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Method Development And Validation Of Ondansetron In Bulk And Pharmaceutical Dosage Form By Stability-Indicating RP-HPLC Method

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Abstract: A precise and feasible high-performance liquid chromatographic (HPLC) method for the analysis of antiemetic drug Ondansetron tablet dosage form has been developed. The analysis was carried out on a zodiac C18 (4.6 x 250mm, 5 mm) column, using a buffer (pH 2.2), acetonitrile (73:27) as the mobile phase using a low pressure gradient mode with flow rate at 1ml/min and analysis was performed at wavelength 246 nm using Photo Diode Array (PDA) detector at ambient temperature. The injection volume was 20µl.The retention time of the drug was 5.2 min. The method produced linear responses in the concentration range of 2.5 to 75µg/ml of Rabeprazole. The LOD and LOQ values for HPLC method were found to be 0.9856 and 2 µg/ml respectively.
Key words: RP-HPLC, Ondansetron, method development and validation.

1.0 Introduction

Ondansetron was developed around 1984 by scientists working at Glaxo's laboratories in London. It is in both the imidazole and carbazole families of heterocyclic compounds. Ondansetron is a serotonin 5-HT3 receptor antagonist and prototype in this class. Used mainly as an antiemetic to treat nausea and vomiting, often following chemotherapy. Its effects are thought to be on both peripheral and central nerves. Ondansetron reduces the activity of the vagus nerve, which deactivates the vomiting center in the medulla oblongata, and also blocks serotonin receptors in the chemoreceptor trigger zone. It has little effect on vomiting caused by motion sickness, and does not have any effect on dopamine receptors or muscarinic receptors[1].

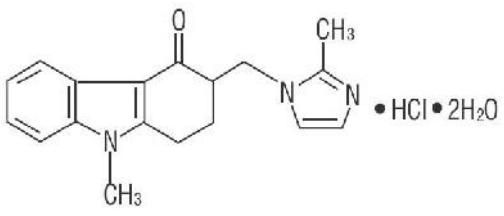

Figure 1: structure of ondansetron

Few derivative UV[2-5], visible[6,7] spectophotometric methods were reported for Ondansetron in pharmaceutical dosage forms. HPLC[8-14] methods for quantitative determination of ondansetron in combination were reported in Literature. HPTLC[15] and LCMS[16] methods were also reported for the estimation of Ondansetron. Majority of these HPLC methods were applied in the determination of ondansetron and its metabolites using buffer solutions and biological fluids. The present work describes a new, simple and accurate reverse phase liquid chromatographic method for the estimation of Ondansetron in pharmaceutical tablet dosage form. The developed method was validated to ensure the compliance in accordance with ICH guidelines[17].

2.0 Materials and Methods

2.1 Chemicals and Reagents

1. Acetonitrile (Merck) : HPLC grade
2. Water : HPLC grade
3. Ortho phosphoric acid : AR grade

2.2 Instrumentation

The HPLC system consisted of Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 solvent delivery module in a quaternary gradient mode and a Waters 2669 PDA detector. Data acquisition was performed by the Empower 2 software. Analysis was carried out at 246 nm with reversed phase Zodiac C18 (4.6 x 250mm, 5 mm) using buffer (water pH adjusted to 2.2 with OPA), acetonitrile (73:27) as the mobile phase by a low pressure gradient mode with flow rate at 1ml/min. The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system.

2.3 Preparation of solutions:

2.3.1 Preparation of Mobile Phase

Accurately measured a 1000 ml of HPLC grade Water, and adjust the pH 2.2 ± 0.5 with ortho phosphoric acid. It was filtered & degassed. HPLC grade Acetonitrile was filtered and degassed.
A mobile phase containing Buffer: Acetonitrile 73: 27v/v was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for Ondansetron.

2.3.2 Diluent Preparation:

Mobile phase as diluent.

2.3.3 Preparation Standard Solution

Accurately weighed 50 mg of the pure drug and dissolved in 70 ml diluent (mobile phase) and sonicated to dissolve it completely, the volume was made up to 100 ml mark with the diluent to get standard solution (500 µg/ml). From this solution 5 ml of solution was pipetted out and transferred in to separate 50 ml volumetric flask. The volume was made up to 50 ml mark with mobile phase to get the concentration of 50µg/ml as second stock solution. This solution was filtered through 0.45 µm Nylon syringe filter. This solution was injected into the column at a flow rate of 1.0 ml/min.

2.3.4 Preparation Sample Solution

10 tablets of Ondansetron were weighed and triturated to get a fine powder. Tablet powder equivalent to 25 mg of Ondansetron was taken into 50 ml volumetric flask. 40 ml of diluent was added, sonicated to dissolve and made up to the volume. Further dilution was carried out to get the concentration of 50µg/ml. This was then filtered through the 0.45 µm pore size nylon syringe filter.

2.4 Method development

2.4.1 Chromatographic parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler
Column : zodiac C18 (4.6 x 250mm, 5 mm)
Flow rate : 1ml per min
Detector : PDA
Wavelength : 246 nm
Injection volume : 20 µl

Column oven : Ambient
Run time : 10 min

2.4.2 System Suitability:
Stock solution-II (50 µg/ml) of Ondansetron standard (20 µl) was injected six times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Acceptance criteria
1. Tailing factor for the peak due to Ondansetron in Standard solution should not be more than 2.0.
2. Theoretical plates for the Rabeprazole peak in Standard solution should not less than 2000.
3.

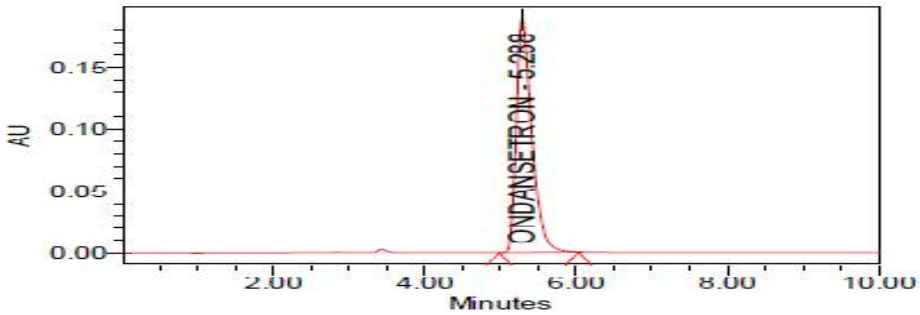

Figure 2: Standard Chromatogram for System suitability

Table 1: System suitability results

Drug name	RT(min)	Area	USP theoretical plates	USP tailing
ondansetron	5.288	2683713	3223.2124	1.5215

2.4.3 Assay
Assay was performed by taking 8 sets of the drug solutions were prepared in diluents containing ondansetron at a concentration range of 2.5–75 µg/ml. Then 20 µl of each standard and sample solution were injected for 4 times separately. The retention time of Ondansetron in bulk drug and pharmaceutical dosage form were found to be 5.2 min. The peak areas of the drug concentration were calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of Ondansetron in tablet dosage form shown in figure: 3.

Calculation:

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

Where:
AT = Peak Area of Rabeprazole obtained with test preparation.
AS = Peak Area of Rabeprazole obtained with standard preparation.
WS = Weight of working standard taken in mg.
WT = Weight of sample taken in mg.
DS = Dilution of Standard solution.
DT = Dilution of sample solution.
P = Percentage purity of working standard.

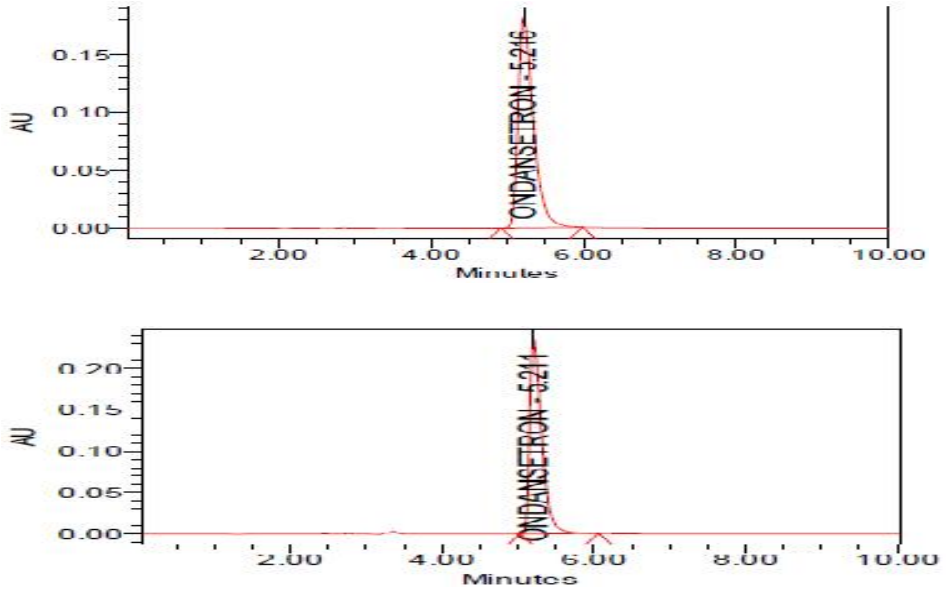

Figure 3: Assay of standard & sample chromatograms for ondansetron

Table: 2 Assay results

S.No	Retention Time (RT)	Peak Area	% Assay
1 (standard)	5.216	2128710	100.8
2 (sample)	5.211	2589223	

2.5 Validation

2.5.1 Linearity

Preparation of stock solution

The linearity of the method was demonstrated over the concentration range of 2.5-75 µg / ml. Aliquots of 2.5, 5, 12.5, 25, 37.5, 50, 62.5 and 75 µg / ml were prepared from stock solution-II and labeled as solution 1, 2, 3, 4, 5, 6, 7 and 8 respectively. Volume of 20 µl of sample was injected for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. The observations and calibration curve and chromatograms of linearity were shown in Table 3, Fig.4 and 5 respectively.

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

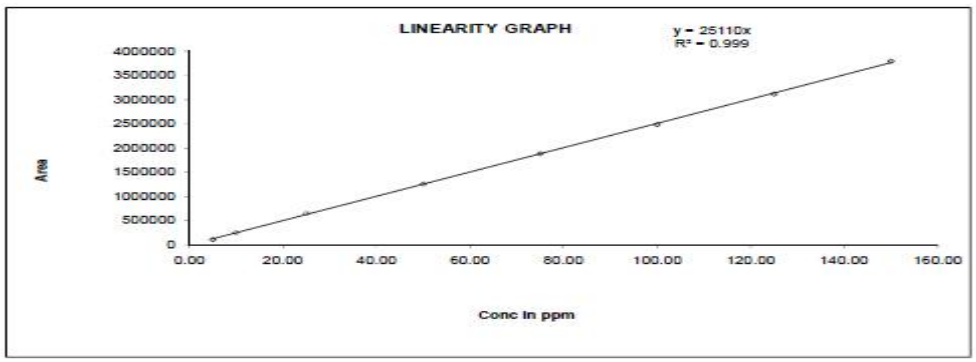

Figure 4: Calibration graph

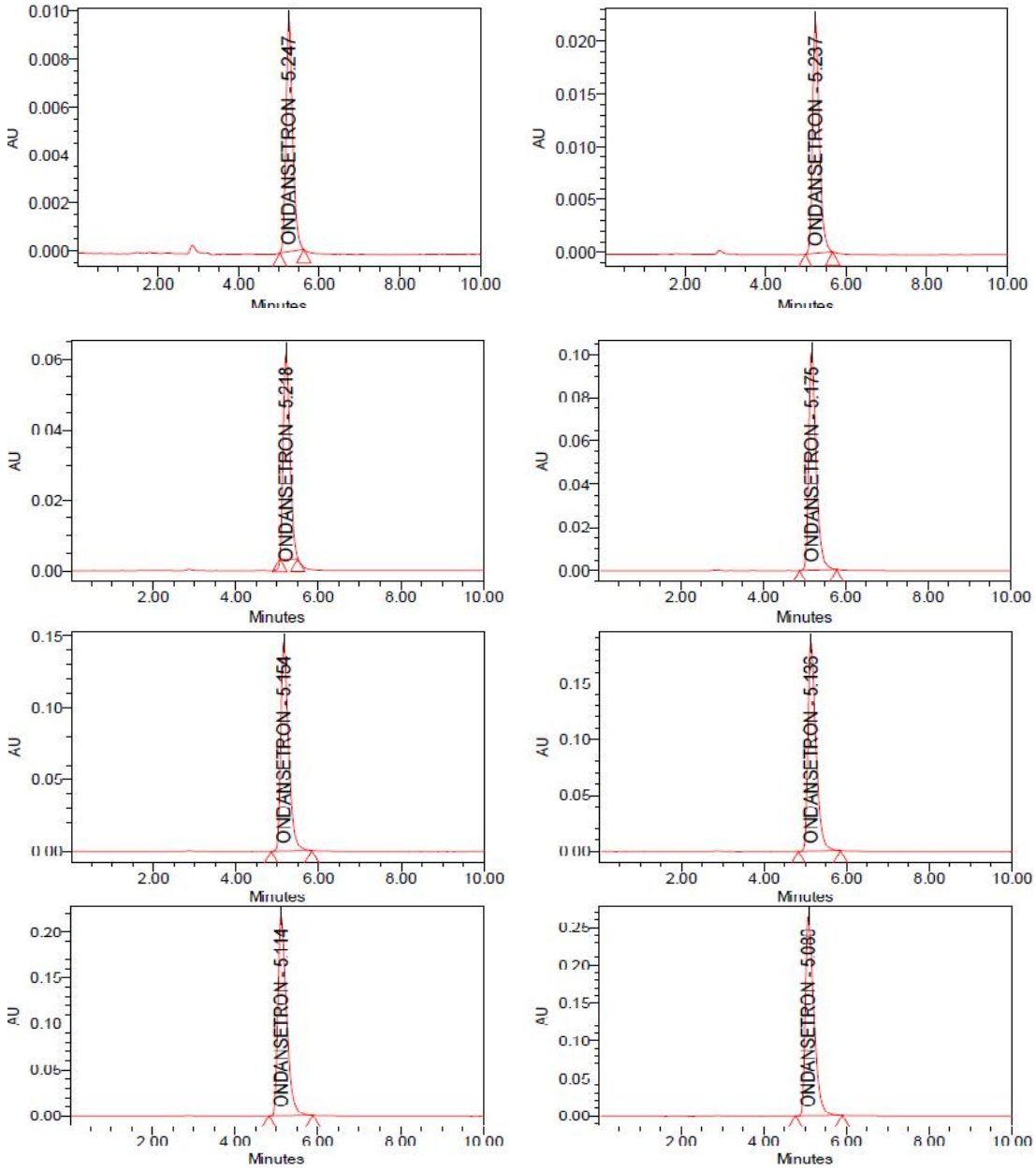

Figure 5: Linearity chromatograms

Table 3: Linearity results

S.No.	*Conc.*(µg / ml)	Rt	Area	USP Tailing
1	2.5	5.247	110947	1.2654
2	5	5.237	255274	1.3416
3	12.5	5.218	649180	1.3529
4	25	5.175	1258645	1.4864
5	37.5	5.154	1884570	1.5228
6	50	5.136	2485752	1.5502
7	62.5	5.114	3116700	1.5466
8	75	5.080	3796470	1.6486

2.5.2 Accuracy

Accuracy and Recovery:

Sample solutions were prepared by spiking at about 50 %, 100% and 150 % of specification limit to Placebo and analyzed by the proposed HPLC method. Chromatograms of 50 %, 100% and 150 % were given in figure 6 respectively. Results are shown in Table-4**.**

Acceptance Criteria:

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

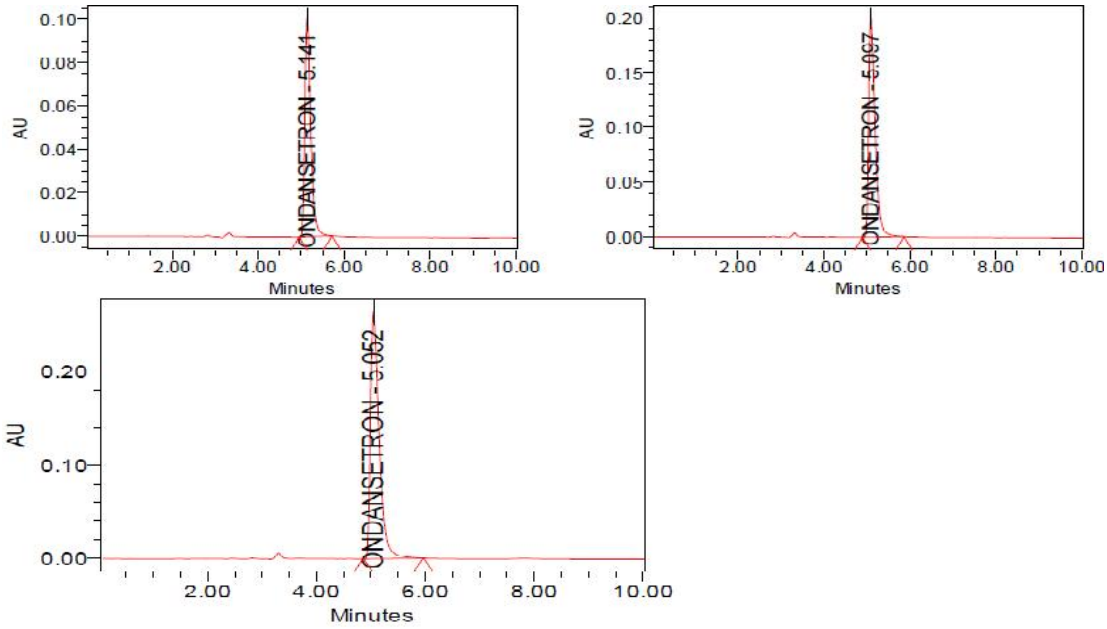

Figure 6: Accuracy chromatograms for ondansetron

Table 4: Accuracy of ondansetron

% ACCURACY	MEAN % RECOVERY	SD	%RSD
50	100.6	0.52	0.510
100	100.4	0.41	0.410
150	100.6	0.30	0.290

2.5.3 Precision:

a. System Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Standard solution of (50µg/ml) were prepared as per test method and injected for 6 times. Results are shown in Table -5.

Acceptance Criteria:

The % RSD for the area of 6 standard injections results should not be more than 2%.

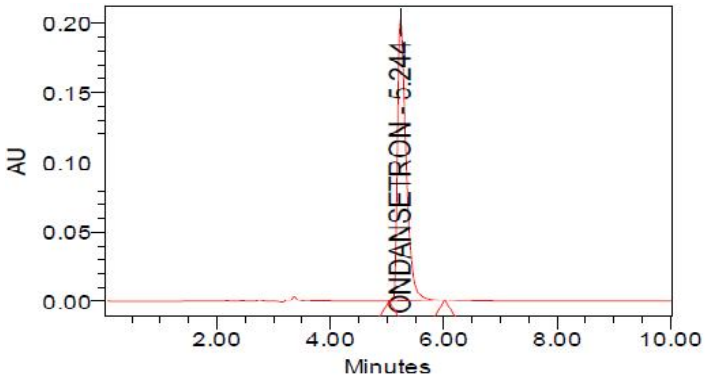

Figure 7: System precision chromatogram for ondansetron

Table 5: system precision of ondansetron

S.No.	Concentration (µg/ml)	Rt	Area
1	50	5.244	2133482
2	50	5.273	2135378
3	50	5.299	2128790
4	50	5.335	2131517
5	50	5.373	2122842
6	50	5.420	2120250
			%RSD = 0.283

b. Method precision

Six sample solutions were prepared and injected into the HPLC system as per test procedure. Results are shown in Table -6.

Acceptance Criteria:

The % RSD for the area of 6 sample injections results should not be more than 2%.

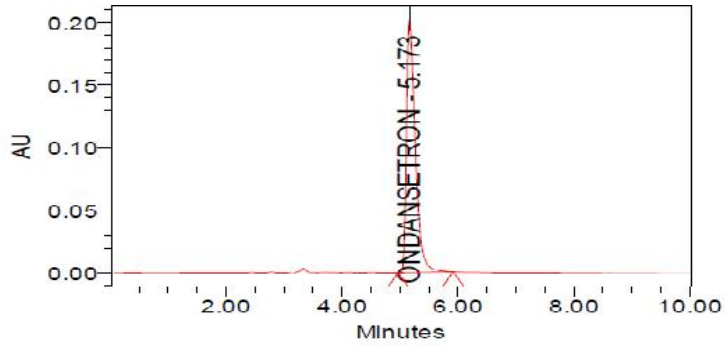

Figure 8: Method precision chromatogram for ondansetron

Table 6: method precision of ondansetron

S.No.	Concentration (µg/m)	Rt	Avg Area
1	50	5.131	2102148
2	50	5.141	2093523
3	50	5.150	2100154
4	50	5.154	2094313
5	50	5.156	2118389
6	50	5.173	2134016
			%RSD = 0.757

c. Intermediate precision

Standard solution of 50 ppm was prepared and injected 6 times into HPLC system on the next day as per test procedure. Results are shown in Table -7.

Acceptance Criteria:

The % RSD for the area of 6 sample injections results should not be more than 2%.

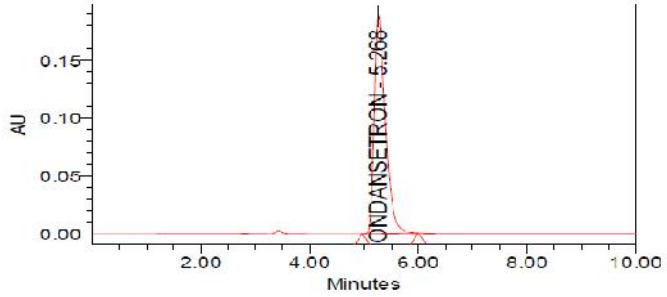

Figure 9: Intermediate precision chromatogram for ondansetron

Table 7: intermediate precision of ondansetron

S.No.	Concentration (µg/m)	Rt	Avg Area
1	50	5.268	2677546
2	50	5.233	2678157
3	50	5.204	2669875
4	50	5.174	2673170
5	50	5.198	2687402
6	50	5.177	2675694
			%RSD = 0.222

2.5.4 Robustness

The robustness of the proposed method was determined by analysis of aliquots from homogenous lots by differing physical parameters like flow rate, wavelength and mobile phase composition which may differ but the responses were still within the specified limits of the assay.

Table 8: robustness values of ondansetron

Proposed variations		USP Plate Count	USP Tailing
Variation in mobile phase composition	10% less	5261	1.721
	*Actual	3223	1.521
	10% more	5866	1.426
Variation in flow rate	0.8 ml/min	5247	1.681
	*1 ml/min	3223	1.521
	1.2 ml/min	5101	1.484
variation in wavelength	241 nm	5786	1.683
	*246 nm	3223	1.521
	251 nm	5329	1.755

Organic Phase Minus

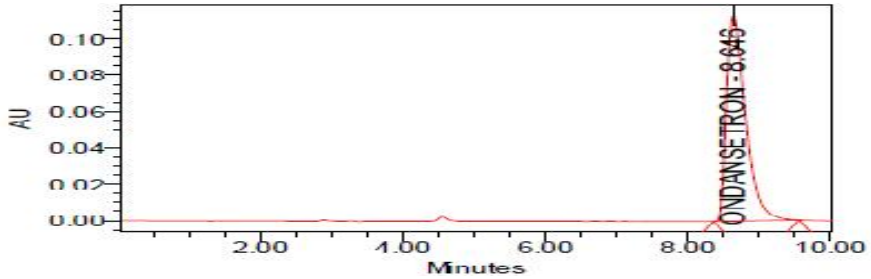

Figure 10: Chromatogram for Robustness (Buffer: ACN = 83:17v/v)

Organic Phase plus

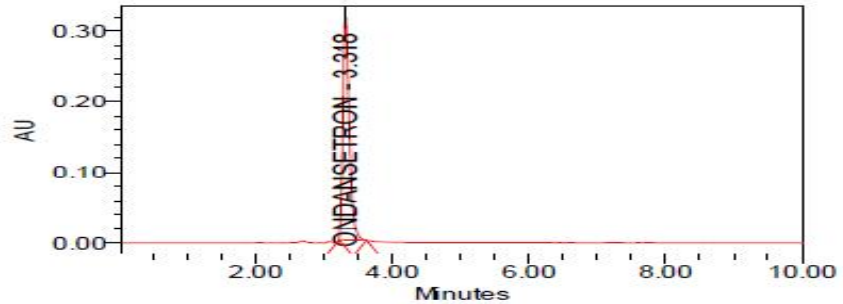

Figure11: Chromatogram for Robustness (Buffer: ACN = 63:37v/v)

Flow rate Minus

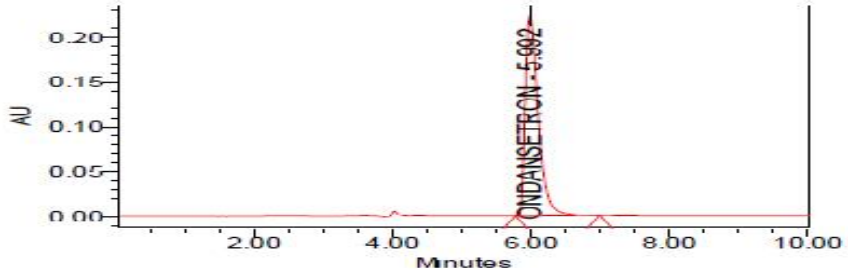

Figure 12: Chromatogram for Robustness (flow rate 0.8 ml/min)

Flow rate plus

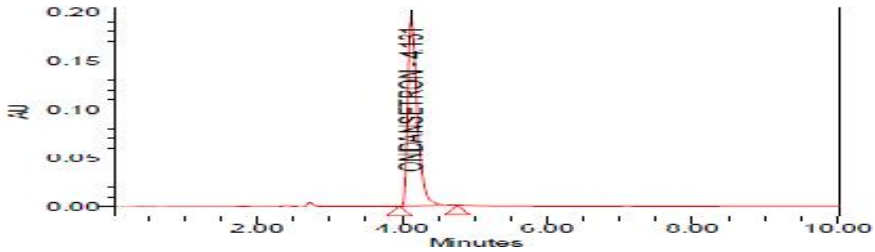

Figure 13: Chromatogram for Robustness (flow rate 1.2 ml/min)

Wave length Minus

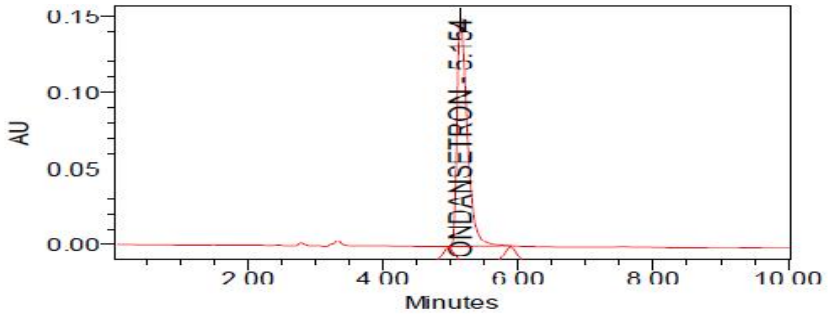

Figure 14: Chromatogram for Robustness (241nm)

Wave length plus

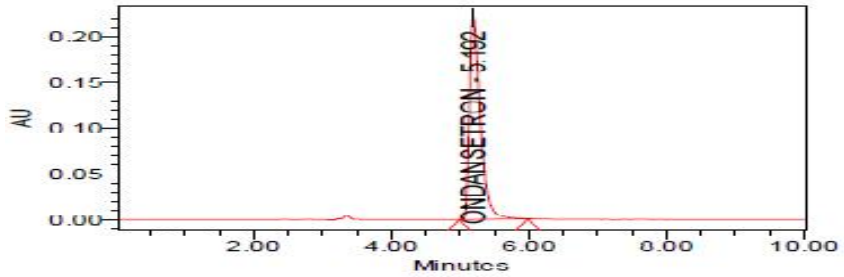

Figure 15: Chromatogram for Robustness (251nm)

2.5.5 Forced degradation

❖ **Acid degradation (5% HCl)**

From the sample stock solution 5 ml of sample was transferred into 20ml volumetric flask and add 2 ml of 5% HCl, A little amount of diluent were added & heated at 80°c for 20 min. Cooled the solution and neutralize the solution by adding 2 ml of 5% NaoH and made up to the volume with diluent. Filtered the solution through 0.45 µm nylon syringe filter by discarding little amount of solution.

Prepare acid degradation blank in the same way without using sample.

❖ **Alkali degradation (5% NaoH)**

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added 2 ml of 5% NaoH, A little amount of diluent were added & heated at 80°c for 20 min. Cooled the solution and neutralize the solution by adding 2 ml of 5% HCl and made up to the volume with diluent. Filtered the solution through 0.45 µm nylon syringe filter by discarding little amount of solution.

Prepare alkali degradation blank in the same way without using sample.

❖ **Peroxide degradation**

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added 2 ml of H_2O_2. A little amount of diluent were added & heated at 80°c for 20 min. Cooled and made up to the volume with diluent. Filtered the solution through 0.45 µm nylon syringe filter by discarding little amount of solution.
Prepare peroxide degradation blank in the same way without using sample.

❖ **Reduction degradation**

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added 2 ml of Sodium bisulphate, A little amount of diluent were added & heated at 80°c for 20 min. Cooled and made up to the volume with diluent. Filtered the solution through 0.45 µm nylon syringe filter by discarding little amount of solution.
Prepare reduction degradation blank in the same way without using sample.

❖ **Thermal degradation**

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added diluent, heated at 105°c for 24 hrs. Filtered the solution through 0.45 µm nylon syringe filter by discarding little amount of solution.
Prepare thermal degradation blank in the same way without using sample.

❖ **Photolytic degradation**

From the sample stock solutions 5 ml of sample was transferred into 20 ml volumetric flask and added diluent, subjected to sun light for 24 hrs. Filtered the solution through 0.45 µm nylon syringe filter by discarding little amount of solution.
Prepare photolytic degradation blank in the same way without using sample.

❖ **Humidity degradation**

From the sample stock solution 5 ml of sample was transferred into 20 ml volumetric flask and added little amount of diluent, subjected to 90% relative humidity at 25°c for 24 hrs. Then the volume was made up with diluent. Filtered the solution through 0.45 µm nylon syringe filter by discarding little amount of solution.
Prepare humidity degradation blank in the same way without using sample.

Acceptance criteria

a. Purity angle is less than purity threshold
b. Peak purity should pass.

Table 9: forced degradation study values of ondansetron

Stress Type	% Degradation	Purity Angle	Purity Threshold	Peak purity
sample		0.098	1.048	Pass
Acid	25.9	0.12	1.035	Pass
Alkali	25.1	0.13	0.12	Pass
Peroxide	24.9	0.092	0.12	Pass
Reduction	26.1	0.085	0.12	Pass
Thermal	30.4	0.11	0.12	Pass
Photolytic	30.1	0.101	0.12	Pass
Humidity	30.8	0.098	0.12	Pass
Hydrolysis	29.3	0.1	0.12	pass

2.5.6 Solution Stability

The solution stability of OND in diluents was determined by leaving 50 ppm sample solution in a tightly capped volumetric flask at room temperature for 24 hrs and measuring the amount at 4, 12, 24 hrs and compared the results with those obtained from freshly prepared solution. The mobile phase was prepared at the beginning of the study period and not changed during the experiment.

Acceptance criteria:
The % assay result should not differ from the initial value by more than ±2.0.

Table10: stability of ondansetron

Solution Stability (Hrs)	% Label Claim	% Deviation
Initial	100.1	
4 hrs	100.3	0.20
12 hrs	100.0	-0.10
24 hrs	100.2	0.10

2.5.7 Limit of Detection (LOD)
Calibration curve was repeated for 3 times and the standard deviation (SD) of the intercepts was calculated. The LOD was determined by the formula:
LOD = 3.3 σ / S, LOD = 0.9856

Where σ = Standard deviation of Intercepts of calibration curves
S = Mean of slopes of the calibration curves
The slope S may be estimated from the calibration curve of the analyte.

2.5.8 Limit of Quantification (LOQ)
Calibration curve was repeated for 3 times and the standard deviation (SD) of the intercepts was calculated The LOQ was determined by the formula:
LOQ = 10 σ / S, LOQ = 2.00.

3.0 Results and Discussion

S.No	Parameter	Acceptance Criteria		Results Obtained	Inference
1	System suitability	% RSD-NMT 2		0.221	Complies
2	Linearity	Correlation coefficient NLT-0.999		0.999	Complies
3	LOD	-		0.9856	Complies
4	LOQ	-		2.00	Complies
5	Precision	%RSD NMT 2	System	0.283	Complies
			Method	0.757	
			Intermediate	0.222	
6	Accuracy	Recovery of the spiked drug (98-102%)	50%	100.6	Complies
			100%	100.4	
			150%	100.6	
7	Specificity	1. No interference of blank, placebo and other degradation products with the main peak.		No interference	Complies
		2.purity angle < Threshold		peak pure	Complies
8	Solution stability	> 12 hour		Stable up to 24 hours	Complies

4.0 Conclusion

The proposed RP-HPLC method was found to be suitable for the estimation of Ondansetron in bulk and tablet dosage form. The method was found to be simple, rapid, precise, accurate, robust, specific. It has the advantage of shorter run time that helps in the rapid analysis of the pharmaceutical dosage forms. Hence, this method is used for the routine quality control analysis.

Acknowledgement

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References

1. http://www.mims.com/USA/drug/info/ondansetron/?q=ondansetron&type=full
2. P. Ravi Kumar, M. Murali Krishna, P. Bhanu Prakash et al, Derivative spectrophotometric estimation of ondansetron and paracetamol. E-Journal of Chemistry, 2006; 3(3): 134-136.
3. Shirish Patel, Dr. LJ Patel, derivative spectrophotometric method for simultaneous determination of ondansetron and Rabeprazole in combined dosage form. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(2).
4. S Pillai, I Singhvi, Spectrophotometric simultaneous estimation of ranitidine hydrochloride and ondansetron hydrochloride from tablet formulation. Indian journal of pharmaceutical sciences, 2007; 69(4): 601-604.
5. S. Ashok Reddy, K.B. Chandra Shekar, UV Spectrophotometric method for simultaneous determination of dexa and ondansetron in bulk and combined dosage form. International Journal of Advances in Pharmaceutical Research, Research Paper, 2230 7583.
6. Asad Raza, Abdul Subhan et al, Spectrophotometric Determination of Ondansetron Hydrochloride in Pharmaceutical Bulk and Dosage Forms. Journal of the Chinese Chemical Society, 2007; 54: 223-227.
7. Lahuerta Zamora et al, Extractive Spectrophotometric Determination of Ondansetron by Ion-Pair Formation with Bromocresol Green. Taylor Francis online, 1996; 29(5):785-792.
8. Ravi sheshala, Yusrida Darwis, Nurzalina Khan, Development and validation of an RP–LC–UV method for the determination of ondansetron, Application to pharmaceutical dosage. Chromatographia, 2009; 70: 75-81.
9. Andreea varvara, Crina - Maria Monciu et al, Application of a selective bonded phase in the liquid chromatographic assay of ondansetron hydrochloride and its impurities. Farmacia, 2009; 57: 5.
10. Kelly JW et al, High-performance liquid chromatographic separation of Ondansetron enantiomers in serum using a cellulose-derivatized stationary phase and solid-phase extraction. J Chromatogr, 1993; 622: 291-295.
11. Siluveru M et al, Enantioselective determination of S-(+) - and R-(-)-ondansetron in human serum using derivatized cyclodextrins - modified capillary electrophoresis and solid-phase extraction. J Chromatogr, 1997; 691: 217-222.
12. Shirish R. Patel, J. Patel, Yogeshvar P et al, Development and Validation of analytical method for the determination of Rabeprazole and Ondansetron in pharmaceutical dosage form by Reversed-phase HPLC. International Journal of ChemTech Research, 2010; 2(3):1531-1536.
13. Zarna Dedania, Ronak Dedania, Vaishali Karkhanis et al, RP-HPLC Method for Simultaneous Estimation of Omeprazole and Ondansetron in Combined Dosage Forms. Asian J. Research Chem, 2009; 2(2).
14. Dotsikas Y, Kousoulos C, Tsatsou G et al, Development and validation of a rapid 96-well format based liquid–liquid extraction and liquid chromatography–tandem mass spectrometry analysis method for ondansetron in human plasma. J Chromatogr 2006; 836: 79-82.
15. P. B. Raval, S. J. Wadher, and P. G. Yeole, A Validated HPTLC Method for Determination of Ondansetron in Combination with Omeprazole or Rabeprazole in Solid Dosage Form. Indian journal of pharmaceutical sciences, 2008; 70(3): 386-390.
16. Lingxia pang, Qing Wang, Youpei Wang et al, Development and Validation of LC-MS/MS Method for Determination of Ondansetron in rat Plasma and its Application. Latin American Journal of Pharmacy, 2012; 31(2): 305-9.
17. ICH Q2 (R1). Validation of analytical procedures: text and methodology International sConference on Harmonization. Geneva 2005; 1-13.

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