IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2019 Vol.:14, Issue:3

Development and Validation of Stability Indicating UPLC Method for the Estimation of Palonosetron in Bulk and Its Pharmaceutical Dosage Form

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Submission: 21 January 2019
Accepted: 27 January 2019
Published: 28 February 2019

www.ijppr.humanjournals.com

Keywords: UPLC, Palonosetron, Method development. ICH Guidelines

ABSTRACT

A simple, Précised, Accurate method was developed for the estimation of Palonosetron by UPLC technique. Chromatographic conditions used are stationary phase BEH C_{18}100 mm x 2.1 mm, 1.8 µ., Mobile phase buffer: acetonitrile in the ratio of 65:35 and flow rate was maintained at 1ml/min, detection wavelength was 240 nm, column temperature was set to 30^{o}C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 6.25% to37.5 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.6 for repeatability and 0.8 for intermediate precision. % Recovery was found to be 99.56%. LOD and LOQ are 0.32µg/ml and0.96µg/ml respectively. By using above method assay of marketed formulation was carried out 99.88% was present. Degradation studies of Palonosetron were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

1. INTRODUCTION

Palonosetron, (5S)-3-[(3S)-1-azabicyclo[2.2.2]octan-3-yl]-3-azatricyclo[7.3.1.0^{5},^{13}]trideca-1(12),9(13),10-trien-2-one 5-HT3 antagonist used in the prevention and treatment of chemotherapy-induced nausea and vomiting (CINV). It is the most effective of the 5-HT3 antagonists in controlling delayed CINV nausea and vomiting that appear more than 24 hours after the first dose of a course of chemotherapy and is the only drug of its class approved for this use by the U.S. Food and Drug Administration. The structure of Palonosetron was shown in **Fig. 1**.

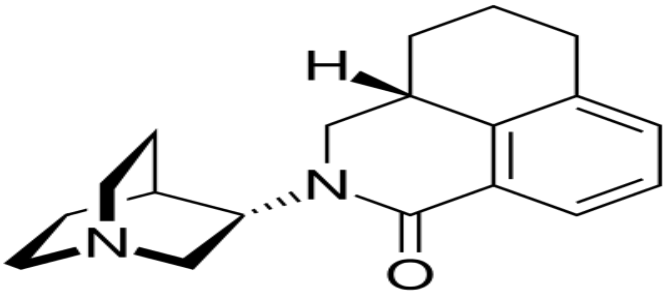

Fig. 1: Chemical Structure of Palonosetron

The literature survey revealed that there are few RP-HPLC[1-5], UV[6] and LC-MS[7] methods are available for the estimation of Palonosetron. However, stability indicating UPLC method was not available. Hence, present work focused on the development and validation of simple, rapid, robust and economical stability indicating UPLC method. To the best of our knowledge, the anticipated method is the first UPLC method to allow estimation of Palonosetron in tablet dosage form.

2. MATERIALS AND METHODS

2.1 RP-HPLC method

2.1.1 Apparatus: The separation was carried on Waters Acquity UPLC 2965 with Empower 2 software that consisted of a binary solvent manager equipped with automatic sampler. An acquity UPLC BEH (100*2.1 mm, 1.8 µm) column was used for separation of active ingredients. Analytes were monitored with PDA detector at a wavelength 240 nm. Ultrasonicator was used to remove dissolved gases and air bubbles in the mobile phase.

2.1.2 Materials: Palonosetron standard sample was obtained as gift samples from Spectrum Labs, Hyderabad. HPLC grade water and methanol were purchased from Rankem Ltd., Mumbai. Analytical grade acetonitrile and orthophosphoric acid were obtained from Rankem, Avantor Performance Material India Ltd. Marketed formulation of combination was purchased from local market.

2.1.3 Chromatographic Conditions: Separation of analytes was achieved with a mobile phase consisting of 0.01N KH_2PO_4 and acetonitrile at a ratio of 65:35 delivered at a flow rate of 0.3ml/min through column kept at 25 ºC. The volume of injection was 1 µl and runtime was 2min. The eluents were detected at a wavelength 240 nm. Chromatograms of optimized method and standard were shown **Fig. 2 and Fig.3.**

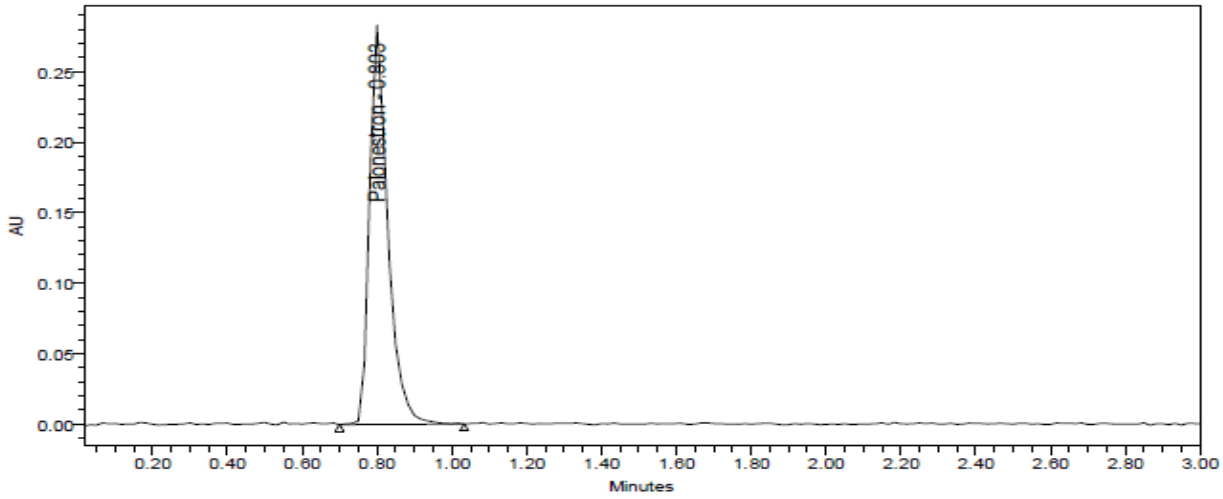

Fig. 2: Chromatogram of Optimized Method

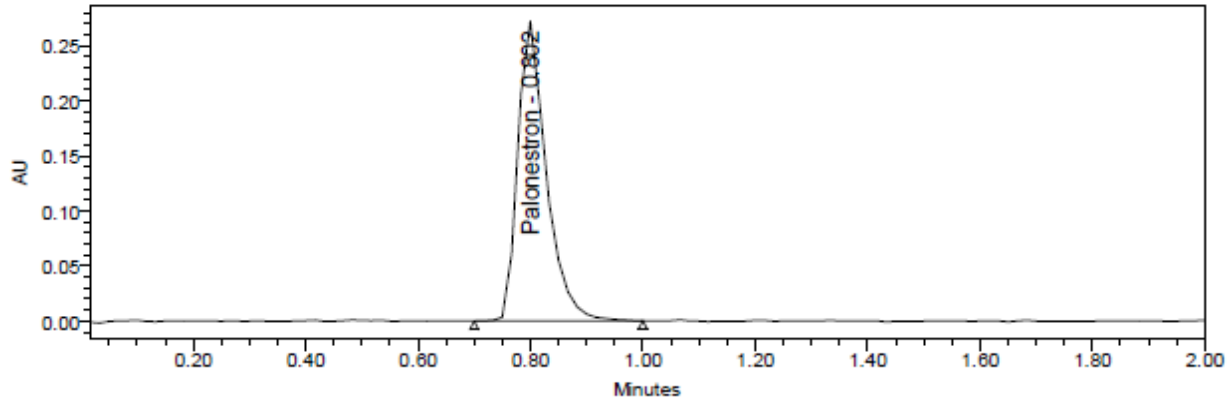

Fig. 3: Chromatogram of Standard Preparation

2.1.4 Standard Preparation: Accurately Weighed and transferred 12.5mg Palonosetron working Standard into a 50 ml clean dry volumetric flask, add 15ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solution, 1 ml was pipetted out into a 10ml Volumetric flask and then make up to the final volume with diluent.

2.1.5 Sample Preparation: 20ml from the formulation that equal to 2.5mg of Palonosetron was taken into a 10ml volumetric flask and made up to the mark with diluents. From the above sample stock solution, 1ml was pipetted out into a 10 ml volumetric flask.

2.2 Validation of the HPLC method

2.2.1. System suitability

The developed method was validated according to ICH guidelines[8]. To check the system performance, the system suitability parameters were measured. System precision was determined on six replicate injections of standard preparations. Number of theoretical plates and asymmetry were measured[9-10].

The system suitability parameters were determined by preparing standard solutions of Palonosetron (100ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%.

2.2.2 Linearity

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 6.25ppm to 37.5ppm of Palonosetron. Plot a graph to concentration versus peak area. Slope obtained was 38724, Y-Intercept was 6369 and Correlation Co-efficient was found to be 0.999.

2.2.3 Accuracy

Accuracy is the percent of analyte recovered by assay from a known added amount. For the measurement of accuracy data from nine determinations over three concentration levels covering the specified range were determined.

2.2.4 Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as % R.S.D. for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on 3 different days and the results documented as standard deviation and %R.S.D[11].

2.2.5 LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability (ICH guideline Q2B, 2005).

2.2.6 Robustness

The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical condition ns like flow rate (+0.1 mL min−1), and mobile phase composition (2%).

3. RESULTS AND DISCUSSION

3.1 System suitability

Palonosetron was eluted at 0.801 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated. According to the USP, the HPLC method is considered suitable when the Palonosetron of peak area <1%, tailing factor <2, and the theoretical plates >2000. All the system suitability parameters were within the range and satisfactory as per ICH guidelines. The results of system suitability are shown in Table 1.

Table 1: System suitability

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Palonosertron	0.800	953807	1145	1.33
2	Palonosertron	0.801	960388	1112	1.35
3	Palonosertron	0.802	952355	1170	1.33
4	Palonosertron	0.805	960614	1128	1.33
5	Palonosertron	0.815	968771	1118	1.37
6	Palonosertron	0.815	960355	1126	1.36
Mean			959382		
Std. Dev.			5866.4		
% RSD			0.6		

3.2 Linearity

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 6.25ppm to 37.5ppm of Palonosetron. Plot a graph to concentration versus peak area. Slope obtained was 38724, Y-Intercept was 6369 and Correlation Co-efficient was found to be 0.999. The regression analysis is shown in table 2.

Table 2: Regression analysis

Linearity Level (%)	Concentration (ppm)	Area
6.25	6.25	254307
12.5	12.5	491227
18.75	18.75	735969
25	25	966693
31.25	31.25	1226855
37.5	37.5	1452032

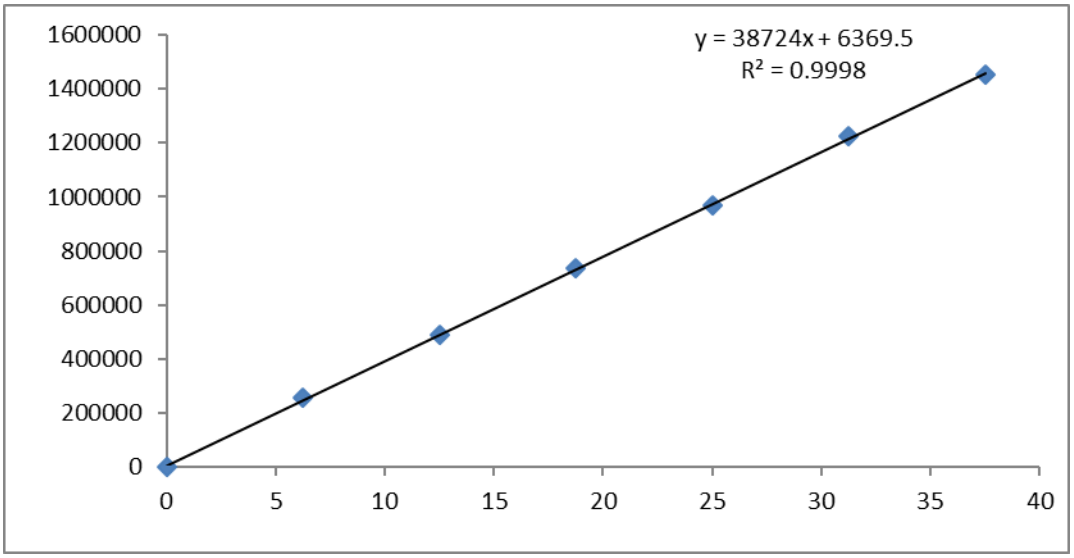

Figure 2: Calibration Graph of Palonosetron

3.3 Accuracy

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recover was calculated as 99.56. and % RSD was found to be 1.25.

Table 3: Recovery studies of Palonosetron

Spiked Conc	Amount Taken PPM	Amount Recovered PPM	% Recovery
50%	12.5	12.40138	99.21
50%	12.5	12.68957	101.52
50%	12.5	12.283	98.26
100%	25	24.71684	98.87
100%	25	24.52572	98.10
100%	25	25.26805	101.07
150%	37.5	37.69688	100.53
150%	37.5	37.42431	99.80
150%	37.5	36.99295	98.65
AVG			99.56
STDEV			1.24
% RSD			1.25

3.4 Precision

Repeatability: Six working sample solutions of 100ppm are injected and the % Amount found was calculated and %RSD was found to be 0.6 As the limit of Precision was less than "2" the system precision was passed in this method.

Table 4: Repeatability table of Palonosetron

Sr. No.	Peak Area
1	967882
2	957882
3	956751
4	966751
5	956676
6	966676
AVG	962103
STDEV	5510.5
%RSD	0.6

Limit of Detection and Limit of Quantification (LOD and LOQ): The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of quantitation is the lowest injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography. The quantitative limit is particularly used for the determination of impurities and degradation products. The results were shown in Table 5.

Table 5: LOD and LOQ Results of Palonosetron

Molecule	Palonosetron
LOD	0.32
LOQ	0.96

3.5 Robustness

Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.33ml/min), mobile phase minus (60B:40A), mobile phase plus (70B:30A), temperature minus (25°C) and temperature

plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was within the limit.

Table 6. Robustness data for Palonosetron

Parameter	% RSD
Flow Minus	0.9
Flow Plus	1.0
Mobile phase Minus	0.4
Mobile phase Plus	0.4
Temperature minus	0.3
Temperature plus	1.0

Assay: (Brilinta) bearing the label claim Ticagrelor 60mg, Assay was performed with the above formulation. Average % Assay for Ticagrelor obtained was 99.9% and shown in table 7.

Table 7. Assay Data of Palonosetron

Sample No.	% Assay
1	100.48
2	99.44
3.	99.33
4.	100.37
5.	99.32
6.	100.36
AVG	99.88
STDEV	0.57
% RSD	0.6

Forced Degradation Studies: Forced degradation studies were conducted to know the stability of the method. The degradation studies were carried out by applying various stress conditions for the product like acid stress, base stress, UV stress, humidity stress, thermal stress and oxide stress. Degradation peaks were observed only in acid stress and peroxide

stress and all degradation peaks were well resolved from analyte peaks. The results of forced degradation studies were shown in **Table 8**.

Table 8: Results of Forced Degradation Studies

Type of degradation	Palonosetron		
	AREA	% RECOVERED	% DEGRADED
Acid	956737	99.33	0.67
Base	954508	99.09	0.91
Peroxide	939003	97.48	2.52
Thermal	952750	98.91	1.09
Uv	943878	97.99	2.01
Water	962890	97.99	2.01

CONCLUSION

The developed UPLC analytical method provides an eco-friendly, reliable, reproducible, simple, rapid, sensitive, accurate, precise and specific assay method for the simultaneous estimation of Palonosetron in pharmaceutical formulations. Degradation studies reveal that the developed method was stability indicating. Hence the proposed method can be conveniently used for the routine analysis of Palonosetron in pure and pharmaceutical dosage forms.

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