International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 2 Mar-Apr 2023, pp: 1423-1436 www.ijprajournal.com ISSN: 2249-7781

Method Development and Validation for the Simultaneous Estimation of Palonosetron and Fosnetupitant in Bulk and Pharmaceutical Dosage Form

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Submitted: 05-04-2023 Accepted: 15-04-2023

ABSTRACT
A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Fosnetupitant and Palonosetron bulk and pharmaceutical formulations. Separation of Fosnetupitant and Palonosetron was successfully achieved by using Dona: Thermo 250X4.6mm, 5µm, C18 or equivalent in an isocratic mode utilizing K2HPO4: Acetonitrile (60:40) at a flow rate of 1.0 mL/min and eluate was monitored at 228nm, with a retention time of 2.554 and 3.657 minutes for Fosnetupitant and Palonosetron respectively. The method was validated and the response was found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Fosnetupitant and 50µg/ml to150 µg/ml for Palonosetron. The values of the correlation coefficient were found to be 1.000 for Fosnetupitant and 0.999 for Palonosetron respectively. The LOD and LOQ for Fosnetupitant were found to be 0.383 and 1.278 respectivly. The LOD and LOQ for Palonosetron were found to be and 0.005 respectively. This method was found to have good percentage recovery for Fosnetupitant and Palonosetron the values were found to be 100 and 101 respectively indicates that the proposed method is highly accurate,so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Fosnetupitant, Palonosetron, High performance liquid chromatography.

I. INTRODUCTION

Palonosetron is an antinauseant and antiemetic agent indicated for the prevention of nausea and vomiting associated with moderately-emetogenic cancer chemotherapy and for the prevention of postoperative nausea and vomiting. Palonosetron is a highly specific and selective serotonin 5-HT3 receptor antagonist that is pharmacologically related to other 5-HT3 receptor antagonists, but differs structurally. Palonosetron has a high affinity for 5-HT3 receptors, but has little to no affinity for other receptors. The serontonin 5- HT3 receptors are located on the nerve terminals of the vagus in the periphery, and centrally in the chemoreceptor trigger zone of the area postrema. It is suggested that chemotherapeutic agents release serotonin from the enterochromaffin cells of the small intestine by causing degenerative changes in the GI tract. The serotonin then stimulates the vagal and splanchnic nerve receptors that project to the medullary vomiting center, as well as the 5-HT3 receptors in the area postrema, thus initiating the vomiting reflex, causing nausea and vomiting.

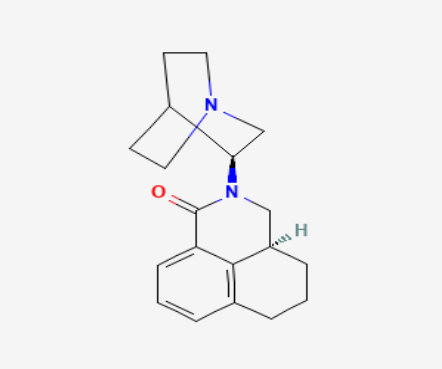

Figure 1:Chemical Structure of Palonosetron

Netupitant, the active moiety of fosnetupitant, is a selective neurokinin 1 (NK1) receptor antagonist with antiemetic activity. Netupitant competitively binds to and blocks the activity of the human substance P/NK1 receptors in the central nervous system (CNS), inhibiting NK1-receptor binding of the endogenous tachykinin neuropeptide substance P (SP), which results in the prevention of chemotherapy-induced nausea and vomiting (CINV). Substance P is found in neurons of vagal afferent fibers innervating the brain-stem nucleus tractus solitariiand the area postrema, which contains the chemoreceptor

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Volume 8, Issue 2 Mar-Apr 2023, pp: 1423-1436 www.ijprajournal.com ISSN: 2249-7781

trigger zone (CTZ), and may be present at high levels in response to chemotherapy. The NK-receptor is a G- protein receptor coupled to the inositol phosphate signal-transduction pathway and is found in both thenucleus tractus solitarii and the area postrema.
Netupitant demonstrated 92.5% NK1 receptor occupancy at 6 hours, with 76% occupancy at 96 hours.

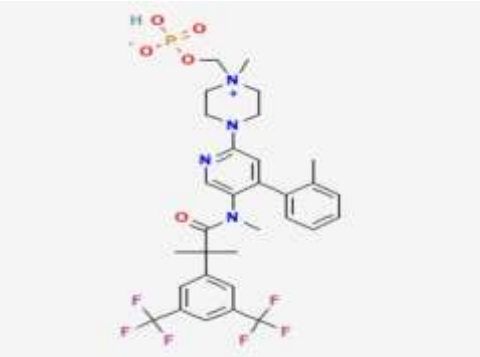

Figure 2: Chemical Structure of fosnetupitant

The literature survey revealed that there are a few HPLC and spectroscopic methods available for the determination of palonosetron and fosnetupitant in combination with other drugs. In view of non- availability of HPLC methods for the selected combination, as per literature, author has aimed to develop a new HPLC method for simultaneous estimation of Netarsudil and latanoprost in combined pharmaceutical dosage form.

EXPERIMENTAL

Chemicals and reagents: Palonosetron, Fosnetupitant, Potassium Dihydrogen Phosphate, Water, Acetonitrile And Na2hpo4. All the above chemicals and solvents are from hetero.

Equipments: Electronic Balance, Ultra-Sonicator, Heating Mantle, Thermal oven, pH Meter and Filter Paper 0.45microns

Chromatographic condition: The mobile phase used was K2HPO4: Acetonitrile (60:40) in the gradient mode employing flow rate at 1.0 ml/min. The analytical column THERMO(C18, 250X4.6mm, 5µm). The detection was carried out at a wavelength of 228 nm with a run time of 7 min.

Preparation of mobile phase: Transfer 1000ml of HPLC water into 1000ml of beaker and K2HPO4 adjust pH 3.8
Transfer the above solution 600ml K2HPO4 of, 400ml of Acetonitrile is used as mobile phase. They are mixed and sonicate for 20min.

Preparation of standard solution: Accurately weighed quantity of 235mg Fosnetupitant and 0.25mg Palonosetron was taken ina 100 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonicate for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. From the above solution 1 ml is taken and further diluted in 10 ml volumetric flasks with mobile phase. To acquire a concentration of 235mg Fosnetupitant and 0.25mg Palonosetron.

Preparation of sample solution: 10 tablets were weighed and crushed, from the powdered tablets, weighed accurately about 235.25mg (235mg Fosnetupitant and 0.25mg Palonosetron) into a 100 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs. Filtered and cooled to room temperature and solution was made up to mark with mobile phase. From the above solution 1 ml is taken and further diluted in 10 ml volumetric flasks with mobile phase. To acquire a concentration of 235 mg Fosnetupitant and 0.25 mg Palonosetron.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, Tailing factor for the peaks due to Fosnetupitant and Palonosetron in standard solution shouldnot be more than 2.0. Theoretical plates for the Fosnetupitant and Palonosetron peaks in standard solution should not be less than 2000. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in Table 1.

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Table 1: System suitability data of Fosnetupitant and Palonosetron

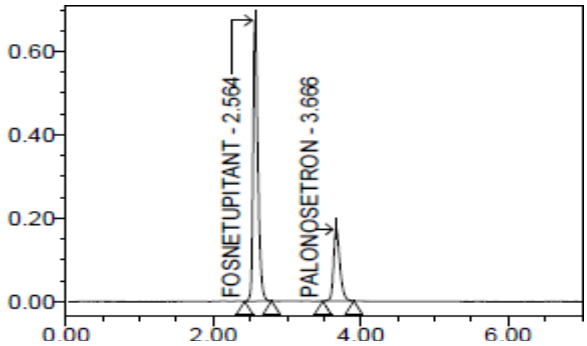
Parameter	Fosnetupitant	Palonosetron	Acceptance Criteria
Retention time	2.554	3.657	+-10
Theoretical plates	7829	8989	>2500
Tailing factor	1.39	1.28	<2.00
% RSD	0.2	0.2	<2.00

Standard Results of Fosnetupitant

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Plate Count	USP Tailing
1	STD-2	FOSNETUPITANT	2.564	3035548	7788	1.37
2	STD-2	FOSNETUPITANT	2.564	3031391	7792	1.38
3	STD-2	FOSNETUPITANT	2.566	3026934	7789	1.38
4	STD-2	FOSNETUPITANT	2.566	3040518	7813	1.38
5	STD-2	FOSNETUPITANT	2.568	3045917	7908	1.38
Mean				3036061.6		
% RSD				0.2		

Standard Results of Palonosetron

	Sample Name	Peak Name	RT	Area (µV*sec)	USP Resolution	USP Plate Count	USP Tailing
1	STD-2	PALONOSETRON	3.666	1037789	7.91	9054	1.29
2	STD-2	PALONOSETRON	3.666	1033349	7.92	9080	1.26
3	STD-2	PALONOSETRON	3.668	1031317	7.92	9052	1.27
4	STD-2	PALONOSETRON	3.670	1033453	7.89	8914	1.26
5	STD-2	PALONOSETRON	3.672	1033292	7.95	9107	1.26
Mean				1033840.1			
% RSD				0.2			



Typical Chromatogram of Standard-2; Injection-1

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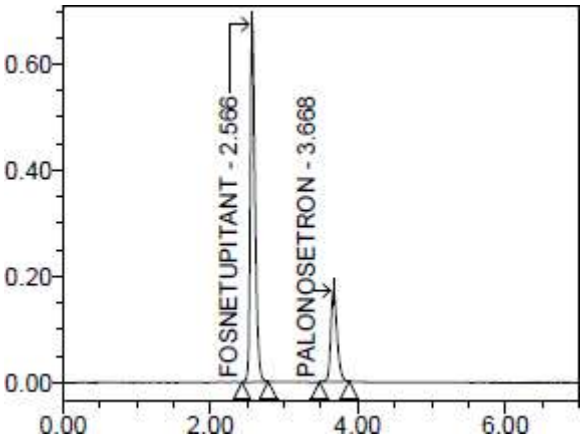
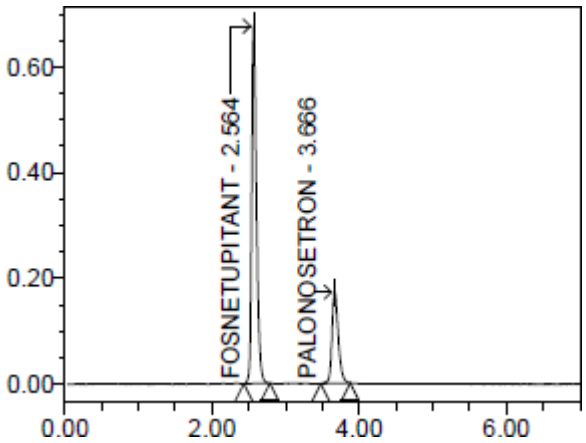
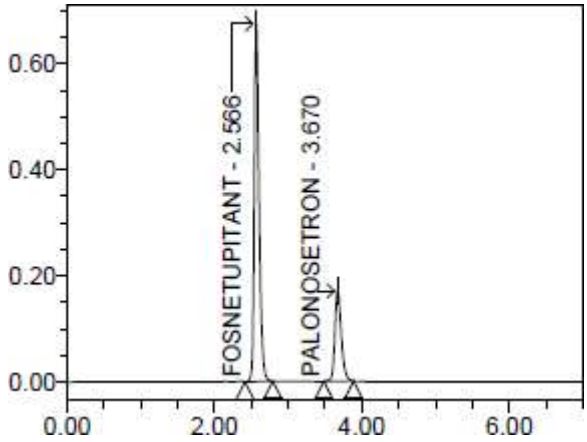


Typical Chromatogram of Standard-2; Injection-2



Typical Chromatogram of Standard-2; Injection-3



Typical Chromatogram of Standard-2; Injection-4

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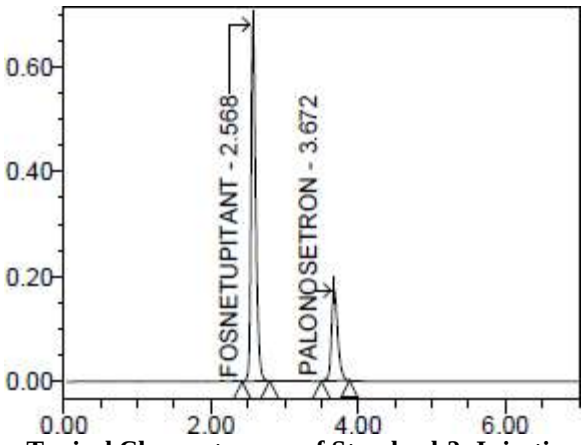
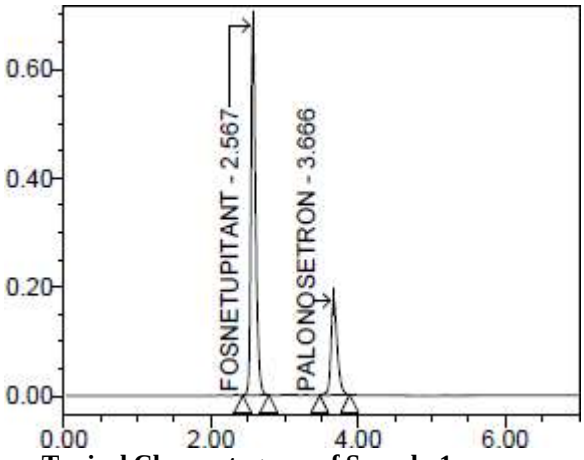


Typical Chromatogram of Standard-2; Injection 5



Typical Chromatogram of Sample-1

Fig 3: System suitability chromatography of Fosnetupitant and Palonosetron

RECOVERY/ACCURACY

Recovery study can be performed in the concentration range of 80% to 120% of the target concentration of the test. Minimum 3 concentrations are recommended.

Acceptance criteria:

The average percentage recovery was between 98-102% and Relative standard deviation of these recovery concentrations was less than 2%.

Table 2: Accuracy (%recovery) results of Palonosetron

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	117.63	0.123	0.12	101	101
		2	117.63	0.123	0.12	102	
		3	117.63	0.123	0.12	101	

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2	100%	1	235.25	0.245	0.25	100	101
		2	235.25	0.245	0.25	100	
		3	235.25	0.245	0.25	101	
3	150%	1	352.88	0.368	0.37	100	101
		2	352.88	0.368	0.37	101	
		3	352.88	0.368	0.37	102	

Table 3: Accuracy (%recovery) results of Fosnetupitant

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	117.63	116.325	115.39	99	99
		2	117.63	116.325	115.45	99	
		3	117.63	116.325	116.30	100	
2	100%	1	235.25	232.650	232.68	100	100
		2	235.25	232.650	232.99	100	
		3	235.25	232.650	233.14	100	
3	150%	1	352.88	348.975	350.34	100	100
		2	352.88	348.975	351.03	101	
		3	352.88	348.975	349.91	100	

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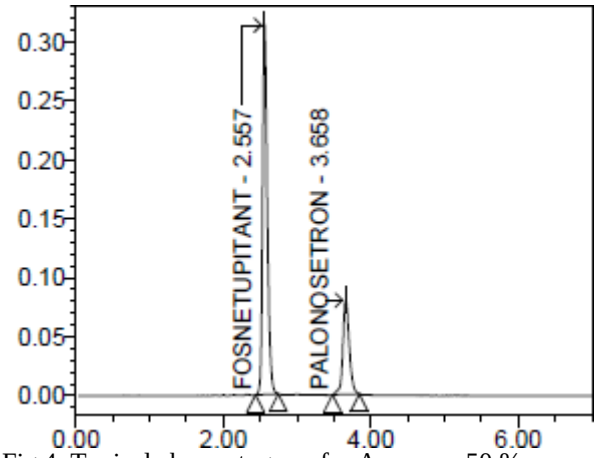

Fig 4: Typical chromatogram for Accuracy 50 %

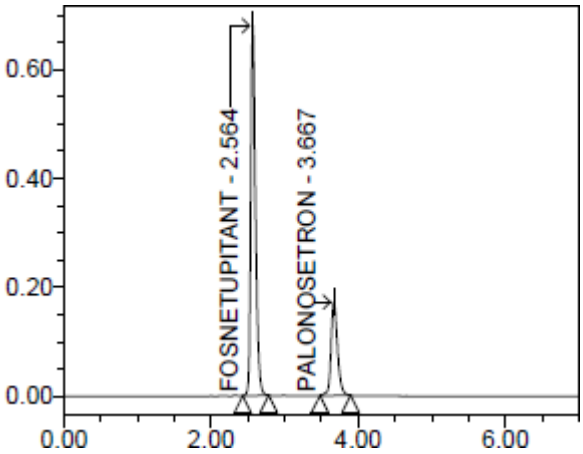

Fig 5: Typical chromatogram for Accuracy 100 %

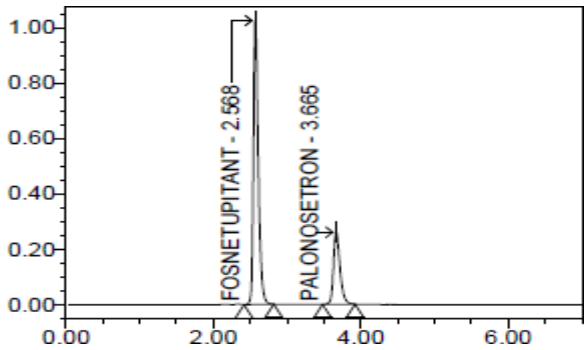

Fig 6: Typical chromatogram for Accuracy 150 %

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RESULT

Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against therecovery amount.

% Recovery was 100% for Fosnetupitant and 101% for Palonosetron. All the results indicate that the method is highly accurate.

Linearity: Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standardVs the actual concentration in µg/ml and determine the coefficient of correlation and basis for100% response.

Table 4: Linearity data for Palonosetron

S.No	Conc (µg/ml)	RT	Area
1.	50	3.648	514297
2.	75	3.656	773090
3.	100	3.664	1018461
4.	125	3.663	1270706
5.	150	3.670	1534826
Correlation coefficient(r^2)			0.999

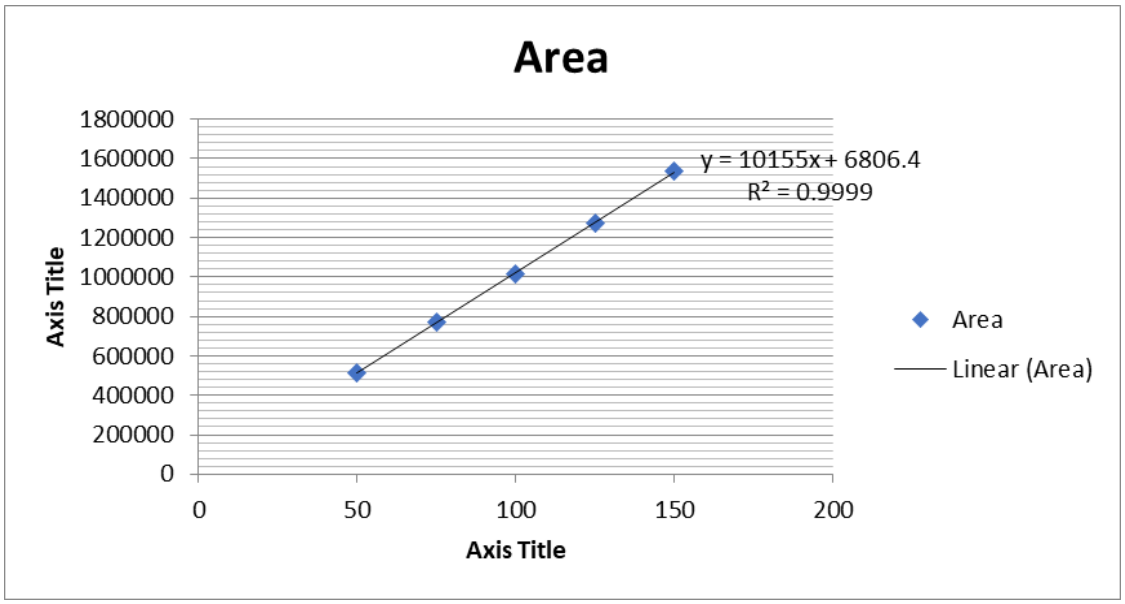

Fig 7: Linearity plot of Palonosetron

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Table 5: Linearity data for Fosnetupitant

S.No	Conc (µg/ml)	RT	Area
1.	50	2.551	1493116
2.	75	2.556	2253263
3.	100	2.563	3014940
4.	125	2.565	3772317
5.	150	2.571	4538564
Correlation coefficient (r^2)			1.000

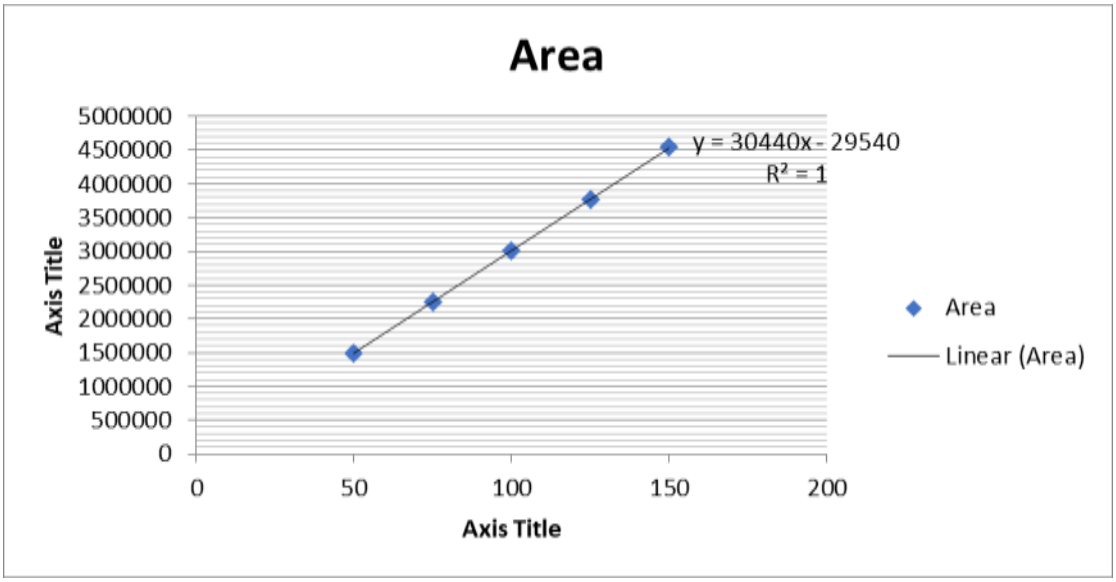

Fig 8: Linearity plot of Fosnetupitant

RESULT

A linear relationship between peak areas versus concentrations was observed forFosnetupitant and Palonosetron in the range of 50% to 150% of nominal concentration. Correlation coefficient was 1.000 and 0.999 for both Fosnetupitant and Palonosetron which prove that the method is linear in the range of 50% to 150%.

Precision: For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The sample was injected in six replicate for intermediate precision and six replicate for precision. The peak area for injections was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed HPLC method was found to be precise. The Precision results were shown in Table-6 and Intermediate Precision were shown in Table-7

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Table 6: Precision data for Palonosetron

S.no	RT	Area	%Assay
injection1	3.666	1018770	98
injection 2	3.670	1015332	98
injection 3	3.655	1020376	99
injection 4	3.665	1013529	98
injection 5	3.658	1025183	99
injection 6	3.661	1012025	98
Mean			98
Std. Dev.			0.47
%RSD			0.48

Table 7: Precision data for Fosnetupitant

S.No	RT	Area	%Assay
injection1	2.567	3018359	99
injection2	2.565	3023383	99
injection3	2.562	3013862	99
injection4	2.564	3018333	99
injection5	2.562	3016014	99
injection6	2.563	3023593	99
Mean			99
Std. Dev.			0.13
% RSD			0.13

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Limit of detection :
Minimum concentration of standard component in which the peak of the standard getsmerged with noise called the LOD
LOD = 3.3* σ/S

Where;
σ = standard deviationS = slope
LOD for Fosnetupitant = 0.383 LOD for Palonosetron = 0.002

Table 8: LOD data for Fosnetupitant and Palonosetron

S.No	Sample name	RT	Area
1	Fosnetupitant	2.539	2487
2	Palonosetron	3.526	12036

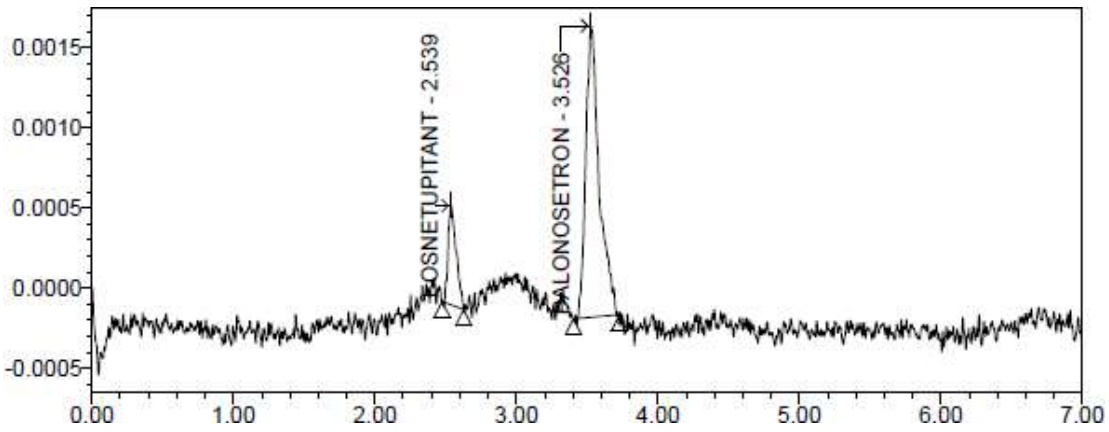

Fig 9: Chromatrogram for LOD

Limit Of Quantification:
Minimum concentration of standard component in which the peak of the standardgets detected and quantification
LOQ = 10*σ/S

Where;
σ = standard deviationS = slope
LOQ for Fosnetupitant = 1.278 LOQ for Palonosetron = 0.005

Table 9: LOQ data for Fosnetupitant and Palonosetron

S.no	Sample name	RT	Area
1	Fosnetupitant	2.542	34696
2	Palonosetron	3.648	23711

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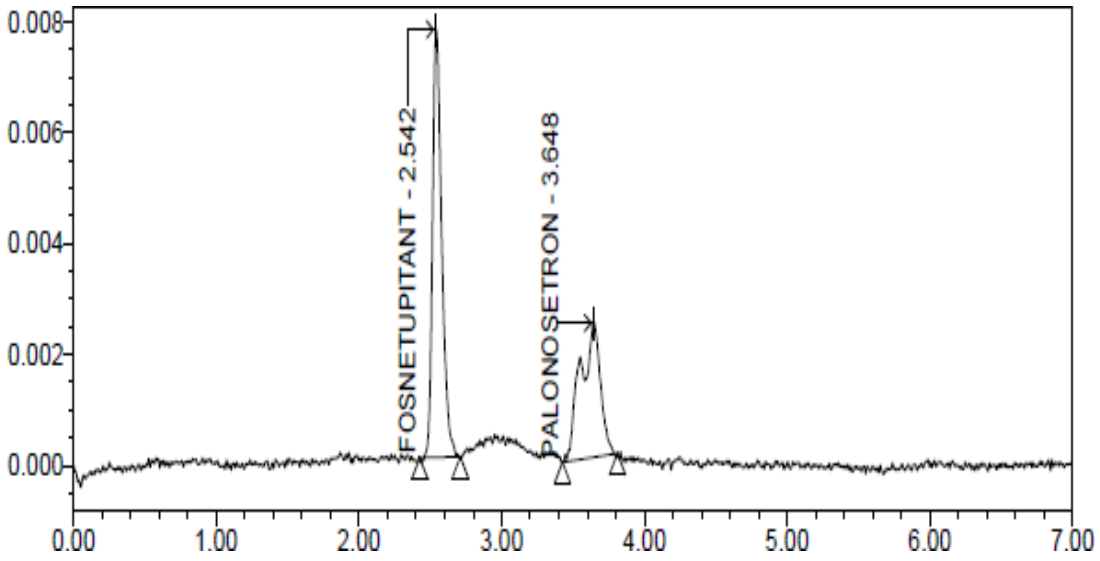

Fig 10: Chromatogram for LOQ

Degradation study:

The tablet sample solution (Fosnetupitant and Palonosetron) was subjected to degradation by acid, base, oxidant, water, thermal and photo conditions. This experiment demonstrates the specificity, stability indicating nature and stability of Fosnetupitant and Palonosetron under different applied conditions.

Table 10: Fosnetupitant and Palonosetron degradation data

Condition	Percent assay		Percent degradation	
	Fosnetupitant	Palonosetron	Fosnetupitant	Palonosetron
0.1 N HCl	89.30	90.52	10.7	9.48
0.1N NaOH	91.95	92.39	8.05	7.61
30% H_2O_2	94.98	95.77	5.02	4.23
105^oC	90.21	89.12	9.79	10.88
Sunlight	93.89	94.34	6.11	5.66
Water	99.20	98.55	0.80	1.45

Fosnetupitant and Palonosetron is more sensitive to acid condition and more resistance to water condition. The degradant peaks were well resolved from the Fosnetupitant and Palonosetron peaks. No interference seen. Therefore, the method is specific and stability indicating.

II. CONCLUSION:

In the present work a new, accurate, precise and robust HPLC method was developed and validated for simultaneous estimation of netarsudil and latanoprost in pharmaceutical dosage form in accordance with the ICH Guidelines. The

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method gives good resolution between both the compounds with a short analysis time (5 min). Both Fosnetupitant and Palonosetron which prove that the method is linear in the range of 50% to 150%. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the netarsudil and latanoprost in combined dosage form without any interference from excipients.

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