

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

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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  $\mu$ L 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

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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  $M\Omega \cdot cm$  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  $\times$  0.53 mm DB-5 column (J&W Scientific, Folsom, CA) with a 1 m  $\times$  0.53 mm guard column was used in GC analysis.

**Gas Chromatograph Operating Parameters.** (a) Column: 30 m  $\times$  0.53 mm i.d. DB-5 (J&W Scientific), with a guard column (approximately 1 m  $\times$  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  $\mu L$  (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  $\mu g/g$  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  $\mu g/g$  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  $\mu\text{g/mL}$  standard was prepared as follows: 250  $\mu\text{L}$  of the 0.2  $\mu\text{g/mL}$  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  $\mu\text{L}$  of the toluene phase of the 0.2  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  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  $-10^\circ\text{C}$ .

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  $\mu\text{g/g}$  standard in 10 g of sample matrix =  
0.050  $\mu\text{g/g}$

0.10 g of the 10  $\mu\text{g/g}$  standard in 10 g of sample matrix =  
0.10  $\mu\text{g/g}$

0.10 g of the 100  $\mu\text{g/g}$  standard in 10 g of sample matrix =  
1.0  $\mu\text{g/g}$

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  $\mu\text{L}$  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  $\mu\text{g/g}$ , and the practical quantitation limit (PQL) for this method is 0.05  $\mu\text{g/g}$ .

## 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  $\mu\text{g/g}$  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  $\mu\text{g/g}$   $Zn_3P_2$  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  $\mu\text{g}$  of  $Zn_3P_2$  when using the 5  $\mu\text{g/g}$  standard). Quantities lower than this gave nonreproducible results. All the calibration standards above 0.025  $\mu\text{g/mL}$  were prepared by weighing out appropriate amounts of the  $Zn_3P_2$ /silica gel mixtures. Standards of concentrations less than 0.025  $\mu\text{g/mL}$  were prepared as described earlier, by diluting the toluene phase of the 0.2  $\mu\text{g/mL}$  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  $\mu\text{g/g}$  (0.1 g of the 5  $\mu\text{g/g}$  standard delivering 0.5  $\mu\text{g}$  of  $\text{Zn}_3\text{P}_2$  to 10 g of sample). However, it is possible to detect quantities of  $\text{Zn}_3\text{P}_2$  (as  $\text{PH}_3$ ) at concentrations as low as 0.005  $\mu\text{g/g}$ . The main problem being the ability to accurately deliver this small a quantity of  $\text{Zn}_3\text{P}_2$  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  $\text{Zn}_3\text{P}_2$  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  $\mu\text{g}$  of  $\text{Zn}_3\text{P}_2$ . With the  $\text{Zn}_3\text{P}_2$ /silica gel standard, the smallest amount that could be accurately delivered was 0.25  $\mu\text{g}$ . Since this was the limiting factor for determining the limit of quantitation, the  $\text{Zn}_3\text{P}_2$ /propylene glycol standard enables detection and quantitation of 0.005  $\mu\text{g/g}$   $\text{Zn}_3\text{P}_2$  in 10 g of sample. Furthermore, for matrixes such as grass, spinach, or other matrixes that irreversibly absorb larger amounts of  $\text{PH}_3$ , 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  $\mu\text{g/g}$  were 62% and 58%, respectively, when 10 g of sample was used. The recoveries went up to 88% (0.05  $\mu\text{g/g}$ ) and 86% (0.1  $\mu\text{g/g}$ ) 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  $\mu\text{g/g}$ , respectively.

The calibration curves obtained by this technique were found to be linear over the range of 0.005–0.2  $\mu\text{g/mL}$  and 0.2–2  $\mu\text{g/mL}$ . Separate calibration curves had to be used to quantitate  $\text{Zn}_3\text{P}_2$  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  $\text{PH}_3$  (gas) between the air and the toluene phases.

Table 1 shows the percent recoveries for  $\text{Zn}_3\text{P}_2$  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  $\mu\text{g/g}$ ) 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  $\text{Zn}_3\text{P}_2$  in the silica gel or propylene glycol. There is a possibility that there are pockets of higher  $\text{Zn}_3\text{P}_2$  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  $\text{Zn}_3\text{P}_2$ /propylene glycol dispersion preparation technique was better than the  $\text{Zn}_3\text{P}_2$ /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 be taken into consideration when drawing the dispersion into the pipet (had to be done slowly).

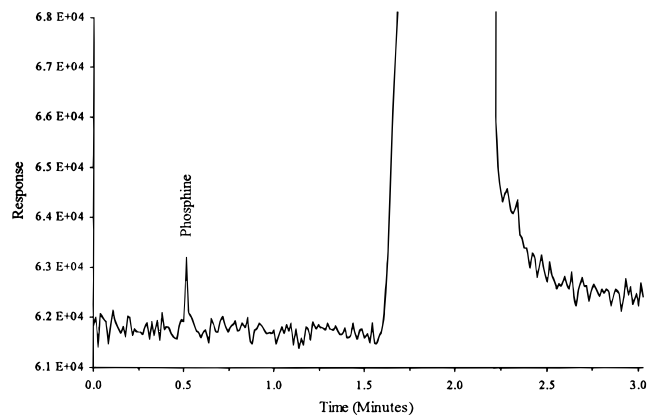
The recoveries for  $\text{Zn}_3\text{P}_2$  are very dependent on the

**Table 1. Method Validation Data for  $\text{Zn}_3\text{P}_2$**

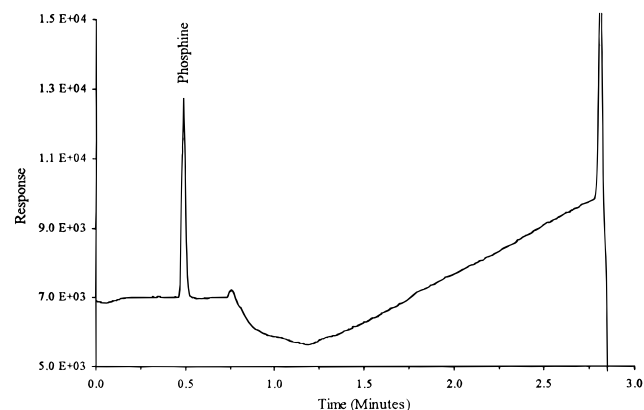
| matrix     | fortification level ( $\mu\text{g/g}$ ) | av recovery (%) | SD  |
|------------|---|-----------------|-----|
| blueberry  | 0.05 ( $n = 3$ )                        | 56.8            | 8.1 |
|            | 0.1 ( $n = 3$ )                         | 52.8            | 10  |
|            | 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  |
|            | 0.05 ( $n = 3$ )                        | 88.0            | 17  |
| spinach    | 0.1 ( $n = 3$ )                         | 85.7            | 7.5 |
|            | 1.0 ( $n = 3$ )                         | 71.1            | 13  |
|            | 0.05 ( $n = 3$ )                        | 75.6            | 8.2 |
| squash     | 0.1 ( $n = 3$ )                         | 93.5            | 28  |
|            | 1 ( $n = 3$ )                           | 68.9            | 1.2 |

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  $\text{Zn}_3\text{P}_2$ , 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  $\text{Zn}_3\text{P}_2$  in a large number of agricultural commodities. Robison and Hilton (1971) observed that about 42% of the total  $\text{PH}_3$  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  $\text{PH}_3$  recovered decreased as the amount of the grass content increased and also that the recoveries were lower for smaller concentrations of  $\text{Zn}_3\text{P}_2$ . Berek and Gunther (1970) also showed that varying amounts of  $\text{PH}_3$  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.

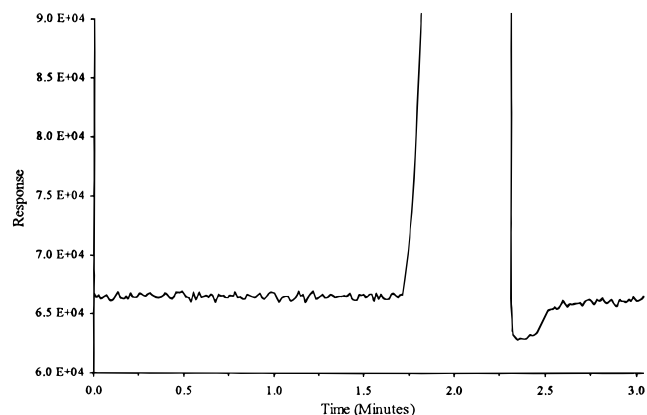
In the course of our studies, we found that the NPD was more sensitive to the detection of  $\text{PH}_3$  than the FPD in the phosphorus mode. Figure 1 shows the chromatogram of the 0.02  $\mu\text{g/mL}$  (0.08 ng on column)  $\text{Zn}_3\text{P}_2$  standards on the FPD, and Figure 2 shows the chromatogram of the 0.005  $\mu\text{g/mL}$  (0.02 ng on column)  $\text{Zn}_3\text{P}_2$  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  $\text{PH}_3$  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  $\mu\text{g/mL}$  (LOD for the FPD)  $\text{Zn}_3\text{P}_2$  standard on the FPD is barely seen above the noise ( $S/N = 3$ ). The response factor (area/concentration) for this peak is  $2.3 \times 10^4$  area units/ng. The signal for the 0.005  $\mu\text{g/mL}$  (LOD for the



**Figure 1.** Chromatogram of a 4- $\mu$ L injection of the 0.02  $\mu$ g/mL  $Zn_3P_2$  (as  $PH_3$ ) standard, FPD in phosphorus mode. Retention time for  $PH_3$  is 0.5 min.

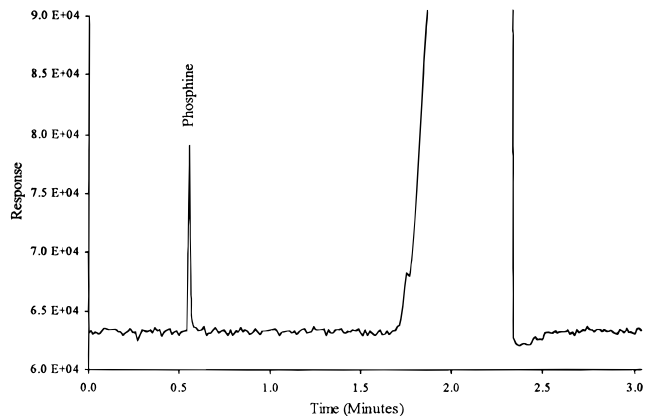


**Figure 2.** Chromatogram of a 4- $\mu$ L injection of the 0.005  $\mu$ g/mL  $Zn_3P_2$  (as  $PH_3$ ) standard, NPD. Retention time for  $PH_3$  is 0.5 min.

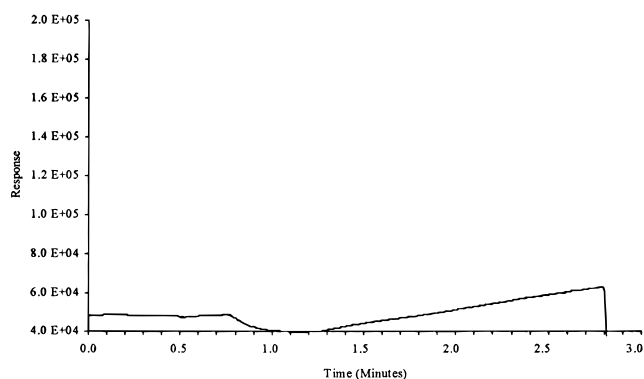


**Figure 3.** Chromatogram of a 4- $\mu$ L injection of untreated control potato sample (4 mg on column), FPD in phosphorus mode. No  $PH_3$  detected.

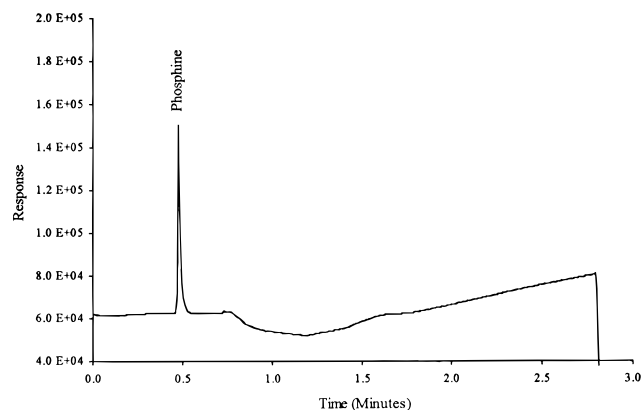
NPD)  $Zn_3P_2$  standard (Figure 2) is much larger than the noise ( $S/N > 5$ ). The response factor for this standard exceeds  $3.5 \times 10^5$  peak area units/ng. We therefore observe that the NPD is approximately 15 times more sensitive for the analysis of  $Zn_3P_2$  as  $PH_3$ . Sample chromatograms of the potato control and the potato control fortified at 0.05  $\mu$ g/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  $\mu$ g/g with  $Zn_3P_2$  on the NPD. These figures show that no chromatographic matrix interferences are ob-



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



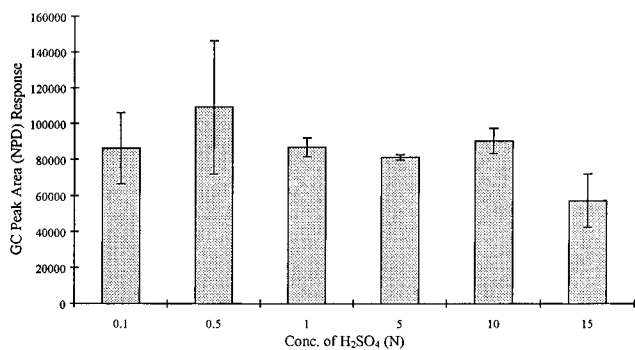
**Figure 5.** Chromatogram of a 4- $\mu$ L injection of untreated control raspberry sample (4 mg on column), NPD. No  $PH_3$  detected.



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

served for any of the matrixes on either the NPD or the FPD. This is true for all the matrixes studied in this work.

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<sub>2</sub>SO<sub>4</sub> on the generation of PH<sub>3</sub> ( $n = 2$ ). 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<sub>2</sub>SO<sub>4</sub>. However there is a decrease in the amount of PH<sub>3</sub> generated by the 15 N H<sub>2</sub>SO<sub>4</sub>, and from a previous study in our laboratory, we found that concentrated H<sub>2</sub>SO<sub>4</sub> does not generate any PH<sub>3</sub>. The percent standard deviations of the GC peak area for the PH<sub>3</sub> generated by the 1.0, 5.0, and 10 N H<sub>2</sub>SO<sub>4</sub> are smaller than those for the 0.1, 0.5, and 15 N H<sub>2</sub>SO<sub>4</sub>. From these data, we concluded that it was best to work with 1 or 5 N H<sub>2</sub>SO<sub>4</sub> for liberating PH<sub>3</sub> from the Zn<sub>3</sub>P<sub>2</sub>. For cucumber, potato, soybean, and squash, we found that both 1 and 5 N H<sub>2</sub>SO<sub>4</sub> generate approximately the same amount of PH<sub>3</sub>; however, for the other matrixes, i.e., blueberry, cantaloupe, raspberry, snap bean, and spinach, the 1 N H<sub>2</sub>SO<sub>4</sub> was more efficient in generating PH<sub>3</sub> than the 5 N H<sub>2</sub>SO<sub>4</sub>. A possible explanation for this is that larger quantities of PH<sub>3</sub> were adsorbed by these matrixes and thus increased the probability of reacting with the H<sub>2</sub>SO<sub>4</sub>. Further studies need to be done to find the optimum concentration of H<sub>2</sub>SO<sub>4</sub> for each matrix.

In conclusion, we feel that the technique described in this work is quite suitable for the analysis of Zn<sub>3</sub>P<sub>2</sub> 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<sub>3</sub> reacting irreversibly with the matrix in the acid medium. This approach may also be applied for the detection of PH<sub>3</sub> and Zn<sub>3</sub>P<sub>2</sub> from animal stomach contents forensic toxicology studies, and we are pursuing studies in that direction.

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