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Utilization of Alizarin Red Sulphonate (ARS) as Chromogenic Reagent for Determination of 2,4-Dichlorophenoxyacetic acid pesticide (2,4-D) Residues

Research Article

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Abstract

Spectrophotometric method has been developed and validated for the determination of 2,4-D residues in water and tomato. The proposed method is based on the derivatization of 2,4-D pesticide with Alizarin Red Sulphonate (ARS) as chromogenic reagent. In slightly acidic medium pH 6, a dark Purple – colored product exhibiting maximum absorption peak (λ_{max}) at 563 nm was formed. The variables that affected the reaction such as pH, temperature, reaction time and the amount of ARS reagent and buffer were carefully studied and optimized. Under the optimum conditions, Beer's law is obeyed in the range 3- 40 µg/mL of 2,4-D at maximum wavelength of 563 nm. The linear regression equation of the calibration curve is A= 0.0795 + 0.0212 c (µg/mL), with a linear regression correlation coefficient of 0.9995. The molar absorptivity was $6.35x10^3$ L/mol cm .The limits of detection (LOD) , limit of quantification (LOQ) and Sandell sensitivity were 0.8911 µg/mL , 2.7003 µg/mL and 0.0348 ug/cm^2 respectively. The recovery in the range of 97.33 - 102.89 % was obtained. The proposed method has been successfully applied to the determination of 2,4-D pesticide residues in tomato and water samples with good accuracy and precision.

Keywords: 2,4-Dichlorophenoxyacetic Acid Pesticide (2,4-D); Alizarin Red Sulphonate (Ars); Spectrophotometry; Pesticide Residues Analysis.

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Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) (Figure 1a), is Phenoxy Acid Herbicides ,it has different trade names such as Dicopur D 870Sl , Safaya 600SL, Jiad 72% SL and Diolina 720 SL. The chlorophenoxy compounds are selective against broad-leaved annual weeds in cereal and grass crops. In general, they have a short persistence in soil. These compounds are active by contact and translocation from leaves to roots of perennial weeds and they are also used in reemergence applications to the soil for the control of young seedlings [1]. Tomato is one of the most popular and widely grown vegetables in the world ranking second in importance to potato in many countries. The tomato fruits are eaten raw or cooked. Tomato in large quantities is used to produce soup, juice ketchup, puree, paste and powder; it supplies vitamin C and adds variety of colours and flavours to the food. Green tomatoes are also used for pickles and preserves. Its many forms are adapted to wide range of soils and climates extending from the tropics to almost the Arctic Circle.

The tomato fruit (*Lycopesicon esculentum*) is one of the most important components of the human diet in various countries, where it is consumed in its raw form. Tomato is an essential food due to the presence of antioxidant molecules such as ascorbic acid, vitamin E, carotenoids, flavonoids, lycopene and phenolic acids, which contribute to human health [2,3].

On the other hand, the tomato crop is susceptible to pest attack throughout the season. Therefore, pesticides are extensively used in its culture at various stages of cultivation to control pests and diseases that may cause yield reduction [2,4-5]. Therefore, residues of pesticide could affect the ultimate consumers, especially when the fruit is consumed uncooked.

Several methods have been reported for the analysis of 2,4-D in food and environmental samples. These methods include gas chromatography (GC) [6-8], High –Performance Liquid Chromatography (HPLC) [9,10] and UV-spectrophotometry [11].

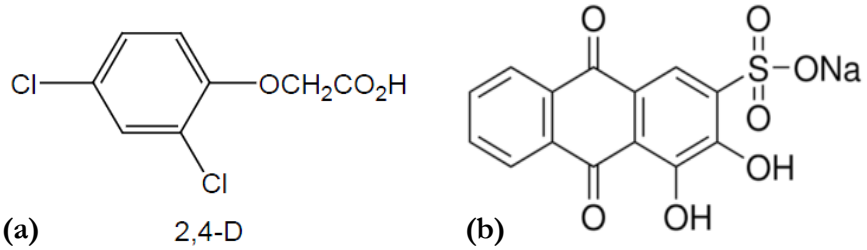
However the chromatographic techniques are expensive and not available in many quality laboratories worldwide. Spectrophotometry is probably the most convenient analytical technique for routine analysis because of its inherent simplicity, low cost and wide

availability in quality control laboratories.

Alizarin red sulphonate (ARS) (Figure 1b) have been used for the extraction spectrophotometric determination of some drugs such as ceterizine hydrochloride [12], trimethoprim [13], promazine [14], antifungal drugs containing an imidazole ring (ketoconazole and clotrimazole) [15] , oxybutynin chloride [16] , histamine H1-antagonists Drugs [17], olopatadine hydrochloride [18], ziprasidone and buclizine hydrochloride [19]. A few methods have been reported for the use of ARS as chromogenic reagent in spectrophotometric determination of pesticides residues [20].

The reaction between ARS and 2,4-D has not been investigated yet. Therefore, this study was devoted to investigate the reaction between ARS and 2,4-D, and use this colored reaction in development of simple rapid and low cost spectrophotometric method for determination of (2,4-D) in residues.

Figure 1. Chemical structure of (a) 2,4D (b) ARS



Materials and Methods

Chemicals

All chemicals used were of analytical-reagent grade. Distilled water was used to prepare all solutions.

Instrumentation

All the spectral measurements were carried out by using a Double beam UV 1800 ultraviolet-visible spectrophotometer model Shimadzu 1800, with quartz cells of 1cm optical path length. pH meter model pH 211(HANNA Italy) was used for pH measurements.

Stock Standard Solution of 2,4-Dichlorophenoxyacetic Acid (2,4-D)

An accurately 0.1 g of 2,4-D standard was dissolved in absolute ethanol, transferred into a 100 mL volumetric flask and diluted to the mark with absolute ethanol and mixed well. This stock solution was further diluted with absolute ethanol to obtain working solutions in the ranges of 3–40 µg/mL.

Alizarin Red Sulphonate (ARS)

1,2-dihydroxyanthraquinone solution (3 x 10^{-3}M) was prepared by dissolving 0.108 g of sodium alizarin sulphonate $C_{14}H_7NaO_7S$.(Purity 98%; MW 342.26; Merck), in ethanol-water mixture (70 + 30) in a 100 mL calibrated flask and mixed well. The solution was freshly prepared and protected from light during use. Buffer solution of pH 6 (Phosphate buffer) was prepared by mixing 100 mL of 0.1 M aqueous solution of sodium dihydrogen phosphate with 11 mL of 0.1M aqueous solution of sodium hydroxide in 100 mL volumetric flask, and adjusted by pH meter.

Procedure of calibration

Accurately measured aliquots of 2,4-D solution containing 3-40 ug/mL were transferred into separate 10 mL volumetric flasks, 2 mL of Alizarin Red Sulphonate (ARS) solution (3 x 10^{-3} M) was added and followed by 2 mL buffer solution pH 6.0 (NaH_2PO_4–NaOH). The reaction mixture was mixed well, completed to volume with distilled water and heated to 60ºC. The absorbance was measured at 563 nm against reagent blank treated similarly.

Procedure for the Determination of the Stoichiometric Ratio of Derivatization Reaction

Under the optimum conditions, the stoichiometry of the reaction between 2,4-D and ARS was investigated by Job's method of continuous variation [21]. Master equimolar (4 X 10^{-3} M) aqueous solution of 2,4-D and ARS were prepared. Series of 10 mL portions of the master solutions of 2,4-D and ARS were made up comprising different complementary proportions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4,7:3, 8:2, 9:1, 10:0) in 10 mL volumetric flask containing 2 mL of buffer solution pH 6, the solutions was further treated as described under the general recommended procedures.

Determination of 2,4-D in water samples

10 ml of distilled water were spiked with known amount of 2,4-D and kept for 30 min. Samples were extracted twice with diethyl ether (10 mL) in a separatory funnel. 15 g of anhydrous sodium sulphate was added to diethyl ether layer to eliminate residues of water. Then, extract was evaporated off under suction to dryness. Residue thus obtained was analyzed using the proposed method. Amount of 2,4-D was computed from standard calibration curve, Table 4.

Determination of 2,4-D in Tomato fruit samples

Samples of Tomato fruit 50 g free from pesticide were taken. Weighed sample was spiked with known amount of 2,4-D and kept for 4 hours. Samples were homogenized in blender with 40 mL diethyl ether for 3 min. at 1800 rpm. The homogenized sample was filtered through 12cm Buchner funnel with filter paper into 100 mL suction flask. The solid residues in blender jar were rinsed with two 10 ml portion of diethyl ether, and rinses were used to wash residues in Buchner funnel. The filtrate was transferred into 250 mL separatory funnel and two 20 mL portions of distilled water were added. 15 g of anhydrous sodium

sulphate was added to diethyl ether layer to eliminate residues of water. Then, extract was evaporated off under suction to dryness. Residue thus obtained was analyzed using the proposed method. Amount of 2,4-D was computed from standard calibration curve Table 5.

Results and Discussion

Absorption spectrum of product

2,4-D is colorless solution, it's absorption spectrum was recorded against absolute ethanol. As can be seen in Figure 2, it was found that 2,4-D exhibits a maximum absorption wave length peak λ_{max} at 286 nm and the ARS was 422 nm . The reaction between 2,4-D and ARS was performed, and the absorption spectrum of the product was recorded against reagent blank. It was found that the product is purple colored exhibiting (λ_{max}) at 563 nm. The λ_{max} of 2,4-D – ARS derivative was red shifted, eliminating any potential interference. Therefore, all measurements were carried out at 563 nm.

Optimization of Reaction Conditions

Effect of pH: The effect of pH on the system of 2,4-D – ARS was examined by varying pH from 4.0 to 11.0 Figure 3. A previous study [22] showed that the wavelength of maximum absorbance of ARS solution is greatly affected by the pH of the solution. As the pH increased, the reading increased and the maximum absorbance was attained when pH is 6.00 and this was occurred may to the ability of the nucleophilic substitution. At pH values more than 6.0, the absorbance of product begins to decrease.

Effect of Temperature: The influence of temperature on the absorbance of the reaction solution was studied at different temperatures (30 – 90ºC). As shown in Figure 4, the absorbance remains constant in temperature range 30-50ºC it means there is no temperature effect in this range. The absorbance of solution was maximal at 70ºC. Then it decrease rabidly with increasing in temperature. But In order to keep the high sensitivity the experiment was carried out at 60ºC, although high absorbance at 70ºC because there is shift in λ_{max} at 70ºC may indicate to complete association of product at this temperature.

Effect of Standing Time: According to the procedure, the effect of reaction standing time on the formation of the reaction product for 2,4-D was investigated by allowing the reaction to proceed for various time periods. The reaction carried out at 60 ºC. The result shows that the absorbance begins to increase and keeps stable after 10 min. In addition, the absorbance of product remains stable for at least 2 h. Therefore 10 min was selected as the optimum condition for 2,4-D, Figure 5.

Effect of Amount of the Buffer Solution: Figure 6 shows the influence of amount of buffer solution on the absorbance of the product, keeping pH at 6.0. It shows that the absorbance of product increases when the amount of the buffer is raised, and becomes maximal when the amount of buffer solution is 2 mL. Then it decreases and becomes stable when the amount of the buffer solution is over 3.0 mL. Therefore, 2.0 mL of buffer solution pH 6 was selected as the optimum experimental condition.

Effect of Amount of the ARS Solution: The effect of amount of ARS solution (3 x 10^{-3} M) on the absorbance of the product was also studied by varying the volume of ARS from 0.5 mL^{-4} mL. The result shows that the absorbance of the product enhances rapidly with rise of amount of ARS solution, and becomes maximal when the amount of ARS solution is 2.0 mL. Then it decreases rapidly with increasing the amount of ARS solution. Therefore, the amount of 2.0 mL ARS solution was selected to ensure the highest absorbance of product, (data not shown).

From all above experiments the optimum conditions were found to be pH 6.0, buffer volume 2.0 mL, ARS concentration 3 x 10^{-3} M, temperature 60ºC and reaction time 10 min.

Stoichiometry of Derivatization Reaction

The Stoichiometry of the reaction between 2,4-D and ARS was investigated by job's method and was found to be 1:1 because 2,4-D molecule contains only one centre (carboxylic group) as shown in Figure 7.

Figure 2. Absorption spectra of the system 2,4-D – ARS. (1) Absorption spectra of 2.4-D (3 ug/ml) against absolute ethanol, (2) ARS against mixture of ethanol and water (70: 30), (3) The reaction product of 2,4-D (3 ug/ml) with ARS against reagent blank.

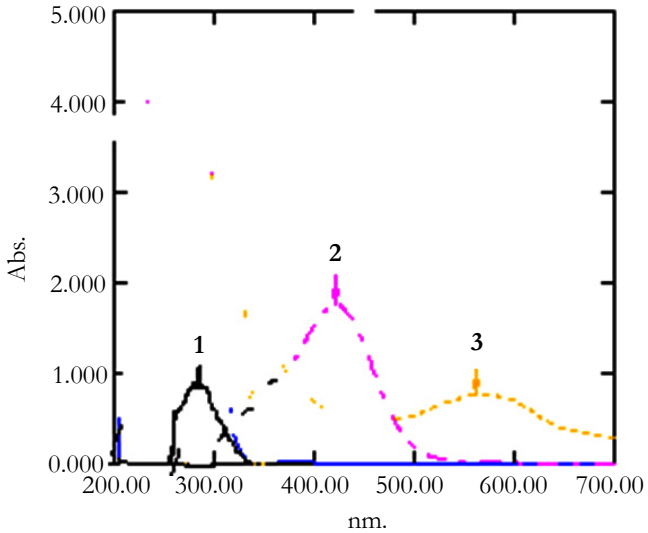


Figure 3. Effect of pH on the reaction of 2,4-D with ARS. 2,4-D (20 ug / ml): 1.0 ml ; buffer solution 2.0 ml ; ARS (3 x 10^{-3} M): 2.0 ml; reaction time: 10 min.

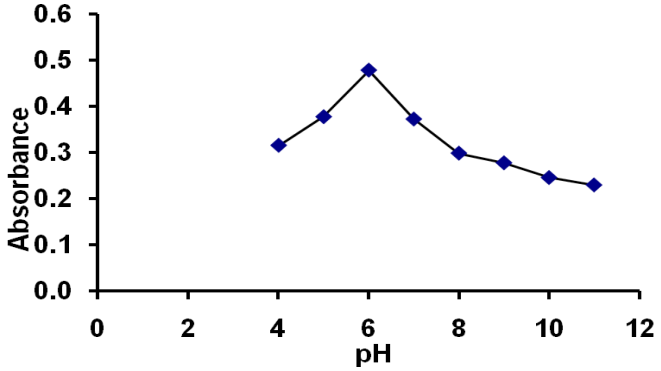


Figure 4. Effect of temperature on the reaction of 2,4-D with ARS. 2,4-D (20 µg/mL): 1.0 mL ; buffer solution pH 6.0 : 2 .0 mL ; (ARS) (3 x 10^{-3} M) : 2.0 mL; reaction time:10 min.

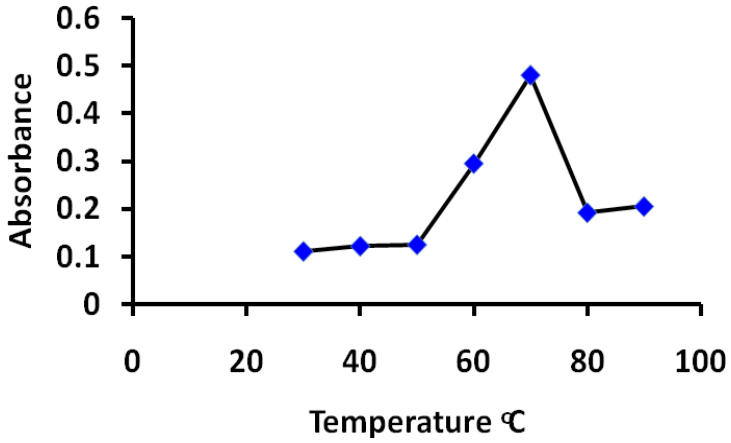


Figure 5. Effect of standing time on the reaction of 2,4-D with ARS. 2,4-D (20 ug / ml): 1.0 mL ; buffer solution pH 6.0 : 2 .0 mL ; (ARS) (3 x 10^{-3} M) : 2.0 mL ; temperature : 60°C.

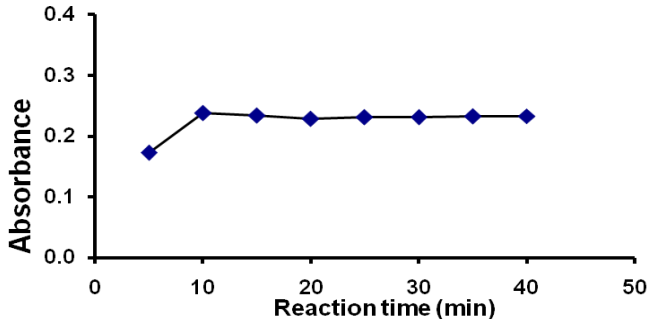


Figure 6. Effect of amount of buffer solution on the reaction of 2,4-D with ARS. 2,4-D (20 ug / ml): 1.0 mL ; buffer solution pH 6.0 ; ARS (3 x 10^{-3} M): 2.0 mL; temperature: 60°C; reaction time: 10 min.

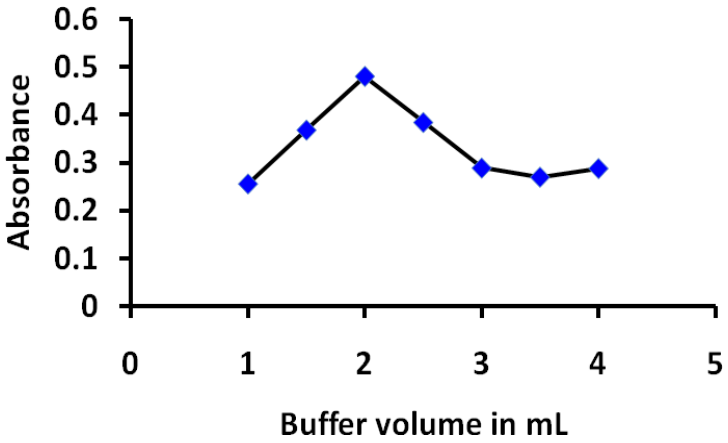
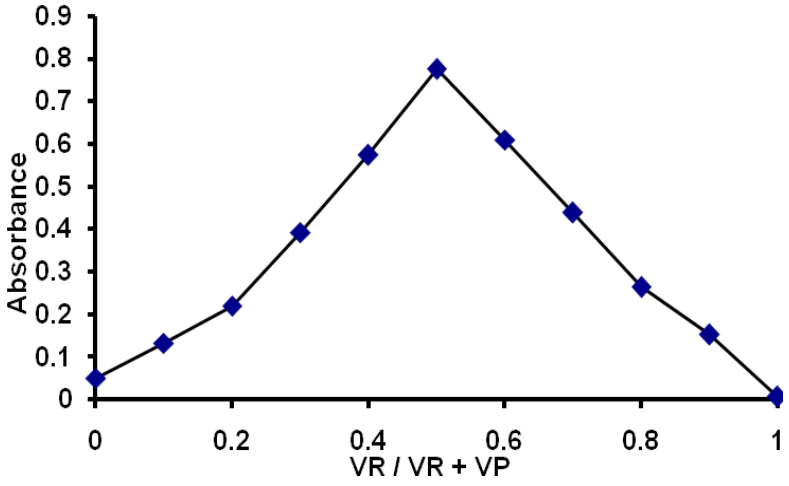


Figure 7. Determination of the product formation by Job's method. V_R: ARS (4 x 10^{-3} M); V_P: 2,4-D (4 X 10^{-3}M);$V_R + V_P$ = 10 mL; total volume of reaction solution 12 mL.



Reaction mechanism

Although the C-SO_3 bond of sulfonation of anthroquinone (ARS) is strong, the (aryl)C-SO_3 bond can be cleaved by certain nucleophiles followed by displacement of the sulfonate group by other nucleophiles, which cannot be installed directly [23]. It was found that the carboxylic group of 2,4-D display nucleophilicity due to the lone electron pair of oxygen atom, trends to attack on the electron – deficient center in (ARS) namely no.3 carbon atom (2,3-C= C bond conjugates with 1,2 – OH, as a result 3-C of (ARS) becomes electron lacking center). So 2,4-D can reacts with ARS in a nucleophilic substitution reaction, but due to present of Cl in position 2 and 4 (2,4-Cl) in 2,4-D molecule these decrease the negativity at oxygen atom therefore the reaction occurs at high temperature. At the same time, it has been proved by Job's method that, the composition of the product is 1:1. So it is concluded that one carboxylic group of substitute the 3- sodium sulfonate of one ARS molecule, to form purple product. The reaction pathway was postulated to be proceeded as shown in scheme 1.

Validation of the Method

***Calibration curve and LOD and LOQ*:** Under the optimized conditions, calibration curve for the determination of 2,4-D by it reaction with ARS was constructed by plotting the absorbance's as a function of the corresponding concentrations. A linear relationship between the absorbance, A, of the product and the concentration, C, of 2,4-D is obtained in the range of 3- 40 µg/mL. The linear regression equation obtained from the calibration curve graph is A = 0.0795 + 0.0212 C with a correlation coefficient, r^2, of 0.9995 and a molar absorptivity of 6.35 X 10^3 L $mol^{-1}cm^{-1}$.

The LOD and LOQ were determined according to the following formula LOD = 3. 3 X $SD^{a/b}$ and LOQ = 10 X $SD^{a/b}$, where: SD^a is standard deviation of intercept, b is the slope. The LOD and LOQ were found to be 0.8911 µg/mL and 2.7003 µg/mL, respectively, Table 1.

Precision: The precision of the proposed method was determined by replicate analysis of five separate solutions of working standard at different concentration levels. The method gave satisfactory results; RSD did not exceed 2% indicating the good precision of the proposed method. This precision level reflecting the usefulness of this method in routine analysis of the 2,4-D in laboratories.

***Recovery of 2,4-D*:** The accuracy and validity of the proposed method was evaluated by the recovery studies for added concentrations. The recovery of each was calculated as the amount found / amount taken X 100. The results of analysis suggest that there is no interference from any excipients, which are present in formulation Table 2.

Scheme 1. Proposed reaction pathway between 2,4-D and ARS

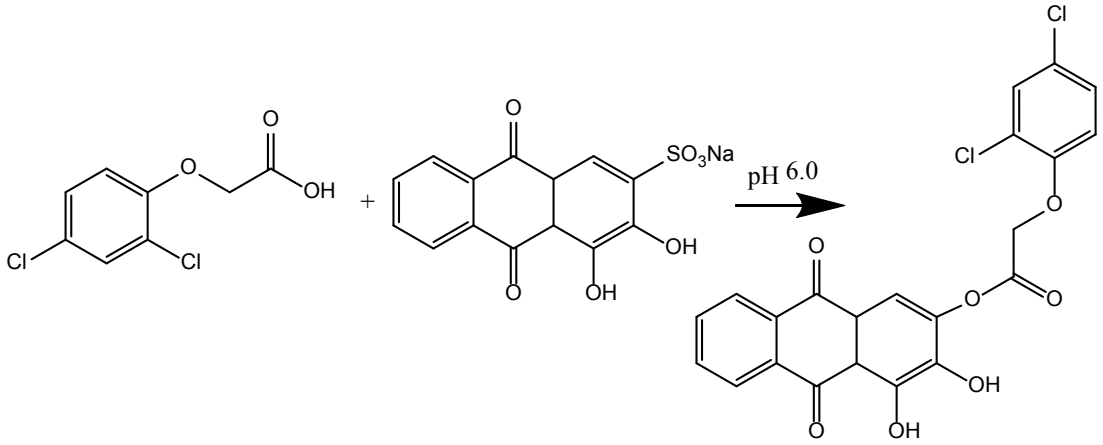


Table 1. Parameters for the performance of the proposed method

Parameter	Value
λ_{max} ,nm (pesticide)	286
λ_{max} ,nm (product)	563
Beer's law limits, (µg/mL)	3- 40
Molar absorptivity, L/mol cm	$6.35x10^3$
Sandell sensitivity, µg/cm^2	0.0348
Limit of detection , (LOD),µg/mL	0.8911
Limit of quantification (LOQ),µg/mL	2.7003
Regression equation ,Y:*	**Value**
Intercept (a)	0.0795
Standard deviation of the intercept (SD^a)	0.0057
Slope (b)	0.0212
Standard deviation of the slope (SD^b)	0.00026
Correlation coefficient (r^2)	0.9995

Table 2. The recovery of the proposed method

Sample content (µg/mL)	Added (standard) (µg/mL)	Found (µg/mL)	Recovery (% ± SD)*
4	4	7.786	97.33 ± 1.59
4	16	19.92	99.6 ± 0.44
4	24	28.81	102.89 ±0.86

●*Recovery was calculated as the amount found / amount taken X 100. Values are mean ± SD: Standard Deviation.

● Mean values of three determinations.

Robustness: Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In this experiment, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were recorded in Table 3. This indicated the reliability of the proposed method in application for the analysis of 2,4-D.

Application of the proposed method to analysis 2,4-D residues in water and tomato fruits

The procedure was applied successfully to the determination of the 2,4-D in water and tomato fruits. The results were listed in Table 4 are indicate the applicability of the procedure to analysis food and environmental samples containing 2,4-D pesticide with simplicity and high accuracy. The recovery of 2,4-D for water and tomato fruits varied from 96% – 102.69 % Table 4.

Table 3. Influence of small variations in the assay conditions on the analytical performance of the proposed spectrophotometric method for determination of 2,4-D using ARS reagent.

Parameters	Recovery (% ± SD)[a]
Recommended conditions[b]	99.8 ± 0.85
Buffer solution (pH) 6.2 5.8	 99..28 ± 0.71 101.63 ± 0.38
Volume of ARS (ml) 2.2 1.8	 97.60 ± 1.23 97.34 ± 0.89
Temperature (°C) 65 55	 100.08 ± 0.47 98.58 ± 0.39
Reaction time (min) 15 5	 101.46 ± 1.05 102.65 ± 0.45
Volume of buffer solution(ml) 2.2 1.8	 99.36 ± 0.66 97.74 ± 0.39

[a] Values are mean of 3 determinations. [b] The recommended conditions see the text

Table 4. Determination of 2,4-D in water and tomato fruits.

Samples	Amount of 2,4-D added (µg/mL)	Amount of 2,4-D found (µg/mL)	Recovery (% ± SD*)
Water[a]	8	7.68	96.00 ± 0.59
	16	16.43	102.69 ± 0.86
	24	23.80	99.17 ± 1.12
Tomato[b]	24	23.17	96.54 ± 1.36
	32	32.25	100.78 ±0.75

* Average of three replicate analysis.
[a] Amount of water sample = 10 mL.
[b] Weight of Tomato sample = 50 g.

Conclusion

The present study described the successful evaluation of ARS reagent in the development of simple and rapid spectrophotometric method for the accurate determination of 2,4-D. The proposed method is superior to the previously reported methods for the determination of 2,4-D in terms of their simplicity. Furthermore, it does not need expensive apparatus, have excellent shell life, all the analytical reagents are inexpensive, are available in any analytical laboratory. The other advantages include that, the method involves the measurement of stable coloured species, have shorter contact time and they are free from the extraction step. Therefore, the method is practical and valuable for routine application in laboratories for the analysis of 2,4-D in food and environmental samples.

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