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Research article ***Analytical research***

RP-HPLC method development and validation for the simultaneous estimation of fosnetupitant and palonosetron in bulk and combined formulation

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Palonosetron and Fosnetupitant, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Altima C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 225 nm. The retention time of the Palonosetron and Fosnetupitant was 2.102, 3.537 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Palonosetron and 12.5-62.5mg/ml of Fosnetupitant. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Palonosetron, Fosnetupitant, RP-HPLC, validation.

INTRODUCTION

HPLC

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good sensitivity.

High Performance Liquid Chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography the use of an instrumentation that was originally developed for gas chromatography. High Pressure Liquid Chromatography was developed in the mid-1970 and was improved with the development of column packing material and the additional convenience of on-line detectors. The various components of HPLC are pumps (solvent delivery system), mixing unit, gradient controller and solvent degasser, injector (manual or automatic), guard column, analytical columns, detectors, recorders and/or integrators. Recent models are equipped with computers and software for data acquisition and processing. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase at a flow rate of 1-5 cm3/min. The mobile phase acts as a carrier for the sample solution. The chemical interactions of the mobile phase and sample with the column determine the degree of migration and separation of components contained in the sample. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

Types of Chromatogphy[1]

Normal-phase chromatography

Mechanism: Retention by interaction with the polar surface of the stationary phase with polar parts of the sample molecules. Stationary phase: SiO2, Al2O3, -NH2, -CN, -Diol,-NO2, etc. Mobile phase: Heptane, hexane, cyclohexane, CHCl3, CH2Cl2, dioxane, methanol, etc.

Application: Separation of non-ionic, non-polar to medium polar substances. Disadvantage: Lack of reproducibility of retention times as water or protic organic solvents change the hydration state of the silica or alumina chromatographic media.

Reversed-phase chromatography

Mechanism: Retention by interaction of the stationary phase's non-polar hydrocarbon chain with non-polar parts of the sample molecules.

Stationary phase: n-octadecyl (RP-18), n-octyl (RP-8), ethyl (RP-2), phenyl, (CH2)n-CN, (CH2)n-diol, etc.

Mobile phase: Methanol, acetonitrile, water, buffer (sometimes with additives of THF or
Dioxane), etc.

Application: Separation of non-ionic and ion forming non-polar to medium polar substances (carboxylic acids, hydrocarbons). If ion forming substances (as carboxylic acids) are to be separated, a pH control by buffers is necessary.

Reversed-phase ion-pair chromatography

Mechanism: Ionic sample molecules are ionically bound to an ion-pair reagent. The ion- pair reagent contains an unpolar part suitable for interaction with the unpolar hydrocarbon chain of the stationary phase.

Stationary phase: Reversed phase materials (RP-18, RP-8, CN), etc.

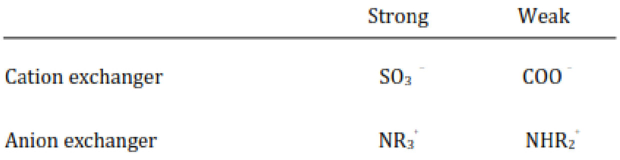
Mobile phase: Methanol, acetonitrile, buffer with added ion-pair reagent in the concentration range of 0.001 to 0.01 M, etc.

Application: Ionic substances often show very poor retention in reversed phase chromatography. To overcome this difficulty an ion-pair reagent is added to the eluent.

Ion-exchange chromatography

Mechanism: Retention of reversible ionic bonds on charged groups of the stationary phase

Stationary phase:



	Strong	Weak
Cation exchanger	SO_3^-	COO^-
Anion exchanger	NR_3^+	NHR_2^+

Mobile phase: Aqueous buffer systems.

Application: Separation of substances which can form ions such as inorganic ions, organic acids, organic bases, proteins, nucleic acids.

Advantages of HPLC[15]

1) It provides specific, sensitive and precise method for analysis of the different complicated sample.
2) There is ease of sample preparation and sample introduction.
3) There is speed of analysis.
4) The analysis by HPLC is specific, accurate and precise.
5) It offers advantage over gas chromatography in analysis of many polar, ionic substances, high molecular weight substances, metabolic products and thermolabile as well as nonvolatile substances.

Applications of HPLC[16]

a) Natural Products: HPLC is an ideal method for the estimation of various components in plant extracts which resemble in structure and thus demand a specific and very sensitive method e.g., analysis of digitalis, cinchona, liquorice, and ergot extracts.

b) Stability studies: HPLC is now used for ascertaining the stability of various pharmaceuticals. With HPLC the analysis of the various degradation products can be done and thus stability indicating HPLC systems have been developed.

c) Bioassays and its complementation: Complex molecules as antibiotics and peptide hormones are mainly analysed by bioassay which suffer from high cost, necessity replicates, poor precision and length of time required. Also bioassay gives an overall estimate of potency and gives no guidance about the composition. Thus HPLC can be used to complement bioassays and give an activity profile. It has been used for analysis of chloramphenicol, penicillins, clotrimoxazole, sulfas and peptides hormones.

d) HPLC has also been used in the cosmetic industry for quality control of various cosmetics.

MATERIALS AND METHODS

Palonosetron from Sura labs, Fosnetupitant from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Palonosetron and Fosnetupitant working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Palonosetron and 0.375ml of the Fosnetupitant stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 40ºC
Column : Altima C18 (4.6×150mm, 5µ)
Buffer : Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Fliter and sonicate the solution by vaccum filtration and ultra sonication.
pH : 4.5
Mobile phase : Methanol: TEA buffer: ACN (50:25:25 v/v)
Flow rate : 1ml/min
Wavelength : 225 nm
Injection volume : 10 µl
Run time : 7 min

Validation

Preparation of buffer and mobile phase

Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the p^H 4.5. Fliter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of mobile phase

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimised Chromatogram (Standard)

Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 225 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 7 minutes

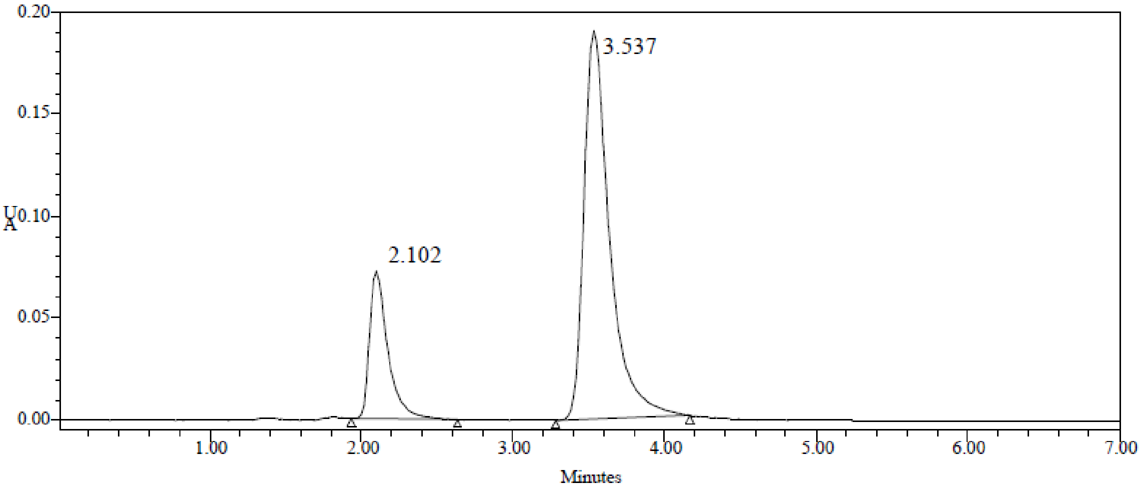


Fig 1: Optimized Chromatogram

Table 1: Peak results for optimized

S. No	Peak name	R$_t$	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Palonosetron	2.102	607323	72100		0.96	5586.0
2	Fosnetupitant	3.537	2231111	190007	2.97	1.22	5371.0

From the above chromatogram it was observed that the Palonosetron and Fosnetupitant peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's Optimised trial.

Optimized Chromatogram (Sample)

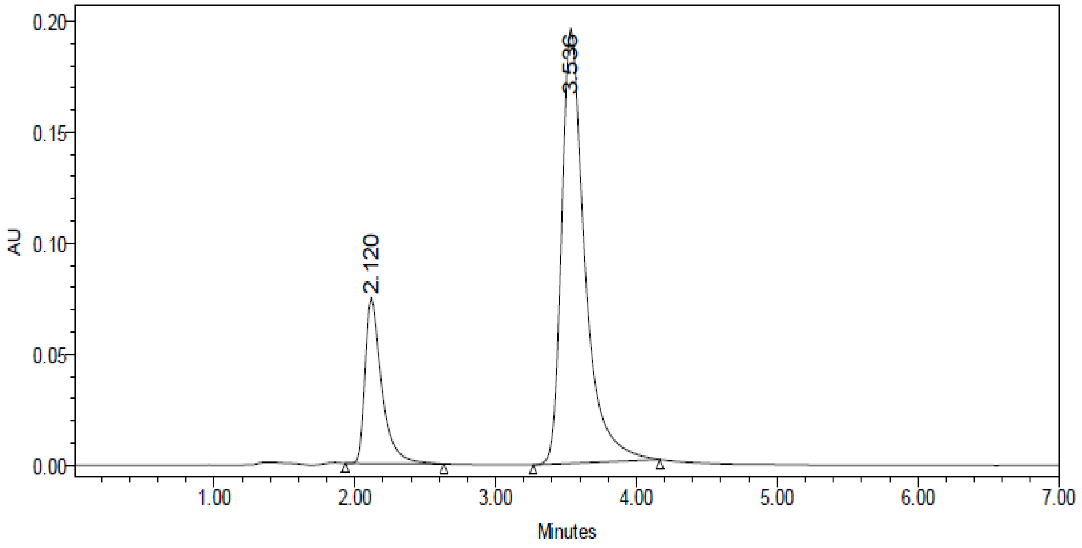


Fig 2: Optimised Chromatogram (Sample)

Table 2: Optimised Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Palonosetron	2.120	775610	130275		0.98	6253
2	Fosnetupitant	3.536	555592	93740	5.06	1.23	7836

- *Resolution between two drugs must be not less than 2*
- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*

Assay (Standard)

Table 3: Results of system suitability for Palonosetron

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Palonosetron	2.117	608452	71498	5643	1.9
2	Palonosetron	2.118	606820	126412	5432	1.6
3	Palonosetron	2.116	608452	126471	5123	1.6
4	Palonosetron	2.109	595267	129859	5207	1.7
5	Palonosetron	2.102	596608	124691	5481	1.6
Mean			603119.8			
Std. Dev			6607.31			
% RSD			1.09			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Palonosetron

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Fosnetupitant	3.547	2234724	188631	5043	1.2	2.07
2	Fosnetupitant	3.539	2240080	2614821	5432	1.4	2.05
3	Fosnetupitant	3.547	2234724	2321451	5987	1.5	2.0
4	Fosnetupitant	3.565	2204466	2324710	5845	1.6	2.01
5	Fosnetupitant	3.537	2209574	2531247	5371	1.6	2.01
Mean			2224714				
Std. Dev			16399.05				
% RSD			0.73				

- *%RSD for sample should be NMT 2*

- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Assay (Sample)

Table 5: Peak results for Assay sample

S.no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Palonosetron	2.120	775610	130275		0.98	7253	1
2	Fosnetupitant	3.536	555592	93740	2.06	1.23	8836	1
3	Palonosetron	2.120	689956	73869		1.05	6530	2
4	Fosnetupitant	3.537	575685	129125	2.04	0.99	7270	2
5	Palonosetron	2.102	607323	128898		1.7	7586	3
6	Fosnetupitant	3.537	558777	2231111	2.04	1.6	8371	3

$$\%ASSAY = \frac{\textbf{Sample area}}{\textbf{Standard area}} \times \frac{\textbf{Weight of standard}}{\textbf{Dilution of standard}} \times \frac{\textbf{Dilution of sample}}{\textbf{Weight of sample}} \times \frac{\textbf{Purity}}{\textbf{100}} \times \frac{\textbf{Weight of tablet}}{\textbf{Label claim}} \times \textbf{100}$$

The % purity of Palonosetron and Fosnetupitant in pharmaceutical dosage form was found to be 99.6%.

Linearity

Chromatographic data for linearity study

Palonosetron

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	205035
66.6	10	381239
100	15	561128
133.3	20	740162
166.6	25	909922

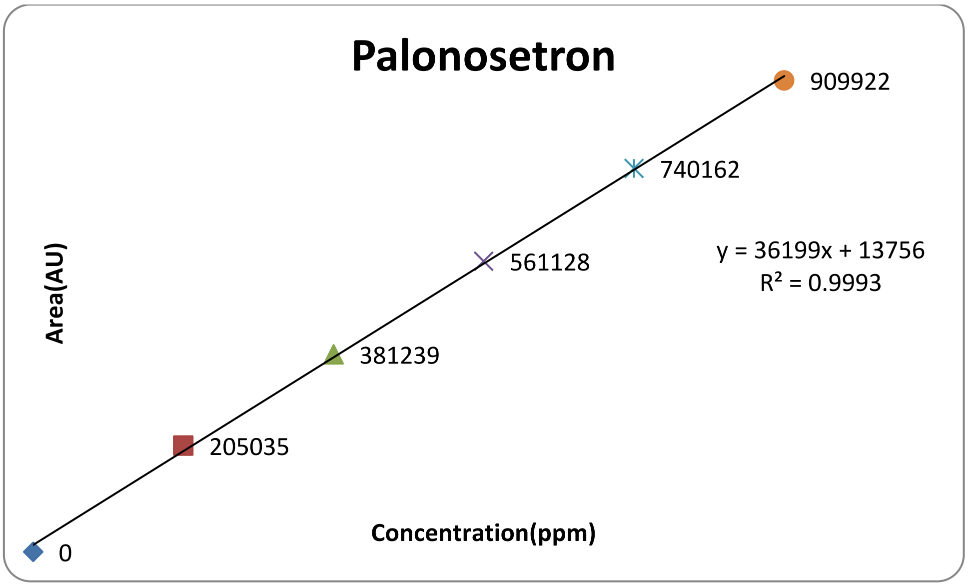


Fig 3: Calibration graph for Palonosetron

Fosnetupitant

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	12.5	757881
66	12.5	757881
100	25	1458941

133	37.5	2132457
166	50	2901811

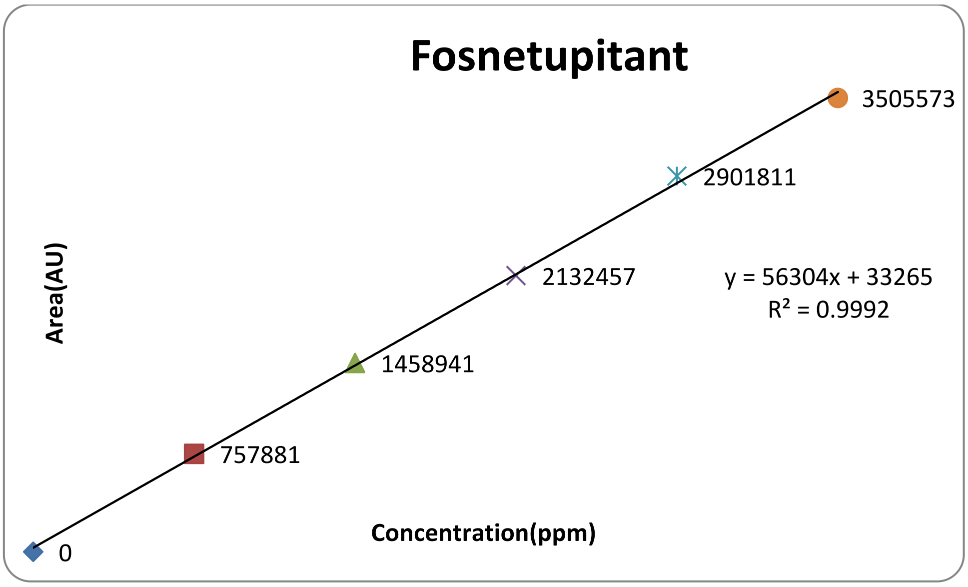


Fig 4: calibration graph for Fosnetupitant

Repeatability

Table 6: Results of repeatability for Palonosetron

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Palonosetron	2.108	602223	128898	2586	1.6
2	Palonosetron	2.105	607748	129233	2947	1.4
3	Palonosetron	2.113	607302	127409	2468	1.6
4	Palonosetron	2.109	608674	127047	2146	1.9
5	Palonosetron	2.109	607376	129859	2307	1.7
Mean			606665			
Std. Dev			2542.3			
% RSD			0.42			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 7: Results of method precession for Fosnetupitant

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Fosnetupitant	3.552	2220333	2231111	1.6	2371
2	Fosnetupitant	3.550	2221573	2674210	1.6	2841
3	Fosnetupitant	3.564	2215483	2231261	1.5	2816
4	Fosnetupitant	3.564	2217379	2421301	1.5	2872
5	Fosnetupitant	3.565	2211255	2324710	1.6	2845
Mean			2217205		1.6	2841
Std. Dev			4100.8			
% RSD			0.18			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 8: Results of Intermediate precision Day 1 for Palonosetron

S.no	**Name**	**Rt**	**Area**	**Height**	**USP plate count**	**USP Tailing**
1	Palonosetron	2.108	596608	128898	2547	1.6
2	Palonosetron	2.105	598959	129233	2944	1.4
3	Palonosetron	2.113	595728	127409	2361	1.6
4	Palonosetron	2.109	594485	127047	2546	1.9
5	Palonosetron	2.109	595267	129859	2207	1.7
6	Palonosetron	2.102	596608	124691	2481	1.6
Mean			596209			
Std. Dev			1718.7			
% RSD			0.29			

- *%RSD of Six different sample solutions should not more than 2*

Table 9: Results of Intermediate precision Day 1 for Fosnetupitant

Sno	**Name**	**Rt**	**Area**	**Height**	**USP plate count**	**USP Tailing**	**USP Resolution**
1	Fosnetupitant	3.552	2207732	2231134	8371	1.5	2.04
2	Fosnetupitant	3.550	2202266	2674210	6841	1.6	2.03
3	Fosnetupitant	3.564	2209375	2247461	7816	1.6	2.01
4	Fosnetupitant	3.564	2204037	2454301	8872	1.6	2.05
5	Fosnetupitant	3.565	2204466	2324710	4845	1.6	2.02
6	Fosnetupitant	3.537	2209574	2531247	8371	1.6	2.03
Mean			2205575				
Std. Dev			2899.8				
% RSD			0.13				

- *%RSD of Six different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 10: Results of Intermediate precision Day 2 for Palonosetron

Sno	**Name**	**Rt**	**Area**	**Height**	**USP plate count**	**USP Tailing**
1	Palonosetron	2.102	602155	127998	5586	1.5
2	Palonosetron	2.105	603662	134844	5636	1.6
3	Palonosetron	2.112	603931	161103	5432	1.6
4	Palonosetron	2.113	607302	127409	5468	1.6
5	Palonosetron	2.109	608674	127047	5146	1.9
6	Palonosetron	2.109	607376	129859	5307	1.7
Mean			605516.7			
Std. Dev			2602.622			
% RSD			0.42			

- %RSD of Six different sample solutions should not more than 2

Table 11: Results of Intermediate precision Day 2 for Fosnetupitant

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Fosnetupitant	3.537	2241579	2263528	2371	1.6	7.98
2	Fosnetupitant	3.552	2236409	2224418	2414	1.6	6.4
3	Fosnetupitant	3.560	2239093	2233725	2384	1.6	8.9
4	Fosnetupitant	3.564	2215483	2231261	2816	1.5	8.3
5	Fosnetupitant	3.564	2217379	2421301	2872	1.5	7.5
6	Fosnetupitant	3.565	2211255	2324710	2845	1.6	5.3
Mean			2226866				
Std. Dev			13567.02				
% RSD			0.60				

- *%RSD of Six different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 12: The accuracy results for Palonosetron

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	287774	7.5	7.56	100.8	99.6%
100%	551495	15	14.8	98.6	
150%	825175	22.5	22.4	99.5	

Table 13: The accuracy results for Fosnetupitant

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1104782	18.75	18.73	100%	100%
100%	2105321	37.5	37.4	99.9%	
150%	3211306	56.25	56.21	100%	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Palonosetron

Table 14: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	607323	2.102	5586	1.7
Less Flow rate of 0.9 mL/min	674735	2.330	5231	1.7
More Flow rate of 1.1 mL/min	1408920	1.950	5234	1.7
Less organic phase	606093	2.290	5643	1.4
More organic phase	603559	1.998	5298	1.5

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Fosnetupitant

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	558777	3.537	5371	1.6
Less Flow rate of 0.9 mL/min	2505636	3.885	5324	1.7
More Flow rate of 1.1 mL/min	1408920	3.263	5098	1.7
Less organic phase	2239255	4.435	5239	1.2
More organic phase	2300346	3.009	5647	1.0

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of palonosetron and fosnetupitant in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

palonosetron and fosnetupitant was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of palonosetron and fosnetupitant in bulk drug and in Pharmaceutical dosage forms.

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