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Development and Validation of Stability Indicating HPTLC Method for Estimation of Palonosetron Hydrochloride

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ABSTRACT

A simple and sensitive stability indicating HPTLC method has been developed and validated for estimation of Palonosetron hydrochloride. Separation of the drug was carried on aluminium plates precoated with silica gel 60 F_{254} using Ethyl acetate: Methanol: Triethylamine (6:3:1 v/v/v) as mobile phase. The retention factor (R_f) for Palonosetron hydrochloride was found to be 0.50 ± 0.04. The detection was carried at 242 nm. Stress testing of Palonosetron hydrochloride was carried out according to the International conference on harmonization (ICH) guideline Q1A (R2). The drug was subjected to acid, base, neutral hydrolysis, oxidation, thermal degradation and photolysis. Palonosetron hydrochloride showed considerable degradation under oxidative condition. The method was successfully validated according to ICH guidelines Q2 (R1). The data of linear regression analysis indicated a good linear relationship over the range of 250–1500ng/band concentrations with correlation coefficient 0.994. The accuracy of the method was established based on the recovery studies. The LOD and LOQ were 11.81ng/band and 35.78ng/band respectively.

Keywords: Palonosetron hydrochloride, High Performance Thin Layer Chromatography (HPTLC), Validation, Stability-Indicating Method.

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INTRODUCTION

Palonosetron HCl is an anti-emetic drug. Chemically (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), with molecular formula of $C_{19}H_{24}N_2O.HCl$ and it has the molecular weight of 332.87. Palonosetron belongs to the class of isoquinolines. Palonosetron is the latest potent and selective second generation 5-HT3 receptor antagonist. It is the only drug of its class approved for prophylaxis against both acute and delayed chemotherapy-induced nausea and vomiting (CINV). Far higher receptor affinity and a much longer half-life (approximately 4Hrs) than other 5-HT3 antagonists confer a prolonged duration of action. Its use for post operative nausea and vomiting (PONV) prophylaxis was approved by the Food and Drug Administration (FDA) in March 2008 following successful Phase III clinical trials[1]. Literature survey reveals the following methods: Estimation of Palonosetron hydrochloride by UV spectrophotometric method[2], estimation in parenterals and bulk dosage form by RP-HPLC[3, 4, 5], stability indicating LC-MS[6], determination in plasma by HPLC and UPLC[7, 8]. The present work describes a simple stability indicating HPTLC method for the determination of Palonosetron hydrochloride, according to the international conference on harmonization (ICH) guidelines[9-11]

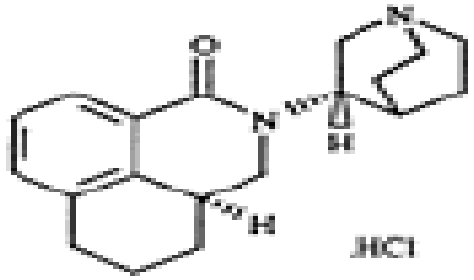

Figure 1: Chemical structure of Palonosetron Hydrochloride

MATERIALS AND METHOD

Chemicals and reagents

Palonosetron hydrochloride was provided as a gift sample by Emcure pahramaceuticals ltd, Pune and used as such, without any further purification. Aluminum sheets precoated with silica gel (60 F_{254}, 20 cm × 20 cm with 250 µm layer thickness) were purchased from E-Merck, Darmstadt, Merck (Germany). Methanol (AR grade), Ethyl acetate (AR grade), Triethylamine were purchased from S. D. fine chemical Laboratories, Mumbai. Hydrochloric acid (HCl), Hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH) were purchased from LOBA CHEMIE PVT. LTD. Mumbai.

Chromatographic conditions and instrumentation

Chromatographic separation of drug was performed on Aluminum plates. Samples were applied on the plate as a band of 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Ethyl acetate: Methanol: TEA (6: 3: 1 v/v/v). 10 cm × 10 cm CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 20mins of chamber saturation time and 10mL of mobile phase was used per run, migration distance was 80mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 400-200 nm, operated by winCATS software (Version 1.4.3, Camag), slit dimensions were 5.00 x 0.45mm and Deuterium lamp was used as a radiation source.

Selection of detection wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200 – 400 nm and the spectrum was obtained. It was observed that the drug showed considerable absorbance at 210 and 242 nm (Figure 2).

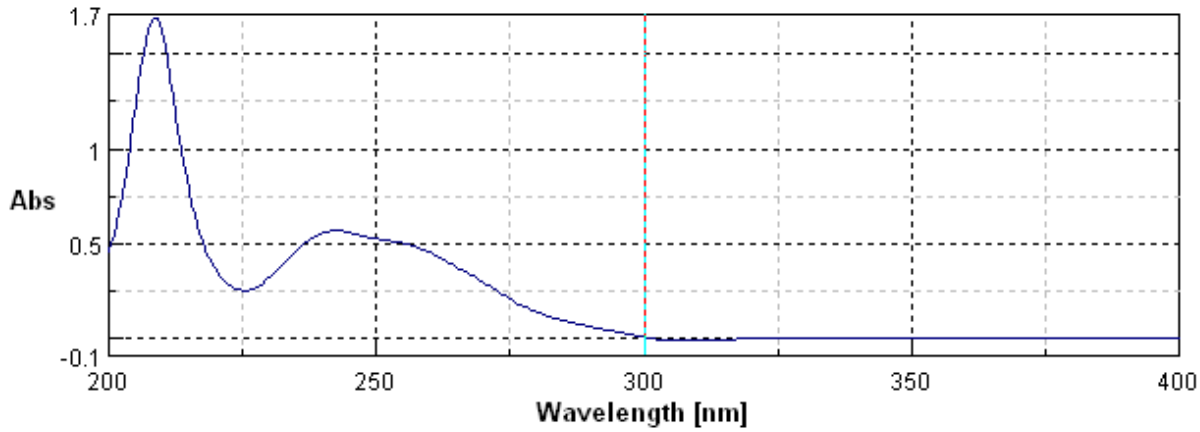

Figure 2: UV Spectrum of Palonosetron Hydrochloride (20µg/ml)

Preparation of standard stock solution

Standard stock solution of Palonosetron hydrochloride was prepared by dissolving 5mg of drug in 10ml of water to get concentration of 500µg/ml. From the standard stock solution, working standard solution was prepared in methanol to contain 50µg/ml of Palonosetron hydrochloride.

Preparation of sample solution

Strength of marketed injection: 0.25mg/5ml

Palonosetron Hydrochloride injection containing 500µg/ml (0.25mg/5ml) of the drug was diluted with methanol to make the final concentration of 50µg/ml and applied on TLC plate (volume 10uL).

Densitogram:

Solution of Palonosetron hydrochloride (50µg/ml) was prepared. 15µl (750ng/band) of solution was applied on pre-activated TLC plate with the help of Hamilton syringe (100µl), using Linomat5 sample applicator. The development chamber was saturated with mobile phase for 20mins. The spotted plate was placed in the saturated chamber and developed up to 80mm distance. The plate was dried and was scanned over 90mm distance at 242nm. The retention factor was found to be: 0.50± 0.04 (Figure 3)

Stress Degradation Study of Bulk Drug

Stress degradation studies were carried under condition of acid/ base/ neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared as follows

1. Palonosetron hydrochloride working standard solution subjected to stress condition
2. The blank subjected to stress in the same manner as the drug solution.

Dry heat and photolytic degradation were carried out in solid state.

Stress conditions were optimized in terms of strength of reagent and time of exposure to achieve 10-30% degradation.

Degradation under alkali catalyzed hydrolytic condition

To 1mL of 500 µg.mL^{-1}solution of Palonosetron hydrochloride, 1mL of 0.2 N NaOH (methanolic) was added. The volume was made up to 10mL with methanol. The above solution was kept for 4 hours at room temperature in dark.

Degradation under acid catalyzed hydrolytic condition

To 1mL of 500 µg.mL^{-1}solution of Palonosetron hydrochloride, 1mL of 0.1N HCL (methanolic) was added. The volume was made upto 10mL with methanol. The above solution was kept for 4 hours at room temperature in dark.

Degradation under neutral hydrolytic condition

5mL of 500 µg.mL^{-1}solution of Palonosetron hydrochloride was taken in a round bottom flask, 5mL of distilled water was added. The volume was again made up to 50mL with distilled water. The above solution was refluxed for 8 hours. After cooling to room temperature the contents were transferred to a 50mL volumetric flask and the volume was again made up to 50mL.

Degradation under oxidative condition

To 1mL of 500 µg.mL^{-1}solution of Palonosetron hydrochloride, 1mL of 30% H_2O_2 was added. The volume was made upto 10mL with methanol. The above solution was refluxed for 2 hours at room temperature

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (100^0 C) for a period of 80 hours.

Photo-degradation studies

The photo degradation study of the drug was carried out by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2, and was subsequently exposed to cool white fluorescence light to achieve1.2million Lux-Hr.

RESULTS AND DISCUSSION

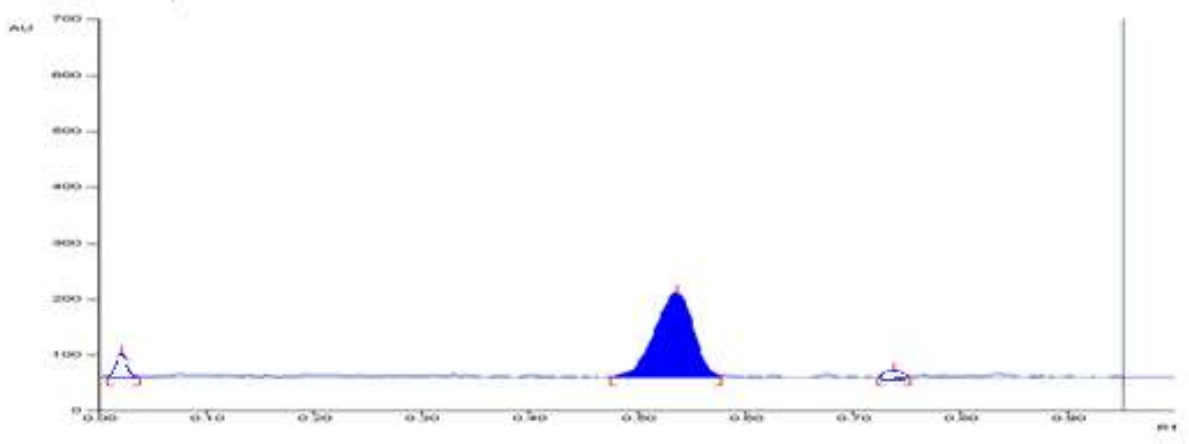

Figure 3: Densitogram of Standard Solution of Palonosetron Hydrochloride (750ng/band).

The drug showed considerable degradation under oxidative (28.68% with two product peaks) and alkaline (5.76% with one product peak) condition. The Densitogram (Fig. 4 and 7) along with the 3D display (Fig. 5 and 8) and overlay spectra (Fig.6 and 9) for oxidative and alkaline degradation are given below respectively.

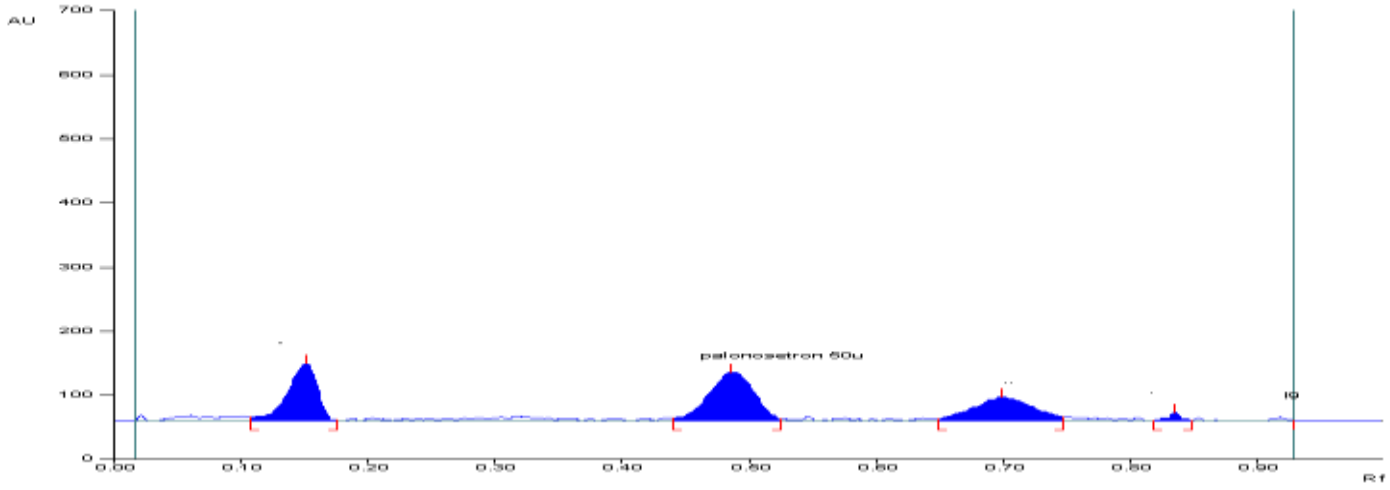

Figure 4: Densitogram of oxidative degradation

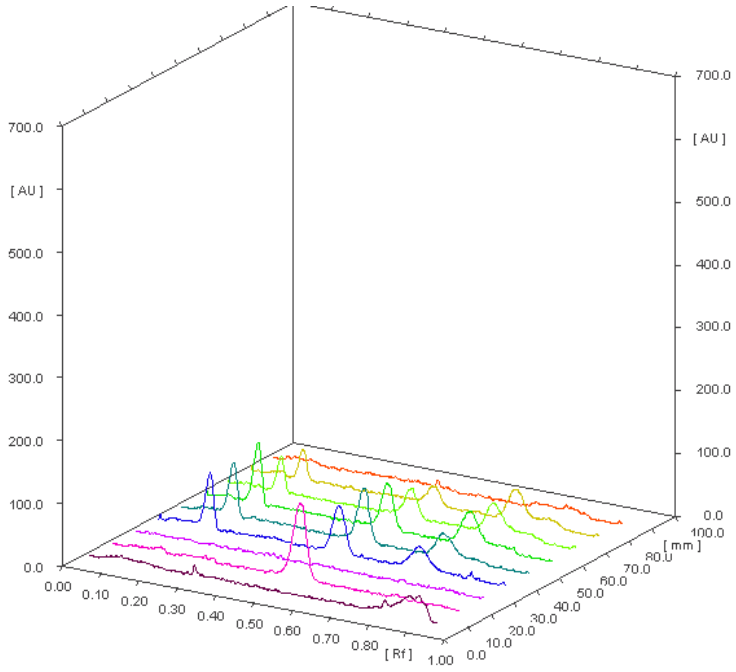

Figure 5: 3D display of oxidative degradation {Track 2: Std. Palonosetron, Track 4: 30% H_2O_2 2hrs stress sample, 750ng spot, Track 5, 6, 7, 8: 30% H_2O_2 4, 6, 8, 10hrs stress sample reply., 750ng spot}

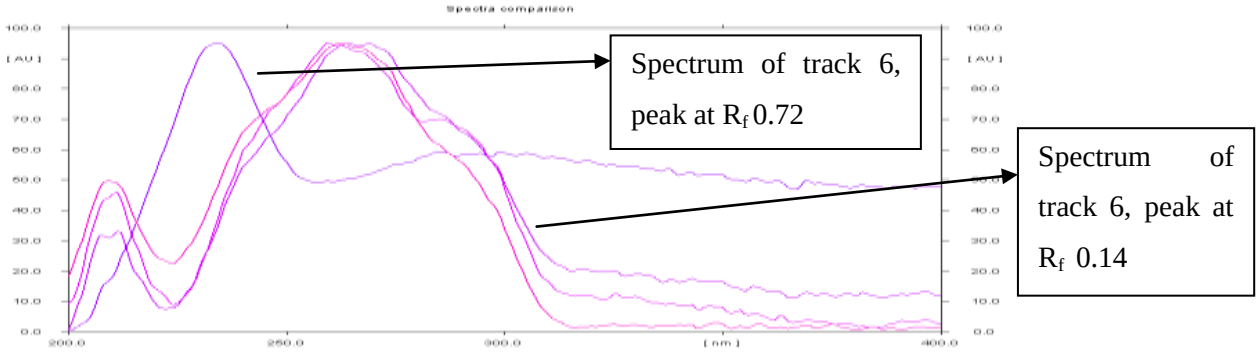

Figure 6: Overlay of Std. and degradation peaks

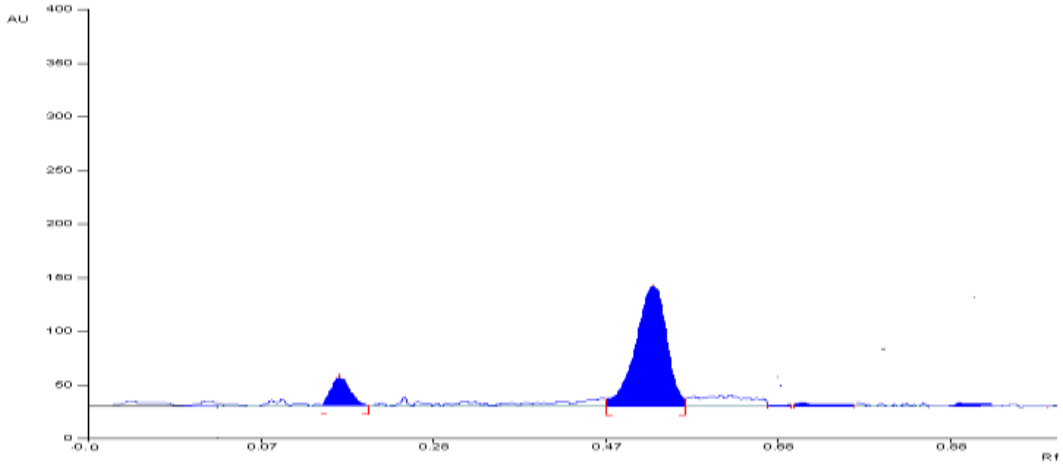

Figure 7: Densitogram of alkaline degradation

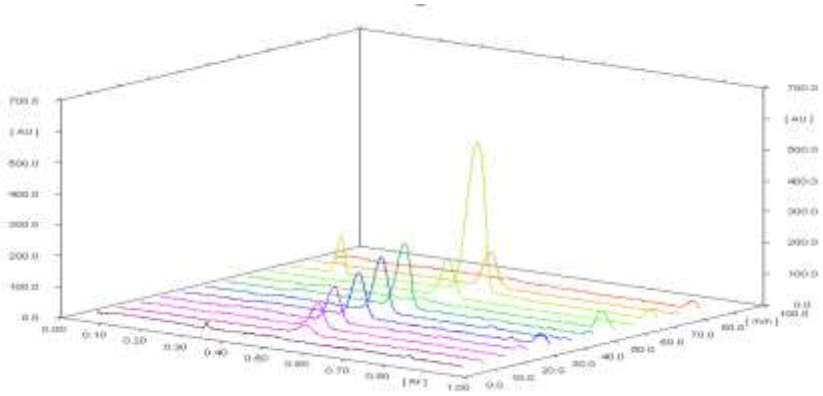

Figure 8: 3D display of alkaline degradation {Track 2-7: Std. Palonosetron linearity, Track 9, 11: 0.2N NaOH stress sample, 750ng spot, Track 10: 0.2N NaOH stress sample, 7500ng spot}

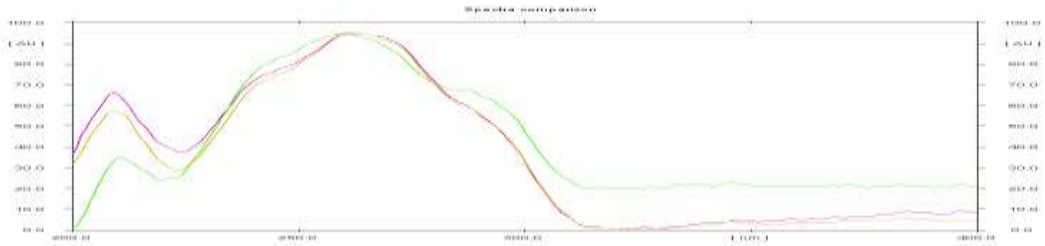

Figure 9: overlay spectra of Std., degradation peak and product peak at R_f 0.18; track 10

Extreme stress conditions for forced degradation

The drug was subjected to

1. 5N HCl and refluxed for 48Hrs at 100^0C
2. 2N NaOH and refluxed for 60Hrs at 90^0C (% degradation remained 5.76% with a product peak at R_f 0.18)
3. Refluxed with water for 48Hrs at 100^0C

However marginal degradation was observed. Hence, the drug is practically stable under acidic, neutral, thermal and photolytic conditions.

Summary of stress degradation studies is shown in table.1

Table 1: Summary of Stress Degradation Studies

Stress Degradation Condition	Palonosetron					
	Peak Area	Percent Recovery (%)	Percent degraded (%)	Rf of the product peak	Peak Purity	
Initial	5063.1	100%	0%	_	R (s,m)	R (m,e)
					0.999898	0.999897
Base (0.2N kept for 4Hrs)	4569.2	94.24%	5.76%	0.18	0.999383	0.997882
Acid (0.1N kept for 4Hrs)	4678.1	96.55%	3.45%	_	0.999384	0.979726
H_2O_2 30% (reflux for 2	3485.8	71.32%	28.68%	0.14, 0.72	0.998884	0.991154

Hrs)						
Neutral (8Hrs reflux)	4799.2	99.11%	0.89%	_	0.998895	0.991155
Dry heat (100^0C, 10 days)	4790.1	98.92%	1.08%	_	0.999646	0.997883
Photo stability(UV, 200 watt hrs/square meter and Florescence 1.2 million Lux. Hrs)	4700.5	97.02%	2.98%	_	0.999484	0.99787

The drug was found to be practically stable under acid, neutral, thermal and photolytic conditions.

Validation of Analytical Method

Specificity

The specificity of the method was ascertained by peak purity profiling studies. Wincats software has provision to compare UV spectrum of peak at start, max and end position peak purity, values are assigned based on this comparison. The peak purity values were found to be more than 0.991, indicating the non interference of any other peak of degradation product or impurity, at the R_f of Palonosetron HCl

Linearity

From the standard stock solution (500µg/ml) of Palonosetron hydrochloride, solution was prepared containing 50µg/ml of Palonosetron hydrochloride. This solution was further used for spotting. Five replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 250-1500ng/band. The equation of the calibration curve was found to be –

y = 6.301x+115.2; the coefficient of determination was found to be 0.994 respectively. (Figure 10)

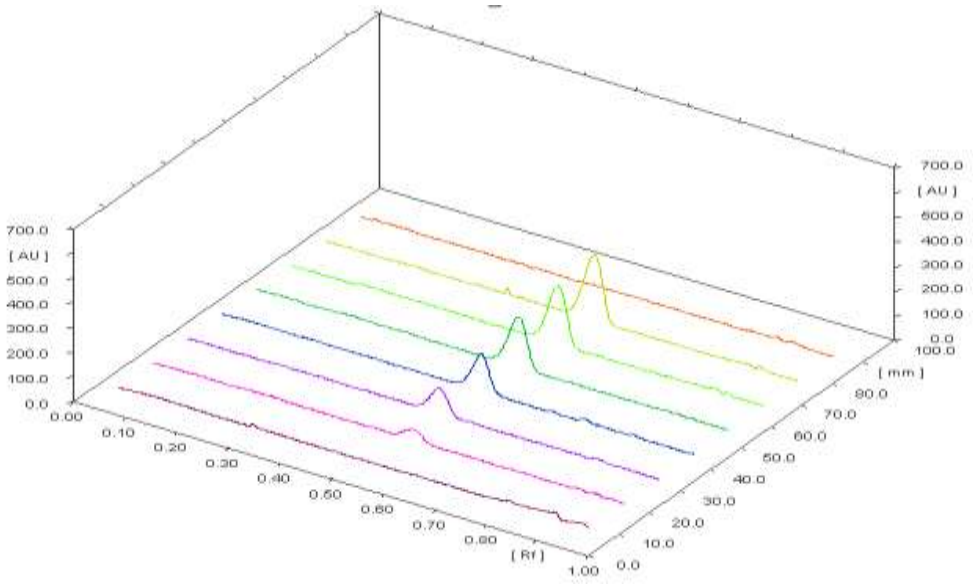

Figure 10: Densitogram of linearity of Palonosetron Hydrochloride (250-1500ng/band) Range

Palonosetron hydrochloride - 250-1500ng/band

Precision

The system precision was demonstrated by Intra-day and Inter-day variation studies. In the Intra-day studies 6 replicates of a concentration were analyzed on the same day, and percentage RSD was calculated. For the inter day variation studies, 3 replicates of 3 concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. For intraday precision % RSD found to be 0.36% (Table.2) and for inter-day precision % RSD found to be 0.86% (Table.3).

Table.2: Intra-day precision of Palonosetron Hydrochloride

Concentration (ng/spot)	Area	SD	Mean % RSD
750	5044.1	18.55	0.36
	5055.8		
	5089.5		
	5047.3		
	5078.5		
	5075.2		

Table 3: Inter-day precision of Palonosetron Hydrochloride

Concentration (ng/spot)	Area	SD	% RSD	Mean % RSD
500	2227.9	11.00	0.49%	0.86%
	2249.9			
	2239.9			
750	3219.9	34.54	1.06%	
	3238.8			
	3286.1			
1000	4174.0	42.77	1.03%	
	4104.1			
	4186.1			

Assay:

For analysis of Palonosetron hydrochloride injection, it was diluted as mentioned under section sample solution. Analysis was repeated six times. 10µl was spotted on TLC plate and it was developed. Peak area was recorded. % assay was determined from linearity equation (Table.4).

*Thus method precision was 1.15%

Accuracy:

To check accuracy of the method, recovery studies were carried by spiking the standard drug in sample formulation, at three different levels 80, 100 and 120 %, Basic concentration of formulation sample chosen was 500ng/band. % recovery was determined from linearity equation. The results obtained are shown in (Table.5)

*Mean area of 3 determination

Table 4: Assay of Palonosetron hydrochloride Formulation

Sr. No.	Peak area of Palonosetron hydrochloride	Amount Recovered (ng/band)	% Recovery
1	3290.9	503.9994	100.7999
2	3295.8	504.777	100.9554
3	3297.8	505.0944	101.0189
4	3250.8	497.6353	99.52706
5	3210.5	491.2395	98.2479
6	3300.5	505.5229	101.1046
Mean	3274.383	501.3781	100.2756
%RSD	1.109505	1.149964	1.149964

Table.5: Recovery studies Palonosetron Hydrochloride

Level	Conc. (ng/band)		Area	Mean	Amount recovered	% Recovery ± SD mm% Recovery ±SD
	Sample	Std.				
80 %	500	400	5850.7 5890.5 5785.5	5842.2	5727.0	100.9±0.92
100 %	500	500	6500.5 6508.5 6480.5	6496.5	6381.3	101.2±0.22
120 %	500	600	7000.5 7010.5 7050.8	7020.6	6905.4	99.62±0.38

Limit of detection and quantification (LOD and LOQ):

From the linearity data the limit of detection and Quantitation was calculated, using the following formula.

LOD= 3.3 σ/ S and LOQ = 10 σ/ S

σ = standard deviation of the lowest response of linearity equation.

S = slope of the calibration curve of the analyte.

LOD=11.81ng/ band

LOQ= 35.78ng/band

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, detection wavelength , chamber saturation time were altered, time was also changed from spotting to development and development to scanning and the effects on the area were noted. It was found that method is robust (Table.6). Comparison of Results with Reported Stability Indicating LC-MS Method (Ref no. 6) was done (table. 7)

Table 6: Robustness Study

Sr. No.	Parameters	Robust condition	% RSD
1.	Saturation time (20min) ± 2 min.	18min	0.16
		22min	0.41
2.	Mobile phase composition Ethyl Acetate: Methanol: TEA (6: 3: 1 v/v/v) ±0.2ml	Ethyl Acetate: Methanol: TEA (5.8: 3.2: 1 v/v/v)	0.67
		Ethyl Acetate: Methanol: TEA (6.2: 2.8: 1 v/v/v)	0.36
3	Time from spotting to development (immediate)	After 30min.	0.43
		After 1hr	0.54
4	Time from development to scanning (immediate)	After 30min.	1.45
		After 1hr	1.78

Table 7: Comparison of Results with Reported Stability Indicating LC-MS Method[6]

Stress degradation condition	% degradation reported	% degradation obtained
Base (2N NaOH and refluxed for 60Hrs at 90^0C)	2.7% with two product peaks	5.76% with one product peak
Acid (5N HCl refluxed for 48Hrs at 100^0C)	1.2% with five product peaks	3.45% with no product peaks
Neutral (refluxed for 48Hrs at 100^0C)	4.7% with four product peaks	0.89% with no product peaks
Dry heat (100^0C 80Hrs)	No degradation	1.08%
Photo stability(UV, 200 watt hrs/m^2, Florescence 1.2 million Lux. Hrs)	No degradation	2.98%
Oxidation	3% H_2O_2 refluxed at 100^0C for 1Hr	30% H_2O_2refluxed at 100^0C for 2Hrs
	5.8% with five product peaks	28.68% with two product peaks

Table.8: Summary of Validation Parameters

Sr. No.	Validation Parameter	Results
1.	Linearity	Y= 6.301x+115.2
2.	Range	250– 1500ng/band
3.	Precision	% RSD
	A) Intra-day precision	0.36%
	A) Inter-day precision	0.68%
4.	Accuracy	% Recovery
	80%	100.9
	100%	101.2
	120%	99.62
5.	LOD	11.81ng/band
6.	LOQ	35.78ng/band
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION

The developed method was found to be simple, sensitive, specific, accurate, and repeatable for analysis of Palonosetron hydrochloride in the formulation without any interference from the excipients. The result of validation parameters are summarized in table.8, The results indicated the suitability of the method to study stability of Palonosetron hydrochloride under various forced degradation conditions.

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