

Novel Analytical Techniques for the Determination of Ondansetron Hydrochloride in Pharmaceutical Dosage Forms by Spectrophotometry

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ABSTRACT

Ondansetron hydrochloride is a 5HT₃-receptor antagonist used as an antiemetic. Ondansetron HCl dihydrate is a white to off-white powder that is soluble in water and normal saline. The 5-HT₃ receptor antagonists are the primary drugs used to treat and prevent chemotherapy-induced nausea and vomiting and radiotherapy-induced nausea and vomiting. Five simple, rapid, sensitive, precise and accurate spectrophotometric methods were developed for the determination of Ondansetron hydrochloride in pharmaceutical formulations. Spectrophotometric analysis was performed at absorption maxima 310 nm in phosphate buffer (pH 3.6), sodium acetate buffer (pH 4.0), phosphate buffer (pH 5.0), phosphate buffer (pH 7.0) and borate buffer (pH 9.0) and shows linearity over the concentration range 0.5-70, 0.1-80, 0.5-60, 0.5-70 and 0.5-60 µg/ml respectively. All the five methods were validated and can be used for the determination of Ondansetron hydrochloride in pharmaceutical formulations.

KEY WORDS: Ondansetron hydrochloride, Spectrophotometry, Validation.

1. INTRODUCTION

Ondansetron hydrochloride (OND), a carbazole derivative is a potent, highly selective competitive 5HT₃-receptor antagonist that has been introduced to clinical practice as an antiemetic for cancer treatment-induced and anesthesia-related nausea and vomiting (Ye, 2001). Chemically it is 9-methyl-3-[(2-methyl-1H-imidazol-1-yl)-methyl]-1, 2, 3, 9-tetrahydro-4H-carbazol-4-one hydrochloride dihydrate with molecular weight 365.86 g/mol (Indian Pharmacopoeia, 2010). It acts both peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the postrema. It is indicated for the prevention of nausea and vomiting associated with cancer chemotherapy, radiotherapy and anesthesia and surgery (Tripathi, 2010).

Ondansetron hydrochloride was determined by different analytical techniques such as Spectrophotometry (Sradhanjali, 2007; Chennaiah, 2012; Kalaichelvi, 2012; Lobhe, 2011; Chauhan Prakash, 2012; Asad, 2007; 2013; Zamora, 1996; Sudhakararao, 2014; Shirish, 2014; Kolte, 2012) and RP-HPLC (Zarna, 2009; Mushabbar, 2013) in pharmaceutical formulations.

In the present study, five novel simple, rapid and cost-effective UV spectrophotometric methods were developed for the routine analysis of Ondansetron hydrochloride in pharmaceutical formulations in phosphate buffer pH 3.6 (Method A), Sodium acetate buffer pH 4.0 (Method B), phosphate buffer pH 5.0 (Method C), phosphate buffer pH 7.0 (Method D) and borate buffer pH 9.0 (Method E) and validated as per the ICH guidelines (2005).

2. EXPERIMENTAL

2.1. Instrumentation: A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm matched quartz cells. For scanning, the wavelength range selected was 400 to 200 nm with medium scanning speed. All weights were taken on electronic balance (Shimadzu).

2.2. Reagents and chemicals: Analytical grade reagents were used. Pure samples of Ondansetron hydrochloride was kindly supplied as gift sample from Symbio Labs (India) India. Ondansetron hydrochloride is commercially available as tablets and injections with brand name Odep Tab® and Odep Inj®, (Label claim: 4 mg/tablet; Plenus Pharmaceuticals Pvt. Ltd., India).

2.2.1. Preparation of Phosphate Buffer (pH 3.6) (Method A): Dissolve 900 mg of anhydrous disodium hydrogen phosphate and 1.298 g of citric acid monohydrate in sufficient water in a 1000 ml. volumetric flask.

2.2.2. Preparation of sodium acetate buffer (pH 4.0) (Method B): 2.86 ml of glacial acetic acid and 1.0 ml of a 50 % solution of sodium hydroxide were taken in a 1000 ml volumetric flask, add diluted up to the mark with distilled water.

2.2.3. Preparation of phosphate buffer (pH 5.0) (Method C): 6.8 grams of potassium di hydrogen phosphate was diluted with water in 1000ml volumetric flask and adjusted pH to 5.0 with 10M potassium hydroxide.

2.2.4. Preparation of Phosphate Buffer (pH 7.0) (Method D): Place 50.0 ml of 0.2 M potassium dihydrogen phosphate and 29.1ml of 0.2 M sodium hydroxide and dilute with water to 1000 ml

2.2.5. Preparation of Borate Buffer (pH 9.0) (Method E): Dissolve 6.20 g of boric acid in 500 ml of water, adjust to pH 9.0 with 1M sodium hydroxide (about 41.5 ml) and dilute with water in a 1000 ml volumetric flask.

2.3. Preparation of stock solutions: Ondansetron hydrochloride stock solution was prepared by dissolving 25 mg

of the drug in methanol in 25 ml volumetric flask (1000 µg/ml) and further dilutions were made from the stock solution as per the requirement with the reagents mentioned for methods A, B, C, D and E respectively.

2.4. Validation

2.4.1. Linearity and Range: A series of Ondansetron hydrochloride solutions were prepared and scanned (200-400nm) against their reagent blank. The absorbance of the solutions was noted from their absorption spectrum in all the five methods A, B, C, D and E. A calibration curve was plotted respectively by taking the concentration of the solutions on the x-axis and the corresponding absorbance on the y-axis.

2.4.2. Accuracy and Precision: The precision and accuracy studies were performed as per the ICH guidelines. Accuracy was evaluated from the percent recovery studies by the addition of 80%, 100% and 120% of pure sample solution to the pre-analysed formulation solution. Ondansetron hydrochloride extracted drug solution from the formulation (10 µg/mL) was spiked with 80%, 100% and 120% of pure drug solution and the % recovery was calculated.

The precision study was done by recording the absorbance of six replicates. The intra-day precision studies were carried out at three different concentration levels (5, 10 and 20 µg/ml) individually on the same day for all the five methods A, B, C, D and E respectively and the % RSD was calculated. The inter-day precision study was also performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (5, 10 and 20 µg/ml) individually on the same day for all the five methods A, B, C, D and E respectively and the % RSD was calculated.

2.5. Assay for marketed formulations: Twenty tablets from each brand Odep Tab® and Odep Inj®, (Label claim: 4 mg/tablet; Plenus Pharmaceuticals Pvt. Ltd., India) were procured from the local pharmacy store, weighed and powdered. Powder equivalent to 25 mg of Ondansetron hydrochloride was transferred carefully in to a 25ml volumetric flask and extracted with methanol. The filtrate so obtained was diluted further with the respective reagents separately for method A, B, C, D and E respectively and the percentage recovery was calculated.

3. RESULTS AND DISCUSSION

The absorption spectrum of Ondansetron hydrochloride in all the reagents mentioned for method A, B, C, D and E respectively have shown absorption maxima values at three λ_{max} values at 310nm, 267nm and 249 nm but 310 nm was selected for all the analytical determinations (Figure 2).

3.1. Validation: A calibration graph was drawn by taking the concentration of the Ondansetron hydrochloride solutions on the x-axis and the corresponding absorbance values on the y-axis for method A, B, C, D and E. The calibration curves were shown in Figure 3. The linear regression equations were found to be $y=0.0508x-0.0045$ ($R^2=0.9998$), $y = 0.0463x-0.0031$ ($R^2=0.9999$), $y=0.0491x+0.0078$ ($R^2=0.9999$), $y=0.0503x-0.002$ ($R^2=0.9999$) and $y=0.0264x+0.0019$ ($R^2=0.9999$) in method A, B, C, D and E respectively.

The % RSD in precision studies was found to be less than 2% in all the method A, B, C, D and E indicating that the methods are more precise. The % recovery in accuracy studies for all the 5 methods A, B, C, D and E were found to be ≤ 98 % indicating that the method is more accurate. The optical characteristics of Ondansetron hydrochloride were shown in Table 1. The % RSD values in precision studies were found to be $<2\%$ indicating that the method is more precise. The % recovery values in accuracy studies were found to be $>98\%$ with % RSD $<2\%$ for method A, B, C and D respectively indicating that the method is more accurate.

The methods have been applied for the determination of Ondansetron hydrochloride marketed formulations and the percentage recovery was found to be in the range of 98.21-98.87, 98.24-99.35, 98.24-99.45, 98.07-99.11 and 98.67-99.02 for method A, B, C, D and E respectively (Table 2) indicating that the proposed methods can be applied for the determination of pharmaceutical formulations successfully.

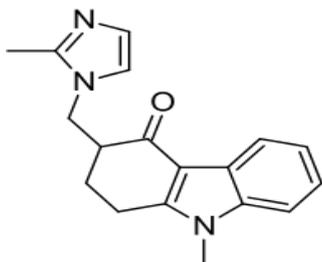
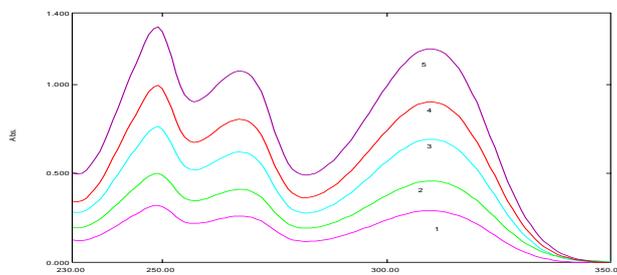
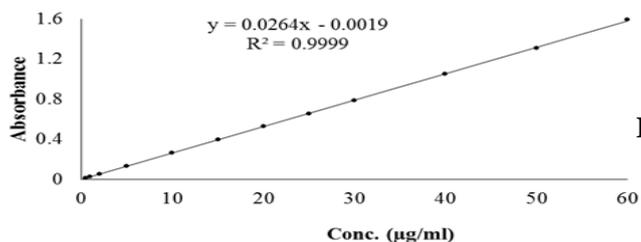
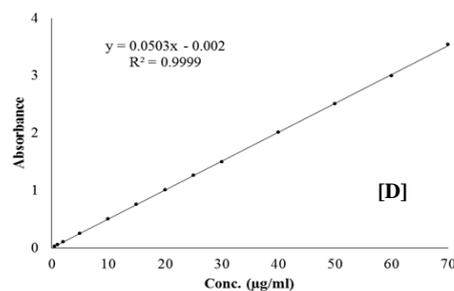
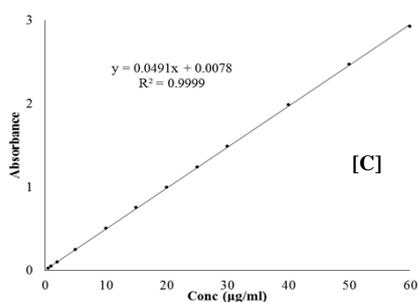
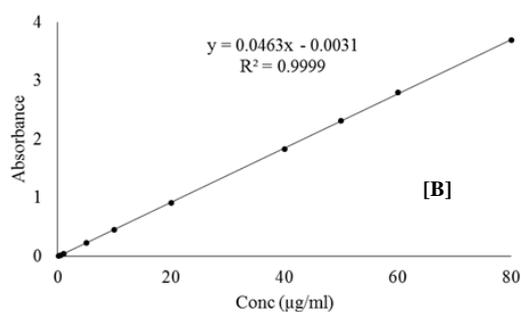
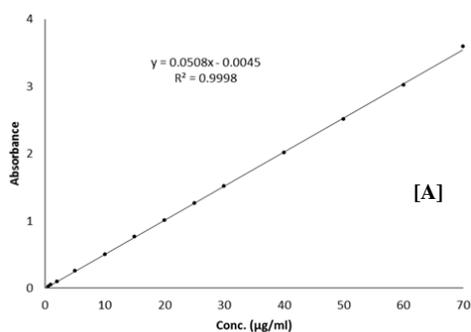
Table.1. Optical characteristics of Ondansetron hydrochloride

Parameters	Method A	Method B	Method C	Method D	Method E
Regression equation ($y=mx + c$)	$y=0.0508x - 0.0045$	$y=0.0463x- 0.0031$	$y=0.0491x+ 0.0078$	$y=0.0503x- 0.002$	$y=0.0264x +0.0019$
Correlation coefficient (R^2)	0.9998	0.9999	0.9999	0.9999	0.9999
Linearity range ($\mu\text{g/ml}$)	0.5-70	0.1-80	0.5-60	0.5-70	0.5-60
Accuracy (% Recovery (%RSD))	99.54-99.87 (0.20-0.43)	99.61-99.89 (0.09-0.25)	99.23-99.61 (0.25-0.47)	99.18-99.43 (0.22-0.34)	99.42-99.88 (0.17-0.65)
Precision: Intra-day (% RSD)	0.17-0.37	0.09-0.21	0.18-0.34	0.09-0.31	0.11-0.35
Inter-day (% RSD)	0.24-0.63	0.15-0.38	0.23-0.68	0.31-0.70	0.25-0.69
Sandell's Sensitivity ($\mu\text{g/cm}^2/0.001$ absorbance unit)	1.996×10^{-2}	2.183×10^{-2}	1.992×10^{-2}	1.996×10^{-2}	3.816×10^{-2}
Molar extinction coefficient (Liter/mole/cm)	1.832×10^4	1.675×10^4	1.836×10^4	1.832×10^4	9.858×10^3

Table 2. Assay of marketed formulations (Tablets)

Brand	Labeled Amount (mg)	*Amount obtained (mg)					% Recovery*				
		Method					Method				
		A	B	C	D	E	A	B	C	D	E
Odep Tab®	4.0	3.928	3.974	3.978	3.964	3.961	98.21	99.35	99.45	99.11	99.02
(Injection)	4.0	3.955	3.930	3.938	3.929	3.947	98.87	98.24	98.44	98.07	98.67

*Each value is average of three determinations

**Figure 1. Chemical structure of Ondansetron hydrochloride****Figure 2. Overlay Absorption spectrum of Ondansetron hydrochloride in Phosphate buffer, pH-3.6 (1), Sodium acetate, pH-4.0 (2), Phosphate buffer, pH-5.0 (3), Phosphate buffer, pH-7.0 (4), Borate buffer, pH-9.0 (5)****Figure 3. Calibration curves of Ondansetron hydrochloride in Phosphate buffer pH-3.6 [A], Acetate buffer pH-4.0 [B], Phosphate buffer pH-5.0 [C], Phosphate buffer pH- 7.0 [D] and Borate buffer pH-9.0 [E]**

4. CONCLUSION

The proposed methods are simple, precise and accurate and can be applied for the determination of Ondansetron hydrochloride in pharmaceutical formulations successfully.

5. ACKNOWLEDGMENT

The authors are grateful M/s GITAM University for providing necessary research facilities and to Symbio Labs (India) for providing the gift samples of Ondansetron hydrochloride.

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