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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE ESTIMATION OF ONDANSETRON HYDROCHLORIDE IN BULK DRUG AND TABLET DOSAGE FORMS

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ABSTRACT

A simple, selective, precise and sensitive high performance thin-layer chromatographic (HPTLC) method has been developed and validated for the analysis of ondansetron hydrochloride both in bulk drug and in tablet dosage forms. The separation was performed on pre-coated silica gel 60 GF^{254} plates using methanol:triethylamine:glacial acetic acid (9.5:0.5:0.1, v/v/v) as mobile phase. Densitometric analysis was performed in reflectance-absorbance mode at 309 nm. The linear regression analysis data for the calibration plot showed good linear relationship with $R^2 = 0.998 \pm 0.00047$ in the range of 300-1100 ng $spot^{-1}$. The minimum amount of ondansetron hydrochloride that could be detected and quantified was 54.60 and 165.46 ng $spot^{-1}$ respectively. The mean retardation factor (R_f) for ondansetron hydrochloride was found to be 0.77 ± 0.01. The developed method was validated according to International Conference on Harmonization (ICH) guidelines for specificity, linearity, range, accuracy, precision, detection limit, quantitation limit and robustness etc.

Keywords: *Ondansetron hydrochloride; HPTLC; Method validation.*

INTRODUCTION

Ondansetron hydrochloride, 9-methyl-3-((2-methyl-1*H*-imidazol-1-yl)methyl)-2,3-dihydro-1*H*-carbazol-4(9*H*)-one hydrochloride (Figure 1) is a serotonin 5-HT_3 receptor antagonist used mainly as an antiemetic to treat nausea and vomiting, often following chemotherapy. Its effects both peripheral and central nerves by reducing the activity of the vagus nerve which deactivates the vomiting center in the medulla oblongata and also blocks serotonin receptors in the chemoreceptor trigger zone. It has little effect on vomiting caused by motion sickness and does not have any effect on dopamine receptors or muscarinic receptors.[1-2]

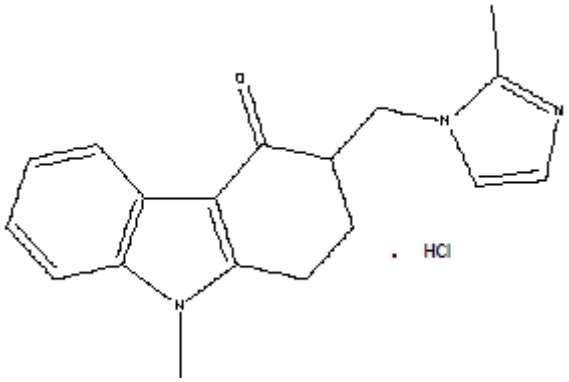

***Fig. 1:** Chemical Structure of Ondansetron hydrochloride*

Literature survey reveals that several analytical methods have been reported for the estimation of ondansetron hydrochloride in pharmaceutical dosage form and biological fluids including ultra violet spectroscopy (UV),[3-6] visible spectroscopy,[7-8] high performance liquid chromatography (HPLC),[9-18] high performance thin layer chromatography (HPTLC),[19-21] liquid chromatography-mass spectrometry (LCMS)[22]. However very few HPTLC methods for simultaneous estimation of ondansetron (free base) with other drugs such as rabeprazole and pantoprazole have been reported[19-20] and there is only one method reported for estimation of ondansetron hydrochloride which bears resemblance to compendial method proposed under USP 35-NF 30.[21] Ondansetron hydrochloride is official in IP 2010, BP 2013 and USP 35-NF 30.[16-18] Thin layer chromatographic method for assessing chromatographic purity proposed under USP 35-NF 30 and BP 2013 make use of a multi-solvent mobile phase system which comprises of solvents with inhalation hazards such as chloroform (carcinogenic) and dichloromethane. The proposed method avoids the use of these solvents and possesses a simple mobile phase system (Table 1). The proposed method is economical and equivalent to the existing methods in terms of symmetrical, well resolved and well defined peak of ondansetron hydrochloride at mean retardation factor (R_f) 0.77 ± 0.01. The developed method was validated to ensure the compliance in accordance with ICH guidelines.[23]

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Table 1: *Comparison of mobile phase system in thin layer chromatographic methods proposed under different Pharmacopoeias, literature and proposed method.*

	Mobile Phase System
IP 2010	Not Available
USP 35-NF 30	Chloroform, Ethyl Acetate, Methanol, and Ammonium Hydroxide (90:50:40:1 v/v/v/v)
BP 2013	Dichloromethane, Ethyl Acetate, Methanol and Concentrated Ammonia (90:50:40:2 v/v/v/v)
Mujtaba A , ***et al.***	Chloroform : Ethyl Acetate : Methanol : Ammonia (9:5:4:0.1 v/v/v/v)
Proposed Method	Methanol, Triethylamine and Glacial Acetic Acid (95:5:1 v/v/v)

EXPERIMENTAL

Materials and Methods

Ondansetron hydrochloride reference standard and ondansetron tablets (Emeset-8) were obtained from Cipla Ltd. Mumbai, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Specialties Pvt. Ltd. Mumbai, India. HPTLC pre-coated Silica gel 60 GF^{254} plates with 0.25 mm thickness, 10 cm x 10 cm sizes were purchased from Merck Specialties Pvt. Ltd. Mumbai, India. Analysis was performed on CAMAG HPTLC instrument with Linomat V applicator, Scanner 3 and Win Cats software (Muttenz, Switzerland supplied by Anchrom Technologists, Mumbai, India).

A standard stock solution of ondansetron hydrochloride (100 µg mL^{-1}) was prepared by dissolving 10 mg of ondansetron hydrochloride reference standard in 100 mL of methanol.

Sample Prep aration

In order to determine the content of ondansetron in ondansetron tablets (label claim: 8 mg ondansetron per tablet), twenty tablets were weighed; average weight was determined and were finely powdered. A quantity of powder equivalent to 50 mg of ondansetron was transferred to a 50-mL volumetric flask containing 20 mL of methanol. The mixture was ultra sonicated for 30 min and the volume was made up with the methanol. Dilute 5.0 mL of this solution to 50.0 mL with the methanol to prepare a final sample stock solution of 100 µg mL^{-1}. The resulting sample stock solution was filtered with Whatman filter paper 41.

HPTLC Analysis

TLC plates were washed with methanol and dried prior to use. The samples were spotted in the form of bands of 6.0 mm width using Linomat V applicator (Muttenz, Switzerland) with 100 µL syringe. A constant application rate of 6 µL s^{-1} was employed, and the space between two bands was 17.5 mm. The slit dimension was kept at 5 × 0.45 mm, and a scanning speed of 80 mm s^{-1} was employed. The mobile phase used was methanol:triethylamine:glacial acetic acid (9.5:0.5:0.1 v/v/v) and 20 mL volume used for the development. Linear ascending development was carried out in 10 × 10 cm twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The length of chromatogram run was 8.5 cm and subsequent to the development, the TLC plates were dried in an oven at 50°C for 5 minutes. Densitometric scanning was performed with CAMAG TLC Scanner 3 in the absorbance-reflectance mode at 309 nm and operated by Win-CATS software. The source of radiation utilized was deuterium lamp. Evaluation was done by linear regression of peak area response against amount of drug.

RESULTS & DISCUSSION

METHOD DEVELOPMENT AND OPTIMIZATION

Different solvent systems containing methanol, ethyl acetate, acetone, triethylamine in different proportions were examined to find out the best combination of solvent system for the separation of ondansetron hydrochloride. The reference standard and test samples were initially tried on different combination of solvents *i.e.* methanol: ethyl acetate, ethyl acetate: acetone, methanol: acetone etc in various proportions, however the retardation factor in these combinations was very low and the spot showed streaking. Ondansetron hydrochloride being in the salt form, the use of base in small proportion is desired to lift up the spot. Solvent system comprising of methanol: triethylamine (9:1 v/v) gave a spot that lacked compactness, possessed tailing and had considerably high R_f value. To the above solvent system, volumes of triethylamine were reduced to half to decrease the R_f and 1 to 2 drop of glacial acetic acid was added to restrain tailing. Mixture of methanol: triethylamine: glacial acetic acid (9.5:0.5:1 v/v/v) resulted in a compact spot with apposite mean retardation factor of 0.77 ± 0.01. Therefore the mobile phase consisting of methanol:triethylamine:glacial acetic acid in the ratio (9.5:0.5:1 v/v/v) was finally optimized, under this optimized condition, ondansetron hydrochloride showed a sharp and symmetrical peak with R_f value of 0.77 ± 0.01 and densitometric scanning was performed at 309 nm (Figure 2). The optimized volume of the mobile phase was 10.0 mL in a twin trough chamber. The spots of reference standard and test samples appeared more compact and the shape of peaks obtained were more symmetrical from the pretreated TLC plates with methanol and also activated at 105°C for 5 min. Well-defined spots of reference standard and test samples were obtained when the saturation time of the chamber was optimized up to 30 min at room temperature.

METHOD VALIDATION

The optimized HPTLC method was validated with respect to parameters including specificity, linearity, range, accuracy, precision, detection limit, quantitation

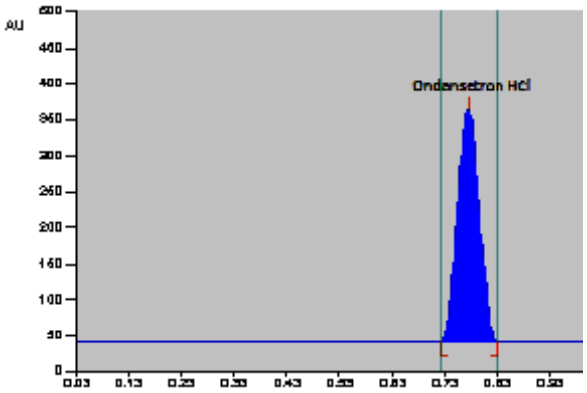

Fig. 2: *A typical HPTLC densitogram of ondansetron hydrochloride reference standard showing R_f 0.77 ± 0.01 at 309 nm using methanol-triethylamine-glacial acetic acid (9.5:0.5:0.1, v/v/v).*

limit and robustness. Figure 3 displays a three dimensional overlay of the HPTLC densitogram of calibration bands. Figure 4 displays a calibration plot of ondansetron hydrochloride reference standard. The figures reveal a good linear relationship over the concentration range studied, signifying the suitability for the analysis of ondansetron hydrochloride.

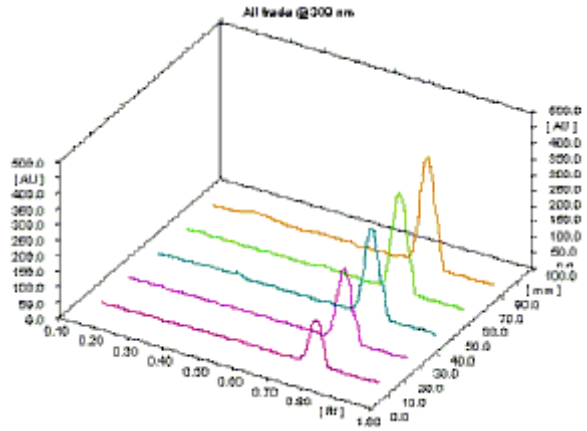

Fig. 3: *Three dimensional overlay of the HPTLC densitogram of calibration bands.*

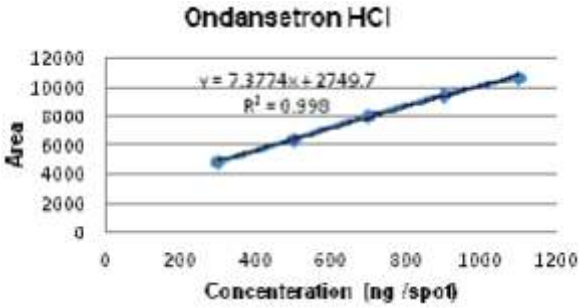

Fig. 4: *Calibration plot of ondansetron hydrochloride reference standard.*

Linearity and Range

A calibration plot was constructed by plotting peak area against concentration of ondansetron hydrochloride and was found linear over the concentration range of 300-1100 ng spot^{-1}. Linear regression analysis was performed and correlation coefficient, slope and intercept were determined and found to be 0.998 ± 0.00047, 7.377 ± 0.25 and 2749.7 ± 13.2 [Values (± SD)] respectively (Table 2).

Table 2: *Linear regression data for the calibration curve (n=6)*

Parameters	Values
Linear range	300 -1100 ng spot^{-1}
Correlation coefficient (R^2) ± SD	0.998 ± 0.00047
Slope ± SD	7.377 ± 0.25
Intercept ± SD	2749.7 ± 13.2
LOD (ng spot^{-1})	54.60
LOQ (ng spot^{-1})	165.46

Detection Limit and Quantit ation Limit

Limit of detection (LOD) and limit of quantitation (LOQ) of ondansetron hydrochloride was estimated using the proposed method and were calculated by using these equations: LOD = 3.3 × ó/S and LOQ = 10 × ó/S, where ó is the standard deviation of the response and S is the slope of the calibration curve. To estimate LOD and LOQ a calibration plot was constructed by plotting peak area against concentration of ondansetron hydrochloride and the regression equation was determined. The residual standard deviation of a regression line may be used as the standard deviation and slope is estimated from the calibration curve. The results are shown in Table 2.

Precision

Precision of the method was estimated with respect to both repeatability (intra-assay) and intermediate precision (intra-day and inter-day). It was performed by using drug sample concentration of 500, 700 and 1000 ng spot^{-1}. Intra-assay precision was assessed by analysis of the same sample concentration in replicate; intra-day precision was assessed by analysis of the same sample concentration thrice a day and inter-day precision was assessed by analysis of the same sample concentration on three different days over a period of one week. The average area and the standard deviations (± SD) were calculated. The results are shown in Table 3. The developed method was found to be precise.

Robustness

The robustness of the method was evaluated by slightly varying the optimized mobile phase composition as

Table 3: *Intra-assay, Intra-day and Inter-day precision by HPTLC method.*

Amount (ng/spot)	Repeatability (Intra-assay) Mean area (AU)[a] ± SD	% RSD	Intermediate Precision			
			Intra-day Mean area (AU)[b] ± SD	% RSD	Inter-day Mean area (AU)[b] ± SD	% RSD
500	5156.16 ± 56.70	1.09	5156.09 ± 14.04	0.27	5163.48 ± 21.34	0.41
700	7446.9 ± 66.06	0.88	7450.86 ± 3.16	0.04	7449.51 ± 3. 21	0.04
1000	9355.84 ± 68.31	1.23	9396.05 ± 28.58	0.30	9310.03 ± 59.29	0.63

[a]*Average of replicate spots.*
[b]*Average of three determinations (in replicate spots).*
SD = standard deviation, RSD = relative standard deviation.

shown in Table 4. The relative standard deviation of peak areas was calculated. The % RSD was found to be less than 2 % which indicates the robustness of method.

Table 4: *Robustness of the method.*

Parameter	Mean area (AU) [a] ± SD	% RSD
Methanol-triethylamine-glacial acetic acid (9.5:0.5:0.1, v/v/v)	5166.02 ± 63.32	1.22
Methanol-triethylamine-glacial acetic acid (9.6:0.4:0.1, v/v/v)	5146.16 ± 83.16	1.61
Methanol-triethylamine-glacial acetic acid (9.4:0.6:0.1, v/v/v)	5187.32 ± 80. 10	1.54

[a]*Average of replicate spots (500 ng spot^{-1})*
SD = standard deviation, RSD = relative standard deviation.

Accuracy

The accuracy of the method was determined by analysis of standard additions at three levels *i.e.* multiple level recovery studies. Ondansetron hydrochloride reference standard corresponding to 80, 100 and 120 % was added to a fixed amount of pre-analyzed tablet sample solution, and the amounts of the drug were analyzed by the proposed method. Results from the recovery studies are given in Table 5.

Table 5: *Recovery studies of ondansetron hydrochloride (n=6).*

Level %	Amount of ondansetron present in the sample (equivalent to free base) (mg)	Amount of ondansetron HCl reference standard added (as Salt) (mg)	Amount of ondansetron Recovered (equivalent to free base) (mg)	% Recovery	% RSD
80	10mg	8.99mg	18.78	98.94	0.23
100	10mg	11.24mg	21.48	101.17	0.45
120	10mg	13.49mg	23.49	100.02	0.32

RSD = relative standard deviation.

Specificity

The specificity of the method was ascertained by analyzing reference standard and test sample. The mobile phase used in the proposed method resolved the drug very efficiently (R_f = 0.77 ± 0.01 at detection wavelength of 309 nm). The peak for ondansetron HCl from the tablet dosage form was identified by comparing its retardation factor with that of ondansetron hydrochloride reference standard.

Analysis of bulk drug and pharmaceutical dosage form

The proposed method was applied for calculating the assay in bulk drugs and estimation of ondansetron in ondansetron tablets. The results obtained for the content of ondansetron in tablets as against the label claims were in good conformity, signifying no interference of any excipients in the assay studies. The results of assay in bulk and tablet dosage form are shown in Table 6 and Table 7. The good performance of the proposed method demonstrates its suitability for routine analysis of ondansetron in pharmaceutical dosage forms.

Table 6: *Assay studies of ondansetron hydrochloride (bulk).*

S.No	Assay %	Average Assay ± SD	% RSD
1	101.01	100.13 ± 0.77	0.769
2	99.58		
3	99.80		

SD = standard deviation, RSD = relative standard deviation.

Table 7: *Assay studies of ondansetron tablets.*

S. No	Labeled Claim (Ondansetron) In mg	Obtained Claim (Ondansetron) In mg	% Claim	Average Assay ± SD	% RSD
1	8	7.86	98.25	98.66 ±0.381	0.387
2	8	7.90	98.75		
3	8	7.92	99.0		

SD = standard deviation, RSD = relative standard deviation.

CONCLUSION

The developed HPTLC method is simple, selective, precise, sensitive and accurate, duly developed and validated on the framework of ICH guidelines. It does not suffer from interference from common excepients present in the pharmaceutical preparations and can be conveniently adopted for the analysis of ondansetron hydrochloride both in bulk drug and tablet dosage forms.

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