Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

https://doi.org/10.36673/AJPCR.2021.v09.i02.A06

A PRECISE RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ONDANSETRON AND RANITIDINE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

New simple isocratic analytical method has been developed and validated for estimation of Ranitidine and Ondansetron simultaneously by RP-HPLC in bulk and pharmaceutical combined dosage form. The mobile phase was pumped at a flow rate of 1.0ml/min and UV detection was found at 225nm. Here two drugs available in Ranitidine (Rt = 2.514 min) and Ondansetron (Rt = 6.354 min) having a good resolution. The developed method was validated as per ICH guidelines. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness.

KEYWORDS

Ranitidine, Ondansetron, RP-HPLC and Validation.

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INTRODUCTION

Pharmaceutical analysis[1] plays a vital role in the pharmaceutical product development. Pharmaceutical analysis is a specialized branch of analytical chemistry. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be undertaken.

Instrumental methods of analysis

Instrumental methods are exciting and fascinating part of chemical analysis that interacts with many other areas of pure and applied sciences. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and environment. This instrumentation provides lower detection limits required to assure safe foods, drugs, water and air. Instrumental methods are widely used by Analytical chemists to save time, to avoid chemical separation and to obtain increased accuracy.

CHROMATOGRAPHIC METHODOLOGY

Method Development

Proper selection of HPLC method development depends upon the nature of the sample, its molecular weight and solubility. For successful method development various Chromatographic parameters such as pH, mobile phase, its composition and proportion, detection wavelength and other factors were exhaustively studied.

Selection of Chromatographic method

The nature of the sample, molecular weight, and solubility. The drug selected for the present study was Non polar.

Non polar compounds can be separated by either normal phase or reverse phase chromatography .Reverse phase chromatography was selected for initial seperations from the knowledge of properties of the compounds**.**

Selection of Diluent

The nature of the drug reveals certain information about the drug such as solubility, pKa. The solvent in which the drug has maximum solubility is selected as the diluents. Ranitidine and Ondansetron are soluble in Methanol. So methanol is used as Diluent.

Selection of detection Wavelength

Standard solutions of Ranitidine and Ondansetron were injected separately as well as in combination into HPLC system then scanned over entire the UV range (190-400nm). The spectra of Ranitidine and Ondansetron was recorded for determination of λmax. The λmax Of Ranitidine was detected at 300nm and the λmax of Ondansetron was detected at 300nm.Detection was carried out at 300nm.

Selection of column

In Reverse phase chromatography non polar stationary phase is used for separation. C8, C18 are the commonly used columns in reverse phase chromatography. Here C18 column of dimensions 150 × 4.6mm and particle size 5µm is used for the separation.

Selection of Mobile phase

A Number of trails were made to find out the mobile phase for eluting the drug. The mobile phase containing Methanol: Water (50: 50), (55: 45) and (45: 55) which contains HPLC grade Methanol+ 0.1% Ortho phosphoric acid + HPLC grade Water in different trails.

Better peak resolution and adequate retention time were obtained with the ratio of Methanol + 0.1% Ortho phosphoric acid + Water (HPLC grade) (50:50).

Mode of separation

Several trails were conducted by changing flow rate, injection volume and other parameters, till satisfactory separation was achieved, the resulting chromatograms were recorded and the chromatographic parameters such as column efficiency, theoretical plates were calculated.

Preparation of Standard Stock Solution

Accurately weigh and transfer 124mg of Ranitidine tablet powder in a 10ml clean dry volumetric flask containing methanol and 10mg of Ondansetron tablet powder ina10ml clean dry volumetric flask containing methanol. The solution were sonicated for about 15mins and then made upto volume with methanol.

Preparation of Working Standard Solution

0.325ml of Ranitidine solution was taken from the prepared standard stock solution in a 4ml volumetric flask and made upto volume with mobile phase.

4mg of Ondansetron tablet powder was taken in a 4ml volumetric flask and made upto volume with mobile phase.

Trail and error method

The following trails were conducted

Trail-1
Mobile phase: Methanol: 0.1% Orthophosphoric acid buffer. (50: 50)
Column : WaterC_{18} 150mm x 4.6mm, 5µm
Detection : 270nm
Flow rate : 0.7ml/min
Injection volume : 20µl/min
Column temperature: Room temperature.

Trail-2
Mobile phase: Methanol: 0.1% Orthophosphoric acid buffer (45: 55)
Column : WaterC_{18} 150mm x 4.6mm, 5µm
Detection : 280nm
Flow rate : 1ml/min
Injection volume : 20µl/min
Column temperature: Room temperature.

Trail-3
Mobile phase: Methanol: 0.1% Orthophosphoric acid buffer (45: 55)
Column : Water C_{18} 150mm x 4.6mm, 5µm
Detection : 290nm
Flow rate : 1ml/min
Injection volume : 20µl/min
Column temperature: Room temperature.

Trail-4
Mobile phase: Methanol: 0.1% Ortho phosphoric acid buffer (45: 55)
Column : WaterC_{18} 150mm x 4.6 mm, 5µm
Detection : 300nm
Flow rate : 1ml/min
Injection volume : 20µl/min
Column temperature: Room temperature.

Trail-5
Mobile phase: Methanol: 0.1% Ortho phosphoric acid buffer (50:50)
Column : Waters C_{18} 150mm x 4.6 mm, 5µm
Detection : 300nm
Flow rate : 1ml/min
Injection volume : 10µl/min
Column temperature: Room temperature.

Trail-6
Mobile phase: Methanol: 0.1% Ortho phosphoric acid buffer (50:50)
Column : Waters C_{18} 150mm x 4.6 mm, 5µm
Detection : 300nm
Flow rate : 0.8 ml/min
Injection volume : 10ul/min
Column temperature: Room temperature.

Trail-7
Mobile phase: Methanol: 0.1% Ortho phosphoric acid buffer (50:50))
Column : Waters C_{18} 150mm x 4.6 mm, 5µm
Detection : 300nm
Flow rate : 0.9ml/min
Injection volume: 10µl/min
Column temperature: Room temperature.

Trail-8
Mobile phase: Methanol: 0.1% Ortho phosphoric acid buffer (50:50)
Column : Waters C_{18} 150mm x 4.6 mm, 5µm
Detection : 300nm
Flow rate : 1.0ml/min
Injection volume: 10µl/min
Column temperature: Room temperature.

Trail-9
Mobile phase: Methanol: 0.1% Ortho phosphoric acid buffer (50:50)
Column : Waters C_{18} 150mm x 4.6mm, 5µm
Detection : 300nm
Flow rate : 0.9ml/min
Injection volume: 10µl/min
Column temperature: Room temperature.

Optimized Methods

Preparation of Buffers

0.1% Ortho phosphoric acid by dissolving Ortho phosphoric acid in 1000ml of water.

Preparation of Mobile Phase

Mobile Phase A

Filtered and degassed Ortho phosphoric acid buffer is used as mobile phase-A.

Mobile Phase B

HPLC Grade Methanol is used as MP-B.

Optimized Chromatographic Conditions

Instrument: HPLC Schimadzu Seperation Module LC- 10AT Liquid chromatograph.
Column: Waters C18 Column 150×4.6mm. 5µm
Column Temperature : Ambient
Flow rate : 0.9ml/min
Wave length : 300nm

Run time : 6mins
Injection volume : 10ul/min
Mobile phase : Methanol: 0.1% Ortho phosphoric acid buffer (50:50).

Analytical Method Validation

The following parameters were considered for validating the developed method as per ICH guidelines.

System Suitability Parameters

Mixed working standard solutions were injected and chromatograms were recorded.

The system suitability studies were carried out as per ICH guidelines.

These parameters include Column efficiency, Resolution, Capacity factor, Theoretical plates and Tailing factor.

Acceptance criteria

The % RSD for the retention times of principal peak from 6 replicate injections of each standard solution should be not more than 2.0%.

The % RSD for the peak area responses of principal peak from 6 replicate injections of each standard solution should be not more than 2.0%.

The number of theoretical plates (N) for the drug peak is not less than 2500.

The Tailing factor (T) for the drug peak is not more than 2.0.

Linearity

Preparation of standard stock solution

Accurately weigh and transfer 124mg of Ranitidine working standard into a 10ml clean dry volumetric flask containing mobile phase and 10mg of Ondansetron working standard into a 10ml clean dry volumetric flask containing mobile phase. The solutions were sonicated for about 10mins and then made upto volume with mobile phase.

Preparation of Working Standard Solution

0.325ml of Ranitidine solution was taken from the prepared standard stock solution in a 4ml volumetric flask and made upto volume with mobile phase.

4mg of Ondansetron working standard solution was taken in a 4ml volumetric flask and made upto volume with mobile phase.

Preparation of 10.02µg/ml of Ranitidine and 9.97µg/ml of Ondansetron

0.02ml of Ranitidine intermediate dilution and 0.02ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Preparation of 20.05µg/ml of Ranitidine and 19.94µg/ml of Ondansetron

0.04ml of Ranitidine intermediate dilution and 0.04ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Preparation of 40.10µg/ml of Ranitidine and 39.88µg/ml of Ondansetron

0.08ml of Ranitidine intermediate dilution and 0.08ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Preparation of 60.15µg/ml of Ranitidine and 59.82µg/ml of Ondansetron

0.12ml of Ranitidine intermediate dilution and 0.12ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Preparation of 80.20µg/ml of Ranitidine and 79.76µg/ml of Ondansetron

0.16ml of Ranitidine intermediate dilution and 0.16ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Preparation of 100.25µg/ml of Ranitidine and 99.70µg/ml of Ondansetron

0.2ml of Ranitidine intermediate dilution and 0.2ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Acceptance criteria

r^2 value should not be less than 0.98

Accuracy

The closeness of agreement between the true value which is accepted either conventional new value or an accepted reference value and the value found.

Preparation of standard stock solution

Accurately weigh and transfer 124mg of Ranitidine working standard into a 10ml clean dry volumetric flask containing mobile phase and 10mg of

Ondansetron working standard into a 10ml clean dry volumetric flask containing mobile phase.

Preparation of working standard solution

0.325ml of Ranitidine solution was taken from the prepared standard stock solution in a 4ml volumetric flask and made upto volume with mobile phase.

4mg of Ondansetron working standard into a 4ml volumetric flask and made upto volume with mobile phase.

Preparation of 25.06µg/ml of Ranitidine and 24.93µg/ml of Ondansetron

0.05ml of Ranitidine intermediate dilution and 0.05ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Preparation of 50.12µg/ml of Ranitidine and 49.85µg/ml of Ondansetron

0.1ml of Ranitidine intermediate dilution and 0.1ml of Onsetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Preparation of 75.18µg/ml of Ranitidine and 74.78µg/ml of Ondansetron

0.15ml of Ranitidine intermediate dilution and 0.15ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Procedure

Inject the standard solution, accuracy 50%, accuracy 100%, accuracy150% solutions. Calculate the amount found and amount added for Ranitidine and Ondansetron and calculate. The individual recovery and mean recovery values.

Acceptance criteria

The % Recovery for each level should be between 98.0 to 102.0%

Precision

Preparation of standard stock solution

Accurately weigh and transfer 124mg of Ranitidine working standard into a 10ml clean dry volumetric flask containing mobile phase and 10mg of Ondansetron in a 10ml clean dry volumetric flask containing mobile phase.

The solutions were sonicated for about 10mins.

0.325ml of Ranitidinne solution was taken from the prepared standard stock solution in a 4ml volumetric flask and made upto volume with mobile phase.

4mg of Ondansetron working standard into a 4ml volumetric flask and made upto volume with mobile phase.

0.05ml of Ranitidine intermediate dilution and 0.05ml of Ondansetron intermediate dilution was taken in 2ml Riavail and make upto volume with the diluent.

Procedure

The %RSD for the area of six replicate injections was found to be within the specified limits.

$$\text{Standard Deviation S.D} = \sqrt{\frac{\sum (x - x_i)^2}{n-1}}$$

Where, x = Sample,

x_i= Mean value of samples.

N = number of samples.

Coefficient of variance / Relative standard deviation:

$$\text{C.V} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

Acceptance criteria

The % RSD for the area of six standard injections results should not be more than 2%.

Assay

The Assay of different formulations available in the market were carried by injecting sample corresponding to equivalent weight into HPLC system and percentage purity was found out by following formulae. Recovery studies was carried out.

Calculate the percentage purity of Ranitidine and Ondansetron present in the tablet using the calibration curve.

Procedure for assay

This combination is not official in any pharmacopoeia. Therefore a general method for the assay of tablets is followed as per the procedure mentioned under tablets of general procedures in the Indian Pharmacopoeia 2010.

Accordingly 20 tablets of marketed formulation containing Ranitidine and Ondansetron were individually weighed and taken into a mortar. The average weight of each tablet is then calculated. The tablets were crushed into a fine powder. Accurately weighed quantity of one tablet equivalent to weight was transferred to a 100ml volumetric flask containing mobile phase. This is named as Solutions 1. Solution 1 is injected and concentration is calculated for Ondansetron.

1ml of the solution 1 is then taken and diluted to 20ml volumetric flask with mobile phase and this solution is named as Solution 2. Solution 2 is injected and concentration is calculated for Ranitidine.

Sonicate it for 10 minutes and then make up the volume up to the mark with mobile phase. Further, suitable volume of the above solution into a 100 ml volumetric flask and made up the volume with mobile phase. This solution is then injected into HPLC system and the assay is calculated.

Procedure

Inject the sample solution in triplicate and take the avg area then calculate the value by using linearity calibration curve equation.

Calculation

Concentration of tablet = dilution factor × sample concentration.

Robustness

Effect of variation of flow rate

A study was conducted to determine the effect of variation in flow rate by injecting 0.8and 1ml/min. The retention time values were measured. The chromatograms are shown in Figure: 35.

Acceptance criteria

Tailing factor of Ranitidine and Ondansetron standard should not be more than 2.0 for variation in flow rate.

The %RSD of Ranitidine and Ondansetron standard should not be more than 2.0 for variation in flow rate.

Effect of variation of Mobile phase composition

A study was conducted to determine the effect of variation in mobile phase composition. Standard solution was prepared and injected into the HPLC system at 45: 55 and 55: 45. The effect of variation in mobile phase composition was evaluated.

Acceptance criteria

Tailing factor of Ranitidine and Ondansetron standard should not be more than 2.0 for variation in mobilephase composition.

The %RSD of Ranitidine and Ondansetron standard should not be more than 2.0 for variation in mobilephase composition.

Ruggedness

It was obtained by the analysis of the same sample under a variety of normal test conditions ie different analysts, laboratories, columns, instruments, reagents, assay temperatures, different days etc. (ie from laboratory to laboratory, from analyst to analyst).

Acceptance Criteria

Overall RSD should not be more than 2.0 %

Analyst to Analyst variation

Procedure

The standard solution is injected for by different analysts and the area for injections in HPLC was measured. The %RSD for the area of replicate injections was found to be within the specified limits.

Column to Column variation

Procedure

The standard solution is injected for by using different columns and the area for injections in HPLC was measured. The %RSD for the area of replicate injections was found to be within the specified limits.

Limit of Detection

This is the lowest concentration in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. The limit of detection is important for impurity tests and the assays of dosages containing low drug levels and placebos.

Based on signal to noise.

Preparation of standard solution of Ranitidine

Accurately weigh and transfer 124mg of Ranitidine working standard into a 10ml clean dry volumetric flask containing mobile phase and. The solution was sonicated for about 10mins and then made upto volume with mobile phase.

0.325ml of Ranitidine solution was taken from the prepared standard stock solution in a 4ml volumetric flask and made upto volume with mobile phase.

From the above solution 0.02ml of Ranitidine solution was taken in 2ml RIA vail and make upto the mark with mobilephase.

Method Procedure

From the above prepared solution take 100µl of solution and make Itupto 1900µl with the diluent. This is called 1ml of LOD sample and from this sample3 injections are injected into the HPLC system.

Preparation of standard solution of Ondansetron

Accurately weigh and transfer 10mg of Ondansetron tablet powder into a 10ml clean dry volumetric flask containing mobile phase and. The solution was sonicated for about 10mins and then made upto volume with mobile phase.

From the above solution 0.02ml of Ondansetron solution was taken in 2ml RIA vail and make upto the mark with mobilephase.

RESULTS AND DISCUSSION

Development of Method and Stability Studies for the Estimation of Ranitidine and Ondansetron

Several trails were conducted during the development of a method for the simultaneous estimation of Ranitidine and Ondansetron in bulk form.

The developed HPLC method allows rapid and precise determinations of Ranitidine and Ondansetron.

the optimization of the chromatographic conditions, to develop RP-HPLC method, A series of mobile phases were tried, among the various mobile phases Methanol and mixed 0.1% Ortho phosphoric acid buffer (50: 50) an ideal mobile phase, since it gave a good resolution and peak shapes with perfect optimization. The flow rate was optimized at 0.9ml/min.

The Linearity and correlation coefficient of Ranitidine and Ondansetron was found to be10-100ug /ml, and 10.02-100.25ug/ml 0.997, and 0.998 respectively.

The limit of detection for Ranitidine and Ondansetron was found to be 0.91and 0.60and the limit of quantification was found to be 1.43 and 1.28.

The method was known to be accurate with the assay method. The % assay was found to be 97.7 and 100.4.

The accuracy and precision study was performed. The developed method showed good accuracy and precision. The % RSD is for Ranitidine and Ondansetron are 1.98 and 0.67.

The isocratic elution technique developed for the determination of Ranitidine and Ondansetron ideally suited for rapid and routine analysis. This method shows good reproducibility of the results. Furthermore this method was simple, sensitive, and accurate.

Degradation studies were done, here the drug stability results were in the range of acceptance criteria 85-115%.

S.No	Parameters	Ranitidine	Ondansetron
1	Area	1991491	2613783
2	Retention time	2.347	3.722
3	Theorotical plates	9631	22492
4	Tailing factor	1.562	1.085
5	Resolution	4.30	

Table No.1: Results for trail

S.No	Name	Ret. Time	Area	Theoretical Plate	Tailing Factor	Resolution
1	Rantidine	1.99	2581776	7316	1.46	0.00

Observation: Proper elution of Ranitidine. Ondansetron not eluted.

Table No.2: Results for trail

S.No	Name	Ret. Time	Area	Theoretical Plate	Tailing Factor	Resolution
1	Ondansetron	2.95	834762	9610	1.41	0.00

Observation: Elution of Ondansetron. Ranitidine not eluted.

Table No.3: Results for trail

S.No	Name	Ret. Time	Area	Theoretical Plate	Tailing Factor	Resolution
1	Ondansetron	2.97	97866	9660	1.41	0.00

Observation: Only ondansetron peak was observed. Ranitidine was not eluted.

Table No.4: For trail Results

S.No	Name	Ret. Time	Area	Theoretical Plate	Tailing Factor	Resolution
1	Rantidine	1.99	1281675	7828	1.44	0.00
2	Ondansetron	3.00	439876	10443	1.38	5.40

Observation: Ranitidine and Ondansetron peaks was not good.

Table No.5: Results for trail

S.No	Name	Ret. Time	Area	Theoretical Plate	Tailing Factor	Resolution
1	Ranitidine	1.98	1268912	8158	1.44	0.00
2	Ondansetron	2.99	434179	10698	1.37	5.53

Observation: Ranitidine and Ondansetron peaks was not properly eluted.

Table No.6: Results for trail

S.No	Name	Ret. Time	Area	Theoretical Plate	Tailing Factor	Resolution
1	Ranitidine	2.48	1752412	8604	1.52	0.00
2	Ondansetron	3.76	561933	10991	1.41	5.71

Observation: Ranitidine and Ondansetron were eluted.

Table No.7: Results for trail

S.No	Name	Ret. Time	Area	Theoretical Plate	Tailing Factor	Resolution
1	Ranitidine	2.53	189200	7188	1.49	0.00
2	Ondansetron	3.89	62325	11242	1.35	5.77

Observation: Multiple peaks was observed.

Table No.8: Observation for trail

S.No	Name	Ret. Time	Area	Tailing Factor	Resolution
1	Ranitidine	2.509	1980201	1.485	3.221
2	Ondansetron	4.678	4831697	1.010	

Observation: Ranitidine and Ondansetron peaks shape was good.

Table No.9: Results for trail

S.No	Name	Ret. Time	Area	Tailing Factor	Resolution
1	Ranitidine	2.308	1740301	1.585	0.000
2	Ondansetron	3.688	2831697	1.020	4.221

Observation: Ranitidine and Ondansetron were properly eluted.

Table No.10: Chromatogram Optimized method

S.No	Name	Ret. Time	Area	Tailing Factor	Resolution
1	Ranitidine	2.259	1885861	1.532	0.000
2	Ondansetron	3.593	2607233	1.012	4.191

Table.No11- System suitability parameters of Ranitidine and Ondansetron

S.No	Parameters	Ranitidine	Ondansetron
1	Area	1991491	2613783
2	Retention time	2.347	3.722
3	Theorotical plates	9631	22492
4	Tailing factor	1.562	1.085
5	Resolution	4.302	

Linearity

Table No.12: Shows Linearity observation of Ranitidine

S.No	Sample ID	Concentration (Microgram/mL)	Retention Time	Peak Area*	Amount Found	% Accuracy
1	CC – 01	10.02	2.339	543088	10.98	109.57
2	CC – 02	20.05	2.40	1006499	21.41	106.77
3	CC – 03	40.10	2.39	1721023	37.48	93.46
4	CC – 04	60.15	2.35	2778170	61.25	101.84
5	CC – 05	80.20	2.31	3438582	76.11	94.90
6	CC – 06	100.25	2.35	4658216	103.54	103.28

* Average of Three readings

Table No.13: Shows Stastical data of the Regression Equation for the Ranitidine

S.No	Parameters	Ranitidine
1	Concentration (µg / ml)	10-100µg/ml.
2	Correlation	0.9978
3	Slope	44462
4	No.of Data Points	6

Table No.14: Shows Linearity observation of Ondansetron

S.No	Sample ID	Concentration (Microgram/mL)	Retention Time	Peak Area*	Amount Found	% Accuracy
1	CC – 01	9.97	3.729	610556	9.11	91.36
2	CC – 02	19.94	3.99	1378273	20.46	102.63
3	CC – 03	39.88	3.819	2654047	39.34	98.64
4	CC – 04	59.82	3.768	3953303	58.56	97.89
5	CC – 05	79.76	3.714	5787937	85.69	107.44
6	CC – 06	99.70	3.724	6478412	95.91	96.20

* Average of Three readings

Table No.15: Shows Statistical data of the Regression Equation for Ondansetron

S.No	Parameters	Ondansetron
1	Concentration (µg/ml)	9.97-99.7µg/ml.
2	Correlation	0.9984
3	Slope	67602
4	No.of Data Points	6

S.No	Parameter	Acceptance Criteria	Results Obtained
1	System suitability	Theoretical Plates-NLT2000	RANT -9631 ONDA-22492
2	Assay	%RSD of RANT NMT2% %RSD of ONDA NMT2%	RANT-0.41 ONDA-0.39
3	Method Precision	%RSD of RANT NMT2% %RSD of ONDA NMT2%	RANT -1.98 ONDA-0.67
4	Limit of Detection	%RSD of RANT NMT2% %RSD of ONDA NMT2%	RANT -0.91 ONDA-0.60
5	Limit of Quantification	%RSD of RANT NMT2% %RSD of ONDA NMT2%	RANT -1.43 ONDA-1.28
6	Linearity	Correlation coefficient NLT 0.996	RANT -0.997 ONDA-0.998
7	Accuracy	Percentage Recovery 98-102%	RANT -98.66 ONDA-101.22

Trail-1

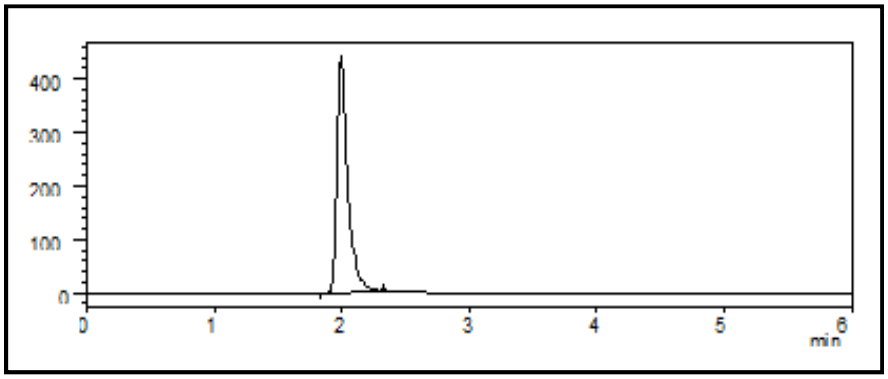

Figure No.1: Chromatogram of trail

Trail-2

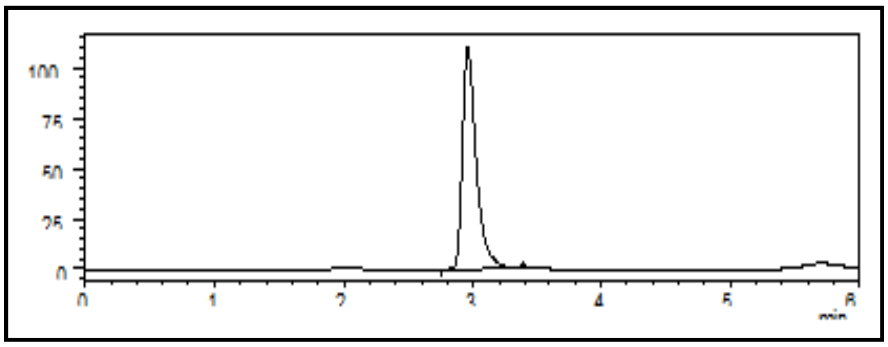

Figure No.2: Chromatogram of trail

Trail-3

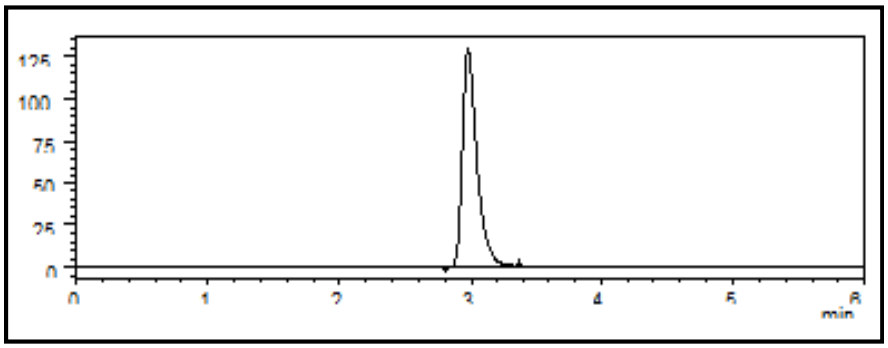

Figure No.3: Chromatogram of trail

Trail-4

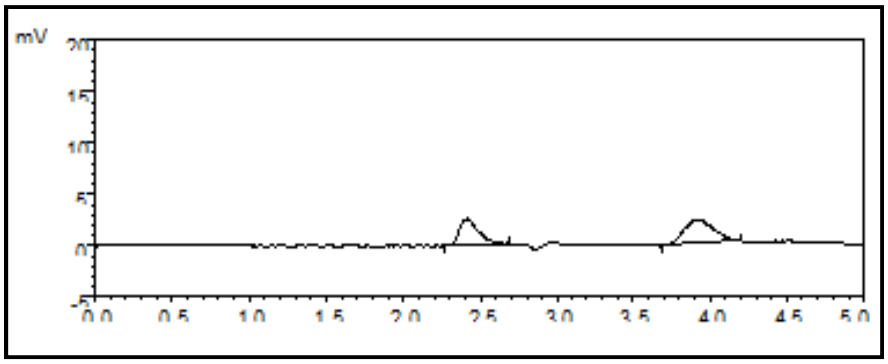

Figure No.4: Chromatogram of trail

Trail-5

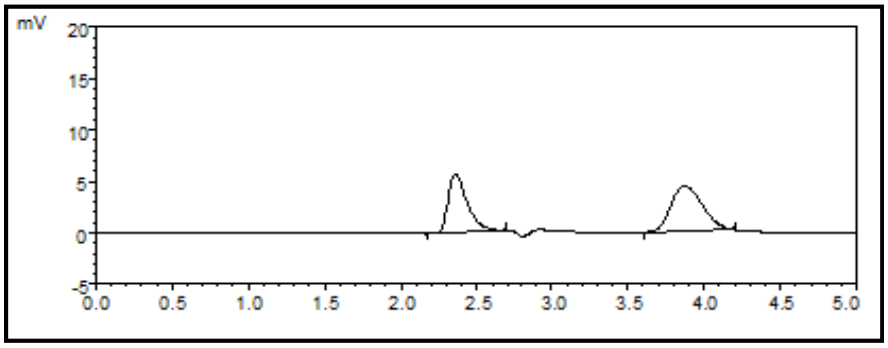

Figure No.5: Chromatogram of trail

Trail-6

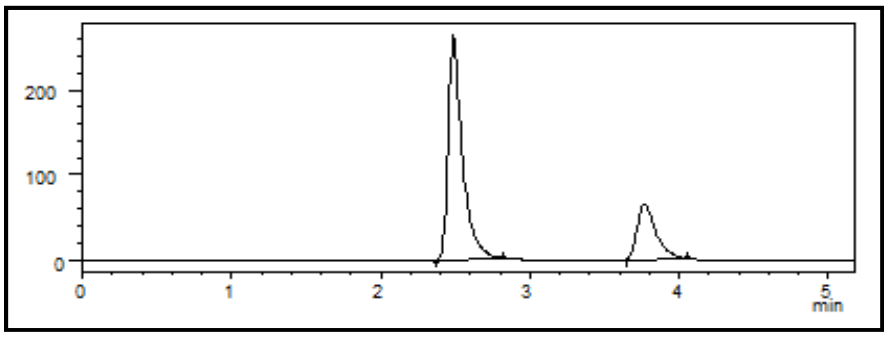

Figure No.6: Chromatogram of trail

Trail-7

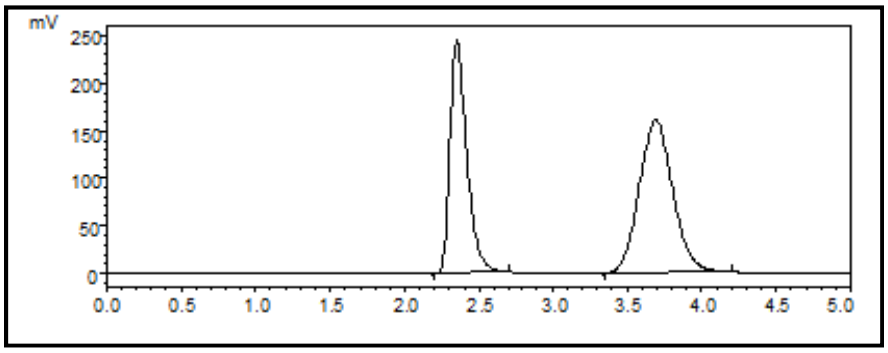

Figure No.7: Chromatogram of trail

Trail-8

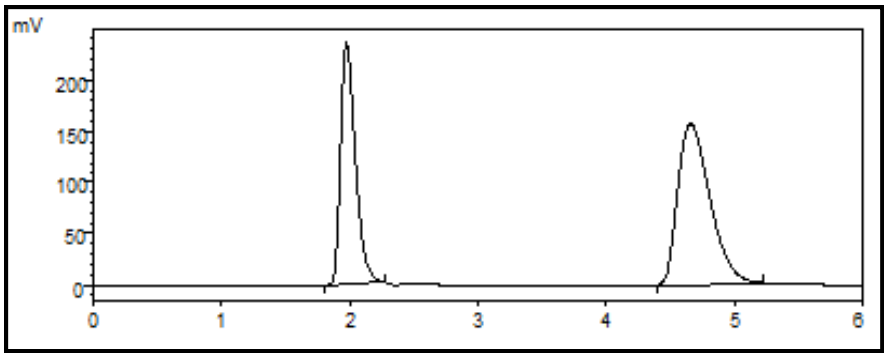

Figure No.8: Chromatogram of trail

Trail-9

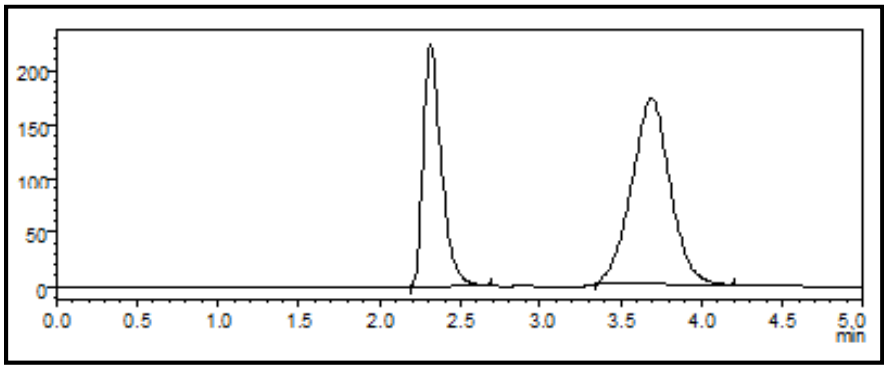

Figure No.9: Chromatogram of trail

Optimized Method

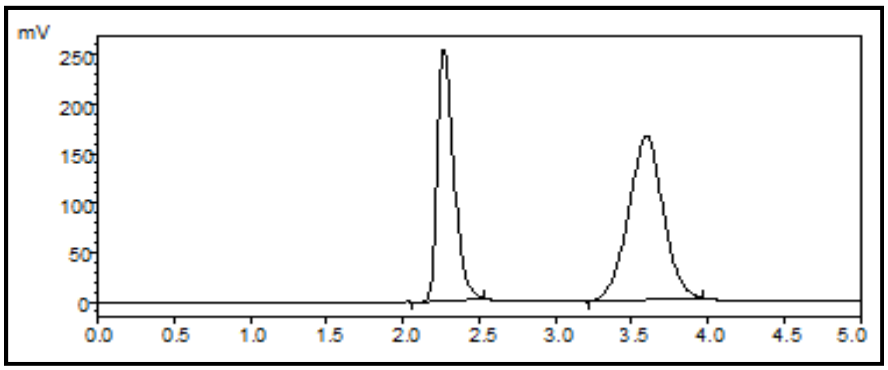

Figure No.10: Chromatogram Optimized method

ANALYTICAL METHOD VALIDATION

System Suitability Parameters

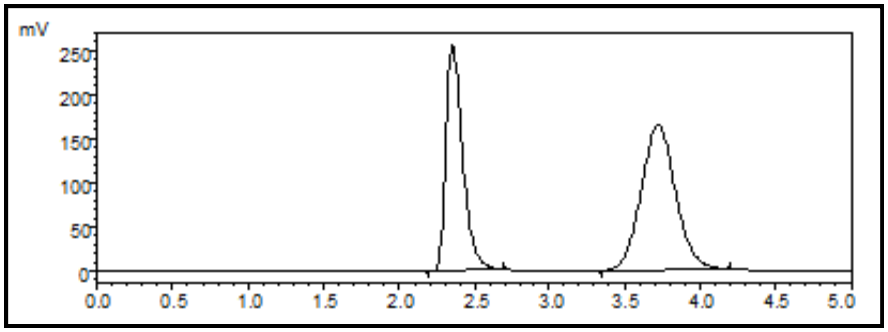

Figure No.11: Chromatogram of System suitability parameters

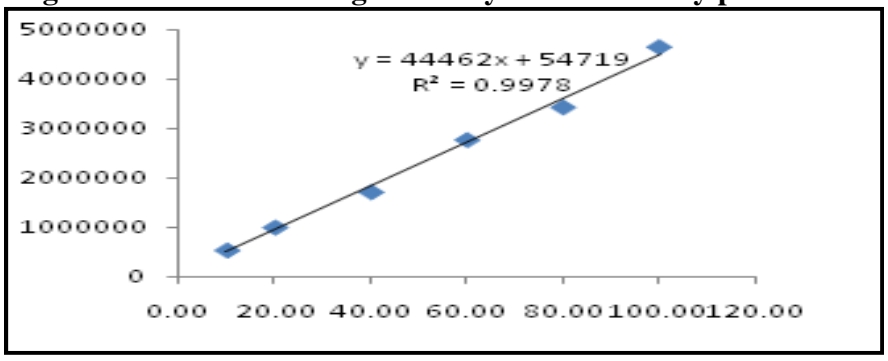

Figure No.12: Calibration curve of Ranitidine

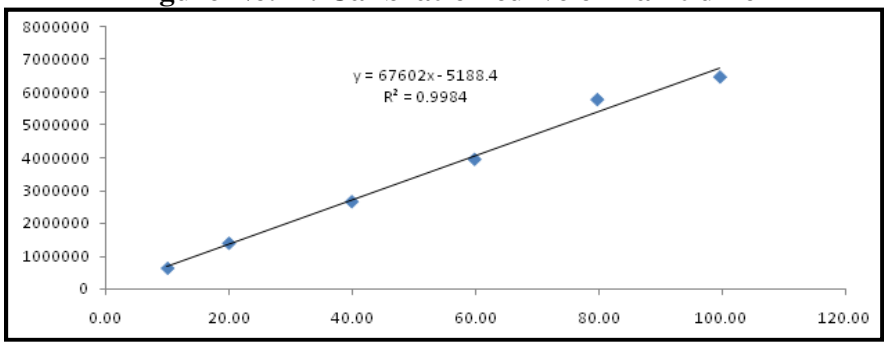

Figure No.13: Calibration curve of Ondansetron

CONCLUSION

The RP-HPLC method for analysis of Ranitidine and Ondansetron was found to be accurate and precise. The proposed method was validated according to ICH guidelines and correlating the obtained values with the standard values, satisfactory results were obtained.

Since this Project can be useful for further research of Ranitidine and Ondansetron.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmaceutics, Vishwa Bharathi College of Pharmaceutical Sciences, Perecherla, Guntur, Andhra Pradesh, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Please cite this article in press as: Sunil Mekala *et al.* A precise RP-HPLC method development and validation for the estimation of ondansetron and ranitidine in pharmaceutical dosage forms, *Asian Journal of Phytomedicine and Clinical Research,* 9(2), 2021, 26-39.