INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 12, December 2025, pp : 22-29
www.ijprems.com
editor@ijprems.com
e-ISSN : 2583-1062
Impact Factor : 7.001

REVIEW ON DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR TRIMETHOPRIM IN PURE AND MARKETED FORMULATION

Mr. Kotkar V.R[1], Dr. Salve M.T[2]
[1,2]Shivajirao Pawar College Of Pharmacy, Pachegaon, India.
DOI: https://www.doi.org/10.58257/IJPREMS45269

ABSTRACT

This review focuses on development and validation of UV spectrophotometric method for Trimethopthoprim in pure and marketed formulation**.** . Trimethoprim is an antibacterial drug with a chemical structure that includes:1). 2,4-diamino pyrimidine moiety 2)1,2,3-trimethoxybenzene moiety 3).A methylene bridge connecting the two moieties . It is useful in the treatment of UTI (Urinary Tract infection). A rapid and sensitive method was developed for the analysis of Trimethoprim in both pure and tablet dosage form. The Methanolic solution of pure drug trimethoprim at room temperature of (25 ± 10C) was measured at a maximum of 285 nm. Under optimum and validated test conditions, Beer's rule was followed within the concentration range of 10 - 60 µg / ml. He experiment for linearity, accuracy, precision(Intra day& interday precision), Range, detection limits, quantification limits,robustness and ruggedness were performed. The preformulation parameters in terms of Melting point of trimethoprim were studied. The method is desirable for evaluating the quality and effectiveness of pure as well as commercial formulations of this significant pharmaceutical constituents.

Keywords: Trimethoprim, Validation, Spectrophotometer, Analytical.

1. INTRODUCTION

Trimethoprim is an antibacterial agent with chemical structure named as 2,4diamino-5-trimethoxybenzyl) pyrimidine. Trimethopthoprim is firstly synthesized by the scientist Bushby and Hitchings . This moleculeTrimethoprim was developed for the treatment of urinary tract infection.Trimethoprim is available in a single dose tablet as well as in combined dosageform i.e. Trimethoprim and sulfadiazine tablet. Its molar mass is 290.32g/molg.mol-1 .Its solubility is in Methanol, Ethanol , n-butanol.This drug stops thefunction of enzyme dihydrofolate reductase for the formation of folic acid which isresponsible for the structured DNA .It can also be used to combat pathogenic bacteria .Trimethoprim is only used in the treatment of simple, symptomatic urinary infections. The combination of Trimethoprim and Sulfadiazine can be active against Acinetobacter, Aeromonas hydrophilla, Bartonella henselae, Burkholderia Pseudomallei, Brucella, Moraxella), Catarrhalis Mycobacterium tuberculosis. From the evidence that emerged it can be pointed out that very few selected spectrophotometer, HPLC-LC-MS and HPLC techniques for the analysis of Trimethoprim and sulfadiazine were reported in relation to other drugs n bulk biological samples. Trimethoprim is acting against most Gram-positive and Gram-negative aerobic bacteria.

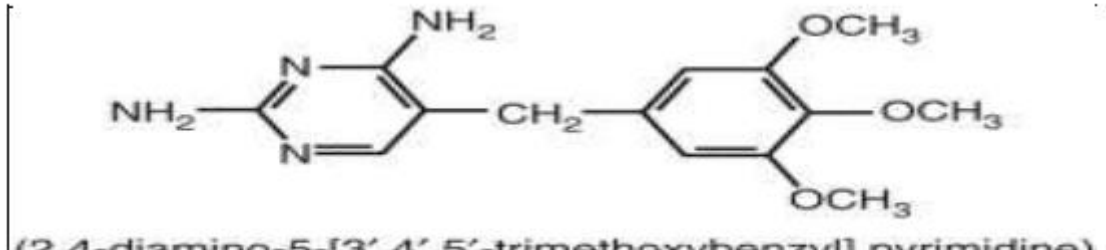

Figure -01 Structure of Trimethoprim (11)

It is readily absorbed by the oral route and is widely distributed in body fluids and tissues. In therapeutic trials, trimethoprim 200 to 400mg daily has been shown to be comparable in efficacy with co-trimoxazole, ampicillin 2g, cephalexin 2g, and nitrofurantoin 200mg daily in the treatment of acute urinary tract infection. Similarly, in long term prophylaxis of urinary tract infection, trimethoprim 100mg daily given as a single dose at night was comparable with nitrofurantoin 50 to 100Omg, methenamine 1g, oxolinic acid 375mg or co-trimoxazole (80mg trimethoprim /1400mg sulphamethoxazole) each given as a single daily dose. Nevertheless, results of serial laboratory surveys suggest that resistance to trimethoprim among enterobacteria is increasing. But as of now, there is no confirmed evidence that the rate of rise will be steeper and come now after the introduction of trimethoprim for the sole purpose of treatment of

urinary tract infection. At the proposed dosages, the agent has been relatively well tolerated and in comparative studies overall, skin rashes and gastrointestinal upset have occurred less frequently with trimethoprim.

Pharmacokinetics:

Trimethoprim is readily absorbed after oral administration. The volume of distribution of trimethoprim is quite large, averaging about 100L, and trimethoprim distributes in body.

Concentrations of the drug in saliva, sputum, lung tissue, prostate gland and fluid are greater than in serum. Concentrations of the drug in cerebrospinal fluid are higher when meninges are inflamed than when normal; such concentrations range from 20-44% of the corresponding serum concentrations. The concentration of trimethoprim in aqueous humour, middle ear fluid, vaginal fluid and in bone is approximately half the corresponding plasma concentration sampled at the same time. In patients with normal renal function, trimethoprim is excreted in urine and there is minimal metabolism. Urinary concentrations of the drug are affected by pH, being elevated by acidification and decreased by alkalinisation. The lack of standardization of pH and fluid intake has resulted in wide variation in urine concentration in pharmacokinetic studies, though the mean value is generally between 50 and 210µg/ml over the first 4 to 12 hours after a single dose of 160 to 200mg in subjects with normal renal function. The half-life of elimination in normal renal function is between 8.8 and 17.3 hours and it increases to 2 to 3 fold value, when the impairment of renal is severe (creatinine clearance less than 10 ml/min). However, when the creatinine clearance is less than 10ml/min, the percentage of trimethoprim excreted via nonrenal mechanisms significantly increases.

Clinical application

Trimethoprim is sold alone, but the combination product is seldom used as a single entity. Trimethoprim is always given with sulfamethoxazole to exploit the synergistic actions of this combination. Besides the antibacterial effects of this drug, this combination agent has antimalarial activity. Alone, trimethoprim is bacteriostatic; however, when combined with sulfonamides, it is bactericidal. Trimethoprim drugs have been applied in combination with sulfonamides for urinary infections, prostatic infections, otitis media in children, and the elimination of Shigella, as well as for the treatment of Pneumocystis carinii pneumonia. Trimmepthoprim has also been applied in combination with polymyxin B for the treatment of acute conjunctivitis. Although trimethoprim is rarely administered as a monotherapy because of bacterial resistance, it may be used to treat uncomplicated urinary tract infections caused by Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enterobacter species, and coagulase-negative Staphylococcus. Even though trimethoprim and sulfonamides inhibit bacterial folate synthesis, patients who are receiving either drug often may take folic acid supplements without the supplements having an effect on the activity of the antimicrobials.For parasitic infections, TMP is administered as a combination with sulfamethoxazole. TMP reduces the secretion of creatinine into the renal tubule, resulting in increased levels without affecting the clearance of creatinine.

Side effects: The adverse effects described include rashes, pruritus, nausea, vomiting, glossitis, raised liver enzymes, cytopenias, megaloblastic anemia, fever, aseptic meningitis and acute renal failure.

Chemicals and reagents

Methanol of analytical grade was used as a solvent in the whole experiment and all other chemicals and reagents used were AR grade.Pure drug, Trimethoprim was obtained from Indo safe pharmaceuticals, Jaipur.Trimethoprim 70mg, purchased marketed tablets.

Instrumentation - A shimadzu UV Spectrophotometer (UV-1800) with a pair of quartz cell were used in experiment.

❖ **Experimental parameter -**

Melting point-

The melting point of drug is calculated using the Thieles tube process, in a capillary tube with one sealed end, a small quantity of drug substances wastaken, using a Bunsen Burner. About 0.5 gm of completely dried and finely powdered compound was filled in capillary tube and was closed. A capillary tube was attached at the lower end of the thermometer. Thermometer was placed along with the capillary tube in a thieles tube containing liquid paraffin. Tube was dipped in thieles tube and recorded the temperature at which the drug melts.

Solubility Study–

To determine the solubility, the drug was dissolved in various solvents. The excess amount of 100mg drug was weighed and dissolved in 5ml of methanol until saturated solution is achieved. The saturated solution was filtered after which the supernatant was analyzed by UV spectrophotometer. The solubility study was repeated thrice for water, 0.1N HCl ,NaOH, Ethanol ,Methanol, Chloroformand water.UV spectrum was taken.

Determination of λ max-

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The given solution was scanned in the range of 200-400nm.Standard calibration curve volumetric flask. The stock solution was diluted with Methanol to get 10 to 50 µg/ml of trimethoprim Methanol was used to determine the absorbance as blankat 285 nm using UV visvible spectrophotometer. Graph between concentration and absorbance is plotted to draw calibration curve.

Standard solution Preparation–

By dissolving 100 mg of Trimethoprim pure drug in 100ml of Methanol in a 100ml volumetric flask, the standard stock solution was prepared and diluted with Sample solution Preparation 100mg of trimethoprim (tablet 70mg) was dissolved in 100ml volumetric flask Analytical ValidationSystem validation of the planned method is validation process established under the International Guideline Harmonization (ICH) conference under section Q2

Linearity

Linearity is the experimental technique to generate results that linearity and concentrations are directly proportionate to each other within a given range of analytes in the sample. Fresh aliquots were prepared ranging from 10-60 ÕJ / ml of standard stock solution and the absorbance of aliquots was recorded at 285 nm. Methanol as the reference solution for this process was used. The calibration curve is drawn between Absorbance and Concentration. The equation generated for trimethoprim obtained a correlation coefficient with the regression line.

Precision

This parameter was carried out and done in order with the same day i.e intraday precision through the analysis of the 6 independent aliquots ranging from 10-60g/ml thrice a day with interval and absorbance was recorded at 284 nm. And the same procedure was carried out in different days i.e Interday precision for 6 aliquots and absorbance was recorded at 285nm. At last % Relative Standard Deviation for inter and intra precision was calculated.

Accuracy

Accuracy of proposed system confirmed by carrying out triplicate restoration studies at three different concentrations of 80%, 100%, 120% each. It was clear In the recovery analysis, it is apparent that the process for quantitative determination of the tablet is very reliable, and the statistics were within the recommended percentage.

Limit of Detection - The detection limit for Trimethoprim by developed system were evaluated using calibration graphs. Limit of detection were analyzed by formula LOD = 3.3 × Standard Deviation Slope LOQ- Limit quantification for Trimethoprim by developed system was The calibration graphs were used for evaluation of the concentration. The detection limits were analyzed through formula. LOQ = 10 × Standard deviationSlope

Robustness- The method's robustness carried out by conducting the observation at different wavelength range . The relevant absorbance were taken and outcome showed through %Relative Standard Deviation.

Ruggedness - This parameter was analyzed by different analyst(two) and the resulted absorption has been noted. Findings were shown by percentage Relative Standard Deviation. Marketed tablet formulation of Trimethoprim tablet Took twenty tablets and powdered them. Accurately weighed 10 mg powder has been transferred to 10ml of volumetric flask, where it was sonicated to fully dissolve the drug. Then it was volumed up to 10 ml with methanol with a standard concentrations of 1 mg/ml solution. Pipette 1.0 ml of the above standard solutions into a 10 ml of volumetric flask and dilute to desired concentration. The absorbance of the sample read at 285 nm, and the amount of drug retrieved has been calculated.

Analysis of Marketed tablet formulation of Trimethoprim tablet

Took twenty tablets and powdered them. Accurately weigh 10 mg and load into a 10 ml volumetric flask, sonicate and dissolve the drug thoroughly. Top up with methanol to make up to 10 mL for stock solutions at a concentration of 1 mg / ml. Add 1.0 ml of the above solutions stock solutions into a 10 ml of volumetric flask, dilute to the required concentration.The absorbance of the sample read at 285 nm, and the amount of drug retrieved has been

2. AIM AND OBJECTIVES

The primary aim of the study was to develop and validate a simple, rapid, selective, precise, and economical UV-spectroscopic method for Trimethopthoprim in pure and marketed formulation .

Primary Objectives

1. To optimize the UV spectrophotometric conditions for the analysis of Trimethoprim.
2. To develop a linear calibration curve for Trimethoprim using UV spectrophotometry.
3. To validate the developed method as per International Conference on Harmonization (ICH) guidelines.

Secondary Objectives

1. To determine the specificity, selectivity, and accuracy of the developed method.

2. To evaluate the precision (repeatability and intermediate precision) of the method.

3. To assess the robustness and ruggedness of the method.

4. To compare the results of the developed method with existing methods (e.g., HPLC).

5. To apply the developed method to the analysis of Trimethoprim in marketed pharmaceutical formulations.

❖ **Plan of Work**

The research paper focuses on the development and validation of a UV-spectroscopic method for Trimethopthoprim in pure and marketed formulation. The following steps were carried out:

Exhaustive literature survey: Conduct a comprehensive review of existing studies on Trimethopthoprim and analytical methods.

Collection of relevant research papers: Gathered key research articles and publications on UV-spectroscopy and validation parameters.

Review on the topic: Analyzed the findings and methods used in prior research related to the UV-spectroscopic analysis for Trimethopthoprim and marketed formulation.

Writing the introduction: Draft a detailed introduction covering the background, significance, and objectives of your review.

Summary of findings: Summarize the findings from the literature survey and how they relate to your research.

Conclusion and future scope: Conclude by addressing the relevance of your research and suggesting areas for further invest

3. LITERATURE REVIEW

1. Salim AM, Haseeb YSZ. Spectrophotometric determination of sulfadiazine via diazotization and coupling reaction-application preparations.

Salim AM and Haseeb YSZ stated that this paper develops a simple, fast, accurate, and sensitive spectrophotometric method for the quantitative determination of sulfadiazine (SDz) in pure as well as its dosage forms. The method is based on diazotization of the primary amine group of sulfadiazine with sodium nitrite and hydrochloric acid followed by coupling with γ-resorsolic acid (2,6-dihydroxybenzoic acid) in alkaline medium. of sodium hydroxide to form a yellow coloured azo dye shows a maximum absorption at 458 nm against reagent blank solution. Beer's law is obeyed over the concentration range of 10-300 µg of SDz / 25 ml (0.4-12 ppm) with a determination coefficient (R2=0.9998) and molar absorptivity 4.38×104 l.mol-1.cm-1 and a relative error in the range of 0.1- 0.64% and it has a relative error of 0.1- 0.64% and the relative standard deviation ranges from 0.27 to 1.21% according to the concentration level of SDz. The method is appropriate for the determination of sulfadiazine in the presence of other ingredients that are usually present in dosage forms. Organic solvents have also been worked out to study the effect on the spectrophotometric properties of the azo dye and the composition of the resulting product. It is found to be 1:2 γ-resorsolic acid: sulfadiazine. The method has successfully been applied for the determination of sulfadiazine in its pharmaceutical preparations, namely, tablet and burn cream.

2. Amina M.A Bass, Sahar et.al Determination of trimethoprim by various analytical techniques A-Review" .

Amina M.A Bass, Sahar et.al stated that few analytical techniques were applied to the determination of trimethoprim that studied Concentration range, correlation coefficient, detection limit, recovery, relative standard deviation, type of column, mobile phase, flow rate, slope, response time, and the range of PH for trimethoprim or sulfamethoxazole.

Trimethoprim chemically its 5-(3, 4, 5-Trimethoxybenzyl) pyrimidine -2, 4-diamine, is C14H18N4O3, representing a molecular weight of 290.3 g/mole. White or yellowish white powder, very slightly soluble in water, slightly soluble in ethanol. Dihydrofolate eductase [1]. Only a few analytical techniques were used in the determination of trimethoprim and concentration range, correlation coefficient, detection limit, recovery, relative standard deviation, type of column, mobile phase, flow rate, slope, response time and the range of PH for trimethoprim or sulfamethoxazole.. Besides the above analytical methods to determine the trimethoprim, there are such analytical methods as square wave voltammetry [3], Photo-Fenton Oxidation Technology [4], formation of charge transfer complexes [5].

3. Goran S, Elizabeta DS, Marija S, Romel V. Optimization, validation and application of UV-Vis spectrophotometric colourimetric methods for determination of trimethoprim in different medicinal products.

Goran S, Elizabeta DS, Marija S, Romel V stated that they have developed and validated simple, sensitive, selective, precise, and accurate methods for the determination of trimethoprim in various sulfonamide formulations to be used in human and veterinary medicine. Both the methods are based on the reaction of trimethoprim with bromcresol green (BCG) and 2,4 dinitro 1 fluo-robezene (DNFB). Extraction solvents used for both the methods were 10% N,N -

dimethyl acetamide in methanol and acetone, respectively. Quantitation of colored products was achieved through visible spectrophotometry at the absorption maxima corresponding to each colored species. The methods were validated for linearity, sensitivity, accuracy, and precision. We applied the method to analyze four different medicinal products in tablet and powder forms containing the combination sulfametrole and sulfamethoxazole with trimethoprim.

4. Nachilobe P, Cassidy RM, Fesser ACE. Determination of trimethoprim in bovine serum high performance liquid chromatography with confirmation by thermospray liquid chromathography-mass spectrometry. J Chromathography

Nachilobe P, Cassidy RM, Fesser ACE stated that a high-performance liquid chromatographic (HPLC) method with a detection limit of 5 ng/ml was developed for the analysis of trimethoprim in bovine serum. Trimethoprim and the internal standard, ormetoprim, under alkaline conditions, were first extracted into dichloromethane and then back-extracted into dilute sulphuric acid (0.15 M) and cleaned-up on a C18 cartridge. Trimethoprim was quantified on a C18 column using a triethylammonium acetate-acetonitrile-methanol (16:3:1, v/v/v) mobile phase at a flow-rate of 1.5 ml/min, with ultraviolet detection at 225 nm. This method was applied to validate the reliability of test responses obtained with the Brilliant Black Reduction test, a rapid screening method, for serum trimethoprim levels in steers treated with Trivetrin.

4. RESULT

The spectroscopic method proposed here was found to be accurate selective, relatively fast, and economical. It was validated about the precision linearity, accuracy and ruggedness. The maximum absorption of Trimethoprim was found at 285nm with good conformity to Beer's law in the concentration range 10 - 60 µg/ml. The proposed method showed linear regression y 0.018x + 0.0821 with a correlation coefficient (R2) of 0.9999. This developed method has been found to be accurate as the % Relative Standard Deviation values for intra day and inter day have been resulted to be 0.031 to 0.223 and 0.011 0.891 respectively. For every concentration added, good recoveries of the drug were observed at (98.99 percent to 99.12 percent) which showed the effective process. The method proved to be robust and rugged as indicated by the %Relative Standard Deviation values 2.82% at 280 nm, 3.10% at 284 nm and 2.22% at 288nm.

SR.NO	VALIDATION PARAMETER	RESULT
1	Melting point	198 °C
2	Solubility 1.methanol 2.water	 1.Freely soluble 2. Soluble
3	Linearity	Obey Beers Law
4	Precision(% RSD)	Interday- 0.011 to 0.891% Intraday - 1.00 to 4.15%
5	Accuracy	99.12 ±0.0 5
6	Robustness(% RSD)	2.22 to 3.10 %
7	ABSORPTION MAXIMA	285 nm

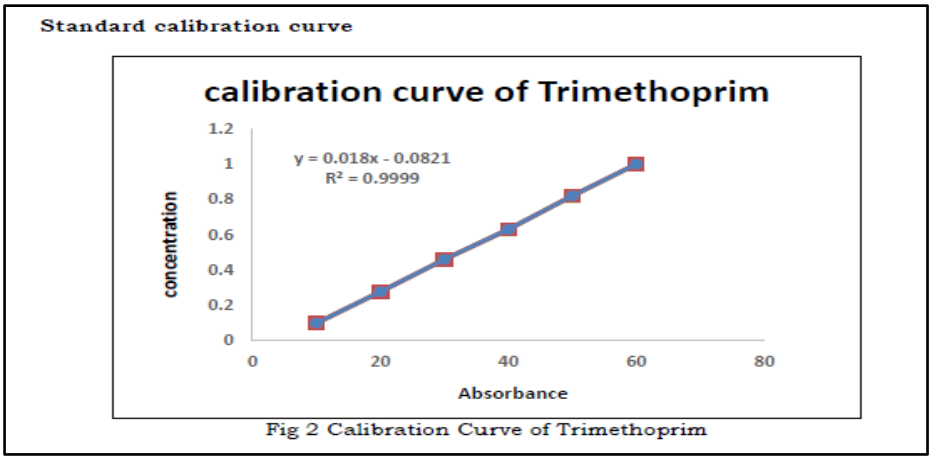

Fig 2 Calibration Curve of Trimethoprim

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Concentration(µg/ml)	Absorbance
10	0.12
20	0.24
30	0.46
40	0.63
50	0.82
60	1.01

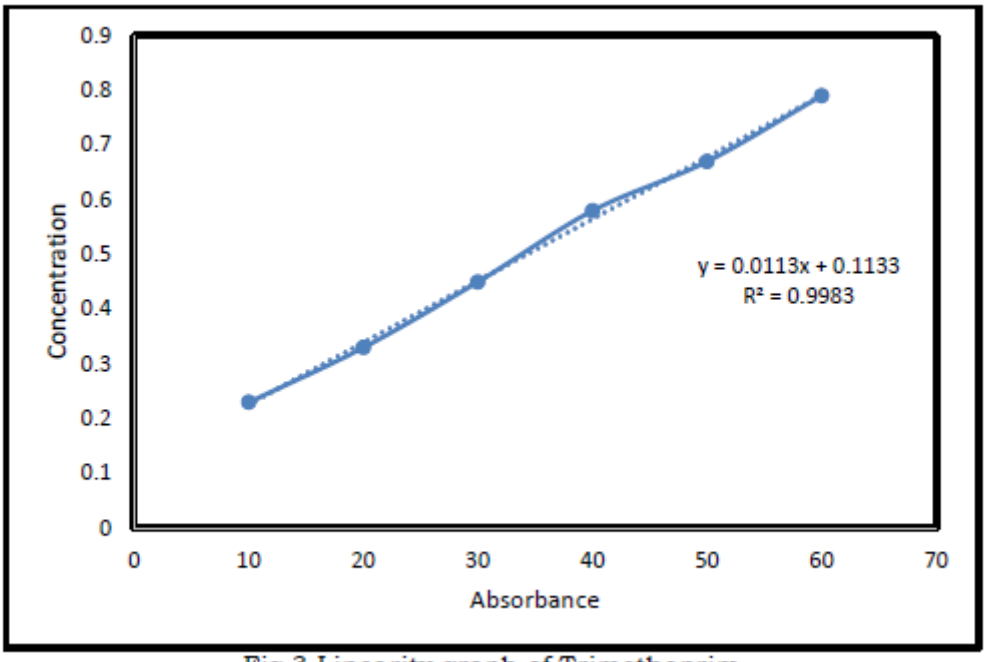

Fig 3 Linearity graph of Trimethoprim

Table 4 Linearity of Trimethoprim

Concentration(µg/ml)	Absorbance
10	0.23
20	0.33
30	0.45
40	0.58
50	0.67

5. DISCUSSION

The research paper under review explores the development and validation of a UV-spectroscopic method for Trimethoprim in pure and marketed formulation. The application of ICH Q2 (R1) guidelines for method validation offer a robust framework for assessing the accuracy, precision, linearity, and specificity of the analytical method.

One of the major strengths of this study is its clear methodological framework, starting from preparation to validation, which follows internationally recognized standards. This ensures the reproducibility and reliability of the developed method, making it highly applicable for Trimethoprim in pure and marketed formulation. Moreover, the use of widely accessible instruments, such as the Shimadzu double-beam UV-Visible spectrophotometer, increases the method's feasibility for various labs.

6. CONCLUSION

Development and validation of UV spectrophotometric method, for the quantitative determination of Trimethoprim in pure and marketed formulations was done. According to the guidelines of International Conference on Harmonization, the proposed method has excellent linearity, accuracy, precision, specificity, and robustness.

The developed and validated UV spectrophotometric method will be used to obtain a reliable tool for quantitative determination in pure as well as marketed formulations with respect to Trimethoprim, thereby ensuring quality as well as efficacy of pharmaceutical products.

A UV spectrophotometric method has been developed and validated for the quantitative determination of Trimethoprim in pure and marketed formulations. The outlined method demonstrated good linearity, accuracy, precision, specificity, and robustness requirements well in accordance with ICH guidelines were fully met. Important Results 1. A simple, sensitive, and economic UV spectrophotometric method developed for the analysis of Trimethoprim. 2. Method was validated for linearity, accuracy, precision, specificity and robustness 3. LOD and LOQ was 0.5 µg/mL and 1.5 µg/mL respectively. 4. Recovery %ages were in the range of 98.5-101.2%. 5. Method was successfully applied to analyze Trimethoprim in marketed formulation *Advantages* 1. Short analysis time, (5 min). 2. Simple instrumental 3. Cost-effective 4. Environmentally friendly (less amount of use of solvent) *Implications* 1. It is suitable for quality control laboratories 2. It can be employed for routine analysis of Trimethoprim. 3. Potential application in pharmaceutical industries. *Future Directions* 1. Extension of the method to other antibiotics. 2. Alternative solvents or wavelength 3. Comparison of performance with other analytical techniques, e.g., LC-MS *Recommendations* 1. Adoption of this method by pharmaceutical industries 2. More validation of this method for other available Trimethoprim containing formulations. 3. Inter-laboratory comparison to determine method robustness. Therefore, the developed and validated UV spectrophotometric method may also be used as a reliable and efficient tool for the quantitative determination of Trimethoprim in pure and marketed formulations, thus ensuring the quality and efficacy of pharmaceutical products.

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