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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RANITIDINE AND ONDANSETRON IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

An accurate, specific and precise UV spectrophotometric method was developed for the simultaneous determination of Ranitidine (RAN) and Ondansetron (OND) in injection dosage form. The optimum conditions for the analysis of the drug were established. The maximum wavelength (λmax) was found to be 230 nm for RAN and 216 nm for OND. The linearity of the proposed method was found in the range of 50-200 μg/ml and 8-32 μg/ml for RAN and OND respectively. Calibration curves showed a linear relationship between the absorbance and concentration. The line equation for RAN Y = 0.0222X - 0.0226 with r2 of 0.9999 and for OND Y = 0.0292X - 0.0149 with r2 of 0.9998 was obtained. Validation was performed as per ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The LOD was found to be 0.0454 and 0.656 μg/ml for RAN and OND and the LOQ was found to be 0.151 and 2.18 μg/ml for RAN and OND respectively. The proposed method was simple, sensitive, precise, accurate, quick and useful for routine analysis of RAN and OND in injection formulations.

KEYWORDS: Simultaneous equation method, Validation, Ranitidine, Ondansetron, UV spectrophotometry.

INTRODUCTION

Ranitidine and Ondansetron is combined dosage form used to treat immediate side effects with cancer chemotherapy are nausea and vomiting & Gastric ulcers exist in patients who following cancer chemotherapy so we need therapy for that. The combination of Ranitidine & Ondansetron drugs are very effective for the treatment of nausea, vomiting & Gastric ulcers. In combination, these drugs do not show any adverse pharmacokinetic interaction.

Ranitidine hydrochloride, chemically 1,1- ethenediamine-N-[2-[[[5[(dimethylamino)methyl]-2-furanyl]-methyl]thio]ethyl]-N.-methyl-2-nitro hydrochloride is an H2-receptor antagonist indicated for the duodenal ulcer1. Literature survey reveals that for ranitidine hydrochloride HPLC3,4, spectrophotometric5 and capillary electrophoresis6,7 methods have been reported for its determination from human plasma and commercial formulation. Ondansetron hydrochloride, chemically 4H-carbazol-4-one-1,2,3,9-tetrahydro- 9-methyl-3-[(2-methyl-1H-imidazole-1-yl) methyl] hydrochloride is a selective 5-HT3 receptor antagonist indicated for the prevention of nausea and vomiting2.

Three HPLC8-10 and one LC11 methods have been reported in literature for estimation of ondansetron hydrochloride from human plasma and commercial formulation. However no spectrophotometric method is yet reported for simultaneous analysis of two drugs from combined pharmaceutical dosage form.

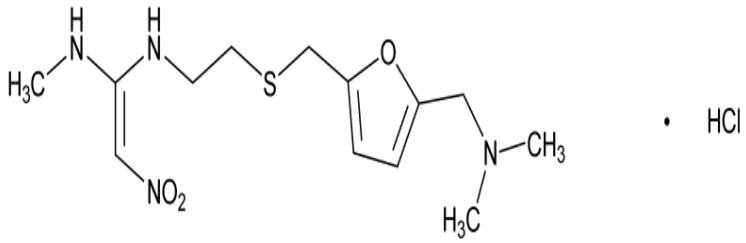

Fig no: 1 Structure of Ranitidine (RAN)

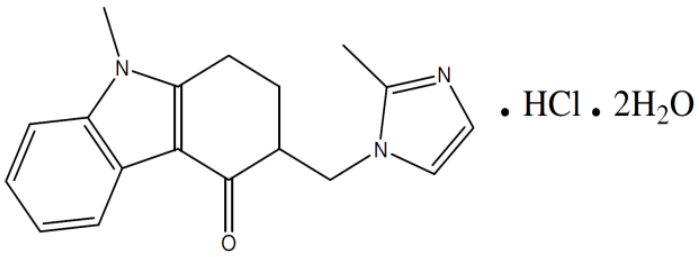

Fig no: 2 Structure of Ondansetron (OND)

The method was further validated as per ICH guidelines (ICH Q2(R1), 2005) for the parameters like precision, accuracy, sensitivity, and linearity. The result of analysis was validated statistically and by recovery studies.

MATERIAL AND METHODS

Samples

Ranitidine (RAN) & Ondansetron (OND) was obtained as gift sample from Ranbaxy Laboratories Limited, Devas, India. The pharmaceutical formulation DORAN-O Injection (Ranitidine - 50 mg and Ondansetron - 8 mg) used in this study was procured from local market, which was manufactured by Bestochem Formulations (India) Ltd.

Reagents

Methanol (GR grade) was obtained from Merck (India) Ltd, Mumbai.

Instruments

UV-Visible double beam spectrophotometer (UV- 3200 LAB INDIA) with 1cm matched quartz cells, digital balance (K- Roy Electronic), hot air oven (CLE-101, coslab) was used in the study.

Standard Stock Solution

Standard stock solution containing Ranitidine (500 µg/ml) and Ondansetron (80 µg/ml) was prepared by transferring 50 mg Ranitidine and 8 mg Ondansetron working standard into a 100 ml volumetric flask. A 40 ml portion of diluent was added, sonicated and cooled to room temperature. The solution was diluted to the mark with diluent. Standard solutions was prepared by pipetting (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 ml) ml stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent. The Aliquot was analysed using HPLC with prior degassing, sonication and filtration through 0.45 µm.

Calibration standards of 50, 75, 100, 125, 150, 175 and 200 µg/ml for Ranitidine & 08, 12, 16, 20, 24, 28 and 32 µg/ml for Ondansetron were prepared from the stock solutions.

Test preparation

Each vial of sample contains 50 mg Ranitidine & 8 mg Ondansetron (2.0 ml) was diluted with methanol, shaken and filtered. The filtrate was evaporated to dryness and the residue was dissolved in mobile phase and volume was made up to 100 ml.

Sample solution containing Ranitidine (100 µg/ml) and Ondansetron (16 µg/ml) was prepared by pipetting 10 ml stock solution into a 50 ml volumetric flask and diluted up to the mark with diluent. and the mixture was sonicated for 15 min with intermittent shaking. The stock solution was filtered through 0.45 µm membrane filter. The concentration of the drug present in formulation was computed from the calibration curve using the equation y= mx + c.

Determination of λmax

The standard solution of RAN and OND were separately scanned at different concentrations in the range of 200-400 nm and the λmax was determined.

Preparation of calibration curve: For each drug, appropriate aliquots were pipetted out from standard stock solution into the series of 10 ml volumetric flask and the volume was made up to the mark with methanol to get concentration of 50-200 μg/ml of RAN and 8-32 μg/ml of OND. Solutions of different concentrations for each drug were analyzed at their respective wavelengths and absorbances were recorded.

Simultaneous equation method

Two wavelengths were selected for the method (230 nm and 216 nm) as the absorbance maxima of RAN and OND respectively in methanol.

Standard stock solution (100 μg/ml for Ranitidine & 16 μg/ml for Ondansetron drugs) were prepare in methanol. The stock solution of both drugs were further diluted with methanol to get series of standard solutions of 50-200 μg/ml for RAN and 8-32 μg/ml for OND. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined.

Concentrations in the samples were obtained by using following equations:

CX = A1ay2 – A2ay1 / ax1ay2 – ax2ay1 Eq. 1

CY = A1ax2 – A2ax1 / ay1ax2 – ay2ax1 Eq. 2

where A1 and A2 are absorbances of mixture at 230 nm and 216 nm respectively, ax1 and ax2 are absorptivities of RAN at λ1 and λ2 respectively, ay1 and ay2 are absorptivities of OND at λ1 and λ2 respectively, Cx and Cy are concentrations of RAN and OND respectively.

Preparation of injection for assay

The volume equivalent to one injection (50 mg RAN and 8 mg OND) were taken into 100 ml clean and dry volumetric flask, about 70 ml of methanol was added. The solution was sonicated for 20 minutes and volume was made up to the mark with methanol. The contents of the solution were mixed well and filtered through Whatman filter paper No. 41. First few ml of filtrate was discarded,5 ml of filtrate was pipetted out and diluted to 50 ml with methanol. The absorbances were recorded at the respective wavelengths.

Recovery study

To check the accuracy of the developed method, recovery study was carried out as per ICH norms where to a reanalyzed sample solution, standard solutions of all the two drugs were added equivalent to 80, 100 and 120% of its drug content. Recovery study was carried by doing replicate study.

Method validation

The analytical method was validated with respect to parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, robustness, and recovery (ICH Q2R1 2003). Linearity was established by least squares linear regression analysis of the calibration curve. Accuracy was studied by adding two different amounts (corresponding to 80%, 100% and 120% of the test preparation concentrations) of RAN and OND to the placebo preparation and comparing the actual and measured concentrations. For each level, three solutions were prepared and each was estimated in duplicate. The precision of the method, as intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the RSD%. The intermediate (interday) precision of the method was checked by performing same procedure on different days by another person under the same experimental conditions. The LOD and LOQ of RAN and OND were **calculated by mathematical equations:**

LOD= 3.3 ´ standard deviation ÷ slope Eq. 3

LOQ=10 ´ standard deviation ÷ slope Eq. 4

Robustness of proposed method was performed by changing UV analyst and keeping the remaining conditions (solvent, dilution, UV spectrophotometer) same.

Statistical analysis: Means, standard deviation (SD), relative standard deviation (RSD) and linear regression analysis were calculated using Microsoft Excel 2007.

RESULTS AND DISCUSSION

The UV scanning showed spectrum exhibiting λmax of 230 nm and 216 nm for RAN and OND respectively (**Figure 3 & Figure 4**).

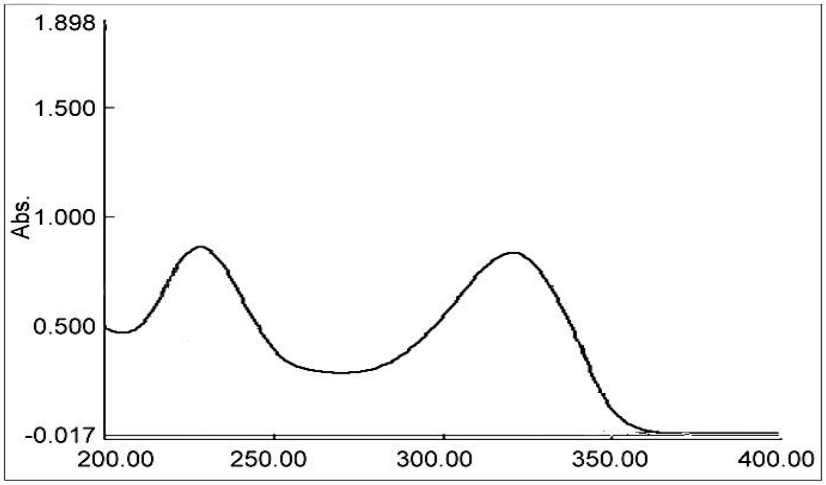

Fig. 3. λmax of Ranitidine (RAN)

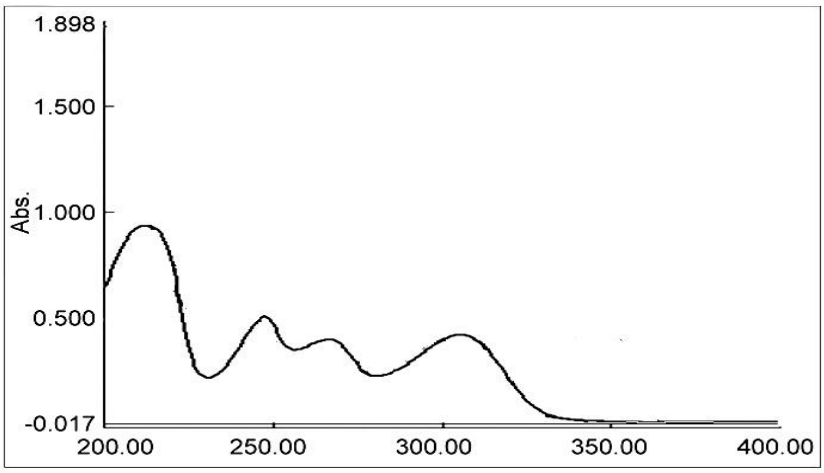

Fig. 3. λmax of Ondansetron (OND)

The linearity of the proposed method was investigated in the range of 50-200 μg/ml and 8-32μg/ml for RAN, OND respectively. Calibration curves showed a linear relationship between the absorbance and concentration. The line equation for RAN y = 0.015x - 0.011 with r2 of 0.999 and for OND y = 0.009x - 0.008 with r2 of 0.999 was obtained. Calibration curves showed a linear relationship between the absorbance and concentration of RAN and OND (**Figure 3a).**

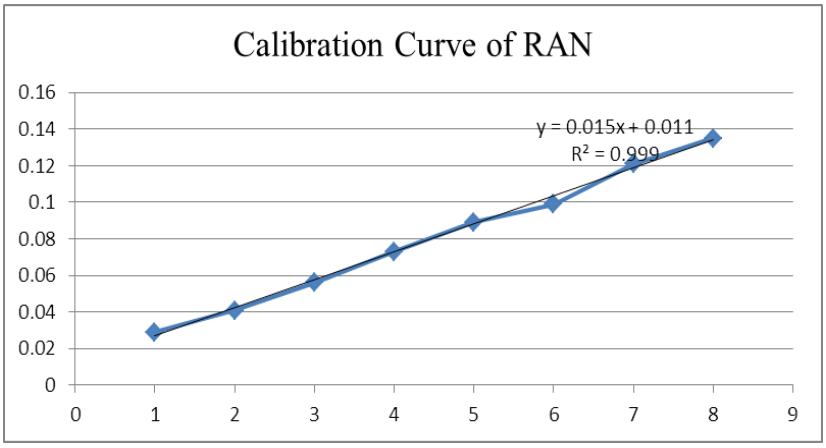

Fig. 3a. Calibration curve of Ranitidine (RAN)

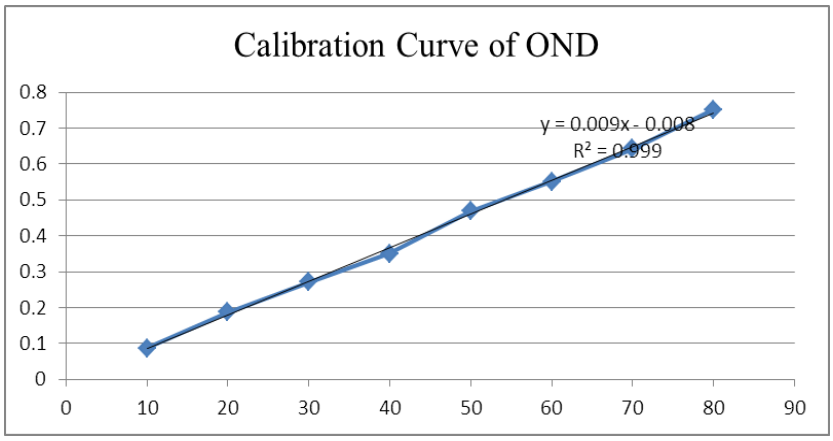

Fig. 4a. Calibration curve of Ondansetron (OND)

The present research work discuss the development of a UV spectrophotometric method for the estimation of RAN and OND in injection dosages form. The optimum conditions for the analysis of the drug were established. During analysis of commercial formulation (Table 1), absorbances were recorded at the respective wavelengths.

The LOD of RAN and OND was 0·454 µg/ml and 0·656 µg/ml and LOQ for RAN and OND was 0·151 µg/ml and 2.18 µg/ml respectively (Table 2).

Table 1. Analysis of injection dosage form

Formulation	Drugs	Label claim	% Label claim (Mean±SD)
Injection	Ranitidine (RAN)	50mg	100·1±0·032481
	Ondansetron (OND)	8mg	99.1±0.000149

Table 2. Validation parameters for Ranitidine (RAN) and Ondansetron (OND)

Validation parameter	Mean±SD	
	Ranitidine	Ondansetron
Linearity & Range	50-200 μg/ml	8-32 μg/ml
Interday Precision		
System Precision	99.5%±0·0015421	100.1±0.000149
Method Precision	98%±0·0086542	99±0.000652
Intraday Precision		
System Precision	99%±0·005241	98.7±0.000927
Method Precision	99.4%±0·009845	99.2±0.000432
Accuracy		
80%	101%±0·00391	99±0.0059
100%	100%±0·00254	98±0.00033
120%	101%±0·00928	99±0.00091
LOD (µg/ml)	0·454 µg/ml	0.657 µg/ml
LOQ (µg/ml)	0·151 μg/ml	2.18 µg/ml
Robustness	101%±0·00389	99±0.000125

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence it can be used in routine analysis for simultaneous determination for ranitidine and ondansetron in bulk as well as in pharmaceutical preparation by UV spectrophotometry. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The relative standard deviation (RSD) for all parameters was found to be less than one, which established the validity of the method. So, the proposed method can be used for routine quantitative simultaneous estimation of both the drug.

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