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A new validated spectrophotometric method for determination of Trimetazidine in Formulation and comparison with UV method

M.Ganesh*, G. Jeraldmaria antony,A.Saravankumar, R.Rajesh, K.Rajasekar

Pharmaceutical Sciences Discipline, Dept .of Nanotechnology, Biotechnology Centre, Anna University, Coimbatore, Tamil Nadu, India

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Abstract

A new validated and more reproducible spectrophotometric method for the determination of trimetazidine (TMZ), a piperizinyl antianginal has been developed and validated. This method was based on the formation of a yellow ion pair complex between TMZ and methyl orange using chloroform as solvent at pH 4 phosphate buffer. The lambda max and molar absorptivity of the chromogen were 427 nm , 5.0216 X 10^3 lit mol cm^{-1} respectively. Chromogen obeys Beer's in the concentration range of 15-50 µg ml^{-1} with linear regression of 0.9993, while the percentage recovery, LOD and LOQ were 99.65-99.98 %., 4.5 µg ml^{-1} and 10 µg ml^{-1} respectively. From the percentage recovery and specificity studies it was concluded that there was no interference of common additives during the estimation. This proves the suitability of this method for the routine quality control analysis of the trimetazidine in formulation.

Key Word: Trimetazidine, Spectrophotometric, Formulation, Methyl orange, Ion pair complex

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Introduction

Trimetazidine hydrochloride(TMZ), 1-(2,3,4-trimethoxybenzyl) Piperazine di-hydrochloride [1], Fig 1, is used in angina pectoris and in ischemia of neurosensorial tissues as in Meniere's disease[2-6]. A number of methods have been reported for the determination of trimetazidine in biological fluids and pharmaceutical preparations. These include HPLC with electrochemical detection [7], GC-MS [8], slow injection chemiluminescence [9], HPTLC [10], UVspectrophotometric method [11] and, voltammetry [12] and by LC-MS [13,14] Until no ion pair colorimetric method reported for its estimation in pharmaceutical dosage forms using methyl orange as ion paring agent.

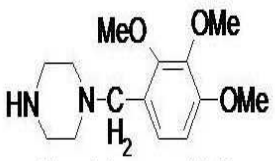

Figure 1.Structure of TMZ

The aim of this study was to develop and validate a ion pair spectrophotometric method for TMZ estimation in formulation. The validation procedures followed the International Conference on Harmonization guidelines for evaluating the parameters specificity, linearity, precision, limits of detection and quantitation, accuracy and comparison of the same with already existed UV spectrophotometric method.

Results and Discussion

In recent years, a spectrophotometry method for determinations of drugs has considerable attention, due to their sensitivity, low cost, and simplicity. In our paper, we developed a method based on the formation of chloroform extractable ion pair complex between TMZ and methyl orange and validated for TMZ in tablets.

The chosen experimental conditions were satisfying the method for the estimation of drug of choice. After testing, the solubility of the drug and dye chloroform is selected as the best solvent for analysis which easily available and less polutent.

Validation of an analytical procedure is necessary before adopting the procedure for routine quality control. The validation proves the suitability of the procedure for the intended purpose. The International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [16], USP 30 2007 and BP[17]guidelines describe the analytical parameters that should be evaluated in a method validation. The type of method and its respective use determine which parameters should be evaluated.

To fix the linearity a calibration curve was constructed by plotting the absorbance as a function of the corresponding concentrations. The regression equation for the results was:

$$A=0.01507X-0.0067C \ (r = 0.\ 0.9993)$$

where *A* is the absorbance at 427 nm, *C* the concentration of TMZ in $\mu g ml^{-1}$ in the range of 15-50 $\mu g\ ml^{-1}$(fig.3) and *r* is the correlation coefficient. The molar absorptivity (*å*) was found to be 5.0216×10^3 lit mol cm^{-1}.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined (ICH 2005) using the formula: LOD or LOQ = κS.D.*a*/*b*, where κ = 3 for LOD and 10 for LOQ, S.D.*a* is the standard deviation of the intercept and *b* is the slope. The LOD and LOQ were 4.5 and 10 $\mu g\ ml^{-1}$, respectively. The detection and quantitation limits determined were 0.10 and 0.29 $mg\ ml^{-1}$ respectively. These low values indicated the high sensitivity of the purposed method.

Precision of the method

Intra-day precision was calculated from results obtained from fivefold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on five consecutive days. The results obtained are

listed in Table 1. The low relative standard deviation (RSD 0.95; 0.87; 1.01 at three different level (intra-day precision), 1.23,0.98,1.45 at three different level (inter-day precision) showed the good precision of the method.

Table .1. Inter and Intra day precision of the method

Concentration (µg ml^{-1})	Intra day		Inter day	
	Mean amount found (µg ml^{-1} n=5)	RSD (%,n=5)	Mean amount Found (µg ml^{-1} ,n=5)	RSD (%,n=5)
20	19.92	0.95	20.12	1.23
40	40.01	0.87	39.99	0.98
60	60.21	1.01	60.09	1.45

Accuracy of the method

Recovery studies were carried out by adding a known quantity of pure drug to a pre-analyzed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated. The results of analysis and recovery studies are given in Table.2. The accuracy expresses the agreement between the accepted value and the true value. The mean percentage recovery was found to be 99.55-100.3% for tablets (Table 2). This value proves the good accuracy of the purposed method.

Table .2. Accuracy of the proposed method (estimated at 427nm)

Sample	Label Claim (mg/tab)	Estimated amount (mg/tab)	Spike Level (%)	Amount of Drug Added (µg ml^{-1} n=5)	Amount of drug recovered (µg ml^{-1} n=5)	% Recovery	RSD (% ,n=5)
Brand I	20	20.01	50	20	19.91	99.55	0.89
			100	40	40.02	100.05	1.01
			150	60	58.98	98.3	0.91
Brand II	20	19.98	50	20	20.02	100.05	1.05
			100	40	40.12	100.3	1.02
			150	60	60.01	100.01	1.03

Application of the proposed method

The method was applied to the analysis of the bulk drug and the mean recovery value was 100.15±0.52 %. It is evident from the aforementioned results that the proposed method gave satisfactory results for determination of TMZ in bulk. For application of the proposed method on marketed dosage forms of the tablet was taken for studies. The tablets were subjected to the analysis for their contents of the active ingredient by both the proposed method and the reported method .The label claim percentage was 99.65±0.18 and 99.98±0.42 for brand I and brand II (Table.3.) by the proposed method ,100.01±0.12 and 99.87±0.43 by the reported UV method . This result was compared with that obtained from the reported UV method by statistical analysis with respect to the accuracy (by *t*-test) and precision (by *F*-test). No significant differences were found between the calculated and theoretical values of *t*- and *F*-tests at 95% confidence level proving similar accuracy and precision in the determination of TMZ by both methods.

Table. 3. Analysis of tablets containing TMZ by the proposed and reported method

Tablets	Recovery [a] (%,±R.S.D)		t-values [b]	F-values [b]
	Proposed	Reported [c]		
Brand I [d]	99.65±0.18	100.01±0.12	1.5	3.3
Brand II [d]	99.98±0.42	99.87±0.43	1.8	3.5

[a] Values are mean of Six determinations; [b] The tabulated values of t and *F* at 95% confidence limit are 2.67 and 6.02 respectively; [c] Reference UV method; [d] Marketed tablets

Study of Interference (Placebo study)

Studies on interference were carried out to explore the effect of common excipients that might be added during formulations. Samples were prepared by mixing known amount (20 mg) of TMZ with various amounts of the common excipients: lactose, starch, talc and magnesium stearate in their recommended percentages [18]. The analysis of these laboratory-prepared samples was carried out using the general recommended procedure, and the recovery values were determined. No interference was found from lactose, starch, talc and magnesium stearate as the percentage recovery value was 98.87–100.35±0.4–0.67%. This indicated the absence of interference liabilities from these excipients (Table.4).

Table.4 Analysis of TMZ in presence of commonly used tablet exicipients(Placebo)

Exipients	Recovery [a] (%±S.D)
Lactose [b](10mg)	98.9±0.67
Starch(5mg)	100.35±0.4
Magnesium Stearate(10mg)	98.87±0.48
Talc(10mg)	99.06±0.43

Table. 5. Robustness and day to day variation of the method

Parameters Studied	**Recovery [a] (%±RSD)**
Recommended Conditions [b]	100.23±0.34
Methyl orange Concentration(%,w/v)	
0.05	100.02±0.11
0.15	99.43±0.54
Change in Wave length	
426nm	98.91±0.43
428nm	99.89±0.41
Ruggedness(day-to-day variation)	
Day 1	100.03±0.43
Day 2	98.95±0.67

Robustness and Ruggedness
Robustness and Ruggedness of the method were also studied by altering wavelength of estimation and changing the dye's concentration which were also within the acceptable limit with respect to % RSD(Table. 5).In case of ruggedness difference in the estimation was studied by means of analyzing the samples in two different days by following same procedure and the results were summarized in Table .5.

Materials and Methods

Chemicals and Reagents
All chemicals used in this study were analytical grade and used without further purification. Chloroform (s.d. finechem., Bombay, India), methyl orange (s.d. finechem, Bombay, India).Pure TMZ was a gift from Micro labs (I) Ltd (Bangalore, India) and tablet formulations (Brand I and BrandII) were purchased from local pharmacy .

Instrument
Shimadzu UV-Visible double beam spectrophotometer UV 160A, with 10 mm quartz cells was used for the measurements.

Standard solutions
1 mg ml^{-1} stock solution of TMZ was prepared by dissolving 100 mg of TMZ in appropriate volume of double distilled water and made up to 100 ml in volumetric flask and used as stock solution.

Sample solution
20 TMZ tablets were powdered and an accurately weighed quantity powder equivalent to 100 mg of TMZ from each brands were dissolved in water. The excipients were separated by filtration using Whatman filter paper (No.40) and the filter paper washed three times with distilled water for effective liberation of drug from the core. Filtrate and washings of the tablet samples were transferred into 100 ml flask and diluted to the mark with chloroform, and the spectrophotometric procedure was followed.

Reagent Preparation
0.1 gm of methyl orange was weighed and transferred into a 100 ml standard flask and the volume was made up to the mark to get the required concentration(0.1%w/v).

Method development
Aliquots of stock transferred into a series of separating funnel then 1 ml of methyl orange reagent and 2 ml phosphate buffer of pH 4 was added, then the solutions were allowed to stand for few minutes, followed by accurately measured quantity (10ml) of chloroform and extracted well to give concentration 15-50 µg ml^{-1}, all the solutions were passed through dried sodium sulphate to remove water. Solution were scanned between 400-800nm which shows λ-max at 427nm (Fig.2). The above λ-max was used for its analysis of TMZ in formulation. Formed ion pair complex was obeying Beer's law in the range of 15-50 µg ml^{-1} (Fig.3).

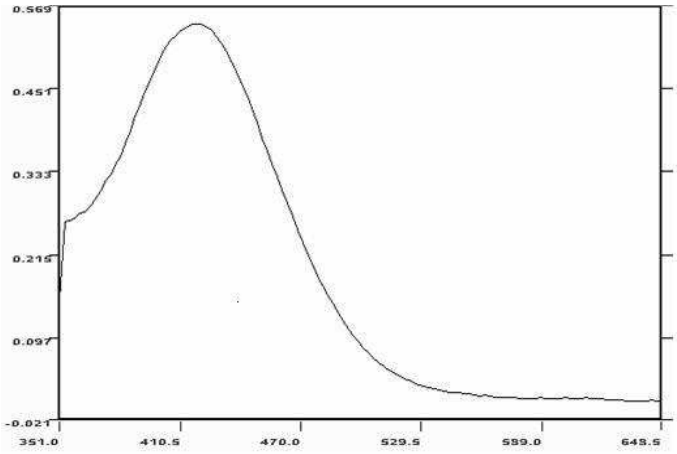

Figure 2.Absorption spectra of TMZ-methyl orange ion paired complex extracted into 10ml chloroform

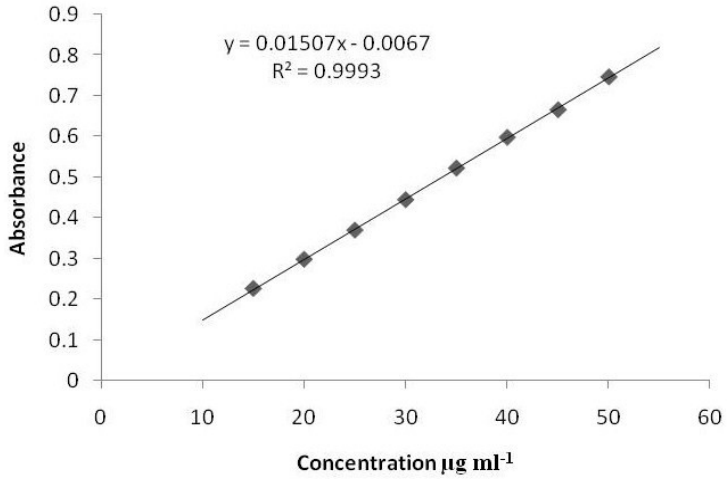

Fig.3. Linearity Plot of TMZ methylorange ion pair complex

Optimization of the method variables method validation

Effects of reagent concentration

The effect of methyl orange concentration on the reaction was checked out at room temperature and away from direct sunlight. As shown in Fig. 4, the reaction of TMZ was dependent on the concentration of dye used. A concentration of 0.1% (w/v) was selected as the optimum reagent concentration. Higher concentrations caused a distinct decrease in the absorbance (Figure.4).

Effect of time

The absorbance of the solution was measured after 10 minutes after adding reagent, and upto 3 hrs. As the results shown in Fig.5.the reaction was slow and the formed color was stable up to 3 hrs (Figure .5.)

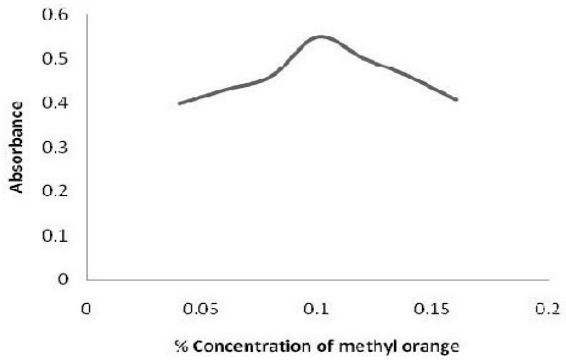

Figure 4. Effect of reagent Concentration

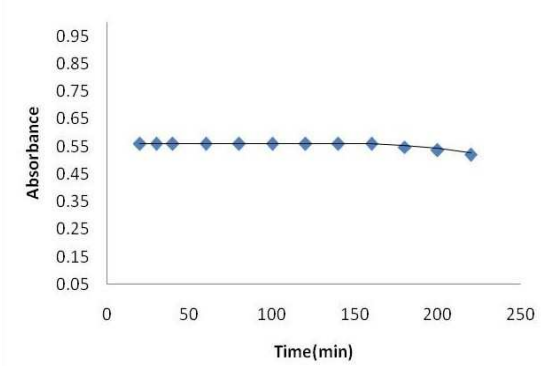

Figure .5 Effect of time on stability of Colour

Stoichiometry and suggested reaction mechanism

Piperazine moiety of the TMZ having secondary amine (loan paired electrons) in it first position is responsible for the formation yellow colored ion pair chloroform extractable chromogen with methyl orange dye. The stoichiometry of the reaction between TMZ and methyl orange was determined by using Job's [16] method for continuous variation and the results revealed that the molar ratio of TMZ and methyl orange was 1:1.

Conclusion

The results demonstrated the successful use of methyl orange in the development of a selective spectrophotometric method for determination of TMZ, a piperizinyl anti-anginal. The proposed method is validated by its sensitivity, which permits the determination of a concentration down to 15µ gml^{-1}, simplicity of the procedure and reliability of the results. Furthermore, the methyl orange is a commonly available reagent and inexpensive, has excellent shelf life and is available in all analytical laboratories. The proposed method can be applied in quality control laboratories for the routine analysis of TMZ in raw material and pharmaceutical formulations; considering that the cited compound is stable in chloroform medium.

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