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Trimetazidine: stability indicating RPLC assay method

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Abstract

An accurate, specific and reproducible reversed phase liquid chromatographic method for the determination of trimetazidine hydrochloride in presence of its degradation products is reported. The mobile phase consisted of water-acetonitrile-triethylamine (90:10:0.1, v/v/v) adjusted with o-phosphoric acid to a pH of 3.3. Chromatography was performed using C-18 column at a flow rate of 1.0 ml/min and the drug along with its degraded products was detected at 270 nm. The calibration curve of trimetazidine hydrochloride in methanol was linear in the range 500–3000 ng. The mean value of correlation coefficient, slope and intercept were 0.99859 & # 61617; 0.001, 17.7986 & # 61617; 0.0709 and 2482.56 & # 61617; 147.03, respectively. The limits of detection and quantitation were 5 and 20 ng, respectively. The recovery of trimetazidine hydrochloride was about 99–100%. This method was utilized to analyze trimetazidine hydrochloride from conventional tablets and controlled release pellets in the presence of commonly used excipients. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Trimetazidine; Stability indicating; RPLC

1. Introduction

Trimetazidine dihydrochloride [1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride] regulates ionic and extracellular exchanges, correcting the abnormal flow of ions across the cell membrane caused by ischemia, and preventing cellular oedema caused by anoxia [1]. Trimetazidine dihydrochloride is official in Japanese Pharmacopoeia [2] and Extra Pharmacopoeia [3]. A number of methods have been reported for the estimation of Trimetazidine. These include UV-visible spectrophotometry [4], TLC [5], HPLC with fluorescent detection [6], GC-MS [7], HPLC with electrochemical detection [8]. These methods are mainly used for the determination of trimetazidine and its metabolites in blood, bile, organs and urine. Spectrophotometric assays, although simple, are not stability indicating and cannot be used for analysis of stability batches.

An ideal stability indicating LC method would quantify the drug per se and also resolve its degradation products. Hence an attempt has been made to develop an accurate, specific and reproducible method for the determination of trimetazidine dihydrochloride in presence of its

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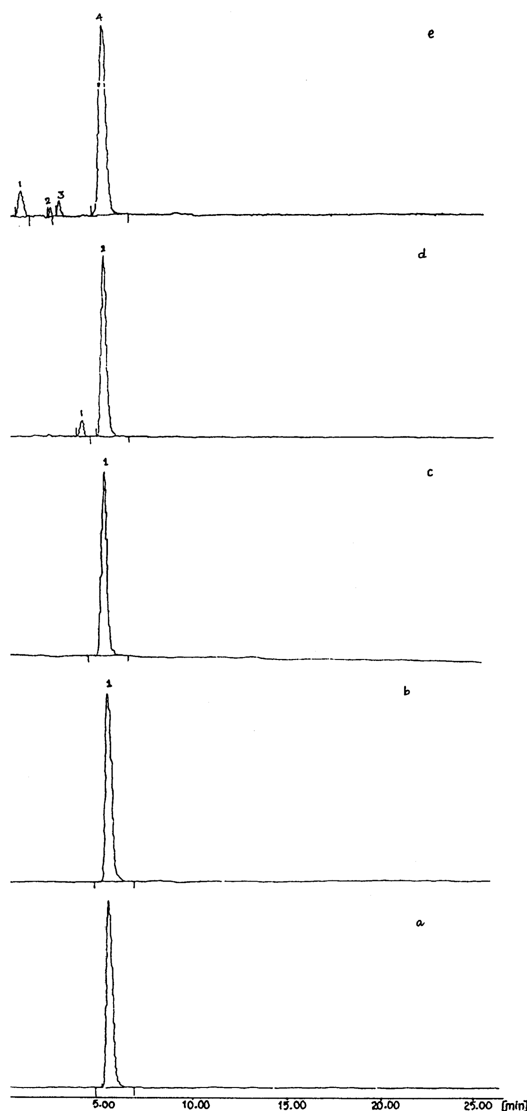

Fig. 1. (a) Chromatogram of 5 μg/ml trimetazidine (pure drug solution): Peak-1 (RT: 5.56 min) is of trimetazidine. (b) Chromatogram of 5 μg/ml trimetazidine degraded with acid: Peak-1 (RT: 5.56 min) is of trimetazidine. (c) Chromatogram of 5 μg/ml trimetazidine degraded with base: Peak-1 (RT: 5.56 min) is of trimetazidine. (d) Chromatogram of 5 μg/ml trimetazidine degraded with hydrogen peroxide: Peak-1 (RT: 4.35 min) is of oxidized product, Peak-2 (RT: 5.56 min) is of trimetazidine. (e) Chromatogram of 5 μg/ml trimetazidine degraded with light: Peak-1 (RT: 0.88 min), Peak-2 (RT: 2.26 min), Peak-3 (RT: 3.18 min) are of light degraded products and Peak-4 (RT: 5.56 min) is of trimetazidine.

degradation products for the assessment of the purity and stability of the bulk drug and of pharmaceutical dosage forms containing the analytes.

2. Experimental

2.1. Materials

Trimetazidine dihydrochloride was a gift from Sharon Pharmaceuticals (Mumbai). Organic solvents for chromatography were of HPLC grade (Ranbaxy laboratories) and double purified water was used. All other chemicals used were of analytical grade and were purchased from Ranbaxy chemicals, India.

2.2. HPLC instrumentation

The liquid chromatograph consisted of a Jasco-PV 980 pump (Jasco, Japan) coupled with a Jasco U-975 UV/VIS intelligent detector. Data integration was done using Borwin software package Version 1.21.

2.3. Chromatography

Chromatography was performed in the reversed-phase (RP) mode. The column was con-

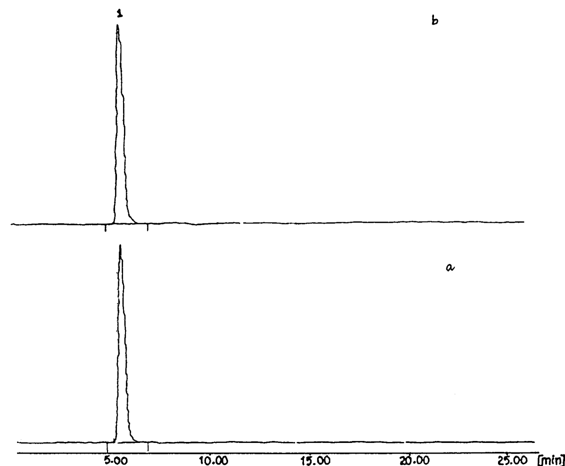

Fig. 2. Chromatogram of 5 μg/ml trimetazidine extracted from formulations. (a) conventional tablets (20 mg trimetazidine per dose); (b) controlled release pellets (60 mg trimetazidine per dose)

Table 1
Polynomial regression data for the standard curve[a]

Linearity range (ng/ml)	'r' ± S.D.	Slope ± S.D.	Intercept ± S.D.
500–3000	0.99859 ± 0.0018	17.7986 ± 0.0709	2482.56 ± 147.03
80–250	0.99549 ± 0.00121	61.14699 ± 0.539	1954.97 ± 2.538

[a] $n = 3$.

structed of stainless steel (250 mm × 4 mm i.d.) and prepacked with Lichrospher 100 RP-18 (5 μm). Manual injections were carried out using a Rheodyne model 7725 injector with a 20 μl sample loop. The mobile phase consisted of water–acetonitrile–triethylamine (90:10:0.1, v/v/v) adjusted with o-phosphoric acid to a pH of 3.3. The mobile phase was filtered through Nylon 66 membrane filter (47 mm, 0.45 μm) and degassed by sonication. A flow rate of 1.0 ml/min was maintained.

2.4. Preparation of standard solution

A stock solution of trimetazidine dihydrochloride (1 mg/ml) was prepared in double purified water and diluted further with mobile phase to obtain a standard of 10 μg/ml.

2.5. Calibration curve of trimetazidine

Appropriate dilutions of the standard solution (10 μg/ml) were made with the mobile phase to obtain solutions of concentrations of 500, 1000, 1500, 2000, 2500, 3000, ng/ml of trimetazidine. A correlation between peak area and the concentration of trimetazidine was established and the calibration curves were obtained. Linearity was also checked over the range of 80–250 ng/ml.

2.6. Method validation

The accuracy and precision of the assay were tested at 500, 2000 and 100 ng/ml of trimetazidine. The accuracy was determined by preparing the samples six times and injecting into the system. Precision was determined by injecting the same solution six times into the chromatographic system. The intra-day variation was evaluated in the range of 500–3000 ng/ml three times a day. The inter-day variation was similarly evaluated over a period of 3 days. The limit of detection and quantitation based on the instrumental parameters were also determined.

2.7. Analysis of pharmaceutical formulations

To determine the content of trimetazidine in conventional tablets (label claim: 20 mg/tablet), samples were prepared by extracting trimetazidine dihydrochloride into water and analyzing the re-

Table 2
Accuracy and precision of HPLC assay of trimetazidine[a]

Tested concentration (ng/ml)	S.D. of areas	RSD (%)
Accuracy		
500	222.89	1.90
2000	200.23	0.533
100	216.1	1.9
Precision		
500	222.76	1.077
2000	245.97	0.989
100	323.4	2.5

[a] $n = 6$.

Table 3
Intra-day and inter-day variation-comparison of slopes and coefficient of regression[a]

	Slope (mean ± S.D.)	Coefficient of regression (mean ± S.D.)
Intra-day variation	17.538 ± 0.379	0.99875 ± 0.006
Inter-day variation	17.798 ± 0.070	0.9985 ± 0.0018

[a] $n = 3$.

Table 4
Applicability of the HPLC method for the analysis of the pharmaceutical formulations[a]

Sample (label claim)	Drug content (%)	RSD (%)
Tablet (20 mg)	99.4	0.91
Controlled release pellets (60 mg)	98.1	1.32

[a] $n = 6$.

sultant solution by the proposed method. The tablets contained trimetazidine, 50%; lactose, 31%; dicalcium phosphate, 12%; starch, 5%; magnesium stearate, 1%; and talc, 1% as excipients. The tablets were crushed and powder equivalent to 1 tablet was weighed accurately, dispersed in water and sonicated for 15 min. The volume was made to 100 ml with purified water, filtered and the filtrate was injected into the chromatographic system after dilution with mobile phase. The controlled release pellets developed in the laboratory were analyzed in a similar manner.

2.8. Recovery studies

Preanalyzed samples were spiked with extra 50, 100 and 150% of the standard trimetazidine dihydrochloride and the mixtures were reanalyzed by the proposed method ($n = 3$).

2.9. Forced degradation of trimetazidine dihydrochloride

The drug was subjected to forced degradation under acidic and basic conditions by refluxing with 1 M HCl and 1 M NaOH respectively at 70°C for a period of 10 h. The stability of the drug to oxidation was studied by stirring 1% w/v drug solution with 30% v/v hydrogen peroxide solution for 2 h. The photochemical stability of the drug was also studied by exposing 1% w/v drug solution to direct sunlight for 4 h. The resultant solutions were appropriately diluted with mobile phase and injected into the chromatographic system.

3. Results and discussion

3.1. Optimization of the LC procedure

The HPLC procedure was optimized with a view to develop a stability indicating method so as to resolve the degraded products from the drug. Various mobile phase compositions were tried so as to obtain a sharp peak and also resolve the peaks of degraded product from the peak of drug. The mobile phase consisting of water–acetonitrile–triethylamine (90:10:0.1, v/v/v) of pH 3.3 adjusted with ortho-phosphoric acid resulted in a retention time of 5.56 min for trimetazidine (Fig. 1a). The chromatogram of the acid degraded sample and base degraded sample (Fig. 1b and c) showed no other peaks indicating that no degradation had occurred with acid and base. The chromatogram of the H_2O_2 degraded sample (Fig. 1d) showed one additional peak at 4.35 min. The photodegraded sample (Fig. 1e) showed three additional peaks at 0.88, 2.26, and 3.18 min. The peaks of all the degraded products were well resolved from the trimetazidine peak (5.56 min).

3.1.1. Calibration curve of trimetazidine

The polynomial regression data for the calibration plots ($n = 3$) (Table 1) showed a linear relationship over the concentration range of 500–3000

Table 5
Recovery studies[a]

Excess drug added to the analyte (%)	Theoretical content (ng/ml)	Recovery (%)	RSD (%)
0	500	99.8	0.04
50	750	99.6	0.21
100	1000	100.1	0.45
150	1250	101.8	0.78

[a] $n = 6$.

ng/ml as well as 80–250 ng/ml. No significant difference was observed in the slopes of standard curves (ANOVA; $P > 0.05$).

3.1.2. Method validation

The results in Table 2 revealed excellent accuracy and high precision of the assay method. The intra-day and inter-day variation was evaluated by comparing the slopes as depicted in Table 3. It is evident that there was no significant variation in the slope values (ANOVA; $P > 0.05$). The limit of detection, with a signal to noise ratio of 3:1, was found to be 5 ng/ml. Here the noise (peak to peak) was 20 U and the signal was 60 U. The limit of quantitation, with a signal to noise ratio of 10:1, was found to be 20 ng/ml where the signal was 200 U.

3.1.3. Analysis of the pharmaceutical formulations

A single peak at 5.56 min corresponding to the drug was observed in the chromatogram of the drug samples extracted from the conventional tablets and controlled release pellets. There was no interference from the excipients commonly present in the conventional tablets as indicated in Fig. 2. It may therefore be inferred that degradation of trimetazidine had not occurred in the conventional formulations and developed controlled release pellets, which were analyzed by this method (Table 4).

3.1.4. Recovery studies

The proposed method when used for extraction and subsequent estimation of trimetazidine from pharmaceutical dosage forms afforded recovery of 98–100% at the levels studied as listed in Table 5.

4. Conclusion

The developed LC technique is precise, specific, accurate and stability-indicating. The statistical analysis proves that the method is reproducible and selective for the analysis of trimetazidine as bulk drug and in pharmaceutical formulations. The run time of less than 8 min was found to be practicably advantageous for use of this method in routine analysis. The method may be extended to study the degradation kinetics of trimetazidine and also for its estimation in plasma and other biological fluids.

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