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R E S E A E C H A R T I C L E

Simultaneous Estimation of Neutipotent and Palonesetron in Its Bulk and Pharmaceutical Dosage Form by RP-HPLC Method

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A B S T R A C T

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Netupitant and Palonosetron in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability.The estimation of Netupitant and Palonosetron was done by RP-HPLC. The Phosphate buffer was pH 2.5 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 80:20 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The detection was carried out using UV detector at 274 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Netupitant and Palonosetron were found to be from 25-125 µg/ml.Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method.The percentage recovery varies from 97-102% of Netupitant and Palonosetron LOD and LOQ was found to be within limit.The proposed method is precise, simple and accurate to determine the amount of Netupitant and Palonosetron in formulation.High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

Keywords: Symmetry C18, Netupitant and Palonosetron, RP-HPLC.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Netupitant, chemically described as 2-[3,5- Bis (trifluoromethyl) phenyl]-N,2-dimethyl-N-[4-(2 methyl phenyl) -6-(4-methyl-1-piperazinyl)-3 pyridinyl] propanamide, is a selective neurokinin 1 receptor antagonist having antiemetic activity. Netupitant is involved in the prevention of chemotherapy induced nausea and vomiting by inhibiting the binding of endogenous tachykinin neuropeptide substance P to the neurokinin 1 receptors in the central nervous system.

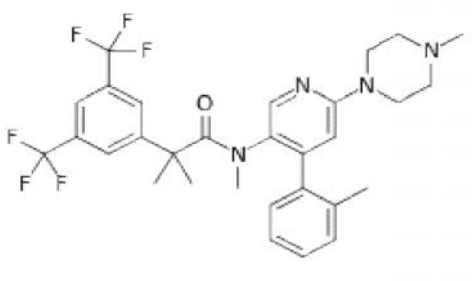

Fig 1: Structure of Netupitant

Palonosetron, chemically known as (3aS)-2-[(3S)-1-Azabicyclo[2.2.2]oct-3-yl]-2,3,3a,4,5,6-hexahydro-1Hbenz[de]isoquinolin-1-one, is a specific and selective serotonin 5-HT3 antagonist with antinauseant and antiemetic activity. It is prescribed for the prevention of nausea and vomiting associated with cancer chemotherapy and postoperative nausea and vomiting. 3-5 Chemotherapeutic agents causes the release of serotonin, which then stimulates medullary vomiting center and 5-HT3 receptors and thus initiating the vomiting reflex, causing nausea and vomiting. The antiemetic activity of palonosetron is brought about by the inhibition of 5-HT3 receptors present both in the medullary chemoreceptor zone and gastrointestinal tract.

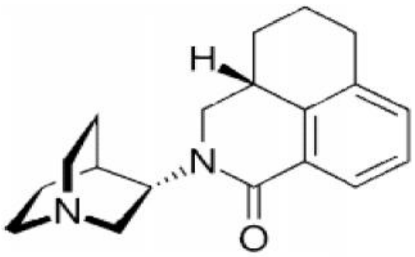

Fig 2: Structure of Palonosetron

2. Materials and Methods

Chemicals

Palonosetron, Netupitant, Water and Methanol for HPLC, Acetonitrile for HPLC, HCl, H2O2, NaOH.

Instrumentation

HPLC-auto sampler –UV detector, Separation module 2695, UV. Detector 2487, Empower-software version-2, Waters, U.V double beam spectrometer, UV 3000+, U.V win software, Lab India.

Chromatographic conditions

Table No 1: Optimized chromatographic conditions

Parameter	Description
Flow rate	1.0ml/min
Column	Kromosil C18 Column(250mm x 4.6mm) 5µm.
Mobile phase	Phosphate buffer pH 2.5: Methanol (65:35%v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Sample temperature	Ambient
Wavelength	254 nm
Injection volume	10µl
Run time	10 mins

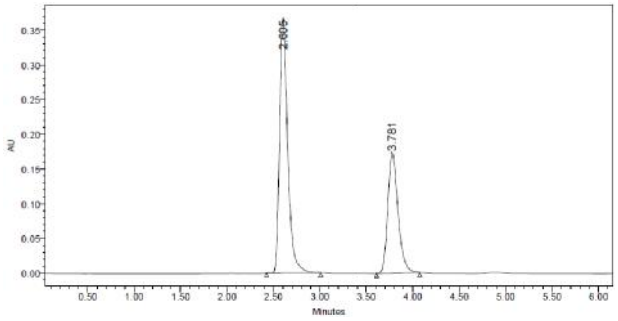

Fig 3: Optimized Chromatogram

Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Standard Solution Preparation

Accurately weighed amount of 50mg Netupitant and 50 mg Palonosetron were taken to a 100 ml clean and dry volumetric flask. This was then diluted with 70 ml of diluent and was sonicated. The volume was made to100 ml with the same solvent. This was taken as stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with the diluent to get final concentration of 75µg/ml.

Sample Solution Preparation

Weight equivalent to50 mg of Netupitant and Palonosetron sample were weighed this was taken into a 100 ml clean dry volumetric flask and about 70ml of diluent was added and sonicated to dissolve it completely and volume made up to the mark with the same solvent. This was taken as stock solution. Further, 1.5 ml of above stock solution was diluted to 10ml with diluent to get final concentration of 75µg/ml.

Method Validation

Method Precision:

Accurately weighed amount of 50mg Netupitant and 50 mg Palonosetron were taken to a 100 ml clean and dry volumetric flask. This was then diluted with 70 ml of diluent and was sonicated.

Intermediate Precision/Ruggedness:

75 µg/ml of the above sample solution was injected five times in five different days and peak areas were recorded.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Linearity:

For determination of linearity five different concentrations i.e. 25%, 50%, 100%, 125%, 150% were prepared and chromatograms are recorded for same.

Limit of Detection (LOD):

Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method.

Limit of Quantitation (LOQ):

Limit of quantitation is the lowest concentration of the substance that can be estimated quantitatively. It can be determined from linearity curve by applying the following formula

Robustness:

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

3. Results and Discussions

Method development

Mobile phase optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and acetonitrile: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer(pH 2.5), acetonitrile in proportion 20: 80 V/V respectively.

Wave length selection:

UV spectrum of 10 µg / ml Netupitant and Palonosetron in diluents was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum, the wavelength of 274 nm was selected. At this wavelength Netupitant and Palonosetron standards shows good absorbance.

Linearity:

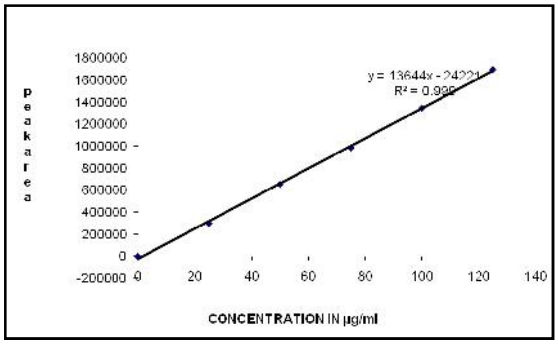

Fig 4: Calibration graph for Netupitant at 274 nm

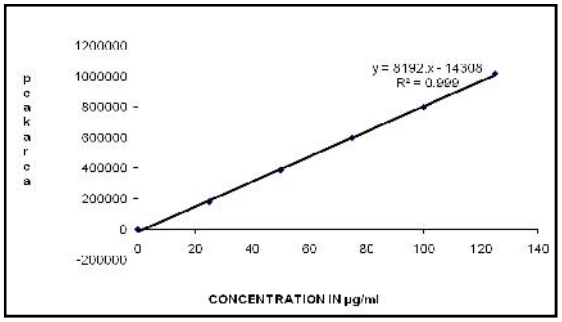

Fig 5: Calibration graph for Palonosetron at 274 nm

Robustness:

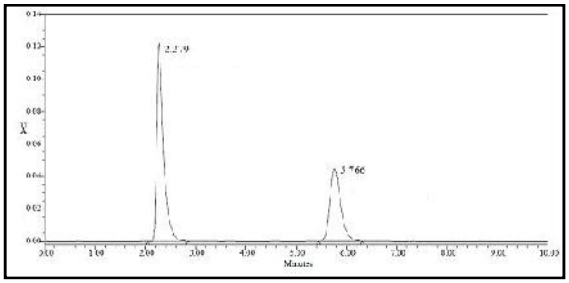

Fig 6: Chromatogram snowing less flow of 0.7ml/min

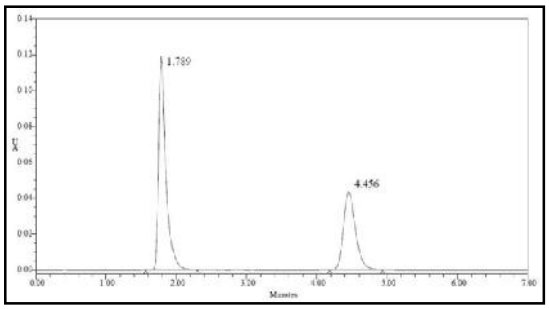

Fig 7: Chromatogram snowing more flow of 0.9 ml/min

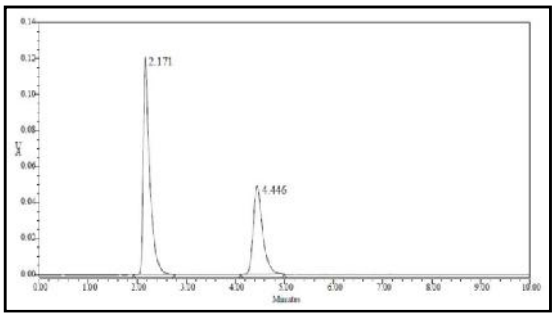

Fig 8: Chromatogram showing less organic composition

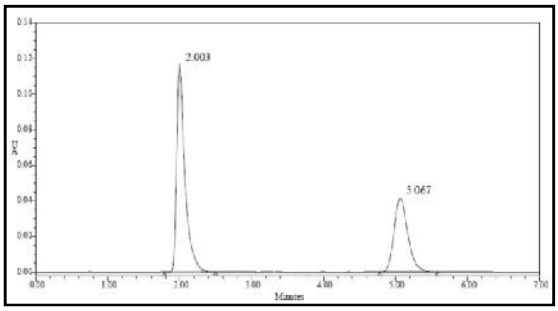

Fig 9: Chromatogram showing more organic composition

Table No 2: Results of system suitability parameters for Netupitant and Palonosetron

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Netupitant	2.003	920101	116666	1.5	1.6	2711.8
2	Palonosetron	5.067	552058	41531	11.0	1.3	3428.2

Table No 3: Results of method precision for Netupitant

S. No	Sample area	Standard area	Percentage purity (%)
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average	**983234**	**976311**	**100.84**
%RSD	**49.5**	**48.2**	**0.304**

Table No 4: Results of method precision for Palonosetron

S. No	Sample area	Standard area	Percentage purity (%)
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average	**592325**	**582755**	**101.24**
%RSD	**29.5**	**28.7**	**0.46**

Table No 5: Results of Intermediate precision for Netupitant

S.No	Sample area	Standard area	Percentage purity (%)
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average	**985321**	**984824**	**99.63**
%RSD	**48.2**	**48.5**	**0.54**

Table No 6: Results of Intermediate precision for Palonosetron

S. No	Sample area	Standard area	Percentage purity (%)
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average	**596537**	**592542**	**99.27**
%RSD	**29.3**	**29.2**	**0.27**

Table No 7: Area of different concentration of Netupitant and Palonosetron

Concentration (µg/ml)	Peak area of Netupitant	Peak area of Palonosetron
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

Table No 8: Analytical performance parameters of Netupitant and Palonosetron

Parameters	Netupitant	Palonosetron
Slope (m)	13644	8192

Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Table No 9: Results of Accuracy

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		NETU	PALO	NETU	PALO	NETU	PALO
50%	1	460064	276931	24.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average Recovery					99.7%	99.7%
100%	1	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					99.6%	99.8%
150%	1	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					99.7%	99.8%

Table No 10: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Netupitant	56	176	3.14
Palonosetron	56	154	2.75

Table No 11: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Netupitant	56	563	10.05
Palonosetron	56	558	9.96

Table No 12: Results for effect of variation in flow

S.No	peak area for Less flow (0.7 ml/min)		peak area for More flow (0.9 ml/min)	
	Netupitant	Palonosetron	Netupitant	Palonosetron
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	**986306**	**582223**	**976755**	**591667**
%RSD	**0.45**	**0.80**	**0.53**	**0.80**

Table No 13: Results for effect of variation in mobile phase composition

S.No	peak area for Less organic(70%)		Peak area for More organic (90%)	
	Netupitant	Palonosetron	Netupitant	Palonosetron
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316
5	995247	585448	994672	587353
Mean	**987286**	**583658**	**986641**	**592877**
%RSD	**0.45**	**0.90**	**0.51**	**0.57**

4. Conclusion

On the basis of experimental results, the proposed method is suitable for the quantitative determination of Netupitant and Palonosetron in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Netupitant and Palonosetron was done by RP-HPLC. The Phosphate buffer was pH 2.5and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 80:20 % v/ v. A Symmetry C18 (4.6 x 150mm, 5µm, Make XTerra) column used as stationary phase. The

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