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Development and validation of a stability indicating RP-HPLC method for the determination of trimetazidine dihydrochloride

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ABSTRACT

A stability-indicating RP-HPLC method was developed and validated for the determination trimetazidine in active pharmaceutical ingredient and tablet dosage form. The chromatographic conditions comprised of a reversed phase Enable C_{18}G column (250mm×4.6µm, 5µ), with a mobile phase consisting of methanol-0.05% formic acid (90:10 v/v) with flow rate of 1.0 ml/min. Detection was carried out at 232 nm. The retention time of trimetazidine was found to be 3.8 min. The linear regression analysis data for the calibration plots showed good linear relationship within the concentration range 10–80 µg/ml. The value of correlation coefficient was found to be 0.999. The recovery of trimetazidine hydrochloride was about 95–105%. Trimetazidine dihydrochloride was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Trimetazidine is more sensitive to wards acidic degradation. The method was validated as per ICH guidelines.

Keywords: Trimetazidine, RP-HPLC, Stability Studies

INTRODUCTION

Trimetazidine is an effective and well tolerated anti-ischaemic agent which, in addition to providing symptom relief and functional improvement in patients with angina pectoris, has a cytoprotective action during ischaemia. The drug is suitable for initial use as monotherapy in patients with angina pectoris and, because of its different mechanism of action, as adjunctive therapy in those with symptoms not sufficiently controlled by nitrates, beta-blockers or calcium antagonists. [1,2]

Trimetazidine is chemically known as 2 [1-(2, 3, 4- trimethoxybenzyl)-piperazine dihydrochloride (**Fig 1**). The orally administered antianginal agent trimetazidine increases cell tolerance to ischaemia by maintaining cellular homeostasis.[3] To date, all analytical methods described in literature for the determination of trimetazidine in API, pharmaceutical dosage form and biological fluids involve spectrophotometric, high performance liquid chromatography, liquid chromatography–mass spectrometry methods and high performance thin layer chromatography.[4-8] In the present work, we developed a simple, precise, accurate, selective and robust liquid chromatographic method for the determination of Trimetazidine in pharmaceutical dosage form as an alternative method.

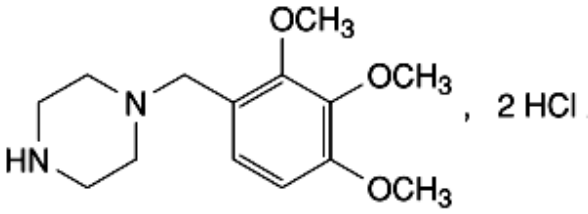

Fig 1: Chemical structure of trimetazidine dihydrochloride

MATERIALS AND METHODS

Trimetazidine was obtained as gift sample from Inogent laboratories private limited, Hyderabad. Methanol and formic acid of HPLC grade were obtained from Sigma Aldrich Chemicals limited, Maharastra. Marketed tablet formulation (Trivedon®) tablets (trimetazidine dihydrochloride, 20 mg) manufactured by Cipla Pharmaceuticals limited were purchased.

Chromatographic conditions

Chromatographic separation was performed with isocratic elution. The following optimised parameters were used as a final method for the estimation of trimetazidine.

Stationary phase	:	Enable C_{18}G (250×4.6mm, 5µm)
Mobile phase	:	Methanol: Formic acid (0.05%) = 90:10
Flow-rate	:	1.0 ml/min
Injection volume	:	20 µL
Detection wavelength	:	232 nm
Temperature	:	Ambient temperature
Run-time	:	10 min

Preparation of standard solution

About 100 mg of trimetazidine dihydrochloride was weighed accurately and dissolved in a 100 ml volumetric flask containing methanol and diluted up to the mark with methanol and sonicated to get the concentration 1000 µg/ml. From this, pipette out 10 ml of above solution into 100 ml volumetric flask and diluted up to the mark with methanol to get the concentration 100 µg/ml. Further dilutions were made with methanol.

Preparation of the sample solution

Twenty tablets were weighed and powdered. An accurate quantity of tablet powder equivalent to 20 mg of trimetazidine was weighed and transferred to a 100 ml volumetric flask and diluted up to the mark with methanol. Then sonicated for 15 min and filtered through 0.45 µm nylon membrane filter to obtain the clear solution. This stock sample solution was diluted quantitatively with methanol to obtain the suitable working sample solutions for chromatographic measurements.

METHOD VALIDATION

The proposed chromatographic method was validated as per ICH guidelines. Peak calibration curve was constructed by plotting peak area Vs concentration. Accuracy was determined by recovery studies with known concentration of drugs and the percentage recoveries of the added drugs were determined. Precision was evaluated in terms of intra-day and inter-day precision. The precision was investigated using six replicates of same concentrations of standard solutions. LOD and LOQ values were calculated from the calibration curve. Robustness of the method was determined by deliberately varying certain parameters like flow-rate, analytical wavelength and column temperature. [9]

FORCED DEGRADATION STUDIES:

The study was intended to ensure the effective separation of trimetazidine and its degradation peaks of formulation ingredients at the retention time of trimetazidine. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[10] Trimetazidine standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N HCl. The resultant solution was analysed for every 24 h

after prior dilution. For alkaline degradation study trimetazidine standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N NaOH. The resultant solution was analysed for every 24 h after prior dilution. Trimetazidine powder was exposed to dry heat at 60^0 C and powder was removed for every 24 h and diluted as mentioned above and analysed for thermal degradation study.

RESULTS AND DISCUSSION

Method development:
Initially wavelength was selected for the method development and different compositions, pH and flow rate of the mobile phase were tried during method development. The 232 nm was selected for the current method since at this wavelength trimetazidine can be selected with high sensitivity. In the course of optimizing the composition of mobile phase, methanol in combination with formic acid in different ratios were tried. After a series of preliminary experiments it was concluded that methanol: 0.05% formic acid (90:10) resulted in better peak shape.

Linearity
The calibration curve was constructed between peak area and respective concentrations. The calibration curve was linear over the range of 10-80 µg/ml. Correlation coefficient was found to be 0.999. The regression equation for calibration curve was found to be y= 59138x + 19600. Results of linearity are shown in **Fig 2.**

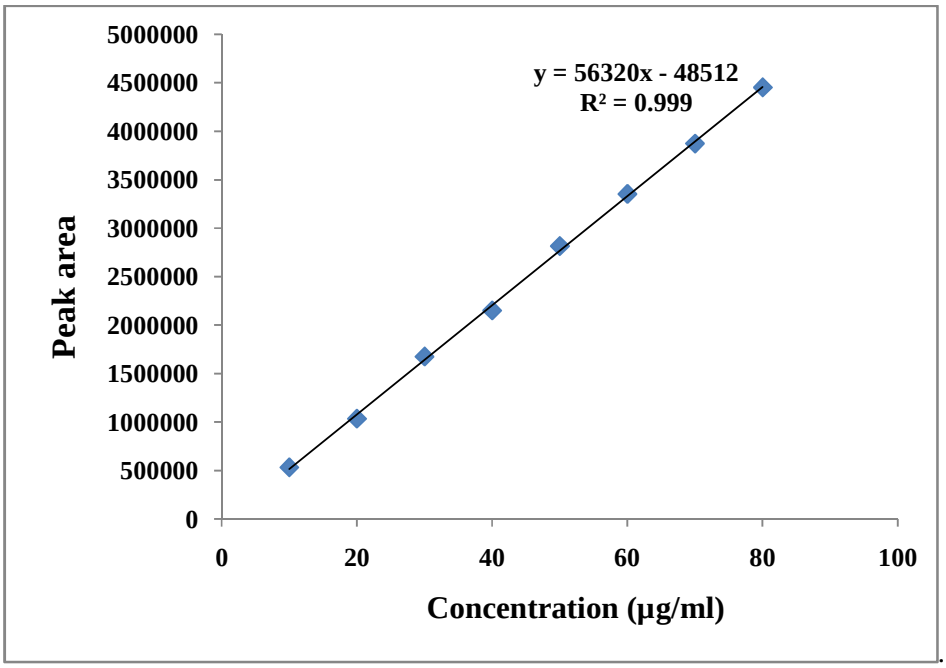

Fig 2: Calibration curve of trimetazidine dihydrochloride

Accuracy and precision
Accuracy of the method was determined by recovery experiments. Two types of recovery studies were performed. They are standard addition and percentage recovery methods. The amount of the each drug present, percentage recovery, percentage relative standard deviation (% RSD) were calculated. The percentage recovery was found to be 99.3%-100.7%. The values of accuracy results are shown in **Table 2**.

Precision of the method was demonstrated by inter day and intraday variation studies. The precision was investigated using six replicates of same concentrations of standard solutions. The intra-day and inter-day precision of the proposed method was determined by analysing the corresponding concentration six times on the same day and six times on the different. The % RSD obtained for intraday and interday precision was less than 2% as shown in **Table 3** .

Table 2: Accuracy results for timetazidine

S. No	Method	Amount present (µg/ml)	Amount recovered (µg/ml)	% Recovery
1	Standard addition (n= 3)	30	29.411	99.3
2		50	49.255	99.5
3	Percentage method (n= 3)	80	79.110	98.8
4		100	101.731	100.7
5		120	120.487	100.4

Table 3: Results for intra-day and inter-day precision values for trimetazidine

S. No	*Concentration* (µg/ml)	*%RSD*	
		Intra-day	*Inter-day*
1	30	0.25	1.00
2	40	0.52	1.23
3	50	0.86	1.76

LOD and LOQ

LOD and LOQ values decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte, while LOQ is the lowest quantifiable concentration. LOD was found to be 4.6 µg/ml and LOQ was found to be 14.2 µg/ml. The results for LOD & LOQ values shows that the method is quite sensitive for trimetazidine compared with previously reported methods.

Robustness:

Robustness of the method was determined by deliberately changing parameters like flow, wavelength and column temperature. Samples were analysed in triplicates and %RSD was calculated from peak areas. Results of robustness are summarized in **Table 4**.

Table 4: Robustness results for trimetazidine

Factor		%RSD
Flow rate (ml/min)	0.8	1.2
	1	0.9
	1.2	1.5
Wavelength (nm)	230	0.6
	232	0.8
	234	0.9
Column temperature (^{0} C)	28	1.1
	30	0.8
	32	1.4

Forced degradation studies:

All the stressed samples in acid, alkaline degradation studies were decomposed to 100 and 50 respectively. No decomposition was seen on exposure of solid drug to dry heat. The forced degradation studies data are summarized in **Table 5, Fig 3a** and **Fig 3b** .

Table 5. Data of forced degradation studies

S:NO	Stress condition	Time	Degradation (%)
1	Acid hydrolysis (1N HCl)	24 h	100
2	Alkaline hydrolysis (1N NaOH)	48 h	50
4	Thermal degradation (60^{0}C)	7 days	Stable

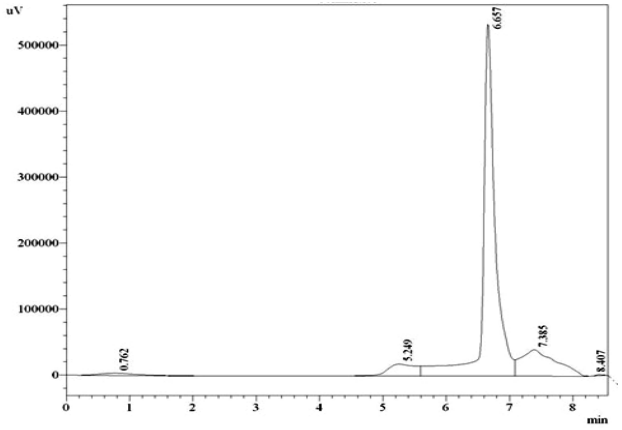

Fig 3a: Chromatogram showing acid degradation of timetazidine

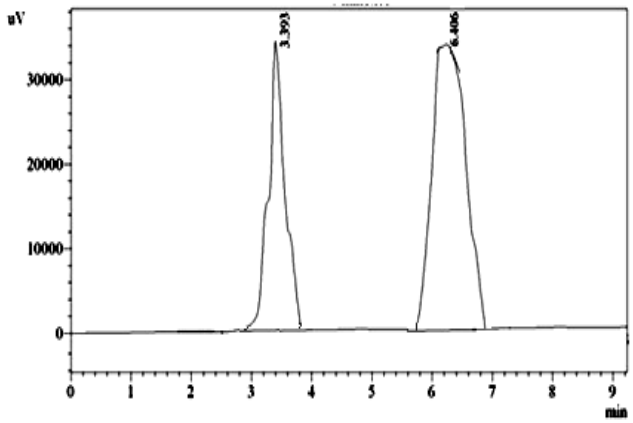

Fig 3b: Chromatogram showing alkaline degradation of timetazidine

ASSAY*:*
The validated method was applied to the determination of timetazidine in commercially available Trivedon® tablets. The percentage assay was found to be 98.75%. The results of assay indicate that the developed method is selective without interference from excipients of tablet. Assay results are shown in **Table 6**

Table 6: %Recovery of timetazidine in marketed formulation (Trivedon®)

Peak areas		Label claim (mg)	Amount found (mg)	Assay (%)
Standard	Drug			
1035165	1034132	20	19.75	98.75

CONCLUSION

A simple, sensitive, specific, accurate and precise stability indicating RP-HPLC method was developed and validated for the routine analysis of bulk and tablet dosage form of trimetazidine. The method is sensitive enough for the detection of analyte in pharmaceutical formulation when compared to the research works found in the literature. The results of forced degradation studies reveal that the method is stability indicating. The proposed method has the capability to separate the analyte from their degradation products obtained during forced degradation studies and excipients found in tablets. The method can be employed for the routine analysis of trimetazidine.

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