International Journal for Research in Engineering Application & Management (IJREAM)
ISSN : 2454-9150 Vol-06, Issue-12, MAR 2021

Development and Validation of Trimethoprim in Pharmaceutical Dosages Form by Spectrophotometric Method

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ABSTRACT - Objective: To develop the sensitive, selective, subjective, accurate and precisely simple UV-visible spectrophotometric method for the quantitative estimation of trimethoprim (TMP) in pure form on parallel with the pharmaceutical dosage forms.

Methods: The method relied on the combination of the diazotized primary amino aromatic group of the analysed compound with Ortho phenylene diamine in both acidic and basic media. The methods suggested have been used in the trimethoprim (TMP) estimation. The resulting product was calculated by UV-visible spectrophotometric method and validated according to ICH guidelines.

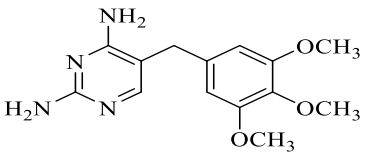
Results: With linearity changes 6-36µgml^{-1} for trimethoprim (TMP) the absorbance was calculated 469 nm in the proposed method. Coefficient of correlation was 0.9997, recovery rate was found to be 98.83-99.93.

Conclusion: The study concludes that UV-visible electrophoretic validation method can be an effective, quick, sensitive, subjective and economically promising technique for quantitative data analysis. It was found that a relationship exists between the results obtained and those obtained by the recorded methods which show that the UV-visible spectrophometric method is very precise. In addition, they may be used in pharmaceutical companies for quantitative measurement of trimethoprim.

Keywords: Trimethoprim, Ortho phenylene diamine, Diazotization, dimethyl sulfoxide, UV-Visible Spectrophotometry, and Validation.

I. INTRODUCTION

Trimethoprim: chemically it is 5-(3, 4, 5-Trimethoxybenzyl) pyrimidine -2,4-diamine, chemical formula $C_{14}H_{18}N_4O_3$, representing a molecular weight of 290.3 gmole^{-1}, yellowish white powder, very slightly soluble in water, ethanol and freely soluble in dimethyl sulfoxide. Trimethoprim is a well-known biological agent, employed as potent metabolic inhibitor of bacterial dihydrofolic acid reductase [1]. This drug is exhibiting very high antibacterial activity against strains resistant to other antibiotics frequently used, e.g. β-lactones. The therapeutic activity of trimethoprim could be attributed to the pyrimidine ring system, also present in the other biologically active substances such as nucleic acids, several vitamins and coenzymes [2]. It was also used to treat and prevent Pneumocystis jiroveci pneumonia.



Trimethoprim

Fig.1 Chemical structure of Trimethoprim

Literature survey reveals that only a few selected spectrophotometric [3-4] HPLC [5] and HPLC-LC-MS [6]

 DOI : 10.35291/2454-9150.2021.0076

methods were reported for the estimation of trimethoprim and also HPLC [7-9] as a combination with other drugs in drugs in bulk and biological samples. There are four HPLC [10] one stability indicating HPLC [11] and one LC-MS [12-14] methods for the estimation of TMP. However, the best of the knowledge of the author no UV-Visible Spectrophotometric method was developed for the estimation of trimethoprim by Ortho phenylene diamine. Hence in the present investigation an attempt was made to develop a simple sensitive and accurate UV-Visible spectrophotometric method for the determination of trimethoprim in pharmaceutical dosage forms.

II. MATERIALS AND METHODS

Thermo (scientific) GENESYS IOS visible Spectrophotometer was used to perform spectral analysis and thermo fisher reported the results. Normal10mm path-length cuvettes are used for analysis. Ultrasonicated sonicate (1.3L) was used to sonicate the standard sample and the formulations.ML-T Analytical Balance (RS232) used to measure normal and sample products.

Chemicals and Reagents

The TMP reference sample was a kind gift from Analog lab, Hyderabad. The ANTRIMA (TMP-160mg) formulation was purchased from the local market, and analytical grade Distilled water (solvent) was purchased from Bross Scientific Pvt. Ltd., A.P., INDIA. **Preparation of standard stock solution**

100 mg of standard drug TMP was correctly weighed and dissolved in 50 ml diluent (Dimethyl Sulfoxide), then moved to a 100 ml volumetric flask, sonicated for 5minutes, and finally, the amount was made up to 1000 µgm1^{-1}stock solution with the same solvent label. From this 10 ml was taken again to a100 ml volumetric flask and diluted to get a 100µgml^{-1} solution of TMP. Then the concentration needed for the trimethoprim calculation is prepared.

Selection of method and wavelength

To achieve the concentration of 10µgml^{-1}the standard stock solution was further diluted with milli-Q water; each solution was scanned against solvent blank in a UV range (200 – 400) in 1.0 cm cell. Drug sample overlain spectrum was recorded and the spectrum analysis showed that TMP displaced individually λ_{max} at 271nm a well-defined λ_{max}. It was found that the overlay spectra for the drug reported at 271 nm are appropriate for the selected drug sample to be λ_{max} . The maximum obtained wavelength for the selected drug was used using spectrophotometric method to estimate trimethoprim.

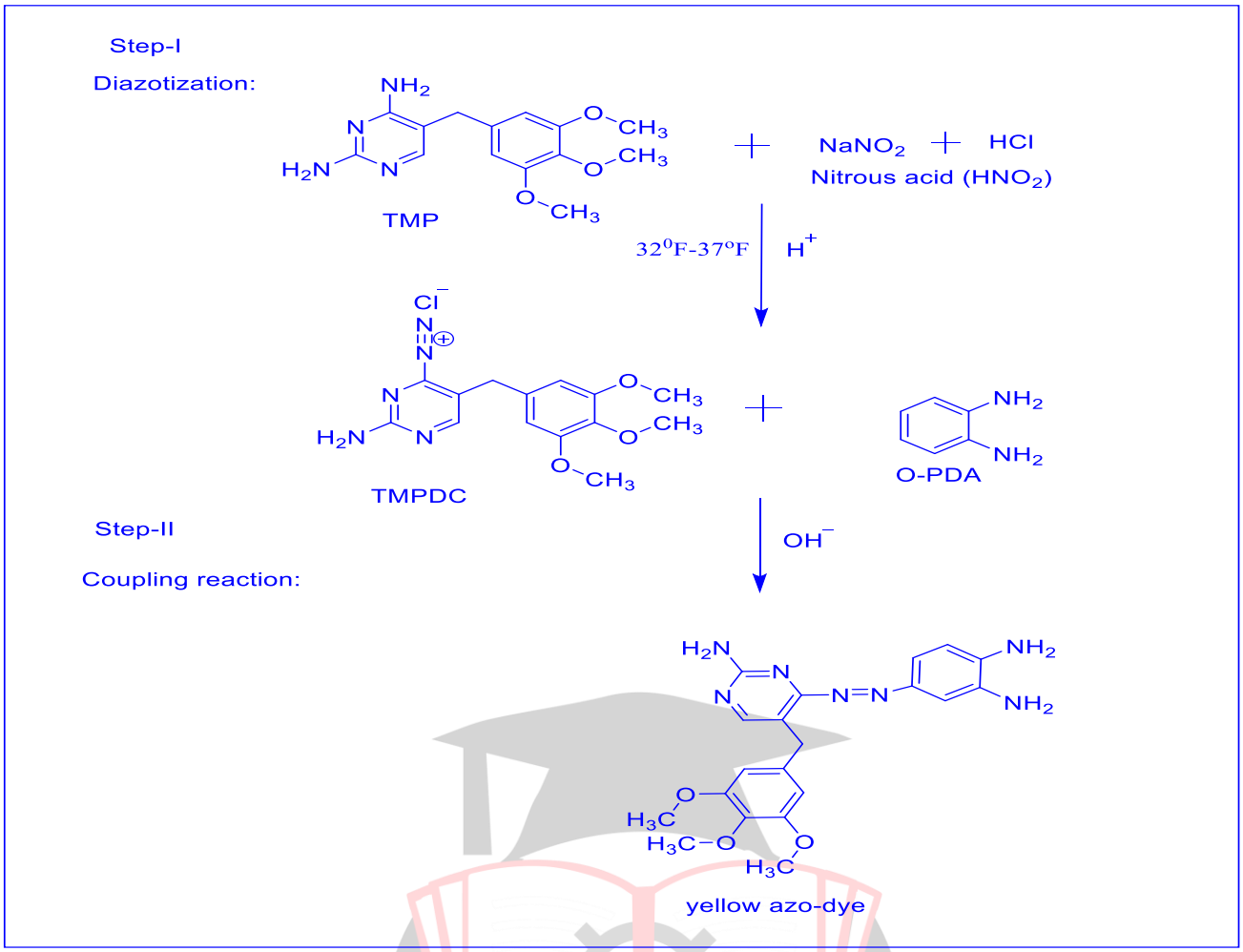
2.4 Preparation of calibration curve

Fresh aliquots of trimethoprim ranging from 0.6 - 3.6ml (6 to 36µgml^{-1}) were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 6 - 36µgml^{-1}. Added 1ml (0.1N) of hydrochloric acid solution to each flask followed by adding 2ml (0.1N) sodium nitrite solution. The resultant was mixed and allowed to stand for diazotization for 5minutes (temperature 0- 5^{0}C). To this solution 1ml (1%) of urea solution has been applied and regularly shaken for evaporation of nitrogen gas. Aded 1 ml of (0.1N) NaOH, 1ml of Ortho phenylene diamine solution and make up to dimethyl sulfoxide level. Yellow chromogen absorbance was measured at against the reagent blank at 469 nm. During 23hrs the colour species was stable. The amount of trimethoprim present in the sample solution was measured using its calibration curve.

2.5 Procedure for Formulations

Twenty tablets weighed and finely coated, containing trimethoprim (TMP). In An accurately measured portion of the powder equal to 100mg of trimethoprim was dissolved in 100ml of dimethyl sulfoxide and mixed for about 5 min and then filtered. The dimethyl sulfoxide was evaporated to dryness. The remaining part of solution was diluted in a 100ml volumetric flask to the volume with dimethyl sulfoxide up to 100ml to get the stock solution A. 10ml of aliquots were piped into 100ml volumetric flask and the volume was rendered up to the mark with dimethyl sulfoxide to obtain the final concentration of 100ml concentration this solution was Subsequently diluted with dimethyl sulfoxide to achieve concentration of 6 to 36µgml^{-1} and prepared as above and analyzed at the specified wavelength, 271nm and statistically conformed results . Scheme 1 depicts trimethoprim reaction mechanism with Ortho phenylene diamine reagent.

 DOI : 10.35291/2454-9150.2021.0076



Scheme 1: Reaction mechanism of Trimethoprim with O-Phenylenediamine (O-PDA) :

III. RESULTS

Results

UV-visible spectrophometric method was developed and validated as per ICH guidelines (ICH committee 2005) for the estimation of in tablet dosage form. The solvent used in this study was dimethyl sulfoxide, and the absorbance was registered at 469 nm, summarizing the result in Fig.2.

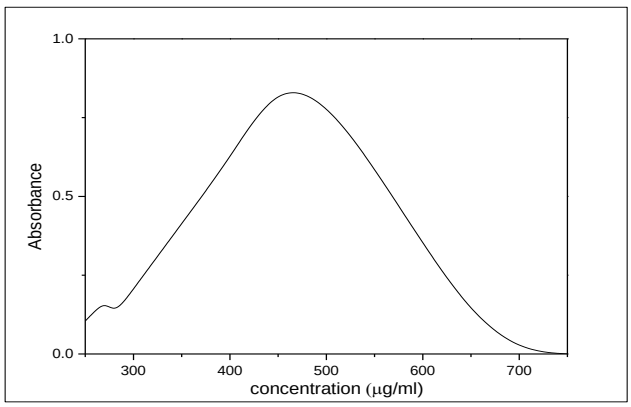


Fig.2 Absorption spectrum of trimethoprim treated with Ortho phenylene diamine

Linearity

Linear relationship between absorbance and concentration of the drug was measured over the concentration range by labelling the concentration range from 6-36 µgml^{-1} trimethoprim and the maximum wavelength of 469 nm. The linearity curve was drawn using the absorbance against concentration obtained. For the selected drug sample in the concentration range tested, a well correlated liner fit graph was observed, and the Linearity finding were shown in table-1, fig.3.

Table .1. Linearity results for Trimethoprim

S.No	concentration in µgml^{-1}	Absorbance
1	6	0.204±0.001
2	12	0.411±0.004
3	18	0.619±0.005
4	24	0.817±0.003
5	30	1.0031±0.001
6	36	1.2011±0.004

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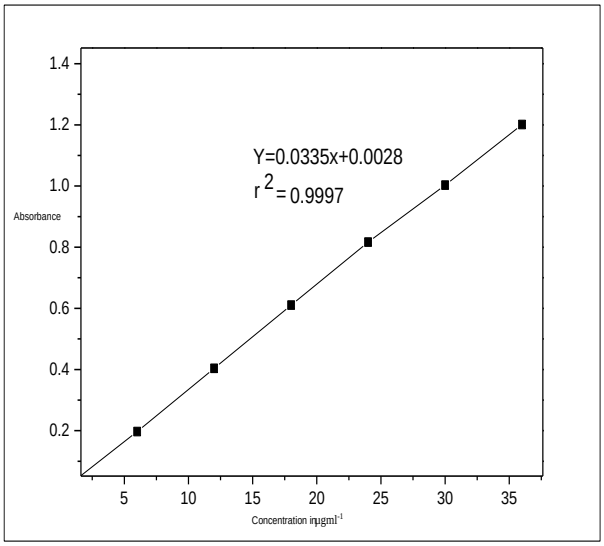

Fig. 3 Linearity graph for Ortho phenylene diamine

Recovery

Studies of recovery were performed by standard procedure. The methods accuracy was calculated by performing three-level recovery studies (10%, 20%, and 30%).The solution resulting was analysed in its corresponding wavelength. Using the absorbance values obtained, percentage recovery and the percentage RSD were determined in each spiked stage. Results were found to be with in the 98-102 acceptance limit and less than 2 percentage RSD percentage. This indicated that the proposed approach was accurate. Recovery results for were given in table-2.

Table .2 Results obtain from recovery studies

S.No	Amount added µgml^{-1}	Amount found* µgml^{-1}	% Recovery
1	6	5.96± 0.02	99.33±0.210
2	12	11.86 ± 0.01	98.83±0.300
3	18	17.93 ± 0.03	99.61±0.331
4	24	23.80±0.01	99.16±0.145
5	30	29.98±0.04	99.93±0.345
6	36	35.89±0.03	99.69±0.146

Precision

The repetitiveness and intermediate precision of the system developed was expressed in terms of the relative standard deviation of the absorbance. Sample application and the absorbance measurement were calculated by conducting six replicate measurement of the same band using a test solution containing trimethoprim at 12µgcm^{-1} the six replicates solutions were tested on the same day for intra-day accuracy and three consecutive days for inter-day accuracy. % 0f RSD found to be 0.506 and 0.514 for intra and inter-day precision, respectively. The finding indicating that the methods precision was considered appropriate. Precision results for intra- and inter-day precision were given in Table-3, respectively.

S.No	Intra Day	Inter Day
1	0.400±0.005	0.393±0.006
2	0.397±0.003	0.392±0.003
3	0.396±0.001	0.390±0.001
4	0.395±0.001	0.389±0.009
5	0.393±0.004	0.387±0.006
6	0.391±0.008	0.386±0.004
7	% RSD=0.506	% RSD=0.514

Table.3 Precision Results for Trimethoprim

Ruggedness and Robustness

The ruggedness and robustness were achieved by evaluating the drug solution with different researchers using the same method comparing the discrepancy between two analysis using percent RSD value was found to be 0.490 for trimethoprim in three absorbance replicates. The low RSD value by percent shows the methods ruggedness. The results of the ruggedness were show in table-4. The proposed approach, although subject to analyst and instrumental variance, was found to be reproducible. We also screened robustness, and summarized the finding in Table5.

Table.4 Ruggedness of the experimental studies

S.No	Variation	(6µgml^{-1})
1	Actual	0.204±0.001
2	Analyst to Analyst	0.203±0.003
3	Instrument to instrument	0.206±0.002
4	%RSD	0.490

Values are given in the table are mean SD of three replicate experiment

Table.5 Robustness study of (12 µgcm^{-1})

S.No	Change in wave length (+2 nm)	Absorbance	Change in Temp. (0^{0c})	Absorbance
1	500	0.410±0.002	At room temp.	0.412±0.004
2	502	0. 416±0.001	Sunlight at Morning	0.418±0.001
3	504	0.416±0.004	Refrigerator	0.424±0.003

Values are given in the table are mean SD of three replicate experiment

Sensitivity of the method

The sensitivity of the method developed has been expressed in terms of the detection limit (LOD) and the quatification limit (LOQ) values. LOD and LOQ have been calculated in accordance with ICH specification and their values are in table-6 The regular solution was prepared, and measured the absorption of the prepared solution. The LOD values for trimethoprim were found to be 0.197µgml^{-1} and 0.246µgml^{-1} respectively. This suggested the approach could be applicable to the spectrum product at the lowest concentration. The LOQ values were found to be 0.597µgml^{-1} and 0.746µgml^{-1}repectively for trimethoprim.

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Table.6 LOD and LOQ

LOD and LOQ were determined in accordance to ICH requirement and their values are present in the table .6.

S.No	Parameter	Present method
1	Intraday	
2	LOD	0.197
3	LOQ	0.597
4	Interday	
5	LOD	0.246
6	LOQ	0.746

Formulation analysis

Tablet- sample solution (formulation solution) was registered at 271nm.The results of the formulation analysis showed that the system can reliably estimate more than 98 percent and the results were found to be in good agreement with the values of the label argument. The percentage assay was found be 99.93. The results of the analysis of the formulations were in Table-7. And the description of approach as a whole was summarized in Table-8.

Table7: Formulation results for trimethoprim (TMP)

S.No	Drug	Brand Name	Label Claim	Amount Prepared	Amount Found	% assay
1.	TMP	ANTRIMA	160 mg	16 mg	15.936 ±0.031	99.60

Table.8 Analytical performance data for the proposed methods

S.No	Parameter	Present method
1	Wavelength(nm)	469
2	Color	Yellow
3	Linearity (µgml^{-1})	6-36
4	Molar absorptivity l mole^{-1} cm^{-1}	1.0016x104
5	Shandell's sensitivity (µgcm^{-1})	0.0345
6	Regression Equation(y=mx+c)	Y=0.0335x+0.0028
7	Slope (m)	0.0335
8	Intercept (c)	0.0028
9	Correlation Coefficient (r^2)	0.9997
10	Intraday Precession	
	%RSD	0.506
11	Interday Precession	
	%RSD	0.514
12	Intraday	
	LOD(µgml^{-1})	0.197
	LOQ(µgml^{-}1)	0.597
13	Interday	
	LOD(µgml^{-1})	0.246
	LOQ(µgml^{-1})	0.746

IV. DICUSSION

UV-Visible spectrophotometric method for the measurement of trimethoprim in tablet dosage form has been developed and validated according to ICH guidelines (ICH committee 2005). The solvent used was 50% v/v aqueous dimethyl sulfoxide. They registered the absorbance at 469 nm. The trimethoprime absorbance was estimated at 469 nm, and calibration curve were plotted. The values for the absorptivity were calculated with the sample wavelength. The absorbance values were measured at a given wavelength. The linearity was found to be within 6-36µgml^{-1} concentration range. Recovery studies determined the exactness of the process. Recovery rate was found to be 98.83-99.93 of trimethroprime with a 0.433 %RSD. The findings were found to be within the approved 98-100 range and less than 2 percent RSD. This indicated that they considered the proposed method to be accurate. Precision experiment was used to tested the repeatability of the process. The percentage of RSD was 0.506 and 0.514; in intra - and inter-day precision, respectively. The percentage RSD value in ruggedness was found to be 0.490 for six absorbance replicates for trimethoprim in pharmaceutical formulations was found to be quick, accurate and rapid. Recovery experiments were carried out to test the validity and reproducibility of the proposed approach in terms of linearity, consistency, precision, specificity and reproducibility.

V. CONCLUSIONS

UV-visible spectrophotometric method for evaluating trimethoprim in a single dosage form using dimethyl sulfoxide as a solvent has been developed and validated according to ICH (International Conference on Harmonization) guidelines. The benefits of the proposed method for analytical purposes are fast determination, cost-effectiveness, assays sample preparation, good reproducibility, simple, economical, accurate and practical. Therefore, the proposed method for evaluating trimethoprim may be recommended in routine quality assurance research in pharmaceutical industries.

ACKNOWLEDGMENTS

The author grateful to the Analog lab, Hyderabad for providing trimethoprime gift sample and also thanks to the Sri Venkateswara University, Department of chemistry, Tirupati-517502, AP, India for providing laboratory facilities and UV-Visible Spectrophotometric instrument.

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