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RESEARCH ARTICLE

A NOVEL VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NETUPITANT AND PALONOSERTRON IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: A stability-indicating reversed-phase high-performance liquid chromatography (Rp-HPLC) method with a high sensitivity was developed for the validation of stability of Netupitant and Palonosetron in bulk and pharmaceutical dosage form.

Methods: The chromatographic separation was achieved on Luna Phenyl Hexyl (250mm×4.6mm, 5mm particle size), Waters Alliance 2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of 0.01M Triethylamine buffer (pH adjusted to 2.5 with orthophosphoric acid) and Acetonitrile (60:40, v/v). The flow rate was set to 1ml/min with the responses measured at 222nm. The reversed-phase HPLC method has been validated as per International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines to determine Netupitant and Palonosetron in pharmaceutical dosage form.

Results: The proposed method showed a good linearity in the concentration range of 120.04-900.30µg/ml for h Netupitant and 0.12-1.56µg/ml for Palonosetron under optimized conditions. The statistical performance of the fully validated HPLC method and the performance results of the proposed HPLC method were considerably satisfactory with reference to the RSD values of validation parameters like linearity, system precision, method precision, robustness, ruggedness etc.,. The validated method was successfully applied to quantify the Netupitant and Palonosetron in tablet form, and the corresponding recovery value was found to be 100 % for both Netupitant and Palonosetron.

Conclusions: The validated HPLC method is one of the promising alternative analytical tool for routine analysis of Netupitant and Palonosetron in pharmaceutical samples.

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INTRODUCTION

Netupitant is a novel antiemetic agent used in cancer chemotherapy. Palonosetron hydrochloride is a antiemetic and antinauseant agent. 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 [1] Bioavailability is estimated to be over 60% for orally taken netupitant. Highest blood plasma concentrations are reached five hours after application. Availability is moderately (10–20%) increased when taken after a fatty meal. Netupitant and its main metabolites (called M1 and M3) are bound to plasma proteins to more than 99%, and M2 protein binding is 97%.

The review of literature revealed that several analytical methods have been reported for netupitant^[2-7] and palonosetron^[12-18] in spectrophotometry, HPLC, HPTLC, LC/MS individually and in the combination.

To date, there are some published reports about the stability indicating studies and simultaneous estimation of netupitant and palonosetron by HPLC in bulk drug and in pharmaceutical dosage forms. This present study report for the first time stability indicating simultaneous estimation of netupitant and palonosetron by RP HPLC in bulk drug and in pharmaceutical dosage form with particular column and buffer specifications.

Netupitant chemically known as 2-[3,5-bis(trifluoromethyl)phenyl]-N,2-dimethyl-N-[4-(2-ethylphenyl)-6-(4-methylpiperazin-1-yl)pyridin-3-yl]propanamide with molecular formula C₃₀H₃₂F₆N₄O. The structural formula of Netupitant is as shown in figure 1^[19]

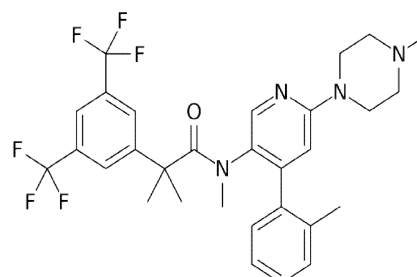


Figure 1 Structure of Netupitant

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Palonosetron is chemically known as (3aS)-2-[(S)-1-Azabicyclo[2.2.2] oct-3-yl]-2,3,3a,4,5,6-exahydro-1-oxo-1Hbenz[de] isoquinoline hydrochloride with molecular formula $C_{19}H_{24}N_2O.HCl$. The structural formula of Palonosetron is as shown in figure 2^[20].

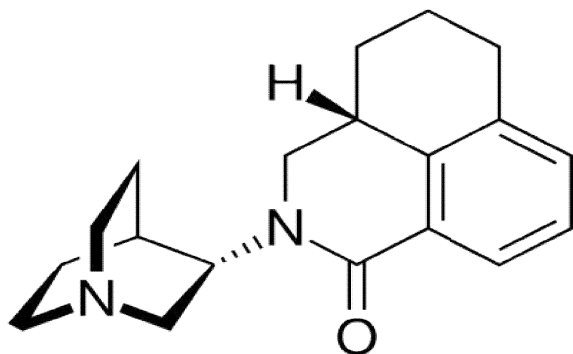


Figure 2 Structure of Palonosetron

Experimental

Chemicals and reagents

All the reagents used in the experimental work were of analytical grade. HPLC grade water was prepared by Milli-Q reverse osmosis (Millipore, Bedford, USA) and meets European Pharmacopoeia requirements. Acetonitrile and *ortho*-phosphoric acid (Sigma–Aldrich, Merk and Rankem) were used for preparing the mobile phase. Mobile Phase was used as solvent.

Working standards of Netupitant and Palonosetron were provided by Glenmark Pharmaceuticals (Mahape, Navi Mumbai). Netupitant and Palonosetron was checked by comparison with European Pharmacopoeia CRS standards. Formulation was obtained from.

Chromatographic conditions (instrumentation and analytical conditions)

An Alliance 2695 (Waters, USA) chromatographic system was used, equipped with a Quaternary pump, and waters 2996 photo diode array detector, Luna Phenyl Hexyl column, auto sampler thermostat and degasser. Chromatographic software Empower was used for data collection and processing. Separations were performed using Luna Phenyl Hexyl analytical column, 4.6 mm × 250 mm packed with 5 μm particle size. A 1m long steel capillary with 0.25 mm internal diameter, was inserted between the injection system and the entrance of the column, and injection volume was 10 μL. Separations and simultaneous determination of Netupitant and Palonosetron were performed using the mixture of Triethylamine(0.1%) : Acetonitrile (60:40, v/v) as a mobile phase. Mobile phase was filtered through a 0.45 μm Millipore filter. The flow rate was 1.0 mL min⁻¹ and the UV detection was performed at 222 nm.

Preparation of solutions

Preparation of Mobile Phase

Take 1ml Tri ethyl amine is dissolved into 1000ml of water. Adjust pH-2.5 with OPA which is mixed with Acetonitrile in the ratio of 60:40. Filter through 0.45μ membrane filter paper. Use the mobile phase as diluent.

Standard solution preparation

Solution-A: Weigh accurately about 600 mg of Netupitant working standard are taken into a 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume with diluent.

Solution-B: Weigh accurately about 10 mg of Palonosetron working standard are taken into a 10 mL volumetric flask. Add 7 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 1ml of the above solution to 50 ml with diluents.

Further dilute each 5mL of solution-A&B standard stock solution to 50 mL with the diluent.

Preparation of Sample solution

Weigh about 640mg of AKYNZEO tablet power was taken into a 100 mL volumetric flask. Add 70 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 5 mL to 50 mL with the diluent. Filter through 0.45μ Nylon syringe filter.

Validation procedure

Chromatographic separation was optimized in the aim to obtain a resolution above 1.5 between all components, with the respect of stationary and mobile phase compositions, flow rate, sample volume, detection wavelength and temperature.

The method was validated for linearity, range, precision (repeatability and intermediated precision), specificity, limit of quantization, limit of detection, robustness and forced degradation.

Linearity and range

Standard calibration curves were prepared with five calibrators over a concentration range of 120.04-900.30μg/ml for Netupitant and 0.12-1.56μg/ml for Palonosetron. The data of peak area versus drug concentration were treated by linear least square regression analysis. The standard curves were evaluated for linearity.

Precision

The precision of the assay was studied with respect to both repeatability and intermediated precision. Repeatability was calculated from six replicate injections of freshly prepared solution in the same equipment on the same day. Repeatability for Netupitant and Palonosetron was realized with a 600 and 1.04 μg mL⁻¹ solution. The experiment was repeated by assaying freshly prepared solution at the same concentration on 2 additionally consecutive days to determine intermediate precision. Precision was expressed by the % of the relative standard deviation (R.S.D.) of the analyte peaks.

Specificity

Specificity of a method can be defined as absence of any interference at retention times of peaks of interest, Specificity of a method can be defined as absence of any interference at retention times of peaks of interest, and was evaluated by observing the chromatograms of blank samples and samples spiked with Netupitant and Palonosetron. The variable number of excipient used in generic versions of Netupitant and Palonosetron, as well

as the lack of information in the composition of some generic formulations makes it difficult to assess selectivity by traditional analysis comparison with a placebo solution.

Limits of detection and quantization

Limits of detection (LOD) and limits of quantization (LOQ) were provided and calculation was made with the following equations:

$$LOD = \frac{3.3 \sigma}{S}$$

$$LOQ = \frac{10 \sigma}{S}$$

When σ was the standard deviation of the response (estimated from the standard deviation of y - intercepts or regression lines) and S was the slope of the standard curve.

Sensitivity

The sensitivity (6σ) of an analytical method is defined by the minimum variation that requires to be applied to the magnitude measured in order to obtain a significant variation in the signal measured.

Robustness

Robustness of method was investigated by varying the chromatographic conditions such as change of flow rate ($\pm 20\%$), organic content in mobile phase ($\pm 2\%$), wavelength of detection ($\pm 5\%$). Robustness of the developed method was indicated by the overall %RSD between the data at each variable condition.

Forced degradation

Forced degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principal peaks shall pass.

Stability

Stability by preparing the analytical solution and injecting at periodic intervals of 24 hours to 48 hours at 3 to 4 hour intervals depending on the instrument utilization and sequence of injection.

RESULTS AND DISCUSSION

In this paper, we developed the reverse phased column procedure for a suitable method for the pharmaceutical analysis of Netupitant and Palanosteron drug and tablets. A typical chromatogram obtained by using the mobile phase. The precision, accuracy and forced degradation of the method was determined from Netupitant and Palanosteron dosage form and obtained. Inter and intra- day studies were performed in three concentrations of the drug was reported on three consecutive days.

Method Validation

The method was validated for linearity, precision, accuracy, robustness, ruggedness, forced degradation and stability of the Netupitant and palanosteron.

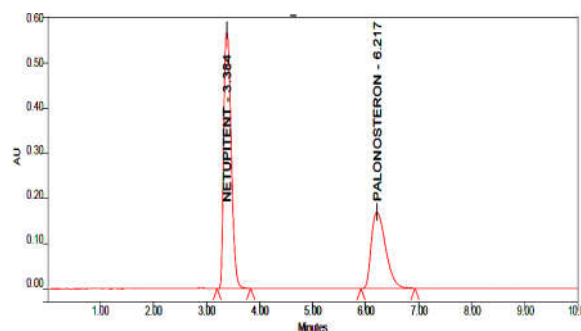


Figure 3 Typical Chromatogram

Linearity was prepared in the range of 120.04-900.30 $\mu\text{g/ml}$ for Netupitant and 0.21- 1.56 $\mu\text{g/ml}$ solutions are analyzed through the high pressure liquid chromatographic technique. The peak area were plotted against concentration was subjected to linear plots shown in figures 4 & 5.

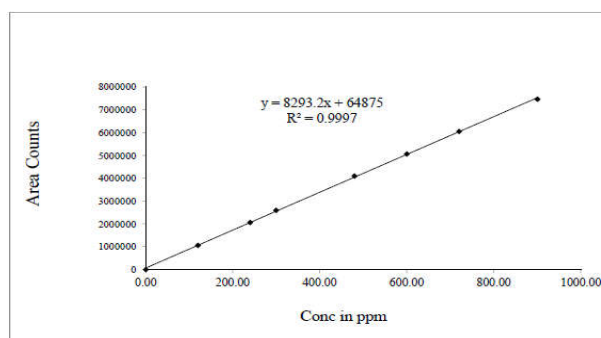


Figure 4 Linearity plot for Netupitant

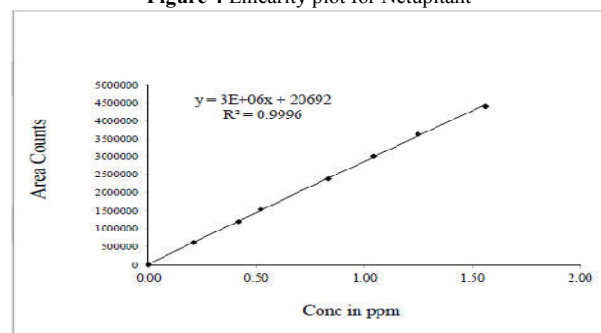


Figure 5 Linearity plot for Palanosteron

Precision of this method was studied in inter day and intra day variation. The precision of intraday studies of six different concentration of the drug was repeated thrice in a day and in the inter day variation studies of six different concentration of the drug was repeated on three consecutive days. The developed method was found to be precise as the percentage of RSD values for inter-day and intra-day precision studies were found to be less than 2%. Good recoveries (99 - 100%) of the drug were obtained at each added concentration, indicating that the method was accurate.

Table 1 Recovery of Netupitant drug

Amount of Netupitant drug mg/ml	Recovery Solution (area) mAU	% drug recovery
300	2582837	99.9
600	5032385	100.1
900	7610863	100.2

Table 2 Recovery of Palanosteron drug

Amount of Palanosteron drug mg/ml	Recovery Solution (area) mAU	% drug recovery
0.5	1515478	100.4
1.0	3016447	100.2
1.5	4635550	100.8

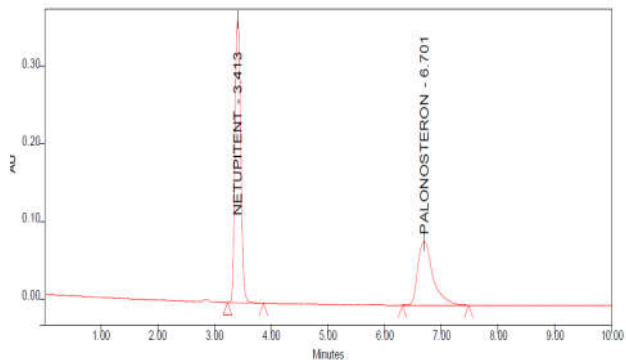


Figure 6 Chromatogram for Accuracy 50%

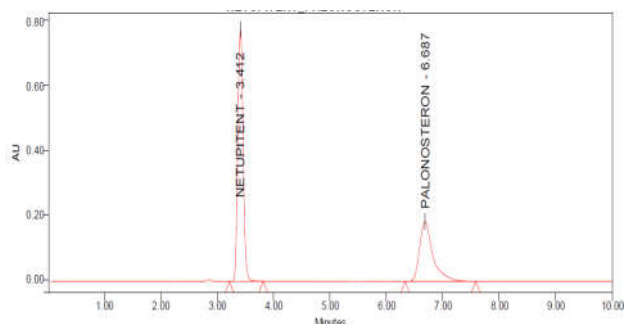


Figure 7 Chromatogram for Accuracy 100%

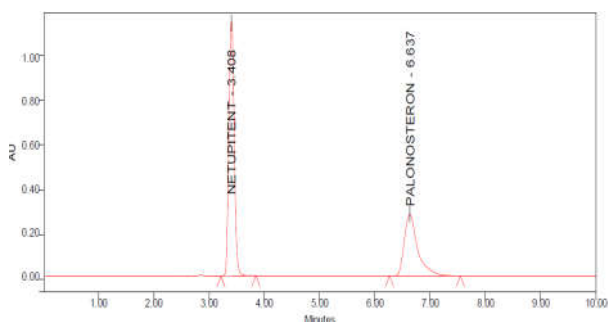


Figure 8 Chromatogram for Accuracy 150%

The limit of detection (LOD) for Netupitant and palanosteron were found to be 1.5005 and 0.0026 µg/mL calculated from related equation (S/N = 3). The similar study claimed that a narrow working range(LOQ) such as 3.001 – 0.0052 µg /mL for Netupitant and palanosteron were obtained at the excitation wavelength of 222 nm.

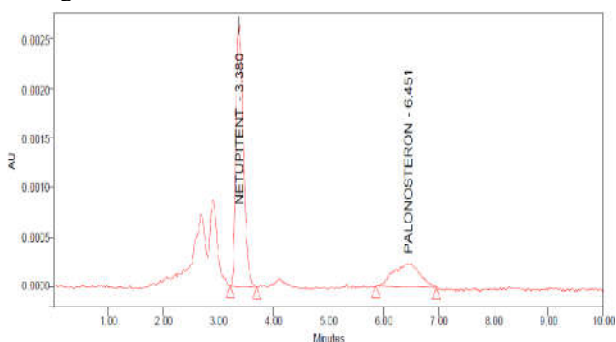


Figure 9 Chromatogram for LOD

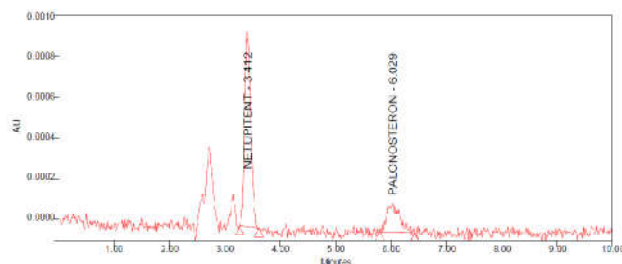


Figure 10 Chromatogram for LOQ

Forced degradation experiments were also performed to evaluate the stability and specificity of the proposed HPLC method in different mediums. The acidic, alkaline and oxidative degradation of Netupitant and Palanosteron were studied by treating with strengths of base (0.05 N and 0.5 N NaOH), acid (0.05 N, 0.5 N and 1 N HCl), 30 % H₂O₂ solutions at 80 °C for 1 h, respectively. The thermal degradation of Netupitant and Palanosteron were also studied by heating the Netupitant and Palanosteron solution at 80 °C for 3 h and photolytic degradation was studied by exposing Netupitant and Palanosteron solution to sunlight for 6 h. The whole degradation products were observed at approximately 3.90 and 5.70 min for Netupitant and Palanosteron, respectively, in all proposed stress conditions as shown in figures 11-17. The stress studies showed no significant difference in terms of retention times of drugs, and no interfering peaks were observed within the retention time under alkaline, acidic, oxidative, thermal and pyrolytic degradation conditions. Considering all these data, Netupitant and Palanosteron were successfully separated from all the degradation products which were confirmed by the resolution values calculated from each chromatogram (Rs > 1.5)

Table 3 Results of force degradation studies

Stress Condition/ duration/solution	Degradation %	
	Netupitant	Palanosteron
Acid degradation (0.5 N HCl, 1 hr)	25.9 %	25.5 %
Alkaline degradation (0.5 N NaOH, 1 hr)	28 %	27.8 %
Oxidative degradation (30 % H ₂ O ₂ , 80°C for 10 min)	28.6 %	29.4%
Reduction Degradation (10% Sod.Bisul, 1hr)	29.6 %	26.7%
Thermal degradation (Solid sample, 80°C, 3 hr)	28.9 %	29.4%
Photolytic Degradation (sample expose sun light 6hr)	21.9 %	23.5%
Hydralysis Degradation	26.8 %	29.5%

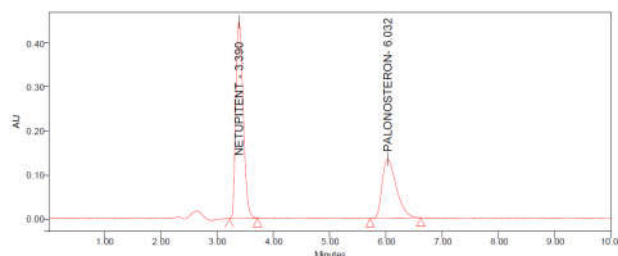


Figure 11 Chromatogram for Acid Deg.

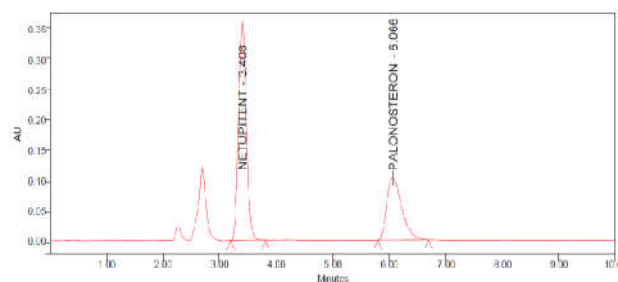


Figure 12 Chromatogram for Alkali Deg.

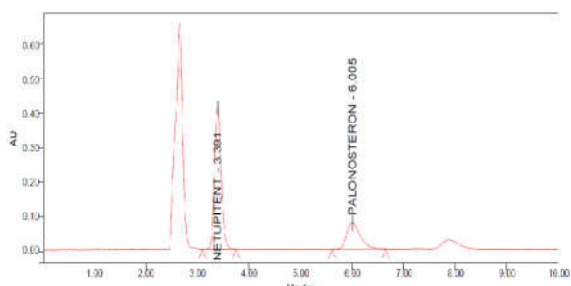


Figure 13 Chromatogram for Peroxide Deg.

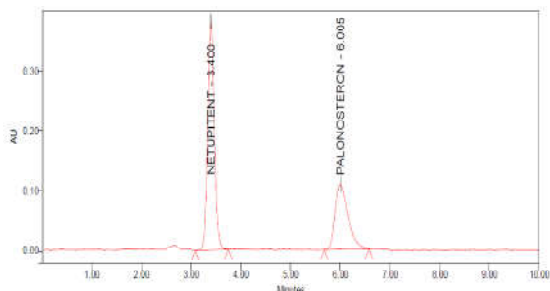


Figure 14 Chromatogram for Reduction Deg.

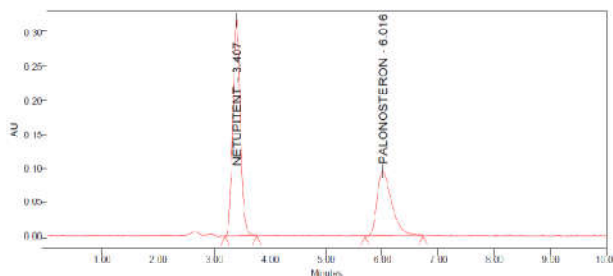


Figure 15 Chromatogram for Thermal Deg.

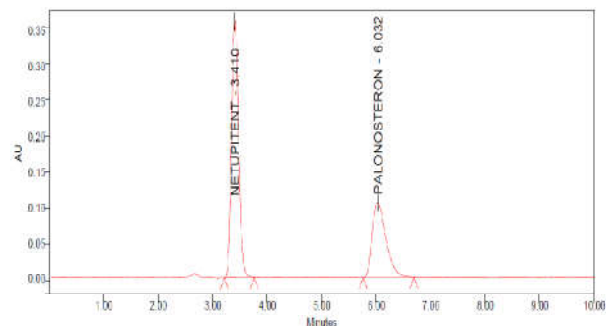


Figure 16 Chromatogram for Photolytic Deg.

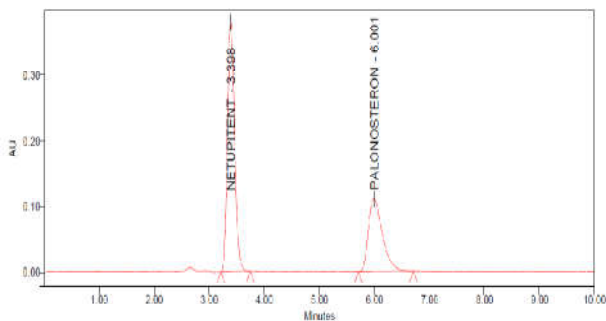


Figure 17 Chromatogram for Hydralysis Deg.

Robustness of the method includes small changes in chromatographic conditions such as change in flow rate ($\pm 20\%$), organic content in mobile phase ($\pm 2\%$), pH (± 0.2) and wavelength of detection ($\pm 5\%$). To determine the robustness of the method for the analysis of FTD and TPI the above

mentioned changes has been undertaken and the RSD values were found to be reliable ($RSD < 1.5\%$).

The influence of changes in chromatographic parameters was shown in table 4. The chromatographic data has been shown in figures 18 to 25.

Table 4 Results for Robustness study

Change in parameter	% RSD
Flow plus (1.2 ml/min)	0.44
Flow minus(0.8 ml/min)	0.1
Wavelength plus (227 nm)	0.1
Wavelength minus (218 nm)	0.4
Organic phase composition (+2%)	0.26
Organic phase composition (-2%)	0.41
pH Variation (+0.2)	0.55
pH Variation (-0.2)	0.35

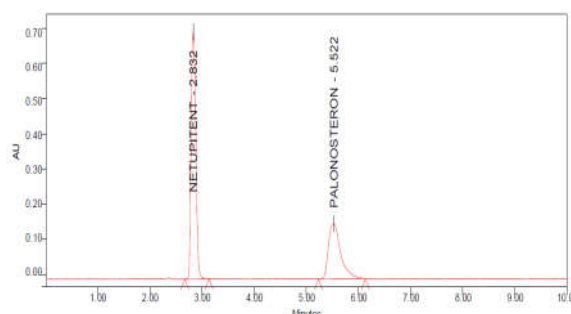


Figure 18 Chromatogram for Flow Plus

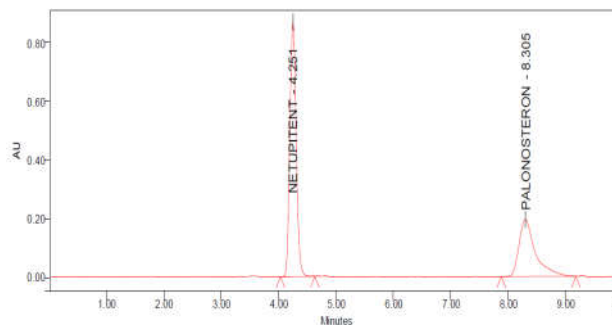


Figure 19 Chromatogram for Flow Minus

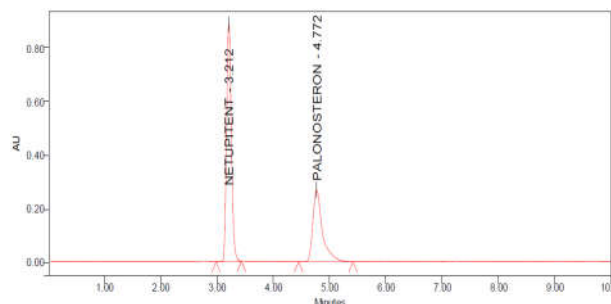


Figure 20 Chromatogram for organic Plus

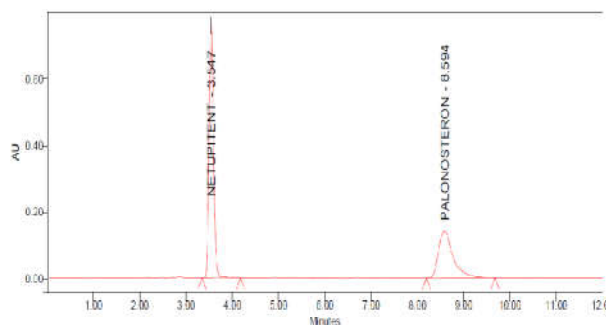


Figure 21 Chromatogram for organic Minus

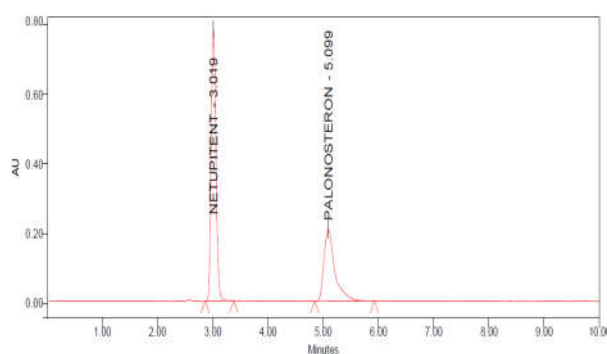


Figure 22 Chromatogram for pH Plus

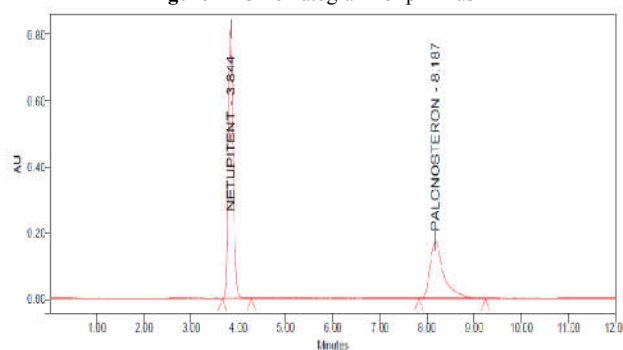


Figure 23 Chromatogram for pH Minus

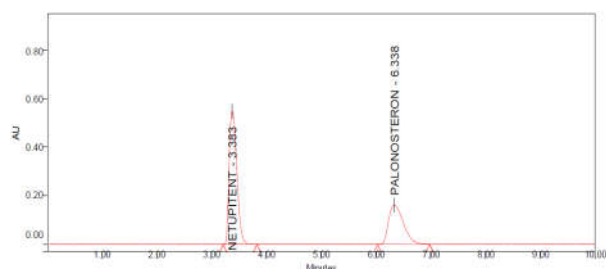


Figure 24 Chromatogram for Wavelength Plus

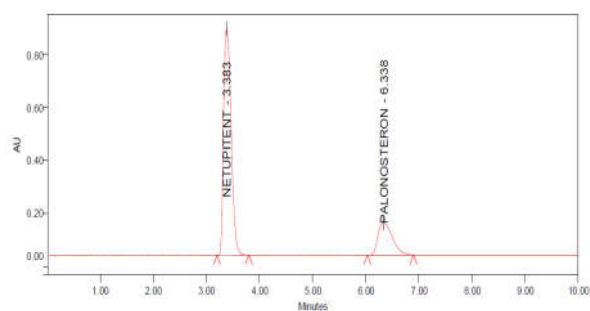


Figure 25 Chromatogram for Wavelength Minus

CONCLUSIONS

A highly sensitive and effective validated reversed-phase HPLC method was successfully developed with a low LOD value for Netupitant and Palonosetron in bulk and pharmaceutical dosage form. The Netupitant and Palonosetron were subjected to forced degradation under several stress conditions. The satisfactory results were achieved from degradation studies, which revealed that the method was stability indicating. Besides This method was validated for linearity, accuracy, precision, robustness of Netupitant and Palonosetron drug. The RSD values for all parameters were found to be less than 2, which indicates the validity of method and results obtained by this method are in fair agreement. Finally this method can be used as better analytical tool for pharmaceutical formulations of Netupitant and Palonosetron drug.

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