EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 5
300-304
Year: 2019

A NOVEL DEVELOPMENT OF RP-HPLC METHOD FOR THE ESTIMATION OF NETUPITANT AND PALONOSETRON IN BULK AND THEIR COMBINED DOSAGE FORM

Shaik Mubeena* and Pavan Kumar K.

Dept. of Pharmaceutical analysis & Quality Assurance, Shadan College of Pharmacy, Perancheruvu, R.R Dist., Telangana. Pin-508284.

***Corresponding Author: Shaik Mubeena**

Dept. of Pharmaceutical analysis & Quality Assurance, Shadan College of Pharmacy, Perancheruvu, R.R Dist., Telangana. Pin-508284.

Article Received on 25/02/2019 **Article Revised on 16/03/2019** **Article Accepted on 08/04/2019**

ABSTRACT

A novel RP-HPLC method has been developed for simultaneous quantitative analysis of Netupitant and Palonosetron in fixed dose pharmaceutical formulations. Waters HPLC with auto sampler and DAD or UV detector was used for HPLC method development and validation of the samples. The technique was developed using a Waters X Terra RP C-18 (4.6mm x 150mm, 5µm) column as a stationary phase. Phosphate Buffer: Methanol (60:40 v/v) **was** used as the mobile phase. The flow rate of the mobile phase was maintained at 0.8 mL/min and the eluted Netupitant and Palonosetron were monitored at 220nm. 20 µL injection volumes were set for both standards and samples. The developed method is more economical and suitable for routine laboratory analysis for the determination of Netupitant and Palonosetron in pharmaceutical formulations.

KEYWORDS: RP-HPLC; Netupitant; Palonosetron; HPLC method; ICH guidelines; Method validation.

1. INTRODUCTION

Description: AKYNZEO (300 mg netupitant/0.5 mg palonosetron) capsules are an oral combination product of netupitant, a substance P/neurokinin 1 (NK-1) receptor antagonist and palonosetron hydrochloride, a serotonin-3 (5-HT3) receptor antagonist. Netupitant is chemically described: 2-[3,5-bis(trifluoromethyl)phenyl]-N, 2 dimethyl-N-[4-(2methylphenyl)-6-(4-methylpiperazin-1-yl)pyridin-3-yl] propanamide. The empirical formula is $C_{30}H_{32}F_6N_4O$, with a molecular weight of 578.61. Netupitant exists as a single isomer. Netupitant is white to off-white crystalline powder. It is freely soluble in toluene and acetone, soluble in isopropanol and ethanol and very slightly soluble in water. Palonosetron hydrochloride is chemically described as (3aS)-2-[(S)-1-Azabicyclo[2.2.2]oct-3-yl]2,3,3a,4,5,6-hexahydro-1-oxo-1H-benz[de]isoquinoline.The empirical formula is $C_{19}H_{24}N_2O.HCl$, with a molecular weight of 332.87. Palonosetron hydrochloride exists as a single isomer. Palonosetron is a white to off-white crystalline powder. It is freely soluble in water, soluble in propylene glycol and slightly soluble in ethanol and 2-propanol.

This combination is used to prevent acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy. Very few methods reported till now for this simultaneous estimation, hence authors proposed this cost effective method with shorter run time.

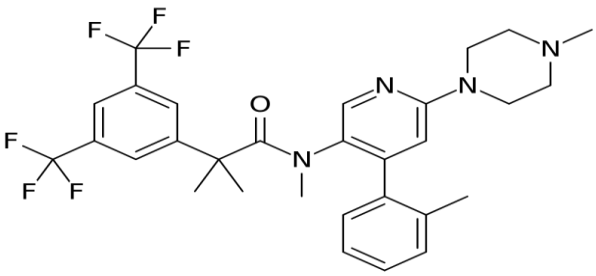

Fig. 1: Structure of Netupitant.

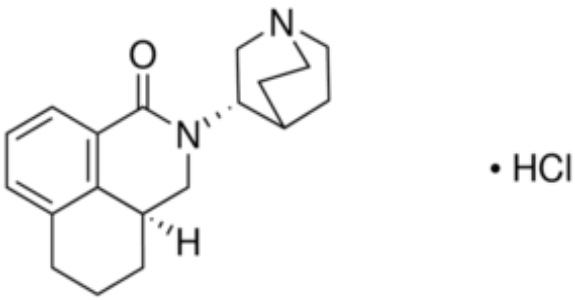

Fig. 2: Structure of Palonosetron Hcl.

2. MATERIAL AND METHODS

Table. 1. Chemicals, reagents.

	Chemicals/Reagents	Manufacturer
1.	Netupitant	Apicore Pharmaceuticals Pvt Ltd
2.	Palonosetron	KPS Chemicals & Pharmaceuticals Pvt Ltd
3.	KH2PO4	Finer chemical ltd.
4.	Methanol for HPLC	Lichrosolv (MERCK)
5.	Water for HPLC	Lichrosolv (MERCK)
6.	HCl / NaOH / H3PO4	MERCK

Table. 2: Instruments Used.

S. No	Instrument	Model
1.	HPLC	Waters 515pump, Detector 2487, WATERS
2.	UV/VIS spectrophotometer	UV Analyst, UV- 2310, TECHCOMP
3.	pH meter	Adwa – AD 1020, ADWA
4.	Weighing machine	Afcoset ER-200A, SHIMADZU
5.	Sonicator	SE60US, ENERTECH

2.2. Chromatographic conditions: Waters HPLC with auto sampler and DAD or UV detector was used for HPLC method development and validation of the samples. The technique was developed using a Waters X Terra RP C-18 (4.6mm x 150mm, 5µm) column as a stationary phase. Phosphate Buffer: Methanol(60:40 v/v) was used as the mobile phase. The flow rate of the mobile phase was maintained at 0.8 mL/min and the eluted Netupitant and Palonosetron were monitored at 220nm. 20 µL injection volumes were set for both standards and samples.

2.3: Preparation of Phosphate buffer: Accurately weighed 7.0 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC grade water and the volume was adjusted to pH 3 with Orthophosphoric acid.

2.4. Preparation of mobile phase: Accurately measured 600 ml (60%) of above buffer and 400 ml of Methanol HPLC grade (40%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

2.5. Diluent Preparation: The mobile phase was used as the Diluent.

2.6 Preparation of standard solutions: Accurately weighed and transferred 10 mg of Netupitant and 6.25 mg of Palonosetron working standard into a 100 mL clean dry volumetric flask and added about 70 ml of Diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent.

Stock solution: From this, 3 ml of the solution was pipetted into another 10 ml volumetric flask and diluted up to the mark with diluent (30,18.75 µg/ml).

2.7 Preparation of sample solution: (ASSAY)
Accurately weighed and transferred Capsule content equivalent to 10 mg of Netupitant and 6.25 mg of Palonosetron into a 100 mL clean dry volumetric flask and added about 70 ml of diluent. It was sonicated to dissolve completely and made volume up to the mark with the same diluent.

Stock solution: From this, 3 ml of the solution was pipetted into another 10 ml volumetric flask and diluted up to the mark with diluent. 20 µL of standard and sample solutions of Netupitant and Palonosetron was injected for three times and the peak areas were recorded.The Assay results, mean and percentage relative standard deviation were calculated from the peak areas.

Formula

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim of drug mg/ml.

3. RESULTS AND DISCUSSION[1-6]

3.1. Method development: The present investigation is to develop a new method and validate the analytical method of Netupitant and Palonosetron in pharmaceutical dosage form by RP-HPLC.

UV spectrum of Netupitant and Palonosetron: UV spectrum of 10 µg/ml of Netupitant and Palonosetron in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. The maximum absorbance was observed at isobestic point 220 nm. Hence the λmax of 220 nm was selected for further HPLC analysis, in the present investigation

Table. 3: Assay results.

Injection	Netupitant			Palonosetron		
	Area	Plate count	Tailing factor	Area	Plate count	Tailing count
Injection 1	8158124	2132	1.1	3119272	4219	1.1
Injection 2	8157418	2116	1.0	3118564	4036	1.0
Injection 3	8146412	2015	1.2	3106732	4175	1.0
Average	8153984.66			3114856.0		
Standard deviation	6567.6151			7044.4906		
%RSD	0.08%			0.23%		

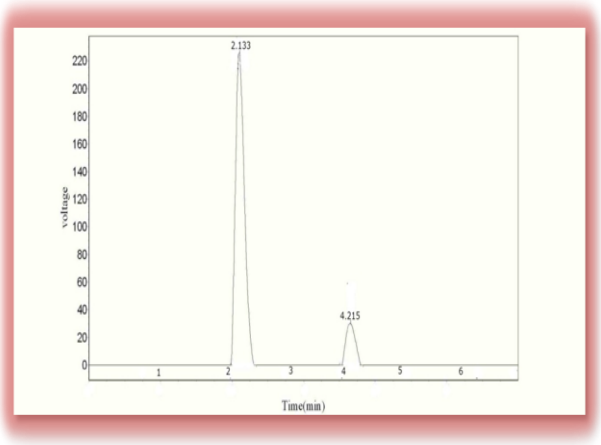

Fig. 3. Sample Chromatogram.

The developed method was validated as per the International Council for Harmonisation (ICH) guidelines[16-18]. The validation parameters namely, precision, accuracy, specificity, robustness, linearity, limit ofquantitation (LOQ) and limit of detection (LOD) are evaluated.

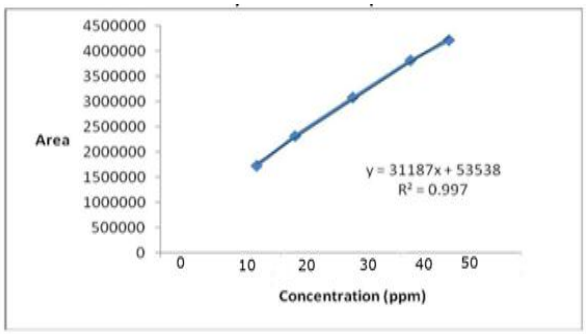

Figure. 4: Calibration curve of Netupitant.

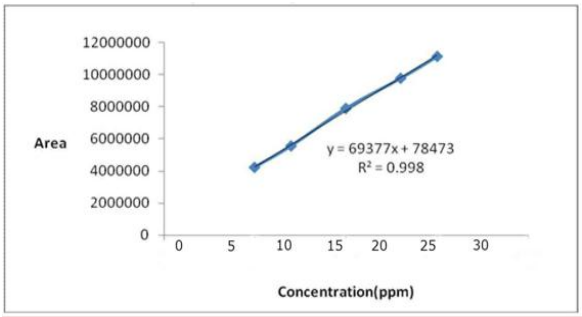

Figure. 5. Calibration curve of Palonosetron.

3.2. Validation [7-15]

3.2.1. Linearity

The linearity range was found to be 10-50 µg/ml for Netupitant and 5-35µg/ml for Palonosetron. Calibration curve was plotted and correlation coefficient for the drug was found to be 0.997 and 0.998 respectively. Hence, the results obtained were within the limits. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by producing serial dilutions. Accuracy was performed on three levels of 50%, 100% and 150%.

3.2.2. Accuracy: It is measured in drug products by spiking known amounts (50%, 100%, 150%) of the analyte into the excipients and calculating the percent recovered.QC samples were taken, intermediate to standard concentrations and area was calculated using the standard graph. Percentage deviation from the theoretical concentration was calculated. Their percentage of deviation are presented in Table 4 and Table 5.

Table 4: Accuracy observation of Palonosetron

% concentration (at specific level)	Area	Amount added (µg)	Amount found (µg)	% recovery	Mean recovery
50%	3092291.66	12.5	13	98.8	
100%	3144756.66	18.75	19.1	99.7	99.3
150%	3114856.0	25	24.8	99.5	

Table. 5: Accuracy observation of Netupitant.

% concentration (at specific level)	Area	Amount added (µg)	Amount found (µg)	% recovery	Mean recovery
50%	8152699.33	20	19.9	98.8	
100%	8147184.33	30	30.4	98.6	98.6
150%	8153348	40	40.7	98.4	

The accuracy studies were shown as % recovery for Netupitant and Palonosetron at 50%, 100% and 150%. The percentage of drug recovered should be between 98 – 102%. The results obtained for both the drugs were found to be within limits. Hence, the method was found to be accurate and also revealed that the excipients present in the formulation did not interfere with the proposed method.

3.2.3: Precision: The standard solution was injected five times and the area was measured for all five injections in HPLC. The %RSD for the areas of the five replicates was found to be within specified limits. For precision studies, 5 replicate injections of formulation (method precision) were injected and %RSD was determined for peak areas obtained. The acceptance limit should not be more than 2% and the results obtained for standard samples were found to be within the acceptance limits This indicates that the method has good repeatability.

Table. 6: System precision observations.

Injection	Peak Area	
	Netupitant	Palonosetron
Injection 1	8147176	3094502
Injection 2	8157419	3147124
Injection 3	8157420	3110638
Injection 4	8157418	3170344
Injection 5	8147421	3064371
Average	8153370.8	3117395.8
Standard deviation % RSD	5543.9029 0.07	42037.7839 1.35

3.2.4 Ruggedness System (Intermediate Precision)
For intermediate precision studies, 5 replicate formulation injections were injected. %RSD was determined for peak areas of sample of Netupitant and Palonosetron. The acceptance limit should not be more than 2% and the results obtained were found to be within acceptance limits i.e. 0.12 and 0.8%. This indicates that the method has good reproducibility (inter-day variation is within specified limits).

3.2.5 Limit of Detection: The LOD was calculated from slope and standard deviation of response and the limit was found to be 0.172 µg/ml for Netupitant and 0.524 µg/ml for Palonosetron. The above value indicates the minimum concentration at which the analyte is detected.

3.2.6 Limit of Quantification
The LOQ was calculated from slope and standard deviation of response and the limit was found to be 0.542 µg/ml for Netupitant and 1.64µg/ml for Palonosetron respectively. The above value indicates the minimum concentration at which the analyte is quantified with acceptable accuracy and precision.

Table. 7. Results of LOD & LOQ.

S. No.	Drug	LOD (µg/ml)	LOQ (µg/ml)
1.	**Netupitant**	**0.172**	**0.542**
2.	**Palonosetron**	**0.524**	**1.641**

3.2.7: Robustness
Robustness was performed with 10% organic variation in mobile phase composition and 0.2 mL/min flow rate. By changing the retention time and chromatographic conditions slightly, the number of theoretical plates and tailing factors were studied and robustness of the developed method was assessed. From the results, minor variations in chromatographic conditions had an insignificant effect on the chromatographic parameters. Hence, from the recovery studies, the developed method was very accurate and appropriate for intended use.

3.2.8 System Suitability
Table 8.Results of system suitability

Drug	Retention time (R_t)	Peak area	Resolution	Tailing factor	Plate count
NTPT	2.1	8156417	-	1.1	2216
PLSN	4.2	3114267	5.42	1.0	4302

4. CONCLUSION

The novel RP-HPLC method was developed for simultaneous quantitative analysis of Netupitant and Palonosetron in their combined dosage form. The present developed method was validated by testing its specificity, accuracy, precision, linearity, stability, limits of detection and limits of quantitation. The shorter runtime enables rapid determination of the Netupitant and Palonosetron. The method was found to be specific and shows an excellent performance in terms of speed and sensitivity. The developed method is more economical and suitable for routine laboratory analysis. Moreover, conventional reported HPLC methods may be replaced by the proposed HPLC method because of its superiority.

5. ACKNOWLEDGEMENT

Authors are thankful to EDPL and Shadan College of Pharmacy, Hyderabad, Telangana, India, authorities for providing valuable support to carry out this research work.

6. CONFLICTS OF INTERESTS

The authors declare that there is no conflict of interests regarding to the publication of the paper.

7. REFERENCES

1. Flamenbaum, W.; Friedman, R.Pharmacotherapy, 1982; 2: 213.
2. Halstenson, C.E.; Matzke, G.R.Drug IntellClin Pharm, 1983; 17: 786.
3. Greger, R.; Wangemann, P.Physiol, 1987; 10: 174–183.

4. Dzhala, V.I.;Talos, D.M.;Sdrulla, D.A.;Brumback, A.C.; Mathews, G.C.;Benke, T.A.;Delpire, E.; Jensen, F.E.; Staley, K.J.Nat Med, 2005; 11: 1205.
5. Dzhala, V.I.;Brumback, A.C.; Staley, K.J.Ann Neurol, 2008; 63: 222.
6. Patel, R.B.; Patel, A.A.; Patel, M.R.; Patel, S.K.; Manakiwala, S.C. Indian J Pharm Sci., 1987; 49(4): 142–43.
7. Nikolic, K.I.; Velasevic, K. J Pharm Belg, 1989; 44(6): 387–390.
8. Gradeen, C.Y.; Billay, D.M.; Chan, S.C. J Anal Toxicol, 1990, 14, 123–26.
9. Wells, T.G.; Hendry, I.R.; Kearns, G.L. J Chromatogr, 1991; 570: 235–42.
10. Dixon, R.; Young, R.L.; Holazo, A.; Jack, M.L.; Weinfeld, R.E.; Alexander, K.; Liebman, A.; Kaplan, S.A. J Pharm Sci., 1976; 65(5): 701–704.
11. Chao, A.C.; Armstrong, W.M. Am J Physiol, 1987; 253(2): C343–47.
12. Feit, P.W.; Roholt, K.; Sorensen, H. J Pharm Sci, 1973; 62(3): 375–83.
13. Lisi, A.M.; Trout, G.J.; Kazlauskas, R. J Chromatogr, 1992; B 581: 57–63.
14. Tsai, T.F.; Lee, M.R. Talanta, 2008; 75: 658–665.
15. Bökens, H. J Chromatogr, 1988; 434: 327–29.
16. International Conference on Harmonisation ICH – Q1B, Photo Stability Testing of New Drug Substances and Products, 1996.
17. International Conference on Harmonisation ICH – Q1A (R2), Stability Testing of New Drug Substances and Products, 2003.
18. International Conference on Harmonisation ICH – Q2 (R1), Guideline on Validation of Analytical Procedure: Text and Methodology, 2005.