IJBPAS, April, 2022, 11(4): 1957-1967

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ONDANSETRON AND RANITIDINE IN BULK AND TABLET DOSAGE FORMS

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**Received 25th April 2021; Revised 26th May 2021; Accepted 30th July 2021; Available online 1st April 2022**

https://doi.org/10.31032/IJBPAS/2022/11.4.6033

## ABSTRACT

Attempts were made to accurate, precise and reproducible develop RP-HPLC method for estimation of Ondansetron and Ranitidine from tablet. For the RP HPLC Agilent (S.K) method Gradient System UV Detector and C18 column with 250mm x4.6 mm i.d and 5µm particle size methanol: 0.1% water with OPA (40:60v/v) was used as the mobile phase for the method. The detection wavelength was 245 nm and flow rate was 0.8 ml/min. In the developed method, the retention time of Ondansetron and Ranitidine were found to be 4.02 and 5.52 min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Ondansetron and Ranitidine bulk drug as well as in formulations.

**Keywords: Ondansetron and Ranitidine, method development, validation, simultaneous estimation, RP- HPLC**

## INTRODUCTION

Ondansetron (OND) is chemically, 9-Methyl-3-[(2-methyl-1 H-imidazol-1-yl) methyl]-1,2,3,9-tetrahydro-4 H-carbazol-4-one. The empirical formula of OND is $C_{18}H_{19}N_3$ and it has a molecular weight of 293.37g/mole. The OND 5-HT3 Receptor antagonists are the primary drugs used to treat and prevent chemotherapy-induced nausea and vomiting**.** Ranitidine (RTD) is chemically N-(2-[(5-[(dimethylamino) methyl] furan-2-yl) methylthio] ethyl)-N'-methyl-2-nitroethene-1,1-diamine; dimethyl (5-{[(2-{[1-(methyl-amino)-2-nitroethenyl] amino}ethyl) sulfanyl] methyl}furan-2-yl) methyl] amine.

It has an empirical formula of $C_{13}H_{22}N_4O_3S$ and a molecular weight of 314.4 g/mole**, (Figure 1)**. RAN is a histamine-H2 receptor antagonist. RAN is used to reduce the amount of acid secreted by the stomach which subsequently reduces ulcer and heartburn pain. It is used to treat stomach and duodenal ulcer and Gastroesophageal reflux Disease **[1, 2]**. The clinical study proved that the combination of OND and RAN is effective in reducing the incidence of nausea and vomiting **[3]**.

Both the drugs are official in Indian Pharmacopoeia, British Pharmacopoeia and United States Pharmacopoeia **[4-6]**. A literature survey regarding quantitative analysis of these drugs revealed that attempts have been made to develop analytical methods for the estimation of OND alone and in combination with other drugs by Spectrophotometric method **[7-9],** HPLC [**10, 11]** and HPTLC **[12]** methods. Literature survey revealed that Spectrophotometric method **[13, 14],** HPLC **[15-17]** and HPTLC **[18]** have been reported for the estimation of RAN.

There are two HPLC **[19, 20]** and Spectrophotometric method **[21],** reported for the estimation of OND and RTD in combined dosage form. Present study involves development and validation of stability indicating liquid chromatographic method for the estimation of OND and RAN in combined dosage form.

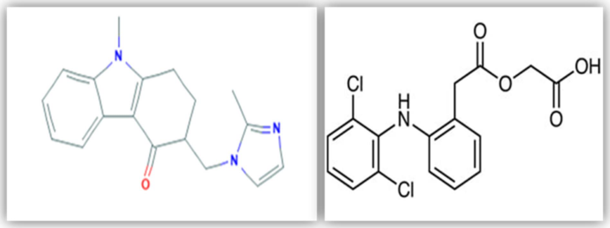

**Figure 1: Structure of Ondansetron and Ranitidine**

## MATERIALS AND METHODS

### Materials and Reagents

The analysis of the drug was carried out on Agilent (S. K.) Gradient System UV Detector. Equipped with Reverse Phase (Grace) C18 column (4.6mm x 250mm; 5µm), a SP930D pump, a 20µl injection loop and UV730D Absorbance detector and running autochro-3000 software. The API of both drugs OND and RTD procured from R.S.I.T.C Jalgaon. Orthophopsphoric acid (OPA), methanol, water (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), 0.45µm filter (Millipore, Bangalore). A combination of OND (150 mg) and RAN (4 mg) in tablet formulation was procured from local pharmacy (Ranidom- O (150:4) mg (Bestochem Formulation).

### Chromatographic Conditions

Column C18 (250 mm× 4.6mm); particle size packing 5µm ; detection wavelength 245 nm; flow rate 0.8 ml/min; temperature ambient; sample size 20 µl; mobile phase methanol: water (OPA 0.1%) (40:60); run time 15 min.

### Preparation of standard stock solution

10 mg of Ranitidine and 4 mg of Ondansetron was weighed accurately and transferred to separate 10 ml volumetric flask dissolved in methanol and diluted to 10ml with the solvent (Methanol**:** Water, 60 : 40v/v) to give a stock solution of 1000 µgm/ml Ranitidine and 4000 µgm/ml Ondansetron **(Table 1 and Figure 2)**.

### Method development and validation:

Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected **[21-22]**.

### Assay preparation for commercial formulation

For analysis of the tablet dosage form, Weigh 20 Ranitidine and Ondansetron combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 5 mg Ranitidine and 10 mg Ondansetron into 10 ml volumetric flask. Add about 10ml MEOH of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (10µg/ml). The simple chromatogram of test Ranitidine and Ondansetron. The amounts of Ranitidine and Ondansetron per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for % Label claim for % RSD Calculated, result is shown **in (Table 2 and Figure 3)**.

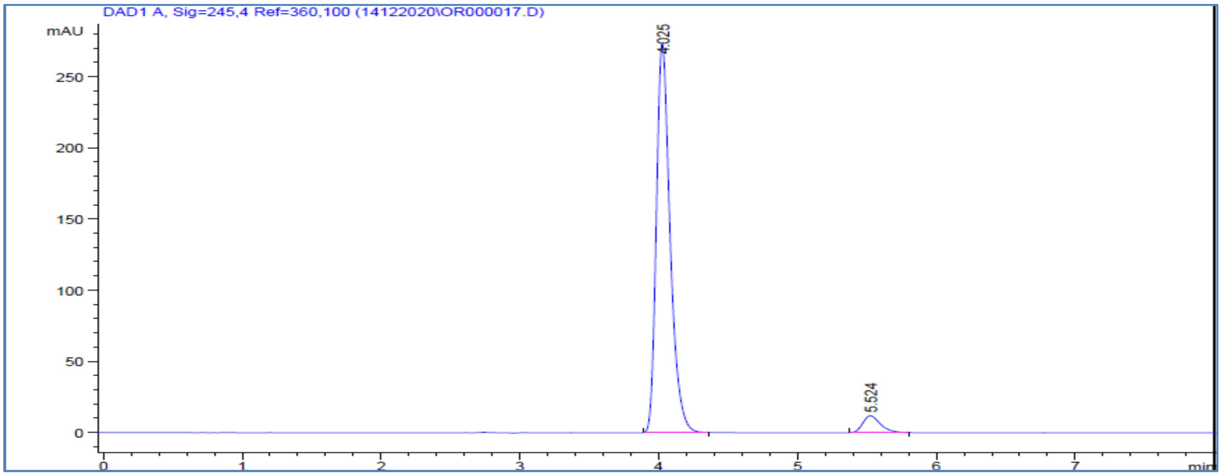

**Figure 2: Chromatogram of standard mixture of Ranitidine and Ondansetron**

**Table 1: Details of chromatogram of standard mixture Ranitidine and Ondansetron**

| Drug name | R.T | AREA | SYMM | TH.PLATES |
|---|---|---|---|---|
| RTD | 4.025 | 1887.48 | 0.69 | 8514 |
| ODN | 5.52 | 106.49 | 0.69 | 9750 |

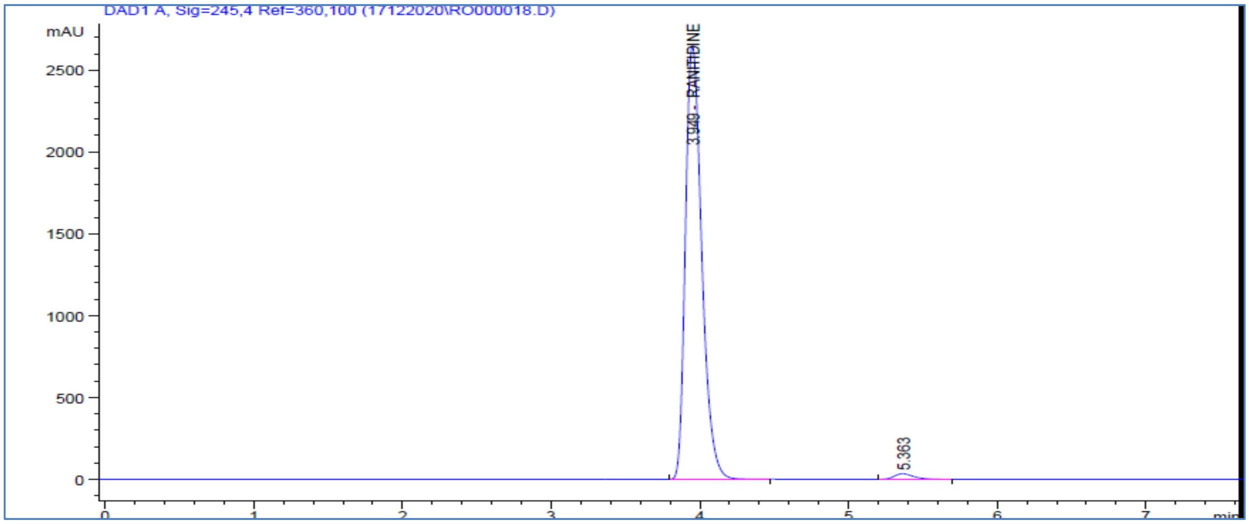

**Figure 3: Chromatogram of Ranitidine and Ondansetron in tablet formulation**

**Table 2: Analysis of marketed formulation**

| Assay | Drug | Amt. Found | % Label Claim | SD | %RSD |
|---|---|---|---|---|---|
| Rp-HPLC Method | RTD | 616.09 | 102.68 | 0.10 | 0.94 |
| | OND | 16.26 | 101.62 | 0.14 | 0.90 |

## RESULTS

### Linearity and Range:

From Ranitidine and Ondansetron standard stock solution, different working standard solutions (20-100μg/ml) were prepared in mobile phase. Likewise from Ranitidine (10-50 μg/ml) and Ondansetron (4-20 μg/mL) standard stock solution different working standard solutions were prepared in mobile phase. 20 μl of sample solution was injected into the chromatographic System using fixed volume loop injector. Chromatograms were recorded. The area for each concentration was recorded **(Table 3 and 4)**. The Calibration curves are shown in **(Figure 4 and 5)**.

### Accuracy:

Recovery studies were performed to validate the accuracy of developed method. To pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) **(Figure 6, 7 and 8)** was added and then its recovery was analyzed**.** Statistical validation of recovery studies shown in **(Table 5 and 6)**.

### System suitability parameters:

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Ranitidine and Ondansetron system suitability parameters were studied. The result shown in below **(Table 7)**.

### Precision:

The method was established by analyzing various replicates standards of RTD and OND. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result. The result obtained for interday and intraday variation are shown in the **(Table 8)**.

### Robustness

The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed **(Table 9)**.

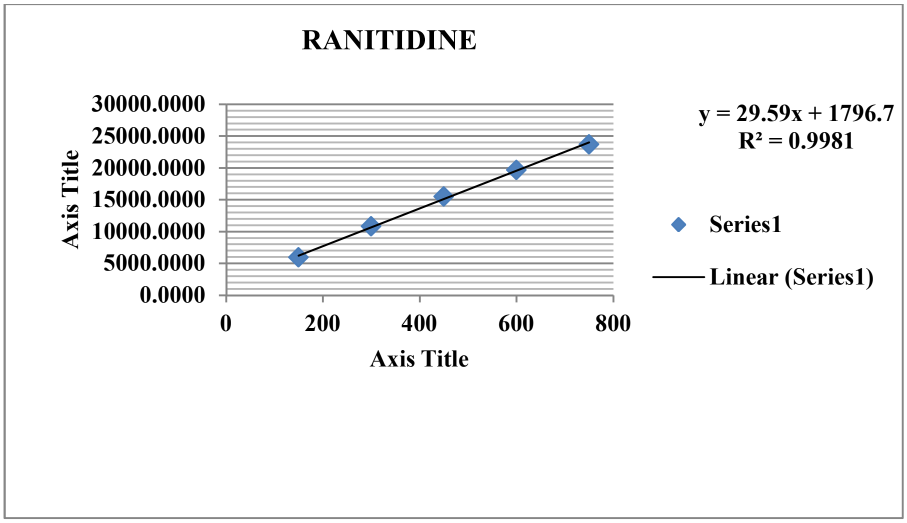

**Figure 4: Calibration curve of Ranitidine**

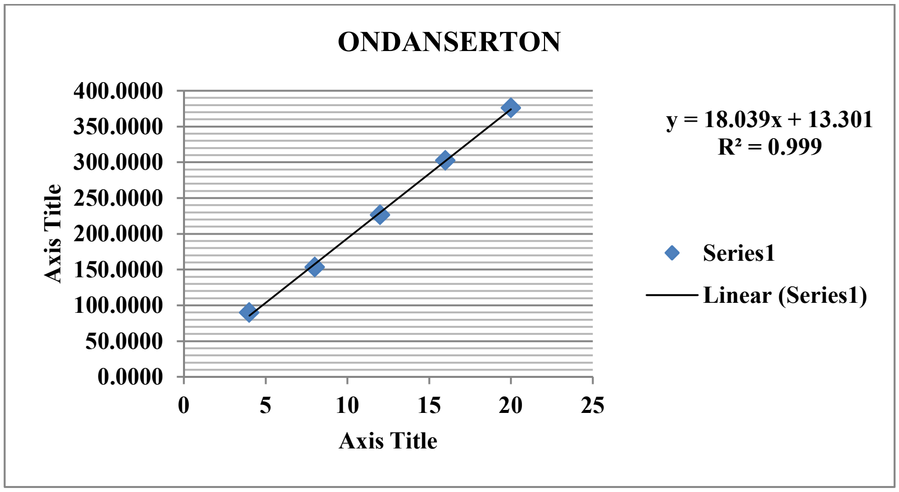

**Figure 5: Calibration curve of Ondansetron**

**Table 3: Linearity data for Ranitidine**

| Method | Conc. µg/ml | Peak area(µV.sec) | | Average peak area (µV.sec) | S.D. of Peak Area | % RSD of Peak Area |
|---|---|---|---|---|---|---|
| | | 1 | 2 | | | |
| UHPLC Method | 150 | 5928.8798 | 5911.0532 | 5919.9665 | 12.6053 | 0.2129 |
| | 300 | 10788.6000 | 10793.6000 | 10791.1000 | 3.5355 | 0.0328 |
| | 450 | 15510.9000 | 15508.9002 | 15509.9001 | 1.4141 | 0.0091 |
| | 600 | 20101.7000 | 20164.5000 | 20133.1000 | 44.4063 | 0.2206 |
| | 750 | 23604.5000 | 23748.9000 | 23676.7000 | 102.1062 | 0.4313 |
| | Equation | | y =18.039 x + 13.301 | | | |
| | $R^2$ | | 0.999 | | | |

**Table 4: Linearity data for Ondansetron**

| Method | Conc. µg/ml | Peak area (µV.sec) | | Average peak area (µV.sec) | S.D. of Peak Area | % RSD of Peak Area |
|---|---|---|---|---|---|---|
| | | 1 | 2 | | | |
| UHPLC Method | 4 | 89.6401 | 89.98993 | 89.8150 | 0.2474 | 0.2754 |
| | 8 | 153.5121 | 153.7319 | 153.6220 | 0.1554 | 0.1012 |
| | 12 | 226.2617 | 226.8419 | 226.5518 | 0.4103 | 0.1811 |
| | 16 | 302.9356 | 302.8251 | 302.8804 | 0.0781 | 0.0258 |
| | 20 | 375.3798 | 376.5409 | 375.9604 | 0.8210 | 0.2184 |
| | Equation | | y = 18.039 X + 13.301 | | | |
| | $R^2$ | | 0.999 | | | |

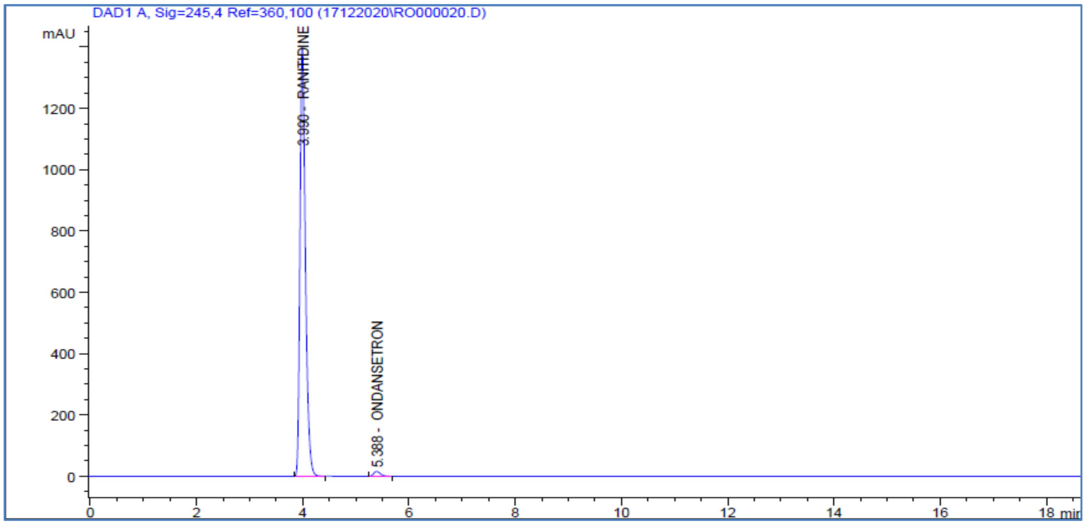

**Figure 6: Chromatogram of Accuracy 80%**

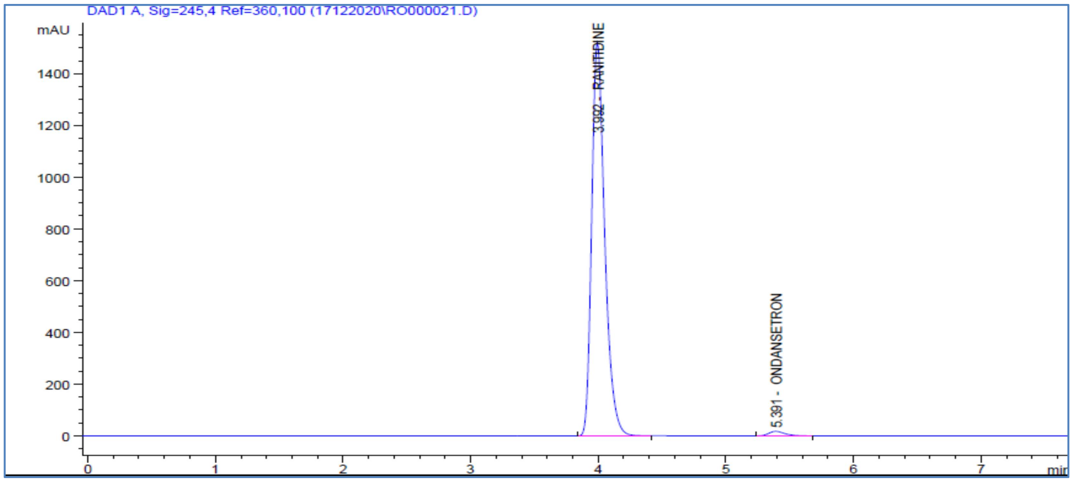

**Figure 7: Chromatogram of accuracy 100%**

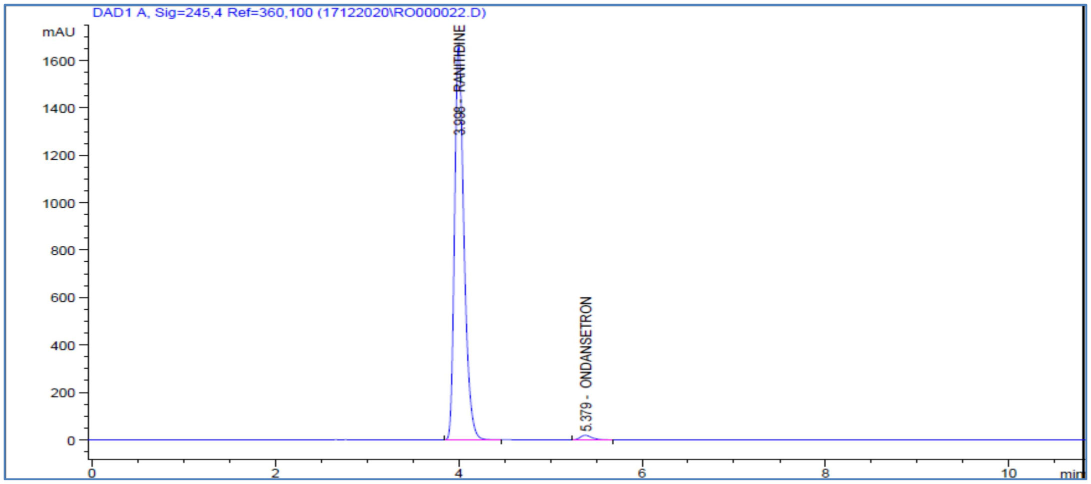

**Figure 8: Chromatogram of accuracy 120%**

**Table 5: Result of Recovery data for Ranitidine and Ondansetron**

| Method | Drug | Level (%) | Amt. taken (µg/ml ) | Amt. Added (µg/ml) | Absorbance Mean* ± S.D. | Amt. recovered Mean *±S.D. | %Recovery Mean *± S.D. |
|---|---|---|---|---|---|---|---|
| RP-HPLC Method | RTD | 80% | 150 | 120 | 272.02±0.23 | 112.02±0.23 | 101.68±0.19 |
| | | 100% | 150 | 150 | 303.87±0.30 | 20.58±0.30 | 102.58±0.20 |
| | | 120% | 150 | 180 | 335.48±0.19 | 20.58±0.18 | 102.54±0.60 |
| | OND | 80% | 4 | 3.2 | 7.23±0.04 | 3.24±0.05 | 101.191.39± |
| | | 100% | 4 | 4 | 8.0.13±0.04 | 20.58±0.04 | 100.034±1.24 |
| | | 120% | 4 | 4.8 | 8.82±0.32 | 20.58±0.32 | 100.46±0.68 |

***mean of each 3 reading for RP-HPLC method**

**Table 6: Statistical Validation of Recovery Studies Ranitidine and Ondansetron**

| Method | Level of Recovery (%) | Drug | % RSD | S.D.* | Mean % Recovery |
|---|---|---|---|---|---|
| RP-HPLC Method | 80% | RTD | 0.19 | 0.19 | 101.68 |
| | | OND | 1.37 | 1.39 | 101.19 |
| | 100% | RTD | 0.19 | 0.20 | 102.58 |
| | | OND | 1.24 | 1.24 | 100.34 |
| | 120% | RTD | 0.58 | 0.60 | 102.54 |
| | | OND | 0.67 | 0.68 | 100.46 |

**Table 7: Repeatability studies on RP-HPLC for Ranitidine and Ondansetron**

| Method | Conc. of RTD and OND (mg/ml) | Peak area | Amount found (mg) | % Amount found |
|---|---|---|---|---|
| HPLC RTD Method | 450 | 15127.8 | 451.6390 | 100.3642 |
| | 450 | 15192.2 | | |
| | Mean | 15160.00 | | |
| | SD | 0.53 | | |
| | %RSD | 0.30 | | |
| | 12 | 226.84 | 11.90 | 99.20 |
| HPLC ODN Method | 12 | 229.02 | | |
| | Mean | 227.93 | | |
| | SD | 1.54 | | |
| | %RSD | 1.16 | | |

**Table 8: Intraday and Inter day Precision studies on RP-HPLC method for RTD & OND**

| Method | Drug | Conc. (µg/ml) | Interday Precision | | Intraday Precision | |
|---|---|---|---|---|---|---|
| | | | Mean± SD | %Amt Found | Mean± SD | % Amt Found |
| Rp-HPLC Method | ONDA | 8 | 154.8657±0.96 | 98.1454 | 157.27±0.65 | 99.8125 |
| | | 12 | 228.1781±0.45 | 99.3150 | 228.84±0.69 | 99.5833 |
| | | 16 | 306.0242±0.27 | 101.6250 | 307.22±0.60 | 101.8856 |
| | RANI | 300 | 10862.1500±0.96 | 102.1307 | 10797.78±0.65 | 101.4056 |
| | | 450 | 15642.8000±0.52 | 102.9900 | 15593.47±0.92 | 102.6196 |
| | | 600 | 20072.4000±0.14 | 102.9424 | 20001.73±0.68 | 102.5443 |

***Mean of each 3 reading for RP-HPLC method**

**Table 9: Robustness study of OND and RTD**

| Parameters | Conc. (µg/ml) | Amount of detected (mean ±SD) | %RSD | Amount of detected (mean ±SD) | % RSD |
|---|---|---|---|---|---|
| | | For ONDA | | For RTD | |
| Chromatogram of flow change 0.7 ml | 8+300 | 135.16±0.87 | 0.61 | 12419.75±0.78 | 0.01 |
| Chromatogram of flow change 0.9 ml | 8+300 | 137.17±0.18 | 0.64 | 9733.88±0.76 | 0.79 |
| Chromatogram of comp change wavelength change 258 nm | 8+300 | 179.1±0.73 | 0.66 | 12505.2±0.95 | 0.04 |
| Chromatogram of comp change wavelength change 260 nm | 8+300 | 155.73±0.54 | 0.47 | 10960.50±1.41 | 0.01 |
| Chromatogram of mobile phase change 69+31 ml | 8+300 | 182.0±0.20 | 0.30 | 12260.8±0.91 | 0.18 |
| Chromatogram of mobile phase change 71+29 ml | 8+300 | 173.91±0.37 | 1.26 | 12447.18±0.52 | 0.79 |

## DISCUSSION

The proposed methods for simultaneous estimation of RTD and OND in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration yielded correlation coefficient ($r^2$) 0.999 & 0.999 for RTD and OND respectively at all the selected wavelengths and the values were average of three readings. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 99-101% for both the drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of RTD and OND in formulations.

## CONCLUSION

The developed HPLC methods were found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of these methods showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The methods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

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