A photo stability indicating HPLC technique for validation of Netupitant and Palonosetron in bulk and formulations

Patta Salomi^{*1}, B. Sireesha¹, K. Ravindra Reddy², S. AfreenSultana¹

¹Department of Pharmaceutical Analysis, P. Rami Reddy Memorial College of Pharmacy, Kadapa. Andhra Pradesh, India.

²Department. of Pharmaceutics, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

ABSTRACT

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Netupitant Delayed emesis has been largely associated with the activation of tachykinin family neurokinin 1 receptors. Palonosetron is a selective serotonin 5-HT₃ receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT₃ receptors present both centrally and peripherally in turn inhibits the visceral afferent stimulation of the vomiting center. The mobile phase used was orthophosphoric and acetate 70% buffer pH 3 and 30% methanol. The assay of Netupitant and Palonosetron was performed with tablets and the % assay was found to be 100.08 and 100.04, The linearity was found to be linear with a correlation coefficient of 0.999, the precision 0.8 and 0.3 for Netupitant and Palonosetron which shows that the method is precise. 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 LOD and LOQ for Netupitant were found to be 3.02 and 9.98 and LOD and LOQ for Palonosetron was found to be 3.00 and 10.00. Thus, it shows that the method is stability indicating, sensitive, accurate, robust and precise. Hence, the developed HPLC method can be successfully applied to the pharmaceutical dosage form and can be used for routine analysis.

Keywords: Netupitant, Tachykinin, Palonosetron, Neurokinin, 5-HT₃ receptor, HPLC.

ISSN: 2582-1970

Research Article

Corresponding Author

Name: Patta Salomi

Email: rayofhope01@gmail.com

Article Info

Received on: 10-08-2020

Revised on: 21-08-2020

Accepted on: 30-08-2020

DOI: <https://doi.org/10.33974/ijrpca.v1i4.220>

Copyright© 2020, Patta Salomi, et al. A Photo Stability Indicating HPLC technique for Validation of Netupitant and Palonosetron in Bulk and Formulations, Production and hosting by Rubatosis Publications.

the activation of tachykinin family neurokinin 1 (NK1) receptors (broadly distributed in the central and peripheral nervous systems) by substance. As shown in vitro and in vivo studies, netupitant inhibits substance P mediated responses [3]. Palonosetron acts as Antiemetic and anti nauseants. Palonosetron is a selective serotonin 5-HT₃ receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT₃ receptors present both centrally and peripherally (GI tract).

Figure 1: Structure of Netupitant

Figure 2: Structure of Glecaprevir

INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials [1]. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity [2]. Netupitant is a neurokinin 1 receptor antagonist. Delayed emesis (vomiting) has been largely associated with

It has been hypothesized that palonosetron's potency and long plasma half-life may contribute to its observed efficacy in preventing delayed nausea and vomiting caused by moderately emetogenic cancer chemotherapy [4]. Literature reveals different methods for their analysis in the following formulations [5-8]. But our present plan is to develop a new, simple, precise & accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validate it according to ICH guidelines [9] and to study photo stability studies according to ICH Q1B guidelines [10].

MATERIALS AND METHODS

The materials used were HPLC, UV/VIS Spectrophotometer, pH meter, weighing machine, Pipettes, Burettes and Beakers. Netupitant, Palonosetron KH_2PO_4 Water and Methanol for HPLC Acetonitrile for HPLC Ortho phosphoric Acid. The Instruments and chemicals used were of HPLC grade.

Wave length selection

UV spectrum of 10 µg/ml Netupitant and Palonosetron in diluents (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.

Figure 3: Scanned Spectrum Curve

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.

Preparation of Standard Solution

Accurately weigh and transfer 300 mg of Netupitant and 0.5 mg of Palonosetron 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.

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 Sample Solution

Accurately weigh and transfer 300 mg of Netupitant and 0.5 mg of Palonosetron 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.

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

Optimized Method

The column used was YMC 4.6×150mm5µ, Mobile phase consists of 70% buffer pH 3 and 30% methanol, Flow rate was 1.0 ml per min, Wavelength detected at 210 nm, Injected volume was 20 µl and Runtime noted as 8min.

Figure 4: Chromatogram of optimized method

Validation of Developed Method

Assay: Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for Netupitant and Palonosetron peaks and calculate the % Assay by using the formulae.

$$\text{Assay}\% = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Table 1: Results of Assay for Netupitant and Palonosetron

Drug name	Label Claim(mg)	% Assay
Netupitant	300	100.08
Palonosetron	0.5	100.04

Linearity

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. Calibration graphs were shown in (Figure 5, Figure 6) and results are shown in (Table 2).

Precision

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. The precision results are represented in (Table 3).

Intermediate Precision (Ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation. The results are represented in (Table 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.

Calculate the Amount found and Amount added for Netupitant & Palonosetron and calculate the individual recovery and mean recovery values. % recovery was calculated and represented in (Table 6, Table 7).

Limit of Detection for Netupitant and Palonosetron

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.

Chromatograms were shown in the (Figure 6) and Results are shown in the (Table 8). The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

LOD: It is calculated by using following formula $\frac{3.3}{SD} \times 100$.

Limit of Quantification for Netupitant and Palonosetron

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. The chromatograms were recorded as show in (Figure 7) and results are shown in (Table 9).

LOQ: It is calculated by using following formula $\frac{10}{SD} \times 100$

Robustness

The deliberate changes were done in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 1.1 ml/min to 0.9ml/min. The Organic composition in the Mobile phase was varied from $\pm 10\%$. The results are shown in (Table 10, Table 11, Table 12, Table 13).

The standard and samples of Netupitant and Palonosetron were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailingfactor, asymmetricfactor and plate count.

Results for actual Mobile phase composition have been considered from Accuracy standard. The Retention time, USP plate count, USP tailing factor obtained

for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

Degradation Studies

The International Conference on Harmonization ICH Q1B 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 result of degradation studies is shown in (Table 14).

Preparation of stock

Accurately weigh and transfer 300 mg of Netupitant and 0.5 mg of Palonosetron 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 60°C 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 60°C 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

Netupitant and Palonosetron sample was taken in petridish and kept in Hot air oven at 1100 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

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.

RESULTS AND DISCUSSIONS

The estimation of Netupitant and Palonosetron was done by RP-HPLC. The assay of Netupitant and Palonosetron was performed with tablets and the % assay was found to be 100.08 and 100.04. The linearity of Netupitant and Palonosetron was found to be linear with a correlation coefficient of 0.999, the method show precision 0.8 and 0.3 for Netupitant and Palonosetron which shows that the method is precise.

Table 2: Linearity for Netupitant and Palonosetron

S. No	Linearity Level	Concentration	Area	Concentration	Area
1	I	300	30018	0.5	2613
2	II	600	58216	1	4969
3	III	900	86174	1.5	7547
4	IV	1200	117088	2	9909
5	V	1500	147293	2.5	12640
Correlation Co-efficient		0.999		0.999

Figure 5: Calibration graph Netupitant

Figure 6: Calibration graph Palonosetron

Table 3: Precision results for Netupitant and Palonosetron

Injection	Area for Netupitant	Area for Palonosetron
Injection-1	87799	7524
Injection-2	86973	7519
Injection-3	86232	7524
Injection-4	87604	7581
Injection-5	85975	7558
Injection-6	87018	7565
Average	86933.8	7545.2

Standard Deviation	723.5	26.2
%RSD	0.8	0.3

Table 4: ID Precision results for Netupitant and Palonosetron

Injection	Area for Netupitant	Area for Palonosetron
Injection-1	86017	7508
Injection-2	86172	7587
Injection-3	86652	7576
Injection-4	86680	7534
Injection-5	86818	7558
Injection-6	86585	7517
Average	86933.8	7546.7
Standard Deviation	723.5	32.1
%RSD	0.8	0.4

The intermediate precision is 0.8 and 0.4 for Netupitant and Palonosetron which shows that the method is repeatable when performed in different days also. The total recovery was found to be 100.43% and 100.50% for Netupitant and Palonosetron. 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.

Table 5: Accuracy (recovery) data for Netupitant and Palonosetron

% Conc	Area	Amount Added (mg)	Amount Found (mg)	% Recovery
50%	43148.6	10	10.01	
100%	86625.0	20	20.09	100.43
150%	130313.3	30	30.23	
50%	3818.7	5	5.04	
100%	7587	10	10.01	100.50
150%	11447	15	15.10	

The LOD and LOQ for Netupitant were found to be 3.02 and 9.98 and LOD and LOQ for Palonosetron was found to be 3.00 and 10.00.

Figure 7: Chromatogram of Netupitant, Palonosetron showing LOD

Table 6: Flow variations for Netupitant and Palonosetron

S. No	Flow Rate (ml/min)	Netupitant		Palonosetron	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.9	3962	1.17	3110	1.13
2	1	3914.29	1.17	3017.92	1.13
3	1.1	3199.71	1.14	2675.77	1.12

Table 7: Organic phase variation for Netupitant and Palonosetron

S. No	Change in Organic Composition in the Mobile Phase	Netupitant		Palonosetron	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	10% less	3591	1.42	2410	1.34
2	*Actual	3914.29	1.17	3017.92	1.13
3	10% more	3340.78	1.17	3341.82	1.18

Table 8: Results of LOD and LOQ

	Drug name	Baseline noise(μV)	Signal obtained (μV)	S/N ratio
LOD	Netupitant	58	175	3.02
	Palonosetron	58	174	3.00
	Drug name	Baseline noise(μV)	Signal obtained (μV)	S/N ratio
LOQ	Netupitant	58	579	9.98
	Palonosetron	58	580	10.00

Table 9: Degradation results for Netupitant and Palonosetron

Sample Name	Netupitant		Palonosetron	
	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

Table 10: Flow variations for Netupitant

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3962	1.17
2	1	3914.29	1.17
3	1.1	3199.71	1.14

Table 11: Flow variations for Palonosetron

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3110	1.13
2	1	3017.92	1.13
3	1.1	2675.77	1.12

Table 12: Organic phase variation for Netupitant

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3591	1.42
2	*Actual	3914.29	1.17
3	10% more	3340.78	1.17

Table 13: Organic phase variation for Palonosetron

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2410	1.34
2	*Actual	3017.92	1.13
3	10% more	3341.82	1.18

Figure 8: Chromatogram of Netupitant, Palonosetron showing LOQ

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 14: Degradation results

Sample Name	Netupitant		Palonosetron	
	Area	% De-graded	Area	% De-graded
Standard	161608.8		747503	
Acid	153252	5.17	719259	3.78
Base	153532	5.00	719321	3.77
Peroxide	152239	5.80	704978	5.69
Thermal	157552	2.51	714851	4.37
Photo	156452	3.19	714789	4.38

CONCLUSION

The developed HPLC method and validation was done according to ICH guidelines and photo stability studies were carried out according to ICH Q1B guidelines. Thus, it shows that the method is stability indicating, sensitive, accurate, robust and precise. Hence, the method can be successfully applied to the pharmaceutical dosage form and can be used for routine analysis.

ACKNOWLEDGEMENT

I am thankful to P.Rami Reddy Memorial College of pharmacy to provide facilities to carry out Research work.

REFERENCES

1. Douglas A.Skoog, F. James Holler & Stanley R. Crouch. Instrumental analysis, India edition, 2007, pg: 13-14.
2. Ahuja S & Dong MW. Handbook of Pharmaceutical Analysis by HPLC. 1st edition, Academic Press Publisher.UK 2005.
3. Www.Drugs.Com
4. Www.Drugbank.Com
5. Panigrahy, U. P., & Reddy, A. S. K. (2015). A novel validated RP-HPLC-DAD method for the simultaneous estimation of Netupitant and Palonosetron in bulk and pharmaceutical dosage form with forced degradation studies. International Journal of ChemTech Research, 8(10), 317-337.
6. Inturi, S., Inturi, R. K., & Venkatesh, G. (2011). A Validated Novel RP-HPLC method development for the estimation of Palonosetron Hydrochloride in bulk and softule dosage forms. Der Pharmacia Sinica, 2(5), 223-234.
7. Mangesh Harole, R. N. Patil and Deepak Gaware, (2016).A Validated Stability Indicating RP-HPLC Method For Simultaneous Determination Of Netupitant and Palonosetron In Pharmaceutical Formulations, World Journal Of Pharmacy And Pharmaceutical Sciences, 5(3),878-887.
8. Bhagavanji, N. V. M. S., Satyanarayana, P. V. V., Sekhar, K., & Naniprasad, D. (2016). Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Netupitant and Palonosetron in Combined Tablet Dosage Form. Int J Pharm Sci Rev Res, 41(1), 81-87.
9. ICH, Harmonized Tripartite Guideline, Validation of Analytical procedure: Text and Methodology, Geneva, Switzerland. 2006.
10. ICH, (Q1B), Harmonized Tripartite Guideline, Stability testing: Photo stability testing of New Drug Substances and Products, in: Proceeding of the International Conference on Harmonization, Geneva. 1996.