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Validated UV-Spectrophotometric methods for Simultaneous Estimation of Rabeprazole and Ondansetron in Bulk drug and Tablet formulation

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Abstract

Three simple, accurate and precise spectrophotometric methods have been developed for simultaneous determination of Rabeprazole (RAB) and Ondansetron (OND) in a laboratory mixture. Simultaneous equation method (Method I) shows absorbance at 284.5 nm (λ1) and 307 nm (λ2) corresponding to the absorbance maxima of RAB and OND respectively. In absorbance ratio method (Method II) isobestic point is observed at 291 nm. Isobestic point 291 nm is considered as (λ1) and absorbance maxima of OND at 307 nm are considered as (λ2). In First order derivative zero crossing method (Method III) (λ1) 247nm (for RAB) and (λ2) 320.5 nm (for OND) was carried out. All dilutions were prepared in distilled water and methanol (50:50). Linearity range was observed in the concentration range of solution 2.5- 12.5 µg/ml for RAB and 4-12 µg/ml for OND. The methods were validated statistically and recovery study was performed to confirm the accuracy of both drugs. The developed methods are simple, economic, accurate, precise and reproducible. They can be adopted for routine quality control analysis of these drugs in pharmaceutical combined dosage form.

Keywords: RAB, OND, Ultraviolet spectroscopy, Simultaneous equation method, Absorption ratio method, First order derivative zero crossing method.

Introduction

Rabeprazole,2-({[4-(3-meyhoxypropoxy)-3-methyl-2-pyridyl]methyl}sulfinyl)-1Hbenzimidazole is used as antiulcerative , it acts by selectively inhibiting the H+/ K+ATPase enzyme in the secretory canaliculus of the simulated parietal cell[1-2] and Ondansetron, 3-[(2-methyl-1H-imidazol-1yl)methyl]-1,2,3,4a-tetrahydro- 4H-carbazol-4-one is an antiemetic, it antagonises 5HT3 receptor present peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone to prevent vomiting radiotherapy[3-4]. In present work we propose to develop and validate UV methods for simultaneous estimation of RAB and OND as per ICH and FDA(USA) guidelines in USP [5-7].

Material and Methods

Instrumentation

UV-visible double beam spectrophotometer, Shimadzu UV-1700 model with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used.

Reagent and chemicals

Standard gift sample of OND and RAB was obtained from Lupin Pvt. Ltd. The synthetic mixture was prepared by using standard active pharmaceutical ingredient RAB (10 mg) and OND(4 mg).

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After assessing the solubility of drugs in different solvents distilled water and methanol (50:50) was used as common solvent for developing spectral characteristics.

Preparation of standard stock solution

The standard stock solutions (25µg/ml) of each of RAB and OND were prepared separately by dissolving accurately about 25 mg of each of drug in 50 ml volumetric flask and volume was made up to the mark with methanol: water (50:50) to made(500µg/ml). From above solution 2.5 ml was pipetted out in 50 volumetric flask and volume was made up to the mark with same solvent producing working standard solutions of 25 µg/ml concentration.

Preparation of calibration curves

Solutions of 25 µg/ml of RAB and OND each were prepared separately. Both the solutions were scanned in the spectrum mode from 200- 400 nm. The maximum absorbance of RAB and OND were observed at 284.5 nm and 307 nm, respectively spectra were shown in Fig. No.1 and Fig. No.2. RAB and OND obey Beer Lamberts law [8-11]. Accurately measured working standard solution of RAB (2.5, 5.0, 7.5, 10.0, 12.5 ml) and OND (4.0, 6.0, 8.0, 10.0, 12.0 ml) were transferred to a separate series of 50 ml of volumetric flasks and diluted to the mark with methanol: water (50:50). The absorbance of each solution was measured at wavelength 284.5 nm and 307 nm for RAB and OND respectively. The coefficient of correlation was found to be 0.999 and 0.999 for RAB and OND respectively. Results are as shown in Table No.1.

Method I. Simultaneous Equation Method

Sample stock solution was appropriately diluted with distilled water to obtain final concentration of 25µg/ml for RAB and OND. These solutions were scanned in the wavelength range of 200 – 400 nm. From the UV spectrum, two wavelengths namely 284.5 nm and 307 nm, λ-max of RAB and OND respectively were selected. The calibration curves were constructed in the concentration range of 2.5- 12.5 µg/ml for RAB and 4- 12 µg/ml OND to maintain the ratio of 10:4 of RAB and OND respectively (Fig. No.3-6). The concentration of drugs was determined by using the equation no. 1 and 2. Results are show in Table. No.1.

$$Cx = A2ay1 - A1ay2 / ax2ay1 - ax1ay2 \quad \text{equation no.1}$$

$$Cy = A1ax2 - A2ax1 / ax2ay1 - ax1ay2 \quad \text{equation no.2}$$

Where, A1 and A2 are absorbance of sample at 284.5 nm and 307 nm respectively; ax1 and ax2 are absorptivities of RAB at 284.5 nm and 307 nm respectively; ay1 and ay2 are absorptivities of OND at 284.5 nm and 307 nm respectively; Cx and Cy are concentrations of and RAB and OND respectively.

Method II. Absorbance Ratio Method

Two wavelengths were selected, from the overlain spectrum of RAB and OND λ1 observed at 291 nm which is the isoabsorptive point (Fig. No.7) for both drugs and λ2 is considered at 307 nm which is λ max of OND. The absorbance of the sample solutions were measured in a similar manner as described in the previous experiment. Wavelengths of absorptions were measured and the absorbance ratio values for both drugs at selected wavelengths were also calculated. The method employs Q- values and the concentration levels of drugs in sample solution were determined by using the equation no.3 and 4. Results are show in Table No.1.

$$Cx = Qm - Qy / Qx - Qy \times A/ax1 \quad \text{equation no.3}$$

$$Cy = Qm - Qx / Qx - Qy \times A/ay1 \quad \text{equation no.4}$$

$$Qm = A2/A1 \,, Qx = ax2/ax1, Qy = ay2/ay1$$

Where, Cx and Cy are concentrations of RAB and OND respectively; Qx = the ratio of absorptivity of RAB at 291 and 307 nm; Qy = the ratio of absorptivity of OND at 291 and 307 nm; Qm = the ratio of absorbance of mixture at 291 and 307 nm; A = the absorbance of mixture at isoabsorptive point; ax = the absorptivity value of RAB at isoabsorptive point; ay = the absorptivity value of OND at isoabsorptive point. Caliberation curves were shown in (Fig. No.8-11)

Method III. First Derivative Zero Crossing Method

For First Derivative Zero Crossing Method, suitable dilutions of the standard stock solutions (25µg/ml) of RAB and OND were prepared separately in distilled water and methanol(50:50). The solutions of drugs were scanned in the range

of 200 nm - 400 nm. For this method, The absorbance of the sample solutions were measured in a similar manner as described in the previous experiment. calibration curves were plotted and the sampling wavelength ranges selected for estimation of RAB and OND are 247 nm -320.5 nm (λ1-λ2) respectively (Fig. No. 12 -14) which showed linear response with increasing concentration hence the same wavelength range were used for estimation of synthetic mixture. By using integrated areas two simultaneous equations were constructed and solved to determine concentrations of analytes. Concentration of two drugs in mixed standard and the sample solution were calculated. Results are shown in Table No.1.

Analysis of laboratory mixture

Accurately weighed quantities of RAB equivalent to 50 mg, and OND equivalent to 20 mg were taken in two separate volumetric flasks and dissolved in methanol: water (50:50) and volume was made up to the mark with same solvent. From above solutions 2.5 ml was pipetted out in two separate 50 ml volumetric flasks for RAB and OND and volume was made up to the mark with same solvent. The sample solution thus prepared was filtered through Whatman filter paper no. 44, diluted with diluent to get the solution containing about 10 µg/ml of RAB and 4 µg/ml of OND.

Validation of Method

Validation of the proposed methods was carried out for its accuracy, precision, specificity and linearity according to ICH guidelines.

Accuracy

Recovery studies were carried out at 80%, 100% and 120% level by adding a known quantity of pure drug to the preanalyzed laboratory mixture and the proposed method was followed. From the amount of drug found, percentage recovery was calculated and results are given in Table a)

Repeatability

Six test sample solutions containing 10 µg/mL of RAB and 4µg/mL of OND were scanned over range of 200-400 nm and absorbance are measured at respective wavelengths of mentioned methods, concentrations were determined with the help of proposed method and % RSD was calculated and results are given in Table a)

Limit of detection

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the equation no.7 and shown in Table a)

LOD = 3.3 (σ / S) Equation no.7

Limit of quantification

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the equation no.8 and shown in Table. No. 1. Where, S = slope of calibration curve, σ = standard deviation of the response. m= Slope, c= Intercepts

LOQ = 10 (σ / S) Equation no.8

Results and Discussion

RAB and OND have estimated at 284.5 nm and 307 nm in distilled water: methanol (50:50), RAB and OND obey Beer-Lamberts law in concentration range of 2.5-12.5µg/ml and 4-12 µg/ml respectively. The method was validated as per ICH and USP guidelines. The result of recovery study was found to be within the prescribed limit of 98 – 101 %. The assay results obtained by proposed methods are as shown in Table. No. 1. The % R.S.D. Linearity was observed by linear regression equation method for RAB and OND in different concentration range. The correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity.

Simultaneous Equation Method

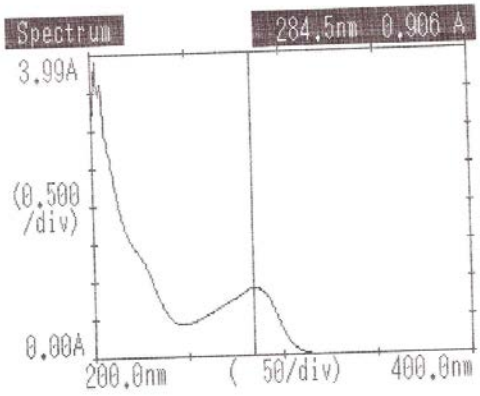

Fig. 1 : UV Spectrum of RAB

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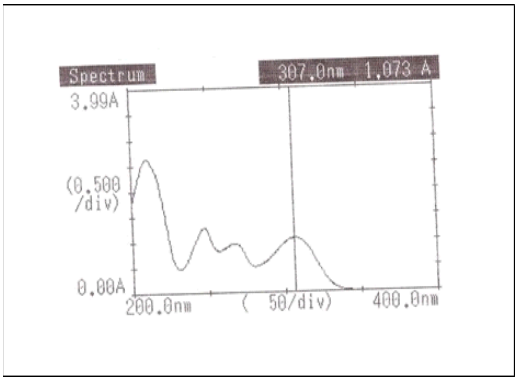

Fig. 2: UV Spectrum of OND

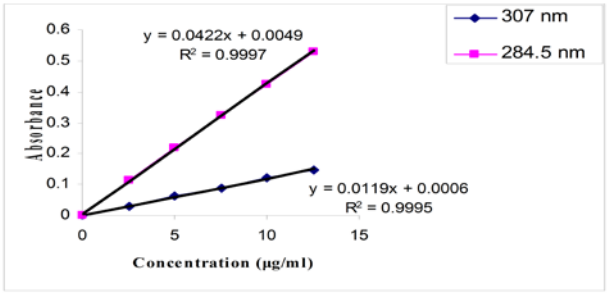

Fig. 3 : Calibration Plot of RAB at 284.5 nm and 307 nm

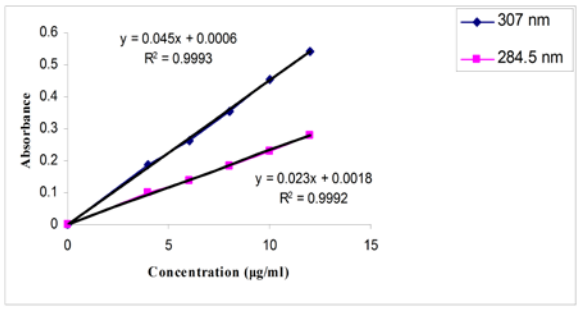

Fig. 4: Calibration Plot of OND at 284.5nm and 307nm

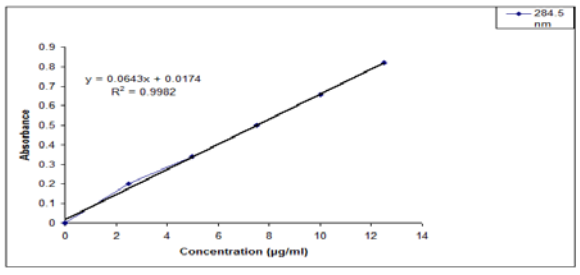

Fig. 5: Calibration Plot of Laboratory mixture at 284.5nm

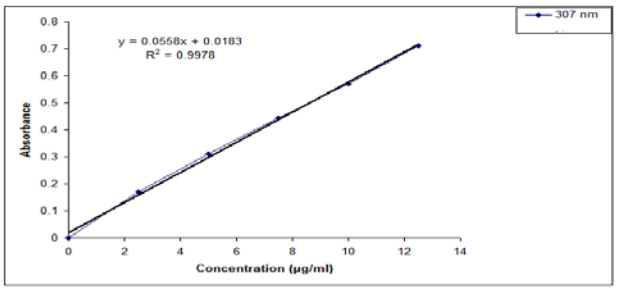

Fig. 6: Calibration Plot of Laboratory mixture at 307nm

Absorption ratio method

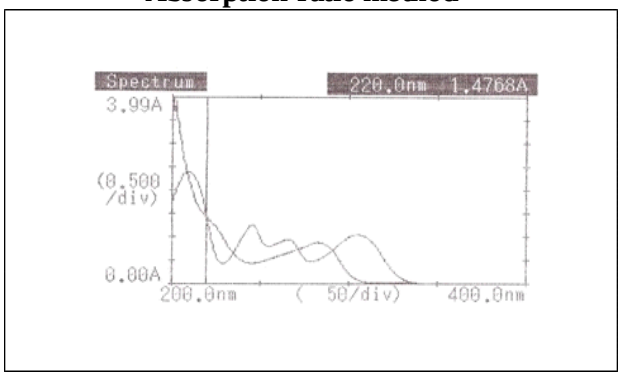

Fig. 7: Overlain spectra of RAB and OND

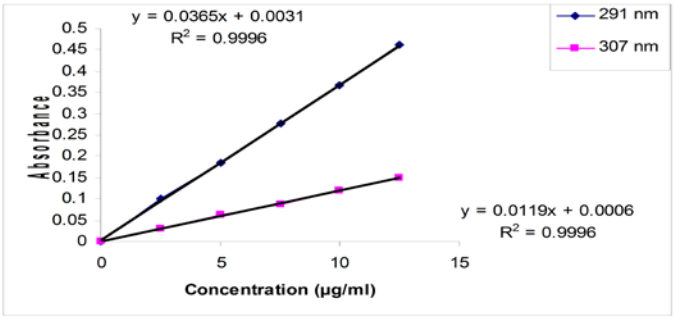

Fig. 8: Calibration Plot of RAB at 291nm and 307nm

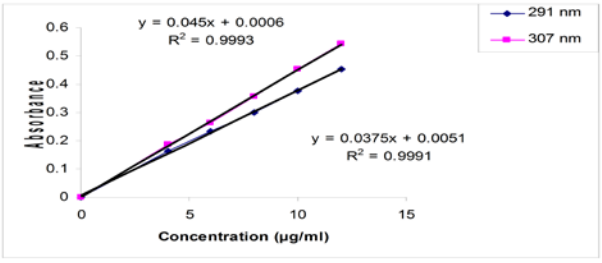

Fig. 9: Calibration Plot of OND at 291nm and 307nm

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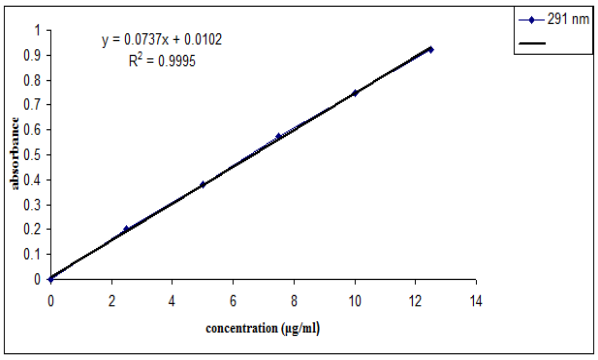

Fig. 10: Calibration Plot of laboratory mixture at 291nm

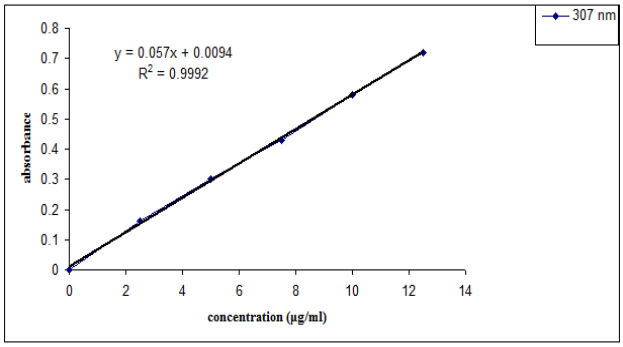

Fig.11: Calibration Plot of laboratory mixture at 307nm

First Derivative Zero Crossing Method

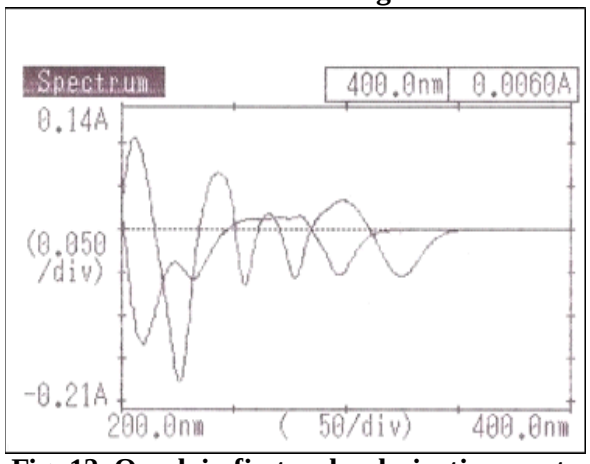

Fig. 12: Overlain first order derivative spectra of RAB and OND

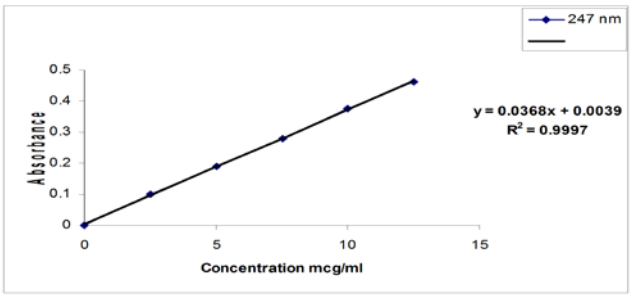

Fig. 13: Calibration plot of RAB at 247 nm

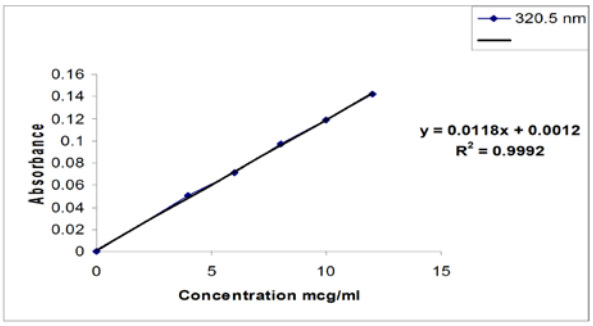

Fig. 14: Calibration plot of OND at 320.5 nm

Table 1: Optical characteristics, Precision study and result of formulation analysis

Parameter		Rabeprazole			Ondansetron		
		Method I	Method II	Method III	Method I	Method II	Method III
Wavelength (nm)		284.5	291.0	247.0	307.0	307.0	320.5
Beers law limit(µg/mL)		2.5-12.5	2.5-12.5	2.5-12.5	4.0-12.0	4.0-12.0	4.0-12.0
Regression equation	m	0.0422	0.0061	0.1009	0.045	0.0033	0.0397
	c	0.0049	0.0035	0.0498	0.0006	0.0013	0.0019
Correlation coefficient(r)		0.999	0.999	0.999	0.999	0.999	0.999
Formulation analysis (%assay)		99.58	100.36	99.81	99.51	100.64	99.98
Recovery(%)		100.66	99.49	99.91	100.21	99.53	100.03
Intra day precision (% RSD,n=3)		0.212	0.698	0.075	0.122	0.512	0.0073
Inter day precision (% RSD ,n=3)		0.224	0.360	0.009	0.123	0.498	0.004
LOD		0.281	0.232	0.202	0.234	0.260	0.080
LOQ		0.853	0.704	0.614	0.711	0.787	0.226

Conclusion

The proposed methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic and can be used for routine quantitative analysis of RAB and OND in pure and synthetic mixture form.

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