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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR ESTIMATION OF ONDANSETRON HYDROCHLORIDE

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ABSTRACT
A simple, precise and sensitive stability indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the analysis of Ondansetron Hydrochloride both in bulk and in tablet dosage form. The seperation was performed on pre-coated silica gel 60 GF_{254} plates using ethyl acetate: methanol (6:4 v/v) as mobile phase. The retention factor (R_f) was found to be 0.43 ± 0.07. The detection of band was carried out at 248 nm. The drug was subjected to different stress conditions like acid, base hydrolysis, oxidation, thermal degradation and photolysis. The method was successfully validated according to ICH Q_2 (R1) guidelines. The linear regression analysis data for the calibration plot showed good linear relationship with R^2 = 0.9928 in the range 500-3000 ng/band. The method found to be accurate as results of the recovery studies are close to the 100%. The developed method was found to be simple, sensitive, selective, accurate and repeatable for analysis of ondansetron hydrochloride and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

KEYWORDS: High performance thin layer chromatography (HPTLC), Ondansetron hydrochloride, method validation, stability indicating.

INTRODUCTION
Ondasetron hydrochloride (OND) is chemically 9-methyl-3-((2-methyl-1Himidazol- 1-yl)methyl)-2,3-dihydro-1H-carbazol-4(9H)- one hydrochloride[1] is a serotonin 5-HT3 receptor antagonist used mainly as an antiemetic to treat nausea and vomiting, often following chemotherapy. Literature survey reveals that several analytical methods have been reported for the estimation of Ondansetron hydrochloride in pharmaceutical dosage form and biological fluids including UV-Visible spectroscopy[2,3], high performance liquid chromatography (HPLC)[4], high performance thin layer chromatography (HPTLC)[5], liquid chromatography-mass spectrometry (LCMS).[6]

Although few reports are available on stability indicating HPLC methods, the information provided is incomplete as well as results are contrast.[7-10] To the best of our knowledge no stability indicating HPTLC method has been reported for estimation of Ondansetron hydrochloride. The present work describes a simple, stability indicating HPTLC method for the determination of Ondansetron hydrochloride in bulk and tablet dosage form (Emeset 4 mg) according to ICH guidelines.

MATERIALS AND METHODS
Reagents and chemicals
The formulation Emeset labeled to contain Ondansetron hydrochloride 4 mg was procured from local market. Methanol (AR grade), Ethyl acetate (AR grade) were purchased from S.D. Fine Chemical Laboratories, Mumbai. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH); all AR grade were purchased from Loba Chemie Pvt. Ltd., Mumbai.

Chromatographic conditions
Chromatographic seperation of drug was performed on aluminum plates precoated with silica gel 60 GF_{254}, (10cm × 10cm with 250µm layer thickness). Sample was 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 (6:4 v/v). 10cm × 10cm Camag twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation conditions and 10 ml of mobile phase was used per run. Migration distance was 80 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 200-400 nm, operated by winCATS software (version 1.4.3), slit dimensions were 4.00×0.45 mm and Deuterium lamp was used as a radiation source.

Selection of detection wavelength

From the standard stock solution (1000 µg/ml) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra was obtained. It was observed that the drug showed considerable absorbance at 248 nm. Representative UV spectrum of Ondansetron hydrochloride is shown in Fig 1.

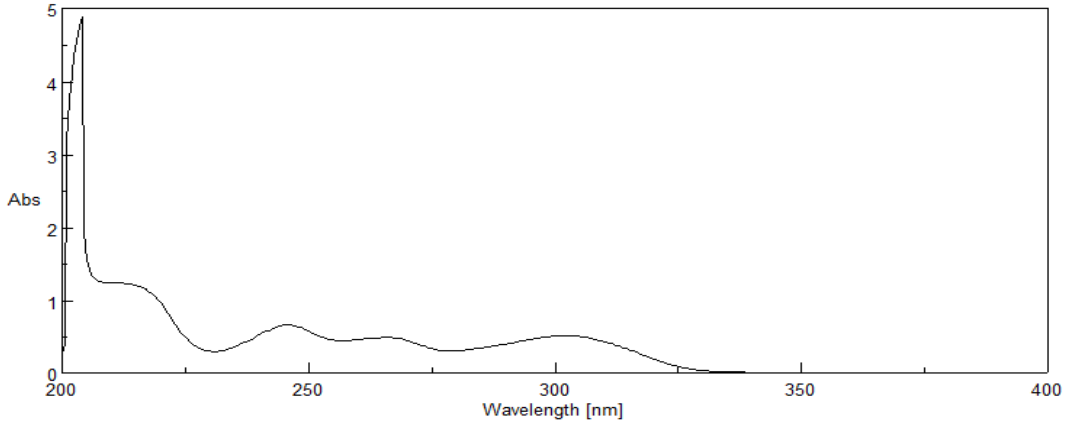

Fig. 1: The UV spectrum of Ondansetron hydrochloride.

Preparation of Standard stock solution

Standard stock solution of drug was prepared by dissolving 10 mg of the drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the standard stock solution, working standard solution was prepared containing 100 µg/ml of ondansetron hydrochloride. Representative densitogram of ondansetron hydrochloride (1000 ng/band) is shown in Fig 2.

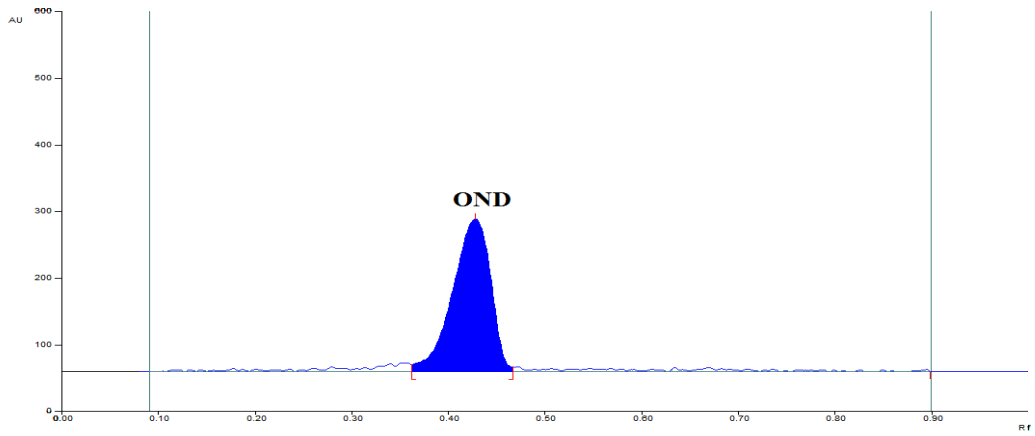

Fig 2: Densitogram of standard solution of ondansetron hydrochloride (1000 ng/band)

Preparation of sample solution

For determination of the content of ondansetron in ondansetron tablets (label claim: 4 mg ondansetron HCl per tablet), twenty tablets were weighed; average weight was determined and were finely powdered. A quantity of powder equivalent to 10 mg of ondansetron was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The mixture was ultra sonicated for 10 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and the volume was made up with the methanol. 5.0 ml of this solution was diluted to 10 ml with the methanol to prepare a final sample stock solution of 500µg/ml.

Stress degradation studies of bulk drug[11]

Stability studies were carried out to provide evidence on how quality of drug varies under the influence of a variety of environmental conditions like acidic, alkaline hydrolysis, oxidation, dry heat and photolytic degradation. Dry heat and photolytic degradation were carried out in the solid state. All studies are carried out at concentration level 2,000 ng/band.

Alkaline hydrolysis

To 1 ml stock solution of ondansetron hydrochloride (1000µg/ml), 1 ml solution of 2.5 N NaOH was added. The resultant solution was kept for 24 hours at room temperature. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 34.43% ondansetron hydrochloride was recovered with no peak of degradant. Representative densitogram is shown in Fig 3.

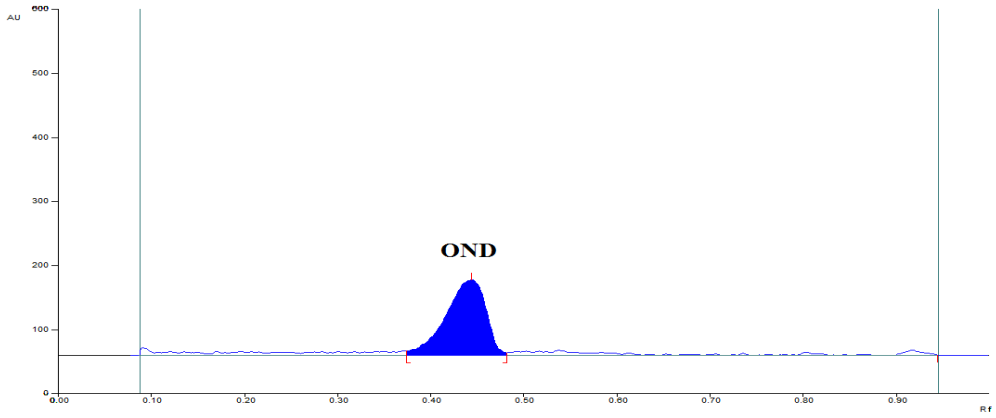

Fig. 3: Representative densitogram of alkali induced degradation of ondansetron hydrochloride (2000 ng/band)

Acid hydrolysis

To 1 ml stock solution of ondansetron hydrochloride (1000µg/ml), 1ml solution of 5 N HCl was added. The resultant solution was kept for 24 hours at room temperature. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 21.62% ondansetron hydrochloride was recovered with peak of degradant (D1) at Rf value 0.20. Representative densitogram is shown in Fig 4.

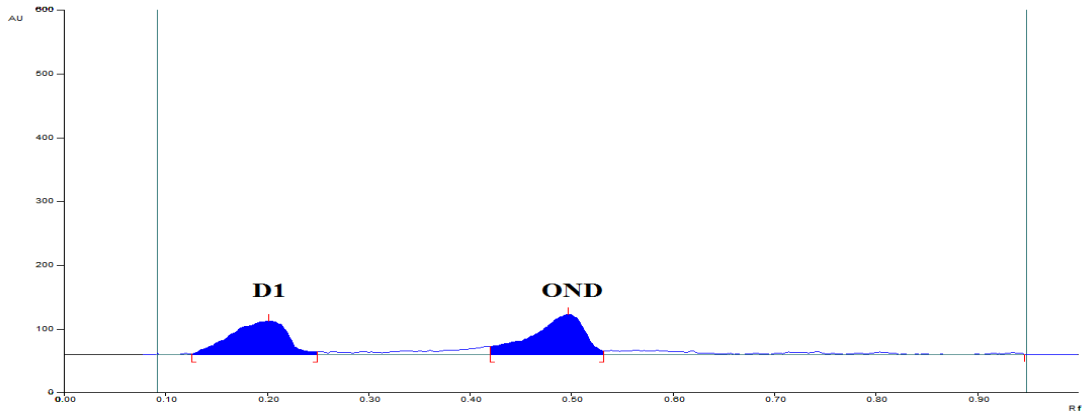

Fig.4: Representative densitogram of acid induced degradation of ondansetron hydrochloride (2000ng/band)

Degradation under oxidative conditions

To 1 ml stock solution of ondansetron hydrochloride (1000 µg/ml), 1 ml solution of 30% H_2O_2 was added. The resultant solution was kept for 24 hours at room temperature. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 16.81% ondansetron hydrochloride was recovered with peak of degradant (D2) at Rf value 0.82. Representative densitogram is shown in Fig 5.

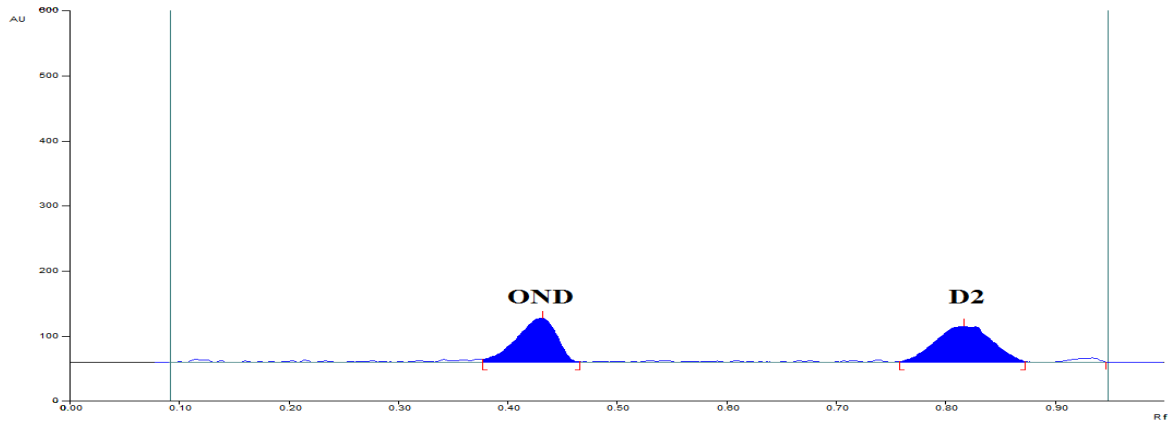

Fig 5:Representative densitogram of peroxide induced degradation of ondansetron hydrochloride(2000ng/band).

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (80℃) for a period of 24 hours. Sample was withdrawn, dissolved in methanol and diluted to get 500 µg/ml. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 38.48 % of ondansetron hydrochloride was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to dry heat is shown in Fig 6.

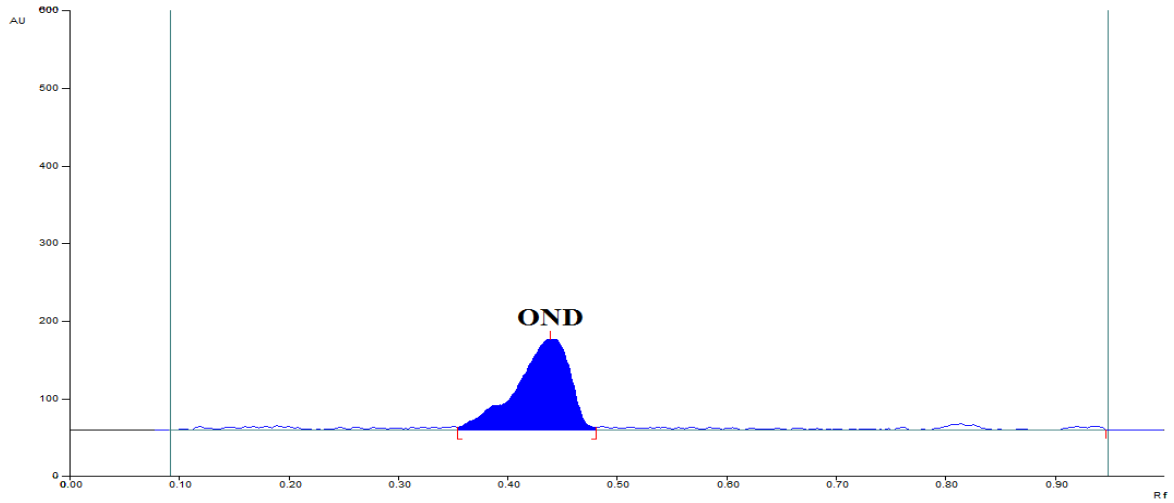

Fig. 6: Representative densitogram of dry heat degradation of ondansetron hydrochloride (2000 ng/band)

Photodegradation studies

1. UV Illumination

The photo degradation study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with methanol to obtain 1000 µg/ml. 5 ml of the resultant solution was then diluted with methanol to get the concentration of 500 µg/ml. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 32.99% of ondansetron hydrochloride was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to UV illumination is shown in Fig 7.

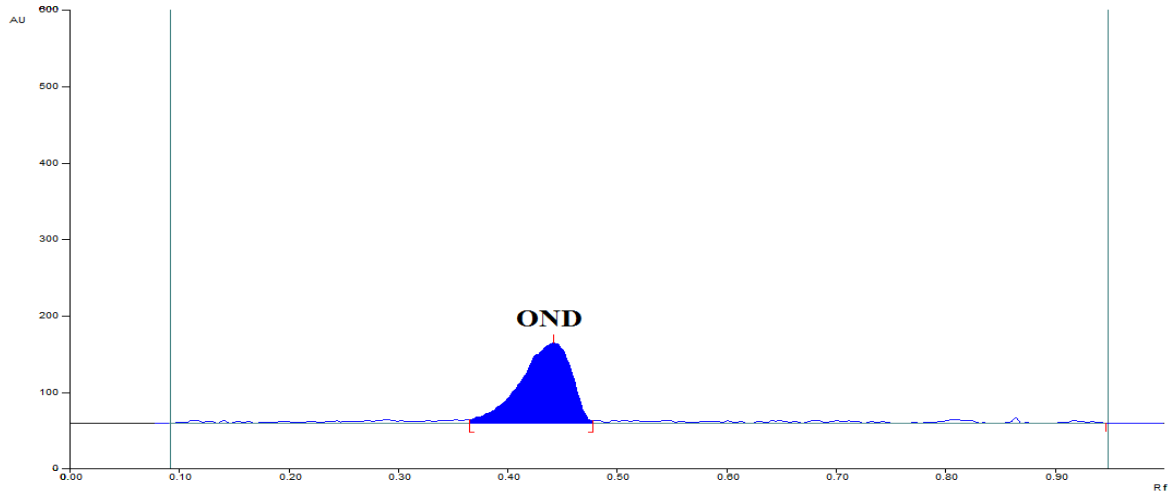

Fig.7: Representative densitogram of photolytic uv degradation of Ondansetron hydrochloride (2000 ng/band)

2. Fluroscent light

The photo degradation study of the drug was studied by exposing the drug to fluroscent light providing illumination of NLT 1.2×10^6 Lux hr of fluroscent light. After exposure accurately weighed 10 mg of drug was transferred to 10 ml volumetric flask; the volume was made up with methanol to obtain 1000 µg/ml. 5 ml of the resultant solution was then diluted with methanol to get the concentration of 500 µg/ml. 4 µl of the resultant solution was then applied at TLC plate and densitogram was developed. Average 32.99% of Ondansetron hydrochloride was recovered with no peak of degradant. Representative densitogram obtained for sample subjected to Fluroscent light is shown in Fig 8.

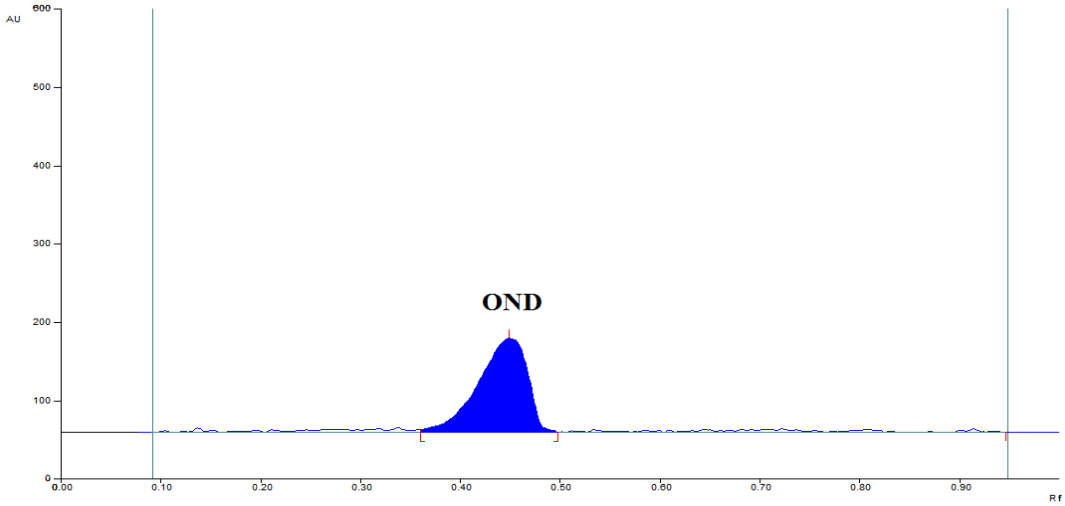

Fig.8: Representative densitogram of photolytic fluroscent light degradation of Ondansetron hydrochloride(2000ng/band)

VALIDATION OF ANALYTICAL METHOD[12]

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.998, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity and Range

From the standard stock solution (1000 µg/ml) of Ondansetron hydrochloride, solution was prepared containing 500 µg/ml of Ondansetron hydrochloride. This solution was further used for spotting. Six replicates per concentration were spotted. The linearity (relationship between peak area and concentration) was determined by analysing six concentrations over the concentration range 500-3000 ng/band for Ondansetron hydrochloride to obtain calibration curve. The results found to be linear with regression equation of y = 5.5173x + 2453.8 and R^2= 0.9928. The calibration curve and three dimensional overlay of the HPTLC densitogram of calibration bands is shown in Fig. 9 and 10 respectively.

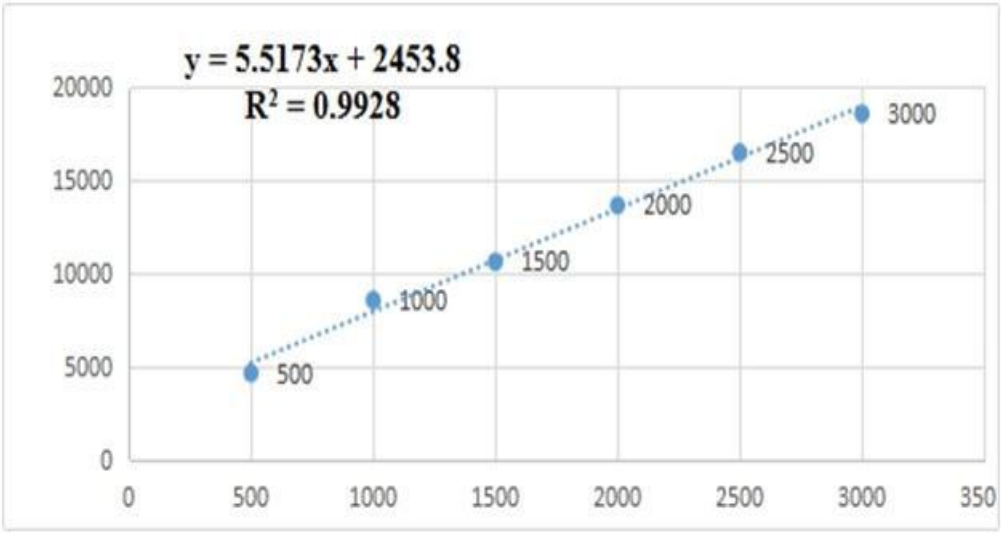

Fig 9: Calibration curve of Ondansetron hydrochloride (500-3000ng/band) reference standard.

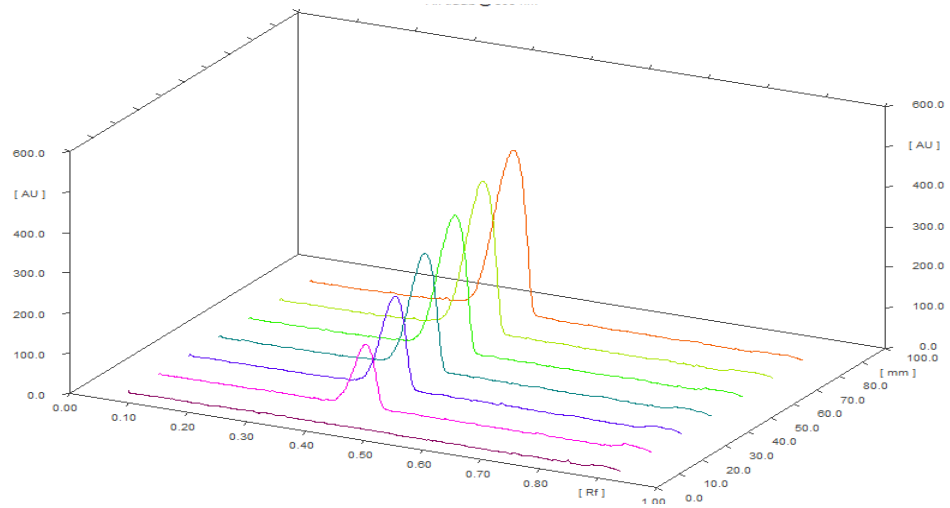

Fig 10: Three dimensional overlay of the HPTLC densitogram of calibration bands.

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day studies 3 replicates of 3 concentrations were analysed on the same day and % RSD was calculated. For the interday variation studies, 3 concentraions were analysed on 3 consecutive days and % RSD was calculated. For intraday precision and interday precision results obtained are shown in Table 1.

Table 1: Intraday and interday variation studies data for ondansetron hydrochloride

Concentration (ng/band)	Intra-day precision			Inter-day precision		
	Area	% recovery	% RSD	Area	% recovery	% RSD
1000	8072.8	101.84	0.60	8017.7	100.84	0.16
	8031.1	101.08		8029.8	101.06	
	8005.9	100.63		8011.4	100.73	
1500	10578.4	98.17	0.52	10625.5	98.74	1.26
	10587.3	98.27		10776.6	100.56	
	10655.9	99.10		10578.4	98.17	
2000	13528.8	100.36	0.66	13595.5	100.97	0.39
	13646.8	101.43		13513.9	100.23	
	13664.2	101.59		13528.8	100.36	

Limit of Detection (LOD) and Limit of quantitation (LOQ)

From the linearity data the limit of detection and quantitation was calculated, using the formula LOD = 3.3 σ / S and LOQ = 10 σ / S where, σ = standard deviation of the response at lowest concentration in range and S = slope of the calibration curve. The LOD and LOQ were found to be 24.10 ng/band and 73.03 ng/band, respectively.

Assay

Emeset 4 tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. 2 µl volume of sample solution was applied and area was recorded. Basic concentration of sample chosen was 1000 ng/band from tablet solution. Concentration and % recovery was determined from linear equation. Assay results obtained are shown in Table 2.

Table 2: Assay of marketed formulation.

Drug	Peak area	Amount recovered (ng/band)	% recovery	% RSD
Ondansetron hydrochloride	7999.8	1005.201	100.520	0.380
	7947.6	995.740	99.574	
	8003.2	1005.818	100.581	
	7993.8	1004.114	100.411	
	7983.4	1002.229	100.222	
	8000.7	1005.364	100.536	

Accuracy

To check accuracy of the method, recovery studies were carried out by spiking the standard drug to the tablet solution, at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 1000 ng/band. % recovery was determined from linear equation. Accuracy results obtained are shown in Table 3.

Table 3: Accuracy studies of ondansetron hydrochloride.

Level	Amount of sample taken (ng/band)	Amount of standard spiked (ng/band)	Area	% recovery	% RSD
50%	1000	500	10784.6	100.662	0.446
			10735.2	100.065	
			10712.2	99.787	
100%	1000	1000	13648.2	101.448	1.227
			13658.5	101.541	
			13417.1	99.353	
150%	1000	1500	16199.5	99.655	0.882
			16433.6	101.352	
			16256.4	100.067	

Robustness

Robustness of the method was determined by carrying out the analysis under conditions during which detection wavelength, chamber saturation time, mobile phase composition, time from spotting to development, time from development to scanning was changed and the effect on the area was noted. It was found that method is robust.

RESULTS AND DISCUSSION

Ondansetron hydrochloride has absorbance maxima at 248 nm. The calibration curve data show good linear relationship in concentration range 500-3000 ng/band. Recovery study is carried out at three different level 50%, 100% and 150% by adding pure drug to the previously analysed test sample. Percentage recovery for drug was determined by linearity equation method and found to be within acceptance criteria. The precision and accuracy was found to be good, which is evident by low standard deviation values. The summary of validation parameters is presented in Table 4.

Table 4: Summary of Validation Parameters.

Sr. No.	Validation parameters	Ondansetron hydrochloride
1.	Linearity equation R^2 Range	y = 5.6292x + 2362.3 R^2= 0.9928 500-3000ng/band
2.	Precision	(%RSD)
	Intraday	0.59
	Interday	0.60
3.	Assay	99.57%-100.53%
4.	Accuracy	
	50	99.78%-100.66%
	100	99.35%-101.54%
	150	99.65%-101.35%
5.	Limit of detection	24.10ng/band
6.	Limit of quantitation	73.03ng/band
7.	Specificity	Specific
8.	Robustness	Robust

CONCLUSION

A simple, precise, accurate, reproducible and stability indicating HPTLC method without interference from the excipients or from degradation products has been developed and validated for the determination of ondansetron hydrochloride as bulk drug and in tablet dosage form. The developed method can be used for quantitative analysis of ondansetron hydrocloride in pharmaceutical dosage form. The method was developed by using easily available and cheap solvents for analysis of drug hence can be considered as economic.

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