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Development and Validation of New Analytical Method for The Simultaneous Estimation of Netupitant And Palonosetron In Pharmaceutical Dosage Form

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Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netupitant and Palonosetron in Pharmaceutical dosage form. The chromatogram was run through Std Discovery C18250 x 4.6 mm, 5m. Mobile phase containing Buffer 0.1% OPA (2.2ph): Acetonitrile taken in the ratio 55:45 was pumped through the column at a flow rate of 1 ml/min. The buffer used in this method was 0.1% OPA. The temperature was maintained at 30°C. The optimized wavelength selected was 220 nm. The retention time of Netupitant and Palonosetron was found to be 2.308min and 3.093min. %RSD of the Netupitant and Palonosetron were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 99.51% and 99.29% for Netupitant and Palonosetron respectively. LOD, LOQ values obtained from regression equations of Netupitant and Palonosetron were 1.84, 0.01, and 5.59, 0.03 respectively. Regression equation of Netupitant is y = 7232.8x + 3439.3., and y = 28857x + 97.732 of Palonosetron. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control tests in Industries.

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Introduction

Netupitant (NTP) is a novel antiemetic [1,2] drug in the combination of NTP/ palonosetron (PLS). It is used to the prevention of acute and delayed chemotherapy-induced nausea and vomiting [3], including highly emetogenic [4] chemotherapy [5] such as with cisplatin [6]. 5-hydroxytryptamine (5-HT3) receptors [7] are located on the nerve terminals [8] of the vagus [9] in the periphery and centrally in the chemoreceptor [10] trigger zone of the area postrema [11]. It is thought that chemotherapeutic agents produce nausea and vomiting by releasing serotonin [12] then activate 5-HT3 receptors located on vagal afferents [13] to initiate the vomiting reflex. Netupitant is chemically called as 2-[3,5-bis (trifluoromethyl) phenyl]-N,2- dimethyl- N- [4-(2-methylphenyl) -6- (4-methylpiperazin-1-yl) pyridin-3-yl] propenamide. It Delayed emesis (vomiting) has been largely associated with the activation of tachykinin family neurokinin 1 (NK1) receptors (broadly distributed in the central and peripheral nervous systems) by substance P. As shown in in vitro and in

vivo studies, netupitant inhibits substance P mediated responses. The structure is shown in figure 01.

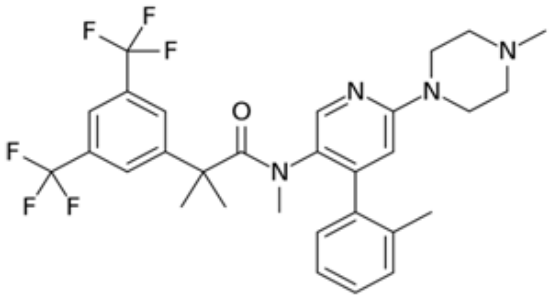

Figure 01: Chemical structure of Netupitant

Palonosetron chemically called as (5S)-3-[(3S)-1-azabicyclo[2.2.2]octan-3-yl]-3-azatricyclo [7.3.1.$0^{5,13}$] trideca- (12), 9(13), 10-trien-2-one.it is a selective serotonin 5-HT_3 receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT_3 receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract). This inhibition of 5-HT_3 receptors in turn inhibits the visceral afferent stimulation of the vomiting center, likely indirectly at the level of the area postrema, as well as through direct inhibition of serotonin activity within the area postrema and the chemoreceptor trigger zone. The chemical structure shown in figure 02

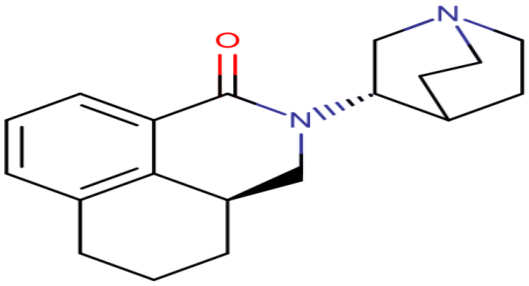

Figure 02: Chemical structure of Palonosetron

The review of literature revealed that several analytical methods have been reported for NTP and PLS in spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatograph y [14-22] individually, and in the combination. To date, there are no reports for stability-indicating simultaneous estimation and forced degradation study of NTP and PLS.

Materials and methods

Materials

Netupitant and Palonosetron pure drugs (API), Combination Netupitant and Palonosetron capsules (Flumed N), Distilled water, Acetonitrile, Phosphate buffer, , Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals andsolvents are from Rankem

Instruments

Electronics Balance-Denver, p^H meter -BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps,Photo Diode Array detector and Auto sampler integrated with Empower 2 Software , UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Netupitant and Palonosetron solutions.

Methods

Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions

Accurately weighed 150 mg of Netupitant , 0.25mg of Palonosetron 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. (3000µg/ml of Netupitant and 5µg/ml of Palonosetron)

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (300µg/mlNetupitant of and 0.5µg/ml of Palonosetron)

Preparation of Sample stock solutions

5 capsules were weighed and the average weight of each capsule was calculated,then the weight equivalent to 1 capsule was transferred into a 100ml 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 (3000µg/ml of Netupitant and 5µg/ml of Palonosetron)

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(300µg/ml of Netupitant and 0.5µg/ml of Palonosetron)

Preparation of buffer

0.1% OPABuffer:1ml of Conc Ortho Phosphoric acid was diluted to 1000mlwith water.

Method Validation [23,24, 26, 27,28,29]

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Netupitant (300ppm) and Palonosetron (0.5ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity

Checking of the interference in the optimized method.We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision

Preparation of Standard stock solutions

Accurately weighed 150 mg of Netupitant , 0.25mg of Palonosetron 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. (3000µg/ml of Netupitant and 5µg/ml of Palonosetron)

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Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(300µg/ml of Netupitant and 0.5µg/ml of Palonosetron)

Linearity

Preparation of Standard stock solutions

Accurately weighed 150 mg of Netupitant , 0.25mg of Palonosetron 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. (3000µg/ml of Netupitant and 5µg/ml of Palonosetron)

25% Standard solution

0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Netupitant and 0.125 µg/ml of Palonosetron)

50% Standard solution

0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (150µg/ml of Netupitant and 0.25µg/ml of Palonosetron)

75% Standard solution

0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (225µg/ml of Netupitant and 0.375µg/ml of Palonosetron)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (300µg/ml of Netupitant and 0.5µg/ml of Palonosetron)

125% Standard solution

1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (375µg/ml of Netupitant and 0.625g/ml of Palonosetron)

150% Standard solution

1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (450µg/ml of Netupitant and 0.75g/ml of Palonosetron)

Accuracy

Preparation of Standard stock solutions

Accurately weighed 150 mg of Netupitant , 0.25mg of Palonosetron 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. (3000µg/ml of Netupitant and 5µg/ml of Palonosetron)

Preparation of 50% Spiked Solution

0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution

1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution

1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102

Robustness

Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Netupitant, Palonosetron , solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Netupitant, Palonosetron , and solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies [25,30,31]

Oxidation

To 1 ml of stock solution of Netupitant and Palonosetron , 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 300µg/ml& 0.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stock ssolution Netupitant and Palonosetron , 1 ml of 2N Hydrochloricacidwasadded and refluxed for 30 mins at 60^0c.The resultant solution was diluted to obtain 300 µg/ml & 0.5µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Netupitant and Palonosetron , 1 ml of 2N sodium hydroxidewasadded and refluxed for 30mins at 60^0c. Theresultantsolutionwas diluted to obtain 300µg/ml& 0.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

Thestandarddrug solution was placed inovenat 105°C for1h tos tudy dry heat degradation.ForHPLCstudy,the resultant solution was diluted to 300µg/ml& 0.5µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 3000µg/ml Netupitant & 5µg/ml Palonosetron solution to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 300µg/ml& 0.5µg/ml solutions and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drugin water for 1 hrs at a temperature of 60^o. For HPLC study, the resultant solution was diluted to 300 µg/ml& 0.5µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Results And Discussion

Optimized method : Chromatographic conditions:

Mobile phase : 55% 0.1% OPA buffer: 450% Acetonitrile

Flow rate : 1ml/min

Column : Discovery C18 (4.6 x 250mm, 5µm)

Detector wave length : 240nm

Column temperature : 30°C

Injection volume : 10µL

Run time : 6min

Diluent : Water and Acetonitrile in the ratio 50:50

Results : Both peaks have good resolution, tailing factor, theoretical plate count and resolution.

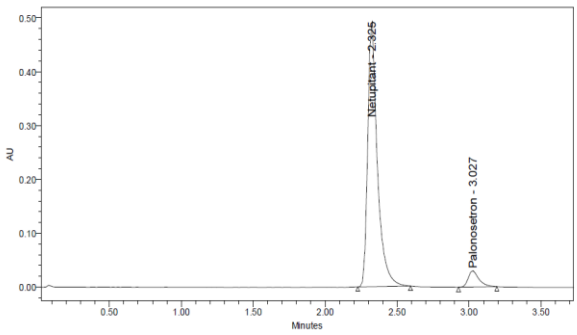

Fig 03 Optimized Chromatogram

Observation

Netupitant and Palonosetron were eluted at 2.325 min and 3.027 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability

All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table: 01 Systemsuitability parameters forNetupitant and Palonosetron

Sno	Netupitant			Palonosetron			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.308	6059	1.47	3.027	8554	1.38	5.4
2	2.325	6110	1.46	3.027	8468	1.38	5.4
3	2.326	6107	1.47	3.028	8479	1.41	5.4
4	2.327	6022	1.47	3.030	8411	1.41	5.3
5	2.327	6049	1.47	3.031	8354	1.48	5.2
6	2.328	6126	1.47	3.093	7946	1.51	5.2

Discussion

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation

Specificity

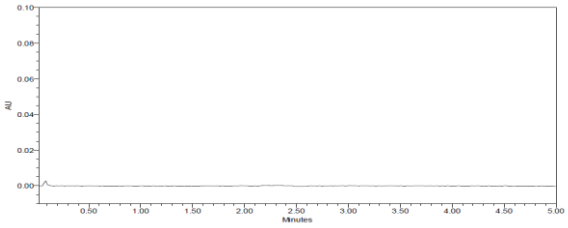

Figure No. 04 Chromatogram of blank

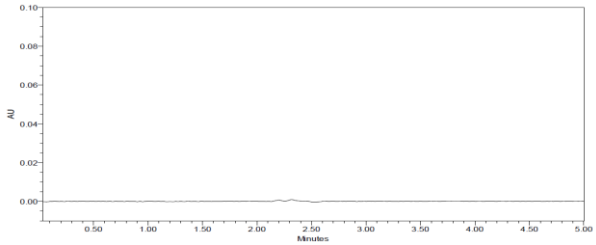

Figure No. 05 Chromatogram of placebo

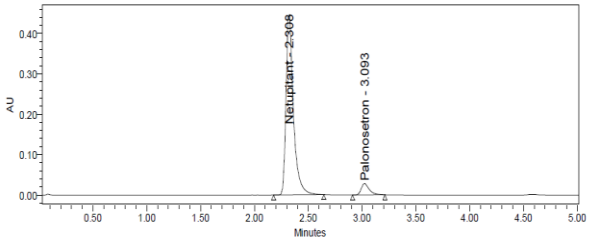

Fig: 06 Typical chromotogram

Discussion

Retention times of Netupitant and Palonosetron were 2.308 min and 3.093 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Table 02: Linearity table forNetupitant and Palonosetron

Netupitant		Palonosetron	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
75	528177	0.125	3559
150	1129538	0.25	7597
225	1642104	0.375	11002
300	2129458	0.5	14542
375	2714082	0.625	18031
450	3272387	0.75	21704

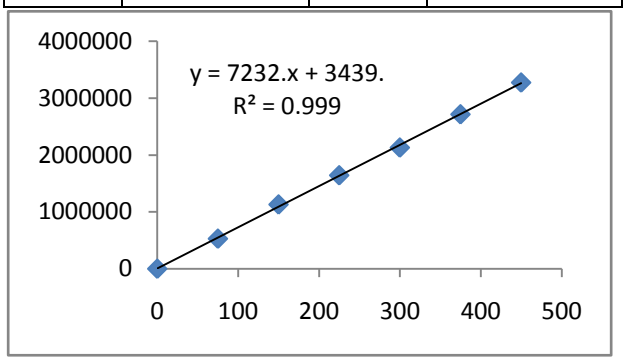

Fig: 07 Calibrationcurve of Netupitant

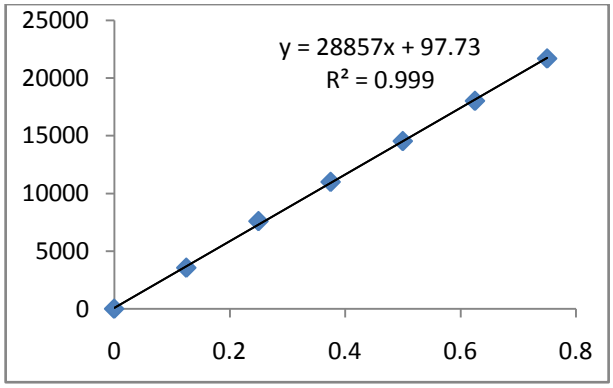

Fig: 08 Calibration curve of Palonosetron

Discussion

Six linear concentrations of Netupitant (150-450µg/ml) and Palonosetron (0.125-0.75µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Netupitant was y = 7232.8x + 3439.3and of Palonosetron was y = 28857x + 97.732Correlation coefficient obtained was 0.999 for the two drugs.

Precision

System Precision

Table 03: System precision table of Netupitant and Palonosetron

S. No	Area of Netupitant	Area of Palonosetron
1.	2123440	14459
2.	2123114	14572
3.	2134157	14541
4.	2097548	14374
5.	2106005	14595
6.	2152700	14571
Mean	2122827	14519
S.D	19714.1	85.3
%RSD	0.9	0.6

Discussion

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs.% RSDobtained as 0.9%and 0.6% respectively for Netupitant and Palonosetron .As the limit of Precision was less than "2" the system precision was passed in this method.

Repeatability

Table: 04 Repeatability table of Netupitant and Palonosetron

S. No	Area of Netupitant	Area of Palonosetron
1.	2107058	14392
2.	2110065	14486
3.	2116355	14486
4.	2109543	14438
5.	2108307	14515
6.	2114707	14518
Mean	2111006	14473
S.D	3693.4	48.8
%RSD	0.2	0.3

Discussion

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.2% and 0.3% respectively for Netupitant and Palonosetron . As the limit of Precision was less than "2" the system precision was passed in this method

Intermediate precision (Day_Day Precision)

Table: 05 Intermediate precision table of Netupitant and Palonosetron

S. No	Area of Netupitant	Area of Palonosetron
1.	2100742	14043
2.	2109756	14045
3.	2102040	14054
4.	2077355	14074
5.	2087633	14049
6.	2131595	14118
Mean	2101520	14064
S.D	18708.3	28.8
%RSD	0.9	0.2

Discussion

Multiple sampling from a sample stock solution was done and six working sample solutions of same

concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.9% and 0.2% respectively for Netupitant and Palonosetron . As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Table: 06 Accuracy table of Netupitant

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	150	148.77	99.18	99.51%
	150	148.80	99.20	
	150	148.94	99.29	
100%	300	296.71	98.90	
	300	297.10	99.03	
	300	299.68	99.89	
150%	450	451.17	99.15	
	450	449.27	99.84	
	450	449.93	99.98	

Table: 07 Accuracy table of Palonosetron

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	0.25	0.25	99.36	99.29%
	0.25	0.25	98.16	
	0.25	0.25	99.51	
100%	0.50	0.50	99.61	
	0.50	0.50	99.72	
	0.50	0.49	98.61	
150%	0.75	0.75	99.45	
	0.75	0.74	99.25	
	0.75	0.75	99.91	

Discussion

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.51% and 99.29% for Netupitant and Palonosetron respectively.

Sensitivity

Table: 08 Sensitivity table of Netupitant and Palonosetron

Molecule	LOD	LOQ
Netupitant	1.84	5.59
Palonosetron	0.01	0.03

Robustness

Table : 09 Robustness data for Netupitant and Palonosetron .

S.no	Condition	%RSD of Netupitant	%RSD of Palonosetron
1	Flow rate (-) 0.9ml/min	0.4	1.1
2	Flow rate (+) 1.1ml/min	0.1	0.8
3	Mobile phase (-) 50B:50A	0.8	0.7
4	Mobile phase (+) 60B:40A	0.7	0.1
5	Temperature (-) 25°C	0.3	0.5

6	Temperatur e (+) 35°C	1.1	0.8

Discussion

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (50B:50A), mobile phase plus (60B:40A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay

Akynzeo, bearing the label claim Netupitant 300mg, Palonosetron 0.5mg.Assay wasperformed with the above formulation. Average % Assay for Netupitant and Palonosetron obtained was 99.34% and 99.58% respectively

Table: 10 Assay Data of Netupitant

S.no	Standard Area	Sample area	% Assay
1	2123440	2107058	99.16
2	2123114	2110065	99.30
3	2134157	2116355	99.60
4	2097548	2109543	99.27
5	2106005	2108307	99.22
6	2152700	2114707	99.52
Avg	2122827	2111006	99.34
Stdev	19714.1	3693.4	0.17
%RSD	0.9	0.2	0.2

Table: 11 Assay Data of Palonosetron

S.no	Standard Area	Sample area	% Assay
1	14459	14392	99.03
2	14572	14486	99.68
3	14541	14486	99.68
4	14374	14438	99.34
5	14595	14515	99.87
6	14571	14518	99.90
Avg	14519	14473	99.58
Stdev	85.3	48.8	0.3
%RSD	0.6	0.3	0.3

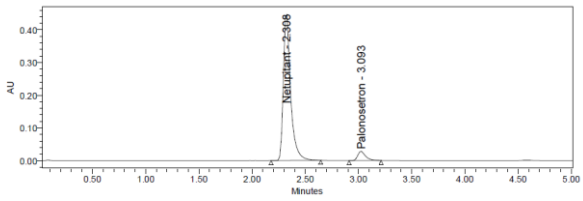

Fig 09 Chromatogram of working standard solution

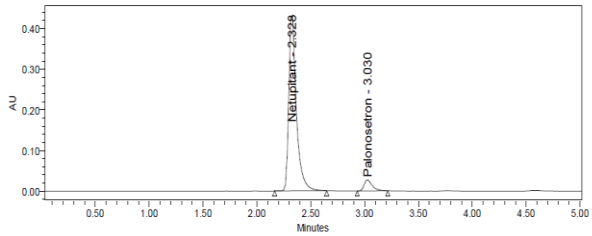

Fig: 10 Chromatogram of working sample solution

Degradation data

Table 12 Robustness data for Netupitant and Palonosetron

Type of degradation	Netupitant			Palonosetron		
	AREA	%RECOVERED	% DEGRADED	AREA	%RECOVERED	% DEGRADED
Acid	2012677	94.72	5.28	13751	94.62	5.38
Base	2032125	95.63	4.37	13801	94.96	5.04
Peroxide	2058647	96.88	3.12	14000	96.33	3.67
Thermal	2075	97.68	2.32	1418	97.58	2.42

	561			2		
Uv	2075561	98.05	1.95	14334	98.63	1.37
Water	2083487	98.05	1.95	14404	99.11	0.89

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netupitant and Palonosetron in pharmaceutical dosage form. Retention time of Netupitant and Palonosetron were found to be 2.308min and 3.093min. %RSD of the Netupitant and Palonosetron were and found to be 0.9 and 0.6 respectively. %Recovery was obtained as 99.51% and 99.29% for Netupitant and Palonosetron respectively. LOD, LOQ values obtained from regression equations of Netupitant and Palonosetron were 1.84, 0.01 and 5.59, 0.03 respectively. Regression equation of Netupitant is y = 7232.8x + 3439.3., and y = 28857x + 97.732 of Palonosetron. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Author Contribution

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References

1. Quinla JD, Hill DA. Nausea and vomiting of pregnancy. Am Fam Physician 2003;68:121-8.
2. Christof S, Peters P, Miller RK. Antiemetics and hyperemesis gravidarum. Drugs During Pregnancy and Lactation: Handbook of Prescription Drugs and Comparative Risk Assessment. New York: Elsevier; 2001.
3. Paula G, Axel G, Loprinzi CL. Nausea and Vomiting in the Cancer Patient. New York: Springer; 2006. p. 1482-96.
4. Judith ET. Emergency Medicine: A Comprehensive Study Guide. New York: McGraw-Hill Companies; 2010. p. 830.
5. Corrie PG, Pippa G. Cytotoxic chemotherapy clinical aspects. Medicines 2008;36:24-8.
6. Levi JA, Aroney RS, Dalley DN. Haemolytic anaemia after cisplatin treatment. Br Med J (Clin Res Ed) 1981;282:2003-4.
7. Barnes NM, Hales TG, Lummis SC, Peters JA. The 5-HT3 receptor-the relationship between structure and function. Neuropharmacology 2009;56:273-84.
8. Purves D, Augustine GJ, Fitzpatrick D. Neuroscience. 4th ed. Sunderland (MA): Sinauer Associates; 2008. p. 11-20.
9. Berthoud HR, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. Auton Neurosci 2000;85:1-7.
10. Saunders CJ, Christensen M, Finger TE, Tizzano M. Cholinergic neurotransmission links solitary chemosensory cells to nasal inflammation. Proc Natl Acad Sci U S A 2014;111:6075-80.
11. Lutz DS Dictonary of Minor Planet Names-Posterma. Berlin Heidelberg: Springer; 2007. p. 118.
12. Pietra S. Indolic derivatives II. A new way to synthesize serotonin. Farmaco Sci 1958;13:75-9.
13. de Lartigue G, Ronveaux CC, Raybould HE. Deletion of leptin signaling in vagal afferent neurons results in hyperphagia and obesity Mol Metab 2014;3:595-607.
14. Pathi PJ, Raju NA The estimation of palonosetron hydrochloride in parenterals by RP-HPLC. Asian J Pharm Tech 2012;2:77-9.
15. Inturi S, Inturi RK, Venkatesh G. A validated novel RP-HPLC method development for the estimation of Palonosetron hydrochloride in bulk and softule dosage forms. Pharm Sin 2011;2:223-34.
16. Murthy MV, Krishnaiah C, Kodithyala J, Katkam S, Mukkanti K, Ramesh K, et al. Enantio separation of palanosetron hydrochloride and its related enantiomeric

impurities by computer simulation and validation. Am J Anal Chem 2011;2:437-46.
17. Jain PS, Chavan RS, Bari PR, Patil SS, Surana SJ. Stability indicating HPTLC method for estimation of palonosetron hydrochloride in tablet dosage form. J Adv Drug Deliv 2015;2:578-86.
18. Patel H, Lava B . Stability indicating HPLC method for estimation of Palonosetron hydrochloride in Tablet dosage form. Int J Pharm Res Sch 2015;4:258-63.
19. Jayesh B, Sharad K, Yoges Y. Development of chromatographic method for the estimation of palonosetron hydrochloride injection. Indian J Pharm Sci 2011;2:24-32.
20. Harole M, Patil RN, Gaware D, Suryawansh G, Pise K. A validated stability indicating RP-HPLC method for simultaneous determination of netupitant and palonosetron in pharmaceutical formulations. World J Pharm Pharm Sci 2016;5:878-87.
21. Shilpa NV, Rajashree C, et al. Simultaneous quantitative estimation of netupitant and palonosetron HCl by HPTLC method development and validation. Eur J Biomed Pharm Sci 2016;3:421-6.
22. Hang TJ, Yu XR, Song M. Direct enantiomeric separation of palonosetron hydrochloride by chiral HPLC. Chin J New Drugs 2008;5:10-6.
23. Rao CP, Rahaman SA, Prasad YR, Reddy PG. RP-HPLC method of simultaneous estimation of amlodipine besylate and metoprolol in combined dosage form. International Journal of Pharmaceutical Research and Development. 2010 Nov;2(9):69-76.
24. Rao CM, Konda R, Ramanjeneeyulu S. Estimation of Nevirapine Anhydrous Bulk Formulation by Using IR, RP-HPLC, GC Methods. Research Journal of Pharmacy and Technology. 2010 Dec 28;3(4):1088-92.
25. Ch M M Prasada et al, Development and Validation of a Novel Stability Indicating RP-HPLC Method for The Estimation of Entecavir In Tablet Formulation, EJBPS, 2017, 4(7):176-180
26. Kumaraswamy.Gandla, R.Lalitha, Dara Varun Kumar, and P.VR Teja Shruthi. "Analytical Method Development & Validation for the Simultaneous Estimation of Ledipasvir and Sofosbuvir in Bulk and it's Dosage Form by Rp-Hplc". International Journal of Pharmaceutics and Drug Analysis, Apr. 2020, pp. 6-15,
27. Gokul S. Sanap, Nilesh S. Zarekar, and Sarita S. Pawar. "review on method development and validation". International Journal of Pharmaceutics and Drug Analysis, May 2017, pp. 177-84,
28. Z. M. Sayyed, Aijaz A. Sheikh, Zakirhussain A. Shaikh, S. A. Shinde, . J. Chaware5, and K. R. Biyani. "development and validation of analytical method for simultaneous estimation of teno-fovir and emtricitabine in pharmaceutical dosage forms by hplc". International Journal of Pharmaceutics and Drug Analysis, Jan. 2016, pp. 24-29.
29. K, V. G., T. K, and S. P. P. "A New Stability Indicating Analytical Method Development And Validation for The Quantitative Determination of Emitricitabine And Lamivudine By RP-HPLC". World Journal of Current Medical and Pharmaceutical Research, Vol. 2, no. 2, May 2020, pp. 184-90, doi:10.37022/WJCMPR.2020.2219.
30. Pemra Raju, K. Thejomoorthy, and P.Sreenivasa Prasanna, "Development and Validation of New Analytical Method for The Simultaneous Estimation of Darunavir And Ritonavir in Pharmaceutical Dosage Form", Int J Indig Herb Drug, pp. 49-57, Apr. 2021.
31. P, S. P., T. K, and D. B. P. "HPLC Method Development And Validation For The Simultaneous Estimation Of Levocetirizine And Phenylephrine In Bulk And Pharmaceutical Dosage Form". International Journal of Pharmacognosy and Chemistry, June 2020, pp. 19-30.