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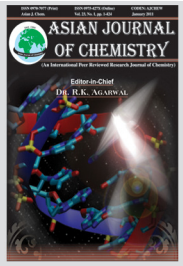

Extractive Spectrophotometric Determination of Ondansetron Hydrochloride in Pure and Dosage Forms by Using Alizarin Red-S and Bromothymol Blue

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Two simple, sensitive and accurate visible spectrophotometric methods have been developed for quantification of odansetron in pure and dosage formulations. These methods (method A & B) are based on the extraction of ion-ion associated complex between ondansetron HCl and acidic dyes such as alizarin red-S (ARS) and bromothymol blue (BTB) into chloroform solvent. The coloured products thus formed showed maximum absorbance at 425 and 420 nm for alizarin red-S and bromothymol blue respectively. Beer's law plots are linear over the range of concentrations 3.3-20.0 and 8.3-50.0 µg/mL with correlation coefficients 0.9997 and 0.9999 for the two methods A and B respectively. Different parameters which are affecting the reaction pathway are studied and optimized. The results thus obtained are in good agreement with those of reference methods. The proposed methods are successfully applied for the determination of ondansetron hydrochloride either in pure or dosage formulations.

Key Words: Ondansetron hydrochloride, Alizarin red-S, Bromothymol blue, Ion-ion associated complex molecule, Spectrophotometry, Dosage forms.

INTRODUCTION

Ondansetron hydrochloride is chemically known as 4*H*-carbazol-4-one,1,2,3,9-tetrahydro-9-methyl-3-(2-methyl-1*H*-imidazol-1-yl)methyl-,monohydrochloride,(±)-,dihydrate or (±)-2,3-dihydro-9-methyl-3-(2-methylimidazol-1-yl)-methylcarbazol-4(1*H*)-one monohydrochloride dihydrate. The empirical formula is $C_{18}H_{19}N_3O{\cdot}HCl{\cdot}2H_2O$ (m.w. 365.9). It is used in the prevention of nausea and vomiting associated with highly emetogenic cancer chemotherapy. The active ingredient in zofran tablet is ondansetron base, the racemic form of ondansetron and a selective blocking agent of the serotonin 5-HT_3 receptor type. Ondansetron HCl is official in the Indian Pharmacopoea, British Pharmacopoea and United States Pharmacopoeia[1-4].

An extensive literature survey is carried out and it is evident that some analytical methods such as reverse phase HPLC, spectrophotometric and derivative spectrophotometric methods are reported for the determination of ondansetron in pure and formulations and mostly in biological fluids. Reverse phase HPLC[5-15] are reported in the literature for the determination of ondansetron mostly in biological fluids[16-25] and in the study of impurities[26]. The reported methods are costly and not applicable at higher concentrations. A few spectrophotometric[17-28] methods are also found in the literature for the determination of ondansetron in pure and formulations. In addition to these methods a derivative spectrophotometric method[29] is also found for the determination of ondansetron in combination with paracetamol.

EXPERIMENTAL

SL159 model, 2 nm high resolution, double beam, 1 cm length quartz coated optics; wavelength range190-1100 nm; high stability, linearity, precision instrument is used for all the spectral measurements. All chemicals and reagents used in the analysis are of analytical grade and doubly distilled water is used for the preparation of all the solutions. 0.2 % solution of alizarin red-S (ARS) is prepared by dissolving about 200 mg of alizarin red-S reagent in 100mL of double distilled water and made up to the mark. 0.1 % solution of bromothymol blue (BTB) is prepared by dissolving about 100 mg of bromothymol blue (BTB) reagent in double distilled water and made up to the mark. HCl solution (0.1 M) is prepared by accurately measuring about 8.6 mL of conc. HCl and is diluted to 100 mL with distilled water. Buffer solution (pH 3.5) is prepared by mixing 50 mL of 0.2 M potassium phthalate, 15.7 mL of 0.1 M HCl and 134.3 mL of distilled water and pH of the solution is adjusted to 3.5.

Standard solution of ondansetron HCl (1 mg/1 mL) is prepared by transferring 50 mg of pure drug into a 50 mL of standard flask and dissolved in methanol and made up to the

mark with double distilled water. Working standard solutions (100 and 250 µg/mL) are prepared by transferring 10 mL and 25 mL of the standard solution into two separate 100 mL standard flasks and diluted up to the mark with water. Sample solution of ondansetron formulation is prepared by accurately weighed portion of table content equivalent to 50 mg of ondansetron HCl, transferred into a 50 mL volumetric flask and about 80 mL of warm methanol is added. Then the contents are mixed well by sonication, filtered and diluted up to the mark with double distilled water.

Assay procedure: Into a series of 125 mL separating funnels containing aliquots of working standard odansetron solution (0.5-3.0 mL. 100 µg/mL for method A and 0.5-3.0mL. 250 µg/mL for method B), 6.0 mL of 0.1 M HCl solution and 1.0 mL of 0.2 % alizarin red-S dye solution (2.0 mL of 0.1 % bromothymol blue solution for method B) are added successively. The total volume of the aqueous phase in each funnel is adjusted to 15 mL with doubled distilled water. To each funnel 10 mL of chloroform is added and the contents are shaken for 2 min and then allowed to stand for some time and then separated. The absorbances of the separated chloroform layer of concentration 2 mL (100 µg/mL) are scanned from 375 to 500 nm and maximum absorbance is found to be at 425 nm for method-A (Fig.1). The absorbances of remaining standard solutions are measured at maximum absorbance and a linear curve is drawn by plotting concentration against absorbances (Fig. 3). The absorbances for the concentration of 2.5 mL (250 µg/mL) in method-B is also scanned from 350 to 550 against a reagent blank and found maximum absorbance is at 420 nm (Fig. 2). Then absorbances of remaining standard solutions are measured at 420 nm, a calibration curve is drawn by plotting concentration against absorbances (Fig. 4).

Optimum conditions: The optimum conditions for the developed methods are fixed based on the study of the effects of various parameters such as type of acid, concentration of the acid, concentration of the dye, choice of the organic solvent and the ratio of the organic phase to the aqueous phase. Control experiments are carried out by measuring absorbance at 425 and 420 nm of series of the solutions varying one and fixing the other parameter for method A and B, respectively.

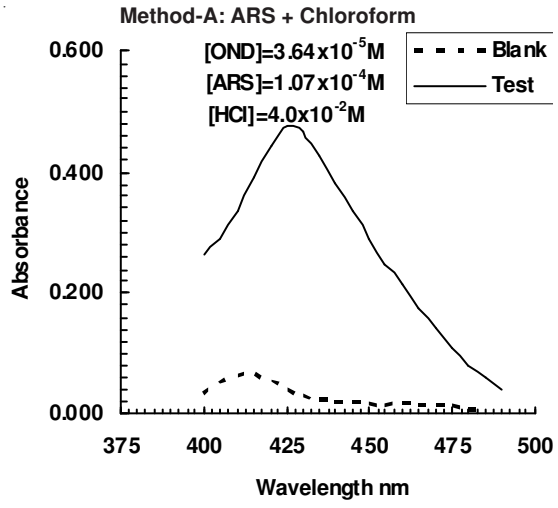

Fig. 1. Absorption spectrum of odansetron with alizarin red-S, chloroform

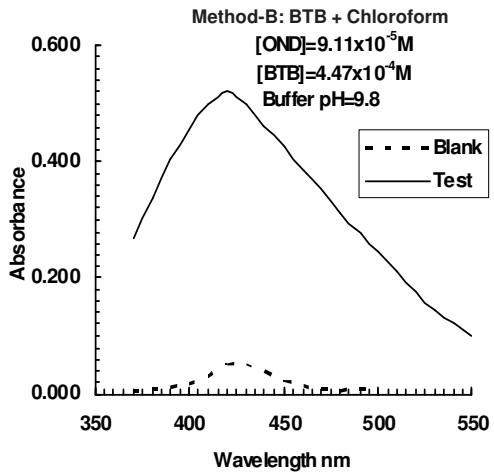

Fig. 2. Absorption spectrum of odansetron with bromothymol blue, chloroform

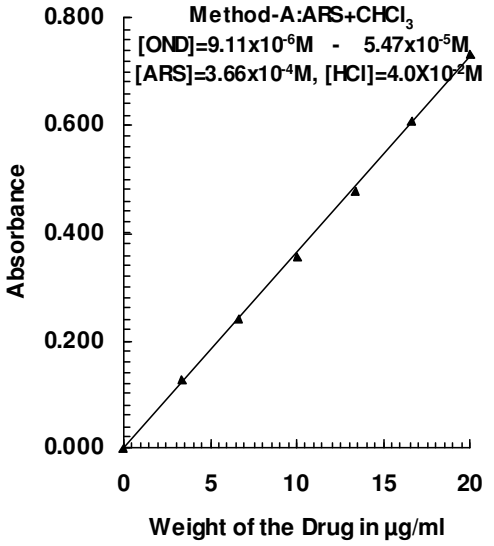

Fig. 3. Beer's law plot of odansetron with alizarin red-S, chlorofrom

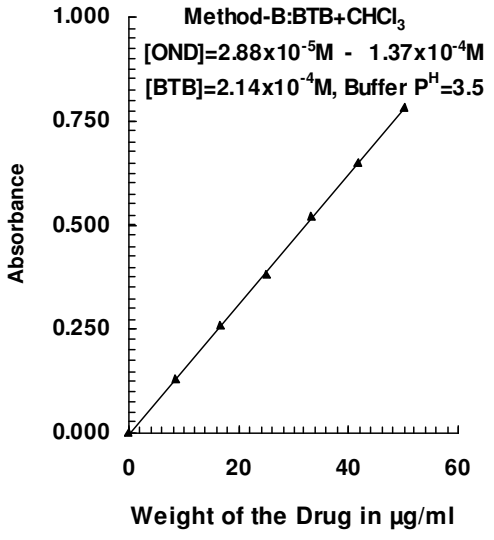

Fig. 4. Beer's Law plot of odansetron with bromothymol blue, chloroform

RESULTS AND DISCUSSION

Ondansetron hydrochloride forms ion-pair complexes in acidic medium with dyestuffs such as alizarin red-S and bromothymol blue and these complexes are quantitatively extracted into chloroform. The absorption spectra of the ion-pair complexes are drawn by plotting absorption against wavelength (Figs. 1 and 2). From the respective absorption spectra the absorption maximum are found to be at 425 and 420 nm respectively.

Optical characteristics: In order to test whether the coloured products formed in these methods adhere to Beer's law, the absorbences at maximum wavelength of a series of six concentrations are plotted against concentration of the drug in μg/mL. Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range are calculated and the values are presented in Table-1. A linear relationship is found between the absorbance and the concentration of the drug in the ranges 3.3-20.0 μg/mL and 8.3-50.0 μg/mL for methods A and B respectively (Figs. 3 and 4). Regression analysis of the Beer's law plots at λ_{max} reveals a good correlation. The graphs show negligible intercept and are described by the regression equation, Y= bC + a (where Y is the absorbance of 1 cm layer, b is the slope, a is the intercept and C is the concentration of the measured solution in μg/mL). The high molar absorptivites of the resulting coloured complexes indicate the high sensitivity of the methods. Linear regression parameters are given in Table-2.

TABLE-1
OPTICAL CHARACTERISTICS OF THE PROPOSED METHODS FOR ODANSETRON

Name of the Parameter	Method-A	Method-B
Maximum Wavelength λ_{max}	425 nm	420 nm
Beer's Law Limits μg/mL	3.3-20.0	8.3-50
Optimum Photometric Range μg/mL	6.7-20.0	25.0-41.67
Sandell's Sensitivity μg/cm^2 / 0.001 Abs	2.62E-02	6.46E-02
Molar absorptivity lt/mole/cm	1.33E+04	5.76E+03

TABLE-2
LINEAR LEAST SQUARE REGRESSION ANALYSIS

Name of the Parameter	Method-A	Method-B
Slope (b)	3.64E-02	1.57E-02
Intercept (a)	-1.53E-03	-4.73E-03
Standard deviation on slope (S_b)	3.67E-04	8.77E-05
Standard deviation on intercept (S_a)	4.76E-03	2.85E-03
Correlation coefficient (r)	0.9997	0.9999
Limit of detection (LOD) μg/mL	0.392	0.542
Limit of quantification (LOQ) μg/mL	1.307	1.808

Precision and accuracy: Precision of the developed methods is ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of the test in total solution. The per cent of relative standard deviation and per cent range of error are calculated and presented in Table-3 for the developed methods. To determine the accuracy of these methods, three different amounts of bulk samples within the Beer's law limits are prepared and analyzed by the developed methods. The results are presented in Table-4. Per cent of relative standard deviation (% RSD) are found to be less than 2, which indicate that the developed methods are precise. The per cent recoveries of the drug by these methods are found to be within the range of 100.01-100.36 and 100.07-100.76 for method A and method B, respectively, indicate that the developed methods are accurate. It is found experimentally that the commonly used additives such as starch, lactose, glucose, sodium chloride, titanium dioxide and magnesium stearate do not interfere in the analysis.

TABLE-3
PRECISION OF THE TEST METHOD

Statistical Parameter Value	Method-A	Method-B
Concentration (μg/mL)	13.33	33.33
Mean (μg/mL)*	13.34	33.31
Standard deviation (s)	0.131	0.165
% relative standard deviation (% RSD)	0.978	0.496
0.05 level confidence limit μg/mL	0.215	0.272

*Mean of six determinations

TABLE-4
ACCURACY OF THE PROPOSED METHODS

Method Number	Method-A			Method-B		
Amount Taken (μg/mL)	10.00	13.33	16.66	25.00	33.33	41.66
Amount Found (μg/mL)*	10.03	13.35	16.66	25.19	33.48	41.69
SD	0.084	0.030	0.112	0.246	0.168	0.201
% of Recovery	100.03	100.15	100.02	100.76	100.45	100.07
% RSD	0.836	0.225	0.675	0.976	0.502	0.479

*Mean of three determinations

Application to the pharmaceutical dosage forms: The developed methods have been successfully applied for the determination of ondansetron hydrochloride in pharmaceutical preparations. Osetron 4, 8 mg and ondem 4, 8 mg are taken for the analysis. The per cent of recovery of the drug is calculated and is compared with a reference method statistically by means of t-test and F-test at 95 % confidence level and found the developed methods are not significantly different. The results obtained by the developed methods are shown in Table-5.

Scheme of the coloured products: As ondansetron HCl contains tertiary nitrogen atom possessing a lone pair of electrons, in the presence of mineral acids or acidic buffers protonation takes place on nitrogen atom and thus protonated cation of the drug is formed. This cation interacts with the anion of the acidic dye to form ion-ion associated complex, which is extractable into chloroform layer. The probable **Scheme-I** of the coloured product is shown below.

Conclusion

The proposed method is simple, sensitive, economic, precise and accurate. This method can be successfully applied for routine quality control analysis.

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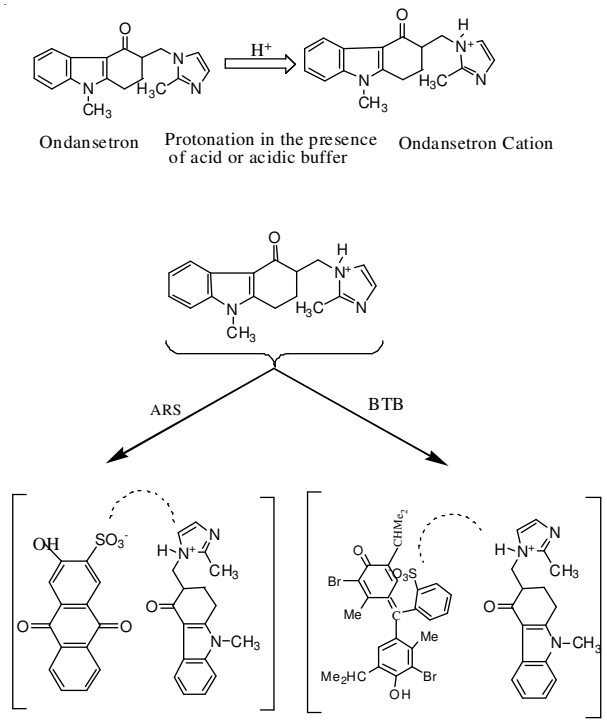
TABLE-5
ASSAY OF FORMULATIONS OF ONDANSETRON HYDROCHLORIDE

Sample	Amount Taken (mg/tablet)		Amount found in proposed methods(mg)*			Percent of Recovery		
						Reference Method	Proposed methods**	
			Method-A	Method-B			Method-A	Method-B
Osetron	4.0	Mean	3.988	3.985	% REC	99.42	99.70	99.63
		SD	± 0.015	± 0.022	% RSD	±0.61	±0.379	±0.549
		F-test	1.389	2.815				
		t-test	0.974	0.640				
Ondem	4.0	Mean	4.008	3.995	% REC	99.91	100.20	99.87
		SD	± 0.036	± 0.031	% RSD	±0.71	±0.905	±0.788
		F-test	1.636	1.231				
		t-test	0.624	0.088				
Osetron	8.0	Mean	7.982	7.975	% REC	100.05	99.77	99.69
		SD	± 0.101	± 0.090	% RSD	±0.66	±1.263	±1.129
		F-test	3.642	2.904				
		t-test	0.391	0.583				
Ondem	8.0	Mean	8.054	8.027	% REC	99.86	100.67	100.34
		SD	± 0.084	± 0.054	% RSD	±0.68	±1.038	±0.670
		F-test	2.368	1.980				
		t-test	1.614	1.250				

*Average of six determinations are considered, AVG=Average, SD=Standard deviation, F=F-test value, t=t-test value; Theoretical values at 0.05 level of confidence limit F = 5.19, t = 1.833.
**% REC = % of Recovery, % RSD = % of Relative standard deviation; Recovery studies 4.0 mg/tablet and 8.0 mg /tablet formulations are analyzed (Average of six determinations)



Scheme-I

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