UPI Journal of Pharmaceutical Medical, and Health Sciences

Content Availabe at www.uniquepubinternational.com **ISSN: 2581-4532**

Open Access **Research Article**

Development and validation of new analytical method for the simultaneous estimation of netupitant and palonosetron in bulk and pharmaceutical dosage form

P.Pushpa Divya*[1], Dr.Raj Kumar kudari[2]

[1] Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur 522002

[2] Professor, Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Guntur 522002

Article History

Received: 22-09-2021
Revised: 13-08-2021
Accepted: 09-11-2021

Keywords

Palonosetron, LOD, LOQ

***Corresponding Author**

P.Pushpa Divya

Email: p.pushpadivya@gmail.com

https://doi.org/10.37022/jpmhs.v4i4.38

Abstract

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netupitant and Palonosetron in pharmaceutical dosage form. Retention time of Palonosetron and Netupitant were found to be 2.266 min and 2.805 min. %RSD of the Netupitant and Palonosetron were and found to be 0.8 and 1.1 respectively. %Recovery was obtained as 100.08% and 100.15% for Netupitant and Palonosetron respectively. LOD, LOQ values obtained from regression equations of Netupitant and Palonosetron were 1.63, 4.94 and 0.003, 0.010. respectively. Regression equation of Netupitant is y =11553x + 9661.and y = 51072x + 152.0. of Palonosetron. 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.

Introduction

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data [1] . Hence, development of ruggedand robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.

The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively. While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.

The wide variety of challenges is encountered while developing the methods for different drugsdepending on its nature and properties. This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives anopportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories. Different physico-chemical methods [1] are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical

methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV- Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Methods such as nuclear magnetic resonance (NMR) and para magnetic resonance (PMR) are becoming more and more popular. The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.

Drug Profile

Netupitant

Description

Netupitant is an anti-emetic 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

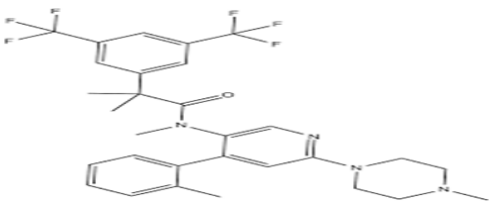

Fig 01: Structure of Netupitant

Palonosetron

Description:

Palonosetron (inn, trade name Aoxi) 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 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 USFDA as of 2008, it is the most recent 5-HT3 antagonist to enter clinical use

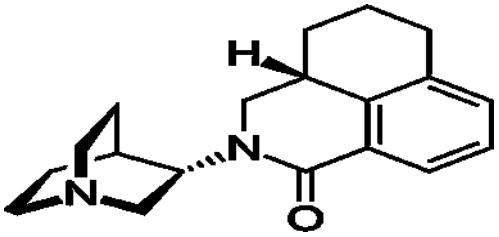

Fig 02: Structure of Palonosetron

Aim, Objective & Plan of Work

Aim

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Netupitant , Palonosetron in bulk ant tablet dosage form.

Objective and Plan

Following are the objectives of the present work:

To develop a new stability indicating HPLC method for simultaneous estimation of `Netupitant and Palonosetron and to develop the validated method accordingto ICH guidelines.

To apply the validated method for the simultaneous estimation of Netupitant and Palonosetron in pharmaceutical formulation

Materials and Methods

Materials

Netupitant and Palonosetron pure drugs (API),

Combination Netupitant and Palonosetron tablets (Akynzeo),

Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid.

Allthe above chemicals and solvents 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 Watertaken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately Weighed and transferred 150mg of Netupitant and 0.25mg of Palonosteron working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. (3000ppm of Netupitant and 5ppm of Palonosteron)

Preparation of Standard working solutions (100% solution): 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (300ppm of Netupitantand 0.5ppm of Palonosteron)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100mlvolumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume wasmade 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

Buffer:0.1% Formic acid buffer

1ml of formic acid was diluted to 1000ml with HPLC milli-Q water to get 0.1% Formic acid(pH-2.2).

Validation

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Netupitant (50ppm) and Palonosetron (25ppm) 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 be not more than 2%.

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

Precision

Preparation of Standard stock solutions: Accurately Weighed and transferred 150mg of Netupitant and 0.25mg of Palonosteron working Standards into a 150ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. (3000ppm of Netupitant and 5ppm of Palonosteron)

Preparation of Standard working solutions (100% solution): 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (300ppm of Netupitantand 0.5ppm of Palonosteron)

Linearity

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Netupitant and 0.125µg/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 0.625µg/ml of Palonosetron)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (450 µg/ml of Netupitant and 0.375µg/ml of Palonosetron)

Accuracy

Preparation of Standard stock solutions: Accurately weighed 150mg of Netupitant, 0.25mg of Palonosetron and transferred to 150ml flasks and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (3000µg/ml of Netupitant and 5µg/ml 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 deliberate changes 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 werenot much effected 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, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies

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 10 µl 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 Hydrochloric acid wasadded and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 30 0µg/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 hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to 300µg/ml& 0.5µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution w as placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, 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 10 µl 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 drug in water for 1h r sat a temperature of 60^o. For HPLC study, the resultant solution was diluted to 300µg/ml& 0.5µg/ml and 10 µl 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: 65% 0.1% Formic acid: 35% Acetonitrile

Flow rate : 1 ml/min

Column : BDS C8 (4.6 x 150mm, 5µm)

Detector wave length : 256nm

Column temperature : 30°C

Injection volume : 10µL

Run time : 5 min

Diluent : Water and Acetonitrile in the ratio 50:50

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

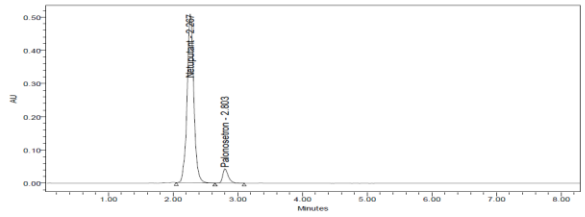

Fig 03: Optimized Chromatogram

Observation: Palonosetron and Netupitant were eluted at 2.267min and 2.803smin 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.

Tab 01: Optimized Chromatogram

S No	Netupitant			Palonosetron			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.257	2590	0.98	2.802	5616	1.35	3.1
2	2.261	2678	0.97	2.803	5671	1.36	3.1
3	2.266	2700	0.97	2.803	5184	1.37	3.0
4	2.270	2554	0.96	2.804	4965	1.41	3.0
5	2.272	2482	1.03	2.804	5220	1.35	3.0
6	2.275	2460	1.00	2.805	5678	1.38	3.1

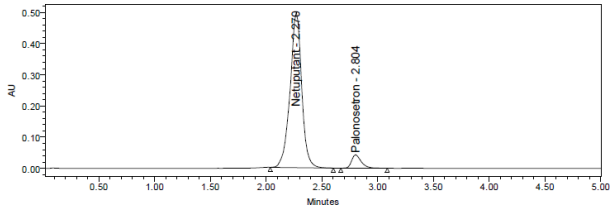

Fig 04: System suitability Chromatogram

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

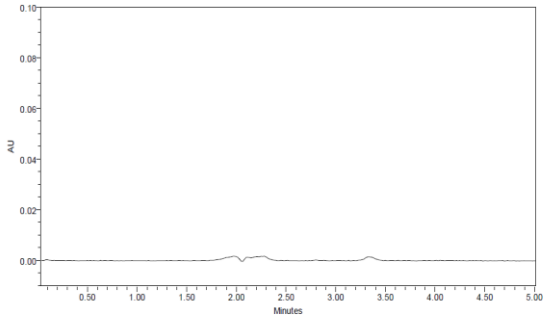

Fig 05: Chromatogram of blank

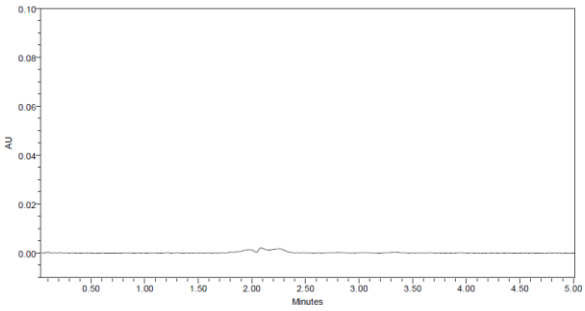

Fig 06: Chromatogram of placebo

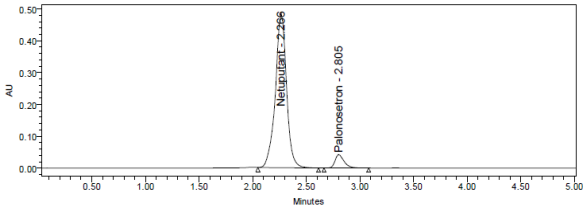

Fig 07: Typical Chromatogram

Discussion

Retention times of Netupitant and Palonosetron were 2.266min and 2.805min 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

Tabl 02: Linearity table for Netupitant and Palonosetron.

Netupitant		Palonosetron	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
75	870863	0.125	6477
150	1738793	0.25	12943

225	2610692	0.375	19501
300	3531707	0.5	25925
375	4325598	0.625	32125
450	5186586	0.75	38158

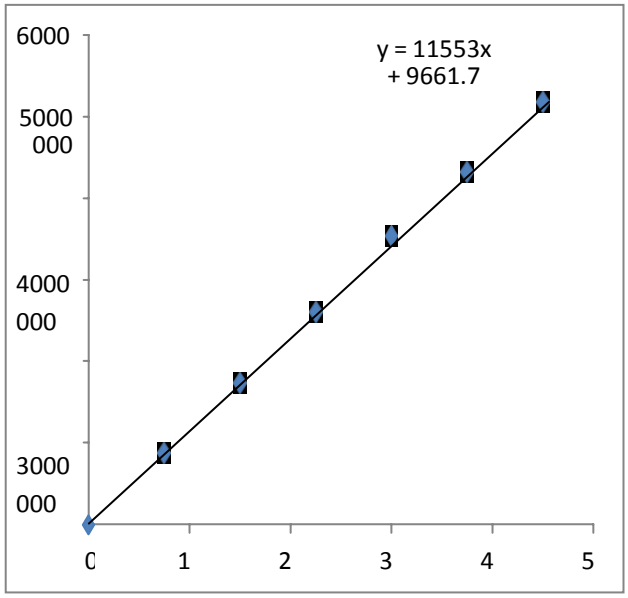

Fig 08: Calibration curve of Netupitant

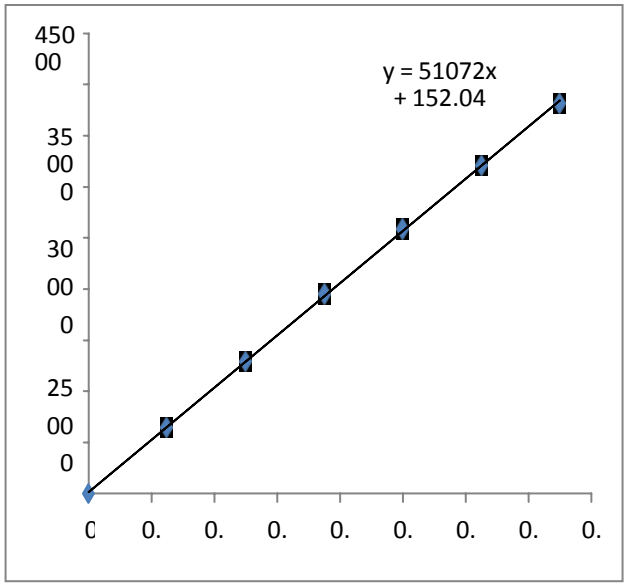

Fig 09: Calibration curve of Palonosetron

Discussion

Six linear concentrations of Netupitant (75_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 = 11553x + 9661. and of Palonosetron was y = = 51072x + 152.0. Correlation coefficient obtained was 0.999 for the both drugs.

Precision

System Precision

Table 03: System precision table of Netupitant and Palonosetron

S. No	Area of Netupitant	Area of Palonosetron
1.	3492593	25875
2.	3526436	26264
3.	3493044	25602
4.	3501522	26041
5.	3460270	25522
6.	3446836	26078
Mean	3486784	25897
S.D	28848.8	288.6
%RSD	0.8	1.1

Repeatability

Tab 04: Repeatability table of Netupitant and Palonosetron

S. No	Area of Netupitant	Area of Palonosetron
1.	3503549	26019
2.	3529833	25948
3.	3487444	25904
4.	3454656	26442
5.	3549807	26294
6.	3522305	26023
Mean	3507932	26105
S.D	33814.4	213.8
%RSD	1.0	0.8

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 1.0% and 0.8% 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)

Tab 05: Intermediate precision table of Netupitant and Palonosetron

S. No	Area of Netupitant	Area of Palonosetron
1.	3492594	24875
2.	3557050	25602
3.	3427412	25348
4.	3460226	25694
5.	3499196	25113
6.	3524965	25957
Mean	3493574	25432
S.D	45905.7	398.1
%RSD	1.3	1.6

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 1.3% and 1.6% respectively for Netupitant and Palonosetron. As the limit of Precision was less than "2" the system precision was passed in this method.

Accuracy

Tab 06: Accuracy table of Netupitant

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
	150	149.7	99.8	
	150	151.2	100.8	
50%	150	151.1	100.7	
	300	299.0	99.7	
	300	301.3	100.4	
100%	300	302.3	100.8	
	450	449.9	100.0	
	450	445.7	99.0	
150%	450	447.7	99.5	100.08%

Tab 07: Accuracy table of Palonosetron

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
	0.25	0.251	100.26	
50%	0.25	0.251	100.32	
	0.25	0.250	99.86	
	0.5	0.503	100.58	
100%	0.5	0.500	100.09	
	0.5	0.498	99.60	
	0.75	0.756	100.76	100.15%
150%	0.75	0.748	99.73	
	0.75	0.751	100.15	

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 100.08% and 100.15% for Netupitant and Palonosetron respectively.

Sensitivity

Tab 8: Sensitivity table of Netupitant and Palonosetron

Molecule	LOD	LOQ
Netupitant	1.63	4.94
Palonosetron	0.003	0.010

Robustness

Tab 09: Robustness table of Netupitant and Palonosetron

S.no	**Condition**	**%RSD of Netupitant**	**%RSD of Palonosetron**
1	Flow rate (-) 0.9ml/min	0.6	1.8
2	Flow rate (+) 1.1ml/min	1.7	1
3	Mobile phase (-) 70B:30A	1	1.0
4	Mobile phase (+) 60B:40A	1.1	1.8
5	Temperature (-) 25°C	0.6	0.7
6	Temperature (+) 35°C	0.6	0.6

Discussion

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (70B:30A), 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 werepassed. %RSD was within the limit.

Assay

Rhodes pharmaceuticals, bearing the label claim Netupitant 300mg, Palonosetron 0.5mg. Assay was performed with the above formulation. Average % Assay for Netupitant and Palonosetron obtained was 100.41% and 100.47%respectively.

Tab 10: Assay table of Netupitant

S.no	Standard Area	Sample area	% Assay
1	3492593	3503549	100.28
2	3526436	3529833	101.03
3	3493044	3487444	99.82
4	3501522	3454656	98.88
5	3460270	3549807	101.60
6	3446836	3522305	100.82
Avg	3486784	3507932	100.41
Stdev	28848.8	33814.4	0.97
%RSD	0.8	1.0	1.0

Tab 11: Assay table of Palonosetron

S.no	**Standard Area**	**Sample area**	**% Assay**
1	25875	26019	100.27
2	26264	25948	100.00
3	25602	25904	99.83
4	26041	26442	101.90
5	25522	26294	101.33
6	26078	26023	100.29
Avg	25897	26105	100.60
Stdev	288.6	213.8	0.8
%RSD	1.1	0.8	0.8

Degradation studies

standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali, and oxidative, photolytic, thermal and neutral analysis.

Tab 12: Assay table of Palonosetron

Type of degradation	**Netupitant**	**Palonosetron**

	%RecoVered	% Degraded	%Recovered	% Degraded
Acid	94.39	5.61	94.36	5.64
Base	95.20	4.80	96.65	3.35
Peroxide	96.02	3.98	97.08	2.92
Thermal	97.30	2.70	97.99	2.01
Uv	98.11	1.89	98.64	1.36
Water	99.02	0.98	99.53	0.47

Conclusion

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netupitant and Palonosetron in pharmaceutical dosage form. Retention time of Palonosetron and Netupitant were found to be 2.266 min and 2.805 min. %RSD of the Netupitant and Palonosetron were and found to be 0.8 and 1.1 respectively. %Recovery was obtained as 100.08% and 100.15%for Netupitant and Palonosetron respectively. LOD, LOQ values obtained from regression equations of Netupitant and Palonosetron were 1.63, 4.94 and 0.003, 0.010. respectively. Regression equation of Netupitant is y =11553x + 9661.and y = 51072x +152.0. of Palonosetron. 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.

References

1. "Burger's Medicinal Chemistry and drug discovery", 6 th edition, Wiley Interscience, NewJersey, 2007.
2. "Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry", 11th edition, Lippincott Williams & Wilkins, New york, 2004.
3. A. Korolkovas. "Essentials of Medicinal Chemistry", 2nd edition, Wiley Interscience, New Jersey, 1988.
4. "Goodman and Gilman's The Pharmacological Basis of Therapeutics", 9th edition, McGraw-Hill health professions division, New york, 1996.
5. Foye's "Principles of Medicinal Chemistry", 6th edition, Lippincott Williams & Wilkins, New york, 2008.
6. Drugs & Cosmetics Act, 1940 & Rules, 1945, 2nd edition, Susmit publishers, Mumbai,India, 2000.
7. Indian Pharmacopoeia, Ministry of Health & Family Welfare, Government of India, New Delhi, 1996.
8. The United States Pharmacopoeia- the National Formulary, United States Pharmacopoeial convention, Rockville, 2007.
9. British Pharmacopoeia, The Stationary Office, London, 2005.
10. "Martindale - The Extra Pharmacopoeia", 33rd edition, The Pharmaceutical Press, London, 2002. 7
11. A. H. Beckett and J. B. Stenlake. "Practical Pharmaceutical Chemistry", Volume I and II, CBS Publishers & Distributors, New Delhi, India, 2000.
12. P. D. Sethi. "Quantitative Analysis of Drugs in Pharmaceutical Formulations". 3 rd edition,CBS Publishers & Distributors, New Delhi, India, 1997.
13. H. H. Willard, L. L. Merrit, J. A. Dean and F. A. Settle. "Instrumental Method of Analysis", 7th edition, CBS Publishers & Distributors, New Delhi, India, 1986.
14. R. A. Day and A. L. Underwood. "Quantitative Analysis", 6th edition, PHI learning private limited, New Delhi, India, 2009.
15. G. Ramana Rao, S. S. N. Murthy and P. Khadgapathi. Gas chromatography to pharmaceutical analysis (Review). Eastern Pharmacist. 30(353): 35 (1987).