INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

DEVELOPMENT AND VALIDATION OF REVERSED-PHASE HPLC METHOD FOR ESTIMATION OF ONDANSETRON HYDROCHLORIDE IN BULK DRUG

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Article Received on: 04/12/11 Revised on: 28/01/12 Approved for publication: 18/02/12

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ABSTRACT
A simple, accurate rapid and precise RP-HPLC method has been developed and validated for determination of Ondansetron hydrochloride in bulk drug. The RP-HPLC separation was achieved on hypersil C_{18} column (216 mm, 4.6 mm, 5µm) using mobile phase methanol: acetonitrile (50:50 v/v) at flow rate of 1.2 ml/min at ambient temperature. The retention times were 2.6 min. for Ondansetron hydrochloride.Calibration plots were linear over the concentration range 10-50µg/ml. Quantification was achieved with photodiode array detection at 216 nm over the concentration range of 10-50 µg/ml. The method was validated statistically and applied successfully for the determination of Ondansetron hydrocloride. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for the routine determination of Ondansetron hydrochloride in bulk drug.
KEYWORDS: Ondansetron hydrochloride, acetonitrile, methanol, validation, HPLC.

INTRODUCTION

Ondansetron hydrochloride (ONDA)1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl]-4H-cabbazol-4-one, monohydrochloride, dihydrate. It represents the class of selective $5HT_3$ antagonists which is commonly employed as anti-emetic drug associated with cancer chemotherapy [1-5], radiotherapy or anesthesia. [6,7]. Literature survey revealed that very few analytical methods have been reported for the estimation of Ondansetron hydrochloride in combination which includes HPLC, HPTLC [8-12], and also spectrophotometric method are reported[13]. Due to the marked polarity of the molecule and the relatively low water solubility, reported methods for the assay of Ondansetron hydrochloride are, mostly, reversed-phase HPLC using nitrile, octylsilyl and phenyl silica stationary phases and mixtures of polar solvents as mobile phases[14-17].

MATERIALS AND METHODS

All the reagents used were of HPLC grade and analytical grade and were purchased from Merck Chemicals, India. Reference standard of Ondansetron hydrochloride was supplied as gift sample from Sun Pharmaceutical Laboratories Limited, Mumbai with purity of 99.987%.

Chromatographic system and condition:

A Younglin (LC -2010TH –Liquid Chromatograph) equipped with PDA detector. Promesil C_{18} ((250 mm, 4.6 mm, 5µm) column and LC software were used. The mobile phase used was methanol : acetonitrile (50:50 v/v) which was filtered through nylon 0.45 µm membrane filter and degassed by ultrasonication for 15 min. The flow rate was 1.2ml/min. Detection was monitored at 216 nm and injection volume was 20 µl. All the experiment were performed at ambient temperature.

Standard solution and calibration graphs for chromatographic measurement:

Stock standard solutions were prepared by dissolving separately 10mg of Ondansetron hydrochloride in 10 ml of methanol. The standard calibration solutions were prepared by appropriate dilution of the stock solution with methanol to reach a concentration range of 10-50 µg/ml. The chromatographed under the optimized conditions described above. The peak area was plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation

Twenty tablet contents were accurately weighed, and their mean weight was determined and they were mixed and finely powered. A portion equivalent to about one tablet was accurately weighed and transferred in to a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to 100 ml methanol. The resulting solution was centrifuged at 3000 rpm for 5 min. supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µm filter. The original stock solution was further diluted to get sample solution of drug concentration of 90 µg/ml. A 20 µl volume of sample solution was injected in to HPLC, six times. The peak area was measured at 216 nm and amount of Ondansetron hydrochloride were determined using related linear regression equation.

RESULT AND DISCUSSION

Linearity of the method was investigated by serially diluting the working standard to give a concentration range of 10-50 µm/ml and 20 µl from this was injected. The flow rate was maintained at 1.2 ml/min. temperature of column was kept ambient and the effluent was monitored at 216 nm. Calibration curve was constructed by plotting concentration against peak area. The method was validated for linearity, precision, accuracy, specificity, limit of detection and limit of quantification as per ICH guidelines.

Assay of tablets of Ondansetron hydrochloride were performed. Twenty tablets of each company of strength 4 mg were weighed and ground to a fine powder. A quantity of tablet powder equivalent to 4 mg of Ondansetron hydrochloride was transferred to 10 ml volumetric flask, dissolved and diluted with acetonitrile and methanol mixture to obtain 1 mg/ml. The solution was sonicated for 15 minute and filtered through 0.45 µm membrane filter. The solution was further diluted to obtain concentration 10 µg/ml. Peak area of the above prepared tablet solutions of Ondansetron hydrochloride were measured by using above mentioned chromatographic conditions and the amount of Ondansetron hydrochloride were found from regression equation.

To optimize the HPLC parameters, several mobile phase compositions were tried. Various mobile phases having different ratios of methanol, water and acetonitrile were tried. Drug was retained in mobile phase consisting of methanol: water (60: 40 v/v) and methanol: acetonitrile (50: 50 v/v). In methanol: acetonotrile (50: 50 v/v) tailing in the peak was observed. Good peak symmetry and satisfactory retention time was obtained with mobile phase consisting of methanol: acetonitrile (50:50 v/v). Quantification was achieved with PDA detection at 216 nm based on peak area. The retention time of Ondansetron hydrochloride obtained was 2.60±0.132 (table 1)

The linear regression date showed a good linear relationship over the concentration range of 10-50 µg/ml as summarized in Table 3.

The limit of detection (LOD) and the limit of quantification(LOQ) of the drug were found by scanning the solution of Ondansetron hydrochloride having different lower concentrations and the LOD and LOQ were found to be 1.7 and 5.26 µg/ml indicates that method is sensitive (Table 5). The intraday and interday precision were determined by analyzing standard solution of Ondansetron hydrochloride at three different concentration levels (6, 8, 10 µg/ml). The % RSD for intraday and interday precision was found to be 0.30% and 0.48% respectively which indicate that method is precise (Table 5). Repeatability of the method was studied by injecting 10 µg/ml solution of Ondansetron hydrochloride for six times and peak area was measured and % RSD was calculated which was found to be 0.195 shows repeatability of the method (Table 5). Accuracy of the method was evaluated by standard addition method in which appropriate portion of stock solutions of Ondansetron hydrochloride were spiked into blank placebo matrix to produce concentrations of 80 100 and 120% of theoretical concentration. The mean recovery of spiked samples obtained was in range of 99.96 to 100.36 reveals no interference of excipients and shows that method is accurate. (Table4). The proposed validated method was successfully applied to determine Ondansetron hydrochloride in bulk drug and tablet form. The results obtained for tablet of Ondansetron hydochloride were comparable with the corresponding labeled amounts (4 mg/tab) (table 4). Robustness of the method was estimated by changing the mobile phase composition (3±3), wavelength ±1 nm, injection volume (20±2µl), column temperature (40 ± 3^0) and RSD values for all these changes calculated were less than 2 indicate that proposed method is robust.

The proposed RP-HPLC method was accurate, precise, sensitive and rapid. The method also can be extended for the routine analysis of Ondansetron hydrochloride in bulk drug and tablet dosage form.

CONCLUSION

It is thus concluded that the proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise and can be successfully employed in the routine analysis of drug. It was successfully validated in terms of system suitability, linearity, range, precision, accuracy, specificity, LOD, LOQ, repeatability and robustness in accordance with ICH guidelines. The proposed method shall prove equally effective to analyze Ondansetron hydrochloride in the corresponding drug sample and may prove to be of great importance in pharmaceutical analysis.

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TABLE 1: HPLC INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS FOR ONDANSETRON HYDROCHLORIDE

Parameters	Description
Instrument	A HPLC instrument (Younglin series) with Model Acme-9000
Column	Promosil C-18, (250 mm, 4.6 mm, 5µm)
Mobile Phase	Different mobile phase used for Trial 1 to 6
Flow Rate	1.2 ml/minute
Detection wavelength	216 nm
Injection Volume	20µL
Run Time	10 minutes

TABLE-2: SYSTEM SUITABILITY TEST PARAMETERS

Parameters	RP-HPLC method
Retention time, min	2.6
Tailing factor	0.81
Asymmetry factor	1.25
Theoretical plates	43264
Resolution	10.2

TABLE 3: LINEARITY PARAMETERS FOR ESTIMATION OF ONDANSETRON HYDROCHLORIDE (N=6)

Parameters	Ondansetron hydrochloride
Linearity range µg/ml	10-50
r^2±SD	0.998±0.36
Slope±SD	0.57±0.21
Intercept±SD	124.2±0.17

TABLE 4: RESULTS OF ACCURACY STUDIES OF ONDANSETRON HYDROCHLORIDE

Label claim mg/tablet	Amount added (%)	Total amount added (mg)	Amount recovered (mg)	% Recovery±SD	%RSD
Ond.Hcl (4)	80	7.2	7.12	99.96±0.30	0.31
	100	8.0	7.81	99.77±0.41	0.40
	120	8.8	8.92	100.36±0.52	0.51

n=6, SD: standard deviation, % RSD: Relative standard deviation

TABLE-5: REGRESSION CHARACTERISTICS AND VALIDATION PARAMETER

Sr. No.	Parameter	Value
1.	λ_{max} (nm)	216
2.	Linearity range	10– 50µg/ml
3.	Correlation coefficient (r^2)	0.998
4.	Regression equation	Y=0.57X+124.2
5.	Intercept (a)	124.2
6.	Slope (b)	0.57
7.	Limit of detection (LOD µg/ml)	1.7
8.	Limit of quantification(LOQ µg/ml)	5.26
9.	Accuracy (%)	99.96-100.36%
10.	Repeatability (RSD, %, n=6)	0.195
11.	Precision (RSD, %), Interday (n=6)	0.30%
12.	Intraday (n=6)	0.48%

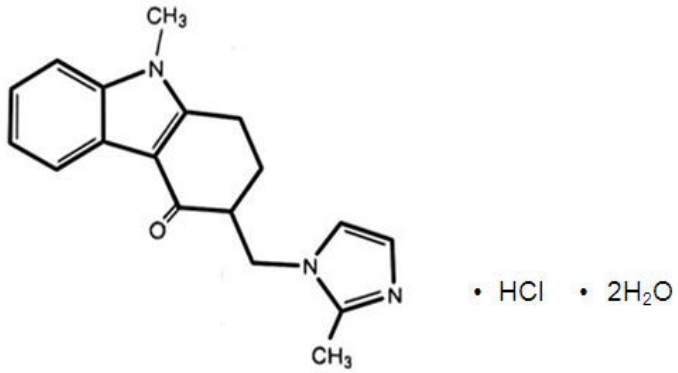


FIG.1: CHEMICAL STRUCTURE OF ONDANSETRON HYDROCHLORIDE

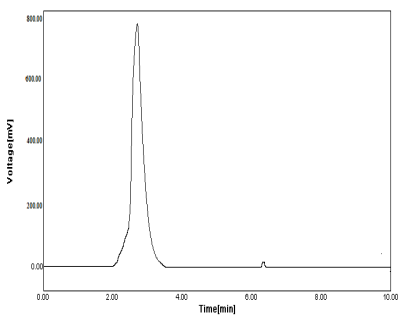


FIG.2: REPRESENTIVE CHROMATOGRAMS OF STANDARD SOLUTION OF ONDANSETRON HYDROCHLORIDE.

Source of support: Nil, Conflict of interest: None Declared