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Extractive spectrophotometric determination of trimethoprim in pharmaceutical formulations

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ABSTRACT

Two new, simple, sensitive, rapid and economical extractive spectrophotometric methods (A, B) have been developed for the determination of Trimethoprim in pharmaceutical bulk and tablet dosage forms. These methods are based on the formation of yellow colored chromogen by ion association reaction of Trimethoprim with Bromophenol Blue (BPB) and Bromocresol purple (BCP) exhibiting maximum absorbance at 422 and 418nm respectively against the corresponding reagent blank. Beer's law is obeyed in the concentration range of 4.0-24 µg/ml for method A and and 5.0-25 µg/ml for method B. The methods have been statistically evaluated and were found to be precise and accurate. The proposed methods have been successfully applied for the analysis of the bulk drug and its tablet dosage forms.

KEYWORDS

Trimethoprim;
Beers law;
Validation;
Accuracy;
Spectrophotometry;
Linearity.

INTRODUCTION

Trimethoprim[1,2] is 5-(3,4,5-trimethoxybenzyl) pyrimidin-2,4-diyldiamine. The empirical formula is $C_{14}H_{18}N_4O_3$, representing a molecular weight of 290.3 g/mole. The molecular structure was presented in Figure.1.Its closed formula $C_{14}H_{18}N_4O_3$ and molecular weight 290.3 g/mol. Trimethoprim has bacteriostatic effect with broad-range of *Gram positive* and *Gram negative* bacteria as it structurally resembles in pytheridine of dihydrofolic acid and is strong inhibitor of *dihydrofolat reductase* which converted dihydrofolate into tetrahydrafolate that in turn blocks purines and finally DNA, RNA and protein synthesis.

Various analytical procedures have been reported in combination forms[3-7] and only two visible spectrophotometric methods in single dosage forms are available for the assay of Trimethoprim[8,9]. Visible spectrophotometric methods involving ion-pair complexes of acidic dyes viz, Bromophenol Blue (BPB), and Bromocresol purple (BCP) have not been reported with this drug and this prompted the authors to develop extractive spectrophotometric methods for the determination of trimethoprim using the above mentioned dyes. The reported methods are simple and sensitive and are based on ion–pair complexation of the drug with acidic dyes {Bromophenol Blue (BPB) and Bromocresol

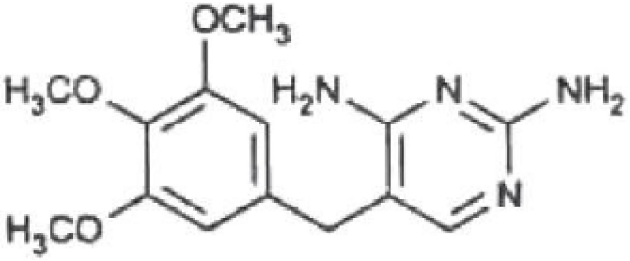

Figure 1: Chemical structure of trimethoprim

purple (BCP)} and subsequent extraction of colored complexes into chloroform and measurement of absorbance of color complexes at their absorption maximum.

EXPERIMENTAL

Instrument

Elico UV Visible spectrophotometer SL 159 with 1.0cm matched quartz cells was used for all spectral measurements. A digital pH meter Model Elico L1 120 was used for pH measurements.

Reagents

All the reagents and solvents used were of analytical reagent grade. Double distilled water was used throughout the investigation. Trimethoprim was received as a gift sample from cipra Lab Limited, sanath nagar, Hyderabad, India.

Solution of Bromophenol Blue (BPB)(0.1%v/v) of Method-A, and Bromocresol Purple solution (0.1%v/v) of Method-B were prepared by weighing and dissolving 100mg of appropriate dyes (BPB and BCP) separately in 100ml of double distilled water.

Buffer solution, (pH 3.0) was prepared by mixing 50mL of 0.2M Glycine acetate solution with 22.4mL of 0.2 M HCl solution and diluted to 200mL with doubly distilled water. The pH of the solution was adjusted to an appropriate value with the aid of a pH meter.

Standard drug solution of trimethoprim was prepared by dissolving 100mg pure trimethoprim into 100ml volumetric flask with to double distilled water obtain 1000µg/ml of stock solution from which desired concentrations 80µg/ml for BPB and 100µg/ml for BCP were prepared.

Procedure for Method A, B

Different aliquots of drug solution were transferred into a series of 100ml separating funnels. To this add 5.0ml of glycine -acetate buffer, 5.0ml of various dye solutions (BPB and BCP), were added and total volume was made upto 15ml with distilled water. To this 10ml of chloroform was added, and the contents were shaken for 5 minutes. The organic layer was separated and the absorbance of yellow colored solution is measured spectrophotometrically 422nm for BPB (Method-A), and 418nm for BCP(Method-B) against blank similarly prepared) which is stable for 24hrs. For the two proposed methods, standard calibration plots were prepared by plotting the absorbance versus drug concentration, and the concentration of the unknown was read from the plotted calibration graphs or computed from the respective regression equations derived using the absorbance concentration data.

Preparation of sample solution

Tablets containing Trimethoprim were successfully analyzed by the proposed methods. Twenty tablets of commercial samples of Trimethoprim were accurately weighed and powdered. Tablet powder equivalent to 100mg of Trimethoprim was dissolved in 50 ml double distal water. The solution was suitably diluted and analyzed as given under the assay procedure for bulk samples. The results were represented in Table.I. None of the excipients usually employed in the formulation of tablets interfered in the analysis of Trimethoprim by the proposed methods.

RESULTS AND DISCUSSION

In the proposed methods(A, B) the drug Trimethoprim in its protonated form reacts with the anionic dyes viz, BPB, and BCP in aqueous solution at pH 3.0 ± 0.01 to form yellow colored ion pair extractable complex. The optimum conditions for the proposed methods were established by varying on parameter at a time and keeping the others fixed and observing the effect on absorbance. The effect of temperature of the reaction, quantity, concentration and addition of various reagents and buffer were studied, optimized after several experiments and incorporated in the procedure. The yellow color developed in methods A, B was stable for more than 24 hours. Wavelength of maximum absorbance for colored ion-pair complexes of Trimethoprim were selected at 422nm for BPB and ND imentaln association complex418nm for BCP and were used for the quantitative determination. Linearity for Trimethoprim was observed in the concentration ranges and the regression analysis of the Beer's law data indicated a linear relationship between absorbance and concentration (TABLE 1) which is corroborated by high values (close to unity) of the correlations coefficients for all three methods. The calculated molar absorptivity

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TABLE 1 : Results of optical characteristics and precision of the proposed methods for trimethoprim assay

Parameter	BPB	BCP
λ_{max} (nm)	422	418
Beer's law limits (μg/ml)	4.0 - 24	5.0 - 25
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	3.416 x 10^3	4.013 x 10^3
Sandell's sensitivity (μg.cm^{-2}/0.001 absorbance unit)	0.03100	0.0178
Optimum photometric range (μg/ml)	5.5 - 22.5	7.5 - 22.5
Regression equation (Y=a+bc); slope (b)	0.0121	0.0190
Standard deviation on slope (S_b)	0.000391	0.000572
Intercept (a)	0.0062	0.0153
Standard deviation on intercept (S_a)	0.000225	0.0003303
Standard error on estimation (S_e)	0.00495	0.00455
Correlation coefficient (r)	0.9969	0.9973
Relative standard deviation (%)*	1.600	0.8593
% Range of error (confidence limits)		
0.05 level	1.338	0.7186
0.01 level	1.982	1.0630

Average of six determinations

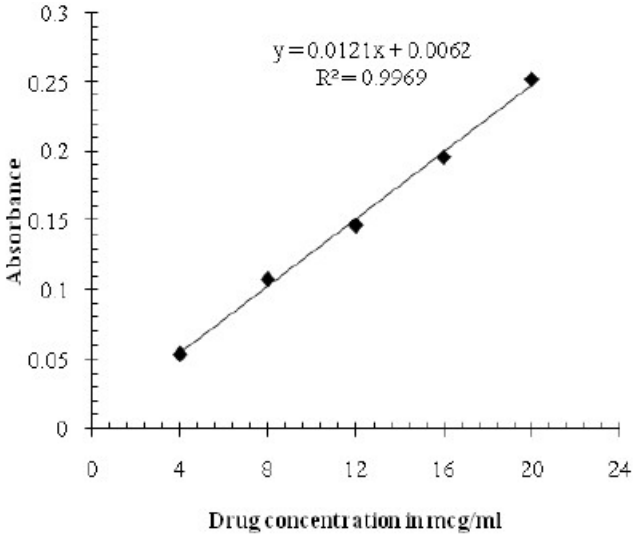
and Sandell sensitivity values are summarized in Table.I. The high values of ε and low values of Sandell sensitivity indicate the high sensitivity of the proposed methods. Precision studies for the proposed methods were carried out by one fixed concentration six times on the same day and the results of this study were summarized in. TABLE 1. The percentage relative standard deviation (%RSD) values indicating high precision of the proposed methods respectively. The accuracy of the proposed methods was determined by the percent mean deviation from known concentration, at one fixed concentration and these results are also presented in TABLE 1. The percent relative error (%RE) values demonstrated the high accuracy of the proposed methods. The proposed methods were applied for the quantification of Trimethoprim in marketed formulations. The results of statistical analysis of did not detect any significant difference between the proposed method and reference method with respect to accuracy and precision as revealed by the Students t-value and variance ratio F-value. The results of assay are given in TABLE 2.

TABLE 2 : Assay of Trimethoprim in dosage forms

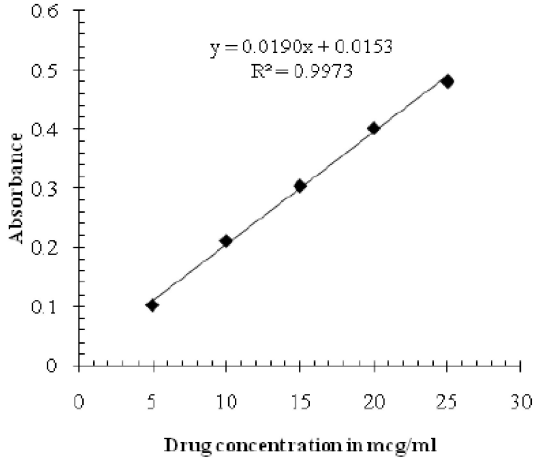
Proposed methods	Labeled Amount (mg)	Proposed Methods			Found by reference method[9] ±S.D	% Recovery by proposed method**
		Amount found* (mg) ±S.D	t (value)	F (Value)		
BPB	100	99.95 ± 0.20	0.314	1.14	199.99 ± 0.24	99.95 ± 0.603
BCP	100	99.93 ± 0.22	0.527	1.19		99.93 ± 0.346

***Average of six determinations**

Linearity graph of Trimethoprim with BPB



Linearity for Trimethoprim with BCP



CONCLUSIONS

The present research work demonstrated the fea-

sibility of the use of visible spectroscopy and ion complexation reaction for the determination of Trimethoprim in pure and its dosage formulations using two acid dyes. The proposed methods make use of simple reagents and are found to be simple, precise, economical and rapid, which an ordinary analytical laboratory can afford. The proposed methods were statistically evaluated and results obtained are accurate, precise, sensitive and free from the interferences of other additives present in the formulation. The proposed extractive visible spectrophotometric methods can be applied for determination of Trimethoprim in pure and dosage forms with high precession and good accuracy in quality control laboratories.

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