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DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ESTIMATION OF GRANISETRON HYDROCHLORIDE IN BULK AND GRANISETRON HYDROCHLORIDE MOUTH DISSOLVING FILM

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ABSTRACT:
A rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for estimation of Granisetron hydrochloride in bulk and pharmaceutical dosage forms. Granisetron hydrochloride was chromatographed on a reverse phase kromasil C18 column (250 x 4.6mm; 5µm) in a mobile phase consisting of 0.05 M potassium dihydrogen phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and acetonitrile in the ratio of 70:30. The mobile phase was pumped at a flow rate of 1.0 mL/min with detection at 301 nm. The retention time for Granisetron hydrochloride was 4.05min.The detector response was linear in the concentration of 1µg/mL-18µg/mL with correlation coefficient of 0.9996. The percentage recovery of granisetron hydrochloride at target concentration was found to be 94.9 %. The limit of detection and limit of quantification was found to be 0.50 µg/ml and 0.25 µg/ml respectively. Method validation parameters were found to be within the specified limits. The proposed method was found to be simple, fast, accurate, precise and reproducible and could be used for routine quality control analysis of granisetron hydrochloride in bulk and Granisetron hydrochloride mouth dissolving film.

Keywords: Granisetron HCL, RP-HPLC, mouth dissolving film, Method Validation

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INTRODUCTION

Granisetron is an antiemetic agent to prevent the nausea and vomiting in conjunction with cancerchemotherapy or with radiation therapy, by blocking 5-HT_3 receptors without having effect onotherreceptors such as dopamine D_2 receptor and 5-HT_4 receptor. It works by blocking serotonin, a naturalsubstance in the body that causes nausea and vomiting due to the anaesthetics[1,2].The hydrochloride salt ofgranisetron is a white to off-white crystalline powder; soluble in water and salivary fluid. Chemically it is endo-N-(9-methyl-9- azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride[3]. 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 Granisetron is presented in Figure-1. A review of the literature revealed that a very few HPLC methods have been reported for determination of granisetron hydrochloride in pharmaceutical dosage forms[4,5]. Hence, in this present investigation an attempt has been made to develop an accurate, precise and economically viable reversed phase HPLC method for the estimation of Granisetron Hydrochloride in bulk and in pharmaceutical dosage form.

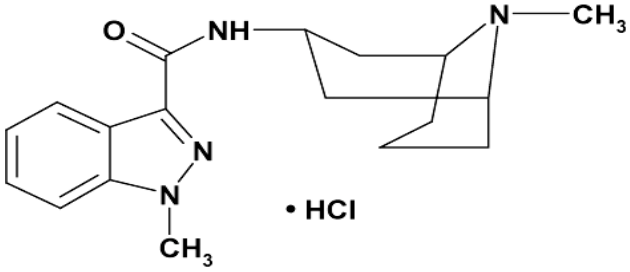

Fig 1: Structure of Granisetron hydrochloride

MATERIALS AND METHODS

Chemicals and reagents

Acetonitrile of HPLC grade was purchased from Merck (India) Ltd. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade was purchased from Qualigens Fine Chemicals Ltd., Mumbai. Granisetron Hydrochloride was received as a gift sample from Wockhardt Ltd, India. Formulation of Mouth dissolving film of granisetron hydrochloride was prepared in-house.

Preparation of mouth dissolving film of Granisetron hydrochloride

Oral strips were prepared by solvent casting technique. In this method, water−soluble ingredients were dissolved to forma clear viscous solution. The active pharmaceutical ingredient and other agents were dissolved in smaller amounts of the solvent and combined with the bulk. The resulting solution was cast as a film and allowed to dry, which was then cut intopieces of the desired size.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu Co, Tokyo, Japan)with UV-Visible dual absorbance detector (PDA), kromasil C18 column (250 x 4.6mm; 5µm).The mobile phase consisting of phosphate buffer (pH 3.0 adjusted with orthophosphoric acid)and acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 70:30 v/v was pumped into the column at aflow rate of 1.0 mL/min. The detection was monitored at 301 nm and run time was 6.0 minutes. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30min. with the mobile phase following through the system. The column and the HPLC system were kept in ambient temperature (25^{o}C).

Preparation of stock solution

About 20 mg of Granisetron hydrochloride was weighed in 200 mL volumetric flask. About 50mL of mobile phase was added, sonicated to dissolve the drug completely and the volume was made up with mobile phase. 5 mL of above solution was diluted to 50 mL with mobile phase (10µg/mL).

Analysis of mouth dissolving film formulation of granisetron hydrochloride

Unit dose of mouth dissolving film (2x2 cm) equivalent to 2 mg of Granisetron hydrochloride in was taken in a clean and dry 200 mL volumetric flask. 80 mL of mobile phase was added, sonicated to dissolve for 5 to 10 minutes and make up the volume with mobile phase and filtered through 0.45µmembrane filter (10 µg/mL).

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines[6].

Selection of detection wavelength

The UV spectrum of diluted solutions for various concentrations of Granisetron hydrochloride in mobile phase was recorded using UV spectrophotometer. The wavelength of maximum absorbance was observed at 301 nm (Figure-2). This wavelength was used for detection of Granisetron hydrochloride.

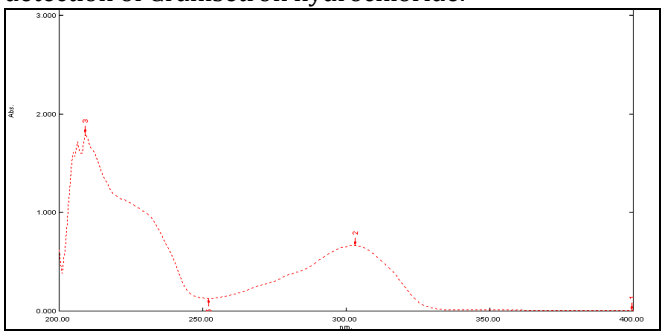

Fig 2: UV Spectrum of Granisetron hydrochloride (10 µg/ml)

Identification and Specificity

The specificity of the HPLC method is illustrated in Figure-3 where complete separation of Granisetron hydrochloride was noticed in presence of mouth dissolving film excipients. In addition there was no any interference at the retention time of in the chromatogram of placebo solution and in peak purity analysis with PDA, purity angle was less than purity threshold observed for the analyte which shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. Hence, Identification and Specificity of granisetron is established.

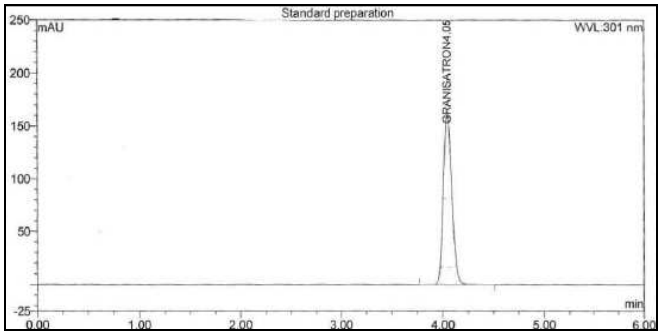

Fig 3: Chromatograms of Granisetron Hydrochloride

System suitability requirement

The chromatographic systems used for analysis must pass the system suitability limits before sample analysis can commence. Set up the chromatographic system; allow the HPLC system to stabilize for 30 minutes. Inject

blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms. System is suitable if tailing factor should NMT 1.5, theoretical plate count should NLT 2000 and % RSD for peak area of six replicate injections of granisetron hydrochloride standard should NMT 2.0. Results of system suitability are presented in Table-1 which shows that all results were within acceptance criteria which proves reproducibility of the method.

Table-1 Results of system suitability

Sr. No.	Injection No.	Peak Area	Theoretical plate count (NLT 2000)	Tailing factor (NMT 1.5)	Retention Time
1	Standard-1	4688.55	4685	1.12	4.05
2	Standard-2	4622.68	4566	1.11	4.04
3	Standard-3	4660.55	4589	1.23	4.05
4	Standard-4	4644.22	4522	1.25	4.04
5	Standard-5	4590.44	4785	1.06	4.05
6	Standard-6	4520.33	4865	1.09	4.05
Mean		4621.13			
% RSD (NMT 2%)		1.29			

Precision

Method precision (Intra-day) was determined from %RSD values carried out by repeating the assay six times. While intermediate precision was obtained by repeating the assay six times on two different days. The intra-day and inter-day precision results were shown in Table-2. The percent relative standard deviation (%RSD) was calculated which is within the acceptable criteria of not more than 2.0 which proves repeatability of methods.

Table-2 Results of Precision Study (Intra-Day and Inter-Day Precision) for Granisetron Hydrochloride

Sr. No.	Intra-Day Precision*		Inter-Day Precision*	
	Injection No.	Peak Area	Injection No.	Peak Area
1	Sample-1	4622.20.	Sample-1	4522.23
2	Sample -2	4520.22	Sample-2	4680.68
3	Sample -3	4550.11	Sample-3	4527.53
4	Sample -4	4570.68	Sample-4	4584.72
5	Sample -5	4480.03	Sample-5	4520.08
6	Sample -6	4562.69	Sample-6	4611.54
Mean		4536.746	**Mean**	4574.46
% RSD (NMT 2%)		0.82	1.40	
Theoretical plate count (NLT 2000)		3890	4250	
Tailing factor (NMT 1.5)		1.18	1.09	

*Concentration of Granisetron Hydrochloride 10 µg/mL

Accuracy

Accuracy is the degree of agreement between a measured value and the accepted reference value. The accuracy of the method was tested by triplicate samples at 3 different concentrations equivalent to 50%, 100% and 150% of the active ingredient, by adding a known amount of granisetron hydrochloride standard to a sample with pre-determined amount of granisetron hydrochloride. The recovered amount of granisetron hydrochloride, %RSD of recovery, % recovery of each concentration is calculated to determine the accuracy. The recovery results for are presented in Table -3. The % mean recovery amount of granisetron hydrochloride was within range of 80% to 120 % and %RSD was below 2.0%. Hence accuracy is established.

Table-3 recovery data of Granisetron hydrochloride

Sr. no.	Recovery Level	Amount taken (mg)	Amount Added (mg)	Total Amount (mg)	Amount Recover (mg)	% recovery	Mean % Recovery	% RSD (NMT 2.0%)
1	50 %	15	5	20	19.75	98.75	94.3	0.83
2					18.25	91.25		
3					18.60	93.00		
4	100 %	20	5	25	24.35	97.40	94.9	0.75
5					23.90	95.60		
6					22.95	91.80		
7	150 %	25	5	30	28.80	96.00	95.2	0.95
8					27.56	91.87		
9					29.33	97.77		

Limit of detection (LOD), Limit of Quantification (LOQ) and Linearity

System suitability was established. LOD, LOQ and Linearity were determined by calibration curve method.20 µl of each calibration standard solutions (0.25, 0.5, 1, 2, 4, 6, 8, 10,12, 14, 16 and 18 µg/mL) were injected into the HPLC system to get the chromatograms. The average peak area and retention time were recorded. Linearity curve was constructed by plotting concentration of granisetron hydrochloride on X-axis and average peak areas of standard granisetron hydrochloride on Y-axis and regression equations were computed. The results are presented in Table-4. Results show that a phenomenal correlation exists between peak area and concentration of drug within the linearity range (1-18 µg/mL). Linearity curve is shown in Figure-4. % RSD till detection of peak response using 0.5 µg/mL was found within range of not more than 2.0% and peak was detected at lowest concentration of 0.25µg/mL. Hence, LOD and LOQ were found 0.5 µg/mL and 0.25 µg/respectively.

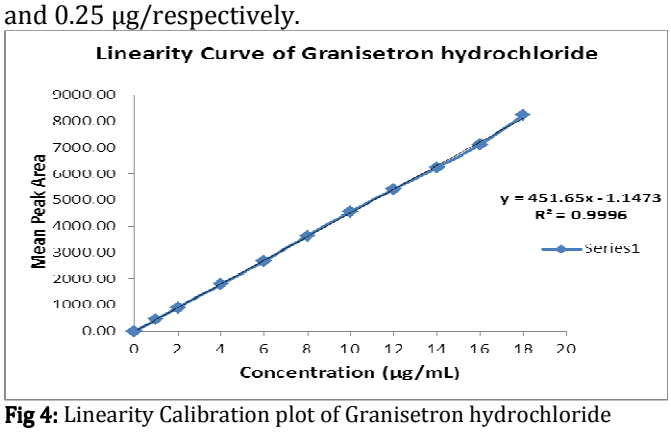

Fig 4: Linearity Calibration plot of Granisetron hydrochloride

Table-4: Results of LOD, LOQ and Linearity

Sr. No.	Concentration (µg/mL)	Mean peak area	% RSD*
1	18.00	8241.33	0.95
2	16.00	7125.65	1.25
3	14.00	6245.35	0.88
4	12.00	5422.12	0.36
5	10.00	4565.55	0.93
6	8.00	3645.54	0.89
7	6.00	2685.84	1.36
8	4.00	1790.65	1.45
9	2.00	910.38	1.08
10	1.00	455.10	0.86
11	0.50	228.63	1.55
12	0.25	115.35	12.36
13	0.13	Not Detected	-
Correlation coefficient (r)		0.9996	
Slope		451.65	
Y intercept		1.15	
LOD (µg/mL)		0.50	
LOQ (µg/mL)		0.25	

Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameters were: Change in flow rate by $\pm$0.2, change in detection wave length by $\pm$2 nm and change in the composition of mobile phase ($\pm$ 5%). The results of robustness study is shown in Table-5 indicated that the small change in the conditions did not significantly affect the determination of granisetron hydrochloride.

Table-5 Robustness Results of granisetron hydrochloride

Sr. No.	Change Parameter	Optimized parameter	Used Condition	Theoretical Plate	Tailing factor	% RSD
1	Flow rate ($\pm$0.2 mL/min)	1.0 mL	0.8 mL/min	3280	1.13	0.88
2			1.2 mL/min	2908	1.25	0.96
3	Detection wavelength ($\pm$2 nm)	301 nm	299 nm	4588	1.14	1.25
4			303 nm	3208	0.98	1.44
5	Mobile phase composition ($\pm$ 5%)	Buffer: acetonitrile (70:30)	Buffer: acetonitrile (68.5:31.5)	3504	0.75	1.22
6			Buffer: acetonitrile (71.5:28.5)	3850	0.88	1.32

CONCLUSION

In this present study an attempt has been made to develop Reverse Phase-HPLC (RP-HPLC) method for the determination of Granisetron hydrochloride in pure and mouth dissolving film dosage form. The results of the validation process showed that the proposed method is authenticated and found within predetermined limits, and fitness for purpose. It can be seen that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations revealed that proposed methods are suitable for their analysis with virtually no interference of the usual additives present in the pharmaceutical formulations. Hence, this method can easily and conveniently adopt for routine quality controlanalysis of Granisetron hydrochloride in bulk and its mouth dissolving film.

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