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

ISSN 2349-8870
Volume: 6
Issue: 2
463-469
Year: 2019

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NETUPITANT AND PALONOSETRON IN PHARMACEUTICAL DOSAGE FORM

Eali Tejakshi[1], Yandamuri Narayudu[1]*, Tummapala Devi Prasanna[1], Valluri Aparna Kumari[1] and P. Abhinava Sri[1]

Departmenet of Pharmaceutical Analysis, Pydah College of Pharmacy, Kakinada, East Godavari District, Andhra Pradesh, India.

***Corresponding Author: Yandamuri Narayudu**
Departmenet of Pharmaceutical Analysis, Pydah College of Pharmacy, Kakinada, East Godavari District, Andhra Pradesh, India.

Article Received on 20/12/2018 **Article Revised on 10/01/2019** **Article Accepted on 30/01/2019**

ABSTRACT
High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Netupitant (NET) and Palonosetron (PAL) was done by RP-HPLC. The separation of NET and PAL was carried on an YMC Pack pro (150 mm x 4.6 mm, 5 µm particle size) c18 column using potassium dihydrogen phosphate: Methanol (70:30 v/v) as the mobile phase with PH (3.0) UV detection was carried out using wavelength 210 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of NET and PAL were found to be from 300-1500 µg/ml of NET and 0.5-2.5 µg/ml of PAL. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The total percentage recovery varies from 100.43% and 100.50% for NET and PAL. LOD and LOQ are found to be within the limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: YMC Pack pro (150 mm x 4.6 mm, 5 µm particle size) C18 column, NET, PAL, RP-HPLC.

INTRODUCTION
Netupitant (NET) is an antiemetic drug widely used in combination with Palonosetron (PAL) in the treatment of cancer chemotherapy including emetogenic chemotherapy. Chemically NET is 2-[3,5-bis(trifluoromethyl)phenyl]- N,2-dimethyl-N-[4-(2-methylphenyl)- 6-(4-methyl-1-piperazinyl)-3-pyridinyl] propanamide. It is a neurokinin receptor antagonist approved in October 2014 by FDA. It has a chemical formula of $C_{30}H_{32}F_6N_4O$ with average molecular weight of 578.603 gm/mol.[1] The molecular structure of the NET is showed in figure-1.

PAL is used to treat chemotherapy induced nausea and vomiting by antagonizing 5-HT_3 receptor both centrally and peripherally and it is also used in postoperative nausea and vomiting. The IUPAC name is (5s)-3-[(3s)-1-azabicyclo[2.2.2]octan-3-yl]-3-azatricyclo [7.3.1.0^{5,13}] trideca-1(12),9(13),10-trien-2-one it has a chemical formula of $C_{19}H_{24}N_2O$ with average weight of 296.414 gm/mol.[2] The molecular structure of PAL is showed in figure 1.

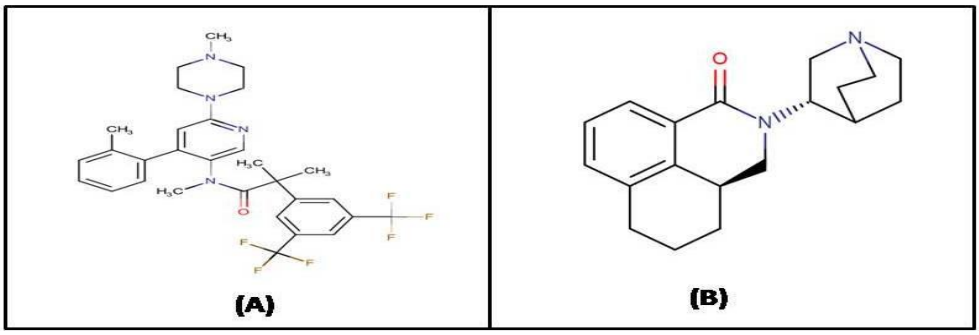

Fig. 1: Chemical Structure of (A) Netupitant (B) Palonosetron.

The literature review shows that the reported methods on NET and PAL in spectrophotometry, HPLC,[3-9] HPTLC,[10] in combination. There have been few methods for simultaneous estimation of NET and PAL in pharmaceutical dosage form. The objectives of this study were, therefore, to develop a simpler, economic, rapid, precise, and accurate RP-HPLC method with good sensitivity for quantitative analysis of NET and PAL in pharmaceutical dosage forms and to validate the method in accordance with International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Samples of NET and PAL were gifted by PHARMA TRAIN LABS, Hyderabad. Potassium dihydrogen phosphate was obtained from FINER chemical LTD and methanol for HPLC was from LICHROSOLV (Merck) all this solvents were HPCL grade.

Instrument and chromatographic condition

Waters HPLC 2695 separation module with Empower software and UV detector was used for this method. For separation of analytes YMC Pack pro (150 mm x 4.6 mm, 5 µm particle size) C18 column is used, Mobile phase consists of potassium di hydrogen phosphate with methanol in the ratio of 70:30v/v at 1.0 ml/min flow rate. It was degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration and the sample was analyzed at 210 nm with injection volume of 20 µl.

Preparation of potassium di hydrogen phosphate buffer

3.4g of KH_2PO_4 was taken into a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC grade water and buffer pH 3.0 was adjusted with sodium hydroxide.

Preparation of mobile phase

Accurately measured 700 ml (70%) of above buffer and 300 ml of Methanol HPLC grade (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 stock solutions

Preparation Standard mixture of NET & PAL (3000 µg/ml NET and 0.5 µg/ ml PAL)

Accurately weigh and transfer 300mg of NET and 0.5 mg of PAL working standard into 100 ml volumetric flasks add about 70ml diluent and sonicate to dissolve it completely and make up to volume with same solvent, 1ml of above stock solution has taken in 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample mixture of NET & PAL (3000 µg/ml NET and 0.5 µg/ml PAL)

Weigh and transfer 300mg and 0.5 mg of NET and PAL sample solution into 100 ml volumetric flask and ad 70ml of diluent and sonicate to dissolve it and make up to the volume with same solvent from this stock solution transfer 1ml into 10 ml volumetric flask and make up to the mark with diluent further dilutions are made by pipette out of 2, 3, 4, 5ml respectively in 10 ml volumetric flasks.

Method Validation

The word validation means assessment of validity or action of proving effectiveness. Method validation is carried out by following ICH guidelines and the parameters are specificity linearity, accuracy, precision, LOD and LOQ.

Linearity

The linearity range was found to be lie from 300 µg/ml to 1500 µg/ml and 0.5 µg/ml to 2.5 µg/ml for NET and PAL respectively and chromatograms are recorded and from this data plot a calibrated graph between peak area versus concentration this calibration plot shows a good linear relationship with high signified correlation coefficient.

Accuracy

It is performed by preparing three different concentrations i.e, (50%, 100%, 150%) and inject this three levels each for three times and calculated the amount found and amount added for NET and PAL and calculated percentage recovery.

Precision

For precision six replicates of 900 µg/ml and 1.5 µg/ml of NET and PAL respectively were prepared and this solutions were analyzed and from the data %RSD was calculated.

Limit of detection

The limit of detection is defined as the lowest concentration of an analyte in a sample that can be detected though not necessarily quantitated. The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Limit of quantitation

The limit of quantitation is defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the sated operational conditions.

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

Robustness

The standard and sample of NET and PAL were injected by changing the conditions of chromatography such as variation in flow, variation of mobile phase organic composition etc.

RESULTS AND DISCUSSIONS

The Linearity of NET and PAL were found to be 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. For the precision % RSD values were 0.8 and 0.3 for NET and PAL respectively which shows that the method is précised. For intermediate precision also the %RSD values were within the limits, 0.8 and 0.4 for NET and PAL respectively, which shows that the method is repeatable when performed in different days also. The % recovery was found to be 100.43% and 100.50% for NET and PAL respectively. 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 are 3 and 10. The LOD and LOQ for NET was found to be 3.02 and 9.98 and LOD and LOQ for PAL 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.

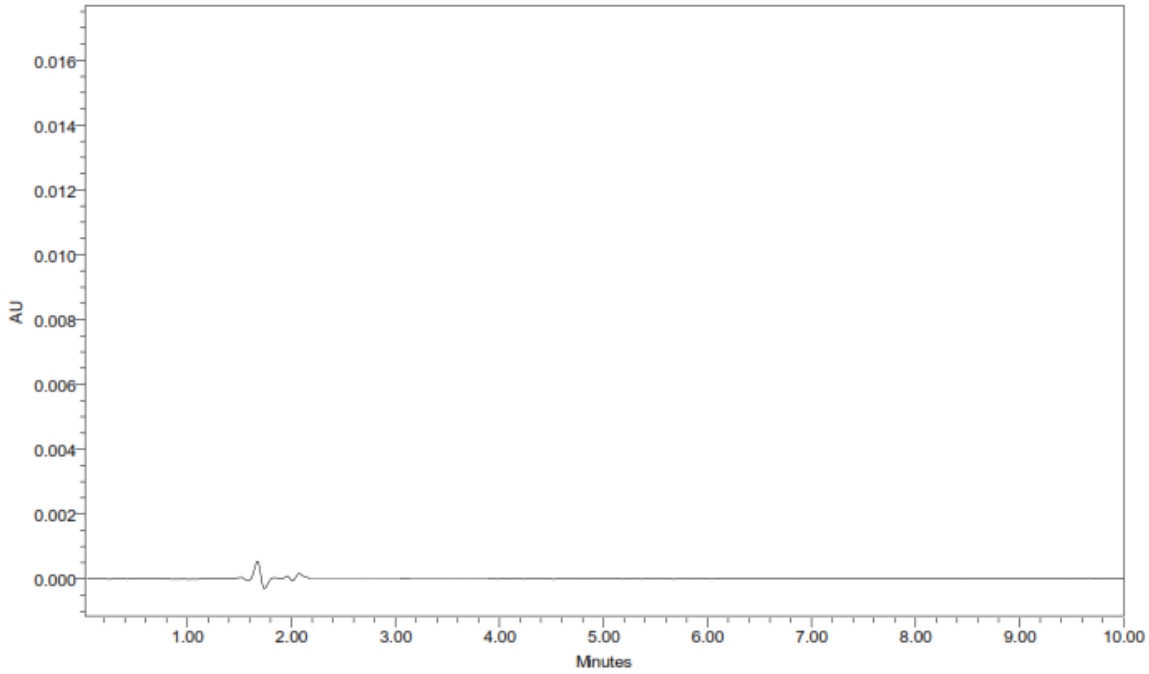

Figure 2: Chromatogram for Blank.

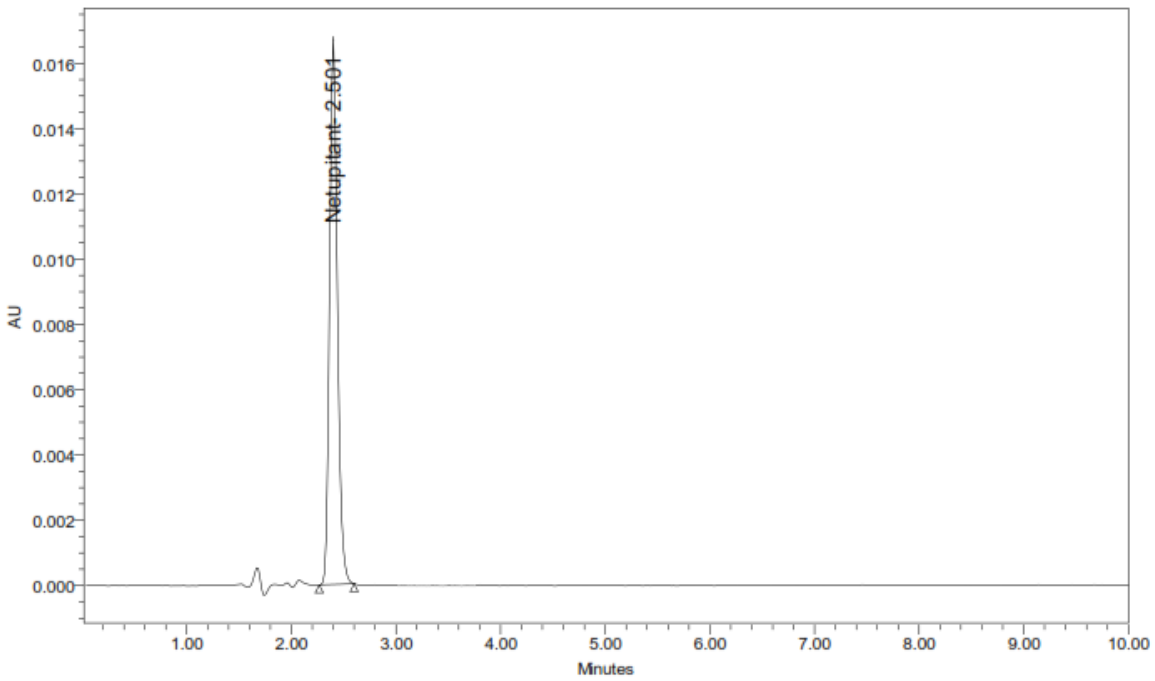

Figure 3: Chromatogram for Netupitant.

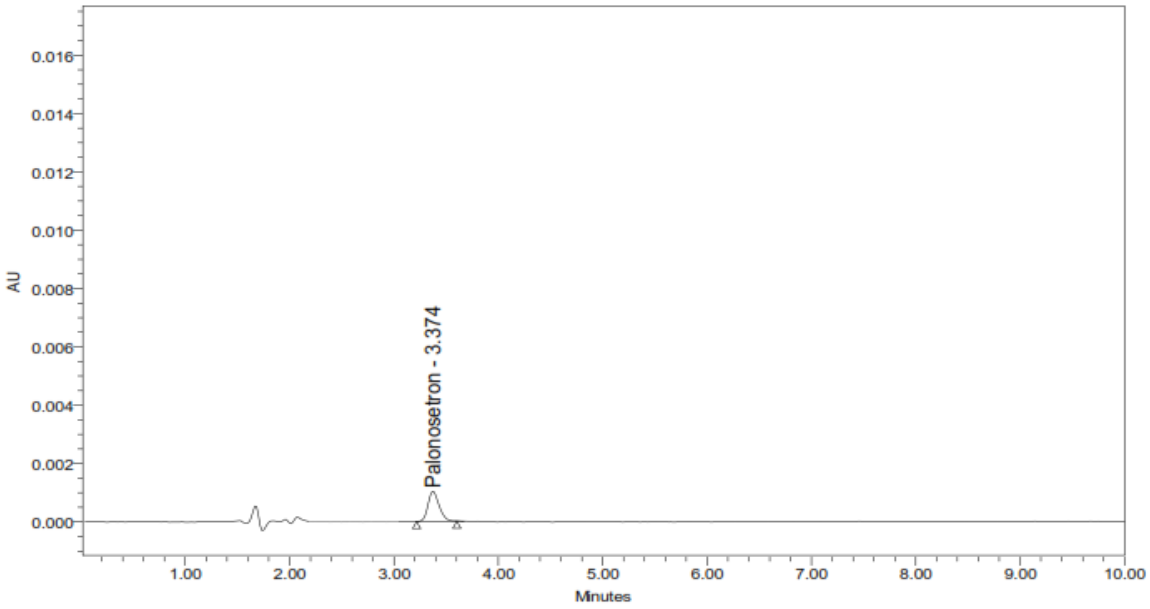

Figure 4: Chromatogram for Palonosetron.

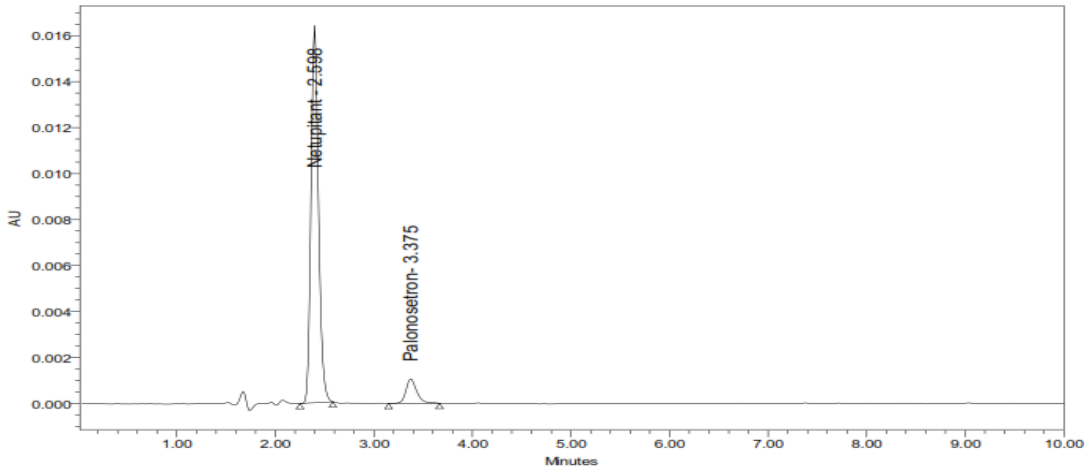

Fig. 5: Chromatogram of standard mixture of NET and PAL.

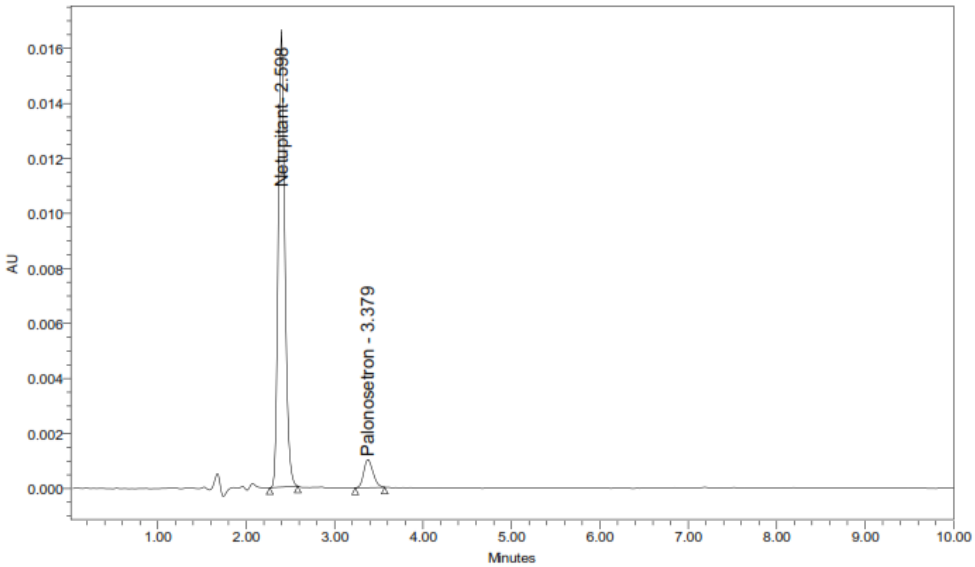

Fig. 6: Chromatogram for sample mixture of NET and PAL.

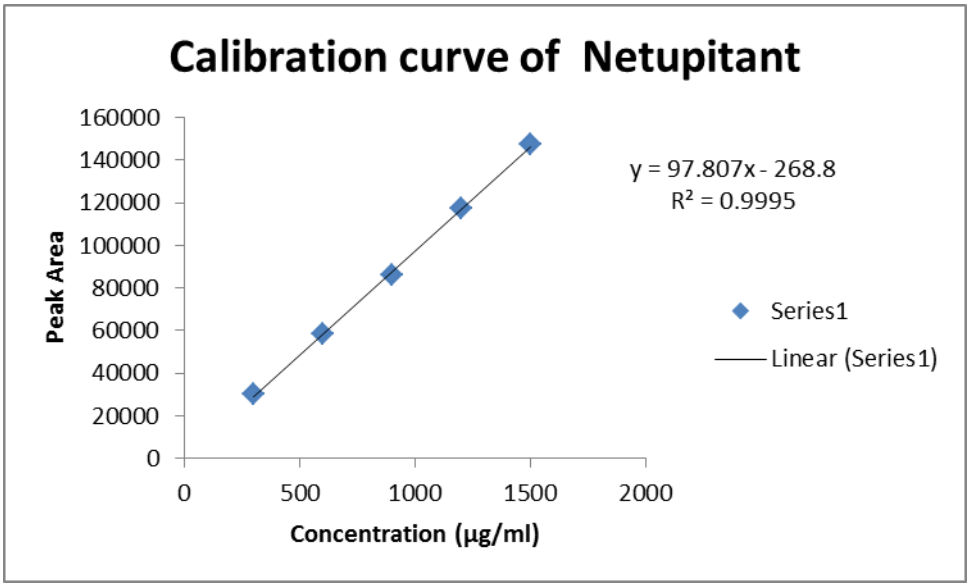

Figure 7: Calibration graph for NET.

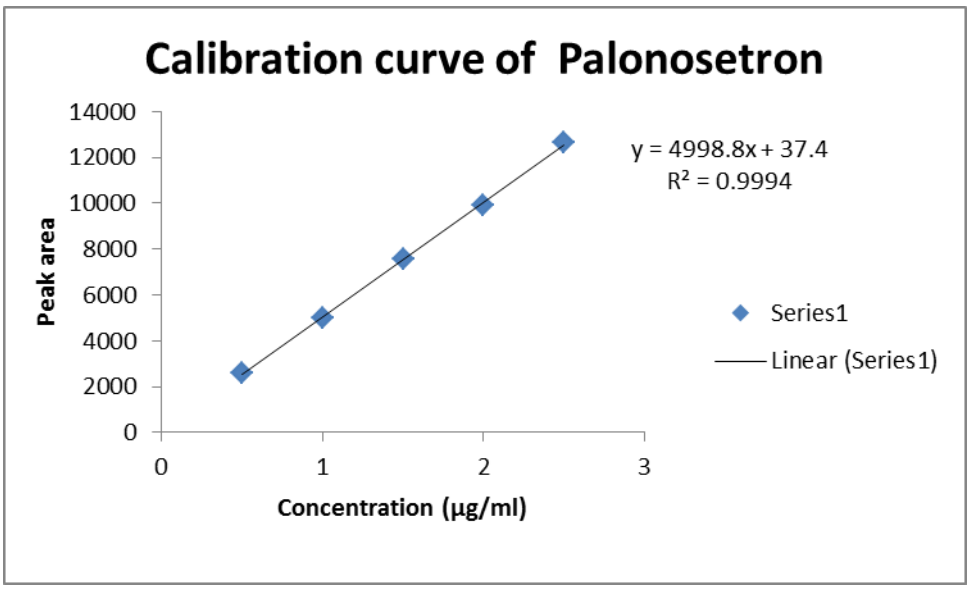

Figure 8: Calibration graph for PAL.

Table 1:

Parameters	Conditions
Instrument used	Waters HPLC with auto sampler and UV detector.
Temperature	Ambient
Column	YMC 4.6*150mm 5µ pack pro C_{18} column
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 per min
Wavelength	210 nm
Injection volume	20 µl
Run time	8 min.

Table 2: Results of system suitability parameters.

S. No	Name	RT(min)	Peak Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Netupitant	2.501	86339	16402	5.75	1.18	4529.07
2	Palonosetron	3.374	7556	1033		1.20	4633.60

Table 3: Linearity Results of standard mixture of NET and PAL.

S.No.	NET		PAL	
	Concentration (µg/ml)	Mean Peak Area	Concentration (µg/ml)	Mean Peak 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
Slope	97.80733		4998.8	
Intercept	-268.8		37.4	
Correlation Coefficient	0.999		0.999	

Table 4: Data of Precision for standard mixture of NET and PAL.

Injection	NET		PAL	
	Concentration (µg/ml)	Mean Peak Area	Concentration (µg/ml)	Mean Peak Area
Injection-1	900 µg/ml	87799	1.5 µg/ml	7524
Injection-2		86973		7519
Injection-3		86232		7524
Injection-4		87604		7581
Injection-5		85975		7558
Injection-6		87018		7565
Mean	**86933.5**		**7545.16**	
Standard Deviation	**723.62**		**26.16**	
%RSD	**0.83**		**0.35**	

Table 5: Summary of validation parameters by HPLC method.

S.No	Validation Parameters		Netupitant	Palonosetron
1.	**ASSAY**	**Label claim**	300 mg	0.5mg
		% Content	100.08	100.04
2.	**Linearity**	**Slope**	293.42	13635
		Intercept	268.8	70701
		Correlation coefficient	0.999	0.999
3.	**Precision**	**%RSD**	0.8	0.3
4.	**Intermediate Precision**	**%RSD**	0.8	0.4
5.	**Accuracy**	**%Recovery for 50%, 100% & 150%**	100.08, 100.46 & 100.75	100.75,100.08 & 100.67
6.	**LOD**	**S/N Ratio**	3.02	3.00
7.	**LOQ**	**S/N Ratio**	9.98	10.00

CONCLUSION

We concluded that our proposed methods for simultaneous estimation of NET and PAL in bulk and pharmaceutical dosage forms are simple, precise, accurate and economical method.

REFERENCES

1. Monograph of Netupitant available on the URL :http://www.drugbank.ca/drugs/DB09048
2. Monograph of Palonosetron available on the URL http://www.drugbank.ca/drugs/00377
3. NVMS Bhagavanji, PVV Satyanarayana, Karanam Sekhar, D. Naniprasad. 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., 2016; 41(1): 81-87.
4. Uttam Prasad Panigrahy, A. Sunil Kumar Reddy. 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, Int. J. Chem Tech Res., 2015; 8(10): 317-337.
5. Mangesh Harole, R. N. Patil and Deepak Gaware, Govind Suryawanshi, Kalyan Pise, 2.A Validated Stability Indicating RP-HPLC Method For Simultaneous Determination Of NET And PAL In Pharmaceutical Formulations, World Journal Of Pharmacy And Pharmaceutical Sciences, 2016; 5(3): 878-887.
6. K.Asha, K.Shalini, Komal Jain, B.Jyothirmayee, Abigail. Simultaneous Estimation Of NET And PAL In Its Bulk And Pharmaceutical Dosage Form By RP-HPLC Method, Journal Of Synthetic And Natural Sciences, 2016; 1(1): 1-6.
7. Kusuma Jogi, Mandava Venkata Basaveswara Rao, Rudraraju Rameshraju. A Novel Validated RP-HPLC Method for the Simultaneous Estimation of

NET and PAL in Bulk and Pharmaceutical Dosage Form, International Journal of Current Science and Technology, 2017; 1(5): 317-323.

8. Vasava Shilpa N, Rajashree C. Mashru. Chemometrics Assisted and RP-HPLC Methods for Quantification of NET and PAL Hydrochloride by Qbd Approach Development and Validation, world Journal Of Pharmaceutical Research, 2016; 5(6): 1173-1197.
9. Xu M, Ni Y, Li S, Du J, Li H, Zhou Y, Li W, Chen H. Development and validation of a rapid LC-MS/MS method for simultaneous determination of netupitant and palonosetron in human plasma and its application to a pharmacokinetic study, J Chromatogr B Analyt Technol Biomed Life Sci., 2016 Aug 1; 1027: 187-93.
10. Vasava Shilpa N, Rajashree C. Mashru1. Simultaneous Quantitative Estimation of NET and PAL HCL by HPTLC Method Development and Validation, European Journal Of Biomedical And Pharmaceutical sciences, 2016; 3(7): 421-426.