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The Estimation of Sarpogrelate Hydrochloride in tablet dosage forms by RP-HPLC

P.Janaki Pathi*[1], N. Appala Raju[2]
[1]Analytical Department, Vishnu Chemicals Limited, Hyderabad.
[2]Department of Pharmaceutical Chemistry, Sultan-Ul-Uloom College of Pharmacy Mount Pleasant, Road # 3, Banjara Hills, Hyderabad-500 034.

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Sarpogrelate Hydrochloride in tablet dosage form. An XTerra MS C18 analytical column (250x4.6 mm, 5 µm partical size) with mobile phase consisting of mixture of buffer 0.03M Potassium Dihydrogen Orthophosphate in water and pH adjusted to 3.20 with Orthophosphoric acid and acetonitrile in the gradient program was used. The flow rate was 1.0 mL/min and the effluents were monitored at 220 nm. The retention time was 12.9 min. The detector response was linear in the concentration of 20-300 mcg/mL. The respective linear regression equation being y= 1143.8x-1149.3. The limit of detection and limit of quantification was 0.6mcg/mL and 1.8mcg/mL respectively. The percentage assay of Sarpogrelate Hydrochloride was 99.4 %. The method was validated by determining its accuracy, precision and linearity. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Sarpogrelate Hydrochloride in bulk drug and in its pharmaceutical tablet dosage form.

Key words: *Sarpogrelate Hydrochloride, RP-HPLC and Tablets.*

INTRODUCTION

Sarpogrelate hydrochloride is a selective 5-HT2A receptor antagonist that is widely used in Japan for the treatment of peripheral arterial disease [1]. Sarpogrelate is a drug which acts as an antagonist at the $5HT_{2A}$ and $5\text{-}HT_{2B}$ receptors. It blocks serotonin-induced platelet aggregation, and has applications in the treatment of many diseases including diabetes mellitus, Buerger's disease, Raynaud's disease, coronary artery disease, angina pectoris, and atherosclerosis [2, 3]. It strongly inhibits the effects of serotonin such as platelet aggregation, vasoconstriction and vascular smooth muscle proliferation Chemically, Sarpogrelate is: 4-[2-(dimethylamino)-1-({2-[2-(3-methoxyphenyl)ethyl] phenoxy}methyl) ethoxy]-4-oxobutanoic acid. The empirical formula is $C_{24}H_{31}NO_6$, with a molecular weight of 429.506. Sarpogrelate Hydrochloride is a white to off-white crystalline powder. It is freely soluble in water,. Literature survey [4, 5] reveals no chromatographic methods for the estimation of Sarpogrelate Hydrochloride from pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Sarpogrelate Hydrochloride in pharmaceutical formulations.

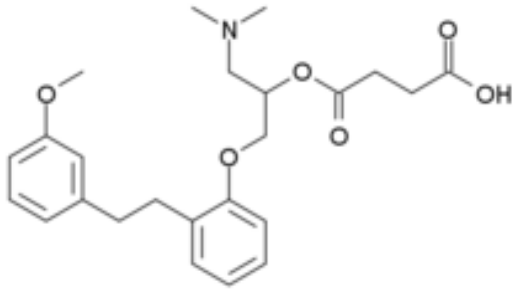

Fig. 1: Structure of Sarpogrelate

EXPERIMENTAL

Materials and Methods:

Sarpogrelate Hydrochloride was obtained as a gift sample from M/s. Vishnu Chemicals Ltd, Hyderabad. Acetonitrile, Potassium Dihydrogen Orthophosphate and water used were of HPLC grade (Qualigens). Commercially available Sarpogrelate Hydrochloride Tablets 100 mg (West - coast pharmaceuticals limited, Gujarat) were procured from local market.

Instrument:

Quantitative HPLC was performed on liquid Chromatograph, Shimadzu LC 2010 dual λ detector equipped with automatic injector with injection volume 20 µl. The HPLC system was equipped with LC solution Software.

HPLC Conditions:

The contents of the mobile phase were mixture of buffer 0.03M Potassium Dihydrogen Orthophosphate in water and pH adjusted to 3.20 with Orthophosphoric acid and acetonitrile in the gradient program was used (shown in table-IV). They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min. The run time was set at 30.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 220 nm.

***Preparation of Standard Stock solution*:**

A standard stock solution of the drug was prepared by dissolving 10 mg of Sarpogrelate Hydrochloride in 10 mL volumetric flask and dissolved in diluent (Acetonitrile and Water:50:50), sonicated for about 15 min and then made up to 10 mL with diluent get 1000 mcg/mL standard stock solution.

***Working Standard solution*:**

2.0 mL of the above stock solution was taken with micropipette in 10 mL volumetric flask and thereafter made up to 10 mL with diluent (Acetonitrile and Water: 50:50) to get a concentration of 200mcg/mL.

***Preparation of Sample solution*:**

Twenty tablets (Sarpogrelate Hydrochloride Tablets 100 mg, West coast pharmaceuticals limited, Gujarat) were weighed, and then powdered. A sample of the powdered tablets, equivalent to 50mg of the active ingredient, was mixed with 30 mL of diluent in 50 mL volumetric flask. The mixture was allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding diluent up 50 mL to obtain a stock solution of 1000mcg/mL. 2 mL of the above solution

***Corresponding author:**
P.Janaki Pathi
Analytical Department, Vishnu Chemicals Limited,
Hyderabad, India.
**E-Mail: pjp02002@yahoo.com*

was taken and further diluted with diluent up to 10 mL to get working sample solution of 200 mcg/mL.

Linearity:

Aliquots of standard Sarpogrelate Hydrochloride stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Sarpogrelate Hydrochloride are in the range of 20-300 µg/mL. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 220 nm and a Calibration graph was obtained by plotting peak area versus concentration of Sarpogrelate Hydrochloride (**Fig. 3**).

The plot of peak area of each sample against respective concentration of Sarpogrelate Hydrochloride was found to be linear in the range of 20-300 mcg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in **Table 1**. The respective linear regression equation being y=1143.8x-1149.3. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table 1**.

Assay:

20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 12.9 minutes. The amount of drug present per parentaral was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in **Table 2**.

Recovery Studies:

Accuracy was determined by recovery studies of Sarpogrelate Hydrochloride, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in **Table 2**. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of Sarpogrelate Hydrochloride. The parameters studied to evaluate the suitability of the system are given in **Table 3**.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The limit of detection (LOD) and limit of quantification (LOQ) for Sarpogrelate Hydrochloride were found to be 0.6 mcg/mL and 1.8 mcg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of Sarpogrelate Hydrochloride as shown in **Fig. 2**, it was found that the retention time was 12.9 min. A mixture of buffer 0.03M Potassium Dihydrogen Orthophosphate in water and pH adjusted to 3.20 with Orthophosphoric acid and acetonitrile in the gradient program was used (shown in **Table 4**) was found To be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2=0.9999) was observed between the concentration range of 20-300 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Sarpogrelate Hydrochloride tablets was found to be 99.4%. From the recovery studies it was found that about 99.0% of Sarpogrelate Hydrochloride was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible.

Thus, the developed method can be easily used for the routine quality control of parental dosage forms of Sarpogrelate Hydrochloride within a short analysis time.

Table No. 1: Linear Regression Data for Calibration curves

Drug	Sarpogrelate Hydrochloride
Concentration range (mcg/mL)	20-300
Slope (m)	1143.8
Intercept (b)	-1149.3
Correlation coefficient	0.9999
% RSD	0.49

Table No. 2: Results of HPLC Assay and Recovery studies

Sample	Amount claim (mg/Tablet)	% found by the proposed method	% Recovery*
1.	100	99.39	98.87
2.	100	99.42	99.21
3.	100	99.37	98.93

*Average of three different concentration levels.

Table No. 3: Validation Summary

Validation Parameter	Results
System Suitability:	
Theoretical Plates (N)	9682
Tailing factor	1.14
Retention time in minutes	12.9
% Area	99.08
LOD (mcg/mL)	0.6
LOQ (mcg/mL)	1.8

Table No. 4: Gradient Program in HPLC method

Time in mins	Buffer	Acetonotrile
0	80	20
5	80	20
12	30	70
20	30	70
25	80	20
30	80	20

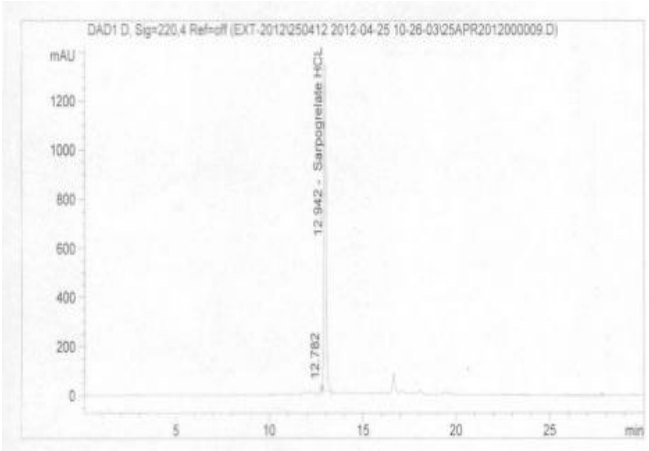

Fig. 2: Typical Chromatogram of Sarpogrelate Hydrochloride by RP-HPLC

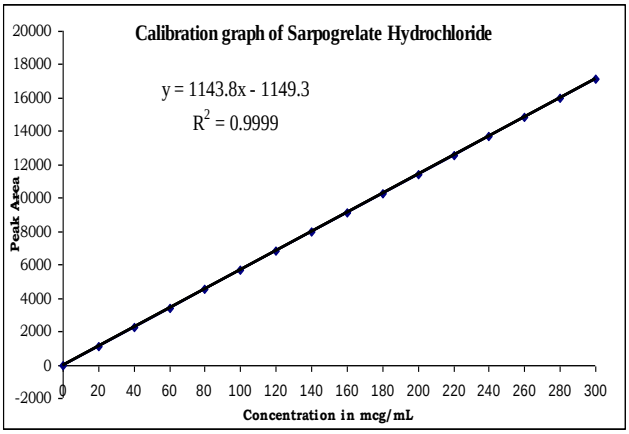

Fig. 3: Calibration curve of the Sarpogrelate Hydrochloride by RP-HPLC

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Conflict of interest: The authors have declared that no conflict of interest exists.

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