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Development and validation of RP-HPLC method for the estimation of Ramosetron hydrochloride in tablet dosage form

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ABSTRACT

A simple, rapid, sensitive, accurate and precise RP-HPLC method has been developed and validated for the estimation of Ramosetron hydrochloride in bulk and tablet dosage form. The method was carried out using Hypersil ODS C18 (150 x 4.6 mm I.D., 5 m particle size) column and mobile phase comprised of buffer pH 3.2 and acetonitrile in proportion of ratio 50:50 v/v and degassed in ultrasonic water bath. The flow rate was 0.8 mL/min and the detection wavelength was at 310 nm. The linearity was observed in the range of 1-5 µg/mL with a correlation coefficient of 0.999. The retention time of Ramosetron hydrochloride was 2.54 min. The method was validated as per the ICH guidelines for its linearity, precision, accuracy, specificity, limit of detection, limit of quantitation and by performing recovery studies. The percentage recovery of the drug Ramosetron hydrochloride was 99.76 % to 100.33 % from the tablet formulation. The proposed method is suitable for the routine quality control analysis for the estimation of Ramosetron hydrochloride in bulk and tablet dosage form.

INTRODUCTION

Ramosetron hydrochloride (Fig. 1) is a serotonin 5-HT_3 receptor antagonist for the treatment of nausea and vomiting [1]. Chemically Ramosetron hydrochloride is (1-Methyl-1*H*-indol-3-yl) (4,5,6,7-tetrahydro-1 *H*-benzo [d] imidazol-6-yl) methanone hydrochloride. Ramosetron is also indicated for a treatment of diarrhea-predominant irritable bowel syndrome in males. Ramosetron was shown in pharmacological assays to inhibit activities mediated by 5-HT3 receptors, such as emesis caused by cisplatin [2]. A few HPLC methods [3-5] were reported earlier for the estimation of Ramosetron hydrochloride in bulk and pharmaceutical dosage form. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of Ramosetron hydrochloride in bulk drug and in tablet dosage form.

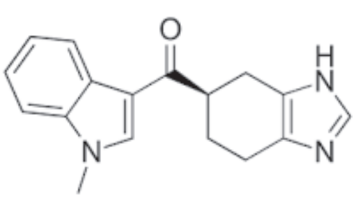

Figure 1: Chemical structure of Ramosetron

MATERIALS AND METHODS

Chromatographic conditions

The analysis of the drug was carried out on a Agilent 1260 Infinity Binary HPLC system equipped with a reverse phase Hypersil ODS C18 (150 x 4.6 mm I.D., 5 m particle size) column, mp, a 20 µL injection loop, rheodyne injector, DAD detector and running on Open Labs EZChrom software.

Chemicals and solvents

The reference sample of Ramosetron hydrochloride (API) was provided as gift sample from Spectrum Pharma Research Solutions, Hyderabad, India. The commercial formulations (IBSET tablets containing 5 mg of Ramosetron hydrochloride) were procured from the local market. Acetonitrile (HPLC grade), ortho phosphoric acid, triethyl amine were purchased from E.Merck (India) Ltd., Mumbai, India. Freshly prepared triple distilled water was used throughout the experiment.

Preparation of buffer

Dissolve 1 mL of ortho phosphoric acid (OPA) in 1000 mL of water. Adjusted the pH to 3.2 by using triethyl amine and the solution is filtered and sonicated for 5 min.

Preparation of mobile phase and diluent

500 ml of the buffer (0.1% OPA) was mixed with 500 ml of

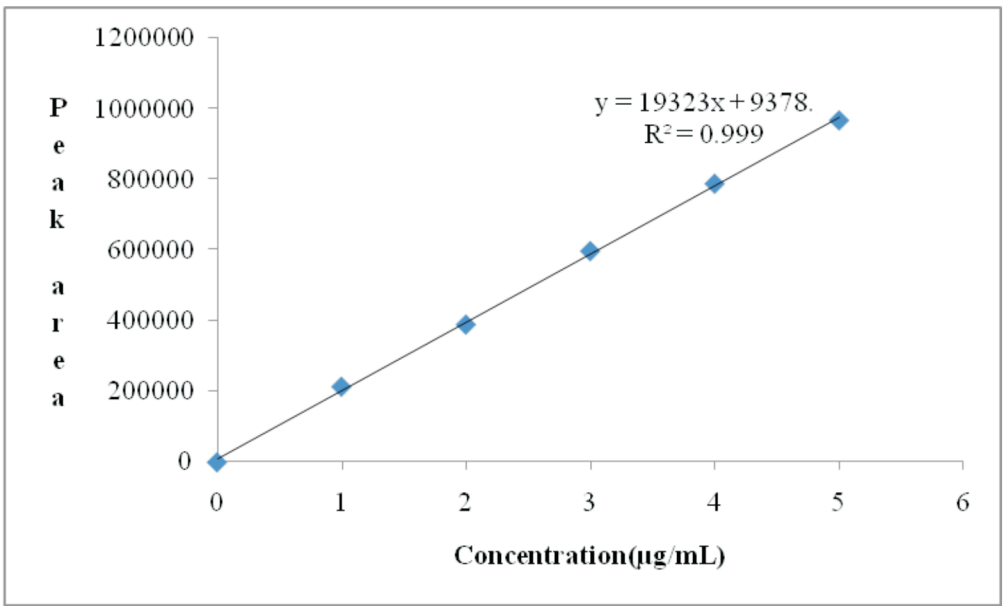

Fig. 2 : Calibration curve of Ramosetron hydrochloride

acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard stock solution

About 50 mg of Ramosetron hydrochloride is accurately weighed and transferred into a 50 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was sonicated for 5 min and the drug was dissolved completely. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ramosetron hydrochloride. Further pipette 0.1 mL of the above stock solution into a 10 mL volumetric flask (100 µg/mL) and the volume was made up to the mark with the mobile phase. Mix well and filter through 0.45 µm filter.

Preparation of sample solution

10 tablets were weighed and finely powdered. An accurately weighed portion of powder sample equivalent to 10 µg of Ramosetron hydrochloride is transferred into a 10 mL (1000 µg/mL) clean dry volumetric flask containing mobile phase. The solution was filtered and sonicated for 5 min. The volume was made up to the mark with a further quantity of the mobile phase to get a stock concentration of Ramosetron hydrochloride. Further pipette 2 mL of the above stock solution into a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. Mix well and filter through 0.45 µm filter.

Table 1: Calibration data of the method

Concentration (µg/mL)	Mean peak area (n=5)
1	213764
2	389351
3	597322
4	787684
5	966721

Calibration plot

About 50 mg of Ramosetron hydrochloride was weighed accurately, transferred into a 50 mL volumetric flask and dissolved in 7 mL of a 50:50 v/v mixture of buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 1000 µg/mL solution. From this, a working standard solution of the drug (100 µg/mL) was prepared by diluting 0.1 mL of the above stock solution into a 10 mL volumetric flask. Further dilutions ranging from 1-5 µg/mL were prepared from the solution in 10 mL volumetric flasks using the above diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 0.8 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area (Fig. 2) was found to be linear in the concentration range of 1-5 µg/mL of the drug. The relevant data are furnished in Table 1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Ramosetron hydrochloride in tablet dosage form.

Procedure

A mixture of buffer (0.1% OPA) and acetonitrile in the ratio of 50:50 v/v was found to be the most suitable mobile phase for ideal separation of Ramosetron hydrochloride. The solvent mixture was filtered through 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.8 mL/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase

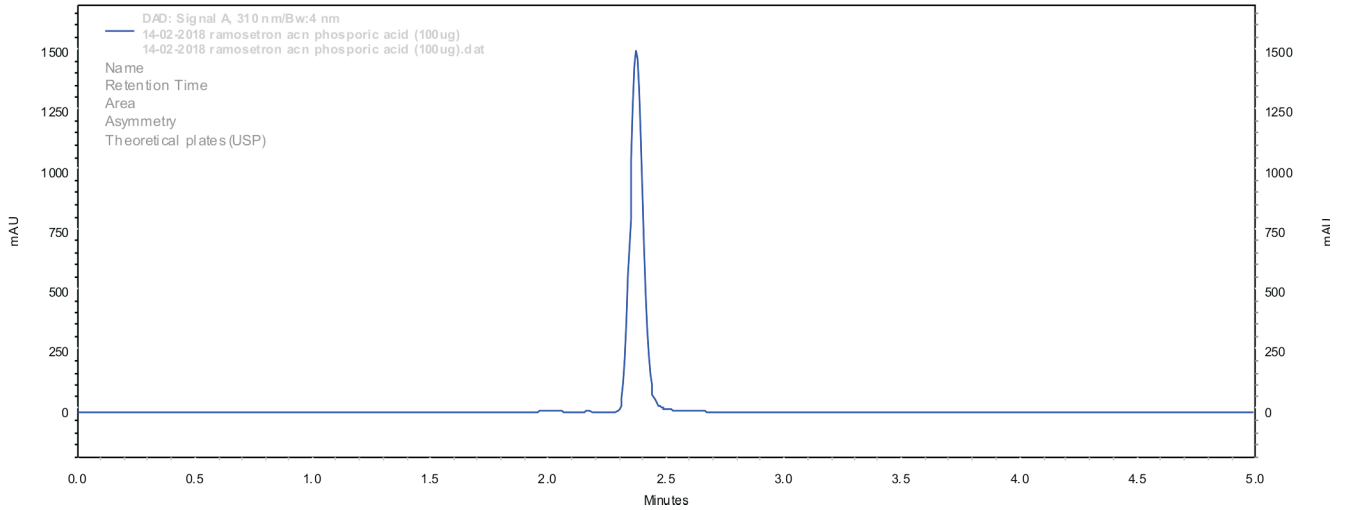

Fig. 3 : Typical chromatogram of Ramosetron hydrochloride

through the column for at least 30 min prior to the injection of the drug solution. Inject 20 L of the standard and sample solutions into the chromatographic system and measure the area for the Ramosetron hydrochloride peak. The detection of the drug was monitored at 310 nm. The run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drug Ramosetron hydrochloride was 2.54 min. A typical chromatogram showing the separation of the drug is given in Fig. 3.

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method as per the ICH guidelines [6] for the estimation of Ramosetron hydrochloride. Solution containing 5 µg/mL solution of Ramosetron hydrochloride was subjected to the proposed HPLC analysis to check intra-day precision and inter-day precision of the method and the results are furnished in Table 2. The accuracy of the HPLC method was assessed by analyzing solutions of Ramosetron hydrochloride at 50 %, 100 % and 150 % concentration levels by the proposed method. The results are furnished in Table 3. The system suitability parameters are given in Table 4.

Estimation of Ramosetron hydrochloride in tablet dosage form

Commercial formulations of Ramosetron hydrochloride tablets were chosen for testing the suitability of the proposed method to estimate Ramosetron hydrochloride in tablet formulation. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 50 mg of Ramosetron hydrochloride was transferred into a 50 mL volumetric flask and dissolved in 5 mL of a 50:50 v/v mixture of buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 3 mL of the diluent was added, the flask

Table 2: Precision data of the proposed HPLC method.

Concentration of Ramosetron (5 mg/mL)	Peak area	
	Intra-day precision	Inter-day precision
Injection-1	963452	963452
Injection-2	961524	964692
Injection-3	961415	959421
Injection-4	959835	954567
Injection-5	959265	956912
Injection-6	955623	956892
Average	960185.7	959322.6
Standard Deviation	2674.317	4005.661
% RSD	0.27	0.42

Table 3: Accuracy studies

% Concentration (at specification level)	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50 %	1.0	0.99	99.0 %	99.66 %
100 %	2.0	2.00	100.0 %	
150 %	3.0	3.00	100.0 %	

Table 4: System suitability parameters

Parameter	Result
Linearity (mg/mL)	1-5
Correlation coefficient	0.999
Theoretical plates (N)	9499
Tailing factor	1.05
LOD (? g/mL)	0.014
LOQ (? g/mL)	0.042

Table 5: Assay studies

Formulation	Label claim (µg)	Amount found (µg)	% Amount found
IBSET	5	4.98	99.6

was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µm membrane filter. Further pipette 1 mL of the above stock solution into a 2 mL volumetric flask and the volume was made up to the mark with the mobile phase. This solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table 5.

RESULTS

In the proposed method, the retention time of Ramosetron hydrochloride was found to be 2.54 min. Quantification was linear in the concentration range of 1-5 µg/mL. The regression equation of the linearity plot of concentration of Ramosetron hydrochloride over its peak area was found to be y=193238x+9378.2 (r^2=0.999), where x is the concentration of Ramosetron hydrochloride (µg/mL) and y is the corresponding peak area. The number of theoretical plates calculated was 9499, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.014 µg/mL and 0.042 µg/mL respectively, which indicate the sensitivity of the method. The use of buffer and acetonitrile in the ratio of 50:50 v/v resulted in peak with good shape and resolution. The high percentage of recovery values ranges from 99.76-100.33 % indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug Ramosetron hydrochloride by the proposed HPLC method.

DISCUSSION

The present study was aimed at developing a simple, sensitive, precise and accurate stability indicating RP-HPLC method for the estimation of Ramosetron from bulk samples and their tablet dosage forms. A non-polar C18 analytical chromatographic column was chosen as the stationary phase for the separation and determination of Ramosetron. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of 0.1%OPA and acetonitrile in the ratio of 50:50 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention time of the Ramosetron was found to be 2.54 min respectively.

The linearity was found satisfactory for in the range of 1-5 µg/mL. The regression equation of the linearity curve for Ramosetron over its peak areas was found to be y=19323x+9378. Precision of the method was studied by repeated injection of

tablet solution and results showed lower %RSD values. This reveals that the method is quite precise. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent recovery and the % RSD at each level were within the acceptable limits. This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific.

The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust. The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive.

CONCLUSION

The proposed HPLC method is rapid, sensitive, accurate and precise for the estimation of Ramosetron hydrochloride and can be reliably adopted for routine quality control analysis of Ramosetron hydrochloride in bulk and its tablet dosage form.

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