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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF PALONOSETRON HYDROCHLORIDE

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Abstract:

A reverse phase HPLC method is described for the determination of Palonosetron hydrochloride. Chromatographic separation achieved isocratically on C_{18} column, (ODS-UG-5, 250 ×4.6 mm,5µ) utilising a mobile phase of phosphate buffer (0.025M sodium dihydrogen phosphate pH adjusted to 6.9 with triethylamine) and acetonitrile (65:35) at a flow rate of 1 ml/min with UV detection at 240 nm. The retention time of palonosetron hydrochloride was 5.6 min. The method is accurate (% recovery 99.35%-100.2%), precise (RSD 0.1%) and linear within the range (0.003-2mg/ml). The detection limit of palonosetron at a signal to noise ratio 3:1 is 1000ng/ml while the quantification limit was 3000 ng/ml. The proposed method is applicable to routine analysis of palonosetron in pharmaceutical dosage forms.

Key words: Palonosetron hydrochloride, RP-HPLC, validation.

Introduction:

Palonosetron hydrochloride is an antiemetic and antinauseant agent .It is a serotonin sub type 3 (5-HT_3) receptor antagonists with a strong binding affinity for this receptor. Chemically Palonosetron hydrochloride is designated as (3aS)-2- [(S)-1-azabicyclo [2.2.2] oct-3-yl]-2, 3, 3a, 4, 5, 6-hexahydro-1-oxo-1*H*benz [*de*]isoquinoline hydrochloride and has the empirical formula $C_{19}H_{24}N_2O.HCl$ with a molecular weight of 332.87.

Various methods have been reported for the estimation of Palonosetron hydrochloride in biological matrices such as plasma, urine which includes use of LC with electron spray ionisation mass spectrometric detection[1], capillary electrophoresis equipped with an air cooling system and UV detector[2], chiral liquid chromatographic method for

enantiomer separation[3], Liquid chromatographic method of separation with diode array detection[4], LC–MS/MS[5,6].

The liquid chromatographic system consists of the following components: WATERS-2695, HPLC instrument containing variable wavelength programmable UVdetector and data handling system, rheodyne injector with 20 µl fixed loop .Chromatographic analysis was performed using Empower software, on a ODS-UG-5, 250×4.6mm, 5µ particle size column. AXIS AGN204 –PO electronic balance was used for weighing purpose. Crest ultra-sonic 257D, degasser is used. Pure samples of Palonosetron hydrochloride were obtained from NATCO pharma ltd. HPLC grade water obtained by double distillation and purification through milli-Q water purification system.

Materials and Methods:

Sodium dihydrogen phosphate was weighed (1.78g) and dissolved in 650 ml of water R and mixed with 1.3ml of triethyl amine, this solution was mixed with 350ml of acetonitrile. This was filtered through the 0.45µm membrane filter paper and sonicated for 10 min. A stock solution of Palonosetron hydrochloride was prepared by accurately weighing 25 mg of drug, transferring to 25ml of volumetric flask and diluting up to the mark with mobile phase.

A reverse phase C-18 column equilibrated with mobile phase acetonitrile: phosphate buffer (sodium dihydrogen phosphate) (35:65) was used. Mobile phase was filtered and degassed .Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 240nm. The sample was injected using a 20 µl fixed loop, and the total run time was 20 min.

Appropriate aliquots of standard Palonosetron hydrochloride stock solution were taken in different 10 ml volumetric flasks followed by dilution up to the mark with mobile phase to obtain final concentration of 0.003,0.02,0.1,0.2,0.4,0.8,1and 2mg/ml(expected limit of quantification 3000ng/ml). The solutions were injected into chromatographic system and chromatograms were developed and peak area was determined for each concentration of drug solution .Calibration curve of Palonosetron hydrochloride was constructed by plotting peak area vs. applied concentration of Palonosetron hydrochloride and regression equation was computed.

The method was validated for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, ruggedness and robustness. The chromatograms were taken by appropriate dilutions and the quantities of drugs were determined.

The linearity of the method was determined at the six concentration levels ranging from 0.003-2mg/ml.The accuracy of the method was determined by calculating recovery of Palonosetron hydrochloride. The recovery studies were carried out over a specified concentration range and the amount of Palonosetron hydrochloride was estimated by measuring the peak area by fitting these values to the straight line equation of calibration curve. From the determination percentage recovery and standard deviation of % recovery were calculated.

The precision study was carried out by estimating the corresponding responses six times on the same day for repeated injections of same concentration and the results were reported in terms of relative standard deviation. Repeatability studies were carried out by estimating the response of replicate determination of single concentration and results were reported in terms of relative standard deviation.

A calibration curve was prepared using concentrations in the range of 0.003-2mg/ml (expected detection limit range) the standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit =3.33 σ/S, quantitation limit =10 σ /S, where σ is standard deviation of Y-intercepts of regression lines and S is the slope of the calibration curve[7,8].

Robustness of the method was studied by changing the pH (7) of the mobile phase by ± 0.7 and by changing flow rate by ± 0.1ml/min. Response of replicate determination of single concentration and results were reported in terms of relative standard deviation.

Ruggedness of the method was studied by analysis of the same samples of Palonosetron hydrochloride by different analysts; the degree of reproducibility was found to be 100 % ± 0.5%. The response of replicate determination of single concentration and results were reported in terms of relative standard deviation.

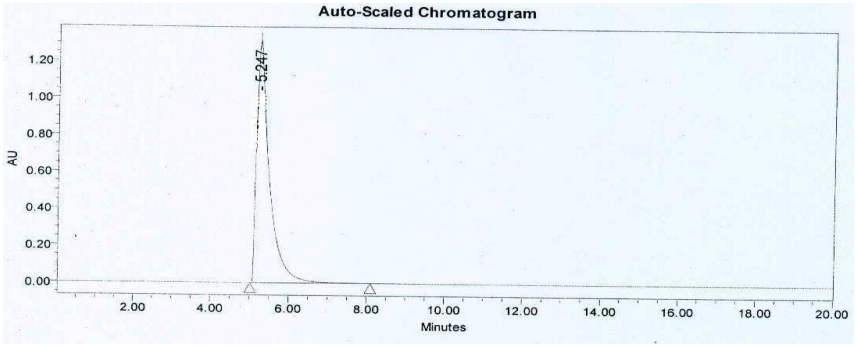

Fig.1: HPLC chromatogram of Palonosetron hydrochloride (RT 5.24min).

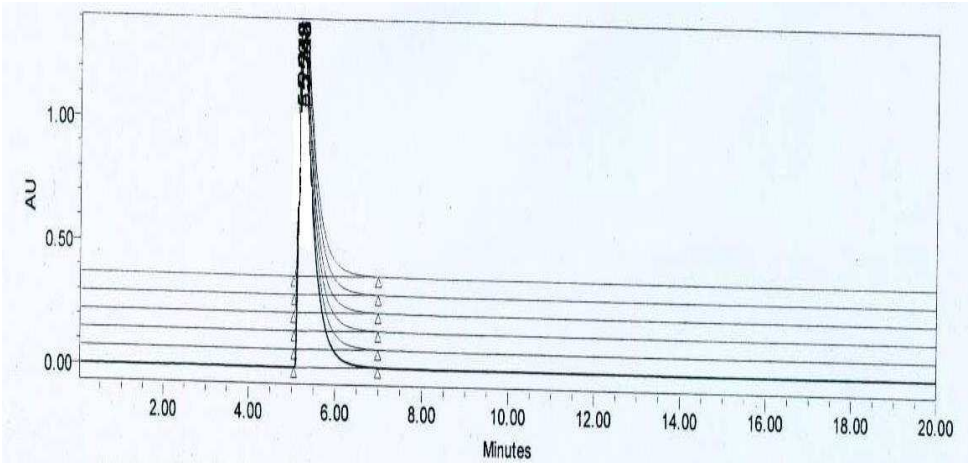

Fig.2: Precision studies: HPLC chromatograms of Palonosetron hydrochloride

Table-1: Summary of validation parameters.

Parameters	Values
Detection limit (µg/ml)	1
Quantitation limit(µg/ml)	3
Accuracy (%Recovery)	99.35-100.2%
Precision (RSD[a], %)	0.1
Linearity(r^2)	0.9999

RSD[a] indicates relative standard deviation

Table-2: Regression analysis of the calibration curve for the proposed method.

Parameters	Values
Calibration range	0.003-2mg/ml
Slope	28734815.51
Intercept	9089.358169
Correlation coefficient	0.9999
Regression equation	y = 28734815.51x + 9089.3581

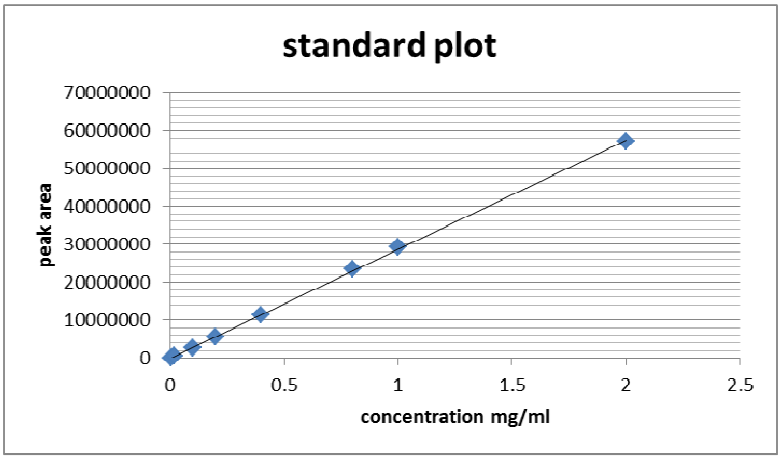

Table-3: System suitability test parameters for Palonosetron hydrochloride by the proposed method.

System suitability test Parameters	Values
Retention time(minutes)	5.24
No. of theoretical plates	1267

UV overlain spectra of Palonosetron hydrochloride shows that the drug absorbs appreciably at 240nm, so 240 was selected as the detection wavelength in liquid chromatography. Optimisation of the mobile phase was performed based on retention times and peak area obtained. Different mobile phases were tried but good symmetric peaks were obtained with the mobile phase acetonitrile: phosphate buffer (0.025M sodium dihydrogen phosphate pH adjusted to 6.9 with triethylamine) (35:65).The retention time of Palonosetron hydrochloride was found to be 5.2 min. Calibration curve of Palonosetron hydrochloride was obtained by plotting the peak area vs. concentration of Palonosetron hydrochloride over the range of 0.0003-2 mg/ml, slope and y-intercept value for calibration curve was y = 28734815.51x + 9089.3581, and it was found to be linear over the entire calibration range studied with r^2 0.9999 .the data of regression analysis of calibration curves are shown in Table 2. Detection limit of Palonosetron hydrochloride was 1000ng/ml and quantitation limit for Palonosetron hydrochloride was 3000ng/ml, which suggests that a Nano gram quantity of it can be estimated accurately and precisely. The validation parameters are summarised in Table 1. Recovery of Palonosetron hydrochloride was found to be in the range of 99.35-100.2%. System suitability test parameters are shown in Table 3. Proposed liquid chromatographic method was applied for the determination of Palonosetron hydrochloride.

Conclusion:

Proposed study describes new RP-HPLC method using simple mobile phases for the estimation of Palonosetron hydrochloride. The method was validated and found to be simple, sensitive, accurate and precise. Percentage recovery shows that the method is free from possible interference. Therefore the proposed method can be used for routine analysis for Palonosetron hydrochloride.

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