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R E S E A R C H A R T I C L E

Method Development, Validation and Stability Studies of Nutipotant and Palanosetran in Pharmaceutical Dosage Form by RP-HPLC

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A B S T R A C T

A new method was established for simultaneous estimation of Nutipatant and Palonosetran by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Olmesertan medoxomil and Chlorthalidone by using YMC 4.6*150mm 5µ, flow rate was 1.0ml/min, mobile phase ratio was 70% buffer and 30% methanol , detection wave length was 210nm. The instrument used was SHIMADZU HPLC Auto Sampler, Separation module 2695, UV Detector 2998, Empower-software version-2. The average retention times for Nutipatant and Palonosetran was found to be 2.2 and 3.0 min, respectively. The estimation of Nutipatant and Palanosetran was done by RP-HPLC. The assay of Nutipatant and Palanosetran was performed with tablets and the % assay was found to be 100.08 and 100.04 which shows that the method is useful for routine analysis. The linearity of Nutipatant and Palanosetran was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.3 for Nutipatant and Palanosetran which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.4 for Nutipatant and Palanosetran which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.43% and 100.50% for Nutipatant and Palanosetran. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Nutipatant was found to be 3.02 and 9.98 and LOD and LOQ for Palanosetran was found to be 3.00 and 10.00.The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Keywords: Nutipatant, Palonosetran, LOD and LOQ, HPLC

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C O N T E N T S

1. Introduction

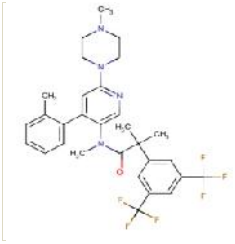

Figure 1: Structure of Nutipatant

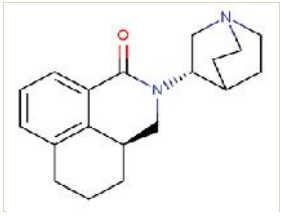

Figure 2: Structure of Palonosetran

Analytical methods

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial). products are developed to reduce the cost and time for better precision and ruggedness[1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available[2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials[3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture[4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements[5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made[6].

Chromatography

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC)..in this system pressure is applied to the column, forcing the mobile phase through at much higher rate[7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution[8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC[9].

2. Materials and Methods

Apparatus:

The instrument used for the study was WATERS, software: Empower, 2695 separation module,UV detector

Reagents and Materials:

The solvents used were Potassium dihydrogen phosphate, Methanol, Orthophosphoric acid, Acetonitril and Water[10].

Selection of detection wavelength:

UV spectrum of 10 µg/ml Nutipatant and Polanosartan indiluent.s (mobile phase composition). was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 210nm. At this wavelength both the drugs show good absorbance.

Preparation of Phosphate buffer:

Accurately weighed 3.4g of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 withNaoH

preparation of mobile phase:

Accurately measured 700 ml (70%). of above buffer and 300 ml of Methanol HPLC (30%). were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration[11].

Diluent. Preparation:

The Mobile phase was used as the diluent.

Optimization Chromatographic trials for Simultaneous Estimation of Nutipatant and Palonosetran by RP-HPLC.

Optimization chromatographic conditions

Instrument used	: Waters HPLC with auto sampler and UV or detector
Temperature	: Ambient
Column	: YMC 4.6*150mm 5µ
Buffer	: 3.4g of KH2PO4 is taken in 1000 ml water pH adjusted with NaoH
pH	: 3.0
Mobile phase	: 70% buffer and 30% methanol
Flow rate	: 1.0 ml/min
Wavelength	: 210 nm
Injection volume	: 20 µl
Run time	: 8 min

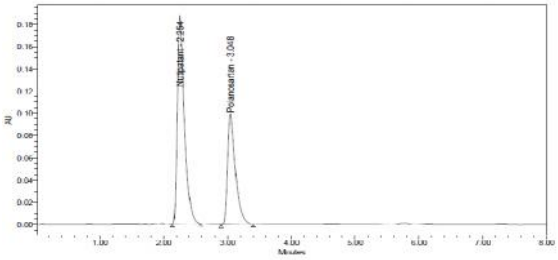

Figure 3: Optimization Chromatogram

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

3. Results and Discussion

1. Assay

Standard Solution Preparation:

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Nutipatant and Polanosartan peaks and calculate the %Assay by using the formulae.

2. Linearity

Preparation of stock solution:

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Preparation of Level – I

0.1 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II

0.2 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III

0.3 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV

0.4 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – V

0.5 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area). and calculate the correlation coefficient

3. Precision

Preparation of stock solution:

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.s[12].

Procedure:

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

4. Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness). of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits[13].

5. Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 150 mg of Nutipatant and 0.25 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 450 mg of Nutipatant and 0.75 mg of Polanosartan

working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.s[14].

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Nutipatant& Polanosartanand calculate the individual recovery and mean recovery values.

6.Limit of Detection: (forNutipatant)

Preparation of 900µg/ml solution:

Accurately weigh and transfer 300 mg of Nutipatant working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 3.200 µg/ml solution:

Accurately weigh and transfer 300 mg of Nutipatant working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent..Further pipette 8.27ml of the above stock solutions into a 0ml volumetric flask and dilute up to the mark with diluent.s. Further pipette 2ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.s. Further pipette 0.43 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Detection: (for Polanosartan)

Preparation of 1.5 µg/ml solution:

Accurately weigh and transfer 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent[15](Stock solution). Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.084 µg/ml solution:

Accurately weigh and transfer 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 10 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 1.65 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 3.38ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

7. Limit of Quantification

Preparation of 900 µg/ml solution:

Accurately weigh and transfer 300 mg of Nutipatant working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 10.575 µg/ml solution:

Accurately weigh and transfer 300 mg of Nutipatant working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.Further pipette 10 ml of the above stock solutions into a 20ml volumetric flask and dilute up to the mark with diluents. Further pipette 5 ml of the above stock solutions into a 20ml volumetric flask and dilute up to the mark with diluents. Further pipette 0.47ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Quantification

Preparation of 1.5µg/ml solution:

Accurately weigh and transfer 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent[17]. (Stock solution).

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.282 µg/ml solution:

Accurately weigh and transfer 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.Further pipette 10 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.Further pipette 10 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. Further pipette 5.62 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure for LOD and LOQ:

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

8.Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 1.1 ml/min to 0.9ml/min.

Standard solution 900ppm of Nutipatant& 1.5 ppm of Polanosartan was prepared and analyzed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate

affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The Organic composition in the Mobile phase was varied from ±10%.Standard solution 900 ppm of Nutipatant & 1.5 ppm of Polanosartan was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method. On evaluation of the above results, it can be concluded that the variation in 10%.Organic composition in the mobile phase affected the method significantly. Hence it Indicates that the method is robust even by change in the Mobile phase ±10

Degradation Studies

The International Conference on Harmonization (ICH). guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Nutipatant and Polanosartan using the proposed method.

Preparation of stock:

Accurately weigh and transfer 300 mg of Nutipatant and 0.5 mg of Polanosartan working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent. and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Hydrolytic degradation under acidic condition

Pipette 0.3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 0.3ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal induced degradation

Nutipatant and Polanosartane sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed[18].

Oxidative degradation

Pipette 0.3ml above stock solution into a 10ml volumetric flask and 1ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation:

Pipette 0.3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Method Validation Parameters

Linearity:

The linearity study was performed for the concentration of 300 ppm to 1500ppm for Nutipatant and 0.5ppm to 2.5ppm for Palonosetran and chromatograms are shown below.

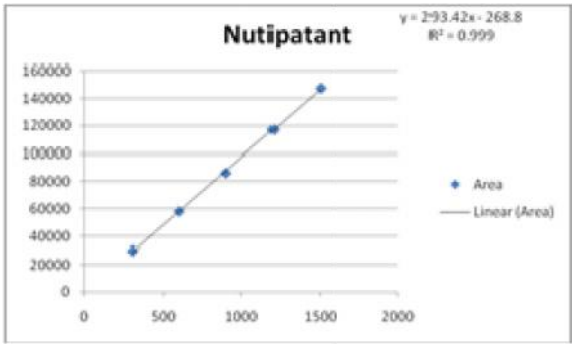

Figure 4: Calibration graph of Nutipatant

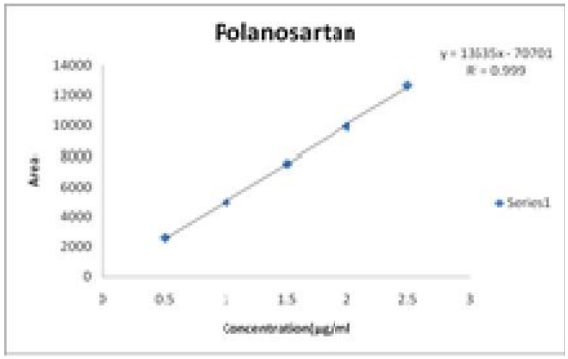

Figure 5: Calibration graph of Palonosetran

4. Conclusion

The estimation of Nutipatant and Palanosetran was done by RP-HPLC. The assay of Nutipatant and Palanosetran was performed with tablets and the % assay was found to be 100.08 and 100.04 which shows that the method is useful for routine analysis. The linearity of Nutipatant and Palanosetran was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.3 for Nutipatant and Palanosetran which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.4 for Nutipatant and Palanosetran which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.43% and 100.50% for Nutipatant and Palanosetran. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Nutipatant was found to be 3.02 and 9.98 and LOD and LOQ for Palanosetran was found to be 3.00 and 10.00. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Table 1: Linearity concentration of Nutipatant and Polanosartan

S. No	Nutipatant		Polanosartan	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	300	30018	0.5	2613
2	600	58216	1	4969
3	900	86174	1.5	7547
4	1200	117088	2	9909
5	1500	147293	2.5	12640
Correlation coeficient			0.999	

Table 2: Accuracy results for Nutipatant and Polanosartan

Drug	%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
Nutipatant	50%	43148.6	10	10.01	100.08	100.43%
	100%	86625.0	20	20.09	100.46	
	150%	130313.3	30	30.23	100.75	
Polanosartan	50%	3818.7	5	5.04	100.75	100.50%
	100%	7587	10	10.01	100.08	
	150%	11447	15	15.10	100.67	

Table 3: precision results for Nutipatant and Polanosartan

	Nutipatant		Polanosartan	
S.no	**Intra day**	**Inter day**	**Intra day**	**Inter day**
Injection-1	87799	86017	7524	7508
Injection-2	86973	86172	7519	7587
Injection-3	86232	86652	7524	7576
Injection-4	87604	86680	7581	7534
Injection-5	85975	86818	7558	7558
Injection-6	87018	86585	7565	7517
Average	86933.8	86933.8	7545.2	7546.7
Standard Deviation	723.5	723.5	26.2	32.1
%RSD	0.8	0.8	0.3	0.4

Table 4: Robustness results forNutipatant and Polanosartan

Robust condition		System suitability results				
		Nutipatant		Polanosartan		USP Resolution
		USP Plate count	USP tailing	USP Plate count	USP tailing	
Change in flow rate	0.9	3962	1.17	3110	1.13	3.60
	1	3914.29	1.17	3017.92	1.13	3.69
	1.1	3199.71	1.14	2675.77	1.12	2.66
Change mobile phase composition	10% less	3591	1.42	2410	1.34	4.01
	*Actual	3914.29	1.17	3017.92	1.13	3.69
	10% more	3340.78	1.17	3341.82	1.18	2.17

Table 5: Limit of Detection for Nutipatant and Polanosartan

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Nutipatant	58	175	3.02
Polanosartan	58	174	3.00

Table 6: Limit of Quantification for Nutipatant and Polanosartan

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Nutipatant	58	579	9.98
Polanosartan	58	580	10.00

Table 7: Results for Degradation studies

Sample Name	Nutipatant		Polanosartane	
	Area	**% Degraded**	**Area**	**% Degraded**
Standard	86056.0		7565.7	
Acid	81872	4.86	7239	4.32
Base	81285	5.54	7298	3.54
Peroxide	82049	4.66	7267	3.95
Thermal	82411	4.24	7245	4.24
Photo	82185	4.50	7264	3.99

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