CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

DEVELOPMENT AND VALIDATION OF A RP - HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF NETUPITANT AND PALONOSETRON IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Abstract:
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 Inertsil C18 (4.6mm ×250mm, 5µm particle size), flow rate was 1.0 ml/min, mobile phase ratio was (55:45% v/v) Methanol: Phosphate buffer pH 4.8 (pH was adjusted with ortho phosphoricacid), detection wavelength was 282nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. The retention times were found to be 1.688mins and 3.282mins. The % purity of Netupitant and Palonosetron was found to be 99.86%. The system suitability parameters for Netupitant and Palonosetron such as theoretical plates and tailing factor were found to be 7586, 1.69 and 6235 and 1.58, the resolution was found to be 10.85. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Netupitant and Palonosetron was found in concentration range of 100µg-500µg and 30µg-70µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.112% and 100.16%, %RSD for repeatability was 0.1702 and 0.043 respectively. The precision study was precise, robust, and repeatable. The LOD value was found to be 2.1µg/ml and 1.28µg/ml, and LOQ value was 6.3µg/ml and 3.84µg/ml for Netupitant and Palonosetron respectively. The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Netupitant and Palonosetron in pharmaceutical dosage form.

Keywords: *Netupitant, Palonosetron, RP-HPLC, Simultaneous estimation.*

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Please cite this article in press ***Angadi Nagesh*** *et al,* ***Development And Validation Of A RP - HPLC Method For The Simultaneous Determination Of Netupitant And Palonosetron In Pure And Pharmaceutical Dosage Form.,****Indo Am. J. P. Sci, 2023; 10(03).*

INTRODUCTION:

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.[1-3] IUPAC name (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. Molecular weight is 296.4. Molecular formula is $C_{19}H_{24}N_2O$. Palanosetron was found to be is easily soluble in water, soluble in propylene glycol, and slightly soluble in ethanol and isopropyl alcohol, Soluble in Methanol.

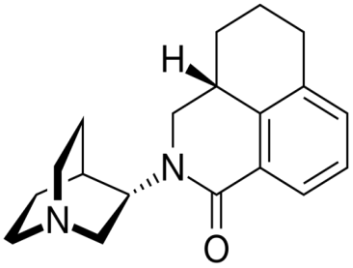

Figure 1: Structure of Palanosetron

Netupitant is an antiemitic 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 is a neurokinin 1 receptor antagonist. The combination drug is marketed by Eisai Inc. and Helsinn Therapeutics (U.S.) Inc. under the brand Akynzeo.[4-6] IUPAC name 2-[3,5-bis(trifluoromethyl)phenyl]-N,2-dimethyl-N-[4-(2-methylphenyl)-6-(4-methylpiperazin-1-yl) pyridin-3-yl] propenamide. Molecular weight is 578.603g/mole. Molecular formula is $C_{30}H_{32}F_6N_4O$. Netupitant was found to be Soluble in DMSO. It is very slightly soluble in water and freely soluble in a range of organic solvents such as acetone, toluene, and methanol, soluble in isopropanol.

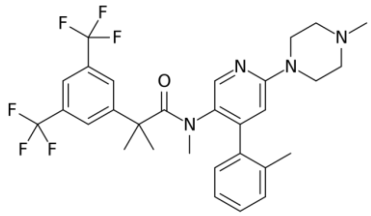

Figure 2: Structure of Netupitant

The literature survey revealed that There are very few methods reported in the literature for analysis of Netupitant and Palonosetron alone or in combination with other drugs in the pure form and pharmaceuticals formulations by HPLC.[7-11] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Netupitant and Palonosetron in API and Pharmaceutical dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Tamsulosin and Dutasteride. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Netupitant and Palonosetron in API and Pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of Netupitant and Palonosetron in API and Pharmaceutical dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Netupitant and Palanosetron were obtained as a gift sample from sura training lab, Hyderabad. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 282 nm with column Phosphate Buffer (pH-4.8): Methanol (55:45% v/v), dimensions at 35^{0}C temperature. The optimized mobile phase consists of. Flow rate was maintained at 1 ml/min and run time for 6 min.

Preparation of solutions:

Preparation of mobile phase: Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation:

Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of Phosphate Buffer (55%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Preparation of the Netupitant and Palanosetron standard solution:

Preparation of standard solution: (Netupitant)

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

Preparation of standard solution: (Palanosetron)

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

Further pipette 3ml of Netupitant, 0.5ml of Palanosetron from stock solutions in to 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.

Preparation of Sample Solution:

Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Netupitant, Palanosetron 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.

Procedure:

Further pipette 1.2ml of Netupitant, Palanosetron from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS C 18 column (4.6 x 250mm, 5µm), the mobile phase of composition Phosphate Buffer (pH-4.8): Methanol (55:45% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Netupitant and Palanosetron in their pharmaceutical dosage form. The result obtained for Netupitant and Palanosetron was comparable with the corresponding labeled amounts and they were shown in Table-2.

RESULTS AND DISCUSSION:

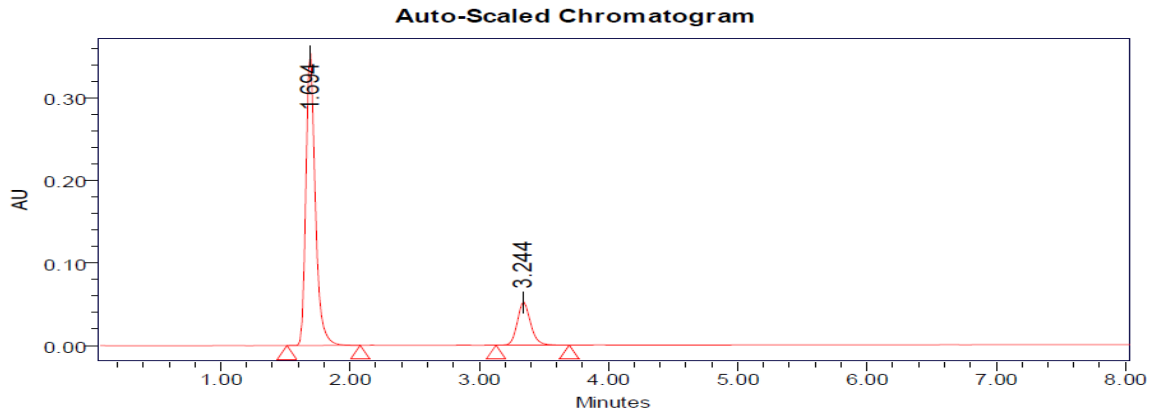

Figure 3: Standard chromatogram

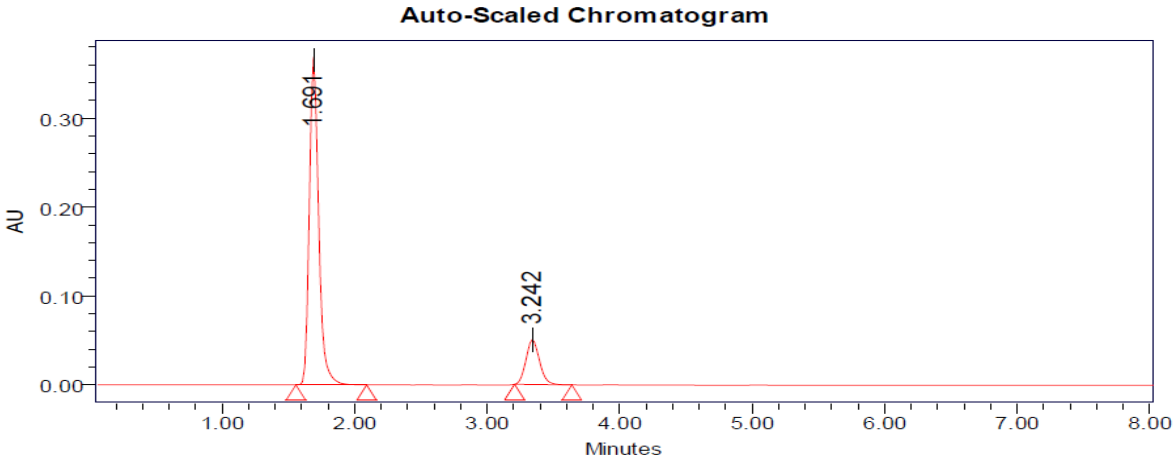

Figure 4: Sample chromatogram

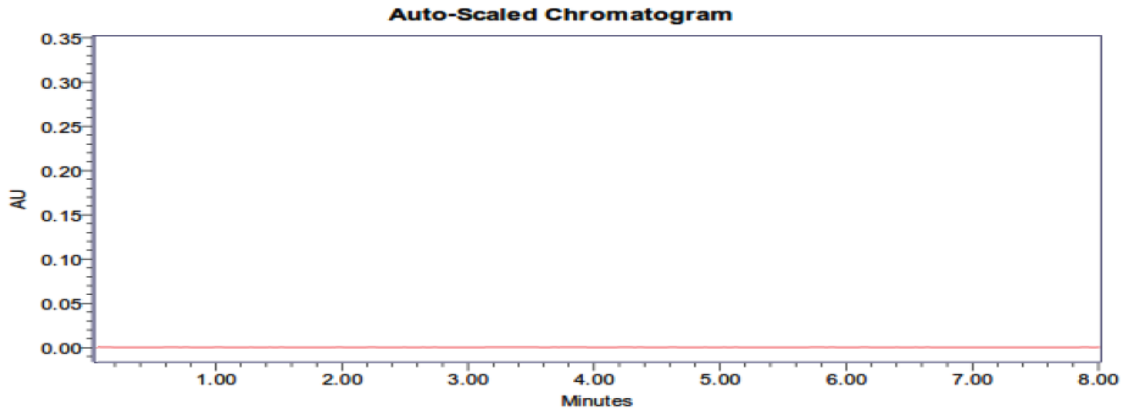

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Netupitant	Palanosetron
Retention time	1.688	3.282
USP Plate count	7586	6235
USP Tailing	1.69	1.58

Table 2: Assay results for Netupitant and Palanosetron

	Label Claim (mg)	% Assay
Netupitant	80	99.86
Palanosetron	20	99.86

Linearity: The linearity study was performed for the concentration of 100ppm to 500ppm and30 ppm to 70 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system 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. The resulte are shown in table 3.

Table 3: Linearity results for Netupitant and Palanosetron

Netupitant		Palanosetron	
Concentration(µg/ml)	**Area**	**Concentration(µg/ml)**	**Area**
100	585985	30	268764
200	1182468	40	356958
300	1768785	50	445631
400	2326852	60	535186
500	2856874	70	624698
Correlation coefficient	0.999	Correlation coefficient	0.999

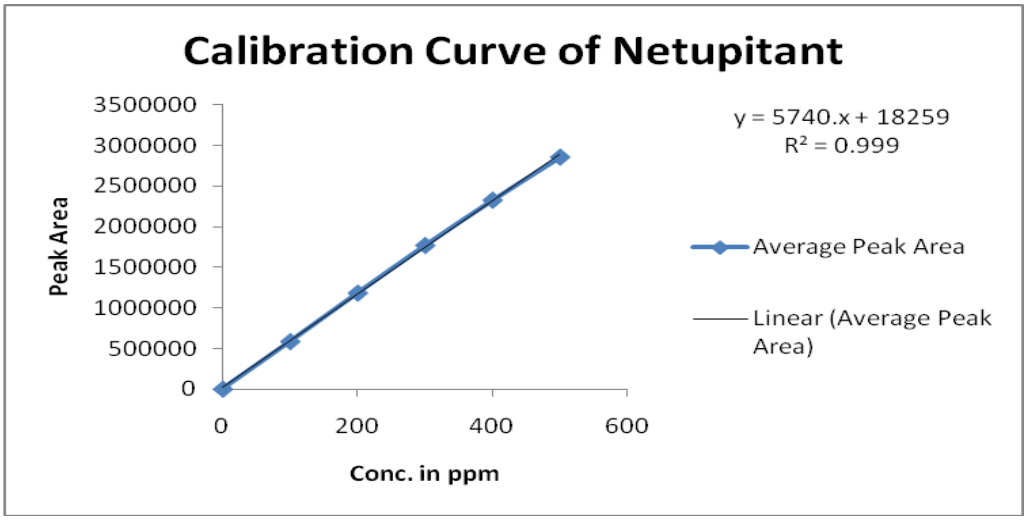

Figure 4: Linearity graph for Netupitant

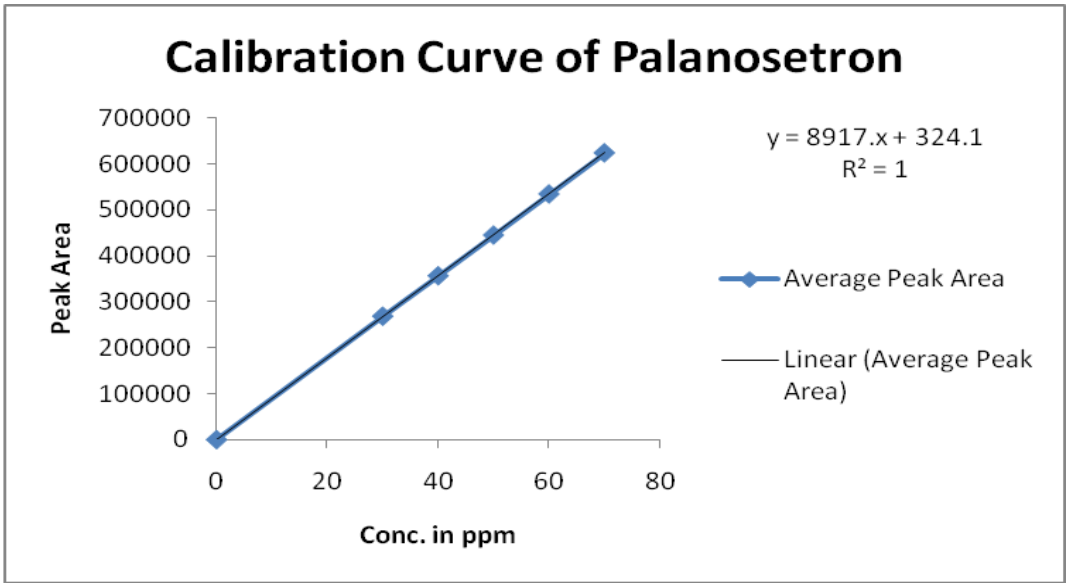

Figure 5: Linearity graph for Palanosetron

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Table 4: Showing accuracy results for Netupitant

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Table 5: Showing accuracy results for Palanosetron

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard 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. The resulte are shown in table 6.

Table 6: Precision results for Netupitant and Palanosetron

S. No	Netupitant	palanosetron
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard 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. The resulte are shown in table 7 and 8.

Table 7: Intermediate precision resultes for Netupitant and Palanosetron on day 1:

S. No	Sample Area 1	Sample Area 2
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

Table 8: Intermediate precision resultes for Netupitant and Palanosetron on day 2:

Injection	Area for Netupitant	Area for Palanosetron
Injection-1	1648598	415985
Injection-2	1642587	415267
Injection-3	1649852	415986
Injection-4	1648754	415265
Injection-5	1645289	415874
Injection-6	1647581	415632
Average	**1647110**	**415668.2**
STD Deviation	**2699.291**	**337.2106**
%RSD	**0.16388**	**0.081125**

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The

Wavelength varied from 243nm to 247nm. The resulte are shown in table 9,10,11,12

Robustness results for Netupitant

Table 9: Organic Composition results for Netupitant:

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	7365	1.62	1.868
Actual Flow rate	1	7586	1.69	1.688
More Flow rate	1.2	7254	1.61	1.544

Table 10: Wavelength variation results for Netupitant:

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Robustness results for Palanosetron

Table 11: Flow variation results for Palanosetron:

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Table 12: Organic Composition results for Palanosetron:

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 13.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 13: LOD, LOQ of Netupitant and Palanosetron

Drug	LOD	LOQ
Netupitant	2.10	6.30
Palanosetron	1.28	3.84

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Netupitant and Palanosetron in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Netupitant and Palanosetron in pure and its pharmaceutical dosage forms.

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