Research Article

Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Netupitant and Palonosetron in Combined Tablet Dosage Form

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ABSTRACT

A stability indicating RP-HPLC method was developed for simultaneous determination of netupitant and palonosetron in bulk and in combined tablet dosage form. The separation of netupitant and palonosetron was achieved within 6 minutes on an YMC Pack pro (150 mm x 4.6 mm, 5 µm particle size) C18 column using 0.1% orthophosphoric acid: Methanol (55:45 v/v) as the mobile phase. UV detection was carried out using wavelength 262 nm. The method showed adequate sensitivity concerning linearity, accuracy and precision over the range 450-1350 µg/ml and 0.75-2.25 µg/ml for netupitant and palonosetron, respectively. Careful validation proved advantages of high sensitivity, accuracy, precision, selectivity, robust and suitability for quality control laboratories. Both the drugs were subjected to acid, alkali, oxidation, thermal and photolytic degradation. The degradation products produced in all the stress conditions were well resolved from the netupitant and palonosetron with significant differences in their retention time values.

Keywords: Netupitant, palonosetron, forced degradation, stability indicating high-performance liquid chromatography.

INTRODUCTION

Netupitant, chemically described as 2-[3,5-Bis(trifluoromethyl)phenyl]-*N*,2-dimethyl-*N*-[4-(2-methylphenyl)-6-(4-methyl-1-piperazinyl)-3-pyridinyl]propanamide, is a selective neurokinin 1 receptor antagonist having antiemetic activity. Netupitant is involved in the prevention of chemotherapy induced nausea and vomiting by inhibiting the binding of endogenous tachykinin neuropeptide substance P to the neurokinin 1 receptors in the central nervous system.[1,2]

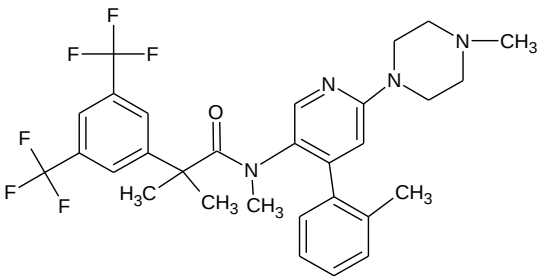

Figure 1: Chemical structure of netupitant

Palonosetron, chemically known as (3aS)-2-[(3S)-1-Azabicyclo[2.2.2]oct-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benz[de]isoquinolin-1-one, is a specific and selective serotonin 5-HT3 antagonist with antinauseant and antiemetic activity. It is prescribed for the prevention of nausea and vomiting associated with cancer chemotherapy and postoperative nausea and vomiting.[3-5] Chemotherapeutic agents causes the release of serotonin, which then stimulates medullary vomiting center and 5-HT_3 receptors and thus initiating the vomiting reflex, causing nausea and vomiting. The antiemetic activity of palonosetron is brought about by the inhibition of 5-HT_3 receptors present both in the medullary chemoreceptor zone and gastrointestinal tract.

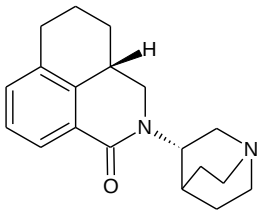

Figure 2: Chemical structure of palonosetron

In the United States and in Europe, the combination of netupitant and palonosetron is approved by the Food and Drug Administration and European Medicines Agency, respectively for the prevention of acute and delayed chemotherapy induced nausea and vomiting, as well as highly emetogenic chemotherapy.[6-8]

Only few analytical methods were reported for the simultaneous assay of netupitant and palonosetron. These methods include chemometry,[9] liquid chromatography-tandem mass spectrometry[10] and high performance thin-layer chromatography.[11] In addition, only 3 reversed phase high performance liquid chromatographic methods were applied for simultaneous determination of netupitant and palonosetron in bulk drug and pharmaceutical dosage form.[9,12,13]

The reported methods liquid chromatography-tandem mass spectrometry[10] and high performance thin-layer chromatography[11] are expensive and sophisticated

HPLC techniques. The chemometry method lacks the sensitivity and selectivity.[9] The reported reversed phase high performance liquid chromatographic methods suffer from low sensitivity,[9] narrow range of linearity,[9] lack of precision & accuracy[9,12,13] and more run time.[9,12,13] Moreover, some of these methods use triple solvent system as mobile phase. The use of triple solvent system and increased run time may increases the utilization of solvents and cost of the analysis.

Therefore, it was thought essential to develop sensitive reversed phase high performance liquid chromatographic method for simultaneous determination of netupitant and palonosetron and applicable to tablet dosage forms.

MATERIALS AND METHODS

HPLC instrumentation

- Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software was used in the current investigation.
- YMC Pack pro 150 mm x 4.6 mm, 5 µm particle size, C18 column was used for separation and simultaneous analysis of netupitant and palonosetron.

Materials and chemicals

All the chemicals and solvents used were of analytical reagent and HPLC grade, respectively.

- Hydrochloric acid, sodium hydroxide, hydrogen peroxide and orthophosphoric acid were all obtained from Sd Fine Chemicals Ltd., Mumbai, India.
- Methanol was from Merck India Ltd., Mumbai.
- Reference standards of netupitant and palonosetron were supplied by Lara Drugs Private Limited, Hyderabad, India.
- Akynzeo tabets, labeled to contain 300 mg of netupitant and 0.5 mg of palonosetron (product of by Helsinn Birex Pharmaceuticals, Ireland). It was purchased from the local pharmacy.
- Milli Q water was used throughout the experiments.

Chromatographic conditions

- Mobile Phase: 0.1% orthophosphoric acid: Methanol (55:45 *v/v*)
- Column: YMC Pack pro 150 mm x 4.6 mm, 5 µm particle size, C18
- Flow Rate: 1.2 ml/min
- Column temperature: 30˚C
- Volume: 10 µl
- Detection wavelength: 262 nm
- Runtime: 6 min

Standard solutions

Stock solution equivalent to 3000 µg/ml of netupitant and 5 µg/ml of palonosetron was prepared by dissolving 300 mg and 0.5 mg of netupitant and palonosetron, respectively in 100 ml mobile phase. Working standard solutions equivalent to 450, 675, 900, 1125 and 1350 µg/ml of netupitant and 0.75, 1.125, 1.5, 1.875 and 2.25 µg/ml of palonosetron was prepared by apt dilution of the stock solution with the same solvent.

Preparation of the degradation samples

For forced degradation studies, tablet powder equivalent to 300 mg of netupitant and 0.5 mg of palonosetron was subjected to acidic, alkaline, oxidative, thermal and photo degradation. The following steps were performed:

For alkaline and acidic degradation

10 ml of 0.1N HCl (acidic degradation) or 0.1N NaOH (alkaline degradation) were added to 100 ml volumetric flasks containing tablet powder. The solutions were mixed well and sonicated for 30 min. After the specified time, the contents of each flask were neutralized and the solutions were then completed to the volume with mobile phase.

For oxidative degradation

10 ml of 30 % H_2O_2 was added to 100 ml volumetric flask containing tablet powder. The solution was then sonicated for 30 min. After the specified time intervals, the volume was completed to the mark with mobile phase.

For thermal degradation

Tablet powder was taken in glass petri dish and placed in oven at 105 °C for 30 min. After specified time, the sample was cooled, transferred into a 100 ml volumetric flask and completed to the volume with mobile phase.

For photo degradation

Tablet powder was taken in glass petri dish and placed in the direct sunlight for 24 h. After specified time, the sample was cooled, transferred into a 100 ml volumetric flask and completed to the volume with mobile phase.

The degraded samples were suitably diluted with mobile phase to give a final concentration of 900 µg/ml (netupitant) and 1.5 µg/ml (palonosetron). The samples were filtered through 0.45 mm membrane filter before injecting into the chromatographic system.

Construction of calibration graphs

Working standard solutions in the concentration range 450-1350 µg/ml of netupitant and 0.75-2.25 µg/ml of palonosetron were prepared from the stock standard solution with mobile phase. 10 µl of each working standard solution is injected into the HPLC system thrice under the chromatographic conditions described. The chromatograms and peak areas were recorded. The mean peak area was then plotted against the final drug concentration in µg/ml to get the calibration graphs of netupitant and palonosetron. The corresponding regression equation was derived.

Analysis of netupitant and palonosetron in combined tablet dosage

The average weight of ten tablets was determined. The tablets were finely powdered using mortar and pestle. An accurately weighed amount of tablet powder equivalent to 300 mg and 0.5 mg of netupitant and palonosetron respectively was transferred into a clean dry 100 ml beaker and about 50 ml of mobile phase was added. The content of the beaker was sonicated for 15 min. The contents were quantitatively transferred into 100 ml volumetric flask, completed to the mark with the same solvent and filtered through 0.45 mm membrane filter. This solution was suitably diluted with mobile phase to give a final concentration of 900 µg/ml (netupitant) and 1.5 µg/ml (palonosetron) for analysis. The procedure described under "construction of calibration graphs" was then applied. The nominal content of the drugs in the tablet was determined either from the previously plotted calibration graph or using the corresponding regression equation.

RESULTS AND DISCUSSION

Method development

RP-HPLC procedure was optimized to develop a stability indicating assay method that can be used for quality control analysis of netupitant and palonosetron simultaneously in laboratories.

For method optimization, various conditions such as different columns and mobile phase mixtures were tried. Two different analytical columns, YMC Pack Pro C18 (250 x 4.6 mm, 5 µm) and Inertsil C8 (250 x 4.6 mm, 5 µm) were tried. For the separation of netupitant and palonosetron and their forced degradation products, YMC Pack Pro C18 (250 x 4.6 mm, 5µm) column maintained at a temperature of 30°C was found to be efficient.

Two different mobile phase mixtures like 0.1% orthophosphoric acid: methanol and 0.1 M NaH_2PO_4: methanol in different ratios and flow rates were tested. Finally, a mobile phase composed of 0.1% orthophosphoric acid and methanol in the ratio of 55:45 v/v with a flow rate of 1.2 ml/min was chosen for analysis that showed proper separation of drug peaks, good peak shape and resolution. For the detection and quantification of netupitant and palonosetron, 262 nm was selected as the optimum detection wavelength. At 262 nm best detector response for both the drugs was obtained.

Under optimized chromatographic conditions, the peaks of netupitant and palonosetron were shaped well and free from tailing. The retention times were 2.216 and 3.131 min for netupitant and palonosetron, respectively (Figure 3).

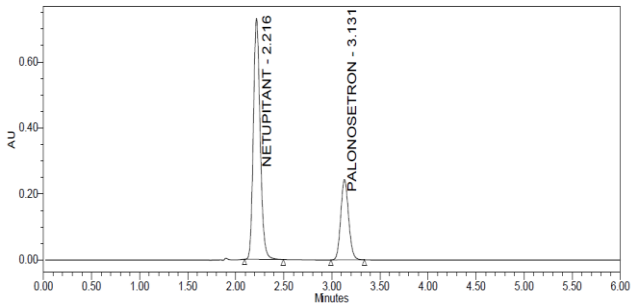

Figure 3: Chromatogram of netupitant and palonosetron

HPLC method validation

Method validation was done in accordance with ICH recommendation.[14]

System suitability

Chromatographic parameters associated to the developed method must pass the system suitability limits before the analysis of sample. The relative standard deviation of peak area, theoretical plates, resolution and tailing factor for netupitant and palonosetron peaks was evaluated using a solution containing 900 µg/ml of netupitant and 1.5 µg/ml of palonosetron. All the results (Table 1) assure the satisfactoriness of the proposed method for routine analysis of netupitant and palonosetron simultaneously.

Selectivity

The selectivity study was assessed to make sure the absence of interference by the excipients commonly found the formulations and components of mobile phase. For this study, standard solution (netupitant-900 µg/ml: palonosetron-1.5 µg/ml), tablet sample solution with concentration similar to standard solution, placebo blank and mobile phase blank solution were injected into the chromatographic system. The chromatograms were recorded and are shown in Figure 4. The chromatogram demonstrated the selectivity of the proposed method, since there were no peaks at the retention time of netupitant & palonosetron in the chromatograms of placebo blank & mobile phase blank and the retention times of the drugs were same in the chromatograms of standard solution & tablet sample solution.

Table 1: System suitability results

Parameters	Netupitant	Palonosetron	Recommended limits
Peak area	3707495 (%RSD – 0.030)	1373988 (%RSD – 0.027)	RSD ≤2
USP resolution	-	6.63	> 1.5
USP plate count	5009	7254	> 2000
USP tailing factor	1.16	1.10	≤ 2

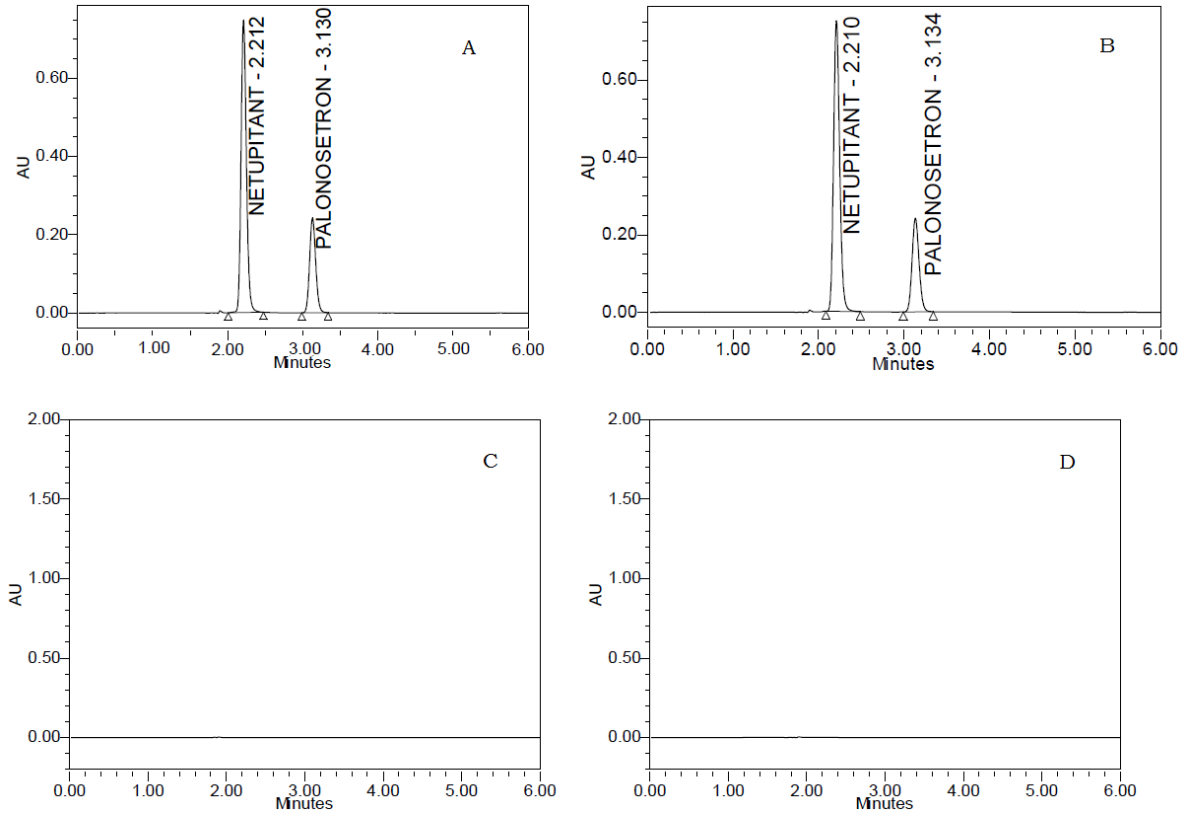

Figure 4: Chromatogram of [A] standard solution [B] tablet sample solution [C] Placebo blank [D] Mobile phase blank

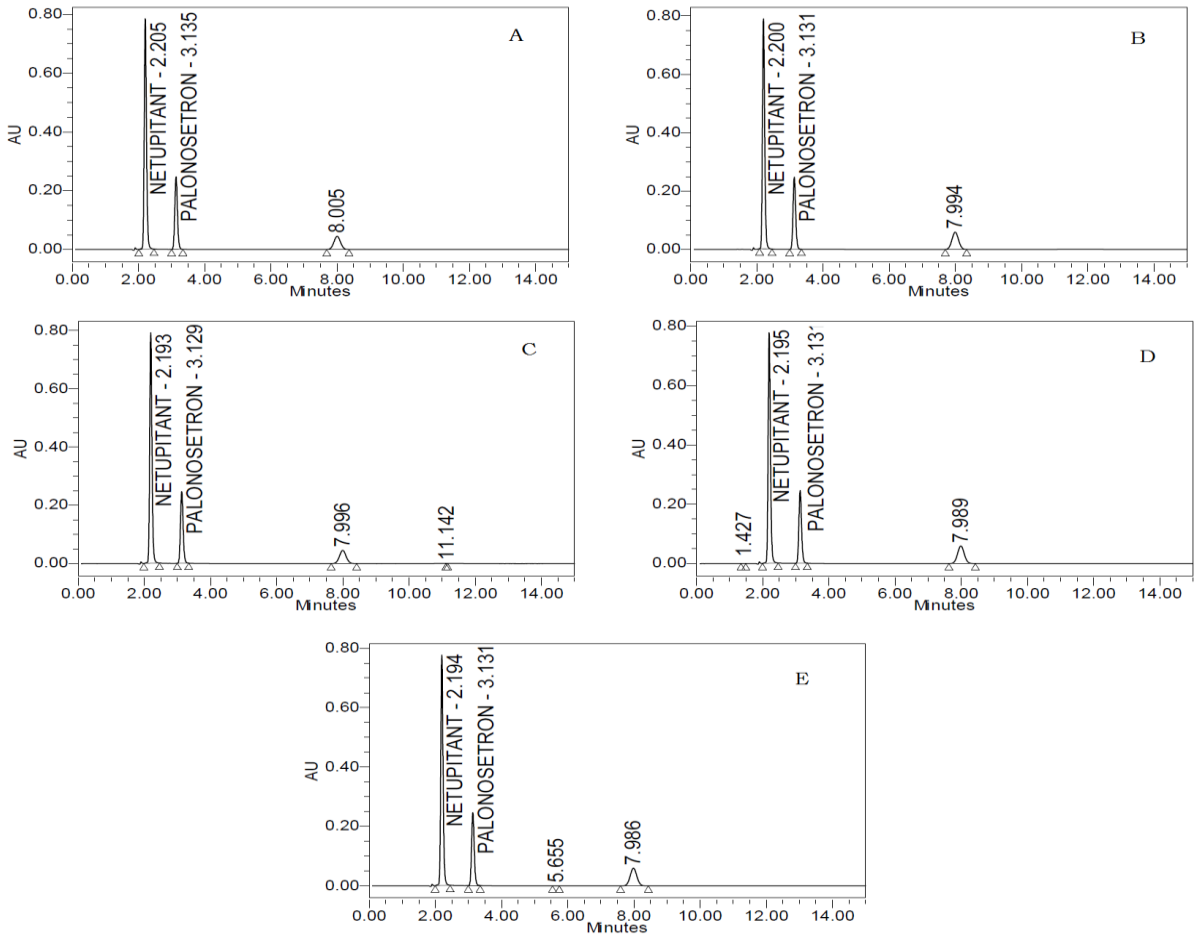

Figure 5: Chromatogram representing results of [A] Acid induced degradation [B] Alkali induced degradation [C] oxidizing agent-induced degradation [D] thermal induced degradation [E] Photo induced degradation

Linearity

Linearity was investigated via replicate analysis of five standard concentrations: netupitant-450, 675, 900, 1125 and 1350 µg/ml; palanosetron-0.75, 1.125, 1.5, 1.875 and 2.25 µg/ml. Calibration graphs of netupitant and palonosetron were constructed by plotting the mean peak area against the drug concentration (µg/ml). The results of the regression equations and correlation coefficients were abridged in Table 2. In present method, good linearity for netupitant and palonosetron was achieved in the range of 450-1350 µg/ml and 0.75-2.25 µg/ml, respectively as indicated by higher value of correlation coefficients (>0.999).

Table 2: Linearity, LOD and LOQ values

Parameters	Netupitant	Palonosetron
Linearity (µg/ml)	450-1350	0.75-2.25
Regression equation (y= mx + c)	y = 4120 x + 888.7	y = 91738 x + 1568
Slope (m)	4120	91738
Intercept (c)	888.7	1568
Regression coefficient (R^2)	0.9999	0.9998
LOD(µg/ml)	0.575	0.0029
LOQ(µg/ml)	1.918	0.0096

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values determine the sensitivity of the method. Both were calculated as signal-to-noise ratio of 3:1 (LOD) and 10:1 (LOQ). The LOD and LOQ values for netupitant and palonosetron were calculated and are presented in Table 2.

Precision

The precision of the developed method was determined using the standard solution with a concentration of 900 µg/ml of netupitant and 1.5 µg/ml of palonosetron. Six injections of the solution were made into the HPLC system. Peak areas and their relative standard deviation were calculated. Small values of the relative standard deviations gave a good indication for the high precision of the proposed method (Table 3).

Table 3: Precision of the method

Netupitant		Palonosetron	
Peak area	**%RSD**	**Peak area**	**%RSD**
3728376	0.272	1374950	0.102
3705596		1371943	
3710842		1375643	
3726636		1374743	
3711451		1373361	
3706180		1372933	

Accuracy

The standard addition technique was used to establish the accuracy of the method. This was performed by adding known amounts of netupitant and palonosetron to a known concentration of the tablet sample solution at three different concentration levels. The percentage recoveries for three replicates were calculated. According to the results revealed in Tables 4, good accuracy was observed for the proposed method and there is no interference from the often encountered tablet excipients.

Table 4: Accuracy determination of netupitant and palonosetron

Spiked Level (%)	Concentration of drug (µg/ml)		Recovery (%)	Mean (%)
	Added	**Found**		
Netupitant				
50	445.500	445.901	100.090	100.090
	445.500	445.878	100.085	
	445.500	445.920	100.094	
100	891.000	891.800	100.090	100.104
	891.000	891.974	100.109	
	891.000	892.008	100.113	
150	1336.500	1337.845	100.101	100.106
	1336.500	1337.946	100.108	
	1336.500	1337.950	100.109	
Palonosetron				
50	0.750	0.7469	99.588	99.619
	0.750	0.7470	99.606	
	0.750	0.7475	99.664	
100	1.500	1.4951	99.675	99.660
	1.500	1.4945	99.634	
	1.500	1.4951	99.671	
150	2.250	2.248	99.911	99.767
	2.250	2.2432	99.696	
	2.250	2.2431	99.694	

Robustness

The robustness of the proposed method was investigated via an analysis of working standard sample under a variety of experimental conditions, such as small changes in column temperature (30±2 °C) or changing the flow rate of mobile phase (1.2±0.1 ml/min). The effect on retention time, peak area, USP plate count, USP tailing and USP resolution was studied. The results are summarized in Table 5. It was found that the method was robust when the column temperature and the mobile phase flow rate were varied.

Table 5: Method robustness results

Parameter varied	Retention time	Peak area	USP plate count	USP Tailing	USP resolution
Netupitant					
Column temperature-29°C	2.471	4173161	5195	1.17	-
Column temperature-31°C	1.980	3268367	4782	1.20	-
Flow rate – 1.1 ml/min	2.476	4192222	5165	1.19	-
Flow rate – 1.3 ml/min	1.974	3272207	4935	1.19	-
Palonosetron					
Column temperature-29°C	3.519	1543891	7693	1.12	6.91
Column temperature-31°C	2.825	1229203	6823	1.12	6.60
Flow rate – 1.1 ml/min	3.522	1547524	7759	1.13	6.89
Flow rate – 1.3 ml/min	2.821	1235000	6882	1.12	6.68

Forced degradation studies

In forced degradation studies, the tablet sample is subjecting to different stress conditions such as acidic/basic hydrolysis, oxidative, thermal and photolytic degradation. The results of degradation study are summarized in Table 6. The chromatograms of degraded samples are shown in Figure 5. Degradation of netupitant and palonosetron was seen in all the applied stress conditions. The percentage of degradation of both the drugs is more in acid induced degradation and less in hydrogen peroxide induced degradation. The degradation products produced due to stress did not interfere with the detection of netupitant and palonosetron. The proposed method can consequently be regarded as stability-indicating.

Table 6: Stress degradation studies

Type of stress	Peak area	% Assay	% Degradation
Netupitant			
Undegraded	3707495	100	0.000
Acid	3515870	93.978	6.022
Base	3555071	95.026	4.974
Peroxide	3591332	95.995	4.005
Heat	3573124	95.508	4.492
Sunlight	3539038	94.597	5.403
Palonosetron			
Undegraded	1373988	100	0.000
Acid	1264520	91.757	8.243
Base	1285373	93.270	6.730
Peroxide	1291195	93.692	6.308
Heat	1288059	93.465	6.535
Sunlight	1299792	94.316	5.684

CONCLUSION

A simple and sensitive stability indicating RP-HPLC method was explored for the simultaneous determination of netupitant and palonosetron in pure form and in commercially available tablet dosage forms. The method was validated as per ICH guidelines. Forced degradation studies were also conducted using different stress condition as per ICH guidelines. The method proved that the selectivity, precision, accuracy and simple mobile phase used provide simple and economic applications. The method was capable to resolve the peak of selected drugs from stress degradation products. Consequently, the stability indicating power of the method can be assessed. Therefore, the method was found to be suitable for the routine quality control analysis of netupitant and palonosetron simultaneously in laboratories with no interference from the excipients or the stress degradation products.

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