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

Development and Validation of RP HPLC Method for Simultaneous Estimation of Netupitant and Palonosetron in Pharmaceutical Dosage Form

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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) 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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1. Introduction

Netupitant is an antiemetic drug. In the United States, the combination drug netupitant/palonosetron (trade name Akynzeo) is approved by the Food and Drug Administration for prevention of acute and delayed chemotherapy-induced nausea and vomiting, including highly emetogenic chemotherapy such as with cisplatin. In Europe, it is approved by the European Medicines Agency for the same indication.

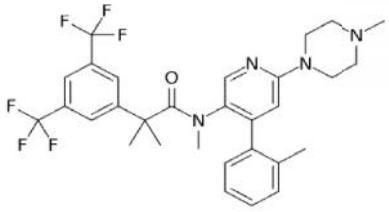

Fig 1: Structure of Netupitant

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 used for the control of delayed CINV—nausea and vomiting and there are tentative data to suggest that it may be more effective than granisetron.Palonosetron is administered intravenously, as a single dose, 30 minutes before chemotherapy, or as a single oral capsule one hour before chemotherapy. It has a longer duration of action than other 5-HT3 antagonists. The oral formulation was approved on August 22, 2008 for prevention of acute CINV alone, as a large clinical trial did not show oral administration to be as effective as intravenous use against delayed CINV.

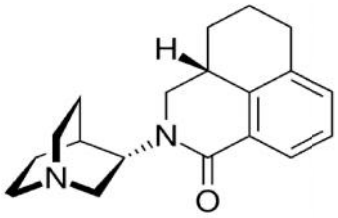

Fig 2: Structure of Palonosetron

2. Materials and Methods

Instrumentation

HPLC Shimadzu LC- SPD-M20A Waters 996 Software LC 20, UV/VIS spectrophotometer, UV 3000 UV Win 5 Lab India, pH meter, Adwa – AD 1020, Weighing machine.

Chemicals

Netupitant and Palonosetron KH2PO4, Water and Methanol for HPLC, Acetonitrile for HPLC, HCl, H2O2, NaOH.

Chromatographic conditions:

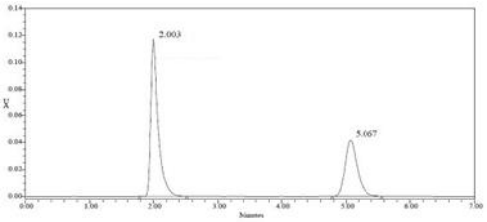

Fig 1: Chromatogram of Trail-6

Table 1: Chromatographic Conditions

Parameters	Description
Flow rate	1ml min^{-1}
Column	Symmetry C18 (4.6 x 150mm), 5µm.
Mobile Phase	Phosphate buffer:Methanol P^H 2.5 (20:80 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	274 nm
Injection volume	20µl
Run time	7min

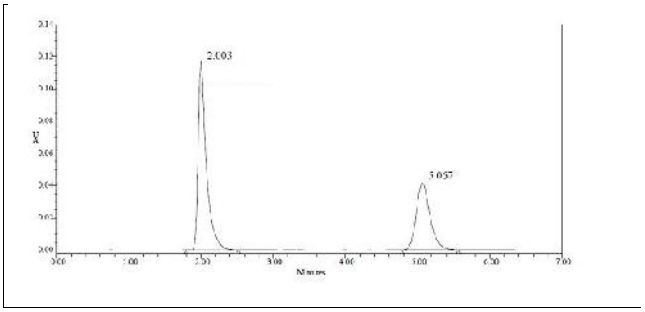

Fig 3: Optimized Chromatogram

Observation:

- From the above chromatogram it was observed that the Netupitant and Palonosetron peaks are well developed.
- Retention time of Netupitant – 2.003 min
- Retention time of Palonosetron - 5.067 min.
- The separation of two analytical peaks was good.
- 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 to 50 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.

Assay

Assay preparation of the Netupitant and Palonosetron standard and sample solution

Sample solution preparation:

1mg of Netupitant and 10 mg Palonosetron tablet powder were accurately weighed and transferred into a 10 ml clean

dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

1mg Netupitant and 10 mg Palonosetron working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Procedure

10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Netupitant and Palonosetron the peaks were used for calculating the % assay by using the formulae.

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. The volume was made to100 ml with the same solvent.

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.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

Linearity:

For determination of linearity five different concentrations i.e. 25%, 50%, 100%, 125%, 150% were prepared and chromatograms are recorded for same.Weight equivalent to 50 mg of sample was weighed in to 100ml volumetric flask, it was dissolved with diluent and the volume was made up to the mark with same diluent (500 µg/ml).

Limit of Detection (LOD):

Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. (Regression statistics). The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the following formula.

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 Discussion

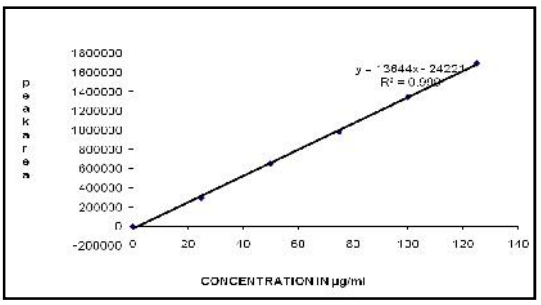

Fig 4: Calibration graph for Netupitant at 274 nm

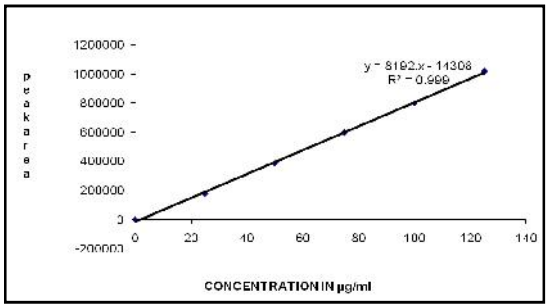

Fig 5: Calibration graph for Palonosetron at 274 nm

Robustness

Flow rate:

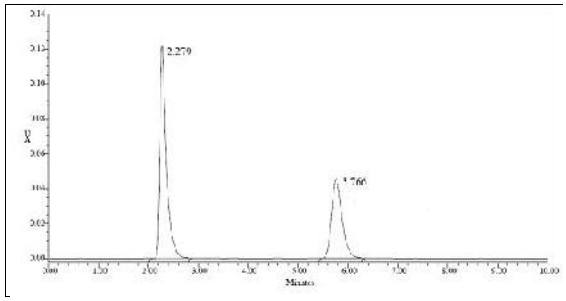

Fig 6: Chromatogram showing less flow of 0.7ml/min

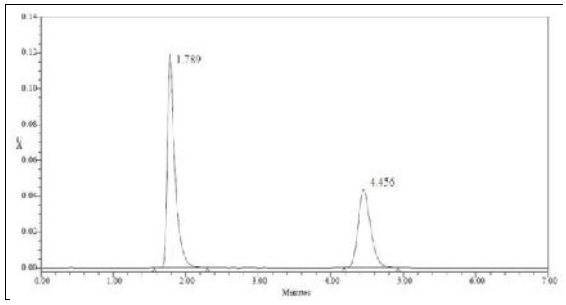

Fig 7: Chromatogram showing more flow of 0.9ml/min

Mobile Phase:

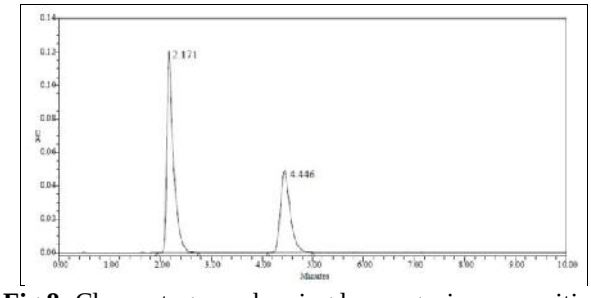

Fig 8: Chromatogram showing less organic composition

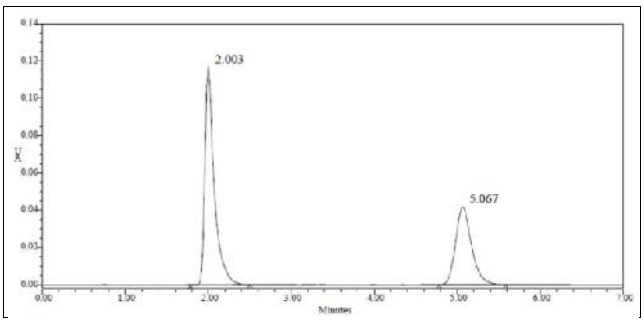

Fig 9: Chromatogram showing more organic composition

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 2.5 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,) 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.

Table 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
	Netupitant	2.004	921023	117523	1.5	1.6	2821.7
2	Palonosetron	5.067	552058	41531	11.0	1.3	3428.2
2	Palonosetron	5.068	553059	41431	11.0	1.3	3448.2

Table 3: 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 4: 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 5: 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 6: 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 7: 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 8: 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 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 10: Details of Standard Injection

	PeakName	RT	Area	Height	USP Plate Count	USP Tailing
1	Netupitant	2.002	1333112	164078	2114.9	1.7
2	Netupitant	2.003	1355521	164511	2127.0	1.7
3	Palonosetron	5.061	462181	44873	2931.4	1.7
4	Palonosetron	5.062	465519	41056	2697.1	1.7

Table 11: 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 12: 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 13: 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 14: 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**

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