

## DERIVATIVE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF ONDANSETRON AND RABEPRAZOLE IN COMBINED DOSAGE FORM

SHIRISH PATEL<sup>\*1</sup>, Dr. LJ PATEL<sup>2</sup>

<sup>1</sup>B S Patel Pharmacy College, Saffrony Institute of Technology, Linch, Gujarat,<sup>2</sup>Shree S K Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Gujarat, Email: shirish\_1984@rediffmail.com

Received: 15 September 2011, Revised and Accepted: 03 January 2012

### ABSTRACT

The present work describes a First order Derivative Spectrophotometric method for simultaneous estimation of Ondansetron Hydrochloride and Rabeprazole Sodium in Capsule formulation. Method was performed on Elico's Double beam UV-Visible Spectrophotometer (SL-191) using methanol as a solvent. Absorbances were recorded at Zero Crossing Point (ZCP) of Ondansetron Hydrochloride (301.5 nm) and ZCP of Rabeprazole Sodium (284 nm) for all the solutions. The selected Spectrophotometric conditions were found to be effectively determined Ondansetron Hydrochloride and Rabeprazole Sodium without prior physical separation. Linearity was found over the range of 2-16 µg/ml for Ondansetron Hydrochloride and over 4-25 µg/ml for Rabeprazole Sodium. The values of Limit of Detection were found to be 0.39 µg/ml for Ondansetron Hydrochloride and 0.777 µg/ml for Rabeprazole Sodium. The values of Limit of Quantification were found to be 1.18 µg/ml for Ondansetron Hydrochloride and 2.35 µg/ml for Rabeprazole Sodium. The proposed method was found to be fast, accurate, precise, reproducible and rugged and can be used for simultaneous analysis of these drugs in combined capsule formulations.

**Key words:** Ondansetron Hydrochloride, Rabeprazole Sodium, Reverse-phase HPLC, Simultaneous

### INTRODUCTION

Ondansetron (ONDA) hydrochloride is chemically 1, 2, 3, 9-tetrahydro-9-methyl-3-[[2-methyl-1H-imidazol-1-yl] methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate. It represents the class of selective 5HT<sub>3</sub> antagonists which is commonly employed as anti-emetic in combination with anti-ulcer and anti-cancer agents<sup>1-7</sup>. Literature survey revealed that very few analytical methods have been reported for the estimation of ONDA which includes HPLC<sup>8-13</sup> and HPTLC<sup>14</sup>. Second drug, Rabeprazole (RABE) a sodium salt of 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole, represents the class of orally active H<sup>+</sup> - K<sup>+</sup> ATPase Inhibitors (Proton Pump Inhibitor) employed in the management of gastric ulcer<sup>15-17</sup>. The individual determination of Rabeprazole has been carried out in formulations by HPLC<sup>18-21</sup>, LC-MS/MS<sup>22</sup> and Derivative Spectroscopy<sup>23</sup>. Literature review did not reveal any method for simultaneous determination of RABE and ONDA 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 derivative spectroscopic method for simultaneous determination of these drugs in combined dosage form.

### MATERIALS AND METHOD

#### Instrumentation:

- A double-beam UV-Visible spectrophotometer, model SL-191 (Elico) having two matched cells with 1-cm light path
- An analytical balance (AX200, Shimadzu)
- Ultra Sonicator (Life care)
- Volumetric flasks - 10ml, 50ml, 100ml
- Pipettes - 1ml, 5ml, 10ml, beakers, measuring cylinders.

#### Chemicals and Reagents:

- Authentic samples of Rabeprazole (RABE) and Ondansetron (ONDA) were supplied by Torrent Research Center (Gandhinagar, India).
- Methanol (AR, Finar laboratories)

#### Preparation of Solutions

##### Standard stock solution (100 µg/ml)

Accurately weighed ONDA (10 mg) and RABE (10 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 100 µg/ml stock solution of both drugs.

##### Working standard solution

Use the Standard stock solutions (100 µg/ml) of ONDA and RABE as working standard solutions.

##### Sample solution

Weigh and collect the powder from 20 capsules. Weigh accurately a quantity of the powder equivalent to about 2 mg of ONDA and 10 mg of RABE in a 10 ml measuring flask and dissolve it in methanol and sonicate for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residues were washed thoroughly with methanol. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol.

#### Selection of wavelengths having the maximum absorbance and wavelength of ZCP for estimation of ONDA and RABE

The working standard solutions were scanned in the range of 200 nm to 400 nm against methanol as a blank. Maximum absorbance was obtained at 246 nm and 284 nm and the ZCP were found to be at 301.5 nm and 284 nm for ONDA and RABE, respectively. (Fig.1)

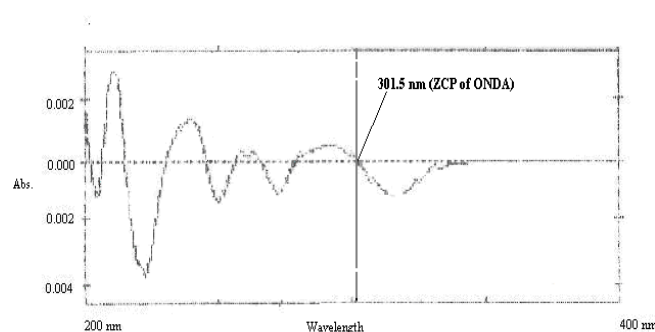
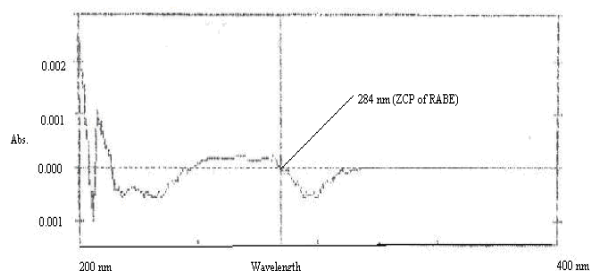


Figure 1(A): First Order Spectra of Onda (10 µg/ml)



**Figure 1 (B): First Order Spectra of Rabe (10 µg/ml)**

#### Calibration curve (Linearity)

Accurately measured working standard solution of ONDA (2, 4, 6, 8, 10, 12, 14 and 16 ml) RABE (4, 6, 8, 10, 12, 16, 20 and 25 ml) was transferred to separate 100 ml volumetric flasks and diluted up to the mark with Methanol. Absorbance was measured at ZCP of ONDA (284 nm) and ZCP of ONDA (301.5 nm) for ONDA and RABE respectively using methanol as a blank. The calibration curve was constructed by plotting absorbance versus concentration corresponding to each working standard solutions. Each reading was average of three determinations. A calibration curve was plotted over a concentration range from 4-25 µg/ml for RABE and 2-16 µg/ml for ONDA.

#### Accuracy and precision

Accuracy was determined in terms of percent recovery. The proposed method was applied to determine ONDA and RABE in capsules. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the capsules with three different concentrations of standards. Precision was determined in terms of intra-day and inter-day precision.

#### Estimation of RABE and ONDA in its formulation

Test solution from capsules which contain ONDA (2, 3 and 4 µg/ml) and RABE (10, 15 and 20 µg/ml) were prepared as described in 4.2.3.3 and solutions were analyzed as described under 4.2.5.

#### Method validation<sup>24-26</sup>

##### Solution stability

Sample solutions were kept at 25°C and 2-8°C for 24 h and three days, respectively. Assay of initial time period was compared with these two time periods. 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 responses of drugs 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 experiment was performed by preparing the standard solution of drug 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 and inter-day precision

Variation of results within same day is called Intra-day precision and variation of results amongst days is called Inter-day precision.

Intra-day precision of the proposed method was evaluated by assaying freshly prepared solutions of both drugs in triplicate at three different concentrations on the same day.

Inter-day precision was evaluated by using freshly prepared solutions of both drugs in triplicates at three different days.

#### Accuracy (% Recovery)

Accuracy of an analysis is determined by systemic error involved. 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 quantified 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.

$$LOD = 3.3 \times N/S$$

Where, N is the standard deviation of the absorbance 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. It is expressed as signal to noise ratio of 10:1.

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$LOQ = 10 \times N/S$$

Where, N is the standard deviation of the absorbance of the drug and S is the slope of the corresponding calibration curve.

## RESULTS AND DISCUSSION

In the proposed method, absorbances were recorded at Zero Crossing Point (ZCP) of Ondansetron Hydrochloride (301.5 nm) and ZCP of Rabeprazole Sodium (284 nm) for all the solutions. The calibration graphs for ONDA and RABE were constructed by plotting the absorbance versus their corresponding concentrations, good linearity was found over the range of 2-16 µg/ml for ONDA and 4-25 µg/ml for RABE. Results obtained by applying the proposed method shown that the concentrations of ONDA and RABE can be simultaneously determined in prepared mixtures. The proposed method has been applied to the assay of ONDA and RABE in pharmaceutical dosage form. The validity of the method 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 (Table 1). The optical and regression characteristics and validation parameters are reported in Table 2. Results of application of proposed method to the pharmaceutical dosage form are shown in Table 3.

**Table 1: Data of recovery study for rabe and onda by derivative spectrophotometric method.**

Drug	Amount taken ( $\mu\text{g/ml}$ )	Amount added ( $\mu\text{g/ml}$ )	Amount found ( $\mu\text{g/ml}$ )	% Recovery $\pm$ S.D (n=3)
RABE	8	6	13.96	99.73 $\pm$ 1.9629
	8	8	16.03	100.19 $\pm$ 1.7205
	8	10	17.77	98.75 $\pm$ 1.5126
ONDA	8	2	9.83	98.32 $\pm$ 1.9367
	8	4	11.75	97.91 $\pm$ 1.04
	8	6	13.55	96.72 $\pm$ 1.0334

**Table 2: Assay parameters and method validation obtained by applying the proposed method for determination of rabe and onda in binary mixtures.**

Parameters	RABE	ONDA
Calibration range	4-25 $\mu\text{g/ml}$	2-16 $\mu\text{g/ml}$
Detection limit	0.777 $\mu\text{g/ml}$	0.39 $\mu\text{g/ml}$
Quantitation limit	2.355 $\mu\text{g/ml}$	1.1819 $\mu\text{g/ml}$
Slope	-0.0021	0.0008
Intercept	0.001	0.0001
Mean	99.7	99.215
Standard deviation	1.6322	1.6409
Coefficient of variance	1.6371	1.6538
Correlation coefficient	0.9956	0.9985
Intraday RSD, %	1.02-2.1739	0.735-1.8929
Interday RSD, %	0.732-3.07692	0.5956-1.8328

**Table 3: Application of the proposed method to the pharmaceutical dosage forms**

RABE			ONDA		
Amount taken ( $\mu\text{g/ml}$ )	Amount found ( $\mu\text{g/ml}$ )	% Amount Found $\pm$ S.D. (n=3)	Amount taken ( $\mu\text{g/ml}$ )	Amount found ( $\mu\text{g/ml}$ )	% Amount Found $\pm$ S.D. (n=3)
10	10	100 $\pm$ 0.5	2	2.0125	100.625 $\pm$ 0.625
15	15.13	100.89 $\pm$ 0.509	3	2.975	99.166 $\pm$ 0.833
20	19.93	99.67 $\pm$ 0.763	4	3.933	98.333 $\pm$ 0.477

## CONCLUSIONS

From the results obtained by applying the suggested procedures, it is obvious that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully in routine analysis for the estimation of RABE and ONDA in pharmaceutical formulations without interference from commonly encountered excipients and additives.

## ACKNOWLEDGEMENT

The authors are thankful to Torrent Research Centre, Gandhinagar for providing samples of pure drugs. The authors are also thankful to Principal of B. S. Patel Pharmacy College for giving permission to work on Spectrophotometer.

## REFERENCES

- Fauci KB, Jameson HL. Harrison's Principle of Internal Medicine. 16<sup>th</sup> ed. New York: Mc Graw- Hill; 2005. p. 221.
- Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5<sup>th</sup> ed. New Delhi: Elsevier; 2005. p. 373-374.
- Tripathi KD. Essentials of Medical Pharmacology. 5<sup>th</sup> ed. New Delhi: J P Brothers Medical Publishers Pvt. Ltd.; 2004. p. 206-207.
- <http://en.wikipedia.org/wiki/Ondansetron>
- <http://www.fda.gov/MedWatch/report.htm>
- <http://www.nlm.nih.gov/Medline Plus/ Drug Info /meds / a601209.html>
- <http://3Dchem.com/molecules.asp?ID=267>

- Venkateshwaran TG, King DT, Stewart JT. HPLC determination of ondansetron-atropine and ondansetron-glycopyrrolate mixtures in 0.9% sodium chloride injection. Journal of Liquid Chromatography 1995; 18(13): 2647-2659.
- Steffen B, Elke S, Rolf K, Pierre-Benoit T, Jurgen B, Ivar R. Simultaneous determination of Ondansetron and Tropisetron in human plasma using HPLC with UV detection. Biomedical Chromatography 2002; 16(3): 187-190.
- Venkateshwaran TG, King DT, Stewart JT. HPLC Determination of a Vincristine, Doxorubicin, and Ondansetron Mixture in 0.9% Sodium Chloride Injection. Journal of Liquid Chromatography 1994; 17(6): 1399-1411.
- Chandrasekara D, Ramakrishna S, Diwan PV. A rapid, sensitive and validated method for the determination of Ondansetron in human plasma by reversed-phase high pressure liquid chromatography. Arzneimittel-Forschung 2004; 54(10): 655-659.
- Liu J, Stewart JT. High performance liquid chromatographic analysis of Ondansetron enantiomers in human serum using a reversed-phase cellulose-based chiral stationary phase and solid phase extraction. Journal of Chromatography B: Biomedical Sciences and Applications 1997; 694(1): 179-184.
- Hailang Z, Lin Y, Stewart JT. Hplc determination of idarubicin-etoposide and idarubicin-ondansetron mixtures in 0.9% sodium chloride injection USP. Journal of Liquid Chromatography and Related Techniques 1998; 21(7): 979-988.
- Tambe S, Kale S, Kulkarni S and Chhajed S. HPTLC analysis of ondansetron in oral solid dosage forms. Abstract No. GP-15; IPC 56<sup>th</sup>; 2004 December 2-4; Kolkata, India.
- Harkishn Singh, VK Kapoor. Medicinal and Pharmaceutical Chemistry. 2<sup>nd</sup> ed. Delhi; Vallabh Prakashan; 2009. p. 234.
- [http://www.nlm.nih.gov/medlineplus/druginfo/meds/a69906\\_0.html](http://www.nlm.nih.gov/medlineplus/druginfo/meds/a69906_0.html)
- <http://en.wikipedia.org/wiki/Rabeprazole>
- Rao AL, Ravikumar BNV, Sankar GG. Development of Rp-HPLC method for the estimation of Rabeprazole in pure and tablet dosage form. E. Journal of Chemistry 2008; 2(S2): 1149-1153.
- Patel BH, Patel MM, Patel JR, Suhagia BN. Simultaneous Determination of Omeprazole and Domperidone in Capsules by RP-HPLC and Densitometric HPTLC. Journal of Liquid Chromatography and Related Techniques 2007; 30(12): 1749 - 1762.
- Uno T, Yasui-Furukori N, Shimizu M, Sugawara K, Tateishi T. Determination of rabeprazole and its active metabolite, rabeprazole thioether in human plasma by column-switching high performance liquid chromatography and its application to pharmacokinetic study. Journal of Chromatography B 2005; 824(1-2): 238-243.
- Ramakrishna NVS, Vishwottam KN, Wishu S, Koteswara M, SureshKumar S. High performance liquid chromatography method for the quantification of rabeprazole in human plasma using solid phase extraction. Journal of Chromatography B 2005; 816(1-2): 209-214.
- Huang J, Xu Y, Gao S, Rui L, Guo Q. Development of a liquid chromatography/tandem mass spectrometry assay for the quantification of rabeprazole in human plasma. Rapid Communications in Mass Spectrometry 2005; 19(16): 2321 - 2324.
- Garcia CV, Sippel J, Steppe M, Schapoval EES. Development and Validation of Derivative Spectrophotometric Method for Determination of Rabeprazole Sodium in Pharmaceutical Formulation. Analytical Letters 2006; 39(2): 341 - 348.
- [www.ich.org.in](http://www.ich.org.in)
- ICH guideline Q2 (R1). Validation of analytical procedures: text and methodology. Geneva: 1996.
- Guidance for industry, Analytical procedure and method validation chemistry, manufacturing and controls documentation, Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM). Rockville, Maryland, U.S.: 2004.