



# Asian Journal of Research in Chemistry and Pharmaceutical Sciences

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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 ( $\lambda_1$ ) and 307 nm ( $\lambda_2$ ) corresponding to the absorbance maxima of RAB and OND respectively. In absorbance ratio method (Method II) isobestic point is observed at 291nm. Isobestic point 291 nm is considered as ( $\lambda_1$ ) and absorbance maxima of OND at 307nm is considered as ( $\lambda_2$ ). In First order derivative zero crossing method (Method III) ( $\lambda_1$ ) 247nm (for RAB) and ( $\lambda_2$ ) 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 $\mu$ g/ml for RAB and 4-12 $\mu$ g/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 and First order derivative zero crossing method.

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### INTRODUCTION

Rabeprazole, 2-([4-(3-methoxy propoxy)-3-methyl-2-pyridyl] methyl) sulfinyl)-1H-benzimidazole is used as anti-ulcerative, it acts by selectively inhibiting the H<sup>+</sup>/ K<sup>+</sup> ATPase enzyme in the secretory canaliculus of the simulated parietal cell<sup>1,2</sup> and Ondansetron, 3-[(2-methyl-1H-imidazol-1yl) methyl]- 1, 2, 3, 4a-tetrahydro-4H-carbazol-4-one is an antiemetic, it antagonises 5HT<sub>3</sub> receptor present peripherally on vagal nerve terminals and

centrally in the chemoreceptor trigger zone to prevent vomiting radiotherapy<sup>3,4</sup>. 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<sup>5-7</sup>.

## MATERIAL AND METHODS

### Instrumentation

UV-visible double beam spectrophotometer, Shimadzu UV-1700 model with spectral bandwidth of 1 nm, wavelength accuracy of  $\pm 0.3$  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). 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 (25 $\mu$ g/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 (500 $\mu$ g/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 $\mu$ g/ml concentration.

### Preparation of calibration curves

Solutions of 25 $\mu$ g/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 284.5nm and 307nm, respectively spectra were shown in Figure No.1 and Figure No.2. RAB and OND obey Beer Lamberts law<sup>8-11</sup>. Accurately measured working standard solution of RAB (2.5, 5.0, 7.5, 10.0, 12.5 ml) and OND (4.0, 0.4, 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 25 $\mu$ g/ml for RAB and OND. These solutions were scanned in the wavelength range of 200 – 400nm. From the UV spectrum, two wavelengths namely 284.5nm and 307nm,  $\lambda$ -max of RAB and OND respectively were selected. The calibration curves were constructed in the concentration range of 2.5-12.5 $\mu$ g/ml for RAB and 4-12 $\mu$ g/ml OND to maintain the ratio of 10:4 of RAB and OND respectively (Figure No.3-6). The concentration of drugs was determined by using the equation No.1 and No.2. Results are show in Table No.1.

$C_x = A_{2ay1} - A_{1ay2} / a_{x2ay1} - a_{x1ay2}$  equation No.1

$C_y = A_{1ax2} - A_{2ax1} / a_{x2ay1} - a_{x1ay2}$  equation No.2

Where, A1 and A2 are absorbance of sample at 284.5 nm and 307 nm respectively;  $a_{x1}$  and  $a_{x2}$  are absorptivities of RAB at 284.5 nm and 307 nm respectively;  $a_{y1}$  and  $a_{y2}$  are absorptivities of OND at 284.5 nm and 307 nm respectively;  $C_x$  and  $C_y$  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  $\lambda_1$  observed at 291 nm which is the isoabsorptive point (Figure No.7) for both drugs and  $\lambda_2$  is considered at 307 nm which is  $\lambda$  max of OND. The absorbance of the sample solutions were measured in a similar manner as described in the previous experiment. By measuring wavelengths of absorptions, 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 No.4. Results are show in Table No.1.

$C_x = Q_m - Q_y / Q_x - Q_y \times A / a_{x1}$  equation No.3

$C_y = Q_m - Q_x / Q_x - Q_y \times A / a_{y1}$  equation No.4

$Q_m = A_2 / A_1$ ,  $Q_x = a_{x2} / a_{x1}$ ,  $Q_y = a_{y2} / a_{y1}$

Where,  $C_x$  and  $C_y$  are concentrations of RAB and OND respectively;  $Q_x$  = the ratio of absorptivity of

RAB at 291 and 307 nm;  $Q_y$  = the ratio of absorptivity of OND at 291 and 307 nm;  $Q_m$  = the ratio of absorbance of mixture at 291 and 307 nm;  $A$  = the absorbance of mixture at isoabsorptive point;  $a_x$  = the absorptivity value of RAB at isoabsorptive point;  $a_y$  = the absorptivity value of OND at isoabsorptive point. Calibration curves were shown in (Figure No.8-11).

### Method III. First Derivative Zero Crossing Method

For First Derivative Zero Crossing Method, suitable dilutions of the standard stock solutions (25 $\mu$ g/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 ( $\lambda_1$ - $\lambda_2$ ) respectively (Figure 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 both 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 concentration was made up to the mark with same solvent. From above solutions 2.5 ml was pipetted out in two separate 50ml volumetric flasks for RAB and OND and volume was made up to the mark with same solvent. The prepared sample solution was filtered through Whatman filter paper No.44, diluted with diluent to get the solution containing about 10 $\mu$ g/ml of RAB and 4 $\mu$ g/ml of OND.

### Validation of Method

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

### Accuracy

By adding a known quantity of pure drug to the preanalyzed laboratory mixture, recovery studies were carried out at 80%, 100% and 120% level and the proposed method was followed. From the amount of drug found, percentage recovery was calculated and results are given in Table No.1.

### Repeatability

Six test sample solutions containing 10 $\mu$ g/mL of RAB and 4 $\mu$ g/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 No.1.

### Limit of detection

The smallest concentration of the analyte that gives the measurable response The Limit of Detection (LOD). LOD was calculated using the equation No.7 and shown in Table No.1.

$$LOD = 3.3 (\sigma / S) \text{ Equation No.7}$$

### 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,  $\sigma$  = standard deviation of the response.  $m$  = Slope,  $c$  = Intercepts

$$LOQ = 10 (\sigma / S) \text{ Equation No.8}$$

## RESULTS AND DISCUSSION

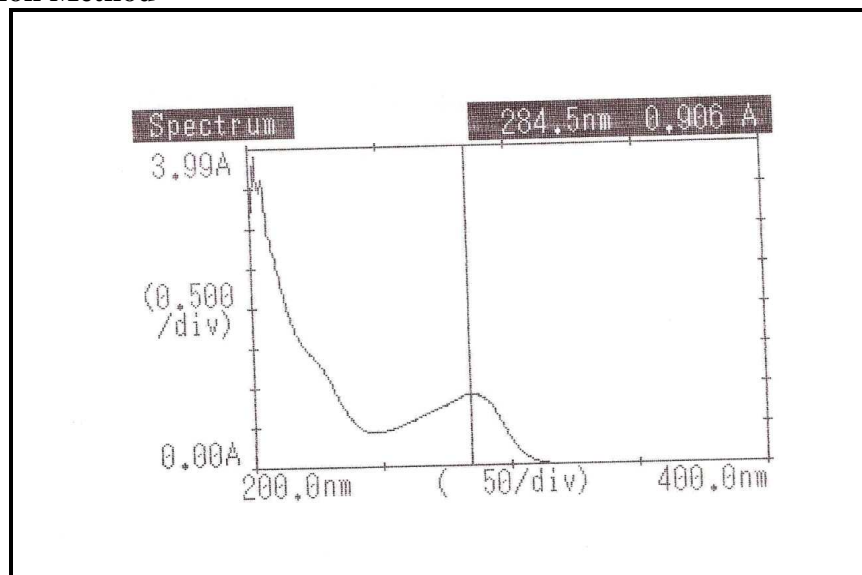
RAB and OND have estimated at 284.5 nm and 307 nm in distilled water: methanol (50: 50), RAB and OND obey Beer-Lamberts law in concentration range of 2.5-12.5 $\mu$ g/ml and 4-12 $\mu$ g/ml respectively. The method was validated as per ICH and USP guidelines. The result of recovery study was found to 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, hence it can be used for routine analysis of two drugs in combined dosage forms. There was no interference from tablet excipients was observed in these methods. The developed methods were accurate, simple, rapid, precise, reliable, sensitive, reproducible and economic.

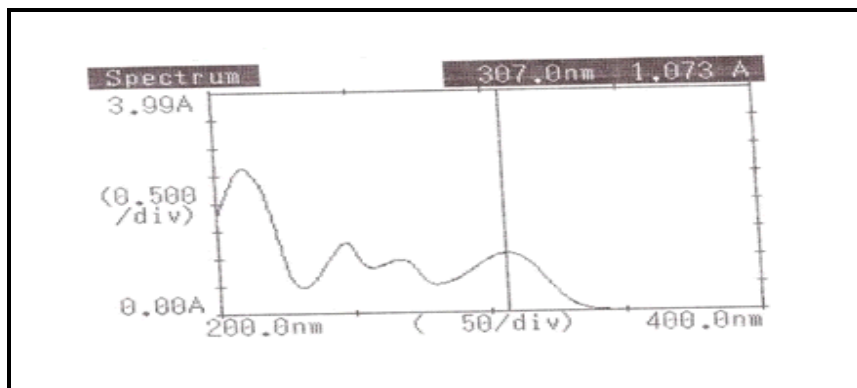
**Table No.1: Optical characteristics, Precision study and result of formulation analysis**

S.No	Parameter	Rabeprazole			Ondansetron			
		Method I	Method II	Method III	Method I	Method II	Method III	
1	Wavelength(nm)	284.5	291.0	247.0	307.0	307.0	320.5	
2	Beers law limit( $\mu\text{g/mL}$ )	2.5-12.5	2.5-12.5	2.5-12.5	4.0-12.0	4.0-12.0	4.0-12.0	
3	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
4	Correlation coefficient(r)	0.999	0.999	0.999	0.999	0.999	0.999	
5	Formulation analysis (%assay)	99.58	100.36	99.81	99.51	100.64	99.98	
6	Recovery (%)	100.66	99.49	99.91	100.21	99.53	100.03	
7	Intraday precision (% RSD, n=3)	0.212	0.698	0.075	0.122	0.512	0.0073	
8	Inter day precision (% RSD, n=3)	0.224	0.360	0.009	0.123	0.498	0.004	
9	LOD	0.281	0.232	0.202	0.234	0.260	0.080	
10	LOQ	0.853	0.704	0.614	0.711	0.787	0.226	

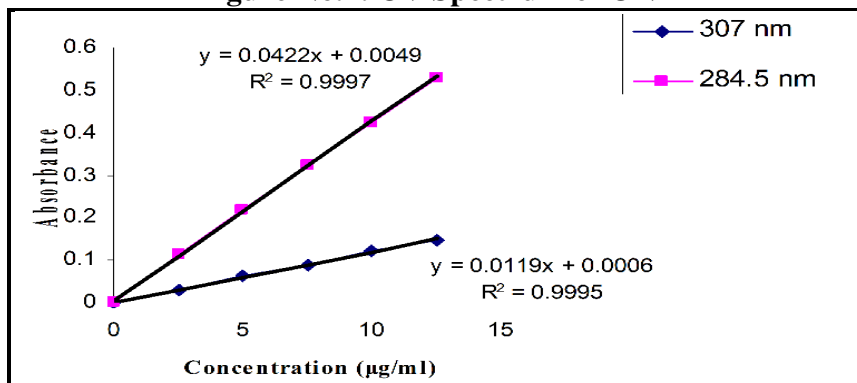
### Simultaneous Equation Method



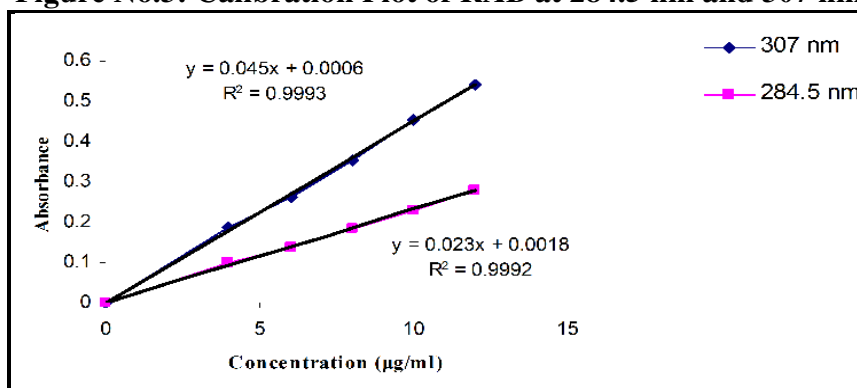
**Figure No.1: UV Spectrum of RAB**



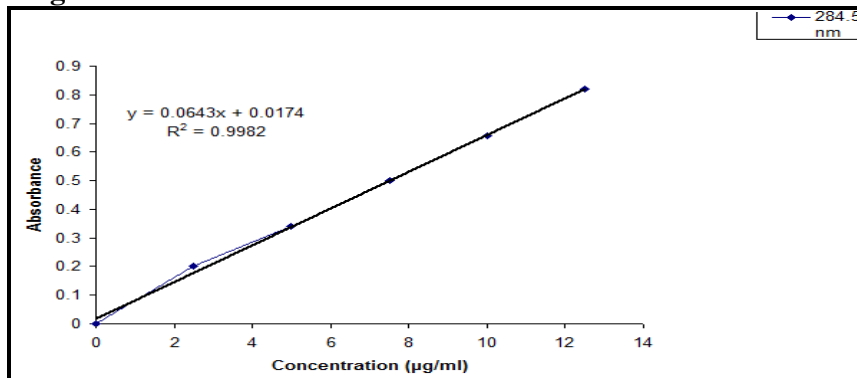
**Figure No.2: UV Spectrum of OND**



**Figure No.3: Calibration Plot of RAB at 284.5 nm and 307 nm**



**Figure No.4: Calibration Plot of OND at 284.5nm and 307nm**



**Figure No.5: Calibration Plot of Laboratory mixture at 284.5nm**

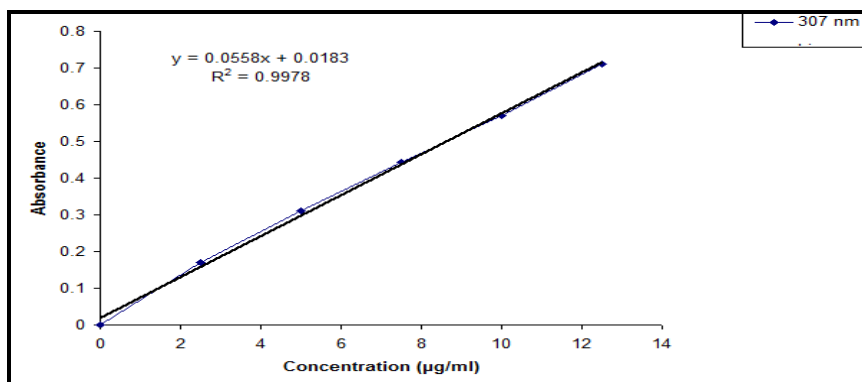


Figure No.6: Calibration Plot of Laboratory mixture at 307nm

Absorption ratio method

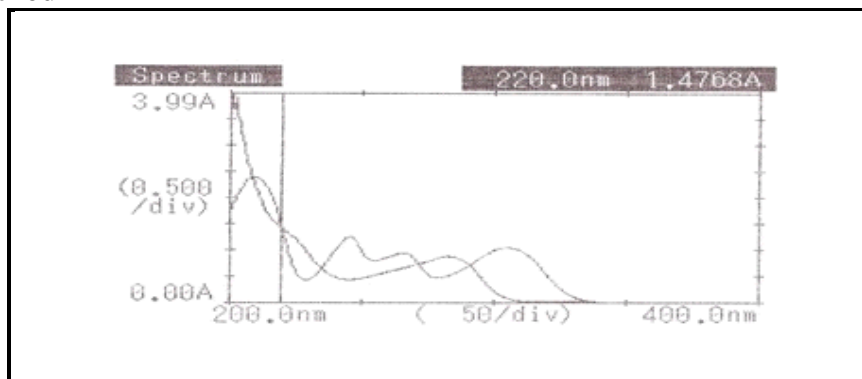


Figure No.7: Overlain spectra of RAB and OND

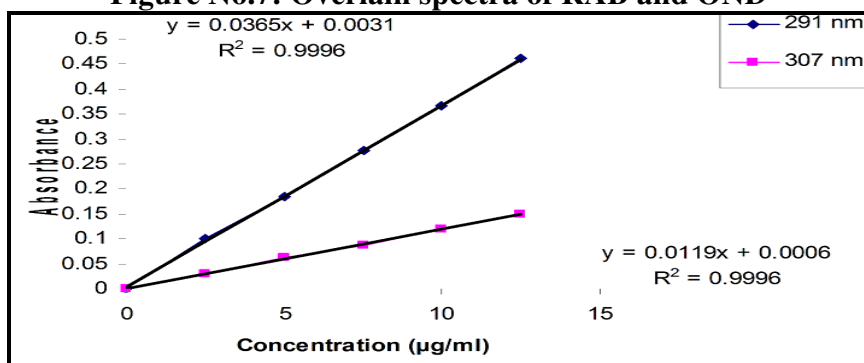


Figure No.8: Calibration Plot of RAB at 291nm and 307nm

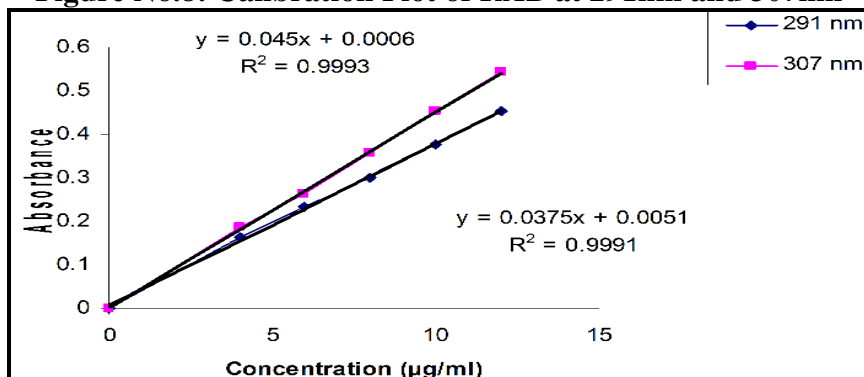


Figure No.9: Calibration Plot of OND at 291nm and 307nm

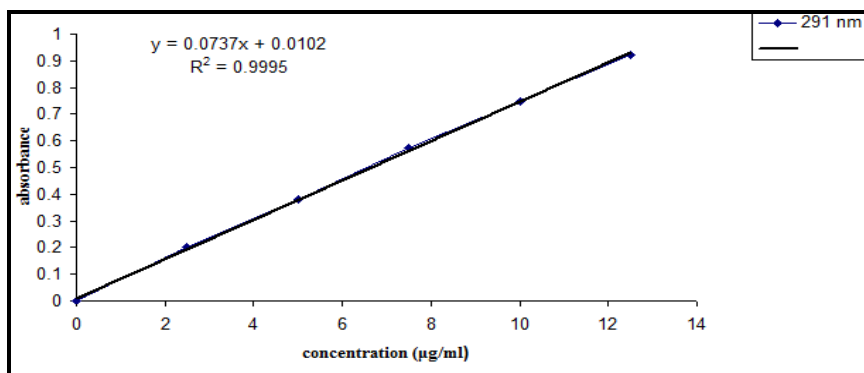


Figure No.10: Calibration Plot of laboratory mixture at 291nm

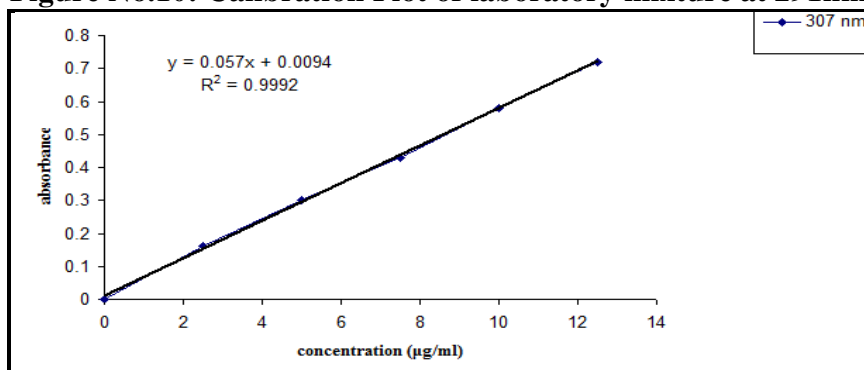


Figure No.11: Calibration Plot of laboratory mixture at 307nm

First Derivative Zero Crossing Method

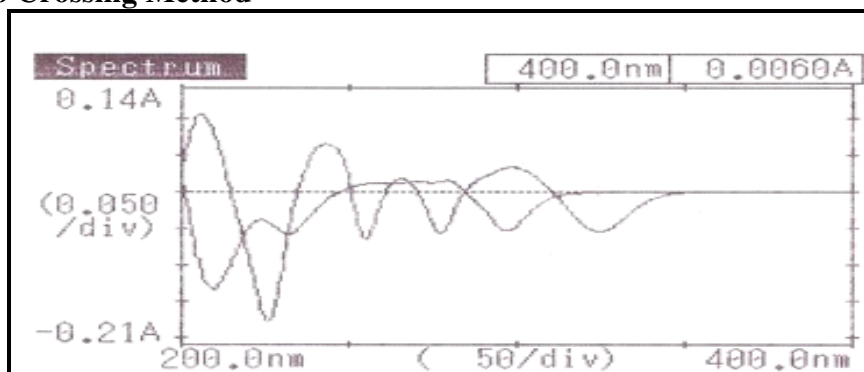


Figure No.12: Overlain first order derivative spectra of RAB and OND

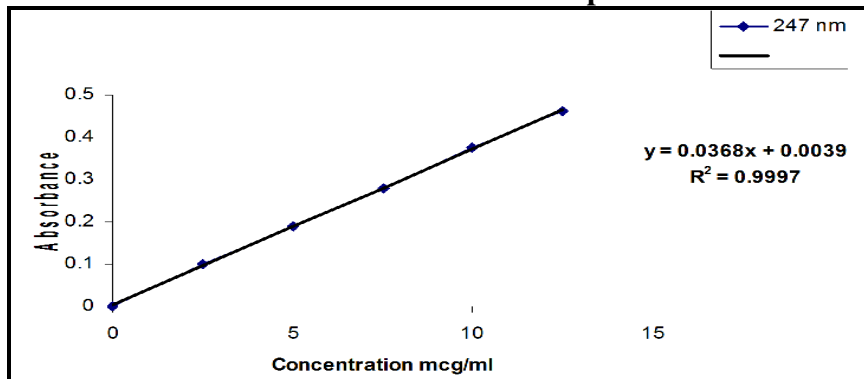


Figure No.13: Calibration plot of RAB at 247 nm

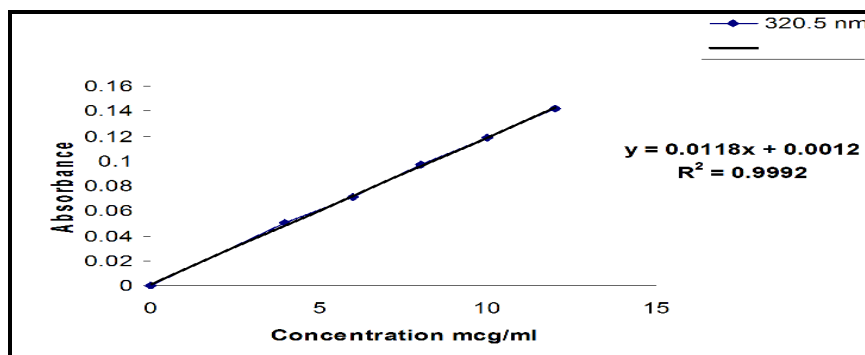


Figure No.14: Calibration plot of OND at 320.5 nm

## CONCLUSION

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

## ACKNOWLEDGEMENT

The authors wish to thank Lupin Laboratories for providing the gift sample of RAB and OND standard.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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**Please cite this article in press as:** Yogeshwari T. Jambhulkar et al. Validated UV-spectrophotometric methods for simultaneous estimation of Rabeprazole and Ondansetron in bulk drug and tablet formulation, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, 7(4), 2019, 999-1006.