evaluated by varying the temperature from 30 to 50ºC. The temperature profile for PH_3 released from fumigated wheat into the headspace and absorption of PH_3 on the fibre (85 µm CAR/PDMS) obtained is shown in Figure 5.

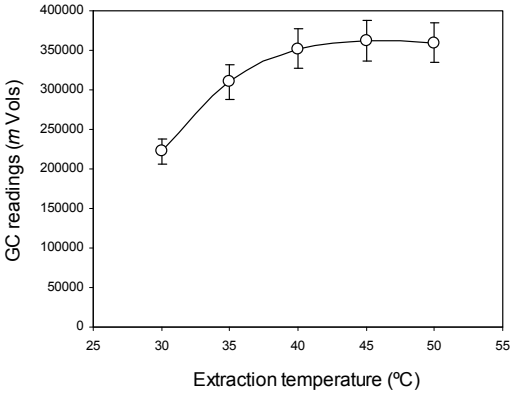

Figure 5 Temperature of extraction with fibre (85 µm CAR/PDMS) for 20 minutes extraction affects the extraction efficacy of PH_3.

The amount of absorbed PH_3 increased with increasing the injector temperature and then reached a maximum at 45ºC. As the temperature increases, the liquid and gas phases equilibrate faster and drive more PH_3 partitioning into the headspace, but increasing fibre temperature causes a competitive effect of

desorption from the fibre, ultimately limiting its analytical sensitivity of PH_3. This is consistent with the results of Vás and Vékey (2004). Therefore, we used the low temperature (45ºC) and long term (30 min) extraction for treatment of wheat samples. The limit of detection (LOD) or level of quantification (LOQ) was estimated based on a signal to noise ratio shown in Figure 5 (B) in the range of 0.005–0.01 ng/g.

Although the determination of PH_3 in grain is well established in the scientific literature, our results have demonstrated that the combination of SPME with headspace sampling is very effective for determining trace levels of PH_3.

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