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ESTIMATION OF GRANISETRON HYDROCHLORIDE BY A NEW RP-HPLC METHOD

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ABSTRACT

A simple, sensitive and specific reverse phase high performance liquid chromatographic method has been developed for the determination of granisetron hydrochloride in injectable dosage forms. Chromatographic separation was achieved on a peerless basic C18 (250×4.6 mm), 5.0 µm column with a diluted 1.6 ml of ortho phosphoric acid in 800 ml water, added 200ml acetonitrile, added 1.0ml of Hexylamine and mixed. Then adjusted the pH to 7.5 with triethylamine as mobile phase, detection was at 304 nm. Response was a linear function of concentration in the range 5-0.02 mg/L for granisetron hydrochloride. LOD and LOQ for granisetron hydrochloride were found 0.01 mg/L and 0.03 mg/L. Accuracy (recoveries 94-97%) and reproducibility were found to satisfactory.

KEY WORDS : Granisetron hydrochloride, RP-HPLC method, method validation.

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INTRODUCTION

Granisetron hydrochloride (Figure 1) is an effective and potent antiemetic drug which is used specifically the treatment of vomiting and nausea from cancer chemotherapy and radiotherapy in adults and children. Chemically it is endo-N-(9-methyl-9-azabicyclo [3.3.1] non-3yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride. Granisetron hydrochloride selectively blocks type 3 serotonin (5-HT3) receptors.

In this paper we describe a simple, sensitive, and validated RP-HPLC method for determination of granisetron hydrochloride in injectable Dosage Forms. The method has been successfully used for quality control analysis of the drugs and other analytical purposes.

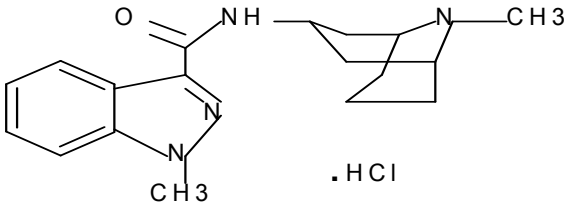

Figure 1
The structure of Granisetron hydrochloride

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a LC-20AT pump and SPD-20A UV-VIS detector with 20µL fixed loop and analyzed by using LC-Solution software.

Peerless basic C_{18} (250×4.6 mm), 5.0 µm make: varian column with a Diluted 1.6 ml of ortho- phosphoric acid in 800 ml water, add 200ml acetonitrile, added 1.0ml of Hexylamine and mix. Then adjusted the pH to 7.5 with triethylamine as mobile phase was delivered at flow rate 1.5 ml/min. The mobile phase was filtered through 0.45µ membrane filter and sonicated for 10min. An external standard method was used. UV detection was performed at 304nm and column oven temperature is 35°C.Peak was confirmed by comparison of spectra and retention time with standard.

REAGENTS AND SOLUTIONS

Preparation of standard solution

Accurately weighed 1.08mg of reference standard of granisetron hydrochloride in 100ml volumetric flask and the volume was brought upto the mark using Mobile phase.

Preparation of sample solution

The commercial samples of injections containing the drug namely Kytril, 1.12 mg (Health biotech Limited) have been chosen for this purpose. Two vails were opened and the contents of the each vail was dissolved in 5 mL of water and transferred into a 100 ml volumetric flask with 30ml diluent (mobile phase) shaken for 5min, and then diluted to volume with diluent to furnish a solution containing 22.40 mg/L granisetron hydrochloride. The solution was diluted with diluent to give a final concentration of 10 mg/L granisetron hydrochloride.

METHOD VALIDATION

Once the HPLC method development was over, the method was validated in terms of parameters like specificity, press ion, accuracy, linearity and range, LOD,LOQ, raggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values ware calculated. The

proposed HPLC method was validated as per ICH guidelines.

Linearity and range

Different known concentrations of granisetron hydrochloride (5.0 mg/L – 0.02 mg/L) were prepared in diluent by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient. The results were shown in Table: 2. The slope, intercept and correlation coefficient values were found to be 28091, 23.54 and 0.9998.

Table 1
Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu system equipped with LC-20 AT pump and SPD-20A interfaced with LC Solution software
Column	Peerless basic C_{18} (250×4.6 mm), 5.0 µm make: varian
Mobile Phase	Diluted 1.6 ml of ortho phosphoric acid in 800 ml water, add 200ml acetonitrile, added 1.0ml of Hexylamine and mix. Then adjusted the pH to 7.5 with triethylamine
Flow Rate	1.5mL/min
Detection	UV at 304nm
Injection Volume	20µL
Column oven temperature	35ºC

Table 2
Validation Parameters

Parameters	Granisetron hydrochloride
Linearity range	0.02-5 mg/L
Correlation coefficient	0.9998
Slope	28091
Y Intercept	23.54

Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise. Results were shown in Table 3-4.

Table 3
Intraday Precession

Concentration (mg/L)	Area	%RSD
0.03	915	
	930	0.82
	924	
0.3	9245	
	9189	0.75
	9327	
1	28845	
	28952	0.31
	29021	

The intraday precision was found to be within 1% RSD for conc.0.03, 0.3, 1.0mg/L

Table 4
Interday Precision

Concentration (mg/L)	Day	Area	% RSD
0.03	1	889	
	2	902	1.35
	3	878	
0.3	1	8974	
	2	8887	1.13
	3	9089	
1	1	27945	
	2	28612	1.21
	3	28414	

Intraday precision was performed for con. Of 0.03, 0.3 and 1.0 mg/L. For about three days and their peak, areas are shown in the table. The %RSD for con. 0.03, 0.3, and 1.0 mg/L was found to be within 2%

Accuracy

To study the reliability, suitability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug was added to the preanalysed sample formulation at the level of 50%, 100% and 200%, dissolved in diluents and made up to 100ml with same solvent. Further dilutions were made so that the each aliquot contained 0.03mg/L of granisetron hydrochloride. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The recovery procedure was repeated 10 times and % RSD was calculated by using the following formula. The contents of granisetron hydrochloride per injection found by proposed method are shown in Table 3; the lower values of RSD of assay indicate the method is accurate. The mean recoveries were in range of 94-97 %

which shows that there is no interference from excipients. Table: 5.

$$\% \text{ recovery} = \frac{b-a}{c}$$

Where,

a-The amount of drug found before the addition of standard drug
b-The amount of drug found after the addition of standard drug
c- The amount of standard drug added

Table 5
Recovery studies

Level (mg/L)	% Recovery	% RSD
0.03	95	0.1
0.3	97	0.12

Recovery studies were performed at 0.03mg/L and 0.3 mg/L levels and the results were found to be within the limits mentioned as per ICH guidelines.

Repeatability of solution

A standard solution of drug substance was injected ten times and corresponding peak areas were recorded. The % RSD was found to be less than 1%.Table:6.

Table 6
Repeatability of injection

Con (mg/L)	Peak area	%RSD
	9352	
	9165	
	9228	
	9301	
0.3	9094	0.97
	9366	
	9249	
	9199	
	9274	
	9356	

Repeatability of injection was performed using 0.3mg/L sample for 10 times and corresponding peak areas were recorded. The % RSD peak was reported.

Specificity

Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peaks shapes.

Assay

20µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area of standard amount of drug in sample were computed. The values are given in Table: 7

Table 7
Analysis of formulation

Amount of drug (mg)		% Label claim	%RSD (n=6)
Labeled	**Estimated**	97	0.41
1.12 mg	***1.09***		

Analysis of formulation was performed using granisetron hydrochloride 1.12 mg of injections and the claim was found to be 97.

Limit of detection and limit of quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the development method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for granisetron hydrochloride found to be 0.01mg/L The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10) The LOQ was 0.03mg/L for granisetron hydrochloride. It was concluded that the developed method is sensitive.

Ruggedness and robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like shimadzu HPLC and Agilent HPLC by different operators using different columns of similar types. The %RSD values with two different instruments shimadzu HPLC and Agilent HPLC, analyst and columns were 0.5-0.5, 0.6-0.5 and 0.4-0.3% respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in mobile phase, flow rate and column temperature. It was observed that ther were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method is rugged and robust. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

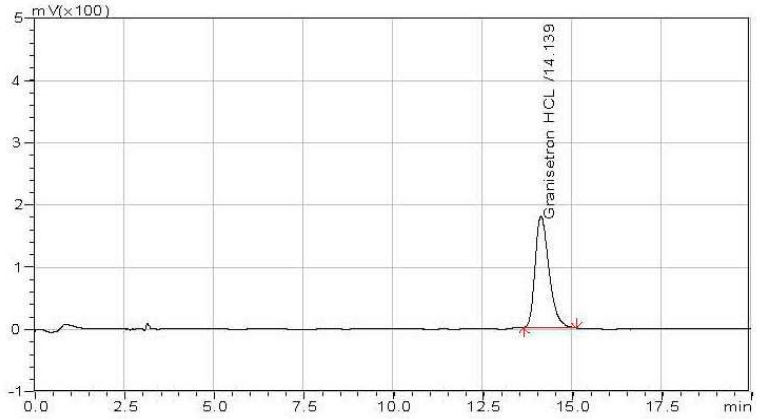

Figure 2
Chromatogram of standard (1.0 mg/L)

RESULTS AND DISSECTION

UV spectrum of granisetron hydrochloride was recorded from which 304nm was selected as wavelength. Flow rate of 1.5ml/min was selected. Diluted 1.6 ml of ortho phosphoric acid in 800 ml water, add 200ml acetonitrile, added 1.0ml of Hexylamine and mix. Then adjusted the pH to 7.5 with triethylamine was selected as mobile phase. The retention time was found to be 14.1min. Granisetron hydrochloride is shown linearity in the range of 0.02-5mg/L, and the co-efficient was found to be 0.9998. Recovery studies were performed at 50%, 100% and 200%, levels. The sensitivity of method LOD and LOQ is shown in Table 2. The stability at room temperature and refrigeration were found to be 3 and 8.5 hrs respectively. Hence the proposed method is simple, accurate, and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

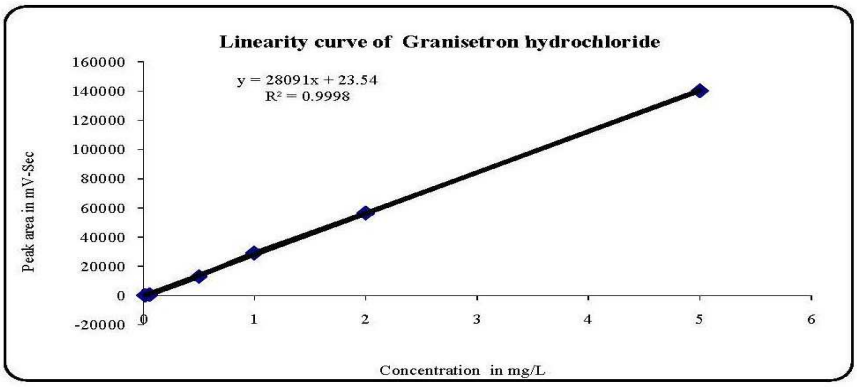

Figure 3
Linearity curve of Granisetron hydrochloride

Regression analysis of the calibration curve for granisetron hydrochloride showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.9998 in all curves assayed.

CONCLUSION

A convenient and rapid RP-HPLC method has been developed for estimation of granisetron hydrochloride in injectable dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % of RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of granisetron hydrochloride in dosage form, bulk drugs as well as for routine analysis in quality control.

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