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ORIGINAL ARTICLE

Stability Indicating RP-HPLC Method Development and Validation for Determination of Anti-Emetic Drug in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A new Stability indicating HPLC method has been developed and validated with different parameters for anti-emetic drug in bulk and dosage form. The chromatographic conditions were optimized using a mobile phase of Acetonitrile: Water (0.1 % OPA) (25:75) and drug flows at a rate of 0.7 ml/min. Column (C18) of 4.6 x 250 mm dimension was used as a stationary phase, 5µm was the particle size capacity of column. The detection was carried out at 233 nm. The method was validated according to ICH guidelines for System suitability, linearity, precision (Intraday & Interday), LOD and LOQ and Robustness of the system. The response was found to be linear in concentration range of 4-20 µgm/ml of Ondansetron. The LOD and LOQ were found to be 0.1415 and 0.4289 respectively for Ondansetron. The method was linear, simple, precise and accurate and therefore suitable for routine analysis of drugs in tablet form. The forced degradation studies were also done through exposure of analyte solution to four different stress conditions.

***KEYWORDS**: HPLC, Ondansetron, Development, Validation, Forced degradation*

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INTRODUCTION

There are two types of HPLC: NP-HPLC and RP-HPLC. For this Chromatographic investigation, RP-HPLC has been chosen. The stationary phase in the RP-HPLC process is non-polar, while the mobile phase is polar. In HPLC, chromatographic separation is the outcome of a particular drug-mobile and stationary

phase interaction. Drug solution is run through the column by the mobile phase. The stationary phase is the column. To analyse various substances, HPLC is composed of several components, including a column, detector, degasser, and mobile phase reservoir. Most logically, ondansetron is an anti-emetic. The purpose of ondansetron is to reduce nausea and emesis, or vomiting, that typically occurs during radiation therapy, chemotherapy, etc.

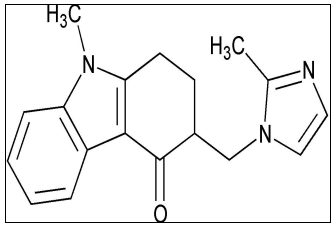

Figure 1: Structure of Ondansetron

MATERIAL AND METHODS

Reagents and Chemicals:

Ondansetron reference standards were supplied by J.B Chemicals, Ankleshwar, India. Pharmaceutical dosage form (Tablet) containing ondansetron was obtained commercially. Methyl alcohol and O-Phosphoric as HPLC grade were used as solvents.

Methodology

Preparation of Mobile phase

A mixture of Acetonitrile and Water (0.1% o-phosphoric acid in water) in the ratio of 25:75 was prepared (filtered and degassed).

Preparation of standard solution

Standard stock solution of Ondansetron was prepared by dissolving 4 mg of Ondansetron in 10 ml MeOH, to get solution containing 400 µgm/ml Ondansetron (STOCK- I). Then, this stock solution is diluted to get solutions containing 4-20 µgm/ml Ondansetron.

Solution Preparation:-

4 ml Injection (containing 4 mg Ondansetron) was transferred into 10 ml volumetric flask and mixed in methanol and then diluted up to the mark. Now, the solution contains 400 µgm/ml Ondansetron (Stock Solution-II).

Assay:-

For assay, take 0.3ml from Stock solution-II and makeup volume upto 10 ml with mobile phase to get 12 µg/ml Ondansetron.

Forced Deg Sample Preparation:

This Study was carried out to check the effective separation of Ondansetron and their degradation product. Forced degradation study was performed to evaluate the stability indicating properties of the method. Forced degradation study was carried out by treating sample with Acid, alkali, Hydrogen peroxide (oxidative degradation), Neutral (Water). These are given below--

1) Acidic Degradation
   0.2 ml sample was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml 1 N Hydrochloric acid (HCL) was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask were removed and cooled to room temperature. The Chromatogram was recorded for this solution.
2) Alkaline degradation
   Sample (0.2 ml) was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml 1 N Sodium hydroxide (NaOH) was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask was removed and cooled to room temperature. The Chromatogram was recorded for this solution.
3) Oxidative degradation
   Sample of 0.2 ml was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml 10 % Hydrogen peroxide (H2O2) was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask was removed and cooled to room temperature. The Chromatogram was recorded for this solution.
4) Hydrolysis
   0.2 ml sample was taken from stock solution-I and transferred in 10 ml volumetric flask. To this, 5 ml water was added, solution was made up to the mark with mobile phase and solution was heated at 60° C for 30 minutes. After 30 minutes flask was removed and cooled to room temperature. The Chromatogram was recorded for this solution.

Method Validation

Method validation was done, to show that the method is suitable for assay and stability studies of Ondansetron. The method validation was carried out as per ICH guidelines for precision, linearity, accuracy, Robustness, LOD and LOQ in analytical solution (ICH 1996, Q2 (R1) ICH, 2005).

i) System Suitability Study

20 µl of standard preparations in five replicates were injected. The chromatogram and peak responses were measured for Ondansetron. System suitability of the method was evaluated in terms of R_t, peak area, theoretical plate, tailing factor and resolution.

ii) Linearity

Linearity of Ondansetron was determined by preparing standard solution at five concentrations in the range of 4-20 µg/ml for Ondansetron from the stock solution. 20 µl of each solution was injected into the HPLC system and the peak area of chromatogram was noted. The standard deviation and % relative standard deviation of peak areas was calculated. Mean AUC was plotted against concentration to obtain the calibration curve. Correlation coefficients were calculated from calibration curve.

iii) Accuracy (Recovery Study)

Recovery experiments were carried out to study the reliability and suitability of standard method at three different levels (80%, 100% & 120%). These samples of Ondansetron were analysed and recovery was calculated.

iv) Precision

- Intraday Precision

Three samples containing known amount of Ondansetron (8, 12 and 16 µg/ml) were put into HPLC system and analysed as per test method and % RSD for Ondansetron were calculated.

- Interday Precision

Three samples containing known amount of Ondansetron (8, 12 and 16 µg/ml) were put into HPLC system and analysed as per test method and % RSD for Ondansetron were calculated.

v) LOD: The LOD value was calculated from calibration curve. SD is the standard deviation of response of minimum detectable drug and slope of calibration curve. Formula for LOD is given below-

Formula- LOD = 3.3×avg S.D/Slope

vi) LOQ

The LOQ value was calculated from calibration curve. SD is the standard deviation of response of minimum detectable drug and slope of calibration curve. Formula for LOQ is given below-

Formula- LOQ = 10×avg S.D/Slope

vii) Robustness

Robustness of the method was studied by varying the chromatographic conditions like change in Mobile Phase Composition, Flow rate and Wavelength. The sample solutions were applied onto the column and the response was determined.

RESULTS AND DISCUSSION

Optimization of Chromatographic condition (Method Development):

Individual drugs and their mixture were taken in various combination of mobile phase for chromatographic study. Proper selection of the method depends upon the nature of the sample (ionic/ionisable/neutral molecule, its molecular weight and solubility). Here, the RP-HPLC method was selected for initial separation because of its simplicity and suitability. Various mobile phases such as acetonitrile and water, acetonitrile and buffer, were tried. Finally, Acetonitrile and Water (0.1% ortho-phosphoric acid in water) were used as mobile phase in the ratio of 25:75 for further chromatographic study. The results of these trials are reported in Table 1.

Table 1: Trials & Optimization of Chromatographic condition

Trial No	Chromatographic condition	Remarks
1	Mobile phase- Acetonitrile: Water (20:80) (0.1 % OPA in water) Flow rate- 0.7 ml/min, λ_{max} –314 nm	Not Sharp Peak **Rejected**
2	Mobile phase- MeOH: Water (30:70) (0.05 % OPA in water) Flow rate- 0.7 ml/min, λ_{max} – 314 nm	Not Sharp Peak **Rejected**
3	Mobile phase- Acetonitrile: Water (25:75) (0.1 % OPA in water) Flow rate- 1 ml/min, λ_{max} – 314 nm	Sharp Peak **Selected**

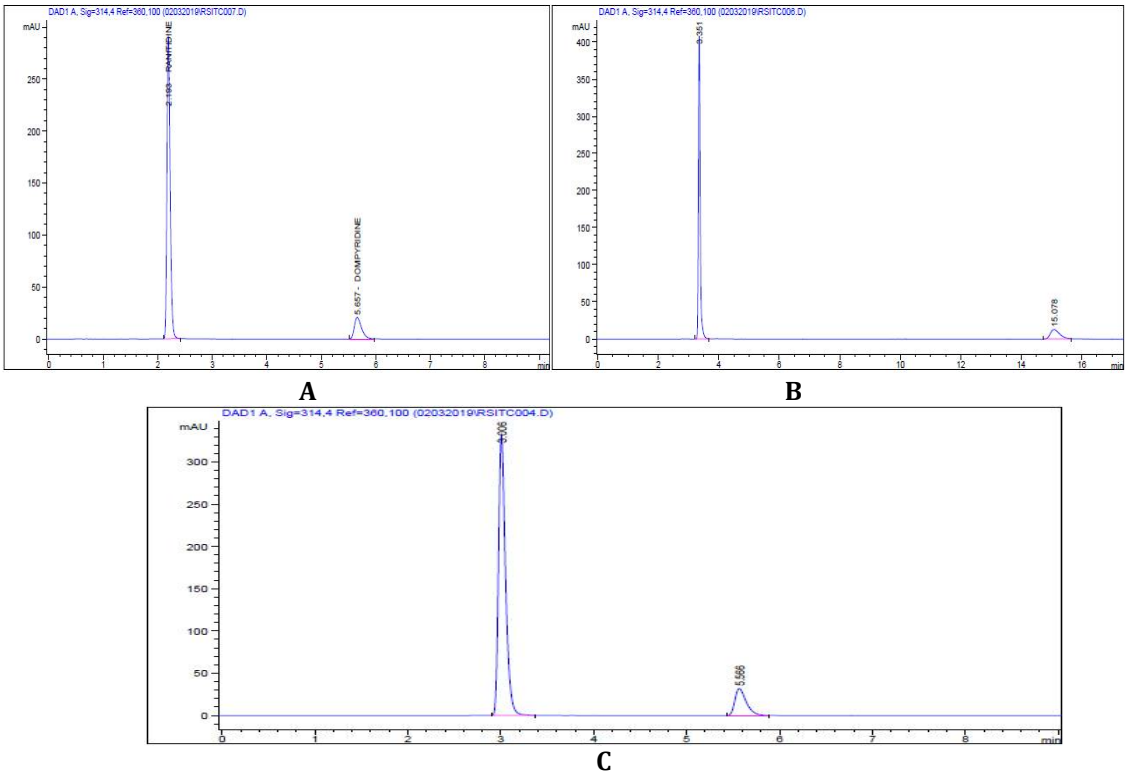

Figure 2: Chromatograms of Trial for Method development

Optimised chromatographic condition:

HPLC : AGILENT (1100) Gradient System with manual injector
Detector : UV
Column : C18 (4.6 x 250 mm id)
Particle size packing :5 µm
Mobile Phase : Acetonitrile: Water (0.1 % OPA) (25:75)
Detection wavelength: 314 nm
Flow rate : 1 ml/min
Temperature : Ambient
Sample Size : 20 µl

i) System suitability test (Repeatability)

System Suitability Tests were carried out on standard solutions containing Ondansetron. System suitability parameters are obtained with 20µl injection volume. Retention time (mean) was found to be 6.536 for Ondansetron. Theoretical plates, tailing factor (peak asymmetry) and resolution were observed. It is summarized in Table 2.

Table 2: System suitability test (Repeatability)

Sr. No	RT[min]	Area[mV*s]	TP	TF	Resolution
Ondansetron					
1	6.536	2554.60	6440	0.41	15.21
2	6.536	2535.63	6448	0.41	15.21
Mean	6.536	2545.12	6444	0.41	15.21

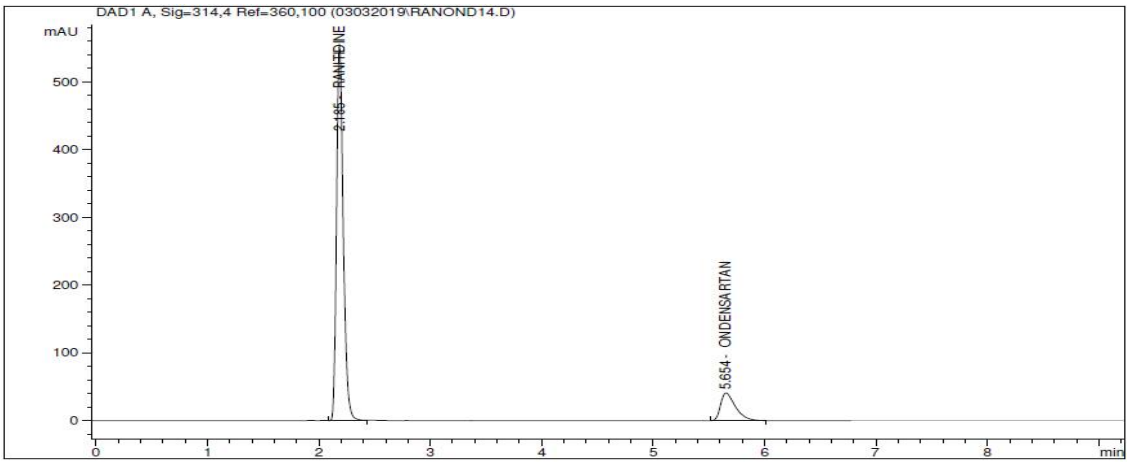

Figure 3: Chromatogram of System suitability Test

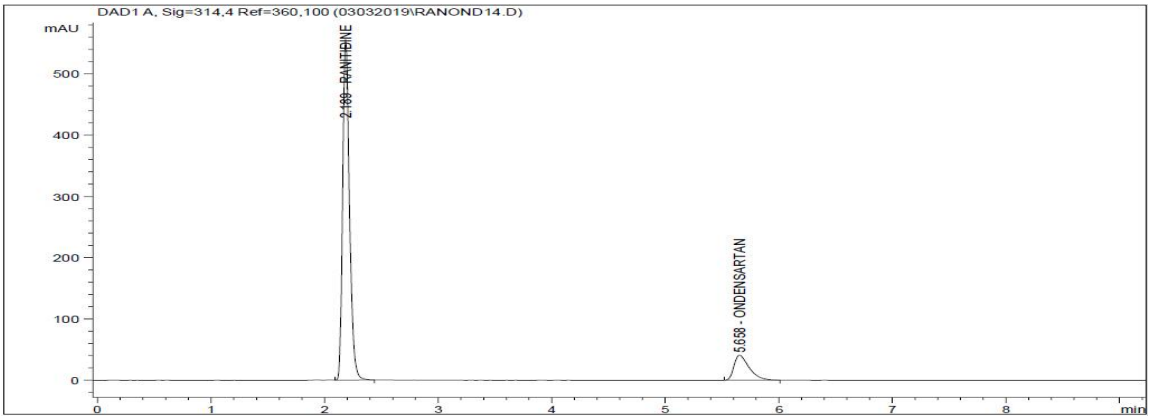

Figure 4:Chromatogram of System suitability Test

Linearity

The concentrations ranging from 4-20 µgm/ml of Ondansetron were injected and peaks were recorded. In these concentrations baseline were obtained for both the drugs. The correlation coefficient (r^2) value was 0.999 for the drug. The graph was plotted as a concentration of drug versus peak area is depicted in figure 6.

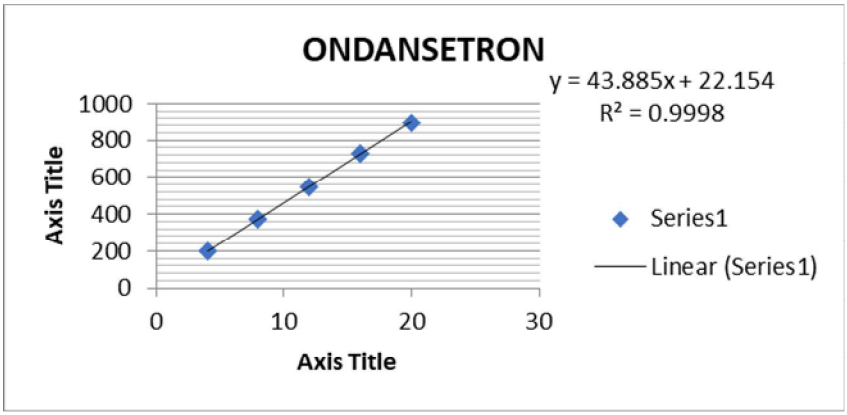

Figure 5 : Standard calibration curve of Ondansetron

Table 3- displayed the linearity study of Ondansetron. Ondansetron used in a concentration range of 4 to 20 µgm/ml. The mean areas of different concentration obtained were 197.07, 375.56, 545.11, 730.13 and 896.70. The %RSD for these concentrations was 0.71, 0.19, 0.27, 0.22 and 0.28 respectively.

Table 3: Linearity study

Sr No.	Conc.	Area I	Area II	Mean	SD	%RSD
Ondansetron						
1	4	198.02	196.12	197.07	1.42	0.71
2	8	375.01	376.11	375.56	0.66	0.19
3	12	544.22	546.23	545.11	1.42	0.27
4	16	729.03	731.22	730.13	1.55	0.22
5	20	895.03	899.06	896.70	2.64	0.28

Precision

Intraday Precision

Table - displayed the study of Interday Precision of Ondansetron. For this, Ondansetron is used in a concentration of 8-16 µgm/ml. The % of amount found for these concentrations were 101.25, 99.33 and 101.25 respectively. The %RSD for these concentrations was 0.89, 0.44, and 0.39.

Table 4: Intraday Precision

Sr No.	Conc.	Area I	Area II	Mean	Amt Found	% Amt Found	SD	%RSD
Ondansetron								
1	8	381.09	375.23	377.81	8.10	101.25	100.45	0.89
2	12	542.05	545.21	545.21	11.92	99.33	2.38	0.44
3	16	731.01	735.26	733.22	16.20	101.25	2.88	0.39

Accuracy

Table 6.42- displayed the Accuracy (% Recovery) study of Ondansetron. For this, Ondansetron also used in a concentration of 80%, 100% and 120%. The mean of % amount recovered of these concentrations was 100.90, 101.56 and 100.58 respectively.

Table 5: Accuracy (% Recovery study)

Sample Conc.	µgm/ml	Amt added	Area	Amt found	Amt rcvd	% rcvd	Mean	SD	%RSD
Ondansetron									
80%	8	6.4	655.65	14.43	6.43	100.57	100.90	0.46	0.46
	8	6.4	657.45	14.47	6.47	101.22			
100%	8	10	730.58	16.14	8.14	101.80	101.56	0.34	0.33
	8	10	728.89	16.10	8.10	101.32			
120%	8	9.6	799.7	17.71	9.71	101.24	100.58	0.93	0.93
	8	9.6	795.14	17.59	9.59	99.92			

Robustness (Ondansetron)

Table 6.41- displayed the Robustness study of Ondansetron. Robustness studies of System were performed by changing the flow rate, M.P concentration and wavelength. The mean found were 606.81 and 496.87 for the flow rate of 0.6 ml/min and 0.8 ml/min respectively. The %RSD found were 0.80 and 0.90 for the flow rate of 0.6 ml/min and 0.8 ml/min respectively. The mean found were 540.9 and 540.76 for Mobile Phase Concentration (74:26) and Mobile Phase Concentration (76:24) respectively. The %RSD found were 0.35 and 0.55 for Mobile Phase Concentration (74:26) and Mobile Phase Concentration (76:24) respectively. The mean found were 562.60 and 523.06 for Wavelength (232 nm) and Wavelength (234 nm) respectively. The %RSD found were 0.66 and 0.35 for Wavelength (232 nm) and Wavelength (234 nm) respectively. This study shows that system is robust and withstand by changing different aspects of system.

Assay of Marketed formulation:

Marketed formulation (Doran-D Injection) of Ondansetron was analysed and % purity was determined. The mean of % assay was found to be 99.51 for Ondansetron respectively in tablet. The result of assay is shown in Table 6.43 and chromatograms are shown in fig. 6.185 and 6.186.

Forced Degradation Study

Standard sample of Ondansetron was undergone acidic, alkaline, Oxidative and Hydrolytic degradation. The sample shows 15%, 14%, 17% and 0.1% degradation in acidic, alkaline, Oxidative and Hydrolysis conditions respectively. The degradation was under acceptance criteria. It shows stability indicating properties of the method. The chromatograms of sample are shown in Fig 6.187 to 6.190.

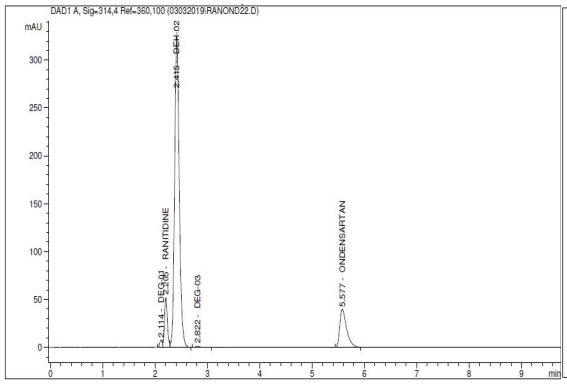

Figure 6: Acidic degradation (0.1 N HCL)

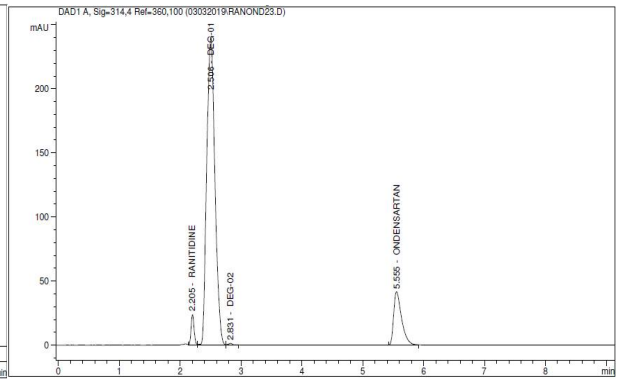

Figure 7: Alkaline degradation (0.1 N NaOH)

CONCLUSION

The aim of current chromatographic study was to develop Stability indicating HPLC method for the estimation of Ondansetron in bulk and dosage form. Hence, Stability indicating RP-HPLC method has been developed and validated for LOD and LOQ. Forced degradation study was also performed under four different stress conditions.From the chromatographic study, we concluded that developed method is more linear, accurate, precise, reliable and reproducible for routine analysis of Ondansetron in bulk and Pharmaceutical dosage form. So one can perform validation and forced degradation study.

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Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

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