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

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Abstract:

The aim of the present study was to develop a validated stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method for estimation of Ondansetron Hydrochloride.

An isocratic, RP-HPLC method was developed using HiQ Sil C_8 (250 x 4.6 mm, 5 µm) column and 10 mM ammonium acetate buffer (pH 3) and methanol (60:40 v/v) as mobile phase at flow rate of 0.8 ml/min at detection wavelength of 250 nm. The retention time (RT) of drug was 12.280 ± 0.034 min . The method was validated with respect to linearity, precision, accuracy and robustness. The data of linear regression analysis indicated a good linear relationship over the range of 5-30 µg/ml concentrations with a correlation coefficient (R^2) of 0.996. Ondansetron hydrochloride was subjected to different stress testing conditions. The developed method was found to be simple, sensitive, selective, accurate, and precise for analysis of Ondansetron hydrochloride and can be adopted for routine analysis of drug in bulk and pharmaceutical dosage form.

Keywords: *High performance liquid chromatography (HPLC), Ondansetron hydrochloride, Stability indicating, Validation.*

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INTRODUCTION:

Ondansetron 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 ultra violet spectroscopy (UV), visible spectroscopy[5,6],high performance liquid chromatography (HPLC)[4],high performance thin layer chromatography (HPTLC)[10], liquid chromatography-mass spectrometry (LCMS)[12]. Although few reports are available on stability indicating HPLC methods, the information provided is incomplete as well as results are contrast [7-9, 11]. Hence we tried to develop stability indicating HPLC method for Ondansetron hydrochloride. The present work describes a simple, stability indicating HPLC method for the determination of Ondansetron hydrochloride in bulk and tablet dosage form 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 (HPLC grade), ammonium acetate (HPLC grade) were purchased from S.D. Fine Chemical Laboratories, Mumbai. HPLC grade water is collected at college using ELGA water purification system. Hydrochloric acid (HCl), acetic acid (CH_3COOH), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH); all AR grade were purchased from Loba Chemie Pvt. Ltd., Mumbai.

Chromatographic condition

HPLC system used was JASCO system equipped with model PU 2080 Plus pump, Rheodyne sample injection port (20 µl), JASCO UV 2075 Plus detector and Borwin chromatography software (version 1.5). A chromatographic column HiQ Sil C_8 (250 x 4.6 mm, 5 µm) was used , for separation at a flow rate of 0.8 ml/min using 10 mM ammonium acetate buffer (pH 3): methanol (60:40 v/v) as mobile phase and detection at 250 nm. The representative chromatogram is shown in Fig. 1.

Preparation of 10 mM ammonium acetate buffer (pH 3) and mobile phase

10 mM ammonium acetate buffer (pH 3) was prepared by dissolving 770 mg of ammonium acetate in 400 ml of HPLC grade water. Solution is filtered through a 0.2µm HPLC certified nylon filter. HPLC grade water was added to 950 ml and pH of the solution was checked and pH was adjusted to 3 by acetic acid. Then voume is made upto 1000ml using HPLC grade water. Mobile phase was prepared by mixing ammonium acetate buffer and methanol in the ratio of 60:40 v/v. It was then filtered and sonicated for 10 min.

Preparation of standard stock solution

Standard stock solution of drug was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From this solution further dilutions were made in methanol to get final concentration of 10 µg/ml.

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 linear, stable and considerable absorbance at 250 nm.

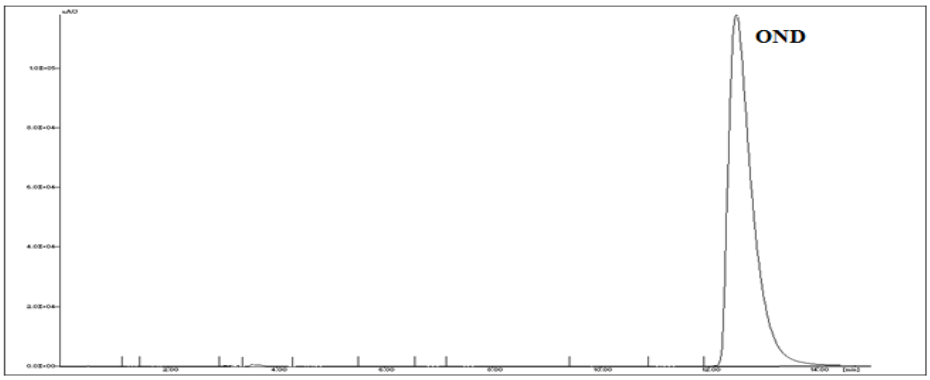

Fig.1: Chromatogram of Ondansetron HCl (10 µg/ml)

Preparation of sample solution-
A tablet containing 4 mg of ondansetron (Emeset 4 mg) was weighed and powdered. A quantity of powder equivalent to 10 mg of ondansetron was transferred to a 10 ml volumetric flask containing 5ml 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 to get concentration of 1000 µg/ml. Further dilution was done to get concentration (10 µg/ml).

STRESS DEGRADATION STUDIES OF BULK DRUG: [2]
Stability studies were carried out to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like hydrolysis, oxidation, temperature, etc. Dry heat and photolytic degradation was carried out in the solid state.

Alkaline hydrolysis
Alkaline sample was prepared by dissolving accurately weighed 5 mg of the pure drug in 10 ml of 2N methanolic NaOH. The drug was insoluble in NaOH hence methanol is used as cosolvent for solublization. This solution is kept at 80˚c for 24 hours. Then solution is cooled and neutralized with HCl and the final dilution is made with mobile phase to get the concentration of 50 µg/ml. Alkali degradation blank is prepared in the same way without using analyte. Under alkaline hydrolysis, percent recovery obtained for Ondansetron HCl was 22.49% with no peak of degradant. The representative chromatogram is shown in Fig. 2.

Acid hydrolysis
Acid sample was prepared by dissolving accurately weighed 5 mg of the pure drug in 10 ml of 5 N HCl. This solution is kept at 80˚c for 24 hours. Then solution is cooled and neutralized with methanolic NaOH and the final dilution is made with mobile phase to get the concentration of 50 µg/ml. Acid degradation blank is prepared in the same way without using analyte. Under acid hydrolysis, percent recovery obtained for Ondansetron HCl was 11.42% with no peak of degradant. The representative chromatogram is shown in Fig. 3.

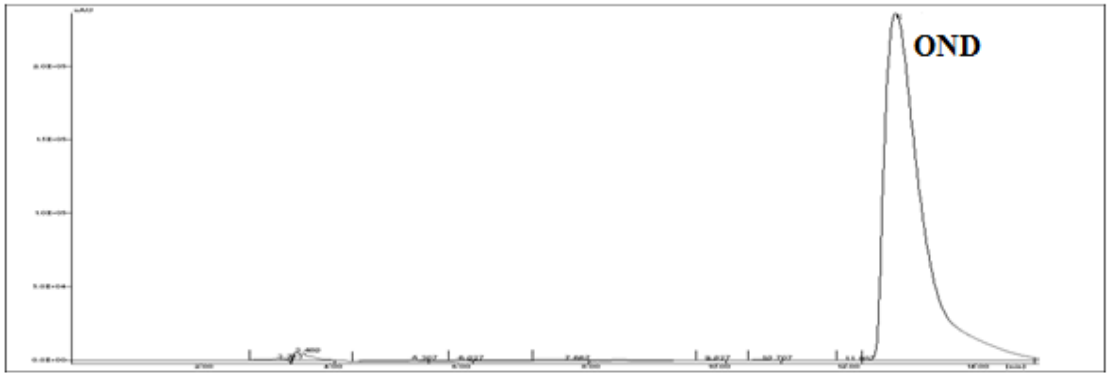

Fig. 2: Chromatogram of OND HCl after alkaline degradation.

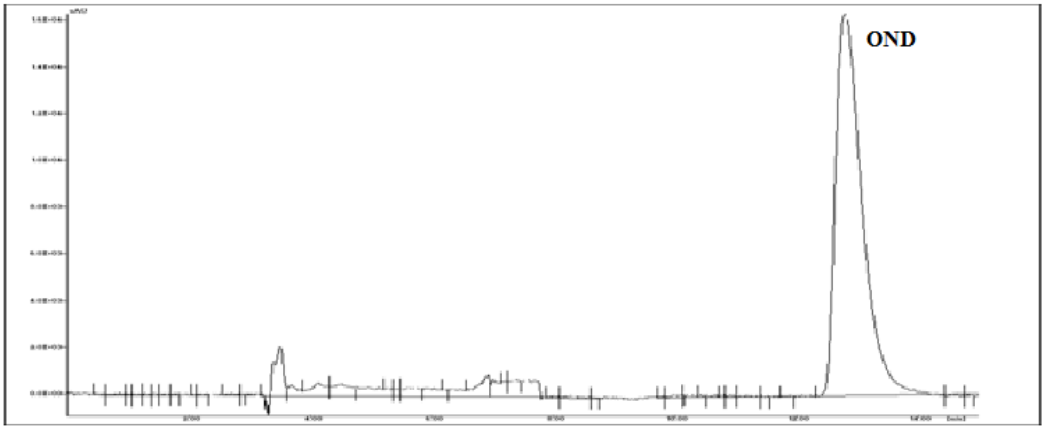

Fig. 3: Chromatogram of OND HCl after acid degradation.

Degradation under oxidative condition

Oxidation sample was prepared by dissolving accurately weighed 5 mg of the pure drug in 10 ml of 30% H_2O_2. This solution is kept for 24 hours. Then the final dilution is made with mobile phase to get the concentration of 50 µg/ml. The blank is prepared in the same way without using analyte. Under oxidative degradation, percent recovery obtained for Ondansetron HCl was 12.85% with no peak of degradant. The representative chromatogram is shown in Fig. 4.

Degradation under dry heat

Dry heat study was performed by keeping drug sample in oven (80^0 C) for a period of 24 hours. A sample was withdrawn after 5 days, dissolved in methanol to get solution of 1000 µg/ml and further diluted with mobile phase to get 50 µg/ml as final concentration and was injected. Under dry heat degradation condition, percent recovery obtained for Ondansetron HCl was 99.63% with no peak of degradant. The representative chromatogram is shown in Fig. 5.

Photo-degradation studies:

The photo degradation stability study of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2 and exposure to cool white fluorescence light of NLT 1.2 million Lux-Hr. After exposure accurately weighed 10 mg of drug was transferred to 10 ml of volumetric flask; the volume was made up with methanol. Further dilution made with mobile phase to get 50 µg/ml as final concentration and was injected. Average 100.49 % of Ondansetron HCl was recovered with no peak of degradant after exposure to UV light and average 99.43 % of Ondansetron HCl was recovered with no peak of degradant after exposure to fluorescence light. Although colour change was seen from white to light brown, no peak of degradant was found. The representative chromatogram is shown in Fig.6 and Fig 7 respectively.

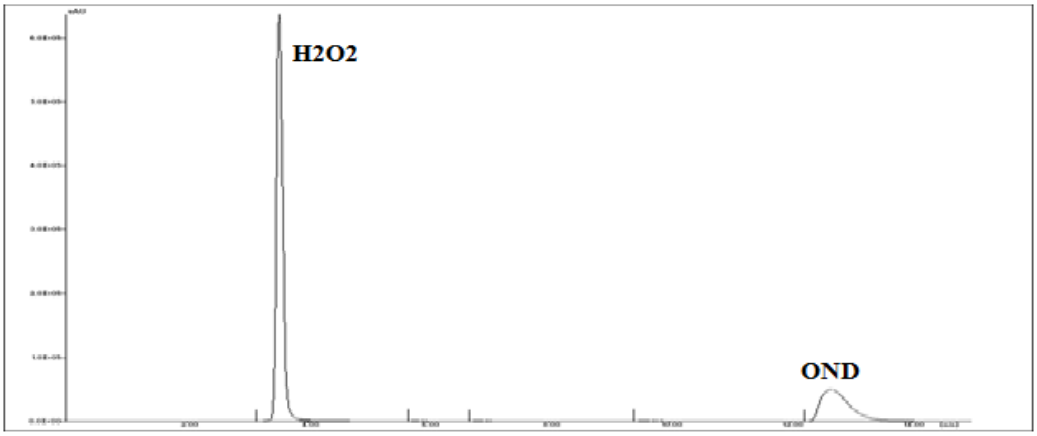

Fig. 4: Chromatogram of OND HCl after oxidation with 30% v/v H_2O_2

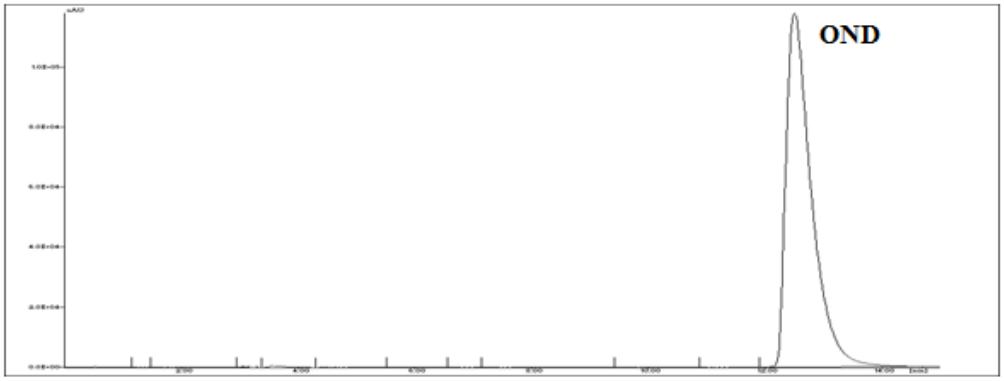

Fig. 5: Chromatogram of OND HCl after dry heat degradation.

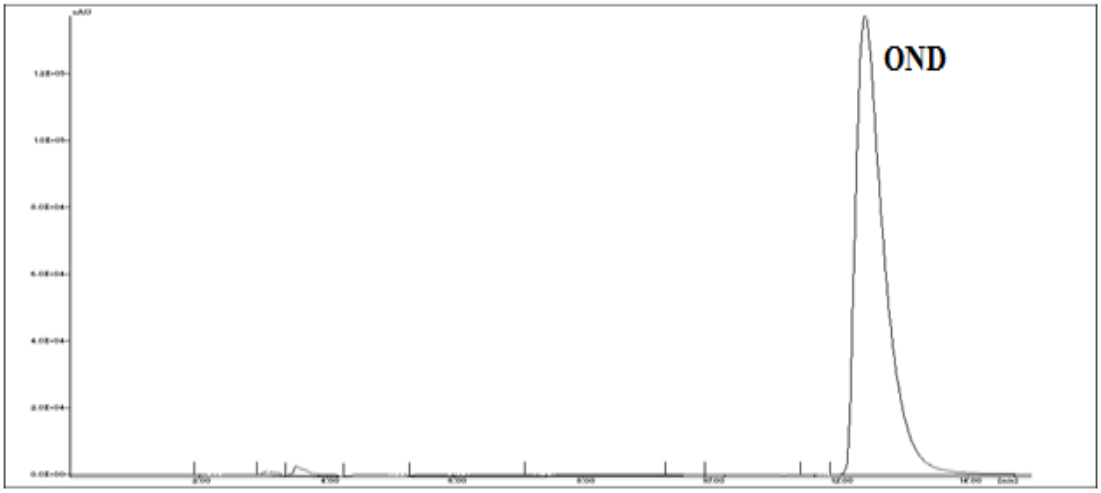

Fig. 6: Chromatogram of OND HCl after UV illumination exposure

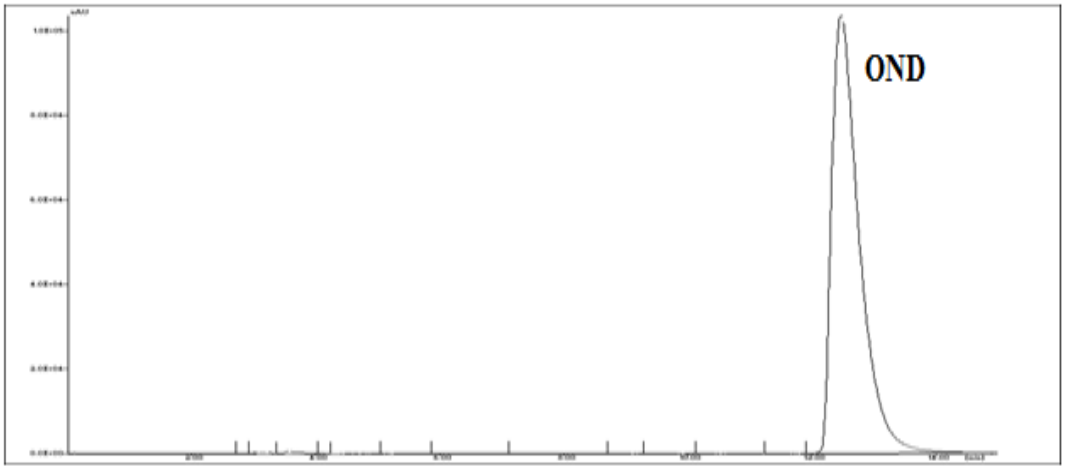

Fig. 7: Chromatogram of OND HCl after fluroscent light exposure

VALIDATION OF ANALYTICAL METHOD: [3]

Specificity

The specificity of the method was ascertained by peak purity profile studies. The peak purity values were found to be more than 0.996, indicating the no interference of any other peak of degradation product, impurity or matrix.

Linearity

From the standard stock solution (1000 µg/ml) of Ondansetron hydrochloride, solution was prepared containing 100 µg/ml. This solution was further used to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml, the equation of calibration curve was found to be y = 65792x + 29924. The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 8

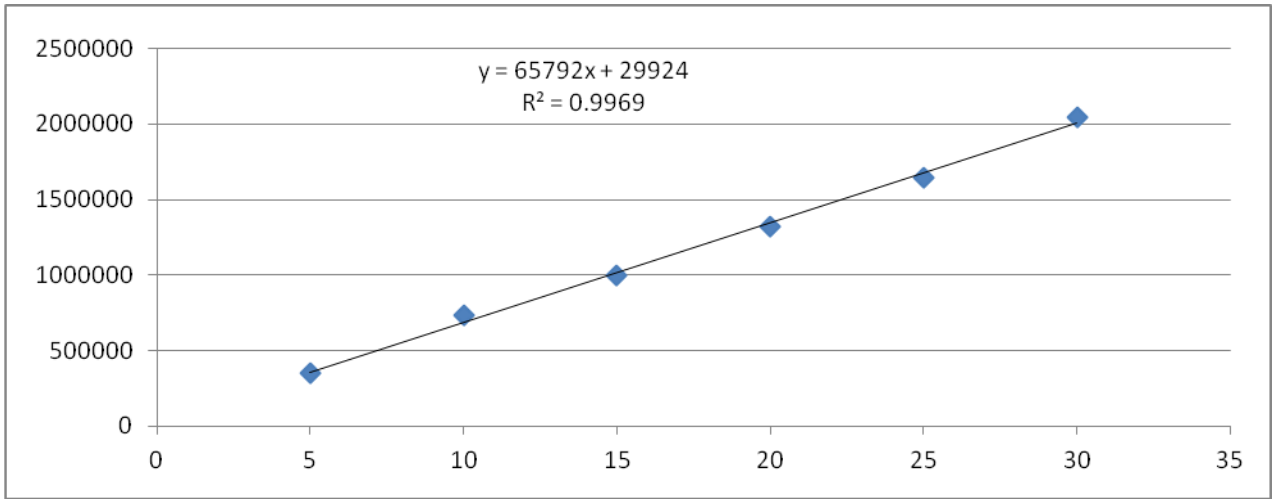

Fig.8: Linearity curve of Ondansetron HCl (5-30 µg/ml)

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 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intraday and inter day variations are shown in Table 1.

Limit of detection (LOD) and limit of quantitation (LOQ)

From the linearity data the LOD and LOQ was calculated, using the formula LOD = 3.3 σ/S and LOQ = 10 σ/S where, σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. LOD was found to be 0.207 µg/ml. LOQ was found to be 0.628 µg/ml.

Assay

Emeset 4 mg tablet formulation analysis was carried out as mentioned under section preparation of sample solution. Procedure was repeated for six times. Sample solution was injected and area was recorded. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table 2.

Table 1: Intraday and Interday variation studies data for Ondansetron HCl

Conc. (µg/ml)	Intra-day precision		Inter-day precision	
	Avg. area	% RSD	Avg. area	% RSD
10	739248.5	1.091	733859.4	0.599
15	1008989	0.516	1008392	0.592
20	1312510	0.304	1307169	0.401

Table 2: Assay of marketed formulation

Sr. No.	Peak Area	Amount Recovered (µg/ml)	%Recovery	Mean ± % RSD
1	703261.8	10.23	102.343	100.92 ± 0.665
2	683495.9	9.93	99.339	
3	690970.6	10.04	100.475	
4	700305.6	10.18	101.889	
5	684735.9	9.95	99.527	
6	700970.6	10.19	101.995	

Table 3: Accuracy of Ondansetron HCl

Level%	Sample (µg/ml)	Standard (µg/ml)	%Recovery (Mean ±%RSD)
50	10	05	99.041 ± 0.436
100	10	10	98.544 ± 1.777
150	10	15	99.991 ± 1.348

Table 4: Summary of Validation Parameters

Sr. No.	Validation parameters	Ondansetron hydrochloride
1.	Linearity equation R^2 Range	y = 65792x + 29924 R^2= 0.9969 5-30 µg/ml
2.	Precision	(%RSD)
	Intraday	0.637
	Interday	0.530
3.	Assay	100.92 ± 0.665
4.	Accuracy	Mean ± %RSD
	50	99.041 ± 0.436
	100	98.544 ± 1.777
	150	99.991± 1.348
5.	Limit of detection	0.207 µg/ml
6.	Limit of quantitation	0.628 µg/ml
7.	Specificity	Specific
8.	Robustness	Robust

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the Emeset 4mg tablet sample solution, at three different levels around 50, 100 and 150 %. Basic concentration of sample solution chosen was 10 µg/ml. % recovery was determined from linearity equation. The results obtained are shown in Table 3.

Robustness-

Robustness of the method was checked by carrying out the analysis under conditions during which mobile phase composition (± 2% Composition), detection wavelength (± 2 nm), flow rate (± 0.05 ml/min) were altered and the effect on the area were noted. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

RESULTS AND DISCUSSION:

The developed method was found to be simple, sensitive, specific, accurate, and repeatable for analysis of Ondansetron HCl in bulk and pharmaceutical dosage form without any interference from the excipients. The results indicated the suitability of the method to study stability of Ondansetron HCl under various forced degradation conditions.

CONCLUSION:

A simple, precise, accurate, reproducible and stability indicating HPLC 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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