Rapid Zinc Phosphide Trace Analysis in Agricultural Commodities by Phosphine Generation, Toluene Trapping, and Gas Chromatography

Johannes Corley,*,† Jennifer Kahl,‡ David Burkhart, Elmer Diaz, and Gregory Möller

Analytical Sciences Laboratory, Holm Research Center and Department of Food Science and Toxicology,
University of Idaho, Moscow, Idaho 83844-2203

A method is described for the analysis of the rodenticide zinc phosphide (Zn_3P_2, CAS Registry No. 1314-84-7) in various agricultural food commodities as well as for certain animal stomach contents. The method involves reacting the sample with aqueous H_2SO_4 and toluene in a volatile organic analysis vial. The H_2SO_4 hydrolyzes Zn_3P_2 to phosphine gas (PH_3), which is trapped in toluene. The sample vials are centrifuged to break the emulsion formed by mixing, and the toluene layer is then analyzed by gas chromatography. PH_3 is detected with a thermionic nitrogen−phosphorus detector or a flame photometric detector. This method is capable of detecting quantities as low as 5 ng of Zn_3P_2/g of sample. The method has been validated for blueberry, cantaloupe, cucumber, hay grass, potato, raspberry, snap bean, soybean, spinach, and squash.

Keywords: *Zinc phosphide; phosphine; residue analysis*

INTRODUCTION

Zn_3P_2 has been widely used as a rodenticide in the United States since 1939 (Johnson and Fagerstone, 1994). Other compounds such as strychnine and sodium monofluoroacetate (compound 1080) replaced Zn_3P_2 for rodenticidal use in the 1950s. However, their use has decreased because of potential environmental hazards to nontarget species. Tietjen (1976a) evaluated several alternative control and management methods to replace these compounds and concluded that Zn_3P_2, because of its efficacy and relative safety, would be appropriate to replace strychnine and compound 1080. Furthermore, Tietjen (1976b) concluded that Zn_3P_2 was largely free of secondary hazards. In this report, he studied the effect of Zn_3P_2 on bird colonies near the treated sites as well as carnivores that fed on these sites and concluded that there was no evidence of either primary (parent compound) or secondary (metabolite) hazards to any species when 2% Zn_3P_2 on rolled oats, formulated with 1% corn oil, was used. This has resulted in an increased interest in the use of Zn_3P_2 for the control of rodents. Matschke et al. (1983) further attested to the efficacy of Zn_3P_2 broadcast bait for the control of Richardson's ground squirrels. Sterner and Mauldin (1995) concluded that undigested Zn_3P_2 poses the main hazard to predators and scavengers consuming poisoned animals (i.e., a primary, not secondary, type hazard).

In animals, Zn_3P_2 reacts with stomach acids to produce poisonous PH_3, which is absorbed into the blood stream. In the field, Zn_3P_2 is applied in the form of 1% and 2% grain (oats, wheat, or cracked corn) bait formulations. It is also used in the tracking powder form for household rodent control.

Developing analytical methods for detecting and quantitating residues of this compound in agricultural commodities is difficult because Zn_3P_2 is practically insoluble in most organic solvents. In aqueous media, it hydrolyzes to form PH_3. The methods available for the analysis of Zn_3P_2 typically involve acid hydrolysis to PH_3. The evolved PH_3 is then analyzed by several different methods. According to an earlier method described in the *Pesticide Analytical Manual Vol. II* (1973), the PH_3 is swept by a stream of N_2 into bromine water where it is oxidized to phosphate. The phosphate is then reacted with ammonium molybdate in the presence of a reducing agent. The resulting molybdenum blue complex is then determined spectrophotometrically. This is the standard method for phosphate analysis. This method, although sensitive, is tedious and time-consuming. It also requires that the glassware used be completely free of phosphates, which would interfere with the determination. Berck et al. (1970) discuss the analysis of phosphine by gas−liquid chromatography. They compared several detectors and concluded that the flame photometric detector in the phosphorus mode had the best sensitivity. Dumas (1978) studied the purge-and-trap method for PH_3 determination. He reported a limit of detection of 0.01 ng of PH_3 per sample by this method. Later on, Saeed and Abu-Tabanja (1985) compared this method with a sulfuric acid treatment for the liberation of PH_3. They concluded that sulfuric acid was much faster than the purge-and-trap method for the desorption of PH_3 from fumigated samples of apricots, figs, beans, walnuts, and dates. Recently, Mauldin et al. (1996) reported a headspace analysis of PH_3 for Zn_3P_2 by gas chromatography. They injected 10 μL of the gaseous headspace into the gas chromatograph and quantitated the PH_3 using a flame photometric detector. Previously, Matschke et al. (1983), Okuno et al. (1975), and Sterner and

---

* Corresponding author [e-mail johannes@uidaho.edu; fax (208) 732-5327].

† Present address: Idaho Food Quality Assurance Laboratory, University of Idaho, P.O. Box 5058, Twin Falls, ID 83303-5058.

‡ Present address: Analytical Laboratories Inc., 1804 N. 33rd St., Boise, ID 83703.

S0021-8561(97)00644-4 CCC: $15.00

Mauldin (1995) used a similar headspace technique for quantitating Zn_3P_2 in animal tissue and vegetable matrixes.

The main problem associated with headspace analysis methods is that the technique is not easily adaptable to typical automated GC injection systems. Since PH_3 is commonly generated in a 250- or 500-mL Erlenmeyer flask, it is difficult to accurately determine the volume of the headspace. Headspace methods can be very tedious, and a single analyst may process only a few samples in the course of an 8-h day. Some of these methods involved weighing out of the undiluted Zn_3P_2 standard onto the sample, making low level determinations difficult or impossible. Regardless of analytical approach, the generation of PH_3 is complicated by many factors including Henry's law considerations and solid-phase sorption.

Robison and Hilton (1971) used toluene to absorb the PH_3 generated from Zn_3P_2 in a method developed for sugar cane. They reported that the absolute recovery of PH_3 in toluene from Zn_3P_2 in sugar cane was 33%. They also reported that, on average, 42% of the PH_3 reacted irreversibly in the acid medium with the chopped sugar cane.

In this work, we use the same principle as Robison and Hilton (1971) for the analysis of PH_3 for Zn_3P_2; however, several modifications are described that increased the recoveries to 50−110%. The wide range in recoveries for different matrixes is probably due to the affinity of the different matrixes toward phosphine as discussed by Berck and Gunther (1970). We validated this method for a variety of different fruit and vegetable matrixes including blueberry, cantaloupe, cucumber, grass, potato, raspberry, snap bean, soybean, spinach, and squash. The advantages of our approach are the rapidity and ease of the analysis, higher recoveries, low limit of detection (5 ng/g), adaptability to auto-injection systems, and the ability to work with small sample sizes (1−10 g). The approach we describe uses minimum amounts of solvents and reagents in a pollution prevention approach to Zn_3P_2 residue determination.

EXPERIMENTAL PROCEDURES

Reagents. Concentrated sulfuric acid (Fisher, reagent grade) was diluted using 18 MΩ·cm water. Baker Resi-Analyzed grade toluene was used to dissolve the PH_3. Liquid nitrogen was used for maintaining the integrity of the frozen samples during grinding. Silica gel, chromatography grade, 200−425 mesh (Applied Science Division, Milton Roy Co., State College, PA) was used for diluting the standards. The silica gel was dried in an oven at 100−150 °C for 1 h, followed by cooling in a desiccator before use. Reagent grade propylene glycol (J. T. Baker Co., Phillipsburg, NJ) was used to dilute the Zn_3P_2 as a suspension. The 40-mL volatile organic analysis (VOA) vials with Teflon septum tops were purchased from Eagle-Picher Environmental Services (Miami, OK). Certified Zn_3P_2 analytical standard was obtained from the manufacturer (Hacco, Inc., Madison, WI).

Instrumentation. A large capacity food processor, Robot Coupe model RSI6Y-1, was used for grinding the frozen raw agricultural commodities (RAC). An ultrasonic mixer (Branson Co.), a magnetic stir plate, and a vortex mixer (VWR Scientific) were used to mix the Zn_3P_2/propylene glycol dispersion. A rotary mixer (Millipore Corp., Bedford MA) at 30 rpm was used to mix the sample matrix with the H_2SO_4 and to extract the PH_3 into the toluene layer. Calibrated solvent pipettor pumps were used to dispense toluene (10 mL minimum capacity) and sulfuric acid (20 mL minimum capacity). The VOA vials were centrifuged in a Beckman model TJ-6 centrifuge. A capillary gas chromatograph (Hewlett-Packard Co., model 5890) equipped with a nitrogen−phosphorus detector (NPD) or a flame photometric detector (FPD) and a 30 m × 0.53 mm DB-5 column (J&W Scientific, Folsom, CA) with a 1 m × 0.53 mm guard column was used in GC analysis.

Gas Chromatograph Operating Parameters. (a) Column: 30 m × 0.53 mm i.d. DB-5 (J&W Scientific), with a guard column (approximately 1 m × 0.53 mm). (b) Helium carrier gas, 15 mL/min flow rate; helium auxiliary gas, 20 mL/min; hydrogen, 3.5−4.5 mL/min; air, 85−95 mL/min. (c) Column temperature: 60 °C isothermal or 30−100 °C gradient. (d) Injector temperature: 120 °C. (e) Detector temperature (NPD): 300 °C. (f) Injection volume: 4 μL (of the toluene layer), manual or auto-injection. (g) Run time is 5 min. Splitless for 1 min. (h) The flame photometric detector was equipped with a phosphorus filter. (i) Two types of beads were used as the nitrogen−phosphorus detector, a quartz bead or rubidium bead. For PH_3, the quartz bead produced better results. The bead power on the NPD was set at 750−900 units.

Preparing Standards. Due to the insolubility of Zn_3P_2 in all organic and aqueous solvents, the standards were diluted and handled either in the solid phase or as a dispersion in propylene glycol.

The solid-phase standard mixtures were prepared by diluting Zn_3P_2 w/w with 200−425 mesh silica gel in a 40-mL amber VOA vial. (The initial concentration of the primary standard was taken into consideration in preparing the necessary dilutions.) In a tared VOA vial, the appropriate amount of finely ground Zn_3P_2 (ground with a mortar and pestle) was weighed out. Oven-dried silica gel was then added to make a total weight of 10.0 g. The mixtures were mixed for a minimum of 30 min on the rotary mixer, so that the vials turned end over end. Initially, a 1000 μg/g standard was prepared, and the more dilute standards were prepared from this mixture. The standards were stored in a desiccator, in labeled 40-mL amber VOA vials. If Zn_3P_2 is kept dry, it is relatively stable and can be stored for extended periods of time.

The Zn_3P_2/propylene glycol suspension was prepared by weighing out 0.0100 g of finely ground (by mortar and pestle) zinc phosphide primary standard in a tared 10-mL volumetric flask. Propylene glycol was added to make the volume up to 10 mL. The slurry was sonicated for 30 min followed by stirring on a magnetic stirrer for 30 min at high speed. The standard was vortex-mixed for 15 s and immediately transferred to a 12-mL amber vial, capped, and stored in a desiccator. Further dilutions were prepared from the 1000 μg/mL standard. The propylene glycol dispersion standards were vortex-mixed for 15 s each time before aliquoting and constantly stirred on a magnetic stirrer during use.

Calibration Standards. GC calibration standards were prepared from either the Zn_3P_2/silica gel mixture or the Zn_3P_2/propylene glycol suspension. When the Zn_3P_2/silica gel mixture was used, the mixture was weighed into VOA vials as described below. Fresh standards were prepared with each set of samples and treated in an identical fashion to the samples:

0.050 g of the 5.0 mg/g standard (in 10 mL of toluene) = 0.025 mg/mL

0.10 g of the 5.0 mg/g standard (in 10 mL of toluene) = 0.050 mg/mL

0.10 g of the 10 mg/g standard (in 10 mL of toluene) = 0.10 mg/mL

0.10 g of the 20 mg/g standard (in 10 mL of toluene) = 0.20 mg/mL

0.05 g of the 100 mg/g standard (in 10 mL of toluene) = 0.5 mg/mL

0.10 g of the 100 mg/g standard (in 10 mL of toluene) = 1.0 mg/mL

0.20 g of the 100 mg/g standard (in 10 mL of toluene) = 2.0 mg/mL.

When the Zn_3P_2/propylene glycol standard was used, the appropriate volumes of the suspension were pipetted out into VOA vials.

Immediately, 10 mL of toluene solvent was added (to trap any phosphine gas generated by the reaction of the Zn_3P_2 standard material with water in the atmosphere) followed immediately by 30 mL of 5 N H_2SO_4 (1 N H_2SO_4 for blueberry, cantaloupe, grass, raspberry, snap bean, and spinach). The vials were capped immediately following the addition of the H_2SO_4. The standards were then mixed by rotating the vials end over end for 1−1.5 h in a rotary mixer.

When the Zn_3P_2/silica gel mixture was used to prepare the calibration standards, the 0.005 μg/mL standard was prepared as follows: 250 μL of the 0.2 μg/mL calibration standard (toluene phase, after mixing) was added to 10 mL of toluene in a VOA vial containing toluene and H_2SO_4. The dilution was carried out in a closed system. A total of 250 μL of the toluene phase of the 0.2 μg/mL calibration standard was measured out using a syringe so as not to expose the standard to air. This was then injected into the sealed vial containing 10 mL of toluene and 30 mL of H_2SO_4. This vial was then shaken to ensure complete mixing. This process of preparing the 0.005 μg/mL calibration standard was not necessary when the Zn_3P_2/propylene glycol standards were used to prepare the calibration standards.

Sample Preparation. For large fruits and vegetables such as cantaloupe, cucumber, squash, potato, etc., the RAC was partially thawed. In these cases, subsamples were taken by sampling alternate quarters. The subsamples were then cut into smaller pieces. This was not done in case of the berries, soybean, spinach, or snap bean that did not need to be cut or subsampled prior to grinding. The sample was placed in the grinder, and liquid nitrogen was added to maintain the frozen integrity of the sample. The RAC was finely ground and stored below −10 °C.

Ten grams (1 g for grass and spinach) of each sample was weighed out into a VOA vial, and the vial was capped. A total of 10 mL of toluene followed by 20 mL (30 mL for grass and spinach) of 5 N H_2SO_4 (1 N H_2SO_4 for blueberry, cantaloupe, grass, raspberry, snap bean, and spinach) were added to the vials. The vials were capped, as soon as the toluene and H_2SO_4 were added, with the Teflon side of septum in contact with sample. The amount of air remaining in the headspace was less than 2 mL.

Fortified Samples. Untreated field samples were prepared as described in the section above. The 10-g aliquots (1 g for grass and spinach) of the untreated sample were weighed out into VOA vials and then spiked either by weighing out the appropriate diluted Zn_3P_2/silica gel standard into aluminum foil and then transferring it on to the matrix or by pipetting out the Zn_3P_2/propylene glycol suspension directly on the matrix.

For the Zn_3P_2/silica gel standard, the untreated matrixes were spiked as described below:

0.10 g of the 5.0 μg/g standard in 10 g of sample matrix = 0.050 μg/g

0.10 g of the 10 μg/g standard in 10 g of sample matrix = 0.10 μg/g

0.10 g of the 100 μg/g standard in 10 g of sample matrix = 1.0 μg/g

A total of 10 mL of toluene and 20 mL (30 mL for grass and spinach) of 5 N H_2SO_4 (1 N H_2SO_4 for blueberry, cantaloupe, grass, raspberry, snap bean, and spinach) were added immediately, and the vials were capped tightly.

All the vials were then loaded onto the rotary mixer. The vials were then mixed by rotating end over end for 1−1.5 h. After mixing, all vials were centrifuged for 10 min at 1800−1900 rpm.

When the auto-injection technique was used, the toluene phase was transferred from the sealed VOA vial to the sealed GC vials using a syringe. A 27-gauge needle was used to let air into the capped VOA vial as well as to vent out the air from the sealed GC vial.

If necessary, the samples were placed in a refrigerator overnight. They were then analyzed by GC equipped with a flame photometric or a thermionic nitrogen−phosphorus detector.

Gas Chromatographic Analysis. The samples to be analyzed were entered into a sequence on the chromatography data station. The standards were run in order of increasing concentration of Zn_3P_2. The samples were run following the standards in order from blanks, to least concentrated, to most concentrated, if known. The fortified samples were run last, in order of least to most concentrated. A check standard was included in the sequence after approximately half of the samples were analyzed, if there were more than 10 samples. The calibration standards were again run following the spikes, thus bracketing the samples. A total of 4 μL of the toluene layer of each sample was injected. In the case of a manual injection, the syringe was rinsed three times each with toluene from two vials, simulating that of the automatic injector.

The retention time of PH_3 (generated from Zn_3P_2) on the GC is approximately 0.5 min. The estimated detection limit (LOD) for this method is 0.005 μg/g, and the practical quantitation limit (PQL) for this method is 0.05 μg/g.

RESULTS AND DISCUSSION

The method described here for the analysis of various RAC is rapid, sensitive, and free of all chromatographic interferences. It is possible for a single analyst to analyze a batch of 20 samples and 3−6 recovery check spikes in 1 day (8 h). This is a significant improvement over other colorimetric and headspace assays, and the relative simplicity of the procedure is another advantage. Unlike colorimetric assays, phosphate impurities from the solvents and apparatus was not a problem since the Zn_3P_2 was analyzed as PH_3 and not as PO_4^{3-}.

The instrumental limit of detection for this method was determined to be 0.005 μg/g by estimating an amount that produced a signal greater than three times the signal-to-noise ratio of the instrument. However, preparing Zn_3P_2 standards at very low levels was difficult because of the variability in the distribution of the Zn_3P_2 particles in the silica gel matrix. It was found that 5.0 μg/g Zn_3P_2 in the silica gel was the lowest concentration level that was practical. Below this concentration, the dispersion of Zn_3P_2 in the silica gel was not reproducible. Also, the smallest quantity of the Zn_3P_2/silica gel standard that could be weighed out to give reproducible results was 0.05 g (delivering 0.25 μg of Zn_3P_2 when using the 5 μg/g standard). Quantities lower than this gave nonreproducible results. All the calibration standards above 0.025 μg/mL were prepared by weighing out appropriate amounts of the Zn_3P_2/silica gel mixtures. Standards of concentrations less than 0.025 μg/mL were prepared as described earlier, by diluting the toluene phase of the 0.2 μg/mL standard into 10 mL of toluene. This had to be done because standards at this low concentration level could not be prepared directly from the silica gel mixtures for the same reason as described above.

The PQL for this technique was estimated at 0.05 μg/g (0.1 g of the 5 μg/g standard delivering 0.5 μg of Zn_3P_2 to 10 g of sample). However, it is possible to detect quantities of Zn_3P_2 (as PH_3) at concentrations as low as 0.005 μg/g. The main problem being the ability to accurately deliver this small a quantity of Zn_3P_2 to the matrix.

A technique suggested by Mauldin (1997) was to prepare a suspension of the finely ground compound in propylene glycol. The suspension of Zn_3P_2 in propylene glycol was much easier to work with and gave more reproducible results. Using this technique, it was possible to accurately deliver quantities as little as 0.01 μg of Zn_3P_2. With the Zn_3P_2/silica gel standard, the smallest amount that could be accurately delivered was 0.25 μg. Since this was the limiting factor for determining the limit of quantitation, the Zn_3P_2/propylene glycol standard enables detection and quantitation of 0.005 μg/g Zn_3P_2 in 10 g of sample. Furthermore, for matrixes such as grass, spinach, or other matrixes that irreversibly absorb larger amounts of PH_3, it is possible to work with 1 g of sample in order to reduce the matrix absorption and increase recoveries. For spinach, the recoveries for the 0.05 and 0.1 μg/g were 62% and 58%, respectively, when 10 g of sample was used. The recoveries went up to 88% (0.05 μg/g) and 86% (0.1 μg/g) when 1 g of sample was used. Similarly for hay grass, the maximum recoveries obtained for 10 g of sample was 30%; however, when 1 g of sample was used, recoveries of 80% and 73% were obtained for 0.05 and 0.1 μg/g, respectively.

The calibration curves obtained by this technique were found to be linear over the range of 0.005−0.2 μg/mL and 0.2−2 μg/mL. Separate calibration curves had to be used to quantitate Zn_3P_2 for these two ranges. The correlation coefficient values for the calibration curves were 0.990 or better most of the time; however, occasionally they did fall as low as 0.986. This was because of the difficulties in diluting the standards in the solid phase. Also, slight differences in the volume of air trapped in the VOA vial causes variation in the partitioning of the PH_3 (gas) between the air and the toluene phases.

Table 1 shows the percent recoveries for Zn_3P_2 and the respective standard deviations on several agricultural commodities. The percent recoveries and standard deviations were calculated at three levels (0.05, 0.1, and 1.0 μg/g) in triplicate, for all matrixes except for potato and soybean. The high standard deviations that were observed for some of the matrixes may result from the uneven distribution of the Zn_3P_2 in the silica gel or propylene glycol. There is a possibility that there are pockets of higher Zn_3P_2 concentrations within the silica gel matrix or the propylene glycol dispersion, and even longer mixing times do not solve the problem. The relative standard deviations for most of the matrixes range from 5% to 20%. Comparing the two techniques for preparing the secondary and tertiary standards, the Zn_3P_2/propylene glycol dispersion prepration technique was better than the Zn_3P_2/silica gel mixture because it was easier and quicker to work with the dispersion. However, care had to be taken to ensure thorough mixing of the dispersion and also when pipeting the dispersion, the viscosity of the propylene glycol had to taken into consideration when drawing the dispersion into the pipet (had to be done slowly).

Table 1. Method Validation Data for Zn_3P^2

matrix	fortification level (μg/g)	av recovery (%)	SD
blueberry	0.05 ($n = 3$)	56.8	8.1
	0.1 ($n = 3$)	52.8	10
	1 ($n = 3$)	69.2	6.8
cantaloupe	0.05 ($n = 3$)	72.6	2.8
	0.1 ($n = 3$)	75.7	7.7
	1 ($n = 3$)	55.5	3.7
cucumber	0.05 ($n = 3$)	68.2	5.6
	0.1 ($n = 3$)	93.9	19
	1 ($n = 3$)	82.6	2.2
grass	0.05 ($n = 3$)	79.8	6.6
	0.1 ($n = 3$)	73.3	1.7
	1 ($n = 3$)	120	4.0
potato	0.05 ($n = 3$)	79.3	11
	0.1 ($n = 7$)	71.7	18
	1 ($n = 3$)	83.8	6.3
raspberry	0.05 ($n = 3$)	77.7	7.2
	0.1 ($n = 3$)	73.5	4.4
	1 ($n = 3$)	93.2	3.0
snap bean	0.05 ($n = 3$)	59.2	7.1
	0.1 ($n = 3$)	62.1	9.4
	1 ($n = 3$)	86.6	1.5
soybean	0.05 ($n = 2$)	93.8	5.6
	0.5 ($n = 1$)	60.8	NA
spinach	0.05 ($n = 3$)	88.0	17
	0.1 ($n = 3$)	85.7	7.5
	1.0 ($n = 3$)	71.1	13
squash	0.05 ($n = 3$)	75.6	8.2
	0.1 ($n = 3$)	93.5	28
	1 ($n = 3$)	68.9	1.2

The recoveries for Zn_3P_2 are very dependent on the matrix. As can be seen from Table 1, the percent recoveries ranged from about 52% for blueberry to 93% for some other matrixes. For most of the matrixes, the recoveries were in the range of 65−75%. These recoveries are fairly good for Zn_3P_2, and even though the standard deviations were high in some cases, this technique is quite reliable and convenient to use for the rapid detection and quantitation of Zn_3P_2 in a large number of agricultural commodities. Robison and Hilton (1971) observed that about 42% of the total PH_3 was irreversibly absorbed by the sugar cane in the acid medium. Furthermore, Mauldin et al. (1996) reported that, for stomachs containing grasses as dietary components, the amount of PH_3 recovered decreased as the amount of the grass content increased and also that the recoveries were lower for smaller concentrations of Zn_3P_2. Berek and Gunther (1970) also showed that varying amounts of PH_3 were absorbed in different matrixes and at different temperatures. These observations highlight the difficulty in achieving good recoveries in fortified agricultural commodities. Analyzing smaller quantities of the RAC may reduce some of these problems.

In the course of our studies, we found that the NPD was more sensitive to the detection of PH_3 than the FPD in the phosphorus mode. Figure 1 shows the chromatogram of the 0.02 μg/mL (0.08 ng on column) Zn_3P_2 standards on the FPD, and Figure 2 shows the chromatogram of the 0.005 μg/mL (0.02 ng on column) Zn_3P_2 standard on the NPD. The solvent peak is observed on the FPD between 1.5 and 2.5 min. The uneven baseline observed on the NPD is as a result of the rising column temperature and the solvent peak (sharp drop at 2.8 min). None of these interfere with the PH_3 peak, which elutes at 0.5 min. The baseline is very stable between 0 and 0.8 min. In Figure 1, the signal for the 0.02 μg/mL (LOD for the FPD) Zn_3P_2 standard on the FPD is barely seen above the noise (S/N = 3). The response factor (area/concentration) for this peak is 2.3×10^4 area units/ng. The signal for the 0.005 μg/mL (LOD for the

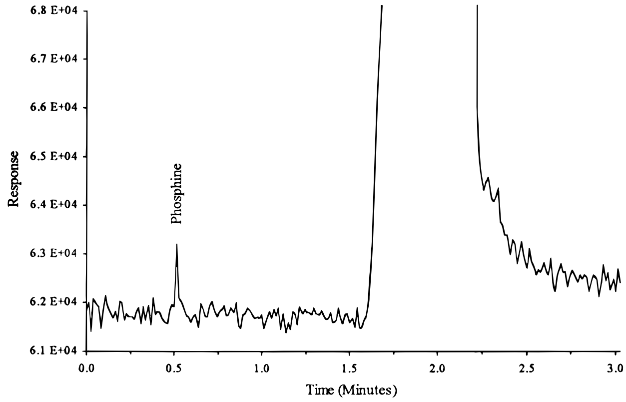

Figure 1. Chromatogram of a 4-μL injection of the 0.02 μg/mL Zn_3P_2 (as PH_3) standard, FPD in phosphorus mode. Retention time for PH_3 is 0.5 min.

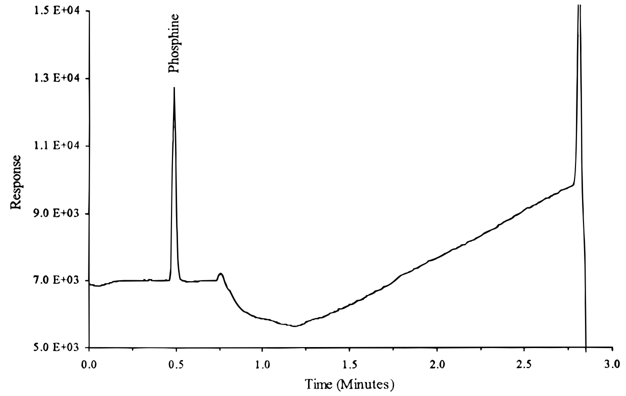

Figure 2. Chromatogram of a 4-μL injection of the 0.005 μg/mL Zn_3P_2 (as PH_3) standard, NPD. Retention time for PH_3 is 0.5 min.

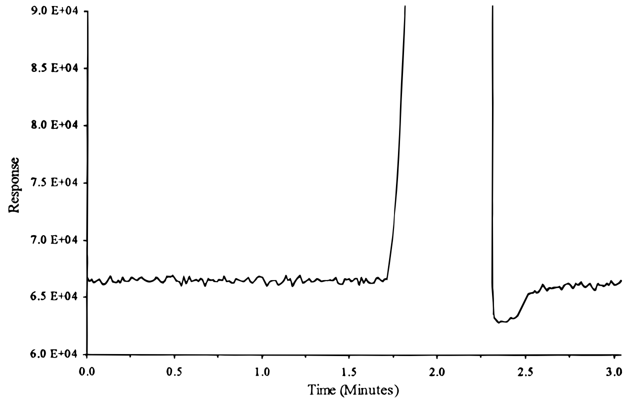

Figure 3. Chromatogram of a 4-μL injection of untreated control potato sample (4 mg on column), FPD in phosphorus mode. No PH_3 detected.

NPD) Zn_3P_2 standard (Figure 2) is much larger than the noise (S/N > 5). The response factor for this standard exceeds 3.5×10^5 peak area units/ng. We therefore observe that the NPD is approximately 15 times more sensitive for the analysis of Zn_3P_2 as PH_3. Sample chromatograms of the potato control and the potato control fortified at 0.05 μg/g with Zn_3P_2 on the FPD are shown in Figures 3 and 4, respectively. Figures 5 and 6 show the sample chromatograms of the raspberry control and the raspberry control fortified at 0.05 μg/g with Zn_3P_2 on the NPD. These figures show that no chromatographic matrix interferences are observed for any of the matrixes on either the NPD or the FPD. This is true for all the matrixes studied in this work.

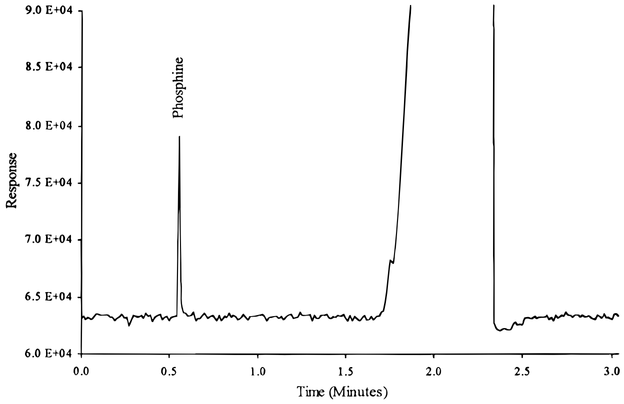

Figure 4. Chromatogram of a 4-μL injection of untreated control potato sample (4 mg on column) fortified at 0.05 μg/g (83.8% recovery), FPD in phosphorus mode. Retention time for PH_3 is 0.5 min.

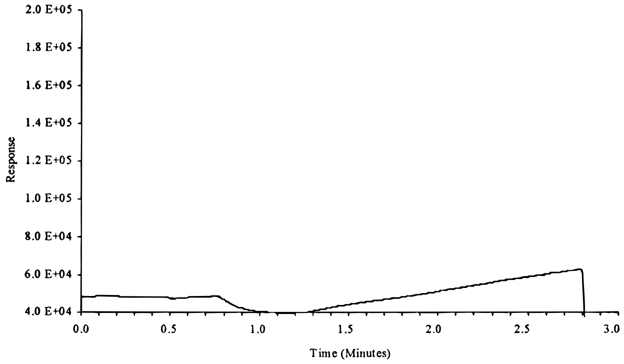

Figure 5. Chromatogram of a 4-μL injection of untreated control raspberry sample (4 mg on column), NPD. No PH_3 detected.

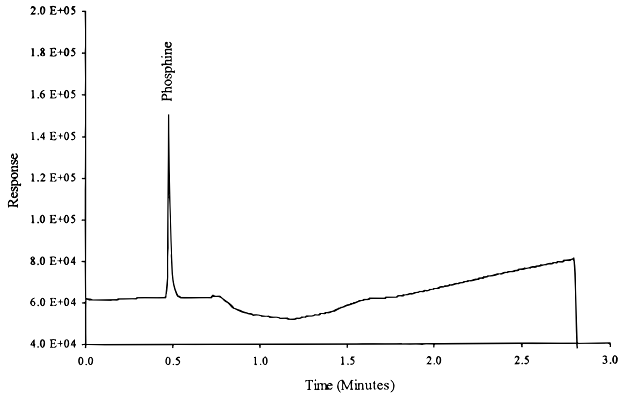

Figure 6. Chromatogram of a 4-μL injection of untreated control raspberry sample (4 mg on column) fortified at 0.05 μg/g (113% recovery), NPD. Retention time for PH_3 is 0.5 min.

Figure 7 shows the effect of the concentration of H_2SO_4 on the amount of PH_3 gas in the toluene phase. This experiment was carried out without any matrix present, so that only the effect of H_2SO_4 concentration on the Zn_3P_2 in generating PH_3 is seen. Data from this table show that, with the exception of 0.5 N H_2SO_4, the amount of phosphine dissolved in the toluene layer remained fairly constant over the range of 0.1–10 N H_2SO_4. Even for the 0.5 N H_2SO_4, the average GC peak area falls within the margin of error and thus cannot

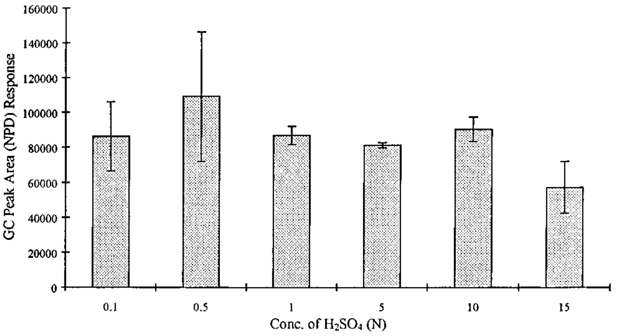

Figure 7. Effect of the concentration of H_2SO_4 on the generation of PH_3 ($n = 2$). Calculated standard deviations are indicated by the error bars.

be considered significantly higher than those for 0.1, 1.0, 5.0, and 10 N H_2SO_4. However there is a decrease in the amount of PH_3 generated by the 15 N H_2SO_4, and from a previous study in our laboratory, we found that concentrated H_2SO_4 does not generate any PH_3. The percent standard deviations of the GC peak area for the PH_3 generated by the 1.0, 5.0, and 10 N H_2SO_4 are smaller than those for the 0.1, 0.5, and 15 N H_2SO_4. From these data, we concluded that it was best to work with 1 or 5 N H_2SO_4 for liberating PH_3 form the Zn_3P_2. For cucumber, potato, soybean, and squash, we found that both 1 and 5 N H_2SO_4 generate approximately the same amount of PH_3; however, for the other matrixes, i.e., blueberry, cantaloupe, raspberry, snap bean, and spinach, the 1 N H_2SO_4 was more efficient in generating PH_3 than the 5 N H_2SO_4. A possible explanation for this is that larger quantities of PH_3 were adsorbed by these matrixes and thus increased the probability of reacting with the H_2SO_4. Further studies need to be done to find the optimum concentration of H_2SO_4 for each matrix.

In conclusion, we feel that the technique described in this work is quite suitable for the analysis of Zn_3P_2 in a wide variety of raw agricultural commodities. It is very sensitive (LOD = 5 ng/g) and free from chromatographic interferences. This method allows for analysis using relatively small sample sizes with low reagent consumption. The technique is rapid, robust, and easily adaptable to auto-injection systems. The use of smaller sample sizes, the lower acid concentrations, and the reproducible volume volatile organic analysis (VOA) vials were primarily responsible for increasing the recoveries and decreasing the amount of PH_3 reacting irreversably with the matrix in the acid medium. This approach may also be applied for the detection of PH_3 and Zn_3P_2 from animal stomach contents forensic toxicology studies, and we are pursuing studies in that direction.

ACKNOWLEDGMENT

We thank Richard Mauldin and Margaret Goodall of the Denver Wildlife Research Center in Denver, CO, and Kim Anderson of the University of Idaho for their valuable suggestions in developing this method. We express our appreciation to Hong Wu for technical assistance in the completion of this work.

LITERATURE CITED

Berck, B.; Gunther F. A. Rapid Determination of Sorption Affinity of Phosphine by Fumigation within a Gas Chromatographic Column. *J. Agric. Food Chem.* **1970**, *18*, 148−153.

Berck, B.; Westlake, W. E.; Gunther, F. A. Microdetermination of Phosphine by Gas−Liquid Chromatography with Microcoulometric, Thermionic, and Flame Photometric Detection. *J. Agric. Food Chem.* **1970**, *18*, 143−147.

Determination of Phosphine Residues in Sugarcane and Related Sugar Products from the Use of Zinc Phosphide. In *Pesticide Analytical Manual, Vol. II*; Pesticide Reg. Sec. 180.284; U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration: Washington, DC, 1973.

Dumas, T. Modified Gas Chromatographic Determination of Phosphine. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 5−7.

Johnson, G. D.; Fagerstone, K. A. In *Primary and Secondary Hazards of Zinc Phosphide to Nontarget Wildlife—A Review of the Literature*; U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Denver Wildlife Research Center Research Report No. 11-55-005; U.S. Government Printing Office: Washington, DC, 1994.

Matschke, G. H.; Marsh, M. P.; Otis, D. L. Efficacy of Zinc Phosphide Broadcast Baiting for Controlling Richardson's Ground Squirrels on Rangeland. *J. Range Manage.* **1983**, *36* (4), 504−506.

Mauldin, R. E.; Goldade, D. A.; Engeman, R. M.; Goodall, M. J.; Carver, R. K.; Johnson, J. J. Determination of Zinc Phosphide Residues in the California Ground Squirrel (*Spermophilus beecheyi*) by Gas Chromatography−Flame Photometric Detection. *J. Agric. Food Chem.* **1996**, *44*, 189−194.

Mauldin, R. E.; Goodall, M. J.;.Volz, S. A.; Griffin, D. L.; Petty, E. J.; Johnson, J. J. Zinc Phosphide Residue Determination in Alfalfa (*Medicago sativa*). *J. Agric. Food Chem.* **1997**, *45*, 2107−2111.

Okuno, I.; Wilson, R. A.; White, R. E. Determination of Zinc Phosphide in Range Vegetation by Gas Chromatography. *Bull. Environ. Contam. Toxicol.* **1975**, *13* (4), 392−396.

Robison, R. H.; Hilton H. W. Gas Chromatography of phosphine derived from Zinc Phosphide in Sugarcane. *J. Agric. Food Chem.* **1971**, *19*, 875−878.

Saeed, T.; Abu-Tabanja, R. Determination of Phosphine: Comparison of Rates of Desorption by Purge-and-Trap Method and by Sulfuric Acid Treatment. *J. Assoc. Off. Anal. Chem.* **1985**, *68* (1), 61−64.

Sterner, R. T.; Mauldin, R. E. Regressors of Whole-Carcass Zinc Phosphide/Phosphine Residues in Voles: Indirect Evidence of Low Hazards to Predators/Scavengers. *Arch. Environ. Contam. Toxicol.* **1995**, *28*, 519−523.

Tietjen, H. P. In *Zinc Phosphide—A Control Agent for Black-Tailed Prairie Dogs*; U.S. Department of the Interior, Fish and Wildlife Service, Wildlife Leaflet 509; U.S. Government Printing Office: Washington, DC, 1976a.

Tietjen, H. P. In *Zinc Phosphide-It's Development as a Control Agent for Black-Tailed Prairie Dogs*; U.S. Department of the Interior, Fish and Wildlife Service, Wildlife No. 9; U.S. Government Printing Office: Washington, DC, 1976b.

Received for review July 25, 1997. Revised manuscript received November 21, 1997. Accepted November 23, 1997. We thank the Idaho Agricultural Experiment Station, the IR-4 Project, and the University of Idaho Analytical Sciences Laboratory for funding the research involved in developing this method.

JF970644Z