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Development and Validation of Stability Indicating RP-LC Modus Operandi for Estimation of Palanosetron in Bulk and Formulations

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-LC) modus operandi has been stride and subsequently validated for the tenacity of Palonosetron in Bulk and its pharmaceutical formulation. Severance was clinch with a Symmetry RP-8 (Make: Waters Corporation; 75 mmx4.6 mm I.D; particle size 5 µm) Column and Prim.- Potassium phosphate monohydrate buffer (pH regulated to 3.0 with lessend orthophosphoric acid): acetonitrile (75:25) v/v as eluent at a flow rate of 0.8 mL/min. UV perception was execute at 210nm. The modus operandi is simple, rapid, and selective. The elucidate modus operandi of Palonosetron is linear over a range of 2.2 µg/mL to 13.6 µg/mL. The modus operandi precision for the tenacity of assay was below 1.0% RSD. The percentage restoration of active pharmaceutical ingredient (API) from dosage forms generation from 99.0 to 100.9%. The results proclaimed that the postulated modus operandi is suitable for the precise, accurate and rapid tenacity of Palonosetron in bulk and its dosage forms.

KEY WORDS: Palonosetron, RP-LC, Validation, Dosage form.

1. INTRODUCTION

Palonosetron hydrochloride is an antiemetic and antinauseant agent. It is a serotonin subtype 3 (5- HT3) receptor antagonist with a mighty binding affinity for this receptor. Chemically, palonosetron hydrochloride is: (3aS)-2-[(S)-1-Azabicyclo [2.2.2] oct-3-yl]-2, 3, 3a, 4, 5, 6- hexahydro-1-oxo-1Hbenz[de]isoquinoline hydrochloride. The empirical formula is $C_{19}H_{24}N_2O.HCl$, with a molecular weight of 332.87. Palonosetron hydrochloride is a white to off-white crystalline powder. It is freely soluble in water, soluble in propylene glycol, and slightly soluble in ethanol and 2-propanol. Palonosetron hydrochloride injection is a sterile, apparent, discolour, non-pyrogenic, isotonic, buffered solution for intravenous administration.

A thorough literature survey has betray that a limited number of spectrophotometric, and chromatographic modus operandis have been proclaim for analysis of Palonosetron.

The authors have stride a new, simple and fast analytical modus operandi by RP-LC to pinhole Palonosetron in bulk and its dosage forms. This validation study is accomplish as per ICH guidelines.

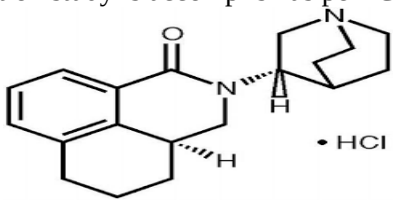

Figure.1. Chemical Structure of Palonosetron

2. EXPERIMENTAL

Instrumentation: The analysis of the drug was accomplish on a waters LC system equipped with 2695 pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Symmetry RP-8 ((Make: Waters Corporation, Ireland); 75 mmx4.6 mm I.D; particle size 5 µm) was used. The spin-off of signal was monitored and integrated using waters Empower 2 software.

Chemicals and solvents: Milli-Q Water, Acetonitrile (HPLC Grade), Methanol (HPLC Grade), Orthophosphoric acid (GR Grade), Potassium dihydrogen phosphate monohydrate (GR Grade) were existent from Qualigens Ltd., Mumbai.

Buffer preparation: Meticulous weigh and demote about 2.72 grams of Prim. - Potassium phosphate monohydrate in 1000 mL of purified water and mix. Adjust pH to 3.0 (±0.05) with lessen orthophosphoric acid solution. Trickle the solution through 0.45µm membrane trickle.

Mobile phase preparation: Prepare a trickled and degassed mixture of Buffer and Acetonitrile in the ratio of 75:25 v/v resultantly.

Diluent preparation: Mobile phase is used as diluent.

Standard preparation: Meticulous weigh and demote about 55.0mg of Palonosetron into a 100 mL volumetric flask, add 60 mL of mobile phase and sonicate to dissolve. Cool the solution to room temperature and lessen to volume with diluent. Demote 2.0 mL of the aloft solution into a 100 mL volumetric flask and lessen to volume with diluent (Mobile Phase).

Sample preparation: Meticulous weigh and demote equivalent to 20 mg of Palonosetron into a 50 mL volumetric flask add about 30 mL of mobile phase, and sonicate for 15minutes with recurrent shaking at restrained temperature and lessen to volume with mobile phase and mix. Trickle the solution through 0.45 µm membrane Trickle. Demote 3.0 mL of the aloft solution into a 100 mL volumetric flask and lessen to volume with diluent.

Chromatographic conditions: An Symmetry RP-8 (Make: Waters Corporation (Ireland); 75 mmx4.6 mm I.D; particle size 5µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 0.8mL/min. The sample injection volume was 20 µL. The photodiode array detector was set to a wavelength of 210nm for the perception and Chromatographic runtime was 15 minutes.

3. RESULTS AND DISCUSSION

Method development: To develop a suitable and robust LC modus operandi for the tenacity of Palonosetron, offbeat mobile phases were hired to clinch the terrific separation and resolution. The modus operandi development was started with Symmetry RP-18 (Make: Waters Corporation (Ireland); 150 mmx4.6 mm I.D; particle size 5 µm) with the following mobile phase. Meticulous weigh and demote about 2.72 grams of Prim.- Potassium phosphate monohydrate in 1000 mL of purified water and mix. Adjust pH to 4.6 (±0.05) with lessen orthophosphoric acid solution. Trickle the solution through 0.45µm membrane trickle. Prepare a trickleed and degassed mixture of Buffer and methanol in the ratio of 500:500 v/v resultantly.

Palonosetron peak was eluted at void volume. For next trial the mobile phase composition was changed slightly. The mobile phase composition was Buffer and methanol in the ratio of 400:600 v/v. aloft trail the peak shape was little broad. Then pH of the buffer was changed to 3.0 from 4.0 and the mobile phase was modified to Buffer: Acetonitrile in the ratio of 75:25 v/v resultantly as eluent at flow rate 0.8 mL/min. UV perception was execute at 210nm. The retention time of Palonosetron is 10.0 minutes (Fig.4) and the peak shape was good.

The chromatogram of Palonosetron standard using the postulated modus operandi is shown in Fig.4. System suitability results of the modus operandi are presented in Table.1. Palonosetron shows significant UV absorbance at Wavelength 210nm. Hence this wavelength has been chosen for perception in analysis of Palonosetron.

Method validation: The stride RP-LC modus operandi extensively validated for assay of Palonosetron using the following Parameters.

Specificity:

Blank and Placebo intervention: A study to establish the intervention of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the delineate aloft chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solutions proclaimed no peaks at the retention time of Palonosetron peak. This indicates that the diluent solution used in sample preparation do not intrude in estimation of Palonosetron in capsules.

The chromatogram of Palonosetron Blank using the postulated modus operandi is shown in Fig.2.

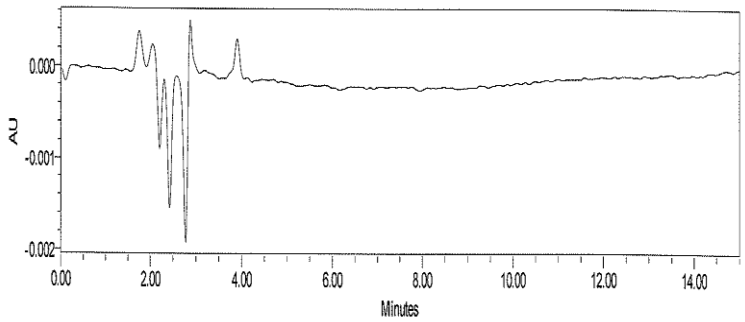

Figure.2. A typical HPLC Chromatogram showing the no intervention of diluent for Palonosetron

The chromatogram of Palonosetron Placebo using the proposed modus operandi is shown in Fig.3.

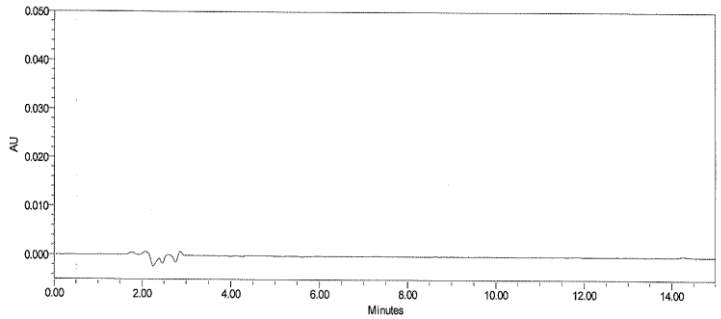

Figure.3. A typical HPLC Chromatogram showing the no intervention of placebo for Palonosetron

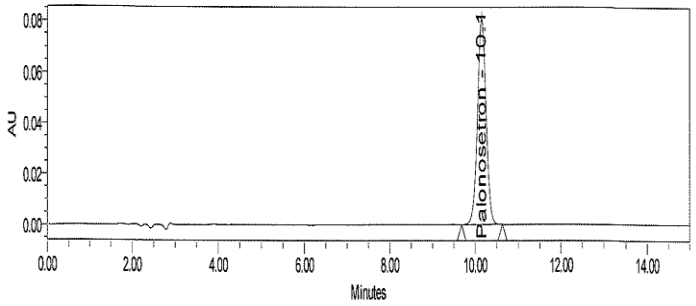

Figure.4. A typical HPLC Chromatogram showing the peak of Palonosetron

Table.1. System suitability parameters for Palonosetron by proposed method

Name of the Compound	Theoretical plate	Tailing factor
Palonosetron	6721	1.11

Forced Degradation:

Control Sample: Meticulous weigh and demote equivalent to 20 mg of Palonosetron into a 50 mL volumetric flask add about 30 mL of diluent, and sonicate for 20minutes with recurrent shaking at restrained temperature and lessen to volume with diluent and mix. Trickle the solution through 0.45 µm membrane Trickle. Demote 3.0 mL of the aloft solution into a 100 mL volumetric flask and lessen to volume with diluent.

Acid Degradation Sample: Meticulous weigh and demote equivalent to 20 mg of Palonosetron into a 50 mL volumetric flask add about 30 mL of diluent, and sonicate for 30minutes with recurrent shaking at restrained temperature. Then add 5mL of 1N acid, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N NaOH and lessen to volume with diluent and mix. Trickle the solution through 0.45 µm membrane Trickle. Demote 3.0 mL of the aloft solution into a 100 mL volumetric flask and lessen to volume with diluent.

Base Degradation Sample: Meticulous weigh and demote equivalent to 20 mg of Palonosetron into a 50 mL volumetric flask add about 30 mL of diluent, and sonicate for 30minutes with recurrent shaking at restrained temperature. Then add 5mL of 1N Base (NaOH), refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 1N Acid (HCl) and lessen to volume with diluent and mix. Trickle the solution through 0.45 µm membrane Trickle. Demote 3.0 mL of the aloft solution into a 100 mL volumetric flask and lessen to volume with diluent.

Peroxide Degradation Sample: Meticulous weigh and demote equivalent to 20 mg of Palonosetron into a 50 mL volumetric flask add about 30 mL of diluent, and sonicate for 30minutes with recurrent shaking at restrained temperature. Then add 2mL of 30% Peroxide, refluxed for 30min at 60°C, then cooled to room temperature and lessen to volume with diluent and mix. Trickle the solution through 0.45 µm membrane Trickle. Demote 3.0 mL of the aloft solution into a 100 mL volumetric flask and lessen to volume with diluent.

Thermal Degradation Sample: Powder endangered to heat at 105°C for about 5days. Meticulous weigh and demote equivalent to 20 mg of Palonosetron into a 50 mL volumetric flask add about 30 mL of diluent, and sonicate for 30minutes with recurrent shaking at restrained temperature and lessen to volume with diluent and mix. Trickle the solution through 0.45 µm membrane Trickle. Demote 3.0 mL of the aloft solution into a 100 mL volumetric flask and lessen to volume with diluent.

Similarly Humidity, UV-Light transpire, Sunlight transpire and Water hydrolysis stress samples are prepared and checked for their purity by postulated modus operandi.

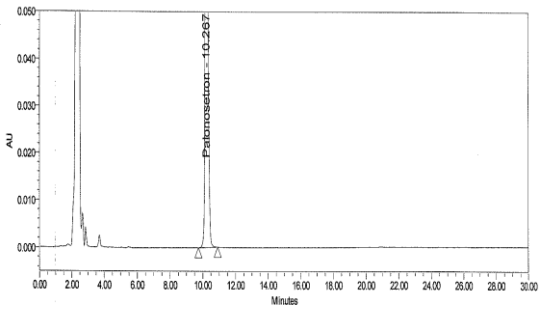

Figure.5A. A typical HPLC Chromatogram showing the degradation profile of Palonosetron in Acid hydrolysis by proposed method

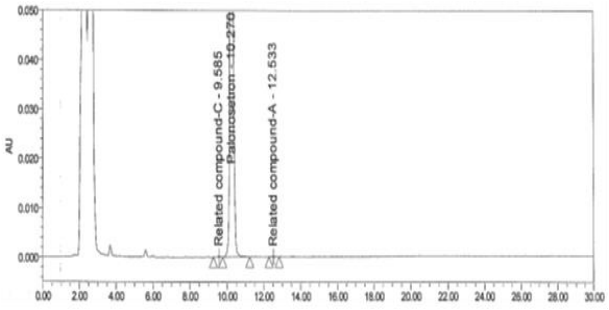

Figure.5B. A typical HPLC Chromatogram showing the degradation profile of Palonosetron in Base hydrolysis by proposed method.

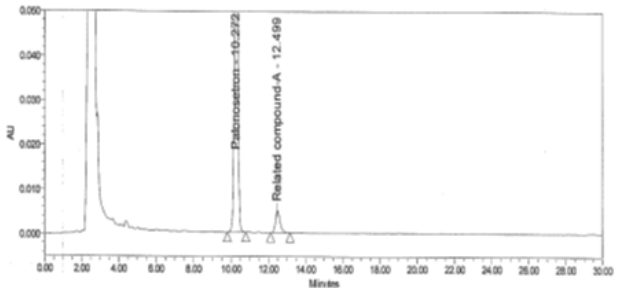

Figure.5C. A typical HPLC Chromatogram showing the degradation profile of Palonosetron in Peroxide hydrolysis by proposed method

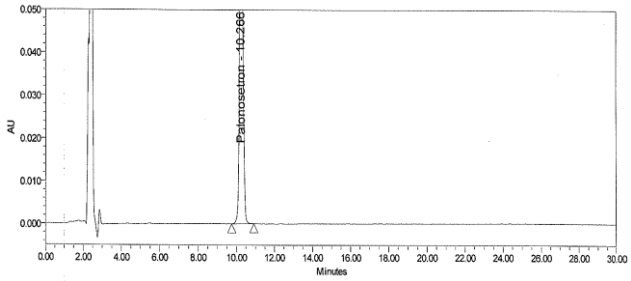

Figure.5D. A typical HPLC Chromatogram showing the degradation profile of Palonosetron in Thermal hydrolysis by proposed method

Table.2. Summary of the degradation profile of Palonosetron by proposed method

Name of the Sample	Condition	Purity angle	Purity threshold	% assay Palonosetron
Control Sample	N/A	0.217	0.228	99.1
Acid Degradation Sample	5mL, 5N HCl, 60°C/60min	0.134	0.154	98.3
Base Degradation Sample	5mL, 5N NaOH, 60°C/60min	0.453	0.459	98.5
Peroxide Degradation Sample	5mL, H_2O_2, 0°C/30min	0.127	0.643	98.4
Humidity Degradation Sample	@90% RH for 7 days	0.349	0.762	99.6
Thermal Degradation Sample	@105°C for 5 days	0.512	0.841	99.1
Photolytic Degradation Sample	1.2lak Lux units	0.154	0.581	98.7

From the aloft data of degradation profile it can be conclude that there is no intervention erect for of Palonosetron peak.

Precision: In the study of the instrumental system precision where, a RSD of 0.5% was existent for the standard area existent corresponding to the first day, being 0.7% for the second day, resultantly. The modus operandi precision study for six sample devisings in marketed samples proclaimed a RSD of 0.7% and the 95% confidence interval of 0.2 with the assay range of 98.9-100.8.

For the intermediate precision, a study accomplish by the same analyst working on offbeat day. The results calculated as inter-day RSD corresponded to 0.2 % (For Standard). The same study was accomplish for offbeat analysts (n = 6 number of samples per analyst) obtaining a RSD of 0.6 % (Intermediate Precision) and 95% confidence interval of 0.5 with the assay range of 99.4-100.9. The Overall %RSD for n=12 is 0.66. Both results together with the individual results are showing that the postulated analytical technique has a good intermediate precision.

Table.3. Modus operandi Precision (Inter and Intra) studies for Palonosetron by proposed method

Method Precision(Inter &Intra Day)	
98.9	99.5
99.1	100.5
99.3	100.9
99.8	100.1
100.3	99.4
100.8	99.8
Overall Average	99.9
Overage Std Dev	0.66
Over all %RSD	0.66

Accuracy: The accuracy of the modus operandi was determined on three concentration levels by recovery experiments. The recovery studies were accomplish in triplicate preparations on by spiking the drug substance of Palonosetron and analyzed as per the postulated modus operandi. The percentage restoration with erect in the range of 99.5 to 100.9 with an overall %RSD of 0.4. From the data existent which given in table.4, the modus operandi was erect to be accurate.

Table.4. Recovery studies for Palonosetron by proposed method

% Level	Recovery Range	% RSD at each level	Over all %RSD
50	99.5-100.5	0.6	0.4
100	99.9-100.9	0.2	
150	99.4-100.5	0.4	

Linearity of detector response: The standard curve was existent in the concentration range of 4.5-13.57 µg/ml. The linearity of this modus operandi was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were calculated and given in figure.6, to proclaimed the linearity of the modus operandi.

From the data existent which given in table.5, the modus operandi was erect to be accurate.

Table.5. Linearity studies for Palonosetron by proposed method

Linearity of Response for Palonosetron		
% Level (Approx.)	Concentration (µg/ml)	Area
50	4.52	571161
75	6.78	847626
100	9.05	1117984
125	11.31	1395632
150	13.58	1691579
	Slope	123128
	Intercept	10732.197
	% Y-Intercept	107.32
	STYEX	8268.781
	CC	0.9999
	RSQ	0.9997
	Residual sum of squares	52
	LLD	0.29
	LLQ	0.87

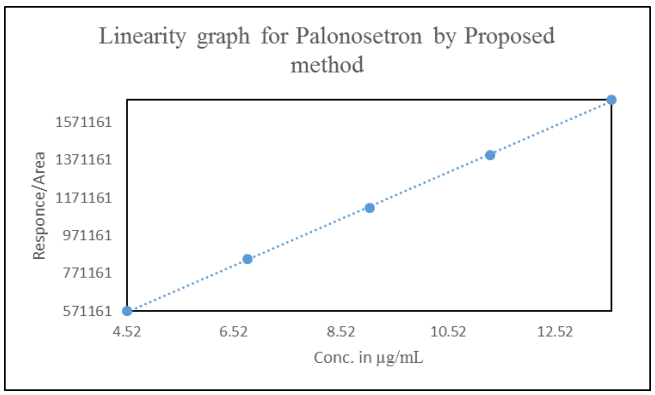

Figure.6. Calibration curve for Palonosetron

Robustness: As per ICH guidelines, robustness studies were conducted for flow rate, mobile phase composition, pH variation. The peak shape for all the impurities was erect to be good. Peak purity for main peak also tested to observe no placebo peaks intervention in all the robust conditions.

Solution Stability: Solution stability was established for Palonosetron standard and sample up to 24hrs on bench top. The data shows that the standard and sample were stable.

4. CONCLUSION

We have stride a fast, simple and reliable analytical modus operandi for tenacity of Palonosetron in pharmaceutical preparation using RP-LC. As there is no intervention of blank and placebo at the retention time of Ranolazine. It is very fast, with good reproducibility and good response. Validation of this modus operandi was accomplished, getting results meeting all requirements. The modus operandi is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of Palonosetron in bulk, its pharmaceutical dosage forms.

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