ISSN: 2456-9909 (Online)

International Journal of Research in AYUSH and Pharmaceutical Sciences

Research Article

STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ONDANSETRON IN BULK AND THEIR PHARMACEUTICAL DOSAGE FORM

A. Lakshmana Rao*, G. Akanksha, G. Neelima, G. Sneha, G.V. Pavan Kumar, G. Lavanya

Department of Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh, India.

ARTICLE INFO

Article history:
Received: Jan 21, 2022
Revised: Feb 02, 2022
Accepted: Feb 12, 2022

Keywords: Ondansetron, Validation, HPLC, Dosage Form.

ABSTRACT

A simple, rapid, precise and accurate stability indicating RP-HPLC method was developed and validated for the estimation of Ondansetron in bulk drug and pharmaceutical dosage form. A Phenomenex C18 (150 mm × 4.6 mm I.D., 5 µm particle size) column was used as stationary phase with mobile phase consisting of 0.1% Formic acid (pH 4.25):Acetonitrile in the ratio of 50:50 V/V. The flow rate was maintained at 0.6 mL/min and effluents was monitored at 250 nm. The retention time was 2.91 min. The linearity of the method was observed in the concentration range of 5-25 µg/mL with correlation coefficient of 0.999. The method developed was validated for linearity, precision, accuracy, system suitability and forced degradation studies like acidic, alkaline, oxidative and hydrolytic stress conditions were performed as per ICH guidelines. The results obtained in the study were within the acceptable limits and hence this method can be used for the estimation of Ondansetron in pure drug and pharmaceutical dosage form.

INTRODUCTION

Ondansetron (Figure 1) is a competitive serotonin type 3 receptor antagonist. It is effective in the treatment of nausea and vomiting [1-3]. Chemically it is 9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-2,3,4,9-tetrahydro-1*H*-carbazol-4-one. Ondansetron is a selective antagonist of the serotonin receptor subtype, 5-HT_3. Cytotoxic chemotherapy and radiotherapy are associated with the release of serotonin (5-HT) from enterochromaffin cells of the small intestine, presumably initiating a vomiting reflex through stimulation of 5-HT_3 receptors located on vagal afferents [4]. Ondansetron may block the initiation of this reflex. Activation of vagal afferents may also cause a central release of serotonin from the chemoreceptor trigger zone of the area postrema, located on the floor of the fourth ventricle [5-6].

Access this article online

Quick Response Code

https://doi.org/10.47070/ijraps.v5i7.118

Literature survey revealed that few HPLC methods [7-14] were reported for the estimation of Ondansetron. Hence a novel, new, sensitive, specific, accurate and precise HPLC method was developed and validated as per ICH guidelines [15-16] for the estimation of Ondansetron in bulk drug and pharmaceutical dosage form.

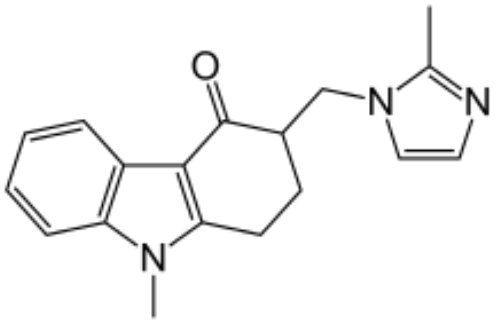

Fig. 1: Chemical structure of Ondansetron

MATERIALS AND METHODS

Instrumentation: To develop a high pressure liquid chromatographic method for estimation of Ondansetron using Agilent Technologies 1260

Infinity Binary HPLC instrument on Phenomenex C18 (150 mm × 4.6 mm I.D., 5 µm particle size) analytical column was used. The instrument is equipped with auto sampler and PDA detector. A 20 µL rheodyne injector port was used for injecting the samples.

Chemicals and solvents: The reference samples of Ondansetron were obtained from Yarrow Chemical Ltd, Mumbai, India. The commercial formulations (Ontoron-MD tablets containing 4 mg of Ondansetron) were procured from the local market. Methanol (HPLC grade), acetonitrile (HPLC grade), formic acid, triethyl amine were purchased from Merck (India) Ltd., Mumbai, India. Freshly prepared triple distilled water was used throughout the experiment.

Chromatographic conditions: A mixture of 0.1% Formic acid (pH 4.25): Acetonitrile in the ratio of 50:50 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Ondansetron. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.6 mL/min. Injection volume was 20 µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 250 nm. The run time was set at 5 min.

Preparation of mobile phase and diluent: 500 mL of 0.1% Formic acid (Dissolve 1 mL of formic acid in 1000 mL of water, adjusted the pH to 4.25 by using triethyl amine) was mixed with 500 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The mixture of water and acetonitrile was used as diluent.

Preparation of standard stock solution: About 10 mg of Ondansetron is accurately weighed and transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was sonicated for 5 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ondansetron. Further pipette 1.0 mL of the above stock solution into a 10 mL volumetric flask (100 µg/mL) and the volume was made up to the mark with the mobile phase.

Preparation of sample solution: 20 tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Ondansetron is transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was filtered and sonicated for 5 min. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ondansetron. Transfer 1.0 mL of the above solution to 10 mL volumetric flask and diluted upto mark with diluents. Further pipette 1mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase.

Linearity: Working standard solutions were prepared for the Ondansetron from the standard solution of 1000 µg/mL. Different aliquots were taken from standard stock solution and diluted with water:acetonitrile separately to prepare 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, and 25 µg/mL solutions respectively. Then the construction of calibration curve was plotted by taking the above prepared solutions of different concentrations ranging from 5-25 µg/mL. Each measurement was carried out in triplicate.

Precision: The concentration used for the precision studies is 10 µg/mL. To study the intra-day and inter-day precision, the analysis of drugs was repeated for six times in the same day and different days. Six replicate mixed standard solution of Ondansetron was measured with the same concentration and the % RSD was calculated.

Accuracy: The accuracy of the method was determined by standard addition method. Recovery study of Ondansetron was determined in the dosage form at three concentration levels. A known amount of standard drug was added to the fixed amount of pre-analyzed drug sample solution. Percent recovery was calculated by comparing the peak area before and after the addition of the standard drug. The standard addition method was performed at three concentration levels in triplicate at 50%, 100% and 150%. Each level was repeated three times.

System suitability: Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like retention time, theoretical plates and tailing factor were calculated and compared with standard values.

Limit of detection and limit of quantitation: The limit of detection (LOD) and limit of quantitation (LOQ) of the developed method were determined by injecting progressively six replicates of the analyte at low concentrations of the standard solution of Ondansetron using the developed HPLC method were measured and quantified.

Ruggedness: The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. Ruggedness of the

method was confirmed by the analysis of samples of Ondansetron at 10 µg/mL concentration were analyzed by different analysts.

Robustness: Robustness of the method was determined by making slight changes in the chromatographic conditions. To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate, wave length and composition of mobile phase.

Estimation of Ondansetron in tablet dosage form: Commercial formulation of tablets was chosen for testing the suitability of the proposed method to estimate Ondansetron in tablet formulation. 20 tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 mg of Ondansetron was transferred to a 10 mL volumetric flask. The contents of the flask were sonicated for about 10 min for complete solubility of the drug and volume made up with further quantity of mobile phase. Further pipette 0.2 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. The standard solutions and sample solutions were determined at 250 nm and the amount of the drugs present in the tablet dosage form was calculated.

Stability studies

Acid degradation studies: To 1 mL of the stock solution of Ondansetron, 1 mL of 0.1N hydrochloric acid was added, refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation studies: To 1 mL of the stock solution of Ondansetron, 1 mL of 2N sodium hydroxide was added, refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation studies: To 1 mL of the stock solution of Ondansetron, 1 mL of 20% hydrogen peroxide was added, refluxed for 30 mins at 60°C. The solutions were kept for 30 mins at 60^{0}C. The resultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of sample.

Hydrolytic degradation studies: Stress testing under hydrolytic conditions was studied by refluxing the standard Ondansetron solution in water for 6 hrs at a temperature of 60ºC. The resultant solution was diluted to obtain 10 µg/mL solution and 20 µL solution were injected into the system and the chromatograms were recorded to assess the stability of the sample.

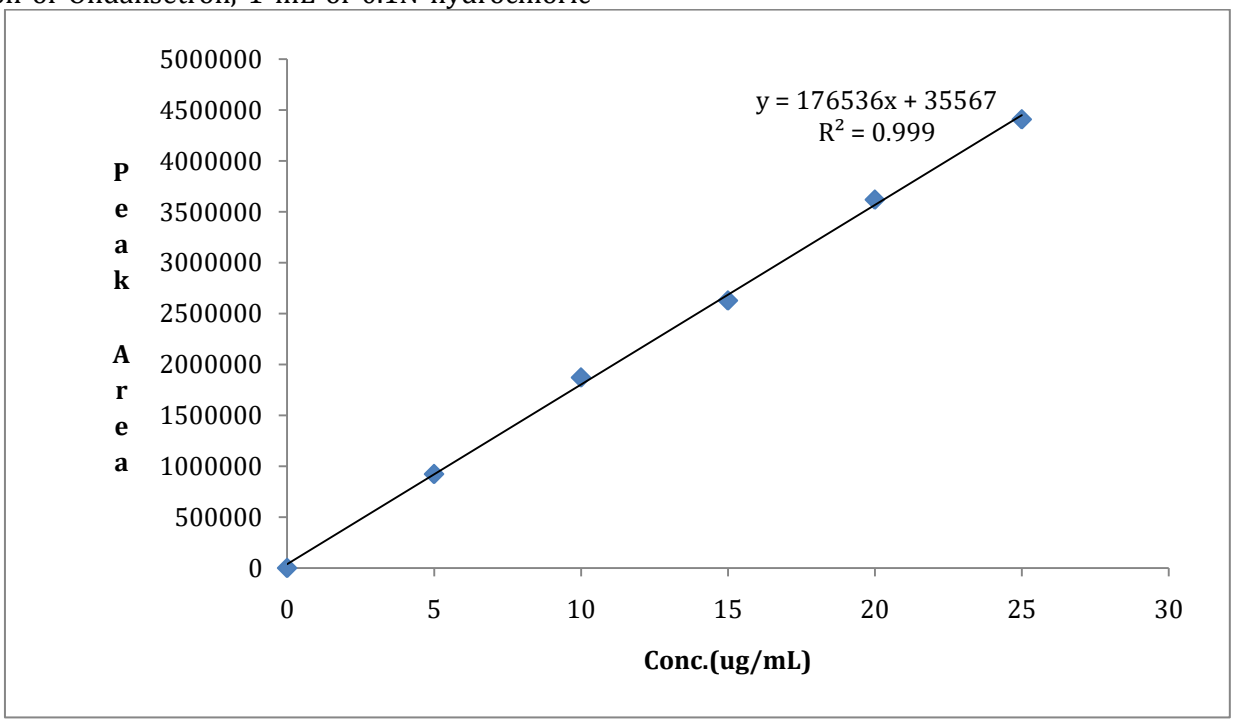

Fig. 2: Linearity curve of Ondansetron

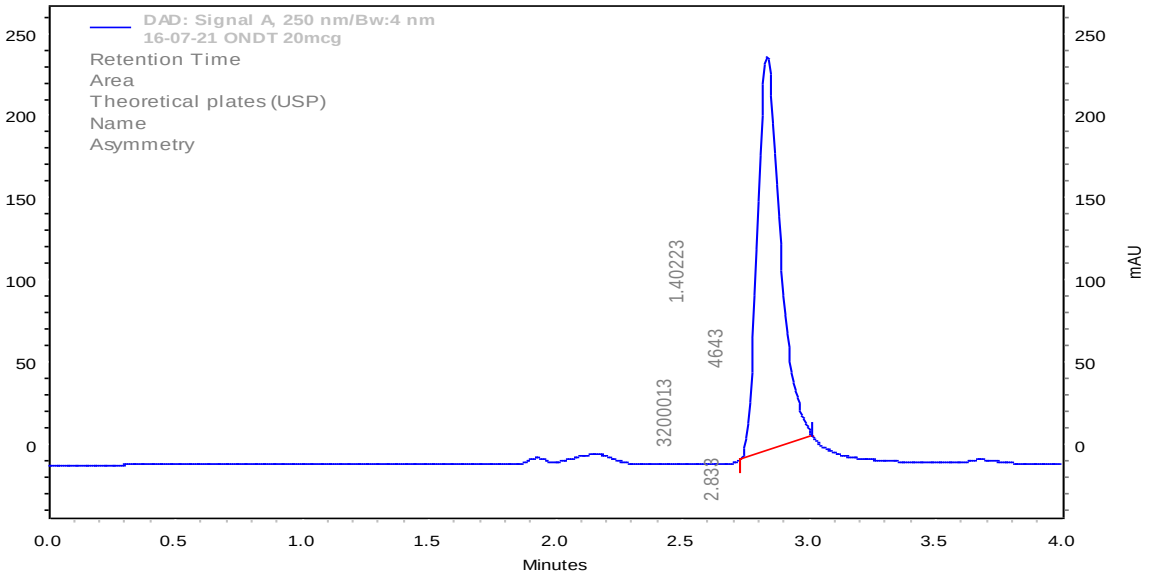

Fig. 3: Typical chromatogram of Ondansetron

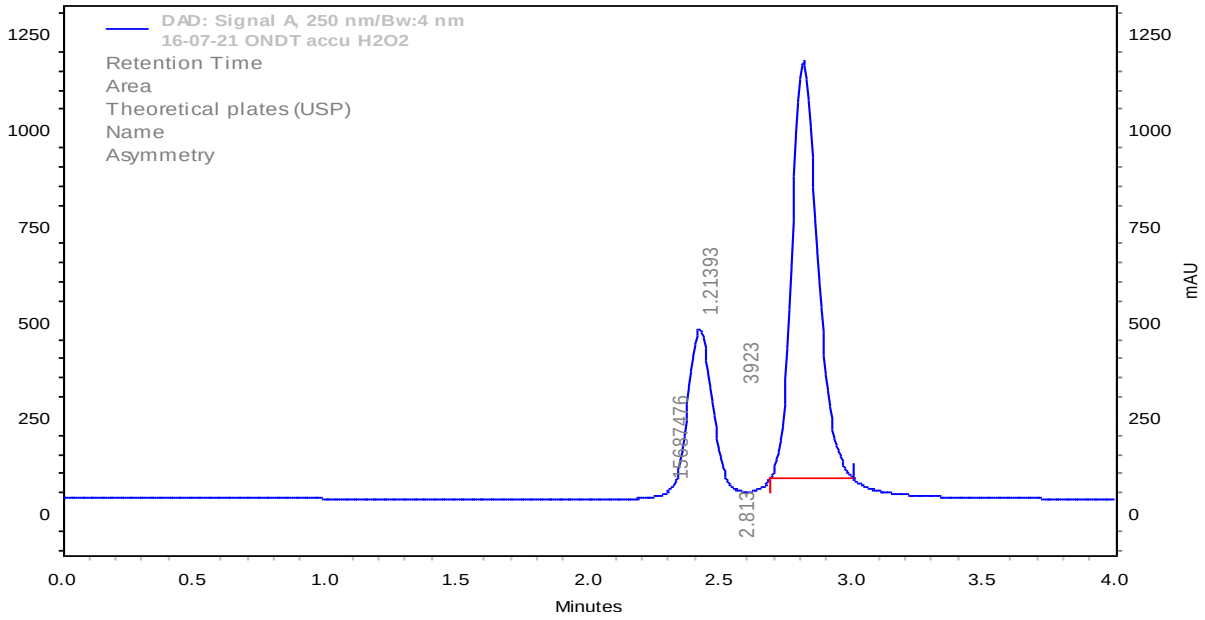

Fig. 4: Chromatogram of Ondansetron in acid degradation

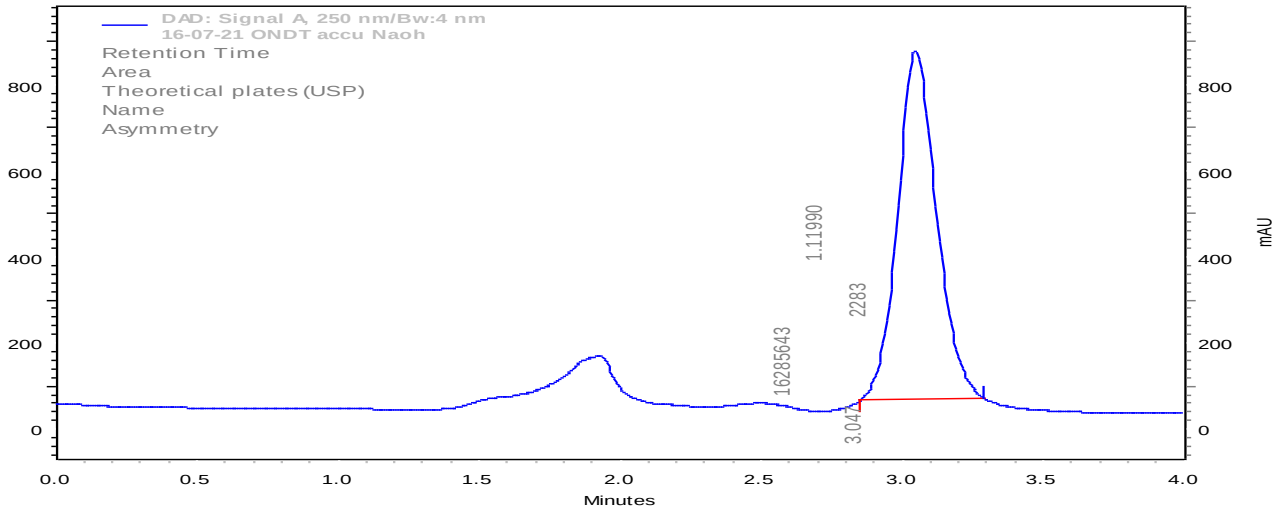

Fig. 5: Chromatogram of Ondansetron in alkali degradation

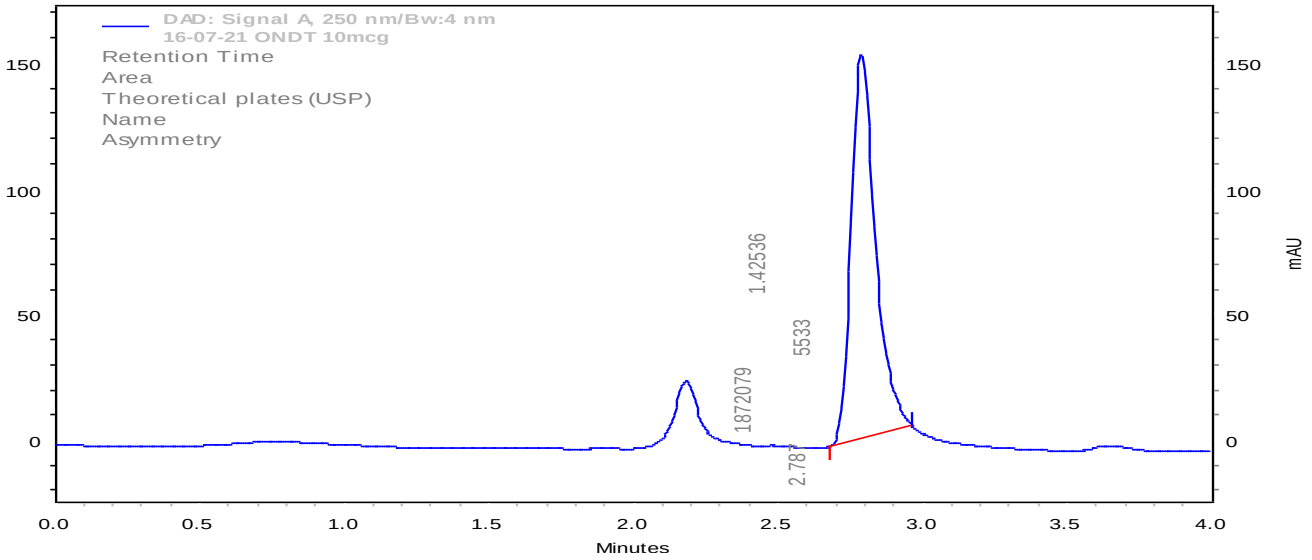

Fig. 6: Chromatogram of Ondansetron in oxidative degradation

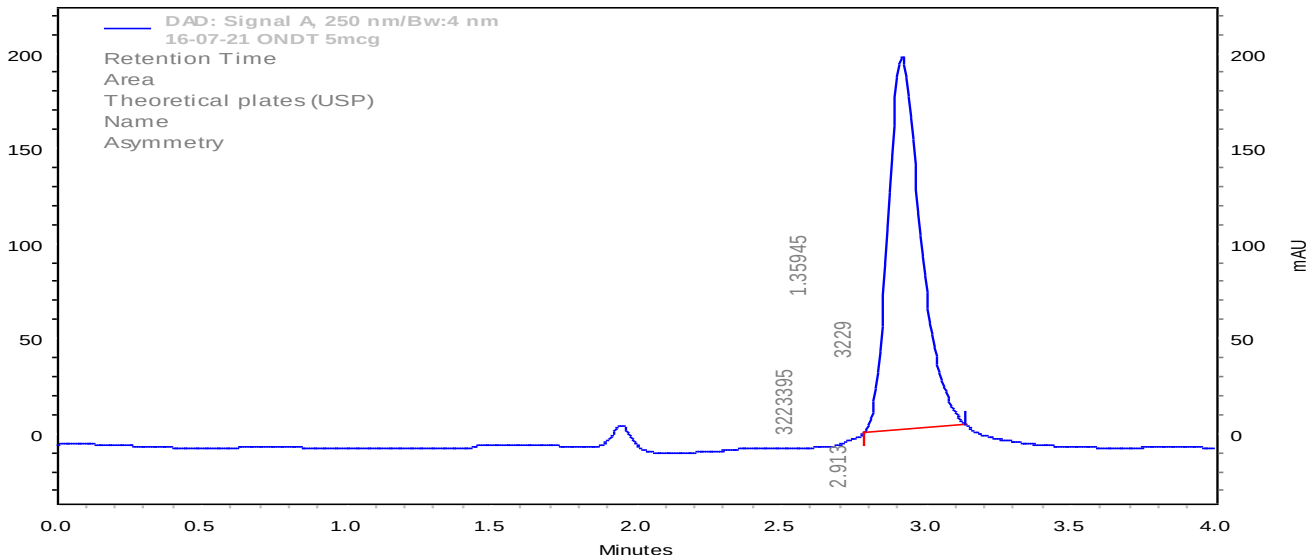

Fig. 7: Chromatogram of Ondansetron in hydrolytic degradation

Table 1: Optimized chromatographic conditions of Ondansetron

Parameter	Condition
Mobile phase	0.1% Formic acid:Acetonitrile (50:50 V/V)
pH	4.25
Diluent	Water:Acetonitrile
Column	Phenomenex C18 (150 mm × 4.6 mm, 5 µm)
Column temperature	Ambient
Wave length	250 nm
Injection volume	20 µL
Flow rate	0.6 mL/min
Run time	5 min
Retention time	2.91 min

Table 2: Linearity results of Ondansetron

S. No.	Concentration (µg/mL)	Peak area
1	0	0
2	5	923395
3	10	1872079
4	15	2628643
5	20	3620013
6	25	4409482
Slope		17653
Intercept		35567
Regression Equation (y)		y = 17653x+35567
Correlation Coefficient		0.999

RESULTS AND DISCUSSION

In the present work, a simple, rapid, novel, precise and accurate stability indicating HPLC method has been optimized, developed and validated for the determination of Ondansetron in pharmaceutical formulation by using Phenomenex C18 (150 mm × 4.6 mm I.D., 5 µm particle size) in isocratic mode with mobile phase composition of 0.1% Formic acid (pH 4.25): Acetonitrile in the ratio of 50:50 V/V resulted the chromatographic peak obtained was in good shape, better resolved and almost free from tailing. The flow rate was 0.6 mL/min and the drug component was measured with PDA detector at 250 nm. The results of optimized HPLC conditions were shown in Table 1.

Linearity was proven by regression analysis by the least square method. The straight line in the calibration curve obeyed linearity in the concentration range of 5-25 µg/mL for Ondansetron with correlation coefficient of 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated. The linearity results were shown in Table 2 and the linearity curve was shown in Figure 2.

The %RSD for intra-day precision and inter-day precision for Ondansetron was found to be 1.59 and 0.39, which are well within the acceptable criteria of not more than 2.0, which indicates the method is precise. The results of precision studies were shown in Table 3.

The %Recovery of Ondansetron were found in the range of 99.49-100.44% and the %Mean recovery was found to be 99.83%, which shows there is no interference from excipients and the lower values of %RSD indicate the method is accurate. The results of accuracy studies were shown in Table 4.

The retention time of Ondansetron was 2.91 min, cuts down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. The number of theoretical plates was 4652 and tailing factor was 1.35 for Ondansetron, which indicates efficient performance of the column. Typical chromatogram of drug Ondansetron was shown in Figure 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug Ondansetron by the proposed HPLC method. Selectivity of the method was demonstrated by the absence of any interfering peaks at the retention time of the drug. The LOD and LOQ for Ondansetron were found to be 1.53 µg/mL and 4.64 µg/mL, which indicate the sensitivity of the method. The summary of system suitability parameters were shown in Table 5.

Ruggedness was observed that there were no marked changes in absorbance, which demonstrated that the developed method was rugged in nature. Robustness was observed that there were no marked changes in chromatograms of Ondansetron, which demonstrated that the developed method was robust in nature. The results of robustness study were showed in Table 6.

Validated method was applied for the estimation of Ondansetron in commercial tablet formulations. The %Assay of Ondansetron was found to be 99.5%. The assay results showed that the drug contents of this product to be in accordance with the labeled claims. The results were furnished in Table 7.

Stability studies of Ondansetron under different stress conditions indicated the following degradation behavior. In acidic degradation, the degradation product was appeared at retention time of 2.918 min and the %Degradation is 14.40%. In alkali degradation, the degradation product was appeared at retention time of 2.894 min and the %Degradation is 22.30%. In oxidative degradation, the degradation product was appeared at retention time of 2.867 min and the %Degradation is 14.63%. In hydrolytic

degradation, the degradation product was appeared at retention time of 2.935 min and the %Degradation is 3.26%. The forced degradation study showed the method was highly specific. The results of forced degradation studies were shown in Table 8. The typical chromatograms of degradation behavior of Ondansetron in different stress conditions are shown in Figure 4 to Figure 7.

CONCLUSION

The present study represents the development and validation of a stability indicating RP-HPLC method for determination of Ondansetron by following the recommendations of ICH guidelines. The proposed method showed acceptable wide linear concentration range, accuracy, precision, selectivity and robustness. The results of analysis proved that the method is suitable for the determination of Ondansetron in bulk and tablet dosage form without any interference from the degradation products and it is recommended for routine quality control analysis of the Ondansetron in pharmaceutical formulation.

REFERENCES

1. https://go.drugbank.com/drugs/DB00904
2. https://www.rxlist.com/consumer_Ondansetron_zofran/drugs-condition.htm
3. https://www.drugs.com/Ondansetron.html
4. https://medlineplus.gov/druginfo/meds/a601209.html
5. K. Chandra Mohan and K. Ravi Kamar. Ondansetron Hydrochloride: A Competitive Serotonin 5-HT_3 Receptor Blocker. Acta Crystallographica. 1995; C51: 2627-2629.
6. I.T. Cohen. Ondansetron for Postoperative Nausea and Vomiting. Therapy. 2006; 3(5): 571-578.
7. A. Imran, R.K. Jat, R. Singh, V. Srivastava and S. Sunny. Development and Validation of Reversed-Phase HPLC for Estimation of Ondansetron Hydrochloride in Bulk Drug. International Research Journal of Pharmacy. 2012; 3(2): 111-113.
8. MD.M. Basha, B. Praveena, M. Srinidhi and SK.A. Rahaman. Method Development and Validation of Ondansetron in Bulk and Pharmaceutical Dosage Form by Stability-Indicating RP-HPLC Method. International Journal of PharmTech Research. 2013; 5(1): 86-98.
9. N. Rajan and K. Anver Basha. HPLC Method Development for Determination of Glyoxal Content in Ondansetron HCl Drug Substances by Derivatization with 2,4-DNPH. Journal of Liquid Chromatography & Related Technologies. 2014; 37(19): 2800-2808.
10. T.B. Deshmukh, S.S. Deo, F. Inam, T.L. Lambat, S.B. Gurubaxani and A.V. Choudhari. Development and Validation of Ondansetron Hydrochloride in Pharmaceutical Dosage Form by RP-HPLC Method. International Journal of Advances in Science Engineering and Technology. 2015; 5(1): 15-20.
11. M.M. Annapurna, B. Venkatesh, J.S.P. Kumar and S.M. Chaitanya. Stability Indicating RP-HPLC Method for Determination of Ondansetron. Journal of Chemical and Pharmaceutical Sciences. 2017; 10(2): 764-767.
12. J. Srinivas, A. Ravi Kumar, P. Srinivas, M. Raveendra Babu and P. Ramyasree. Novel Stability Indicating RP-HPLC Method for the Determination of Ondansetron Impurities in Ondansetron Injection. International Journal of Current Trends in Pharmaceutical Research. 2018; 6(4): 103-109.
13. V.G. Santosh, S.R. Madhuri and A.P. Chaudhari. Development and Validation of Stability Indicating HPLC Method for Estimation of Ondansetron Hydrochloride. Indo American Journal of Pharmaceutical Sciences. 2018; 5(1): 584-591.
14. J.K.D. Tejaswi. Estimation of Ondansetron Hydrochloride by RP-HPLC. International Journal of Research and Development. 2020; 5(5): 29-34.
15. ICH Harmonised Tripartite Guideline, Q2(R1) (2005). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, Geneva; 1-13.
16. ICH Harmonised Tripartite Guideline, Q1A(R2) (2003). Stability Testing of New Drug Substances and Products. International Conference on Harmonization, Geneva; 1-18.

Cite this article as:
A. Lakshmana Rao, G. Akanksha, G. Neelima, G. Sneha, G.V. Pavan Kumar, G. Lavanya. Stability Indicating RP-HPLC Method Development and Validation for the Estimation of Ondansetron in Bulk and their Pharmaceutical Dosage Form. International Journal of Research in AYUSH and Pharmaceutical Sciences, 2021;5(7):557-563.
https://doi.org/10.47070/ijraps.v5i7.118
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. A. Lakshmana Rao
Professor and Principal,
V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh, India.
Email: dralrao@gmail.com