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Research Article

Method Development and Validation for the Simultaneous Estimation of Ondansetron and Rabeprazole in Bulk and Pharmaceutical Dosage Form by RP-HPLC Method

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ABSTRACT

A rapid, sensitive, and accurate RP-HPLC method was developed for the identification and quantification of Ondansetron and Rabeprazole using a Waters HPLC system equipped with a PDA detector. Chromatographic separation was achieved on an Inertsil ODS-C18 column (250 × 4.6 mm, 5 µm particle size) with a flow rate of 1.2 mL/min. The mobile phase consisted of degassed and filtered Ammonium acetate buffer: water: methanol (25:15:60). Detection was performed at 275 nm, providing reliable resolution and measurement of both analytes.

INTRODUCTION

Ondansetron (6-7), a competitive antagonist of serotonin type 3 receptors, is used to treat nausea and vomiting induced by cytotoxic chemotherapy drugs, including cisplatin. In addition to its antiemetic properties, ondansetron has shown anxiolytic and neuroleptic effects. Ondansetron has evolved with the introduction of orally soluble films. These films offer a more discreet and patient friendly option, especially during episodes of vomiting.

Rabeprazole (8-9) is an antiulcer medication belonging to the proton pump inhibitor class. It acts as a prodrug, converting to its active sulphonamide form in the acidic environment of parietal cells. Rabeprazole works by

inhibiting the H⁺, K⁺ ATPase enzyme in the gastric lining cells, thereby reducing both basal and stimulated gastric acid secretion in a dose dependent manner.

MATERIALS AND METHODS

Preparation of Stock solution: 40 mg each of Ondansetron and Rabeprazole was accurately weighed and is transferred into neat and dry volumetric flasks of 100ml. About 60ml of methanol was added and allow to sonicate to remove the air bubbles formed in it, which is again make up to mark with methanol to give 1000µg/ml or 1mg/ml.

Preparation of working standard solution: From the stock solutions of both standards, each 4 ml was

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pipetted out and transferred in to 50ml volumetric flasks, then diluted up to the mark to get 32µg/ml for both the drugs.

RESULTS AND DISCUSSION

Method validation: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness and assay (1-5).

Specificity: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Ondansetron and Rabeprazole were injected into the HPLC system. The peak purity data of Ondansetron and Rabeprazole were compared. There should not be any interference at the retention time of the main peaks.

Linearity: Linearity for the drugs Ondansetron and Rabeprazole (10-13) was determined by preparing the standard solutions at five concentrations levels in range of 16 to 48µg/ml of Ondansetron and 16 to 48µg/ml of Rabeprazole from stock solution. The linearity charts of Ondansetron and Rabeprazole was shown in the figure no 2&3. The correlation coefficient was found to be 0.9974 and 0.9981 for Ondansetron and Rabeprazole respectively. Linearity results were tabulated in table 2.

Accuracy: Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analyzed for %RSD which should not be more than 2.0. The % recovery was calculated and the results was reported in table no. 3 & 4.

Precision: The precision (14-18) of the analytical method was studied by injecting six replicates of standard containing 32µg/ml of Ondansetron and 32µg/ml of Rabeprazole which were injected into HPLC system.

The % RSD was calculated and the results were reported in the table no.5

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection was defined as the concentration which yields a signal - to - noise ratio 3:1 whereas the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to - noise ratio 10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no 6.

Robustness: The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease in flow rate (1.0 ml/min), increase in flow rate (1.4 ml/min) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no 7.

Assay: Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 8.

Table 1: Optimised Chromatographic Conditions

Parameter	Method
Stationary Phase (column)	Inertsil -ODS C18 (250 x 4.6 mm, 5 µ)
Mobile Phase	Ammonium acetate buffer: water: methanol (25:15:60)
Flow rate (ml/min)	1.2 ml/min
Run time (minutes)	15 min
Temperature in the column (°C)	Ambient
Volume of injection loop (µl)	20
Wavelength of detection (nm)	275nm
Drug RT (min)	4.918 for Ondansetron and 6.561 for Rabeprazole



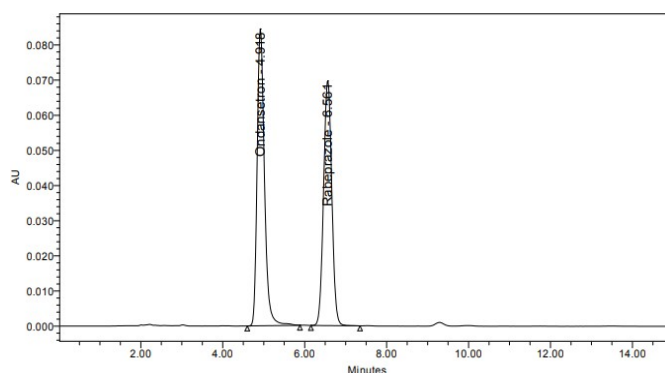


Figure 1: Optimized Chromatogram

Table 2: Linearity Data of Ondansetron and Rabeprazole

Ondansetron		Rabeprazole	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
16	499407	16	450945
24	779751	24	710474
32	1025303	32	965603
40	1195365	40	1133888
48	1487048	48	1396831

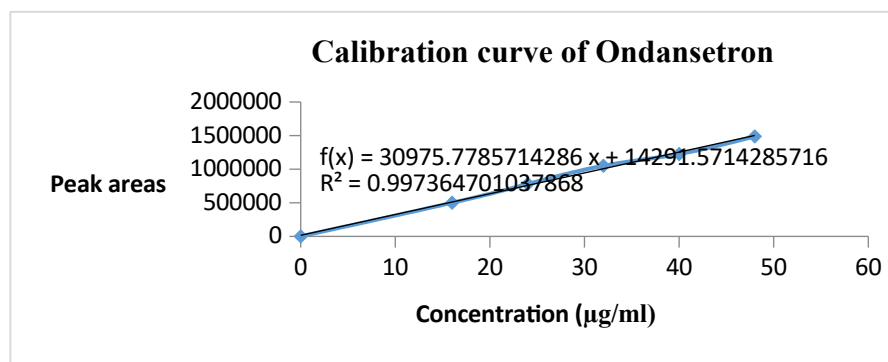


Figure 2: Calibration Curve of Ondansetron

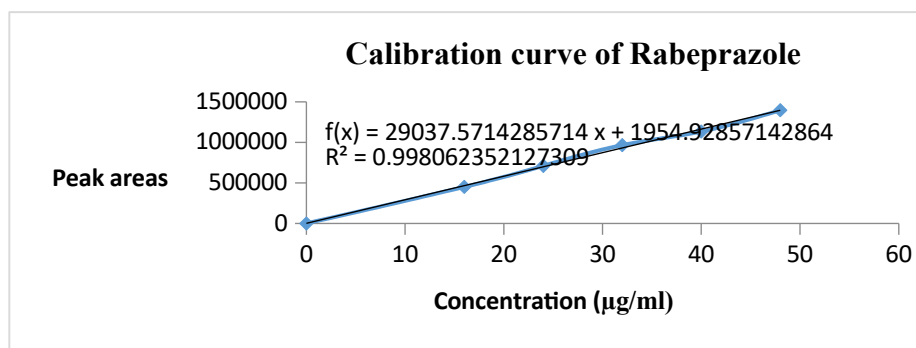


Figure 3: Calibration Curve of Rabeprazole

Table 3: Accuracy Data of Ondansetron

Concentration % of spiked level	Area	RT	Statistical Analysis of % Recovery	
50% - 1	498173	4.954	MEAN	98.84
50% - 2	498919	4.957	%RSD	0.48
50% - 3	485240	4.96		
100 % - 1	1058995	4.956	MEAN	99.81
100 % - 2	1075045	4.958	%RSD	0.5
100% - 3	1072698	4.961		
150% - 1	1486752	4.964	MEAN	98.87
150% - 2	1484370	4.965	%RSD	0.2
150% - 3	1480898	4.968		

Table 4: Accuracy Data of Rabeprazole

Concentration % of spiked level	Area	RT	Statistical Analysis of % Recovery	
50% - 1	454998	6.623	MEAN	98.81
50% - 2	454813	6.629	%RSD	0.32
50% - 3	452545	6.634		
100 % - 1	986499	6.637	MEAN	99.47
100 % - 2	978944	6.642	%RSD	0.31
100% - 3	977737	6.646		
150% - 1	1416722	6.656	MEAN	98.51
150% - 2	1413967	6.658	%RSD	0.15
150% - 3	1409179	6.663		

Table 5: Precision Data for Ondansetron and Rabeprazole

S. No	Peak areas of Ondansetron	Peak areas of Rabeprazole
1	1058995	986499
2	1075045	978944
3	1072698	977737
4	1068268	973847
5	1071754	976937
6	1046255	972390
Mean	99.8	99.2
SD	0.42	0.35
% RSD	0.4	0.4

Table 6: LOD and LOQ Data of Ondansetron and Rabeprazole

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Ondansetron	2.628	7.963
Rabeprazole	2.252	6.826

Table 7: Robustness Data of Ondansetron and Rabeprazole

S No	Drug Name	Condition	Peak area	% RSD	
1	Ondansetron	Decreased Flow rate of 1.0 ml/min	1058342	1.234	



S No	Drug Name	Condition	Peak area	% RSD	
2		Increased Flow rate of 1.4 ml/min	1045647	0.624	
3	Rabeprazole	Decreased Flow rate of 1.0 ml/min	979365.9	0.785	
4		Increased Flow rate of 1.4 ml/min	970738.8	0.942	

Table 8: Assay Data of Ondansetron and Rabeprazole

S. No	Peak area of Ondansetron	% Assay	Peak area of Rabeprazole	% Assay
Sample- 1	1030610	98.49	969447	99.37
	1058342		979366	
Sample- 2	1049227		965439	
	1058342		979366	

CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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