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Development and Validation of a Stability indicating HPLC Assay Method for determination of Ticlopidine Hydrochloride in Tablet Formulation

V.R.Ram[1],G.J.Kher[1], K.L.Dubal[1], B.L.Dodiya[1], G.P.Pandya[1] and H.S.Joshi[1]*

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

***Corres. Author: drhsjoshi49@gmail.com**

Tel. +91 281 2578512(O), Fax. +91 281 2576802

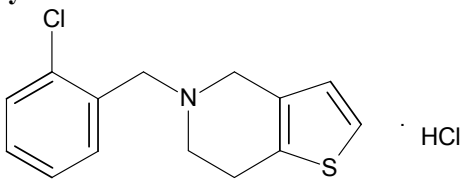
Abstract : The objective of the current study was to developed simple, precise and accurate isocratic reversed-phase stability indicating HPLC assay method and validated for determination of ticlopidine hydrochloride in solid pharmaceutical dosage forms. Isocratic RP-HPLC separation was achieved on a Phenomenex Luna C8 (2) column (250 mm × 4.6 mm i.d., 5 µm particle size) using mobile phase of methanol- ammonium buffer (80:20, v/v) at a flow rate of 1.0 ml/min 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 40-160 µg/ml with a correlation coefficient 0.9999. The precision (RSD) amongst six-sample preparation was 0.41 % for repeatability and the intermediate precision (RSD) amongst six-sample preparation was 0.61 %. The accuracy (recovery) was between 99.78 and 99.95 %. 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.

Key words: Ticlopidine hydrochloride, Stability indicating assay, Method validation.

1. Introduction

Stress testing is a part of developmental strategy under the 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[1-3]. It is suggested that stress testing should include the effect of temperature, light, oxidizing agents as well as susceptibility across a 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.

Figure: 1 Chemical structure of ticlopidine hydrochloride



Ticlopidine hydrochloride is an inhibitor of platelet aggregation used in the management and prevention of thromboembolic disorders[4]. Ticlopidine hydrochloride is chemically 5-[(2-chlorophenyl) methyl]-4, 5, 6, 7-

tetrahydrothieno [3, 2-c] pyridine hydrochloride [Fig.1]. Its molecular formula is $C_{14}H_{14}ClNS.HCl$ having molecular weight 300.25 g/mole. It is used as adenosine diphosphate (ADP) receptor antagonists in an antiplatelet therapy[5]. It is aslo significantly reduces restenosis after endovascular therapy in femoropopliteal lesions[6].

Quantitative nondestructive methods for the determination of ticlopidine in tablets using reflectance near-infrared and Fourier transform Raman spectroscopy was published[7] but HPLC method has many advantages over reflectance near-infrared and Fourier transform Raman spectroscopy method for quantization. Moreover HPLC method can be the first choice of chromatographers among the HPLC, reflectance near-infrared and Fourier transform Raman spectroscopy methods. So, development is based on HPLC method. Ticlopidine quantification in human plasma by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry. Application to bioequivalence study was reported[8]. Liquid chromatographic method for the determination of ticlopidine in human plasma was also reported[9]. So far to our present knowledge, no validated stability indicating HPLC assay method for the determination of ticlopidine hydrochloride in pharmaceutical formulation was available in literature. This paper deals with the forced degradation of ticlopidine hydrochloride under stress condition 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 from its dosage form (tablets).

2. Experimental

2.1. Materials

Ticlopidine hydrochloride hydrochloride standard of was provided by Aarti Drugs Ltd., Boisar (India). Ticlopidine hydrochloride tablets containing 250mg ticlopidine hydrochloride and the inactive ingredient used in drug matrix were obtained from market. Analytical grade ammonium acetate was purchased from Sisco Research Pvt. Ltd., Mumbai (India). HPLC 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 Chemical, New Delhi (India).

2.2. Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10AT*vp* binary pump, a SPD-M10A*vp* photodiode-array detector and a rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) connected to a multi-instrument data acquisition and data processing system (Class-*VP* 6.13 SP2, Shimadzu).

2.3. Chromatographic conditions

Chromatographic analysis was performed on a Phenomenex Luna C8 (2) (250mm × 4.6mm i.d., 5 µm particle size) column. The mobile phase consisted of methanol – 0.01M ammonium acetate buffer pH 5.0 (80: 20, v/v). To prepare the buffer solution, 0.7708 g ammonium acetate were weighed and dissolve in 1000 ml HPLC grade water and then adjusted to pH 5.0 with glacial acetic acid acid. Mobile phase was filtered through a 0.45 µm nylon membrane (Millipore Pvt. Ltd. Benglore, India) and degassed in an ultrasonic bath (Spincotech Pvt. Ltd., Mumbai). The flow rate of the mobile phase was adjusted to 1.0 ml/min and the injection volume was 20 µl. Detection was performed at 235nm.

2.4. Standard preparation

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

2.5. Test preparation

Twenty tablets were weighed and the average weight of tablet was determined. From these, five tablets were weighed and transfer 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 0.45µm nylon syringe filter. The concentration obtained was 250 µg/ml of ticlopidine hydrochloride.

2.6. Method validation

2.6.1. Specificity study

The evaluation of the specificity of the method was determined against placebo. The interference of the excipients of the claimed placebo present in pharmaceutical dosage form was derived from placebo solution. Further the specificity of the method toward the drug was established by means of checking the interference of the degradation products in the drug quantification for assay during the forced degradation study.

2.6.1.1. 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 concentrations. Specific conditions were described as follows.

2.6.1.1.1. Acidic condition

Acidic degradation study was performed by heating the drug content in 0.1 N HCl at 80˚ C for 1.5 and mixture was neutralized.

2.6.1.1.2. Alkaline condition

Alkaline degradation study was performed by heating the drug content in 1 N NaOH at 80˚ C for 2.0h and mixture was neutralized.

2.6.1.1.3. Oxidative 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 condition

Thermal degradation was performed by exposing solid drug at 80˚ C for 72 h.

2.6.1.1.5. Photolytic condition

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

2.6.2. Linearity

Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration (40, 60, 80, 100, 120, 140 and 160 µg/ml). The peak areas versus concentration data were evaluated by linear regression analysis.

2.6.3. Precision

The precision of the assay method was evaluated in terms of repeatability by carrying out six independent assays of ticlopidine hydrochloride test sample preparation and calculated the % RSD of assay (intraday). Intermediate precision of the method was checked by performing 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 ($\pm$ 0.1 ml/min), mobile phase composition [methanol-buffer (78: 22 and 82: 18, v/v)], buffer pH ($\pm$ 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 h, 24 h, 36 h and 48 h. The responses for the aged solution were evaluated using a freshly prepared standard solution.

3. Result and discussion

3.1. Development and optimization of the HPLC method

To develop a rugged and suitable HPLC method for the quantitative determination of ticlopidine hydrochloride, the analytical condition 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. By using 0.01M ammonium acetate 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. Figure 2 and Figure 3 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 then 2.0, theoretical plate was greater than 5000 and % RSD of peak area was less then 2.0 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 condition. There was no interference of any peak of degradation product with drug peak. Major degradation was found in oxidative condition that product was degraded up to 7.76 %. The major impurity peaks was found at 3.49 min (Fig. 4). In alkali degradation, it was found that around 5-6 % of the drug degraded (Fig. 5) and in photolytic condition around 4-5 % of the drug degraded (Fig. 6). Ticlopidine hydrochloride was found to be slightly degraded in acidic while it was stable under the thermal degradation.

Figure 2: Chromatogram of standard preparation

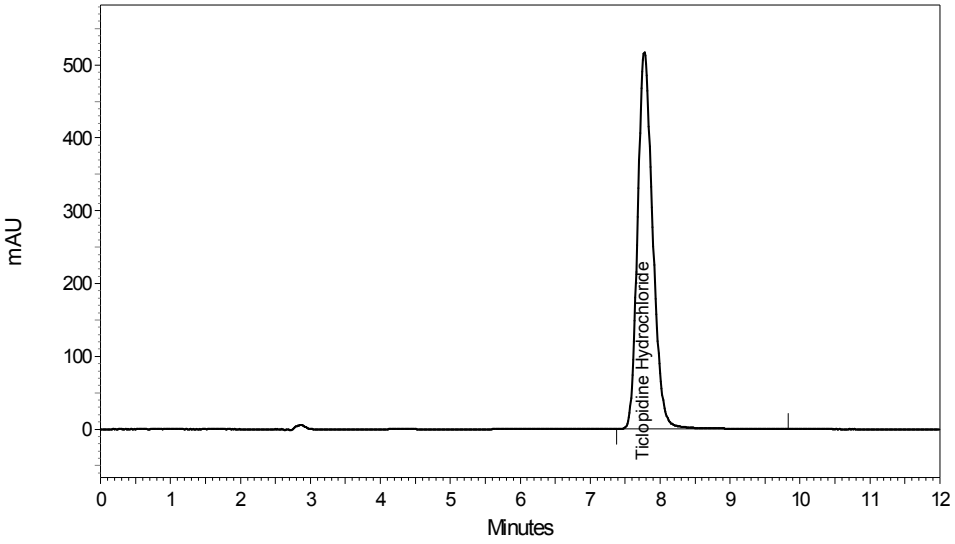


Figure 3: Chromatogram of test preparation

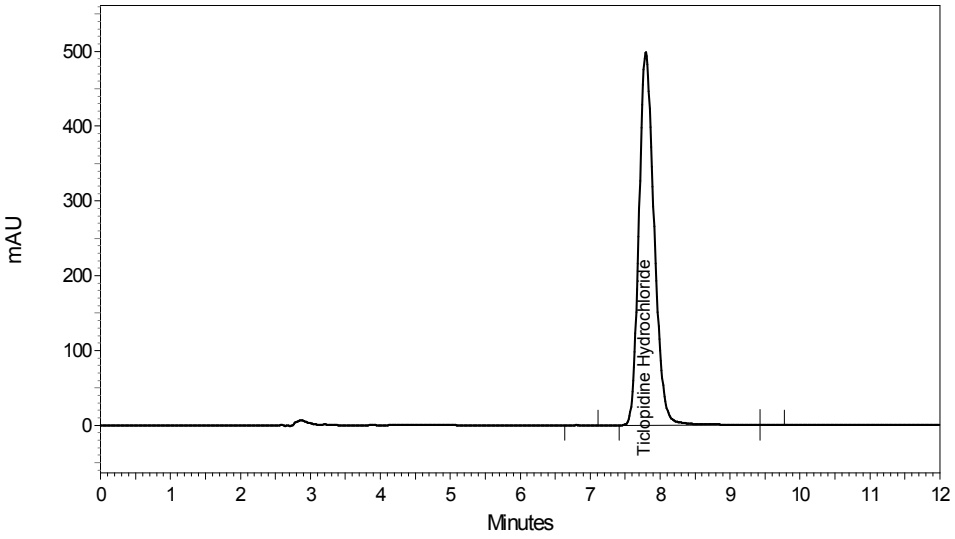


Figure 4: Chromatogram of oxidative forced degradation study

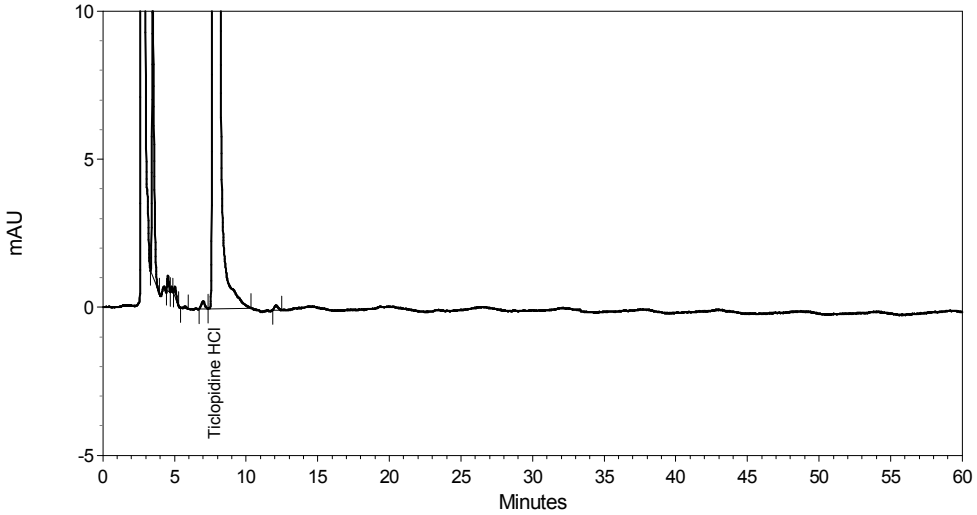


Figure 5: Chromatogram of alkali forced degradation study

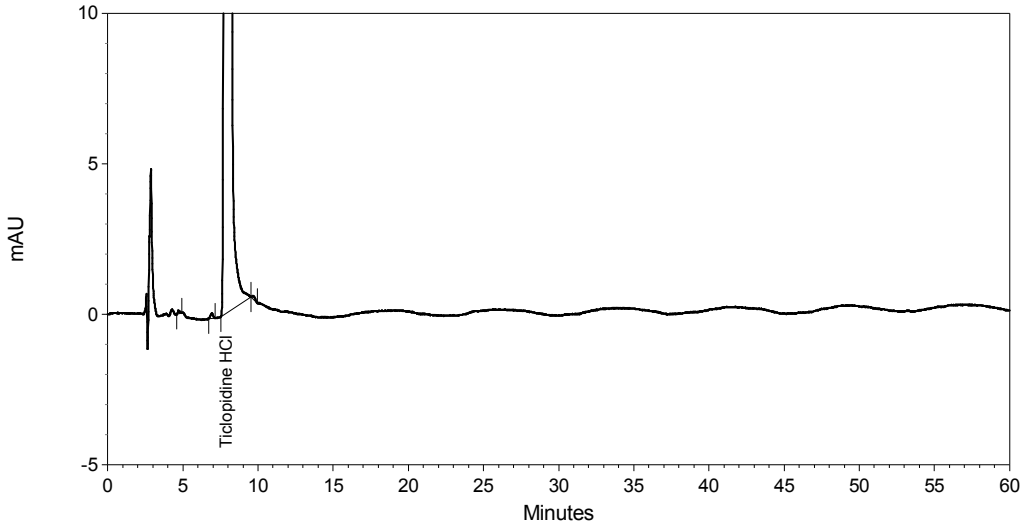
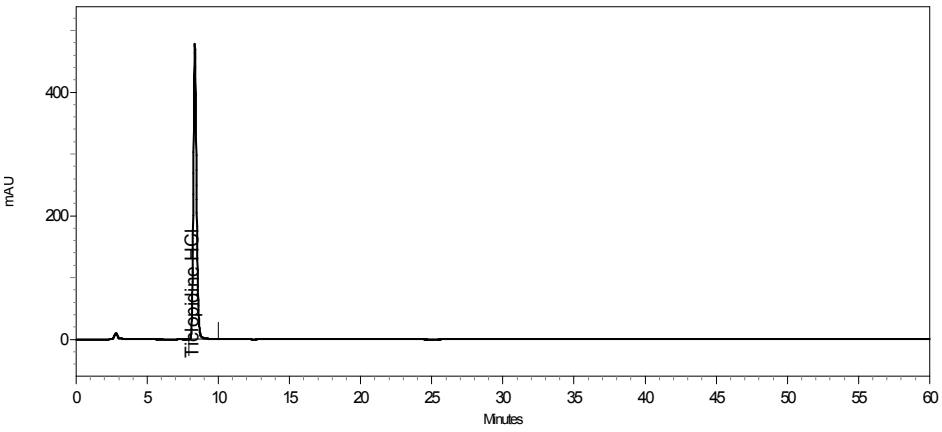


Figure 6: Chromatogram of Photoyitic degradation study



3.4. Linearity

Seven points calibration curve were obtained in a concentration range from 40-160 µg/ml for ticlopidine hydrochloride. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was Y=30375861.79x+ 76638.19 with correlation coefficient 0.9999.

3.5. Precision

The result of repeatability and intermediate precision study are shown in Table I. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were < 0.41 % and < 0.61 %, respectively, which confirm that method was precise.

Table I: Evaluation data of precision study

Set	Intraday (n = 6)	Interday (n = 6)
1	100.3	100.5
2	100.8	101.6
3	101.4	101.3
4	101.3	101.7
5	100.8	100.5
6	100.6	100.3
Mean	100.9	101.0
Standard deviation	0.42	0.62
% RSD[a]	0.41	0.61

[a]RSD= Relative Standard Deviation

3.6. Accuracy

The HPLC area responses for accuracy determination are depicted in Table II. The results shown that best recoveries (99.78-99.95 %) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table II: Evaluation data of Accuracy Study

Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml)	% Recovery	% RSD
50	12.70	12.69	99.95	0.41
100	25.13	25.09	98.83	0.23
150	37.13	37.05	99.78	0.28

3.7. Solution stability study

Table III shows the results obtain 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 ambient temperature as during this time the result was not decrease below the minimum percentage.

3.8. Robustness

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

Conclusion

A new analytical method has been developed to be routinely applied to determine ticlopidine hydrochloride in pharmaceutical dosage form. In this study, stability of ticlopidine hydrochloride in present dosage form was established through employment of ICH recommended stress condition. The developed procedure has been evaluated over the specificity, linearity, accuracy, precision and robustness in order to ascertain the stability of the analytical method. It has been proved that it was specific, linear, precise, accurate and robust and stability indicating. Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

Table III: Evaluation data of solution stability study

Intervals	% Assay for test preparation solution stored at 2˚ C-8˚ C	% Assay for test preparation solution stored at ambient temperature
Initial	100.1	100.1
12 h	100.2	100.0
24 h	99.9	99.8
36 h	99.9	99.9
48 h	99.6	99.4

Table IV: Evaluation data of robustness study

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Flow 0.9 ml/min	100.6	6662	1.12
Flow 1.1 ml/min	101.2	6349	1.18
Buffer pH 4.8	100.1	5201	1.14
Buffer pH 5.2	100.2	5630	1.24
Buffer-ACN (78:22,v/v)	100.2	6.89	1.12
Buffer-ACN (82:18,v/v)	100.0	5446	1.29
Column change	99.7	5741	1.08

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