A NEW SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF TRIMETHOPRIM USING ALKALINE POTASSIUM PERMANGANATE

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A modest and sensitive method for the determination of trimethoprim has been suggested in pure and pharmaceutical formulations. The method is based on the reaction of trimethoprim with alkaline $KMnO_4$ which leads to the formation of a green coloured complex having a λ_{max} at 520nm. The system obeyed Beer's law and the Sandell's sensitivity. The preferred method was successfully applied to direct the elected trimethoprim with high precision and accuracy compared to standard method as revealed by t-values and F-values.

Keywords: Trimethoprim, Alkaline Potassium permanganate, Spectrophotometery.

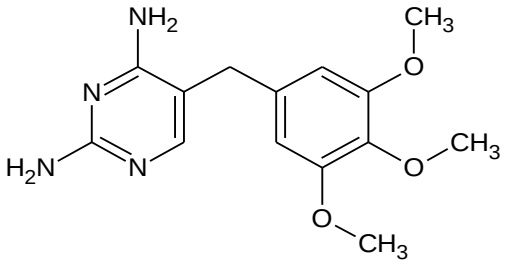
Trimethoprim(2,4-diamino-5-(3'4'5'-trimethoxybenzyl) pyrimidine) (Figure-1) is a synthetic antibiotic agent, employed as a potent metabolic inhibitor of bacterial dihydrofolic acid reductase [1]. Trimethoprim is an antibiotic that interferes with the production of tetrahydrofolic acid, a chemical that is required for bacteria and human cells to produce proteins. Trimethoprim prevents the production of tetrahydrofolic acid by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid. Trimethoprim inhibits the bacterial enzyme more than the corresponding human enzyme. Therefore, trimethoprim has less effectual on the production of tetrahydrofolic acid by humans. Trimethoprim is more potent against a wide variety of bacteria's [2]. Many analytical methods have been employed for the analysis and determination of trimethoprim. The foremost method being ion-selective piezoelectric sensor [3]. Spectrofluorometry was used in the determination of trimethoprim [4]. The drug was analysed by differential pulsed polarography and cyclic voltammetry [5]. The transforms for simultaneous spectral determination of trimethoprim was done by continuous wavelet transforms [6]. The Solid-phase extraction and TLC quantification of trimethoprim was carried out [7, 8]. Micellar Electro Kinetic Capillary Chromatography [9]. The determination of trimethoprim and other drugs in biological systems have been reported by HPLC method [10-12]. Trimethoprim was also determined by ion selective electrode [13].

Few spectrophotometric methods have been described for the determination of trimethoprim. The first publication had described an application of various reagents as π-acceptors for obtaining adduct products i.e., bromothymol blue, bromocresol green, and alizarin red S. For the extraction of solvents, the authors have been proposed chloroform, methylene chloride and chlorobenzene [14]. One of the methods describes the spectrophotometric determination of trimethoprim based on the reaction of NH_2 group with 2, 4-dinitro-1-fluorobenzene reagent in acetone medium [15]. Other spectrophotometric methods are used for the determination of trimethoprim in a mixture with sulphametoxazole depending on derivatives [16-18] extractive [19] and bivariate calibrations [20] spectrophotometric methods. The present study describes the spectrophotometric determination of trimethoprim based on the reaction of the amine group with potassium permanganate reagent in alkaline medium.

Figure 1: Chemical structure of Trimethoprim

2. EXPERIMENTAL

2.1 Apparatus



LABMAN – UV-1200 spectrophotometer with 1.0 cm matched quartz cells was used for all spectral measurements. A digital pH meter (Systronics) was used for the pH measurements. During all spectrophotometric measurements, temperature controller was used to maintain the temperature.

2.2 Materials and 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 Cipla Lab Limited, Sanath Nagar, Hyderabad, India. Weighing was carried out on a balance type of Mettler H54 AR. 2M NaOH was prepared by dissolving 8g of NaOH in 100ml of double distilled water. Potassium permanganate 1×10^{-3}M solution was freshly prepared by dissolving 0.0395g of $KMnO_4$ in 100ml of distilled water. 2M of perchloric acid was prepared by dissolving 17.5ml of HClO4 in 100ml of distilled water. 2M $NaClO_4$ was prepared by dissolving equal proportions of 2M NaOH and $2HClO_4$. The pH of the solution was adjusted to an appropriate value with the aid of a pH meter.

2.3 Standard stock solution

Stock solution of Trimethoprim was prepared by dissolving 0.01g standard trimethoprim in 5ml of ethanol and then transferred into 100ml volumetric flask and made up to the mark using distilled water.

2.4 Recommended analytical procedure

Different aliquots of drug solution were transferred into a series of 100ml volumetric flask. To this 2ml of $KMnO_4$, 0.2ml of 2M NaOH and 0.2ml of 2M $NaClO_4$ were added and shaken well for about 2minutes. The total volume was made up to 100ml with distilled water. An aliquot of the solutions was quickly transferred into a quartz cell and then it was held in the cell chamber (kept at 25^0C) of the spectrophotometer and the absorbance was measured at λ=520nm against a reagent blank prepared similarly. A calibration graphs were drawn by plotting the absorbance against the drug concentration.

2.5 Determination of the Studied Drug in Pharmaceutical Formulations

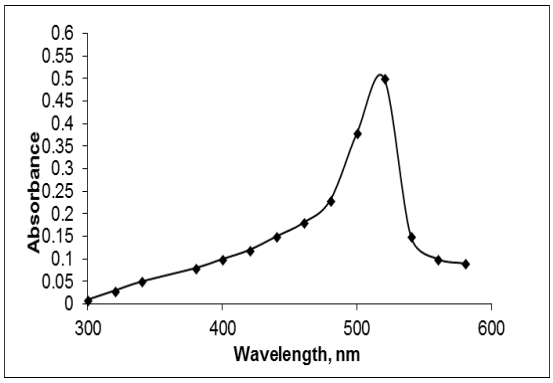
About ten tablets were accurately weighed and finely powdered. An amount of the powdered equivalent Trimethoprim (50 mg) was dissolved in 5ml of Ethanol and transferred into 100ml volumetric flask. The contents of the calibrated flask were shaken well with distilled water to obtain the desired concentration of Trimethoprim. Working solutions were prepared freshly by successive dilution with distilled water and analysed by the recommended analytical procedure.

3. RESULTS AND DISCUSSION

Potassium permanganate reacts with trimethoprim in acetone medium resulting in the oxidation which depends upon the functional group (-OH) present in trimethoprim. $KMnO_4$ oxidises in the presence of NaOH and gets reduced to manganate ion. The absorption spectra of the reaction product measured against reagent blank show λ_{max} value as shown in figure 2. Potassium permanganate is a strong oxidising agent and the manganese containing products from the redox reactions which depend on the pH. The solution of permanganate gets impulsively reduced to faintly pink coloured complex $[Mn(H_2O)^6]^{+2}$ and in alkaline medium it is spontaneously reduced to bluish green coloured K_2MnO_4, were the Mn is in +6 oxidation state. The results obtained by the recommended analytical procedure are in accordance with the results obtained by that of the reference method.

The performance of the proposed method was further verified by applying Students t-test for accuracy and F-test for precision. The calculated t-values and F-values suggest that the recommended procedure is accurate and precise as the reference method. The method developed for quantification of the drug has been justified in terms of Limit of quantification, Limit of detection, linearity and selectivity. To ingress the precision and reproducibility, the experiment was repeated at least 4-5 times. The accuracy and validity of the recommended method is further established by performing recovery studies. The recovery studies are good. These analytical views have been effectively employed in developing sensitive spectrophotometric method for the assay of trimethoprim in pure and pharmaceutical formulations

Fig 2. The absorption spectrum of alkaline potassium permanganate



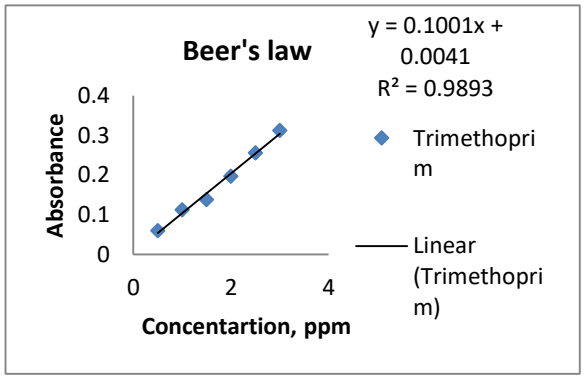
4. Study on optimization of reaction conditions for achieving coloured products of maximum sensitivity and stability.

The effect of several parameters on the absorption intensity of the coloured product was studied and the reaction conditions were optimised.

4.1 Effect of solvent

Several solvents such as acetonitrile, chloroform, ethanol, acetone and water were inspected for the sensitivity of the coloured product formed. It was analysed that acetone was given a green coloured product with the maximum absorption at 520nm of which other solvents was given with maximum absorption less than 400 nm. Consequently, acetone was chosen as the best organic solvent for the drug.

Fig 3. Calibration graph of Trimethoprim



4.2 Effect of pH and buffer solutions

The effect of pH on the absorption of the coloured product was designed using different pH of HCL or NaOH. It was observed that the coloured product formed with maximum absorption in the presence of NaOH. Different buffers namely barbiton, phosphate, bicarbonate and borate were prepared and their effects on the sensitivity were studied. It was examined that all the buffers decrease the absorbance.

4.3 Effect of potassium permanganate concentration

The behaviour of the concentration of potassium permanganate on the colour evolution was examined using different concentration ranging 2×10^{-4} to 6×10^{-4}M. The most favourable concentration was obtained with 2×10^{-4}M. The higher concentrations of potassium caused disrupted in the colour development. Hence, the optimum concentration was formed to be 2.0ml of 2×10^{-4}M.

4.4 Effect of NaOH

The influence of NaOH concentration was studied by taking fixed concentration of the drug, 2.0ml of 0.0001M KMnO4 solution and varying the volumes between 0.1-0.3ml of 5M NaOH. Of all these observations, it was found that the maximum absorbance was acquired with 0.2ml of 5M NaOH. A stable absorbance was noticed with further increase in the volume of NaOH.

4.5 Effect of elongated time

The effect of time on the reaction between the drug and potassium permanganate was examined. It was noticed that the absorbance of the reaction mixture increased with increase in time. But the solutions turned turbid after 20-25 minutes.

4.6 Effect of temperature

I It was noticed that, suppose if the temperature of the reaction rate is increased, it causes precipitation of MnO_2. At room temperature, the reaction rate of the drug increased significantly as the colour development increased. Therefore, it was apparent to execute all the spectral measurements at room temperature.

4.7 Absorption maximum of the coloured species

In order to find out the λ_{max} of the coloured product, a solution containing of about 10ppm of $KMnO_4$ was taken and the reaction product was employed by following the procedure as described earlier. The absorption spectrum was scanned on the UV-Visible spectrophotometer in the wavelength region between 200-800nm against reagent blank. The absorption curve of the coloured product exhibits maximum absorbance at 520nm where the reagent blank solution conveyed negligible absorbance by those permitting good analytical conditions for the assay of $KMnO_4$.

4.8 Standardisation of reaction conditions

The favourable reaction conditions required for rapid and quantitative formation of oxidative coloured product of maximum sensitivity and stability were probed. This is being done by differing one at a time and fixing other parameters at $\lambda_{max.}$

4.9 Optical characteristics of the coloured product

The Beer's law limits, correlation coefficient, regression equation and Sandells's sensitivity values for the system are given below in table1. A linear relation correlated between absorbance at consisting λ_{max} and concentration of the coloured complex species. Regression analysis of Beer's law plots at their particular λ_{max} values disclosed a good correlation. Graph of absorbance and the concentration revealed zero intercept which is described by regression equation, Y=a+bX (where Y is absorbance, b is slope, a is the intercept and X is the concentration of drug) acquired by least-square method.

4.10 Studies of interferences of excipients and additives

In order to evaluate the feasible analytical applications of the proposed method, the effect of excipients and additives that often lead to the drug (trimethoprim) in several pharmaceutical formulations were examined by adding different of these to known amounts of the drug. It was observed that the studied excipients do not interfere in the current method, even when present in large excess. Errors of about 2.0% in the absorbance values were bearable. It was noticed that the hydroxyethyl cellulose, polyvinylpyrrolidone, talc and magnesium stearate do not interfere in the determination of trimethoprim in the synthetic mixtures at the following levels (mg)

1. Trimethoprim (10), hydroxyethyl cellulose (80), polyvinylpyrrolidone (10), talc (20), magnesium stearate (5)
2. Trimethoprim (10), hydroxyethyl cellulose (200), polyvinylpyrrolidone (20), talc (10), magnesium stearate (5)
3. Trimethoprim (10), hydroxyethyl cellulose (100), polyvinylpyrrolidone (20), talc (10), magnesium stearate (5)

5. Recovery studies

Recovery studies were accompanied by examining each pharmaceutical formulation in the example for the active ingredient by the proposed method. Three varying amounts of pure active constituent were added to the formerly analysed formulation and the amount of the drug (trimethoprim) was once again determined by the suggested method following the active constituent concentration within Beer's law limits.

6. Statistical analysis of the results in contrast with the reported method

The outcome of the analysis of Trimethoprim in various pharmaceutical preparations were compared statistically by Student t-test and by the variance ratio F-test with those acquired by the reported method. The Student t-test values did not overreach the theoretical value recommending that there was no notable difference between the proposed method and the reported method. It was also observed that the variance ratio F-test deliberated did not exceed the theoretical value specifying that there was no such difference between the proposed method and the reported method.

7. Method validation

Each method employed for the quantification of drug have been validated in terms of accuracy, precision, linearity, limit of quantification, limit of detection and selectivity. To evaluate the precision and to know the reproducibility, the experiment was carried out repeatedly for at least 4-5 times in a day and was done for day1 and day2. To estimate the accuracy, the experiment was determined in terms of percentage recovery and relative standard deviation (RSD). Exceptional results of recovery and RSD were formed. Later t-test and F-test have been calculated using standard reference methods which gave the permissible range results.

8. Conclusion

From an analytical opinion, it is concluded that the outlined procedure permits for the determination of Trimethoprim in pure and pharmaceutical dosage forms. The great advantage is that the results obtained are reproducible. Unlike HPLC, gas chromatography and spectroflourometery, the UV-Visible spectrophotometer is simple, sensitive, stable, inexpensive with sensible accuracy and precision. The results also certainly recommend the utility of the proposed method for the analysis of the drug. Additionally, the reported method is free from the interference by various excipients. Consequently, the official method would be securely suggested for regular quality control in pharmaceutical industries.

Table 1: Optical characteristics of the drug:

Parameters	**Values**
λ_{max}	520
Intercept	0.04
Slope	0.10
Regression coefficient(r)	0.994
LOD	0.02
LOQ	0.26
Sandell's Sensitivity	0.014
Regression equation	Y=a+bx
Confidence level	95%

Table 2: Analytical of Trimethoprim in pharmaceutical formulations

Drug	**Labelled**	**Found (X± RSD)**

		Proposed method	Reference method
Antima	50mg/Tab	49 ± 0.84 t= 0.92, F=1.49	40± 0.6
Bactrim	200mg/Tab	198 ± 0.6 t= 0.42, F=1.01	90.27±0.4
Metheprim	100mg/Tab	97.1±0.77 t= 0.11, F=0.58	100.2±0.44

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Dedication

I dedicate this research paper to my mother K Papamma.

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