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METHOD DEVELOPMENT AND VALIDATION OF ONDANSETRON BY UV-VISIBLE SPECTROPHOTOMETRY

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Abstract:

A simple, accurate, precise, reproducible, highly sensitive, economic spectrophotometric method has been developed for the estimation of Ondansetron[1]. UV spectrophotometric method is based on measurement of absorption at maximum wavelength 419nm. The developed method was validated with respect to linearity, accuracy (recovery), Precision.

Beer's law was obeyed in the concentration range of 0.5-2.5 mg/ml with correlation coefficient of 1. Results of the analysis were validated statistically and by recovery study. Hence the developed and validated method can be used for estimation of Ondansetron.

Key words: *Ondansetron , UV spectrophotometry ,Method development ,Validation ,Phenol red.*

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INTRODUCTION

Ondansetron hydrochloride, chemically 4H-carbazol- 4-one-1, 2, 3, 9-tertahydro-9-methyl-3-[(2-methyl-1H-imidazole-1-yl) methyl] hydrochloride is selective 5-HT3 antagonist[1]. 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[2]. The IUPAC name of ondansetron is (RS)-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-2,3-dihydro-1H-carbazol-4(9H)-one.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.

Ondansetron is the subject of monograph in Indian Pharmacopoeia, United States Pharmacopoeia, and British Pharmacopoeia [3]. Present work describes simple, accurate, reproducible, rapid and economical methods for simultaneous estimation[4] of Ondansetron. The HPLC, LC and spectrophotometric methods are available in literature for the determination of the cited drug. HPLC and LC methods involve costly equipment and are tedious.[5] However methods on spectrophotometric determination of this drug involving ion pair complexes with common and versatile acidic [4] dye *viz.*, eosin are not reported yet. This prompted the authors to develop extractive spectrophotometric methods for the determination of Ondansetron using the above mentioned dyes [5]. In this paper we report simple and sensitive extractive spectrophotometric methods for the assay of ondansetron. The methods are based on ion-pair complexation of drug with dye eosin and subsequent extraction into chloroform and measure the absorbance of colour complex.

MATERIALS AND METHODS

Phenol red is a water-soluble dye used as a pH indicator, changing from yellow to red over pH 6.6 to 8.0, and then turning a bright pink colour above pH 8.0.Molecula formula of phenol red is $C_{19}H_{14}O_5S$.[6] As such, phenol red can be used as a pH indicator dye in various medical and cell biology tests. Phenol Red possesses wide range of applications, such as bromination catalysts, pH indicator, estrogenic properties and screening test [7]. Phenol Red is a compound widely used in culture media which identifies pH changes from neutral (red) to acidic (yellow) values. Typically a change in colour from red to yellow indicates high rates of cellular death when used in tissue cultures. In the culture of breast cancer cells Phenol Red has been observed to act as a weak estrogen. In human MCF-7 cells, phenol red has reduced Roscovitine mediated cell cycle arrest and apoptosis. Phenol red has also induced cogenesis from cells scraped from the surface of adult ovaries.

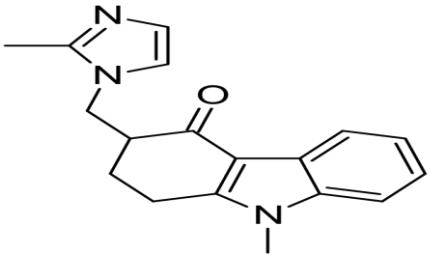

Fig 1: Ondansetron hydrochloride structure

Mechanism of Action

Mechanism not fully characterized; selective 5-HT_3 receptor antagonist; binds to 5-HT_3 receptors both in periphery and in CNS, with primary effects in GI tract. Has no effect on dopamine receptors and therefore does not cause extra pyramidal symptoms.

Instrument:

Elico double beam ultra violet – visible double beam spectrophotometer SL-244 with 1 cm matched quartz cells was used for all spectral measurements**.**

Materials:

Reagents: All the chemicals used were of *Analytical Reagent* grade [8].

Preparation of Phosphate Buffer pH 2.5:

Dissolved 100 gm of potassium dihydrogen phosphate in 800ml of water; adjust the pH to 2.5 with hydrochloric acid and add sufficient water to produce 1000ml [9].

Preparation of 0.2% Phenol red dye solution for 10ml:

Dissolve 0.1gm of phenol red in 2.82 ml of 0.1 M sodium hydroxide and 20 ml of ethanol (955).After solution is affected; add sufficient water to produce 100 ml [9].

Chloroform AR grade

Preparation of standard solution of 1mg/ml stock solution:

Weigh 50 mg of bulk drug (Ondansetron) and dissolved in 50ml of ethanol, shake well till it dissolves and make upto 25ml

Chromogenic reagents used: Phenol red.

Method development:

In this the chromogenic reagent is added to the drug i.e. test solution and blank solution (here

ethanol is taken as blank) and it is shaken. To it suitable amount of chloroform and phosphate buffer is added and shaken vigorously, so that the drug is extracted in chloroform layer then this is tested for absorbance in UV spectro-photometer. So, in one test tube we have taken 1 ml drug solution , 1 ml chromogenic reagent (Phenol red),1 ml phosphate buffer 2.5 and 2 ml chloroform and in another test tube without drug solution. The drug was extracted in chloroform layer which appeared in yellow colour and the buffer solution appeared colorless. This solution was scanned over a range of 400 – 800 nm using a UV spectrophotometer. From the result obtained the λ max was fixed at 419nm (absorbance 6.526 x 10^{-3}). Various drug concentrations were prepared varying from 0.5 – 2.5mg/ml and scanned at λmax 419 and finally the drug concentration was optimized to 1 mg /ml.

Assay Procedure:
Method A:

The methods were extended for the determination of Ondansetron from tablet formulations. Ten tablets of ondansetron were accurately weighed and powdered. Tablet powder equivalent to 1000 mg of ondansetron was dissolved in 50 ml of ethanol, sonicated for 15 mins and filtered. The filtrate is combined and the final volume was made to 100 ml with ethanol for the above method. The solution was suitably diluted and analyzed as given under the assay procedure for bulk sample. The analysis procedure was repeated three times with tablet formations and the results of analysis are shown in Table 1.

Recovery Studies:

To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug to the previously analyzed formulate samples and these samples were reanalyzed by the proposed method and also performed recovery experiment. The percentage recoveries thus obtained were given in Table 1.

Table 1: Assay of Ondansetron In Tablet Formation:

Tablet formation	Labeled Amount (mg)	Amount obtained(mg)*By the Proposed Method Method A	% Recovery By the Proposed Method Method A
1	4	3.84	96 %
2	4	3.91	97.75%
3	4	3.99	99.90 %

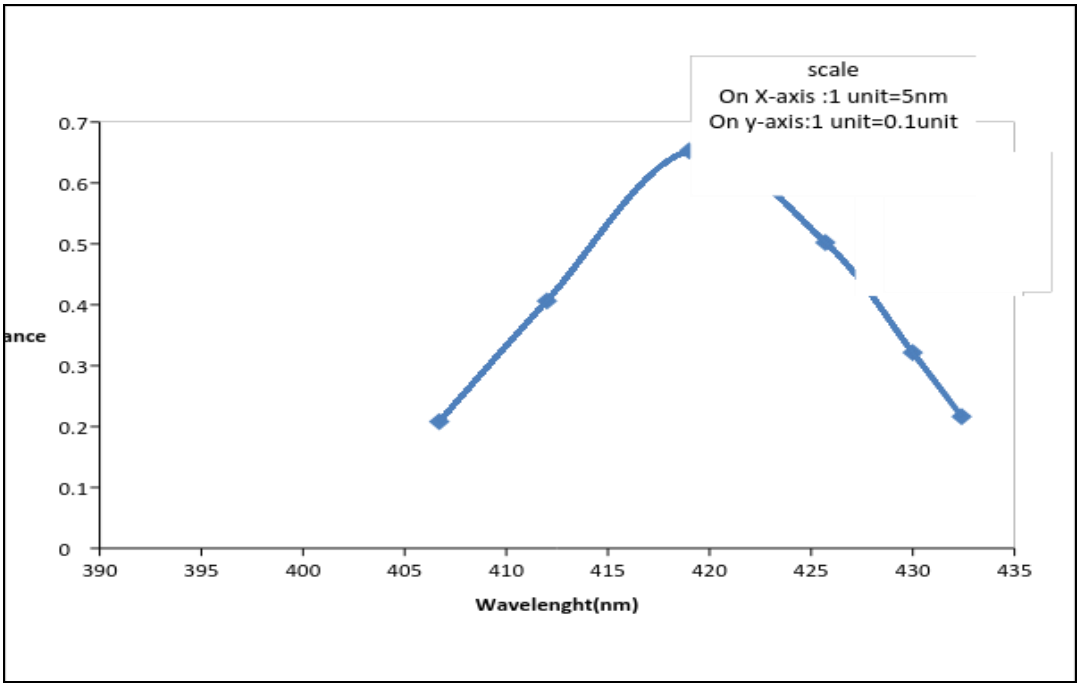

Fig 2: Absorption spectrum of Ondansetron with Phenol red

Table 2: Absorbance of Ondansetron In Presence of Reagent (Phenol red):

Concentration(µg/mL)	Absorbance
500	0.0781
1000	0.1428
1500	0.2175
2000	0.3032
2500	0.4034

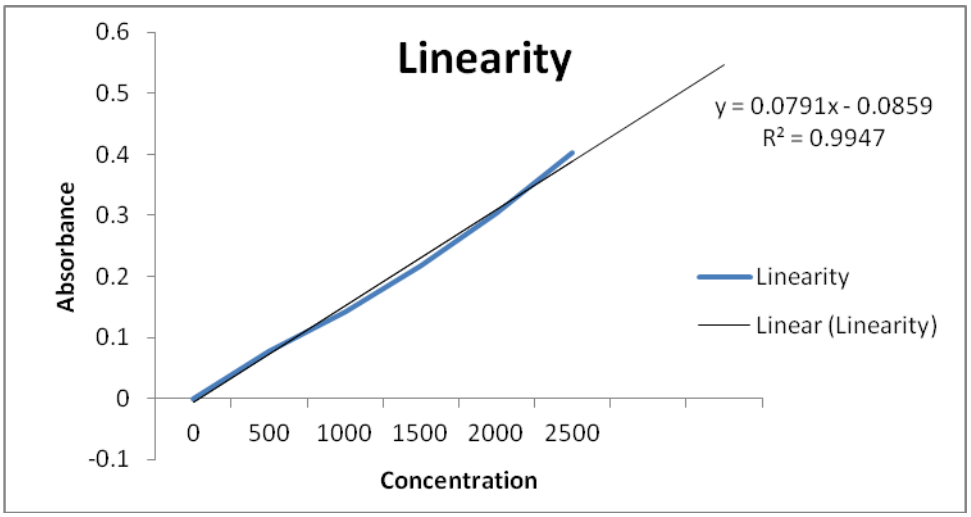

Fig 3: Calibration curve of Ondansetron using Phenol red

RESULTS AND DISCUSSION:

Development and Optimization

The solubility [10] of Ondansetron was tested in water, ethanol, and methanol. Based upon the free solubility of Ondansetron in ethanol it was selected as solvent for method development Ondansetron estimation. The analyte was estimated at 419 nm and respective UV spectrum was depicted in Figure 2

Validation [10]

The developed UV spectrophotometric method was validated for linearity range, accuracy, precision, and limit of detection, limit of quantification and robustness parameters as per ICH guidelines.

Accuracy

Accuracy of developed method was determined by a recovery study at 3 concentration levels by replicate analysis (n=3). Standard drug solutions were added to a pre-analyzed sample solution and percentage of total drug content was calculated. The results of accuracy studies were reported in Table 1.

Precision

Precision was determined by studying the repeatability and intermediate precision. The standard deviation and relative standard deviation were calculated for the drug. Repeatability was determined by five estimations of Ondansetron 0.5 – 2.5 mg/ml and %RSD was calculated. The results of precision studies were reported in Table 3.

LOD and LOQ

The LOD and LOQ of ondansetron were found to be 0.28 µg/mL and 0.85 µg/mL respectively.

Table 3: Optical Characteristics and Precision Data:

Parameters	Phenol red
λ max	419
Beer's law limit (µg/ml)	1000-2000 µg/ml
Molar absorptivity (micrograms/cm²/0.001 Absorbance unit)	6.526 x 10^{-3}
Sandell's sensitivity (micrograms/cm²/0.001 Absorbance unit)	1.53
Regression equation 'y' Slope (m) Intercept (c)	 1.4 x 10^{-3} 0.0068
Correlation coefficient	1
Precision (% relative standard deviation)	0.008
Standard error of estimate	0.14
LOD	0.28
LOQ	0.85

CONCLUSION:

A simple spectrophotometric method for the determination of Ondansetron in pure as well as in its dosage form were developed in presence of reagent Phenol red. The absorbance of chromogen was found to be maximum at 419 nm against the corresponding reagent blank. The method was found to be simple, precise, economic and less time consuming. The method has been statistically evaluated and results obtained were accurate, precise and insensitive and free from the interference of other additives present in the formation.

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