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Development and Validation of Spectrophotometric Methods for Estimating Sulfamethoxazole in Pharmaceutical Preparations

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ABSTRACT

Four simple, sensitive, accurate, and rapid visible spectrophotometric methods (A, B, C and D) have been developed for the estimation of sulfamethoxazole in pharmaceutical Preparation. They are based on the diazotization of sulfamethoxazole with sodium nitrite and hydrochloric acid followed by coupling with *N*-(1-naphthyl ethylenediamine dihydrochloride (Method A) to form pink coloured chromogen, diphenylamine (Method B) to form pink coloured chromogen, *β*-napthol (in alkaline medium) (Method C) to form a orange yellow coloured chromogen and Resorcinol (in alkaline medium) (Method D) to form orange red coloured chromogen and exhibiting absorption maxima λ_{max} at 536 nm, 516 nm, 477 nm and 502 nm respectively. The coloured chromogens formed are stable for more than 2 h. Beer's law was obeyed in the concentration range of 4 -12 µg mL^{-1} in method A , 2 – 10 µg mL^{-1} in method B, 5 – 25 µg mL^{-1} in method C and 1 – 5 µg mL^{-1} in method D respectively. The Results of the four analysis have been validated statistically and by recovery studies. The results obtained in the proposed methods are in good agreements with labeled amounts, when marketed pharmaceutical preparations are analyzed.

Keywords: Sulfamethoxazole, Diazotization, Visible spectrophotometric, Chromogen.

INTRODUCTION

Sulfamethoxazole [1] is chemically 4-amino-*N*-(5-methylisoxazol-3-yl)-benzenesulfonamide (Molecular mass 253.279 g mol^{-1}). It is a sulfonamide bacteriostatic antibiotic. It is most often used as part of a synergistic combination with trimethoprim in a 5:1 ratio in co-trimoxazole, which is also known as Bactrim, Septrin, or Septra (also abbreviated SMX/TMP). Its primary activity is against susceptible forms of *Streptococcus, Staphylococcus aureus, Escherichia coli, Haemophilus influenzae*, and oral anaerobes. It is commonly used to treat urinary tract infections. In addition can be used as an alternative to amoxicillin-based antibiotics to treat sinusitis. It can also be used to treat toxoplasmosis. It is official in United State Pharmacopoeia, British Pharmacopoeia and European Pharmacopoeia. Literature survey reveals the estimation of Sulfamethoxazole in pharmaceutical formulations by various spectrophotometric [2-7], HPLC [8-11], HPTLC [12], Capillary zone electrophoresis [13], Micellar electrokinetic chromatography [14], Derivative ratio spectrometry [15], Flow injection sensor [16], Sulfamethoxazole-imprinted polymer [17], Spectrofluorometry [18], Fluorescence Spectrophotometric [19] and NMR [20] methods.

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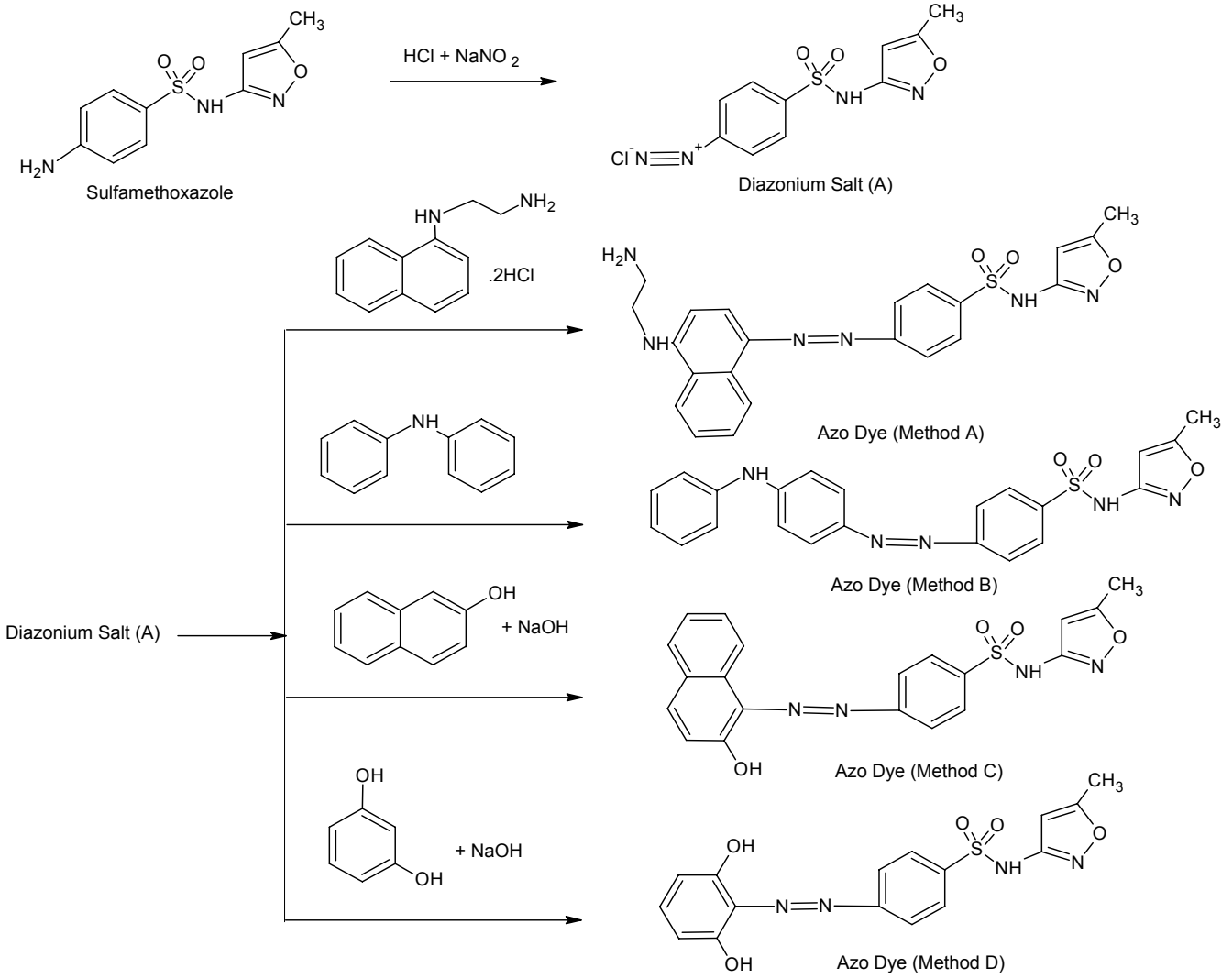
The present work deals with the development of four simple and sensitive visible spectrophotometric methods for the quantitative estimation of Sulfamethoxazole in bulk and pharmaceutical preparations.

The aromatic amino group present in Sulfamethoxazole is diazotized [21] with nitrous acid ($NaNO_2$ / HCl) at room temperature and diazonium salt thus formed is coupled with the *N*-(1-napthyl) ethylenediamine dihydrochloride (0.2% w/v; Bratton Marshall Reagent) in method A, diphenylamine in method B, *β*-naphthol (in alkaline medium) in method C and resorcinol (in alkaline medium) in method D to formed colored chromogens and exhibiting absorptions maxima λ_{max} at 536 nm, 516 nm, 477 nm and 502 nm, respectively. The coloured chromogens formed in method A, B, C and D are stable for more than 2 h. Beer's law limits are 4-12 µg mL^{-1} in method A, 2-10 µg mL^{-1} in method B, 5-25 µg mL^{-1} in method C and 1-5 µg mL^{-1} in method D, respectively. Spectrophotometric parameters and statistical analysis of data were established for standardization of these four methods. They have been successfully extended to pharmaceutical preparations containing Sulfamethoxazole.

MATERIALS AND METHOD

Materials

A Shimadzu UV / VIS double beam spectrophotometer (model 1700 PC) with 1 cm matched quartz cells used for all spectral measurements.



Scheme 1: Proposed reaction scheme for methods A, B, C and D.

Sulfamethoxazole, Trimethoprim bulk drug and excipients were obtained form Molecule Analytical Laboratory, Ahmedabad, India. Bactrim d.s. (Nicholas Piramal India Ltd) and Septran (GlaxoSmithKline Pharm. Ltd.) tablets were purchased from the market. All chemicals used are of analytical grade. Sodium nitrite, hydrochloric acid, sodium hydroxide, resorcinol and diphenylamine were obtained from E. Merck. *β*-naphthol, sulphamic acid, *N*-(1-napthyl) ethylenediamine dihydrochloride which obtained from Otto kemi, Qualigens and Acros organics, respectively. Conductivity water (pH 6.32, Conductivity 0.92 µS cm^{-1}) was used for dilution and preparation of all reagents.

Preparation of standard stock solution

About 100 mg of Sulfamethoxazole weighed accurately and dissolved in 30 ml of 2 mol L^{-1} hydrochloric acid in a 100 ml volumetric flask and diluted up to the mark with water (1000 µg ml^{-1}). The final concentration of Sulfamethoxazole was brought to 100 µg ml^{-1} with water.

Preparation of sample stock solution

Two brands of commercial tablets were analyzed by the proposed methods. 20 tablets each containing 800 mg and 400 mg Sulfamethoxazole are taken and average weight was calculated, tablets were cursed thoroughly in a mortar. Tablets powder equivalent to 100 mg of the drug weighed accurately and dissolved in 30 ml of 2 mol L^{-1} hydrochloric acid in a 100 ml volumetric flask and allowed to sonicate with intermittent shaking for 10 min, cooled and diluted up to the mark with water (1000 µg ml^{-1}). The solutions were filtered through Whatman filter paper No. 41 and the final concentration of Sulfamethoxazole was brought to 100 µg ml^{-1} with water.

Method A

For Method A aliquots of Sulfamethoxazole ranging from 0.4-1.2 ml (100 µg ml^{-1}) were transferred into a series of 10 ml volumetric flasks. To each flask 1 ml of ice cold sodium nitrite (0.1% w/v) and 1 ml of 2 mol L^{-1} hydrochloric acid were added at room temperature. After 5 min, 1 ml of sulphamic acid (0.2% w/v) and 1 ml of Bratton Marshall Reagent ware added. The volumes were made up to the mark with distilled water. The absorbance of a pink-coloured chromogen was measured at 536 nm against a reagent blank. The amount of Sulfamethoxazole present in the sample was computed from calibration curve.

Method B

For Method B aliquots of Sulfamethoxazole ranging from 0.2-1.0 ml (100 µg ml^{-1}) were transferred into a series of 10 ml volumetric flasks. To each flask 1 ml of ice cold sodium nitrite (0.1% w/v) and 1 ml of 2 mol L^{-1} hydrochloric acid were added at room temperature. After 5 min, 1 ml of sulphamic acid (0.2% w/v) and 0.25 ml of alcoholic

diphenylamine (0.3% w/v) were added. The volumes were made up to the mark with distilled water. The absorbance of a pink-coloured chromogen was measured at 516 nm against a reagent blank. The amount of Sulfamethoxazole present in the sample was computed from calibration curve.

Method C

For Method C aliquots of Sulfamethoxazole ranging from 0.5-2.5 ml (100 µg ml^{-1}) were transferred into a series of 10 ml volumetric flasks. To each flask 1 ml of ice cold sodium nitrite (0.1% w/v) and 1 ml of 2 mol L^{-1} hydrochloric acid were added at room temperature. After 5 min, 1 ml of sulphamic acid (0.2% w/v), 1.0 ml of aqueous *β*-naphthol (0.2% w/v) and 1 ml sodium hydroxide (20% w/v) were added. The volumes were made up to the mark with distilled water. The absorbance of a orange yellow-coloured chromogen was measured at 477 nm against a reagent blank. The amount of Sulfamethoxazole present in the sample was computed from calibration curve.

Method D

For Method D aliquots of Sulfamethoxazole ranging from 0.1-0.5 ml (100 µg ml^{-1}) were transferred into a series of 10 ml volumetric flasks. To each flask 0.5 ml of ice cold sodium nitrite (0.1% w/v) and 1 ml of 2 mol L^{-1} hydrochloric acid were added at room temperature. After 5 min, 1 ml of sulphamic acid (0.2% w/v), 0.5 ml of aqueous resorcinol (0.5% w/v) and 1 ml sodium hydroxide (20% w/v) were added. The volumes were made up to the mark with distilled water. The absorbance of a orange red-coloured chromogen was measured at 502 nm against a reagent blank. The amount of Sulfamethoxazole present in the sample was computed from calibration curve.

RESULTS

The presence of an aromatic amino group in Sulfamethoxazole led to diazotization of the drug with nitrous acid, and coupling of the resulting diazonium salt with *N*-(1-naphthyl ethylenediamine dihydrochloride (Method A) and diphenylamine (Method B) to form pink-coloured chromogen, *β*-napthol (in alkaline medium) (Method C) to form a orange yellow-coloured chromogen and resorcinol (in alkaline medium) (Method D) to form a orange red-coloured chromogen. The proposed chemical reactions are shown in [Scheme-1].

The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity (*ε*) and Sandell's sensitivity (*S*) are presented in Table 1. A regression analysis using the method of least squares was studied; the slope (*m*), intercept (*a*) and correlation (*r*) obtained from different concentrations are summarized in Table 1. The percent relative standard deviation and percent range of error (0.05 and 0.01 level of confidence limits) calculated from the eight measurements. The result shows that these methods have reasonable precision. In accordance with ICH recommendations, the precision was determined at two levels i.e. repeatability and intermediate precision. The repeatability (intra day) and intermediate precision (inter day) in terms of the percent relative standard deviation are reported in Table 1.

Effect of the reagent concentration

The obtained results show that at least 1.0 ml of *N*-(1-napthyl) ethylenediamine dihydrochloride and *β*-naphthol were required for the maximum color development in methods A, and C, respectively (Fig. 1). In methods B and D at least 0.25 ml of diphenylamine and 0.5 ml resorcinol were required, respectively (Fig. 2). The amount of sodium nitrite and hydrochloric acid required for optimum color development was 1.0 ml for all of the methods (Fig. 3 and Fig. 4).

Table 1: Optical characteristics and statistical data

Parameters	Method A	Method B	Method C	Method D
λ_{max} (nm)	536	516	477	502
Beer's law limits, *x*/µg mL^{-1}	4-12	2-10	5-25	1-5
Molar Absorptivity, *ε*/L mol^{-1} cm^{-1}	5.06182 × 10^4	1.09257 × 10^4	2.53279 × 10^5	3.19113 × 10^4
Sandell's sensitivity, *S*/µg cm^{-2} /0.001 A	0.005	0.023	0.021	0.008
Regression equation[a] (*y*)				
Slope (*m*)	0.1776	0.0402	0.0489	0.1270
Intercept (*a*)	0.2202	0.0233	-0.0149	-0.0040
Correlation coefficient (*r*)	0.9996	0.9997	0.9995	0.9995
Repeatability [b], RSD, %	0.3541	0.2600	0.3677	0.2462
Intermediate precision [c], RSD, %	0.65	0.41	0.44	0.26
Standard error of mean	± 0.124	± 0.091	± 0.128	± 0.871
Range of errors [b]				
95% confidence level	± 0.292487	± 0.214643	± 0.303360	± 0.202937
99 % confidence level	± 0.432855	± 0.317653	± 0.448946	± 0.300329

[a] $y = mc + a$ where c is the concentration of Sulfamethoxazole in µg mL^{-1} and y is the absorbance at the respective λ_{max}, [b] Calculated by analysis performed within the same day for eight replicates of samples, [c] Calculated by analysis performed in two different days and with different analyst, RSD: Relative standard deviation.

Effect of excess nitrous acid

The interference of excess nitrous acid and its effect on the colors of the chromogens are shown in Fig. 5. This interference was minimized by adding sulfamic acid before the coupling reaction.

A stability study of the chromogen was carried out by measuring the absorbance values at time intervals of 10 min, and it was found to be stable for more than 2.0 h in all of the selected methods. Moreover, to check the validity of the proposed optimized methods, the standard addition method by adding Sulfamethoxazole to the previously analyzed tablets was used. The recovery of each drug was determined at three levels (50, 100 and 150%), and the accuracy was calculated by comparing the concentration obtained from the spiked mixtures with those of pure drugs. The mean of nine determinations (three from each level) are summarized in Table 2. The proposed method was successfully applied for the determination of Sulfamethoxazole in pharmaceutical dosage forms. Interference studies revealed that Trimethoprim and additives like common excipients (Starch, Lactose, HPMC, colloidal silica and magnesium stearate) that are usually present in tablets did not interfere at their regularly added levels.

DISCUSSION

The developed visible spectrophotometric methods are simple, sensitive, accurate, precise, reproducible, economical and can be successfully applied for routine estimations of

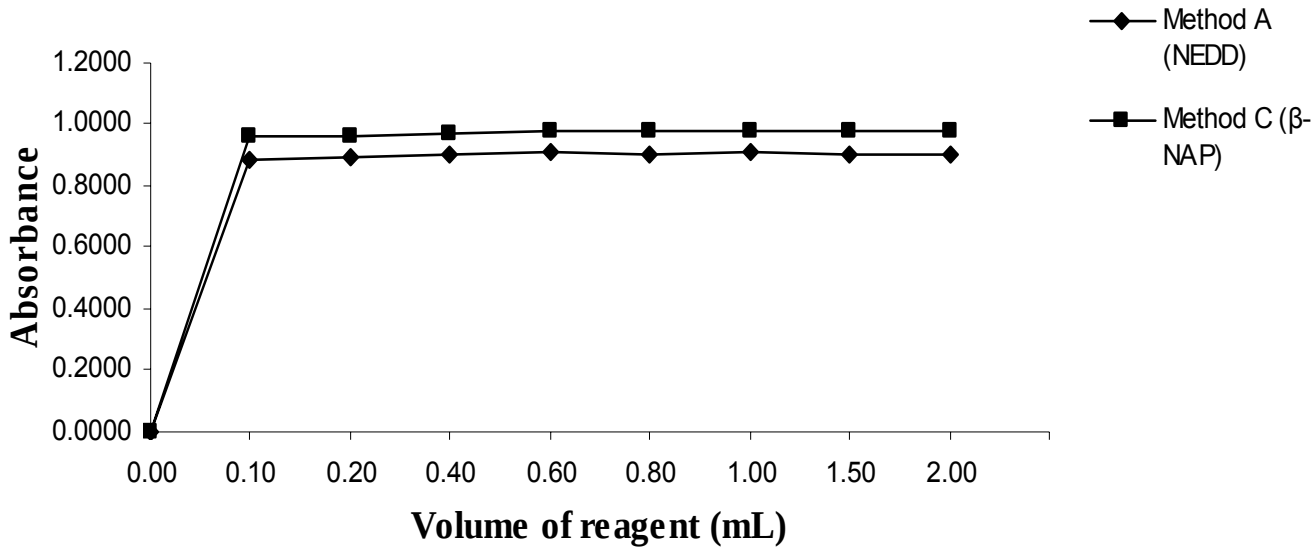


Fig. 1: Effect of *N*-(1-napthyl) ethylenediamine dihydrochloride (NEDD) and *β*-naphthol (*β*-NAP)

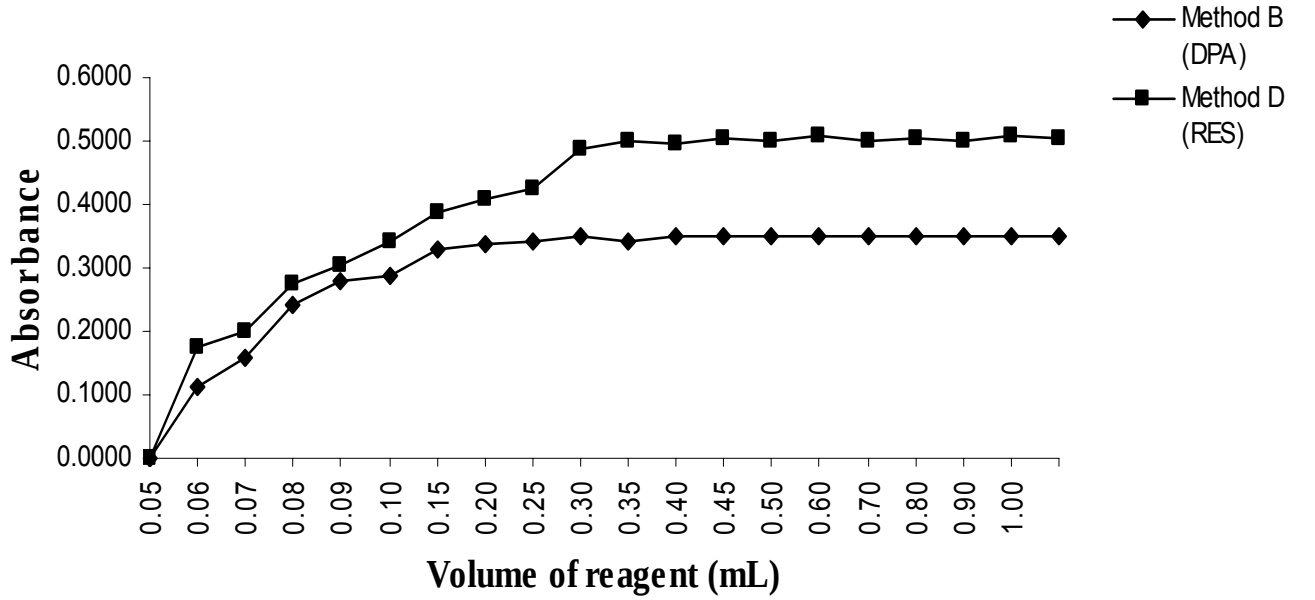


Fig. 2: Effect of resorcinol (RES) and diphenylamine (DPA)

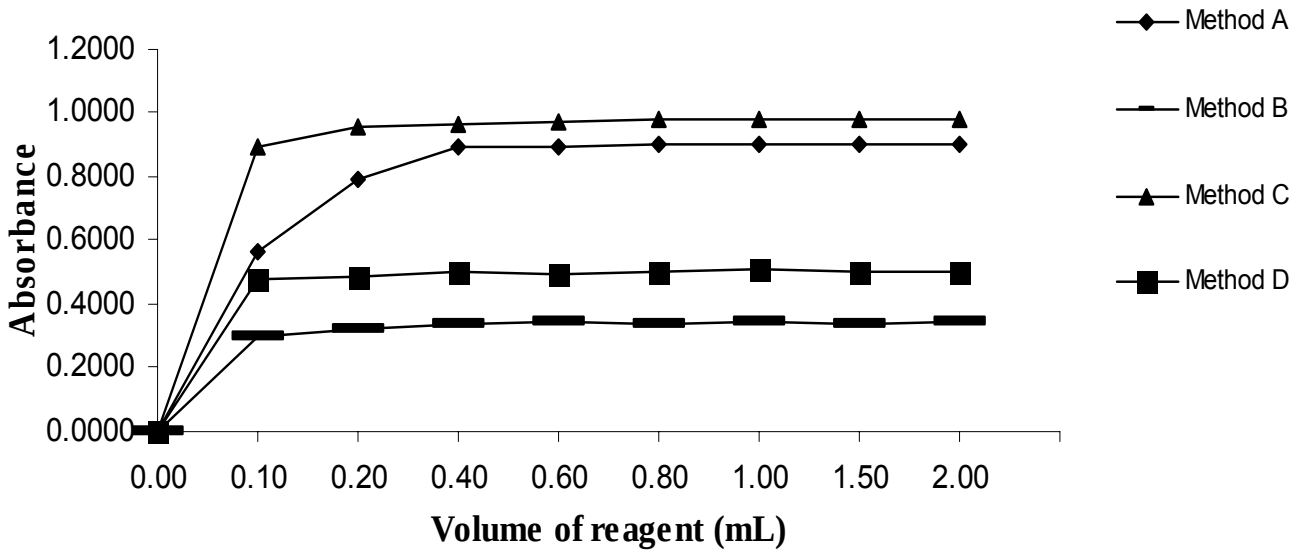


Fig. 3: Effect of sodium nitrite in Method A , Method B, Method C and Method D

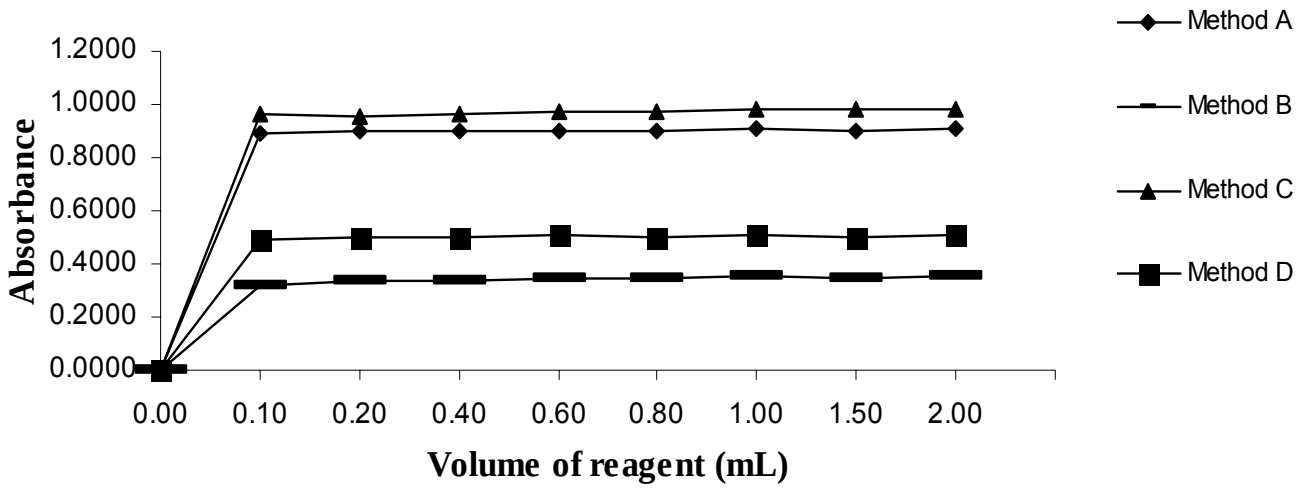


Fig. 4: Effect of hydrochloric acid in Method A, Method B, Method C and Method D

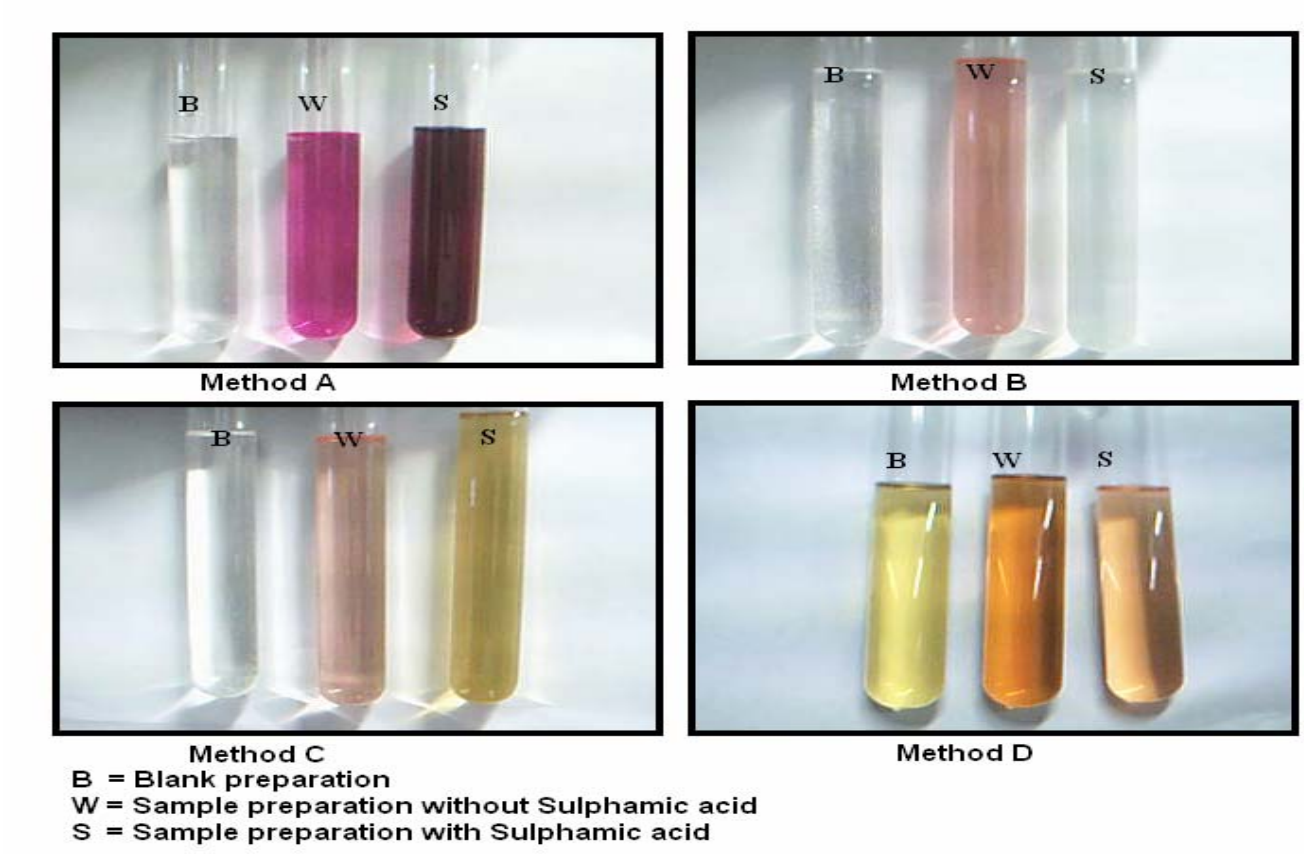


Fig. 5: Final color of the sample and interference of excess sodium nitrite

Table 2: Evaluation of Sulfamethoxazole in pharmaceutical preparations

Method	Labeled Amount mg tab^{-1}	Sample[a]	Amount Obtained (%) ± SD[b]	% Recovery ± SD[c]
A	800	1	98.79 ± 0.35	99.45 ± 0.34
	400	2	98.33 ± 0.36	99.51 ± 0.29
B	800	1	98.72 ± 0.26	99.54 ± 0.50
	400	2	98.23 ± 0.30	99.52 ± 0.56
C	800	1	98.66 ± 0.36	99.17 ± 0.41
	400	2	98.41 ± 0.40	99.21 ± 0.41
D	800	1	98.54 ± 0.24	99.35 ± 0.88
	400	2	98.50 ± 0.22	99.41 ± 0.69

[a]Tablets from different Manufacturers, [b]Mean of eight determinations, [c]Mean of nine determinations (three from each level 50, 100 and 150%), SD: Standard deviation

Sulfamethoxazole in bulk and pharmaceutical dosage forms. The values of the standard deviation were satisfactorily low, and the recovery was close to 100%, which indicates the reproducibility and accuracy of the four methods (Table 2).

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