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Development and Validation of Rapid RP-HPLC Method for Estimation of Ondansetron in the Tablet Dosage Form

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Abstract
The developed reverse phase high-performance liquid chromatographic (RP-HPLC) method for the analysis of Ondansetron tablet dosage form is precise and feasible. The separation was carried out on a Fortis C18 (4.6 x 100 mm, 2.5 µm) column, using Methanol: 0.1 % OPA (Ortho-phosphoric acid) in ratio of 50: 50 % v/v as the mobile phase with flow rate at 0.7 mL/min and analysis was performed at wavelength 248 nm at ambient temperature. The injection volume was 20 µL. The retention time of the drug was 4.77 min. The method produced linear responses in the concentration range of 10 to 50 µg/mL. The LOD and LOQ values for HPLC method were found to be 0.11 and 0.32 µg/mL respectively. The method was validated as per ICH norms. The use of short column made method consumable. The method is also cost effective. The proposed method is useful for rapid analysis of Ondansetron in pharmaceutical dosage forms.

Keywords
RP-HPLC, Ondansetron, method development and validation.

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INTRODUCTION:
Ondansetron hydrochloride is chemically 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl1H-imidazol-1-yl) methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate. It represents the class of selective 5-HT3 antagonists which is commonly employed as anti-emetic in combination with anti-ulcer and anti-cancer agents. Ondansetron reduces 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].

The spectroscopic methods such as UV [2-5], visible [6, 7] were reported for Ondansetron in pharmaceutical dosage forms. The quantitative determination of Ondansetron in combination by HPLC [8-14] technique were reported in Literature. HPTLC [15] and LC- MS [16] methods were also reported for the estimation of Ondansetron. Generally shorter columns are used for simple separation and large column used for separation of complex sample. Both the separation and efficiency affected by the column length. The column efficiency tends to decrease with length however; shorter column results in shorter analysis time [17]. The present work describes a new, simple and accurate reverse phase liquid chromatographic method for the estimation of Ondansetron in

pharmaceutical tablet dosage form. The developed method was validated to ensure the compliance in accordance with ICH guidelines [18].

MATERIAL & METHOD:

Chemical

Ondansetron standard is procured as a gift sample from Ipca Laboratories Limited, Mumbai. The HPLC Grade solvents such as Methanol and water were purchased from Merck (India) Ltd. Ortho-phosphoric acid of analytical Grade was purchased from Merck, Mumbai. The marketed formulation (Vomiz 4 mg- ZYDUS CADILA) was purchased from retail pharmacy in Mumbai (Maharashtra, India).

Instrumentation

Chromatographic analysis was performed on Agilent Technologies- 1100 Gradient System equipped with UV (DAD) G13148 Detector controlled by CHEMSTATION 10.1 Software, with auto injector. The column Fortis C18 (4.6 x 100 mm, 2.5 µm) is used.

Chromatographic conditions

The chromatographic separation of Ondansetron was carried out on column Fortis C18 (4.6 x 100 mm, 2.5 µm) by using mobile phase vary in the ratio for the development of the HPLC method. The suitability of the solvent system ratio was decided on the basis of the sensitivity of the assay, retention time and tailing factor.

Preparation of standard stock solution

Weighed accurately about 10 mg of Ondansetron working standard into a 10 mL volumetric flask and added 7 mL of Methanol, sonicated to dissolve and make up the volume with Methanol. Further dilutions were made from 0.1 mL to 0.5 mL upto the volume 10 mL with the diluent.

Preparation of sample solution

Twenty tablets were weighed and crushed. The powdered drug equivalent to 135 mg was transferred in a single vial. 5 mL of methanol was used to dissolve the powder completely and the volume was made to 10 mL.

Selection of analytical wavelength

An appropriate dilution of standard stock solution with mobile phase were prepared. The solutions were scanned between the wavelength range 400-200 nm using the UV spectrophotometer

Methodology [19- 24]

The optimized chromatographic separation of Ondansetron was achieved on column Fortis C18 (4.6 x 100 mm, 2.5 µm) by using mobile phase composed Methanol: 0.1 % OPA (ortho phosphoric acid) in ratio of 50: 50 % v/v; at a flow rate of 0.7 mL/min. Detection of drug was carried out at 248 nm by using diluent as mobile phase.

Method validation

The validation parameters like limit of detection, limit of quantitation, linearity, accuracy, precision, robustness were evaluated as per ICH guidelines.

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

The LOD and LOQ were separately determined based on the signal to noise ratio. For LOD the S/N ratio was 3:1 and for LOQ the ratio was 10:1

Linearity

Under the optimized conditions, a calibration curve was prepared for Ondansetron. Standard mixture of different concentration were prepared for determining the working range. To assure the concentration range studied is linear the regression equation and correlation coefficient were evaluated.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analysed sample solution of Ondansetron, a known amount of standard drug powder of Ondansetron was added to 80, 100, 120% level.

Precision

The precision was determined by repeatability and intermediate precision through intra-day precision and inter-day precision study of the method. The intra-day precision was examined by replicating the assay thrice for two levels in the same day whereas the inter-day precision was obtained by assay of three sample sets on different days. The results of precision study were stated in terms of % RSD.

Robustness

Robustness of the method was tested by small but deliberate variations of flow rate, mobile phase composition and temperature. Effects of variation in the flow rate and wavelength were studied at two different concentrations.

Analysis of marketed formulation

The content of the drug was estimated in tablet formulation by the developed and validated HPLC method. Twenty tablets of Vomiz (4 mg) from Zydus Cadila were weighed and crushed to the fine powder. The powdered drug equivalent to 135 mg was transferred in a 10 mL volumetric flask. 5 mL of methanol was added and sonicated for 5 min to dissolve the powder completely and the volume was made to 10 mL. The content of Ondansetron in tablet formulation was determined.

RESULTS AND DISCUSSION:

Method development

The proposed chromatographic method was found to be suitable for effective separation of Ondansetron (Rt- 4.77) with good resolution, peak shape given in the figure 1. The mobile phase

composed of Methanol: 0.1 % OPA (Ortho-phosphoric acid) in ratio of 50: 50 % v/v, at a flow rate of 0.7 mL/min was selected as it gave well resolved peaks of standard Ondansetron. The optimum wavelength 248 nm selected for detection and quantitation.

Method validation

Linearity

The calibration curves were found be linear for the concentration range of 10-50 µg/mL. The standard working curve equation for drug was found to be y = 108.69 x + 87.827 with correlation coefficient value r^2 = 0.9999. The results of linearity are given in Table-1 and Figure- 2.

Recovery studies

The mean % recovery at 80, 100, 120 % of the test concentration along with its statistical validation for Ondansetron given in Table-2. The % recovery at 80, 100, and 120 % was found to be 100.41, 99.82, and 101.58. It was found that the method was accurate as the percent recovery was in the range of 100% for Ondansetron.

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % RSD and was found to be less than 2.0%. The % RSD of intra-day precision was found to be 0.06, 0.07 and 0.02 % RSD of interday precision was found to be 0.11, 0.21 and 0.18. The results of precision studies are shown in Table-3 and 4.

Limit of detection and Limit of quantitation

It was calculated by standard deviation of the response and the slope of calibration curve. LOD and LOQ of the method were calculated and found to be 0.11 µg/mL and 0.32 µg/mL.

Robustness

It was measured by multiple injections of a homogenous sample containing Ondansetron by changing flow rate 0.6 mL/min and 0.8 mL/min that indicates the performance of the HPLC instrument under chromatographic conditions by changing Wavelength i.e 247 nm and 249 nm. The method was found to be robust in the range of deliberate changes made. (Table- 5, 6)

Estimation of content of drugs in tablet

The developed and validated HPLC method estimated that the percent content of Ondansetron in tablet formulation was 99.57%. The detailed data is mentioned in Table- 7.

Table- 1 Linearity data of Ondansetron

Concentration µg/mL	Area
10	1179.15
20	2276.04
30	3317.91
40	4435.15
50	5533.95

Table-2 Recovery data of Ondansetron

Level (%)	Drug Conc (mg)	Amt added (mg)	Total Amt (mg)	Amt recovered (mg)	% Recovery
80%	10	8	18.03	8.03	100.41
100%	10	10	19.98	9.98	99.82
120%	10	12	22.03	12.03	101.58

a) Conc= Concentration, Amt= Amount

Table- 3 Precision study (intra- day) of Ondansetron

Conc µg/Ml	Area	AVG	SD	%RSD
	1179.54			
10	1178.96	1179.63	0.72	0.06
	1180.40			
	3313.68			
30	3316.89	3316.38	2.48	0.07
	3318.56			
	5531.37			
50	5534.12	5533.16	1.55	0.02
	5533.98			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table- 4 Precision study (inter-day) of Ondansetron

Conc µg/mL	Area	AVG	SD	%RSD
	1067.42			
10	1064.91	1065.83	1.13	0.11
	1065.15			
	3242.58			
30	3251.47	3250.08	6.91	0.21
	3256.20			
	5381.89			
50	5375.67	5373.32	9.95	0.18
	5362.40			

a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-5: Robustness study with change in flow rate of Ondansetron

Flow rate mL/min	Conc µg/mL	Area	AVG	%RSD
0.6		3957.32		
0.6	30	3965.23	3955.88	0.26
0.6		3945.10		
0.8		2960.67		
0.8	30	2965.87	2966.31	0.20
0.8		2972.40		

a) Conc= Concentration; b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-6: Robustness study with change in Wavelength of Ondansetron

Wavelength nm	Conc µg/mL	Area	AVG	%RSD
247		3290.41		
247	30	3294.82	3293.80	0.09
247		3296.30		
249		3426.78		
249	30	3452.69	3440.15	0.38
249		3440.98		

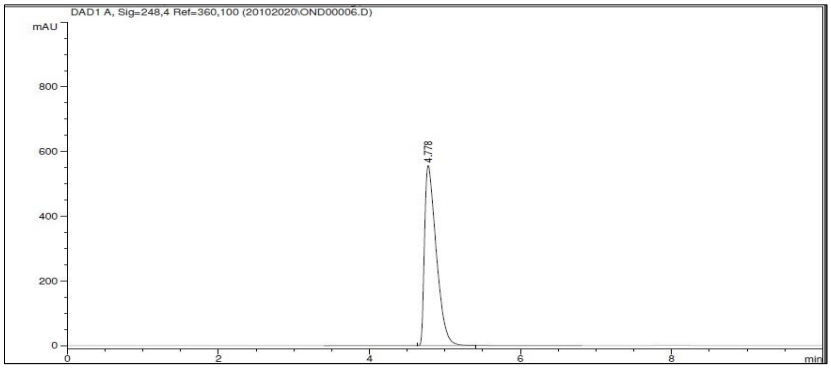
a) Conc= Concentration

b) AVG= average, SD= Standard deviation, RSD= Relative standard deviation

Table-7: Assay Results of Tablet Dosage Form

Parameter	Ondansetron
Label claim amount (mg)	4
Amount found (mg)	3.98
% Content	99.57%

Figure-1: HPLC Chromatogram with resolved peak of Ondansetron



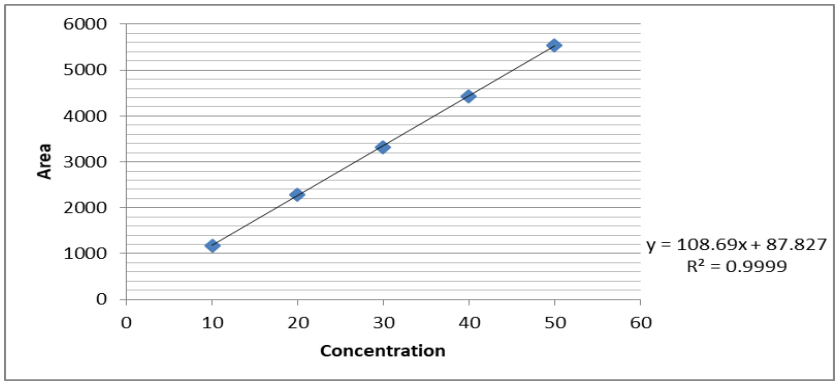


Figure-2: Linearity curve of standard Ondansetron

CONCLUSION:

The proposed developed method was validated as per ICH guidelines. The use of shorter column results in shorter analysis time (Rt- 4.77). The standard deviation and % RSD calculated of the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy of the proposed method. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be successfully used for the estimation of Ondansetron in bulk and marketed formulations.

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