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RESEARCH ARTICLE

HPLC Analytical Method Development and Validation Studies of Ondansetron and Rabeprazole.

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ABSTRACT

Objective: Present investigation involves development and validation of chromatographic method for ondansetron and rabeprazole estimation by HPLC.

Methods: The present work describes a validated reverse phase high performance liquid chromatographic method for simultaneous estimation of Rabeprazole and Ondansetron in bulk and pharmaceutical dosage form. Chromatography was performed on a XTerra RP column C_{18} (150 mm x 4.6 mm i.d., 5 µm) column with mobile phase containing Buffer (phosphate buffer pH 5.5): Water: Methanol (30: 10: 60 v/v/v). The flow rate was 0.6 ml/min and the eluent was monitored at 274 nm. Rabeprazole and Ondansetron were studied for their estimation by RP-HPLC method development and the method was also studied for validation parameters.

Results: The selected chromatographic conditions were found to effectively separate Rabeprazole (RT-5.919 min) and Ondansetron (RT- 4.382 min). Linearity for Rabeprazole and Ondansetron were found in the range of 20-100 µg/ml. The values obtained of LODs were 3.06 and 0.06 µg/ml; LOQs were 9.90 and 0.21 µg/ml for Rabeprazole and Ondansetron, respectively. The proposed method was found to be fast, accurate, precise, reproducible and rugged and can be used for simultaneous analysis of Rabeprazole and Ondansetron in combined pharmaceutical formulations.

Conclusion: Developed HPLC method was conveniently used for the estimation of ondansetron and rabeprazole in bulk and formulations and the results obtained in this study were uniform, sensitive and reproducible within the limits. Therefore the method is suitable for its intended use for routine analysis of both drug candidates.

KEYWORDS: Rabeprazole, Ondansetron, Reversed-Phase HPLC.

INTRODUCTION:

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity. Reversed-phase chromatography refers to the use of a polar eluent with a non polar stationary phase in contrast to normal-phase chromatography, where a polar stationary phase is employed with a non-polar mobile phase. In reverse-phase liquid chromatography, the stationary phase is prepared by chemically bonding a relatively non-polar group on to the stationary phase support. The most frequent non-polar group on to the stationary phase support is octadecylsilane (ODS), which gives a highly lipophilic stationary phase [1-4].

The rate of elution of the components is controlled by the polarity of the organic modifier and its proportion in the mobile phase. Degassing is quite important with reversed-phase mobile phases. HPLC provides reliable quantitative precision and accuracy, along with a linear dynamic range sufficient to allow for the determination of the active pharmaceutical ingredient (API) and related substances in the same run using a variety of detectors along with excellent reproducibility and is applicable to a wide array of compound types by judicious choice of HPLC column chemistry. Major modes of HPLC include reverse phase and normal phase for the analysis of small (<2000 Da) organic molecules[5].

ANALYTICAL METHOD VALIDATION:

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use before their introduction into routine use. Whenever the condition changes for which the method has been validated.

VALIDATION PARAMETERS: SPECIFICITY:

In practice this can be done by spiking the drug substance or product (placebo formulation, excipients degradation product, process impurity) with appropriate level and demonstrating the assay result is unaffected by the presence of these extraneous materials[6]. **Precision:** The precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogenous sample to

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be able to calculate statistically valid estimates of standard deviation or relative standard deviation for observing the amount of scatter in the results[6].

ACCURACY:

Accuracy for analytical synthetic mixtures of the drug components to which the known amount of analyte have been added within range of the method was calculated as percentage recovery or as the difference between the mean and the accepted true value together with confidence intervals. The ICH recommends that accuracy should be assessed during the minimum of nine determinations over a minimum of three concentrations levels, covering a specified range[7].

LINEARITY:

ICH recommended that, for the establishment of linearity, a minimum of 5 concentrations. It is also recommended that the following minimum specified range should be considered. For assay of a drug substance or a finished product 80-120% of the concentration should be taken. Acceptability of the linearity data is often judged by examining the correlation coefficient. The correlation coefficient of > 0.999 is generally considered as evidence of acceptable fit of the data to the regression line the analyte at to target level[8].

ROBUSTNESS:

The robustness of the methods was determined by performing the assay of the triplicate by deliberately alternating parameters and that the results are not influenced by different changes in the above parameters[9]. like change in column temperature: $\pm$ 2^0C, change in flow rate: $\pm$ 0.2ml/min, change in organic phase: $\pm$ 10% and change in pH: $\pm$ 0.2

RUGGEDNESS:

The ruggedness of an analytical method is determined by the analysis of aliquots form homogeneous loss in different laboratories by different analyst using operational and environmental condition that may differ but or still within the specified parameters of the assay and dissolution. The degree of reproducibility of the result is then determined as a function of assay and dissolution variables this reproducibility was compared to precision of assay to obtain a measure of the ruggedness of the analytical method[9].

LIMIT OF DETECTION (LOD):

It is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio[10]. In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level. S/N = 2/1 or 3/1.

LIMIT OF QUANTIFICATION (LOQ):

LOQ is expressed as a concentration at a specified signal to noise ratio[10]. In chromatography, limit of quantification is the injected amount gives a peak with a height; ten times as high as base line noise level. S/N = 10/1.

Ondansetron[11] is a serotonin 5-HT_3 receptor antagonist used as antiemetic to treat nausea and vomiting. It reduces the activity of the vagus nerve, which deactivates the vomiting center in the medulla oblongata, and also blocks serotonin receptors in chemoreceptor trigger zone. It has 60% oral bioavailability, 70-76% Protein binding with 5.7 h half-life. The drug is administered 1-3 times daily, depending on the severity of nausea and/or vomiting[12]. The normal oral dose for adults and children over the age of 12 is 8 mg initially, followed by a second dose of 8 mg eight hours later. The drug is then administered once every 12 h, usually for not more than 2-3 days. Following oral administration, it takes about 1.5 to 2 hours to reach maximum plasma concentrations[13]. This is effective in controlling post-operative nausea and vomiting (PONV), to prevent chemotherapy-induced nausea and vomiting. It is also used to treat cyclic vomiting syndrome. Rabeprazole is an antiulcer drug in the class of proton pump inhibitors with fastest acid suppression activity. It has oral bioavailability of 52% with half-life 1-1.5 h. Rabeprazole is used for healing of duodenal ulcers, treatment of symptomatic GERD, pathological hypersecretory conditions (zollinger-ellison syndrome), and *helicobacter pylori* eradication to reduce risk of duodenal ulcer recurrence[14-15].

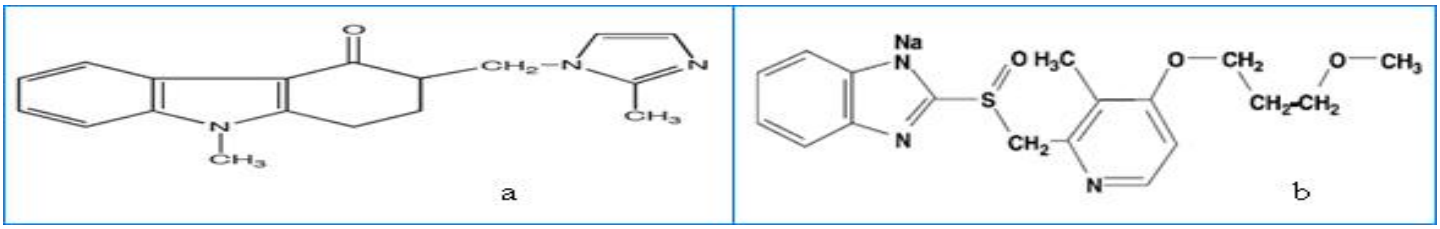

Figure No. 1: Sturcture of a) ondansetron and b) Rabeprazole

In the present study we developed simple HPLC chromatographic method for estimation of ondansetron and rabeprazole in bulk and pharmaceutical formulations. The developed method was also studied for their validation parameters.

METHODOLOGY:

MATERIALS:

Ondansetron obtained as complimentary sample from Alkem labs, Rabeprazole was obtained from Torrent Pharmaceuticals Ltd, Sodium di hydrogen orthophosphatewith AR grade purchased from Merck, Acetonitrile HPLC Grade procured from Rankem, HPLC Grade Methanol from Merck, Orthophosphoric acid and Triethyl amine of AR Grade was purchased from Merck. All other chemicals used were of AR Grade.

CHROMATOGRAPHIC PARAMETERS:

HPLC (Waters) equipped with auto Sampler and DAD or UV detector fitted by Symmetry C18 column (4.6 x 150mm, 5µm, Make: XTerra) operated at 0.6 mL per min flow rate at 274 nm with injection volume 20 µl and 8min runtime.

PREPARATION OF MOBILE PHASE:

Mix 3:6:1 ratio of phosphate buffer pH 5.5(300ml): methanol (600ml): HPLC water (100ml).This mixture was degassing in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration. The mobile phase is also used as diluent.

PREPARATION OF STANDARD SOLUTION:

Accurately weighed 10 mg of ondansetron and rabeprazole working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the diluent. Further pipette out 5ml each from the above stock solution into two 50ml volumetric flask of dilute up to the mark with diluent. Further pipette out 6ml each into separate 10ml volumetric flask and dilute up to the mark with diluent.

SAMPLE SOLUTION PREPARATION:

Accurately weigh and transfer to 266 mg of capsule powder of ondansetron and rabeprazole sample into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette out 0.4ml of from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Procedure: Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for the ondansetron and rabeprazole peaks and calculate the % assay by using the formulae.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg.WT}}{\text{Lable Claim}} \times 100$$

Where, AT - Area of the peak due to ciprofloxacin in sample preparation, AS is Area of the peak due to ciprofloxacin in standard preparation, WS is Weight of working standard (mg), P is Potency of working standard in%, LC is Label claim (mg). Acceptance limit: 90 % to 110% of the labeled amount**.**

METHOD VALIDATION[16]: PREPARATION OF STANDARD STOCK SOLUTION:

Accurately weigh and transfer 10 mg of ondansetron and rabeprazole working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent (standard stock solution-1). Further pipette out 5ml of standard stock solution-1 into a 50ml volumetric flask and dilute up to the mark with diluent (standard stock solution-2). Further pipette out 6ml of the standard stock solution-2 into a 10 volumetric flask and dilute up to the mark with diluent.

PRECISION[16]:

Pipette out 6ml of the standard stock solution-2 into a 10 volumetric flask and dilute up to the mark with diluent. This solution was injected for five times and

measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was recorded. The % RSD for the area of five standard injections should not be more than 2%.

ACCURACY[17]: PREPARATION SAMPLE SOLUTIONS (WITH RESPECT TO TARGET ASSAY CONCENTRATION): PREPARATION OF 50% SOLUTION:

Accurately weigh and transfer 5.18mg of ondansetron and 5.12mg of rabeprazole working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent (sample stock solution-1). Further pipette out 5ml of the stock solution into a 50ml volumetric flask and dilute up to the mark with diluent (sample stock solution-2). Further pipette out 6ml of the stock solution-2 into a 10ml volumetric flask and dilute up to the mark with diluent.

PREPARATION OF 100% SOLUTION:

Accurately weigh and transfer 10.2 mg of ondansetron and 9.8mg of rabeprazole working standards into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent (sample stock solution-1). Further pipette out 5ml of the sample stock solution-1 into a 50ml volumetric flask and dilute up to the mark with diluent (sample stock solution-2). Further pipette out 6ml of the sample stock solution-2 into a 10ml volumetric flask and make up the mark with diluent.

PREPARATION OF 150% SOLUTION:

Accurately weigh and transfer 15.1mg of ondansetron and 15.3mg of rabeprazole working standards into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent (sample stock solution-1). Further pipette out 5ml of the sample stock solution-1 into a 50ml volumetric flask and dilute up to the mark with diluent (sample stock solution-2). Further pipette out 6ml of sample stock solution-2 into a 10ml volumetric flask and make up to the mark with diluent. Inject the standard solution, accuracy -50%, accuracy -100% and accuracy -150% solutions. Calculate the amount found and amount added for ondansetron & rabeprazole and calculate the individual recovery and mean recovery values. The % Recovery for each level should be between 98.0 to 102.0%.

LINEARITY[17]:

Accurately weigh and transfer 10 mg of ondansetron and rabeprazole working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicated to dissolve it completely and make volume up to the mark with the diluent (standard stock solution-1). Further pipette out 5ml of standard stock solution-1 into a 50ml volumetric flask and dilute up to the mark with diluent (standard stock solution-2). Pipette out 2, 4, 6, 8, 10ml of standard stock solution-2 in to a series of 10ml clean dry volumetric flask and dilute up to the mark with diluent to get five levels of solutions 20, 40, 60, 80 and 100ppm respectively. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y axis peak area) and calculate the correlation coefficient. Correlation coefficient should be not less than 0.9

LIMIT OF DETECTION[18]: PREPARATION OF 60µG/ML SOLUTION:

Accurately weigh and transfer 10mg of ondansetron working standard into a 10ml clean dry volumetric flask add about 7ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent (standard stock solution-1). Further pipette out 5ml of the standard stock solution-1 into a 50ml volumetric flask and dilute up to the mark with diluent (standard stock solution-2). Further pipette out 6ml of the standard stock solution-2 into a 10ml volumetric flask and dilute up to the mark with diluent (standard stock solution-3). Preparation of 0.1% solution at specification level (0.06µg/ml solution): Further pipette out 1ml of the standard stock solution-3 into a 10ml volumetric flask and dilute up to the mark with diluent (standard stock solution-4). Pipette out 0.1ml of standard stock solution-4 into a 10 ml of volumetric flask and dilute up to the mark with diluent. Calculation of S/N ratio: Average baseline noise obtained from blank is 44 µV, Signal obtained from LOD solution (0.1% of target assay concentration) is 135 µV. S/N= 135/44 = 3.06. The S/N ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION[18]:

Preparation of 0.35% solution at specification level (0.21µg/ml solution): Further pipette out 1ml of the standard stock solution-3 into a 10ml volumetric flask and dilute up to the mark with diluent (standard stock solution-4). Pipette out 0.35ml of standard stock solution-4 into a 10 ml of volumetric flask and dilute up to the mark with diluent. Calculation of S/N Ratio: Average baseline noise

obtained from blank is 44 µV, Signal obtained from LOQ solution (0.35% of target concentration) at 436µV, The S/N= 436/44 = 9.90. S/N ratio value shall be 10 for LOQ solution.

ROBUSTNESS[19-20]**:**

As part of the robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to evaluate the impact on the method. The flow rate was varied at 0.5 ml/min to 0.7ml/min. Standard solution 60ppm of ondansetron and rabeprazole was prepared and analysed using the varied flow rates along with method flow rate. The organic composition in the mobile phase was varied from 65% to 55%. Standard solution 60 µg/ml of ondansetron and rabeprazole was prepared and analysed using the varied mobile phase composition along with the actual mobile phase composition in the method.

RESULTS:

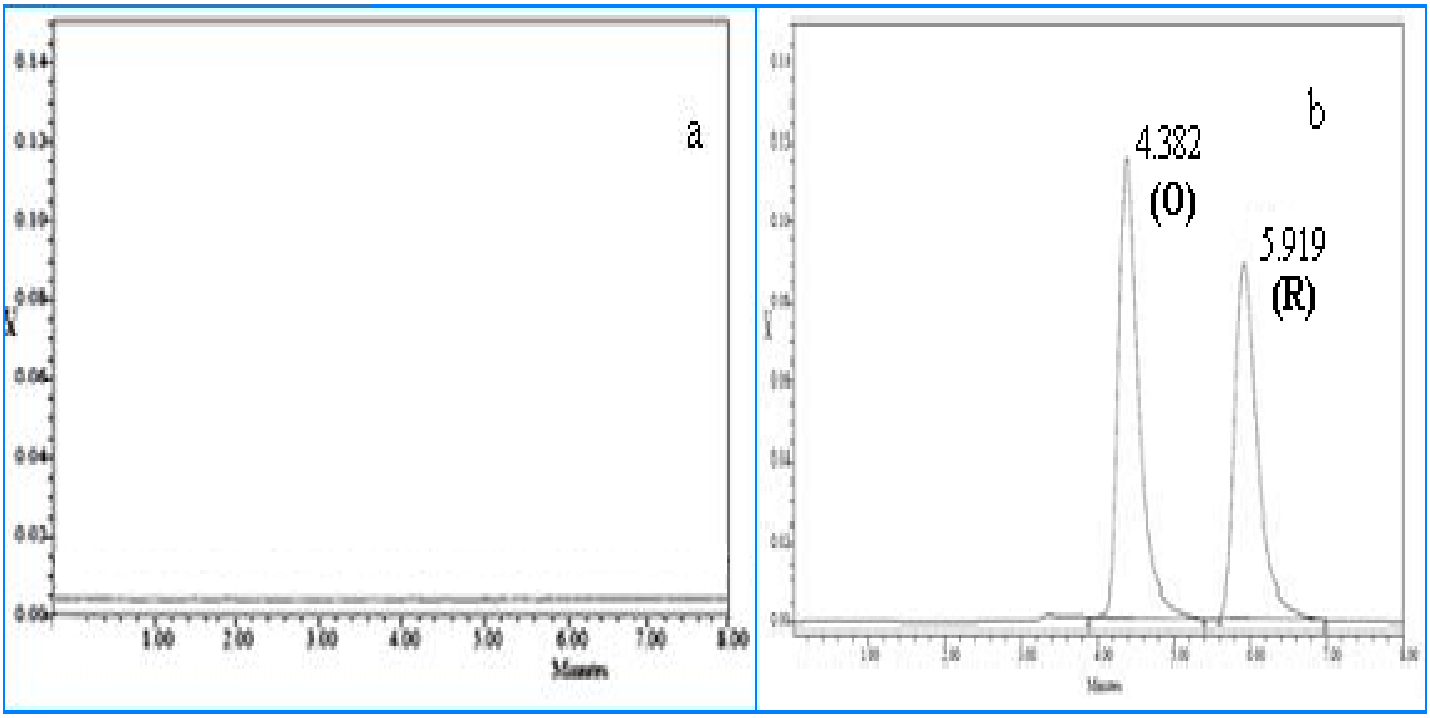

Figure No. 2: a) Blank and b) ondansetron, rabeprazole standard chromatogram

Sr. No.	Drug analyte	Retention time (min)	Area (µV*sec)	USP plate count	USP Tailing
1	Ondansetron	4.382	2152997	2393.5	1.6
2	Rabeprazole	5.919	1869758	2976.1	1.5

Table 1: System suitability parameters of working standard

Ondansetron linearity		Rabeprazole linearity	
concn (ppm)	Area	concn (ppm)	Area
0	0	0	0

20	792037	20	547966
40	1453247	40	1126388
60	2116730	60	1733110
80	2765666	80	2281240
100	3451223	100	2926733
y = 34058x + 60259		y = 29200x - 24109	
(r^2 = 0.9999)		(r^2 = 0.9999)	

Table No. 2: Linearity data of Ondansetron Rabeprazole by HPLC (n=3)

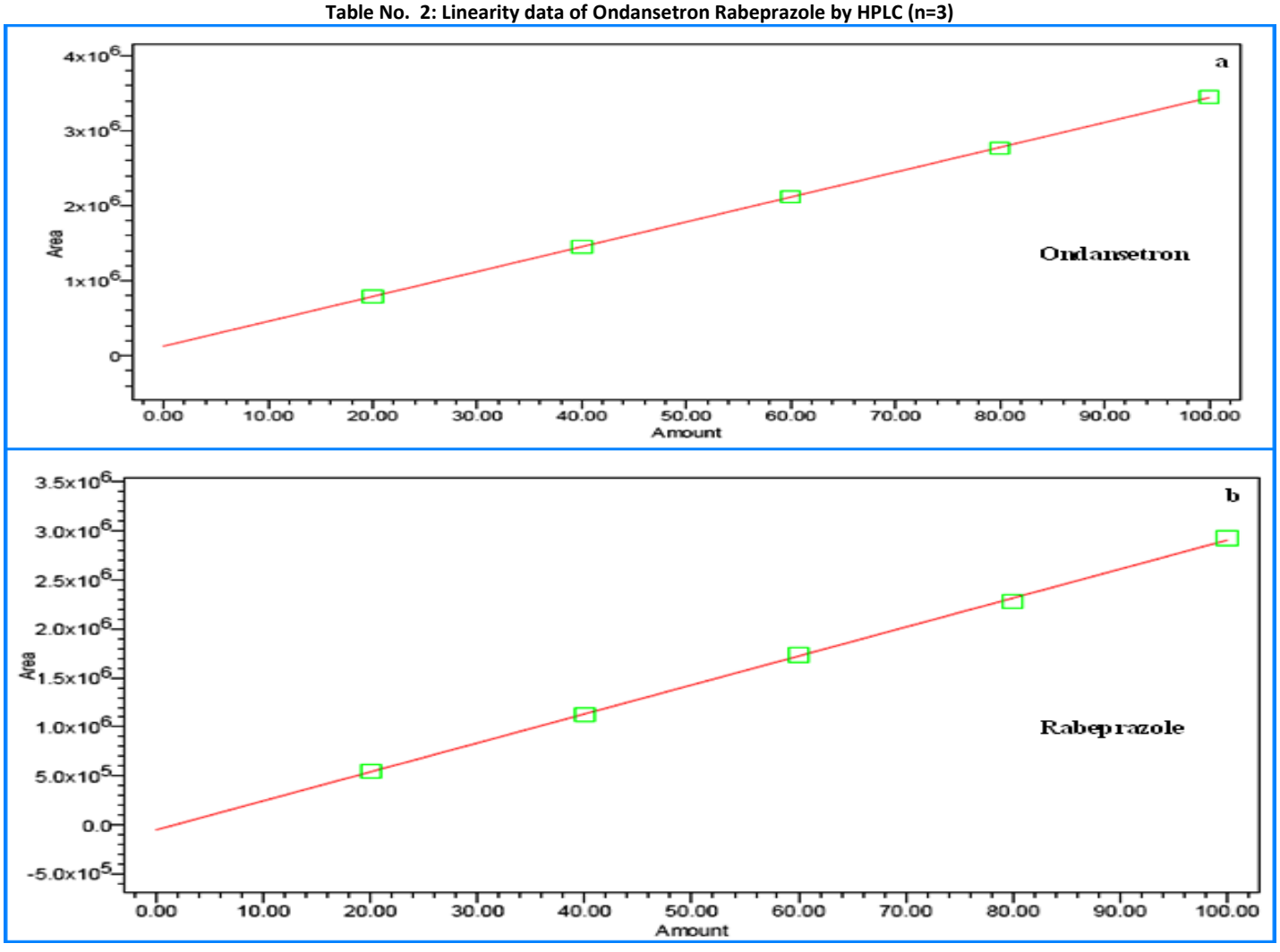

Figure No. 3: Calibration curves of a) Ondansetron and b) Rabeprazole

Inj. No	Peak	RT	Area	Mean	SD	% RSD
1	Ondan	4.375	2156669	2147852	27442.0	0.28

2	setron	4.376	2169051			
3		4.380	2162695			
4		4.381	2100336			
5		4.380	2150510			
1	Rabe prazole	5.805	1957592	1913913	30406.8	0.59
2		5.816	1927813			
3		5.865	1911816			
4		5.889	1880993			
5		5.907	1891349			

Table No. 3: Method precision study of Ondansetron and Rabeprazole by HPLC (n=5)

Inj. No	Peak	RT	Area	Mean	SD	% RSD
1	Ondan setron	4.371	2148720	2176091	26447.5	0.22
2		4.369	2160783			
3		4.367	2162871			
4		4.369	2197825			
5		4.361	2210256			
1	Rabe prazole	5.980	1671464	1662853	11040.7	0.66
2		5.983	1662891			
3		5.973	1675097			
4		5.979	1657165			
5		5.965	1647649			

Table No. 4: Intermediate precision study of Ondansetron and Rabeprazole by HPLC (n=5)

Robustness parameter	Name	Retention time (min)	Area (µv*sec)	plate count	Tailing factor
Less flow rate	Ondansetron	5.224	2630487	2423.4	1.7
	Rabeprazole	7.129	2000564	3115.8	1.6
Less organic composition	Ondansetron	4.744	2219833	2349.6	1.6
	Rabeprazole	7.810	1639154	3186.8	1.4
More flow rate	Ondansetron	3.731	1848995	2339.3	1.6

	Rabeprazole	5.097	1410318	2872.2	1.5
More organic composition	Ondansetron	4.095	1905817	2492.2	1.5
	Rabeprazole	4.901	1508745	2908.5	1.6

Table No. 5: HPLC robustness optimization studies of Ondansetron and Rabeprazole

Drug	**Flow rate (ml/min)**	System suitability results	
		Plate count	**Tailing factor**
Ondansetron	0.5	2423	1.7
	0.6*****	2387	1.6
	0.7	2339	1.6
Rabeprazole	0.5	3115	1.6
	0.6*****	2966	1.5
	0.7	2872	1.4
***** Results of actual flow (0.6ml/min) have been considered from Assay standard			

Drug	**Change in organic composition of mobile phase**	**System suitability results**	
		Plate count	**Tailing factor**
Ondansetron	10% less	2349	1.6
	Actual*	2387	1.6
	10% more	2492	1.5
Rabeprazole	10% less	3186	1.5
	Actual*	2966	1.5
	10% more	2908	1.6
***** Results for Mobile phase (60:30:10 Methanol: Buffer: Water)			

Table No. 6: Robustness studies of Ondansetron and Rabeprazole by HPLC method

Drug	**Concentration level**	**Area**	**Amount added (mg)**	**Amount found (mg)**	**Recovery**	**Mean recovery**
Ondansetron	50%	1083959	5.18	5.26	101.6%	101.2%
	100%	2130002	10.2	10.3	101.4%	
	150%	3138518	15.1	15.2	100.8%	
Rabeprazole	50%	971270	5.12	5.19	101.5%	100.5%
	100%	1807732	9.8	9.67	98.7%	
	150%	2897817	15.3	15.5	101.4%	

Table No. 7: Recovery studies of Ondansetron and Rabeprazole at 50, 100 and 150%

Sr. No	**Parameter**	**Ondansetron**	**Rabeprazole**
1	Retention time (Rt)	4.382	5.919

2	Peak area	2152997	1869758
3	Theoretical plates	2393.5	2976.1
4	Tailing factor	1.6	1.5
5	Absorption maxima (λ max) (nm)	274	274
6	Beer's range (µg/ml)	20-100	20-100
7	Regression equation	y = 34058x + 60259	y = 29200x - 24109
8	Correlation coefficient (r^2)	0.9999	0.9999
9	Limit of detection (µg/ml)	0.06	3.06
10	Limit of quantification(µg/ml)	0.21	9.90
11	Linearity (µg/ml)	10-100	10-100
12	Assay (%)	100.8 – 101.6	98.7 – 101.5
13	Precision (RSD, %), Method (n=5)	0.28	0.59
14	Precision (RSD, %), Intermediate (n=5)	0.22	0.66
15	Robustness(RSD, %), Flow rate (0.5 ml/min)	0.237	0.452
16	Robustness(RSD, %), Flow rate (0.7 ml/min)	0.340	0.369

Table No. 8: Analytical data of Ondansetron and Rabeprazole by HPLC estimation method

DISCUSSION:

The development of an analytical method for the estimation of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. A rapid and sensitive HPLC method for the analysis of Ondansetron and Rabeprazole in bulk and formulation dosage forms was developed and validated. There is no interference peaks to diluent and placebo at the retention time of Ondansetron and Rabeprazole, the results suggest analytical procedure for the assay of Ondansetron and Rabeprazole is specific. The limit of detection for Ondansetron and Rabeprazole is 0.06 µg/ml and 3.36 µg/ml respectively. Where the limit of quantitation of Ondansetron and Rabeprazole is 0.21µg/ml and 9.90µg/ml respectively. The linearity of the developed method showed correlation coefficient 0.9998. The method was found to be linear between 10 – 100 µg/ml for both the analytes. Average recovery of Ondansetron and Rabeprazole were found to be within the acceptable limits. The developed HPLC method was accurate. The % RSD of method precision for Ondansetron and Rabeprazole was found to be 0.28% and 0.59% respectively. The relative standard deviation was found to be within the acceptable limit. The % RSD of robustness was found to be 0.237% and 0.4525 (0.5 ml/min), 0.340% and 0.369% (0.7 ml/min) for Ondansetron and Rabeprazole respectively.

The robustness of an analytical method was found to be within the acceptable limit. The relative standard deviation at each level was less than 2%. It can be concluded that the analytical method is robust towards the above designed changes. The % RSD, Tailing factor, Theoretical plates was found to be reproducible and uniform for both Ondansetron and Rabeprazole. System suitability parameters were found to be within acceptable limits. The HPLC method developed and validated for estimation of Ondansetron and Rabeprazole was sensitive and reproducible with consistency and could be used for routine sample analysis.

CONCLUSION:

A UV Absorption maximum for Ondansetron and Rabeprazole was determined and was found to be 274 nm. Further it is used as a UV detector in HPLC studies. The HPLC method developed and validated for Ondansetron and Rabeprazole in bulk and dosage forms as per ICH guidelines was accurate and precise; hence it can be employed for both drugs estimation studies. The proposed method was found to be specific, accurate, linear, system precise, method precise. The method showed repeatability of results with respect to precision studies, robustness and system suitability conditions. In all cases % RSD was found to be less than 2% and standard deviation is within the limits. From the above studies it could conclude that the

Ondansetron and Rabeprazole drugs were estimated accurately by using the above developed HPLC method in bulk and formulation samples.

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