METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF NETUPITANT AND PALONOSETRON IN BULK FORM AND MARKETED COMBINED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

Analytical Method Development and Validation for Netupitant and Palonosetron in bulk and Combine Dosage Form by RP-HPLC, New method was established for simultaneous estimation of Netupitant and Palonosetron by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Netupitant and Palonosetron by using Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size, flow rate was 1.0 ml/min, mobile phase ratio was Methanol: Tri Ethyl Amine Buffer (35:65% v/v), detection wavelength was 261nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. The retention times were found to be 2.256mins and 5.427mins. The % purity of Netupitant and Palonosetron was found to be 99.72%. The system suitability parameters for

Netupitant and Palonosetron such as theoretical plates and tailing factor were found to be 7589, 1.08 and 6354 and 1.04, the resolution was found to be 5.85. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Netupitant and Palonosetron was found in concentration range of 60µg-140µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.36% and 100.15%, %RSD for repeatability was 0.212 and 0.064, % RSD for intermediate precision was 0.611 and 0.296 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.63 and 7.92, and LOQ value was 3.84 and 11.54 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Netupitant and Palonosetron in API and Pharmaceutical dosage form.

Keywords: Netupitant and Palonosetron, Method Development, Validation, Accuracy.

INTRODUCTION

Netupitant is an antiemetic drug approved by the FDA in October 2014 for use in combination with palonosetron for the prevention of acute and delayed vomiting and nausea associated with cancer chemotherapy including highly emetogenic chemotherapy. Netupitant[1] is a neurokinin 1 receptor antagonist. The combination drug is marketed by Eisai Inc. and Helsinn Therapeutics (U.S.) Inc. under the brand Akynzeo. Delayed emesis (vomiting) has been largely associated with the activation of tachykinin family neurokinin 1 (NK1) receptors (broadly distributed in the central and peripheral nervous systems) by substance P. As shown in in vitro and in vivo studies, Netupitant[2] inhibits substance P mediated responses. It is used to prevent nausea and vomiting caused by cancer drug treatment (chemotherapy). Netupitant[3] and Palonosetron work by blocking natural substances in the body (such as substance P/neurokinin 1, serotonin) that cause vomiting. The IUPAC Name of Netupitant is 2-[3,5-bis(trifluoromethyl)phenyl]-N,2-dimethyl-N-[4-(2-methylphenyl)-6-(4-methylpiperazin-1-yl) pyridin-3-yl] propanamide. The Chemical Structure of Netupitant is as following

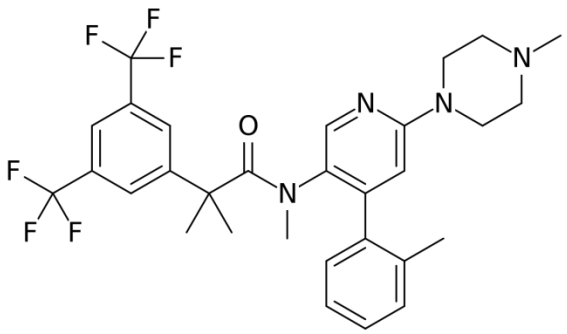

Fig-1: Chemical Structure of Netupitant

Palonosetron (INN, trade name Aloxi) is a 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. As of 2008, it is the most recent 5-HT3 antagonist to enter clinical use. Palonosetron[4] is an anti-nauseant and antiemetic agent indicated for the prevention of nausea and vomiting associated with moderately-emetogenic cancer chemotherapy and for the prevention of postoperative nausea and vomiting. Palonosetron is a highly specific and selective serotonin 5-HT_3 receptor antagonist that is pharmacologically related to other 5-HT_3 receptor antagonists, but differs structurally. Palonosetron[5] has a high affinity for 5-HT_3 receptors, but has little to no affinity for other receptors. The serontonin 5-HT_3 receptors are located on the nerve terminals of the vagus in the periphery, and centrally in the chemoreceptor trigger zone of the area postrema. It is suggested that chemotherapeutic agents release serotonin from the enterochromaffin cells of the small intestine by causing degenerative changes in the GI tract. The serotonin then stimulates the vagal and splanchnic nerve receptors that project to the medullary vomiting center, as well as the 5-HT3 receptors in the area postrema, thus initiating the vomiting reflex, causing nausea and vomiting. The IUPAC Name of Palonosetron[6] is (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. The Chemical Structure of Palonosetron is as follows

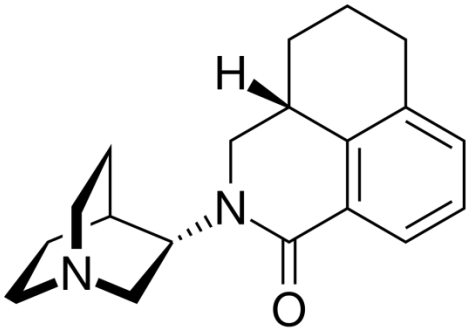

Fig-2: Chemical Structure of Palonosetron

MATERIALS AND METHODS

Table-1: Instruments used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module. 996 PDA detector, software: Empower 2
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Digital ultra sonicator	Labman

Table-2: Chemicals used

S.No	Chemical	Brand Names
1	Netupitant	Akynzeo
2	Palonosetron	Akynzeo
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Netupitant and Palonosetron working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 1ml of the above Netupitant and 3ml of Palonosetron stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Netupitant and Palonosetron sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1ml of Netupitant and 3ml Palonosetron above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[30-31].

Preparation of Mobile Phase:

Accurately measured 350ml (35%) of Methanol, 650ml of Tri Ethyl Amine Buffer (65%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration[7].

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Netupitant and 3ml of Palonosetron from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1ml of Netupitant and 3ml of Palonosetron from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Take average weight of the Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Netupitant and Palonosetron sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1ml of Netupitant and 3ml Palonosetron above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay[8] by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (60ppm of Netupitant & 100ppm of Palonosetron):

Pipette out 0.6ml of Netupitant and 1ml of Palonosetron stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80ppm of Netupitant & 200ppm of Palonosetron):

Pipette out 0.8ml of Netupitant and 2ml of Palonosetron stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (100ppm of Netupitant & 300ppm of Palonosetron):

Pipette out 1ml of Netupitant and 3ml of Palonosetron stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (120ppm of Netupitant & 400ppm of Palonosetron):

Pipette out 1.2ml of Netupitant and 4ml of Palonosetron stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (140ppm of Netupitant & 500ppm of Palonosetron):

Pipette out 1.4ml of Netupitant and 5ml of Palonosetron stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system[9] and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[10].

Precision

Repeatability

Preparation of Netupitant and Palonosetron Solution for Precision:

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Netupitant and 3ml of Palonosetron from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The sample solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision[11] (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1:

The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2:

The sample solution was injected for six times and measured the area for all six injections in HPLC. The %RSD[12] for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Sample Stock solution:

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.5ml of Netupitant and 1.5ml of Palonosetron from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Sample Stock solution:

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Netupitant and 3ml of Palonosetron from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Sample Stock solution:

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working Sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5ml of Netupitant and 4.5ml of Palonosetron from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses.

Calculate the Amount found and Amount added for Netupitant and Palonosetron and calculate the individual recovery and mean recovery values[13].

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:

Accurately weigh and transfer 10 mg of Netupitant and 10mg of Palonosetron working sample into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1ml of Netupitant and 3ml of Palonosetron from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Tri Ethyl Amine (35:65% v/v) was taken in the ratio and 40:60, 30:70 instead (35:65% v/v) remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Method Development

Optimized Chromatogram

Mobile phase : Methanol: Tri Ethyl Amine Buffer (35:65% v/v)

Column : Phenomenex Luna C18 (4.6mm×150mm, 5µm) Particle size

Flow rate : 1 ml/min

Wavelength : 261 nm

Column temp : 38ºC

Injection Volume : 10 µl

Run time : 10 minutes

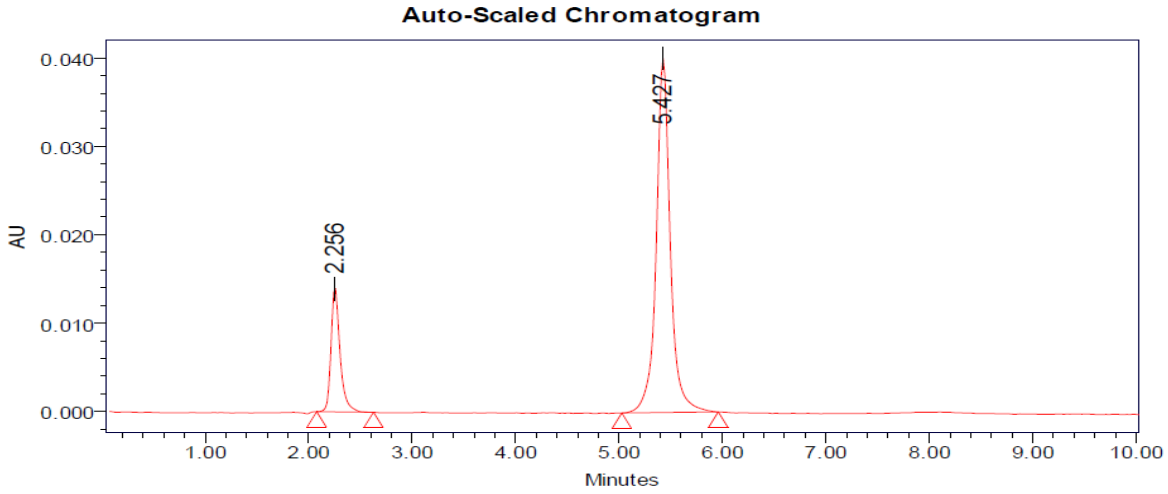

Fig-3: Optimized Chromatographic Condition

Validation of Analytical Method

The method was validated for linearity, accuracy, precision and limit of detection, and limit and quantitation.

System Suitability

Tailing factor for the peaks due to Netupitant and Palonosetron in Standard solution should not be more than 2.0. Theoretical plates for the Netupitant and Palonosetron peaks in Standard solution should not less than 2000.

Table-3: Results of System Suitability for Netupitant

S.No.	Name	Rt	Peak Area	Height	USP plate Count	USP Tailing
1	Netupitant	2.247	105698	18652	7592	1.08

2	Netupitant	2.246	105874	18754	7584	1.09
3	Netupitant	2.248	105698	18698	7562	1.08
4	Netupitant	2.252	105465	18689	7549	1.08
5	Netupitant	2.248	105236	18695	7591	1.09
Mean			**105594.2**			
Std. Dev			**247.4049**			
% RSD			**0.234298**			

Table-4: Results of System Suitability for Palonosetron

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Palonosetron	5.452	1856985	63659	6359	1.05	5.86
2	Palonosetron	5.484	1856754	63598	6384	1.04	5.85
3	Palonosetron	5.491	1856985	63845	6395	1.05	5.86
4	Palonosetron	5.482	1856574	63989	6345	1.04	5.86
5	Palonosetron	5.491	1854735	63895	6395	1.05	5.85
Mean			**1856407**				
Std. Dev			**950.2696**				
% RSD			**0.051189**				

Specificity

The ICH documents define specificity[14] as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method[15] was tested for specificity to measure accurately quantitates Netupitant and Palonosetron in marketed formulation.

Table-5: Peak Results for Assay Standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Netupitant	2.256	105865	18569		1.05	7598	1
2	Palonosetron	5.427	1856982	63658	5.86	1.09	6359	1
3	Netupitant	2.249	105874	18698		1.04	7564	2
4	Palonosetron	5.430	1856475	63458	5.85	1.08	6375	2
5	Netupitant	2.248	105698	185695		1.05	7549	3
6	Palonosetron	5.443	1856985	63598	5.86	1.09	7369	3

Table-6: Peak Results for Assay Sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Netupitant	2.247	116532	19865		1.08	7659	1
2	Palonosetron	5.452	196585	19724	5.89	1.05	6359	1
3	Netupitant	2.246	116859	19356		1.09	7625	2
4	Palonosetron	5.461	196898	19874	5.88	1.04	6349	2
5	Netupitant	2.243	116758	19862		1.09	7643	3
6	Palonosetron	5.466	196898	19453	5.89	1.05	6347	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity[16] of Netupitant and Palonosetron in pharmaceutical dosage form (marketed formulation) was found to be 99.72%.

Linearity

Working dilutions of Netupitant and Palonosetron in the concentration ranges of 60-140µg/ml and 100-500µg/ml was prepared by taking suitable aliquots of working standard solutions of drug in different 10ml volumetric flask and diluting up to the mark with mobile phase. 10µl quantity of each dilutions was injected into the column at a flow rate of 1.0ml/min. The drugs in the elutes was monitored at 261nm. The calibration curve[17,18] of analytical method was assessed by plotting concentration versus peak area and represented graphically in Figure 4 and 5 and shown in Table 7 and 8.

Table-7: Chromatographic Data for Linearity Study of Netupitant

Concentration µg/ml	**Average Peak Area**
60	648743
80	856982
100	1068542
120	1268984
140	1469853

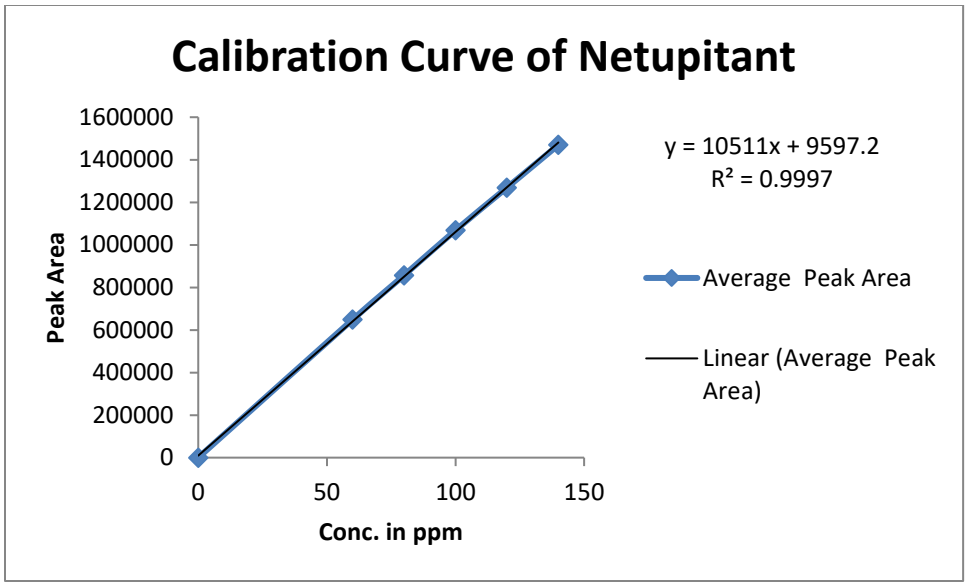

Fig-4: Calibration Graph for Netupitant

Linearity Plot:

The plot of Concentration[19] (x) versus the Average Peak Area (y) data of Netupitant is a straight line.

Y = mx + c

Slope (m) = 10511

Intercept (c) = 9597

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity[20] is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 9597. These values meet the validation criteria.

Table-8: Chromatographic Data for Linearity Study of Palonosetron

Concentration µg/ml	Average Peak Area
100	667564
200	1268547
300	1868598
400	2465487
500	3085864

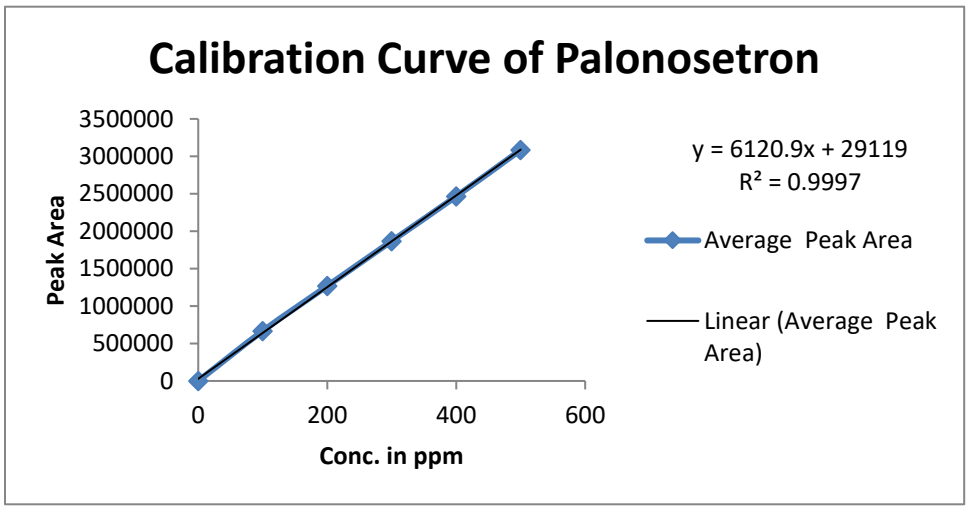

Fig-5: Calibration Graph for Palonosetron

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Palonosetron is a straight line.

$Y = mx + c$

Slope (m) = 6120

Intercept (c) = 29119

Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 29119. These values meet the validation criteria.

Precision:

The instrument precision[21] was evaluated by determining the absorbance of the standard solution five times repeatedly. The results are reported in terms of relative standard deviation[22]. The intra- and inter-day variation[23] for the determination was carried out in triplicate for the standard solution.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-9: Results of Repeatability for Netupitant

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Netupitant	2.269	105698	18569	7598	1.08
2	Netupitant	2.255	105684	18547	7546	1.09
3	Netupitant	2.252	105421	18594	7549	1.09
4	Netupitant	2.267	105879	18574	7538	1.08
5	Netupitant	2.260	105326	18563	7582	1.08
Mean			105601.6			
Std. Dev			224.5023			

% RSD			0.212594			

Table-10: Results of Method Precision for Palonosetron

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Palonosetron	5.274	1856985	63598	6359	1.05	5.86
2	Palonosetron	5.266	1857458	63579	6357	1.04	5.85
3	Palonosetron	5.265	1854795	63547	6358	1.04	5.86
4	Palonosetron	5.278	1857469	63592	6357	1.05	5.86
5	Palonosetron	5.305	1857685	63569	6345	1.04	5.85
Avg			1856878				
Std. Dev			1192.4				
% RSD			0.064215				

Intermediate Precision:

Day 1:

Table-11: Results of Intermediate Precision for Netupitant

S. No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Netupitant	2.248	115246	19685	7698	1.09
2	Netupitant	2.245	116985	19654	7685	1.09
3	Netupitant	2.242	115847	19675	7645	1.09
4	Netupitant	2.239	116985	19682	7682	1.09

5	Netupitant	2.243	115848	19654	7691	1.09
6	Netupitant	2.246	116582	19647	7642	1.10
Mean			**116248.8**			
Std. Dev			**710.3091**			
% RSD			**0.611025**			

Table-12: Results of Intermediate Precision for Palonosetron

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Palonosetron	5.284	1948592	64582	6459	1.05	5.96
2	Palonosetron	5.293	1958245	64256	6475	1.06	5.95
3	Palonosetron	5.306	1947584	64598	6498	1.05	5.96
4	Palonosetron	5.319	1948675	64785	6472	1.06	5.95
5	Palonosetron	5.346	1959854	64585	6493	1.05	5.96
6	Palonosetron	5.352	1958246	64924	6438	1.06	5.96
Mean			**1953533**				
Std. Dev			**5792.661**				
% RSD			**0.296522**				

Day 2:

Table-13: Results of Intermediate precision Day 2 for Netupitant

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Netupitant	2.255	102658	62584	6259	1.03
2	Netupitant	2.260	102856	62359	6276	1.02
3	Netupitant	2.242	102658	62451	6215	1.03
4	Netupitant	2.245	102698	62584	6285	1.02
5	Netupitant	2.260	102451	62758	6235	1.03
6	Netupitant	2.255	102368	62154	6298	1.02
Mean			**102614.8**			
Std. Dev			**176.9592**			
% RSD			**0.17245**			

Table-14: Results of Intermediate precision for Palonosetron

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Palonosetron	5.266	1798952	62859	6265	1.03	5.42
2	Palonosetron	5.265	1789854	62985	6289	1.02	5.43
3	Palonosetron	5.306	1798659	62895	6279	1.03	5.42
4	Palonosetron	5.293	1789898	62785	6285	1.02	5.43
5	Palonosetron	5.265	1796856	62354	6249	1.03	5.42

6	Palonosetron	5.266	1798568	62589	6245	1.02	5.43
Mean			**1795465**				
Std. Dev			**4390.879**				
% RSD			**0.244554**				

Accuracy:

The accuracy[24] of the method was assessed by determination of the recovery[25] of the method at 3 different concentrations (50%, 100% and 150% concentration) by addition of known amount of standard to the placebo. For each concentration three sets were prepared.

Table-15: The Accuracy Results for Netupitant

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	539070	50	50.373	100.746%	100.36%
100%	1063578	100	100.274	100.274%	
150%	1587149	150	150.085	100.056%	

Table-16: The accuracy results for Palonosetron

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	949127	150	150.328	100.218%	100.15%

100%	1867824	300	300.441	100.147%	
150%	2785321	450	450.359	100.079%	

Limit of Detection & Quantification:

LOD and LOQ[26] were calculated using following equation as per ICH guidelines. LOD[27] = 3.3 × σ/ S and LOQ = 10×σ /S, where σ is the standard deviation of response and S is the slope of the calibration curve.

LOD:

Netupitant: 2.63µg/ml

Palonosetron: 3.84µg/ml

LOQ:

Netupitant: 7.92µg/ml

Palonosetron: 11.54µg/ml

Robustness:

Robustness[28] of the method was done by the deliberate changes in flow rate, column oven temperature, and mobile phase composition[29] and wave length.

Table-17: Results of Robustness Test for Netupitant

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	105265	2.256	7589	1.08
Less Flow rate of 0.9 mL/min	109898	2.505	7256	1.05
More Flow rate of 1.1 mL/min	102365	2.046	7469	1.07

Less organic phase	101548	2.505	7358	1.06
More organic phase	104645	2.046	7659	1.02

Table-18: Results of Robustness Test for Palonosetron

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1858475	5.427	6354	1.04
Less Flow rate of 0.9 mL/min	1925684	5.599	6253	1.05
More Flow rate of 1.1 mL/min	1863525	4.576	6248	1.03
Less organic phase	1825471	5.599	6415	1.02
More organic phase	1836594	4.576	6529	1.06

SUMMARY AND CONCLUSION

The developed HPLC method for the estimation of selected drug is simple, rapid, accurate, precise, robust, and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive, and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested noninterference of formulation excipients in the estimation and can be used in laboratories for the routine analysis of selected drugs. Since the system validation parameters of HPLC method used for estimation of selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose. The present work concluded that assay method by RP-HPLC was simple, accurate, precise, and specific and has no interference with the place and degradation products. Hence, these can be used for routine analysis of Netupitant and Palonosetron.

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