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Spectrophtometric determination of Sulfadiazine in Various Sample by coupling with 2,5-dimethoxy aniline

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Abstract

A simple, accurate and sensitive spectrophotometric method is developed for the quantitative determination of sulfadiazine (SDZ) in both pure and dosage forms. The method is based on diazotization of primary amine group of sulfadiazine with sodium nitrite and hydrochloric acid followed by coupling with 2,5-dimethoxy aniline to form a orang colored azo dye which shows maximum absorption at (478) nm. Beer's law is obeyed over the concentration range of (0.1-5) $\mu g.ml^{-1}$ of sulfadiazine, with molar absorptivity of ($8,26\times10^4$) L mol^{-1} cm^{-1}, and Sandell sensitivity index of (0.003) $\mu g/cm^2$. The method does not need to temperature control. The optimum conditions for all color development are described and proposed methods were successfully applied to the determination of sulfadiazine in its pharmaceutical preparations (burn cream).

Key words: Spectrophotometric determination, Diazotization and coupling, Sulfadiazine , 2,5- dimethoxy aniline.

الخلاصة

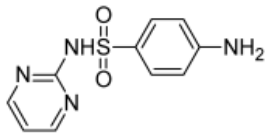
تم تطوير طريقة طيفية سهلة ، سريعة وحساسة لتقدير السلفاديازين في حالته النقية وفي مستحضراته الصيدلانية . تعتمد الطريقة على أزوتة السلفادايازين بواسطة نتريت الصوديوم وحامض الهيدروكلوريك ثم اقترانه مع الكاشف 2,5-dimethoxy aniline لتكوين صبغة ذات لون برتقالي ذائبة بلماء ومستقرة لها امتصاص اعلى عند الطول الموجي (478) نانوميتر . وجد انها تطاوع قانون بير عند مدى

التراكيز (0.1-5) مايكروغرام .مل$^{-1}$ من السلفاديازين ، وان الامتصاصية المولارية ($8,26\times10^4$)لتر.مول$^{-1}$.سم$^{-1}$ ودلالة ساندل للحساسية (0.003) مايكروغرام\سم^2 .الطريقة لاتحتاج الى السيطرة على درجات الحرارة. تم دراسة الظروف المثلى لتكوين المركب الملون وطبقت الطريقة المقترحة بنجاح لسلفاديازين في مراهم معالجة حروق الجلد كما وجد انه لايوجد تأثير للمضافات في هذه الطريقة .

مفتاح الكلمات: التقدير الطيفي ، الاقتران والازوتة ، السلفاديازين ، ٢,٥-داي ميتوكسي أينلين

Introduction

Sulfadiazine (SDZ), 4-amino-N-pyrimidin-2-yl-benzenesulfonamide, $C_{10}H_{10}N_4O_2S$,whereas its chemical structure is[1]



is a sulfonamide group of antibiotic drug ,which is one of the oldest and still widely used sulfonamides, a group of synthetically produced antibiotics, which was introduced in 1939[2], and has been used in veterinary and human therapy over 60 years[3]. A number of analytical methods for the determination of SDZ has been reported in the literature. These included high performance liquid chromatography coupled with on- line atmospheric pressure chemical ionization mass spectrometry (HPLC,APCI-MS)[4], cloud point extraction /flow injection-flame atomic absorption (CPE/FI-FAAS) spectrometry)[5], capillary zone electrophoresis[6], inductively coupled plasma-atomic emission spectroscopy (ICP-AES)[7], liquid chromatography[8], UV–spectrophotometry[9], immune chromatographic assay[10], flow injection chemiluminescence[11], ion selective electrode [12], Many UV-Visible spectrophotometric methods for the determination of SDz have been developed. Most of them included diazotization of SDZ and then coupling with different coupling reagents, such as : α-naphthylamine[13], 8-hydroxyquinoline [14], iminodibenzyl [15], histidine[16], γ-resorsolic acid[17], thymol[18], Other methods are either based on the formation of charge transfer complex with alizarin derivatives[19],and with phenosa-phranine[20], or an oxidative coupling reaction of SDZ with 4-amino-N,Ndimethylaniline in the presence of dichromate [21], N,N-diethylp-phenylenediamine sulphate and KIO4 [22]. In the present work, we succeeded in developing a novel

coupling agent for sensitive and selective spectrophotometric determination of the SDZ drugs based on the coupling of diazotized form with 2,5-dimethoxy aniline which results in the formation of orange product was spectrophotometrically measured at (478) nm. that has been proved successfully for the determination of SDZ in both pure form and its pharmaceutical preparations.

Experimental

Apparatus

- A UV/VIS spectrophotoeter digital double–beam recording spectrometer / Shimaduz, Japan, model UV-1650PC which connected has the software UV-Prob version, with 1 cm matched quartz cells were used.
- sencetive balance/ Mettler Toledo, Switzerland.
- Water bath / Julabo F12, Germany.

Materials

All Chemicals used are of the highest purity. A provided from different commercial company.

Sulfadiazine solution (250) µg mL^{-1}: prepared by dissolving (0.0125)g of SDZ in (50) mL ethanol.

2,5-dimethoxyaniline (0.05) mol mL^{-1}: prepared by dissolving (0.1912)g of SDZ in (25)mL ethanol.

Sodium nitrite (0.01) mol mL^{-1}: prepared by dissolving (0.0173)g of sodium nitrite (BDH) in (100) mL distilled water.

Sulfamic acid (0.2) mol mL^{-1} :prepared by dissolving (0.485)g of sulfamic acid in 100 ml distilled water.

Hydrochloric acid (1) mol mL^{-1}: prepared by diluting suitable amount of concentrated hydrochloric acid to (25) mL with distilled water.

Procedure:

An aliquot sample containing (0.01-0.5) mL of pure sulfadiazine (250)µg.mL^{-1} was transferd into a series of (25) mL standard volumetric flask. followed by (0.1) mL hydrochloric acid, and (1) mL of sodium nitrite (0.01) mol mL^{-1} were added. The solutions were allowed to stand for (5) min, then (1) mL of sulfamic acid was added to remove of nitronuim ion and the solutions were allowed to stand for (2) min, then

(2) mL of 2,5-dimethoxyaniline was added. The contents are mixed well and diluted to the mark with distilled water. The absorbances are measured After (20) min against the corresponding reagent blank at (478)nm using 1-cm quartz cells.

Procedure for dosage forms

floumizin cream (Ag.SDZ) (100 µg Ag.SDZ/ml): prepared by take (1) g from drug (containing 0.01g of Ag.SDZ) was transferred in to separation funnel and shaking with (50) mL ether, then extracted the Ag. SDZ with (25) mL of distilled water (three times). filtered and diluted up to the mark (100) mL with distilled water[23]. The concentration of SDZ is obtained by calibration curve already, made and described above.

Results and Discussion

Study of the optimum reaction conditions : The effect of various variables factors on the color development of azo day was studied to get the optimum conditions to determine the SDZ.

1- Effect of Reagent concentration: The effect of reagent concentration (0.005-0.1) mol mL^{-1} on the intensity of the absorbance, has been studied and (0.05) mol mL^{-1} was found to be optimum. shown no (Fig.1).

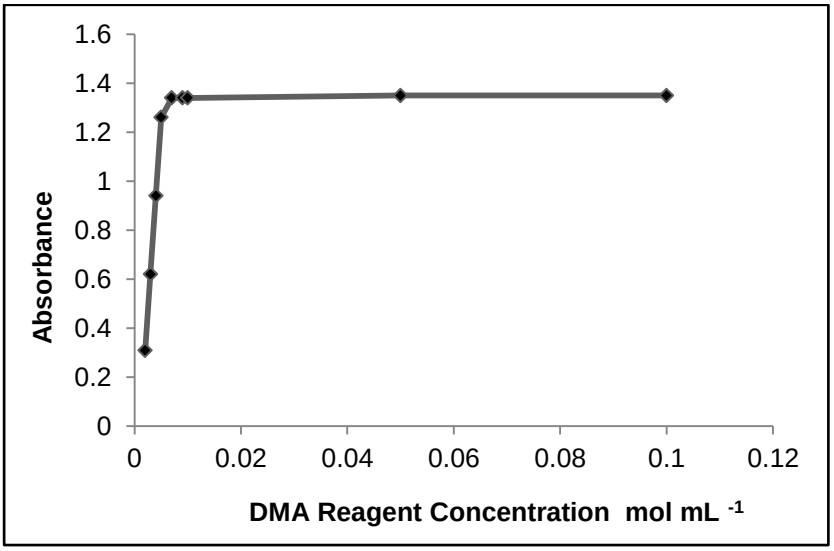


Fig (1):Effect of 2,5-dimethoxyaniline concentration mol mL^{-1}

2– Effect of Reagent volume: The effect of reagent (0.05) mol mL^{-1} volume (0.1 -5) mL on the intensity of the absorbance, has been studied and (2) mL was found to be optimum.(Fig.2).

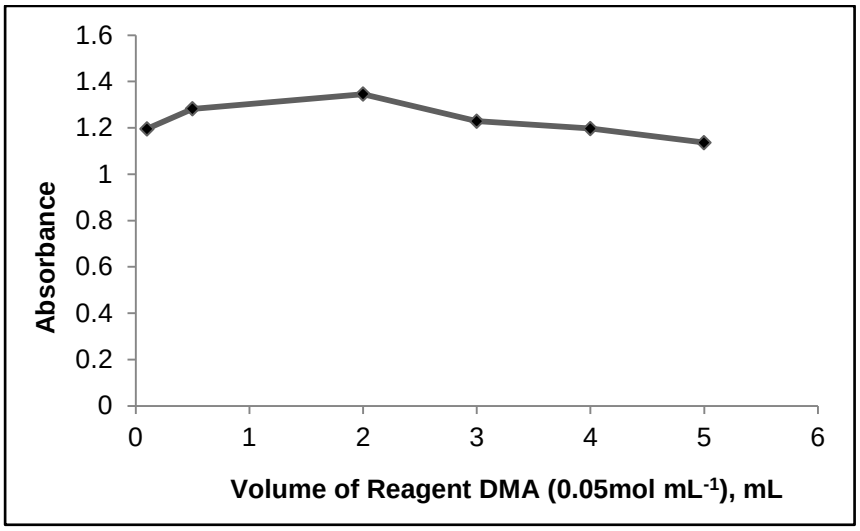


Fig (2): Effect of volume (0.05 mol mL^{-1}) reagent , mL

3- Effect of acid: It was found that the presence of acid caused increase the intensity of the produced color product, therefore some acids such as HCl, HNO_3 and CH3COOH (1)
mol mL^{-1}, were examined and was found .The maximum diazotization was obtained in presence of HCl, so; HCl was selected and the effect acid volume (0.05-3) mL on the intensity of the absorbance has been studied and (o.1)mL was found to be optimum. (Fig.3).

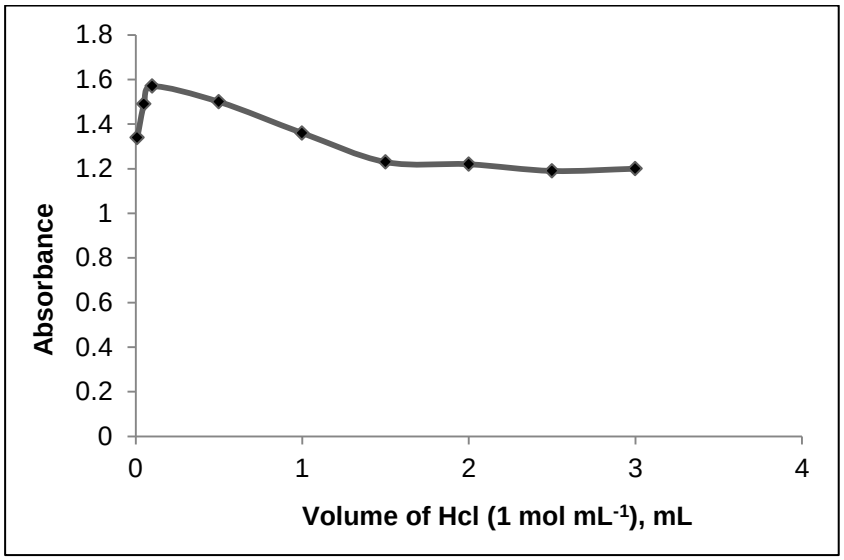


Fig (3) : Effect of volume (1 mol mL^{-1}) Hcl, mL

4-Effect of nitrite volume and time : The effect of (0.01) mol mL^{-1} nitrite volume (0.1- 3) mL on the intensity of the absorbance has been studied and (1) mL was found to be optimum. (Fig 4) and the time was found to be (5) min enough to complete diazotization of SDZ.

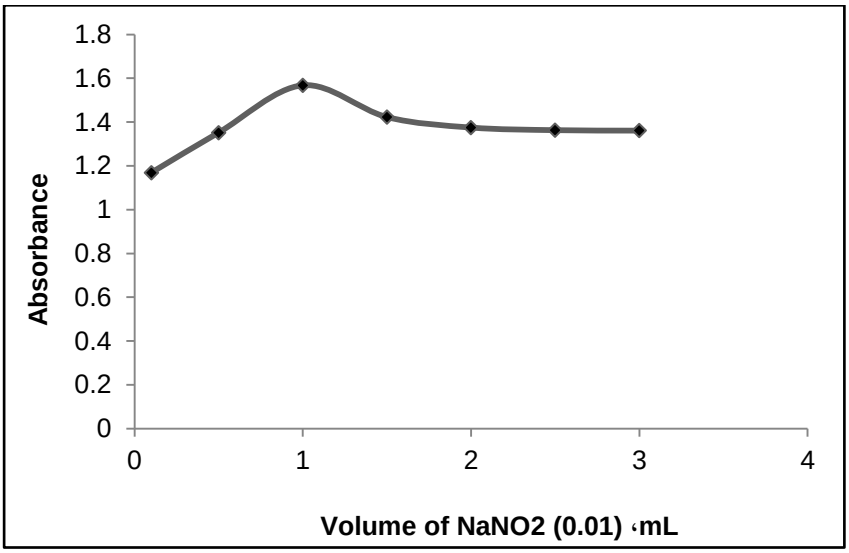


Fig (4) : Effect of volume of (0.01 mol mL^{-1}) $NaNo_2$, mL

5-Effect of sulfamic acid volume: The excess of nitrite must be removed by the addition of sulfamic acid solution to prevent reaction with reagent, so that the effect of sulfamic acid volume was studied and (1) mL was found to be optimum (Fig 5) and the time was found (2) min enough for removing the excess of nitrite.

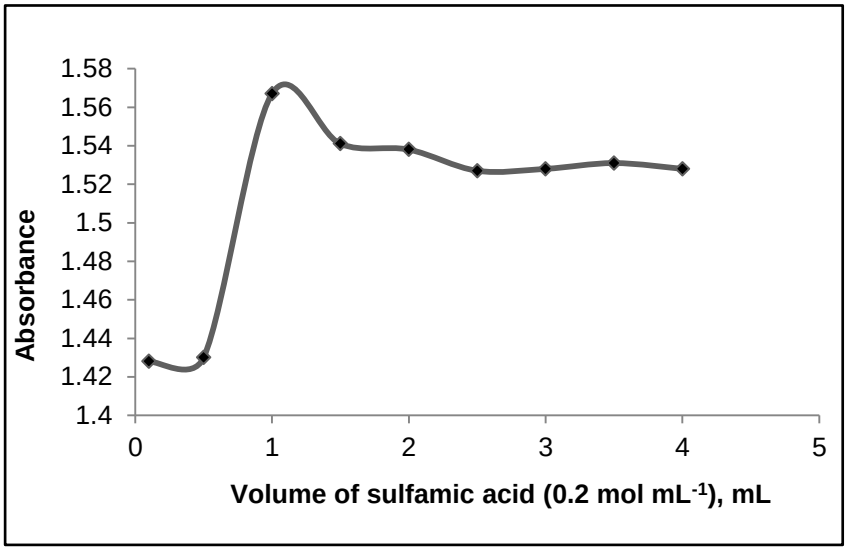
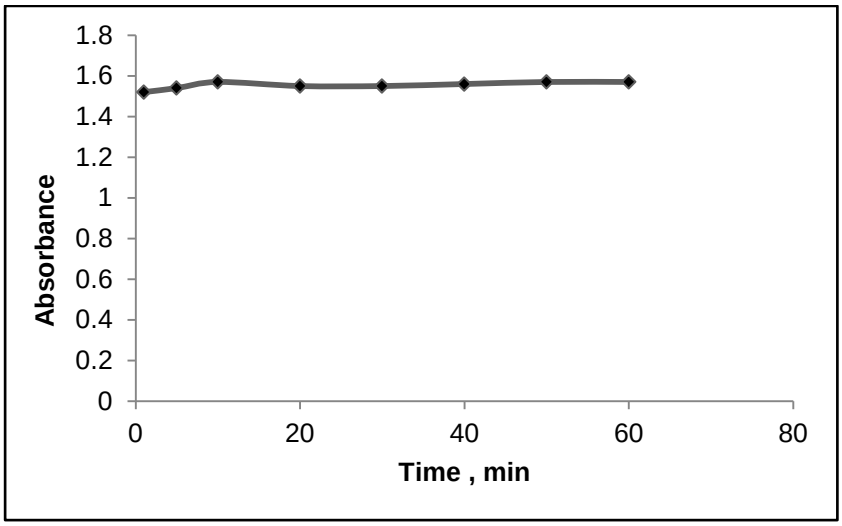


Fig (5) : Effect of volume of (0.2 mol mL^{-1}) sulfamic acid, mL

6-Effect of Reaction Time: The color intensity reached its maximum and became stable after (20) min. therefore (20) minutes were selected as optimum in the general procedure. (Fig 6)



Fag (6) : Effect of reaction time, min

7- Effect of Temperature: The effect of temperature on the resulting product was studied. It Was found the coloured product was stable at room temperature (20-30)ºC at higher temperatures the absorbance decrease, and attributed to the dissociation of the product on prolonged heating . (Fag 7).

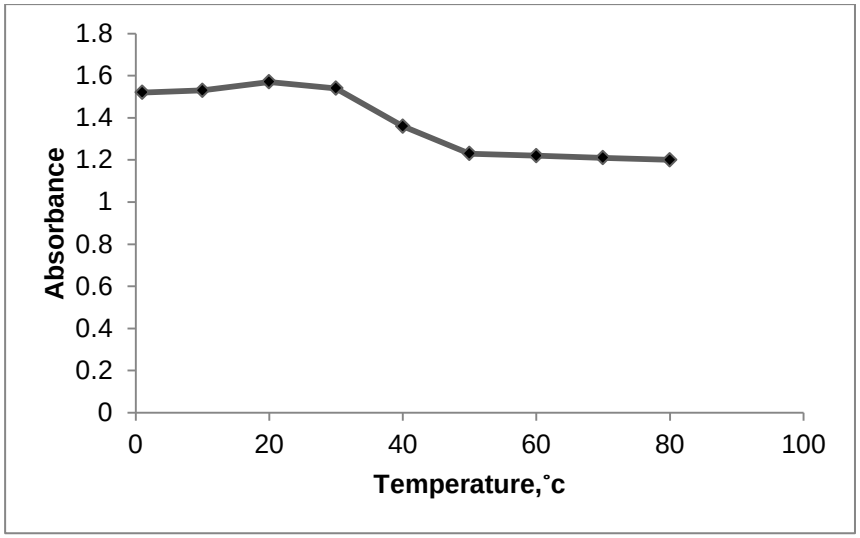


Fig (7) : Effect of temperature ,˚c

Absorption spectra

Sulfadiazine was reacted with 2,5-dimethoxyaniline, under the above-established conditions producing orange colored product with maximum absorption at (478) nm, while the reagent blank shows no absorption at this wavelength. (Fig.8) shows the absorption spectra.

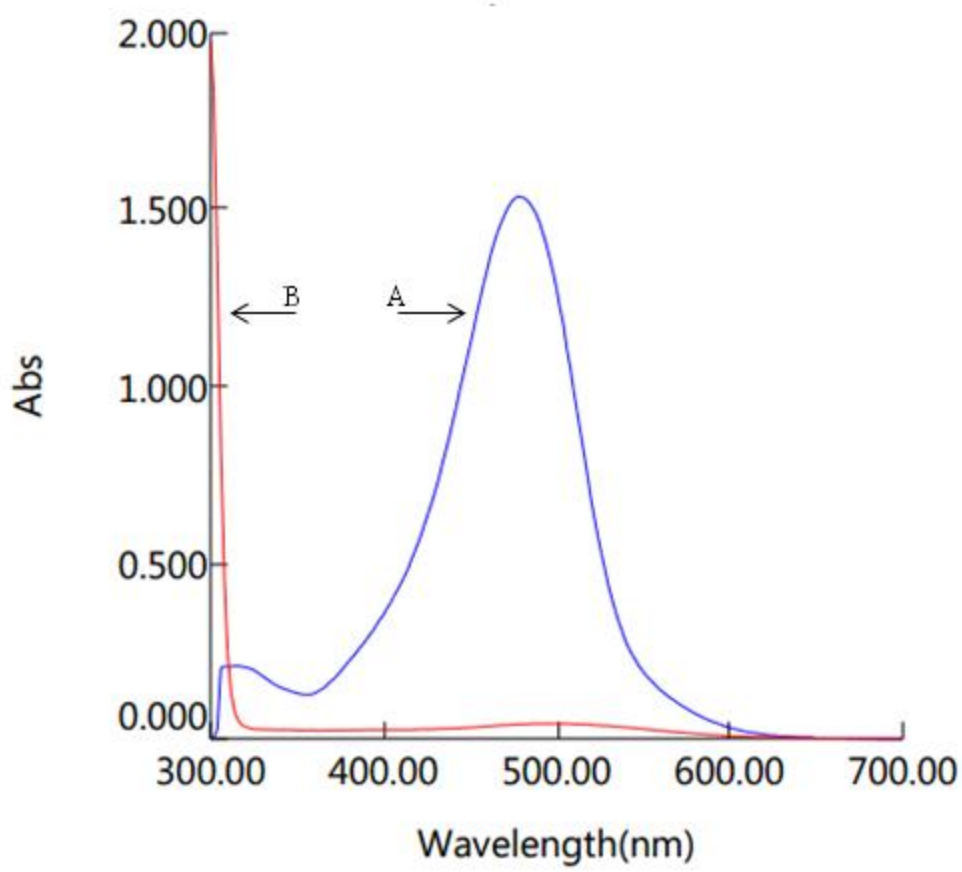


Fig (8) : Absorption spectra :
A : Sulfadiazine (5 µg mL^{-1}) with 2,5-dimethoxyaniline (0.05 mol mL^{-1}) product versus reagent blank.
B : Reagent blank versus D.W.

Calibration curve:
Under the optimum operating conditions, a linear calibration curve (Fig 9) is obtained over the concentration range of (0.1-5) µg.mL^{-1} of SDZ in a final volume of (25) mL. with a correlation coefficient of (0.9956) and intercept of (0.0114). A negative deviation from Beer's law was observed

above (5) μg.ml^{-1} concentration of SDZ. The apparent molar absorptivity of the azo dye has been found to be (8.264×10^{4}) L.mol^{-1}.cm^{-1}.

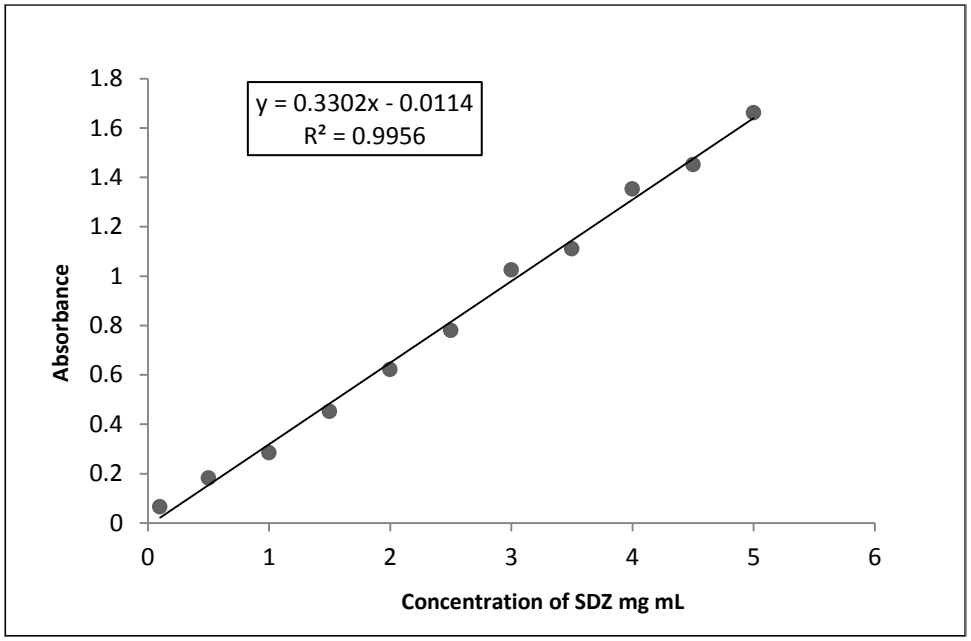


Fig (9) : calibration curve of Sulfadiazine with 2,5-dimethoxy aniline

Accuracy and precision

To determine the accuracy and precision of the calibration graph, sulfadiazine was determined at three different concentrations. The results shown in Table (1) indicate a satisfactory precision and accuracy.

Table (1): Accuracy and precision of proposed method

	Conc. of SDZ mg 25ml^{-1}		Error %*	Recovery* %	RSD*%
SDA	Present	found			
	0.5	0.496	-0.8	99.20	1.197
	2	1.977	-1.1	98.89	0.734
	4	3.98	-0.29	99.70	0.973

*Average for Three time

Nature of product and reaction mechanism

he stoichiometry of the azo dye was studied under the established conditions, by applying the continuous variations (Job's method) and mole-ratio methods. The experimental data in both methods (Fig. 10a and 10b) show that the azo dye has been formed by a 2:1 combining ratio of diazotized SDZ to 2,5-dimethoxyaniline.

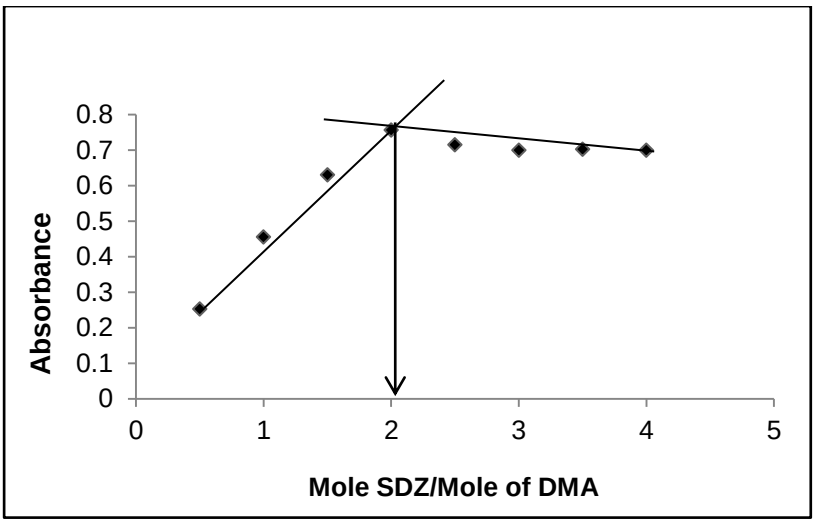
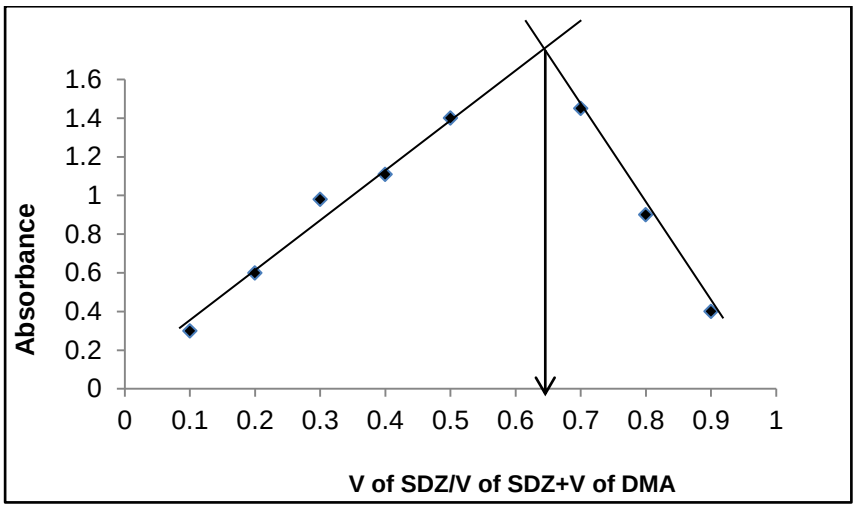
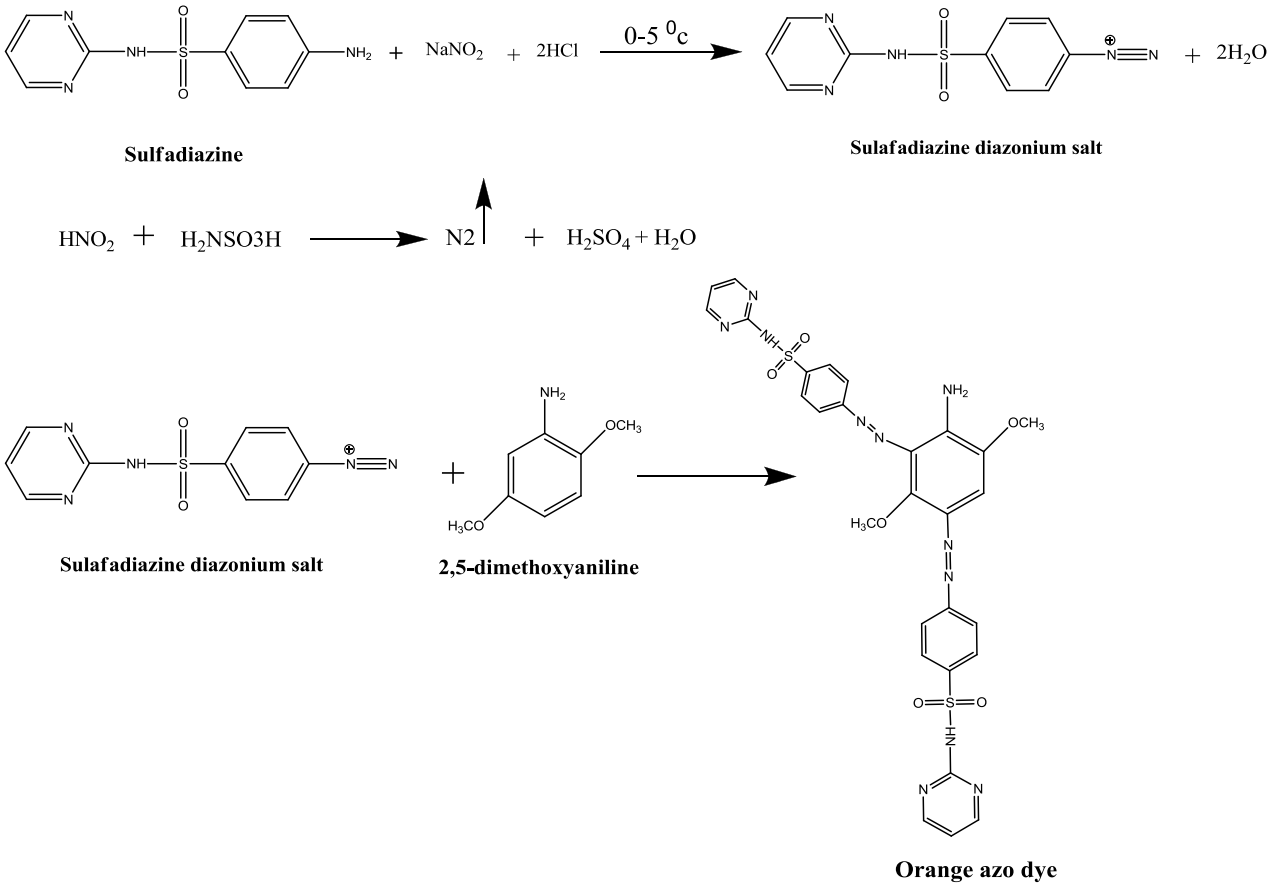


Fig. 10: (a) mole-ratio method



(b) Continuous variations method

The reaction of the DMA with SDZ was represent by the following scheme :



The apparent stability constant of the azo dye in aqueous solution, under the conditions of experimental procedure, has been calculated, and found to be (2.5×10^6) $L^2.mole^{-2}$ The regression equation obtained, and the analytical features of the procedure are summarized in (Table 2).

Table 2: Analytical characteristics of the procedure developed for the determination of sulfadiazine

Parameters	Present method
Regression equation	Y=0.3302x- 0.0114
Linear range (µg ml^{-1})	0.1-5
Correlation coefficient, r^2	0.9956
L.O.D (µg ml^{-1})	0.023
L.O.Q(µg ml^{-1})	0.078
Average of RSD) %	0.968
Average of recovery %	99.26
Molar absorptivity L. mol^{-1} .cm^{-1}	8.26×10^4
Sandell's sensitivity (µg.cm^{-2})	0.003

Application of the method

The proposed method is applied to the determination of sulfadiazine in four types of Burn cream (containing 1% Ag-sulfadiazine):The results which are shown in (Table 3)indicate that a good recoveries were obtained ,the proposed method was compared successfully with the official method[24].

Table 4: Application of the proposed and standard methods for the determination of Burn cream containing sulfadiazine

Pharmaceutical preparation	Rec.* % proposed method	Rec.* % standard method
SDZ pure	99.26	101.3
Flamazine (LOB)	99.57	99.60
Flaumizin (SYR)	98.90	99.30
Flamazine (KSA)	98.64	99.60
No-Burn (JOR)	98.85	100.80

* Average for three determinations

Conclusions

The proposed method was found to be very simple, accurate and sensitive spectrophotometric method, did not require temperature control, and pH control. The proposed method was applied to determine sulfadiazine (SDZ) in both pure and its dosage forms and can be used for the routine analysis.

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