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Validated stability indicating RP-HPLC method for simultaneous determination of netupitant and palanosetron in combined pharmaceutical formulations

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Abstract
A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of Netupitant and palanosetron using reverse phase Kromasil C18 (4.6 x 150mm, 5µm) with UV detector at 240nm. The mobile phase consisting of ACN, Potassium disodiumhydrogen phosphate in the ratio of 40:60 (v/v) and at a flow rate of 1 mL/min. The method was linear over the concentration range of 75-450µg/m for Netupitat and 0.125-0.75µg/m for palanosetron. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Netupitant and Palanosetron in combined tablet dosage form.

Keywords: Netupitant, palanosetron, HPLC, validation

Introduction

Netupitant [1] is an antiemitic 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. Palonosetron [2] 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 CINV 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 U.S. Food and Drug Administration. The stability indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product. The chemical structures of both drugs were given in fig. 1. Literature survey revealed few analytical methods is reported for estimation both the drugs by using HPLC [3, 7], UPLC [8]. The aim of the present study was to develop a simple, precise, reliable, sensitive and selective stability indicating RP- HPLC method simultaneous analysis of Netupitat and Palanosetron in combined dosage forms.

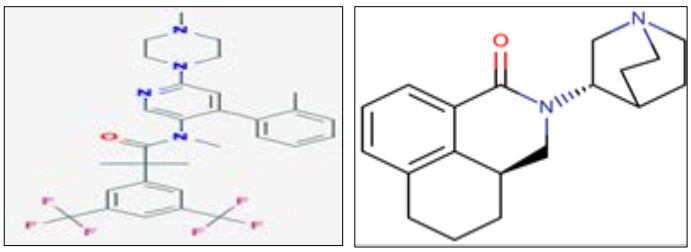

Fig 1: Chemical structure of Netupitant (a) and Palanosetron (b)

Experimental

Chemicals and reagents

Netupitant and Palonosetron was separated using Kromasil C18 column (250mm×4.6mm, 5mm particle size), Waters Alliance e2695 HPLC system with 2998 PDA detector and the mobile phase contained a mixture of 0.01M Ammonium acetate buffer (pH adjusted to 3.5 with orthophosphoric acid) and Acetonitrile (65:35 v/v). The flow rate was set to 1ml/min with the responses measured at 265nm.

Apparatus and chromatographic condition

The chromatographic separation was performed on a HPLC system (WATERS) Series Alliance e2695 Software EMPOWER-2, integrated with Auto Sampler and 2998 PDA detector. The mobile phase consisting of disodium hydrogen phosphate and ACN in the ratio of 60:40 (v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1mL/min and the detector wavelength was set at 240nm. The injection volume was 10 µL. Based up on the solubility of the drugs, diluent was selected, as acetonitrile and Water taken in the ratio of 50:50

Preparation of standard and sample solutions

Mixed Standard solution

Accurately weighed 150 mg of Netupitant, 0.25mg of Palonosetron and transferred to 50ml and 50ml individual volumetric flasks and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (3000µg/ml Netupitant of and 5µg/ml of Palonosetron).

Sample Solution

5 capsules were weighed and the average weight of each capsule was calculated, then the weight equivalent to 1 capsule was transferred into a 100 ml volumetric flask, 50 ml 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 Palonoestron)

Procedure

Inject 10µL of the standard and sample solution into the chromatographic system separately and measure the peak areas of Netupitant and Palanosetron and calculate the % assay value.

Results and Discussion

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines. Obtained validation parameters are presented in Table 1.

Table 1: Analytical validation parameters (System suitability and Linearity)

Parameter	Netupitant	Palanosetron
Linearity	75-450µg/ml	0.125-0.75 µg/ml
Slope	7143.9	34990
Intercept	9757.6	1880.7
Correlation coefficient	0.9991	0.9996
LOD	1.24	0.002
LOQ	3.76	0.007
Theoretical Plates	8683	10051
Tailing Factor	1.3	1.0
Retention Time (min)	2.413	2.998

Table 2: Recovery studies of Netupitant and Palanosetron

Recovery data of Netupitant				
Concentration (at specification level)	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % Recovery
50%				99.66
100%				
150%				
Recovery data of Palnosetron				
Concentration (at specification level)	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean % Recovery
50%				
100%				
150%				

Linearity

Accurately weighed 150 mg of Netupitant, 0.25mg of Palonosetron and transferred to 50ml and 50ml individual volumetric flasks and 3/4 th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (3000µg/ml Netupitant of and 5µg/ml of Palonosetron). The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated.

Recovery

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 100.01% and 100.37% for Netupitant and Palonosetron respectively.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.24 and 3.76 for Netupitant and 0.002µg/ml and 0.007µg/ml for Palanosetron. The LOD and LOQ showed that the method is sensitive for Netupitant and Palanosetron.

System suitability test

The specificity of this method was determined by complete separation of Netupitant and Palanosetron as shown in Fig. 2 with parameters like retention time, resolution and tailing factor. Retention time of Netupitant and Palonosetron were found to be 2.413min and 2.998min factor for peaks of Netupitant and Palanosetron was less than 2% and resolution was satisfactory. The peaks obtained for Netupitant and Palanosetron were sharp and have clear baseline separation. Analysis was also performed for active Netupitant and palanosetron, placebo sample (All the ingredients except active Netupitant and palanosetron,) both at stressed and unstressed condition. After analysis it was found that there is no interference of peak in the amlodipine and metoprolol region for the stressed, placebo & active sample. Hence the developed method was specific for the analysis of this product**.**

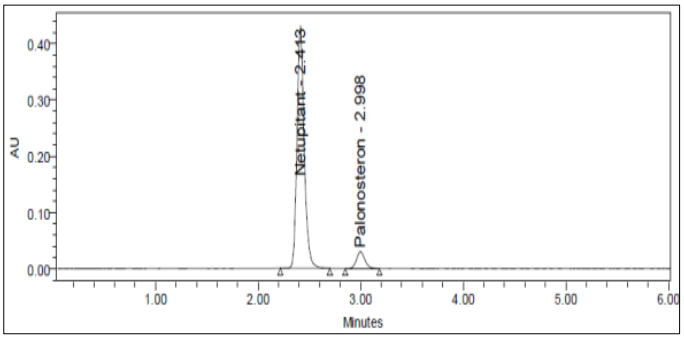

Fig 2: Typical chromatogram of Netupitant and Palanosetron

Precision

The method precision study was performed for five sample preparations of marketed formulations. A study was carried out for intermediate precision with the same analyst on the different day for five sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, temperature and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The assay results of tablet dosage formulation by the proposed method are presented in Table 3.

Table 3: Assay result of tablet dosage formulation

Drug	Label strength (mg)	Amount found (mg)	% Assay
Netupitant	300	299.61	99.86
Palanosetron	0.5	0.5	100.17

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Netupitant and Palanosetron remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table 4.

Table 4: Forced degradation studies of Netupitant and Palanosetron

Stress Conditions	Peak Area		% Degradation		% of Active drug present after degradation	
	Netupitant	Palanosetron	Netupitant	Palanosetron	Netupitant	Palanosetron
Control	2087273	2696175	-	-	-	-
Acid	1975897	164339	5.40	4.79	94.60	95.21
Base	1953226	163341	6.48	5.37	93.52	94.63
Peroxide	1986167	165489	4.91	4.12	95.09	95.88
Water	2072536	171653	1.55	0.55	98.45	99.45
Thermal	2037785	168339	2.43	2.47	97.57	97.53

Control sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 10 mg of Netupitant and 10 mg Palanosetron into a 100 mL clean dry volumetric flask, add about 75 mL of methanol and sonicate to dissolve it completely and make volume up to the mark with the diluent. Filter the solution through 0.45µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with diluent.

Acid degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask, add about 10 mL of 0.1N acid (Hydrochloric acid), refluxed for 30 minutes at 60°C, then cooled to room temperature, neutralize with 10 ml of 0.1N base (Sodium hydroxide) and make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of acid degradation was given in Fig. 3.

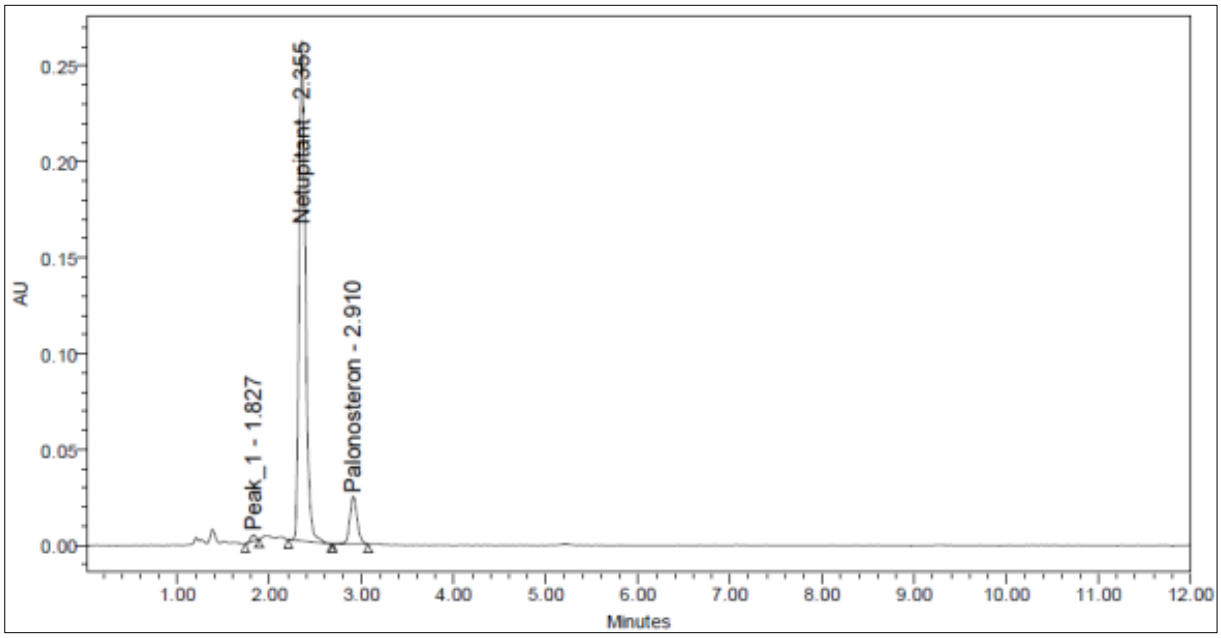

Fig 3: Acid degradation chromatogram of Netupitant and Palanosetron

Base degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of 0.1N base (Sodium hydroxide), refluxed for 30 minutes at 60°C, then cooled to room temperature, neutralize with 10 ml of 0.1N acid (hydrochloric acid) and make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of base degradation was given in Fig. 4.

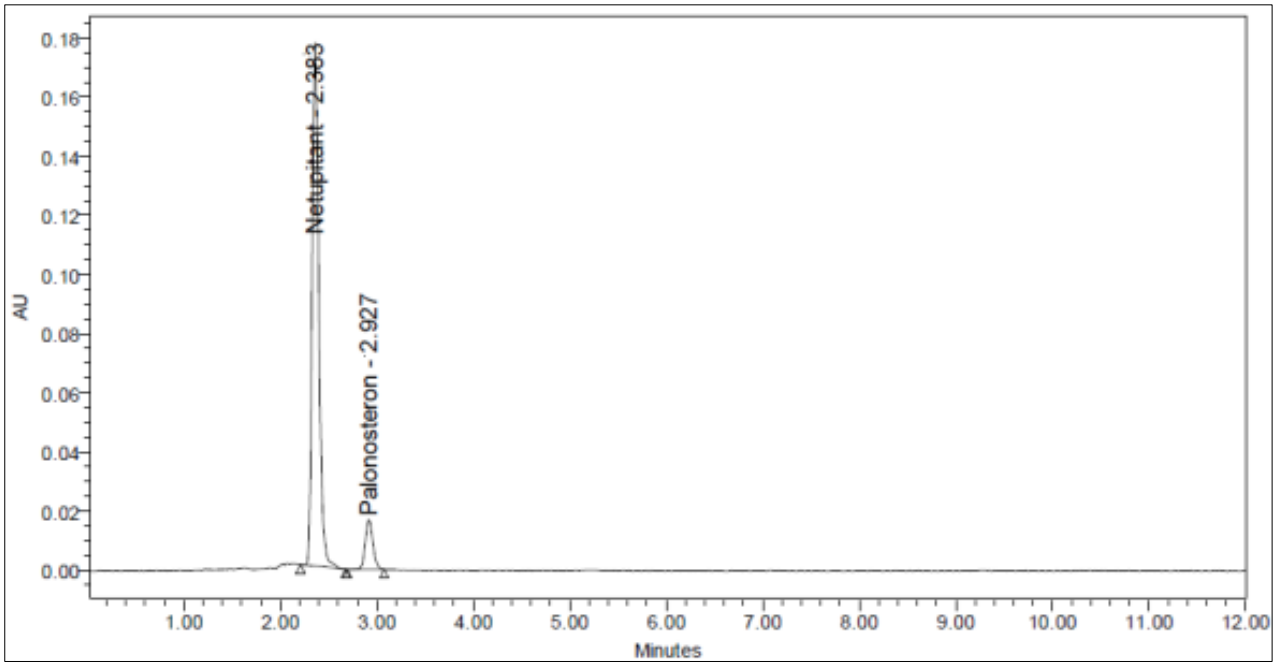

Fig 4: Base degradation chromatogram of Netupitant and Palanosetron

Peroxide degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of 1% H_2O_2, refluxed for 30minutes at 60°C, then cooled to room temperature, make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of oxidative degradation was given in Fig. 5.

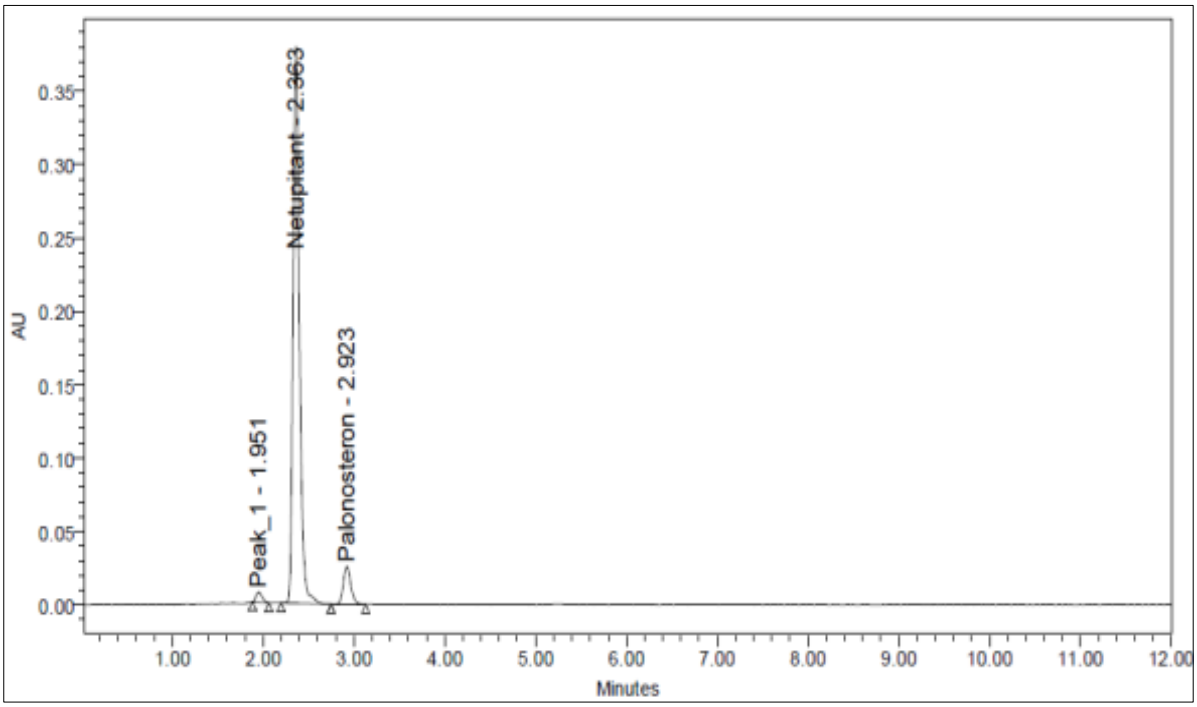

Fig 5: Peroxide degradation chromatogram of Netupitant and Palanosetron

Water degradation sample

Weigh and finely powder not fewer than 2 tablets. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask add 10 ml of H_2O, refluxed for 30minutes at 60°C, then cooled to room temperature, make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of oxidative degradation was given in Fig. 6.

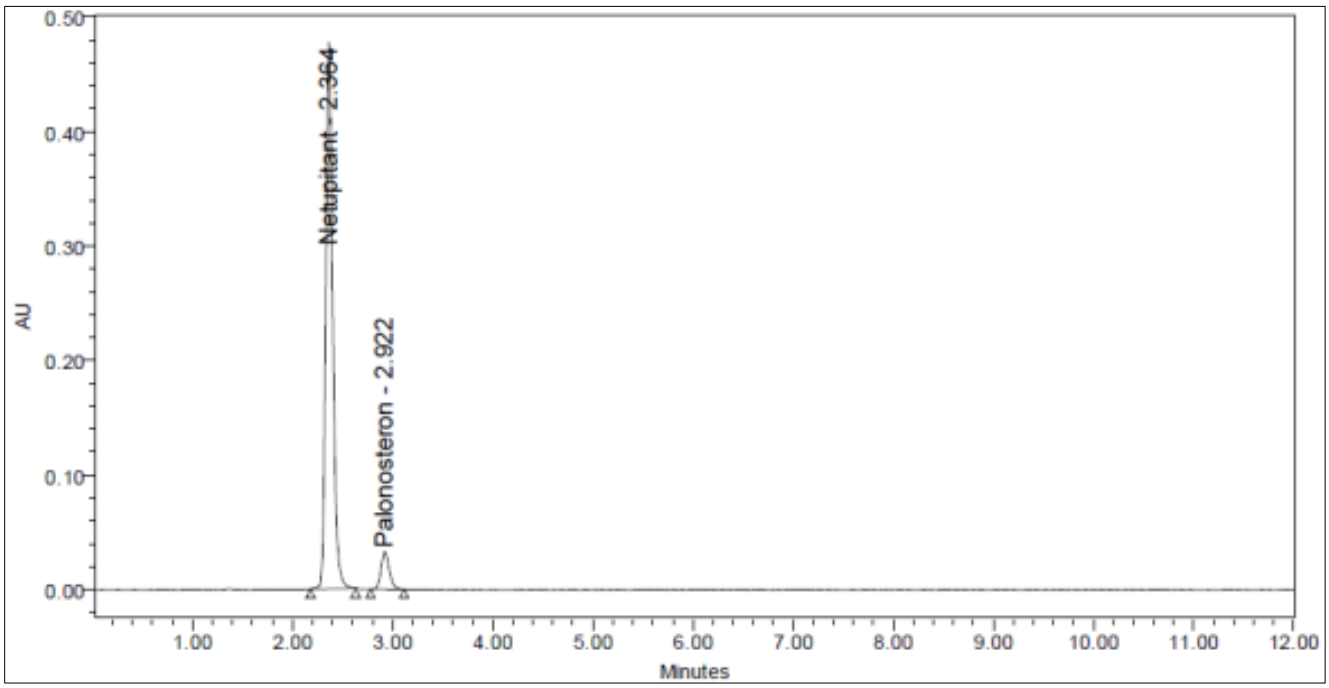

Fig 6: Water degradation chromatogram of Netupitant and Palanosetron

Thermal degradation sample

Weigh and finely powder not fewer than 2 tablets, this powder is exposed to heat at 105°C for about 2 days. Accurately weigh and transfer sample equivalent to 173 mg into a 100 mL clean dry volumetric flask. Add about 75 mL of methanol and sonicate to dissolve it for about 30minutes with intermittent shaking at controlled temperature. Then make volume up to the mark with methanol and mix. Filter the solution through 0.45 µm membrane filter. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and dilute up to the mark with methanol. The typical chromatogram of thermal degradation was given in Fig. 7.

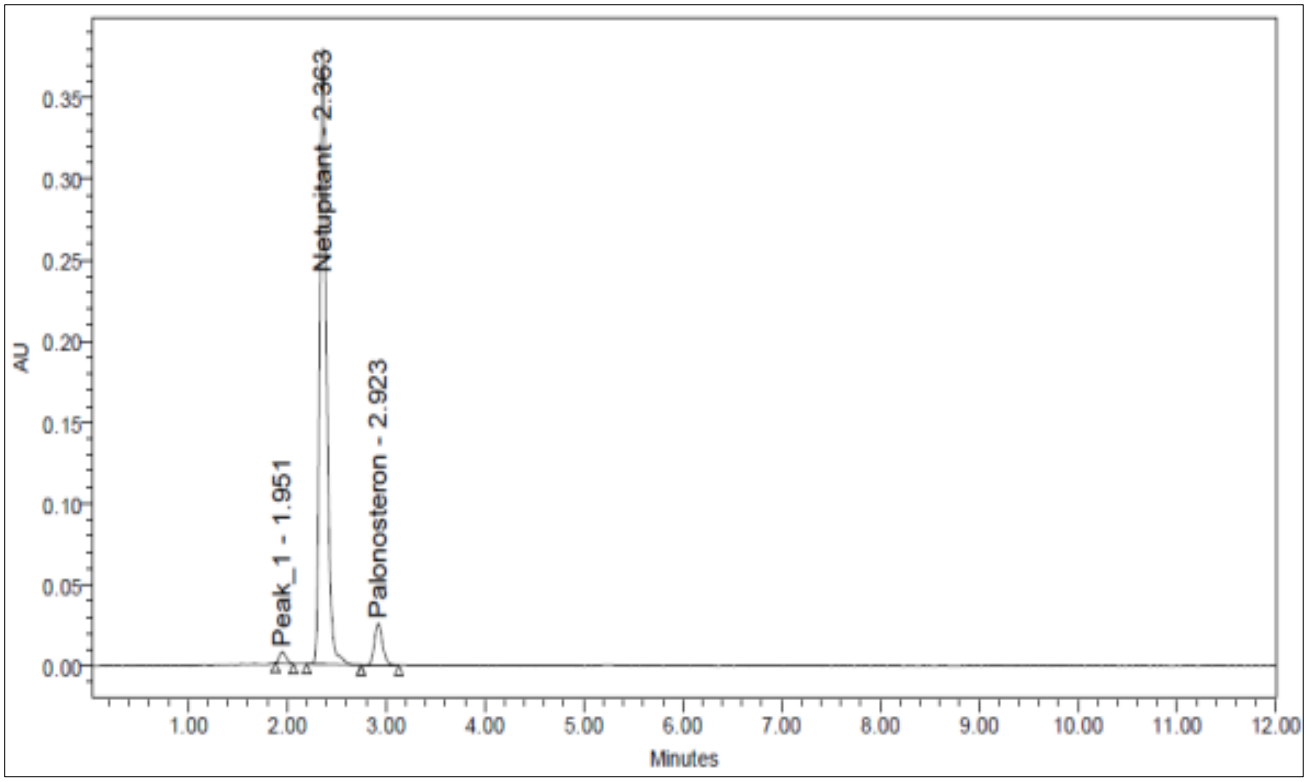

Fig 7: Thermal degradation chromatogram of Netupitant and Palanosetron

Conclusion

The findings of the present investigation are summarized as follows

1. A suitable chromatographic method was developed through optimization by changing various parameters such as the mobile phase, injection volume, flow rate etc.
2. In the present method a column Kromasil (4.6 x 150mm, 5µm) has been used for Netupitant & Palanosetron drugs respectively.
3. Mobile phase used was Acetonitrile: Phosohate buffer (40:60%v/v) for drugs Netupiant & Palanosetron respectively, Retention of Netupitant & Palanosetron has more dependence on the mobile phase.
4. The separation of the two peaks was also dependent on the buffer and the percentage of mobile phases. Netupitant & Palanosetron were eluted at acceptable retention times and got good resolution.
5. Several assay methods has been developed for the determination of Netupitant & Palanosetron in formulations and biological fluids but this method is most economic and accurate so this method is very useful for the determination of Netupitant & Palanosetron in tablet formulations. This method was validated as per ICH-Q2 (R1) guidelines and met the regulatory requirements for selectivity, accuracy and stability. Considering the obtained data, it was possible to affirm that the proposed method was fast, simple and suitable for the accurate determination of drug Netupitant & Palanosetron in tablet formulation

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