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Estimation of Ondansetron Hydrochloride in Pharmaceutical Dosage Forms by HPLC

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High pressure liquid chromatographic method (HPLC) has been developed for the estimation of ondansetron hydrochloride in its pharmaceutical tablet dosage forms using RP C-18 column. The mobile phase consisted of methanol and 0.02 M phosphate buffer in the ratio of 60 : 40 and doxazocin was used as internal standard. The detection was carried out at 213 nm and the linearity was found to be in the range of 0.01 to 59 μg/mL. The method is simple, precise, specific, less time consuming and accurate for the estimation of ondansetron hydrochloride in tablet dosage forms.

Key Words: HPLC, Estimation, Ondansetron hydrochloride, Pharmaceutical dosage.

INTRODUCTION

Ondansetron hydrochloride, a selective 5-HT_3 receptor antagonist used as antiemetic in chemotherapy-induced emesis[1]. Several analytical methods have been reported for the estimation of ondansetron hydrochloride in pharmaceutical dosage forms by HPLC[2-3] and spectrophotometric methods[4]. In the present study a sensitive, accurate and precise HPLC method has been developed for the estimation of ondansetron hydrochloride in pharmaceutical dosage forms.

EXPERIMENTAL

Ondansetron hydrochloride was a gift sample from M/s Natco Fine Chemicals Pvt. Ltd., Hyderabad, India and doxazocin was a gift sample from M/s. Cipla Ltd., Mumbai, India. The methanol used was of HPLC grade (E. Merck), and triple distilled water was used. All other reagents (potassium dihydrogen phosphate) used in the study were of AR quality (E-Merck).

A gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), guard column (Pelliguard™, LC-18, 2 cm, Supelco, Inc., Bellefonte, PA.) and RPC-18 column (150 mm × 4.6 mm I.D.;

particle size 5 μm; Flexit Inc., India) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

Preparation of stock solution of internal standard: Doxazocin was used as internal standard for the estimation of ondansetron hydrochloride. About 100 mg of doxazocin was accurately weighed, transferred to 100-mL volumetric flask, dissolved in methanol and made up to volume with methanol so as to give a stock solution of 1000 μg/mL (Stock-I). 5 mL of this stock solution was diluted to 100 mL with methanol to give 50 μg/mL solution (stock-II). 1 mL of stock-II solution is added to standard ondansetron hydrochloride sample solutions.

Preparatin of stock solutions of ondansetron hydrochloride: About 100 mg of ondansetron hydrochloride was accurately weighed and transferred to 100 mL volumetric flask. It was dissolved in triple distilled water and the solution was made up to volume with triple distilled water. Each mL of this stock solution (Stock-I) contained 1000 μg fo ondansetron hydrochloride. 10 mL of stock-I solution (1000 μg) were diluted to 100 L with triple distilled water to give a stock solution containing 100 μg/mL (Stock-II).

Chromatographic conditions: Both methanol and 0.02 M potassium dihydrogen phosphate were filtered before use through 0.4 μm PFTE membrane filter. The flow rate of the mobile phase was maintained at 1 mL/min in the ratio of 60 : 40 (methanol : 0.02 M KH_2PO_4). The column temperature was maintained at 40°C and concentration of drug was detected by UV detector at 213 nm. The data were acquired, stored and analyzed with the software Class-VP series version 5.03 (Shimadzu).

Procedure: A series of 10 mL volumetric flasks were taken. From Stock-II solution of ondansetron hydrochloride 0.01 to 5 mL quantities of the solution were transferred to 10 mL volumetric flasks. To these solutions 1 mL of doxazocin (internal standard) containing 50 μg/mL was added and the volume was made up to 10 mL with mobile phase so as to give 0.01 to 50 μg/mL of ondansetron hydrochloride and 5 μg/mL of doxazocin respectively. The standard solutions prepared as above were filtered through 0.4 μm membrane filter and the filtrate was injected five times into the column at a flow rate of 1 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of ondansetron hydrochloride in pharmaceutical formulations.

Ondansetron hydrochloride solutions containing 10 μg/mL, 20 μg/mL and 40 μg/mL were subjected to the proposed HPLC analysis for finding out the intra- and interday variations. The recovery studies were carried out by adding known amount of ondansetron hydrochloride to the preanalysed samples and subjecting them to the proposed HPLC method.

Estimation of ondansetron hydrochloride in tablet dosage forms: Twenty tablets were weighed and powdered. An accurately weighed portion of the powder, equivalent to 10 mg of Ondansetron hydrochloride was transferred to a 100 mL volumetric flask containing about 30 mL of mobile phase. The contents

of the flask were sonicated to dissolve ondansetron hydrochloride, made up to volume and the resulting mixture was filtered through a 0.45 μ filter. 2 mL of this solution were added to 10 mL volumetric flask containing a solution equivalent to 50 μg of internal standard (doxazocin) and were made up to volume with mobile phase. This solution (20 μL) was injected five times into the column. The mean values of peak area ratio of drug to internal standard of five such determinations were calculated and the drug content in the tablet dosage form was quantified using the regression equation obtained above. The same procedure was followed for the estimation of ondansetron hydrochloride in three different batches of tablets dosage forms.

RESULTS AND DISCUSSION

The present study was carried out to develop a specific, sensitive, precise and accurate HPLC method for the analysis of ondansetron hydrochloride in pharmaceutical tablet dosage forms. A typical chromatogram is shown in Fig. 1. The column pressure varied from 165–166 kg/cm^2. The retention times for ondansetron hydrochloride and internal standard (doxazocin) were 2.92 min and 6.61 min respectively. Each of the samples was injected 5 times and the same retention times were observed in all cases. The ratio of peak area of ondansetron hydrochloride to peak area of internal standard for different concentrations set up as above were calculated, and the average values for 5 such determinations are shown in Table-1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (2.56%). A good linear relationship (r = 0.99996) was observed between the concentration of ondansetron hydrochloride and the respective ratio of peak areas. The calibration graph was found to be Y = −0.00012 + 0.2751X (where Y = ratio of peak area of drug to that of internal standard; X = concentration of ondansetron hydrochloride) in the range of 0.01 to 50 μg/mL.

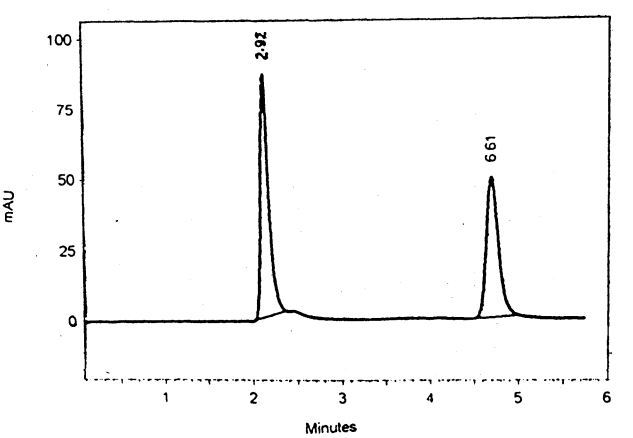

Fig. 1. A typical chromatogram for ondansetron hydrochloride

TABLE-1
CALIBRATION OF THE HPLC METHOD FOR THE ESTIMATION OF ONDANSETRON HYDROCHLORIDE

Concentration of ondansetron hydrochloride (μg/mL)	Mean (±s.d) peak-area ratio (n = 5)	% CV
0	0	0
0.01	0.0040	2.10
0.1	0.0312	1.12
0.5	0.1414	1.00
1.0	0.2687	0.98
5.0	1.3707	2.56
10.0	2.8120	1.28
25.0	6.7820	1.36
50.0	13.7910	2.01

Regression equation (from 0.01 to 59 μg/mL: Y = 0.00012 + 0.2751X (r = 0.99996)

When ondansetron hydrochloride solutions containing 10, 20 and 40 μg/mL were analyzed by the proposed HPLC method for finding out intra and inteday variations, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise. The amounts of ondansetron hydrochloride from the preanalysed samples containing known amounts of the drug are shown in Table-3. About 99.95% of ondansetron hydrochloride could be recovered from the preanalysed samples indicating the high accuracy of the proposed HPLC method.

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Ondansetron hydrochloride concentration (μg/mL)	Concentration of ondansetron hydrochloride (μg/mL) found on			
	Intra-day		Inter-day	
	Mean (n = 5)	(%) CV	Mean (n = 5)	(%) CV
10	10.01	1.89	9.99	2.50
20	20.10	1.25	19.98	4.80
40	40.15	1.25	40.00	1.80

TABLE-3
RECOVERY OF ONDANSETRON HYDROCHLORIDE

Amount of drug added (μg)	Mean (± s.d.) amount (μg) found (n = 5)	Mean (± s.d.) % of recovery ((n = 5)
10	9.98 ± 0.12	99.80 ± 1.20
20	19.99 ± 0.18	99.95 ± 0.90
40	39.95 ± 0.51	99.87 ± 1.28

The drug content in the tablets was quantified using the proposed analytical method. The mean content of ondansetron hydrochloride in threee different batches of tablet dosage form is shown in Table-4. The absence of additional peaks indicates no interference of the excipients used in the tablets. The tablets were found to contain 99.8 to 100.5% of the labelled amount. The low % CV indicates the reproducibility of the assay of ondansetron hydrochloride in the tablet dosage form. Thus the developed HPLC method was found to be simple, precise, highly accurate, specific for the estimation of ondansetron hydrochloride in pharmaceutical tablet dosage forms.

TABLE-4
ASSAY OF ONDANSETRON HYDROCHLORIDE IN TABLET DOSAGE FORMS

Batch number of tablets	Labelled amount of drug (mg)	Mean (±SD) amount found (mg) by the proposed method [n = 5]	Mean (±SD) % labelled amount [n = 5]
I	10	9.98 ± 0.01	99.80 ± 0.06
II	10	10.05 ± 0.31	100.50 ± 0.07
III	10	10.01 ± 0.02	100.10 ± 0.07

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