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A NEW ISOCRATIC RP-HPLC METHOD DEVELOPMENT FOR THE ASSAY OF GRANISETRON HCl IN API AND DOSAGE FORMS

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ABSTRACT

An isocratic RP-HPLC method has been developed and validated for the assay of Granisetron HCl in active pharmaceutical ingredient (API) and pharmaceutical dosage forms. The chromatographic determination was performed on Shimadzu HPLC (LC2010AHT) system equipped with an LC-10A VP quaternary pump, a variable-wavelength programmable UV-Visible detector, 20-µL injection loop and a Gemini NX C_{18} (250mm × 4.6mm, 5µm) reversed phase column using 0.01M sodium dihydrogen phosphate buffer of pH 7.5 and acetonitrile in the ratio 80:20 v/v as mobile phase at a flow rate of 1.5mL.min^{-1}. The chromatographic data was acquired by using Class-VP 5.032 software. The eluent was monitored at wavelength 305nm and found a sharp and symmetrical peak with retention time of 7.466min, USP plate count of 2944.62 and tailing factor of 1.3. The linearity of the proposed method was in the range of concentration 2.0-10.0µg.mL^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were found to be 0.1502µg.mL^{-1} 0.4553µg.mL^{-1} respectively. The proposed method was found to be precise, accurate and sensitive. Hence, this method can be applied as an alternative for the assay of Granisetron HCl in quality control.

Keywords: Granisetron HCl, API, Linearity, LOD, LOQ, Assay.

INTRODUCTION

Granisetron is an antiemetic agent to prevent the nausea and vomiting in conjunction with cancer chemotherapy or with radiation therapy, by blocking 5-HT_3 receptors without having effect on other receptors such as dopamine D_2 receptor and 5-HT_4 receptor. It works by blocking serotonin, a natural substance in the body that causes nausea and vomiting due to the anaesthetics[1,2] .The hydrochloride salt of granisetron is a white to off-white crystalline powder; soluble in water and saline; administered intravenously. Chemical designation is endo-N-(9-methyl-9- azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride. The molecular formula and its gram molecular weight are $C_{18}H_{24}N_4O{\cdot}HCl$ and 348.87grams/mole respectively. The chemical structure of the Granisetron is presented in Fig.1. Granisetron dosage forms are not yet official in USP[3] and BP[4]. An extensive literature survey is carried out and found that some HPLC methods have been reported for the determination of Granisetron HCl mostly in plasma and a few in pharmaceutical formulations[5-13] and an UV spectrophotometric method[14] in dosage forms. The objective of the present work is to develop a simple, precise and accurate RP-HPLC method for the assay of Granisetron HCl in pharmaceutical dosage forms.

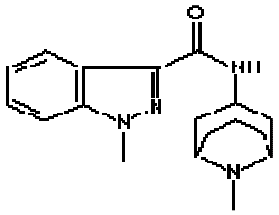

Fig.-1: Chemical structure of Granisetron

EXPERIMENTAL

Instrumentation and chromatographic process

Chromatographic determination was performed on Shimadzu HPLC (LC2010AHT) equipment comprising an LC-10A VP quaternary pump, a variable-wavelength programmable UV-Visible detector, an SPD-10AVP column oven, an SCL 10AVP system controller and a Rheodyne injector fitted with a 20-µL loop. The separation was carried at ambient temperature (25 ± 2°C) on a 250mm × 4.6mm i.d., 5-µm particle, Gemini NX C_{18} reversed phase column using 0.01 M sodium dihydrogen phosphate buffer of pH-7.5 and acetonitrile in the ratio 80:20 v/v as mobile phase at a flow rate of 1.5mL.min^{-1}. The mobile phase was filtered through a 0.22-µm Nylon filter prior to use and the chromatographic data was acquired by using Class-VP 5.032 software.

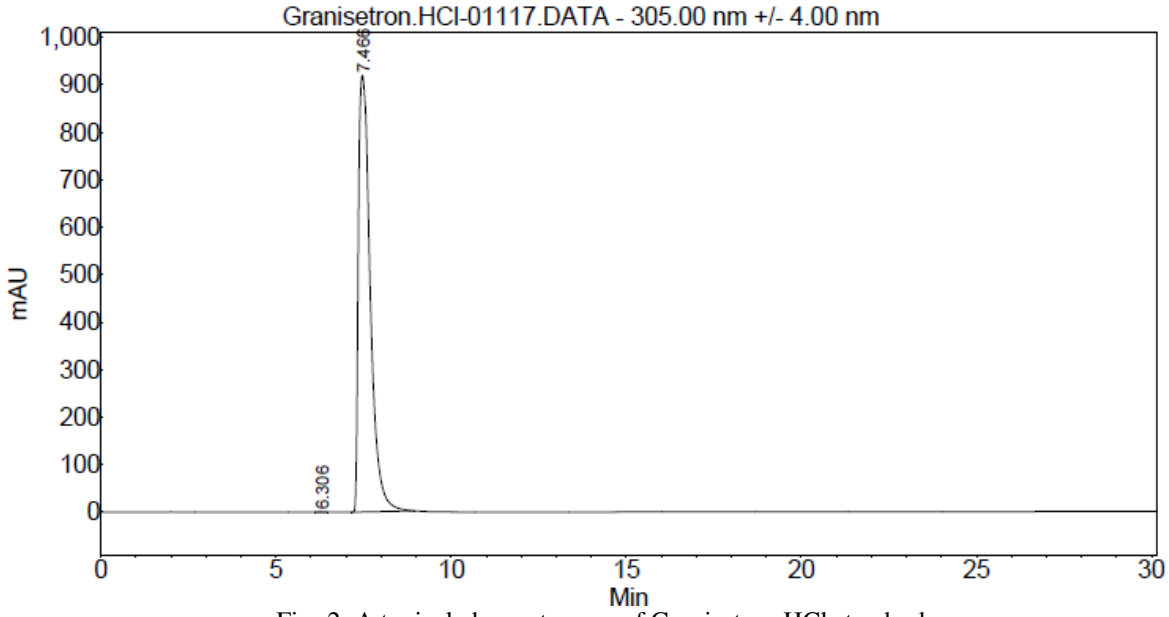

Fig.-2: A typical chromatogram of Granisetron HCl standard

Materials and methods

Granisetron HCl was obtained as a gift from Hetero drugs, Hyderabad. Granisetron tablets were procured from local pharmacy each containing 5.0mg Granisetron, respectively. HPLC-grade sodium dihydrogen phosphate, acetonitrile and ortho phosphoric acid were purchased from E. Merck (Mumbai, India). A stock solution of Granisetron (1.0mg.mL^{-1}, calculated as the free base) was prepared in HPLC grade water. Standard solutions in the concentration range 2.0 - 10µg.mL^{-1} were prepared by dilution of the stock solution with mobile phase.

The HPLC procedure was optimized with a view to develop a suitable method. Granisetron HCl in pure form was run in different mobile phase compositions by using different C18 columns such as Kromacil (25cm x 4.6mm i.d.,5µ), Gemini NX 100 C_{18} (25cm x 4.6mm i.d., 5µ) and Phenomenex C18 column (25cm x 4.6mm i.d., 5µ).The flow rate was also varied from 0.5 ml to 2.0ml.min. Finally, Gemini NX C_{18} column (25cm x 4.6mm i.d., 5µ) with a mobile phase of sodium dihydrogen phosphate buffer (pH 7.5) and acetonitrile in the ratio of 80:20 v/v at a flow rate of 1.5ml.min with a detection wavelength at 305nm gave a sharp and symmetrical peak with retention time of 7.466, USP plate count of 2944.62 and tailing factor of 1.3. A typical chromatogram of standard was shown in Fig. 2.

Validation

The linearity of the proposed method was determined at five different concentrations ranging from 2.0-10.0µg.mL^{-1} of Granisetron HCl. The calibration curve was constructed by plotting response factor

against concentration of drug in µg.mL^{-1} and found to be in a straight line passing through origin (Fig.3)with slope, intercept and squared correlation coefficient of 10789, -46.38 and 0.9990 respectively. The results showed that an excellent correlation exists between response factor and concentration of drug within the concentration range. The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions found to be 0.1502µg.mL^{-1} 0.4553µg.mL^{-1} respectively. The results of linear regression analysis were shown in Table-1.

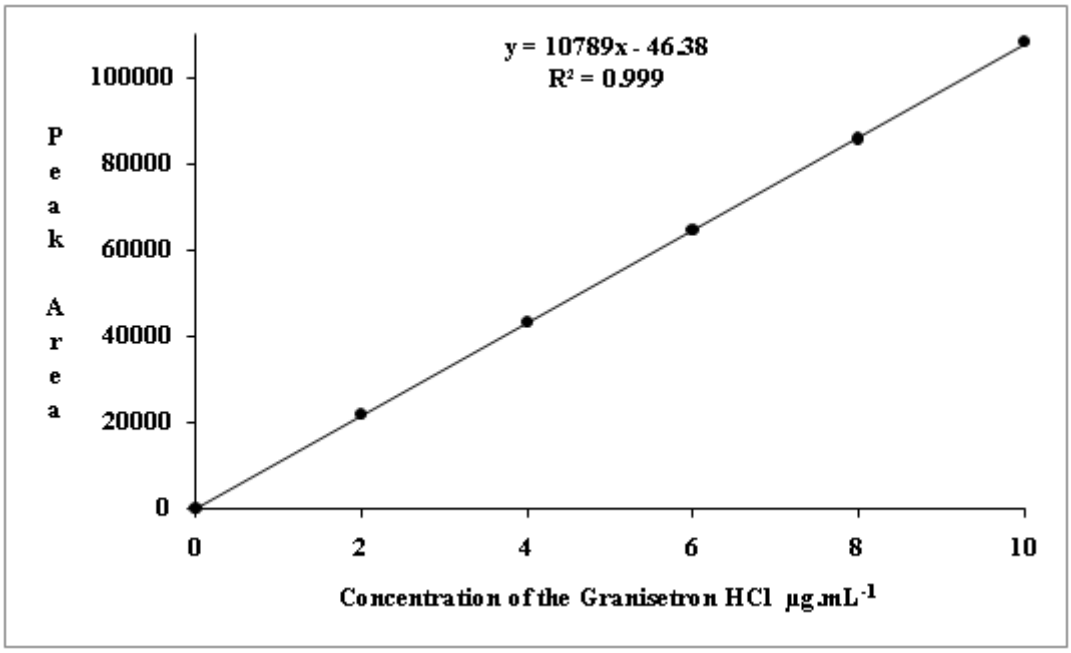

Fig.-3: A calibration plot of response against concentration Granisetron HCl

Table-1: Linearity of the drug concentration to response

Concentration µg.mL^{-1}	Peak Area
2.0	21672
4.0	43114.8
6.0	64523.6
8.0	85675.3
10.0	108361.5
Slope (b)	10789.0
Intercept (a)	-46.38
Correlation coefficient	0.9990
LOD	0.1502
LOQ	0.4553

The precision of the method was demonstrated by inter day and intra-day variation studies. In the present investigation the intra-day precision expressed in terms of percent of relative standard deviation (%RSD) was calculated form the area of the peak for six replicate injections of standard and found to be within the specified limits i.e. not more than 2.0. The accuracy of the proposed method was determined by recovery experiments. Recovery of the drug was carried out by spiking the known standard drug into powdered

formulations. The results of the recovery analysis were calculated and found to be 99.61% to 101.5% respectively.The results of precision and accuracy were presented in Table-2 and Table-3 respectively.
The ruggedness of the proposed method was studied by carrying out the experiment on different instruments like Agilent HPLC and Water's Breeze HPLC, by using different columns of similar type like Kromacil (25cm x 4.6mm i.d.,5µ), Gemini NX 100 C_{18} (25cm x 4.6mm i.d., 5µ) and Phenomenex C18 column (25cm x 4.6mm i.d., 5µ). A study of robustness of the method was carried out by making slight variation in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust.
This procedure was repeated for the sample solutions obtained from the marketed formulations. The peak area, percent of recovery and standard deviation of the percent of recovery of individual drug found in formulation was calculated and presented in Table-4. From the results of analysis it was evident that the proposed procedure for the assay of Granisetron HCl was in good agreement with the label claim of the formulations.

Table-2: Precision of the proposed method

S.No.	Concentration µg.mL^{-1}	Peak Area
1	2.0	21672.3
2	2.0	22421.4
3	2.0	21893.7
4	2.0	20996.5
5	2.0	21521.6
6	2.0	22008.2
	Average*	21603.78
	% RSD	2.142

*Average values of six determinations

Table-3: Accuracy of the developed method at three different concentrations

Labeled amount µg.mL^{-1}	Amount added µg.mL^{-1}	Total amount µg.mL^{-1}	Amount found µg.mL^{-1}	% of Recovery*	Mean
10.0	4.0	14.0	14.21	101.5	
10.0	6.0	16.0	15.96	99.75	
10.0	8.0	18.0	17.93	99.61	100.22%

All the values are the averages of three determinations

Table-4: Analysis of pharmaceutical dosage forms Granisetron HCl

Pharmaceutical formulation	Amount of Granisetron HCl(mg)		% of recovery
	Labeled	Found*	
Tablet – 1	1.0	0.994	99.4

*Average of three determinations

RESULTS AND DISCUSSION

An isocratic RP-HPLC method has been developed and validated for the assay of Granisetron HCl in pure and pharmaceutical formulations. Based on peak purity results obtained for the proposed method, it was found that there were no co-eluting peaks along with the main peak of Granisetron HCl which indicate that the proposed method is specific and selective for the estimation of Granisetron HCl. The drug obeys

linearity (Fig.3) in the range of 2.0-10.0 $\mu g.mL^{-1}$, and the correlation coefficient is found to be 0.9990. The Limit of detection and limit of quantification values reveal that the developed method shows very good sensitivity (Table-1). The precision of a method was expressed in terms of statistical parameters such as standard deviation and %RSD. The %RSD was calculated for six replicate measurements and found to be less than 2.0. The results were given in Table-2. The percentage recovery of the drug at three different concentration levels is presented in Table-3. Repeatability and reproducibility studies reveal that the developed RP-HPLC method was highly precise and accurate. The developed RP-HLC method for the determination of Granisetran HCl was rugged and robust.

CONCLUSSIONS

The developed method is simple, precise, accurate, rapid, robust and rugged, therefore the proposed method can be applied for routine analysis of Granisetron HCl in pharmaceutical formulations.

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