Spectrophotometric method for the Determination of Sulfathiazole Drug by Schiff's base Formation

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Abstract

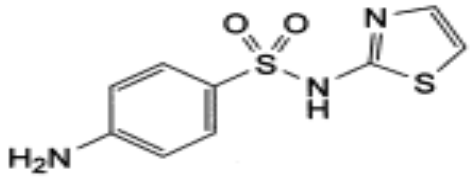
This research involves the development of simple, sensitive and rapid spectrophotometric method for determination of sulfathiazole (STHZ) in aqueous solution. The method is based on the Schiff's base formation between the primary amino group of sulfathiazole and aldehyde group of the p-dimethyl aminobenzaldehyde reagent (p-DMAB) to produce a yellow colored complex having maximum absorption at 451 nm at pH 2.2. Beer's law is obeyed in the concentration range 2-24µg/ml of sulfathiazole , with a molar absorptivity 11259 $L.mol^{-1}.cm^{-1}$ and sandal index of 0.023 $\mu g.cm^{-2}$. The average recovery is 99.925%, relative standard deviation 0.68-0.81% and D.L of 0.1534 µg/ml . This method has been applied successfully to determination of sulfathiazole in veterinary injection liquid solution (bio prime).

Keywords: Spectrophotometric, Sulfathiazole, p-DMAB reagent.

Introduction

Schiff's bases are those organic compounds that contain azomethine group (C = N), it is active group and are often characterized by the yellow colored product and these compounds disintegrate rapidly or polymerize[1,2].

The scientific name for the sulfathiazole[3] is 4-amino-*N*-(thiazol-2-yl) benzenesulphonamide



The molecular structure for the sulfathiazole is $C_9H_9N_3O_2S_2$ and the molecular weight of 255.3 g/mol, A white or slightly yellowish, crystalline powder, slightly soluble in alcohol, practically insoluble in methylene chloride. It dissolves in dilute solutions of alkali hydroxides and in dilute mineral acids.[3,4] Soluble 1g in 2500 ml of water and 1g in 120 ml of ethanol; practically insoluble in chloroform and ether; soluble in acetone.The drug is used as an anti-bacterial[4,5].

Many different analytical methods were used for the determination of sulfathiazole such as spectrophotometric methods[6-9], high performance liquid chromatographic methods (HPLC)[10-18], electrochemical method[19-20], chemiluminescence method[21], flow-injection method[22-23], FT-Raman technique[24-25] and gas-liquid chromatographic method (GLC)[26] . In this research a simple, accurate and sensitive spectrophotometric method for determining of sulfathiazole in pure form as well as in veterinary injection liquid solution (bioprime) based on the formation of Schiff's base using p-dimethylaminobenzaldehyde reagent.

Experimental

Apparatus

1-Shimadzu UV-Visible Spectrophotometer UV-160.
2- Jenway pH/mv meter 3310.
3- Ultrasonic with water bath, UNISONICS.
4- Hot Plate with Magnetic Stirrer (BIOSAN MSH 300).
5- Sartorius BL210 S AG GOTTINGEN.

Reagents and chemicals used

All chemicals and analytical reagents used in this research are of high purity and provided by two companies, Fluka and BDH.

Preparation of solutions (3.9×10^{-4}M) 1- Standard sulfathiazole solution,100 µg/ml

This solution is prepared by dissolving 0.01 g of sulfathiazole in amount of distilled water and the volume is diluted to 100 ml with distilled water in a volumetric flask. This solution is used for not more than one month.

2- p-dimethylaminobenzaldehyde reagent solution (5×10^{-2}M)[27].

This solution is prepared by dissolving 0.7455g of p-dimethylaminobenzaldehyde in 1 ml of concentrated hydrochloric acid and the volume is completed to 100 ml in a volumetric flask with distilled water.

3- Hydrochloric acid solution (approximate,0.1 M)

This solution is prepared by appropriate dilution of 0.85 ml of the concentrated hydrochloric acid (11.8 M) solution to 100 ml with distilled water in a volumetric flask.

4- Sodium hydroxide solution (approximate,0.1 M)

This solution is prepared by dissolving 0.400 g of pure sodium hydroxide in amount of distilled water and the volume is completed to 100 ml in a volumetric flask with distilled water.

5- Interference solutions, 1000 µg/ml

A 0.1000 g of (starch, glucose, fructose, maltose and sucrose) compounds is dissolved in distilled water then the volume is completed to 100 ml in a volumetric flask with distilled water.

6-Solution of STHZ injection formulation (100µg/ml)

Veterinary injection liquid solution (bio prime) (Bioagripharm GmbH-germany), every 1.0 ml contains 40 mg of trimethoprim, 40 mg of sulfathiazole, 60 mg of sulfadiazine and 100 mg of sulfamerazine , the solution was prepared as follows:

This solution is prepared by taking the equivalent of 0.100 g from sulfathiazole and the volume has been completed to 100 ml with distilled water to obtain a solution with a concentration of 1000 µg/ml. A solution of 100 µg/ml is prepared by dilution of 10 ml of the above solution by distilled water in a volumetric flask of 100 ml .

Preliminary Investigations

A 3.0 ml of p-dimethylaminobenzaldehyde reagent is added to 3 ml of standard sulfathiazole solution consists of yellow color solution. Absorption spectrum of the colored dye(after dilution to 25 ml with distilled water in a volumetric flask) against its corresponding blank reagents shows maximum absorption at 451 nm in contrast to blank reagent which shows no absorbance at this wavelength. The optimal conditions for the coupling reaction are investigated.

Optimization of the experimental conditions

The effect of various variables on the color intensity of 3.0 ml of standard sulfathiazle (STHZ) solution (100µg/ml) and 3.0 ml of p-dimethylaminobenzaldehyde reagent(p-DMAB) has been studied to establish the optimum conditions.

Selection of the coupling reagent

Different types of coupling reagents(5×10^{-2}M) were investigated to select the best reagent that gives the highest color intensity, the results are shown in Table(1).

Table (1) Selection of the coupling reagent

Reagent(5×10^{-2}M)		Colour	$_{max}\lambda$ nm	$\Delta\lambda$ nm	Abs.
4- Dimethylaminobenzaldehyde	SB	Yellow	451	82	0.396
	BW	Colourless	379		2.216
	BW*	------	451		0.000
3 .Methoxy-4- hydroxy benzaldehyde	SB	Colourless	345	26	0.023
	BW	Colourless	319		0.552
	BW*	------	345		0.000
4-Aminoacetophenone	SB	Colourless	358	22	0.018
	BW	Colourless	336		1.234
	BW*	------	358		0.000
P . Hydroxybenzaldehyde	SB	Colourless	337	50	0.011
	BW	Colourless	287		1.233
	BW*	------	337		0.000
Salicyladehyde	SB	Colourless	338	43	0.009
	BW	Colourless	295		1.331
	BW*	------	338		0.000
Benzaldehyde	SB	Colourless	345	38	0.026
	BW	Colourless	307		1.036
	BW*	------	345		0.000

SB : Absorption spectrum of STHZ solution versus reagent blank.
BW: Absorption of reagent blank versus distilled water.
BW*: Absorption of reagent blank at λ_{max} of STHZ absorption.

The results illustrated in Table (1) indicate that p-DMAB reagent gives the highest color intensity and a good color contrast $\Delta\lambda$ in comparison with other reagents. so this reagent is chosen in subsequent experiments.

Effect of Acid

The effect of acid was studied by adding 0.3-2.5 ml of 0.1M hydrochloric acid solution to a series of volumetric flasks (25ml) containing 3.0 ml of STHZ (100 µg/ml) and 3.0 ml of p-DMAB reagent, the solutions are diluted to the mark with distilled water, the absorption is measured against blank reagents at 451 nm and pH is measured, the results are shown in Table (2).

Table (2) Effect of acid.

ml of HCl (0.1M)	Absorbance	pH
without	**0.396**	**1.6**
0.3	0.372	1.5
0.5	0.345	1.4
0.7	0.317	1.3
1.0	0.289	1.2
1.5	0.271	1.1
2.0	0.255	1.1
2.5	0.239	1.0

Table (2) shows that the addition of the acid leads to a decrease in the absorption of the color complex, so it is avoided in subsequent experiments.

Effect of the base

This study has been carried out by the addition of different volumes (0.3 - 3.5 ml) of sodium hydroxide (0.1M) to a series of volumetric flasks (25ml) containing 3.0 ml of STZH (100 µg/ml) and 3.0 ml of p-DMAB, the solutions are diluted to the mark with distilled water, the absorption is measured against blank reagents at 451 nm and pH is measured. The results are shown in Table (3).

Table (3) Effect of base.

ml of base (0.1M)	Absorbance	pH
without	0.396	1.6
0.3	0.417	1.8
0.5	0.455	1.9
1.0	**0.539**	**2.2**
1.5	0.477	2.5
2.0	0.429	2.8
2.5	0.368	3.0
3.0	0.256	3.2
3.5	0.233	3.5

Table (3) shows that the best pH is in the range of 1.9-2.5, so the pH of 2.2 and 1.0 ml of sodium hydroxide solution is adopted in subsequent experiments because it gave the highest absorption.

Effect of the buffer solution

The formed yellow color product gives the highest absorption at pH 2.2, therefore; the effect of the some buffer solutions has been studied and note their impact on the absorption of the formed product[28]. The results are shown in Table (4).

Table (4) Effect of the buffer solution

Buffer solution (1.0 ml)	Absorbance
Hydrochloric acid – potassium chloride	0.547
Hydrochloric acid - potassium phthalate	0.509

The results shown in Table (4) indicate that the buffer solution (HCl – KCl) gave the highest absorbance, so it is used in subsequent experiments.

Effect of the amount of buffer solution

The effect of the amount of buffer solution(HCl – KCl) has been studied using increased volumes from the buffer solution ranging from 0.5-3.5 , the results are shown in Table (5).

Table (5)Effect of the amount of buffer solution.

ml of buffer solution	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Absorbance	0.537	0.541	0.549	0.558	0.563	0.550	0.545	0.532
pH	2.2	2.1	1.9	2.1	2.2	2.1	1.9	1.8

Table (5) indicates that the best volume of the buffer solution which gives the highest absorption is in the range of 0.5 - 3.0 ml, so the 2.0 ml of the buffer solution is adopted in subsequent experiments.

Effect of the amount of coupling reagent

The effect of the amount of coupling reagent has been studied by adding different volumes (1.0 - 6.0 ml) of p-DMAB reagent (5×10^{-2}M) to the volumetric flask containing 3.0ml of sulfathiazole solution (100 µg/ml), 2.0 ml of buffer solution (HCl-KCl) and the volume was completed to 25ml with distilled water. Table (6) is clear that the volume of 4.5 ml of coupling reagent (5×10^{-2} M) is the optimum amount because it gave the highest absorption, so it is adopted in subsequent experiments.

Table (6) Effect of the amount of coupling reagent

ml of Reagent (5×10^{-2}M)	Absorbance
1.0	0.394
1.5	0.429
2.5	0.531
3.0	0.564
3.5	0.587
4.0	0.623
4.5	**0.649**
5.0	0.583
5.5	0.544
6.0	0.498

Effect of time

a series of volumetric flasks, each containing 3.0 ml of sulfathiazole (100µg/ml), 4.5 ml of p-DMAB (5×10^{-2}M) and 2.0 ml of the buffer solution (HCl-KCl), the solutions are left for different periods of time, then the volume was completed to 25ml with distilled water, and the absorption of solutions was measured at a wavelength of 451 nm versus blank, the results are shown in table (7).

Table (7) Effect of time.

Time(min)	5	10	15	20	25	30
Absorbance	0.377	0.651	0.649	0.649	0.648	0.649
Time(min)	35	40	45	50	55	60
Absorbance	0.648	0.647	0.647	0.645	0.641	0.638

Table (7) shows that the absorption of the formed color product is increased after 5 min and then stabilized for a period of at least 60 min, this period is sufficient to perform many experiments, so it is adopted in the subsequent experiments.

Effect of temperature

The effect of temperature on the absorption of the formed colored product were studied by using different temperatures (10-60°C), the results are shown in Table (8).

Table (8)Effect of temperature.

Temp (°C)	10	20	25	30	35
Absorbance	0.387	0.575	0.651	0.592	0.559
Temp (°C)	40	45	50	55	60
Absorbance	0.446	0.307	0.264	0.156	0.104

The optimum temperature is 20 - 35°C , so 25°C was adopted in the subsequent experiments.

Effect of time on stability of the colored product

The stability time of the formed colored product has been studied by adding 4.5 ml of p-DMAB (5×10^{-2}M) to 3.0 ml of sulfathiazole (100 µg/ml), then 2.0 ml of buffer solution was added and the volume is

completed to 25 ml in a volumetric flasks with distilled water .It was observed that the absorption is increased after 10 min of dilution and the solution remains stable for 60 min after dilution at least and the results are shown in Table (9).

Table (9) Effect of time on stability of the colored product.

Time (min.)	10	15	20	25	30
Absorbance	0.376	0.598	0.649	0.651	0.652
Time (min.)	35	40	45	50	60
Absorbance	0.651	0.649	0.647	0.646	0.599

Effect of the solvents

The effect of the solvents on the formed colored product has been studied, the dilution is carried out by different organic solvents instead of water. The results are shown in Table (10).

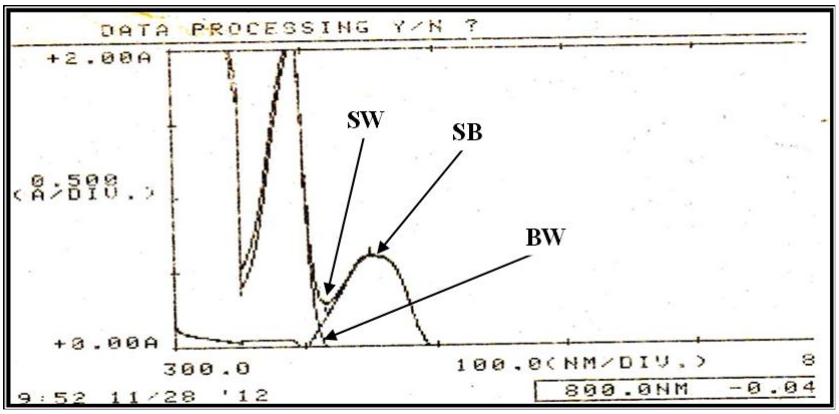
Table (10) Effect of the solvents.

Solvent	Water	Methanol	Ethanol	Aceton	DMF
Absorbance	0.649	0.426	0.339	0.145	0.011
$\lambda_{max,}$ nm	451	450	452	450	457

The results shown in table (10) indicate that the water is a good medium for reaction and gives high absorption value at the wavelength of 451 nm and due to its availability, it has been used as the best solvent in the subsequent experiments.

Final absorption spectrum

The spectrum of the formed colored product by coupling of sulfathiazole with p-DMAB (5×10^{-2}M) in the presence of buffer solution (pH 2.2) and temperature 25°C against its corresponding reagent blank show a maximum absorption at 451 nm in contrast to the blank reagent of zero absorbance at λ_{max}. The spectra are shown on Fig. (1).



Figure(1) Final absorption spectrum of the determination sulfathiazole.

SB : Absorption spectrum of sulfathiazole solution versus blank reagent.
SW: Absorption spectrum of sulfathiazole solution versus distilled water.
BW: Absorption of blank reagent versus distilled water.

Procedure for construction of calibration curve

To a series of volumetric flasks (25ml), 0.5- 6.0 ml of (100 µg/ml) of sulfathiazole are transferred, 4.5 ml of p-DMAB (5×10^{-2} M) and 2.0 ml of buffer solution(pH 2.2) are added at 25°C . After that the solutions have been left for 5 minute to complete the reaction, then the volumes are completed to the mark with distilled water and the solutions have been left for 10 minunt to complete the reaction. The absorbance has been measured at 451 nm against the blank reagent. Fig.(2) illustrate that the calibration curve is linear over the concentration range of 2.0 – 24.0 µg /ml while higher concentrations show a negative deviation from Beer's law. The molar absorptivity value is 1.1259×10^4 liter. $mol^{-1}.cm^{-1}$and the Sandell's sensitivity index 0.023 $\mu g/cm^2$.

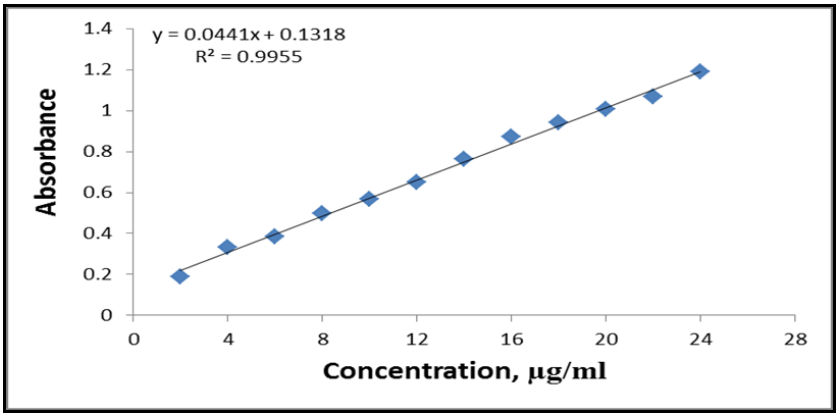


Fig. (2) Calibration curve for determination sulfathiazole by Schiff's base with p-DMAB reagent

Accuracy and precision

Accuracy and precision have been studied by measuring absorption (n=6) at 451 nm for two different concentrations of the drug within the limits of Beer's law, the average recovery (99.925 %) and the RSD <0.81% indicate that the method is of high accuracy and precision . The results are shown in table (11).

Table (11) Results of accuracy and precision.

Conc. of STHZ (ppm)	RSD %	Recovery* %	Average recovery %	RE %
4.0	0.81	99.70	99.925	-0.30
12.0	0.68	100.15		0.15

* Average of six determinations

Detection limit

Detection limit has been calculated by measuring the absorption for the lower concentration 2.0 µg/ml at optimal conditions (n=10) at 451 nm. The results are shown in table (12).

Table (12) Detection limit.

Concentration µg/ ml	$\overline{X}$	S.D	L.O.D (µg/ml)
2.0	0.189	0.0045	0.1534

* Average of ten determinations

The nature of the formed product

To know the nature of the formed yellow color product (stoichiometry of drug with the reagent), Job's method and molar ratio method have been applied. In both methods, the concentration of each of the standard sulfathiazole solution and p-DMAB reagent solution is equal to 3.9×10^{-4} M. In Job's method, in a series of volumetric flasks (25 ml), different volumes of the drug solution ranging from 1-9 ml and different volumes (9-1 ml) of reagent solution are mixed. A 2.0 ml of buffer solution is added and volumes have been completed to the mark with distilled water. The absorbance was measured at 451 nm against the blank reagent. The results in Fig. (3) show that the ratio is 1:1.

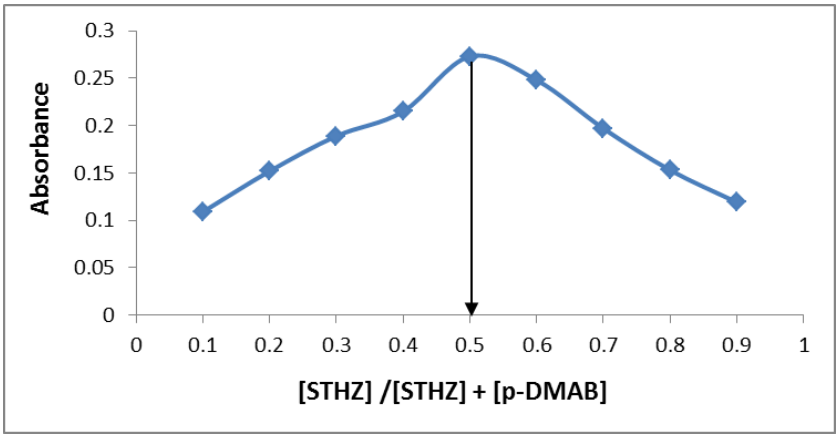


Fig. (3) Job's method of formed product by Schiff's base of sulfathiazole with p-DMAB reagent.

In molar ratio method, 3 ml of the standard drug solution in a series of volumetric flasks (25 ml) are transferred and different volumes 1.0 -5.5 ml of p-DMAB reagent solution, 2.0 ml of buffer solution has been added. The volumes have been completed to the mark with distilled water and the absorbance was measured at 451 nm against the blank reagent. Molar ratio was found to be 1:1. The results are shown in Fig. (4) which is in agreement with the Job's method results.

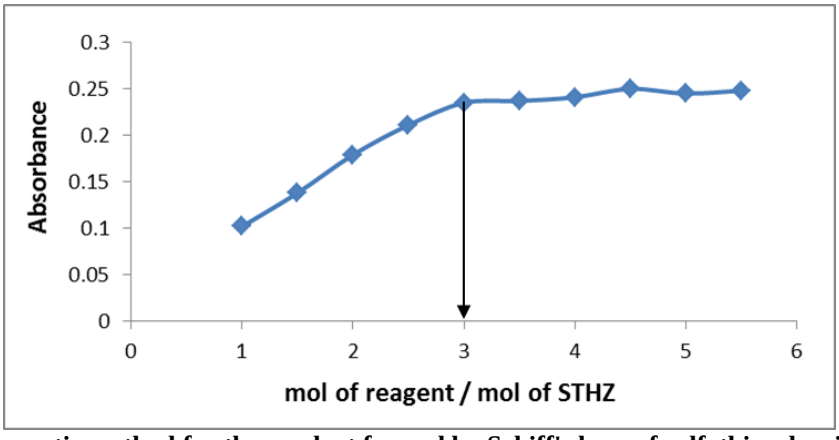
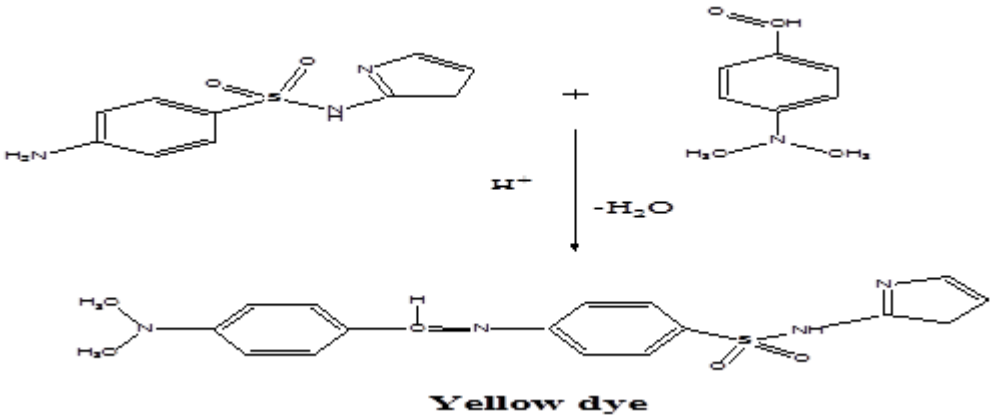


Fig. (4) Molar ratio method for the product formed by Schiff's base of sulfathiazole with p-DMAB reagent.

The proposed equation for reaction can be written as follows:



Yellow dye

Effect of interferences

In order to test the efficiency and selectivity of the proposed method, the effect of some foreign substances (starch, glucose, fructose , maltose and sucrose) that usually present in dosage forms are studied by taking volumetric flasks (25 ml) containing 3.0 ml of sulfathiazole (100 µg /ml), then different volumes (2.5, 5.0 ,7.5 ml) of foreign substances (1000 µg / ml) have been added resulting in a final concentration of (100, 200 ,300 µg / ml). The optimum conditions have been applied and the volumes have been completed to the mark with distilled water.The absorbance was measured at 451 nm versus blank reagent and recovery is calculated. The results showed that there is no interferences (13).

Table (13) Effect of interferences.

Foriegn compound	**Recovery (%) of 12 µg /ml of STHZ per µg /ml foreign compound added**		
	100	200	300
Starch	99.56	100.23	99.15
Glucose	98.24	98.96	97.31
Fructose	99.28	99.23	99.67
Maltose	100.23	98.26	100.25
Sucrose	98.54	99.45	98.65

Applications

This method has been applied for the determination of sulfathiazole in its pharmaceutical formulation bio prime injection (40 mg).

Direct method

In this method, different volumes (0.5, 1.0 ml) of a pharmaceutical formulation solutions (100 µg/ml) are transferred to 25 ml volumetric flasks and the resulting concentrations (2.5 ,5 µg/ml) and are treated as in construction of calibration curve. The absorbances was measured at 451 nm for six times(n=6). Recovery and RSD are calculated and the results are shown in table (14).

Table (14) Direct method for determination of STHZ in bio prim injection.

STHZ present µg/ml	STHZ measured µg/ml	RE, %	RSD, %	Recovery*, %
2.0	1.99	-0.50	1.11	99.50
4.0	4.05	1.25	1.66	101.25

* Average of six determinations

Table (14) shows the efficiency and success of the developed method for the determination of STHZ in its pharmaceutical formulation, the average recovery is 100.38 %.

standard additions method

To prove that the developed method is free from interferences , method of standard additions is applied for determining of sulfathiazole in its pharmaceuticals. Different volumes (0.5, 1.0 ml) of a pharmaceutical formulation solutions (100 µg/ml) are transferred to seven volumetric flasks (25 ml) for each volume, then increasing volumes (0.5-3.0 ml) of 100 µg/ml sulfathiazole standard solution were added with leaving the seventh flask without addition. The solution was treated as in construction of calibration curve. The absorbances were measured at 451 nm (Fig.5) the measured concentration was calculated from the equation of the straight line and the results of Recovery and RE shown in the table (15).

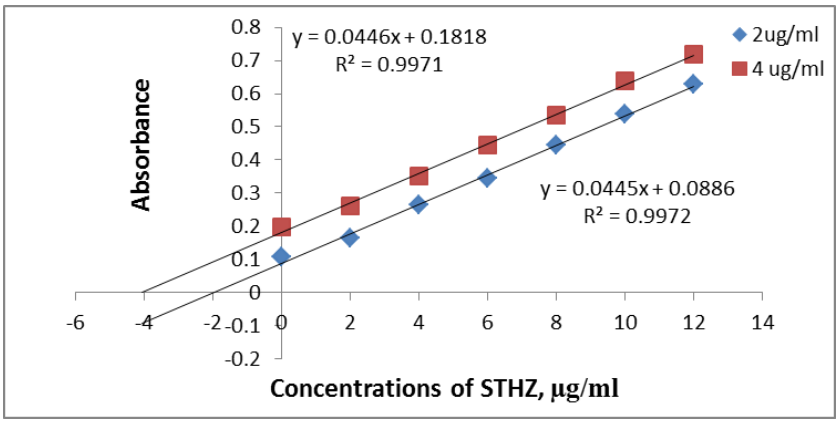


Fig. (5) Standard additions curve for the determination of STHZ in Bio prim injection

Table (15) Results of standard additions method.

Type of Drug	STHZ present µg/ml	STHZ measurd µg/ml	RE%	Recovery, (%)
Injection	2.0	1.99	-0.50	99.50
	4.0	4.08	1.96	102.00

The results shown in table (15) indicate that method of standard additions is in consistent with the direct method within the acceptable range of error, indicating that the method is satisfactory and free from interferences.

Conclusions

The results obtained confirm that the proposed method is simple, rapid and of good sensitivity for the determination of sulfathiazole. The method is based on Schiff's base formation between sulfathiazole and p-dimethylaminobenzaldehyde reagent (p-DMAB) to form yellow colored dye which is water soluble, stable and shows a maximum absorption at 451 nm. This method does not require temperature control, nor use of organic solvents and it can be applied successfully for determination of sulfathiazole in veterinary pharmaceuticals formulation with recovery of not less than 100.75%.

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التقدير الطيفي للسلفاثايازول بتكوين قواعد شيف

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الملخص

يتضمن البحث تطوير طريقة طيفية سهلة وسريعة وحساسة لتقدير عقار السلفاثايازول في المحلول المائي ،أذ تعتمد الطريقة على تكوين قواعد شيف بين مجموعة الامين الأولية في السلفاثايازول ومجموعة الالديهايد في الكاشف بارا- ثنائي مثيل امينو بنزلديهايد ليعطي ناتج لونه اصفر يظهر اعلى امتصاص له عند الطول الموجي451 نانوميتر عند الدالة الحامضية 2.2، كانت حدود قانون بير في مدى التراكيز 2-24 مايكروغرام/مل من السلفاثايازول، والامتصاصية المولارية 11259 لتر.مول-1 .سم-1 ودلالة ساندل 0.023 مايكروغرام .سم-2، ومعدل الاسترداديةً99.925 %، وتراوحت قيمة الانحراف القياسي النسبي 0.68- 0.81% ، وحد كشف 0.1534 مايكروغرام/مل. تمّ تطبيق هذه الطريقة بنجاح لتقدير السلفاثايازول في محلول سائل الحقن البيطري (بايوبرايم).