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ESTIMATION OF ONDANSETRON HYDROCHLORIDE BY RP-HPLC

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ABSTRACT
A RP-HPLC method was developed for the estimation of Ondansetron Hydrochloride in Bulk drug using high performance liquid chromatography. Ondansetron was a serotonin 5-HT_3 receptor antagonist used mainly as an antiemetic drug to treat nausea and vomiting after cancer chemotherapy. The separation was achieved by Promosil C-18 (250 mm x 4.6 mm x 10 µm) column because it allows higher separation and Acetonitrile: Methanol (50:50) as mobile phase with a flow rate of 1.2 ml/min. The detection was carried out at 216 nm. The retention time of drug was found 2.64 min. The developed method was validated. The proposed method shall prove equally effective to analyze Ondansetron Hydrochloride in the corresponding drug sample and may prove to be of great importance in pharmaceutical analysis.

KEYWORDS: *Ondansetron, Acetonitrile, Antiemetic, Methanol.*

INTRODUCTION

Ondansetron [1-2] was a serotonin 5-HT_3 receptor antagonist used mainly as an antiemetic drug to treat nausea and vomiting after cancer chemotherapy by reduces the activity of Vegas nerve which deactivates the vomiting centre in medulla oblongata and also blocks serotonin receptor in the chemoreceptor trigger zone. [3-4] It was chemically 4H-Carbazol-4-one, 1, 2, 3, 9-tetrahydro-9-methyl-3-(2-methyl-1H-imidazol-1-yl)methyl-monohydrochloride,(±)-,dihydrate.(±)-2,3-Di hydro-9-methyl-3-(2-methylimidazol-1yl)methylcarbazol-4(1H) onemonohydrochloride dihydrate. After through literature survey, the present method was developed as per ICH Guidelines [5-7]. In the present work, an attempt was made to provide a newer, simple, accurate and low cost HPLC method based on solubility for the determination of Ondansetron Hydrochloride as an active pharmaceutical ingredient as shown in **Fig. 1.**

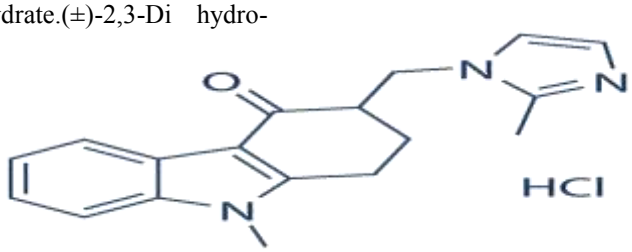

Fig. 1: Structure of Ondansetron Hydrochloride.

MATERIAL AND INSTRUMENT

HPLC method was developed and validated on Younglin HPLC model (Acme-9000. Double beam UV-Visible Spectrophotometer of company-Systronic, Model (2101) with a 1 cm matching quartz cell was used. Ondansetron Hydrochloride was obtained from different companies as gift samples for research and was authenticated by Symed Lab. Ondansetron HCl Tablet (Zofron 4mg) of Cipla company was used. Methanol and

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Acetonitrile were of Merck Company. Potassium di hydrogen phosphate was of Rankem company.

STANDARD SOLUTION PREPARATION

Dissolved 9 mg of Ondansetron Hydrochloride working standard into 100 ml volumetric flask and volume was made up to the mark with mobile phase and dilute quantitatively with mobile phase to obtain a solution having concentration of about 90 µg/ml and Filtered through 0 .45µ.

ABSORPTION MAXIMUM (λ_{Max})

Selection of Ondansetron Hydrochloride (10µg/ml) was prepared in mobile phase. The max was determined on Shimadzu UV – visible spectrophotometer (Model UV – 1800 PC) in the range of 200-400nm and λ_{Max} was found to be 216nm as shown in **Fig. 2.**

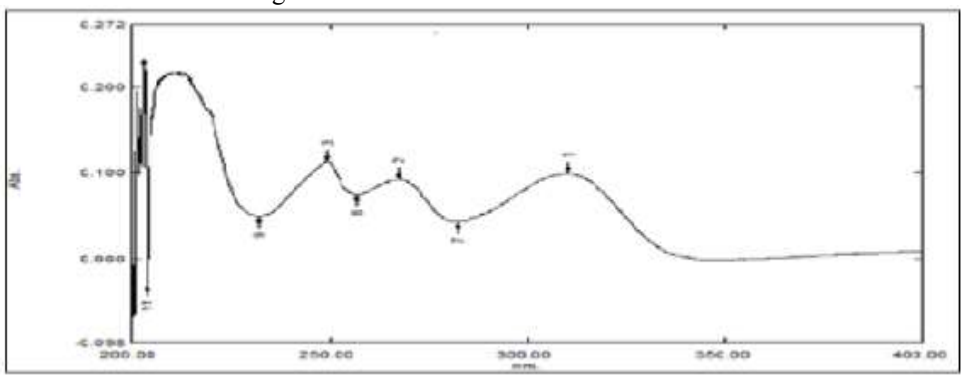

Fig. 2: UV – Absorption Spectra of Ondansetron Hydrochloride.

METHOD OPTIMISATION

Optimum condition of mobile phases was investigated in the development of an HPLC method suitable for analysis of Ondansetron hydrochloride in the bulk drug. The same solvent mixture was used for extraction of the drug from the formulation containing excipients as shown in **Fig. 3** and **Table 1**.

Table 1: HPLC Chromatographic Conditions of Ondansetron Hydrochloride.

S. No.	Parameters	Description
1.	Instrument	A HPLC instrument (Younglin series) with Model Acme-9000.
2.	Column	Hypersil C_{18} column (216 mm, 4.6 mm, 5 µm).
3.	Mobile Phase	Acetonitrile: Methanol (50:50).
4.	Flow Rate	1.2 ml/min.
5.	Detection wavelength	216 nm.
6.	Injection Volume	10µL.
7.	Auto Sampler Temperature	5°C.
8.	Run Time	10min.

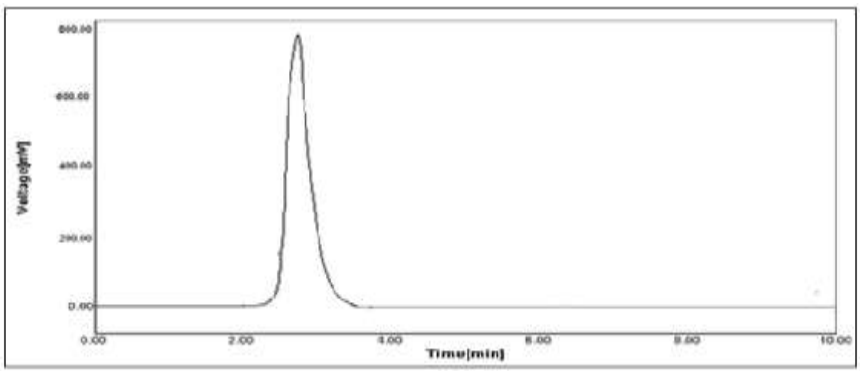

Fig. 3: Optimised Chromatogram of Ondansetron Hydrochloride.

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ASSAY PROCEDURE

Twenty tablets were weighed and average weight was determined. It was finely powdered and mixed thoroughly. Accurately weight tablet power equivalent to 9 mg of Ondansetron hydrochloride and was transferred to dry stopped 100 ml volumetric flask and made up with mobile phase and filtered through Whatmann filter paper. Further dilution was done with mobile phase to get concentration of 90 µg/ml. The chromatogram was recorded and response i. e peak areas of major peaks were measured as shown in **Fig. 4** and **Table 2.**

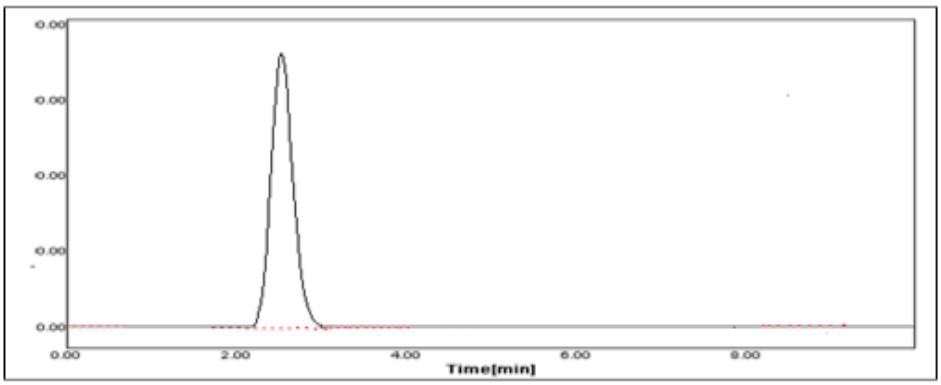

Fig. 4: Assay Chromatogram obtained by using marketed preparation.

Table 2: Analysis of Marketed Tablet Formulation

S.No.	Weight Taken (mg)	Standard Peak Area	Sample Peak Area	Amount Estimated (mg)	Labeled Claim (%)
1.	495	48807	48897	4.21	100.18
2.	495	48807	48857	4.03	100.10
3.	495	48807	48604	3.98	99.58
4.	495	48807	48402	4.11	99.17
5.	495	48807	48381	4.10	99.13
Mean					99.63
S.D					0.496
%R.S.D					0.50

METHOD VALIDATION

Linearity

Accurately weighed quantity of 10 mg Ondansetron hydrochloride was transferred to 10 ml volumetric flask and from the standard stock solution 1,2,3,4 & 5 ml was transferred to 100 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain concentration 10, 20, 30,40 & 50 µg/ml. Then dilution was injected and peak area was recorded. The graph concentration of drug Vs peak area depicted in **Fig. 5** and **Table 3**.

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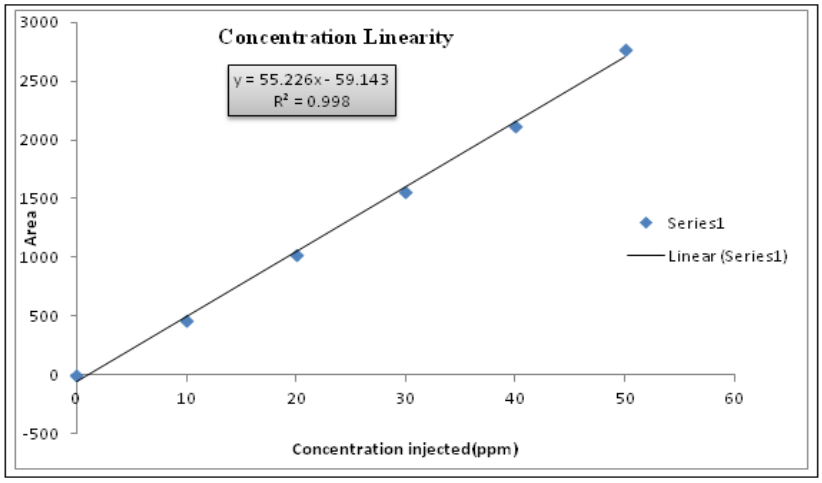

Fig. 5: Standard curve of Ondansetron Hydrochloride.

Table 3: Linearity Results of Ondansetron Hydrochloride.

S.No.	Concentration (µg/ml)	Peak Area
1.	10	465
2.	20	1018
3.	30	1561
4.	40	2122
5.	50	2763

System Suitability

Accurately weighed quantity of 9 mg of Ondansetron Hydrochloride was transferred to 100 ml volumetric flask and dissolved in mobile phase and volume was made up to the mark with mobile phase to obtain solution of 90 µg/ml. A 20µl standard solution was injected separately and their system suitability parameters were recorded. The tests were performed by collecting from five replicate injection of standard drug solution as shown in **Fig. 6** and **Table 4.**

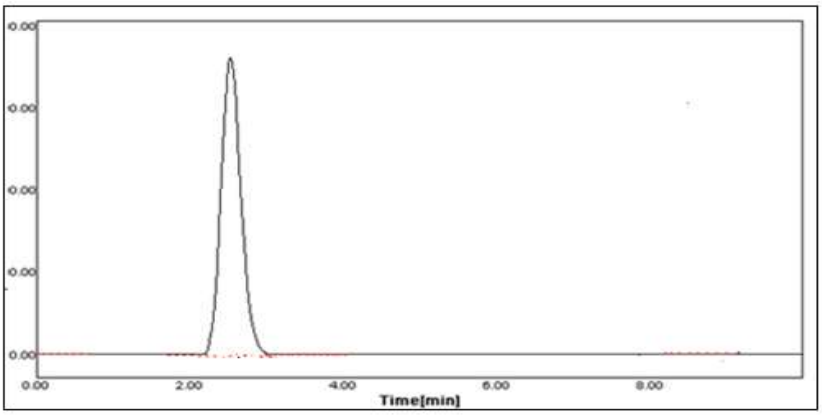

Fig. 6: System Suitability Chromatogram of Ondansetron Hydrochloride.

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Table 4: System Suitability Results of Ondansetron Hydrochloride.

S.No.	Rt (Min)	Peak area	Tailing Factor	Resolution	Theoretical Plates	Asymmetry Factor
1.	2.64	48901	0.81	10.2	43264	1.25
2.	2.64	48904	0.81	10.2	43270	1.25
3.	2.62	49402	0.80	10.3	43560	1.29
4.	2.64	48504	0.80	10.3	43106	1.23
5.	2.63	47910	0.79	10.1	42897	1.20
MEAN	2.634	48772.8	0.802	10.22	43219.4	1.234
S.D	0.0089	629.77	0.008	0.083	243.6	0.02
%RSD	0.338	1.29	1.0	0.81	0.56	1.62

Accuracy

Recovery of the method was evaluated by standard addition method in which appropriate portion of stock solutions of Ondansetron hydrochloride were spiked into blank placebo matrix to produce concentrations of 80, 100 and 120% of theoretical concentration. The mean recovery of spiked samples obtained was in range of 99.96 to 100.36 reveals no interference of excipients. The total amount of drug estimated using formula given below and results of recovery studies are shown in **Fig. 7 – 9** and **Table 5.**

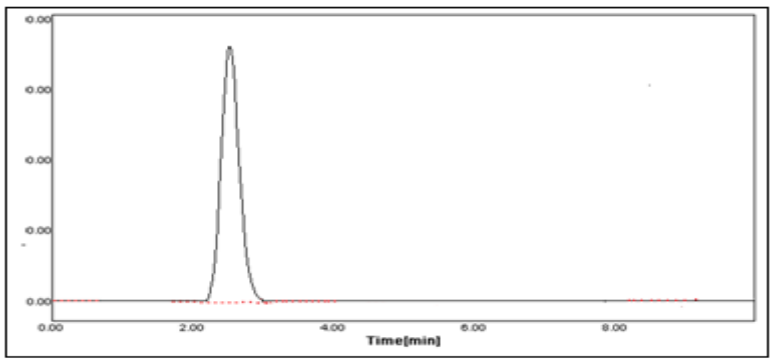

Fig. 7: Accuracy at 80 % Chromatogram of Ondansetron Hydrochloride.

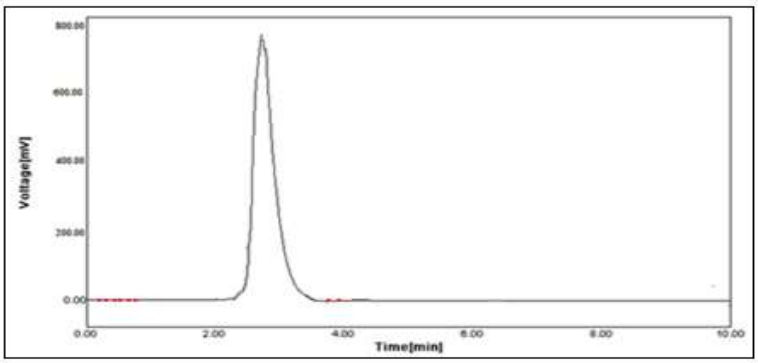

Fig. 8: Accuracy at 100 % Chromatogram of Ondansetron Hydrochloride.

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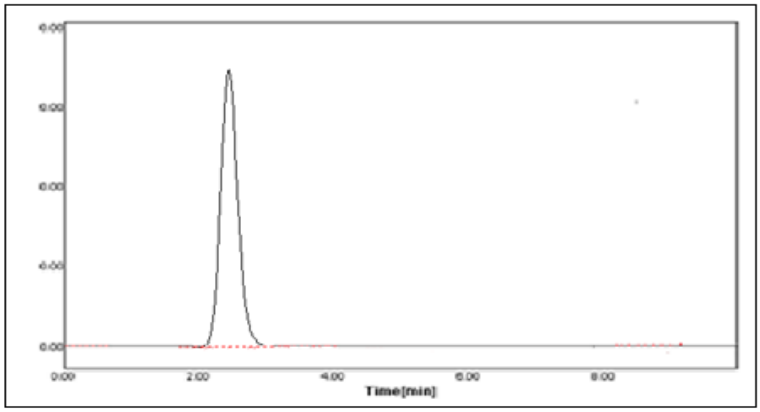

Fig. 9: Accuracy at 120 % Chromatogram of Ondansetron Hydrochloride.

Table 5: Results of Accuracy studies of Ondansetron Hydrochloride

Amount Added (%)	Total amount Added (mg)	Amount Recovered (mg)	%Recovery ± SD	%RSD
80	7.2	7.12	99.96±0.30	0.31
	7.2	7.13		
	7.2	7.11		
100	8.0	7.81	99.77±0.41	0.40
	8.0	7.80		
	8.0	7.82		
120	8.8	8.92	100.36±0.52	0.51
	8.8	8.90		
	8.8	8.94		

SD: standard deviation, % RSD: Relative standard deviation

Limit of Detection and Limit of Quantitation

LOD and LOQ were estimated from single to noise ratio using 3.3 σ/s and 10 σ/s respectively. LOD and LOQ were found to 1.7 and 5.26 µg/ml.

CONCLUSION

The developed method was validated for various parameters and found to be reliable and accurate. The detection was carried out at 216 nm. The retention time of drug was found 2.64 min. The proposed method shall prove effective to analyze Ondansetron Hydrochloride in the corresponding drug sample.

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