Biosciences, Biotechnology Research Asia Vol. 4(2), 463-470 (2007)

Spectrophotometric determination of trimethoprim in pure form and pharmaceutical preparations using tropeolin OO and 2,3-dicloro-5,6-dicyano -p-benzoquinone

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(Received: November 11, 2007; Accepted: December 20, 2007)

ABSTRACT

Two simple and sensitive specrophotometric methods are described for the quantitative determination of trimethoprim (TMP) in pure forms as well as in its pharmaceutical formulations. The first method (Method – A) is based on the formation of an ion association complex between the drug and an acidic dye, tropaeolin OO (TPOO), which is extractable into chloroform. A violet colour, peaking at 540 nm was produced and its absorbance is linear with the concentration over the range 2.9-13.3 µg ml^{-1} with correlation coefficient (n=6) of 0.9998. The second method (Method – B) is subjected to charge transfer complexation reaction with 2,3-dicloro-5,6-Dicyano-p-benzoquinone (DDQ) as a p-electron acceptor. A redish brown colour peaking at 458 nm was obtained and its absorbance is linear with concentration over the range 5-130µg ml^{-1} with correlation cofficient (n=8) of 0.9998. All variables in these methods are optimized. No interferences were observed from pharmaceutical formulations additives and the applicability of the methods was examined by analyzing pharmaceutical formulations containing TMP. The quantities determined were 98.5-101.6% of the expected value.

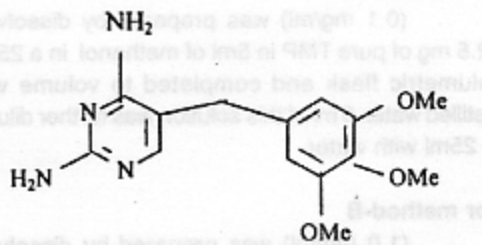
Key words: Trimethoprin, Ion association and charge transfer complexes,Tropaeolin OO, 2,3-dicloro-5,6-Dicyano-p-benzoquinone, Pharmaceutical analysis, Spectrophotometry.

INTRODUCTION

Trimethoprim (TMP): 5- (3,4,5-Trimethoxybenzyl) pyrimidine-2,4-diamine, its structure is shown in scheme 1, is a diaminopyrimidine antibacterial that is used for the treatment of infections due to sensitive organisms, including gastro-enterties, respiratory-tract infections, and in particular the treatment and prophylaxis of urinary-tract infections[1].

Trimethoprim is the subject of a monograph in each of the British Pharmacopoeia, BP[2]; and the United States Pharmacopoeia, USP[3]. Both the BP and USP recommended a non-aqueous titration with potentiometric detection.

The therapeutic importance of trimethoprim initiated several reports on its determination, both in formulations and biological fluids, vis titrimetry[4-5], spectrophotometry[6-13], fluorimetry[14-16], flow-injection analysis, FIA[17-19], chemiluminescene-FIA[20-21], voltammetry[22-25], near infrared spectroscopy, NIR[26,27], electropray MS[28], HPLC[29-36], LC-MS[37-42], TLC[43-45], HPTLC[46,47], GC-MS[48-49], micellar electrokinetic capillary chromatography, MEKCC[55] and cacillary zone electrophoresis, CZE[51-54].



Scheme 1: Structural formula of trimethoprim

The aim of the present work was to develop new, simple, sensitive and reliable methods for the fast control analysis of the drug in pure form and in dosage forms. The results obtained were satisfactorily accurate and precise.

EXPERIMENTAL

Apparatus

Ultrospec 2100 pro/80-2112-21/Amersham Bioscience spectrophotometer with matched 1cm quartz cuvetts was used for all spectra and absorbance measurements.

Materials and reagents

Trimethoprim was obtained from Janssen Pharmaceutica Co. The pharmaceutical formulations were obtained from local markets, as Septrin injections (80 mg TMP/ ampule), produced by Glaxo Smithkline Co. and Bacterium tablets (80mg TMP/ tablet), produced by Roche Co.

All chemicals used were of analytical reagent-grade quality and solvents were of spectroscopic grade. Water was always double distilled. Tropaeolin OO (Carlo Erba Division Chimica Industrial), 0.001 M solutions was prepared in distilled water. DDQ (FLUKA), 0.04M solution was freshly prepared in acetonitrile (BDH). Britton and Robinson Buffer of pH = 3 was prepared[55]. Acid mixture was prepared by mixing 1ml of concentrated H_2SO_4, 98% (BDH) and 100 ml of methanol (BDH). Other chemicals used were anhydrous sodium sulfate (BDH) and chloroform (BDH).

Procedures

Standard solutions

Standard stock solutions of pure TMP must be freshly prepared for each method.

For method-A

(0.1 mg/ml) was prepared by dissolving 12.5 mg of pure TMP in 5ml of methanol in a 25-ml volumetric flask and completed to volume with distilled water. 5 ml of this solution was further diluted to 25ml with water.

For method-B

(1.0 mg/ml) was prepared by dissolving 25mg of pure TMP in acetonitrile in a 25-ml volumetric flask and was further diluted with same solvent to the mark.

Recommended procedures for pure form

Method - A

Accurately measured aliquots of the standard TMP solution equivalent to 2.9-13.3 µg/ml, were transferred into a series of 25-ml separating funnels. 3 ml of 0.001 M TPOO solution and 1ml of pH3 buffer solutions were added to each separating funnel. Then 10ml of chloroform was added to each and the contents were shaken for 2min. The two phases were allowed to separated and the separate chloroform layer was filtered through anhydrous sodium sulfate. 0.5 ml of acid mixture (1ml conc. H_2SO_4/100ml methanol) was added to each solution and the absorbance of the chloroform layer was measured at 540 nm against a reagent blank. The calibration curve obtained by plotting the absorbance against the concentration of TMP was utilized to determine the amount of TMP contained in a given sample.

Method - B

In a series of 10-ml volumetric flasks, different aliquots of TMP standard solution equivalent to 5-130 µg/ml were transferred. 3ml of 0.04 M DDQ solution were added. The volumes completed with acetonitrile and the absorbance was measured to each solution at 458 nm against a reagent blank.

The amount of TMP present in the sample solutions was computed from the calibration curve, which was obtained by plotting the absorbance against the concentration of TMP.

Procedures for pharmaceutical formulations

Tablets

Ten tablets were weighed and powdered.

For method - A

The powder equivalent to 12.5 mg of active ingredient was dissolved in 5ml of methanol and filtered. The filtrate was transferred quantitatively to a 25-ml volumetric flask and completed to volume with water. Then 5ml of this solution was diluted to 25ml with water to get the stock solution of 0.1 mg/ml and proceeded using the procedure for the pure form.

For method - B

The tablet powder equivalent of 25 mg of active ingredient was dissolved in 5ml of acetonitrile and filtered quantitatively in a 25-ml volumetric flask then completed to volume with acetonitrile to get the stock solutions of 1.0 mg/ml. The filtrate was diluted to the required concentration and analysed using the procedure for the pure form.

Injections

For method - A

The contents of three injections were emptied and mixed. 12.5 ml of this solution was measured accurately and transferred into a 100-ml volumetric flask and completed to volume with water. 6.25 ml of this solution was transferred to a 25-ml volumetric flak, 5,ml of methanol was added and the volume was completed with water. Then 5ml of this solution was transferred to a 25-ml of volumetric flask, water was added to the mark to get a solution of 0.1 mg/ml and analysed using the procedure for the pure form.

For method - B

The content of one injection was transferred quantitatively into a 25-ml separationg funnel. The solution was extracted with 3 × 10 ml chloroform. The chloroform extract was collected and evaporated under nitrogen and an amount equivalent to 25 mg from the residue was dissolved in 25ml acetronitrile to get a solution of 1.0 mg/ml and then the procedure for pure form was allowed.

RESULTS AND DISCUSSIONS

The optimum conditions for the development of method A and B were established by varying the parameters one at a time keeping the other fixed, and observing the effect produced on the absorbance of these solutions.

Method - A

The reaction of TMP with TOO in chloroform results in the formation of a violet product absorbing maximally at 540 nm.

Chloroform was chosen as the suitable solvent used for the extraction of the ion association complex formed between TMP and the acidic dye TPOO with respect to sensitivity, minimum doubtful colour and selectivity of extraction.

In order to establish the optimum volume of the buffered solution of pH = 3 for the method, the drug was allowed to react with TPOO in range of volumes between 0.5-3 ml of the aqueous buffered solution (pH=3), and the complex formed was extracted into the chloroform layer for measurements. A volume of 1.0 ml of pH 3.0 buffer solutions was required for higher absorbance value of the complex in the organic phase at the wavelength of maximum absorbance. The efficiency of extraction was also affected by dye concentration. A3 ml portion of TPOO solution (0.001 M) was found to be optimal. Constant absorbance was obtained for shaking periods between 0.5-3 min; hence, a shaking time of 1min was selected. The effect of volume of acid mixture (1ml conc. H_2SO_4/ 100 ml methanol) was also studied in the range of 0.1 – 1.0 ml. NO coloured species were formed without adding this mixture to the reaction[57]. 0.5 ml of the acid mixture was found to be the optimal volume for development of colour of the complex, which formed immediately after addition. There were no measurable changes in the absorbance of the extract even after standing 24 hr in a glass-stoppered flask at room temperature. The absorption spectrum of the coloured species formed in method-A is shown in Fig. 1.

Method - B

π- acceptors are known to yield charge transfer complexes with a variety of electron donors[58]. In non-polar solvent, the molecular charge transfer complexes are formed, where as in polar solvent, the radical anions are the predominant species[59]. TMP is considered as a basic nitrogenous compound which can act as n-donor when mixed with DDQ solution. A radical anions is formed as in the following equation:

$$\underset{\text{Donour}}{D} + \underset{\text{Acceptor}}{A} \rightarrow (D-A) \xrightarrow[\text{solvent}]{\text{polar}} \underset{\text{Radical anion}}{D^+ + A^-}$$

The optimum reaction time was determined by following colour development at ambient temperature (30°C), complete colour development was attained immediately and was stable for more than 25 min. The effect of reagent concentration with respect to maximum sensitivity and minimum blank

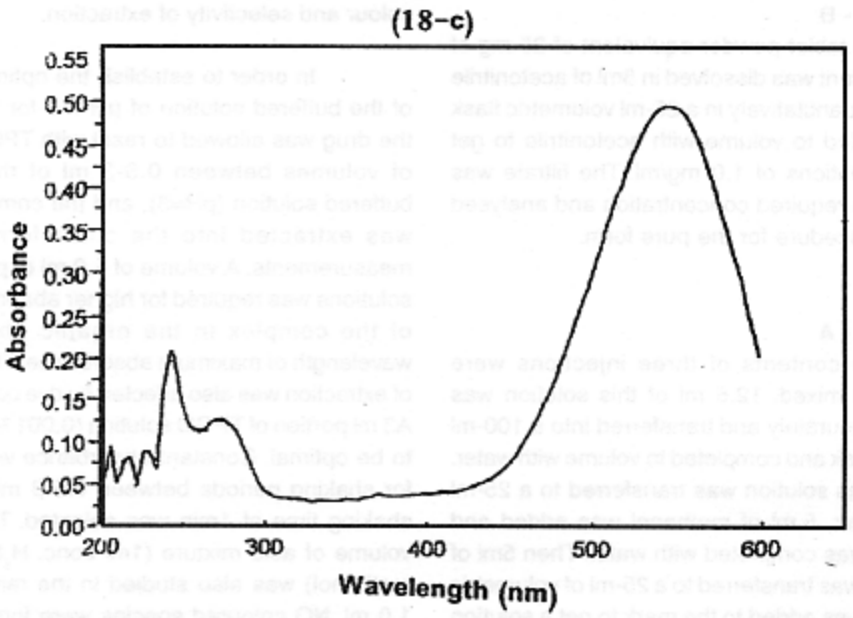


Fig. 1: Absorption spectrum of trimethoprim (9.5 µg/ml) / TPOO (3×10^{-4} M) ion pair complex in chloroform

reading, adherence to linearity and stability of coloured species was studied through controlled experiments. For colour development, 3ml of DDQ solution (0.04M) was found to be optimal. The spectrum of the coloured species produced by the suggested procedure is shown to possess maximum absorbance at 458 nm, as shown in Fig. 2.

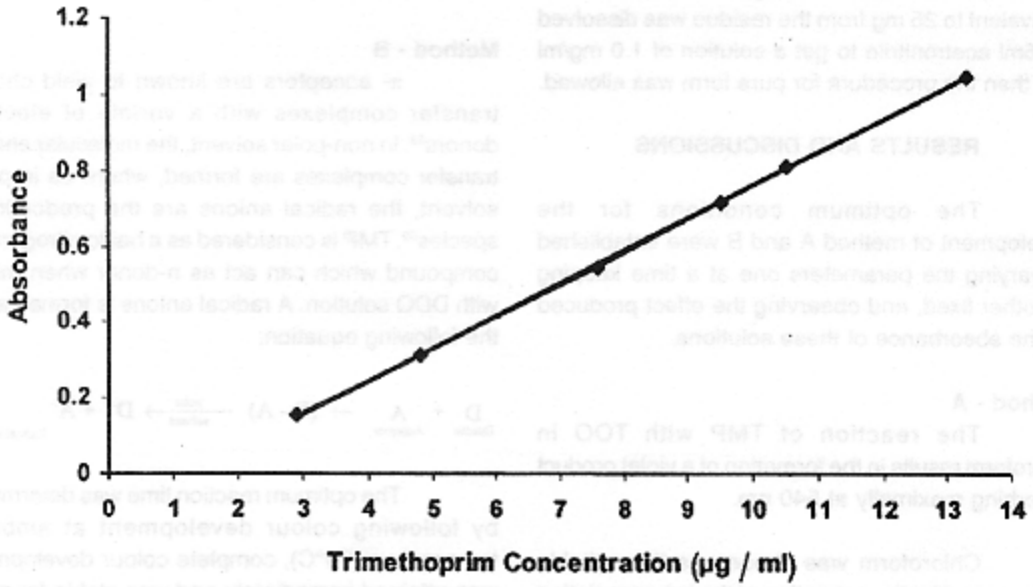


Fig. 2: Calibration curve for Method A.

The above optimum experimental conditions were incorporated in recommended procedure for colour development. Beer's law was found to be valid over the concentration ranges given in Table 1. LOD and LOQ were calculated and cited in Table 1. Spectral data and statistical evaluation of the experimental data regarding standard deviation of the slope (s_b) and standard of the intercept (s_a) gave the values given in Table 1.

Table 1: Selected spectral data for the reaction of trimethoprim with the mentioned reagents

Parameter	Proposed methods using TPOO (Method-A)	DDQ (Method-B)
λ_{max}(nm)	540	458
Linearity range (µg/ml)	2.9-13.3 (n=6)	5-130 (n=8)
Regression equation:		
Intercept (a)	-0.0966	0.0177
S_a	0.007	0.003
Slope (b)	0.0858	0.0051
S_b	0.001	0.000
Correlation coefficient(r)	0.9998	0.9998
LOD (µg/ml)	0.95	1.5
LOQ (µg/ml)	1.4	2.5
%RSD (n=10)	0.792 (10 µg/ml)	0.685 (20 µg/ml)

S_a= Standard deviation of teh intercept.
S_b= Standard deviation of the slope

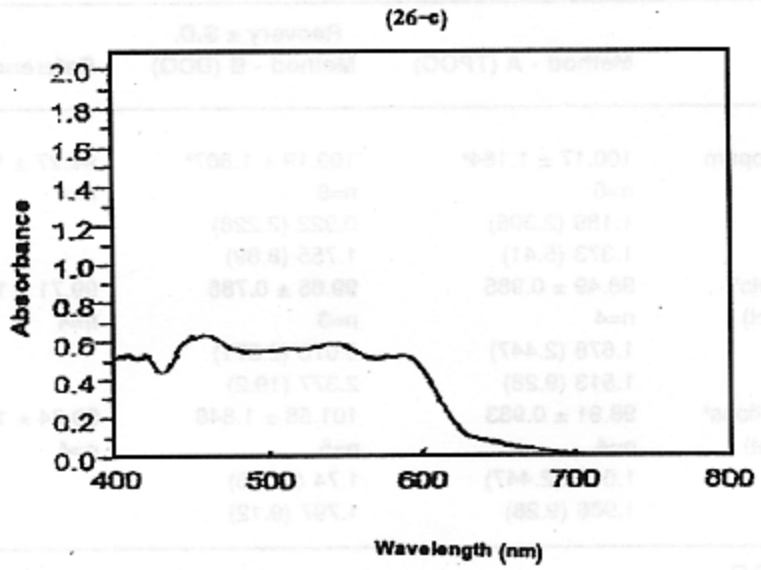


Fig. 3: Absorption spectrum of trimethoprim (11 µg/ml) / DDQ (0.012 M) charge transfer complex.

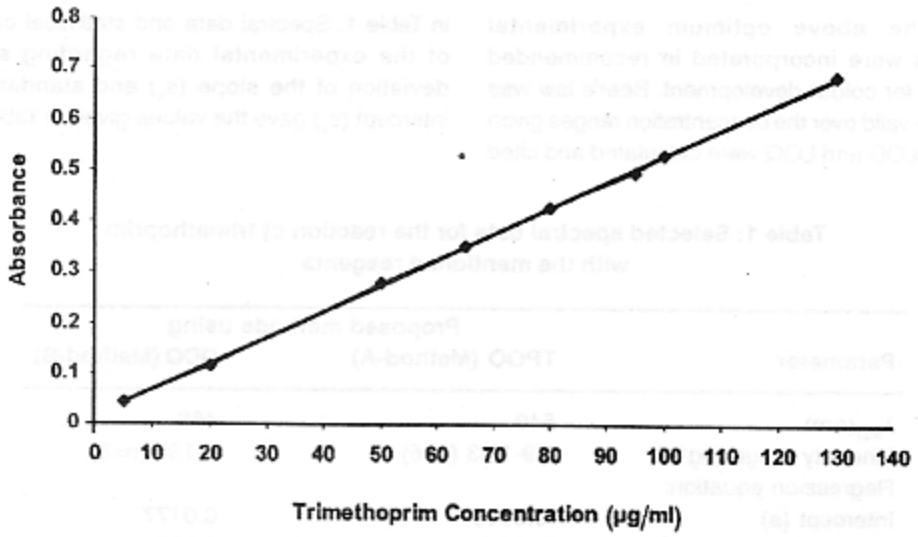


Fig. 4: Calibration curve for Method B.

The precision of the method was tested by percentage of standard deviation of ten multiple analysis of solutions containing 10 µg/ml and 20 µg/ml TMP for both methods A and B respectively.

The validity of the methods could be proved by analyzing authentic samples of the drug. The results obtained in Table 2 were in good agreement with those given by the comparison *spectrophotometric method*[12].

Table 2: Determination of trimethoprim in pure form and pharmaceutical formulations by the proposed and reference method.

Preparation	Reovery ± S.D. Method - A (TPOO)	Method - B (DDQ)	References Method[12]
Pure trimethoprim	100.17 ± 1.164[a]	100.19 ± 1.807[a]	99.27 ± 1.364[a]
	n=6	n=8	n=4
t-value	1.189 (2.306)	0.922 (2.228)	
F-value	1.373 (5.41)	1.755 (8.89)	
Bactrim tablets[b]	98.49 ± 0.985	99.65 ± 0.786	99.71 ± 1.211
(80) mg/tablet)	n=4	n=3	n=4
t-value	1.678 (2.447)	0.078 (2.571)	
F-value	1.513 (9.28)	2.377 (19.2)	
Septrim injections[c]	98.91 ± 0.983	101.58 ± 1.848	99.74 ± 1.378
(80) mg/tablet)	n=4	n=5	n=4
t-value	1.0408(2.447)	1.74 (2.365)	
F-value	1.966 (9.28)	1.797 (9.12)	

[a]% Found ± S.D.
[b]Product of Roche Co.
[c]Product of Glaxo Smithkline Co.
Figures in parentheses are the theoretical t and F values at P = 0.05 confidence limit.

The specificity of the methods was investigated by observihng that no interference was encountered from common tablet excipients. The simplicity of the method and the stability of the reaction product permitted the determination of trimethoprim in injections and commercial tablets. The results obtained (Table 2) were statistically comparable with those given using the previously mentioned comparison method[12]. There was no significant difference between the two methods regarding accuracy and precision as revealed by applying the t- and F- tests, respectively[60].

Conclusion

The proposed methods are simple, accurate, inexpensive, less time consuming, sensitive and requires minimum equipments and chemicals. TPOO and DDQ method utilize a single step reaction. The results are reproducible. These methods can be used as general methods for spectrophotometric determination of TMP in bulk powder and in dosages from, have many advantages over other separation techniques such as HPLC, are reduced cost, and speed with high accuracy. The proposed methods are suitable for routine quality control.

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