# A Simple and Sensitive Kinetic Method for Determination of Dichlorvos

## Sunita Kurup* and Ajai K. Pillai

**Department of Chemistry, Govt. V. Y. T. P G. Autonomous College, Durg (C.G.) - 491001, India.**

**\*Corres. author: sunik09@gmail.com**

**Abstract:** A simple kinetic catalytic method for the determination of dichlorvos is described. The method is based on its catalytic effect on oxidation of rhodamine B by potassium bromate in sulfuric acid medium. The reaction has been followed spectrophotometrically by measuring the decrease in the absorbance of rhodamine B at 555 nm. A fixed time method of 240 s from initiation of the reaction was used. Under optimum conditions, the proposed method allows the determination of dichlorvos concentrations in the range 0.04-0.4 µg mL$^{-1}$ with molar absorptivity of $1.97 \times 10^5$ L mol$^{-1}$cm$^{-1}$. The limit of detection and quantification were 0.018 and 0.055 µg mL$^{-1}$, respectively. The method was found to be sensitive, selective and was applied for the determination of dichlorvos in food materials with satisfactory results.
**Keywords:** Dichlorvos, Catalytic, Kinetic, Rhodamine B, Bromate.

## Introduction

Pesticides have made major contributions to agriculture and disease control, but widespread use has created serious concerns regarding their effects on the environment and on human health. During recent years, organophosphorus pesticides have been widely used in agriculture because of their low environmental persistence and high effectiveness[1]. Organophosphorous pesticides are widely used in agriculture and their properties provide numerous benefits in terms of production and quality increase[2]. Their toxicology is important because of the frequent accidental exposure to humans during their widespread use in farming and because of the non occupational exposure through environmental routes[3, 4]. The organophosphorous insecticides and their activated metabolites are potent anti-cholinesterases (anti- ChEs) which display a wide range of acute toxicity levels in mammals.

Dichlorvos, 2,2-dichlorovinyl-*O*,*O*-dimethyl phosphate (DDVP), is a chlorinated organophosphate insecticide (Scheme 1) that is extensively used in many countries for controlling insect pests on agricultural, commercial, domestic and industrial sites[5]. The common trade name of DDVP are Dedevap, Nogos, Nuvan and Vapona, etc. Compared to other pesticides, dichlorvos is generally preferred because of its cost effectiveness and wide range of bioactivity. It is a systemic insecticide and acaricide, used to control sucking, chewing and boring insects and spider mites on a very wide range of crops. It is of great value for fumigation of ware houses and other enclosed places. The formulation is used in apple and pear orchards to control leaf miners, apple moths and on cabbage to control leaf eating caterpillars, etc.
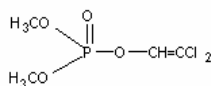


**Scheme 1** Structure of dichlorvos

Dichlorvos is toxic to fish and aquatic arthropods, and highly toxic to birds and honeybees. Concerns were expressed over acute and chronic toxicity in human beings[6]. It is readily absorbed through the skin, because it is volatile. Inhalation is the most common route of exposure. Dermal absorption and ingestion also occurs causing acute toxicity[7]. Detection limit for DDVP is 6.8 µg/mL[8]. Trace amounts of this insecticide may remain in foodstuffs or drinking water that threatens human health. Therefore, simple, fast and accurate methods are needed to monitor the pesticides residue in food, water sources, so as to minimize the possible health risk[9, 10].

A number of methods have been developed for the detection of dichlorvos. Various analytical method such as flow injection[11], tandem mass spectrometry[12], pH sensitive fluorescence probe[13], GC-ECD[14], GC-FPD[15], GC-MS[16], GC[17, 18], partial least square discriminant analysis[19], HPLC[20-21], biosensor analysis[22], TLC[23-24], oxidation by hydrogen peroxide[25]. Various methods have been reported for spectrophotometric determination of DDVP[26-30]. The method described are generally based on its hydrolysis to produce dichloroacetaldehyde and its reaction with reagents like resorcinol[26], J-acid[27], phloroglucinol[28], 2,4-dinitrophenyl hydrazine[29] and diphenyl semicarbazide[30]. In the present work, a simple catalytic kinetic method for determination of dichlorvos has been described based on redox reaction between rhodamine B and potassium bromate in acidic medium. Sensitivity, selectivity, rapidity and easy availability of apparatus and reagents and the simplicity of analytical procedure are advantages of this method.

## Experimental

### Apparatus and reagents

A Systronics Visiscan 167 Spectrophotometer was used for spectral measurements. All the reagents were of analytical grade. Distilled water was used throughout the experiments for preparation and dilution of reagents as well as samples.

Dichlorvos (76 % Hindustan Ciba-geigy Bombay, India) stock solution (0.1316, 1mg mL$^{-1}$) was prepared in ethanol (100 mL). Working standard solutions were prepared by appropriate dilution of the stock.
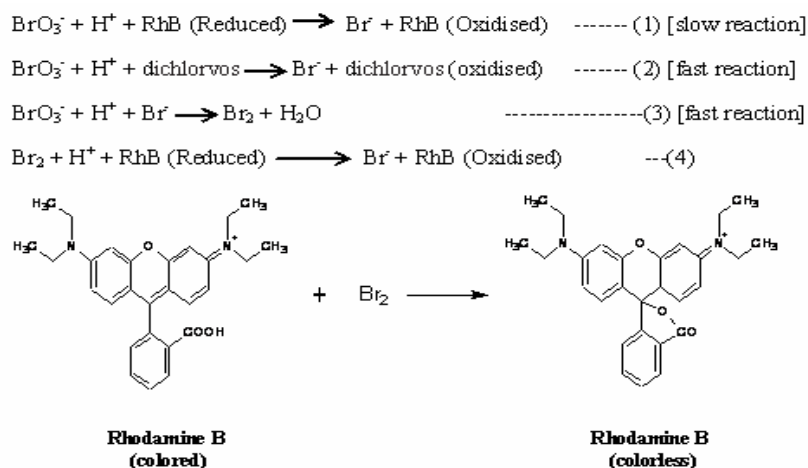
Potassium bromate (Merck, Mumbai, India) solution (1.0354 g, $6.2\times10^{-2}$ mol L$^{-1}$) was prepared in water (100 mL). Rhodamine B (BDH) solution (0.0201 g, $4.2\times10^{-4}$ mol L$^{-1}$) was prepared in water (100 mL). Sulfuric acid solution (2 mol L$^{-1}$) was prepared by diluting the appropriate volume of concentrated acid.

### Procedure

To a series of 10 mL volumetric flask, 1.0 mL of $4.2\times10^{-4}$ mol L$^{-1}$ of rhodamine B solution, 1.4 mL of 2.0 mol L$^{-1}$ sulphuric acid and different concentrations of dichlorvos were added in sequence. The solution was diluted to *ca.* 9 mL with water. Then 1.0 mL of $6.2\times10^{-2}$ mol L$^{-1}$ potassium bromate solution was added to the reaction mixture. Time was measured just after the addition of the bromate solution. After thorough mixing a portion of this solution was transferred to a quartz cell within 30 s for measurement. The decrease in absorbance of rhodamine B by bromate in acidic medium was monitored using the "fixed time method" by measuring the change in absorbance at 555 nm for the first 30-240 s from the start of the reaction ($A_S$). The blank reaction was performed according to the same procedure without addition of dichlorvos and the change in absorbance was labeled as ($A_b$). The standard curve was constructed between the difference between sample and blank absorbance change ($\Delta A_s - \Delta A_b$) *versus* dichlorvos concentration.

## Results and Discussion

Rhodamine B (RhB) is a dye which can be oxidized with strong oxidizing agent at very slow reaction rate. However, this reaction undergoes rapid oxidation when dichlorvos was added at trace level. The possible mechanism of the reaction may be attributed in its simplest form as (Scheme 2):

$$BrO_3^- + H^+ + RhB \text{ (Reduced)} \longrightarrow Br^- + RhB \text{ (Oxidised)} \quad \text{------ (1) [slow reaction]}$$

$$BrO_3^- + H^+ + dichlorvos \longrightarrow Br^- + dichlorvos \text{ (oxidised)} \quad \text{------ (2) [fast reaction]}$$

$$BrO_3^- + H^+ + Br^- \longrightarrow Br_2 + H_2O \quad \text{---------------(3) [fast reaction]}$$

$$Br_2 + H^+ + RhB \text{ (Reduced)} \longrightarrow Br^- + RhB \text{ (Oxidised)} \quad \text{--(4)}$$



**Rhodamine B** (colored)  + $Br_2$ →  **Rhodamine B** (colorless)

**Scheme 2** Mechanism of the reaction

The rate of reaction depends on concentration of sulfuric acid, bromate and rhodamine B. But, under the chosen conditions, the sulfuric acid, bromate, and rhodamine B concentrations are greater than dichlorvos concentration, and their concentrations are constant as the reaction proceeds. Therefore, the variation of signal is proportional to the dichlorvos concentration where the other variables and the reaction time and temperature are held constant for the given system.

**Effect of reagent concentration**

The reaction variables were optimized in order to maximize the sensitivity and precision of the proposed catalytic kinetic method. The effect of reagents concentration, temperature and time on the rate of reaction was studied, where each variable was changed in turn keeping all other constant. The optimum condition used for the final working procedure in order to obtain best figures of merit.

*Effect of sulphuric acid concentration*

That effect of sulfuric acid concentration on obtaining maximum sensitivity was investigated for catalyzed and uncatalyzed reaction (Fig. 1). The results show that the sensitivity increases with increase in acid concentration up to 2.8 mol $L^{-1}$ whereas; at higher acid concentration decrease in sensitivity is observed. Therefore 2.8 mol $L^{-1}$ of sulphuric acid was selected as the optimum working concentration.
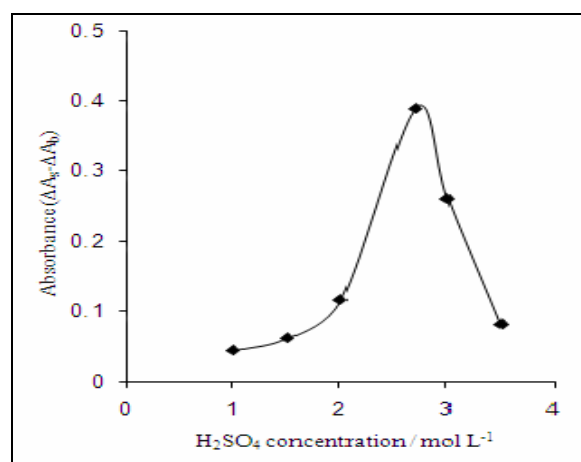


**Figure 1** Effect of $H_2SO_4$ concentration on the sensitivity.

*Effect of RhB concentration*

The effect of rhodamine B concentration on the sensitivity was investigated in the range of $2.1\times10^{-4}$-$5.0\times10^{-4}$ mol $L^{-1}$. The results shows that by increasing the rhodamine B concentration up to $4.2\times10^{-4}$ mol $L^{-1}$ the

sensitivity increases and at higher concentration decrease in sensitivity is observed (Fig. 2). According to the results, $4.2 \times 10^{-4}$ mol $L^{-1}$ rhodamine B was selected as the optimum working concentration.
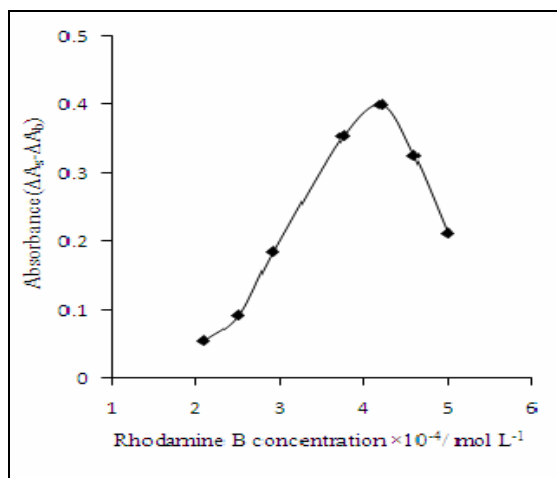


**Figure 2** Effect of rhodamine B concentration on the sensitivity.

### *Effect of bromate concentration*

The effect of bromate concentration on the reaction rate was investigated in the range of $3.5 \times 10^{-2} - 7.4 \times 10^{-2}$ mol $L^{-1}$. The results show that by increasing the bromate concentration up to $6.2 \times 10^{-2}$ mol $L^{-1}$ the sensitivity increased, whereas higher concentration of bromate caused decrease in sensitivity (Fig. 3). Therefore, $6.2 \times 10^{-2}$ mol $L^{-1}$ bromate concentration was chosen as the optimum concentration.
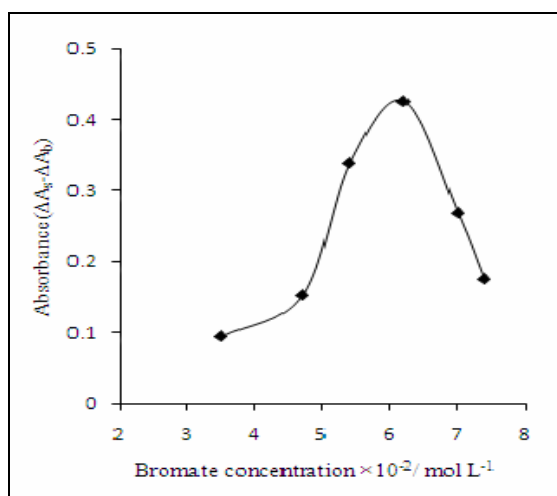


**Figure 3** Effect of $KBrO_3$ concentration on the sensitivity.

### *Effect of temperature and time*

The kinetic characteristics of the proposed method were studied by measuring absorbance change as a function of time (Fig. 4). Under the conditions were the other experimental variables were kept optimum, effect of time was investigated by measuring the absorbance change during 30-300 s after initiation of reaction. 240 s was chosen as optimum time, because it provided the best correlation coefficient and sensitivity. The temperature of the reaction mixture was varied over the range 10–50 °C. An increase in temperature caused an increase in the rate of both catalyzed and uncatalyzed reactions. However, the rate of the blank reaction increased much faster with increase in temperature causing a net decrease in the signal-to-blank ratio. Thus, 30 °C was chosen as the optimum temperature for dichlorvos determination.
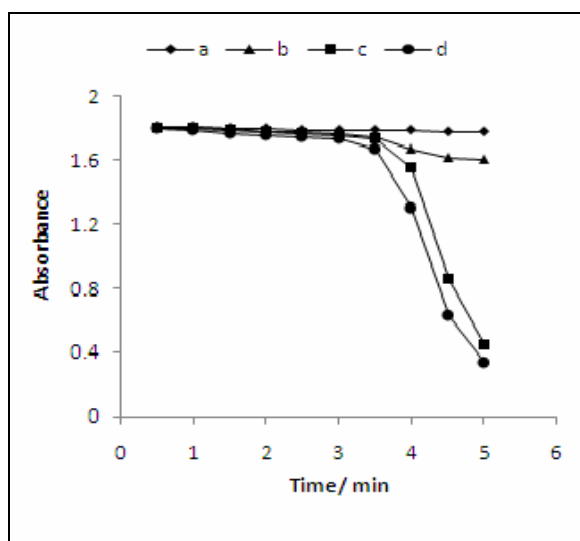
**Figure 4** Kinetic behavior of proposed reaction in presence of $H_2SO_4$: 2.8 mol $L^{-1}$; rhodamine B: $4.2 \times 10^{-4}$ mol $L^{-1}$; $KBrO_3$: $6.2 \times 10^{-2}$ mol $L^{-1}$. (a) Blank (b) 0.05 µg $mL^{-1}$ (c) 0.1 µg $mL^{-1}$ (d) 0.2 µg $mL^{-1}$ dichlorvos.

## Analytical characteristics

Under the optimum experimental conditions mentioned above, the calibration graph was obtained by plotting $(\Delta A_s - \Delta A_b)$ *versus* dichlorvos concentration. From the results of experiments, fixed-time of 240 s was chosen as the optimal time, because it provided the best correlation coefficient and sensitivity. The calibration graph was linear in the range of 0.04-0.4 µg $mL^{-1}$ of dichlorvos (Fig. 5). The molar absorptivities and Sandell's sensitivity of dichlorvos are given in Table 1. The slope, intercept, and the correlation coefficient were calculated by least square regression analysis (Table 1).The limits of detection and quantification (LOD = 3.3 $\sigma$ /S and LOQ = 10 $\sigma$ /S, where $\sigma$ is the slope of the calibration curve), SD of slope and intercept calculated are given in Table 1. The correlation coefficient was 0.9993, which indicated a good linear correlation between $(\Delta A_s - \Delta A_b)$ and dichlorvos concentration. Each point in the calibration graph was the average of five replicates. The relative standard deviation for seven replicate determination of 0.2 and 0.3 µg $mL^{-1}$ of dichlorvos solution was 1.91 % and 2.05 % respectively. Comparisons of the proposed method with published spectroscopic methods are summarized in Table 2.

**Table 1** Analytical and method validation parameters

| Parameter | Dichlorvos |
|---|---|
| $\lambda_{max}$ (nm) | 555 |
| Limit of Beer's Law (µg $mL^{-1}$) | 0.04-0.4 |
| Molar absorptivity (L $mol^{-1}cm^{-1}$) | $1.97 \times 10^5$ |
| Sandell's sensitivity (µg $cm^{-2}$) | 0.0011 |
| Limit of detection (µg $mL^{-1}$) | 0.018 |
| Limit of quantification (µg $mL^{-1}$) | 0.055 |
| Regression equation(y = bx + a)* | |
| Correlation coefficient (r) | 0.999 |
| Slope (b) | 1.99 |
| SD of slope | 0.019 |
| Intercept (a) | 0.015 |
| SD of intercept | 0.004 |
| *Concentration in µg $mL^{-1}$ | |

**Table 2** Comparisons of the proposed method with published spectroscopic methods

| S.No | Reagent used | $\lambda_{max}$ (nm) | Dynamic range ($\mu g\ mL^{-1}$) | Remarks |
|------|--------------|---------|----------------|---------|
| 1. | Resorcinol[26] | 490 | 1-20 | Dye unstable and high reagent blank |
| 2. | J-acid[27] | 470 | 1-10 | Less sensitive |
| 3. | Phloroglucinol[28] | 475 | 0.4-4.0 | Less sensitive |
| 4. | 2,4-dinitrophenyl hydrazine[29] | 450 | 0.21-1.68 | Less sensitive |
| 5 | Diphenyl semicarbazide[30] | 490 | 0.18-1.36 | Less sensitive |
| 6 | Rhodamine B [Present method] | 555 | 0.04-0.4 | High sensitivity |
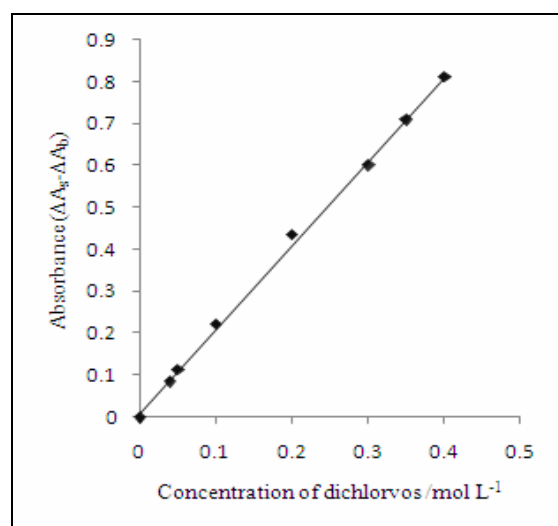


**Figure 5** Calibration line for determination of dichlorvos.

**Interference study**

To study the selectivity of the proposed method, the effect of foreign ions and pesticides expected to exist in field samples of dichlorvos studied by adding known amount of different foreign ions and pesticides to the test solution containing 0.2 $\mu g\ mL^{-1}$ dichlorvos. The tolerance limit was defined as the concentration of the added ion causing not more than ± 2 % relative error for the determination of 0.2 $\mu g\ mL^{-1}$ dichlorvos. The results are summarized in Table 3, which clearly indicates that most common ions and pesticides do not interfere with the catalytic determination.

**Table 3** The effect of foreign ions and pesticides on reaction

| Interfering ions and pesticides | Tolerance limit ($\mu g\ mL^{-1}$)[a] |
|---|---|
| $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, $CH_3COO^-$, $Al^{3+}$ | 1000 |
| ziram, propoxur, dinocap, atrazine, benzene | 1000 |
| $Cd^{2+}$, $NO_3^-$, $NH_4^+$ | 800 |
| Formaldehyde, Aniline, glucose | 500 |
| $Mn^{2+}$, $Zn^{2+}$, $CO_3^{2-}$ | 450 |
| $Cr^{3+}$, $Fe^{2+\,b}$, $Sn^{2+}$, $Cu^{2+}$ | 100 |

[a]Causing(±) 2 % variation in absorbance value;
[b]masking by the addition of 0.2 mol $L^{-1}$ EDTA

**Application**

*Determination of dichlorvos in vegetable samples*

To check the applicability of the method, the amount of dichlorvos was analyzed in vegetable samples collected from agriculture fields. The samples were found to be free from dichlorvos. Therefore known amount of dichlorvos was spiked to 20 g of cabbage and spinach and left for 48 h. The vegetable leaves were cut into

slices. The slices were put into a conical flask and thoroughly mixed with dichloromethane (30 mL) and sodium carbonate (15 g). Then the mixture was allowed to stand for 12 h in a well-sealed conical flask. The mixture was filtered through a filter paper and then the flask was washed with dichloromethane. The filtered liquid phase was taken in an open watch glass. When dichloromethane was dried out, methanol (5 mL) was added to extract dichlorvos. The extraction was repeated twice with methanol (2 mL). These extractions were mixed and diluted by methanol to 10 mL and analyzed by the proposed as well as reported method[30] and the results are shown in Table 4.

**Table 4** Determination of dichlorvos in vegetable samples

| Sample | Dichlorvos added (µg) | Proposed method | | Reported method | |
|---|---|---|---|---|---|
| | | Total dichlorvos recovery found [a](µg) | % | Total dichlorvos recovery found [a](µg) | % |
| Cabbage | 2 | 1.95 | 97.50 | 1.93 | 96.50 |
| | 4 | 3.86 | 96.50 | 3.88 | 97.00 |
| Spinach | 2 | 1.91 | 95.50 | 1.92 | 96.00 |
| | 4 | 3.78 | 94.50 | 3.74 | 93.50 |
| [a] Mean of three observations. | | | | | |

### *Determination of dichlorvos in citrus juice (orange juice)*

Citrus juice was fortified with known amount of pesticides and extracted with 2×10 mL of acetone. The crude extract was partitioned between 5% sodium chloride solution and dichloromethane and the dichloromethane layer was concentrated by evaporating to dryness. The residue was dissolved in a mixture of acetone and n-hexane and made up to the mark and the aliquot was taken, separated and analyzed by the proposed as well as reported method[30]. The results obtained by the proposed method were compared statistically with the reported method[30] by applying Student's t-test for accuracy and F-test for precision (Table 5).

The results shows that the t and F-values at 95% confidence level are less than the theoretical values[31], which confirmed that there is a good agreement between the results obtained by the proposed method and the reported method[30] with respect to accuracy and precision.

**Table 5** Determination of dichlorvos in orange juice

| Sample | Dichlorvos added (µg) | Proposed method | | Reported method | |
|---|---|---|---|---|---|
| | | Total dichlorvos recovery found [a](µg) | % | Total dichlorvos recovery found [a](µg) | % |
| Orange juice | 5 | $4.876 \pm 0.023$ <br> t = 1.391 <br> F = 1.278 | 97.52 | $4.898 \pm 0.026$ | 98.96 |
| | 10 | $9.836 \pm 0.024$ <br> t = 1.787 <br> F = 1.19 | 98.36 | $9.746 \pm 0.022$ | 97.46 |

[a] Mean of five observations. Tabulated t-value at the 95 % confidence level is 2.306. Tabulated F value at the 95 % confidence level is 5.05.

## Conclusions

A simple and sensitive kinetic method for determination of dichlorvos has been described. The method is based on its catalytic effect on oxidation of rhodamine B by bromate in sulfuric acid media, without the need for extraction, preconcentration and preseparation. It offers advantages like reliability and reproducibility in addition to its simplicity and suffers from less interference.

It has been successfully applied to the determination of dichlorvos in food materials with satisfactory results. The statistical parameter and recovery study data clearly indicates the precision and accuracy of the method.

## Acknowledgements

## References

1. Everett W.R. and Rechnitz G.A., Anal. Chem., 1998, 70, 807-810.
2. Russo M.V., Campanella, L. and Avino, P., J. Chromatogr., B, 2002, 780, 431-441.
3. Yamashita, M., Yamashita, M., Tanaka, J. and Ando, Y., Vet. Hum. Toxicol., 1997, 39, 84-85.
4. Azaroff, L.S., Environ. Res., 1999, 80, 138-147.
5. Gan Q., Singh R.M., Wu T. and Jans U., Environ. Sci. Technol., 2006, 40, 5717-5723.
6. Oncescu T., Stefan M.I. and Oancea P., Environ. Sci. Pollut. Res. Int., 2010, 17, 1158-1166.
7. Gallo M.A. and Lawryk N.J., Organic Phosphorous Pesticides, In Handbook of Pesticide Toxicology, By Hayes Jr. W.J., Laws Jr. E.R., Academic Press: New York, 1991.
8. Tomsej T. and Hajsloa J., J. Chromatogr., A, 1995, 704, 513-517.
9. Patil V.B. and Shingare M.S., Talanta, 1994, 41, 367-369.
10. Jimenez A.M. and Navas M.J., Crit Rev Anal. Chem., 1997, 27, 291-305.
11. Wang J., Zhang C., Wang H., Yang F. and Zhang X., Talanta, 2001, 54, 1185-1193.
12. Nakazawa H., Takahashi N., Inoue K., Ito Y., Goto T., Kato K., Yoshimura Y. and Oka H., Talanta, 2004, 64, 899-905.
13. Jin S., Xu Z., Chen J., Liang X., Wu Y. and Qian X., Anal. Chim. Acta, 2004, 523, 117-123.
14. Chen Y.I., Su Y.S. and Jen J.F., J. Chromatogr., A, 2002, 976, 349-355.
15. Nobuyuki Y., Jutaro T., Kumiko S., Masatake T., Ryuji N. and Kikuyoshi Y., J. Food Hygienic Soc. Japan, 2002, 43, 196-201.
16. Nordgren I., Fundam Appl Toxicol, 1981, 1, 230-237.
17. Guven H., Tuncok Y.,Gidener S., Gelal A., Demetci M., Fowler J., Apaydin S. and Keskin M., J. Toxicol. Clin. Toxicol., 1994, 32, 157-163.
18. Hall G.L., Mourer C.R. and Shibamoto T., J. Agric. Food Chem., 1997, 45, 145-148.
19. Wang S., Xiang B., Su Y. and Tang Q., Environ. Chem. Lett., 2012, 10, 383-387.
20. Huang G., Ouyang J., Baeyens W.R.G., Yang Y. and Tao C., Anal. Chim. Acta, 2002, 474, 21-29.
21. Wang S., Xiang B. and Tang Q., J. Chromatogr. Sci., 2012, 50, 702-708.
22. Vidal J.C., Esteban S., Gil J. and Castillo J.R., Talanta, 2006, 68, 791-799.
23. Janghel E.K., Rai J.K., Khan S., Rai M.K. and Gupta V.K., J. Environ. Sci. Eng., 2007, 49, 133-138.
24. Mali B.D., Garad M.V., Patil V.B. and Padalikar S.V., J. Chromatogr., A, 1995, 704, 540-543.
25. Lu M.C., Chen J.N. and Chang C.P., J. Hazard. Mater., 1999, 65, 277-288.
26. Rangaswamy J.R. and Muthu M., Microchim. Acta, 1984, 84, 433-443.
27. Shivhare P. and Gupta V.K., J. Ind. Chem. Soc., 1991, 68, 287-289.
28. Asthana A., Pillai A.K. and Gupta V.K., Ind. J. Chem. Technol., 2003, 10, 96-98.
29. Dhundhel R.S., Rai M.K., Gupta V.K., Janghel E.K. and Rai J.K., J. Ind. Chem. Soc., 2006, 83, 1282-1284.
30. Janghel E.K., Rai M.K., Gupta V.K. and Rai J.K., J. Chin. Chem. Soc., 2007, 54, 345-350.
31. Christian G.D., Analytical Chemistry; 6th Ed., John Wiley and Sons, New York, 2004.

\*\*\*\*\*