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Gas Chromatographic Method Development and Validation of Assay Method for the Determination of Ticlopidine Hydrochloride in Tablets Formulation

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The objective of the current study was to developed simple, precise and accurate Gas Chromatographic [GC] assay method and validated for determination of Ticlopidine hydrochloride in solid pharmaceutical dosage forms. Separation was achieved on a SGE C5 BPX50 30m x 0.25mm i. d. x 0.25µ capillary column, injector temperature was 290 °C, Nitrogen gas used as a Carrier gas with Isothermal Column oven temperature 270 °C, the injection volume was 3µl and the detection was carried out at 310 °C by using Flame ionization 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 1000-4000 µg ml^{-1} with a correlation coefficient 0.9980. The precision (relative standard deviation- RSD) amongst six-sample preparation was 0.83 % for repeatability and the intermediate precision [RSD] amongst six-sample preparation was 0.42 %. The accuracy (recovery) was between 99.33 and 101.37 %. 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.

Keyword: Ticlopidine hydrochloride, Stability indicating assay, GC method development and validation.

1. Introduction

Gas chromatography has found important applications in the fingerprinting of oil spills, where the pattern of peaks obtained from a sample can pinpoint the petroleum source, and can also be utilised to determine the long-term fate of the petroleum hydrocarbons [1]. Other common environmental examples of quantitative GC are in the determination of pesticides in water[2], dioxin levels in soil[3] and air pollutants. It is routinely used to examine levels of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). It is also a very important technique in the food industry, where it is used extensively for assay of fatty acids, flavors [4], sterols and residues such as insecticides, herbicides, preservatives, solvents [5] and veterinary drugs. Through chemical dramatization, more polar food compounds can be analyzed, including sugars and carboxylic acids. Tedetti et al. reported the analysis of low molecular weight carboxylic acids by GC with prior dramatization to dibutyl esters [6].

Ticlopidine hydrochloride is an inhibitor of platelet aggregation used in the management and prevention of thromboembolic disorders [7]. Ticlopidine hydrochloride is a white crystalline solid. It is freely soluble in water and self-buffers to a pH of 3.6. It also dissolves freely in methanol, is sparingly soluble in methylene chloride and ethanol, slightly soluble in acetone and insoluble in a buffer solution of pH 6.3.

Ticlopidine hydrochloride is chemically 5-[(2-Chlorophenyl) methyl]-4, 5, 6, 7-tetrahydrothieno [3, 2-c] pyridine hydrochloride. Its molecular

formula is $C_{14}H_{14}ClNS$. HCl having molecular weight 300.25 g/mole. Its CAS Registry Number is 55142-85-3. It is used as adenosine diphosphate [ADP] receptor antagonists in an antiplatelet therapy [8]. It is also significantly reduces restenosis after endovascular therapy in femoropopliteal lesions [9].

In the literature review, Reflectance near-infrared and Fourier transform Raman spectroscopy [10], Colorimetric [11], Volta metric [12], High-performance Liquid Chromatography method [13], and Ultra performance Liquid Chromatography methods [14], High performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry [15], UV-Detection [16], Mass Spectrometry [17] for the determination of ticlopidine hydrochloride in pharmaceutical dosage forms or in biological fluids are reported or in combine dosage form [18]. HPLC methods applied to the pharmacokinetic studies of ticlopidine hydrochloride. So far to our present knowledge, no validated GC assay method for the determination of ticlopidine hydrochloride in pharmaceutical formulation was available in literature. This work deals with the validation of the developed method for the assay of ticlopidine hydrochloride from its formulation (tablets). Hence, the method is recommended for routine quality control analysis and also stability sample analysis.

2. Experimental

2.1 Materials

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

2.2 Instrumentation

The gas chromatographic system used to perform development and validation of this assay method was a Shimadzu GC- 14B equipped with flame ionization detector (FID) (Shimadzu, Kyoto, Japan) connected to an instrument data acquisition and data processing system (winchrom99, Indtech Instruments).

2.3 Standard Preparation

A standard solution containing 2500 µg/ml Ticlopidine hydrochloride was prepared in a 100 ml volumetric flask by dissolving 250 mg of ticlopidine hydrochloride and then diluted to volume with methanol as diluent.

2.4 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 ambient temperature and diluted to volume with methanol. The sample was filtered through 0.45µm nylon syringe filter. The concentration obtained was 2500 µg/ml of ticlopidine hydrochloride.

2.5 Chromatographic Conditions

Injector Temperature	**:**	**290 °C**
Column	:	SGE C5 BPX50 30m x 0.25mm i. d. x 0.25µ capillary column
Carrier gas	:	Nitrogen
Carrier gas Pressure	:	200 kPa
Column oven temperature	:	Isothermal at 270 °C
Injection volume	:	3µl
Detector	:	Flame ionization detector
Detector temperature	:	310 °C
Diluent	:	Methanol

3. Result and discussion

3.1 Development and Optimization of the GC Method

Proper selection of the methods depends upon the nature of the sample (volatile or nonvolatile molecule) its molecular weight, solubility and melting point. Ticlopidine hydrochloride is soluble in polar solvent hence gas chromatography was selected to estimate them. To develop a rugged and suitable GC method for the quantitative determination of ticlopidine hydrochloride, the analytical condition were selected after testing the different parameters such as diluents, melting point and other chromatographic conditions.

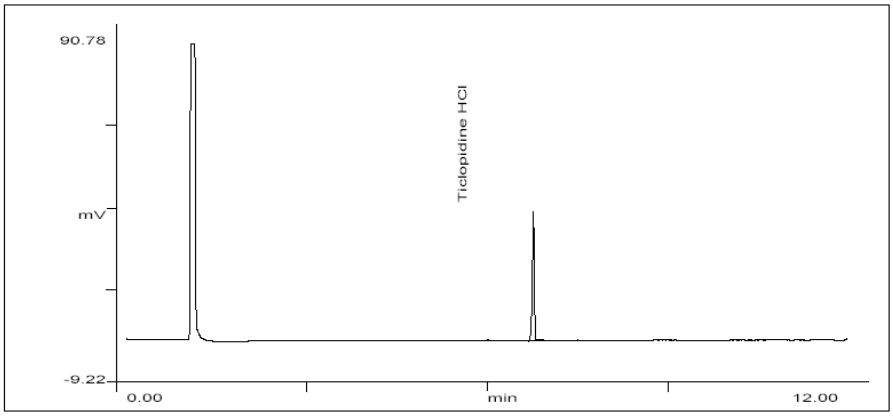

Fig 1: Chromatogram of standard preparation

3.2 Method validation

3.2.1 Specificity

The specificity of the method was determined by checking the interference of placebo with analyte and with the proposed method. There was no interference of any peak of excipients or any impurities with the analyte peak.

3.2.2 Linearity

Seven points calibration curve were obtained in a concentration range from 1000-4000 µg/ml for ticlopidine hydrochloride. A stock Solution of 12000 µg/ml was used for preparation of linearity range solution. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 412,673.29x - 61,103.79 with correlation coefficient 0.9980. (Figure 2)

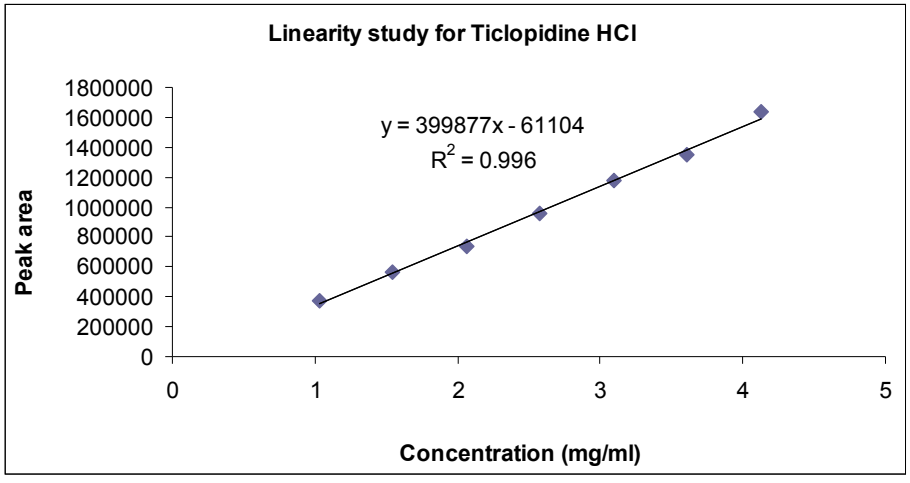

Fig 2: Linearity curve for ticlopidine hydrochloride

3.2.3 LOD and LOQ

The limit of detection and limit of quantification were evaluated by serial dilutions of ticlopidine hydrochloride stock solution in order to obtain signal to noise ratio of 3:1 for LOD and 10:1 fro LOQ. The LOD value for ticlopidine hydrochloride was found to be 0.025 µg/ml and the LOQ value 0.25 µg/ml. Chromatogram of LOD and LOQ study were shown in Figure 3 and 4.

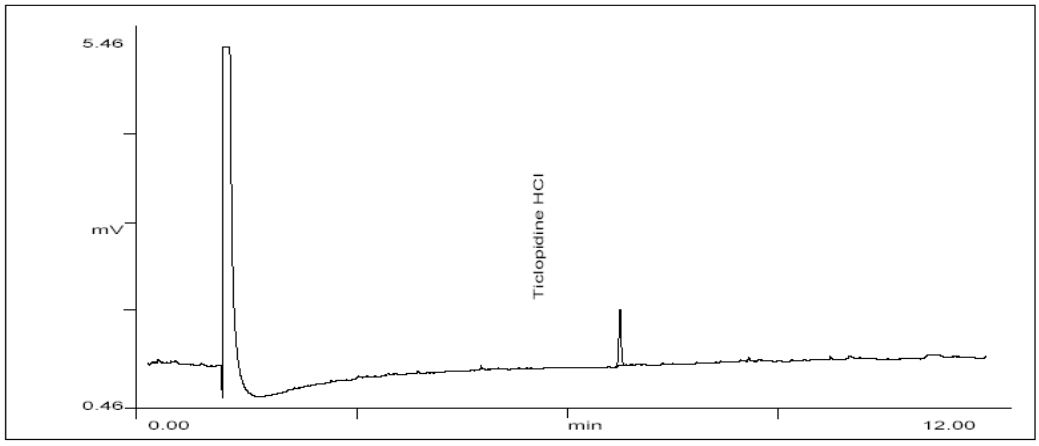

Fig 3: Chromatogram of LOD Study of ticlopidine hydrochloride

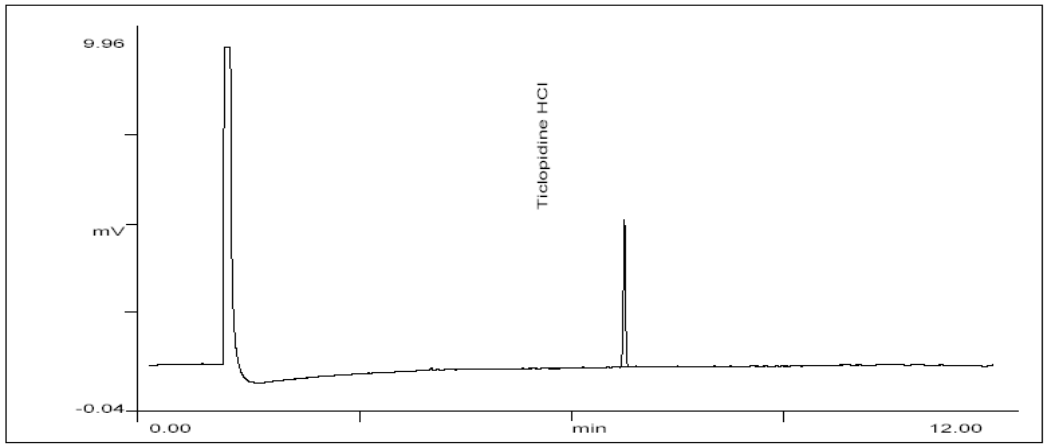

Fig 4: Chromatogram of LOQ study of ticlopidine hydrochloride

3.2.4 Precision

The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were 0.83 % and 0.42 %, respectively, which confirm that method was precise.

3.2.5 Accuracy

The GC area responses for accuracy determination are depicted in Table 1. accuracy study was conducted by using standard addition method. A known concentration of standard substance (analyte) was added to blank preparation of sample matrix and recovery of analyte is calculated on the basis of area obtained in the chromatogram The result shows that best recoveries (99.33-101.37 %) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Table 1: Evaluation data of accuracy study

Level (%)	Amount added concentration [a] (mg/ml)	Amount found concentration [a] (mg/ml)	% Recovery	% RSD
50	1.25100	1.24257	99.33	0.38
100	2.50133	2.53567	101.37	0.42
150	3.75167	3.79454	101.14	0.38

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

3.2.6 Solution stability study

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 – 8 ˚C and ambient temperature, as during this time the result was not decrease below the minimum percentage.

3.2.7 Robustness

The result of robustness study of the developed assay method was established in Table 2. 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.

Table 2: Evaluation data of robustness study

Robust conditions	% Assay	System suitability parameters	
		Theoretical plates	Asymmetry
Column oven temp. at 260°	101.8	220068	0.92
Column oven temp at 280°C	101.5	201245	0.95
Carrier gas pressure at 210kPa	99.9	181471	1.02
Carrier gas pressure at 190kPa	102.1	145630	0.88
Injection volume 2.5µl	98.9	232101	0.74
Injection volume 3.5 µl	101.4	216544	0.89
Column change	99.2	155741	1.08

3.2.8 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. Acceptance criteria for system suitability, asymmetry not more than 2.0, theoretical plate not less then 5000 and % RSD of peak area not more then 10, were found to be satisfactory, during all validation parameter.

4. Conclusion

This GC method for assay of Ticlopidine hydrochloride in a tablet formulation was successfully developed and validated for its intended purpose. The method was shown to specific, linear, precise, accurate, and robust. Because the method separates Ticlopidine hydrochloride and all the degradation products formed under variety of stress conditions it can be regarded as stability indicating. This method is recommended to the industry for quality control of drug content in pharmaceutical preparations

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