ISSN: 2457-0400

Impact Factor: 7.675

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

www.wjahr.com

Volume: 10, Issue: 4

Page N. 50-57

Year: 2026

Original Article

Coden USA: WJAMA3

METHOD DEVEOPMENT AND METHOD VALIDATION FOR SIMULTANEOUS EVALUATION OF ONDANSETRON AND RABEPRAZOLE SOLID DOSAGE FORM USING RP-HPLC TECHNIQUE

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Article Received: 17 February 2026 **Article Revised:** 07 March 2026 **Article Published:** 01 April 2026

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DOI: https://doi.org/10.5281/zenodo.19329633

How to cite this Article: J. K. V. Sastry*[1], Dr. K. Atchuta Kumar[2], Mr. K. Charan Sai[3], Mrs. P. Prathyusha[4], Miss. N. Poojitha[5] (2026). Method Deveopment And Method Validation For Simultaneous Evaluation Of Ondansetron And Rabeprazole Solid Dosage Form Using Rp-Hplc Technique. World Journal of Advance Healthcare Research, 10(4), 50–57.

ABSTRACT

Validation is an important concept in several industries, particularly those related to manufacturing, pharmaceuticals, and quality assurance. It entails a systematic approach to ensure that processes, systems, procedures, and equipment consistently execute their intended functions and provide dependable outcomes. Validation is critical for ensuring quality, adhering to regulatory standards, and reducing production and operational risks. The developed method was novel and simple for the simultaneous estimation of Ondansetron & Rabeprazole by RP-HPLC. The two peaks were well resolved at 275nm in isocratic mode at retention times 4.918 and 6.561 min for Ondansetron and Rabeprazole respectively at a run time of 15 min and flow rate 1.2ml/min with 250mm x 4.6mm, 5µm column & Ammonium acetate buffer: water: methanol (25:15:60) as mobile phase. % Assay values for Ondansetron and Rabeprazole were found to be 98.49% & 99.37% respectively. Linearity was obtained in the range of 16-48 ppm and linearity correlation coefficient was found to be 0.9995 & 0.9997 for Ondansetron and Rabeprazole respectively. This new approach was verified using ICH guidelines and found to be specific, sensitive, precise, accurate, and linear.

KEYWORDS: Method development and validation, ICH guidelines, Ondansetron, Rabeprazole, and RP-HPLC.

1. INTRODUCTION

1.1 DRUG PROFILE OF ONDANSETRON

Ondansetron, a competitive antagonist of serotonin type III receptors, is used to alleviate nausea and vomiting induced by cytotoxic chemotherapy drugs, including cisplatin. In addition to its antiemetic properties, Ondansetron has shown anxiolytic and neuroleptic effects. Developed by GlaxoSmithKline in the 1980s, it received approval from the US FDA in January 1991 and has since maintained a strong track record of effectiveness. Available in various forms such as oral tablets, orally disintegrating tablets (ODT), injections, and generics, 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. The FDA has revoked approval for intravenous Ondansetron hydrochloride products containing more than 16 mg per dose due to the risk of QT interval prolongation. IUPAC name of the drug is 9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-2,3-dihydro-1H-carbazol-4-one with the chemical formula $C_{18}H_{21}N_3O_3S$.[11-13] The molecular mass is 293.370 g/mol. The half-life of ondansetron is around 34 hours following an 8 mg oral or intravenous dose, and this duration can extend to 68 hours in elderly individuals.[1-10]

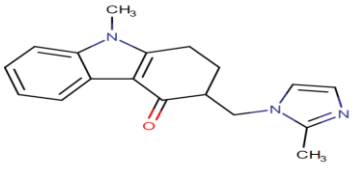


Figure 1: Chemical Structure of Ondansetron.

1.2 DRUG PROFILE OF RABEPRAZOLE

Rabeprazole is an antiulcer medication belonging to the proton pump inhibitor class. It acts as a prodrug, converting to its active sulphenamide form in the acidic environment of parietal cells. Rabeprazole acts by blocking the H+, K+ ATPase enzyme in gastric lining cells, which reduces basal and induced gastric acid output in a dose-dependent manner. Rabeprazole is one of the antisecretory drugs known as substituted benzimidazole protonpump inhibitors. Unlike anticholinergics and histamine H2receptor antagonists, rabeprazole lowers stomach acid production by blocking the H+/K+ ATPase (hydrogen potassium adenosine triphosphatase) enzyme on the secretory membrane of gastric parietal cells.

This enzyme is required for acid (proton) pumping in these cells, hence rabeprazole acts as a gastric proton pump inhibitor. It efficiently inhibits the final stage in stomach acid generation. Rabeprazole is protonated in parietal cells, where it accumulates and becomes an active sulfonamide. In vitro studies demonstrate that rabeprazole becomes chemically active at pH 1.2 and has a half-life of 78 seconds. The IUPAC designation for Rabeprazole is 2-{[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl}-1H-benzimidazole. Its molecular weight is 359.40 g/mol and molecular formula $C_{18}H_{21}N_3O_3S$.[11-15]

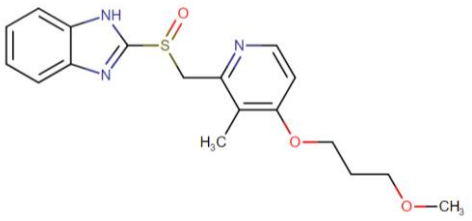


Figure 2: Chemical Structure of Rabeprazole.

After an exhaustive literature study.[14-20] the authors concluded that a very few methods for simultaneous quantification of Ondansetron and Rabeprazole have been assessed using spectroscopy and liquid chromatography. Therefore, there is a need to design an innovative, fast, exact, sensitive, and selective approach for simultaneous estimation of Ondansetron and Rabeprazole by RP-HPLC in its tablet dosage forms.

2. MATERIALS AND METHODS

2.1 APPARATUS & CHEMICALS

Table 1: List of apparatus.

S.no	Name	Model	Manufacturer
1	HPLC	Waters 2690	ALLIANCE
2	pH meter	Model 152	RI
3	Weighing Balance	SAB 203 L	Scale tech
4	Pipettes, Beakers and Burettes	NA	Borosil Class-A
5	Ultra Sonicator	PSA-10A	DIGITAL PRO

Table 2: List of chemicals.

S.no	Name	Grade	Batch No
1	Water (Milli Q / HPLC Grade water)	HPLC	P24E100596
2	Ammonium acetate	HPLC	J058A24
3	Methanol	HPLC	R276G24

2.2 PREPARATION OF SOLUTIONS

Mobile phase: Ammonium acetate buffer, water and methanol was mixed and sonicated well and prepared in the ratio of 25:15:60.

Buffer preparation: Ammonium acetate buffer: 2.5 grams of ammonium acetate was properly weighed and put into a 500 ml volumetric flask. Water was added to fill the flask and filtered through a 0.45 µm membrane filter.

Diluent preparation: Methanol was used as a diluent throughout the study.

Standard preparation: 40.12 mg of Ondansetron and 40.15 mg of Rabeprazole were precisely weighed and transferred to two separate 100 ml volumetric flasks. 60 mL of diluent was added and sonicated for 5 minutes. The volume was increased to the mark using diluent. Then, 4ml of each solution was put into a 50ml volumetric flask, and the volume was adjusted to the mark using the same diluent.

Sample preparation: Equivalent powder from 20 tablets was accurately taken each from EMESET-4 containing Ondansetron and RABEVA-20 containing Rabeprazole respectively and transferred to two separate 100 ml volumetric flasks. 60 mL of diluent was added and sonicated for 5 minutes. The volume was made up to the mark using diluent. Then, 4ml of each solution was placed into a 50ml volumetric flask, and the final volume was produced to the mark using the same diluent.

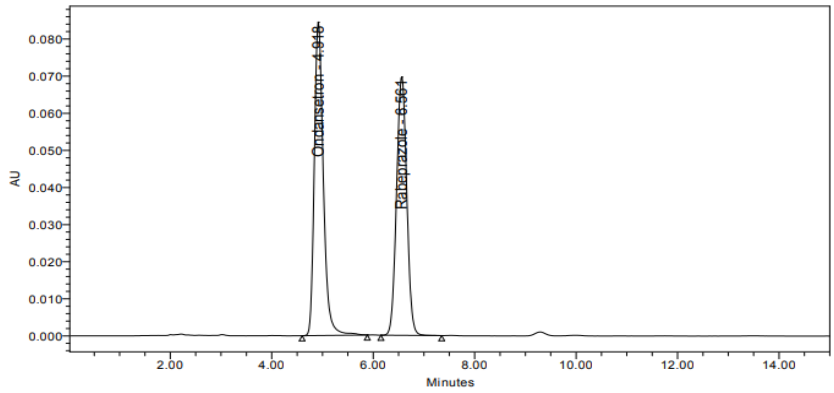
Optimized chromatographic conditions: After performing various trials in isocratic mode, the optimized chromatogram was obtained at 275nm with 1.2ml/min flow rate using Ammonium acetate buffer: water: methanol (25:15:60). The Sample temperature

was maintained at 20°± 5°C. Peaks were well resolved using Welchrom 250mm x 4.6mm, 5µm column at an ambient temperature for 15 min run time.

3. RESULTS AND DISCUSSION

3.1 SYSTEM SUITABILITY

This refers to a set of tests performed to confirm that the chromatographic system (including equipment, electronics, analytical processes, and samples) is operational prior to beginning an analysis. These tests are necessary to establish that the system's performance is appropriate for the intended application and to assure the dependability of analytical results in compliance with the ICH requirements.[21-24]



	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing	USP Resolution	K Prime
1	Ondansetron	4.918	1042636	51.79	84512	3821	1.3		4
2	Rabeprazole	6.561	970386	48.21	69801	4984	1.0	5	6

Figure 3: System suitability for standard chromatogram.

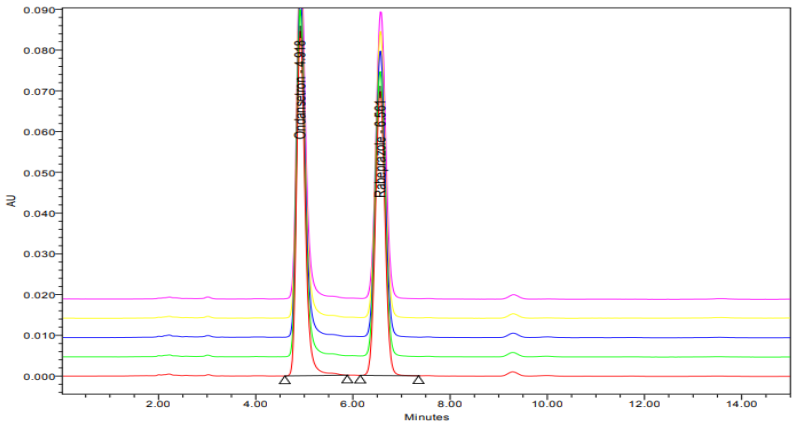


Figure 4: Overlay of System suitability for standard chromatograms.

Table 3: System suitability results.

		Ondansetron		Rabeprazole	
		Retention Time	Area	Retention Time	Area
1	Mean*	4.919	1058342	6.565	979365.9
2	Std. Dev	0.003	13098	0.005	7687.3
3	% RSD	0.05	1.2	0.08	0.8

***** Average of five replicate injections

Discussion: The developed method successfully passed the system suitability criteria, as evidenced by a theoretical plate value exceeding 2000, tailing factor not exceeding 2.0, and % RSD remaining below 2.0%.

3.2 SPECIFICITY

A high level of specificity refers to the chromatographic process can correctly and precisely extracts and identifies the target chemical while avoiding interference from other compounds in the sample.

Blank

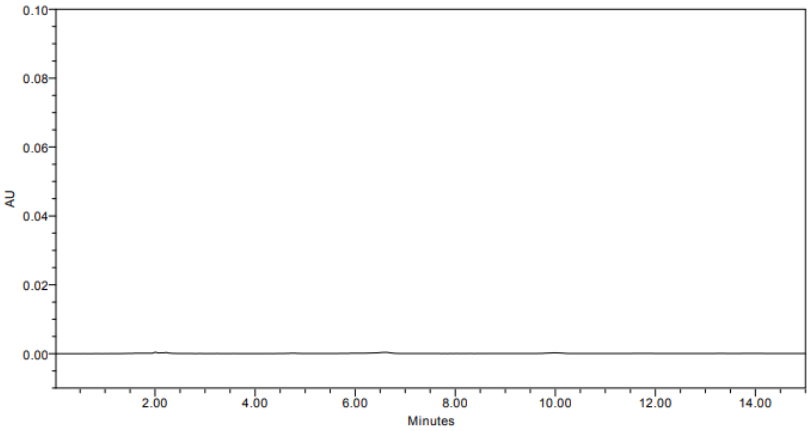


Figure 5: Blank chromatogram.

Discussion: The specificity chromatogram for the blank showed no interference with the primary peak, indicating that the technique is specific.

3.3 ACCURACY

The accuracy of an analytical technique relates to how closely the test results reflect the true value. To test accuracy, sample solutions with concentrations of 50%, 100%, and 150% of the target analyte are injected into the system, and the percentage recovery is computed using the predicted values.

Table 4: Results for Accuracy.

S.No	Sample solution concentration*	Ondansetron		Rabeprazole	
		% RSD	Recovery %	% RSD	Recovery %
1	50% sample solution	1.6	98.87%	0.3	98.51%
2	100% sample solution	0.8		0.5	
3	150% sample solution	0.2		0.3	

***** Average of three replicate injections

Discussion: The RSD percentage does not exceed 2.0%. The approach is considered accurate because the percentage recovery acceptability criterion for Ondansetron and Rabeprazole ranges between 98.0% and 102.0%.

3.4 PRECISION

Consistency and reproducibility relates to getting the same findings while examining the same sample several times under the same conditions. Precision quantifies the variability or dispersion in these data and is commonly stated in statistical terms such as Coefficient of variation (CV), Standard deviation (SD) and Relative standard deviation (RSD).

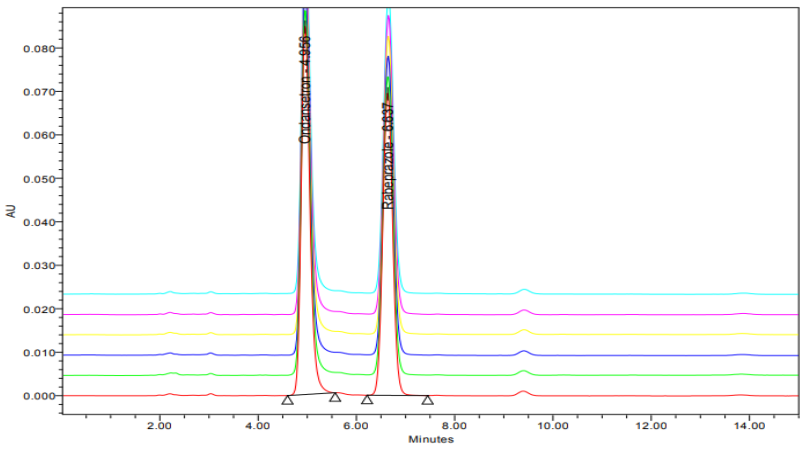


Figure 6: Precision chromatograms overlay of Ondansetron and Rabeprazole.

Table 5: Method precision results for Ondansetron and Rabeprazole.

S. No	Peak Name*	Average	SD	% RSD
1	Ondansetron	1065502	0.42	0.4
2	Rabeprazole	977725	0.35	0.4

***** Average of six replicate injections

Discussion: The RSD percentage does not exceed 2.0%. The approach is regarded as precise since the precision values are within the acceptable range for Ondansetron and Rabeprazole.

3.5 LINEARITY

Linearity in chromatography is the relationship between the concentration or quantity of analyte given and the detector response. It describes how closely the detector response corresponds with variations in analyte concentration over a certain range. A linear chromatogram shows that as the analyte concentration increases or decreases, so does the detector response (peak area or height). This property is required for the exact and reliable measurement of compounds in samples using chromatographic techniques. Ondansetron and Rabeprazole were made in five concentrations, and each concentration was injected three times to test linearity. A graph was plotted taking concentration of analyte on x-axis and peak area on y-axis.

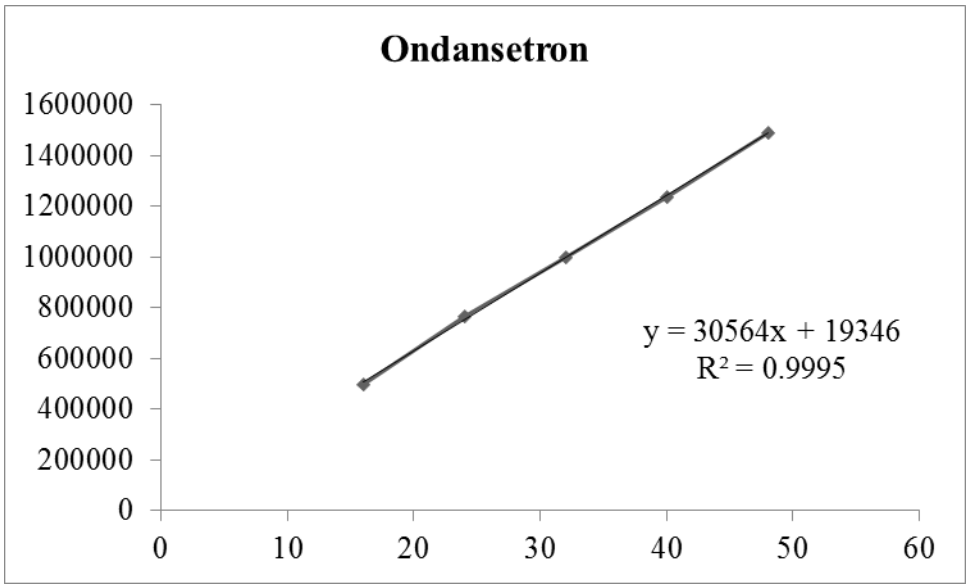


Figure 7: Linearity graph for Ondansetron.

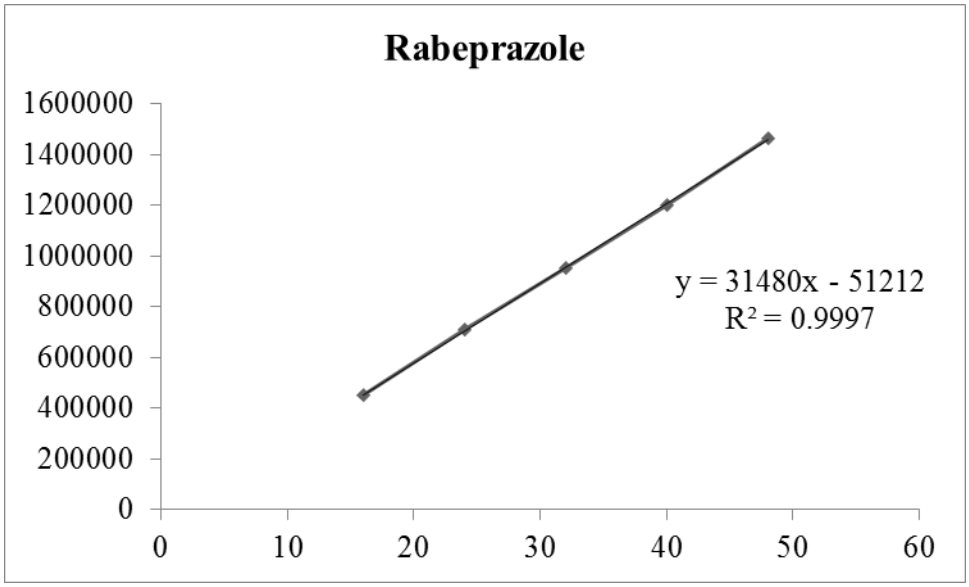


Figure 8: Linearity graph for Rabeprazole.

Table 6: Results for linearity.

	Ondansetron	Rabeprazole
Conc. in PPM*	**Peak Area**	**Peak Area**
16	499407	450945
24	765845	710474
32	998987	954545
40	1235682	1198786
48	1487048	1465989
Regression Equation	y = 30564x + 19346	y = 31480x – 51212
Linearity Correlation Coefficient (R^2)	0.9995	0.9997

***** Average of three replicate injections

Discussion: The R^2 values are within the acceptability standards, i.e. NLT 0.99 for ondansetron and Rabeprazole, indicating that the method is linear.

3.6 RANGE

The range is the interval between the highest and lowest analyte concentrations in the sample across which the technique has been demonstrated to be exact, accurate, and linear.

Table 7: Range values for Ondansetron & Rabeprazole.

Percentage of solution	% RSD for Ondansetron	% RSD for Rabeprazole
50%	1.84%	0.37%
100%	0.03%	0.19%
150%	0.13%	0.20%

Bracketing Standard

Bracketing is an analytical approach in which samples are evaluated at the higher and lower limits of a specified range to ensure accuracy and precision over the whole range.

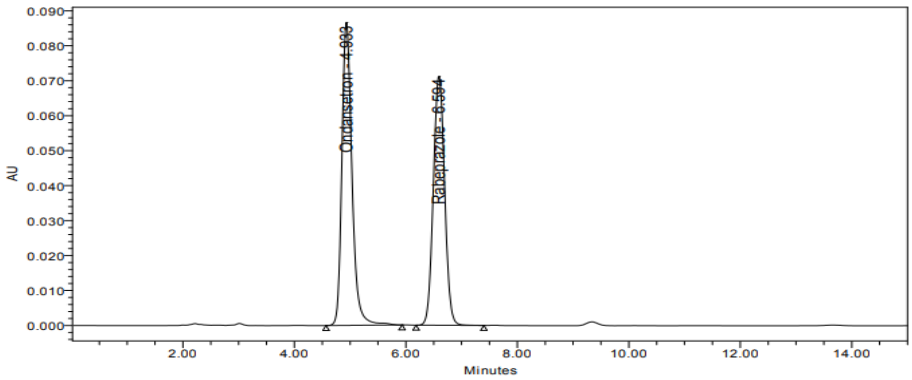


Figure 9: Sample solution Bracketing Standard chromatograms.

METHOD APPLICATION TO THE ANALYSIS OF ONDANSETRON AND RABEPRAZOLE

The proposed and verified technique was used to simultaneously determine Ondansetron and Rabeprazole in commercially available tablet dosage forms EMESET-4 and RABEVA-20. The assay findings are reported in the table below. It was discovered that no dosage form excipients interfered with their analysis, indicating that the approach is suitable for routine quality control work.

Table 8: %Assay of Ondansetron and Rabeprazole.

Ondansetron EMESET-4		Rabeprazole RABEVA-20	
Labeled Claim (mg)	**% Assay***	**Labeled Claim (mg)**	**% Assay***
4	98.49	20	99.37

***** Average of six replicate injections

SUMMARY AND CONCLUSION

The study successfully established and validated a particular, new, and accurate RP-HPLC technique for simultaneously estimating Ondansetron and Rabeprazole in Tablet Dosage Forms.

\Parameters	Ondansetron	Rabeprazole
% Recovery in Accuracy	98.87%	98.51%
% RSD in Precision	0.40%	0.40%
Linearity Correlation coefficient	0.9995	0.9997
% Assay	98.49%	99.37%

The RP-HPLC method employed provided satisfactory resolution between Ondansetron and Rabeprazole within a reasonable runtime, demonstrating the method's efficiency in routine pharmaceutical analysis. Both drugs exhibited good linearity over the tested range with a high correlation coefficient ($R^2 > 0.99$ for both drugs), and the precision expressed as relative standard deviation (RSD) was well within the acceptable limits of less than 2%.

The validation parameters such as system suitability, specificity, accuracy, linearity, and range were systematically assessed and found to comply with the stringent quality requirements of pharmaceutical analytical practices. This validated method can be implemented in pharmaceutical laboratories for the routine analysis of these compounds, facilitating quality assurance and control. This method provides a foundation for further analytical studies and can be adapted or expanded for other similar compounds in multi-component pharmaceutical formulations.

ACKNOWLEDGMENT

All authors are thankful to Principal and management of Srinivasararao College of Pharmacy for providing facilities for this work.

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