World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

Method development and validation for simultaneous estimation of netupitant and palonosetron in bulk and pharmaceutical dosage form by using RP-HPLC

Sridhar Siddiraju[*1], Akshay[2], M. Sudhakar[3]

[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmaceutical Analysis, [3]Department of Pharmaceutics, Malla Reddy College of Pharmacy, Osmania University, Secunderabad - 500010, Telanagana, India

Received: 02-11-2021 / Revised Accepted: 30-11-2021 / Published: 01-12-2021

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netupitant and Palanosetron in Tablet dosage form. Chromatogram was run through Std Discovery C18 250 x 4.6 mm, 5□. Mobile phase containing Buffer 0.1% OPA (2.2ph): Acetonitrile taken in the ratio 55:45 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1% OPA. Temperature was maintained at 30°C. Optimized wavelength selected was 230 nm. Retention time of Netupitant and Palanosetron were found to be 2.325min and 3.026min. %RSD of the Netupitant and Palanosetron were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 100.28% and 99.76% for Netupitant and Palanosetron respectively. LOD, LOQ values obtained from regression equations of Netupitant and Palanosetron were 1.01, 3.05 and 0.001, 0.003 respectively. Regression equation of Netupitant is y = 13900x + 26511, and y = 563617x + 1740.8 of Palanosetron. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

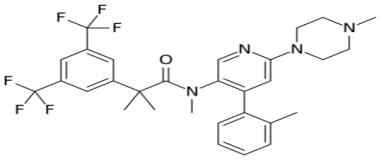
Keywords: Netupitant, Palonosetron, Method development, RP-HPLC.

INTRODUCTION

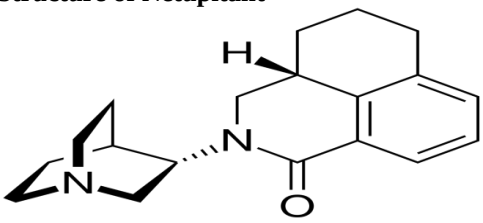
Netupitant is an antiemitic drug approved by the FDA in october 2014 for use in combination with palonosetron for the prevention of acute and delayed vomiting and nausea associated with cancer chemotherapy including highly emetogenic chemotherapy. Netupitant is a neurokinin 1 receptor antagonist. The combination drug is marketed by eisai inc. And helsinn therapeutics (u.s.) Inc. Under the brand akynzeo. **PALONOSETRON** (inn, trade name aloxi) is an antagonist of 5-ht3 receptors that is indicated for 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 clinicaluse [(10-14)].

Address for Correspondence: Sridhar Siddiraju, Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Affiliated to Osmania University, Maisammaguda, Dhulapally Secunderabad-500100, Telangana, India; **drss.chem@gmail.com**

How to Cite this Article: Sridhar Siddiraju, Akshay and M. Sudhakar. Method development and validation for simultaneous estimation of netupitant and palonosetron in bulk and pharmaceutical dosage form by using RP-HPLC. World J Pharm Sci 2021; 9(12): 218-225; https://doi.org/10.54037/WJPS.2021.91212



Structure of Netupitant



Structure of Palonsetron

Figure-1: Structures of Netupitant and Palonsetron.

Several analytical methods have been documented, according on a thorough review of the literature In the literature, there is no method reported for the stability indicating estimation. hence a simple, cost-effective stability-indicating simultaneous estimation of Netupitant and Palonosetron by RP-HPLC in pharmaceutical dosage form has to be develop and validated as per the guidelines of ICH (Q2 specification) [15,21].

MATERIALS AND REAGENTS

Spectrum Pharma research solutions, Hyderabad, provided the Netupitant and Palonsetron pure pharmaceuticals. The Netupitant and Palonsetron. (AKYNZEO) combo tablet was purchased from a local pharmacy. Rankem in India provided all of the chemicals and buffers utilised in this Method.

Instrumentation

Waters HPLC System series with Binary pumps and Photo diode array detector was used for the development and method validation, with an manual sample injector with software Empower 2.

CHROMATOGRAPHIC CONDITIONS:

Flow rate: **1**ml/min
Column : Symmetry C18 (4.6 x 150mm, 5µm)
Mobile phase: 55% 0.01N Na2hpo4 buffer: 45% Acetonitrile
Detector: 230.0 nm
Temperature: Ambient
Injection volume : 10.0□L
Run time : 6.0 mins
Diluent : Water: Acetonitrile (50:50)

Preparation of solutions

Preparation of 0.01N Sodium hydrogen phosphate Buffer: Accurately weighed 1.42gm of Sodium hydrogen phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water..

Preparation of Standard solution: Accurately weighed 150 mg of Netupitant, 0.25mg of Palanosetron and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (150µg/ml Netupitant of and 0.25µg/ml of Palanosetron)

Preparation of Sample solution: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (150µg/ml of Netupitant and 0.25µg/ml of Palanosetron)

Method Validation

The validation of HPLC method was carried out for the simultaneous estimation of Netupitant and Palanosetron drug substance as per the ICH guidelines to demonstrate that the method is proposed for the routine analysis.

System suitability: The system suitability was performed for each validation parameters by injecting standard solution containing Netupitant 150µg/ml, Palanosetron 0.25µg/ml. System suitability chromatogram was shown in figure 2 and values are mentioned in the table 1.

Specificity (Selectivity): Checking of the interference in the optimized method. We haven't found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2.

S no	Netupitant	Palanosetron

Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	RS
1	2.325	6059	1.47	3.026	8554	1.38	5.4
2	2.325	6110	1.46	3.027	8468	1.38	5.4
3	2.326	6107	1.47	3.027	8479	1.41	5.4
4	2.327	6022	1.47	3.028	8411	1.41	5.3
5	2.327	6049	1.47	3.030	8354	1.48	5.2
6	2.328	6126	1.47	3.031	7946	1.51	5.2

Table 1: System suitability results

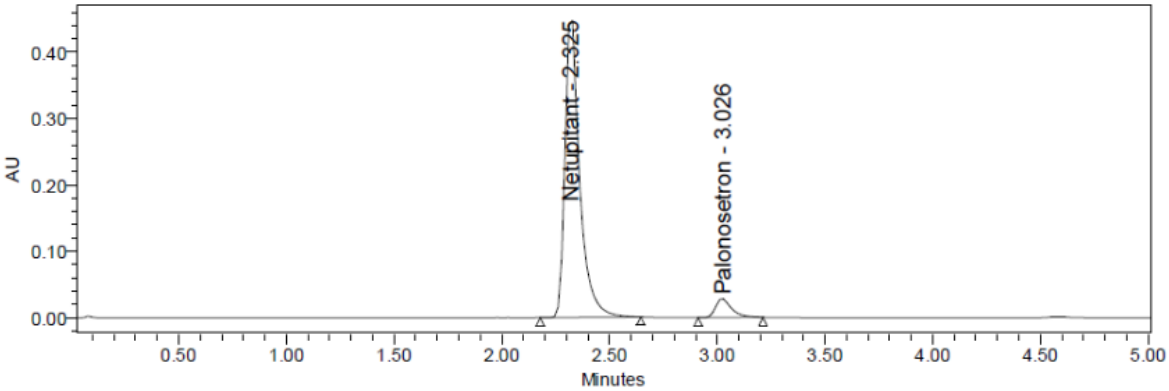
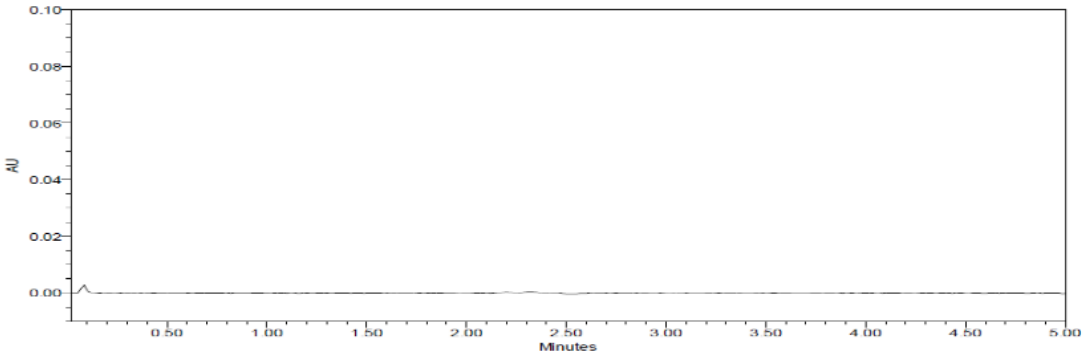


Figure 3: System suitability Chromatogram of Netupitant and Palanosetron

Sample name	**Retention time(mins)**	**Area**
Netupitant	2.328	2108082
Palonosetron	3.030	146577

Table 2: Specificity data



Blank Chromatogram

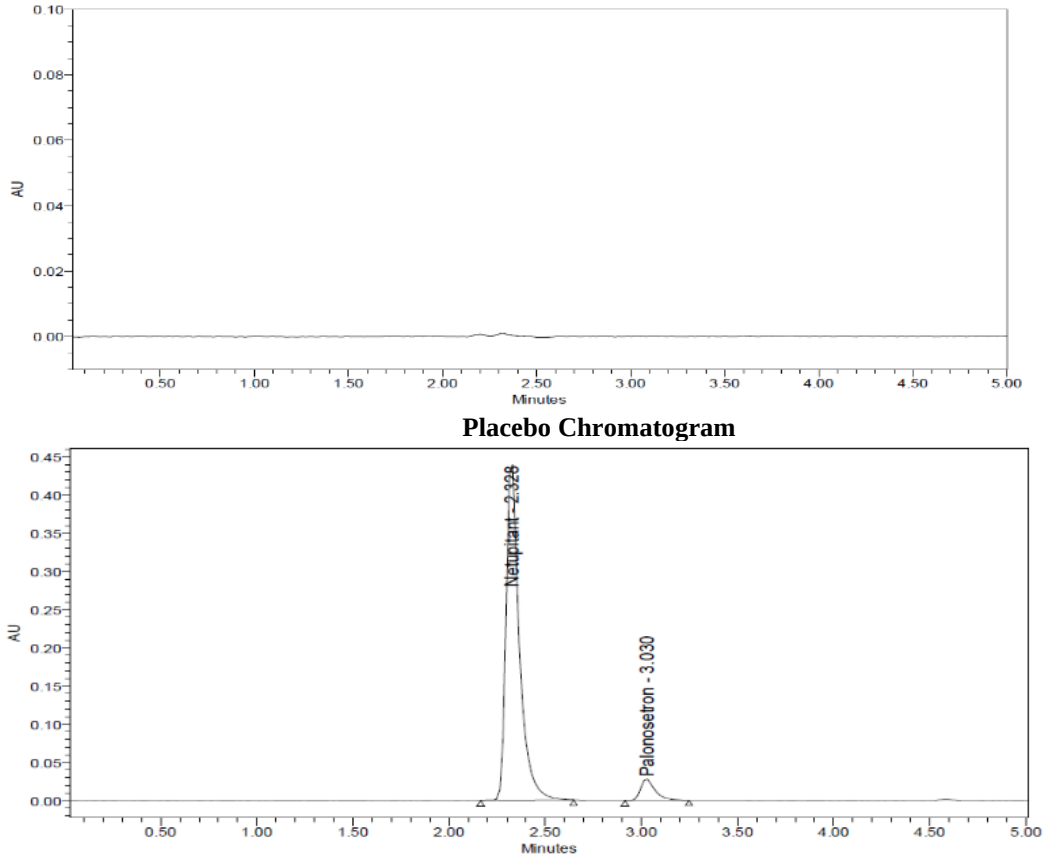


Figure 4: Specificity Chromatograms of Netupitant and Palonosetron.

Table 3: Netupitant Linearity

% Level	CONC	Area
0	0	0
25%	37.5	533177
50%	75	1109538
75%	112.5	1592104
100%	150	2150458
125%	187.5	2619082
150%	225	3127387
R^2 value		**0.999**

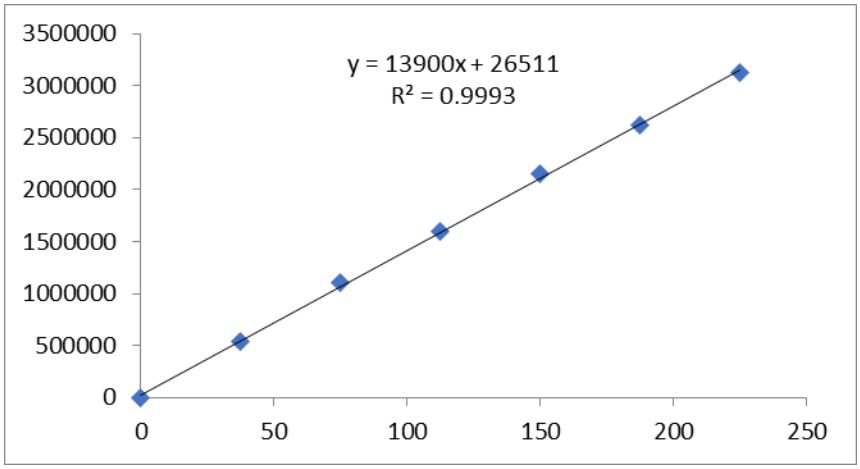


Figure 5: Netupitant Calibration curve

Table 4: Palonosetron Linearity

% Level	CONC	Area
0	0	0
25%	0.0625	35587
50%	0.125	74593
75%	0.1875	109017
100%	0.25	145422
125%	0.3125	175271
150%	0.375	212044
R^2 value		**0.999**

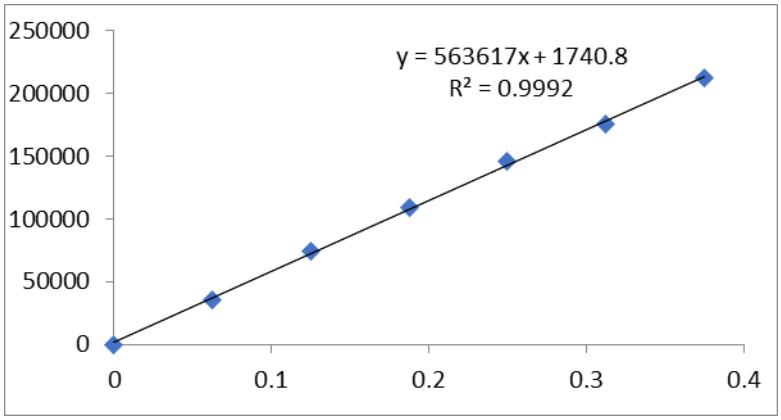


Figure 6: Palonosetron Calibration curve

Table 5: Accuracy (%Recovery data)

%Level	%Recovery					
	Netupitant			Palonosetron		
	Amt added	Amt found	%Rec	Amt added	Amt found	%Rec
50% Level	75	74.66	99.55	0.125	0.12	99.78
	75	75.56	100.75	0.125	0.12	99.73
	75	75.63	100.84	0.125	0.12	99.66
	150	149.72	99.81	0.25	0.25	100.97

	150	149.20	99.47	0.25	0.25	100.45
100%Level	150	149.82	99.88	0.25	0.25	100.68
	225	227.10	100.93	0.375	0.38	100.72
	225	227.02	100.90	0.375	0.38	100.02
150%Level	225	225.93	100.41	0.375	0.37	99.76
Mean%			**100.0**			**99.76**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Netupitant and Palonosetron. Results of peak area are summarized in Table 7.

Table 6: System precision data

Inj	**Netupitant**	**Palonosetron**
1	2123440	144590
2	2123114	145723
3	2134157	145411
4	2097548	143740
5	2106005	145965
6	2152700	145701
Avg	**2122827**	**145188**
Std dev	**19714.1**	**855.2**
%RSD	**0.9**	**0.6**

The % RSD for the peak areas of Netupitant and Palonosetron obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Netupitant and Palonosetron (Six individual sample preparations). Data obtained is summarized in Table 8.

Table 7: Method precision data

Injection	**Netupitant**	**Palonosetron**
1	2107058	146925
2	2110065	144866
3	2146355	144826
4	2109543	144398
5	2128307	145195
6	2114707	145158
Avg	**2119339**	**145228**
Std dev	**15261.3**	**879.5**
%RSD	**0.7**	**0.6**

From the above results, the % RSD of method precision study was within the limit for Netupitant and Palonosetron.

Table 8: Robustness results

Chromatographic condition	Netupitant (RSD)	Palonosetron (RSD)
Flow(-)	0.4	0.8
Flow(+)	0.1	0.7
Temp(Ambient-)	0.8	0.6
Temp(Ambient+)	0.7	0.2
Mobile phase(-)	0.3	0.5
Mobile phase (+)	1.1	0.8

Table 9: Forced degradation conditions for Netupitant and Palonosetron.

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^0c	30 mins
Base	2N NAOH	60^0c	30 mins
Oxdation	20% H_2O_2	60^0c	30 mins
Thermal	Diluent	105^0c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^0c	

From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 10: Degradation profile results

Degradation condition	Netupitant % Degraded	Palonosetron % Degraded
Acid	5.38	5.44
Base	4.46	5.09
Oxidation	3.22	3.73
Thermal	2.42	2.52
Photolytic	2.05	1.45
Hydrolytic	0.93	1.01

Table 11: Assay results for Netupitant and Palonosetron

Drug name	Label claim dose	%Assay
Netupitant	300mg	99.64%
Palonosetron	0.5mg	99.83%

CONCLUSION

The RP-HPLC methodology was used to create and evaluate a new stability indicating analytical approach. The sample preparation is straightforward, uses less mobile phase, and takes very little time to analyse. The results of the study will be highly beneficial for quality monitoring of Netupitant and Palonosetron in pharmaceutical dosage forms. The assay examination of two medications from a combination dosage form using this devised method yielded results that were nearly 100 % accurate. The results of the recovery studies were good, indicating that there was no interference from excipients.

ACKNOWLEDGEMENT

The authors are thankful to the principle of Malla reddy college of pharmacy, Hyderabad and spectrum Pharma research solutions, Hyderabad, India, for providing Netupitant and Palonosetron drugs as gift samples.

REFERENCES

1. B.k Sharma, Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication , Meerut, (2007)
2. Lindholm.J, Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis, pg . 13-14, (2004).
3. Rashmin, An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences , Vol.2 , Issue 2, Pg 191-196 (2012).
4. Malvia R, Bansal V , Pal O.P and Sharma P.K. A Review of High Performance Liquid Chromatography. Journal of Global Pharma technology (2010)
5. Douglas A Skoog, F. James Holler, Timothy A. Niemen, Principles of Instrumental Analysis Pg 725-760.
6. Dr.S. Ravi Shankar, Text book of Pharmaceutical analysis, Fourth edition, Pg 13.1-13.2
7. IUPAC. Compendium of Chemical Terminology, 2nd edn. (The Gold Book). PAC69, 1137 (1997). Glossary of terms used in computational drug design (IUPAC Recommendations.
8. K. D. Tripathi, Essentials of Medical Pharmacology, 6th Edition, Jaypee brother's medical publishers (P) LTD, p-254-255.
9. Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India, 2 (2010) 1657-1658https://www.drugbank.ca/drugs/DB09048
10. https://www.drugbank.ca/drugs/DB00377
11. https://www.scbt.com/p/netupitant-290297-26-6
12. https://pubchem.ncbi.nlm.nih.gov/compound/Netupitant
13. https://www.scbt.com/p/palonosetron-hydrochloride-135729-62-3
14. https://pubchem.ncbi.nlm.nih.gov/compound/Palonosetron
15. Potluri surendra et al.., development and validation of new analytical method for the simultaneous estimation of netupitant and palonosetron in pharmaceutical dosage form, international journal of pharmacognosy and chemistry, vol. 2, no. 2, may 2021, pp. 50-59.
16. NVMS Bhagavanji1*, PVV Satyanarayana2, Karanam Sekhar3, D. Naniprasad4. Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Netupitant and Palonosetron in Combined Tablet Dosage Form.Int. J. Pharm. Sci. Rev. Res., 41(1), November - December 2016; Article No. 17, Pages: 81-87.
17. P. Sri Haritha [1] *, Dr. S. Shobha Rani [2], Dr. M. Ajitha [3], K. Rambabu 4. Stability Indicating Method Development and Validation for The Simultaneous Estimation of Palonosetron And Netupitant By Rp-Hplc In Its Bulk Form.J Pharma Res, 2017; 6(2):101-106.
18. Manoranjani M et al.., Method development and validation for simultaneous quantification of netupitant and palonosetron in bulk and pharmaceutical dosage form and their forced degradation study by RP-HPLC.Asian journal of pharmaceutical and clinical research,2019;12(2):119-23.
19. Uttam Prasad Panigrahy1 *, A. Sunil Kumar Reddy2.A novel validated RP-HPLC-DAD method for the simultaneous estimation of Netupitant and Palonosetron in bulk and pharmaceutical dosage form with forced degradation studies.International Journal of ChemTech Research.2015;8(10):317-337.
20. Mangesh Harole*, R. N. Patil and Deepak Gaware, Govind Suryawanshi and Kalyan Pise.A Validated stability indicating rp-hplc method for simultaneous determination of netupitant and palenoserton in pharmaceutical formulations. Wjpps.2016;5(3):878-887.
21. Kalavati. T1 *, Shyamala1, J V C Sharma2. Development and Validation of Stability Indicating UPLC Method for the Estimation of Palonosetron in Bulk and Its Pharmaceutical Dosage Form. 2019;14(3): 144-154.