ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 15–20

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Stability indicating HPTLC determination of Trimetazidine as bulk drug and in pharmaceutical formulations

Simmy O. Thoppil, Rita M. Cardoza, P.D. Amin *

Pharmaceutical Division, Department of Chemical Technology (Autonomous), University of Mumbai, Nathalal Parikh Marg, Matunga, Mumbai 400 019, India

Received 25 April 2000; received in revised form 9 August 2000; accepted 5 September 2000

Abstract

A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of trimetazidine hydrochloride both as a bulk drug and in formulations is reported. The mobile phase composition was n-butanol-water-methanol-ammonia (20%) (14:0.2:0.2:2, v/v/v/v). Densitometric analysis of trimetazidine hydrochloride was carried out in the absorbance mode at 254 nm. The calibration curve of trimetazidine hydrochloride in methanol was linear in the range 400 – 2400 ng. The mean value of correlation coefficient, slope and intercept were 0.99815 & # 61617; 0.001, 0.4849 & # 61617; 0.001 and 31.633 & # 61617; 5.996 respectively. The limits of detection and quantitation were 50 and 80 ng respectively. The recovery of trimetazidine hydrochloride was about 98 – 100%. This method was utilized to analyze trimetazidine hydrochloride from conventional tablets and controlled release pellets in the presence if commonly used excipients. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Trimetazidine; HPTLC; Stability indicating

1. Introduction

Trimetazidine dihydrochloride [1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride] (Fig. 1) regulates ionic and extracellular exchanges, correcting the abnormal flow of ions across the cell membrane caused by ischaemia, and preventing cellular oedema caused by anoxia [1]. A number of methods have been reported for the estimation of trimetazidine. These include UV–visible spectrophotometry [2], TLC [3], HPLC with fluorescence detection [4], GC-MS [5], HPLC with electrochemical detection [6]. These methods are mainly used for the determination of trimetazidine and its metabolites in blood, bile, organs and urine. These methods are often time-consuming, expensive and cumbersome.

An ideal stability indicating method is one that quantifies the drug per se and also resolves its degradation products. A very viable alternative for stability indicating analysis of trimetazidine is High-Performance Thin-Layer Chromatography (HPTLC). Over the past decade HPTLC has been

* Corresponding author. Tel. +91-22-4145616; fax: +91-22-4145614.

E-mail address: dramin@bom7.vsnl.net.in, pda@pharma.udct.ernet.in (P.D. Amin).

0731-7085/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(00)00440-4

successfully used in the analysis of pharmaceuticals, plant constituents and biomacromolecules [7–10]. Several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. It also facilitates automatic application and scanning in situ.

The objective of the present work was to develop an accurate, specific and reproducible method for the determination of trimetazidine in presence of its degradation products and related impurities for the assessment of the purity of the bulk drug and stability of its pharmaceutical dosage forms.

2. Experimental

2.1. Materials

Trimetazidine dihydrochloride was a gift from Cipla India Ltd. All chemicals and reagents used were of analytical grade and were purchased from Ranbaxy chemicals, India.

2.2. HPTLC instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60 F-254, (20 × 10 cm with 250 μm thickness; E. Merck, Germany) using a Camag Linomat IV (Switzerland). A constant application rate of 15 μl/s was employed. The mobile phase consisted of *n*-butanol–water–methanol–ammonia (20%) (14:0.2:0.2:2, v/v/v/v). Linear ascending development of chromatogram was carried out in a Camag twin trough glass chamber saturated with the mobile phase. The chamber saturation time for mobile phase was optimized at 50 min. The length of chromatogram run was 9 cm. Subsequent to the development, the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner III in the absorbance mode at 254 nm. The source of radiation utilized was deuterium lamp.

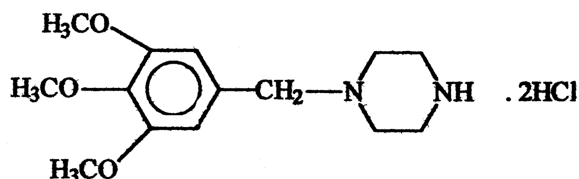

Fig. 1. Structure of trimetazidine dihydrochloride.

2.3. Calibration curves of trimetazidine

A stock solution of trimetazidine dihydrochloride (100 μg/ml) was prepared in methanol. Four, 8, 12, 16, 20, and 24 μl of the stock solution were spotted on the TLC plate to give concentrations of 400, 800, 1200, 1600, 2000, 2400 ng of trimetazidine dihydrochloride respectively. The data of spot area vs. drug concentration was treated by linear least square regression analysis. Linearity was also determined over the range of 80–250 ng.

2.4. Method validation

2.4.1. Accuracy and precision of the assay

The accuracy and precision of the assay were tested at 120, 800 and 2000 ng of trimetazidine dihydrochloride.

2.4.2. Ruggedness of the method

The intra-day variation was evaluated in the range of 400–2400 ng three times a day. The inter-day variation was similarly evaluated over a period of 3 days.

2.4.3. Limit of detection and limit of quantification

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times following the same method as explained in Section 2.2. The noise level was determined. The limit of detection was calculated to be three times the S.D. while ten times the S.D. value gave limit of quantification.

2.4.4. Recovery studies

The analyzed samples were spiked with extra 50, 100 and 150% of the standard trimetazidine dihydrochloride and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulations.

2.5. Analysis of the marketed formulation

To determine the content of trimetazidine of conventional tablets (label claim: 20 mg/tablet), the tablets were powdered and weight equivalent to one tablet was extracted in water. To ensure complete extraction of the drug it was sonicated for 15 min and the volume was made up to 100 ml. The resulting solution was filtered and the filtrate was analyzed for the drug content. Ten microlitres of the filtered solution was spotted onto the plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate. The marketed formulation excipients included lactose, microcrystalline cellulose, dicalcium phosphate, starch and polyvinylpyrrolidone. A placebo tablet was subjected to the same extraction process as discussed above and spotted. The possibility of excipient interference in the analysis was studied.

2.6. 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 forced degradation in acidic and basic media were performed in the dark in order to exclude the possible degradative effect of light on the drug. The stability of the drug to oxidation was studied by stirring the drug solution with hydrogen peroxide solution for 2 h. The photochemical stability of the drug was also studied by exposing the drug solution to direct sunlight for 4 h. The resultant solutions were appropriately diluted and spotted and the chromatograms run as described in Section 2.2.

2.7. Detection of the related impurities

The related impurities were determined by spotting higher concentrations of the drug so as to detect and quantify them. 0.5 g of trimetazidine dihydrochloride was dissolved in 10 ml of methanol, and this solution was termed as sample solution (50 mg/ml). One ml of the sample solution was diluted to 100 ml with methanol and this solution was termed as standard solution (0.5 mg/ml). Ten microlitres of both the standard and the sample solution was spotted on the plate and chromatograms run as described in Section 2.2.

3. Results and discussion

3.1. Development of the optimum mobile phase

The TLC procedure was optimized with a view to develop a stability indicating assay method. The mobile phase was modified from the reported procedure [2]. A solvent system that would give dense and compact spots with appropriate and significantly different R_f values for trimetazidine and its degraded products was desired for quantification of trimetazidine in the pharmaceutical formulations. Both the pure drug and the degraded products were spotted on the TLC plates and run in different solvent systems. The mobile phase consisting of *n*-butanol–water–methanol–strong ammonia (14:0.2:0.2:2, v/v/v) gave a R_f value of 0.3 for trimetazidine (Fig. 2a). The chromatogram of the acid degraded sample (Fig. 2b) showed two spots at R_f of 0.02 and 0.06 indicating only base spots. These spots were found to be fluorescent, indicating the formation of salt. The chromatogram of the base degraded sample (Fig. 2c) also showed spot at 0.09 as a base spot. In both the cases the concentration of the drug was found to be not changing from the initial concentration indicating that trimetazidine does not undergo degradation under acidic and basic conditions. The sample degraded with hydrogen peroxide (Fig. 2d) showed an additional peak at R_f of 0.21. The photodegraded sample (Fig. 2e) showed additional spots at R_f values of 0.26 and 0.42. This indicates that trimetazidine undergoes

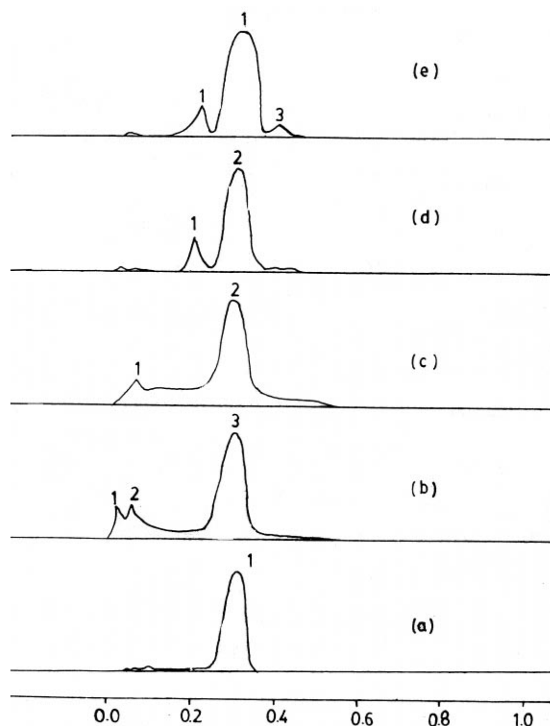

Fig. 2. Chromatograms of trimetazidine and its degraded products: (a) pure drug: peak 1 (R_f: 0.3) is of trimetazidine; (b) acid degraded: peak 1 (R_f: 0.02) and peak 2 (R_f: 0.06) are of base spots. Peak 3 (R_f: 0.3) is of trimetazidine; (c) base degraded: peak 1 (R_f: 0.09) is of base spot. Peak 3 (R_f: 0.3) is of trimetazidine; (d) degraded with hydrogen peroxide: peak 1 (R_f: 0.21) is of degraded product. Peak 2 (R_f: 0.3) is of trimetazidine; (e) photodegraded: peak 1 (R_f: 0.26) and peak 3 (R_f: 0.42) are of degraded products. Peak 2 (R_f: 0.3) is of trimetazidine.

Table 1
Polynomial regression data for the calibration curves[a]

Linearity range (ng)	'r' ± S.D.	Slope ± S.D.	Intercept ± S.D.
80–250	0.99466	0.055	21.366
	± 0.027	± 0.001	± 4.101
400–2400	0.99815	0.4849	31.633
	± 0.001	± 0.001	± 5.996

[a] $n = 3$.

oxidation. The spots of the degraded products were well resolved from the trimetazidine spot (R_f of trimetazidine was 0.3).

3.2. Calibration curves

The polynomial regression data for the calibration curves ($n = 3$) as shown in Table 1 showed a good linear relationship over a concentration range of 400–2400 ng as well as over the concentration range of 80–250 ng. No significant difference was observed in the slopes of standard curves (ANOVA; $P > 0.05$).

3.3. Validation of the method

3.3.1. Accuracy and precision of the assay

The precision and accuracy of the developed HPTLC method were expressed in terms of (R.S.D.). The results depicted in Table 2 revealed excellent accuracy and high precision of the assay method.

3.3.2. Ruggedness of the method

The intra-day and inter-day variation were evaluated by comparing the slopes of the calibration curve over the concentration range of 400–2400 ng. There was no significant variation in the slope values (ANOVA; $P > 0.05$). The R.S.D. was found to 0.24 and 0.28% respectively for intra-day analysis and inter-day analysis.

3.3.3. LOD and LOQ

The limit of detection, with a signal-to-noise ratio of 3:1, was found to be 50 ng. Here the noise

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

Tested concentration (ng/ml)	S.D. of areas	R.S.D. (%)
Accuracy		
800	2.06	0.48
2000	9.36	0.46
120	0.89	0.9
Precision		
800	2.43	0.61
2000	9.56	0.48
120	1.08	1.1

[a] $n = 6$.

Table 3
Recovery studies[a]

Excess drug added to the analyte (%)	Theoretical content (ng)	Recovery (%)	R.S.D. (%)
0	800	99.8	0.3
50	1200	99.6	0.14
100	1600	98.9	0.78
150	2400	98.3	0.65

[a] $n = 6$.

gave an area of six units and the signal was 18 units. The limit of quantitation, with a signal-to-noise ratio of 10:1, was found to be 80 ng where the signal was 60 units.

3.3.4. Recovery studies

The proposed method when used for extraction and subsequent estimation of trimetazidine from pharmaceutical dosage forms after spiking with 50, 100 and 150% of additional drug afforded recovery of 98–100% as listed in Table 3.

3.4. Analysis of the marketed formulation

A single spot at R_f of 0.3 was observed in the chromatogram of the drug samples extracted from the conventional tablets. There was no interference from the excipients commonly present in the conventional tablets, as evidenced from the chromatogram of the placebo formulation. The drug content was found to be 99.4% with a R.S.D. of 0.23%. It may therefore be inferred that degradation of trimetazidine had not occurred in the marketed formulations that were analyzed by this method. The low R.S.D. value indicated the suitability of this method for routine analysis of trimetazidine in pharmaceutical dosage forms.

3.5. Detection of the related impurities

The spots other than the principal spot and the spot of the starting point from the sample solution were not intense than the spot from the standard solution. The sample solution showed three additional spots at R_f of 0.13, 0.71 and 0.88. However the area of these spots were found to be much less as compared to the spot of the standard solution as indicated in Table 4.

Table 4
Related impurities

Concentration of drug (μg)	R_f value	Area
5	0.3	2777.2
Related impurities		
500	0.13	398.7
	0.71	652.8
	0.88	1195.1
	Total area	2246.6

4. Conclusion

The developed HPTLC technique is precise, specific, accurate and stability indicating. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. The statistical analysis proves that the method is reproducible and selective for the analysis of trimetazidine as bulk drug and in pharmaceutical formulations. It may be extended to study the degradation kinetics of trimetazidine and also for its estimation in plasma and other biological fluids.

Acknowledgements

The authors thank Cipla India Ltd. for gift sample of trimetazidine dihydrochloride. Two of the authors are grateful to University Grants Commission, New Delhi for awarding Senior Research Fellowship.

References

[1] C. Harpey, P. Clauser, C. Labrid, J.-L. Freyria, J.-P. Poirier, Cardiovasc. Drug Rev. 6 (1989) 92–312.

[2] A.C. Moffat (Ed.), Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Post-Mortem Materials, second ed., The Pharmaceutical Press, 1986, p. 1048.

[3] S.-I. Naito, S. Osumi, K. Sekisshiro, M. Hirose, Chem. Pharm. Bull. 20 (1972) 682–688.

[4] S. Courte, N. Bromet, J. Chromatogr. Biomed. Appl. 224 (1981) 162–167.

[5] L. Fay, G. Michel, P. Goupit, C. Harpey, M. Prost, J. Chromatogr. Biomed. Appl. 490 (1989) 198–205.

[6] V.R. Bari, U.J. Dhorda, M. Sunderesan, Indian Drugs 36 (1995) 289–292.

[7] F. Bonte, P. Pinguet, J.M. Chevalier, A. Meybeck, J. Chromatogr. B Biomed. Appl. 664 (1995) 311–316.

[8] G.A. Nores, R.K. Mizutamari, D.M. Kremer, J. Chromatogr. A686 (1994) 155–157.

[9] K. Datta, S.K. Das, J. AOAC Int. 77 (1994) 1435–1438.

[10] S. Tammilehto, H. Salomies, K. Torniainen, J. Planar, Chromatogr. Mod. TLC 7 (1994) 368–371.