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Validated Analytical Method Developed for Estimation of Ondansetron by Spectroscopy in Pharmaceutical Dosage Form

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ABSTRACT

The present research was undertaken to develop a spectroscopic method for determination of Ondansetron in pharmaceutical dosage forms. This paper describes a simple, rapid, accurate and precise UV-spectroscopic method for the assay of Ondansetron in bulk and marketed tablet dosage forms. The method is based on UV spectroscopic technique. Ondansetron shows the maximum absorbance at 302 nm in absorption maxima method. Drug followed the linearity in the range of 4-24 µg/ml for this method with correlation coefficient (r^2) of 0.999. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and sensitive.

Keywords: Validation, Ondansetron, Ethanol, Precision.

INTRODUCTION

Ondansetron is chemically named as 9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-2,3,4,9-tetrahydro-1H-carbazol-4-one, is official in IP, BP and USP. It is 5-HT3 receptor antagonist used mainly as an antiemetic (to treat nausea and vomiting). The antiemetic activity of the drug is brought about through the inhibition of 5-HT3 receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract) [1,2]. Literature review shows that there are developed methods including spectroscopic, HPLC and HPTLC methods for the estimation of Ondansetron alone and in combination of other drugs like Omeprazole, Rabeprazole etc. There are developed Spectroscopic methods of analysis in single or in combination. Ondansetron shows absorption in UV-visible range in acidic media produced by hydrochloric acid was measured in absorption maxima method. Inthe present investigation simple and sensitive UV spectroscopic method have been developed for the quantitative estimation of Ondansetron in bulk and its marketed formulations with good accuracy and economy [3].

MATERIALS AND METHODS

Active pharmaceutical ingredient used

Ondansetron drug from Sample from Nitin Pharmaceuticals Company is used along with Ondem drug from Alkem laboratories Ltd. The Instruments and chemicals used are shown in Table 1.

Method of development

Preparation of standard stock solution: Stock solutions of Ondansetron was prepared by transferring 50 mg of the drug in 50 ml volumetric flask and dissolved in 30 ml of ethanol and the volume was made up to the mark with ethanol. 2.5 ml of this solution was transferred to additional 25 ml volumetric flask and further diluted up to 25 ml mark with ethanol .This standard solution contained 100 µg of drug per ml.

Selection of wavelength maxima (λ_{max}): Pipette out 1 ml of working standard solution and transfer into 10 ml volumetric flask and the volume was made up to the mark with solvent to get the concentration 10 µg/ml. The resulted 10 µg/ml solution was scanned in UV-Spectrophotometer between 200-400 nm using ethanol as blank. The wavelength maxima were found at 302 nm [4].

Preparation of Calibration curve: Pipette out 0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 working standard solution and was transferred into eight separate 10 ml volumetric flasks and the volume of all of them was made to 10 ml with ethanol to get the concentrations 4, 8, 12, 16, 20, 24 µg/ml respectively. Absorbance of the resultant solution was measured at 302nm using ethanol as blank. A graph was plotted between the concentrations and their respective absorbance. The response of the drug was found linear in the entire investigational range of 4-24 µg/ml [5]. The calibration equation was obtained y=0.0397x + 0.0008 with 0.999 correlation coefficient. The Beer's-Lambert law was obeyed in the concentration range of (4-24 µg/ml) at 302 nm as shown in the Figure 1. Result was shown in Table 2.

Determination of optical parameters

The molar absorptivity and Sandell's sensitivity were calculated as

Molecular absorptivity=AM/CT

A=Absorbance

M=Molecular weight

C=Concentration

B=Path length

Sandell's sensitivity=M/E

M=Molecular weight

E=Molecular absorptivity

Other optical parameter that is beer's limit, slope, intercept and correlation coefficient were calculated from calibration curve.

The table shows the optical and regression characteristics of ondansetron. This shows that the method is linear and obeys Beer's law in the concentration range from 4-24 (µg/ml) with the molar absorptivity of 11617.169 litre $mole^{-1}cm^{-1}$ and sandell's sensitivity of 25.255×10^{-3} $\mu g/cm^2/0.001$ absorbance unit (Tables 3 and 4).

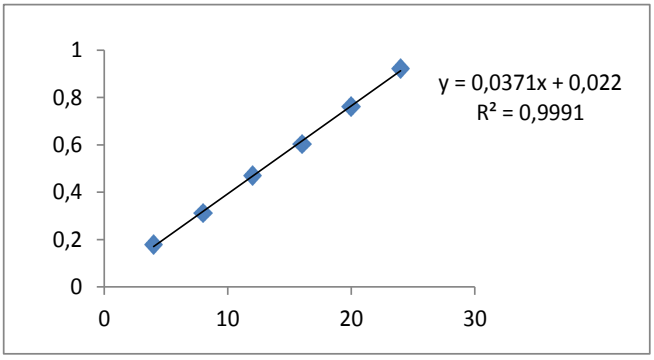

Figure 1: Calibration curve of ondansetron.

Table 1: Instruments and chemicals used.

S.No	Name	Model	Supplier/manufacturer
Instruments			
1	Single beam UV spectrometer	Cary 60 UV Visible	Agilent tech
2	Digital weight balance	TX323L	Shimadzu Instrument Pvt.Ltd
Chemicals			
1	Ethanol		Central Drug House Pvt.Ltd

Table 2: Data for calibration curve of Ondansetron.

S. No.	Concentration (µg/ml)	Absorbance*
1	4	0.179366
2	8	0.3117
3	12	0.470466
4	16	0.6045
5	20	0.761133
6	24	0.9220333
Note: *Average of three reading		

Table 3: Linearity, E1%1 CM, Absorptivity (L gm^{-1} cm^{-1}), Molar Absorptivity (L mol^{-1} cm^{-1}).

S. No	Conc. (µg/ml)	Absorption			Mean	E1%	Absorptivity	M.Absorptivity
		A1	A2	A3				
1	4	0.1787	0.187	0.1727	0.1793	448.25	44.82	13150.188
2	8	0.3111	0.31	0.3145	0.3117	389.62	38.96	11430.846
3	12	0.4753	0.4628	0.4853	0.4744	395.33	39.533	11600.449
4	16	0.6067	0.6057	0.6011	0.6045	377.81	37.781	11084.945
5	20	0.7662	0.751	0.7662	0.7611	380.55	38.055	11165.337
6	24	0.924	0.9144	0.9277	0.922	384.16	38.416	11271.254
Note: Mean: -11617.169								

Table 4: Optical parameter of ondansetron.

Parameters	Observation
Beer's law limit	04-32
Molar absorpivity	11617.169
Sandell's sensitity (mg/cm/0.001 absorbance unit)	0.025255
Regression equation (y=mx+c) m=slope, c=intercept	0.037
	0.022
Correlation coefficient	0.999

RESULTS AND DISSCUSSION

Validation of proposed method according to ICH guidelines

Precision Repeatability: Pipette out 1.2 ml standard solution and was transfer into a series of nine 10 ml volumetric flasks. It was diluted to 10 ml with ethanol to get 12 µg/ml solutions [6,7]. Absorbance of the resultant solutions was measured at 302 nm using ethanol as blank. The result was obtained and summarized in the Table 5.

Keeping the concentration of the drug same procedure was repeated 6 times. The calculated RSD for repeatability study is 0.082258, which is acceptable; this shows a good repeatability of method.

Intra-day precision: Pipette out 0.8,1.2,1.6 ml working solution and was transferred into separate 10 ml volumetric flasks and the volume was made up to 10ml with ethanol to get the concentrations 8,12,16 µg/ml . Absorbance of the resultant solutions was measured at 302 nm using ethanol as blank. Such 6 revisions were performed within a day at 3 and 6 hrs interval. The result was summarized in the Table 6. The calculated mean RSD was 0.08683

Inter-day precision: Pipette out 0.8,1.2,1.6 ml working solution and transfer into separate 10 ml volumetric flasks. Dilute all of them to 10 ml with ethanol to get solution of concentrations 8,12,16 µg/ml. Absorbance of the resultant solutions was measured at 302 nm using

Table 5: Study of repeatability.

Conc. (µg/ml)	Absorbance	Observed Conc. (µg/ml)	Mean Conc. (µg/ml)	SD	RSD
12	0.4656	12	12.08	0.00041	0.082258
	0.4657	12			
	0.4679	12.1			
	0.4785	12.3			
	0.4623	11.9			
	0.4727	12.2			

Table 6: Study of intraday precision.

Conc. (µg/ml)	Absorbance			Observed Conc. (µg/ml)			Mean Conc. (µg/ml)	SD	% RSD
	0 hr	3 hrs	6 hrs	0 hr	3 hrs	6 hrs			
8	0.3451	0.3283	0.2808	8.4	8.2	7.2	7.93	0.000143	0.04288
12	0.4785	0.4727	0.453	12.3	12.2	11.6	12.03	0.000473	0.10136
16	0.6174	0.6092	0.5996	15.9	15.7	15.5	15.7	0.0007	0.11625
Note: Mean :-0.08683									

Table 7: Study of Interday precision.

Recovery at	Nominal	Absorbance	Observed Conc. (µg/ml)	% Recovery
80%	18=10+8	0.6981	17.8	98.88
80%	18=10+8	0.698	17.9	99.44
80%	18=10+8	0.6983	18	100
100%	20=10+10	0.7326	19.6	98
100%	20=10+10	0.7908	20	100
100%	20=10+10	0.7461	19.8	99
120%	22=10+12	0.8478	22	100
120%	22=10+12	0.8424	21.8	99.09
120%	22=10+12	0..8475	21.9	99.54
Note: The % Recovery was found to be 99.327 (Mean: 99.327)				

Table 8: Study of accuracy.

Nominal conc.	Without excipients		With excipients		% Interference
	Absorbance	Observed Conc. (µg/ml)	Absorbance	Observed Conc. (µg/ml)	
12	0.4755	12.1	0.476	12.2	1.01
12	0.4458	11.5	0.4465	11.9	0.99
12	0.4753	12	0.5986	15.4	1.28
12	0.4555	11.7	0.4727	12.1	1
12	0.4623	11.9	0.4675	12	1
12	0.4785	12.3	0.469	11.7	0.97
Note: Mean: -1.20					

Table 10: %Assay of ondansetron in pharmaceutical dosage form (ondem, 4 mg).

Sr. No.	Absorbance	Conc. (µg/ml)	Dil. Factor	Weight taken (mg)	Avg. weight (mg)	Label claim (mg)	Assay%
1	0.6765	15.9	2500	40	40	4	99.37
2	0.6514	16.1	2500	40	40	4	
3	0.6846	16.2	2500	40	40	4	
4	0.6297	15.8	2500	40	40	4	
5	0.6247	16.1	2500	40	40	4	

Table 11: Summary of validation parameters of ondansetron.

Validation parameters	Observation
Linearity and range	4-24
Correlation coefficient (f)	0.999
Precision (RSD)	
• Repeatibility	0.082258
• Intraday	0.08683
• Interday	0.06222
%Recovery	99.327
%Interference	1.2
%Assay	99.37

ethanol as blank. Such six studies were performed for day one day two day three intervals. The result was summarized in the Table 7.

Accuracy: Pipette out 1 ml standard solution and transfer into 10 ml volumetric flasks. Nine such transfers were made. Spike three of volumetric flask with the solutions with 0.8 ml of working solution (Prepared from Formulation) and dilute each to 10 ml with ethanol to get 18 µg/ml solutions. Spike another three of the solutions with 1 ml of working solution and dilute each to 10 ml with ethanol to get 20 µg/ml solutions. Spike last three of the solutions with 1.2 ml of working solution and dilute each to 10 ml with ethanol to get 22 µg/ml solutions. Absorbance of the resultant solutions was measured at 302 nm using ethanol as blank. The obtained results were summarized in the Table 8.

Specificity: Specificity study was carried out by observing any interference in absorbance of drug in the presence of common excipients like starch, talc, lactose, magnesium stearate etc. Absorbance of 10 µg/ml drug solution with and without excipients was measured at 302 nm using ethanol as blank [8]. The results obtained were summarized in the Table 9.

Estimation of ondansetron in pharmaceutical dosage form (Ondem: 4 mg)

20 tablets were weighed and the average weights of the tablets were calculated. The tablets were powdered and weighed accurately [9]. A quantity of powdered containing about 20 mg of ondansetron was transfer into 50 ml volumetric flask and 15 ml ethanol was added. Sonicated for 15 minutes and the volume were made upto 50 ml with solvent then was mixed and filtered. 2.5 ml of the filtrate was taken and was made up to 25 ml with ethanol. Further 1.6 ml of the resulting solution was diluted to 10 ml with ethanol. The absorbance of this resulting solution was measured at 302 nm. The above procedure was repeated for three times. The result obtained is summarized in the Tables 10 and 11.

CONCLUSION

A simple and sensitive spectroscopic method for quantitative determination of ondansetron in either pure form or in pharmaceutical dosage for was developed. Ondansetron showed maximum absorbance at 302nm in solvent. It has linear response in the entire range of 4to 24 µg/ml with correlation coefficient of 0.999, with molar absorptivity of 11582.332 litre $mole^{-1}cm^{-1}$ and Sandell's sensitivity of 0.025255 $microgram/cm^2/0.001$ absorbance units. The linear regression equation obtained is y=0.037x+0.022. The method has good precision <2% and accuracy is 99.37 ± 0.50. No significant interference was observed in the absorbance of the drug in the presence of common excipients. The method was statistically validated according to ICH.

The method was employed for the quantitative determination of tablet dosage form. In conclusion, the developed spectroscopic methods are simple, accurate, and reproducible, and can be used in routine analysis of Ondansetron in bulk.

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