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Spectroscopic Determination of Taiopronin by Oxidative Coupling Method Using Dimethyl Phenylene Diamine

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***Abstract:** This article presents the spectrophotometric determination of taiopronin using an oxidative coupling reaction with dimethyl phenylene diamine. The UV-visible spectrophotometric method was developed to analyze taiopronin in pure drugs and pharmaceutical formulations. The process is easy to use, rapid, accurate, sensitive, and selective. It has the maximum absorption at 552 nm in wavelength. The method involves the combination of a drug solution with a reagent and an oxidizing agent, resulting in a purple solution that is dissolved in water and exhibits the highest absorption at a specific wavelength. The concentration range for the Beer's law is 5–30 µg/mL. The oxidation and conjugation process can be completed in two minutes at 25°C, with a stable absorption value for an hour. The process has high accuracy, linearity, and a recall rate of 99.42%. The detection limit, quantitative limit, and recovery rate are all high, demonstrating its precision and compatibility. The recovery studies generating up to 100% and a relative standard deviation of less than 2 demonstrated how accurate and precise the procedure was found to be, indicating excellent results. The proposed method has been effectively utilized in analyzing preparations that contain taiopronin.*

***Keywords:** Oxidative coupling, Pharmaceutical, Taiopronin, Spectrophotometer.*

1. INTRODUCTION

For the treatment of cystinuria, a drug known by the brand name Thiola, is used to regulate the pace at which cystine precipitates and is excreted from the body.[3] [4] As a generic drug, it is accessible [1]. Tiopronin is recommended for the prevention of cystine stone formation in individuals with severe homozygous cystinuria weighing 20 kg (44 lb) or more,

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who do not respond to these treatments alone. It should be used in conjunction with high fluid intake, alkali, and dietary modification [2]. When the solubility limit is reached and urine becomes supersaturated with endogenous cystine, kidney stones might develop.[3]. An active reducing agent called timopronin forms a water-soluble mixed disulfide complex with cystine through a thionyl sulfide exchange. This lowers the quantity of cystine that is sparingly soluble. Tiopronin aids in the prevention of cystine stone formation by lowering urine cystine concentrations below the solubility limit [4]. Wilson's illness, which is caused by an excess of copper in the body, may also be treated with tiopronin to bind metal nanoparticles [5]. It has been researched for usage as a neuroprotective agent in aneurysmal subarachnoid hemorrhage and for the treatment of arthritis [6]. 2-mercaptopropionic acid is the main metabolite of tiopronin (2-MPA). By hydrolysis, 10–15% of the medication is converted to 2-MPA[7].After consumption, tiopronin absorbs slowly and reaches its maximal plasma concentration three to six hours later. The bioavailability of total and unbound tiopronin was determined to be 63% and 40%, respectively, in a study including healthy participants[8]. In plasma, tiopronin extensively binds to proteins. This is believed to happen as a result of a disulphide bridge forming between albumin's free thiol group and itself[8, 9]. The drug's medical significance has led to its estimation by several analytical techniques, including high-performance liquid chromatography technology. HPLC [10]. Techniques such as flow injection [11], electrolysis and spectroscopy [12].The current study aims to identify a straightforward, accurate, and focused spectrophotometric technique and establish its validity for salbutamol determination from pharmaceutical formulations[13]. The work [14] that created a novel organic reagent, Schiff's base, in an oxidative coupling process to make medicinal bure form using a straightforward, sensitive, and quick spectrophotometric approach.

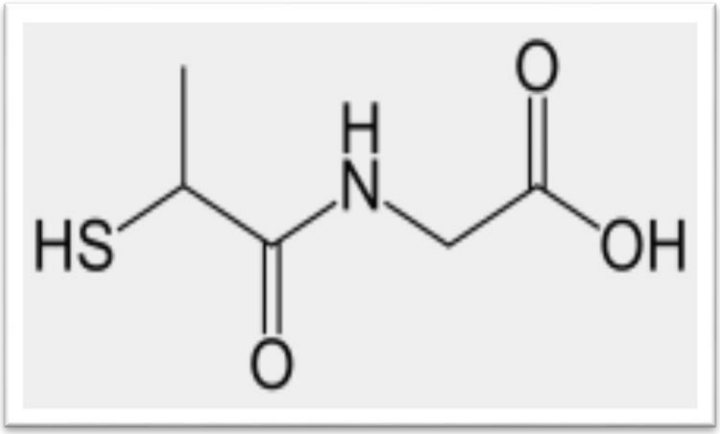

Fig.1 Chemical structure of tayopronin.

2. RELATED WORKS

In study of the [15], novel organic reagents were prepared and used to the spectrophotometric detection of trimethoprim (TMP). This process resulted in the development of a quick, easy,

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and sensitive spectrophotometric technique for the measurement of trimethoprim in aqueous solutions. The technique relies on Schiff's base formation, which is produced by combining the medication with an organic reagent that has been synthesized in an acidic medium to produce a colored result with a maximum absorbance at 573 nm. The suggested approach has several advantages for the quick determination of TMP in both pharmaceutical formulation and pure form. Compared to some of the published colorimetric techniques, the procedure was found to be somewhat selective, quick, inexpensive, and easy to follow. The study of [16] shows how basic kinetic spectrophotometric techniques may be used to assess penicillamine, tiopronin, and glutathione in pharmaceutical formulations. The novel technique relies on a redox process in which Cu-neocuproine complex is reduced to Cu^{1}-neocuproine complex by the RSH. It may be used for routine RSH measurement without the need for pricey reagents and equipment because it is sensitive and precise enough. The wide linearity range, increased sensitivity, and speed of the suggested approach surpass those of the kinetic spectrophotometric methods that have already been reported. While potassium dichromate discoloring spectrophotometry is used to provide an alternative approach for tiopronin, the results are in good agreement with the conventional method. It is obvious that the measurement of tiopronin using potassium dichromate discoloring spectrophotometry has some importance and application potential [17]. Tiopronin's hydrosulfuryl group may convert Fe^{3+} to Fe^{2+}, generating an orange-red complex with maximum absorbance at 522 nm. This approach introduces a novel spectrophotometric method for determining tiopronin content; the content of tiopronin is 91.40 mg tablet using this method, while the traditional method yields 90.57 mg tablet. As may be seen, the results are consistent [18].The research of [19] looks at the spectrophotometer technique for detecting acetylcysteine level in medicine. The yellow complex generated by the reaction between Cu(I) and neocuproine shows a maximum absorption at 453 nm, and the findings are compared to those obtained using the pharmacopoeia approach. The recovery test for standard addition produced good results. Obviously, this approach may be used to determine acetylcysteine. These research agree with the present study. The technique and the theory of oxidative coupling are accurate, and the given approaches have benefits such as sensitivity, fast analysis time, simplicity of application, and low cost.

3. METHODOLOGY

Instrument

Ultraviolet spectroscopy and dual-beam visible Shimadzu UV-1700 Japanese , with 1cm matched quartz cells, Sensitive scale Kem Acj/Germany, Heater HPL-248 China, BS-11 water bath German.

Materials

The chemicals utilized in this research were of high purity (Fluka, bdh, SDI).
Preparation of Solutions
Tiopronin standard solution 1000 µg/ml 0.006127 M The solution was prepared by taking 0.1000 grams of taiopronin and combining it with distilled water and adding more distilled water to fill a 100 ml volumetric container, from which other diluted solutions were prepared.

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DMPD Reagent Solution 0.01 M Prepare this solution by weighing 0.3405 grams of the dimethylphenylenediamine reagent, combining it with distilled water to dissolve it, then adding more distilled water to a 100 ml volumetric container to bring the volume up to par.

Fecl$_3$ Reagent Solution 0.01 M
This solution prepared by weighing 0.4055 g of the $FeCl_3$ reagent, combining it with distilled water to dissolve it, then adding more distilled water to a 100 ml volumetric container to bring the volume up to par.

20 Mg Tablet Solution at a Concentration of 100 µg/Ml
The pharmaceutical preparation containing taiopronin is available in the form of pills produced by sigma aldrich Company. It contains 20 mg of tayopronin in each pill. The solution was prepared by weighing 10 tablets, and their weight was 216.2 grams before crushing. After crushing, 501 grams of the substance were weighed. dissolved it in distilled water, then filtered and washed the precipitate more than once with the same solvent. It was placed in a 100 ml volumetric bottle and the volume was completed to the mark with distilled water. Thus, a 1000 µg/ml solution was prepared, and the required solution, which is 100 µg/ml, was prepared from it by taking 10 ml of it in a 100 ml volumetric bottle and completing the volume with distilled water to the mark.

Method Principle
The method's basic idea is to mix a tayopronin drug solution with DMPD reagent in the presence of $FeCl_3$, an oxidizing agent, to create a purple solution that dissolves in water and has the highest absorption at a wavelength of (552) nm when compared to a blank solution.

Preliminary Test
One millilitre of 100 µg/ml taiopronin solution was found to produce a purple colour when combined with one millilitre of 0.01M DMPD reagent and one millilitre of 0.01M $FeCl_3$, and this was observed after the mixture was diluted with distilled water to the appropriate level in a bottle. The solution was allowed to settle in a volumetric capacity of 10 ml for a while before the colour spectrum was measured. This resulted in the highest absorbance at 552 nm wavelength when compared to the mock solution.

Study the Optimal Conditions for the Reaction
To develop a dye with high absorption intensity and sensitivity, a study was conducted to determine the best circumstances that result in the most absorption of the colored product using a 10 mL volumetric flask.

4. RESULTS AND DISCUSSIONS

Results
Choosing the Best Coupling Reagent
1 ml of several reagents were used at a concentration of 0.01M each, by adding 2 ml of tayopronin at a concentration of 100 µg/mL. Then add 1 ml of the oxidizing agent $FeCl_3$ at a

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concentration of 0.01M. After adding distilled water to the appropriate amount in a 10-milliliter volumetric container . The colored solutions' absorbance was then compared to the blank solutions, as indicated in table 1.

Table 1 Choosing the best coupling reagent

Reagent (0.01M)	Variable	Absorbance	Λmax
DMPD	SB BW	0.379 0.063	552 400
3-amino phenol	SB BW	0.143 0.025	395 378
4-nitro aniline	SB BW	0.111 0.016	385 347

The Effect of the Volume of Reagent

Two millilitres of taiopronin 100 µg/ml and various quantities (0.1-3.0) of DMPD 0.01M reagent were added to examine the impact of the reagent quantity. Then fill 10 ml volumetric vials to the brim with distilled water after adding 1 ml of $FeCl_3$, at a concentration of 0.01M. table 2 displays the results of measuring the solutions' absorbance at 552 nm in comparison to their blank solutions.

Table 2 Effect of the coupling reagent volume

NO	volume (ml) DMPD 0.01M	SB	Absorbance BW
1	0.1	0.173	0.060
2	0.2	0.210	0.053
3	0.5	0.214	0.042
4	1	0.362	0.055
5	2	0.218	0.043
6	3	0.211	0.026

Study the Best Oxidizing Agent

To 2 ml of 100 µg/ml tiopronin, 1 ml of various oxidizing agents (0.01Meach), 1 ml of 0.01M DMPD reagent to 10 ml volumetric vials and fill to the mark with distilled water. The absorbance of various colored solutions was measured at 552 nm, and the findings are reported in table 3.

Table 3 Choosing the best oxidizing agent

oxidizing agent 0.01M	chemical formula	Absorbance		λ max (nm)
		Blank	sample	
Iron (III) Chloride	$FeCl_3$	0.379	0.066	552
Iron (III) nitrate hydride	$Fe(No_3)_3.9H_2o$	0.282	0.045	420
Iron (III) sulfate	$Fe_2(So_4)_3$	0.278	0.026	400

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Effect of the Quantity of the Oxidizing Agent

To each 10 ml container containing 2 ml of the medication taiopronin at a concentration of 100 µg/ml, 0.01M oxidizing agent $FeCl_3$ was added in increments of 0.04 ml. Then, 1 ml of DMPD dual concentration reagent 0.01M was added to the bottles until the volume reached the level of the distilled water. Color solutions were compared with blank solutions. Table 4 shows the full findings.

Table 4 Effect of the oxidizing agent volume

No.	ml of $FeCl_3$ 0.01M	Absorbance SB
1	0.4	0.184
2	0.6	0.229
3	1	0.378
4	2	0.210
5	3	0.134

Order of Addition

Due to the significant impact of the sequence of adding the solutions used on the absorption of the resulting compound, a number of experiments were conducted with different sequences of additions, as shown in table 5. However, all the volumes and concentrations used were the same in all cases, which was reached from previous tests, and it was noted that The results obtained are that order 1 gives the highest absorption.

Table 5 Choosing the best base to be used for coupling

No	order of addition	Absorbance
1	D+O+R	0.378
2	R+O+D	0.231
3	D+R+O	0.202

The effect of acid and base

It was observed that when adding acid to the reaction medium, this led to the disappearance of the color of the product, and when adding the base, a turbidity occurred in the color of the resulting solution.

The effect of time of oxidizing

To study the reaction time, several 10 ml volumetric bottles were filled with 2 ml of taiopronin at 100 µg/ml concentration.1 ml of the oxidizing agent $FeCl_3$ (0.01M) was added to it, followed by 1 ml of the DMPD reagent (0.01M), and the bottles were filled to the mark with distilled water. The absorbance of the colored products was then measured at a wavelength of 552 nm in comparison to the fake solutions, the results are shown in table 6.

Table 6 The effect of changing time

Time/ min	2	4	6	8
Absorbance	0.378	0.367	0.362	0.360

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The Effect of Temperature

To study the impact of temperature on color absorption, a 10 ml volumetric vial was filled with 2 ml of taiopronin at 100 µg/ml, 1 ml of $FeCl_3$ at 0.01M, and 1 ml of DMPD reagent at 0.01M. Distilled water was added to the mark. After two minutes, the colored product's absorption was measured, as shown in table 7.

Table 7 The effect of changing temperature

No.	Temprature C°	Absorbance
1	10	0.255
2	15	0.360
3	25	0.377
4	30	0.370
5	40	0.354
6	45	0.233
7	50	0.161

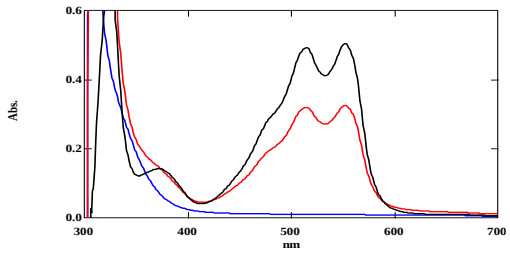
Stability Effect of the Product

Stability effect of the product shown in table 8 below.

Table 8 The reaction product's stability

No.	Time (min)	Absorbance of tiopronin
1	2	0.378
2	4	0.381
3	6	0.382
4	8	0.382
5	15	0.385
6	20	0.386
7	25	0.387
8	30	0.388
9	40	0.387
10	50	0.387
11	60	0.387

Final spectrum absorption



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Final spectrum absorption shown in Fig. 2 below.Fig. 2 Final absorption spectrum for determining taiopronin by a: Absorption spectrum of the product vs the photo solution, distilled water, and b: Absorption spectrum of the product versus the distilled water. c: Absorption spectrum of the light solution compared to pure water.

The Optimal Working Method and Calibration Curve Numbers
The optimal working method and calibration curve numbers shown in table 9below.

Table 9 Summary of optimum condition

No.	experimental conditions	Results
1	λ Max (nm)	552
2	Volume (ml) of ($6.127*10^{-3}$ M) Tiopronin	2ml
3	Volume (ml) of (0.01M) $FeCl_3$	1 ml
4	Volume (ml) of (0.01M) DMPD	1 ml
5	Temprature C°	25C°
6	time (minte)	2 minte
7	Solvent	Water

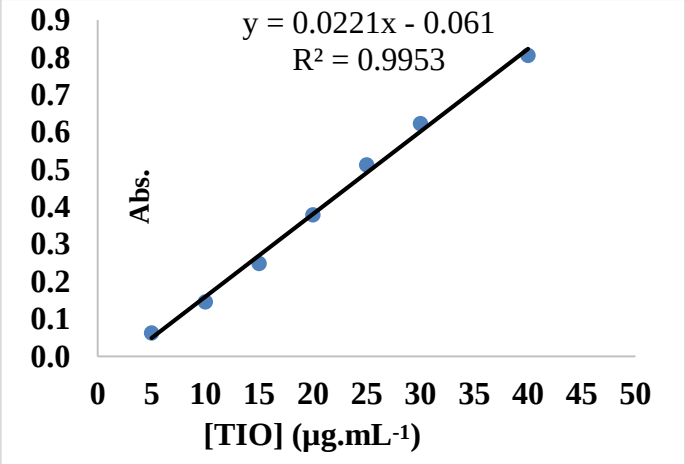

Fig. 3 Calibration curve for the determination of taiopronin

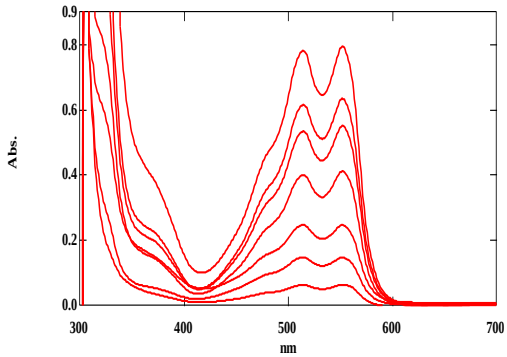

Fig. 4 Absorption spectrum of taiopronin (5-40)

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Accuracy and Precision

The optimal conditions for the calibration curve of the working method were used to test the accuracy and compatibility of this method by calculating the relative standard deviation and recall, by taking five readings for three different concentrations of taiopronin drug solution (30, 15, 5) µg/mL. The average of these readings for each concentration was taken and it was shown from table 10. that the average relative standard deviation is between (0.164% - 0.322%) and that the recall rate is (99.42%) and the relative error does not exceed (0.411 - 0.481). This indicates that this method is accurate and compatible.

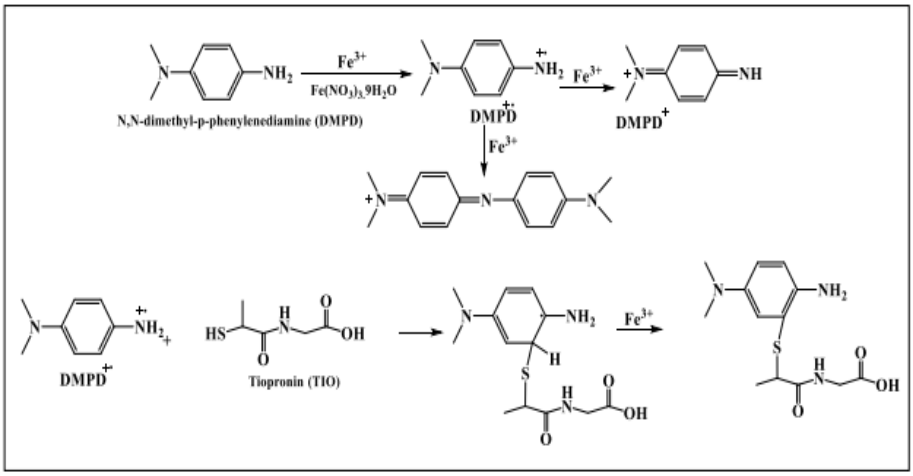

Fig. 5 The proposed reaction equation for the drug with the reagent, which resulted in the compound in purple.

Table 10 The accuracy and compatibility of the method

Conc. of Tio present µg m/ml	Conc. of Tio found µgm/ml	RE %	Recovery %	Average Recovery %	RSD
5	5.005	- 0.316	100.1		0.311
				99.042	
15	14.52	- 0.481	96.79		0.322
30	30.41	0.411	101.37		0.164

Determination Limits and Limits of Quantitation

The detection limit and quantitative limit for the determination of the drug taiopronin were determined by measuring the absorbance of the lowest concentration in the calibration curve. of 5 µg/mL by taking the average of five readings and according to the optimal conditions, and the results are shown in table 11 .

Table 11 Limits of Quantitation

Conc. of Tio. µg m/ml	∂	SD	LoD	LoQ
5	0.0221	0.0007	0.0950	0.3167

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The Nature of the Product Formed

To determine the nature of the produced product and the ratio of drug binding capacity to the reagent, the continuous changes technique (Job's method) and the molar ratio method were used . In both techniques, the reagent solution and taiopronin solution have concentrations of 1 × 10−2 M each. A volumetric flask with a 10 mL capacity was filled with various amounts of the drug solution (0.1–0.9 mL); reagent was added in decreasing volumes to the 10 mL; the remaining additions were finished with the ideal volumes in accordance with the work technique, and then they were diluted with distilled water to the mark limit; lastly, the absorbance of these solutions was measured at a wavelength of 552 nm in comparison to respective blank solutions . Fig. 6 shows that the ratio is 1:1. The molar ratio method was used to make sure that the drug taiopronin and the reagent had a 1:1 reaction ratio. Two milliliters of the drug solution were added to a series of ten milliliter volumetric flasks, along with varying amounts of the reagent solution (0.1–3 mL). Distilled water was used to dilute the mixture to the appropriate level, and each solution's absorption was measured at a wavelength of 552 nm in comparison to the blank solution. The molar ratio was discovered to be consistent with the continuous changes technique. The 1:1 ratio between the reagent DMFD and the taiopronin is verified by Fig. 7. Consequently, the following will be the suggested equation.

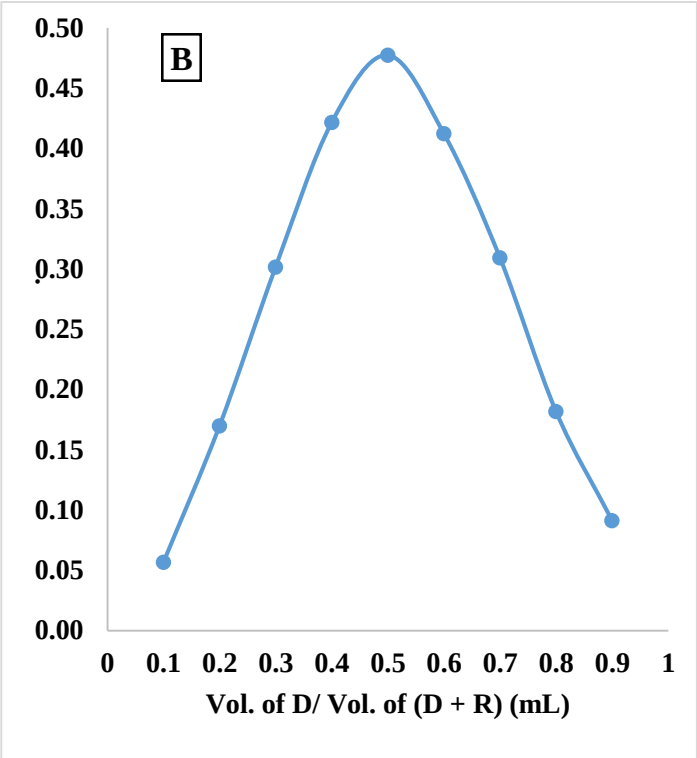

Fig. 6 Job's method for the determination of taiopronin

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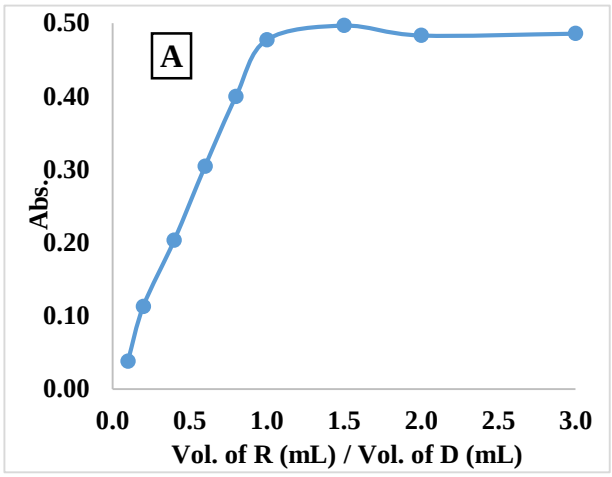

Fig. 7 The molar ratio method shows that the ratio is 1:1 between taiopronin and DMPD

Applications

The proposed method was applied to the pharmaceutical preparation that contains taiopronin in the form of pills in the following way:

The Direct Method

The direct method was studied by taking three different concentrations of the pharmaceutical solution prepared in (5.3.1.2), which is (20.10.5) mcg/ml. These solutions are treated with the same steps The calibration curve was followed according to the optimal conditions and the absorbance was measured against the mock solutions at a wavelength of 522 nm. Then calculate the average of five readings for each concentration, calculate the relative error and recall, and the results are shown in table 12.

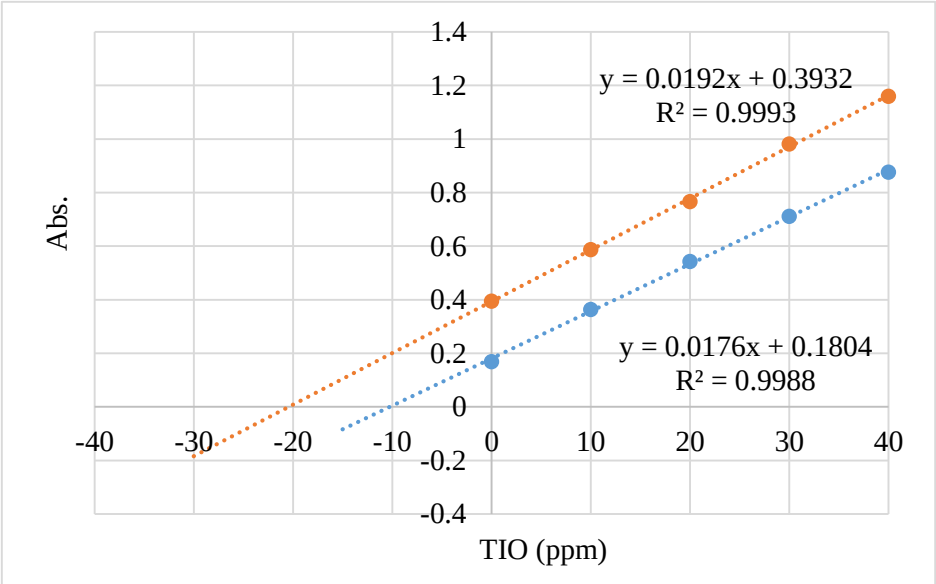

Fig. 8 Standard addition curve for the determination of taiopronin in pharmaceutical tablets.

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Table 12 The direct method

Drug of Tio.	Conc. of Tio. present µg m/ml	Conc. of Tio. found µg m/ml	RE %	Recovery%	
Tablet 20 mg	5	5.02	0.46	100.46	
					101.21
	10	10.22	0.23	102.23	
	20	20.19	0.94	100.94	

Standard additive method

The standard additive approach was used to determine the amount of thaipronin in pharmaceutical formulations in order to guarantee that the suggested method is free from interference.The results showed that the standard additive method agreed well with the direct method within the acceptable range of error. The method involves adding fixed amounts (2 ml), which is equivalent to (10-20 µg/ml.) of 100 mcg/ml pharmaceutical solutions. In two series of volumetric bottles with a capacity of 10 ml for the drug tiaberone (1-2-3-4) ml at a concentration of 100 mcg/ml. The solutions were treated with the same method of work used in the calibration curve. The absorbance of the solutions was measured after they were processed using the same methodology as the titration curve. contrasted with the dummy solution at 552 nm in wavelength, and the results are shown in table 13.

Table 13 Standard addition method for determination of taiopronin in tablets

Amount taken mg/ml	Amount Measurd	Recovery%
10	10.25	102.50
20	20.48	102.40

Discussion

The study thoroughly examined the optimal conditions for conjugation reagents, oxidizing agents, and the media in which analytical reactions take place. To obtain optimal analytical results for the measurement of taiopronin, we thoroughly examined the impact of volumes, time, sequence of additions, temperature, and product stability**.** The best reagent was discovered to be dimethyl phenylene diamine., which had the maximum absorbance at 552 nm when compared with the other reagents at concentrations of 0.01M. It is also worth noting that the reagent's optimal volume is 1mL, and the best oxidizing agent is $FeCl_3$ as shown in table 3 $FeCl_3$ of concentration 0.01M and volume 1 mL because it gave the highest absorbance**.** The best addition sequence number (1) as in table 5 because it give the highest absorbance, and the best temperature was 25°C because it gave a highest absorbance as shown in table 7. The results indicate that the suggested approach has high sensitivity and is not poorer in quality than other spectrum methods. Some physical variables of the proposed method were compared with some variables of spectroscopic methods in the literature used to estimate tio. Figure 6 [20].Spectrophotometry methods for drug assessment have improved greatly in recent years due to their relevance in pharmaceutical analysis. Because of this, it is frequently used in research due to its increased solubility in distilled water and HCl [21].

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Both the standard deviation and coefficient of variance were low enough. The accuracy of the approach was indicated by the percentage recovery range of 96.79 %–101%. taiopronin linearity (0.9953 within the concentration range of 10-250g/ mL was discovered using the proposed approach. The RSD% was determined to be less than 2, indicating that the procedure is highly reproducible [22].

5. CONCLUSIONS

Of by employing the oxidative coupling technique with the dimethyl phenylene diamine reagent. Based on the drug's oxidative coupling reaction with dimethylphenylene diamine, a straightforward, sensitive, and easy spectroscopic approach has been devised to determine taiopronin. Reagent reacts with the oxidizing agent $FeCl_3$ to produce a purple solution that dissolves in water, has the maximum absorbance at 552 nm, and follows Beer's law between 5 and 30 µg/mL of taiopronin. The correlation value was 0.9993, the sandal index was 0.0453µg/cm2, and the molar absorption coefficient was 3606 L•mol−1•cm−1. With a 99.42% recovery rate, the technique was effectively used to estimate taiopronin in medicinal preparations (tablets).

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