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SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF TICLOPIDINE HYDROCHLORIDE IN TABLET FORMULATION

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Abstract:

The objective of the current study was to develop a simple, precise and accurate Spectrophometric assay method and validated for determination of Ticlopidine hydrochloride in solid pharmaceutical dosage forms. Standard tock solution was prepared in methanol and distilled water in the ratio of 70:30. The λmax of Ticlopidine Hydrochloride was found to be 233 nm. 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^{-1} with a correlation co-efficient 0.9972. The precision (relative standard deviation- 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 99.65 and 100.61 %.

Keywords: Ticlopidine hydrochloride, Assay method, Spectrophotometry, Development and Validation.

Introduction

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

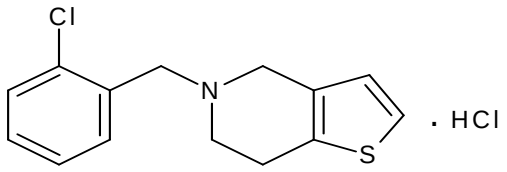

Figure: 1 Molecular structure of Ticlopidine hydrochloride

Quantitative methods for the determination of Ticlopidine in tablets using reflectance Near-infrared and Fourier transform Raman spectroscopy [4], high-performance liquid chromatography coupled to electro spray tandem mass spectrometry was reported [5,6]. Spectrophotometric methods for the estimation of Ticlopidine Hydrochloride in bulk and dosage form [7]. The spectrophotometric method has many advantages over reflectance near-infrared and fourier transform Raman spectroscopy method for quantization. So far to our present knowledge, no such validated stability indicating spectrophometric assay method for the determination of Ticlopidine hydrochloride in pharmaceutical formulation was available in literature. Moreover spectrophometric method can be the first choice of chromatographers among the High performance liquid chromatography; reflectance near-infrared and Fourier transform Raman spectroscopy methods. So, development is based on spectrophometric method. This paper deals with the validation of the developed method for the assay of Ticlopidine hydrochloride from its dosage form (tablets).

Experimental

Materials

Ticlopidine hydrochloride standard of 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 market. Analytical grade methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai (India).

Instrumentation

The spectrophotometer system used to perform development and validation of this assay method was UV-Visible double beam spectrophotometer PharmaSpec 1700 (Shimadzu, Kyoto, Japan) with matched quartz cells (1cm).

Diluent Preparation: Mixture of methanol and water (70:30, v/v) used as a diluent.

Standard preparation: A Ticlopidine hydrochloride standard stock solution containing 100 μg mL^{-1} was prepared in a 100 mL volumetric flask by dissolving 10.00 mg of Ticlopidine hydrochloride and then diluted to volume with methanol as diluents.

Further 10 mL of this stock solution in 50 mL volumetric flask and make up to mark with diluents. (Final concentration of standard solution is 40μg mL^{-1})

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 diluents 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 2500 μg mL^{-1} of Ticlopidine hydrochloride.

Further take 0.8 mL of this filtered solution in 50 mL volumetric flask and make up to mark with diluents. The concentration obtained was 40 μg mL^{-1} of ticlopidine hydrochloride.

Method validation

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.

Linearity

Linearity test solutions for the assay method were prepared at seven concentration levels from 40 to 160 % of assay analyte concentration (100, 150, 200, 250, 300, 350, 400 μg mL^{-1}). The peak areas versus concentration data were evaluated by linear regression analysis.

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.

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.

Robustness

The robustness of study was carried out to evaluate the influence of small but deliberate variations in the spectrophotometric conditions. The factors chosen for this study were the change in diluent composition [methanol-water (78: 22 and 82: 22, v/v)], and by different analyst study.

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 hours. The responses for the aged solution were evaluated using a freshly prepared standard solution.

Result and discussion

The λ maxima of Ticlopidine hydrochloride in standard and test preparation was found to be 233 nm from its spectrum (Fig. a, b).

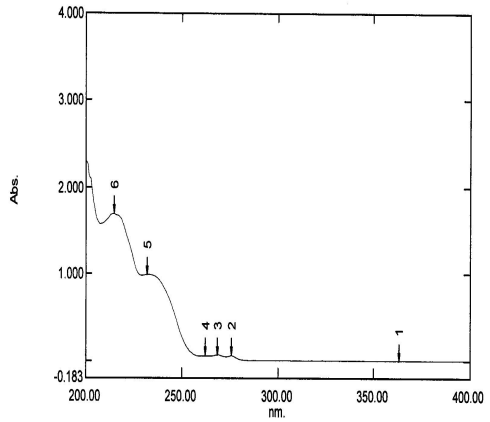

Fig. a: UV spectrum of Ticlopidine hydrochloride in Standard solution

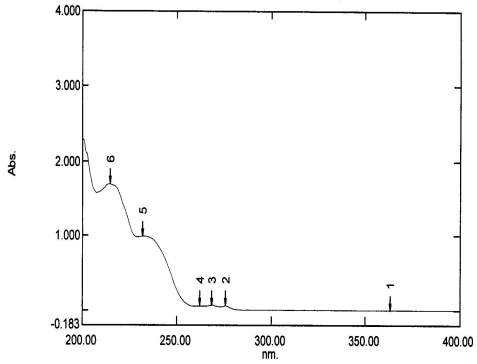

Fig. b: UV spectrum of Ticlopidine hydrochloride in Sample solution

Ticlopidine Hydrochloride showed linear absorption from 140-160 µg/mL. The correlation coefficient (r) was found to be 0.9972.(Fig. c) The stability of solutions of formulation was determined by measuring the absorbance at 233 nm at periodic intervals. There was no considerable change in the absorbance at this wavelength up to 3 hours indicating that the solution was stable for at least 3 hours. Commercial formulations containing Ticlopidine hydrochloride were analyzed by proposed method. The precision (relative standard deviation- RSD) amongst six-sample preparation was 0.41 % for repeatability and the intermediate precision [RSD] amongst six-sample preparation was 0.61 % indicating that the method has required precision. The accuracy (recovery) was between 99.65 and 100.61 %. The validation results are presented in Table 1-4. 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.

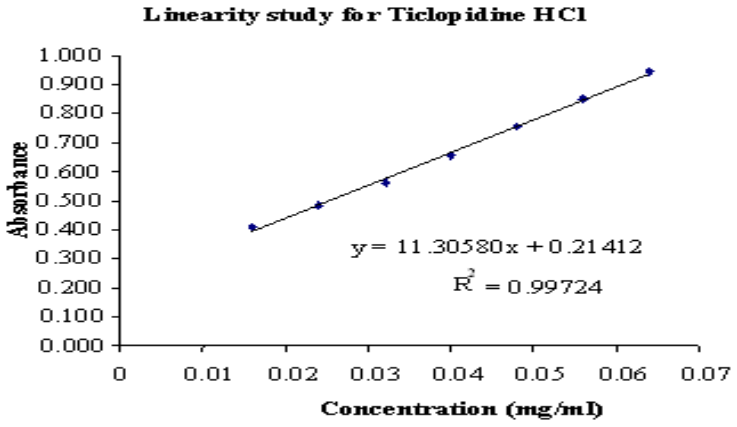

Fig. c: Linearity curve for Ticlopidine hydrochloride

Table 1: Evaluation data of precision study.

Set	Intraday (n = 6)	Interday (n = 6)
1	101.8	100.5
2	101.2	99.0
3	99.1	98.2
4	101.3	98.6
5	100.6	99.0
6	101.5	99.3
Mean	101.1	99.1
Standard deviation	0.99	0.78
% RSD	0.98	0.79

Table 2: Evaluation data of accuracy study.

Level (%)	Amount Added Concentration [a] (mg/mL)	Amount Found Concentration [a] (mg/mL)	% Recovery	% RSD
50	0.01960	0.01953	99.65	1.82
100	0.04040	0.03992	98.82	1.13
150	0.06067	0.06104	100.61	0.20

[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	100.2
12 h	98.7	98.7
24 h	100.1	101.0
36 h	100.8	100.5
48 h	101.3	101.8

Table 4: Evaluation data of robustness study.

Robust conditions	% Assay
Methanol: Water(78:22,v/v)	98.6
Methanol: Water(78:22,v/v)	100.3
Analyst change	100.4

Conclusion

A new analytical method has been developed to be routinely applied to determine Ticlopidine hydrochloride in pharmaceutical dosage form. In this study, 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.

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