Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (5): 223-234

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

A Validated Novel RP-HPLC method development for the estimation of Palonosetron Hydrochloride in bulk and softule dosage forms

Srikanth Inturi[*1], RaviKanth Inturi [2], G.Venkatesh[3]

[1]*Department of Pharmaceutical, Andhra Analysis, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam Pradesh, India*
[2]*Department of Organic Chemistry, Hindu College of P.G Courses, Guntur, Andhra Pradesh, India*
[3]*Department of Pharmacology, PSG College of Pharmacy, Coimbatore, Tamilnadu, India*

ABSTRACT

A simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method was developed and validated for the determination of Palonosetron Hydrochloride in bulk and softule dosage forms. It was found that the excipient in the tablet dosage forms does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on ODS-UG-5 column (250×4.6mm) with a mobile phase composed of Buffer (0.025M sodium dihydrogen orthophosphate pH adjusted 6.9 with triethyl amine) : acetonitrile (65:35 %v/v) in isocratic mode at a flow rate of 1.0ml/min. The detection was monitored at 240nm. The calibration curve for Palonosetron Hydrochloride was linear from the concentrations of 0.03µg/ml to 2µg/ml. The interday and intraday precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility and specificity for the determination of Palonosetron Hydrochloride in bulk and its softule dosage forms. LOD and LOQ for Palonosetron Hydrochloride were found to be 20ng/ml and 60ng/ml. Accuracy (recoveries: 98.76-100.2%) and reproducibility were found to satisfactory.

Keywords: Palonosetron Hydrochloride; Novel RP-HPLC ; Acetonitrile; Softule dosage forms; Validation.

INTRODUCTION

Palonosetron hydrochloride (PALO), chemically known as (3aS)- 2-[(S)-1-azabicyclo [2.2.2] oct-3-yl]-2,3,3aS,4,5,6-hexahydro-1Hbenz[de] isoquinolin-1-one hydrochloride (Fig. 1) Palonosetron is an Antiemetic; selective inhibitor of serotonergic (5-HT3) receptors. It is used in prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy also in prevention of acute nausea and vomiting

associated with initial and repeat courses of highly emetogenic cancer chemotherapy. Palonosetron is administered intravenously, as a single dose, 30 minutes before chemotherapy, or as a single oral capsule one hour before chemotherapy. The oral formulation was approved on August 22, 2008 for prevention of acute CINV alone, as a large clinical trial did not show oraladministration to be as effective as intravenous use against delayed CINV[1-4]

High Performance Liquid Chromatography (HPLC) It is also called as liquid chromatography (LC). It is one of the most widely used analytical techniques today, among the different chromatographic procedure due to the significant evolution in LC instrument, providing superior qualitative and quantitative16 results. HPLC is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high speed and wide range of sensitivity make it ideal for analysis of many drugs in dosage forms as well as in biological fluids. HPLC is a separation technique where separation is accomplished by partitioning between a mobile phase (solvent) and a Stationary phase (column). HPLC differ from other types of liquid chromatography with regards to packing material of small, uniform particles utilized .The small size of particles give high column efficiencies that also results in high pressure drop across the columns, and therefore higher pressures are utilized to achieve desired flow rates. Hence it is also called as “high pressure liquid chromatography”.[5-9]

Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development. Development of methods to achieve the final goal of ensuring the quality of drug substances and drug products must be implemented in conjunction with an understanding of the chemical behavior and physicochemical properties of the drug substance. This determination requires highly sophisticated methods and instruments like HPLC.[10-12]

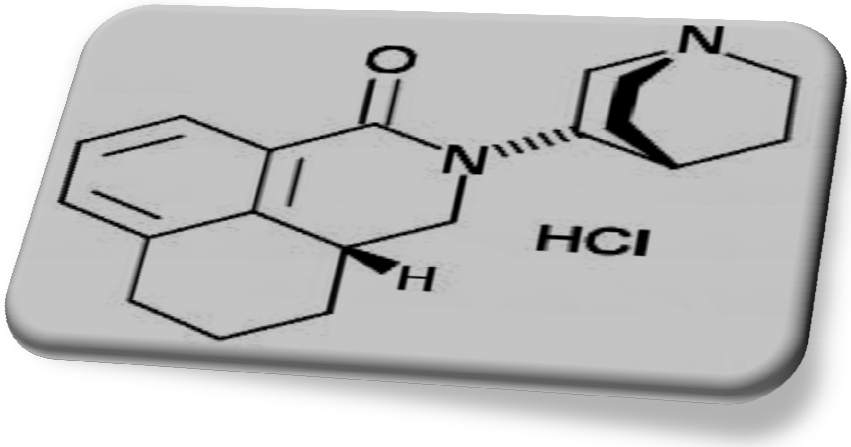

Figure 1: Chemical structure of Palonosetron Hydrochloride

Palonosetron Hydrochloride is evaluated in LCMS and HPLC method by using different solvents and columns that shows not much predicted values in current scenario and there is no method was reported for the estimation of this drug in bulk and oral pharmaceutical dosage forms (Softules). Hence, the purpose of this study was to develop simple, rapid, precise, specific and accurate RP - HPLC method for the estimation of the drug in pure and in oral pharmaceutical dosage forms (Softules) [19-27].

MATERIALS AND METHODS

2.1 Reagents and Solutions

Pure sample of Palonosetron Hydrochloride was kindly supplied by the Natco Pharma limited, Hyderabad. Acetonitrile, triethyl amine and water used were of HPLC grade. All other reagents used in this study were of AR grade. Optimized chromatographic conditions are listed in Table: 1.

2.2 Apparatus and Chromatographic conditions

Chromatographic separation was performed on a Alliance-Waters 2695 chromatographic separation module with variable wavelength programmable Waters 2996 photodiode array detector and Rheodyne with 20µL fixed loop and data analyzed by using Empower software. ODS-UG-5 column (250 × 4.6 mm) was used for separation. Mobile phase consisting of a mixture of buffer (0.025M sodium dihydrogen orthophosphate pH adjusted 6.9 with triethyl amine) : acetonitrile (65:35 v/v) was delivered at a flow rate of 1ml/min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min at 100rpm. Analysis was performed at ambient temperature.

Standard solution

Weigh accurately and transfer about 25 mg of Palonosetron Hydrochloride into a 25 ml volumetric flask. Dissolve and make up to volume with mobile phase and mix. The solution was shaken for 5 min and sonicated for 15min at 100 rpm

Sample solution

For the analysis of softgelatin capsule dosage form, twenty capsules (ALOXI) were weighed and their average weight was determined. The powder equivalent to 25 mg of Palonosetron Hydrochloride was transferred to a 25 ml of volumetric flask and dissolve with mobile phase. The solution was shaken for 5 min and sonicated for 15min at 100 rpm. The solution was filtered through Whatman filter paper #41. This filtrate was further diluted with mobile phase. 20 µl of the sample solution was injected into the chromatograph for quantitative analysis. Then record the chromatogram.

3. Method Validation

Once the HPLC method development was over, the method was validated in terms of parameters like specificity, precision, accuracy, linearity and range, LOD, LOQ, ruggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values were calculated. The proposed HPLC method was validated as per ICH guidelines (ICH Guidelines; 2006)

3.1Accuracy

To study the reliability, system suitability, accuracy and recovery experiments were carried out Prepare separately 0.5, 1.0 and 1.5 mg/ml solution of Palonosetron Hydrochloride standard as well as test samples with mobile phase injected separately two replicate volumes of 20µl of each of standard as well as test solutions into the chromatograph and record the chromatograms. Purity of Palonosetron Hydrochloride test sample was calculated from the areas obtained. The mean recoveries were in range of 99.8-101.20 % which shows that there is no interference from excipients. The concentration of the drug product in the solution was determined using assay

method. The % RSD was calculated by using the following formula. The mean recoveries were in the range of 98.76-100.2 % which shows that there is no interference from excipients Table: 4

$$\text{Absolute Recovery} = \frac{\text{Response of analyte spike into matrix (processed)}}{\text{Response of analyte of pure standard (unprocessed)}} \times 100$$

3.2.Linearity and Range

The linearity of measurement was evaluated by analyzing different concentrations of the standard solutions of Palonosetron Hydrochloride. The Beer lamberts concentration was found to be 0.03µg/ml to 2µg/ml. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed. The results were shown in Table: 2.The slope, intercept and correlation coefficient values were found to be 2.8711, 0.1346 and 0.99987. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above regression graph was shown at Fig: 7.

3.3.Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise.

3.4.Repeatability of solution

A standard solution of drug substance was injected six times and corresponding peak areas were recorded. The % RSD was found to be less than 1%. Table: 5.

3.5.Specificity

Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc was changed. Inspite of above changes no additional peaks were found, although there were shift retention times or little changes in peak shapes.

3.6.Assay

20 µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area of standard amount of drug in sample were computed. The values are given in Table: 8.

3.7.Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Palonosetron Hydrochloride found to be 20ng/ml. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 60ng /ml for Palonosetron Hydrochloride. It was concluded that the developed method is sensitive.

3.8. Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different analysts the degree of reproducibility was found to be 100%±0.5.

1.0 mg/ml solution of Palonosetron Hydrochloride was prepared with mobile phase. Separately injected six (6) replicate volumes of 20µl each solution into the chromatograph and record the chromatogram

3.9. Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in mobile phase, flow rate, column temperature and pH of the mobile phase. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The robustness limit for mobile phase variation, flow rate variation, temperature variation and pH of the mobile phase are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

RESULTS AND DISCUSSION

UV spectrum of Palonosetron Hydrochloride was recorded from which 240nm was selected as wavelength. Flow rate of 1ml/min was selected. buffer (0.025M sodium dihydrogen orthophosphate pH adjusted 6.9 with triethyl amine) : acetonitrile (65:35%v/v) was selected as mobile phase. The retention time was found to be 5.6 and Palonosetron Hydrochloride shown linearity in the range of 0.03 - 2µg/ml, and the coefficient was found to be 0.99987.

Recovery studies were performed at 50, 100, 150 % levels. The sensitivity of method LOD and LOQ is shown in Table 3. Hence the proposed method is simple, accurate, precise and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method. Regression analysis of the calibration curve for Palonosetron Hydrochloride showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.99987 in all the curves assayed.

Table 1: Optimized chromatographic conditions

Parameter	**Optimized condition**
Chromatograph	HPLC (Alliance-Waters with 2996 PDA)
Column	ODS-UG-5 column (250 × 4.6 mm)
Mobile Phase	0.025M sodium dihydrogen orthophosphate pH adjusted 6.9 with triethyl amine : acetonitrile (65:35%v/v)
Flow rate	1 ml/min
Detection	240 nm
Injection volume	20µl
column Temperature	Ambient

Table 2: Validation Parameters

Parameter	Palonosetron Hydrochloride
Linearity range	0.03 - 2 µg/ml
Correlation coefficient	0.99987
Slope	2.8711
Y Intercept	0.1346

Table 3: System suitability parameters

Parameter	Palonosetron Hydrochloride
Calibration range	0.03 - 2 µg/ml
Theoretical plates	1267
Resolution	-
Tailing factor	2.498
LOD	20ng/ml
LOQ	60ng/ml

Table 4: System suitability, Accuracy and Recovery studies

<table>
<tr><th colspan="2">System suitability</th><th colspan="4">Sample analysis</th></tr>
<tr><th>Injection No</th><th>Area</th><th>Description</th><th>At 50%</th><th>At 100%</th><th>At 150%</th></tr>
<tr><td>1</td><td>29424858</td><td rowspan="2">Area in inj 1</td><td rowspan="2">16010072</td><td rowspan="2">29747691</td><td rowspan="2">43473608</td></tr>
<tr><td>2</td><td>29411624</td></tr>
<tr><td>3</td><td>29439346</td><td rowspan="2">Area in inj 2</td><td rowspan="2">16011414</td><td rowspan="2">29486779</td><td rowspan="2">43536307</td></tr>
<tr><td>4</td><td>29413050</td></tr>
<tr><td>5</td><td>29393912</td><td rowspan="2">Average</td><td rowspan="2">16010743</td><td rowspan="2">29617235</td><td rowspan="2">43504957</td></tr>
<tr><td>6</td><td>29386386</td></tr>
<tr><td>Average</td><td>29411529.2</td><td rowspan="2">Actual assay (%w/w)</td><td rowspan="2">98.76%</td><td rowspan="2">100.2%</td><td rowspan="2">99.8%</td></tr>
<tr><td>%RSD</td><td>0.1</td></tr>
<tr><td rowspan="2">Acceptance criteria</td><td rowspan="2">%RSD should not be more than 2.0</td><td>% Recovery</td><td>49.38%</td><td>100.2%</td><td>149.7%</td></tr>
<tr><td colspan="4">Acceptance criteria %RSD of assay results should not be more than 2.0</td></tr>
</table>

Recovery studies were performed at 50%, 100%, 150% levels and the results were found to be within the limits mentioned as per ICH Guidelines.

Table 5: Repeatability of injection

InjectionNo	RT	Area	
1	5.242	29424858	%RSD was found to be 0.1
2	5.245	29411624	
3	5.248	29439346	
4	5.248	29413050	
5	5.248	29393912	
6	5.250	29386386	
Average		29411529.2	
Std .Dev.		19474.1	

Repeatability of injection was performed using 1.0mg/ml sample for six times and corresponding peak areas were recorded. The % RSD peak was reported

Table 6: Ruggedness

ANALYST-1			ANALYST-2		
Injection No.	**Area**	**RT**	**Injection No.**	**Area**	**RT**
1	31316188	5.314	1	29182682	5.405
2	31339084	5.314	2	29189572	5.407
3	31310225	5.317	3	29173946	5.408
4	31369462	5.319	4	29145518	5.415
5	31335878	5.320	5	29170000	5.417
6	31261192	5.322	6	29177065	5.419
Mean	31322004.9		Mean	29173130.4	
Std. Dev.	36351.5		Std. Dev	15165.2	
% RSD	0.1		% RSD	0.1	

Flow rate								
Flow rate 1.0 ml/min			**Flow rate 0.9ml/min**			**Flow rate 1.1ml/min**		
Inj No.	**Area**	**RT**	**Inj. No.**	**Area**	**RT**	**Inj. No.**	**Area**	**RT**
1	31316188	5.314	1	35263911	5.793	1	28712233	4.848
2	31339084	5.314	2	35289054	5.797	2	28736727	4.857
3	31310225	5.317	3	35397920	5.798	3	28795130	4.859
4	31369462	5.319	4	35325821	5.803	4	28876115	4.861
5	31335878	5.320	5	35303580	5.814	5	28743136	4.865
6	31261192	5.322	6	35204436	5.837	6	28737215	4.868
Mean	31322004.9		Mean	35297453.5		Mean	28766757.9	
Std.Dev	36351.5		Std.Dev	64472.8		Std.Dev	60117.6	
%RSD	0.1		%RSD	0.2		%RSD	0.2	

Table 7: Robustness

pH of the mobile phase					
pH 6.9			**pH 7.1**		
Injection No.	**Area**	**RT**	**Injection No.**	**Area**	**RT**
1	27880288	5.183	1	29625250	5.925
2	27814874	5.185	2	29669343	5.927
3	27825683	5.200	3	29700119	5.927
4	27860721	5.207	4	29660604	5.929
5	27833893	5.250	5	29719307	5.933
6	27789073	5.312	6	29749853	5.935
Mean	27834088.7		Mean	29687412.7	
Std.Dev.	32588.3		Std.Dev.	44692.0	
%RSD	0.1		%RSD	0.2	

Table 8: Analysis of formulation

Amount of drug (mg/capsule)			
Labeled	**estimated**	**%label claim**	**%RSD**
0.5	0.494	98.8	0.1

Analysis of formulation was performed using Palonosetron Hydrochloride 0.5 mg soft gelatin capsules and the % label claim was found to be 98.8%.

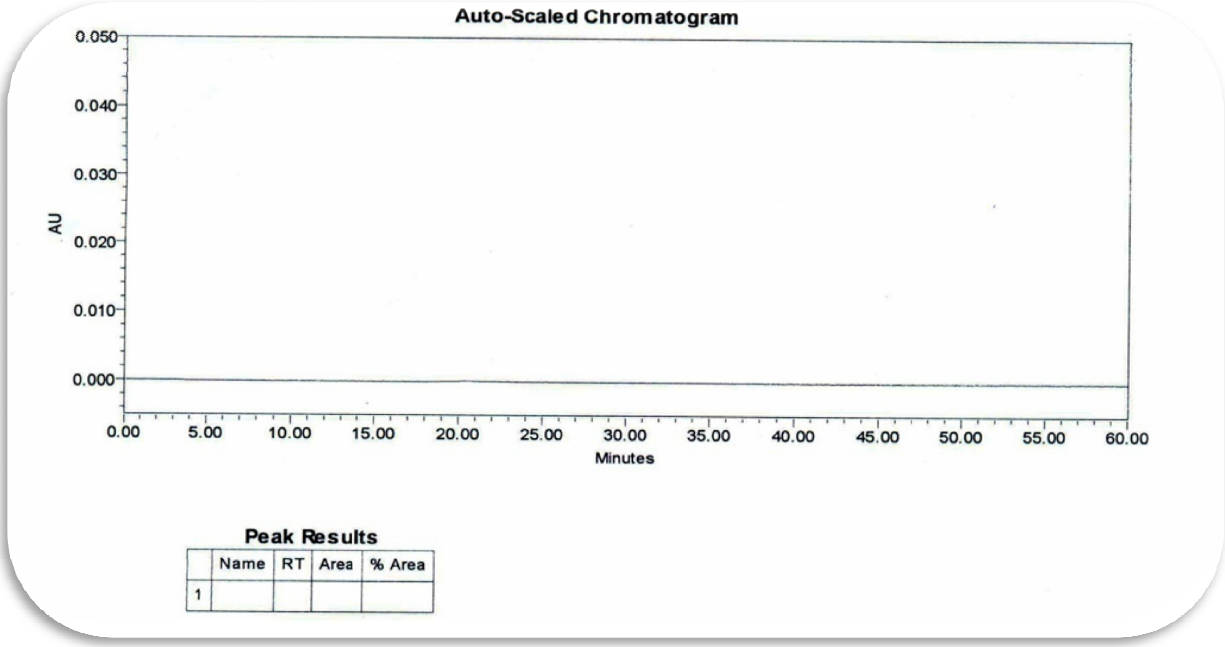

Figure 2: A Representative Chromatogram of Palonosetron Hydrochloride (Placebo)

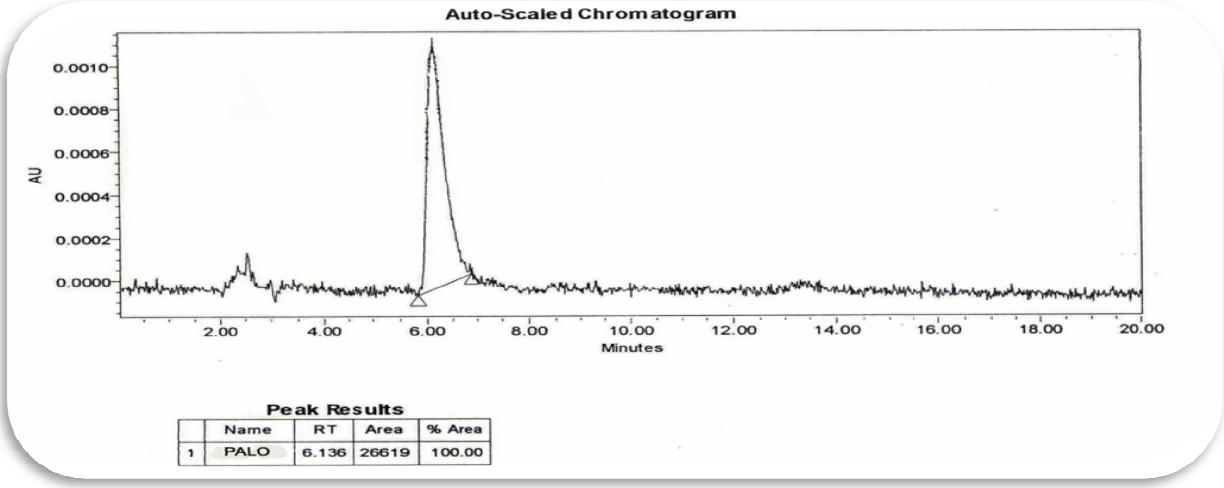

Figure 3: A Representative Chromatogram of Palonosetron Hydrochloride (Standard)

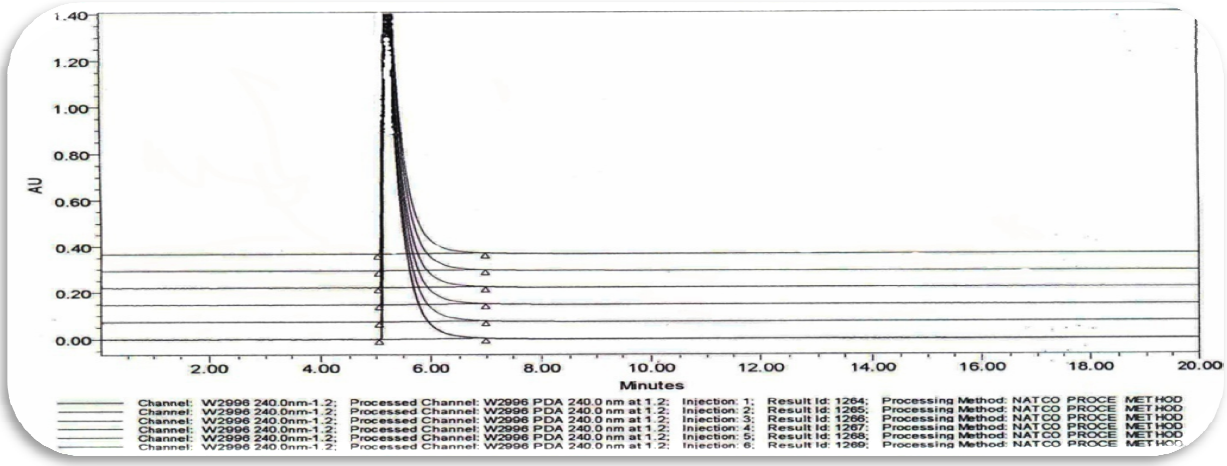

Figure 4: A Representative chromatogram of Palonosetron Hydrochloride (Precision)

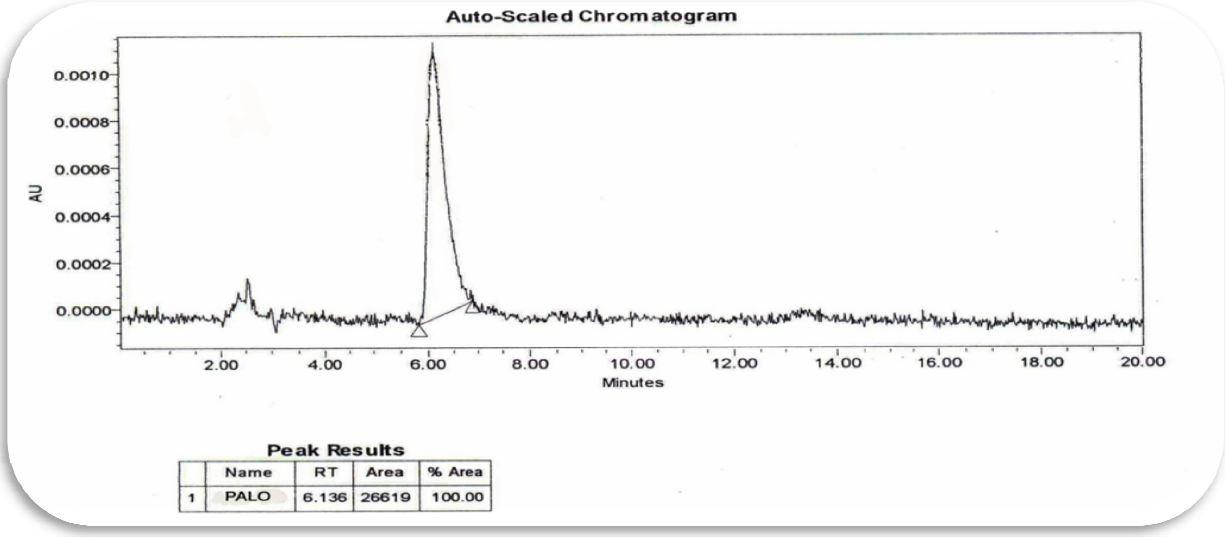

Figure 5: A Representative chromatogram of Palonosetron Hydrochloride (LOD)

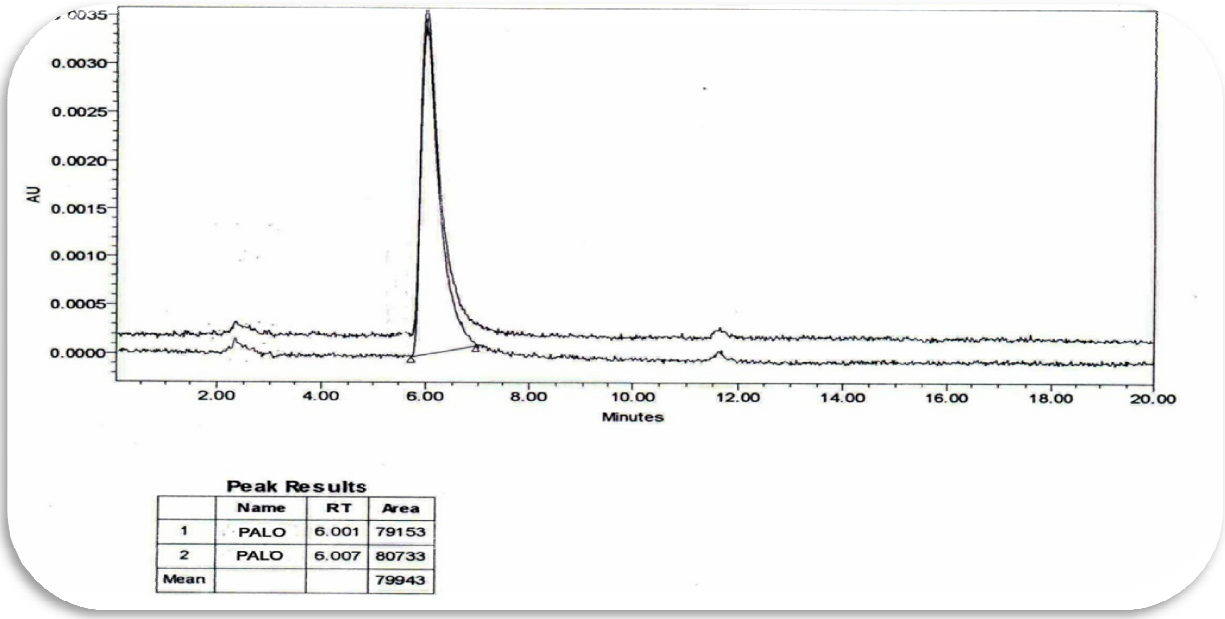

Figure 6: A Representative chromatogram of Palonosetron Hydrochloride (LOQ)

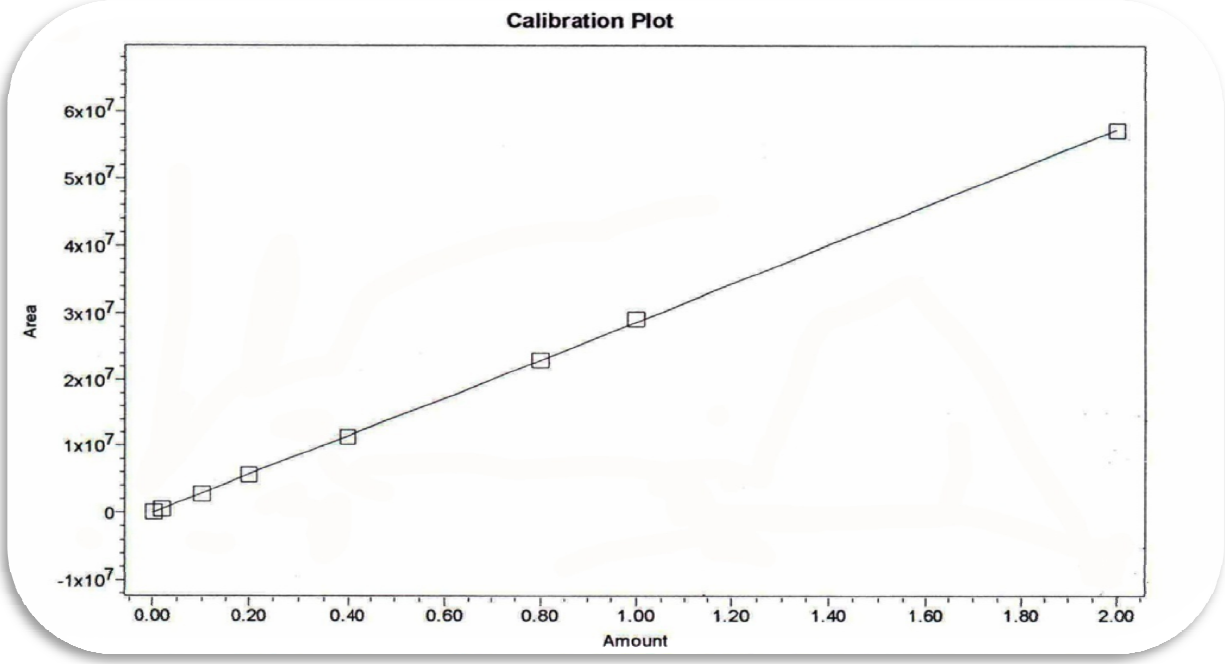

Figure 7: Linearity plot of Palonosetron Hydrochloride

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of Palonosetron Hydrochloride in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The

proposed method is simple, fast, accurate and precise for the simultaneous quantification of Palonosetron Hydrochloride in dosage form, bulk drugs as well as for routine analysis in quality control.

Acknowledgement
The authors wish to thank the NATCO PHARMA LIMITED for providing the samples of Palonosetron Hydrochloride bulk and softule dosage forms. The authors are also thankful to the management, University College of pharmaceutical sciences, Visakhapatnam, for providing necessary facilities and constant encouragement.

REFERENCES

[1] Willard Merritt Dean settle, Instrumental method of analysis 7th edition New delhiCBS publication and distributors **1986**. P. 3-5, 684-689.
[2] 2.. Gennaro AR, Remington: The Science and Practice of Practice of Pharmacy, twenty first edition volume II Lippincort, Williams and welkins Mack Publisheing Company, P. **1680**-1682.
[3] Sharma B.K., Instrumental Methods of Chemical Analysis, seventeenth edition, Goel Publishing Housing, Krishna Prakashan Ltd, 3-6, chromatographic section, 4,5.
[4] Daniel C, Harris, Quantitative chemical analysis 6th edition W.H. Free man and company New York **2004**. P. 726.
[5] USP N.F. "The official compendia of standard" Asian edition **2004**. Printed in Canada by web.com.ltd. Torontao Ontoriao P. 2622-2624.
[6] MGI Pharma. Aloxi (palonosetron hydrochloride) injection prescribing information. Bloomington, MN; **2006** Jan.
[7] McKeage MJ. Drug Saf. **1995**; 13:228-44.
[8] Cubeddu LX, Hoffmann IS. *J Clin Pharmacol*. **1993**; 33:691-7.
[9] Hesketh PJ, Gandara DR. *J Natl Cancer Inst*. **1991**; 83:613-20.
[10] Gralla RJ. Adverse effects of treatment: antiemetic therapy. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. Cancer: principles and practice of oncology. 4th ed. Philadelphia: J.B. Lippincott Company; **1993**:2338-48.
[11] Plosker GL, Goa KL, *J.Pharmacology* **1991**; 42:805-24.
[12] Di Vall MV, Cersosimo RJ. Palonosetron. *Formulary*. **2003**; 38:414-30.
[13] Barger AM, Clark-Snow RA. Adverse effects of treatment. In: VT DeVita, S Hellman, SA Rosenberg, eds. Cancer: principles & practice of oncology. 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins; **2001**: 2869-80.
[14] Kealey D, Haines PJ. Instant notes Analytical chemistry. UK: BIOS Scientific Publishers Ltd; **2002**. p. 1.
[15] Jeffery GH, Bassett J, Mendham J, Denny RC. Vogel's Text book of Quantitative Chemical Analysis. 5th ed. New york: John wiley & sons, Inc; **1989**. p. 3, 668-9.
[16] Ravi Sankar S. Pharmaceutical Analysis. 3rd ed. Rx Publishing House; **2001**. p. 1-1, 2-2.
[17] Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi: CBS Publishers and Distributors; **1997**. p. 296-99.
[18] David WG. Pharmaceutical Analysis A text book for pharmacy students and pharmaceutical chemists. Toronto: Churchill Livingstone; **1999**. p. 92.
[19] Ding L, Chen Y, Yang L, Wen A, *J. Pharm. Biomed. Anal*. 44 (**2007**) 575–580.
[20] Zhang W, Feng F, *Chromatographia* 68 (**2008**) 193–199

[21] Tian K, Chen H, Tang J, Chen X, Hu Z, *J. Chromatographia. A* 1132 (**2006**) 333–336.
[22] Radhakrishnanand P, Subba Rao DV,. Hima bindu V, *Chromatographia* 69 (**2009**) 369–373.
[23] Xiao-rong YU, Min SONG,. Tai-jun HANG, *Chin. J. New Drugs* 10 (**2008**).
[24] Xi SHEN,. Hui ZHOU,. Wan-qing WANG,. Hua-fang CAI, 24 (**2007**) 307–308.
[25] Xiao-xia SHEN,. Xiao-dong LIU, Qiang O, *Chin. J. Pharm. Anal*. 01 (**2008**).
[26] Vishnu Murthy M, Katkam Srinivas, Ramesh Kumar,. Mukkanti K, *J. Pharm. Biomed. Anal* 56 (**2011**) 429– 435.
[27] Jayesh D. Bodar, Sharad Kumar, Yogesh Chand Yadav,. Seth A K, Gajanan J. Deshmukh, Tejas K. Ghelani, Arti Zanwar , Patel Divyakant, Surendra Nath Yadav. *J.Pharma science monitor* (**2011**) (1009-1016).