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Improved Spectrophotometric Estimation of Sulphadiazine as Pure Form and in Its Pharmaceutical Preparations Via Condensation Reaction.

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Highlights

- **4-Dimethylaminobenzaldehyde was used as a suitable reagent to assay sulfadiazi spectrophotometrically via a condensation reaction in an acid medium.**
- **A good linear calibration graph was obtained and obeyed to Beer's law in the concentration range of 0.5 to 15 ppm with a coefficient of determination (r^2=0.9995) and molar absorptivity 2.9×10^4 l/mol.cm.**
- **The suggested procedure was successfully applied for the estimation of SDz in its some drugs.**

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ABSTRACT

This research includes the development of a sensitive, swift and low-cost spectrophotometric procedure for estimating sulfadiazine in the form of pure substance and in its pharmaceutical dosage forms via condensation reaction. The method was involved the reaction of sulphadiazine with 4-dimethylaminobenzaldehyde in an acidic medium of HCl to produce a yellow soluble product has maximum absorption intensity at the wavelength 452 nm. The calibration graph was linear and compatible to Beer's low over the range of concentration 0.5 to15 ppm with a determination coefficient (r^2= 0.9995) and molar absorptivity 2.90×10^4 l/mol.cm. The detection limit (DL), quantification limits (QL) and the sensitivity of Sandell were calculated and found to be 0.0543, 0.1810 µg/ml and 0.00860 µg/cm^2, respectively. A relative error percentage and the relative standard deviation were calculated and found to be in the range −3.8% to 0.36% and ±0.33 to ±1.26%, respectively. The method has been successfully applied to assay sulfadiazine in burn cream and veterinary drugs (injection and powder).

1. Introduction

Sulphadiazine (4-amino-N-pyrimidin-2-ylbenz-enesulfonamide) (SDz) is one of sulpha drugs. It is widely used as antibiotic medicine because it can effect the growth of many bacteria. SDz inhibits the formation of folic acid inside the bacteria and destroy it (Vandana *et al.*, 2011). Many spectrophotometric procedures have been developed for the determination of SDZ in Pharma-ceutical preparations and in biological fluids. Most of these procedures are based on the formation of diazotized SDz and coupling with 4-amino-2-hydroxy acetophenon (Al-Rufaie *et al.,* 2016), 2,6-dihydroxybenzoic acid (Mohammed and Zebary, 2013), 2,5-dimethoxy aniline (Mahdi and Kadim, 2015), histidine (Othman and Kadder, 2006), 4-(4-sulphophenyl azo) pyrogallol (Naser *et al.*, 2018), 8-hydroxyquinoline in basic medium (Nagaraja *et al.,* 2011), phloroglucinol (Azeez and Mohammed, 2022), α-naphthylamine using flow injection analysis (Fan *et al.*, 2003). Others based on the oxidative coupling reaction of SDz with 2,4-dinitrophenylhydrazine in the presence of potassium perchlorate (Ahmed and Khaleel, 2018) and o-aminophenol in the presence of potasium periodate (Abd *et al.*, 2019). p-N,N-diethylphen-ylenediamine sulphate using KIO4 as oxidizing agent (Nagaraja *etal.*, 2010). In addition, determination of SDz via charge transfer complex formation with phenolsaphranine (Al-Attas, 2003) and oxidation with $KMnO_4$ in alkaline medium (Siddiqui *et al.*, 2013) have been also reported.

Several analytical techniques have been published for direct or indirect SDz estimation, these techniques include: Fluorometry (Afsharipour *et al.*, 2019), liquid chromatography-mass spectrometry (LC-MS) (Zonaras *et al.*, 2016), flame atomic absorption spectroscopy with flow injection (FI-FAAS) (Dadfarnia *et al.*, 2011), ion selective electrode using PVC membrane with tetraphenylporphyrin-manganese(III) chloride (Almeida *et al.*, 2011), microchip electrophoresis (Zhang *et al.*, 2013), nanozyme-labeled biomimetic immunoassay (He *et al.*, 2020), tandem mass spectrometric detection, (Forti and Scortichini, 2009), Inductively coupled plasma coupled with atomic emission spectroscopy (ICP-AES) (Xiao-Ling *et al.*, 2010). However, most of these procedures either require sophisticated, several manipulation steps, cloud point extraction and expensive devices, which may not be available in any laboratory.

The present investigation involves a condensation reaction of SDz with 4-dimethyl-aminobenzaldehyde (4-DMAB) reagent in an acid medium to form a yellow product solution has been employed to determine SDz in some pharmaceutical preparations.

2. Experimental

2.1. Apparatus

A Jasco V-630 double beam UV-Vis spectro-photometer (Japan) with 1.0-cm matched quartz cells and Bp3001 professional bench top pH meter devices were used for all absorption spectra recording and pH measurements, respectively.

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2.2. Chemical materials

High pure chemical materials were used in all experiments. They were obtained from (Fluka and BDH) companies, while the pure SD_Z compound was obtained from the (state company for drug industries and medical appliances, SDI), Samarra-Iraq.

Stock SD_Z solution (500 µg/ml) :

A little amount of distilled water (DW) was added to dissolve 0.0500 g of pure SD_Z and the volume was then completed to 100 ml with the same solvent in a calibrated flask.

Working SD_Z standard solution (50 µg/ml = 1.9976×10^{-4} M) :

A 10 ml of the stock solution of SDz was diluted to 100 ml with DW using a calibrated flask.

4-DMAB solution (1%w/v):

A 1.0 g of 4-DMAB reagent was dissolved in a 100ml of ethanol using a calibrated flask. The solution was transferred and kept in a dark bottle.

Hydrochloric acid solution (1M) was also prepared.

2.3. Recommended procedure

To a series of 10 ml calibrated flasks a suitable volume containing 5-150 µg of working SDz solution, 2 ml of 4-DMAB reagent (1%) and 1 ml of hydrochloric acid (1M) solution were added. The contents of all flasks were mixed thoroughly and kept constant at laboratory temperature (25±2 C°) for 15 minutes to complete the reaction. After that, the volumes of all flasks were diluted to the mark with DW. The absorbance of each solution was then recorded at 452 nm versus the black solution prepared in the same manner but without SDz.

2.3.1. Analysis of SDz in drug

2.3.1.1. *Vapcotrim injection solution (100 ppm):*

To prepare stock SDz solution (2000 ppm), a 1ml of injection solution was taken and diluted with ethanol to 100 ml in a calibrated flask and 10 ml of it was then transferred quantitatively to a 100 ml calibrated flask and with ethanol the volume was completed to the mark. A working injection solution containing 100 ppm of SDz was prepared by diluting 5 ml of the stock solution (2000 ppm) with 100 ml of ethanol. An aliquot of the working injection solution drug solution was then treated as the recommended procedure.

2.3.1.2. *Flama life cream solution(100 ppm):*

A 1.00 g of the cream was weighed and dissolved in 50 ml of ether into a separation funnel. The SDz was then removed three times with 25 ml DW. The extracted SDz layer was then separated, filtered, and completed to 100 ml with DW in a calibrated flask (United States Pharm-acopeia, 1995). An aliquot of diluted solution of the drug was then analysed using the recommended procedures.

2.3.1.3. *Intertrim-480 powder solution(100 ppm):*

An accurately weighed 0.025g of the drug powder was dissolved completely in about 20 ml of DW. The solution was then mixed vigorously, filtered and finished in a 100 ml calibrated flask with ethanol. Each ml of this solution is equivalent to 100 µg of SDz. The recommended procedure was then followed for the determination of SDz by taking an aliquot of the dilute solution of the drug.

3. Results and discussions

3.1. Absorption spectra

A yellow colure product was produced on the treatment of a solution containing SDz with 4-DMAB reagent in an acidic medium of hydrochloric acid (1M) in final volume 10 ml. The yellow resulting product exhibits maximum absorption at wavelength 452 nm versus the blank solution (Fig. 1). The corresponding reagent blank shows a negligible absorbance at this wavelength. The intensity of the colour is directly proportional to the amount of SDz that originally exist in the solution.

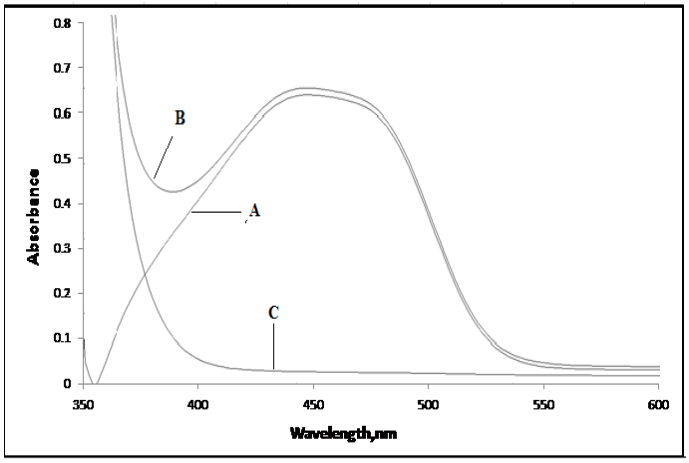


Fig. 1. Absorption spectra of 5 ppm SDz treated according to the recommended procedure Vs. reagent blank (A), distilled water (B), and blank solution measured Vs. distilled water

3.2. Effect of acids on absorbance

The influence of different amounts of 0.2-2.0 ml of various strong and weak acid solutions (1M) has been studied on the absorbance of yellow products. The results shown in Fig. 2 indicated that 1ml of HCl (1M) acid is optimum; therefore, it has been established for the subsequent experiments.

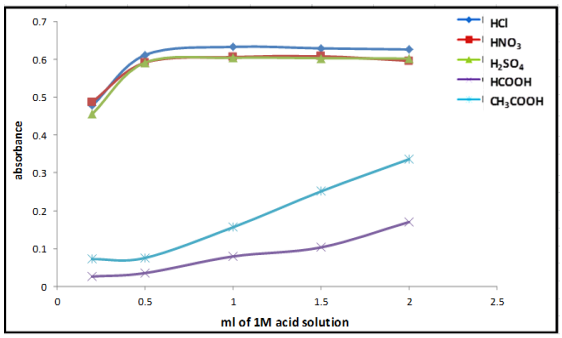
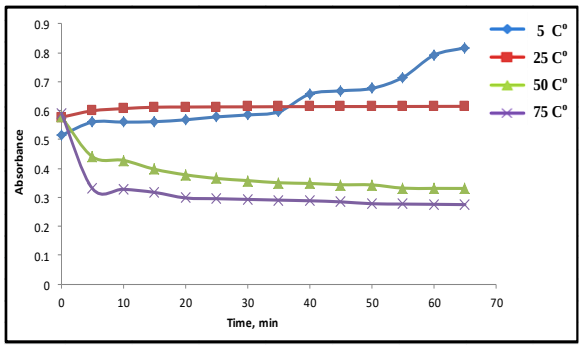


Fig. 2. Effect of 1M acid solutions on absorbance

3.3. Effect of reaction time and temperature

The influences of temperature and the time of reaction on the absorption intensity and the development of the colour product were studied by carrying out the reaction of SDz and 4-DMAB at different temperatures using a thermostatic water bath at different periods of time and the results are listed in Fig. 3.



*RT. (laboratory temp.)= 25±2

Fig. 3. Effect of temperature and time on absorbance

The results in Fig. 3 revealed that when the reaction was carried at lower temperatures 5C° and high temperature 50 and 70C° a noticeable instability in the sensitivity of the product formed with increasing time were observed, while the measurements at

laboratory temperature were found to be the most appropriate for doing all measurements after waiting about 15 minutes as standing time. The colour of the pigment formed was stable for at least 60 minutes. Therefore, 15 minutes have been adopted in the approved method for the subsequent studies.

3.4. Effect of 4-DMAB amount

The influence of different increasing amounts of 0.5-3.0 ml of the reagent 4-DMAB (1%) on the absorption intensity of the yellow product was investigated. The results in Table 1 are indicated that 2 ml of 4-DMAB reagent can be considered the optimum because they gave a high absorbance value and good determination coefficient (r^2=0.9992). Therefore, the volume of 2ml 4-DMAB was chosen for the subsequent measurements.

Table 1

4-DMAB amount effect on absorbance

	Absorbance/µg of SDZ added						r^2
	5.0	10	30	40	50	75	
0.5	0.040	0.082	0.155	0.309	0.444	0.596	0.9689
1.0	0.044	0.178	0.347	0.465	0.606	0.779	0.9703
1.5	0.072	0.268	0.355	0.491	0.613	0.849	0.9858
2.0	0.142	0.258	0.371	0.504	0.622	0.897	0.9992
2.5	0.134	0.249	0.369	0.502	0.621	0.884	0.9982

3.5. Reproducibility and Validity of Beer's law

Under the optimum conditions of the recommended procedure, a linear standard calibration graph was obtained which is compatible with Beer's low over the concentration range of 5.0 to 150 µg of SDz in a final volume of 10 ml (i.e. 0.5-15 ppm) with determination coefficient (r^2=0.9995) (Fig. 4).

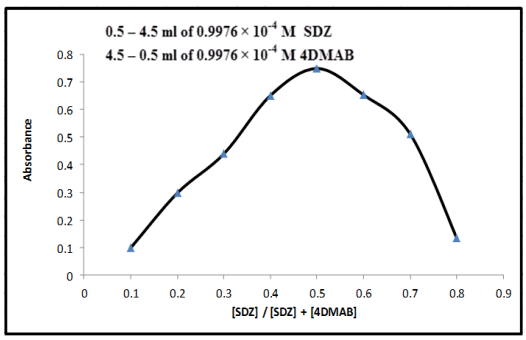


Fig. 4. Calibration curve for SDZ determination

The apparent molar absorptivity was estimated and is equal to 2.9×10^4 l/mol.cm, which corresponds to Sandell's sensitivity index of .00086 µg/cm². The detection and quantification limits (DL) and (QL) were calculated and their values are equal to 0.0543 and 0.1810 µg/ml, respectively (International Conference on Harmonization, 2005). A relative error percentage and the relative standard deviation (RSD) were also calculated and their values are in the ranges of -3.8% to 0.36% and ±0.33 to ±1.26%, respectively. The stability constant (Ks) of the colour product was also estimated and equal to 0.26×10^6 l/mol (Hargis, 1988).

3.6. Stoichiometry of the product

The composition of the resulting product which formed by the reaction of 4-DMAB reagent with SDz has been estimated under the established conditions by applying the continuous variations (Job's) and mole-ratio methods (Delevic, 1997). In the molar ratio method, different volumesof 0.2-4.0 ml of 0.9976×10^{-4}M (4-DMAB) solution (V_R) were added to a 1 ml of 0.9976×10^{-4} M of SDz (Vs) in the presence 1 ml of 1M HCl, and the absorbance was measured at 452 nm after dilution to the mark with distilled water. In Jop's method, volumes 0.5–4.5 ml of 0.9976×10^{-4} M portions of SDz (Vs) were coupled according to analytical procedure with the corresponding complementary volume of 0.9976×10^{-4}M (4-DMAB) solution(V_R) to give a total volume of 5 ml for V_S+ V_R in presence of 1ml of 1M HCl and diluted to 25 ml with distilled water. The results obtained in Fig. 5a and b show that the ratio of SDz to 4-DNAB reagent was found to be 1:1. Therefore, the chemical composition of the resulting product which formed through the condensation reaction can be written as (scheme 1).

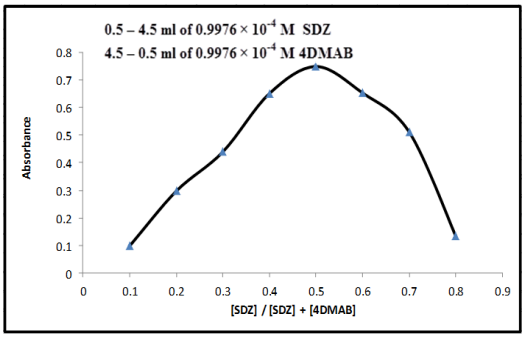


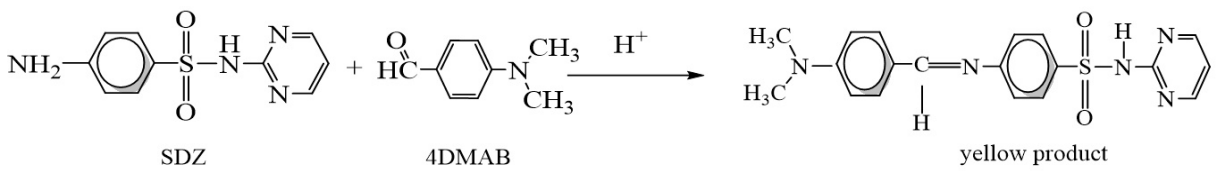
Fig. 5. (a) Continuous variations (Job) plot

4. Applications

The present method has been successfully applied for the estimation of SDz in three pharmaceutical preparations (burn cream, injection and powder). The results listed in Table 3 reveal that the proposed method is suitable for determining SDz with acceptable recovery results. To evaluate the results of the recommended procedure a t-test has been investigated. The results of t-experimental in Table 3 are less than the tabulated-t value (2.776) at the 95% confidence level and for four degrees of freedom (N=4) (Christian, 2004). These results of (t) indicate that the difference is statistically not significant, which confirms the success of applying the recommended procedure to estimate SDz in its pharmaceutical preparations.

5. Standard additions method

To check the efficiency of the recommended method for assaying SDz in its pharmaceutical preparations due to the unavailability of the requirements of the standard method, a standard additions method was applied for this purpose and the results were illustrated and summarized in Fig. 5 and Table 3.



Scheme (1)

Table 2.

Determination of SDz in pharmaceutical preparations

Drug	Certified value	SDz found (µg)	Re. error (%)*	Recovery (%)*	RSD*	Measured value (mg)*	t-expt.[a]
Vapcotrim injection (Jordan)	200 mg of SDz/1 ml	25.05 75.13	0.20 0.17	100.20 100.17	0.64 0.33	200.40 200.34	0.74 1.14
Intertrim-480 veterinary powder (Holland)	400 mg of SDz/1 g	25.09 74.72	0.36 -0.37	100.36 99.63	1.19 0.49	401.44 398.52	1.75 1.98
Flamalife cream (Holland)	1g of SDz/100g	19.24 48.69	-3.80 -2.62	96.20 97.38	0.92 1.26	0.9620[b] 0.9738[b]	1.76 2.03

*average of five estimations, [a] $t \pm = (\bar{x} - \mu)\frac{\sqrt{N}}{S}$, [b] g/100g

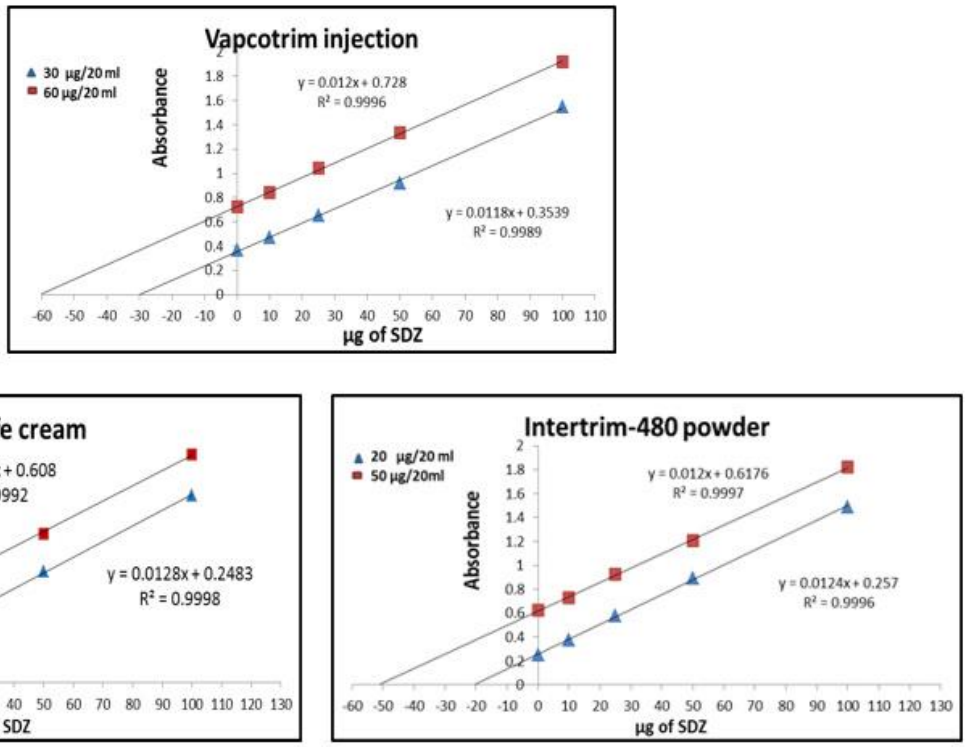


Fig. 6. Calibration graphs of standard additions method for the estimation of SDz in (a) injection, (b) powder, and (C) burn cream

Table 3.

The results of SDz estimation by using standard additions methods

Drug	Certified value	SDz Present (µg)	Recovery (%)	Re. error (%)	Measured value (mg)
Intertrim-480 veterinary powder (Holland)	400 mg of SDz/g	20 50	104.63 102.93	4.65 2.92	209.26 205.86
Vapcotrim injection (Jordan)	200 mg of SDz/ml	30 60	99.97 101.11	-0.03 1.12	399.88 404.44
Flamalife cream (Holland)	1g of SDz/100g	20 50	96.95 98.06	-3.01 -1.94	0.9695* 0.9806*

6. Conclusion

An inexpensive reagent of 4-mimethylamino-benzaldehyde was used to develop a simple and sensitive spectrophotometric method for the estimation of sulphadiazine as pure form and in some drugs through a condensation reaction. The method does not need solvent extraction steps or temperature control. The method is also accurate and precise enough to be successfully applied for the estimation of SDz in burn cream and veterinary injection and powder.

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