

Stability-indicating HPTLC Method for Estimation of Palonosetron Hydrochloride

P. S. Jain*, R. S. Chavan , P. R. Bari, S. S. Patil and S. J. Surana

Abstract -A simple, selective, precise and Stability-indicating High-performance thin-layer chromatographic method for analysis of palonosetron hydrochloride both in a bulk and in pharmaceutical formulation has been developed and validated. The method employed, HPTLC aluminium plates precoated with silica gel as the stationary phase. The solvent system consisted of methanol: dichloromethane: ethyl acetate: glacial acetic acid (3: 7: 1: 0.5 v/v). The system was found to give compact spot for palonosetron hydrochloride (R_f value of 0.23 ± 0.05). Densitometric analysis of palonosetron hydrochloride was carried out in the absorbance mode at 241 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.994 \pm 0.0005$ with respect to peak area in the concentration range 400 - 2400 ng per spot. The method was validated for precision, recovery and robustness. The limits of detection and quantification were 76.12 and 230.65 ng per spot, respectively. Palonosetron hydrochloride was subjected to acid and alkali hydrolysis, oxidation and thermal degradation. The drug undergoes degradation under acidic, basic, oxidation and thermal conditions. This indicates that the drug is susceptible to acid, base, oxidation and thermal conditions. The degraded product was well resolved from the pure drug with significantly different R_f value. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of investigated drug. The proposed developed HPTLC method can be applied for identification and quantitative determination of palonosetron hydrochloride in bulk drug and parenteral dosage form.

Keywords- Palonosetron hydrochloride; HPTLC; Validation; Stability; Degradation.

1. Introduction

Palonosetron hydrochloride is a potent and highly selective 5-HT₃ receptor antagonist recently approved by the US Food and Drug Administration (FDA) for the prevention of acute and delayed chemotherapy-induced nausea and vomiting.^[1] It is chemically known as (3aS)-2-[(S)-1-azabicyclo [2.2.2] oct-3-yl]-2,3,3a,4,5,6-hexahydro-1H-benz [de] isoquinolin-1-one hydrochloride^[2], has the empirical formula $C_{19}H_{24}N_2O \cdot HCl$ with a molecular weight of 332.87.^[3] Palonosetron hydrochloride 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. Palonosetron hydrochloride injection is a sterile, clear, colorless, non-pyrogenic, isotonic, buffered solution for intravenous administration.^[4] Palonosetron hydrochloride is administered intravenously, as a single dose, 30 minutes before chemotherapy, or as a single oral capsule one hour before chemotherapy. Literature survey reveals that palonosetron hydrochloride can be estimated by HPLC method.^[5]

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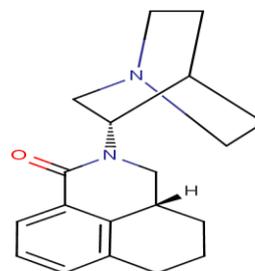


Fig. 1: Chemical structure of Palonosetron HCl

Various methods have been reported for the estimation of palonosetron hydrochloride in biological matrices such as plasma, urine which includes radioimmunoassay was high sensitive and selective but radiation was harmful to operators and environment Literature survey reveals that palonosetron hydrochloride can be estimated by HPLC method.^[5] Various methods have been reported for the estimation of palonosetron hydrochloride in biological matrices such as plasma, urine which includes radioimmunoassay was high sensitive and selective but radiation was harmful to operators and environment , Some LC-MS/MS methods were developed to determine palonosetron hydrochloride in human plasma,^[6] ultra performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS) method.^[7] However to our knowledge no information related to the stability indicating high performance thin-layer

chromatography (HPTLC) determination of palonosetron hydrochloride in pharmaceutical dosage forms has ever been mentioned in literature. HPTLC is a widely used analytical technique due to its advantages of low operating cost, high sample throughput, and minimum sample preparation requirement. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus reducing the analysis time and cost per analysis.^[8]

Hence, the objective of the present study was to develop a stability-indicating HPTLC method^[9] for estimation of palonosetron hydrochloride as bulk drug and in formulations and to perform stress studies under a variety of ICH-recommended test conditions.^[10] The proposed method was validated for linearity, accuracy (recovery studies), specificity, precision, robustness, ruggedness, LOD (limit of detection), LOQ (limit of quantitation), and repeatability according to the ICH guidelines^[11,12] and its updated international convention.^[13]

2. Experimental

2.1 Chemicals and reagents

Palonosetron hydrochloride working standard was obtained as gift sample from Dr. Reddy's Pvt. Ltd. The drug was used without further purification. A parenteral formulation containing 0.25 mg of palonosetron hydrochloride was purchased from local market. Analytical grade solvents were used for the experiment.

2.2 HPTLC instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on precoated silica gel aluminium plates 60 RP-18 F₂₅₄ (10 × 10 cm with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The plates were prewashed with methanol and activated at 60 °C for 5 min prior to chromatography. The slit dimension was kept at 6.00 × 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of methanol: dichloromethane: ethyl acetate: glacial acetic acid (3: 7: 1: 0.5 v/v), and 10 ml of mobile phase was used. Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C±2). The length of the chromatogram run was approximately 8 cm. Subsequent to development; the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by WINCats software.

2.3 Preparation of standard solution and linearity study

An accurately weighed quantity of 5 mg palonosetron hydrochloride was transferred to 100 ml volumetric flask, dissolved in ethanol and volume was made up to mark with the same solvent to obtain concentration 50 ng/μl. Aliquots of standard solutions 8, 16, 24, 32, 40 and 48 μl of palonosetron hydrochloride was applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtained the concentration of 400, 800, 1200, 1600, 2000 and 2400 ng per spot. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated six times.

2.4 Method validation

2.4.1 Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (1200 ng per spot of palonosetron hydrochloride). The intra and inter-day variation for the determination of palonosetron hydrochloride was carried out at three different concentration levels of 800, 1200 and 1600 ng per spot.

2.4.2 Limit of detection (LOD) and limit of quantification (LOQ)

In order to determine detection and quantification limit, palonosetron hydrochloride concentrations in the lower part of the linear range of the calibration curve were used. palonosetron hydrochloride solutions of 400, 500, 600, 700 and 800 ng/spot were prepared and applied in triplicate. The LOQ and LOD were calculated using equation $LOD = 3.3 \times N/B$ and $LOQ = 10 \times N/B$, where, N is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

2.4.3 Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for palonosetron hydrochloride in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of palonosetron hydrochloride was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex (M) and peak end (E) positions of the spot.

2.4.4 Ruggedness

Ruggedness of the method was performed by spotting 1200 ng of palonosetron hydrochloride by two different analyst keeping same experimental and environmental conditions.

2.4.5 Accuracy

The analysed samples were spiked with extra 80, 100 and 120% of the standard palonosetron hydrochloride and the mixture were analysed by the proposed method. At each level of the amount, six determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

2.4.6 Robustness

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of methanol: dichloromethane: ethylacetate: glacial acetic acid (3.3:7:1:0.5 and 2.8:7:1:0.5, v/v) were tried and chromatograms were run. The amount of mobile phase, mobile phase volume and saturation time was varied in the range of $\pm 5\%$.

2.5 Application of proposed method to parenteral formulation

To determine the concentration of palonosetron hydrochloride in parenteral dosage form (labeled claim: 0.25 mg/5ml), The above solution (1200 ng per spot) was applied on TLC plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate.

2.6. Forced degradation of Paonosetron Hydrochloride

2.6.1. Acid and base induced degradation

The 10 mg of palonosetron hydrochloride was separately dissolved in 10 ml of ethanolic solution of 5 N HCl and 2 N NaOH. These solutions were kept for 6 h at 70°C temperature in the dark in order to exclude the possible degradative effect of light. The 1ml of above solutions was taken and neutralized, then diluted up to 10 ml with ethanol. The resultant solution were applied on HPTLC plate (12 μ l each, i.e. 1200 ng per spot). The chromatograms were run as described in Section 2.2.

2.6.2. Hydrogen peroxide-induced degradation

The 10 mg of palonosetron hydrochloride was separately dissolved in 10 ml of ethanolic solution of hydrogen peroxide (3.0%, v/v). The solution was kept for 1 h at 70°C temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution was applied on HPTLC plate in triplicate (12 μ L, i.e. 1200 ng per band). The chromatograms were run as described in Section 2.2.

2.6.3 Photochemical Degradation

The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 6 h. The resultant solution (12 μ L, i.e. 1200 ng per band) was applied on a HPTLC plate and chromatograms were run as described in section 2.2.

2.6.4 Dry heat degradation Product

The powdered drug was stored at 70°C for 6 h under dry heat condition. No degradation was observed. In

all degradation studies, the average peak areas of palonosetron hydrochloride after application (12 μ L, i.e. 1200 ng per band) of three replicates were obtained.

3. Results and discussion

3.1 Development of optimum mobile phase

TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, methanol: dichloromethane: ethylacetate: glacial acetic acid (2: 8: 1: 0.5v/v) gave good resolution with R_f value of 0.20 for palonosetron hydrochloride but typical peak nature was missing. Finally, the mobile phase consisting of methanol: dichloromethane: ethylacetate: glacial acetic acid (3: 7: 1: 0.5v/v) gave a sharp and well defined peak at R_f value of 0.29 (Fig. 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

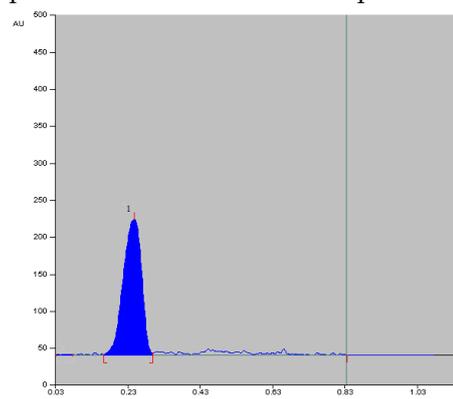


Fig. 2: A typical HPTLC chromatogram of Palonosetron Hydrochloride ($R_f = 0.29$) in methanol: dichloromethane: ethyl acetate: glacial acetic acid (3: 7: 1: 0.5 v/v) at 241 nm.

3.2 Calibration curve

The linear regression data for the calibration curves showed good linear relationship over the concentration range 400-2400 ng/spot. (Table 1)

Table 1 Linear regression data for the calibration curves^a

Linearity range (ng per spot)	400 -2400
$R^2 \pm S.D.$	0.994 ± 108.99
Slope $\pm S.D$	4.088 ± 108.99
Intercept $\pm S.D$	1320 ± 108.99

^a $n = 3$.

3.3 Validation of method

3.3.1 Precision

The precision of the developed HPTLC method was expressed in terms of % relative standard deviation

(% R.S.D.). The results depicted revealed high precision of the method is presented in Table 2.

Table 2: Intra-day and Inter-day precision of HPTLC method

Drugs	Conc. ng/spot	Inter-day		Intra-day	
		S.D.	% R.S. D.	S.D.	% R.S.D
PALONOSETRON	800	58.854	1.295	67.925	1.494
	1200	53.146	0.838	69.901	1.097
	1600	116.45	1.448	116.076	1.455

* mean of three estimations

3.2.2 LOD and LOQ

Detection limit and quantification limit was calculated by the method as described in section 2.4.2. The LOQ and LOD were found to be 76.12 and 61.68 ng respectively. This indicates that adequate sensitivity of the method.

3.2.3 Recovery studies

The proposed method when used for extraction and subsequent estimation of palonosetron hydrochloride from the pharmaceutical dosage form after over spotting with 80, 100 and 120 % of additional drug; afforded good recovery of palonosetron hydrochloride. The amounts of drug added and determined and the % recovery are listed in Table 3.

Table 3: Recovery study

Drug	Label claim (mg/tablet)	Amount of standard drug added (%)	Drug Recovered	%R.S.D.
PALONOSETRON	0.25	80	100.49	0.80
		100	100.58	0.97
		120	100.54	1.33

* mean of three estimations at each level

3.3.4. Specificity

The peak purity of palonosetron hydrochloride was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., $r_2(S, M) = 0.9998$ and $r_2(M, E) = 0.9988$. Good correlation ($r^2 = 0.9989$) was also obtained between standard and sample spectra of palonosetron hydrochloride (Fig. 3).

3.3.5. Robustness of the method

The standard deviation of peak areas was calculated for each parameter and %R.S.D. was found to be less than 2%. The low values of %R.S.D. values as indicated are shown in Table 4 indicated robustness of the method.

Table 4: Robustness of the method ^a

Parameter	S.D. of peak area	% R.S.D.
Mobile phase composition	33.8	0.82
Mobile phase volume	48.47	1.18
Duration of saturation	39.02	0.97

^a n=3

3.4 Analysis of the marketed formulation

A single spot at R_f 0.23 was observed in the chromatogram of the drug samples extracted from

tablets. There was no interference from the excipients commonly present in the tablet. The % drug content and % RSD were calculated. The low

% RSD value indicated the suitability of this method for the routine analysis of palonosetron hydrochloride in pharmaceutical dosage forms.

3.5 Force degradation

The chromatogram of the acid degraded samples for palonosetron hydrochloride showed additional peak at R_f value of 0.25 (Fig. 4a), base degraded drug shows at 0.27 (Fig. 4b), hydrogen peroxide shows at 0.27 (Fig. 4c) respectively. The chromatograms of photo-degraded drugs and dry heat degraded drugs was not shows degradation. The spot of the degraded product was well resolved from the palonosetron hydrochloride spot. In both cases, the concentration of the drug was changing from the initial concentration, indicating that palonosetron hydrochloride undergoes degradation under acidic, basic and oxidative conditions. The lower R_f values of degraded components indicated that they were less polar than the analyte itself. The results are listed in Table 5.

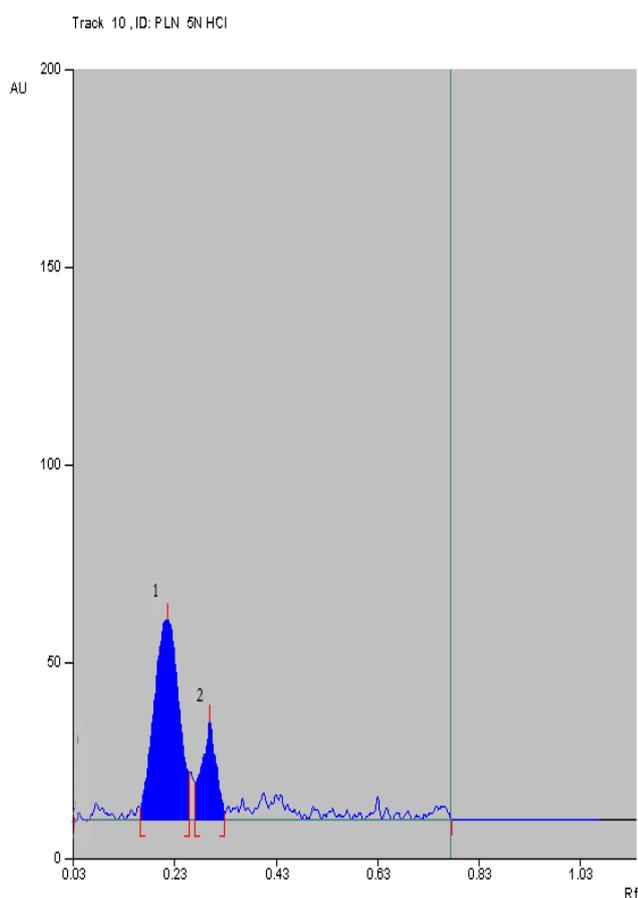


Fig. 4a: HPTLC Chromatogram of acid (5 N HCl, 6h, 70°C.) treated Palonosetron Hydrochloride; peak 1 (Palonosetron Hydrochloride) (R_f : 0.21), peak 2 (impurity) (R_f : 0.25).

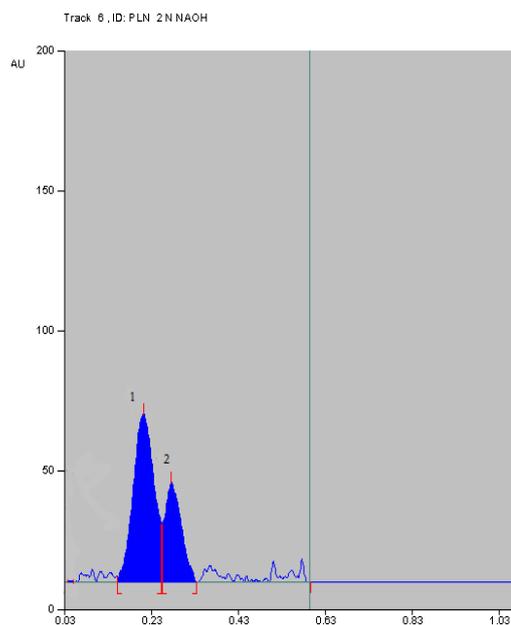


Fig. 4b: HPTLC Chromatogram of acid (2N NaOH, 6h, 100°C.) treated Palonosetron Hydrochloride; peak 1 (Palonosetron Hydrochloride) (R_f : 0.22), peak 2 (impurity) (R_f : 0.27).

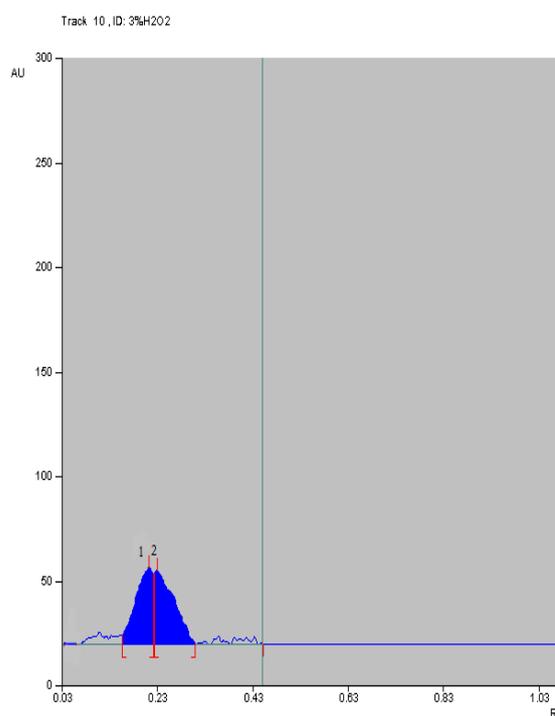


Fig. 4c: HPTLC Chromatogram of hydrogen peroxide (3% w/v, 1 h, 70°C.) treated Palonosetron Hydrochloride (R_f : 0.21), peak 2 (impurity) (R_f : 0.27).

Table 5. Degradation of Palonosetron Hydrochloride

Sr no.	Sample exposure condition	No. of degradation product (R_f)	Palonosetron Hydrochloride remained (ng/1200ng) (\pm drug remained)	Recovery (%)
1	5 N HCl, 6h, 70°C	1 (0.25)	1103.91	91.99
2	2 N NaOH, 6h, 70°C	1(0.27)	990.09	82.51
3	3% H ₂ O ₂ , 1h, 70°C	1 (0.27)	1079.79	89.98
4	Heat, 6h, 70°C	-	-	-
5	Photo, 6h	-	-	-

4. CONCLUSION

The developed HPTLC method was precise, specific, and accurate and stability indicating and validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of palonosetron hydrochloride as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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