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Spectrophotometric determination of dimethoate and deltamethrin insecticides in their formulations, environmental and biological samples using cerric (IV) ammonium sulfate

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Abstract

Two simple, sinsitive and accurate spectrophotometric methods have been developed for the determination of two insecticides (dimethoate and deltamethrin) in pure forms, formulations and environmental samples. In this technique we investigated the development of indirect redox spectrophotometric reaction for determination of deltamethrin or dimethoate with known excess of cerric(IV) ammonium sulfate as an oxidizing agent in acidic medium, followed by determination of unreacted cerric (IV) by adding a fixed amount of Amaranth (AM) or Rhodamin 6G (Rh6G) and measuring the absorbance at λ_{max} 520 and 525 nm for AM and Rh6G methods, respectively. The beer's law was obeyed in the concentration ranges 0.5-4.0 and 0.5-4.5 µg mL^{-1} for deltamethrin and 0.5-5.0 and 0.5-6.0 µg mL^{-1} for dimethoate using AM and Rh6G methods, respectively with correlation coefficients ≥ 0.9993. The molar absorptivity and Sandell's sensitivity, detection and quantification limits were calculated. Intra-day and inter-day accuracy and precision of the methods have been evaluated. No interference was observed from the additives. The proposed methods have been successfully applied for the determination of deltamethrin and dimethoate in its formulations and environmental samples.

Keywords: dimethoate; deltamethrin; cerric (iv) ammonium sulfate; amaranth; rhodamin 6g; spectrophotometry; formulations; environmental samples

1. Introduction

Pyrethroid insecticides are derived from natural compounds (the pyrethrins), isolated from *Chrysanthemum* genus of plants. Pyrethroid pesticides, used on crops like cotton, appear to be a significant source of sediment toxicity in urban and agriculturally dominated streams. Pyrethroids act as neurotoxins and target the central nervous system of insects [1]. Pyrethroid pesticides are toxic to man and animals, carcinogenic to human and exert genotoxic, mutagenic and embryotoxic effects [2]. The use of pyrethroid insecticides is increasing for agriculture, commercial pest control and residential consumer use. The insecticides are highly stable under acidic conditions, though unstable at pH 8.0. Synthetic pyrethroids containing nitrile group such as, deltamethrin: (*S*)-cyano-3-phenoxybenzyl (1*R*, 3*R*)-3-(2, 2-dibromovinyl)-2,2-dimethyl cyclopropane carboxylate (Scheme 1) is synthetic pyrethroid that has been used for the management of a variety of insect pests [3]. Deltamethrin kills insects on contact and through digestion. It works by paralyzing the insect's nervous system and therefore giving a quick knock down effect.

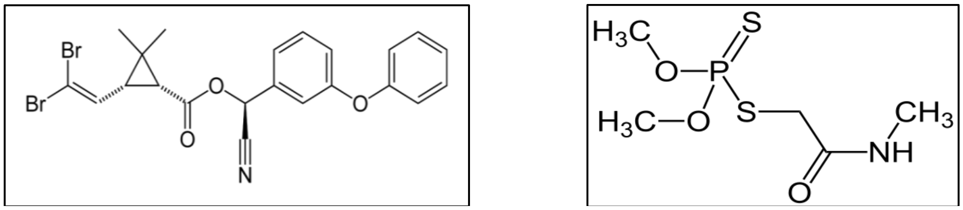

Deltamethrin Dimethoate

Fig 1: The chemical structure of deltamethrin and dimethoate.

Organophosphates are organic esters of phosphoric acid, thiophosphoric acid and other phosphoric acids, which are widely used as insecticides and aca-ricides. Organophosphorus compounds exhibit a wide range of toxicity to mammals. They work on central nervous system inhibiting its normal function resulting in convulsions, paralysis and death. Dimethoate is O, O-dimethyl S-[2-(methylamino)-2-oxoethyl] dithiophosphate, a widely used organophosphate insecticide used to kill insects on contact. Like other organophosphates, dimethoate is an anticholinesterase which disables cholinesterase, an enzyme essential for the central nervous system function [4].

Because of its significance, several analytical methods have been developed for the determination of synthetic pyrethroid deltamethrin based on the use of chromatographic techniques which include high performance liquid chromatography (HPLC) [5-9], LC-MS, [10], GC-ECD [11, 12] and solid phase extraction [13] and electrochemical methods [14-17]. These techniques required large number of solvents for the extractions and also some limitations in terms of high cost of instruments used in routine analysis and matrix effects.

Also several analytical methods have been developed for the determination of dimethoate based on liquid chromatography with mass spectrommetry (LC-MS) [18], gas chromatography [19], Electrophoresis [20], Fluorimetrry [21], spectrophotometry [22-25] 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 [26]. 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.

Spectrophotometric method is still one of the important analytical techniques in most quality control and clinical laboratories and the determination of pesticides n formulations and environmental samples because it is less expensive and easy to use. The availability of spectrophotometric apparatus and the simplicity of analytical procedures made this technique very attractive for a wide range of applications. Various spectrophotometric methods have also been reported for determination of deltamethrin and dimethoate [27-30].

From the foregoing paragraphs, it is clear that cerium (IV) ammonium sulphate despite their strong oxidizing power, versatility, and high oxidation potential and stability in solution has not been applied for the assay of deltamethrin and dimethoate in pure forms, formulations and environmental samples.

The aim of the present work is to develop facile, rapid, low cost, accurate, sensitive and validated spectrophotometric method for the determination of deltamethrin and dimethoate based on the oxidation of the studied insecticides with slight excess of ceric (IV) ammonium sulfate in acidic medium. The unconsumed ceric (IV) is then estimated by adding a fixed amount of AM and Rh6G dyes to form colored species which absorbs maximally at 520 and 525 nm, respectively. The proposed methods have been successfully applied for the determination of deltamethrin and dimethoate in pure forms, formulations and environmental samples.

2. Materials and Methods

2.1 Apparatus

All the absorption spectral measurements were made using Jenway 6405 (UV-Visible) spectrophotometer (British) with scanning speed 400 nm min^{-1} and band width 5.0 nm equipped with 10 mm matched quartz cells.

2.2 Materials and reagents

All chemicals and reagents used were of Analytical reagnet grade or of the best available quality and all solvents were for spectroscopic grade. Double distilled water was used throughout the experiment. Also all solutions were prepared fresh daily.

2.3 Standard solution

Stock solutions (100 µg mL^{-1}) of insecticides (dimethoate and deltamethrin) were prepared by dissolving 10 mg of insecticide (technical forms and formulations) in minimum amount of glacial acetic acid (Merck) and then diluting to 100 mL with distilled water. The standard solution were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use.

2.4 Reagents

Cerium (IV) ammonium sulphate (3.0×10^{-3} mol L^{-1})

A stock solution of cerric (IV) ammonium sulfate $Ce(NH_4)_4(SO_4)_4.2H_2O$ (Sigma Aldrich Co. St. Louis, USA) (3.0×10^{-3} mol L^{-1}) was prepared by dissolving 189.7 mg of [$CeN_4H_{20}S_4O_{18}$, M.Wt.= 632.55 g mol^{-1}] in the least amount of H_2SO_4 (1.0 mol L^{-1}) then completed to the mark in a 100 mL calibrated flask with the same acid and kept in a dark bottle and a refrigerator when not in use.

Sulfuric acid (H_2SO_4) (1.0 mol L^{-1})

A stock solution of 1.0 mol L^{-1} H_2SO_4 was prepared by adding 5.4 mL of concentrated acid (Merck, Darmstadt, Germany, 98%, Sp. Gr. 1.84) to bidistilled water, cooled to room temperature, transfer to 100 mL with measuring flask, diluted to the mark and standardized as recorded.

Dyes (1.0×10^{-3} mol L^{-1})

A stock solutions of amaranth (AM) and rhodamine 6G (Rh6G) (1.0×10^{-3} mol L^{-1}) were prepared by dissolving accurately weighed 112 mg of each dye (Sigma-aldrish, 90 % dye content) in bidistilled water and diluting to volume in a 100 mL calibrated flask.

2.5 Recommended procedures

Different aliquots (0.1-0.9 and0.1-0.8 mL) for deltamethrin (50 µg mL^{-1}) and (0.1-1.2 and 0.1-1.0 mL) for dimethoate (50 µg mL^{-1}) using AM and Rh6G, respectively were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 5.0 mL by adding adequate quantity of bidistilled water. To each flask, 2.0 mL of 1.0 mol L^{-1} H_2SO_4 and 2.0 mL of (3.0×10^{-3} mol L^{-1}) cerric(IV) ammonium sulfate were added. The flasks were stoppered and the contents were mixed well and the flasks were kept in boiled water bath for 5.0 min with occasional shaking. Finally, the solution was cooled and 1.0 mL of (1.0×10^{-3} mol L^{-1}) AM dye and 0.7 and 1.0 mL (1.0×10^{-3} mol L^{-1}) of Rh6G, for deltamethrin and dimethoate, respectively wwere added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The decrease in color intensity of AM and Rh6G dye were measured after 5.0 min against reagent blank solution treated similarly at their corresponding λ_{max} 520 and 525 nm for AM and Rh6G dye, respectively. The concentration of unknown was determined from calibration graph which obtained by plotting the concentration of deltamethrin or dimethoate against the absorbance of dye at the corresponding λ_{max} or computed from the regression equation derived using Beer's law data.

2.6 Procedure for formulations

The contents of three bottles of deltamethirn or dimethoate formulations were mixed and the average volume for one bottle was determined. An aliquot of the solution equivalent to 20 mg each insecticide was transferred to 100 mL calibrated flask and dissolved in minimum amount of glacial acetic acid and made up to the mark with bidistilled water. Aliquot of the cited solutions was taken and analyzed as described under the above spectrophotometric methods using cerric(IV) ammonium sulphate for construction of calibration curves. For the proposed methods, the concentration of the studied insecticide was calculated using the corresponding regression equation of the appropriate calibration graph. The method of standard addition was used for the accurate determination of deltamethirn or dimethoate contents.

2.7 Procedure for environmental samples

2.7.1 Water samples

A volume of 100 mL runoff water (tap and river water) were collected in PTHE bottles from different agriculture fields where dimethoate and deltamethrin had been sprayed. The samples were filtered and 1.0 mL of 5.0% EDTA was added to each of them to remove various metal ions. Each sample was extracted with chloroform (2.0 x 100 mL). The extracts were combined and washed with 20 mL of 0.1 M K_2CO_3 solution to break any emulsions. The chloroform extracts were dried over anhydrous sodium sulfate in a filter funnel and the filtrate was collected in a 250 mL calibrated flask. The filter funnel was washed with 20 mL of chloroform and the volume of the filtrate was made up to the mark, known aliquots of chloroform extracts were taken and evaporated on a water bath at about 50°C. The residue was dissolved in 10 mL methanol. The solution was then transferred into a separating funnel with 25 mL of water [31] and to it sodium hydroxide was added and analyzed as described above.

2.8 Vegetables and fruits samples

Various samples of vegetables (tomatoes and cauliflower) and fruits (apples), each of 25 g, were collected from agricultural fields, where dimethoate (40%) or deltamethrin (5%) had been sprayed as a n insecticide. The samples were macerated with two 20 mL portions of ethanol-distilled water (1 : 1), filtered through a thin cotton cloth and the filtrate was centrifuged. The filtrate was quantitatively transferred into 25 mL volumetric flask and made up to the mark with 50% ethanol. Aliquots of supernatant were taken in a 25 mL graduated cylinder and then analyzed. Filtrate from foliages were passed through a silica gel N extracted sample [31]. The column was washed with 10 mL of 50% ethanol, washings were collected in a 25 mL volumetric flask and analyzed as recommended by the above procedures.

3. Results and Discussion

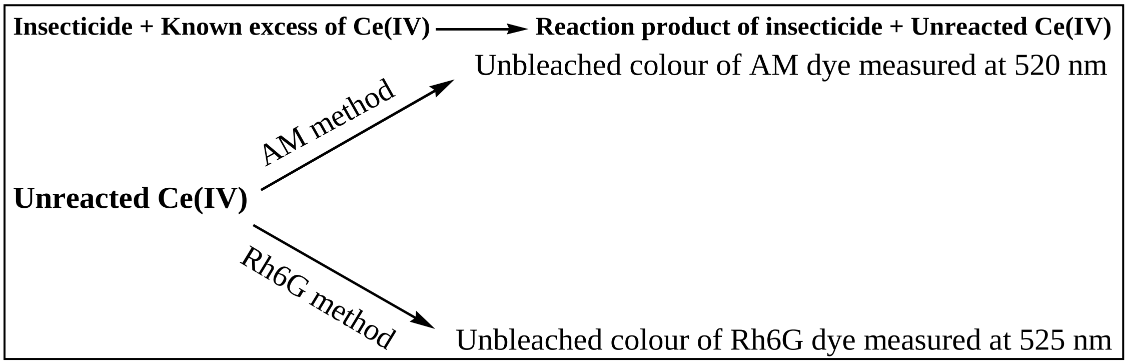
3.1 Absorption spectra

Cerric (IV) ammonium sulfate is a versatile oxidimetric reagent. Since of its high oxidation potential and excellent solutions stability, it was used for the quantitative determination of many compounds.

Cerium (IV) ammonium sulphate, because of its high oxidation potential and excellent solution stability, has been widely used as an effective analytical reagent in spectrophotometric determination of many compounds [32,33]. The analytical reactions involves two steps namely:

1. Reaction of dimethoate or deltamethrin with a known excess of oxidant Ce(IV) in acidic medium under the effect of heating.
2. Estimation of the residual unreacted oxidant Ce(IV) via its reaction with a fixed amount of AM or Rh6G dyes and measuring the decrease in colour of AM or Rh6G at λ_{max} 520 or 525 nm, respectively.

The tentative reaction scheme of spectrophotometric methods is shown in Scheme 1. In all methods, the absorbance increased linearly with increasing concentration of dimethoate or deltamethrin. The latter methods make use of the bleaching action of oxidant on dyes, the discoloration being caused by the oxidative destruction of the dye.



Scheme 1: The suggested reaction pathway for the proposed spectrophotometric methods using Ce(IV) and AM and Rh6G dyes

3.2 Optimization of the reaction conditions

The optimum conditions for the assay procedures and color development for each method have been established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

3.2.1 Effect of acid type and concentration

In order to investigate the effect of acid concentration, different types of acids were examined (HCl, H_2SO_4, H_3PO_4, HNO_3 and CH_3COOH) to achieve maximum yield of redox reactions. Better results were suitable in sulphuric acid (H_2SO_4) (1.0 mol L^{-1}) with Ce(IV) as oxidant. The effect of H_2SO_4 concentration on the reaction between insecticide and Ce(IV) was studied by varying the volume of H_2SO_4 (1.0 mol L^{-1} H_2SO_4) from 0.25-3.0 mL, keeping the concentration of oxidant and insecticide fixed. The results indicated that, at 1.0-3.0 mL of H_2SO_4 (1.0 mol L^{-1}), there were almost same absorbance values were obtained in the presence of dimethoate or deltamethrin (Fig. 2.). At the acid volumes less than 2.0 mL, reaction led to go slower and incomplete. Therefore, 2.0 mL of H_2SO_4 (1.0 mol L^{-1}) was the optimum volume for subsequent studies.

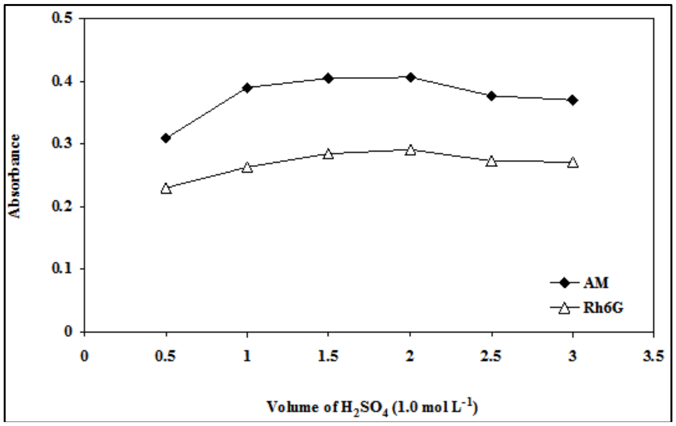


Fig. 2. Effect of volume of H_2SO_4 (1.0 mol L^{-1}) of the oxidation product of deltamethrin with Ce(IV) and dyes.

3.2.2 Effect of Ce (IV) reagent concentration

To investigate the optimum concentration of Ce(IV), different volumes of oxidant were treated in the range of 0.25–3.0 mL with a fixed concentration of dyes in optimum H_2SO_4 medium (2.0 mL, 1.0 mol L^{-1}) and the absorbance measured at 520 and 525 nm for AM and Rh6G, respectively. The results indicate that constant and maximum absorbance resulted with 2.0 mL of (3.0 x $l0^{-3}$ mol L^{-1}) Ce(IV) solution; hence, different concentrations of dimethoate or deltamethrin were treated with 2.0 mL of (3.0 x $l0^{-3}$ mol L^{-1}) Ce(IV) in H_2SO_4 medium before determining the residual Ce(IV) by reacting with AM or Rh6G (Fig. 3).

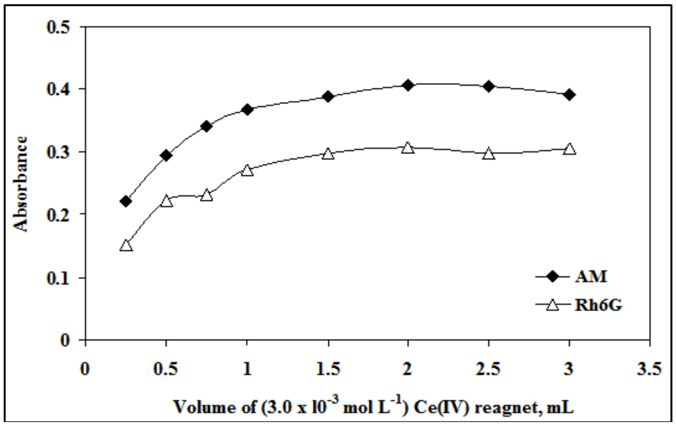


Fig 3: Effect of volume of Ce(IV) (3.0x 10^{-3} mol L^{-1}) of the oxidation product of dimethoate in presence of H_2SO_4 (2.0 mL, 1.0 mol L^{-1}) and dyes.

3.2.3 Effect of dye concentration

The effect of dye concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of Ce(IV). The effect of dye concentration was studied using different volumes (0.25–3.0 mL) of (1.0×10^{-3} mol L^{-1}) AM and Rh6G dyes (Fig. 4). It was observed that maximum color intensity of the oxidation products was achieved with 1.0 mL of AM dye solution for deltamethrin or dimethoate and0.8 and 1.0 mL of Rh6G dye solution for deltamethrin and dimethoate, respectively (Fig. 4). The color was found to be stable up to 12 h.

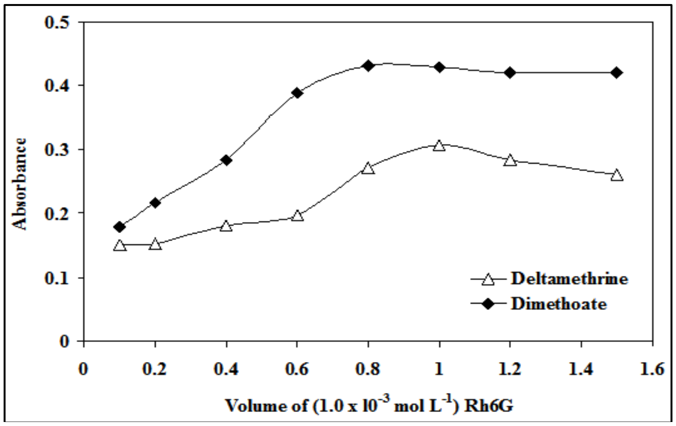


Fig. 4. Effect of volume of Rh6G (1.0x 10^{-3} mol L^{-1}) of the oxidation product of deltamethrin and dimethoate in presence of Ce (IV) (3.0x 10^{-3} mol L^{-1}) and H_2SO_4 (1.0 mol L^{-1}).

3.2.4 Effect of temperature and time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 30 to 100 °C in water bath. It was found that raising the temperature accelerate the oxidation process and give reproducible results, so maximum color intensity was obtained in boiling water bath. The effect of mixing time required completing oxidation of insecticide and for reducing the excess oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0–20 min. It was found that the contact times gave constant and reproducible absorbance values at 5.0 min in boiling water bath (Fig. 5). After oxidation process, 5.0 min standing time was found necessary for the complete bleaching of the dye color by the residual Ce(IV) and the absorbance of the unreacted dye was stable for at least 12 h, thereafter.

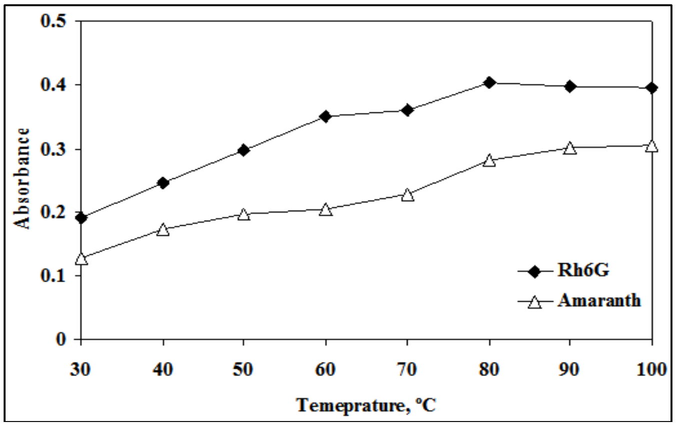


Fig. 5. Effect of temperature on the oxidation of dimethoate in presence of Ce (IV) (3.0 x 10^{-3} mol L^{-1}) H_2SO_4 (1.0 mol L^{-1}) and dyes.

3.2.5 Effect of sequence of addition

After optimizing all other experimental variables, further experiments were performed to ascertain the influence of sequence of addition of reactants on the color development by measuring the absorbance. The optimum sequence of addition was Insecticide –H_2SO_4– Ce(IV)–dye. Other sequences gave lower absorbance values under the same experimental conditions.

3.3 Method of validation

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

3.3.1 Linearity and sensitivity

Under the optimum conditions a linear correlation was found between absorbance at λ_{max} and the concentration of deltamethrine and dimethoate in the ranges of 0.5-4.5 and 0.5-6.0 µg mL^{-1}, respectively. The calibration graph is described by the equation:

$$A = a + b\,C \qquad (1)$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg mL^{-1}, obtained by the method of least squares. Correlation coefficient, intercept and slope of the calibration data are summarized in Table 1. For accurate determination, Ringbom concentration range [34] was calculated by plotting log concentration of drug in µg mL^{-1} against transmittance % from which the linear portion of the curve gives an accurate range of microdetermination of the studied insecticides and represented in Table 1. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines [35] and illustrated in Table 1. The high molar absorptivity and lower Sandell's sensitivity values reflects the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis [36] between the results achieved from the proposed methods and that of the reported methods [24, 37]. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 1), there is no significant difference between the proposed and reported method [25] regarding accuracy and precision.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas [35, 36]:

$$LOD=3.3\sigma/s \text{ and } OQ=10\sigma/s \qquad (2)$$

Where σ is the standard deviation of five reagent blank determinations, and s is the slope of the calibration curve.

Table 1: Analytical and regression parameters of proposed oxidation spectrophotometric methods for determination of deltamethrin and dimethoate.

Parameters	Deltamethrin		Dimethoate	
	AM	**Rh6G**	**AM**	**Rh6G**
Beer's law limits, µg mL^{-1}	0.5-4.0	0.5-4.5	0.5-5.0	0.5-6.0
Ringboom limits, µg mL^{-1}	1.0-3.0	1.0-3.5	1.0-4.0	1.0-5.0
Molar absorptivity, x 10^4 L mol^{-1} cm^{-1}	3.9855	5.0351	1.433	1.7442
Sandell sensitivity, ng cm^{-2}	12.68	10.03	16.0	13.14
Regression equation,[a]				
Intercept (a)	-0.0044	0.002	0.0013	0.0042
Standard deviation of intercept ($S_{a)}$	0.0028	0.0011	0.0096	0.0054
Slope (b)	0.084	0.0978	0.0612	0.0722
Standard deviation of slope (S_b)	0.0053	0.0037	0.0075	0.0062
Correlation coefficient, (r)	0.9995	0.9993	0.9997	0.9993
Mean ± SD	99.45 ± 1.20	99.60 ±0.80	99.50 ±0.90	99.20 ±0.75
RSD%	1.21	0.80	0.90	0.74
RE%	1.27	0.84	0.94	0.78
Limit of detection, µg mL^{-1}	0.146	0.133	0.14	0.124
Limit of quantification, µg mL^{-1}	0.487	0.443	0.47	0.41
Calculated *t*-value [b]	0.37	0.49	0.27	0.72
Calculated *F*-value [b]	1.59	1.43	1.80	2.64

[a] $A = a + bC$, where *C* is the concentration in µg mL^{-1}, *A* is the absorbance units, *a* is the intercept, *b* is the slope.

[b] The theoretical values of *t* and *F* are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (*p*= 0.05).

3.3.2 Accuracy and precision

In order to evaluate the precision of the proposed methods, solutions containing three different concentration levels of deltamethrin or dimethoate within the linearity range were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 2. The low values of the relative standard deviation (% R.S.D) and percentage relative error (% RE) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation

$$\%\,R.E. = \left[\frac{found - taken}{taken}\right] \times 100 \qquad (3)$$

The assay procedure was repeated six times, and percentage relative standard deviation (% R.S.D) values were obtained within the same day to evaluate repeatability (intra-day precision), and over five different days to evaluate reproducibility (inter-day precision).

For the same concentrations of insecticides inter- and intra-day accuracy of the methods were also evaluated. The percentage recovery values with respect to found concentrations of each insecticide were evaluated to ascertain the accuracy of the methods. The recovery values close to 100% as compiled in Table 2 shows that the proposed methods are very accurate.

Table 2: Evaluation of intra-day and inter-day accuracy and precision for deltamethirn and dimethoate obtained by the proposed methods.

Method	**Taken ($\mu g\ mL^{-1}$)**	**Intra-day**				**Inter-day**			
		Recovery %	**Precision RSD % [a]**	**Accuracy RE %**	**Confidence Limit [b]**	**Recovery %**	**Precision RSD % [a]**	**Accuracy RE %**	**Confidence Limit [b]**
Deltamethrin									
AM	1.0	99.10	0.64	-0.90	0.991 ± 0.007	99.40	0.63	-0.60	0.994 ± 0.007
	2.0	98.30	1.30	-1.70	1.966± 0.027	98.90	1.10	-1.10	1.978± 0.023
	4.0	98.70	1.50	-1.30	3.948± 0.062	98.50	1.40	-1.50	3.94 ± 0.058
Rh6G	1.0	98.60	0.74	-1.40	0.986 ± 0.008	99.20	0.90	-0.80	0.992 ± 0.009
	2.0	99.00	1.40	-1.00	1.98 ± 0.029	99.00	1.30	-1.00	1.980 ± 0.027
	4.0	98.80	1.20	-1.20	3.952±0.05	98.70	1.70	-1.30	3.948± 0.070
	Dimethoate								
AM	1.0	98.10	0.57	-1.90	0.981 ± 0.006	98.60	0.70	-1.40	0.986 ± 0.007
	2.0	99.00	0.93	-1.00	1.98 ±0.019	99.20	0.95	-0.80	1.984 ±0.02
	4.0	98.50	1.25	-1.50	3.94 ± 0.052	98.30	1.15	-1.70	3.932 ± 0.047
Rh6G	1.0	99.10	0.70	-0.90	0.991 ± 0.007	99.30	0.66	-0.70	0.993 ± 0.007
	2.0	99.40	1.05	-0.60	1.988 ±0.022	98.40	1.35	-1.60	1.968 ±0.028
	4.0	98.90	1.60	-1.10	3.956 ± 0.066	99.00	1.50	-1.00	3.96 ± 0.062

[a] RSD%, percentage relative standard deviation; RE%, percentage relative error.

[b] Mean ± standard error.

3.3.3 Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables, including volume of H_2SO_4 and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. The analysis was performed with altered conditions by taking three different concentrations of insecticide and it was found that small variation of method variables did not significantly affect the procedures as shown by the RSD values in the range of 0.75-2.40% and 0.88-2.40% for deltamethirn and dimethoate, respectively. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of and so the proposed spectrophotometric methods are considered robust. Method ruggedness was expressed as the RSD and was also tested by applying the proposed methods to the assay of deltamethirn and dimethoate using the same operational conditions but using three different instruments as well as three different anaysts. The inter-analysts RSD were in the ranges 0.85-2.30% and 0.80-2.60% for deltamethirn and dimethoate, respectively whereas the inter-instruments RSD ranged from 0.90 - 1.50% and 1.10 - 2.50% for deltamethirn and dimethoate, respectively suggesting that the developed methods were rugged. The results are shown in Table 3.

Table 3: Results of method robustness and ruggedness (all values in %RSD) studies.

Method	**Nominal amount concentration ($\mu g\ mL^{-1}$)**	**RSD%**			
		Robustness		**Ruggedness**	
		Variable alerted [a]			
		Acid volume (n=3)	**Reaction time (n=3)**	**Different analysts (n=3)**	**Different instruments (n=3)**
Deltamethirn					
AM	1.0	0.75	1.10	1.20	0.90
	2.0	1.10	1.50	1.70	1.10
	4.0	2.40	1.90	2.30	1.70
Rh6G	1.0	1.04	0.90	0.85	1.12
	2.0	1.80	1.40	1.20	1.60
	4.0	2.20	1.75	1.70	2.50
Dimethoate					
AM	1.0	0.88	0.65	0.80	1.10
	2.0	1.25	1.35	1.50	1.68
	4.0	1.90	2.20	2.30	2.05
Rh6G	1.0	1.07	0.78	1.0	1.20
	2.0	1.80	1.60	1.95	1.80
	4.0	2.20	2.40	2.60	2.50

[a] Volume of 1.0 mol L^{-1} H_2SO_4 is (2.0 ± 0.2 mL) and reaction time is (5.0 ± 1.0 min) (after adding Ce(IV)) were used.

3.3.4 Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure insecticide (50, 100 and 150% of the level present in the insecticide formulations) to a fixed amount of insecticides in formulation (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\% \text{ Recovery} = \frac{[C_F - C_T]}{C_p} \text{ x } 100 \qquad (2)$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the formulation; C_P is a concentration of analyte (pure insecticide) added to formulations. The results of this study presented in Table 4 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in formulations which did not interfere in the assay.

Table 4: Results of recovery experiments by the standard addition method

Formulations	**Taken Conc. from formulation ($\mu g\ mL^{-1}$)**	**Pure pesticide added ($\mu g\ mL^{-1}$)**	**Total found ($\mu g\ mL^{-1}$)**	**Recovery% ^{a} ± SD**
AM method				
DeltaGard SC (0.5 %)	1.0	1.0	1.972	98.60 ± 0.22
		2.0	2.970	99.00 ± 0.85
		3.0	3.952	98.80 ± 1.10
Deltamethrin (5.0% EC)	1.0	1.0	1.974	98.70 ± 0.38
		2.0	2.949	98.30 ± 0.74
		3.0	3.924	98.10 ± 0.90
Delta kil (2.5% EC)	1.0	1.0	1.982	99.10 ± 0.17
		2.0	2.946	98.20 ± 0.40
		3.0	3.940	98.50 ± 0.85
Dimethoate (40% EC)	1.0	1.0	1.976	98.80 ± 0.26
		2.0	2.976	99.20 ± 0.43
		4.0	3.976	99.40 ± 1.05
Rh6G method				
DeltaGard SC (0.5 %)	1.0	1.0	1.976	98.80 ± 0.16
		2.0	2.973	99.10 ± 0.32
		3.0	3.936	98.40 ± 0.59
Deltamethrin (5.0% EC)	1.0	1.0	1.984	99.20 ± 0.20
		2.0	2.961	98.70 ± 0.39
		3.0	3.944	98.60 ± 0.60
Delta kil (2.5% EC)	1.0	1.0	1.980	99.00 ± 0.240
		2.0	2.973	99.10 ± 0.480
		3.0	3.944	98.60 ± 0.78
Dimethoate (40% EC)	1.0	1.0	1.982	99.10 ± 0.30
		2.0	2.955	98.50 ± 0.52
		4.0	3.932	98.30 ± 0.90

[a] Mean value of three determinations

3.4 Application to formulations

The proposed methods were applied to the determination of deltamethirn and dimethoate in their formulations. The results in Table 5 showed that the methods are successful for the determination of deltamethirn or dimethoate and the excipients in the formulations do not interfere. The results obtained from the assay of deltamethirn and dimethoate by the proposed methods and reference methods [24, 37] for deltamethirn and dimethoate, respectively for the same batch of material is presented in Table 5. The results agreed well with the label claim and also were in agreement with the results obtained by the reference methods. When the results were statistically compared with those of the reference methods by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values of 2.57 and 5.05, respectively, for five degrees of freedom [36]. Hence, no significant difference existed between the proposed methods and the reference methods at the 95 % confidence level with respect to accuracy and precision.

Table 5: Application of the proposed methods for the determination of deltamethirn and dimethoate in their formulations

Samples	**Proposed method**		**Reference methods**
	AM	**Rh6G**	
DeltaGard SC (0.5 %)			[37]
Recovery ± RSD ^{a}	96.80 ± 0.93	97.20 ± 0.69	96.40 ± 0.85
t-Value ^{b}	0.71	1.63	
F-Value ^{b}	1.20	1.52	
Deltamethrin (5.0% EC)			[37]
Recovery ± RSD ^{a}	96.87 ± 1.13	96.83 ± 0.87	96.0 ± 1.05
t-Value ^{b}	1.26	1.36	

F-Value [b]	1.16	1.46	
Delta kil (2.5% EC)			[37]
Recovery ± RSD [a]	98.10± 0.86	97.90 ± 0.96	97.30 ± 0.74
t-Value [b]	0.78	1.107	
F-Value [b]	1.35	1.68	
Dimethoate (40% EC)			[24]
Recovery ± RSD [a]	99.03± 0.41	98.73 ± 0.59	98.40 ± 0.66
t-Value [b]	1.77	0.83	
F-Value [b]	2.59	1.25	

[a] Mean for six independent analyses.

[b] The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

3.5 Applications to environmental samples

The proposed methods were used for the determination of deltamethrin and dimethoate in water samples (tap and river), grains (rice and wheat) samples vegetables (tomatoes and cauliflower) and fruit (apples**)** samples (Table 6).

Table 6: Estimation of deltamethrin and dimethoate in fortified water, grain, vegetables and fruits samples using the proposed methods

Samples	**Added amount (µg)**	**Deltamethrin**				**Dimethoate**			
		AM method		**Rh6G method**		**AM method**		**Rh6G method**	
		Found amount[a] (µg)	**Recovery (%)**	**Found amount[a] (µg)**	**Recovery (%)**	**Found amount[a] (µg)**	**Recovery (%)**	**Found amount[a] (µg)**	**Recovery (%)**
Tap water[b]	1.0	0.97	97.0	0.93	93.0	0.94	94.0	0.95	95.0
	2.0	1.90	95.0	1.92	96.0	1.90	95.0	1.84	92.0
	4.0	3.88	94.0	3.76	94.0	3.76	94.0	3.84	96.0
River water[b]	1.0	0.95	95.0	0.94	94.0	0.96	96.0	0.93	93.0
	2.0	1.92	96.0	1.92	96.0	1.88	94.0	1.90	95.0
	4.0	3.84	96.0	3.72	93.0	3.72	93.0	3.68	92.0
Rice[c]	2.0	1.90	95.0	1.88	94.0	1.88	94.0	1.88	94.0
	4.0	3.78	94.50	3.80	95.0	3.80	95.0	3.72	93.0
Wheat[c]	2.0	1.90	95.0	1.92	96.0	1.92	96.0	1.92	96.0
	4.0	3.84	96.0	3.68	92.0	3.84	96.0	3.72	93.0
Tomatoes[c]	2.0	1.86	93.0	1.86	93.0	1.88	94.0	1.90	95.0
	4.0	3.80	95.0	3.84	96.0	3.80	95.0	3.76	94.0
Cauliflower[c]	2.0	1.88	94.0	1.88	94.0	1.86	93.0	1.92	96.0
	4.0	3.80	95.0	3.68	92.0	3.76	94.0	3.72	93.0
Apples[c]	2.0	1.94	97.0	1.88	94.0	1.84	92.0	1.88	94.0
	4.0	3.76	94.0	3.84	96.0	3.88	97.0	3.68	92.0

[a] Mean of six replicates analyses.

[b] Water sample 25 mL, 1.0 mL aliquots of sample was analyzed, after treatment as described in the procedure.

[c] Sample 25 g (sample taken from a field where deltamethrin had been sprayed).

4. Conclusions

A new, useful simple, rapid and cost-effective spectrophotometric methods have been developed for determination of deltamethrin and dimmethoate in pure forms, formulations and environmental samples using ceric (IV) ammonium sulfate as oxidizing agents and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous method reported for deltamethrin and dimmethoate. The assay methods have some additional advantages involve, less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of deltamethrin and dimmethoate in pure forms, formulations and environmental samples.

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