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SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF GRANISETRON HYDROCHLORIDE IN BULK AND INJECTIONS

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Keywords:

Granisetron hydrochloride, UV Spectrophotometry, RP-HPLC, Validation, Small volume parenteral

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ABSTRACT: The present UV Spectrophotometric and RP-HPLC method was simple, accurate, precise, specific, and sensitive. Spectrophotometric method was developed in water and very useful for analysis of bulk drug and injection formulation. RP-HPLC method was developed in Phosphate buffer (pH 3.0 adjusted with orthophosporic acid) and acetonitrile (70:30) using column HiQsil C18 column (250 × 4.6 mm; 5 µm). Flow Rate: 1.0 ml / min. The method was validated for the determination of granisetron hydrochloride in bulk and parenteral dosage form. The standard solution of granisetron hydrochloride in water showed maximum absorption at 301 nm with correlation, slope, and intercept 0.9998, 0.04198, and 0.01255, respectively and the percentage recovery of the formulation was 99.965% by UV spectrophotometry. By RP-HPLC, the % RSD of intraday and interday precision was 0.82% and 1.40 %, respectively. The % means recovery amount of Granisetron hydrochloride in 80 %, 100%, 120% was 94.3%, 94.9%, 95.2%, respectively, which reflect that the method was free from the interference of the impurities and other additives during the estimation of drug in the formulation. The proposed method can be successfully used for analysis of granisetron hydrochloride in marketed preparations. The results of analysis have been validated statistically and by recovery studies. This method was found suitable for quantitative and qualitative estimation of granisetron hydrochloride in bulk and parenteral dosage form.

INTRODUCTION: Granisetron hydrochloride, endo - 1 - methyl - N - (9 - methyl - 9 - azabicyclo 3.3.1 non - 3 - yl) H-indazole-3-carboxamide **Fig. 1**, is a selective 5-HT3 receptor antagonist [1]. Granisetron hydrochloride is an effective and potent antiemetic drug which is used in the postoperative treatment of vomiting and nausea resulting from cancer chemotherapy and radio-therapy in adults and children [2-6].

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Granisetron hydrochloride is also effective in the management of postoperative nausea and vomiting due to anesthetics [7]. Granisetron hydrochloride exhibits its antiemetic effects through central and peripheral 5-HT3 receptors [18]. Granisetron, as Granisetron hydrochloride, is used both in oral tablets and injection [1].

Literature survey reveals that the drug has been estimated by HPLC [8-13], HPTL C [20], tandem LC-MS [15,] and Bioanalytical [16-17] methods in biological fluids like plasma and pharmaceutical dosage forms but no simple UV Spectro-photometric and RP-HPLC method in pharmaceutical formulations of injection has been reported so far. A thorough literature survey reveals that only few analytical methods have been reported for the determination

of granisetron hydrochloride in bulk and pharmaceutical formulations, including RP-HPLC stability-indicating and bioanalytical method. The present work deals with the estimation of granisetron hydrochloride in injection by UV spectrophotometry and by RP-HPLC. Experimentation was carried out in MET's institute of pharmacy Nashik.

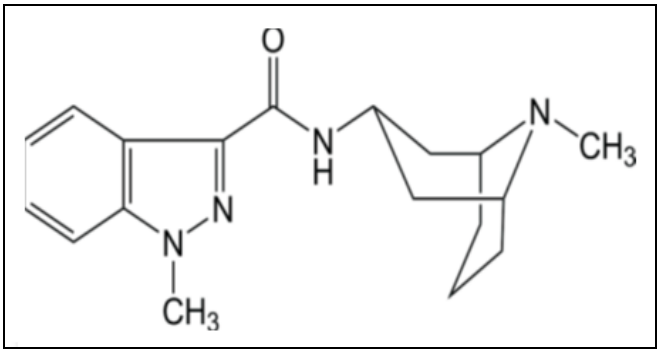

FIG. 1: CHEMICAL STRUCTURE OF GRANISETRON

MATERIALS AND METHODS:

Instruments: A Jasco UV-Visible Spectrophotometer (UV-1700) with a matched pair cell of 10 mm quartz was used for experimental purpose. The HPLC system used consisted of a pump PU- 2080 plus (JASCO Corporation, Japan) fitted with 20 µL Rheodyne loop injector (7725*i*). Detection was carried out on UV -2075 detector (JASCO Corporation, Japan). The data acquisition was done on BORWIN chromatography software (version 1.50).

Materials: Pharmaceutical grade Granisetron hydrochloride was kindly provided as gift sample from Sunpharma Ltd., and it was used without further purification. All analytical grade reagents were purchased from S.D Fine chemicals, Mumbai. Granisetron hydrochloride injection (KYTRIL) was purchased from market. Each 1 mL contains 1.12 mg granisetron hydrochloride equivalent to 1mg of granisetron. Double distilled water was freshly prepared by all double distillation glass Assembly (Borosil, Mumbai, India) for the preparation of solutions and further used in analysis after filtering through 0.45 × 47 mm membrane filter papers purchased from PCI Analytics Ltd. Was used for degassing the prepared mobile phase.

Preparation of Standard Stock Solution and Calibration Curve: The standard stock solution was prepared by dissolving granisetron hydrochloride in distilled water to make the final concentration of 100 µg/ml. Different aliquots were taken from the stock solution and diluted with distilled water separately to prepare a series of concentrations from 5-30 µg/ml.

The λ_{max} was found by the UV spectrum of granisetron hydrochloride in distilled water in the range of 200-400 nm, and it was found to be 301 nm. Absorbance was measured at 301 nm against distilled water as blank. The calibration curve was prepared by plotting absorbance versus concentrations of granisetron hydrochloride.

Analytical Method Validation by UV and RP-HPLC: The developed method was validated in terms of specificity and selectivity, linearity and range, precision, accuracy, the limit of detection, the limit of quantitation, and robustness as per USP and ICH guidelines [21-23].

Linearity and Range: The calibration graph of the absorbance versus concentration was found to be linear over the range of 5-30 µg/ml. The calibration graph was constructed after an analysis of 6 different concentrations. Each point of the calibration graph corresponded to the mean value obtained from three independent measurements. The regression equation was y = 0.04198 × - 0.01255, where y is the absorbance, and x is the concentration in µg/ml. (r^2 = 0.9998).

Precision and Accuracy: Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as %RSD for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on three different days, and the results are documented as the standard deviation and % RSD. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

Sensitivity: The limit of quantification (LOQ) is the lowest concentration of Granisetron hydrochloride on the calibration curve that can be quantified with acceptable precision and accuracy. The LOQ was found as 1.35 µg/ml for the proposed method. The limit of detection (LOD) was found to be 0.4465 µg/ml. The results indicate that the proposed method is sensitive to detect and quantify 0.4465 µg/ml and 1.35 µg/ml respectively.

Specificity: The spectra obtained from the test solution were identical with that obtained from a standard solution containing an equivalent concentration of Granisetron hydrochloride indicating that the wavelength of maximum absorbance of Granisetron hydrochloride did not change.

It was concluded that the excipients did not interfere with the quantification of Granisetron hydrochloride in pharmaceutical formulations by the proposed method. Thus, the proposed method can be used for the determination of Granisetron hydrochloride in the presence of excipients.

Robustness: The terms robustness refer to the ability of an analytical method to remain unaffected by small variations in method parameters (mobile phase composition, column age, column temperature, change in wavelength, *etc*.) and influential environmental factors (room temperature, air humidity, *etc*.) and characterize its reliability during normal usage. It was making small deliberate changes in the wavelength of detection used for testing the robustness of method.

Analysis of Marketed Formulation: Granisetron hydrochloride injection (KYTRIL) (containing Granisetron equivalent to 1 mg per ml) was purchased from the market. 0.15 ml of this solution was diluted with 10 ml distilled water (15 µg/ml) and subjected to UV and RP-HPLC analysis. The amount of Granisetron hydrochloride was obtained from the regression equation of calibration curve.

Specificity: Complete separation of Granisetron hydrochloride was noticed in the presence of excipients of the tablet formulation. In addition there was no any interference at the retention time of in the chromatogram of placebo solution, which shows that the peaks of analyte were pure and excipients does not interfere the analyte. Hence, Identification and Specificity of Granisetron is established.

System Suitability: The chromatographic systems used for analysis must pass the system suitability criteria before sample analysis can commence. Set up the chromatographic system; allow the HPLC system to stabilize for 30 min. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms.

RESULTS AND DISCUSSION:

UV Spectrophotometry Selection of Detection Wavelength: The UV spectrum of diluted solutions for various concentrations of Granisetron hydrochloride in the mobile phase was recorded using UV spectrophotometer. The Wavelength of maximum absorbance was observed at 301 nm.

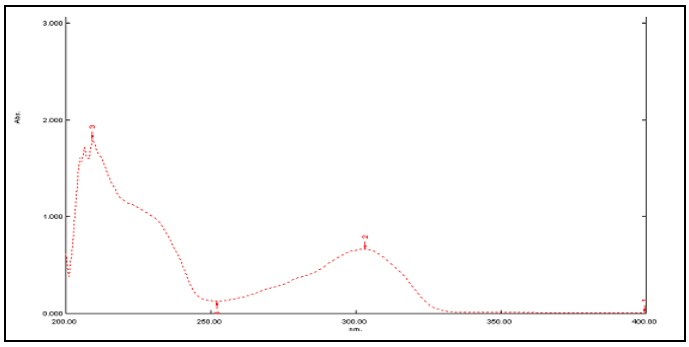

FIG. 2: UV SPECTRUM OF GRANISETRON HYDROCHLORIDE (10 µg/ml)

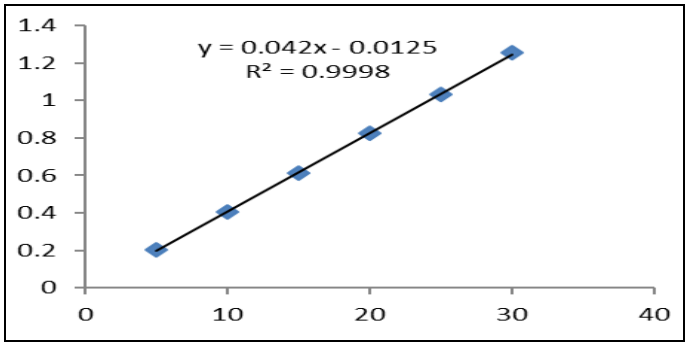

FIG. 3: CALIBRATION CURVE OF GRANISETRON HYDROCHLORIDE

Linearity: The response for the drug was linear in the concentration range of 5-30 µg/ml. The regression equation was y = 0.04198 × -0.01255, where y is the absorbance, and x is the concentration in µg/ml. (r^2 = 0.9998). The regression data, values of correlation coefficient (r) and other statistical parameters are listed in **Table 1**.

TABLE 1: ANALYTICAL CHARACTERISTICS OF GRANISETRON HYDROCHLORIDE BY PROPOSED UV SPECTROPHOTOMETRIC METHOD

S. no.	Parameter	Result
1.	λmax nm	301nm
2	Linearity range µg/ml	5-30
3	Regression equation	y =0.04198x-0.01255
4	Correlation coefficient(r2)	0.9998
5	Limit of detection (LOD) µg/ml	0.4465
6	Limit of quantification (LOQ) µg/ml	1.3532
7	Number of data points	6
8	95% Confidence interval	0.9992 to 1.000

TABLE 2: REPEATABILITY

Sample no.	Conc. (µg/ml)	Obtained conc. (µg/ml)
1	15	14.89
2	15	14.92
3	15	14.95
4	15	14.67
5	15	14.88
6	15	14.83
mean		14.85
Standard deviation		0.0999
%RSD		0.6227%

Precision and Accuracy: The results of the repeatability listed in **Table 2**. The developed method was found to be precise as the % RSD values for repeatability, and intermediate precision studies were found < 2. From accuracy data, as shown in **Table 3** the % recovery of the drug was 102.1% to 101.2% found.

Analysis of Marketed Formulation: The % recovery of drug content in the marketed formulation was found to be 99.965% with 0.6227 % RSD; the low RSD value indicated the stability of method for the routine analysis of granisetron hydrochloride in pharmaceutical formulation.

Validation using HPLC:

Specificity: There was no any interference at the retention time of in the chromatogram of the drug in the presence of placebo, which shows that the peaks of analyte were pure, and excipients do not interfere with the analyte.

TABLE 3: ACCURACY AND PRECISION TABLE

Amount Added (mg)	Amount Found (mg)	Within Mean Square			Between Mean Square	F
80% (20 mg)	Day1	Day 2	Day 3	0.1864	0.1661	0.8910
	19.96	20.003	19.97			
	20.18	20.10	20.81			
	21.11	19.9	20.42			
Mean	20.42	20.001	20.4			
% RSD	2.98	0.5025	2.060			
100%(25 mg)	24.81	25.36	25.41	0.07490	0.3037	4.054
	24.66	24.84	25.71			
	25.3	25.36	25.55			
Mean	24.92	25.18	25.55			
%RSD	1.343	1.192	0.5874			
120%(30 mg)	30.53	31.20	31.15	0.1723	0.1440	
	30.05	30.5	30.77			0.8360
	30.58	30.76	30.06			
Mean	30.38	30.82	30.66			
%RSD	0.9631	1.147	1.804			

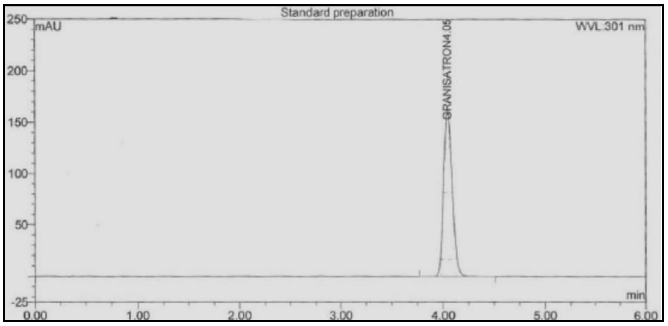

FIG. 4: CHROMATOGRAMS OF GRANISETRON HYDROCHLORIDE STANDARD

System Suitability: System is suitable if the 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 the reproducibility of the method.

Precision: The intraday and inter-day precision results were shown in Table. The percent relative standard deviation (% RSD) was calculated, which is within the acceptable criteria of not more than 2.0, which proves the repeatability of the method.

TABLE 4: SYSTEM SUITABILITY BY HPLC

S. no.	Injection No.	Peak Area	Theoretical Plate Count (NLT) 2000	Tailing Factor (NMT 1.5)	Retention Time
1	Standard-1	4588.55	4785	1.02	4.05
2	Standard-2	4422.68	4346	1.11	4.04
3	Standard-3	4560.55	4589	1.13	4.05
4	Standard-4	4434.22	4231	1.05	4.04
5	Standard-5	4580.44	4489	1.06	4.05
6	Standard-6	4420.33	4178	1.09	4.05
Mean	4501.12				
% RSD	1.26				

TABLE 5: PRECISION BY HPLC

S. no.	Intraday Precision		Interday Precision	
	Injection no	Peak Area	Injection no	Peak Area
1	Sample-1	4622.20.	Sample-1	4522.23
2	Sample-2	4520.22	Sample-1	4680.68
3	Sample-3	4550.11	Sample-1	4527.53
4	Sample-4	4570.68	Sample-1	4584.72
5	Sample-5	4480.03	Sample-1	4520.08
6	Sample-6	4562.69	Sample-1	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	

TABLE 6: ACCURACY BY HPLC

Recovery Level (% of Sample Concentration)	Amount Taken (mg)	% Recovery	Mean % Recovery	% RSD (NMT 2.0%)
80%	20 mg	93.00	94.3	0.83
		98.75		
		91.25		
100%	25 mg	97.40	94.90	0.75
		91.8		
		95.60		
120%	30 mg	96.00	95.2	0.95
		97.77		
		91.87		

CONCLUSION: The proposed methods can be successfully applied for granisetron hydrochloride assay by UV Spectrophotometry and RP- HPLC in small volume parenteral dosage forms without any interference in quality control. Analysis of the small volume parenteral by this method was reproducible, reliable, and in good agreement with the labeled claim of the drug.

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CONFLICTS OF INTEREST: Nil

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