

Spectrophotometric Determination of Some Organophosphorus Pesticides Based on Reaction with Cerium (IV) Sulfate

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ABSTRACT

A simple and sensitive spectrophotometric method has been described for the assay of some organophosphorus pesticides, (diazinon, chlorpyrifos and temefos) either in pure form or in dosage forms. The proposed method was based on the oxidation of the studied pesticides by a known excess of cerium (IV) sulfate in acidic medium and estimating the unreacted oxidizing agent by adding a known amount of methyl orange in the same acid medium and measuring the absorbance of the resulted solution at $\lambda_{\max}=508$ nm. Different variables affecting the reaction were studied and optimized. Beer's law is obeyed in the concentration range of 1-8 $\mu\text{g ml}^{-1}$ for diazinon and chlorpyrifos and 2-15 $\mu\text{g ml}^{-1}$ for temefos. The proposed method was applied successfully for determination of the examined pesticides either in pure or dosage forms with good precision. No interferences were observed from excipients and the results obtained were in good agreement with those obtained using reported methods.

Keywords : Spectrophotometry; Diazinon; Chlorpyrifos; Temefos; Cerium Sulphate; Methyl Orange.

I. INTRODUCTION

Organophosphates (OPP) are organic esters of phosphoric acid, thiophosphoric acid, which are widely used as insecticides and acaricides. These compounds exhibit a wide range of toxicity to mammals as they work on central nervous system. They are anticholinesterases that disable cholinesterase, an enzyme essential for the central nervous system function. [1-3]

Among organophosphorus pesticides are diazinon, chlorpyrifos and temefos, their chemical structures are shown in Fig.1

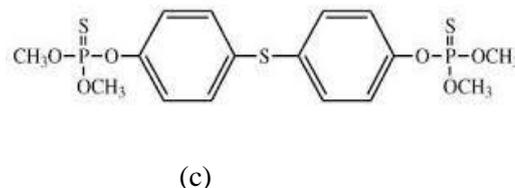
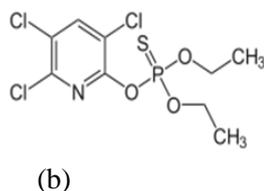
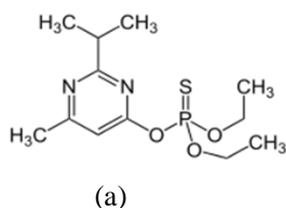


Figure 1: The chemical structures of (a) diazinon, (b) chlorpyrifos and (c) temefos.

These compounds are widely used and show severe toxicity so several methods were reported for their assay like LC-MS [4-6], titrimetry[7], voltammetry[8,9], GC [10-13], HPLC[14], atomic absorption spectrophotometry(AAS) [15] and spectrophotometry [16-19] have been reported in the literature for the determination of organophosphates. Most spectrophotometric procedures involve the determination of organophosphorus insecticides by total phosphorus measurement, based on the formation of molybdenum blue using various reducing agents [17-19].

Some of these methods suffer from poor sensitivity, instability of color or involve extraction where as others suffer from the interference with arsenic and copper,

blank absorption or longer time required for color development. To overcome these drawbacks, a rapid and sensitive method has been proposed for the determination of the studied organophosphorus insecticides by their reaction with cerium(IV) sulfate which is a powerful oxidimetric reagent. Due to its high oxidation potential and excellent solution stability, it was used for the quantitative determination of many compounds [20-23]. Because of its simplicity, speed, sensitivity, reasonable accuracy, precision, and cost-effectiveness, visible spectrophotometry continues to be the preferred technique in laboratories of developing and underdeveloped nations which can afford expensive chromatographic and other related techniques. The present method is based on the oxidation of the organophosphorus insecticides with slight excess of cerium (IV) sulfate to form oxidation products and the determination of the unreacted oxidant by treatment with methyl orange (MO).

II. METHODS AND MATERIAL

A. Instrumentation

All absorbance measurements were made with a double beam UV-1601PC (SHIMADZU, Japan) ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells and Temperature Controller was used for all spectrophotometric measurements.

B. Chemicals

All chemicals used were of analytical reagent grade. Chemicals (suppliers) were as follows: Diazinon(98.2%, Wenzhou Lucheng Dongon Dyestuff, Intermediate Plant, China), Chlorpyrifos (97.5%, JiangSu Knaida Agrochemical Co.,LTD, China), Temefos (99.1%, JiangSu Knaida Agrochemical Co.,LTD, China), Cerium(IV) sulphate (Scharlau Co., Spain), Methyl orange (Fluka Chemika Sigma-Aldrich), sulphuric acid (S.d.Fine Chem, Mumbai, India), Doubly distilled water was used to prepare all solutions.

C. Pharmaceutical Formulations

The following available commercial preparations were analyzed: Diazinon sand bait (Egychem) labeled to

contain 0.5g % diazinon, Insect stop E.C (Egychem) labeled to contain 25 g% chlorpyrifos , Temo larvae (egychem) labeled to contain 50g % temefos.

D. Reagents

Cerium (IV) Sulphate (250 $\mu\text{g ml}^{-1}$)

A stock cerium (IV) sulphate solution was prepared by dissolving 0.5 g of cerium (IV) sulphate in 1.0 M sulphuric acid and transfe into 50-ml volumetric flask. The volume was completed to the mark with the same acid. Stock solution was diluted appropriately with 1 M sulphuric acid to yield 250 $\mu\text{g ml}^{-1}$ cerium (IV) sulphate solution.

Methyl Orange (500 $\mu\text{g ml}^{-1}$)

It was prepared by dissolving 50 mg of methyl orange in water and transfe into a 100-ml volumetric flask. The volume was completed to the mark with water.

Sulphuric acid (5 M)

This was prepared by adding 274 ml of concentrated sulphuric acid to 726 ml water with cooling.

Preparation of stock and sample solutions

Stock Standard Solutions:

A stock standard solutions (1 mg ml^{-1}) of diazinon, chlorpyrifos and temefos, were prepared by accurately weighing 10 mg of pure sample of each pesticide, and transferring it separately into 10-mL volumetric flasks, with addition of methanol to make up to volume.

Diazinon Sample Solution:

A mass of 10 g of sand was transferred into 250-ml stoppered conical flask, 30 ml of methanol were added and shaken mechanically for about two hours. The prepared solution was filtered through Whatman No. 42 filter paper into 50-ml volumetric flask and completed to volume with methanol to obtain stock solution with a concentration of 1 mg ml^{-1} .

Chlorpyrifos Sample Solution:

A volume of 0.4 ml of insect stop E.C solution was transferred into a 100-ml volumetric flask, completed to volume with methanol to obtain stock solution with a concentration of 1 mg ml⁻¹.

Temefos Sample Solution:

A volume of 0.2 ml of temo larvae solution was transferred into a 100-ml volumetric flask, completed to volume with methanol to obtain stock solution with a concentration of 1 mg ml⁻¹.

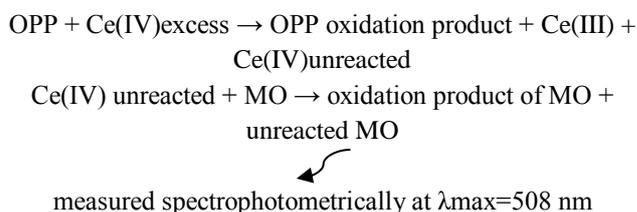
C. General Procedure:

Aliquots of each of diazinon, chlorpyrifos and temefos stock solutions were added, separately into different series of 10-mL volumetric flasks to give final concentrations of 1–8, 1-8 and 2-15 µg ml⁻¹, respectively. 1 ml of sulphuric acid (5 M) and 1 ml of cerium (IV) sulphate solution (250 µg ml⁻¹) were added to each flask. After mixing, flasks were allowed to stand at room temperature for 10 minutes with occasional shaking. Finally, 0.5 ml of methyl orange solution (500 µg ml⁻¹) was added. Each solution was diluted to the mark with water and mixed. After 5 minutes, UV scan was done and the absorbance of each solution was examined and measured at 508 nm against a reagent blank prepared in the same manner using 1 ml water instead of 1 ml methyl orange solution.

III. RESULTS AND DISCUSSION

The proposed method is an indirect spectrophotometric one based on the ability of cerium (IV) sulfate to oxidize the studied pesticides and to bleach the colour of the indicator methyl orange. The studied pesticides are allowed to react with a known excess of cerium (IV) sulfate in acidic media. The unreacted cerium (IV) sulphate found in excess over the pesticide is quantified by adding methyl orange and monitoring the absorbance of the solution at λ_{max} = 508 nm. In the presence of the oxidant the absorbance of the indicator solution decreases. Assuring that the oxidation reaction is complete, the unreacted cerium (IV) amount is proportional to the drug amount in the sample. According to scheme 1, with increased drug concentration in the

sample, the amount of unreacted Ce (IV) bleaching the dye is less and thus the solution absorbance measured at λ_{max}=508 nm increases linearly with the pesticide concentration (Fig. 2,3).



Scheme 1: Reaction scheme of the indirect determination of OPP by oxidation with cerium (IV) sulfate.

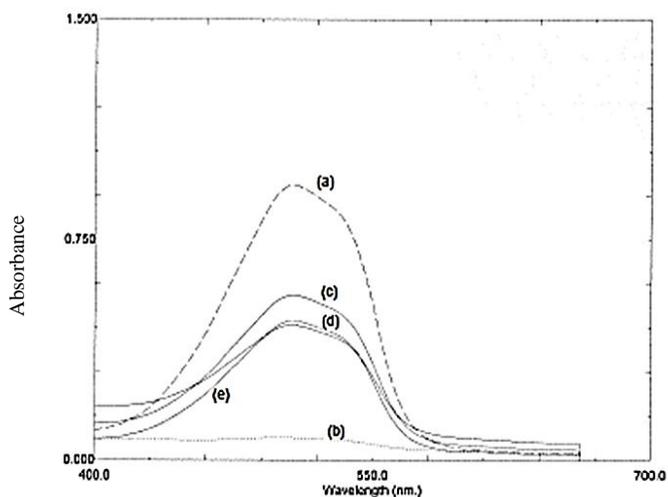


Figure 2 : Absorption spectra of solutions containing 0.5 M H₂SO₄ and

- (a) 25 µgml⁻¹ methyl orange (MO);
- (b) (a) + 1ml of 250 µg ml⁻¹ Ce(IV) sulfate;
- (c) (b) + 7 µg ml⁻¹ diazinon.;
- (d) (b)+7µg ml⁻¹ chlorpyrifos;
- (e) (b)+ 10µg ml⁻¹ temefos.

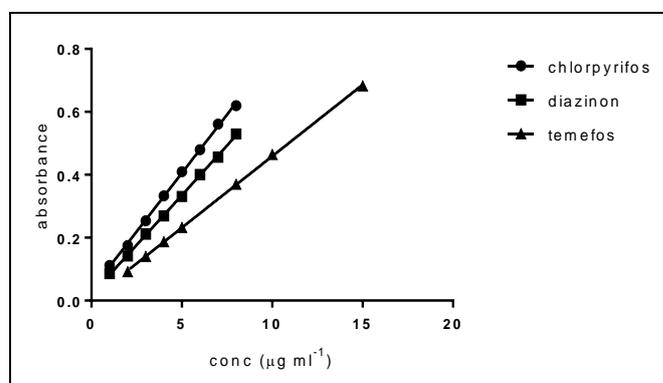


Figure 3: Calibration curves of diazinon, chlorpyrifos and temefos.

A. Method Optimization

1) Selection of methyl orange concentration:

The absorbance variation at $\lambda_{\max}=508$ nm with methyl orange concentration was investigated in the range 10-90 $\mu\text{g ml}^{-1}$ MO. The linear range was 5-30 $\mu\text{g ml}^{-1}$ MO. In order to minimize the photometric error for further investigations a concentration of 25 $\mu\text{g ml}^{-1}$ MO was selected which presented an absorbance of 0.907.

2) Optimisation of cerium (IV) sulfate concentration:

The absorbances of solutions containing a fixed concentration of MO (25 $\mu\text{g ml}^{-1}$) and different concentrations of cerium (IV) sulfate comprised in the range 2.5-25 $\mu\text{g ml}^{-1}$ Ce(IV) in 5 M H₂SO₄ have been measured and it was observed that a concentration of Ce(IV) higher than 25 $\mu\text{g ml}^{-1}$ Ce(SO₄)₂ bleaches completely the solution containing 25 $\mu\text{g ml}^{-1}$ MO (Fig. 4).

3) Influence of the order of reagents addition:

It was observed that regardless of OPP amount added, methyl orange is almost totally bleached if the reagents addition order was dye+ drug+ oxidant or dye+oxidant+drug. The reason of this observation is that the cerium (IV) sulfate didn't have enough time to oxidize the drug because it rapidly bleaches methyl orange. In conclusion, the drug and oxidant solutions respectively must be added first, their addition order doesn't influence the reaction and methyl orange have to be added after a given period of time during which the drug is totally oxidized by Ce(SO₄)₂.

4) The influence of time on the oxidation reaction of OPP by Ce(SO₄)₂:

It was observed that if methyl orange is added immediately to the solution containing OPP and Cerium (IV) sulfate in acidic medium the resulted solution is bleached rapidly and the absorbance is very low. This can be explained by the fact that the drug oxidation by cerium (IV) sulfate is a time developing reaction and thus the influence of the reaction time was studied. In this respect, separate solutions containing 7 $\mu\text{g ml}^{-1}$ diazinon,

7 $\mu\text{g ml}^{-1}$ chlorpyrifos and 10 $\mu\text{g ml}^{-1}$ temefos are allowed to react with 25 $\mu\text{g ml}^{-1}$ Cerium (IV) sulfate at darkness different times before adding the indicator and measuring the absorbance. It was observed that the absorbance of this solutions increases with the time up to 10 minutes remaining then constant (Fig. 5). Thus, for further measurements a reaction time of 10 minutes was selected.

5) The effect of sulphuric acid concentration:

The reaction was carried out in sulphuric acid medium. The highest absorption intensity was obtained at 5 M H₂SO₄ for diazinon, chlorpyrifos and temefos (Fig. 6). One mL of acid was used in the assay procedure.

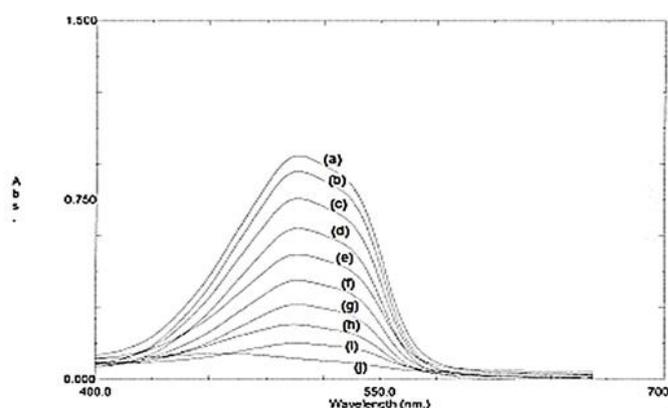


Figure 4 : The influence of the cerium (IV) sulfate concentration on the absorbance of a solution containing 25 $\mu\text{g ml}^{-1}$ MO.

Concentration of Ce(SO₄)₂ solution:(a)0, (b)2.5 μgml^{-1} , (c)5 μgml^{-1} , (d)7.5 μgml^{-1} , (e)10 μgml^{-1} , (f)12.5 μgml^{-1} , (g)15 μgml^{-1} , (h)17.5 μgml^{-1} , (i)20 μgml^{-1} and (j)25 μgml^{-1} .

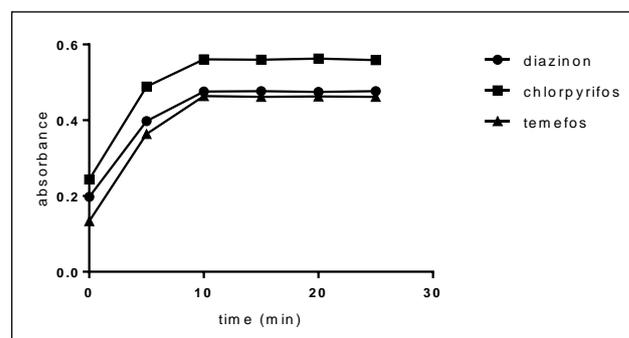


Figure 4 : Influence of time on the reaction of diazinon, chlorpyrifos and temefos oxidation by Ce(SO₄)₂ in 5 M H₂SO₄

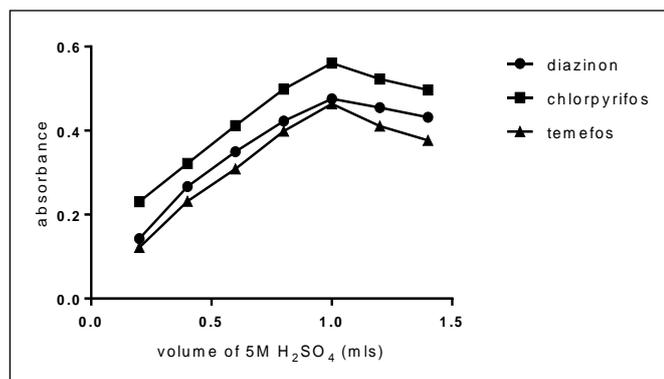


Figure 5 : Influence of volume added of 5M H₂SO₄ on oxidation of diazinon, chlorpyrifos and temefos by Ce(SO₄)₂

B. Method Validation

1) Analytical Parameters of the Method:

A linear relation was found between absorbance at λ_{max} and concentration of diazinon, chlorpyrifos and temefos in the ranges shown in Table 1. The calibration graphs are described by the equation:

$$Y = a + b X$$

(Where Y = absorbance, a = intercept, b = slope and X = concentration in $\mu\text{g ml}^{-1}$) obtained by the method of least squares. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to The International Conference of Harmonization (ICH) guidelines for validation of analytical procedures (ICH Guideline 2005). The following formula was used: LOD or LOQ = $kSDa/b$, where k = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The apparent molar absorptivity and Sandell sensitivity values together with the limits of detection and quantification compiled in Table 1 are indicative of the high sensitivity of the proposed method.

2) Accuracy and Precision

The accuracy and precision of the method were evaluated by performing five replicate analyses on pure drug solutions at three different amount/ concentration levels (within the working ranges). The RSD (%), an indicator of accuracy did not exceed 0.244, 0.752 and 1.048% for diazinon, chlorpyrifos and temefos respectively, and intraday precision which is also called

the repeatability expressed in relative standard deviation (RSD) (%) was within 0.072-0.085 for diazinon, 0.087-0.232 for chlorpyrifos and 0.109-0.213 for temefos, indicating the high accuracy and precision of the method. The results of this study are compiled in Table 3. The reproducibility of the method, also called the day-to-day precision or intermediate precision, was assessed by performing replicate analyses on pure drug solutions at three levels over a period of five days preparing all solutions afresh each day. The day-to-day RSD values were less than 1% reflecting the usefulness of the method in routine analysis of the investigated pesticides in quality control laboratories.

C. Recovery Studies of the Proposed Method

The accuracy and precision of the method were further assessed by performing recovery experiments. To a fixed amount of each drug in the dosage form, pure drug was added at three different levels and the total was found by the proposed method. Each test was performed in triplicate. The percent recoveries of the added pure diazinon, chlorpyrifos and temefos were in the range of 100.75-101.5, 100.5-101.67 and 101-101.67% respectively revealing good accuracies and non-interference from excipients and diluents. This was further confirmed by the fact that no more than the stoichiometric amount of cerium (IV) was consumed when the pesticides solutions or bait were treated with cerium (IV) under the described experimental conditions.

D. Application of the proposed method to analysis of diazinon, chlorpyrifos and temefos in dosage forms

Commercial dosage forms containing diazinon, chlorpyrifos and temefos were successfully analyzed by the proposed method. Co-formulated substances did not interfere. It is evident from the above-mentioned results that the proposed method gave satisfactory results with the three pesticides. Thus their dosage forms were subjected to the analysis of their diazinon, chlorpyrifos and temefos contents in each case by the proposed method. The label claim percentages were 100.42, 99.19 and 100.61% for diazinon, chlorpyrifos and temefos respectively. This result was compared with that obtained from reported methods (24, 25, 26) by statistical analysis with respect to the accuracy (by t-test) and precision (by F-test). No significant differences

were found between the calculated and theoretical values of t- and F-tests at 95% confidence level proving similar accuracy and precision in the determination of diazinon, chlorpyrifos and temefos by both methods (Table 5).

Table (1) : Regression equations and validation parameters for the proposed spectrophotometric method for the determination of diazinon, chlorpyrifos and temephos.

Item	Diazinon	Chlorpyrifos	Temephos
Linearity range ($\mu\text{g ml}^{-1}$)	1-8	1-8	2-15
Molar absorbitivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	19.238×10^3	26.049×10^3	21.196×10^3
Sandell's sensitivity* ($\mu\text{g cm}^{-2}$)	0.01580	0.01346	0.02200
Regression equation*:			0.04544
Slope	0.06321	0.07430	0.004678
Intercept	0.01879	0.03379	0.9998
Regression coefficient (r)	0.9993	0.9990	0.0002856
Standard error of the slope	0.0006946	0.0009368	$0.04544 \pm 7 \times 10^{-4}$
Confidence limit of the slope	$0.06321 \pm 1.70 \times 10^{-3}$	$0.07430 \pm 2.29 \times 10^{-3}$	0.00221
Standard error of the intercept	0.003507	0.004731	$0.004678 \pm 5.41 \times 10^{-3}$
Confidence limit of the intercept	$0.01879 \pm 8.58 \times 10^{-3}$	$0.03379 \pm 11.57 \times 10^{-3}$	
LOD**	0.213	0.315	0.623
LOQ***	0.645	0.954	1.888

* $Y = a + bX$, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in $\mu\text{g ml}^{-1}$.

**LOD is limit of detection = $\frac{3.3 \times \sigma}{S}$ where σ is the standard deviation of 5 replicate determinations under the same conditions as for the sample analysis in the absence of the analyte and S is the sensitivity, namely the slope of the calibration graph.

***LOQ is the limit of quantification = $\frac{10 \times \sigma}{S}$.

Table (2): Determination of diazinon, chlorpyrifos and temephos in pure form by the proposed spectrophotometric method.

Diazinon			Chlorpyrifos			Temephos		
Taken $\mu\text{g ml}^{-1}$	Found* $\mu\text{g ml}^{-1}$	Recovery %	Taken $\mu\text{g ml}^{-1}$	Found* $\mu\text{g ml}^{-1}$	Recovery %	Taken $\mu\text{g ml}^{-1}$	Found* $\mu\text{g ml}^{-1}$	Recovery %
3.00	3.04	101.33	3.00	2.98	99.33	3.00	3.02	100.67
5.00	5.09	101.80	5.00	4.95	99.00	5.00	4.98	99.60
7.00	7.10	101.43	7.00	7.03	100.43	7.00	7.12	101.71
Mean		101.52	Mean		99.59	Mean		100.66
SD		0.248	SD		0.749	SD		1.055
RSD%		0.244	RSD%		0.752	RSD%		1.048

* Average of five determinations.

Table (3): Intra and inter-day validation for the determination of diazinon, chlorpyrifos and temephos using the proposed spectrophotometric method.

Investigated drug	Precision items				
	Concentration ($\mu\text{g ml}^{-1}$)	Repeatability		Intermediate precision	
		Recovery % \pm SD	RSD%	Recovery % \pm SD	RSD%
Diazinon	2	100.90 \pm 0.076	0.075	101.50 \pm 0.143	0.141
	4	101.59 \pm 0.086	0.085	99.50 \pm 0.209	0.210
	8	100.38 \pm 0.072	0.072	101.75 \pm 0.095	0.093
Chlorpyrifos	3	99.67 \pm 0.087	0.087	100.87 \pm 0.121	0.120
	6	100.93 \pm 0.167	0.165	101.33 \pm 0.095	0.094
	8	98.50 \pm 0.229	0.232	100.63 \pm 0.356	0.354
Temephos	4	99.90 \pm 0.109	0.109	101.00 \pm 0.151	0.149
	8	100.83 \pm 0.215	0.213	101.10 \pm 0.350	0.346
	12	101.87 \pm 0.180	0.177	99.90 \pm 0.252	0.252

Table (4): Statistical analysis of the results obtained by applying the proposed spectrophotometric and reported methods^(26,27,28) for the determination of pure diazinon, chlorpyrifos and temefos.

Drug	Proposed method	Reported methods ^(24, 25, 26)	n	Student's t-test	F value
Diazinon	100.23 \pm 0.390	100.34 \pm 0.453	5	0.412	1.349
Chlorpyrifos	99.65 \pm 0.800	99.97 \pm 0.912	5	0.589	1.299
Temefos	100.18 \pm 0.460	100.50 \pm 0.322	5	1.274	2.041

The theoretical values of t-test= 2.780 and of F value= 6.390 at 95% confidence level

Table (6): Statistical analysis of the results obtained by applying the proposed spectrophotometric and reported methods^(26,27,28) for the determination of diazinon, chlorpyrifos and temefos in dosage forms.

Drug	Proposed method	Reported methods ^(24, 25, 26)	n	Student's t-test	F value
Diazinon in Diazinon 0.5g% sand bait Batch no. 0066/2016	100.42 \pm 0.750	100.23 \pm 0.730	5	0.406	1.056
Chlorpyrifos in Insect stop 25g% E.C Batch no.088/2016	99.19 \pm 0.260	99.418 \pm 0.190	5	1.583	1.873
Temefos in Temo larvae 50g% Batch no.0088/2015	100.61 \pm 0.920	100.41 \pm 0.522	5	0.423	3.106

The theoretical values of t-test= 2.780 and of F-value=6.390 at 95% confidence level

IV. CONCLUSION

The present study described the successful development of new, simple, sensitive, selective, accurate and rapid spectrophotometric method for the accurate determination of diazinon, chlorpyrifos and temefos; each one in its dosage forms using cerium (IV) sulphate as the oxidimetric reagent. The proposed method is superior to the previously reported spectrophotometric methods for the determination of diazinon, chlorpyrifos and temefos in terms of their simplicity. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. The other advantages include that, the method involve the measurement of stable coloured species, have shorter contact times and free from extraction and boiling step compared to many of the previously reported procedures. Therefore, the method is practical and valuable for routine analysis in quality control laboratories for analysis of each investigated pesticide

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