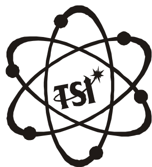

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Application of certain π-acceptors for the spectrophotometric determination of ondansetron in pharmaceutical formulations

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ABSTRACT

In this study, two simple, fast, accurate and sensitive spectrophotometric methods have been developed for the determination of ondansetron in pharmaceutical formulations. These methods(A and B) are based on the charge-transfer reaction of ondansetron base as n-electron donor with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone(DDQ) and 7,7,8,8-tetracyano quinodimethane(TCNQ) as π-acceptors to give highly colored complex species which absorb maximally at 465 and 742 nm, respectively. Beer's law was obeyed in the concentration limit of 4-120μg/ml and 10-350μg/ml respectively for method A and B. The proposed methods were successfully applied for the determination of ondansetron hydrochloride in pharmaceutical formulations. The results demonstrated that the procedures are accurate, precise and reproducible (relative standard deviation <2%), while being simple, cheap and less time consuming and can be suitably applied for the estimation of ondansetron in different dosage forms and dissolution studies with out interferences from common additives encountered. © 2007 Trade Science Inc. - INDIA

KEYWORDS

Spectrophotometry;
Ondansetron;
Pharmaceutical preparations;
Charge-transfer reactions.

INTRODUCTION

Ondansetron is used as a selective serotonin 5-HT_3 receptor antagonist, is well established in patients with nausea and vomiting associated with cancer chemotherapy, radiotherapy or anesthesia and surgery. Preliminary data have shown ondansetron to have clinical benefit in patients with nausea and vomiting associated with drug over dosage or poisoning, anti-infective or antidepressant therapies, uremia or neurological trauma, and in patients with pruritus[1].

An extensive literature survey was carried out and it is evident that ondansetron is official in British Pharmacopoeia[2] and United State Pharmacopoeia[3]. Many HPLC methods are reported[4,5]. A number of spectrophotometric methods[6-12] are available in literature for the determination of ondansetron in pharmaceutical formulations. Most of these spectrophotometric methods are tedious and time consuming. Therefore, the need for a rapid, economical and selective method is obvious, especially for a routine quality control analysis of pharmaceutical products containing ondansetron. Aim of this study was to develop fast, sensitive, economical and easy spectrophotometric methods for the determination of ondansetron in raw and pharmaceutical formulations.

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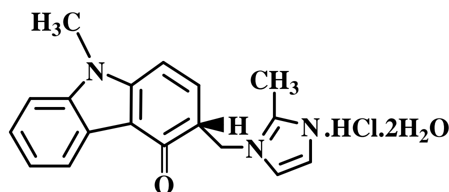


Chemical structure of ondansetron hydrochloride dihydrate

EXPERIMENTAL

Apparatus

All spectrophotometric measurements were carried out using a spectrophotometer(U 1100 Hitachi, Japan) with silica glass cell of 1cm thickness. Officially calibrated Pyrex glassware was used throughout this study.

Reagents and standard solutions

First, 0.5 %(w/v) ondansetron hydrochloride(Bio Fine Pharmaceuticals Pvt. Ltd Multan, Pakistan) in water, 0.15 %(w/v) 7,7,8,8-tetracyano-quinodimethane (Fluka, Switzerland) solutions in acetonitrile, 0.2 %(w/v) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (Fluka, Switzerland) solutions in acetonitrile and 0.1N aqueous sodium bicarbonate were prepared. All chemicals used were of Analytical Reagent grade. Double distilled water was used for aqueous solution preparation.

Preparation of ondansetron base solution

An ondansetron base solution was prepared by transferring 100ml of 0.5%(w/v) ondansetron hydrochloride solution into 250ml separating funnel, followed by 25ml 0.1N sodium bicarbonate solution. The contents of separating funnel were mixed well and shaken for two minutes. The two phases were allowed to separate. The chloroform layer was separated and dried over anhydrous sodium sulphate.

Recommended analytical procedure

Method A

An aliquot of a sample containing 4-120µg/ml of the ondansetron base solution was transferred into a series of 10ml standard volumetric flasks. A volume of 1 ml of a 0.2 %(w/v) DDQ solution was added. The absorbance was measured after two minutes with in the stability period of one and half hours after dilution with acetonitrile at 465nm against reagent blank.

Method B

An aliquot of a sample containing 10-350µg/ml of the ondansetron base solution was transferred into a series of 10ml standard volumetric flasks. A volume of 1 ml of a 0.15 %(w/v) TCNQ solution was added and heated the reaction mixture at 60^0C for 15 minutes. The absorbance of the resulting solutions was measured at the wavelength of maximum absorbance(742nm) against reagent blank treated similarly.

Procedure for pharmaceutical preparations

Twenty tablets were accurately weighed and powdered, a quantity equivalent to 250mg of ondansetron hydrochloride was stirred well with 20ml chloroform and left standing for five minutes. The residue was filtered on Whatman filter No.42 paper and washed with chloroform. The filtrate and washings were diluted to the volume in 50ml measuring flask with chloroform. The ondansetron hydrochloride solution was converted into ondansetron base following the procedure given under the head "preparation of ondansetron base solution", and the subjected to the recommended procedures for determination.

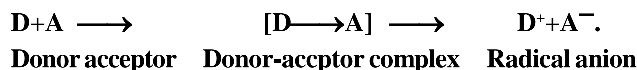
For injection, an appropriate volume of sample (equivalent to 50mg of ondansetron) was diluted to 50ml measuring flask with chloroform. The ondansetron hydrochloride solution was converted into ondansetron base following the procedure given under the head "preparation of ondansetron base solution", and the subjected to the recommended procedures for the determination.

RESULTS AND DISCUSSION

The charge-transfer(CT) reactions have been widely studied recently. Many drugs are easy to be determined by spectrophotometry based on color charge-transfer (CT) complexes formed with electron acceptors[13,14]. The charge-transfer(CT) complexes are formed between electron donors, having sufficiently low ionization potential, and acceptors, having sufficiently high electron affinity. The transfer of an electron from a donor to an acceptor is readily possible in the charge-transfer process[15]. DDQ is π-electron acceptors as a result of the strong electron withdrawing halo and cyano groups conjugated with the π-system[16,17]. DDQ

reacts instantaneously with basic nitrogenous compounds to form charge-transfer complexes of n-π type. The absorption spectrum of DDQ in acetonitrile shows a characteristic band peaking at 350nm. The addition of ondansetron solution to this solution causes an immediate change in the absorption spectrum, with a new characteristic band peaking at 465nm. This band may be attributed to the formation of DDQ radical anions, which probably resulted from the dissociation of the donor-acceptor complex in a highly polar solvent like acetonitrile.

TCNQ is also used for quantitative determination of pharmaceutical drugs in dosage forms by charge-transfer complex formation[18,19]. Interaction with TCNQ in acetonitrile solution was found to yield a deep color causing characteristic long wavelength absorption band. The predominant chromagen with TCNQ is blue colored radical anion, which probably resulted through the dissociation of an original donor-acceptor complex with the drug. This complex is formed by the lone pair of electron donated by the ondansetron as n-donor and the charge-transfer reagent as an electron acceptor, which a partial ionic bond ($D^+ A^-$) is assumed to be formed.



The dissociation of the complex was promoted by the high ionizing power of acetonitrile solvent[20].

Optimization of variables

The spectrophotometric properties of the colored species formed with DDQ and TCNQ as well as different parameters affecting the color development, were extensively studied. The optimum conditions for the assay procedures(Method A and B) have been established by studying the reaction as function of the concentration of the reagent, the nature of the solvent, heating time and stability of the colored species.

Effect of color producing reagent and time

For method A, the effect of volume of 0.2 %(w/v) DDQ solution was studied over the range of 0.2-2.0 ml, in a solution containing 40µg/ml ondanetron. The results revealed the fact that 1 ml of DDQ solution was required to achieve the maximum intensity of the color (Figure 1). Therefore 1ml was the optimum value and

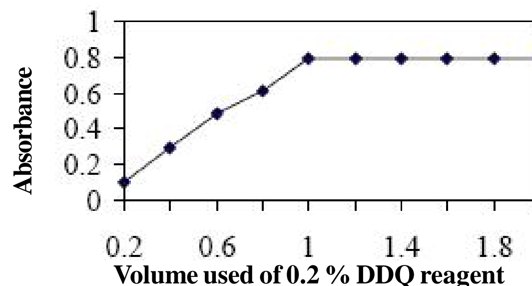


Figure 1 : Effect of reagent concentration on color development

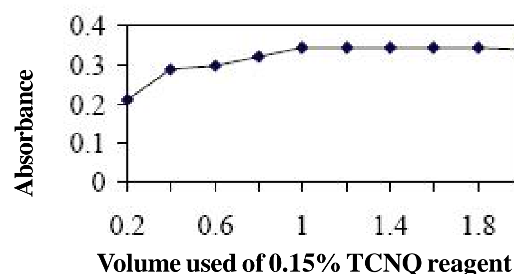


Figure 2 : Effect of regent concentration on color development

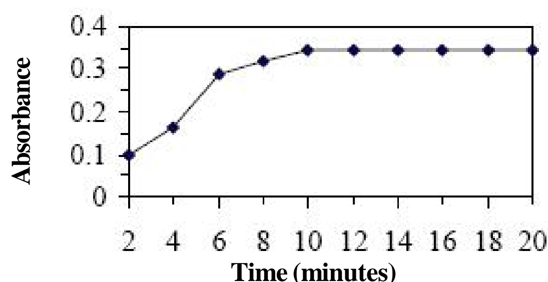


Figure 3 : Effect of heating time on color development

maintained throughout the experiment. The reaction gets stabilized with in the 5minutes of mixing at room temperature and absorbance remained constant for 1.5hours.

For method B, the effective variables are the concentration of TCNQ and temperature. To study the influence of the volume of 1.5%(w/v) TCNQ solution, we pipetted an aliquot of the drug solution containing 50µg/ml into a series of 10ml volumetric flasks, followed by varying volumes of 1.5 %(w/v) TCNQ solution(0.2-2.0 ml). The contents were diluted to the volume with chloroform. The highest absorbance was obtained with volume 1ml of 1.5 %(w/v) TCNQ solution (Figure 2). Further addition of TCNQ solution cause no change in the absorbance, so 1 ml was selected the optimum volume for all determinations. The intensity of

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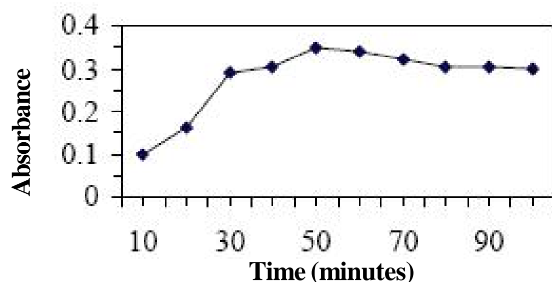

Figure 4 : Effect of temperature of color development

TABLE 1 : Optical characteristics and statistical data for the regression equation of the proposed method

Parameter	Method A	Method B
λ_{max} (nm)	465	742
Beer's law verification range (µg/ml)	4-120	10-350
Molar absorptivity (L $mole^{-1}$ cm^{-1})	7.3×10^3	2.5×10^3
Sandell's sensitivity (µg/ml per 0.001A)	4.9×10^{-2}	1.5×10^{-1}
Color stability	1.30	1
Regression equation (Y*)	0.02	0.00706
Slope (b)	-0.0104	-0.01018
Intercept (a)	0.9999	0.9998
Correlation coefficient (r)	0.47	0.51
RSD** (%)	2.2	7.2
Limit of detection(µg/ml) Limit of quantification(µg/ml)	7.5	23.5

Y*=a+bC(Where C is the concentration of analyte (µg/ml) and Y is absorbance); **=Calculated from five determinations

TABLE 2 : Determination of ondansetron in the presence of excipients(50µg/ml of ondansetron was taken for interferences studies)

Excipient	Amount taken (µg/ml)	% Recovery ± RSD (N=5) method A	% Recovery ± RSD (N=5) method B
Microcrystalline cellulose	300	101.2 ± 0.40	101.5 ± 0.30
Pregelatinized starch	300	99.26 ± 0.40	99.8 ± 0.45
Lactose	100	101.7 ± 0.20	100.7 ± 0.25
Magnesium stearate	50	99.52 ± 0.48	99.9 ± 0.40
Colloidal silicon dioxide	300	98.95 ± 0.30	99.5 ± 0.30
Povidone	50	99.1 ± 0.39	99.6 ± 0.30

the color formed on mixing the reagent reached maximum with in 10minutes(Figure 3) by heating the mixture at 60^0C in water bath(Figure 4) and was stable up to one hour at room temperature. Therefore, it is recommended that absorbance should be measured with in this period.

Interference study

To study the potential interference problems from the commonly used excipients and other additives such as microcrystalline cellulose, talc, lactose, sodium starch glycolate, povidone, gelatin, starch and magnesium stearate, recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug (ondansteron 50 µg/ml), excipients in different concentrations were added and analyzed. Results of the recovery analysis are presented in TABLE 2. Excipients up to the concentrations shown in the TABLE 2 do not interfere with the assay. In addition recoveries in most cases were around 100 % and the lower values of the RSD indicate the good precision of the method.

Sensitivity

The results for the determination of ondansetron are shown in TABLE 3 and 1, which show the sensitivity, validity and reproducibility of the proposed methods. These are also reasonably precise and accurate, as the amount taken from identical samples is known and the amount found does not exceed a relative standard deviation of 0.47 and 0.51(N=5).

APPLICATION

The applicability of the proposed methods for the determination of ondansetron in commercial dosage forms was examined by analyzing marketed products. The results of the proposed methods were statistically

TABLE 3 : Determination of ondansetron in pharmaceutical preparations by the proposed and reference[2] method

Formulation	Method A				Method B				Reference method	
	Recovery*(%)	RSD(%)	t-value	F-value	Recovery*(%)	RSD(%)	t-value	F-value	Recovery*(%)	RSD(%)
Tablets										
Zofran	99.42	0.51	0.27	2.96	100.45	0.51	0.75	3.6	100.31	0.57
Ondanles	99.91	0.64	0.42	3.12	99.21	0.61	0.61	2.4	100.00	0.49
Setron	100.05	0.58	0.36	2.85	101.05	0.49	0.41	3.3	100.14	0.34
Onset	99.86	0.48	0.31	2.14	99.09	0.49	0.73	2.5	100.09	0.47
Injection										
Zofran	100.05	0.71	0.38	3.18	100.20	0.58	0.98	3.6	100.89	0.29
Ondanles	100.45	0.68	0.52	2.98	100.29	0.49	0.82	2.7	99.89	0.48

*Average of 3 independent analyses.

compared with reference method[2] and summarized TABLE 3. It is evident from the table that the calculated t and F values[21] are less than the theoretical ones at 95% confidence level, indicating no significant difference between the methods compared. The proposed methods are sensitive, simple, and accurate and are successfully applied for the quality control of pure ondansetron in pharmaceutical dosage forms.

CONCLUSION

It may be concluded that the newly developed spectrophotometric methods for the determination of ondan setron are reliable, simple, sensitive, accurate, rapid, and economical. The results are in good agreement with reference method.

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