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A new UPLC method development and validation for simultaneous estimation of netiputant and palonosetron using bulk and pharmaceutical dosage form using UPLC

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***Abstract*---**A simple and selective LC method is described for the determination of Netupitant and Palonosetron in tablet dosage forms. Chromatographic separation was achieved on a Inertsil ODS (C18) RP Column (250mm x 4.6 mm. 5µm) column using mobile phase consisting of a 55 volumes of mixed phosphate buffer and 45 volumes of acetonitrile were prepared with detection of 253nm. Linearity was observed in the range 25-125 µg/ml for Netupitant (r 2 =0.995) and 50-150 µg /ml for Palonosetron (r 2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

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***Keywords*---**palonosetron, netupitant, acetonitrile, inertsil ODS, repeatability.

Introduction

Netupitant competitively binds to and blocks the activity of the human substance P/NK1 receptors in the central nervous system (CNS), thereby inhibiting NK1-receptor binding of the endogenous tachykinin neuropeptide substance P (SP), which may result in the prevention of chemotherapy-induced nausea and vomiting (CINV). It is -N,2-dimethyl-N-[4-(2-methylphenyl)-6-(4- methylpiperazin-1-yl)pyridin-3-yl]propenamide. Its molecular weight is 578.603 mg/mol. (M, manoranjani.2019)

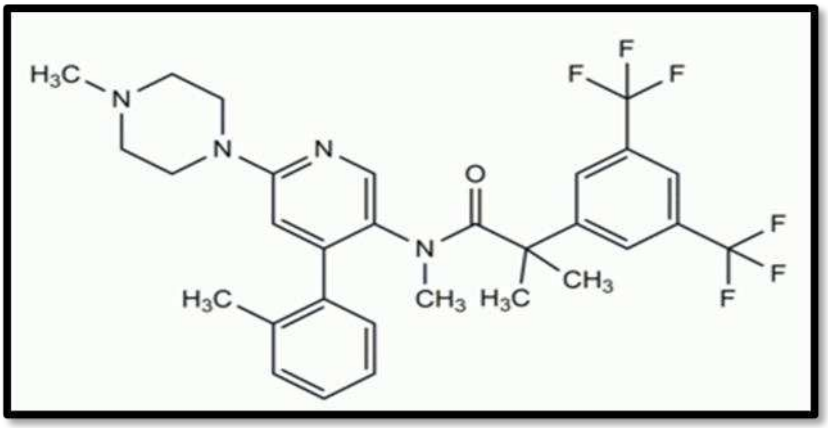

Fig 1. Structure of Netupitant

Palonosetron is a 5-HT3 receptor antagonist with a strong binding affinity for this receptor and little or no affinity for other receptors. Cancer chemotherapy may be associated with a high incidence of nausea and vomiting, particularly when certain agents, such as cisplatin, are used. It is (5S)-3-[(3S)-1-azabicyclo[2.2.2]octan-3-yl]-3-azatricyclo[7.3.1.0]trideca-1(12),9(13),10-trien-2-one.its molecular weight is 296.407 g/mol (Suman et al 2020).

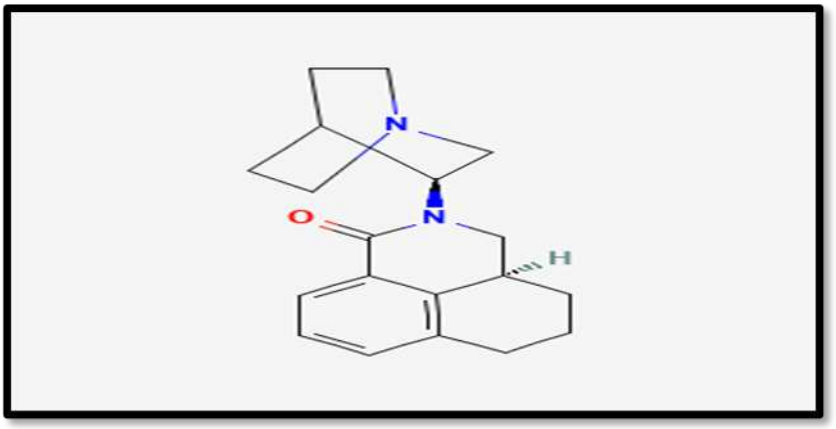

Fig 2. Structure of Palonosetron

In the literature, it was observed that there are not many approaches for simultaneous measurement of Netupitant and Palonosetron. The work aims were to find an optimized new way to determine Netupitant and Palonosetron on a reduced period and with less solvent used. This research aims to improve simple, reliable, and effective methods for the determination of Netupitant and Palonosetron

Materials and Methods

Instruments used

UV-Visible Spectrophotometer Nicolet evolution 100, UV-Visible-pectrophotometer software. Vision Pro, UPLC software OPEN LABS, UPLC AZILENT 1290, Column Inertsil ODS 3V(250x4.6mm) 5µm were used.

Reagents used

Water HPLC Grade, Methanol HPLC Grade, Potassium dihydrogen rthophosphate AR Grade. Dipotassium hydrogen orthophosphate AR Grade, Acetonitrile HPLC Gradewere obtained from MERCK laboratories Mumbai.

Determination of Working Wavelength (λ_{max})

In simultaneous estimation of two drugs, Isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same two substances that are interconvertible. So this wavelength (253 nm) is used in simultaneous estimation to estimate both drugs accurately (Swartz, M 2009).

Preparation of Standard stock solution of Netupitant and Palonosetron

10 mg of Netupitant was weighed into a 50 mL volumetric flask, to this 50 mL (200 µg/mL) of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase. 10 mg of Palonosetron was transferred into a 50 mL volumetric flask, to this 50 mL (200 µg/mL) of mobile phase was added,

sonicated and the volume was made up to mark with the mobile phase. (Satinder, A 2005)

Dilutions

About 0.5 ml was transferred from standard stock solution (200 µg/mL) to get the concentration range of 10 µg/mL of Netupitant and 10 µg/mL of Palonosetron. The wavelength of maximum absorption (λ_{max}) of the solution of the drugs in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against mobile phase as blank. The absorption curve shows characteristic absorption maxima at 220 nm for Netupitant, 248 nm for, Palonosetron and at 222nm and same absorbance for both the drugs, i.e., Isobestic point. Thus, 222nm was the selected as detector wavelength for the chromatographic method.

Mobile Phase

A mixture of 55 volumes of mixed phosphate buffer and 45 volumes of acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Preparation of mixed phosphate buffer

Weigh accurately 1.625 gms of potassium di hydrogen phosphate (KH_2PO_4) and 0.3 gms of Dipotassium hydrogen phosphate was weighed and dissolved in 1000 ml of water and volume was made up to 1000ml with water. Sonicate it for 10 mins to remove air bubbles and gases.

Method Development of Palonosetron and Netupitant

Table 1
Optimized chromatographic conditions

Mobile phase	Mixed phosphate buffer: Acetonitrile
Ratio	55: 45
Column	Inertsil ODS 3V column, C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	253
Injection volume	20 µl
Run time	5 min

Assay
Preparation of samples for Assay

10 tablets (each tablet containing Akynzeo (Netupitant and Palonosetron – 300 mg and 0.5 mg) were weighed and taken into a mortar and crushed to a fine powder and uniformly mixed. Tablet stock solutions were prepared by dissolving weight equivalent to 10 mg of Netupitant and Palonosetron and dissolved in sufficient mobile phase. After that filter, the solution using a 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with the mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Netupitant and 50µg/ml of Palonosetron was made by adding 1.5 ml of stock solution to 10 ml of mobile phase.

Preparation of mixed standard solution

Weigh accurately 10mg of Netupitant and 10 mg of Palonosetron in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From the above stock solution 50 µg/ml of Netupitant and 100 µg/ml of Palonosetron is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram (Snyder, R. L 1997)
Method Validation

System Suitability& System precision

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by Netupitant and Palonosetron were injected six times and the chromatograms were recorded for the same.

Specificity

The terms selectivity and specificity are often used interchangeably. Term specific generally refers to a method that produces a response for a single analyte only while the term selective refers to a method which provides responses for a number of chemical entities that may or may not be distinguished from each other.

Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are. directly proportional to the concentration (amount) of analyte in the sample.

Preparation of standard stock solution

Standard stock solutions of Netupitant and Palonosetron (10 microgram/ml) were prepared by dissolving 10 mg of Netupitant and Palonosetron dissolved in sufficient mobile phase and dilute to 10 ml with mobile phase.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre- analyzed sample solution at three different levels 50%, 100%, 150%.

Precision

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. It is measured by injecting a series of standards or analyzing series of samples from multiple samplings from a homogeneous lot.

Method precision

Prepared sample preparations of Netupitant and Palonosetron as per test method and injected into the column.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Ruggedness

The ruggedness of the method was studied by the determining the analyst-to-analyst variation byperforming the Assay by two different analysts.

Results and Discussion

Method development

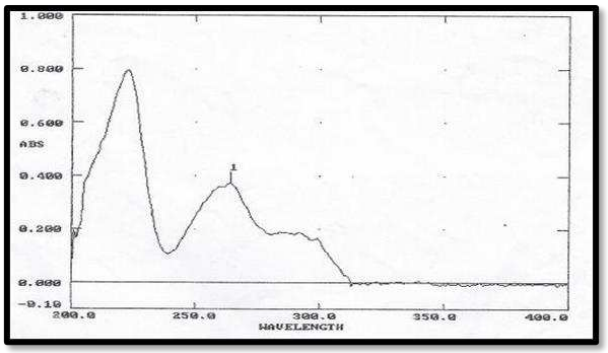

Figure 3. UV-VIS spectrum of Palonosetron (264 nm)

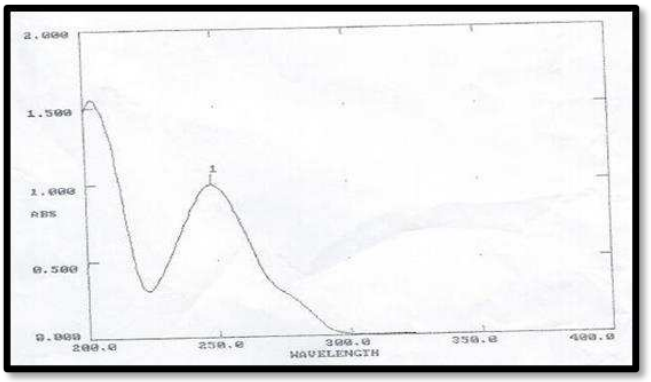

Figure 4. UV-VIS spectrum of Netupitant (248 nm)

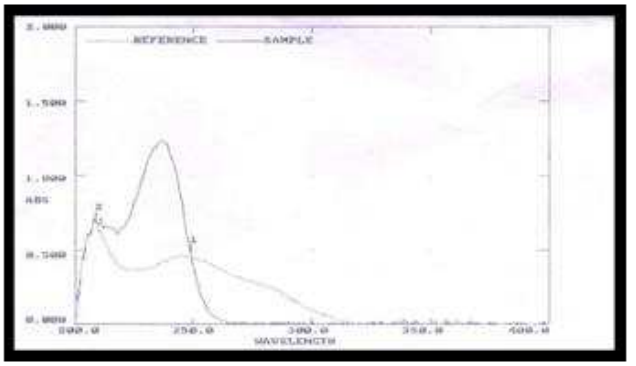

Figure 5. UV-VIS spectrum of Palonosetron and Netupitant (253nm)

Optimized method

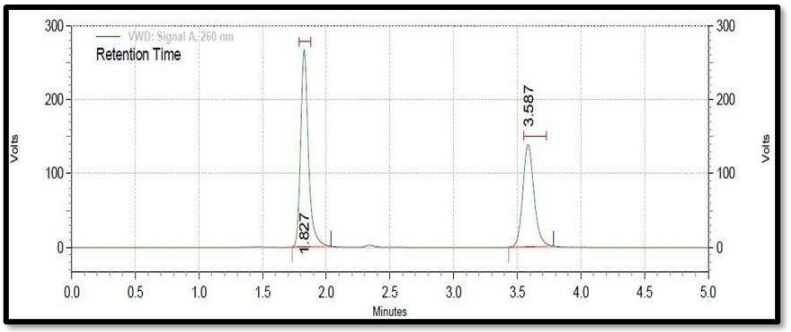

Figure 6. Optimized Chromatogram

Table 2
Optimized chromatographic conditions

Mobile phase	Mixed phosphate buffer: Acetonitrile
Ratio	55: 45
Column	Inertsil ODS 3V column,C18(150x4.6 ID)

	5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	253
Injection volume	20 µl
Run time	5 min

Method validation

Table 3
Results for system suitability of Netupitant.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.832	5634025	4445	1.21
2	1.834	5600158	4542	1.17
3	1.835	5603347	4549	1.18
4	1.838	5610171	4499	1.14
5	1.833	5592387	4441	1.15
6	1.830	5578014	4432	1.14
Mean	1.834	5603017.0	-	-
SD	0.003	18756.89	-	-
%RSD	0.15	0.33	-	-

Table 4
Results for system suitability of Palonosetron.

Injection	Retention time	Peak area	Theoretical plates	Tailing factor	Resolution
1	3.535	4413425	7587	1.14	12.25
2	3.545	4340572	7902	1.16	12.85
3	3.555	4363971	7664	1.18	12.72
4	3.575	4349470	7828	1.19	11.84
5	3.585	4368127	7886	1.19	11.95
6	3.567	4350561	7912	1.17	11.90
Mean	3.560	4364354.3	-	-	-
SD	0.019	26075.69	-	-	-
%RSD	0.53	0.60	-	-	-

Specificity

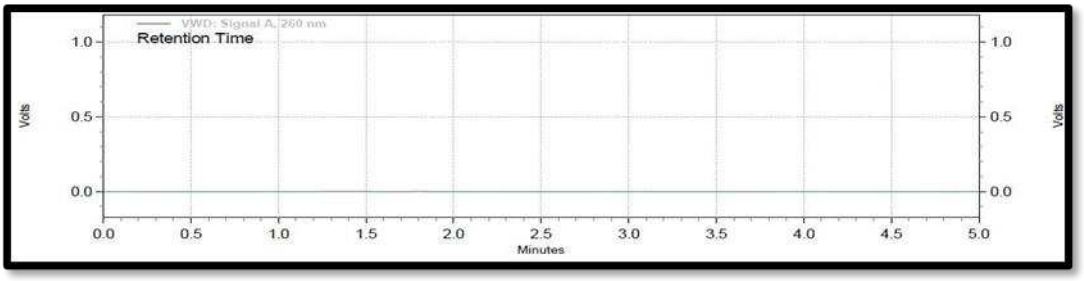

Figure 7. Chromatogram for specificity of Blank

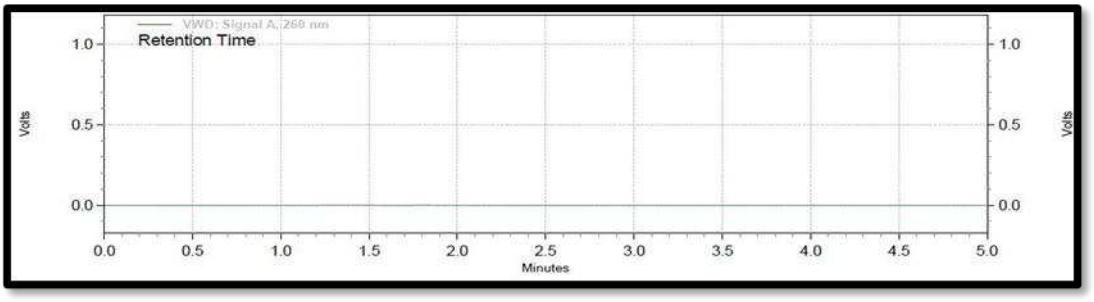

Figure 8. Chromatogram for Specificity of Placebo

Linearity and range

Table 5
Linearity Preparations

Preparations	Volume from standard stock transferred inml		Volume made up in ml (with mobile phase)	Concentration of solution (µg /ml)	
				Netupitant	Palonosetron
Preparation 1	0.25	0.5	10	25	50
Preparation 2	0.50	0.8	10	50	80
Preparation 3	0.75	1.0	10	75	100
Preparation 4	1.0	1.2	10	100	120
Preparation 5	1.25	1.5	10	125	150

Table 6
Linearity of Netupitant

S.No.	Conc. (µg/ml)	Area
1	25	3236.788
2	50	4409.861
3	75	5560.106
4	100	6760.326

5	125	7803.508

Table 7
Linearity of Palonosetron

S.No.	Conc. (µg/ml)	Area
1	50	347.912
2	80	520.885
3	100	657.488
4	120	780.529
5	150	892.314

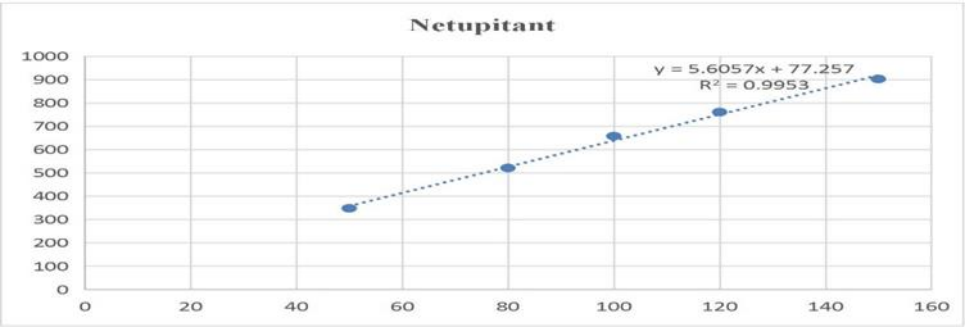

Figure 9. Linearity graph of Netupitant

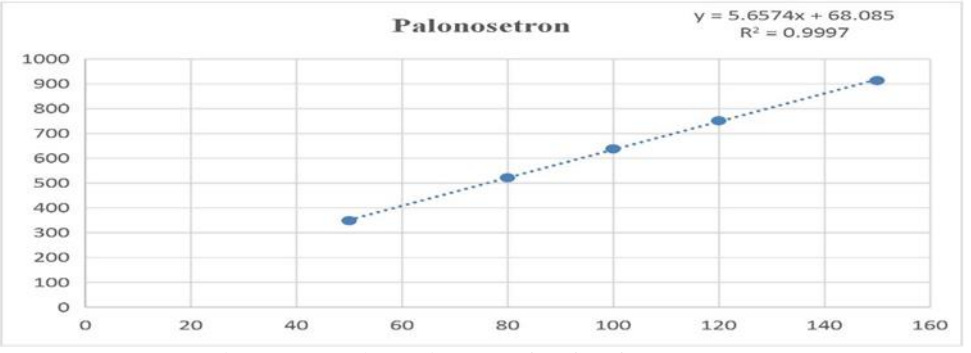

Figure 10. Linearity graph of Palonosetron

Table 8
Recovery results for Netupitant

Recovery level	Accuracy Netupitant				Average Recovery
	Amount taken(mcg/ml)	Area	Average area	%Recovery	
50%	256	4462.113	4604.360	99.52	101.93
	256	4682.749			
	256	4668.22			
100%	320	5595.271	5589.47	103.57	
	320	5588.966			

	320	5584.193			
150%	384	6296.468	6313.25	102.73	
	384	6324.06			
	384	6319.242			

Table 9
Recovery results for Palonosetron

Recovery level	Accuracy Palonosetron				Average Recovery
	Amount taken(mcg/ml)	Area	Average area	%Recovery	
50%	48	558.057	560.812	99.95838	
	48	563.349			
	48	561.03			
100%	60	657.465	657.722	104.8264	
	60	659.972			100.5
	60	655.729			
150%	72	751.964	757.928	108.1861	
	72	755.857			
	72	759.963			

The percentage mean recovery of Netupitant and palonosetron is101.93% and 100.5%respectively.

Precision

Table 10
Results for Method precision of Netupitant and Palonosetron

Netupitant		
S.No.	Rt	Area
1	1.827	5599365
2	1.830	5626643
3	1.833	5600893
4	1.833	5606288
5	1.830	5593455
6	1.843	5593358
Avg	1.833	5603333.7
Stdev	0.006	12416.62
%RSD	0.30	0.22

Palonosetron		
S.No.	Rt	Area
1	3.573	707341
2	3.593	692140
3	3.593	687020

4	3.607	696712
5	3.620	700091
6	3.640	695011
Avg	3.604	696385.8
Stdev	0.024	6949.62
%RSD	0.65	1.00

Limit of Detection

$$LOD = 3.3\sigma / S$$

Where, σ = the standard deviation of the responseS = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

= (3.3)*(12.85)/15.74
= 2.69µg/ml (Netupitant)
= (3.3)* (8.98)/26.94
=1.10µg/ml (Palonosetron)

Limit of Quantification

$$LOQ = 10\sigma / S$$

Were,
σ = the standard deviation of the responseS = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

= (10)*(12.85)/15.74
= 8.16µg/ml (NETUPITANT)
= (10)* (8.98)/26.94
=3.33µg/ml (PALONOSETRON)

Robustness

Table 11
Result of Robustness study

Parameter	Netupitant		Palonosetron	
	Retention time(min)	Tailing factor	Retention time(min)	Tailing factor
Flow Rate				
0.8 ml/min	1.353	1.37	2.643	1.41
1.2 ml/min	2.823	1.29	5.683	1.38
Wavelength				
251nm	1.833	1.42	3.610	1.41
255nm	1.827	1.41	3.580	1.43

Ruggedness

Table 12
Results for Ruggedness

Netupitant	%Assay	Palonosetron	%Assay
Analyst 01	100.01%	Analyst 01	99.37%
Anaylst 02	100.82%	Anaylst 02	99.58%
% RSD	0.27	% RSD	1.13

Table 13
Assay Results

	Netupitant		Palonosetron	
	Standard Area	Sample Area	Standard Area	Sample Area
Injection-1	558996	556320	640249	648536
Injection-2	560893	559691	647102	645717
Injection-3	560837	563445	642088	648013
Injection-4	557645	559365	648819	647341
Injection-5	556714	557488	642237	642566
Average Area	559017	559261.8	644099	646434.6
Standard deviatuion	1872.058		3661.628	
%RSD	0.33		0.56	
Assay(%purity)	100.04		100.36	

Conclusion

From the above experimental results and parameters, it was concluded that this newly developed method for the simultaneous estimation of Netupitant and Palonosetron was found to be simple, precise, accurate, and high resolution and shorter retention time makes this method more acceptable and cost-effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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