UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF ONDANSETRON HYDROCHLORIDE IN BULK AND FORMULATION

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ABSTRACT

UV Spectrophotometric Method Development and Validation for quantitative estimation of Ondansetron Hydrochloride (OND). UV Spectrophotometric method has been widely employed in the determination of individual components in a mixture of fixed-dose combinations. We aim to develop a spectroscopic method for estimation of the Ondansetron HCL in ternary mixture by using UV spectrophotometry. The method was validated as per ICH guidelines. The drug obeyed Beer's law and showed a good correlation. It showed absorption maxima at 309 nm in simulated saliva. The recovery studies confirmed the accuracy and precision of the method. It was successfully applied for the analysis of the drug in bulk and could be effectively used for routine analysis.

KEYWORDS: UV spectrophotometry, Ondansetron hydrochloride, Simulated saliva, Tablets.

INTRODUCTION

Ondansetron hydrochloride, chemically 4Hcarbazol- 4-one-1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazole-1-yl) methyl] hydrochloride is selective 5-HT$_3$ antagonist. It acts both, peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. It is indicated for the prevention of nausea and vomiting associated with cancer chemotherapy, radiotherapy, or anesthesia and surgery. It is penicillinase-resistant penicillin, used in the treatment of bacterial infections such as pneumonia and bone, ear, skin,

and urinary tract infection. The IUPAC name of ondansetron Hydrochloride is (RS)-9-methyl-3-[(2-methyl-1H-imidazol-1-   yl)methyl]-2,3-dihydro-1H-carbazol-4(9H)-one.   The molecular formula and Molecular weight is $C_{18}H_{19}N_3O$ and 293.4g/mol respectively and belongs to the class of anti-emetics.[1]

Ondansetron Hydrochloride is the subject of a monograph in Indian Pharmacopoeia, United States Pharmacopoeia, and British Pharmacopoeia. Present work describes simple, accurate, reproducible, rapid, and economical methods for simultaneous estimation of Ondansetron Hydrochloride.[2]

MATERIAL AND METHODS

Material

The reference standard of Ondansetron HCl API was supplied as a gift sample by Anugraha Chemical, Bangalore, India.

Instruments

A double beam UV Visible Spectrophotometer (Systronic 2201), Electronic Weighing Balance (SHIMADZU AY220), and Sonicator (Oscar Ultrasonicator micro clean-103) were used to perform this experiment.

Methods

Preparation of Simulated Saliva fluid pH 6.8

Simulated saliva fluid of pH 6.8 used in the analysis was prepared as per the composition described by Mashru et al. 2005. It contains 2.38 g of disodium hydrogen phosphate Na_2HPO_4, 0.19 g of potassium dihydrogen phosphate (KH_2PO_4), and 8.00 g of sodium chloride (NaCl) per 1000 ml of distilled water. The pH of the solution was adjusted to 6.8 using orthophosphoric acid.[3]

Preparation of standard stock solution

An accurately weighed 10 mg of Ondansetron hydrochloride pure drug was dissolved in 100 ml of simulated saliva using a 100 ml volumetric flask. The solution was then sonicated for 10 min and the final volume was adjusted up to the mark with the same solvent, to give the final concentration of 100μg/ml. Out of this stock, 6.25ml was pipetted and diluted up to 25ml by simulated saliva (2.5- 12.5μg/ml) and examined between 200-400 nm. The

maximum absorbance was determined using UV Vis Spectrophotometer (Systronic 2201) to confirm the λmax of the drugs.

Selection of wavelength

Ondansetron hydrochloride standard solution (25 µg/ml) was prepared by appropriate dilution of standard stock solution and then scanned in the UV range (200-400 nm). Ondansetron hydrochloride showed the absorption maxima at 309 nm (Fig.no.1).

Fig. no.1: UV-VIS spectra of Ondansetron Hydrochloride.

Calibration curve

Five concentrations of Ondansetron hydrochloride standard solution (2.5, 5, 7.5, 10, 12.5 µg/ml) were prepared by pipetting out 1 ml, 2 ml, 3 ml, 4 ml, 5 ml of standard stock solution and transferring into a series of 10 ml volumetric flask. The volume was then adjusted up to the mark with simulated saliva. The absorbance of each solution was recorded at 309nm using simulated saliva as a blank. A calibration graph was prepared by plotting absorbance vs respective concentration.

Validation of method

The method was validated as per ICH guidelines for different parameters like Linearity, Precision, Accuracy, LOD, LOQ, and Robustness.

Linearity and Range

Various aliquots of Ondansetron Hydrochloride were prepared from a stock solution in the range of 2.5-12.5 µg/ml. The samples were scanned in UV-VIS Spectrophotometer and the calibration graph plotted absorbance versus respective concentration. Beer's law was obeyed over the concentration range of 2.5-12.5 µg/ml. (Table 1)

Precision

Precision study of the developed method was performed as inter-day precision and intraday precision. Intraday precision (Table 2) was performed by analyzing the solution of known concentration i.e. 2.5 µg/ml six times a day. Inter-day precision (Table 3) was performed by analyzing the solution of the same concentration for 2 days. The % RSD was calculated.

Accuracy

The accuracy study of the developed method was carried out by calculating the recovery of the drug by the standard addition method. In this, a known amount of ondansetron hydrochloride standard solution was added to the sample solution (tablet). The recovery study was performed at three different concentration levels i.e. 80%, 100%, and 120% of the working concentration of the sample. The percentage recoveries were calculated (Table 4).

Limit of detection (LOD) and Limit of quantification (LOQ)

Limit of detection (LOD) is the lowest concentration of analyte in the sample that can be detected but not necessarily be quantified, under a stated experimental condition, and Limit of Quantification (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental condition. The sensitivity of Posaconazole by the developed method was estimated by the limit of detection (LOD) and Limit of Quantification (LOQ). The LOD & LOQ were calculated using the following Formula-

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where 'σ' is the standard deviation of response and 'S' is the slope of the corresponding calibration curve.

Robustness

The robustness of the developed method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. Robustness was determined by recording the absorbance of the solution at two different wavelengths i.e. at 310 ± 1 nm. (Table 5)

RESULTS

Determination of wavelength of maximum absorption

The wavelength of maximum absorption (λmax) of Ondansetron hydrochloride was found to be 309nm.

Calibration curve of Ondansetron Hydrochloride

The calibration curve showed the linearity in the range of 2.5-12.5 µg/ml with a correlation coefficient of 0.9986 and regression equation as y = 0.0659x-0.0028(Fig. no.2).

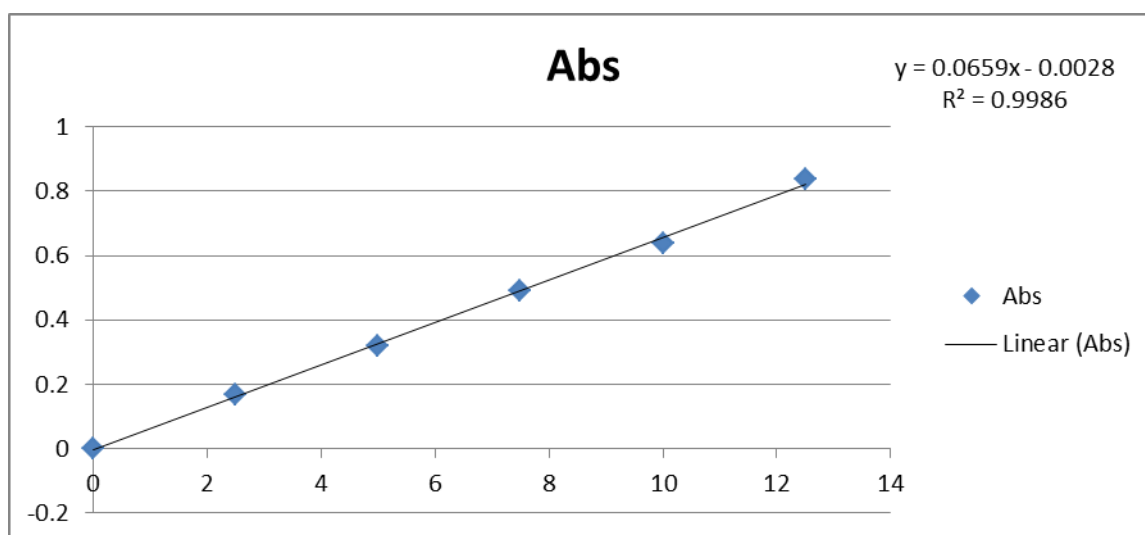**Fig. no.2: Calibration curve for Ondansetron Hydrochloride.**

Validation parameters

Linearity

The linearity was found to be in the range of 2.5-12.5 µg/ml. The regression coefficient was found to be 0.9986.

Table 1: Linearity of Ondansetron hydrochloride.

Sr.No.	Concentration (µg/ml)	Absorbance
1	0	0
2	2.5	0.169
3	5	0.319
4	7.5	0.491
5	10	0.639
6	12.5	0.837

Precision

The % RSD values for inter-day and intraday precision were found to be less than 2 %.

Table 2: Intraday precision.

Sr.No.	Concentration (µg/ml)	Absorbance
1	2.5	0.169
2	2.5	0.171
3	2.5	0.172
4	2.5	0.169
5	2.5	0.171
6	2.5	0.173
	AVERAGE	0.170833
	SD	0.001602
	% RSD	0.937806

Table 3: Inter-day precision.

Sr.No.	Concentration (µg/ml)	Absorbance (DAY 1)	Absorbance (DAY 2)
1	2.5	0.169	0.168
2	2.5	0.171	0.171
3	2.5	0.172	0.171
4	2.5	0.169	0.172
5	2.5	0.171	0.169
6	2.5	0.173	0.173
	AVERAGE	0.170833	0.17066
	SD	0.001602	0.001862
	% RSD	0.937806	1.09099

Accuracy

The % recoveries were found to be in the range of 98 – 101 % indicating that the method was accurate.

Table 4: Accuracy of Ondansetron hydrochloride.

Concentration (µg/ml)	% of standard spiked to sample	Amount added (µg/ml)	Amount found (µg/ml)	Mean % Recovery
100	80	8	8.08	101
100	100	10	9.86	98.6
100	120	12	11.9	99.6

Limit of Detection & Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.6482 and 1.9645 respectively.

Robustness

The robustness was calculated at two different wavelengths 309 nm and 312 nm and it gave reliable results as the % RSD was found to be less than 2 %.

Table 5: Robustness studies.

Wavelength	309	312
Concentration	2.5(μg/ml)	2.5(μg/ml)
Absorbance	0.169	0.174
	0.170	0.174
	0.170	0.173
Average	0.16966	0.17366
SD	0.00577	0.000577
%RSD	0.340298	0.33246

CONCLUSION

A simple, sensitive, accurate, and precise UV-Visible spectrophotometric method was developed and validated as per ICH guidelines. All the parameters were found to be within the standard range. The method can be applied for routine analysis of Ondansetron hydrochloride in bulk and pharmaceutical dosage form.

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