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VALIDATED RP-HPLC AND DERIVATIVE SPECTROSCOPIC METHODS FOR SIMULTANEOUS DETERMINATION OF ONDANSETRON AND OMEPRAZOLE IN PHARMACEUTICAL COMBINED DOSAGE FORM

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ABSTRACT

The present work describes fast, accurate, precise, reproducible, and rugged RP-HPLC and derivative spectroscopic methods for simultaneous estimation of Ondansetron Magnesium and Omeprazole Sodium in capsule formulation. The first method was performed on a gradient HPLC instrument using Water-Methanol (10:90 v/v) as a mobile phase. Areas were recorded at 301 nm for both the drugs and retention times were found at 3.41 min and 5.31 min for Ondansetron and Omeprazole respectively at 1.0 ml/min flow rate. Linearity was found over the range of 1-5 µg/ml for Ondansetron and 5-25 µg/ml for Omeprazole. The values of Limit of Detection were found to be 0.0712µg/ml and 0.239µg/ml for Ondansetron and Omeprazole, respectively. The values of Limit of Quantification were found to be 0.215µg/ml for Ondansetron and 0.725µg/ml for Omeprazole. Second method uses derivative spectroscopy and Zero Crossing Points for measurements of both the drugs. Proposed methods were found to be applicable for simultaneous analysis of these drugs in combined capsule formulations.

KEYWORDS

Ondansetron, Omeprazole, RP-HPLC, Capsule formulation

INTRODUCTION

Ondansetron a Magnesium salt of 6-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl) methyl sulfinyl)-1H-benzo[d]imidazole, represents the class of orally active H^+ - K^+ ATPase Inhibitors (Proton Pump Inhibitor) employed in the management of gastric ulcer [1-4]. The individual determination of Ondansetron has been carried out in formulations by HPLC [5-14] and Spectroscopy [15-16]. Second drug, Omeprazole a sodium is chemically 2- {2-[(2, 6-dichlorophenyl) amino] phenyl} acetic acid. It represents the class of non-steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic actions which is commonly employed for the acute and chronic treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis [17-20]. Literature survey revealed that very few analytical methods have been reported for the estimation of Omeprazole which includes HPLC [21-24]. Literature review did not reveal any method for simultaneous determination of Ondansetron and Omeprazole in combined pharmaceutical dosage form. So, we decided to work towards development and validation of simple, sensitive, accurate, precise, rugged, and economic method for simultaneous determination of these drugs in combined dosage forms. The present work describes a validated reverse phase HPLC and first order derivative spectroscopy methods for simultaneous determination of these drugs in combined dosage form.

MATERIALS AND METHOD

Instrumentation

The first method uses A Youngling's HPLC (YL-9100) instrument with UV Detector, Manual injector of 20-µl loop, Column - Phenomenex C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size), Software – YL Clarity. Second method was performed on Shimadzu's UV-1700 Double beam UV-Visible spectrophotometer. Proposed methods also uses Digital pH meter (Elecon), Corning volumetric flasks (10ml, 50ml and 100 ml), AX 200 analytical balance (Shimadzu), Ultrasonic bath (Frontline FS 4 ultrasonic cleaner, Mumbai). Vacuum pump, Pipettes – 1ml, 5ml, 10ml, beakers, measuring cylinder.

Chemicals and Reagents

The drug samples of Ondansetron and Omeprazole were kindly supplied by Torrent Research Center (Gandhinagar, India), Methanol and Water (HPLC

grade) was kindly supplied by S.D. Fine Chemicals Ltd., Mumbai.

Chromatographic Conditions

Chromatographic separation was achieved on Phenomenex C_{18} column (250 mm x 4.6 mm, 5 µm) at 25 ± 3^0 C temperature using mobile phase Acetonitrile: Methanol: Water: (15:80:5 v/v/v) at a flow rate of 0.8 ml/min. Detection was carried out at 254 nm. The mobile phase after preparation was filtered through a Nylon 0.22 µm membrane filter and sonicated for 10 min; injection volume used for assay was 20 µl.

Preparation of solutions

Preparation of standard stock solution (500 µg/ml)

Accurately weighed Ondansetron (50 mg) and Omeprazole (50 mg) were transferred to two separate 100 ml volumetric flask. 50 ml methanol was added to the flask. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to prepare a 500 µg/ml stock solution of both drugs.

Preparation of working standard solution (100 µg/ml)

From the above stock solution (500 µg/ml) of both drugs, transfer an accurately measured 20 ml volume of the stock solution into a 100 ml volumetric flask and make the final volume with methanol to prepare 100 µg/ml working solutions.

Preparation of the mobile phase

The mobile phase was Acetonitrile: Methanol: Water: (15:80:5 v/v/v). The mobile phase was filtered through a nylon 0.22 µm membrane filter and was degassed before use.

Preparation of calibration curves

A calibration curves were plotted over a concentration range 2-10 µg/ml for both Ondansetron and Omeprazole in RP-HPLC method and 4-15 µg/ml for Ondansetron and 4-20 µg/ml for Omeprazole in Spectroscopic method. Calibration curves were constructed for Ondansetron and Omeprazole by plotting peak area versus concentration at 254 nm for HPLC method and by plotting absorbance versus concentration in Spectroscopic method. Each reading was average of three determinations.

Quantification of Ondansetron and Omeprazole in formulation

Weigh and collect the powder from 20 tablets. Weigh accurately a quantity of the powder equivalent to about 4 mg of Ondansetron and 10 mg of Omeprazole into 100 ml measuring flask and mixed with mobile phase and sonicate for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with mobile phase. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with mobile phase to get a final concentration of 40 µg/ml of Ondansetron and 100 µg/ml of Omeprazole. For final diluted test solutions were diluted up to the mark with mobile phase. Same above-mentioned method was repeated for the Spectroscopic method using methanol as a solvent. The resulting solutions were analyzed as per respective methods.

METHOD DEVELOPMENT

Determination of wavelength

In RP-HPLC method standard solution of Ondansetron and Omeprazole were scanned in the range of 200-400 nm against mobile phase as a blank. Ondansetron and Omeprazole showed maximum absorbance at 254 nm. So the wavelength selected for the determination of Ondansetron and Omeprazole was 254 nm. In derivative spectroscopic method absorbance was recorded at 248 nm (ZCP of Ondansetron) and 274 nm (ZCP of Omeprazole) for Omeprazole and Ondansetron solutions in the first derivative spectra of the respective solutions.

Method Validation [25]

Solution stability

Sample solutions were kept at 25°C and 2-8°C for 24 hour and three days, respectively. Assay of initial period was compared with these two time points. The falls in the assay values were evaluated. The difference between assays should not be more than 2 % for formulation, and 0.5% for API.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation proportional to the concentration of analyte in samples within a given range. The range of analytical method is the interval between upper and lower level of analyte including levels that have been demonstrated to be determining with precision and accuracy using the method. The linear response of Ondansetron and Omeprazole were determined by analyzing five independent levels of the calibration curve. Result should be expressed in terms of Correlation co-efficient.

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative

standard deviation, or coefficient of variance of a series of measurements.

Repeatability (Precision on replication)

It is a precision under a same condition (Same analyst, same apparatus, short interval of time and identical reagents) using same sample. Method precision of experiment was performed by preparing the standard solution of Ondansetron and Omeprazole for six times and analyzed as per the proposed method.

Intermediate precision (Reproducibility)

It expresses within laboratory variations as on different days analysis or equipment within the laboratory. Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions of Ondansetron and Omeprazole in triplicate at three different concentrations. Inter-day precision was evaluated by using freshly prepared solutions of Ondansetron and Omeprazole in triplicates at three different days.

Accuracy (% Recovery)

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the capsules with three different concentrations of standards.

Limit of Detection

It is the lowest amount of analyte in sample that can be detected but not necessarily quantitated under the stated experimental conditions. It is expressed as signal to noise ratio of 2:1 or 3:1. Limit of detection can be calculated using following equation as per ICH guidelines. LOD = 3.3 × N/S where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy condition. It is expressed as signal to noise ratio of 10:1. Limit of quantification can be calculated using following equation as per ICH guidelines. LOQ = 10 × N/S where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System suitability

System suitability parameter is establishing to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyst. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

RESULTS AND DISCUSSION

The proposed methods can determine Ondansetron and Omeprazole in combined dosage form and the validity of this method was confirmed in accordance with the ICH guidelines. In proposed RP-HPLC method retention times were recorded at 2.75 min and 5.80 min at 0.8 ml/min. flow rate for Ondansetron and Omeprazole respectively, as shown in Figure1. First order derivative spectra for Ondansetron and Omeprazole are shown in Figure 2. The calibration graphs for Ondansetron and Omeprazole were constructed by plotting the area versus their corresponding concentrations for HPLC method and absorbance versus concentration for spectroscopic method. Results obtained by applying the proposed methods shown that the concentrations of Ondansetron and Omeprazole can be simultaneously determined in prepared mixtures. The proposed methods have been applied for the assay of Ondansetron and Omeprazole in pharmaceutical dosage form. The validity of the methods was further assessed by applying the standard addition technique. The results obtained indicate that the additives present do not interfere with analysis of the studied mixtures, see Table 1. The optical and regression characteristics and validation parameters are reported in Table 2. Results of application of proposed methods to the pharmaceutical dosage form are shown in Table 3.

Table 1: Data of Recovery Study for Ondansetron and Omeprazole

Drug	% Recovery ± S.D (n=3) RP-HPLC method	% Recovery ± S.D (n=3) Spectroscopic method
ONDANSETRON	100.56 ± 0.824	101.25 ± 0.835
	99.74 ± 0.597	100.52 ± 0.6509
	100.38 ± 0.923	103.58 ± 0.3818
OMEPRAZOLE	98.91 ± 0.408	100.77 ± 0.813
	100.83 ± 0.213	101.27 ± 0.702
	98.99 ± 0.276	99.86 ± 0.57

Table 2: Optical and Regression Characteristics and Validation Parameters

Parameters	RP-HPLC method		Spectroscopic method	
	Ondansetron	Omeprazole	Ondansetron	Omeprazole
Calibration range	2-10 µg/ml	2-10 µg/ml	4-15 µg/ml	4-20 µg/ml
Detection limit	0.14 µg/ml	0.08 µg/ml	0.255 µg/ml	0.772 µg/ml
Quantitation limit	0.424 µg/ml	0.244 µg/ml	0.772 µg/ml	2.16 µg/ml
Slope	348.8	588.2	-0.004	-0.0036
Intercept	86.2	220.5	-0.0033	0.0022
Mean	100.7716934	98.76176	102.83	100.964
Standard deviation	0.861782392	0.574911	0.6055	0.4946
Coefficient of variance	0.855183001	0.58212	0.5888	0.4899
Correlation coefficient	0.998	0.994	0.9993	0.9994
Intra-day RSD, %	0.593-0.837	0.337-0.452	0.639-1.858	1.315-1.953
Inter-day RSD, %	0.408-0.776	0.331-0.779	1.062-1.8886	0.576-1.713

Table 3: Application of Proposed Methods to Pharmaceutical Dosage Forms

RP-HPLC method		Spectroscopic method	
ONDANSETRON	OMEPRAZOLE	ONDANSETRON	OMEPRAZOLE
% Amount found S.D. (n=3)	% Amount found S.D. (n=3)	% Amount Found ± S.D. (n=3)	% Amount Found ± S.D. (n=3)
97.11 ±0.954	98.92±0.690	99.79±1.3	101.85±0.699
100.26 ±0.636	98.68 ±0.631	99.72±0.867	99.13±0.932
100.56 ±0.824	99.10 ±0.448	100.10 ±0.65	98.75 ±0.694

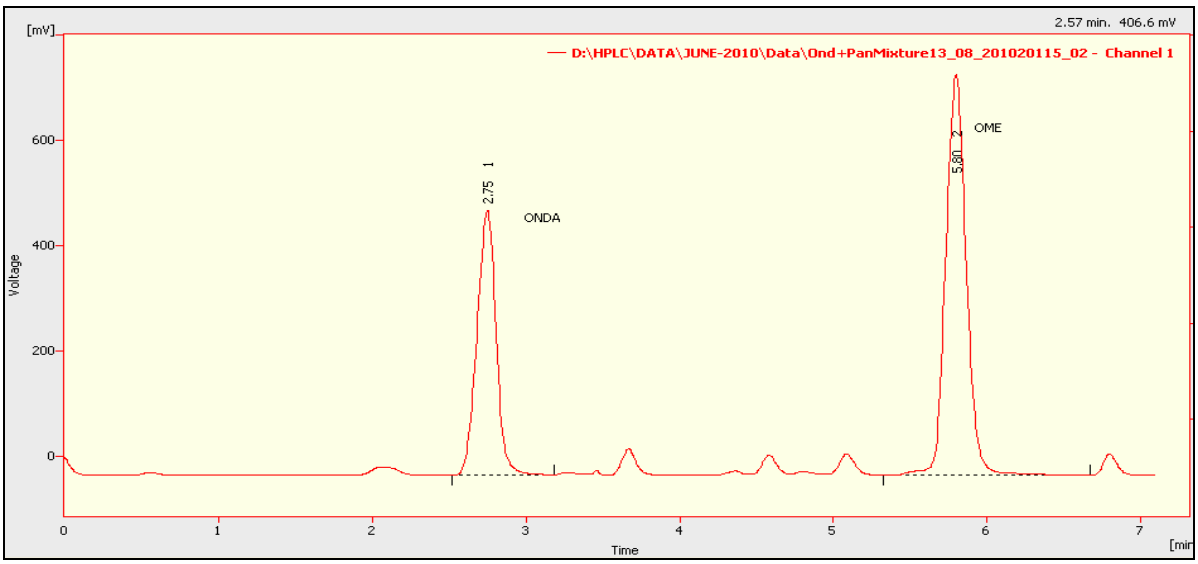

Figure 1: Chromatogram of Ondansetron and Omeprazole

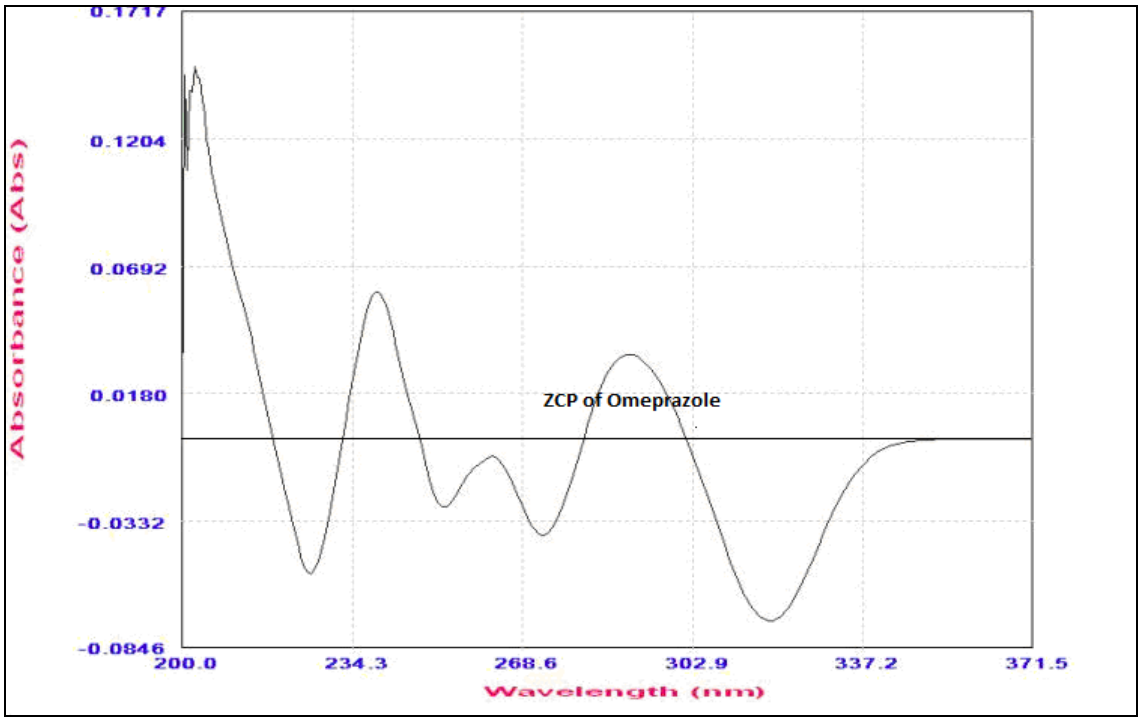

Figure 2 (a): First order spectra of Ondansetron

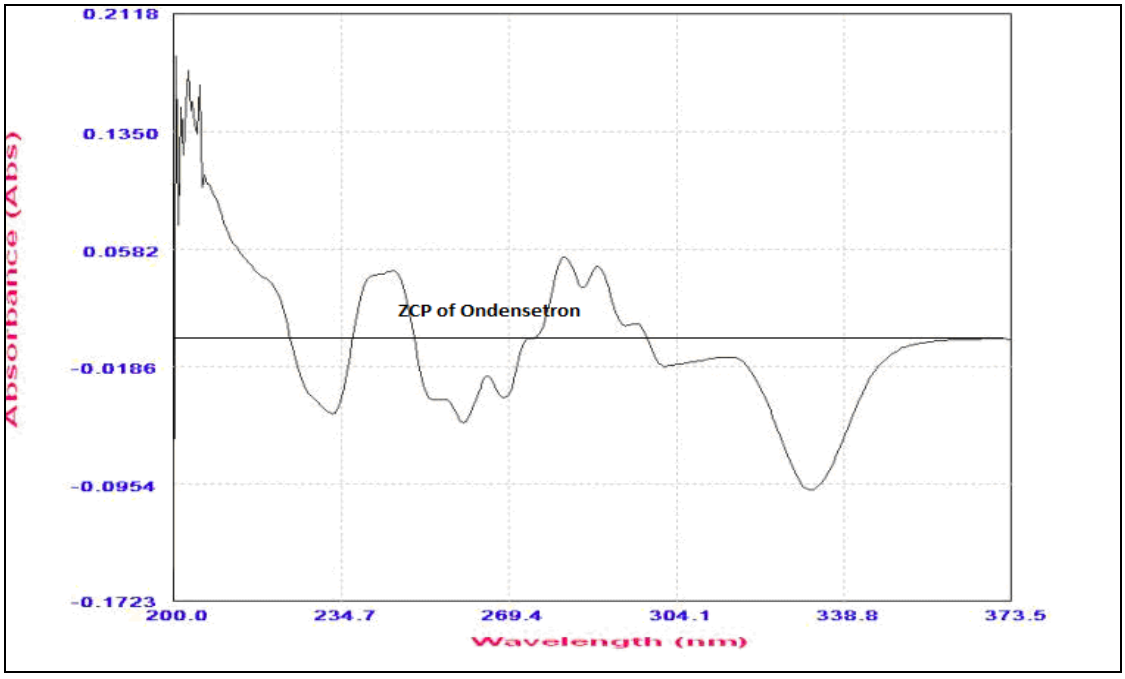

Figure 2 (b): First order spectra of Omeprazole

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