Research Article

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Stability indicating RP-HPLC method development and validation for the determination of palonosetron in API and pharmaceutical dosage form

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ABSTRACT

A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Palonosetron in bulk and pharmaceutical dosage form dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cm x 4.6mm i.d. column with UV detection at 254 nm and Methanol: Phosphate Buffer (0.05M) pH-3.8 with OPA (60:40) ratio at a flow rate of 1.0 ml/ min. The proposed method was successfully applied to the determination of Palonosetron in bulk and pharmaceutical dosage form. The method was linear over the range of 6-16µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.006 µg/ml and quantification was found to be 0.018 µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.

Keywords: RP-HPLC, Palonosetron, Method development and validation, ICH Guidelines.

INTRODUCTION [1]

Palonosetron (INN, trade name Aloxi) is a 5-HT3 antagonist used in the prevention and treatment of chemotherapy-induced nausea and vomiting (CINV). It is used for the control of delayed CINV—nausea and vomiting and there are tentative data to suggest that it may be more effective than granisetron. Palonosetron is administered intravenously, as a single dose, 30 minutes before chemotherapy,[2] or as a single oral capsule one hour before chemotherapy.[3] It has a longer duration of action than other 5-HT3 antagonists. The oral formulation was approved on August 22, 2008 for prevention of acute CINV alone, as a large clinical trial did not show oral administration to be as effective as intravenous use against delayed CINV. The oral combination netupitant/palonosetron is approved for both acute and delayed CINV. Palonosetron does not relevantly inhibit or induce cytochrome P450 liver enzymes. There are case reports about serotonin syndrome when the drug is combined with serotonergic substances such as selective serotonin

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reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs), two common types of antidepressants.

The IUPAC Name of Palonosetron 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.

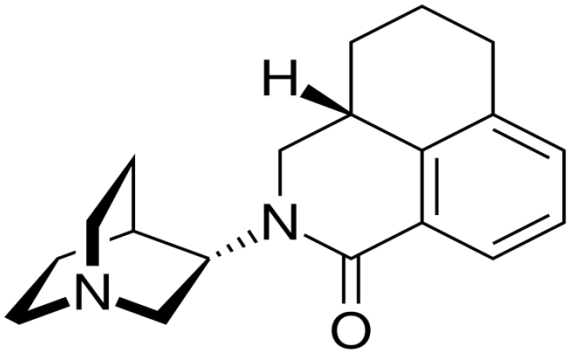


Fig 1: Chemical Structure of Palonosetron

MATERIALS AND METHODS

HPLC Instrumentation & Conditions

The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector. [4]

Standard & sample preparation for UV-spectrophotometer analysis [2]

25 mg of Palonosetron standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.2 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase. The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Palonosetron, so that the same wave number can be utilized in HPLC UV detector for estimating the Palonosetron. While scanning the Palonosetron solution we observed the maxima at 254 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.

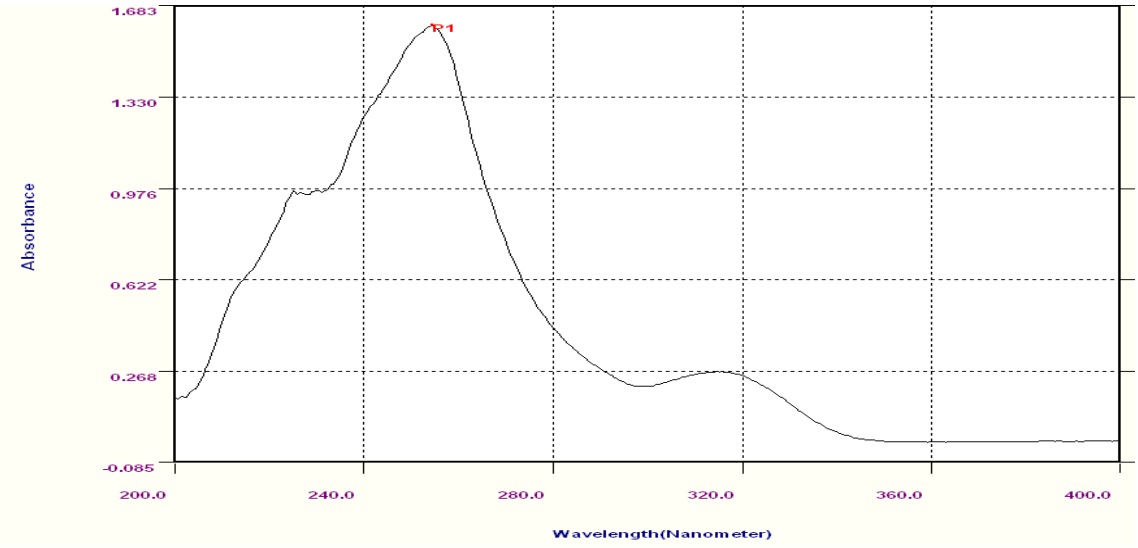


Fig 2: UV Spectrum

Optimized Chromatographic Conditions [3]

- **Column** **:** Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm.
- **Mobile Phase** **:** Methanol : Phosphate Buffer (0.05M) pH-3.8 with OPA (60:40).
- **Flow Rate** **:** 1.0ml/minute
- **Wave length** **:** 254 nm
- **Injection volume** **:** 20µl
- **Run time** **:** 07 mins.
- **Column temperature :** Ambient
- **Sampler cooler** **:** Ambient

MOBILE PHASE PREPARATION

Mobile phase was prepared by taking Methanol: Phosphate Buffer (0.05M) pH-3.8 with OPA (60:40). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

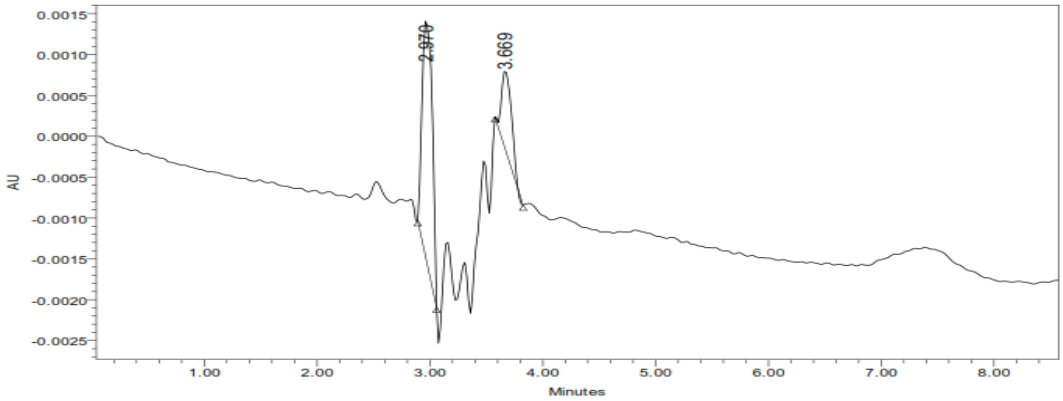
SAMPLE & STANDARD PREPARATION FOR THE ANALYSIS

25 mg of Palonosetron standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

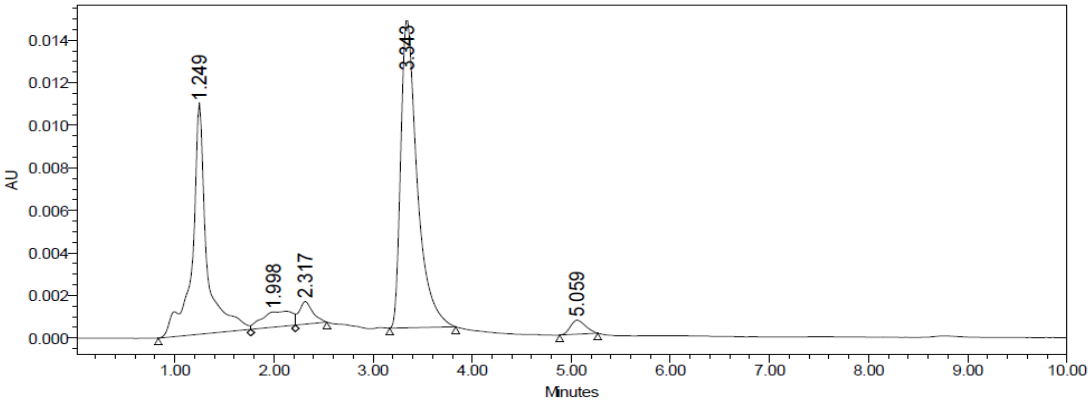
RESULT AND DISCUSSION

Table-1:Trials for Method Development[4]

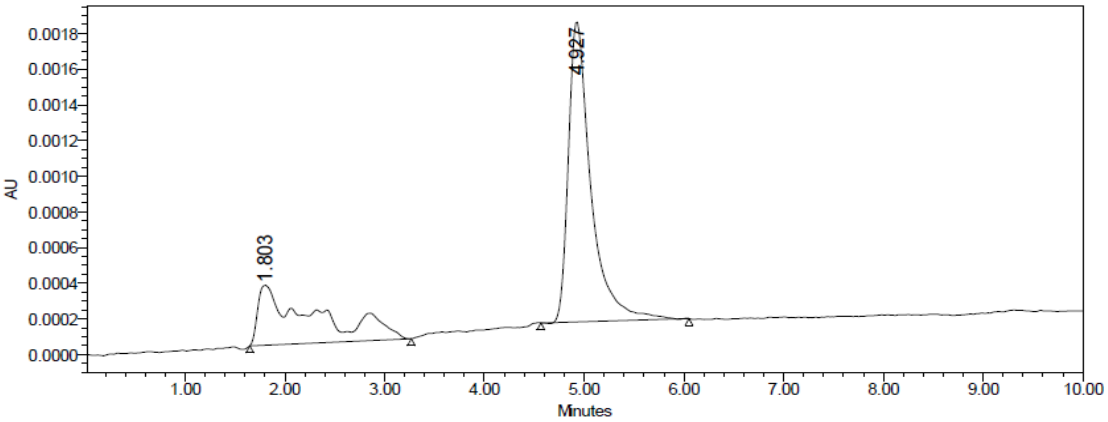
Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Methanol : Acetonitrile (70:30)	1.0 ml/min	254 nm	Broad Peak	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Methanol : Acetonitrile (40:60)	1.0 ml/min	254 nm	Peak broken at the end	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Acetonitrile : Water (70:30)	1.0 ml/min	254 nm	Splitting of peak	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Acetonitrile : Acetate Buffer (55:45)	1.0 ml/min	254 nm	Tailing peak	Method rejected
Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm	Methanol : Phosphate Buffer (0.05M) pH-3.8 with OPA (60:40)	1.0 ml/min	254 nm	Splitting of peak	Method rejected



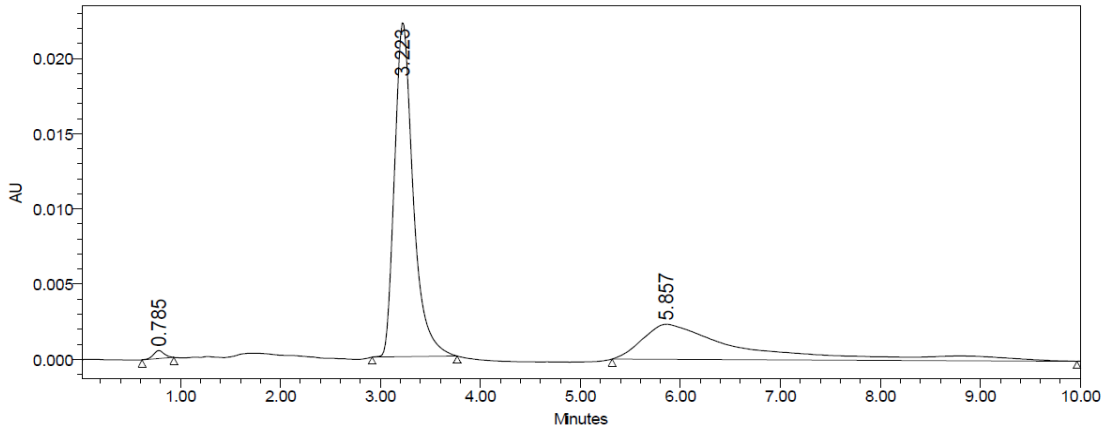
Trial-1



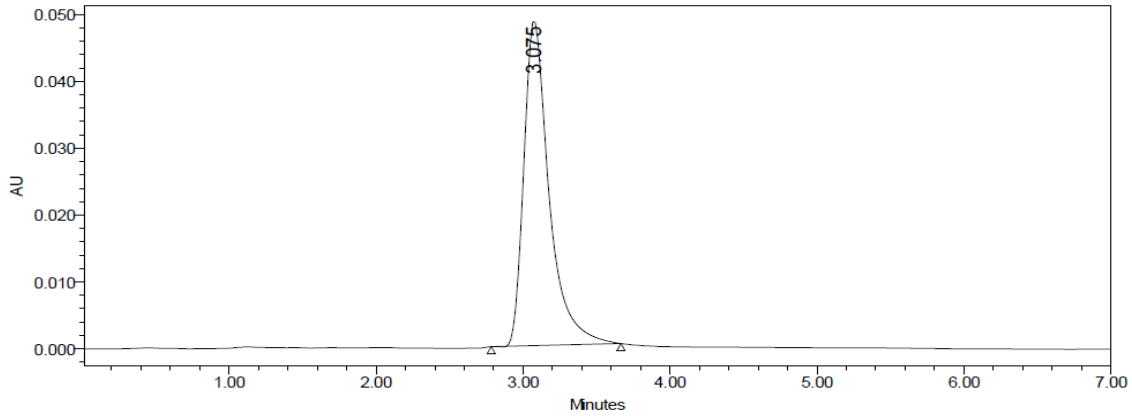
Trial-2



Trial-3



Trial-4



Trial-5

Table 2: Peak results

Name	Rt	Peak Area	Theoretical Plates	Tailing Factor
Palonosetron	3.075	1024536	4569	1.36

METHOD VALIDATION [5]

Accuracy: Recovery study

To decide the exactness of the proposed strategy, recuperation thinks about were completed by including diverse sums (80%, 100%, and 120%) of unadulterated medication of Palonosetron were taken and added to the pre-dissected detailing of fixation 50µg/ml. From that rate recuperation esteems were ascertained [14,5,16]. The outcomes were appeared in Table-3.

Table-3: Accuracy Readings [6]

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S1 : 80 %	8	8.105	93435	101.312	Mean= 100.0163%
S2 : 80 %	8	7.898	91287	98.725	S.D. = 1.293505
S3 : 80 %	8	8.001	92356	100.012	% R.S.D.= 1.293294
S4 : 100 %	10	10.195	115135	101.95	Mean= 101.4033%
S5 : 100 %	10	10.152	114687	101.52	S.D. = 0.613379
S6 : 100 %	10	10.074	113879	100.74	% R.S.D.= 0.60489

S7 : 120 %	12	12.171	135647	101.425	Mean= 100.6053%
S8 : 120 %	12	12.044	134324	100.366	S.D. = 0.730041
S9 : 120 %	12	12.003	133897	100.025	% R.S.D. = 0.725649

Precision

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug [17,18,19]. Palonosetron (API) the percent relative standard deviations were calculated for Palonosetron is presented in the Table-4[7].

Table-4: Repeatability Results of Precision [8]

HPLC Injection Replicates of Palonosetron	Area Under the Curve
Replicate – 1	1013546
Replicate – 2	1025824
Replicate – 3	1012351
Replicate – 4	1036584
Replicate – 5	1015419
Replicate – 6	1028572
Average	**1022049**
Standard Deviation	**9781.365**
% RSD	**0.957035**

Intra day & Inter day: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Palonosetron revealed that the proposed method is precise.

Table-5: Results of Intra day & Inter day

Conc. Of Palonosetron (API) (µg/ml)	Observed Conc. of Palonosetron (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.96	0.86	8.07	0.93
10	10.08	0.76	10.03	0.47
12	12.06	0.57	12.03	0.83

Linearity and Range [9]

Linearity range was found to be 0-70µg/ml for Palonosetron. The correlation coefficient was found to be 0.999, the slope was found to be 10380 and intercept was found to be 9304 for Palonosetron.

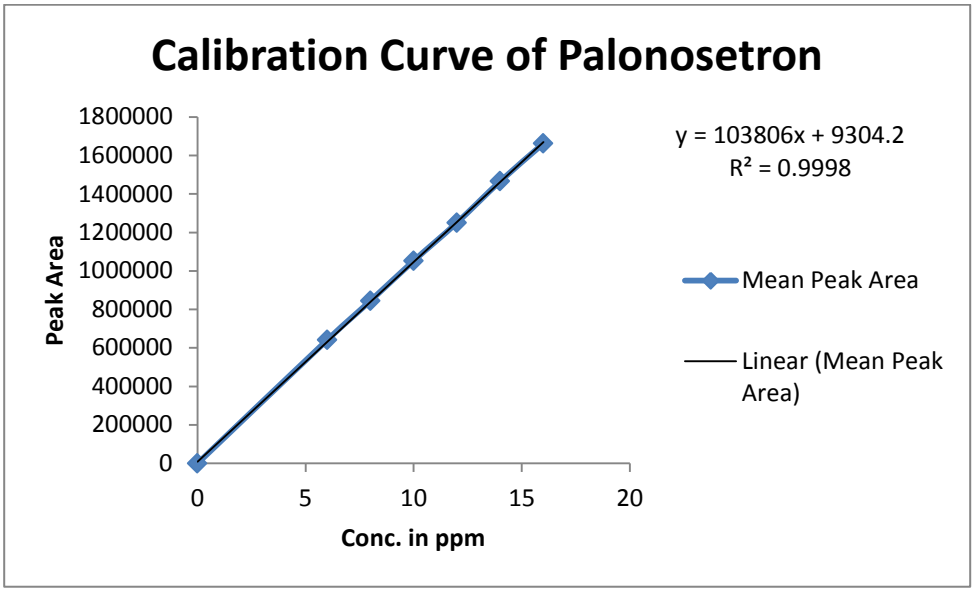


Fig-3: Calibration curve of Palonosetron (API)

Table-6: Linearity Results of Palonosetron[10]

S. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	641233
3	8	844610
4	10	1052647
5	12	1250435
6	14	1465354
7	16	1662043

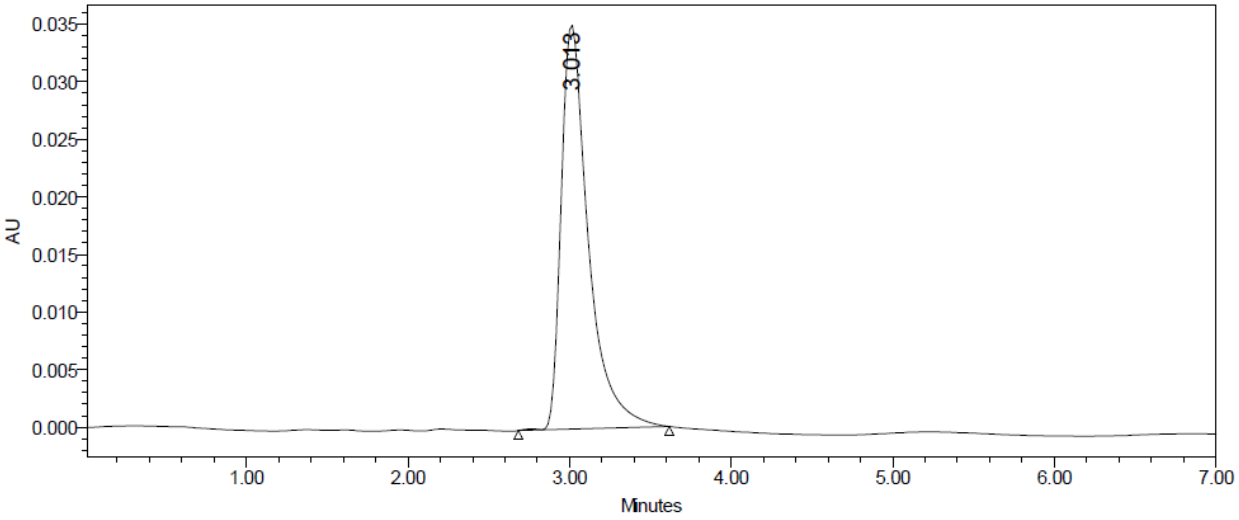


Figure-4: Chromatogram for Linearity-6

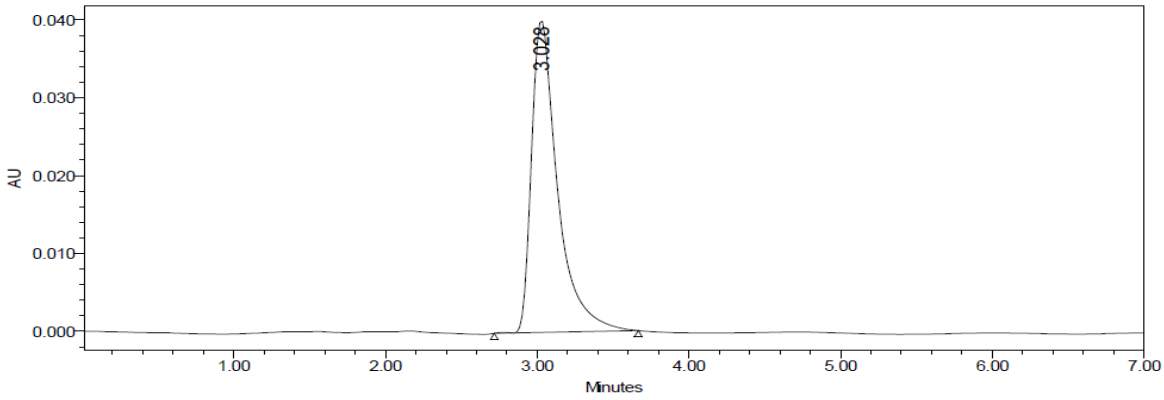


Figure-5: Chromatogram for Linearity-8

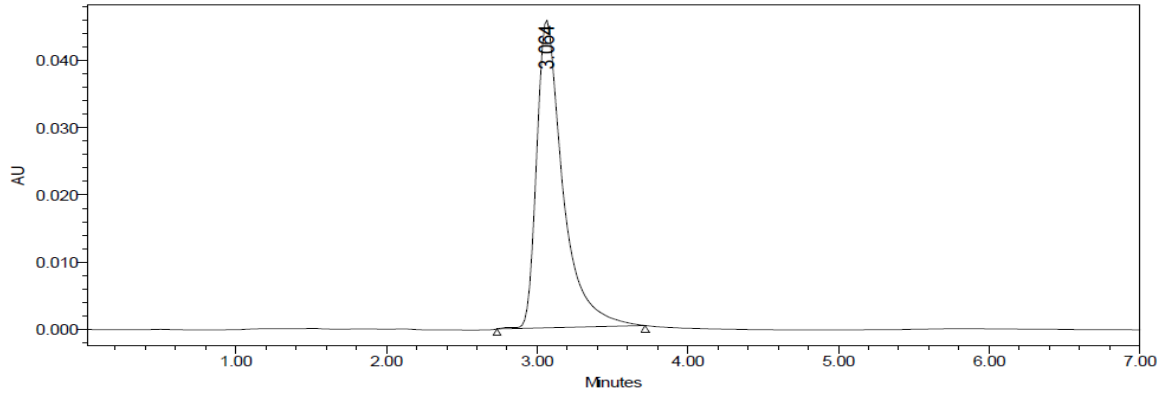


Figure-6: Chromatogram for Linearity-10

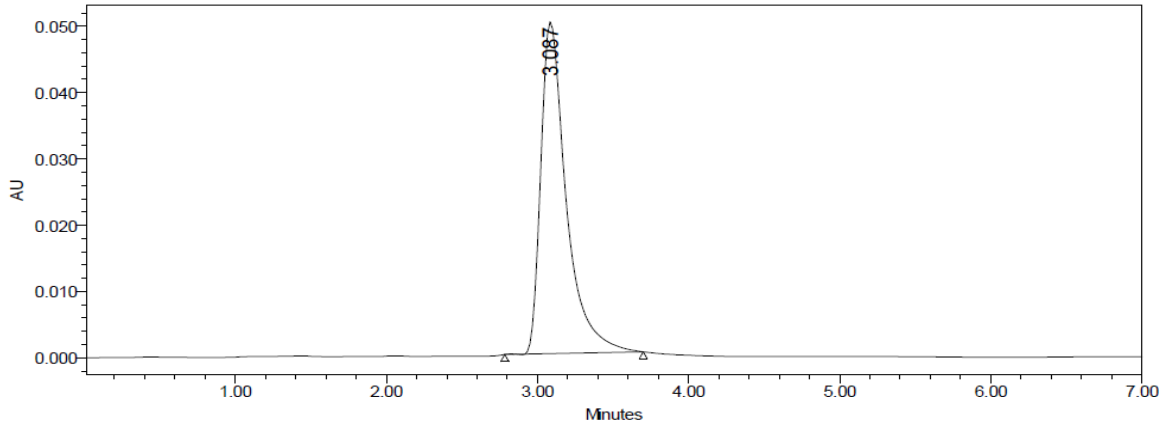


Figure-7: Chromatogram for Linearity-12

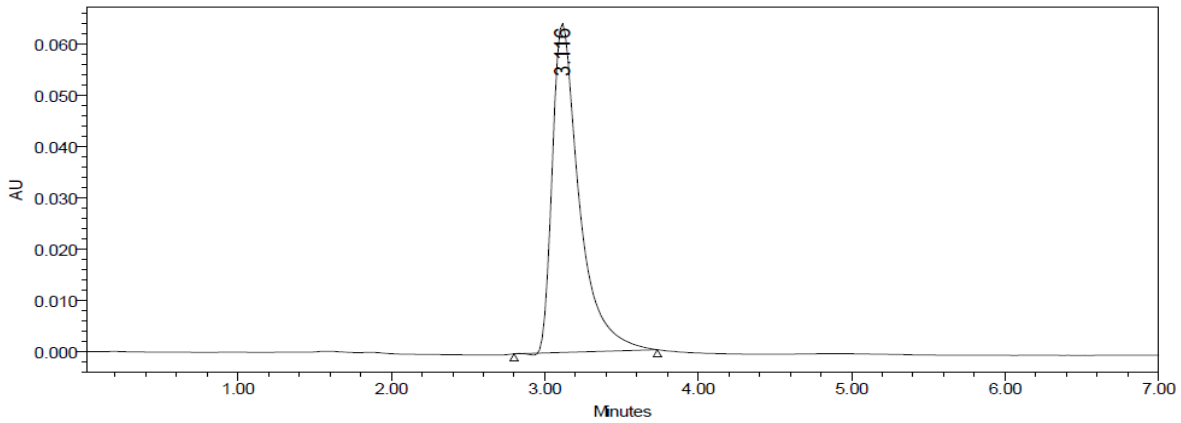


Figure-8: Chromatogram for Linearity-14

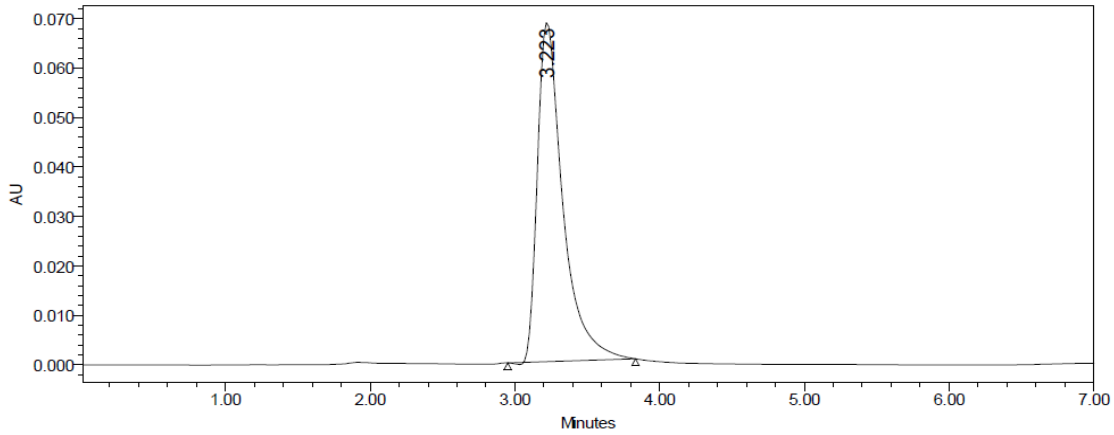


Figure-9: Chromatogram for Linearity-16

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.006 & 0.018 µg/ml respectively[11].

STABILITY STUDIES

ACID DEGRDATION [12]

A accurately estimated 10 mg of unadulte rated prescription was traded to a spotless and dry round base conta iner. 30 ml of 0.1 N HCl was added to it and it was reflfuxed in a water shower at 600C for 4 hours. Allowwed to cool to rooom cliamte. The case was then killed utilizing we.aken NaOH arrangement and last volume of the example was made up to 100ml with water to plan 100 µg/ml arrangem ent. It was infused into the HPLC framework against a clear of moveable stage (following to adva ncing the flexible stage creations). This examination was rehashed a few times utilizing same grouping of HCl (0.1N) and watched its debase.ment profile. The run of the mill chromatogram appeared underneath is the debase-ment pro-file of Palonosetron in 0.1N HCl.

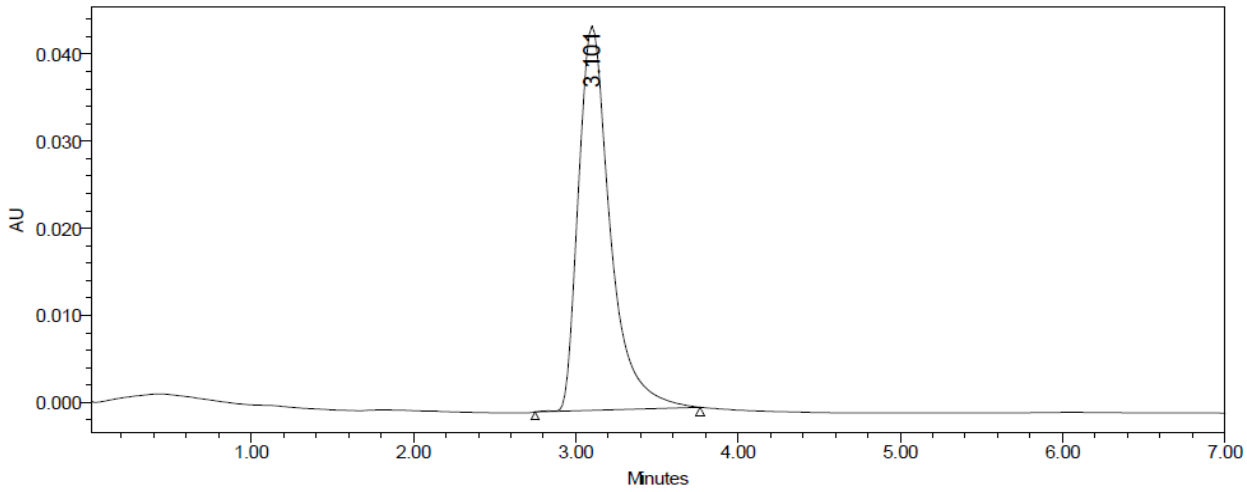


Fig-9: Chromatogram of acid hydrolysis of Palonosetron

BASIC DEGRADATION [13]

An accurately gauged ten mg of pure medication was changed to a clean and dry spherical base vessel. 30 ml of 0.1N NaOH was accessorial to that. And it had beenrefluxed in a very water shower at 600C for four hours. Permissible to chill to are a temporary worker. The arrangement was then killed by mistreatment 2N HCl arrange and last volume of the model was created up to one hundred ml to organize 100 µg/ml course of action. it had been imbued into the HPLC system against a transparent of versatile stage subsequent to streamlining the convenient stage associations. This trial was rehashed many times utilizing same convergence of NaOH, for instance, 0.1N to observe its debasement profile.

The recording appeared beneath is that the debasement profile of Palonosetron in zero.1N NaOH. Fig-40: recording of base chemical reaction of Palonosetron in 0.1N NaOH.

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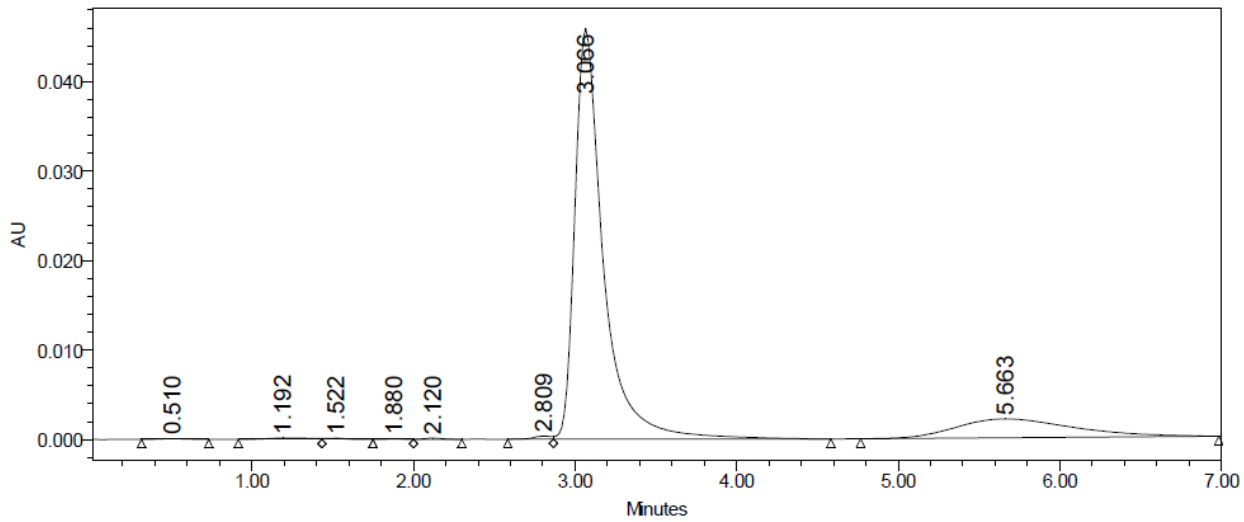


Fig-10: Chromatogram of basic hydrolysis of Palonosetron

THERMAL DEGRADATION [14]

An accurately gauged 10 mg of pure medication was modified to a clean and dry spherical base vessel. 30 ml of 0.1N NaOH was another to it. and it completely was refluxed throughout a water shower at 600C for four hours. Allowable to

relax to space employee. The preparement was then killed by practice 2N HCl arrange and last volume of the model was created up to at least one hundredml to prepare one hundred µg/ml course of action. it completely was imbued into the HPLC system against a clear of versatile stage following streamlining the convenient stage associations. This trial was rehashed some of times utilizing same convergence of NaOH, as associate example, 0.1N to seem at its debasement profile. The recording appeared to a lower place is that the debasement profile of Palonosetron in zero.1N NaOH. Fig-40: recording of base reaction of Palonosetron in zero.1N NaOH. arrangement. It was infused into the HPLC framework against a clear of versatile stage .

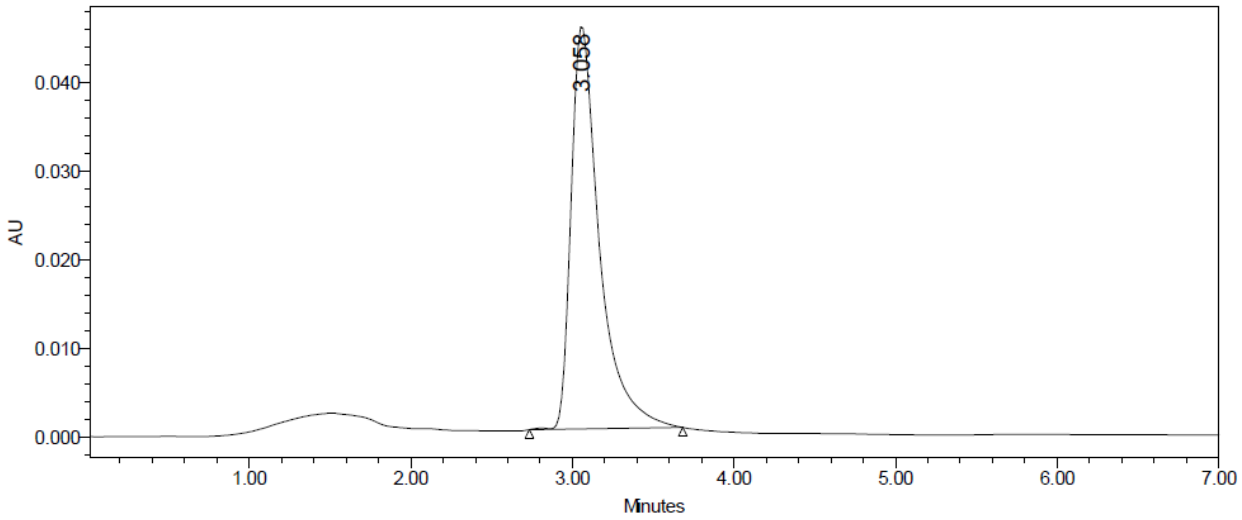
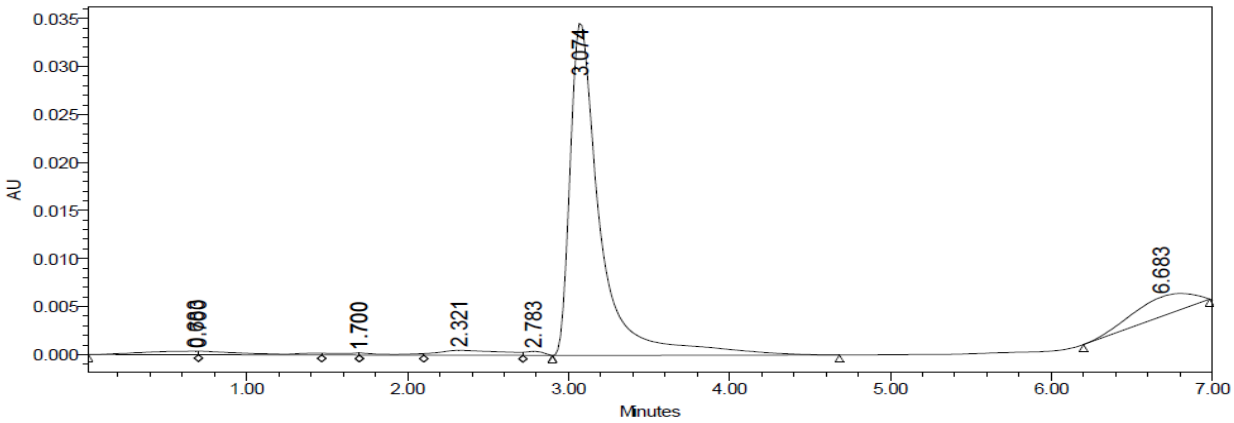


Fig-11: Chromatogram showing thermal degradation studies

PHOTOLYTIC DEGRADATION [15]

Approximately ten mg of pure drug was taken during a clean & dry dish. it absolutely was unbroken in Associate in Nursing ultraviolet light bureau at 254 nm wavelength for twenty-four hours while not intrusion. exactly measured one mg of the ultraviolet light uncovered medication was changed to a clean and dry ten milliliter meter cup. Initial the ultraviolet light exposed drug was dissolved in wood alcohol up to the mark with mobile part to urge one hundred µg/ml answer. Finally this answer was injected into the HPLC system against a blank of mobile part and recording was obtained.



Fig–12: Chromatogram showing photolytic degradation

OXIDATION WITH (3%) H^2O^2 [16]

Correctly estimated 10 mg of unadulterated prescription was taken in a perfect and dry 100 ml volumetric container. 30 ml of 3% H2O2 and a little methanol was added to it to make it dissolvable and then kept in that capacity in dim for 24 hours. Last volume was made up to 100 ml. Utilizing water to get ready 100 µg/ml arrangement. The above example was infused into the HPLC framework.

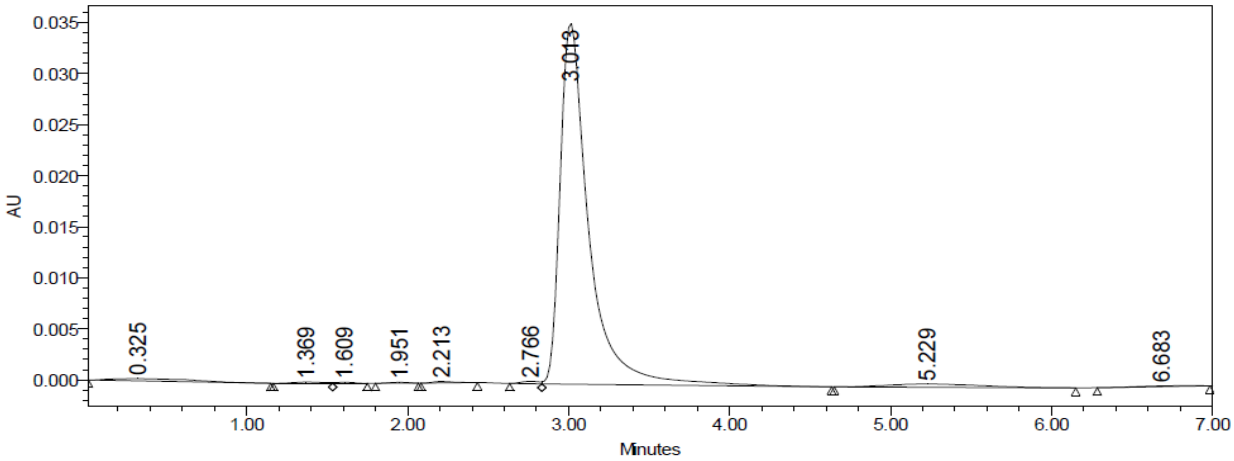


Fig-13: Chromatogram showing oxidative degradation.

Table-7: Results of forced degradation studies of Palonosetron API[17].

Stress condition	Time (hours)	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1N HCl)	24Hrs.	95.32	4.68	100.00
Basic Hydrolysis (0.IN NaOH)	24Hrs.	90.13	9.87	100.00
Thermal Degradation (50 0C)	24Hrs.	94.32	5.68	100.00
UV (254nm)	24Hrs.	84.71	15.29	100.00
3% Hydrogen peroxide	24Hrs.	73.16	26.84	100.00

CONCLUSION [18]

A delicate and specific, sensitive RP-HPLC strategy has been created and approved for the investigation of Palonosetron API.

Facilitate the proposed RP-HPLC strategy has amazing affectability, exactness and reproducibility.

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