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Simultaneous method development and validation for estimation of fosnetupitant and palonosetron using HPLC

G Karuna*, R.V. Valli Kumari, M. Sathish Kumar, S Markatham**

Depertment of pharmaceutical analysis Mallareddy Institute of pharmaceutical Sciences, Maisammaguda Dhulapally, Secunderabad-500014 Telangana State

***Corresponding Author: G Karuna**

E-mail: gurigallakaruna@gmail.com

ABSTRACT

Akynzeo injection dosage form containing 0.25 mg and 235 mg of palonosetron and fosnetupitant, respectively is recently approved in US, used in adults for the avoidance of delayed and acute nausea and vomiting during chemotherapy in emetogenic cancer. In this investigation, a reverse phase high pressure liquid chromatography method for quantification of palonosetron and fosnetupitant simultaneously is developed and validated. The separation of palonosetron and fosnetupitant was accomplished using 0.1M NaH2PO4 - methanol (60:40, v/v) as mobile phase and Kromosil, C18 (25 cm × 4.6 mm, 5µm) as analytical column. Quantitation of palonosetron and fosnetupitant was by photodiode array detector at 225 nm in the amount range of 235-705 µg/ml (fosnetupitant) and 0.25-0.75 µg/ml (palonosetron). Using described conditions of chromatography, the method shown good resolution and separation of fosnetupitant and palonosetron with retention time of 3.034 min and 4.779 min, respectively. Detection & quantification limits for palonosetron and fosnetupitant were calculated as 0.0245 µg/ml & 0.0817 µg/ml and 1.219 µg/ml & 4.062 µg/ml, respectively. For accuracy and precision that are expressed as relative standard deviation and percent respectively, the found values are satisfactory and acceptable as given by ICH.Recovery assay carried out for samples gave recoveries of 99.65% and 99.29% 102.5% and 95.2% for fosnetupitant and palonosetron, respectively. In conclusion, a reliable and suitable and reliable procedure for simultaneous estimation of fosnetupitant and palonosetron using RP-HPLC technique was developed & validated successfully.

Keywords: Fosnetupitant, Palonosetron, RP- HPLC.

INTRODUCTION
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography, alias high pressure liquid chromatography, a latest technique of analysis is used to separate, detect and estimate every ingredient from the mixture. The liquid solvent of sample mixture is pressurized and infiltrated and passed into a column of solid adsorbant using HPLC pumps. This technique enables the column to flow under high pressure by which the different constituents of the sample are isolated by the difference of their affinities.A mass exchange process incorporating adsorption is seen in chromatography technique. In HPLC, the partition of specimen segments is prompted by sending a pressurized fluid and an example that gets brewed in a section containing adsorbent with the help of a pump. As usual, the adsorbent comprises of a granular material made of solid particles (e.g. silica, polymers, etc.) 2 µm to 50 µm in size forms that is the dynamicsegment [1-4].

As usual, the adsorbent comprises of solid particles of granular nature such as silica, polymers, etc 2 µm to 50 µm in size forms that is the dynamic segment. The connection with retentive particles having distinctive degrees forms the segments of example mixture/blend that has to be freed from each other. The pressurized fluid possessing a combination of solvents (e.g. acetonitrile, water and/or methanol) is 'mobile phase'. A vital role is played by the organization and temperature affecting connections among sample segments and adsorbent of the partition procedure plays a vital role.As basically pressure at which the method performed (50 to 350 bar) are more, the traditional ("low weight") liquid chromatography perceived is HPLC, where as orderly liquid chromatography confide in gravity potential to forward the portable stage through its segment. Only tiny sample amount has to be isolated in scientific HPLC, measurements of column section across are 2.1 mm to 4.6 mm distance and 30 mm to 250 mm along its length. Further, segments of HPLC posses much tinier adsorbent particles (2 to 50 µm in normal molecule size) which increase resolving power of HPLC (the capacity to identify and separatecomponents) through mixture's isolation,to make it an outstanding chromatography method.The HPLC instrumentation has pump, injector, column, detector and display system. The parts are inclusive of the following [5-10].

FOSNETUPITANT

2018 April by Food and Drug Administration. Fosnetupitant is a netupitant's prodrug form. Substance P or neurokinin 1 receptor antagonist. Option for treating patients who have chemotherapy induced vomiting and nausea. 4-(5-{2-[3,5-bis(trifluoromethyl)phenyl]-N,2-dimethylpropanamido}-4- (2- methylphenyl) pyridin- 2-yl)-1- [(hydrogenphosphonooxy) methyl]-1- methylpiperazin-1-iumC31H35F6N4O5P 688.608g/mol

CHEMICAL STRUCTURE

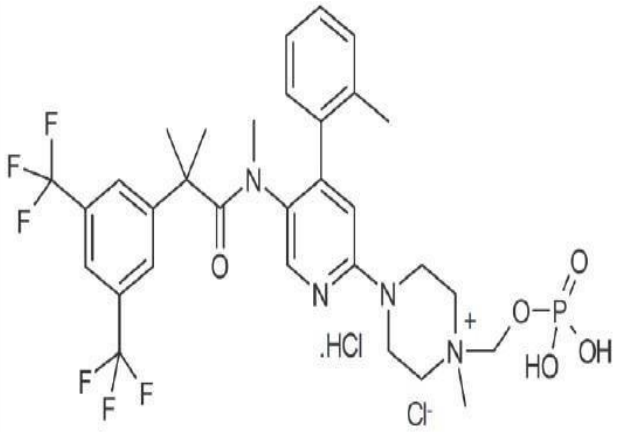

Figure 1: Fosnetupitant structure

PALONOSETRON

2003 July by Food and Drug Administration. Antiemetic, serotonergic antagonist and 5 hydroxytryptamine Type 3 receptor antagonist.Option for treating patients who have chemotherapy induced vomiting and nausea. (3aS)-2-[(3S)-1-Azabicyclo [2.2.2] oct-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benz [de] isoquinolin-1- one. C19H24N2O. 332.87 g/mol

CHEMICAL STRUCTURE

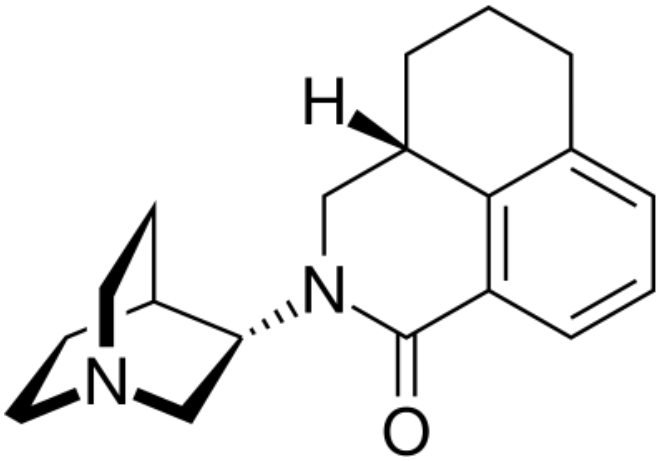

Figure 2: Palonosetron structure

MATERIALS AND METHODS

Materials

Material	Obtained from
Fosnetupitant reference drug	Lara Drugs Private Limited, Telangana, India
Palonosetron reference drug	-do-
Milli-Q water	Milli-Q water system, USA
NaH2PO4 (AR)	Sd. Fine Chemicals Ltd, Mumbai, India
Methanol (HPLC grade)	Merck specialities Ltd, Hyderabad, India

Instrumentation

Name	Specification
Chromatography system	Waters Alliance system (2695 Module)
Detector	Photodiode array detector (2998 Module)
Injector	Rheodyne ten microliter
Data processorsoftware	Empower 2 version
Column	Kromosil, C18, 25 cm × 4.6 mm, 5 µm
pH meter	Digisun pH meter 7007 model
Membrane filter	Tarsons, India

PREPARATION OF MOBILE PHASE

M NaH2PO4 and methanol are mixed in ratio 60:40 volume/volume

Chromatography conditions

Parameter		Optimized condition
Flow rate		1.0 ml/min
Injector volume		10 microlitres
Temperature		25˚C
Wavelength	for detection	225 nm
Run time ofanalysis		7 min

PREPARATION OF STOCK SOLUTION OF FOSNETUPITANT AND PALONOSETRON

The mixed stock solution was prepared freshly by dissolving 235 mg of fosnetupitant and 0.25 mg of palonosetron in 100 ml of mobile phase. Concentration of stock solution is 235.0 µg/ml of fosnetupitant and 2.5 µg/ml of palonosetron.

RESULTS AND DISCUSSION

Method development

Optimization is initiated after a reasonable chromatogram is obtained. A reasonable chromatogram is one that has less or more symmetrical peaks of the selected drugs, fosnetupitant and palonosetron, in the chromatogram. The detection of all selected compounds has to be achieved. The mobile phase components, analytical column, detection wavelength, flow rate were chosen on the basis of better separation, peak symmetry, peak area, theoretical plates and retention time. To optimize the conditions in the current investigation, the following trails weredone.

Mobile Phase :0.1% orthophosphoric acid and methanol (60:40, vol/vol) Analytical column: Lichrocart, C18, 25 cm × 4.6 mm, 5 µm

Flow Rate : 1ml/min

Temperature of column : 25˚C

Volume of sample : 10 µl Run

Time for analysis :10min Wavelength for detection : 225 nm

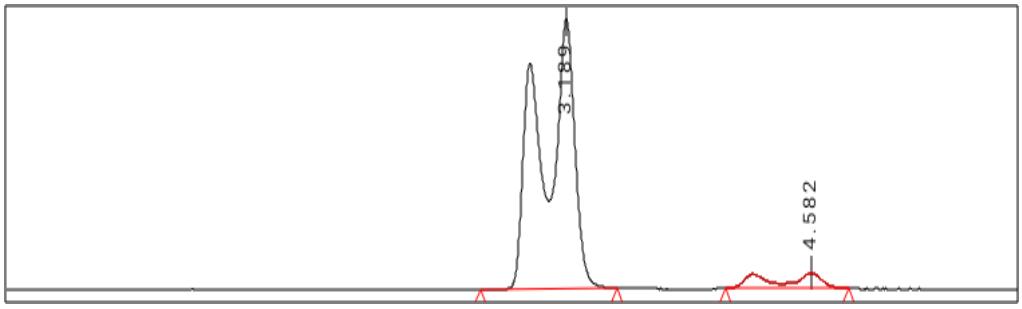

Figure 3: Chromatogram obtained in trail one

Data obtained for trail 1

Name	Retention Time	Area	Resolution	Tailing	Plate Count
Fosnetupita nt	3.189	4963541	-	0.71	1166
Palonosetron	4.582	407839	3.04	0.67	1179

METHOD VALIDATION

The developed method for fosnetupitant and palonosetron simultaneous quantification by RP-HPLC technique with photodiode array detector was validated for system suitability, linearity, selectivity, precision, accuracy, limit of detection and limit of quantification and robustness.

SYSTEM SUITABILITY

Working solution with concentration 470 µg/ml fosnetupitant and 0.50µg/ml palonosetron is prepared and assayed by applying the proposed method five times. Relative standard deviation of peak area response and retention time of fosnetupitant and palonosetron were determined. Also the values of theoretical plates, tailing factor and resolution are determined. Recommended limits are given below:

- %RSD of peak area - ≤ 2%
- % RSD of retention time - ≤ 2%;
- Tailing factor - ≤2
- Plate count - >2000
- Resolution - >1.5.

As values are inside the acceptance limits (Table 1 and 2), the method passes the system suitability test.

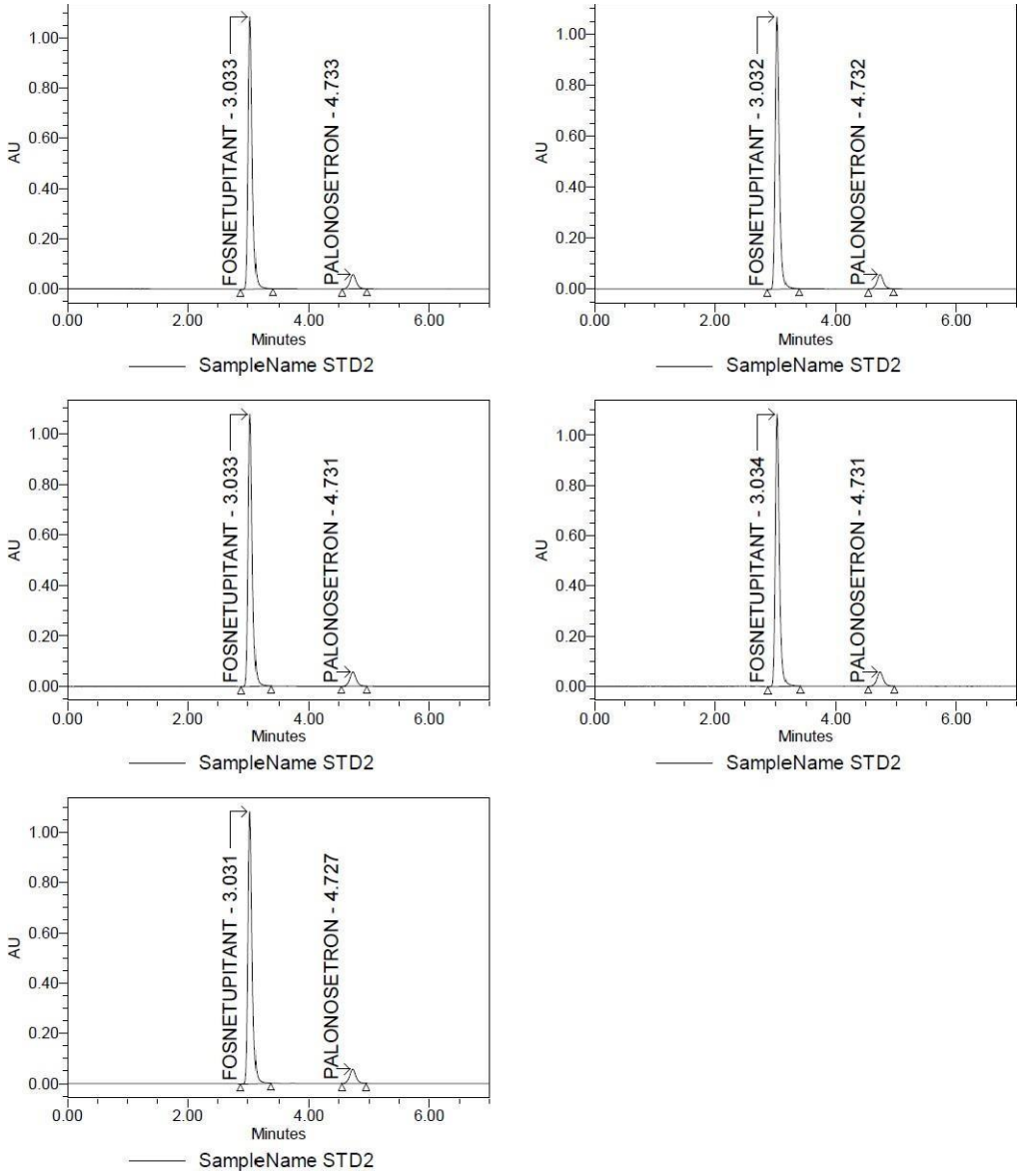

Figure 8: Chromatograms obtained in system suitability test

LINEARITY

Linearity study was performed with five calibration solutions (fosnetupitant from 235 µg/ml to 705 µg/ml and palonosetron from 0.25 µg/ml to 0.75 µg/ml). The peak area of fosnetupitant and palonosetron in all calibration solutions were determined using proposed method. The study showed linear relationship with concentration range of 235 µg/ml – 705 µg/ml for fosnetupitant and 0.25 µg/ml - 0.75 µg/ml for palonosetron. The linearity of a fosnetupitant and palonosetron calibration curves are confirmed by good regression coefficient (R^2 is > 0.999) and small y-intercept value in the regression equation.

Table 3: Fosnetupitant and palonosetron linearity results

Fosnetupitant concentration (µg/ml)	Fosnetupitant peak area (mAU)	Palonosetron concentration (µg/ml)	Palonosetron peak area (mAU)
235	2521917	0.25	208806
352	3787886	0.375	312577
470	5045499	0.5	417202
587	6309105	0.625	521712
705	7568990	0.75	625300

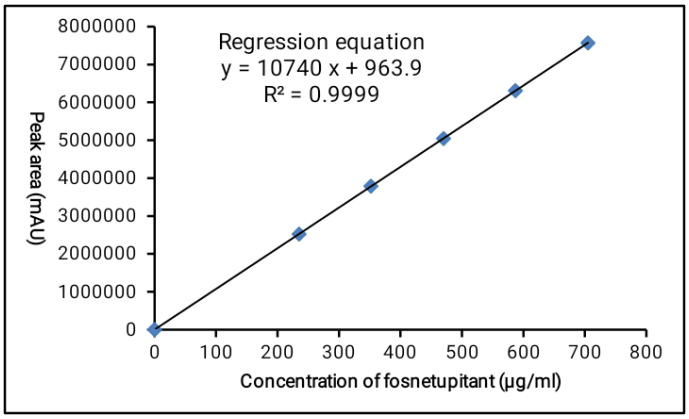

Figure 9: Fosnetupitant linearity graph

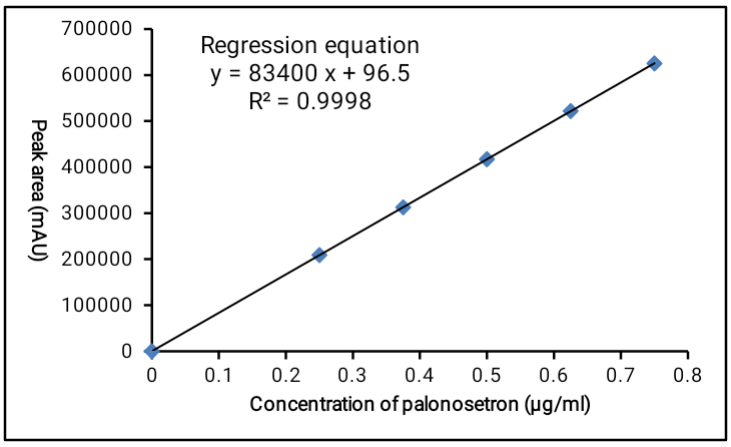

Figure 10: Palonostron linearity graph

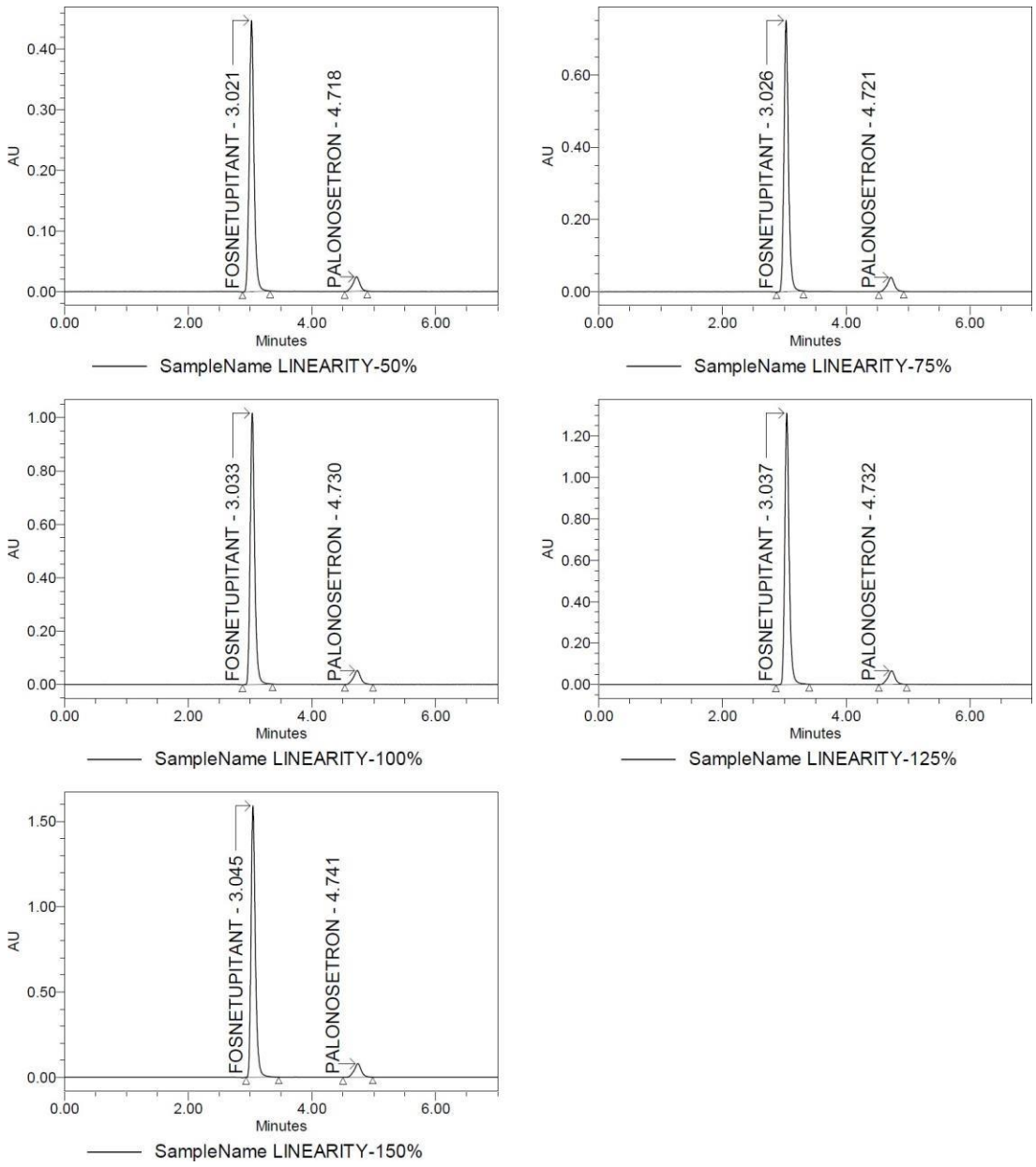

Figure 11: Chromatograms obtained in linearity test

SELECTIVITY

Selectivity was weighed up through comparison of standard solution (470 µg/ml fosnetupitant and 0.50 µg/ml palonosetron), mobile phase, placebo blank and spiked placebo (470 µg/ml fosnetupitant and 0.50 µg/ml palonosetron) chromatograms. Nonappearance of peaks interfering at the retention time of fosnetupitant and palonosetron was taken as evidence for method's selectivity.

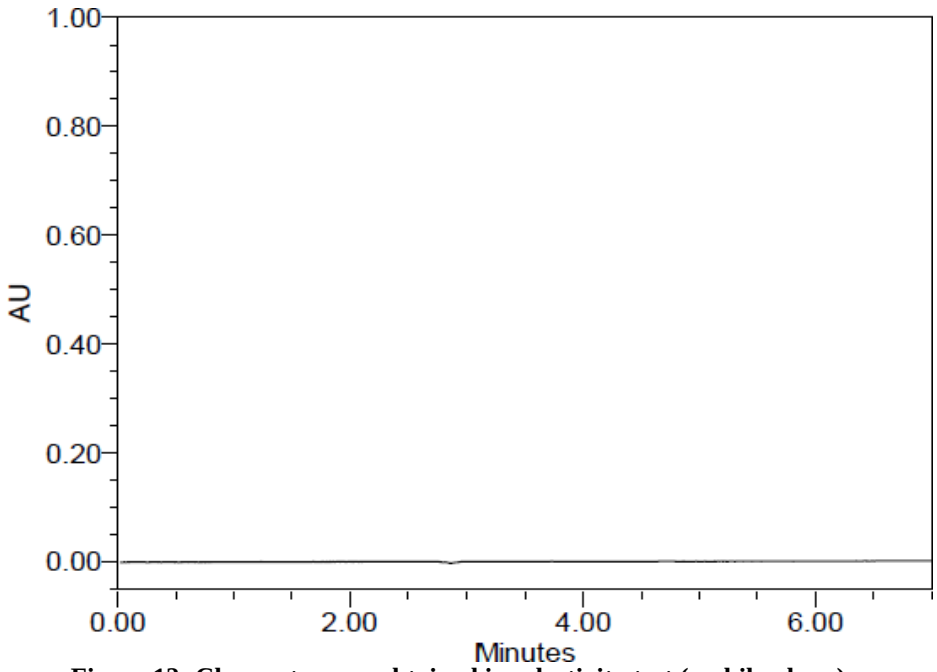

Figure 12: Chromatogram obtained in selectivity test (mobile phase)

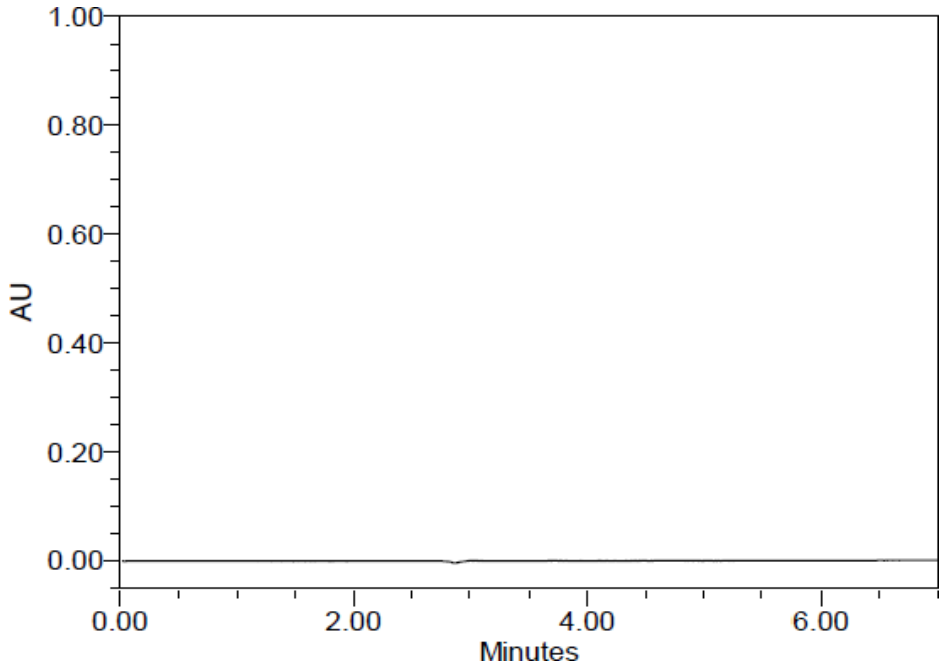

Figure 13: Chromatogram obtained in selectivity test (placebo blank)

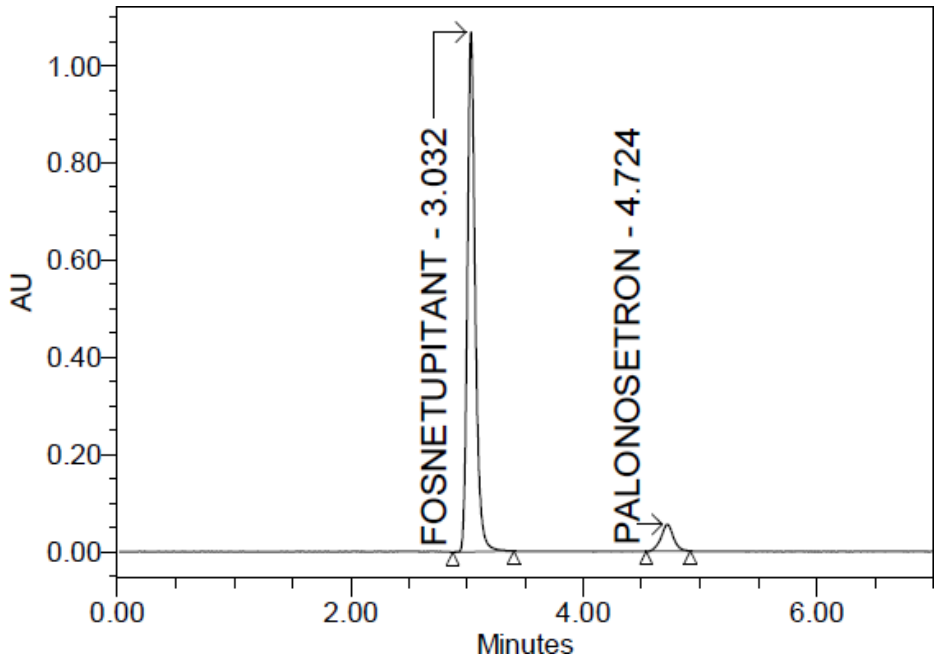

Figure 14: Chromatogram obtained in selectivity test (placebo spiked with drugs)

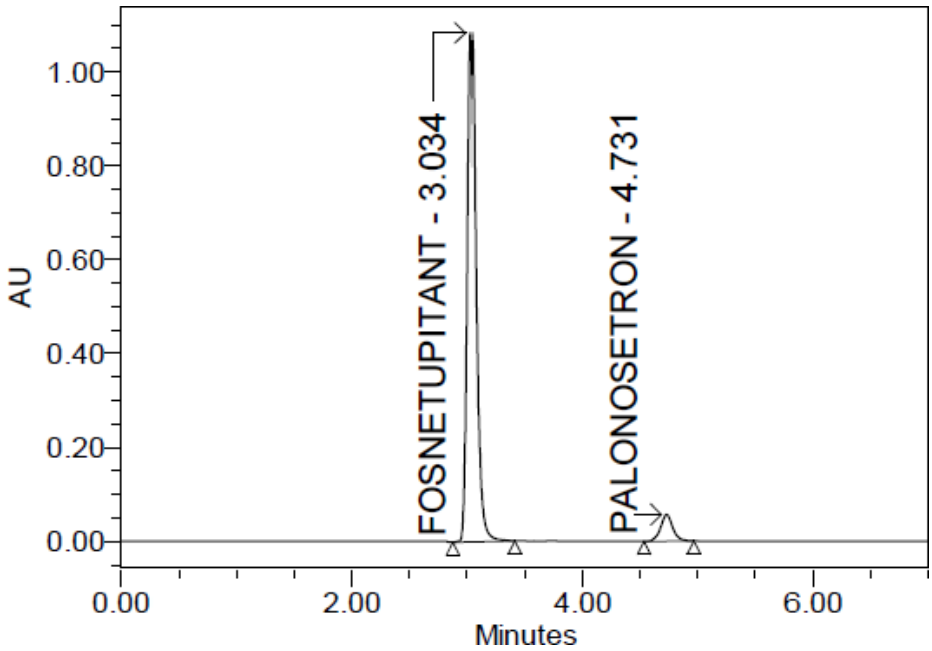

Figure 15: Chromatogram obtained in selectivity test (standard solution)

SENSITIVITY

Limit of detection (LOD) and Limit of quantification (LOQ) are used to represent the method sensitivity. Signal to noise ratio of 3.1 and 10.1 is determined to calculate the values of LOD and LOQ. For this, low concentration solutions of fosnetupitant and palonosetron were injected into the system and proposed method is applied. The LOD value and LOQ value determined for fosnetupitant were 1.219 and 4.062 µg/ml, respectively. The LOD value and LOQ value determined for palonosetron were 0.0245 and 0.0817 µg/ml, respectively. The values got were suitable enough.

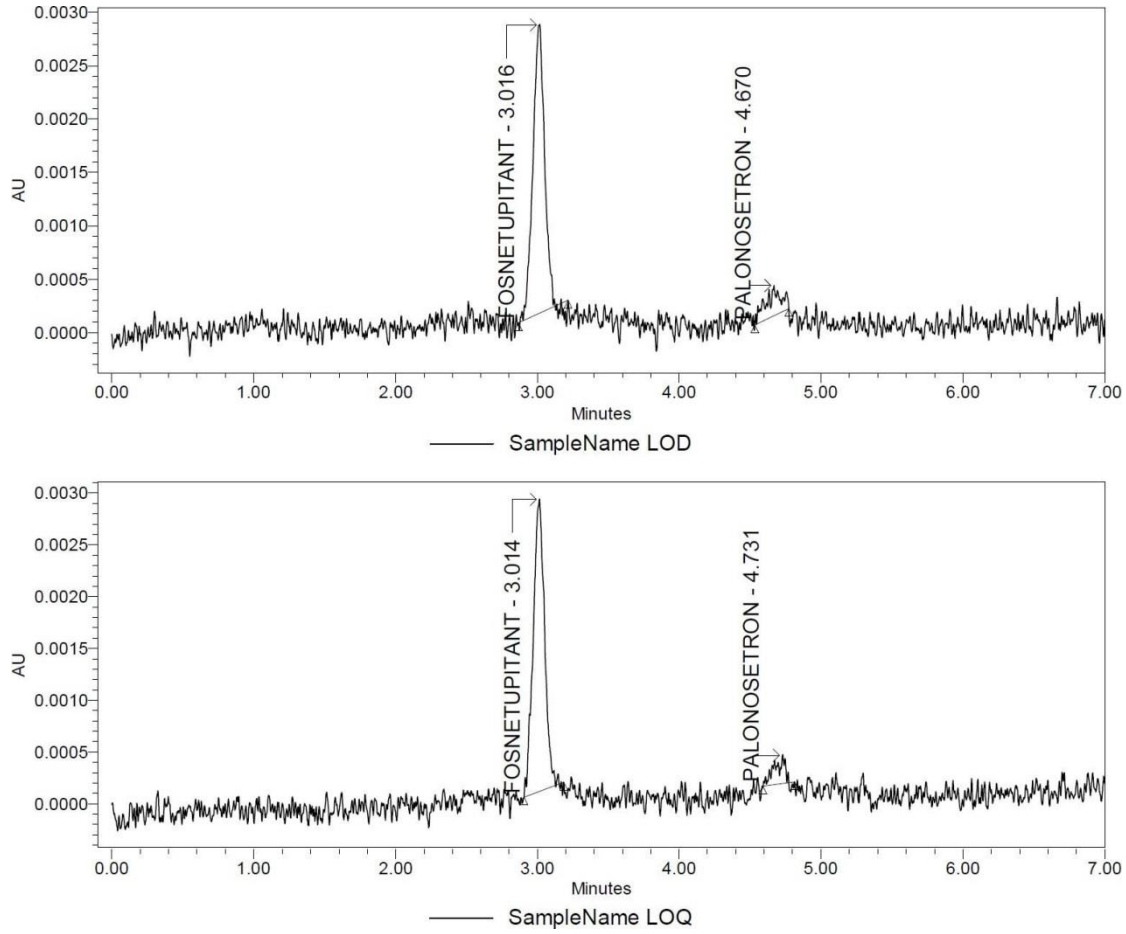

Figure 16: Chromatogram obtained in sensitivity test

PRECISION

Precision was examined through analyzing 6 replicates of working standard sample (470 µg/ml fosnetupitant and 0.50 µg/ml palonosetron) on the similar day. The peak areas and relative standard deviation of fosnetupitant and palonosetron are determined. The relative standard deviation less than 2%, showed method preciseness.

Table 4: Fosnetupitant and palonosetron precision results

S.No	Fosnetupitant peak area (mAU)	Statistical values for fosnetupitant	Palonosetron peak area (mAU)	Statistical values for palonosetron
A	5044401	Average:	417184	Average:
B	5044099	5044990	417520	417578
C	5042900	SD:	417692	SD:
D	5042095	2999.167	417742	125.225
E	5049118	RSD:	417532	RSD:
F	5047328	0.059	417798	0.030

S.No. – Sample number; SD – Standard deviation; RSD – Relative standard deviation

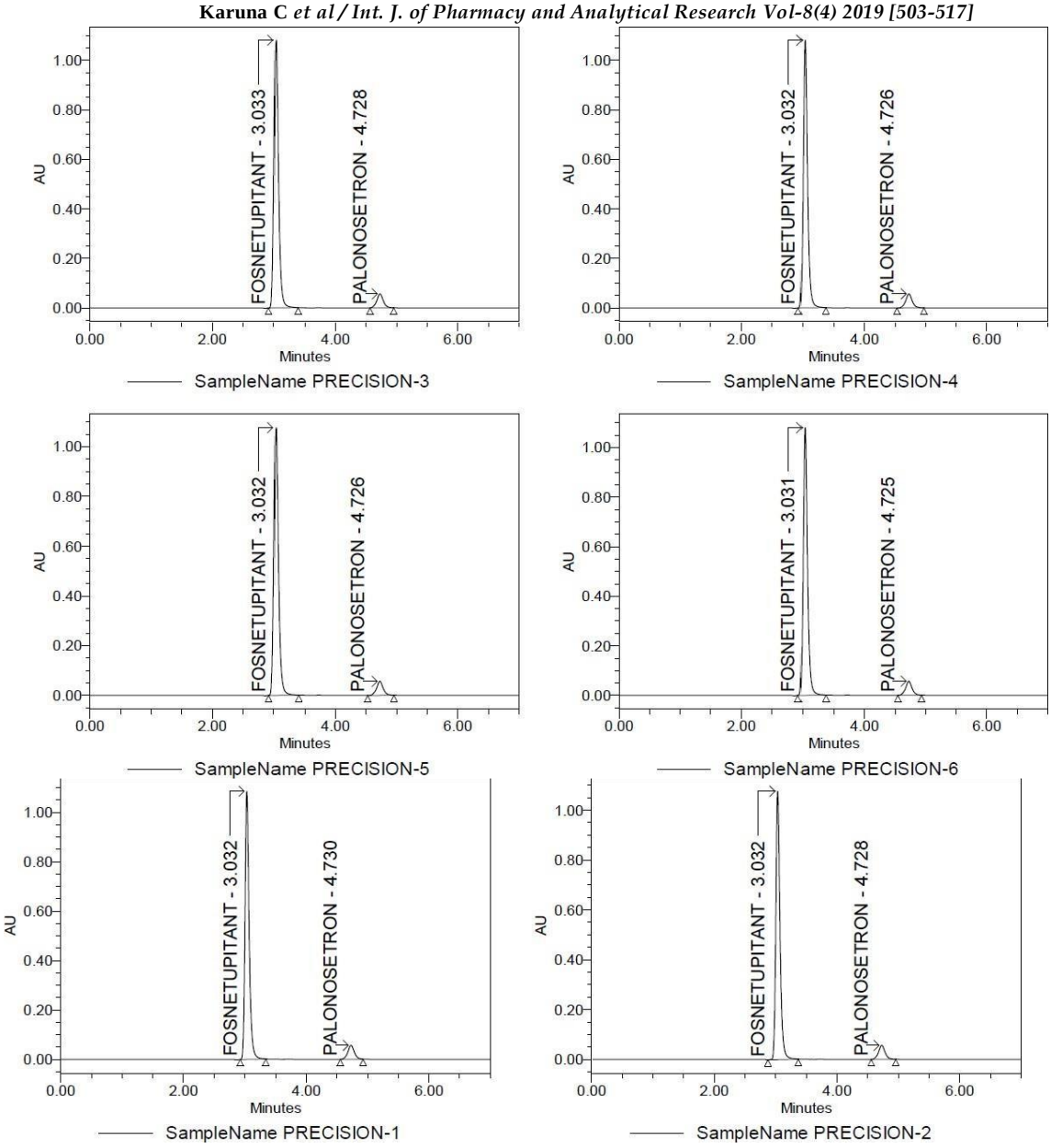

Figure 17: Chromatograms obtained in precision test

RECOVERY TEST

Three replicates of three samples (50%, 100% and 150%) were prepared by spiking pure fosnetupitant palonosetron in placebo for determination of accuracy by recovery experiment. The recovery percent of fosnetupitant and palonosetron was determined using the proposed method. The recovery achieved for fosnetupitant and palonosetron was inside the range 80% to 120 as recommended by ICH. There is no hindrance from the excipients used in the placebo. Hence the accuracy of method is proved.

Table 5: Fosnetupitant accuracy and recovery results

Percentage spiked (%)	Peak area (mAU)	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	Statistical values
50%	2524312	235	234.34	99.72	Mean: 99.79%
	2528442	235	234.72	99.88	SD: 0.0808%
	2525953	235	234.49	99.78	RSD: 0.0810%

100%	5042907	470	468.15	99.61	Mean: 99.61%
	5045615	470	468.40	99.66	SD: 0.0451%
	5041045	470	467.98	99.57	RSD: 0.0453%
150%	7568672	705	702.62	99.66	Mean: 99.60%
	7563011	705	702.10	99.59	SD: 0.0513%
					RSD: 0.0515%

Table 6: Palonosetron accuracy and recovery results

Percentage spiked (%)	Peak area (mAU)	Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	Statistical values
	208560	0.248	0.248	100.19	Mean: 100.29%
50%	208841	0.248	0.248	100.32	SD: 0.0889%
	208932	0.248	0.248	100.36	RSD: 0.0886%
					Mean:
	417444	0.495	0.496	100.26	100.26%
100%	417036	0.495	0.496	100.17	SD: 0.0900%
	417795	0.495	0.497	100.35	RSD: 0.0898%
150%	625066	0.743	0.743	100.09	Mean: 100.14%
	625230	0.743	0.743	100.11	SD: 0.0757%
	625927	0.743	0.744	100.23	RSD: 0.0756%

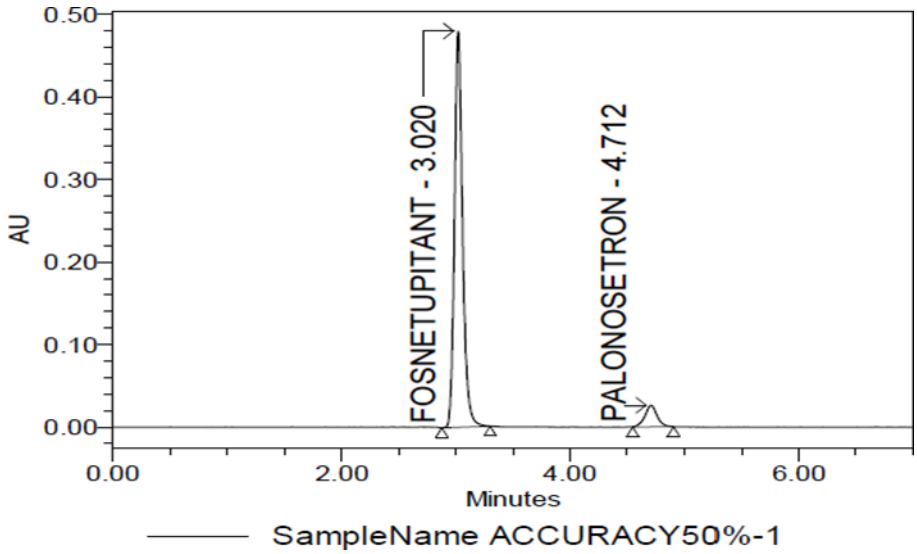

Figure 18: Chromatogram obtained in accuracy and recovery test at50% level

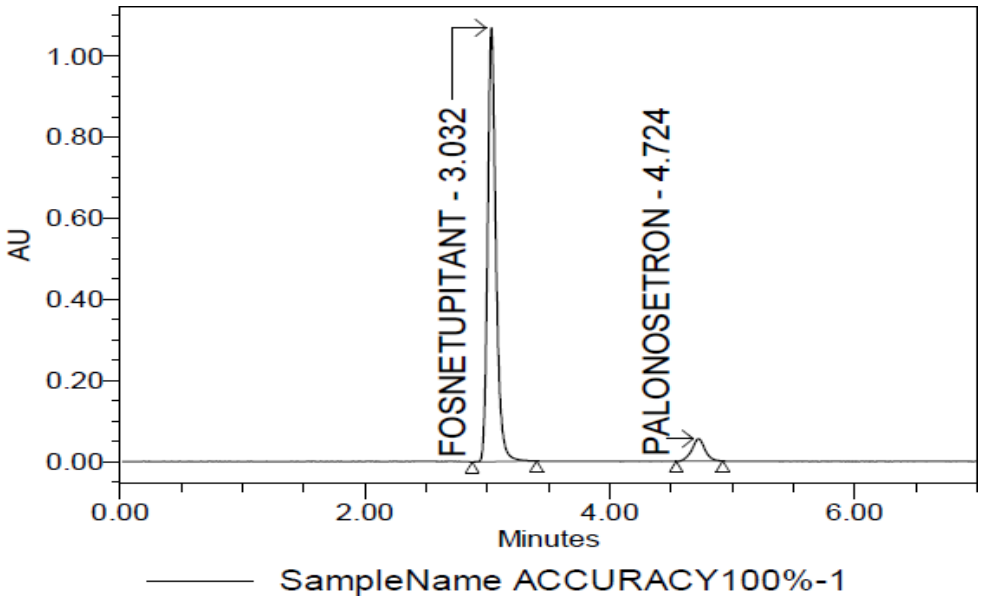

Figure19: Chromatogram obtained in accuracy and recovery testat100% level

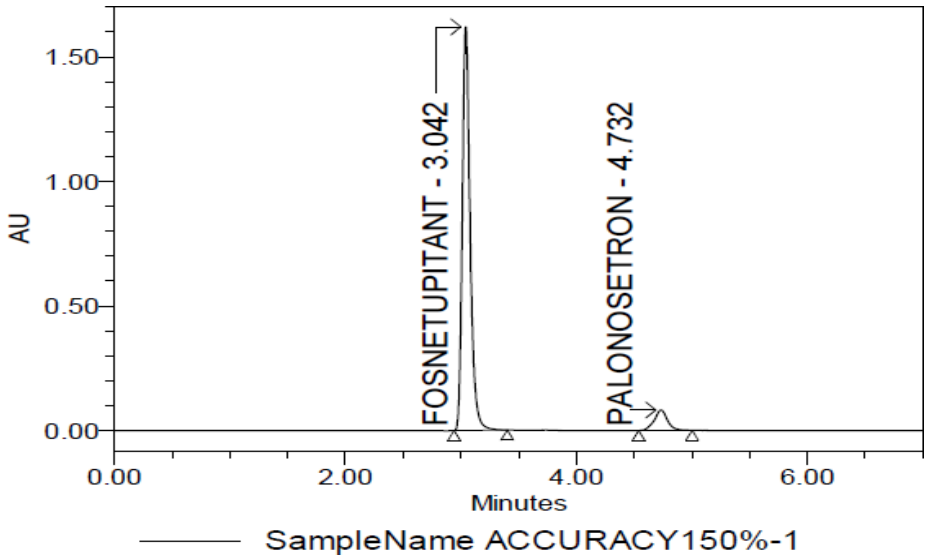

Figure20: Chromatogram obtained in accuracy and recovery testat150% level

ROBUSTNESS

Robustness is done with working standard sample (470 µg/ml fosnetupitant and 0.50 µg/ml palonosetron). Influence of slight or small modifications in HPLC conditions on system suitability parameters in the proposed method is evaluated to assess method robustness. The robustness results tested showed a slight or small change in method conditions, such as ratio of components in mobile phase (± 5%), temperature (±2 ^{O}C), flow rate (±0.1 ml/min), wavelength (±2 nm) and pH (±0.2 units) is robust and found within the tolerable limits. The resolution, peak tailing and theoretical plates were found within the tolerable limits.

Table 7: Fosnetupitant Robustness Data

Condition tested	Tested value	Theoretica l plate	Peak tailing	Resolution
Temperature in column (^{o}C)	23	9050	1.17	-
	27	11071	1.23	-
Flow rate (ml/min)	0.9	6227	1.13	-
	1.1	6726	1.12	-
Mobile phase pH (units)		10581	1.29	-
		10423	1.27	-
Ratio of methanol in mobile phase (%)	35	6227	1.13	-
	45	9050	1.17	-
Analytical wavelength (nm)	223	10481	1.30	-
	227	10588	1.29	-

Table 8: Palonosetron robustness data

Condition tested	Tested value	Theoretical plate	Peak tailing	Resolution
Temperature in column (^{o}C)	23	7554	0.94	9.02
	27	9839	0.98	10.50
Flow rate (ml/min)	0.9	4884	0.92	7.38
	1.1	4893	0.92	7.60
Mobile phase pH (units)		10070	1.03	10.65
		10019	1.05	10.57
Ratio of methanol in mobile phase (%)	35	4884	0.92	7.38
	45	7554	0.94	9.02
Analytical wavelength (nm)	223	10275	1.06	10.63
	227	10375	1.05	10.73

CONCLUSION

A novel robust and simple reverse phase high performance liquid chromatography method was developed for assay of fosnetupitant and palonosetron, simultaneously. Proposed method shown good resolution and separation of fosnetupitant and palonosetron with retention time of 3.034 min and 4.779 min, respectively. The method proposed was validated for selectivity, system suitability, accuracy, robustness and precision. The developed & validated method provides reward of being economic, simple andconsumes less time for more number of analyzing samples.

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