NEW ANALYTICAL METHODS FOR THE ASSAY OF TRIMETAZIDINE DIHYDROCHLORIDE IN PURE AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Two simple and sensitive Spectrophotometric methods (A and B) for the determination of Trimetazidine dihydrochloride in Bulk samples and pharmaceutical formulations are described. Method A is based on oxidative coupling reaction using 2,6 dichloro Quinine -4 – Chlorimde (DCQC). Which yield reddish coloured complex is measured at λ_{max} 520 nm. And method B is based on the formation of the blue coloured species, which the drug is treated with folin-ciocltеu (FC) reagent under alkaline conditions with an absorption maximum of λ_{max} 770nm. The results obtained by the proposed methods were good agreement with the labelled amounts.

Key words: Trimetazidine, dihydrochloride, Dictloro quinine – 4 chloramide (DCQC), folin-ciocltеu (FC) reagent.

Introduction:

Trimetazidine dihydrochloride (TMZH) Piperazine, 1- [(2,3,4-Trimethoxy phenyl) Methyl] is an anti – ischemic agent,[1-6] widely used in the treatment of Ischemic heart disease. TMZH is official in B.P[7] methods appeared in the literature.

A number of methods have been reported for the determination of TMZH in biological fluids and pharmaceutical formulations. These include HPLC[8], GC-MS[9], HPCTLC[10] UV spectrophotometric method[11], slow injection chemiluminescene[12], voltammetry[13], and by LC-MS[14] and visible spectrophotometric[15-24] methods, most of these methods are less sensitive and more time consuming than the proposal methods. The present communication reports two new visible spectrophotometric methods for the determination of TMZH in pure and pharmaceutical formulation. Method A is based on the oxidative coupling reaction of the drug with the reagent 2, 6 Dictloro quinine – 4 chloramide[25-29], and the resulting coloured species is measured at 520nm. Method B is based on the reduction of FC[30] reagent by TM2H in alkaline medium to from a blue coloured chromogen that exhibits λmax 770nm.

Materials and Methods:

A Milton Roy spectronic and systronic 106 spectrphotometers with 1cm matched quartz cells were used for all spectral and absorbance measurements.

Preparation of Reagents:

All the chemicals were prepared were of analytical grade.

DCQC Solution (Loba; 0.3% 1.90 x 10^{-2}M)	-	DCQC solution was prepared by dissolving 300mg in 100ml of isopropanol.
FC Reagent (Loba, 2N)	-	Folin – Ciocalteau reagent (2N) supplied by Loba chemie was used directly in the investigation.
Na_2CO_3 solution (Merck, 10%)	-	Prepared by dissolving 10mg of sodium carbonate analydunus in 100ml of distilled water.

Preparation of solutions:

A stock containing 1mg/ml of pure TMZH was prepared by dissolving 100mg of the drug in 10ml of distilled water. The stock solution was further diluted with distilled water to get the 1mg/ml (Method A) and 100 µg/ml (Method B).

Analytical procedure for method A:

In to a series of (0.5 – 3.5ml) 15ml calibrated flasks containing aliquots of the TMZH solution (1mg/ml), 1.0ml of 1.90 x 10^{-3}M DCQC solution was added and the total volume in each flask was brought to 10ml with distilled water. Then the contents were kept on a water bath for 30min. Cooled and the flasks were made upto the volume with distilled water and the absorbances were measurement at 520mm against a reagent blank. The coloured species was stable for 12hours. The drug content was determined with a standard plot prepared under identical conditions.

Method B:

In to a series of 20ml graduated test tubes continuing aliquots (1.0 – 7.0ml of TMZH solution (100µg/ml), 2.0ml of F.C. Reagent and 9.0ml of Na_2CO_3 solution were added successively and allowed to react for 10mn at laboratory temperature. The solution was made upto the mark with distilled water and the absorbance of the solution was measured at 770nm against a reagent blank prepared simultaneously with in the stability period (5min – 1hrs). The TMZH content was determined with standard plot.

Analysis of pharmaceutical formulations:

Tablet powder equipment to 100mg was weighted accurately and transferred in to a 100ml volumetric flasks and the contents were dissolved with distilled water and volume was made upto 100ml and filtered. The above solution was further diluted to the requisite concentrations for methods A and B were analysed as described under the procedure for pure samples.

Results and Discussion

The optical characteristics such as Beer's law limits, molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope and intercept data from linear least squares treatment and percent relative standards deviation (from six replicate samples) were summarized in Table – 1.

Table 1
Optical and regression
Characteristics, precision and accuracy of proposed methods for TMZH

Parameters	Method A	Method B
λmax (nm)	520	770
Beer's law limits (μg/ml)	33 - 200	5.0 – 35.0
Molar absorptivity ($1mol^{-1}/cm^{-1}$)	1.05×10^{3}	4.07×10^{3}
Sandell's sensitivity ($\mu g.cm^{2}$/0.001 Abs. Unit)	3.1×10^{-1}	8.3×10^{-2}
Regression equation (y = a+bc) slope (b)	3.15×10^{-3}	1.0×10^{-2}
Intercept (a)	-1.6×10^{-3}	7.7×10^{-3}
Correlation coefficient (γ)	0.9999	0.9998
Relative standard deviation (%)*	0.58	0.66
% range of error (confidence limits) 0.05 levels	0.61	0.70

* calculated from 6 determinations

The accuracy of the method was ascertained by comparing the results from proposed and reported methods statistically by the t and F tests and found not to differ significantly. In order to justify the reliability and suitability of the proposed methods, known quantities of pure drug was added to its pre-analysed dosage forms and the mixtures were analysed by the proposed methods and the values are listed in table 2. There is no interference from other ingredients present in the assary methods.

In method A TMZH is estimated on the basis of oxidative coupling reaction of the drug with 2, 6-dichloro quinine-4-chlorimide 1.0 to 1.50ml of DCQC on boiling water bath gave a maximum and reproducible absorbance of the coloured species was studied by conducting the reaction at different temperature for different time intervals. Prolonging the time beyond 30min and increasing the temperature gave erratic results. Stability of the coloured complex was determined by measuring absorbance values at time intervals 15min. and was found to be stable for 3hrs.

Method B is based on the reduction of FC reagent by TMZH in alkaline medium to form a blue coloured chromogen that exhibits λmax 770m and stable for 60min.

The proposed methods are simple and sensitive with good precision and accuracy and can be used for the routine quality control analysis of TMZH in pure form as well as in pharmaceutical formations.

Table 2

Assay of Trimetazidine dihydrochloride in Pharmaceutical formulation (TMZH)

Pharmaceutical formulations	Labelled amount (mg)	Amount found by proposed method** %	Found reference method	% Recovery by proposed methods***	
		METHOD A	METHOD B	METHOD A	METHOD B
Tablet I	20	99.5 ± 0.54 T = 0.99 F = 1.65	100.1 ± 0.86 T = 0.75 F = 1.91	99.3 ± 0.25	100.3 ± 0.19
Tablet II	20	99.2 ± 0.88 T = 0.62 F = 1.56	99.1 ± 1.05 T = 1.20 F = 2.15	99.3 ± 0.57	100.1 ± 0.81
Tablet III	20	99.8 ± 0.67 T = 0.89 F = 3.67	99.9 ± 0.69 T = 0.15 F = 3.84	99.9 ± 0.39	100.2 ± 0.81
Tablet IV	20	99.2 ± 1.12 T = 0.75 F = 1.29	100.6 ± 0.39 T = 0.51 F = 1.19	99.6 ± 0.39	100.1 ± 0.32

* Formulations that are manufactured by four different Pharmaceutical companies

** Average ± Standard deviation of six determinations; the t and F values refer to comparison of the proposed method with reference method.

Theoretical values at 95% confidence limit, F = 5.05, t = 2.57

*** Recovery of 10 mg added to the Pharmaceutical formulations (average of three determination)

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