



## Analysis of phorate in vegetable samples by spectrophotometric method

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### ABSTRACT

A spectrophotometric method for the analysis of phorate (organophosphorus insecticide) is described. The analysis is based on the oxidation of organophosphorus pesticide with slight excess of N-bromosuccinimide (NBS) and the unconsumed NBS was determined with rhodamine B on a spectrophotometer (Genesis IOS V1.200) at  $\lambda_{\max}$ . 553nm. Beer's law was obeyed in the concentration range of 0.10 to 1.00ppm. The method was successfully applied for the determination of phorate in vegetable samples. The validity of the proposed method was assessed by comparing it with the high performance liquid chromatographic method (HPLC) through statistical analysis. There was no significant difference between the two methods. In the absence of sophisticated equipment like high performance liquid chromatograph and gas liquid chromatograph, the proposed method could be used for pesticide residues analysis.

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### Introduction

Pesticides are reliable and effective chemicals used for controlling pests and diseases that could lead to loss of crops on the field and in storage (Cremlyn, 1979). In recent times, synthetic pesticides have been gaining ground at the expense of naturally occurring pesticides (Buchel, 1983). Synthetic pesticides are classified broadly into insecticides, fungicides and herbicides.

Organophosphorus pesticides are widely used in agriculture, both in pre-and post-harvest pest control (Hassall, 1983). These pesticides are preferred to organochlorine pesticides because of their broad range of activity. Most organophosphorus pesticides degrade fairly quickly after application but some however, persist in one form or another for a long period. Crops with such persistent pesticides create a potential source of harm when consumed by either human beings or animals (Janice et al., 2004). For the safety of the environment, it is pertinent that the levels of these pesticides in the environment are closely monitored. This would ensure that the tolerance level of the pesticides set by the Food and Drug Administration is not exceeded (Judicate et al., 2005). Among the analytical methods available for determining organophosphorus pesticides are gas-liquid chromatography (Ronald, 1988), high-performance liquid chromatography (Akiko et al., 2000), Voltametric (Randhir et al., 2005), thin layer chromatography (Akmal et al., 1996), mass spectrophotometric (James et al., 1995), FT – Raman spectroscopy (Stavroula et al., 2008) and spectrophotometric (Sunitha et al., 2006).

Spectrophotometric method is one of the most widely used methods of analysis. It is widely used in clinical and environmental chemistry because many substances can be selectively converted to a coloured derivative. The instrumentation is readily available and easy to operate. In spectrophotometric method, the sample solution absorbs electromagnetic radiation from an appropriate source, and the amount absorbed is related to the concentration of the analyte in the solution (Gary, 1980).

A spectrophotometric method based on the oxidation of the organophosphorus pesticide (phorate) with slight excess of oxidizing agent (N-bromosuccinimide) is hereby reported. With this spectrophotometric method, work on pesticide residue analysis could still continue even in the absence of more sophisticated analytical equipment like gas – liquid chromatograph and high – performance liquid chromatograph.

### Materials and methods

#### Reagents and equipment

All the reagents used were of analytical grade. Phorate (organophosphorus pesticide), N-bromosuccinimide and rhodamine B were AR grade chemicals (Sigma – Aldrich, Germany).

#### Procedure

##### Preparation of calibration graph

Stock solution of phorate (100ppm) was prepared by dissolving 0.01g of phorate in minimum amount of acetic acid in 100cm<sup>3</sup> standard flask. The resultant solution was made to mark with distilled water. Working standards were prepared by serial dilution. Aqueous solutions of N-bromosuccinimide (0.01% w/v), rhodamine B (0.02% w/v), HCl (2 mol moldm<sup>-3</sup>) and acetic acid (1 mol dm<sup>-3</sup>) were prepared.

Into series of 100-cm<sup>3</sup> standard flasks were added by means of a Measuring pipette, 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00-cm<sup>3</sup> of phorate. Into another 100-cm<sup>3</sup> standard flask was placed 50cm<sup>3</sup> of stock solution of distilled water as blank. Into each flask was added 6.0cm<sup>3</sup> of N-bromosuccinimide (NBS), 4.0cm<sup>3</sup> of acetic acid and 2.0cm<sup>3</sup> of HCl. The solution was allowed to stay for about 10min at 30°C with occasional shaking. After which 3.0cm<sup>3</sup> of rhodamine-B solution was added and mixed thoroughly. Each solution was diluted with distilled water to exactly 100cm<sup>3</sup>. The standards corresponded to 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00ppm of phorate respectively. The absorbance of each of the standard solutions was measured against blank at the wavelength of maximum absorbance (553nm). Calibration graph was prepared by plotting absorbance of each of the standard solutions against concentration of phorate in ppm.

### Application of the recommended method

To check the validity of the proposed method, analyses were carried out on pesticide-free samples, as well as phorate containing samples. The results obtained from both experiments were compared with that carried out by high performance liquid chromatography (HPLC). The extraction and cleanup methods of Liao et al., (1991) were adopted.

The high performance liquid chromatographic analysis was carried out at the Research Laboratory of Obafemi Awolowo University, Ile – Ife, Nigeria, using Hewlett – Packard HPLC equipment with a photodiode detector at 230nm; while the spectrophotometric analysis was carried out at the Central Research Laboratory (CRL) of Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

### Application of the method on pesticide – free samples (Recovery experiment)

Seeds of tomatoes, garden egg and pepper were planted without application of pesticides in a green-house. After twelve weeks of planting, a sizeable amount of the vegetable (tomatoes) was plucked, washed, chopped into small pieces and thoroughly mixed with a food chopper. A 25g portion of the chopped vegetable and 5g of anhydrous  $\text{Na}_2\text{SO}_4$  were ground together and then transferred to a 100 $\text{cm}^3$  beaker. The mixture fortified by spiking with 1 $\text{cm}^3$  of 0.25ppm of phorate pesticide standard. This was followed by addition of 50 $\text{cm}^3$  of acetonitrile. The mixture was extracted according to Liao et al. method. The extraction process was followed by drying of the extract with anhydrous  $\text{Na}_2\text{SO}_4$ . Florisil – cleanup and concentration of the extract were carried out. The final volume of the sample extract was reduced to 1 $\text{cm}^3$  under a gentle stream of nitrogen and analysed on HPLC equipment.

The extraction procedure was repeated for the spectrophotometric analysis. The concentrated spiked vegetable sample (1 $\text{cm}^3$ ) was transferred quantitatively into 100 $\text{cm}^3$  standard flask. This was followed by the addition of N-bromosuccinimide (6.0 $\text{cm}^3$ ), acetic acid (4.0 $\text{cm}^3$ ) and HCl (2.0 $\text{cm}^3$ ). The solution was allowed to stay before the addition of rhodamine – B solution (3 $\text{cm}^3$ ). The flask was made to mark with distilled water. The absorbance of the solution was measured against blank. The concentration corresponding to the absorbance was extrapolated from the calibration graph.

The recovery experiments were repeated for both chromatographic and spectrophotometric methods by spiking 25g tomato sample with 1 $\text{cm}^3$  of 0.50ppm and 0.75ppm of phorate pesticide standard respectively.

The recovery experiments were also carried out on other vegetable samples.

### Application of the method on phorate containing samples

Vegetable samples (tomatoes, garden egg and pepper) were collected from the University Agricultural farm. Phorate had been previously used on this farm as insecticide. The samples were extracted according to earlier procedure but without fortification. The extracted samples were then analysed by HPLC, as well as the proposed method.

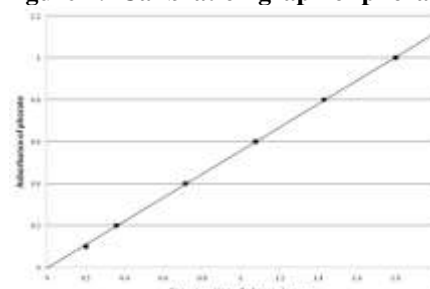
### Statistical analyses

The precision of the proposed method was determined through relative standard deviation (RSD) while accuracy of the method was expressed through relative accuracy. F-test was carried out to determine whether there was a significant difference between the proposed method and the standard method (i.e high performance liquid chromatographic method), based on their standard deviations.

## Results and Discussion

The wavelength at maximum absorption was 553nm. The graph of absorbance against concentration of phorate in ppm (i.e calibration graph) was linear and therefore Beer's law was obeyed (Table 1 and Figure 1).

Figure 1: Calibration graph of phorate



The percentage recovery of phorate pesticide from fortified vegetable samples for both high performance liquid chromatographic and spectrophotometric methods are shown in Table 2. The mean recovery percentage and mean relative standard deviation for the proposed method were 90.2 and 3.1% respectively; while the mean recovery percentage and mean relative standard deviation for HPLC were 95.0 and 2.2% respectively. (Table 2). The precision of the HPLC was higher than that of the proposed method since its mean relative standard deviation was lower. The relative accuracy of the recovery of the proposed method when compared with the HPLC – method (assumed to be the accepted true value). With the high value of the relative accuracy (94.9%), the proposed method was comparable with the HPLC – method.

In order to confirm that the two methods were comparable, their variances were subjected to statistical F-test. Since the calculated F-value (2.07) was lower than the tabulated value (3.44) at 95% Confidence Level, it could be concluded that there was no significant difference in the precision of the two methods. For more confirmation the two methods were further subjected to 'the student t-Test'. There was no significant difference in the two methods based on the result obtained from the 'Student t-Test'.

The proposed method was used for the analysis of pesticide (phorate) in pesticide containing vegetable samples and the results obtained were compared with the HPLC method (Table 3). The results of the statistical analysis showed that the two methods were comparable. The results obtained from the two methods however showed that the residues levels were higher than the CODEX Maximum Residue Limit (MRL) for phorate (WHO, 1997). MRL is the maximum concentration of pesticide residues recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in food, agricultural commodity and animal feed. MRL values for agricultural products are usually lower than that of the Codex Value.

This study showed some degree of contamination of the analyzed vegetable samples by phorate. It is therefore recommended that farmers were educated on the proper use of pesticides so as to protect consumers from being exposed to pesticide contamination and ensuring that pesticides were correctly applied to food crops.

### Conclusion

The proposed method was found to be comparable with the HPLC-method that is commonly used for pesticide residue analysis. The proposed method was found to be simple, rapid,

sensitive and reproducible. The method had been successfully used for the determination of pesticide residues in vegetable samples.

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**Table 1: Measurement of absorbance at various concentrations of phorate**

Concentration of phorate (ppm)	Absorbance of phorate*	R.S.D
0.10	0.200	3
0.20	0.358	2
0.40	0.714	4
0.60	1.075	3
0.80	1.429	2
1.00	1.800	3

\* Mean of three determinations.

**Table 2: Recovery of phorate pesticide from fortified vegetable samples by the proposed method and HPLC**

Vegetable samples	PROPOSED METHOD			HPLC		
	Fortification level (ppm)	Mean Rec. %	RSD %	Fortification Level (ppm)	Mean Rec. %	RSD %
Tomatoes	0.25	92	3	0.25	90	2
	0.50	88	4	0.50	94	1
	0.75	90	3	0.75	98	3
Garden egg	0.25	91	2	0.25	97	2
	0.50	90	4	0.50	99	2
	0.75	95	3	0.75	98	1
Pepper	0.25	90	4	0.25	92	4
	0.50	87	2	0.50	96	3
	0.75	89	3	0.75	91	2
Mean Rec. % and RSD:		90.2	3.1		95	2.2

$$\left( \text{Relative accuracy} = \frac{90.2}{95} \times 100 \right) = 94.9\%$$

**Table 3: Determination of phorate residue in vegetable samples by the proposed method and HPLC**

Vegetable samples	Mean of pesticide residue (mg/kg)		CODEX – MRL (mg/kg)
	Proposed Method	HPLC	
Tomatoes	0.15	0.12	0.01
Garden egg	0.08	0.10	0.01
Pepper	0.04	0.05	0.01