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Spectrophotometric Method for Determination of Trimetazidine in Formulation Using Chloranil as Chromogenic Agent

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Abstract

A new, simple, selective 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 blue colored chromogen between trimetazidine and chloranil acetaldehyde reagent in DMSO. The chromogen exhibit maximum absorbance at 627 nm with molar absorptivity of 5.205 x 10^4 lit mol^{-1} cm^{-1} in methanol. Beer's law was found to be obeyed in the concentration range of 30 - 65 µg mL^{-1} with linear regression of 0.9984, while the percentage recovery and the limit of detection 98.81-99.91%, 5.5 µg mL^{-1} respectively. From the percentage recovery and placebo studies it was concluded that there was no interference of additives during the estimation. This shows the suitability of this method for the routine analysis of the trimetazidine in tablet formulation.

Keywords:

Trimetazidine; Chloranil; Spectrophotometric; Formulations

1. Introduction

Trimetazidine hydrochloride (TMZ), 1-(2,3,4-trimethoxybenzyl) piperazine di-hydrochloride (Fig. 1.) is used in angina pectoris and in ischemia of neurosensorial tissues as in Meniere's disease[1-6]. 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], HPTLC [9], UV spectrophotometric method [10], slow injection chemiluminescence [11], voltammetry [12], and by LC-MS [13,14]. These methods are often time-consuming, expensive, lack selectivity and cumbersome. Until now no colorimetric methods using chloranil and acetaldehyde for the analysis of TMZ in its pharmaceutical dosage forms have been reported. In this work, a reaction of TMZ with chloranil acetaldehyde reagent in di methyl sulphoxide (DMSO) is taken into consideration for the development of new simple, rapid , reliable and validated visible spectrophotometric method for the determination of TMZ in bulk and in formulation. The proposed method was successfully applied for the analysis of drug in raw material of TMZ and its formulations and the results are in good agreement with those of already existed UV method.

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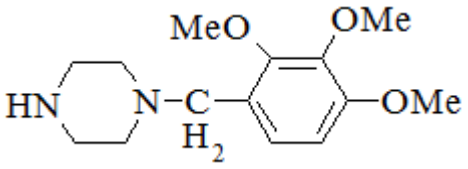


Fig.1. Chemical structure of TMZ

2. Experimental

2.1. Instrument

Elico (Bombay, India) UV-Visible double beam spectrophotometer SL-164 with 10 mm matched quartz cell was used for all spectrum measurement.

2.2. Reagents

All chemicals used in this study were analytical grade and used without further purification. Methanol (SD. Finechem., Bombay, India), DMSO (S.D. Finechem, Bombay, India), acetaldehyde (SD Fine chem.) and chloranil (SD Finechem, Bombay, India.). Pure TMZ was a gift from Micro labs (I) Ltd (Bangalore, India) and tablet formulations were purchased from local pharmacy (A& B).

2.3. Standard solutions

1 mg mL^{-1} stock solution of TMZ was prepared by dissolving 100 mg TMZ in appropriate volume of methanol and made up to 100 mL in volumetric flask and used as stock solution.

2.4. Sample solution

An accurately weighed quantity of powder equivalent to 100 mg of TMZ from well powdered sample of 20 TMZ tablets from each brands were dissolved in methanol. The insoluble excipients were filtered through Whatmann filter paper No. 41 and the filter paper was washed three times with methanol. Filtrate and washings of the tablet samples were transferred into 100 mL flask and diluted to the mark with methanol.

2.5. Reagent preparation

Various concentration of chloranil acetaldehyde reagent was prepared by dissolving 0.1, 0.2, 0.3, 0.4 and 0.5 mg of chloranil and 8 mL of acetaldehyde in 100 mL DMSO and was used for this study.

2.6. General procedure

0.6 mL of standard or sample solution, containing appropriate amount of the TMZ was pipetted into a 10 mL volumetric flask and 3 mL of 0.3% chloranil acetaldehyde reagent was added, then the solution was allowed to stand for 30 min ,final volume was made up to the mark with methanol. The maximum absorbance of the solution was determined by taking the spectrum of the complex against the reagent blank and it was shown at 627 nm (Fig.2) This λ_{max} was used for the further studies.

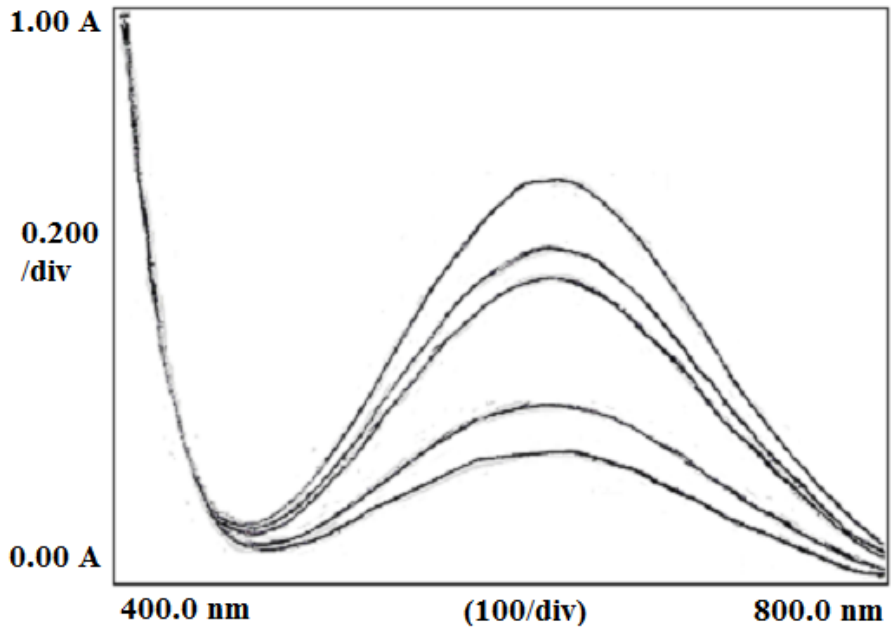


Fig. 2. Visible spectra of reaction product

3. Results and Discussion

3.1. Spectroscopic study of reaction product

The secondary amine part of the piperazine ring in TMZ reacts with acetaldehyde in chloranil acetaldehyde reagent to form vinylamine complex which again reacts with chloranil in DMSO and produce blue colored chromogen. DMSO is selected as solvent for reagent preparation because the reagent is stable only in this solvent [15]. The formation of this colored product was utilized in the development of the proposed method for determination of TMZ in bulk and its marketed formulations. Principle behind the reaction is summarized in Fig.3.

3.2. Optimization of the method variables

3.2.1. Effects of reagent concentration

The effect of chloranil 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 chloranil solution. A concentration of 0.3% (w/v) in DMSO was selected as the optimum reagent concentration. Higher concentrations caused a distinct decrease in the absorbance.

3.2.2. Effect of time

The absorbance of the solution was measured after 30 minutes of adding reagent, and up to 6 h. The results were shown that the reaction was slow and the formed color stable up to 6 h (Fig.5.).

3.2.3. Stoichiometry and suggested reaction mechanism

The stoichiometry of the reaction between TMZ and chloranil was determined using Job's[16] method for continuous variation and the results revealed that the molar ratio of TMZ: Chloranil was 1:1. Based on this data, and the previously reported knowledge, the

reaction between TMZ and Chloranil can be postulated to proceed according to the pathway given in Fig. 3.

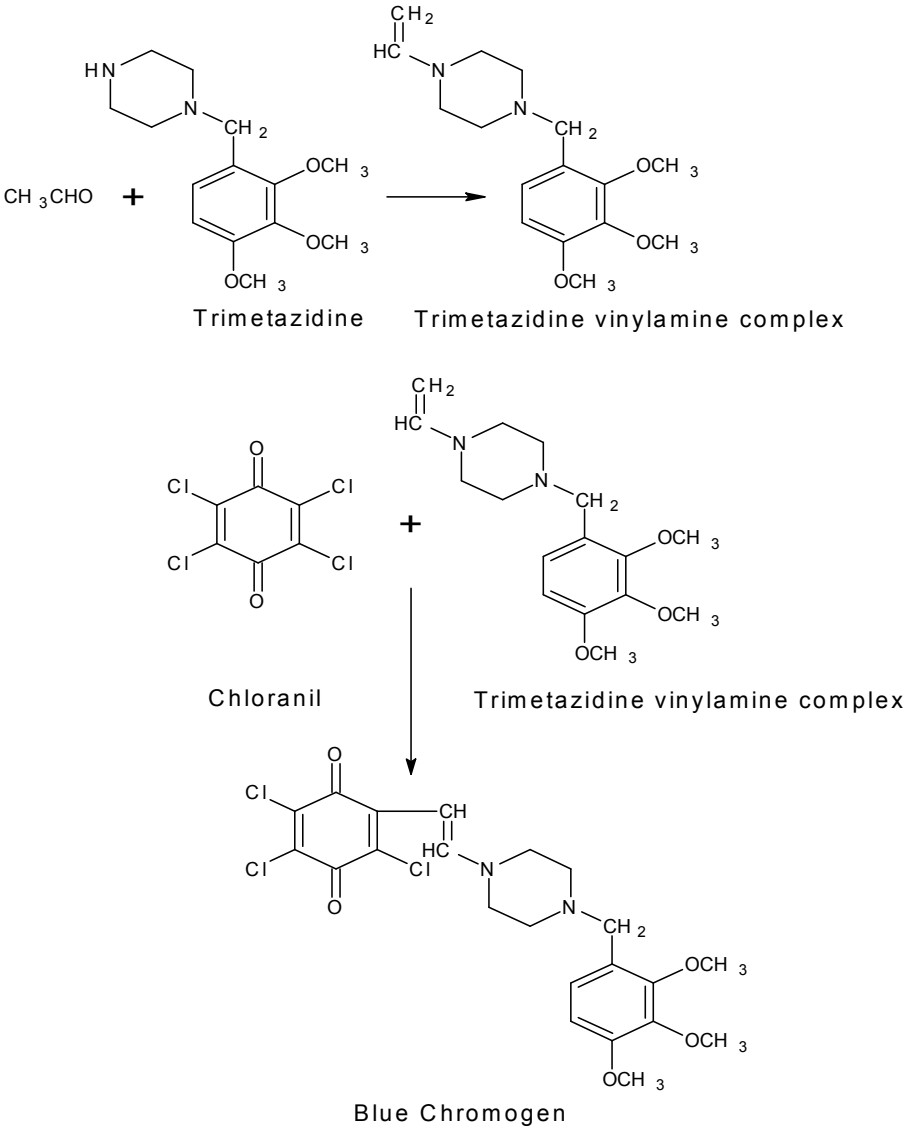


Fig.3. Reaction mechanism

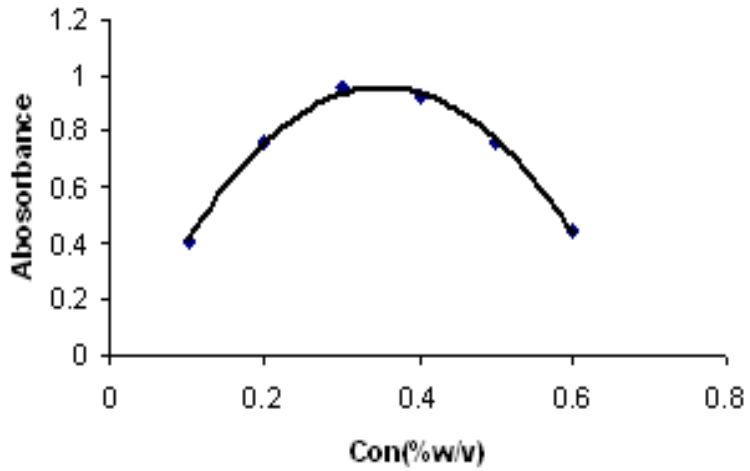


Fig. 4. Effect of reagent concentration

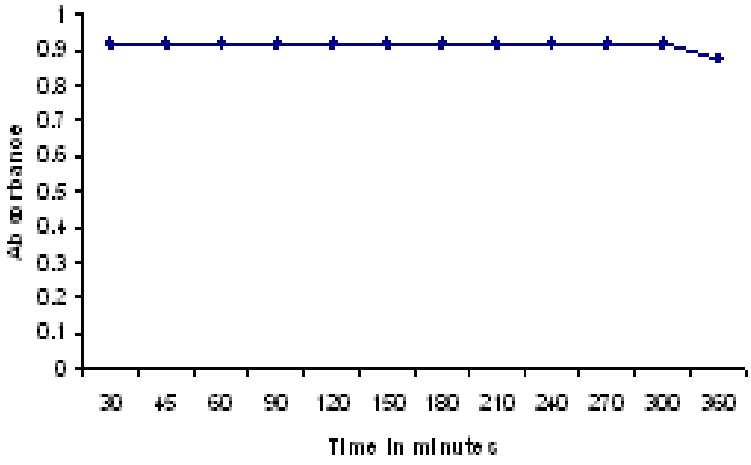


Fig. 5. Effect of time

3.2.4. Method development and method validation

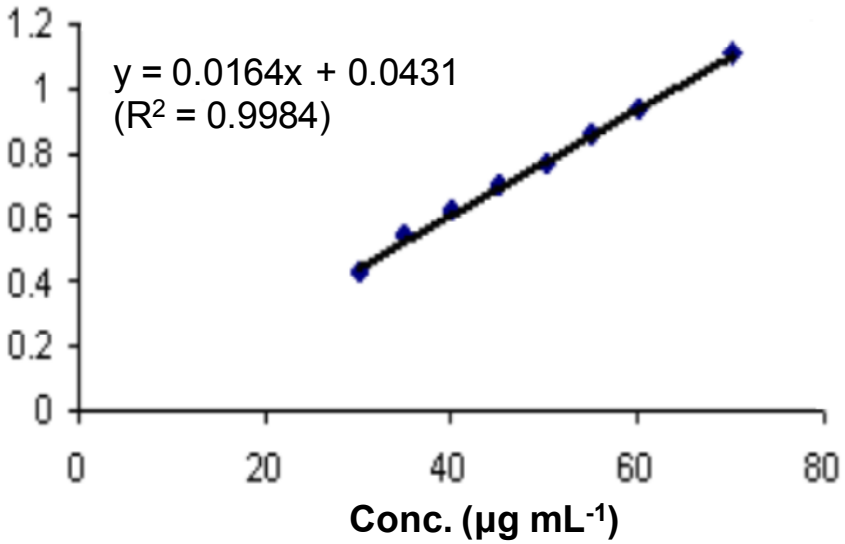
Validation of an analytical procedure is important before transferring the procedure for routine quality control which proves the suitability of the procedure for the intended purpose. The International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [17] and USP 30 2007[18] 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.

3.2.5. Calibration, sensitivity and precision

Calibration curve for the analysis of TMZ by its reaction with chloranil acetaldehyde reagent was constructed by plotting the absorbance as a function of the corresponding concentrations. The regression equation for the results was:

$A = 0.0164 + 0.0431C$ ($r = 0.9984$)

where A is the absorbance at 627 nm, C is the concentration of TMZ in µg mL^{-1} in the range of 30–65 µg mL^{-1}(Fig.6) and r is the correlation coefficient. The molar absorptivity ($å$) was found to be 5.205 x 10^4 mol^{-1} cm^{-1}. The limit of detection (LOD) and limit of quantitation (LOQ) were determined using the formula: LOD or LOQ = κ S.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 5.5 and 15µg mL^{-1}, respectively. The precision of the proposed method was determined by analyzing six replicate samples of standard TMZ solution at one concentration level. The assay gave satisfactory results; the relative standard deviation (RSD) was less than 2%. The placebo test demonstrated that there was no interference in the determination of the drug by the common excipients. The accuracy expresses the agreement between the accepted value and the true value. The mean percentage recovery was found to be 99.55-100.4% for tablets (Table 1). These values prove the good accuracy of the purposed method.



ig. 6. Beer's law Standard Plot

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

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)
20	20.01	50	20	19.91	99.55	0.99
		100	40	40.02	100.05	1.03
		150	60	59.89	98.14	0.94
20	19.98	50	20	20.02	100.05	1.00
		100	40	40.16	100.4	1.05
		150	60	60.00	100.0	1.01

obustness of the method were also studied by altering wavelength of estimation, oncentration which were also within the acceptable limit with respect to %RSD). In case of ruggedness the difference in the estimation was studied by means of g the samples in two different days by following same procedure and the results were ized in Table 2.

: Robustness and day to day variation of the method

ers studied	Recovery (%±S.D)
nended conditions	100.23±0.34
l concentration(%,w/v)	
	99.02±0.11
	99.43±0.54
in wave length	
	99.91±0.43
	99.80±0.61
ness (day-to-day variation)	
	100.03±0.43
	98.95±0.67

3.2.6. Study of Interference

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 [18,19]. 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 98.87–100.15±0.4–0.67%. This indicated the absence of interference liabilities from these excipients Table 3. Moreover, the proposed has the advantage that the measurements were performed at 627 nm in the visible region away from the UV-absorbing capabilities of interferents that might be co-extracted from laboratory-prepared tablet admixture.

Table 3: Analysis of TMZ in presence of commonly used tablet excipients

Exipients	Recovery [a] (%±S.D)
Lactose [b](10 mg)	99.09±0.67
Starch(5 mg)	100.15±0.4
Magnesium stearate (10 mg)	98.87±0.58
Talc(10 mg)	99.16±0.34

[a] values are average of six determination
[b] The amount of exipients added per 20 mg of TMZ

3.2.7. 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 the 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 [10]. The label claim percentage was 99.98±0.18 and 99.95±0.42 for brand I and brand II (Table 4). This result was compared with that results 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 4: 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]	99.98±0.18	100.01±0.12	1.6	3.4
Brand II [d]	99.95±0.42	99.87±0.43	1.8	3.5

[a] Values are mean of six determinations
[b] The tabulated values of t-test and *F-test* at 95% confidence limit are2.67 and 6.02 respectively
[c] Reference [11]
[d] Marketed tablets

4. Conclusions

The results demonstrated the successful use of chloranil in the development of a selective spectrophotometric method for determination of TMZ, a piperizinyl anti-anginal. The proposed method is characterized by its sensitivity, which permits the determination of a concentration down to 15µg mL^{-1}, simplicity of the procedure and reliability of the results. Furthermore, the chloranil as an analytical reagent is inexpensive, has excellent shelf life, and is available in any analytical laboratory. 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 DMSO medium.

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