World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: https://wjpsonline.com/
Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF ONDANSETRON BY UHPLC

R. Saran [1], Dr. K.L. Senthil Kumar [2], Dr. P.D. Gokulan [3]

[1] M.Pharm Final Year Student, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri affiliated to The TN Dr MGR Medical University, Chennai, Tamil Nadu.
[2] Principal, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri affiliated to The TN Dr MGR Medical University, Chennai, Tamil Nadu.
[3] Head of Department, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri affiliated to The TN Dr MGR Medical University, Chennai, Tamil Nadu.

Received: 01-03-2024 / Revised Accepted: 25-03-2024 / Published: 16-04-2024

ABSTRACT

The developed Reverse phase-Ultra High performance liquid chromatographic (RP-UHPLC) method for the analysis of Ondansetron solid dosage form is precise and feasible. The separation was carried out on a Phenomenex C18; 3mm X 50 mm; 2.1microns column, using Mobile phase: Mixture of Buffer: Acetonitrile (500:500) with flow rate at 0.3 mL/min and analysis was performed at wavelength 216 nm. The injection volume was 10 µL. The retention time of the drug was 3.571 min. Robustness conditions like Flow minus (0.2ml/min), Flow plus (0.4ml/min), Wavelength minus and Wavelength plus was maintained. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. The method was validated as per ICH norms. The use of short column made method consumable. The method is also cost effective. The proposed method is useful for rapid analysis of Ondansetron in pharmaceutical dosage forms.

Key Words: Reverse phase-Ultra High performance liquid chromatographic (RP-UHPLC), Ondansetron.

INTRODUCTION

Ondansetron [8] is used to prevent nausea and vomiting that is caused by cancer medicines (chemotherapy) or radiation therapy. It is also used to prevent nausea and vomiting that may occur after surgery. Ondansetron works in the stomach to block the signals to the brain that cause nausea and vomiting.

Address for Correspondence: R. Saran**,** M. Pharmacy, M. Pharm Final Year Student, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri affiliated to The TN Dr MGR Medical University, Chennai, Tamil Nadu, **E-Mail:** saranraj3842@gmail.com

How to Cite this Article: R. Saran. Analytical Method Development and Validation of Ondansetron By UHPLC. World J Pharm Sci 2024; 12(01): 18-36; https://doi.org/10.54037/WJPS.2022.100905

Structure[12]:

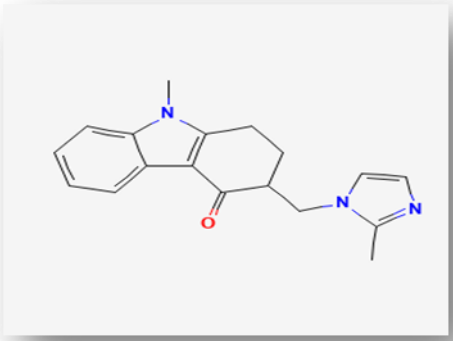


Figure no.1 Structure of Ondansetron

Pharmaceutical Analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical Analysis derives its principle from various branches of sciences like Chemistry, Physics, Microbiology, Nuclear Sciences, Electronics, etc. [1]

Quantitative analysis is the primary way that pharmaceutical analytical techniques are used, while there are many additional uses as well [2].

Pharmaceuticals and drugs are chemicals or similar substances that can be inorganic, organic, or have a different origin. Regardless of their source, we quantify or subjectively assess them using a feature of the medicinal substance [2].

Chromatography [3]:

- The term "chromatography" is derived from Greek, chroma meaning "colour" and graphy meaning "to write".
- Chromatography is the separation of a mixture into individual components using a stationary phase and a mobile phase.

TYPES OF CHROMATOGRAPHY[4]:

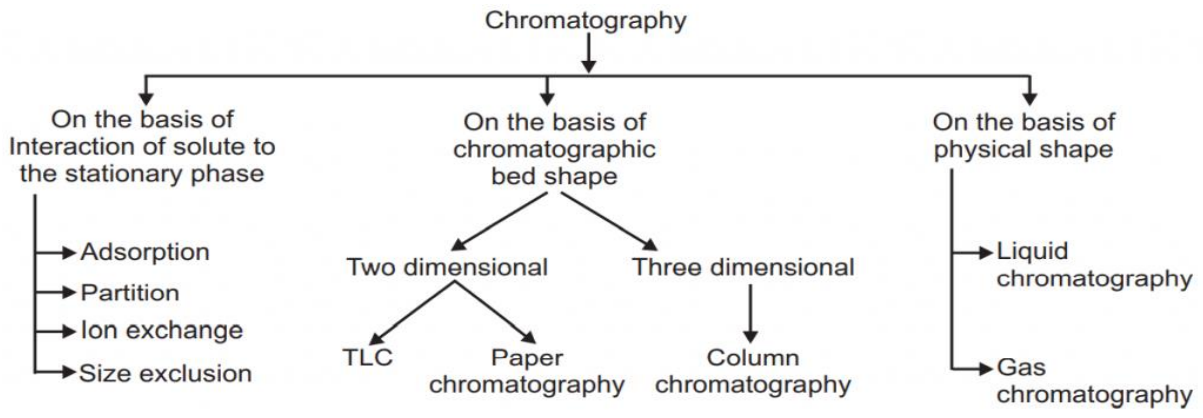


Figure no.2 Chromatography types

ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY [5]:

UHPLC, Ultra-High-Performance Liquid Chromatography is similar to HPLC, in that it is a technique used to separate different constituents of a compound. Used predominately to identify, quantify and separate components of a mixture by using high pressure to push solvents through the column. In UHPLC, particle sizes less than 2um can be used, providing better separation than HPLC where particle size is limited to 5um. These smaller particles require higher pump pressures (100MPa vs.40 MPa, making this technique very efficient with fast analysis and higher resolution.

ANALYTICAL METHOD DEVELOPMENT[6]:

There are more and more new medications coming into the market each year. These medications could be brand-new creations or a partial structural alteration of already-existing ones. The date a medicine is first introduced to the market and the date it is included in pharmacopoeias frequently coincide. This occurs as a result of the potential risks associated with continuing to use these medications more widely, reports of new toxicities that prompt their removal from the market, the emergence of patient resistance, and the launch of more effective medications by rival companies. Studies on specificity, linearity, accuracy, precision, range, detection limit, quantization limit, and robustness are often required for methods for regulatory submission. These studies guarantee that the analytical approach in issue provides timely, accurate, repeatable, and reliable data sufficient for the intended application. The goal of the evolutionary process of HPLC and UPLC technique development and application at every level of the drug development process is to satisfy a pharmaceutical company's business, regulatory, and scientific demands.

ANALYTICAL METHOD VALIDATION[7]:

Validation: A systematic study that serves to demonstrate that processes and systems carry out their intended functions in an appropriate and consistent manner is known as validation.

Validation Parameters:

a) Accuracy

b) Precision

c) Linearity Range

d) Limit of Detection and limit of Quantification

e) Selectivity and Specificity

f) Ruggedness

g) Robustness

h) System suitability.

Principle:

RP-UHPLC are the reported analytical methods for compounds either individually or in combination with other dosage form. Hence, it was felt that, there is a need of new analytical method development for the estimation of Ondansetron by UHPLC in pharmaceutical dosage form.

Chromatographic conditions:

Table No.1 conditions of chromatography

Column	Phenomenex C18; 3mm X 50 mm; 2.1microns
Wavelength	216 nm
Flow Rate	0.3 ml/min
Injection volume	10 µl
Mobile Phase	Mixture of Buffer: Acetonitrile (500:500)

Buffer preparation:

Weigh 2.7 g of potassium dihydrogen phosphate and transfer into clean and dried 1000 ml beaker and add 600 ml water , mix well and make upto volume with water then adjust the pH 5.4 with 1 M sodium hydroxide.

1. Diluents:

Based up on the solubility of the drugs, diluents were selected equal volume of methanol and mobile phase.

2. Preparation of Standard solution:

Weigh accurately and transfer about 40 mg of Ondansetron hcl working standard into a 50 ml volumetric standard flask. Dissolve with Diluents and makeup to the volume with Diluents and further dilution 5 ml in to a 50 ml volumetric standard flask with makeup to the volume with diluents

3. Preparation of Sample solution:

Weigh accurately equivalent to 4mg of sample into 50 ml volumetric standard flask. Dissolve with Diluents and makeup to the volume with diluents.

4. VALIDATION:

System suitability parameters:
The system suitability parameters were determined by preparing standard solution of Ondansetron were injected five times and the parameters like RSD% for Retention time & Area were determined.
The % RSD for the area of five standard injections results should not be more than 2%.

4.1. Specificity:

Checking of the interference in the optimized method. We should not find any interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

4.2. Precision:

4.2.1. Preparation of standard solution:

Weigh accurately and transfer about 40 mg of Ondansetron HCl working standard into a 50 ml volumetric standard flask. Dissolve with Diluents and makeup to the volume with Diluents and further dilution 5 ml in to a 50 ml volumetric standard flask with makeup to the volume with diluents

4.2.2. Preparation of sample solution:

Weigh accurately equivalent to 4 mg of sample into 50 ml volumetric standard flask. Dissolve with diluents and makeup to the volume with Diluents.

4.3. Linearity (for Ondansetron):

4.3.1. Preparation of standard stock solution:

Weighed accurately and transferred about 40mg of Ondansetron hcl standard into 50 ml volumetric standard flask Dissolve with diluents and makeup to the volume with Diluents.

4.3.2. 50% Ondansetron Standard solution:

2.5ml standard stock solutions were pipette out and made up to 50 ml.

4.3.3. 75% Ondansetron Standard solution:

3.75 ml standard stock solutions were pipette out and made up to 50 ml.

4.3.4. 100% Ondansetron Standard solution:

5 ml standard stock solutions were pipette out and made up to 50 ml.

4.3.5. 125% Ondansetron Standard solution:

6.25ml standard stock solutions were pipette out and made up to 50ml.

4.3.6. 150% Ondansetron Standard solution:

7.5ml standard stock solutions were pipette out and made up to 50ml.

4.4. Accuracy (for Ondansetron):

4.4.1. Preparation of Standard solution:

Weigh accurately and transfer about 40 mg of Ondansetron HCl working standard into a 50 ml volumetric standard flask. Dissolve with Diluents and makeup to the volume with Diluents and further dilution 5 ml in to a 50 ml volumetric standard flask with makeup to the volume with diluents.

4.4.2. For preparation of 80% solution:

Weigh accurately and transfer powdered sample equivalent to about 3.2 mg of Ondansetron into 50 ml volumetric standard flask. Dissolve with diluents and makeup to the volume with diluents.

4.4.3. For preparation of 100% solution:

Weigh accurately and transfer powdered sample equivalent to about 4 mg of Ondansetron into 50 ml volumetric standard flask. Dissolve with diluents and makeup to the volume with diluents.

4.4.4. For preparation of 120% solution:

Weigh accurately and transfer powdered sample equivalent to about 4.8 mg of Ondansetron into 50 ml volumetric standard flask. Dissolve with diluents and make up to the volume with diluents.

Procedure:

The standard solution, Accuracy -80%, Accuracy -100% and Accuracy -120% solutions were injected. The amount found and amount added for Ondansetron mean recovery values were calculated and the results were summarized.

Acceptance Criteria:

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

4.5. Robustness:

Small deliberate changes in method like Flow rate and Wavelength were made but there were no recognized change in the result and were within range as per ICH[13] Guide lines.

Robustness conditions like Flow minus, Flow plus, Wavelength decreasing and Wavelength increasing was maintained and samples were injected in duplicate manner. System suitability parameters were not affected and all the parameters were passed. %RSD was within the limit.

SYSTEM SUITABILITY:

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

System suitability parameter for Ondansetron

Table No.2 for system suitability parameter for Ondansetron

Sample ID	ONDANSETRON	
	RT	**AREA**
Injection -01	3.571	3798.294
Injection -02	3.572	3759.254
Injection -03	3.580	3799.625
Injection -04	3.521	3792.451
Injection -05	3.586	3796.257
Average:	**3.566**	**3789.176**
SD:	**0.03**	**16.945**
% RSD:	**0.73**	**0.45**

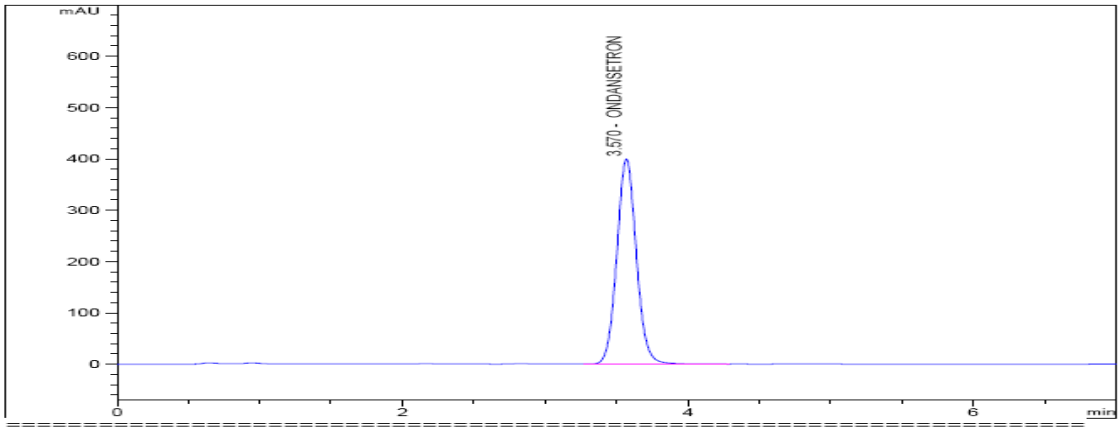


Figure No.3 System suitability Chromatogram

Discussion:

According to ICH guidelines % RSD for Retention and Area should not more than 2.0, all the system suitable parameters were passed and were within the limits.

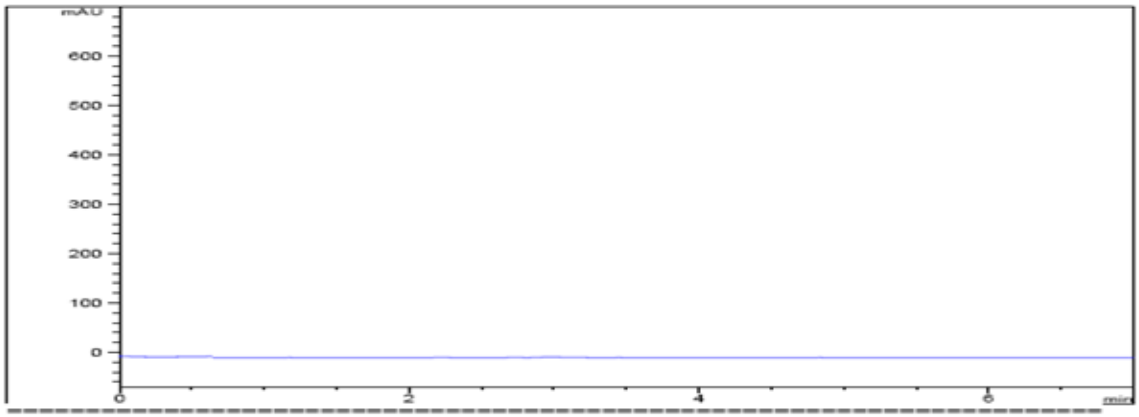


Figure No.4. Chromatogram of blank.

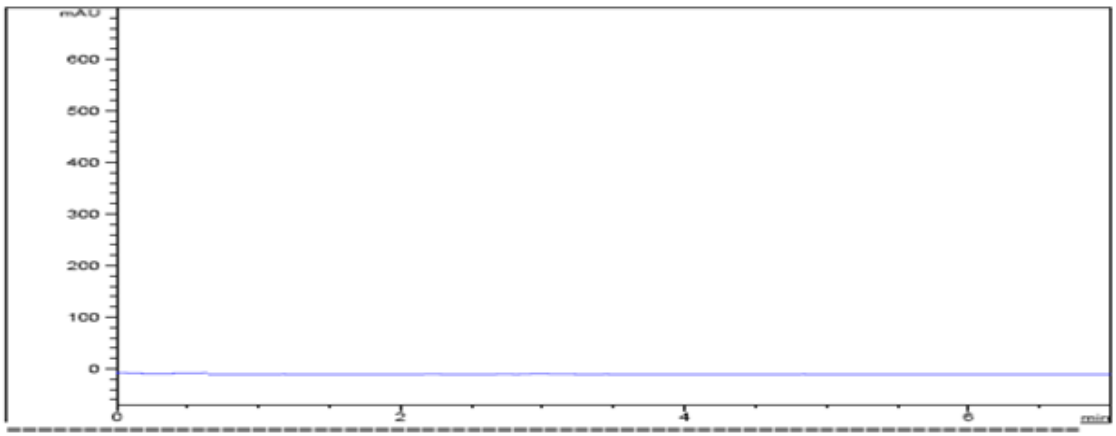


Figure No.5 Chromatogram of placebo

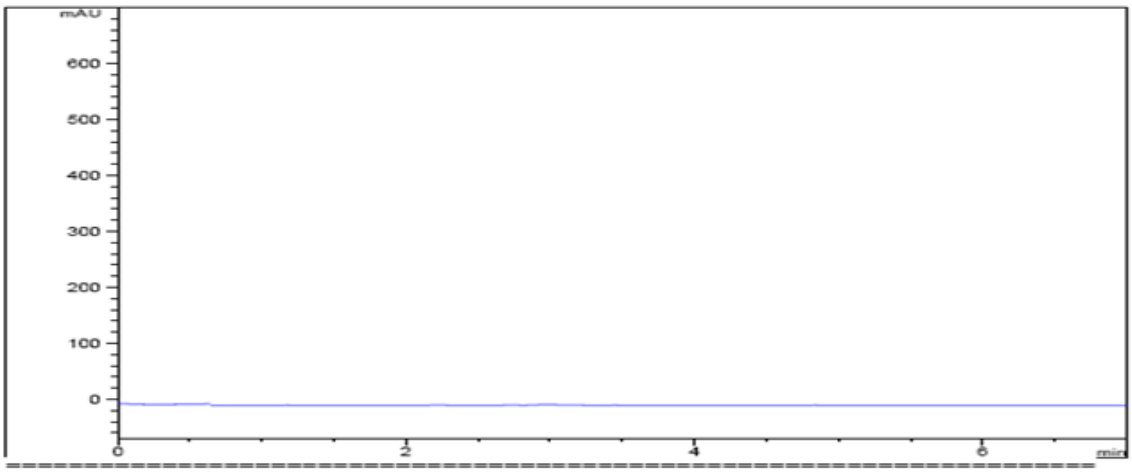


Figure No.6 Typical Chromatogram

Discussion:

Retention time of Ondansetron was eluted at 3.571min. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

PRECISION:

SYSTEM PRECISION:

System precision of Ondansetron

Table No 3. for System Precision of ondansetron

VALIDATION PARAMETER - METHOD PRECISION

LABEL CLAIM: Average weight of tablet : **138.38 mg**

ONDANSETRON 4 mg

Factor : 1.0000

STANDARD DILUTIONS : *Purity of std.* : 90.23 mg

40.36 mg diluted to 50 ml, further 5 ml diluted to 50 ml.

STANDARD VALUES :

3798.294	3759.254	3799.625	3792.451	3796.257

Average : 3789.1762

Standard Deviation : 16.945

% RSD : 0.45

SAMPLE DILUTIONS :

Sample diluted to 50 ml, further 1 ml diluted to 1 ml.

Content in mcg

$$\frac{\text{Spl. Area}}{3789.176} \times \frac{40.36}{50} \times \frac{5}{50} \times \frac{50}{\text{Spl. Wt.}} \times \frac{1}{1} \times \frac{90.230}{100.000} \times 138.38 \times$$

Conc. level	Sample ID	Sample wt. (mg)	Sample Area	Calculated Assay (in mg)	Calculated Assay (in percentage)
MIDDLE LEVEL (100%)	Sample -01	135.69	4158.321	4.076	101.90
	Sample -02	137.21	4100.235	3.974	99.35
	Sample -03	138.47	4124.876	3.962	99.05
	Sample -04	136.25	4129.635	4.031	100.78
	Sample -05	137.44	4198.210	4.062	101.55
	Sample -06	138.26	4187.320	4.028	100.70
			Average :	**4.022**	**100.56**
			SD. :	**0.046**	**1.148**
			% RSD :	**1.14**	**1.14**

Discussion: From a single volumetric flask of working standard solution five injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD was calculated for Ondansetron. % RSD obtained for six different sample solutions as **1.14%** for Ondansetron. As the limit of Precision was less than "2" the system precision was passed in this method.

ACCURACY:

Accuracy of Ondansetron

Table No.4 for Accuracy of Ondansetron

VALIDATION PARAMETER - ACCURACY

LABEL CLAIM: Average weight of tablet: 138.38 mg

ONDANSETRON 4 Factor : 1.0000

STANDARD DILUTIONS : *Purity of std.* : *90.23* IU

40.36 mg diluted to 50 ml, further 5 ml diluted to 50 ml.

STANDARD VALUES :

3798.294	3759.254	3799.625	3792.451	3796.257

Average : 3789.1762

Standard Deviation : 16.945

% RSD : 0.45

SAMPLE PREPARATIONS :

80% sample -01:	110.55	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
80% sample -02:	109.87	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
80% sample -03:	109.96	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
100% sample -01:	138.62	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
100% sample -02:	137.96	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
100% sample -03:	138.26	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
120% sample -01:	165.64	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
120% sample -02:	165.03	mg diluted to	50	ml, further	1	ml diluted to	1	ml.
120% sample -03:	164.27	mg diluted to	50	ml, further	1	ml diluted to	1	ml.

Sample ID	Sample wt. (mg)	Sample Area	Calculated Content (in mg)	Calculated Content (in %)
80% sample -01:	110.55	3356.231	4.038	100.95
80% sample -02:	109.87	3325.610	4.026	100.65
80% sample -03:	109.96	3307.895	4.001	100.03
100% sample -01:	138.62	4166.784	3.998	99.95
100% sample -02:	137.96	4199.320	4.048	101.20
100% sample -03:	138.26	4152.001	3.994	99.85
120% sample -01:	165.64	5000.221	4.015	100.38
120% sample -02:	165.03	4985.210	4.017	100.43
120% sample -03:	164.27	4926.345	3.988	99.70
			Average :	100.35
			SD :	0.514
			% RSD :	0.51

LINEARITY:

Table No.5 for Linearity data of Ondansetron

VALIDATION PARAMETER : LINEARITY

LABEL CLAIM:

ONDANSETRON 4 mg

Std purity : 90.23 *mg*

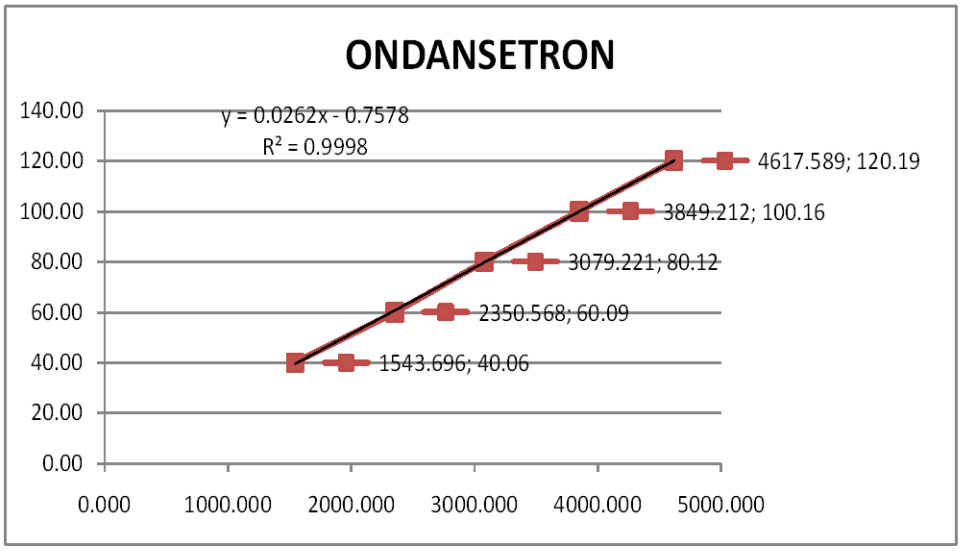
Factor : 1

ONDANSETRON linearity stock dilution:

44.40 mg standard diluted to 50 ml (Stock Soln.)

Soln. ID		*Linearity solution dilutions:*		*Concentrations*
50.0% :	2.50 ml	Stock soln diluted to	50 ml	40.06 mcg/ml
75.0% :	3.75 ml	Stock soln diluted to	50 ml	60.09 mcg/ml
100.0% :	5.00 ml	Stock soln diluted to	50 ml	80.12 mcg/ml
125.0% :	6.25 ml	Stock soln diluted to	50 ml	100.16 mcg/ml
150.0% :	7.50 ml	Stock soln diluted to	50 ml	120.19 mcg/ml

earity Concentrations-->	40.06	60.09	80.12	100.16	120.19
Observed Area-->	1543.696	2350.568	3079.221	3849.212	4617.589



Discussion:

Five linear concentrations of Ondansetron were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Ondansetron y = 0.0262x–0.7578 Correlation coefficient obtained wasR^2 =0.9998 for the Ondansetron respectively.

ROBUSTNESS:

Robustness for Ondansetron

Table No.6 for Robustness data of Ondansetron

S.No	Condition	%RSD of Ondansetron Assay
1	Flow rate (-) _0.2	0.50%
2	Flow rate (+) _0.4	0.79%
3	Wavelength (-)_215	0.95%
4	Wavelength (+)_217	0.52%

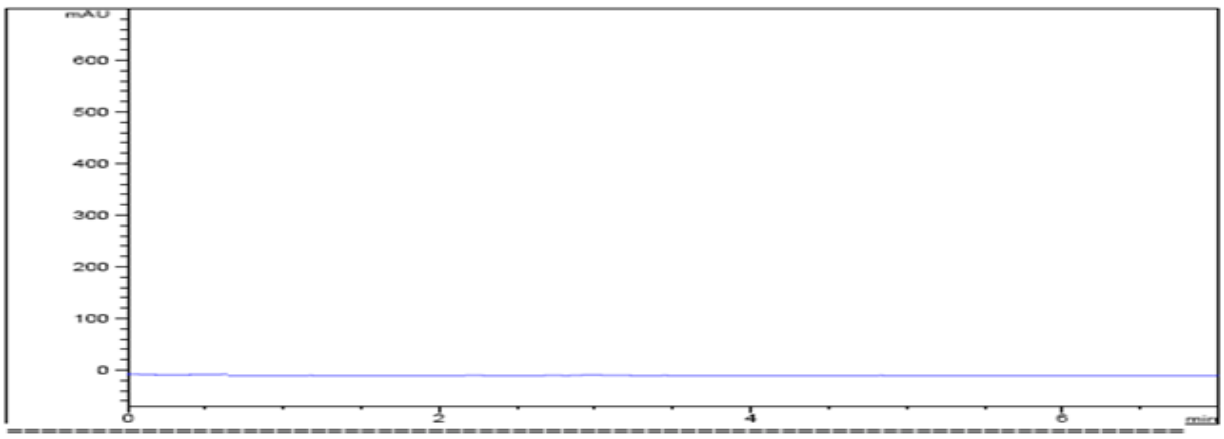


Figure No.7 Flow minus blank Chromatogram of Ondansetron

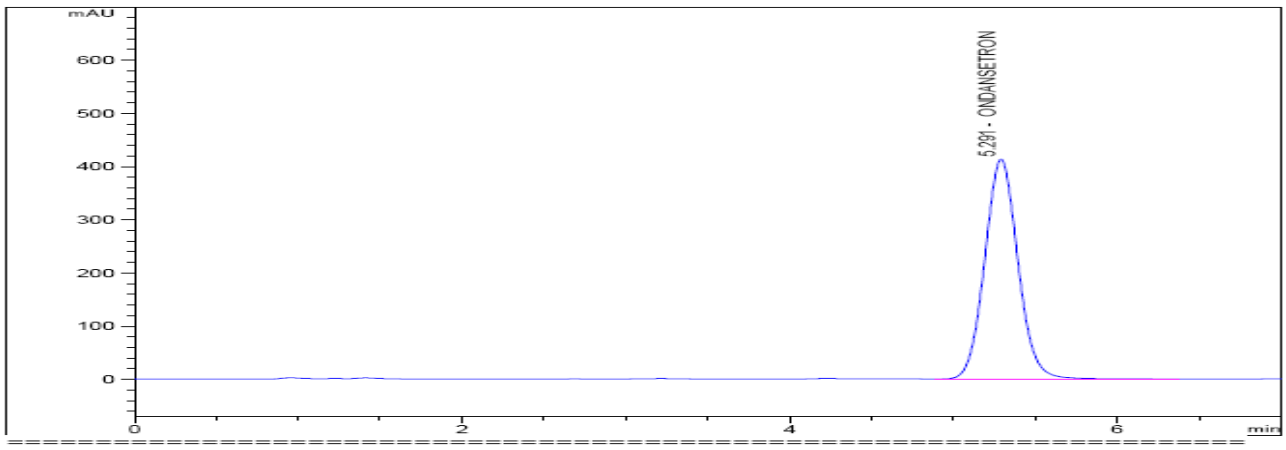


Figure No.8. Flow minus standard Chromatogram of Ondansetron

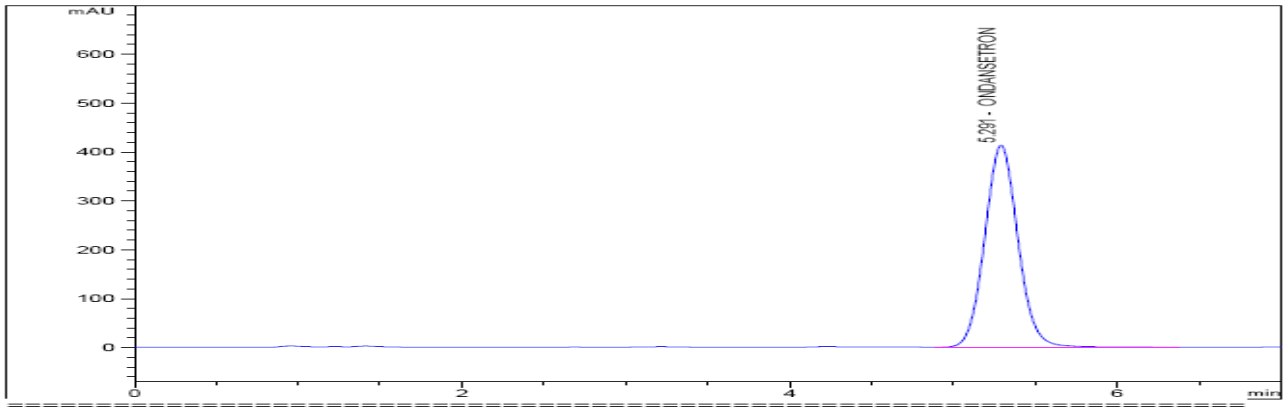


Figure No.9. Flow minus sample Chromatogram of Ondansetron

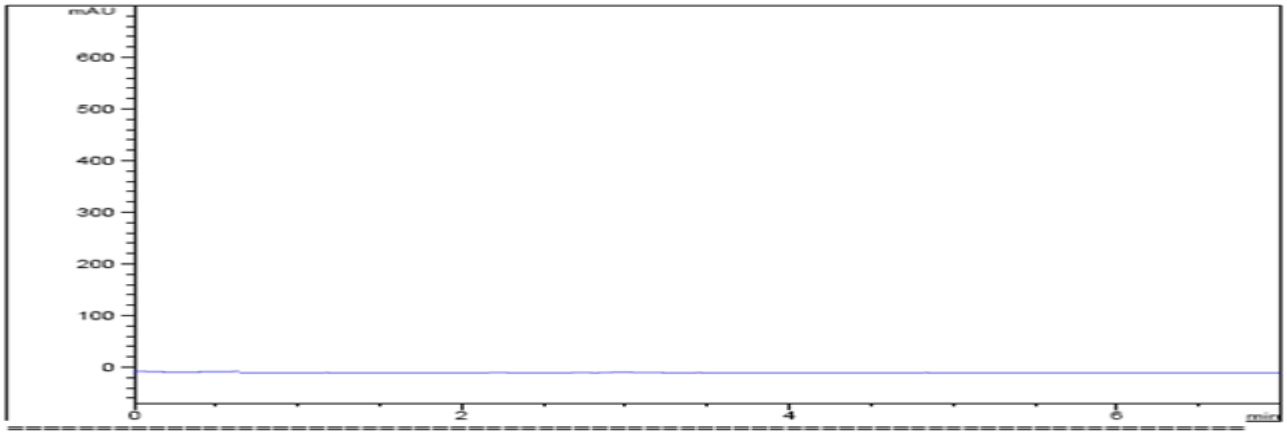


Figure No.10 Flow plus blank Chromatogram of Ondansetron

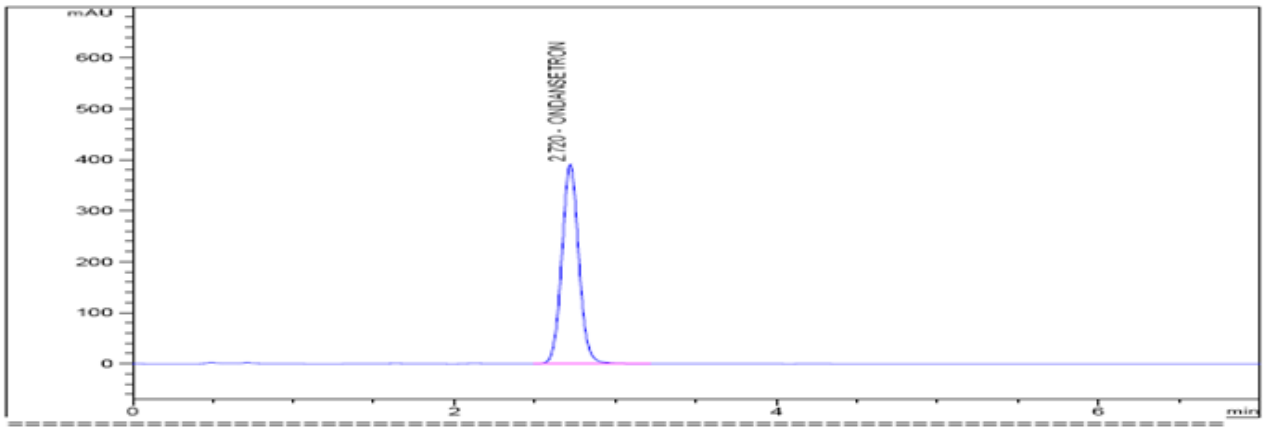


Figure No.11 Flow plus Standard Chromatogram of Ondansetron

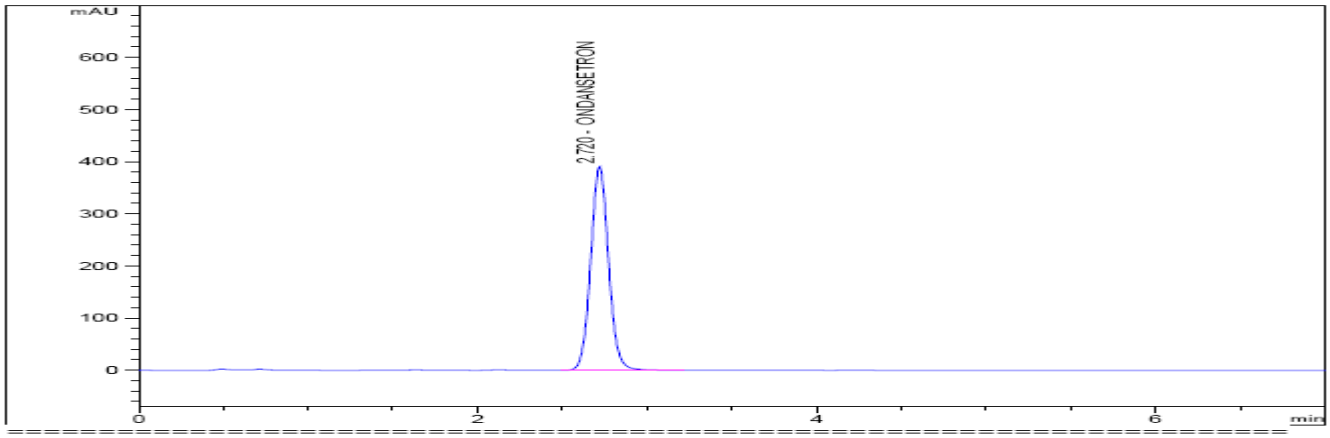


Figure No.12. Flow plus sample Chromatogram of Ondansetron

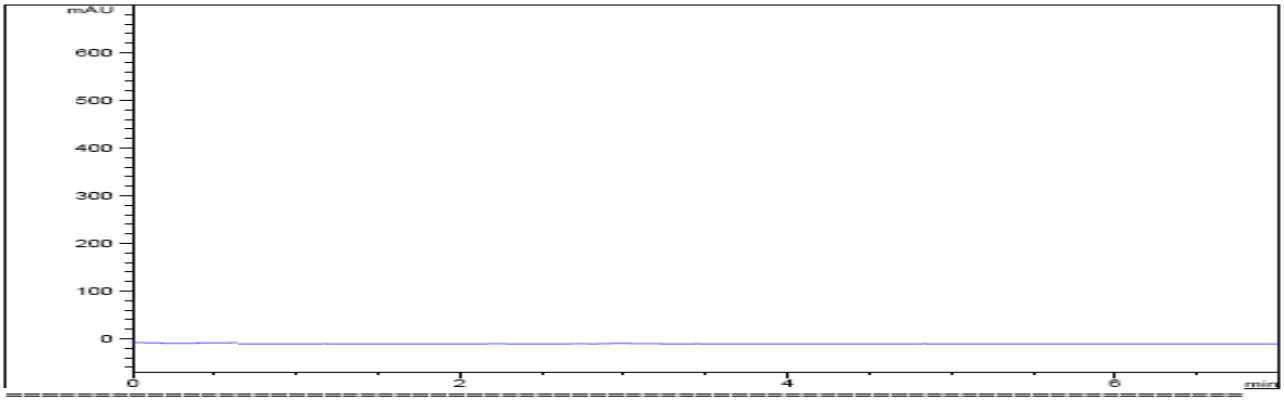


Figure No.13.Wavelength minus blank Chromatogram of Ondansetron

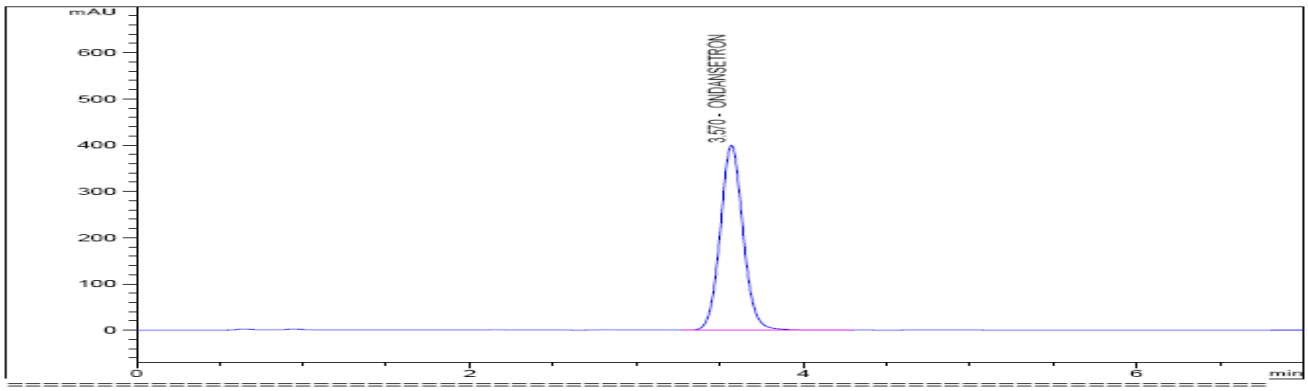


Figure No.14. Wavelength minus standard Chromatogram of Ondansetron

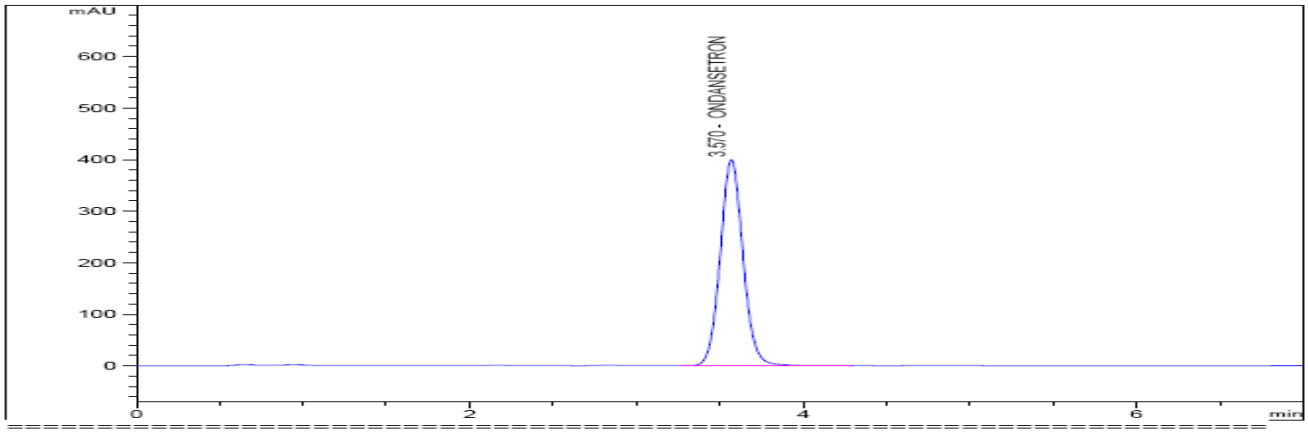


Figure No.15. Wavelength minus sample Chromatogram of Ondansetron

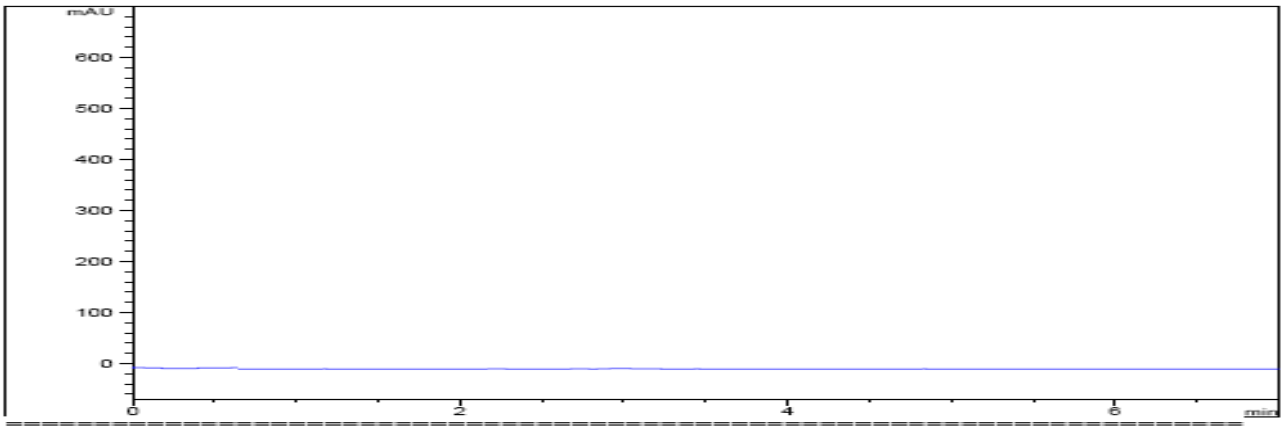


Figure No.16. Wavelength plus blank Chromatogram of Ondansetron

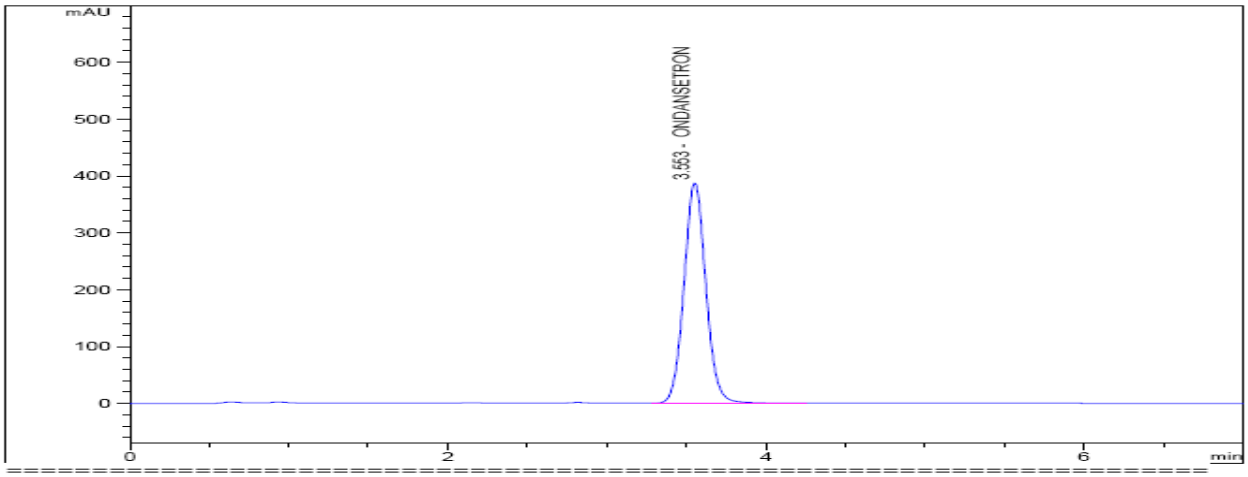


Figure No.17. Wavelength plus standard Chromatogram of Ondansetron

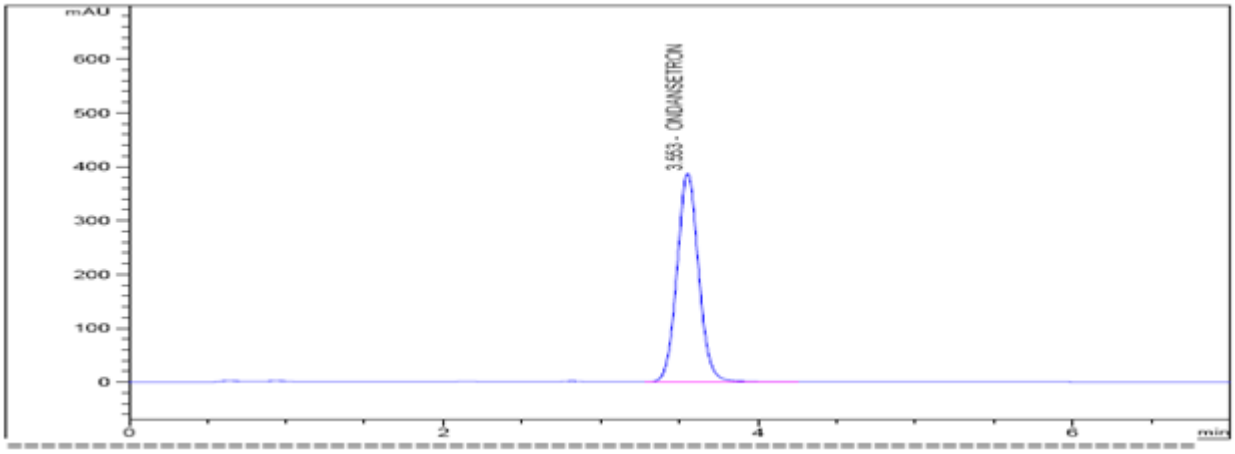


Figure No.18. Wavelength plus sample Chromatogram of Ondansetron

Discussion:

Robustness conditions like Flow minus (0.2ml/min), Flow plus (0.4ml/min), Wavelength minus and Wavelength plus was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

ASSAY:

Assay of Ondansetron

Table No.7 for Assay of Ondansetron

VALIDATION PARAMETER : ASSAY

LABEL CLAIM:

ONDANSETRON 4 mg
Average weight of tablet: 138.38 mg

STANDARD DILUTIONS :

Name of standard : **ONDANSETRON**
Purity of standard : *90.23* *mg (as such)*

40.36 mg diluted to 50 ml, further 5 ml diluted to 50 ml.

SAMPLE DILUTIONS :

138.62 mg diluted to 50 ml, further 1 ml diluted to 1 ml.

STANDARD VALUES :

3798.294	3759.254	3799.625	3792.451	3796.257

Average : 3789
SD : 16.945
% RSD : 0.45

SAMPLE VALUES :

4198.220	

Average : 4198

Content in mg

$$\frac{4198}{3789} \times \frac{40.36}{50} \times \frac{5.0}{50} \times \frac{50}{138.62} \times \frac{1}{1} \times \frac{90.23}{100.00} \times 138.38$$

.= **4.0278 mg**

Content in % *Limits :* *%* *to* *102* *%*

.= **100.70 %**

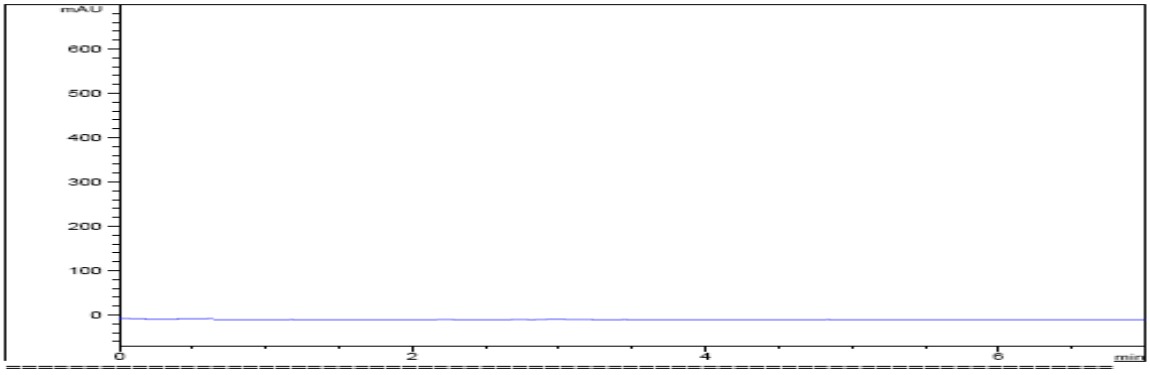


Figure No.19 Assay blank Chromatogram of Ondansetron

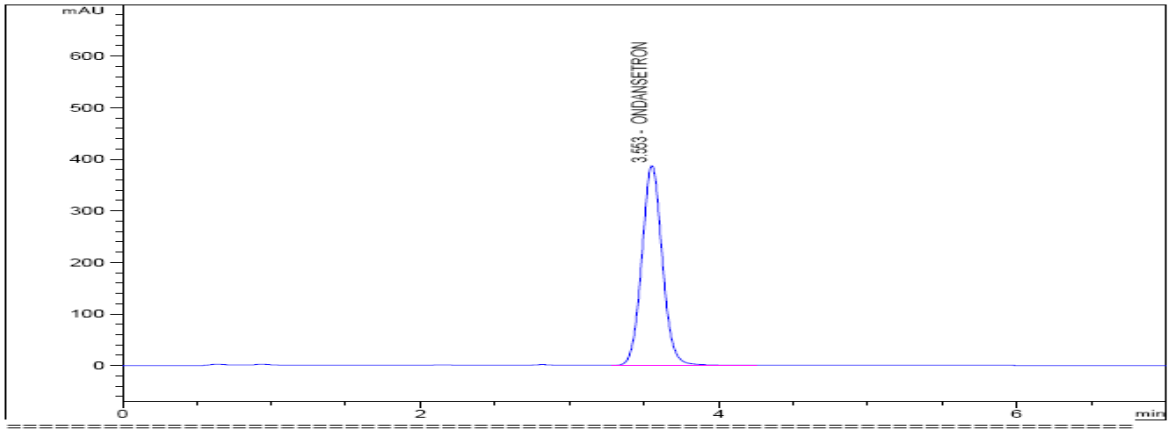


Figure No.20 Assay standard (injection 1) Chromatogram of Ondansetron

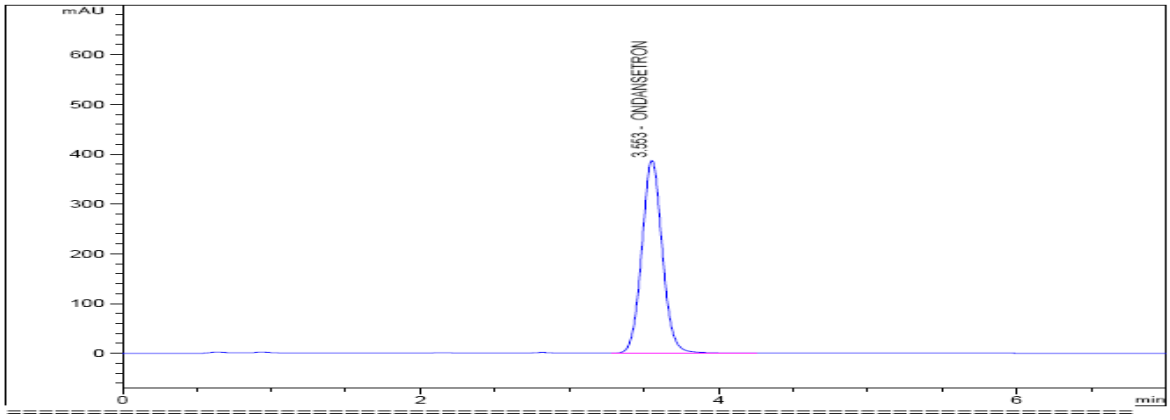


Figure No.21 Assay standard (injection 2) Chromatogram of Ondansetron

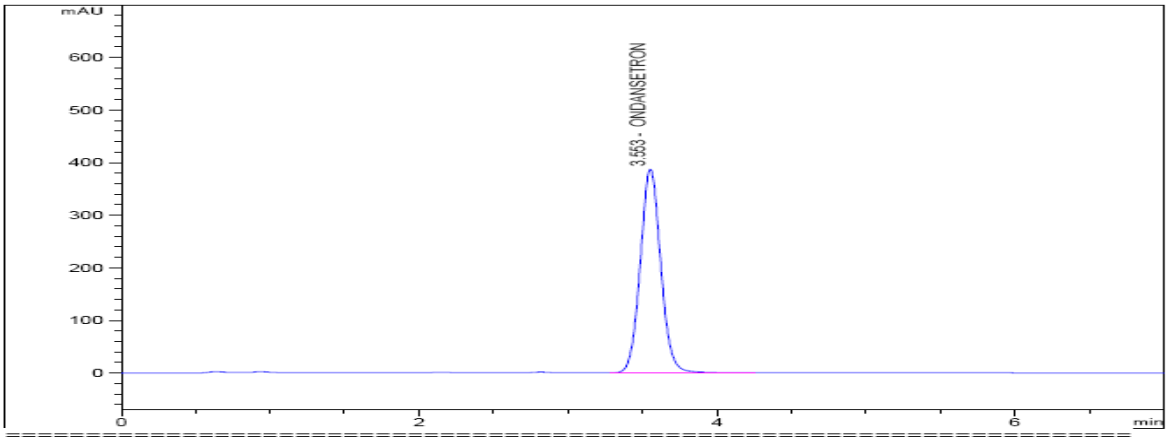


Figure No.22. Assay standard (injection 3) Chromatogram of Ondansetron

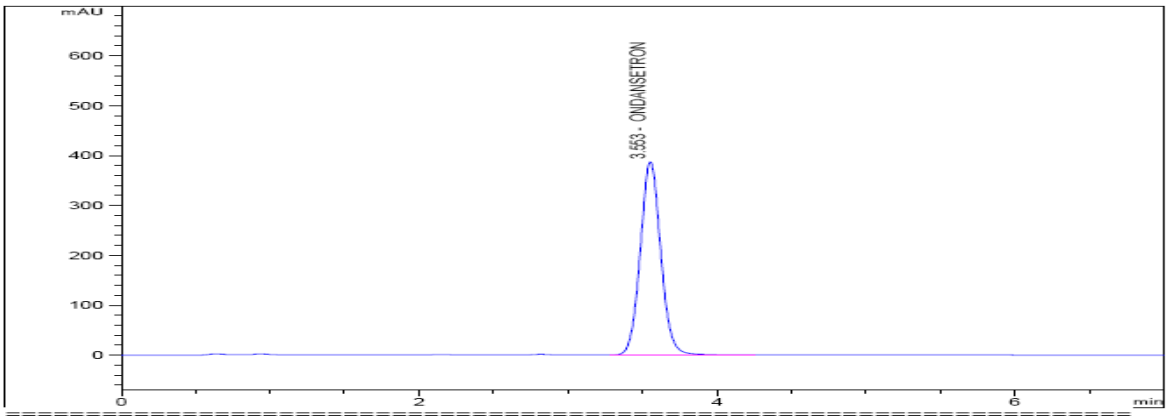


Figure No.22. Assay standard (injection 4) Chromatogram of Ondansetron

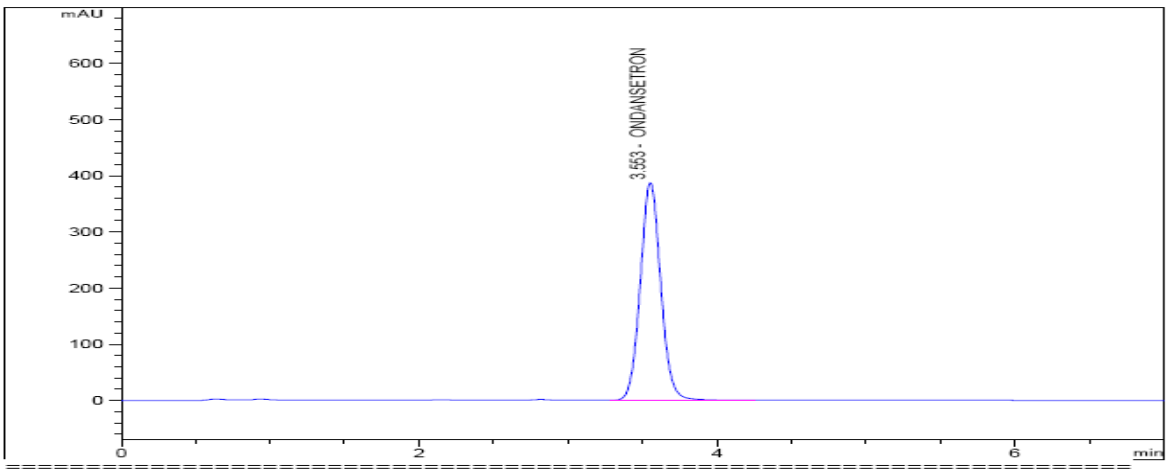


Figure No.23. Assay standard (injection 5) Chromatogram of Ondansetron

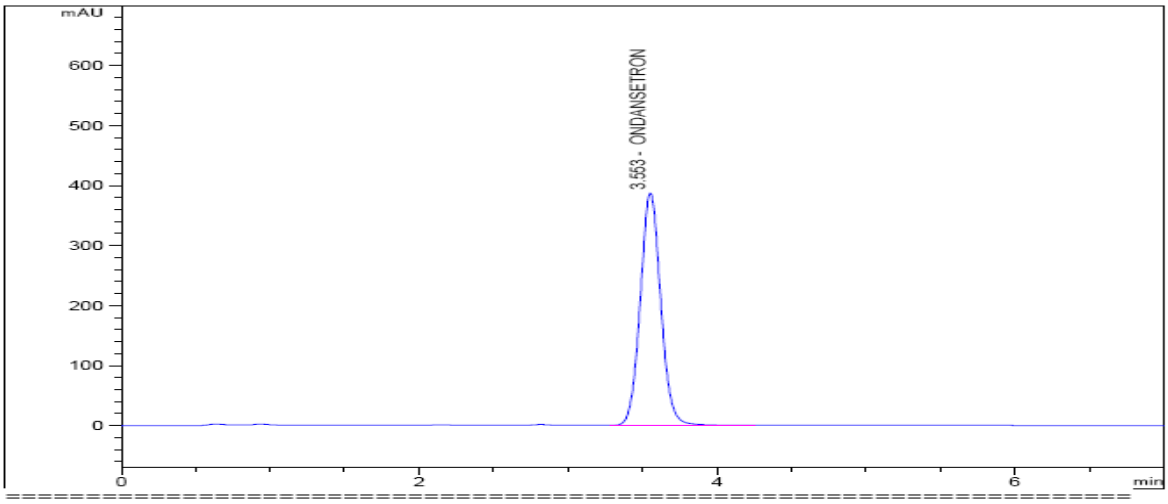


Figure No.24. Assay sample (injection 1) Chromatogram of Ondansetron

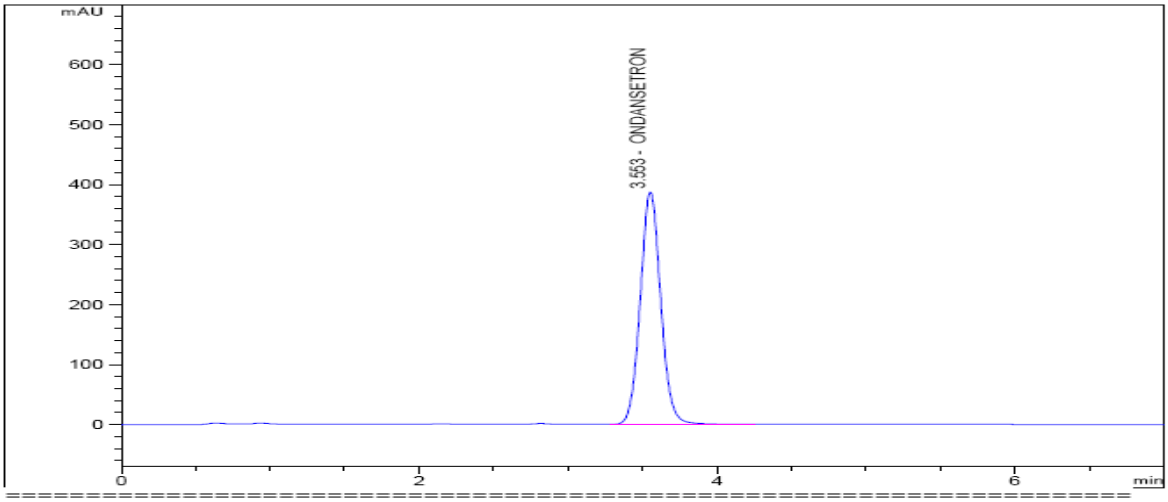


Figure No.25. Assay sample (injection 2) Chromatogram of Ondansetron

Assay:

Assay was performed with the above formulation. Average percentage of Assay for Ondansetron 100.70% respectively.

SUMMARY AND CONCLUSION

Table No.8 for Results of test parameters

Parameters		**Ondansetron**	**Limit**
Linearity: Regression equation(Y=mx+c)		y = 0.0262x–0.7578 (r^2 =0.9998)	r^2 not less than 0.99
Assay **(% mean assay)**		100.70%	98%-102%
Specificity		Complies	No interference of any peak
Method precision %RSD		1.14 %	RSD NMT 2.0%
Intermediate precision day-01 %RSD		0.66%	RSD NMT 2.0%
Intermediate precision day-02 %RSD		1.21%	RSD NMT 2.0%
Accuracy %		99.70% to 101.20%	98-102%
Robustness	**FM**	0.50%	%RSD NMT 2.0
	FP	0.79%	
	WM	0.95%	
	WP	0.52%	

Conclusion:

Method development & validation of ondansetron was done by using UHPLC method. The estimation was done by using Phenomenex C18; 3mm X 50 mm; 2.1microns, mobile phase as Acetonitrile, methanol (500:500) at a flow rate 0.3ml/min. Accuracy parameter is considered accurate if the average recovery is not less than 98% and not more than 102%. Precision parameter RSD of six replicate injections should be NMT 2%. The linearity range of Ondansetron was found to be r^2 =0.9998 in UHPLC. Linear regression was not more than 0.999.the values of %RSD was <2. Specificity for the ondansetron is complies to the result that should not interfere in the peak. Robustness conditions like Flow minus (0.2ml/min), Flow plus (0.4ml/min), Wavelength minus and Wavelength plus was maintained. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. A simple, Accurate, precise method was developed for the simultaneous estimation of the Ondansetron in solid dosage form. Retention time of Ondansetron was found 3.571min. %RSD of the Ondansetron found 0.73%. %Assay was obtained as 100.70% for Ondansetron Regression equation of Ondansetron is y=0.0262. x-0.7578 & r^2 =0.9998. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

BIBLIOGRAPHY:

1. https://www.ipl.org/essay/Pharmaceutical-Analysis-Essay-PCCCP2WVVG
2. Masoom Raza Siddiqui, Zeid A. AlOthman, Nafisur Rahman Analytical techniques in pharmaceutical analysis: A review https: //www.sciencedirect. com/science/article/pii /S1878535213001056.

3. Introduction to pharmaceutical analysis, (internet) cited on 20/03/18 available online at http://en.wikipedia.org/wiki/analysischemistry.https://en.wikipedia.org/wiki/Analysi#:~:text=A)%20Qualitative%20Analysis%3A%20It%20is,a%20given%20sample%20or%20compound.&text=B),find%20concentration%20by%20uv%2Dspectrophotometer.

4. Applications _of _Chromatography. https://byjus.com/chemistry/differential-extraction-chromatography/.

5. https://www.eag.com/app-note/ultra-high-performance-liquid-chromatography-uhplc/.

6. ICH, "Validation of analytical procedures: methodology Q2 (R1)," in Proceedings of The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, IFPMA, November 1996. View at: Google Scholar
7. ICH, "Validation of analytical procedures: methodology Q2 (R1)," in Proceedings of The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, IFPMA, November 1996. View at: Google Scholar
8. http://en.wikipedia.org/wiki/Zofran
9. Acquity UPLC column solution. https://www.waters.com//UPLCColumns 2009.
10. Ondansetron @ 3Dchem.com
11. ICH (International Conference on Harmonization) – Guidelines Q2B.
12. Ondansetron @ 3Dchem.com
13. Long and J.D. winefordnes "Limit of detection". "Anal chem. 1983. 55. 713A.
14. Christian GD. Analytical chemistry. 4th Edn. John Wiley and Sons Publications. New York. 1986.
15. Foye WO. Principles of medicinal chemistry. 6th Edn. Wolter Kluwer Pvt. Ltd. New Delhi. 2008.
16. Douglas A. Skoog, Donal M. West, James Holler F, Stanley R. Crouch. Fundamentals of analytical chemistry. 8th Edn. Cengage Learning India Pvt. Ltd. New Delhi. 2010.