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ORIGINAL ARTICLE

Development and validation of a stability indicating UPLC method for determination of ticlopidine hydrochloride in its tablet formulation

Vijay Ram, Govind Kher, Kapil Dubal, Bhavesh Dodiya, Hitendra Joshi *

Department of Chemistry, Saurashtra University, Rajkot 360 005, Gujarat, India

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Abstract The objective of the current study was the development of a simple, precise and accurate isocratic reversed-phase stability indicating Ultra Performance Liquid Chromatography [UPLC] assay method and validated for determination of ticlopidine hydrochloride in solid pharmaceutical dosage forms. Isocratic separation was achieved on a Zorbax SB-C18 (50 mm × 4.6 mm, 1.8 μm) column using mobile phase of methanol–0.01 M ammonium acetate buffer, pH 5.0 (80:20, v/v) at a flow rate of 0.8 ml min^{-1}, the injection volume was 4.0 μl and the detection was carried out at 235 nm by using photo-diode array detector. The drug was subjected to oxidation, hydrolysis, photolysis and heat to apply stress condition. The method was validated for specificity, linearity, precision, accuracy, robustness and solution stability. The method was linear in the drug concentration range of 62.5–375 μg ml^{-1} with a correlation coefficient of 0.9999. The precision (relative standard deviation – RSD) of six samples was 1.31% for repeatability and the intermediate precision [RSD] among six-sample preparation was 0.77%. The accuracy (recovery) was between 98.80% and 101.50%. Degradation products produced as a result of stress studies did not interfere with detection of ticlopidine hydrochloride and the assay can thus be considered stability indicating.

* Corresponding author. Tel.: +91 281 2578512; fax: +91 281 2576802.
E-mail address: drhsjoshi49@gmail.com (H. Joshi).

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1. Introduction

Stress testing is a part of development strategy under the International Conference on Harmonization [ICH] requirements and is carried out under more severe conditions than accelerated conditions. These studies serve to give information on drug's inherent stability and help in the validation of analytical methods to be used in stability studies (Bakshi and Singh, 2002, 2004; Bakshi et al., 2001; Pecanac et al., 2000). It is suggested that stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a

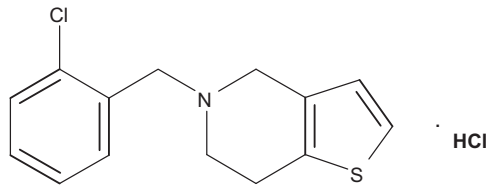

Figure 1 Chemical structure of ticlopidine hydrochloride.

wide range of pH values. It is also recommended that analysis of stability sample should de done through the use of a validated stability testing methods.

Ticlopidine hydrochloride is an inhibitor of platelet aggregation used in the management and prevention of thromboembolic disorders (Braunwald et al., 2008). Ticlopidine hydrochloride is chemically named as: 5-[(2-chlorophenyl) methyl]-4,5,6,7-tetrahydrothieno [3,2-c] pyridine hydrochloride (Fig. 1). Its molecular formula is $C_{14}H_{14}ClNS{\cdot}HCl$ having molecular weight of 300.25 g mol^{-1}. It is used as adenosine diphosphate [ADP] receptor antagonists in an antiplatelet therapy (Papathanasiou et al., 2008). It is also significantly reduces restenosis after endovascular therapy in femoropopliteal lesions (Iida et al., 2008).

Quantitative methods for the determination of ticlopidine in tablets using reflectance near-infrared and Fourier transform Raman spectroscopy (Markopoulou et al., 2008). UPLC coupled to electrospray tandem mass spectrometry was reported (Borges et al., 2004; Rona et al., 1997). The UPLC method has many advantages over reflectance near-infrared and Fourier transform Raman spectroscopy method for quantitation. So far to our present knowledge, no validated stability indicating UPLC assay method for the determination of ticlopidine hydrochloride in pharmaceutical formulation was available in literature. Moreover UPLC method can be the first choice of chromatographers among the UPLC, reflectance near-infrared and Fourier transform Raman spectroscopy methods. So, development is based on UPLC method. This paper deals with the forced degradation of ticlopidine hydrochloride under stress conditions like acid hydrolysis, base hydrolysis and oxidation, thermal and photolytic stress. This paper also deals with the validation of the developed method for the assay of ticlopidine hydrochloride in its dosage form (tablets).

2. Experimental

2.1. Materials

Ticlopidine hydrochloride standard was provided by Aarti Drugs Ltd., Boisar (India). Ticlopidine hydrochloride tablets containing 250 mg ticlopidine hydrochloride and the inactive ingredient used in drug matrix were obtained from (Trede name: Ticlop, Manufactured by Vapi Care Pharma Pvt. Ltd., Vapi, India) market. Analytical grade ammonium acetate was purchased from Sisco Research Pvt. Ltd., Mumbai (India). UPLC grade methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai (India). Analytical grade hydrochloric acid, glacial acetic acid, sodium hydroxide pellets and 30% (v/v) hydrogen peroxide solution were obtained from Ranbaxy Fine Chemicals, New Delhi (India).

2.2. Instrumentation

The LC system of Waters Acquity UPLC with Photo diode array detector was used for this study and chromatographic separation was achieved on Zorbax SB-C18 (50 mm × 4.6 mm, 1.8 μm) column as stationary phase with binary gradient mode.

2.3. Chromatographic conditions

Chromatographic analysis was performed on Zorbax SB-C18 (50 mm × 4.6 mm, 1.8 μm) column. The mobile phase consisted of methanol–0.01 M ammonium acetate buffer pH 5.0 (80:20, v/v). To prepare the buffer solution, 0.7708 g ammonium acetate was weighed and dissolved in 1000 ml HPLC grade water and then adjusted to pH 5.0 with glacial acetic acid. Mobile phase was filtered through a 0.20 μm nylon membrane (Millipore Pvt. Ltd., Bangalore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai). The flow rate of the mobile phase was adjusted to 0.8 ml min^{-1} and the injection volume was 4.0 μl. Detection was performed at 235 nm.

2.4. Standard preparation

A ticlopidine hydrochloride standard solution containing 250 μg ml^{-1} was prepared in a 100 ml volumetric flask by dissolving 25.00 mg of ticlopidine hydrochloride and then diluted to volume with methanol as diluents.

2.5. Tablet sample preparation

The whole 20 tablets had to be crushed together and finely powdered. Then a definite weight of the powder can be taken for further manipulation. From these, 1444.0 mg powder was weighed and transferred into a 500 ml volumetric flask. About 50 ml methanol was added and sonicated for a minimum 30 min. with intermittent shaking. Then content was brought back to room temperature and diluted to volume with methanol. The sample was filtered through 0.22 μm nylon syringe filter. The concentration obtained was 250 μg ml^{-1} of ticlopidine hydrochloride.

2.6. Method validation

2.6.1. Specificity study

The specificity of the method was determined by checking the interference of placebo with analyte which was eluted by checking the peak purity of ticlopidine hydrochloride during the force degradation study. The peak purity of the ticlopidine hydrochloride was found satisfactory under different stress conditions.

2.6.1.1. Design of the force degradation study. The degradation samples were prepared by transferring powdered tablets, equivalent to 250 mg ticlopidine hydrochloride into a 250 ml

round bottom flask. Then prepared samples were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with mobile phase to attain 250 μg ml^{-1} concentrations. Specific conditions were described as follows.

2.6.1.1.1. Acidic degradation condition. Acidic degradation study was performed by heating the drug content in 0.1 N HCl at 80 °C for 2.0 h and mixture was neutralized.

2.6.1.1.2. Alkali degradation condition. Alkaline degradation study was performed by heating the drug content in 1 M NaOH at 80 °C for 2 h and mixture was neutralized.

2.6.1.1.3. Oxidative degradation condition. Oxidation degradation study was performed by heating the drug content in 3% (v/v) H_2O_2 at 80 °C for 1 h.

2.6.1.1.4. Thermal degradation condition. Thermal degradation was performed by exposing solid drug at 80 °C for 72 h. Ticlopidine hydrochloride is found to be stable under thermal degradation condition.

2.6.1.1.5. Photolytic degradation condition. Photolytic degradation study was performed by exposing the drug content in UV-light for 72 h.

2.6.2. Linearity

Five points calibration curve was obtained in a concentration range from 62.5 to 375 μg ml^{-1} for ticlopidine hydrochloride.

2.6.3. Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of test sample preparation and the % RSD of assay (intraday) was calculated. Intermediate precision of the method was checked by performing the same procedure on the different day (interday) by another person under the same experimental condition.

2.6.4. Accuracy

An accuracy study was performed by adding known amounts of ticlopidine hydrochloride to the placebo preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50%, 100% and 150% of test preparation concentration). For each concentration level, three sets were prepared and injected in duplicate.

2.6.5. Robustness

The robustness of study was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions. The factors chosen for this study were the flow rate (± 0.1 ml min^{-1}), mobile phase composition [0.01 M ammonium acetate buffer pH 5.0–acetonitrile (78:22 and 82:18, v/v)], buffer pH (± 0.2 pH) and using different lot of LC column.

2.6.6. Solution stability

The stability of solution for test preparation was evaluated. The solution was stored at ambient temperature and 2–5 °C and tested at interval of 12, 24, 36 and 48 h. The responses for the aged solution were evaluated using a freshly prepared standard solution.

3. Results and discussion

3.1. Development and optimization of the HPLC method

Proper selection of the methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and solubility. Ticlopidine hydrochloride dissolves in polar solvent thus RP-UPLC was selected to estimate them. To develop a rugged and suitable UPLC method for the quantitative determination of ticlopidine hydrochloride, the analytical conditions were selected after testing the different parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water with methanol or acetonitrile, did not give good peak shape.

The mobile phase consisted of methanol–0.01 M ammonium acetate buffer pH 5.0 (80:20, v/v). By using 0.01 M ammonium acetate buffer in 1000 ml of buffer, adjusted to pH 5.0 with glacial acetic acid and keeping mobile phase composition as methanol–ammonium acetate buffer (80:20, v/v), best peak shape was obtained. For the selection of organic constituent of mobile phase, methanol was chosen to reduce the longer retention time and to attain good peak shape. Fig. 2(A) and (B) represent the chromatograms of standard and test preparation, respectively.

3.2. System suitability

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. For all system suitability injections, asymmetry was less than 2.0, theoretical plates were greater than 10,000 and % RSD of peak area less than 2.0 was found.

3.3. Specificity

The specificity of the method was determined by checking the interference of placebo with analyte and the proposed method was eluted by checking the peak purity of ticlopidine hydrochloride during the force degradation study. The peak purity of the ticlopidine hydrochloride was found satisfactory under different stress conditions. There was no interference of any peak of degradation products with the drug peak. Major degradation was found in the oxidative condition and that product was degraded up to 7.0–8.0%. The major impurity peaks were found at 9.01 min (Fig. 2(C)). In alkali degradation, it was found that around 5–6% of the drug degraded (Fig. 2(D)) and in photolytic condition around 4–5% of the drug degraded. Ticlopidine hydrochloride was found to be slightly degraded in acidic while it was stable under the thermal degradation.

3.4. Linearity

Five points calibration curve was obtained in a concentration range from 62.5 to 375 μg ml^{-1} for ticlopidine hydrochloride.

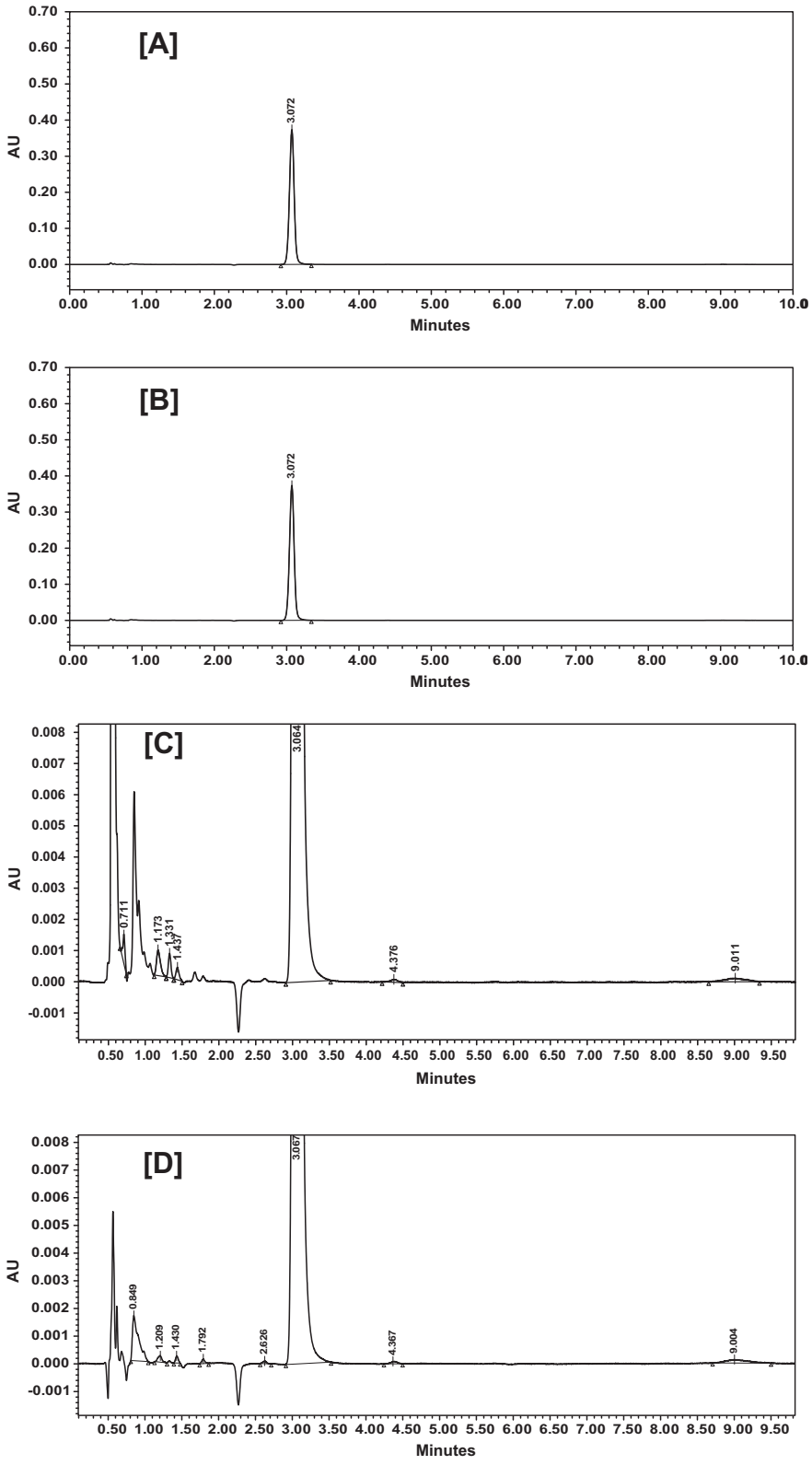

Figure 2 (A) Chromatogram of standard preparation; (b) chromatogram of test preparation; (c) chromatogram of oxidative forced degradation study; (d) chromatogram of alkali forced degradation study.

The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was $y = 7165671.78378x - 34207.26$ with correlation coefficient 0.9999.

Table 1 Evaluation data of precision study.

Set	Intraday ($n = 6$)	Interday ($n = 6$)
1	101.4	100.2
2	98.8	99.3
3	101.6	98.3
4	99.4	99.7
5	101.5	100.3
6	99.2	100.2
Mean	100.3	99.7
Standard deviation	1.31	0.77
% RSD	1.31	0.77

Table 2 Evaluation data of accuracy study.

Concentration (%)	Amount added concentration[a] (mg/ml)	Amount found concentration[a] (mg/ml)	% Recovery	% RSD
50	0.1227	0.1207	98.37	0.27
100	0.2513	0.2499	99.45	0.22
150	0.3713	0.3759	101.22	0.87

[a] Each value corresponds to the mean of three determinations.

Table 3 Evaluation data of solution stability study.

Intervals	% Assay for test preparation solution stored at 2–5 °C	% Assay for test preparation solution stored at ambient temperature
Initial	100.2	101.0
12 h	101.1	101.7
24 h	98.5	99.8
36 h	99.6	99.3
48 h ± SD	98.6 ± 1.10	98.4 ± 1.32

Table 4 Evaluation data of robustness study.

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.7 ml/min	98.2	10,244	1.08
Flow 0.9 ml/min	98.8	10,365	1.18
Buffer pH 4.8	100.3	10,400	1.10
Buffer pH 5.2	100.5	10,388	1.20
Buffer–ACN (78:22, v/v)	98.4	10,354	1.09
Buffer–ACN (82:18, v/v)	100.7	10,300	1.15
Column change different lot No. Zorbax SB-C18 (50 mm × 4.6 mm, 1.8 μm)	99.7	10,270	1.12

3.5. Precision

The results of repeatability and intermediate precision study are shown in Table 1. The developed method was found to be precise as the % RSD values for the repeatability and intermediate precision studies were $< 1.31\%$ and $< 0.77\%$, respectively, which confirm that the method was precise.

3.6. Accuracy

The UPLC area responses for accuracy determination are depicted in Table 2. The results show that best recoveries (98.80% and 101.50%) of the drug were obtained at each added concentration, indicating that the method was accurate.

3.7. Solution stability study

Table 3 shows the results obtained in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 h at 2–5 °C and was ambient temperature as during this time the result was not decreased below the minimum percentage.

3.8. Robustness

The result of robustness study of the developed assay method was established in Table 4. The result shows that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of the actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

4. Conclusion

A new stability-indicating UPLC method has been developed to be routinely applied to determine ticlopidine hydrochloride in pharmaceutical dosage form. The method was validated by employment of ICH recommended stress condition. The method has been proved to be specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

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