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Analysis of Stability of Granisetron Hydrochloride in Nasal Formulations by Stability-Indicating RP-HPLC Method

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ABSTRACT

The objective of current study was to develop a stability indicating reversed-phase liquid chromatographic method for the quantitative determination of granisetron hydrochloride in bulk sample and pharmaceutical dosage form (in-situ gelling nasal formulation). Chromatographic separation was achieved using reversed phase, Column Agilent (TC-C18 (150 mm x 4.6 mm i.d., 3.5 5µ particle size) at ambient temperature with acetonitrile: 0.05 M potassium dihydrogen phosphate solution (pH 3.0 adjusted using 1% orthophosphoric acid) (65: 35 v/v) as a mobile phase, at a flow rate of 1.0 ml/min. The eluted compounds were monitored at 302nm. Granisetron hydrochloride was subjected to the stress conditions of acid, base hydrolysis along with thermal degradation. The degradation products were well resolved from main peak proving the stability-indicating power of the method. The retention time for granisetron hydrochloride was found to be 3.07 min. Linear regression analysis revealed a good linear relationship (r^2 = 0.9970±0.0023) between peak area and concentration in the range 5-50µg/mL. The method was successfully employed for the estimation of granisetron as a bulk drug, and from in situ nasal gel formulations (developed in –house). The results of assay, recovery studies and its statistical validation data indicate high degree of precision and accuracy of the method.

Keywords: granisetron hydrochloride, RP-HPLC method, stability indicating, nasal formulation

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INTRODUCTION

Nausea and vomiting are typical side effects of chemo therapy, radiotherapy and some surgical procedures. Current treatment guidelines recommend the use of 5-HT3 receptor antagonists for highly and moderately emetogenic chemotherapy and radiotherapy. Granisetron (Fig.1), [endo-1-methyl –N- (9-methyl-9-aza-bicyclo [3.3.1] non-3-yl)-1 H-indazole-3-carboxamide] is a potent and highly selective serotonin (5HT3) receptor antagonist causes antiemetic effect by blocking 5-HT_3 receptor in brain and peripherally. It is safe in children and geriatric patients [1, 2, 3, 4]. Orally administered granisetron is subjected to hepatic first pass effect and may be associated with certain unwanted gastrointestinal effects. Since cancer patients under chemo- or radiotherapy already have distressed stomach as well as nausea and vomiting, orally administered drug tends to be expelled by vomiting. Intravenous route is also not desirable due to the invasiveness and not suitable for self-medication. Thus, an alternative approach is required to make the treatment more convenient and effective in cancer patients. In this perspective, nasal administration of granisetron hydrochloride (GRH) represents an interesting alternative administration route [5]. In the literature, a number of methods have been described for the determination of granisetron hydrochloride in bulk, formulations and in biological fluids employing various techniques such as HPLC coupled with UV Detection[6], fluorescence detection[1,7-12**]** ,electrochemical[8], and DAD detection[13-16]. There are several publications involving use of hyphenated techniques as LC-MS or LCMS- MS [17-19]. Q1A on Stability Testing of New Drug Substances and Products [20] emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability-indicating testing methods.

It also recommends carrying out stress testing on the drug substance to elucidate its inherent stability characteristics and hence supporting the suitability of the proposed analytical procedure [21]. So far to our knowledge, no HPLC method has been reported for simultaneous determination of granisetron hydrochloride and its degraded products in nasal formulation therefore, the aim of the present study is to develop and validate purposely a simple, rapid stability-indicating HPLC method to separate GRH from its stress-formed degradation products. The proposed HPLC method is validated and thereafter, the method has been successfully applied for routine estimation of granisetron hydrochloride from bulk and pharmaceutical dosage forms such as in-situ gelling formulations and to estimate them in stability sample.

MATERIALS AND METHODS

GRH, pharmaceutical grade (Natco Pharma Ltd.) was used and certified to contain 99.67%. Acetonitrile (HPLC grade) was obtained from Qualigen (Germany). Orthophosphoric acid solution (85%) and potassium dihydrogen phosphate (HPLC grade) were obtained from Merck (Darmstadt, Germany). Sodium hydroxide pellets and hydrochloric acid (33%) were of analytical grade (Merck, Darmstadt, Germany). HPLC water was generated in-house using Water still distillation assembly (Pharma Lab) and double distillation unit (J-Sil). Ultipor N Nylon 6.6 membrane (poresize0.45 µm and 0.22 µm) filter were purchased from Pall Life Sciences. Pluronic F127 (Poloxamer407, Signet Chemicals, Mumbai, India) Chitosan

(Research Lab., Islampur, India)) was procured as gift sample. All other chemicals and solvents were of analytical reagent grade. Degradation experiments in acid, alkaline and neutral conditions were performed using a water bath shaker (Modern Industrial Corporation 1402). Ultrsonic cleaner (Wensar), Filtration assembly (Tarsons Rockyvac), Electronic balance (Denver Instruments) and pH meter (Toshniwal, Ajmer) were used. A Refrigerator (Samsung) and stability chamber (Remi) was used for stability studies.

HPLC

A HPLC Quaternary gradient system (Lachrom 2000) consisting of L-7100 Merck Hitachi Pump, UV visible detector (L-7400), Rhenodyne injection system with 20µl loop was used for analysis. HPLC Column Agilent (TC-C18 (150 mm x 4.6 mm i.d., 3.5 5µ particle size) with guard column Thermo Scientific (BDS-Hypersil-C-18) was used for analysis.

Chromatographic conditions

The mobile phase consisting of acetonitrile: 0.05 M potassium dihydrogen phosphate solution (pH 3.0 adjusted using 1% orthophosphoric acid) (65: 35 v/v) was filtered through a Ultipor N Nylon 6.6 membrane (0.45 mm) filter (Pall Life Sciences), degassed using ultrasonicator and pumped by the reciprocating pump (L-7100 Merck Hitachi) at a flow rate 1 ml/min.The column temperature was maintained at 29^0C. The sample (20µl) was injected through a Rheodyne injector and was analyzed by variable wavelength detector set at 302.0 nm. The data was acquired, stored and analyzed with Winchrome software. The separation was carried out on Agilent C18 column (Hypersil, 250mm x 4.5mm, 5µ).

Preparation of gel formulation

Preparation of In situ gel of PF127 (Formulation A)

In situ gels were prepared by cold method using 18%w/v Poloxamer 407 (PF127). Drug and other ingredients were added and mixture was cooled to 4^oC.

Method of preparation of In situ gel of chitosan (Formulation B)

Aqueous gels of chitosan – β glycerophosphate were prepared by mixing slowly required amount of acidic solution of chitosan with solution of β glycerophosphate in distilled water with continuous stirring. Drug & other ingredient were added and mixture was cooled to 4^oC. For stability testing formulations were kept at 4^0C and 25^0C for 6 months and analyze at 1st day, 15 th day, and after 6 month.

Preparation of standard stock and sample solution of granisetron hydrochloride

About 10 mg of granisetron hydrochloride was accurately weighed and transferred to 10 ml volumetric flasks. It was dissolved in methanol and the solution was made up to volume with methanol to obtain 1000µg/ml of stock solution. The sample solution was further diluted with mobile phase to obtain standard solutions of different concentrations

containing 5-50µL of GRH. The solution was filtered through 0.45µm nylon membrane filter and 20µl aliquots were injected in six times into the sample injector of HPLC system under the chromatographic condition as described above. The area under the curve of each peak was measured at 302.0 nm. The amount of drug present in the sample solutions was determined using the prepared calibration curves of standard GRH. The correlation coefficients, slopes and Y-intercepts of the calibration curve were determined.

Forced degradation studies

Stress testing of the drug substance can help to identify likely degradation products, which can, in turn, help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. All stress degradation studies were performed at an initial drug concentration of 1mg/mL. Samples were withdrawn at appropriate times and subjected to LC analysis to evaluate the capacity of the method to separate GRH from its degradation products. In all degradation studies, the average peak area of granisetron hydrochloride (20 µg/mL) after application of six (n=6) replicates was obtained.

Acid-induced hydrolysis, alkaline-induced hydrolysis and neutral hydrolysis

Hydrolytic degradations were carried out by preparing the drug solutions in 1M HCl, 1M NaOH and water, respectively. All the solutions were protected from light. The prepared solutions were heated in a water bath for 2 h at 80^0C. The solutions were neutralized, diluted with mobile phase to contain 20µg/mL and 20µL was injected in to the LC system.

Optimization of stability-indicating HPLC method

The HPLC procedure was optimized with a view to develop the stability-indicating assay method. Force-degraded samples were analysed by HPLC using HPLC Column Agilent (TC-C18 (150 mm x 4.6 mm i.d., 3.5 5µ particle size) and a mobile phase initially, composed of acetonitrile: water (50:50v/v). As the separation and the shape of the peaks were not good, the % of the organic modifier was changed from 50% to 25% but no improvement was observed. Hence water was replaced by 0.05 M potassium dihydrogen phosphate solution (pH 3.0 adjusted using 1% orthophosphoric acid) in 50:50 v/v ratios. To reduce peak broadening, acetonitrile concentration was increased. Finally it was found that a mixture of acetonitrile: 0.05 M potassium dihydrogen phosphate solution (with pH 3.0 adjusted using 1% orthophosphoric acid) (65: 35 v/v) as a mobile phase at a flow rate of 1mL/min gave acceptable retention time (tR), and good resolution of the drug and degradation products (Fig.1, Fig. 2 and Fig. 3).

Fig. 1 Structure of granisetron hydrochloride

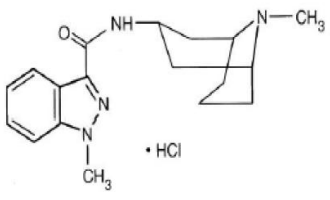

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Fig. 2: Typical Chromatogram of Standard Granisetron hydrochloride (50µg/mL)

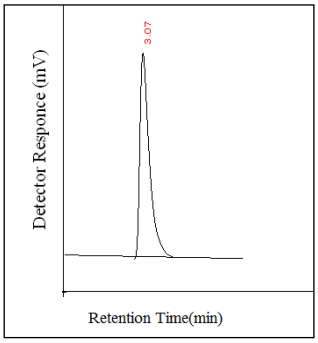


Fig. 3: Typical Chromatogram of granisetron hydrochloride and degradation product in 1M HCL

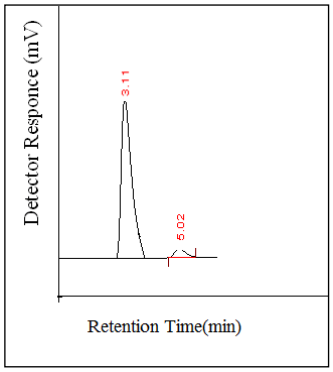
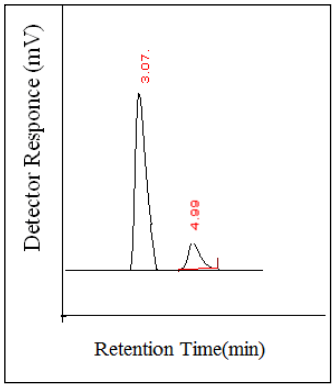


Fig. 4: Typical Chromatogram of granisetron hydrochloride and degradation product in 1M NaOH



Validation procedure- Validation of HPLC method was done with respect to following parameters.

Linearity and Range

The linearity of the detector response with the concentrations of the investigated drugs was evaluated. Stock standard solution of GRH, was prepared at strength of 1.0 mg /mL. Dilution with the mobile phase was carried out to obtain solutions containing concentrations ranged from 5-50µg/mL, of GRH. The solutions were injected in triplicate into the HPLC system keeping the injection volume constant (20 µL). The Peak areas were

plotted versus the corresponding concentration to obtain calibration graphs. The equations were obtained by least squares linear regression analysis of the peak area versus the concentration.

Precision

The precision of the proposed HPLC method was verified by repeatability and intermediate precision studies. For determination of repeatability, three different concentrations (20, 40, and 60 µg/mL) of the drug solutions were prepared and analyze in hexplicate (n=6) on the same day. The inter-day precision studies were done by repeating the studies on three consecutive days.

Limit of detection (LOD) and quantification (LOQ)

The LOD and LOQ for granisetron hydrochloride were determined at a signal-to-noise ratio of 3:1and 10:1, respectively, by injecting a series of dilute solutions with known concentrations and calculating the RSD of the area.

Robustness of the method

To evaluate robustness of the HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate (0.9, 1.0 and 1.1mL), percentage of acetonitrile in the mobile phase (63, 65 and 67%), and pH of mobile phase (2.9, 3.0 and 3.1).

Specificity

The specificity of the proposed method was evaluated through study of resolution factor of the drug peak from the nearest resolving peak. The specificity of the proposed HPLC method for determination of GRH, in the investigated pharmaceutical preparations in presence of the ICH stress-formed degradation products was proved by comparison of retention times for such peaks on the chromatograms of recovery experiments to that of standard solutions. The absence of peaks on the chromatograms of placebo solution indicated the absence of interference from co-formulated excipients.

Analysis of the gel Formulation

To determine the GRH content of gels, an amount of formulation equivalent to 1mg of granisetron was transferred to a 10 ml volumetric flask, extracted with methanol, sonicated for 30 min, diluted to volume with same solvent. It was further diluted with mobile phase. The solution was filtered through 0.45µm nylon membrane filter and 20µl aliquots were injected in six times into the HPLC system under conditions described above. The peak areas were measured at302nm and concentrations in the samples were determined using multi level calibration developed on the same LC system under the same conditions using linear regression equation. To study the effect of excipients, two gel formulations with different excipients were prepared and analyzed.

Analysis of stability sample

Stability samples were removed at fixed interval and analyzed as above.

Accuracy

Accuracy of the method was evaluated by carrying the recovery of the drug at three different levels in formulations. Gels were prepared with 80%, 100%, and 120% of granisetron and were analysed by the proposed method. The experiment was conducted in triplicate. The percentage recoveries were calculated from the slope and Y-intercept of the calibration curve.

RESULTS AND DISCUSSION

Results of forced degradation studies

Hydrolytic degradation studies

The drug was found to be labile to basic degradation as compared to that of acid while it is less sensitive to neutral pH. Initially 0.1M hydrochloric acid and 0.1M NaOH was used at 80 ^{0}C for 3h but negligible degradation was observed hence the strength of acid and alkali was increased. 5–20% degradation was observed by heating drug solution with1M hydrochloric acid and 1M NaOH at 80 ^{0}C for 2h and associated with rise in a major degradation product at retention time 5.02min in HCL and 4.99 in NaOH. However, the drug was found to be stable towards hydrolysis in neutral solution. The chromatograms of the acid-induced degradation and the alkaline-induced degradation were shown in Figure 2 & Figure 3

Validation of the method

The results of validation studies on the stability-indicating method developed for granisetron hydrochloride by using mixture of acetonitrile: 0.05 M potassium dihydrogen phosphate solution (with pH 3.0 adjusted using 1% orthophosphoric acid) (65: 35 v/v) as a mobile phase at a flow rate of 1mL/min are given below

Linearity

The response for the drug was linear in the concentration range of 5-50µg/mL. The mean (±RSD) values of slope, and correlation coefficient were 332803 (±1.30) and 0.9970(±0.0023), respectively. The regression data, values of correlation coefficient (r) and other statistical parameters are listed in Table 1.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 2. The developed method was found to be precise as the RSD values for repeatability

and intermediate precision studies were <2%, respectively, as recommended by ICH guideline.

LOD and LOQ

The signal: noise ratios of 3:1and10:1were considered as LOD and LOQ, respectively. The LOD and LOQ were found to be 0.046µg/mL and 0.139 µg /mL, respectively.

Robustness

To evaluate the robustness of the developed RP-HPLC method, deliberate variations were made in the method parameters such as change in the pH of the mobile phase, flow rate and the ratio of organic: aqueous composition of the mobile phase. The robustness of the method was evaluated by determining the effect of the modified parameters on retention time and peak area. Insignificant differences in peak areas and less variability in retention time were observed (Table 3).

Specificity

The specificity of the LC method is illustrated in Fig. 2& 3, where complete separation of granisetron hydrochloride in the presence of its degradation product was seen. The peaks obtained were sharp and had clear base line separation. The resolution factor for the drug from its nearest peak was >3.

Applications to pharmaceutical preparations

The proposed validated stability-indicating HPLC method was applied to the determination of GRH in bulk and in nasal formulations and stability samples of nasal formulations. The mean percentage drug found and the RSD% values (Table 4) indicated that the proposed validated stability-indicating HPLC method could be adopted for the selective determination of the investigated drugs in their pharmaceutical preparations without interference from either GRH degradation products formed under ICH-recommended stress conditions, or co-formulated adjuvant. From analysis of stability sample it was found that the two formulations were stable up to 6 month (Table 5).

Accuracy (Recovery Studies)

As shown from the data in Table 6 good recoveries of the drug in the range from 99.56%to100.96%were obtained from formulations.

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Table 1 Statistical Parameters of method development

Parameter	Granisetron hydrochloride
Linearity range (µg/mL)	5-50µL
Detection Wavelength	302 nm
Retention time (min)	3.07
Resolution	2.54
LOD (µg/ml)*	0.046µg/ml
LOQ (µg/ml)*	0.139µg/ml
Linear Regression equation	332803X+413044
Slope ± S.D*	332803 ± 4470
Correlation coefficient ± S.D*	0.9970 ± 0 .0023

* Mean of six determinations
S.D: Standard Deviation

Table 2 Precision studies of granisetron hydrochloride

Concentration µg/mL	Intraday precision(n=6)		Intermediate Precision(n=6)			
	Measured Conc.	%RSD	%Recovery	Measured Conc.	%RSD	%Recovery
20	19.95	1.395	99.75	19.93	1.39	99.65
40	39.84	1.50	99.60	40.08	1.139	100.2
60	59.74	1.41	99.56	59.98	1.42	99.96

Table 3: Statistical Validation Data of Robustness

Flow Rate (ml/min)	*RT of GRH(min)	*Tailing factor	*Area
0.9	3.17	1.8	7967191
1.0	3.07	1.2	7966073
1.1	2.84	1.6	7966532
% of acetonitrile in the mobile phase (v/v)	***RT of GRH(min)**	***Tailing factor**	***Area**
63	3.16	1.6	7967342
65	3.07	1.1	7965432
67	3.01	1.4	7966653
pH of the Mobile Phase	***RT of GRH(min)**	***Tailing factor**	***Area**
2.9	3.01	1.8	7965976
3.0	3.07	1.2	7967395
3.1	3.11	1.4	7966503

* Mean of three determinations

Table 4 Analysis of Drug solution and Nasal Formulation

Formulation	LabelClaim (mg)	Amount Found (mg, mean ± SD)	%RSD	%Recovery
F1(Drug Solution)	1.0	0.9972±0.0104	1.042	99.72
Formulation A	1.0	0.9965±0.00118	0.1184	99.65
Formulation B	1.0	0.9985±0.0096	0.961	99.85

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Table 5 Analysis of Formulation in Stability Sample

Formulation		0 DAY		15th DAY		6 Month	
	Temp	4°C	25°C	4°C	25°C	4°C	25°C
Formulation A	%Assay	99.65	98.37	97.94	98.63	99.67	99.49
	±SD	1.18	0.9619	0.8492	0.738	0.5717	0.6702
	%RSD	1.184	0.977	0.867	0.748	0.573	0.673
Formulation B	%Assay	99.85	99.7	99.50	99.65	99.53	99.77
	±SD	0.96	1.105	0.533	0.6782	0.6783	0.6844
	%RSD	0.961	1.108	0.535	0.680	0.6815	0.685

Table 6: Statistical Validation Data of Recovery Studies

Formulation	Level of % recovery	% Recovery*	S.D*	% R.S.D*	S.E*
		GRH	GRH	GRH	GRH
Formulation A	80	99.63	1.11	1.114	0.453
	100	100.96	0.8915	0.8830	0.3640
	120	100.12	1.346	1.344	0.5497
Formulation B	80	99.098	0.7486	0.755	0.3056
	100	99.85	0.5212	0.521	0.2128
	120	99.56	0.9961	1.00	0.4067

* Mean of three determinations
S.D: Standard Deviation, R.S.D: Relative Standard Deviation, S.E: Standard Error

CONCLUSION

A validated stability-indicating HPLC method has been developed for determination of GRH, in nasal formulations. The method has been proved to be selective for determination of GRH in presence of GRH degradation products formed under ICH-recommended stress testing conditions. The developed robust method is simple, selective, specific, accurate and precise for determination of the investigated drug without interference from co-formulated adjuvant of the tested pharmaceutical preparations. The method has been applied successfully for determination of GRH in nasal formulations and nasal formulations in stability studies.

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