INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Article

Method Development and Validation of Netupitant and Palonosetron in Bulk and Capsule Dosage Forms Using Rp-Hplc

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ARTICLE INFO

Published: 16 Jun. 2026
Keywords:
Netupitant, Palonosetron, Method development, RP-HPLC.
DOI:
10.5281/zenodo.20719864

ABSTRACT

For the simultaneous estimate of Netupitant and Palanosetron in tablet dose form, a straightforward, accurate, and exact approach was created. The Std Discovery C18 column (250 mm x 4.6 mm, 5 µm particle sizes) was used to run the chromatogram. Mobile phase comprising acetonitrile (65:35, v/v) and 0.01 M ammonium acetate buffer (pH adjusted to 3.5 with orthophosphoric acid) The temperature was kept at 30°C while the flow rate was 1 mL/min. 265 nm was the chosen optimal wavelength. Netupitant and Palanosetron were shown to have retention times of 2.439 and 3.718 minutes, respectively. The Netupitant and Palanosetron percent RSDs were determined to be 0.06 and 0.19, respectively. For Netupitant and Palanosetron, recovery rates were 100.16% and 99.86%, respectively. Netupitant and Palanosetron regression equations yielded LOD and LOQ values of 1.02, 3.06, and 0.002, 0.004, respectively. Netupitant's regression equation is y = 11003x + 686, while Palanosetron's is y = 968863x + 1760. Because retention times and run times were reduced, the devised approach was straightforward and cost-effective, making it suitable for use in routine quality control testing in industries.

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

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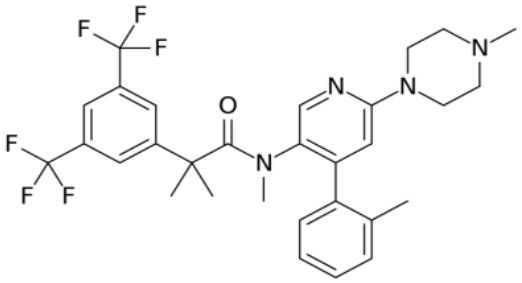
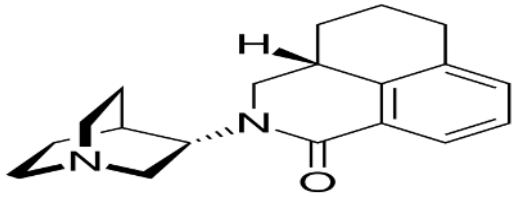
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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.



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.

Experimental Investigations

An isocratic RP-HPLC method was performed on a Waters Alliance e2695 HPLC system with 515 HPLC pump, equipped with 2998 Photo Diode Array (PDA) detector and Empower 2 software for processing and data collecting. Kromasil C18 column (250 mm×4.6 mm ID, 5 □m particle size) is used as a stationary phase. An ultrasonic bath sonicator (Frontline FS 4, Mumbai, India), semi-micro analytical balance (India) and Whatman filter paper No. 41 is used in the study.

Preparation of mobile phase

An accurately weighed quantity of 0.77 g of Ammonium acetate was taken into a 1000 mL beaker and diluted to 1000 mL with HPLC grade water and degassed in ultrasonic water bath and filtered through 0.45µm nylon membrane filter using vacuum filtration gives required buffer concentration of 0.01 M Ammonium acetate buffer and the pH was adjusted to 3.5 with orthophosphoric acid. 0.01 M Ammonium acetate buffer with pH adjusted to 3.5 with orthophosphoric acid were mixed with HPLC grade Acetonitrile in the proportion of 65:35, v/v and it was filtered through 0.45µm nylon membrane filter and degassed by ultrasonication.

Preparation of NET and PAL mixed standard drug stock solutions

The mixed standard drug stock solutions of Netupitant and Palonosetron were prepared by dissolving 300 mg of Netupitant and 0.5 mg of Palonosetron in 100 mL of the mobile phase into a 100 mL of volumetric flask and then sonicated to dissolve it completely to get a concentration

of 3000 µg/mL of Netupitant and 5 µg/mL of Palonosetron

Preparation of linearity solutions

The mixed standard working solutions for linearity were prepared by pipette out an aliquots of 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mL from the mixed standard drug stock solutions of 3000 µg/mL of Netupitant and 5 µg/mL of Palonosetron and transferred into the series of 10 mL of volumetric flask and volume make upto 10 mL with the mobile phase to get a concentration of 75, 150, 225, 300, 375 and 450 µg/mL of Netupitant and 0.125, 0.25, 0.375, 0.5, 0.625 and 0.75 µg/mL ofPalonosetron respectively. All the above solutions were filtered through 0.45 µm nylon membrane filter before injection into the HPLC system.

Preparation of sample solution

Sample solution was prepared from Akynzeo® capsules. Twenty capsules of Akynzeo® were taken and weighed individually and the average weight of twenty capsules was calculated. From this calculation the weight of each capsule is determined. Each capsule of Akynzeo® contains 300 mg of Netupitant and 0.5 mg of Palonosetron. After weighing, twenty capsules of Akynzeo® were the body and cap of the capsule is separated out and the capsule powder is collected. An accurately weighed quantity of capsule powder equivalent to 300 mg of Netupitant and 0.5 mg of Palonosetron were transferred into a clean and dry 100 mL volumetric flask and then mobile phase was added and sonicated to dissolve it completely and filtered through 0.45 µm nylon membrane filter and volume was made up to the mark with the same mobile phase to get the concentration of 3000 µg/mL of Netupitant and 5 µg/mL of Palonosetron. An aliquot of 1 mL was pipette out from the above solution and transferred into a 10 mL volumetric flask and diluted up to the mark with mobile phase to get a concentration of 300 µg/mL of Netupitant and 0.5 µg/mL of Palonosetron solution.

Method validation

Method validation for bio-analytical studies consist of procedures that shows a suitable method for quantitative analysis of drug analytes present in the biological fluids such as blood, plasma, serum and urine was reproducible and reliable for the future purpose. The essential factors for bio-analytical method validation consist of: (1) Accuracy (2) Precision (3) Selectivity (4) Sensitivity (5) Reproducibility and (6) Stability.

RESULTS AND DISCUSSION

Method optimisation

For the optimisation of RP-HPLC method several parameters and mobile phase compositions were tried. A satisfactory separation and good peak symmetry for NET and PAL were obtained with Kromasil C18 column (250 mm×4.6 mm, 5 □m particle size) and mobile phase containing a mixture of 0.01 M Ammonium acetate buffer (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (65:35, v/v) was delivered at aflow rate of 1 mL/min to get better reproducibility and repeatability. Both NET and PAL were scanned in the wavelength region of 200-400 nm by using photo diode array (PDA) detector. Quantitation was attained with a PDA detector at 265 nm depends on peak area. Therefore 265 nm was selected as detection wavelength in the present study. The retention time of NET and PAL was found to be 2.438 min and 3.718 min respectively. A typical chromatogram of blank, standard and sample solution of NET and PAL is shown in Figure 1.

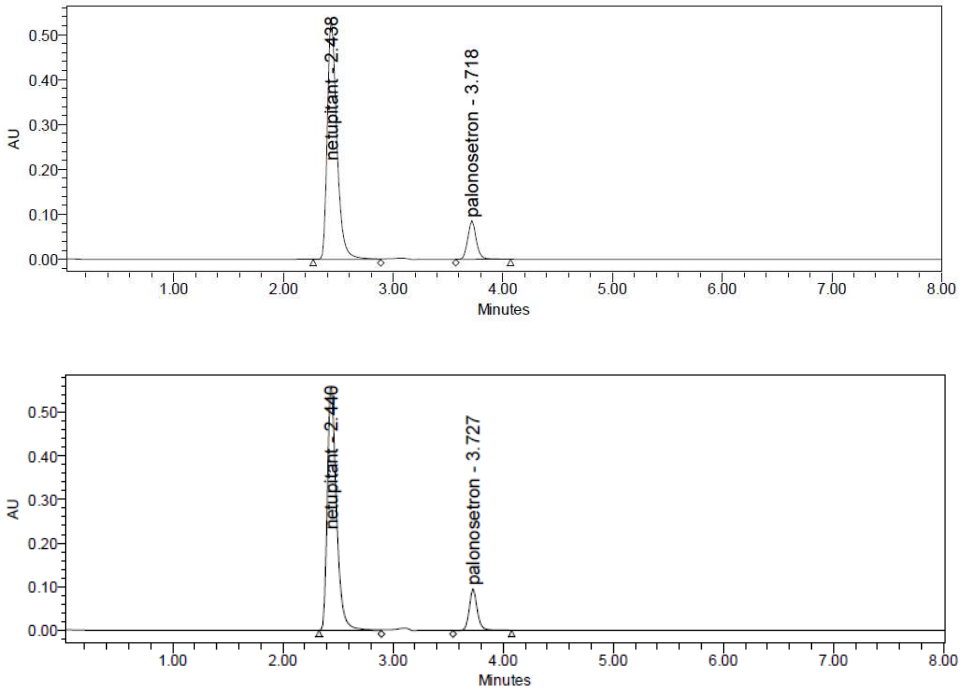


Figure 1 Chromatogram of blank, standard and sample solution of NET and PAL

Method validation:

Specificity

The effect of excipients and other additives usually present in the combined dosage form of NET and PAL in the determination under optimum conditions was investigated and confirms that there is no interference. The specificity of the RP-HPLC method was established by injecting the placebo solution into the HPLC system. The representative chromatogram of placebo was shown in Figure 2.

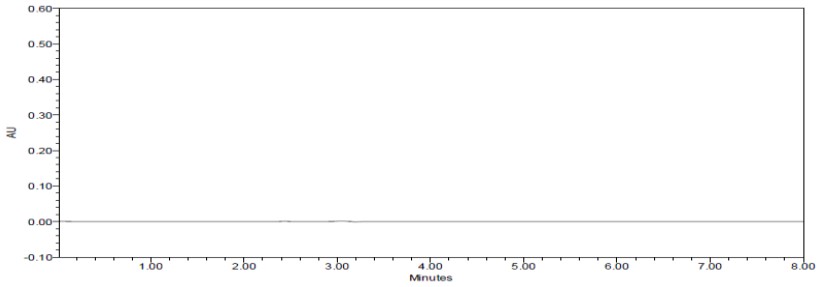


Figure 2 Chromatogram of placebo for NET and PAL

Table 1 Performance calculations and system suitability parameters of NET and PAL

Parameters	NET	PAL	Acceptance limits
Retention time (min)	2.438	3.718	-----
Theoretical plates (N)	3871	10816	Not less than 2000
Asymmetry factor	1.1	1.1	Not more than 2
Resolution		8.08	More than 2

Linearity range (µg/mL)	75-450	0.125-0.75	-----
Limit of detection (LOD) (µg/mL)	0.06	0.01	-----
Limit of quantification (LOQ) (µg/mL)	0.18	0.03	-----

Linearity

An aliquots of 0.25, 0.5, 0.75, 1, 1.25 and 1.5 mL from the mixed standard drug stock solutions of 3000 µg/mL of Netupitant and 5 µg/mL of Palonosetron was pippetted out and transferred into the series of 10 mL of volumetric flask and volume make upto 10 mL with the mobile phase to get a concentration of 75, 150, 225, 300, 375 and 450 µg/mL of Netupitant and 0.125, 0.25, 0.375, 0.5, 0.625 and 0.75 µg/mL of Palonosetron respectively. All the above solutions were filtered through 0.45 µm nylon membrane filter and then 20 □L of each solution was injected three times into the HPLC system. Least square regression analysis was carried out for the slope, intercept and correlation coefficient.

Table 2 Linearity of NET and PAL

Concentration of Netupitant (µg/mL)	**Peak Area**	**Concentration of Palonosetron (µg/mL)**	**Peak Area**
75	864115	0.125	128061
150	1612752	0.25	245238
225	2466709	0.375	364102
300	3249231	0.5	474414
375	4226134	0.625	612356
450	4915001	0.75	730816

Table 3 Optical and regression parameters of NET and PAL

Optical and regression parameters	**NET**	**PAL**
Detection wavelength (nm)	265	
Linearity range (µg/mL)	75-450	0.125-0.75
Regression Equation (y=mx+C)	11003x+686	968863x+1760
Slope (m)	11003	968863
Intercept (C)	686	1760
Correlation coefficient (r)	0.999	0.999
Limit of detection (µg/mL)	0.06	0.01
Limit of quantification (µg/mL)	0.18	0.03

Accuracy

The accuracy of the proposed method was determined by calculating the recoveries of NET and PAL by standard addition method. Recovery studies were carried out by adding concentration level of 50 %, 100 % and 150 % of standard drug solution of NET and PAL to the pre-analysed

sample solution of Akynzeo® capsule powder and the mixtures were reanalyzed by the proposed method.

Table 4 Results of accuracy studies of NET

Concentration Level in %	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery	RSD %
S_1:50%	75	75.03	100.04	99.85	0.36
S_2:50%	75	75.06	100.08		
S_3:50%	75	74.58	99.44		
S_4:100%	150	149.97	99.98	100.04	0.21
S_5:100%	150	149.79	99.86		
S_6:100%	150	150.41	100.27		
S_7:150%	225	224.94	99.97	99.92	0.12
S_8:150%	225	225.03	100.01		
S_9 :150%	225	224.51	99.78		

Table 5 Results of accuracy studies of PAL

Concentration Level in %	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	% Mean Recovery	RSD %
S_1:50%	0.125	0.1252	100.16	100.03	0.17
S_2:50%	0.125	0.1251	100.08		
S_3:50%	0.125	0.1248	99.84		
S_4:100%	0.25	0.251	100.40	99.73	0.61
S_5:100%	0.25	0.249	99.60		
S_6:100%	0.25	0.248	99.20		
S_7:150%	0.375	0.3734	99.57	99.79	0.33
S_8:150%	0.375	0.3756	100.16		
S_9 :150%	0.375	0.3736	99.63		

Precision

The precision of the proposed method was performed to express the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the optimized conditions. Precision are of three levels they are repeatability intermediate precision and reproducibility. Repeatability was carried out by calculating method and system precision. Method precision was performed by injecting six times of a homogenous sample preparation of 300 µg/mL of Netupitant and 0.5 µg/mL of Palonosetron of a single batch sample solution of Akynzeo® capsule powder into the HPLC system to ensure that the analytical method is working properly.

Table 6 Method precision of Netupitant

Injection No.	Name of the drug	Concentration (µg/mL)	Retention time (min)	Peak Area	Assay %
1	NET	300	2.438	3227906	99.66
2	NET	300	2.438	3258393	100.60
3	NET	300	2.439	3227518	99.65
4	NET	300	2.439	3265378	100.82
5	NET	300	2.440	3252181	100.41
6	NET	300	2.442	3239480	100.02
Average			2.439	3245143	100.19
SD			0.00151	15964.81	0.492913
RSD %			0.06	0.49	0.5

Table 7 Method precision of Palonosetron

Injection No.	Name of the drug	Concentration (µg/mL)	Retention time (min)	Peak Area	Assay %
1	PAL	0.5	3.713	479376	100.18
2	PAL	0.5	3.713	476760	99.63
3	PAL	0.5	3.717	481643	100.65
4	PAL	0.5	3.717	478012	99.89
5	PAL	0.5	3.718	477938	99.88
6	PAL	0.5	3.732	479413	100.19
Average			3.718	478857	100.07
SD			0.00703	1690.934	0.3534
RSD %			0.19	0.35	0.35

Limit of detection and Limit of quantitation

Limit of detection is a smallest concentration of an analyte which gives a measurable response. Limit of quantitation is a smallest concentration of an analyte that gives a measurable response which can be quantified accurately. LOD and LOQ are calculated by using following formula andthe results of LOD and LOQ of Netupitant and Palonosetron were reported in Table 8.

Robustness

Robustness of the method was carried out by deliberately changing the mobile phase composition by altering the proportion of organic phase by ±10 % and flow rate by ±0.1 mL. There are no marked variations were observed in the system suitability parameters and the results of robustness were reported in Table 8 and Table 9 ensures that the developed analytical method remain unaffected by small, but deliberate variations in chromatographic method parameters and provides an indication of its reliability during normal usage.

Table 8 Robustness data of Netupitant

Variations in method parameters	Retention Time (mins)	Average peak area*	RSD %	System suitability parameters	
				Theoretical Plates	Asymmetry

Buffer : ACN (69:31,v/v)	2.423	3264268	0.22	3986	1.46
Buffer : ACN (61:39,v/v)	2.432	3224224	0.3	3879	1.49
0.9 mL/min Flow rate	2.726	3641636	0.37	4043	1.48
1.1 mL/min Flow rate	2.198	2920162	0.11	3562	1.48

Table 9 Robustness data of Palonosetron

Variations in method parameters	**Retention Time (mins)**	**Average peak area***	**RSD %**	**System suitability parameters**	
				Theoretical Plates	**Asymmetry**
Buffer : ACN (69:31,v/v)	3.623	478099	0.03	11948	1.2
Buffer : ACN (61:39,v/v)	3.690	473333	0.4	11670	1.2
0.9 mL/min Flow rate	4.141	513373	0.33	12020	1.2
1.1 mL/min Flow rate	3.340	422857	0.15	10861	1.2

Solution stability study

Solution stability was carried out to ensure that the sample solutions of 300 µg/mL of Netupitant and 0.5 µg/mL of Palonosetron were found to be stable upto 48 hrs at room temperature. Solution stability was performed by injecting six times of a homogenous sample preparation of 300 µg/mL of Netupitant and 0.5 µg/mL of Palonosetron of a single batch sample solution of Akynzeo® capsule powder in different time intervals i.e. 0, 8, 16, 24, 32 and 48 hrs at room temperature into the HPLC system.

CONCLUSION:

In conclusion, the developed RP-HPLC method for the simultaneous estimation of Netupitant and Palonosetron was successfully validated according to ICH guidelines and demonstrated excellent specificity, accuracy, precision, linearity, robustness, and stability-indicating capability. The method provided efficient chromatographic separation with satisfactory recovery and low detection limits, making it suitable for the reliable quantification of both drugs in bulk materials and pharmaceutical dosage forms. Therefore, the proposed method can be effectively employed for routine quality control analysis and stability studies of Netupitant and Palonosetron in combined pharmaceutical formulations.

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HOW TO CITE: Kampalli Triveni, M. Ramakrishna Reddy, Chandra sekhar Naik.D.*, Method Development and Validation of Netupitant and Palonosetron in Bulk and Capsule Dosage Forms Using Rp-Hplc, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 6, 3918-3927. https://doi.org/10.5281/zenodo.20719864