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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF GRANISETRON IN API AND FINISHED FORMULATIONS BY RP UPLC METHOD

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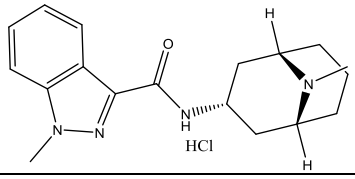
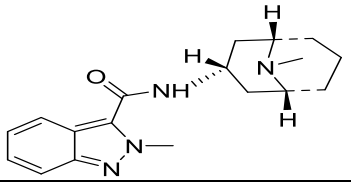
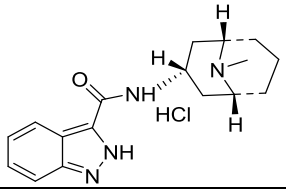
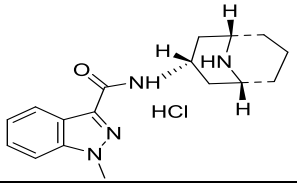
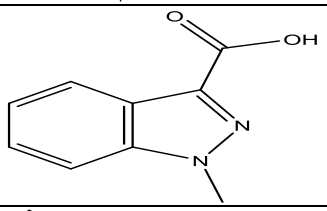
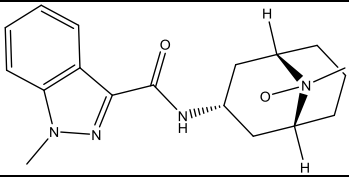
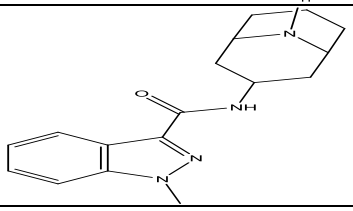
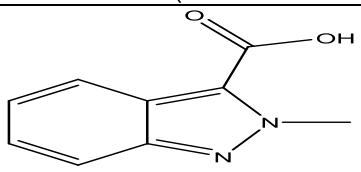
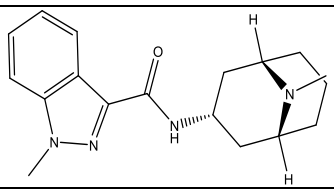
ABSTRACT
A simple, sensitive and stability indicating reverse phase Ultra performance liquid chromatography method (UPLC) was developed and validated for the determination of Granisetron in bulk and finished dosage forms. The newly developed method was assessed using Acquity BEH C18 (1.7 µm, 100 mm *2.1 mm) UPLC column with pH- 6.5 ammonium acetate buffer and acetonitrile as mobile phase in gradient elution mode with a run time of 4 minutes and a flow rate of 0.4 ml/min. UV detection was carried out at a wavelength of 305 nm with an injection volume of 2 µL. The developed method was validated as per the ICH guidelines with respect to precision, accuracy, linearity, robustness, specificity and system suitability. The LOD values and LOQ values for Granisetron was found to be 0.25 and 0.77 µg/ml respectively. The average recovery value of Granisetron was found to be in the range of 97.5-101.2 %. The developed method was linear over a range of 0.77-350 µg/ml for Granisetron. The proposed method was found to be suitable and accurate for the determination of Granisetron in bulk and finished formulations. Granisetron was degraded to n-Oxidative impurity under peroxide degradation but it was not degraded in acid hydrolysis, base hydrolysis, thermal condition, UV and visible condition.

KEYWORDS: Granisetron, UPLC, Antiemetic, Validation, LOD, LOQ.

INTRODUCTION
Granisetron (GRA) is a potent and selective 5-hydroxytryptamine (5-HT3) receptor antagonist with a little or no affinity for other serotonin receptors, including 5-HT1, 5-HT1A; 5-HT1B/C; 5-HT2; for alpha1-, alpha2-, or beta-adreno receptors; for dopamine-D2; or for histamine-H1; benzodiazepine; picrotoxindf or opioid receptors. Granisetron has antiemetic activity indicated for prevention and treatment of nausea and vomiting associated with cytotoxic chemotherapy and radiotherapy, and postoperative nausea and vomiting. Granisetron has had little effect on blood pressure, heart rate or ECG.[1-5]

Literature survey reveals that HPLC-UV[6-10], LC-MS/MS, UPLC/MS-MS methods have been reported for the estimation of Granisetron in bulk, finished formulations and in biological samples. Granisetron Hydrochloride is official in United States pharmacopeia (USP), British Pharmacopeia (BP) and Indian Pharmacopeia (IP) for its qualitative and quantitative determination. Recently stability indicating HPLC method for the determination of Granisetron in bulk and its degraded products was reported. The objective of the present work is to develop a stability indicating UPLC method and validated as per ICH and USP validation guidelines[11-15] for the estimation of Granisetron in quality control laboratories with respect to specificity, limit of detection and quantification, linearity, precision, ruggedness and robustness with shorter run time. The Chemical name of Granisetron hydrochloride (GRA) is endo-N-(9-methyl-9-azabicyclo 10 [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride with a molecular weight of 348.9 and free base is 312.4. Melting point of Granisetron Hydrochloride is 219°C. Its empirical formula is $C_{18}H_{24}N_4O$•HCl. UV spectrum of Granisetron in water at 37 °C was determined by using UV-Visible spectrophotometer. Granisetron is highly soluble BCS class-III drug and is formulated as a film coated tablets with 1 mg and 2 mg strengths and Resolutions. Granisetron is marketed under the trade name KYTRIL.[16-26]

Table. 1: Granisetron and its impurities Structures and Chemical Names.

Name of the compound	Structure	Chemical Name	Molecular weight
Granisetron		1-Methyl-N-((1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)-1H-indazole-3-carboxamide hydrochloride.	348.9
Imp-A		2-methyl-N-((1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)-2H-indazole-3-carboxamide	312.4
Imp-B		N-((1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)-2H-indazole-3-carboxamide hydrochloride.	298.38
Imp-C		N-((1R,3r,5S)-9-azabicyclo[3.3.1]nonan-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride.	298.38
Imp-D		1-methyl-1H-indazole-3-carboxylic acid	176.18
Imp-E		N-Oxide impurity	328.42
Imp-F		1-methyl-N-(9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)-1H-indazole-3-carboxamide	312.41
Imp-G		2-methyl-2H-indazole-3-carboxylic acid	176.17
Imp-H		1H-indazole-3-carboxylic acid	162.15

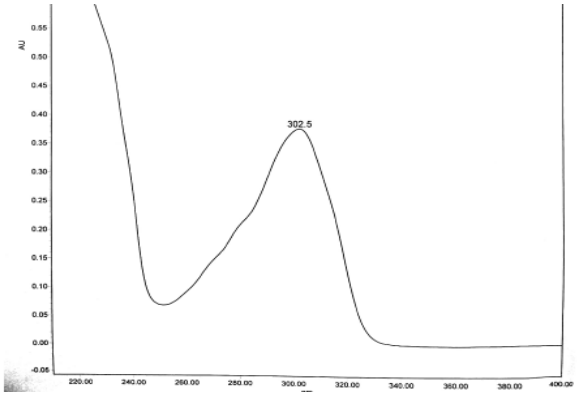


Fig. 1: UV spectrum of Granisetron in water.

MATERIALS AND METHODS

Chemicals, reagents and sample: Granisetron drug substance is from synthetic division of Dr Reddys labs, Hyderabad, India. KYTRIL 2 mg marketed tablets of Granisetron drug product was purchased from Macleods. Acetonitrile of HPLC grade was purchased from Rankem chemicals (Mumbai, India). Ammonium acetate, sodium hydroxide, formic acid, hydrochloric acid and hydrogen peroxide were purchased from Merck chemicals (Darmstadt, Germany). HPLC grade water was obtained from milli-Q water purification system (Millipore, Milford, USA).

Equipments: The UPLC system used for the chromatographic method development, forced degradation and validation was Waters quaternary pump separation module with a PDA detector. UPLC system consisted of quaternary pump and photodiode array detector. The signal output was monitored and processed using Empower-3 software on a Lenovo computer. Chromatographic separation was achieved on Waters Acquity BEH C18-100*2.1 mm, with particle size of 1.7 µm was used. pH of the mobile phase was adjusted on a microprocessor water proof pH tester (pH tester 20, Eutech instruments, Oakton, USA). Thermal degradation study was carried out in a dry hot air oven (Ultra Biotech, Bangalore, India). Ultrasonic bath sonicator was purchased from Cole-parmer (Mumbai, India) and photolytic degradation was carried out on photo stability chamber purchased from Atlas suntester CPS+ (Illinois, USA).

Chromatographic conditions: The objective of the present study is to develop a rapid stability indicating UPLC method for the estimation of Granisetron with proper peak shape, shorter run timeand LC-MS compatibility. Chromatographic separation was performed on Waters UPLC with Acquity BEH C18 100*2.1 mm, 1.7 µm column. Mobile phase A was 10 mM ammonium acetate pH adjusted to 6.5 and mobile phase B was acetonitrile. Diluent was prepared by mixing water and acetonitrile in the ratio of 50:50 (v/v). Injection volume was 2 µl, Flow rate was 0.4 ml/min, column oven temperature 40°C, analysis was carried out at a wavelength of 305 nm with data acquisition time of 4 min.

Preparation of buffer: Dissolved accurately 0.77 g of ammonium acetate in 1000 ml of milli-q water and mixed well further adjusted the pH of the solution to 6.5 ± 0.05 with diluted ammonia. The buffer solution was filtered through 0.22 µm nylon membrane filter.

Preparation of standard solution

A working standard stock solution of Granisetron Hydrochloride was prepared by dissolving standard equivalent to 20 mg of Granisetron into 100 ml volumetric flask, to this added 30 ml of diluent and sonicated for 10 minutes at a temperature not exceeding 20°C. Allowed the solution to attain room temperature and then diluted to the volume with diluent to have a solution with concentration of 200 µg/ml.

Preparation of system suitability solution

The standard solution was exposed to sun light for 8 Hrs or exposure the solution to UV light for 24 Hrs. During the light exposure Granisetron converts into its Impurity-C. The resolution between the peak of Granisetron and its Impurity -C should be not less than 3.5.

Preparation of sample solution

Weighed 20 tablets and determined the average weight of the tablets and crush them to a fine powder by using mortar and pestle. Transfer crushed powder equivalent to 20 mg of Granisetron into 100 ml volumetric flask and added 30 ml of diluent and sonicated in ultrasonic bath for 20 minutes with intermediate shaking at a temperature not more than 20°C. Allowed the flask to attain room temperature and diluted to the volume with diluent. Filter the solution through 0.45 µm nylon membrane filter by discarding 4 ml of filtrate and injected the same solution (0.2 mg/ml).

Preparation of placebo solution

Weighed accurately 10 mg of placebo powder into 100 ml volumetric flask added 30 ml of diluent and sonicated in ultrasonic bath for 20 minutes with intermediate shaking at a temperature not more than 20°C. Allowed the flask to attain room temperature and diluted to the volume with diluent. Filter the solution through 0.45 µm nylon membrane filter by discarding 4 ml of filtrate and injected the same solution.

Method validation

Specificity

Specificity is the ability of the method to measure the analyte response in presence of its potential known impurities. Specificity of the developed UPLC method for Granisetron was carried out in the presence of blank, placebo and its known impurities i.e., Imp-A, Imp-B, Imp-C and Imp-D for the accurate measurement of Granisetron present in the sample. As a part of specificity, stress studies were carried out for Granisetron drug substance, drug product and placebo under stress conditions like oxidation, acid, base, photolytic and thermal (105°C). These stress samples were analysed using the proposed method at a

concentration of 200 µg/ml of Granisetron to separate all the Granisetron impurities from Granisetron peak. In these stress conditions the peak purity test was verified for the Granisetron peak and by using diode array detector.

Precision

Precision of the analytical method is the closeness agreement for a series of measurement from multiple samplings as mentioned in ICH Q2 (R1). As per the guidelines, method precision and intermediate precision were analysed on the homogeneous sample and the % RSD for precision and intermediate precision was calculated and reported.

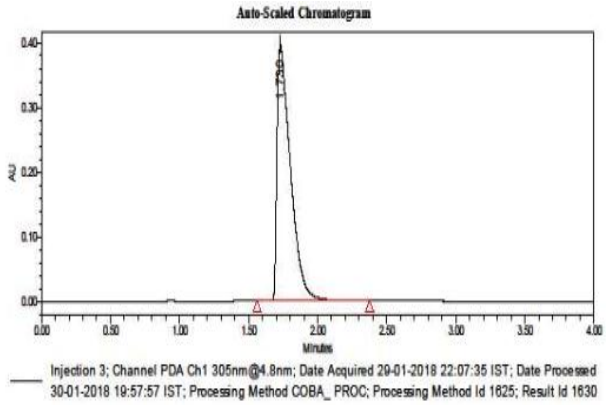


Fig. 2: Typical Granisetron chromatogram.

LOD and LOQ: The detection limit (LOD) and quantification limit (LOQ) for Granisetron were established by means of linearity method. The solutions from concentration ranging from 0.07 ppm to 350 ppm with 7 different levels were prepared and injected. Based on the peak response and STEYX value, the least concentration up to which can Granisetron be identified and quantified were calculated and verified.

Linearity: Linearity of the detector response was established for Granisetron peak with concentration ranging from LOQ to 150 % of the Assay concentration level. The samples were analyzed as per the described test method. A linearity graph was plotted between the responses of impurity (Y-axis) against actual concentration in ppm (X-axis) and determined the correlation co-efficient and Y-intercept at 100 % response.

Accuracy: Accuracy of the analytical method is the closeness of agreement between the true value and experimental value. Accuracy of the method was performed at 3 different levels ranging from 50% to 150 % of the Assay concentration level. The % recovery was calculated the % Assay at each level of spiked sample with as such sample.

Robustness: The robustness of the method was evaluated to establish the capability of the method by changing the experimental conditions and studying its impact on the system suitability. Robustness was performed by changing the method parameters like mobile phase flow rate and column temperature.

RESULTS AND DISCUSSION

Method development and Optimization

As there was no stability indicating UPLC method available for the determination of Granisetron in bulk and drug product with proper peak shapes and shorter run time. The objective of the current method is to separate all the potential impurity peaks arise during the forced degradation study from Granisetron peak. For the optimization of the UPLC method, forced degradation sample was taken as reference. Initial trials were taken on pH-4.5 Ammonium format buffer with acetonitrile as mobile phase and test concentration of 200 ppm in mobile phase was injected in which there was no clear separation between Granisetron and its related compound-C. Further trials were taken by varying the pH value of the mobile phase buffer from 4.5 to 6.5. i.e., ammonium acetate buffer was selected as a mobile phase as it LC-MS compatible and also having maximum buffering capacity at its pKa.

Forced degradation samples were injected and found that all the four known impurities (Impurity-A, Impurity-B, Impurity-C and Impurity-D) were separated with longer run time and broader peak shapes in isocratic mode. In order to shorten the run time gradient separation mode was optimized with satisfactory separation. Optimal separation was achieved on Acquity BEH C18 100*2.1 mm, 1.7 µm UPLC column maintained at 40°C. Gradient elution was performed using the mixture of 10 mM ammonium acetate buffer pH-6.5 (pH was adjusted with Ammonia) and acetonitrile as organic modifier at a flow rate of 0.4 mL/min. Detection was carried out at a wavelength of 305 nm. Sample cooler compartment was maintained at a temperature of 5°C. Gradient programme was mentioned in table-1.

Mobile phase A is 10 mM Ammonium Acetate in milli-q water, mobile phase B is 100 % Acetonitrile.

Table. 2: Gradient programme for UPLC method.

Time (minutes)	Flow rate (ml/min)	% of mobile phase-A	% of mobile phase-B
0	0.4	50	50
2	0.4	30	70
3	0.4	30	70
3.1	0.4	50	50
4	0.4	50	50

System suitability

System suitability solution was prepared and injected to evaluate the system suitability of the method and found that Granisetron was separated from its four known impurities with good resolution. The system suitability results were given in table-2. The developed UPLC method was found to be specific for determination of Granisetron from its known impurities namely Fig.3 shows separation of all the seven known impurities from Granisetron in the proposed method.

Precision

Method precision was performed by preparing six different samples as per the test concentration and analysed as per the developed method. The % Assay was calculated by using the area and concentration of the sample against 200 ppm standard sample. The % RSD was calculated for these six samples and was found to be less than 0.2. Intermediate precision was performed on Homogeneous samples on a different day using different UPLC system and UPLC column by a different analyst. The % Assay was calculated for these six samples and % RSD was calculated. The cumulative % RSD was calculated for the twelve samples and the % RSD was found to be 0.3 and the average Assay was found to be 98.5

Linearity and range

Linearity of the developed method was evaluated by injecting the Granisetron at seven different levels ranging from 0.75 ppm to 350 ppm. The concentrations ranged from LOQ to 150 % of sample concentration. The respective peak area was recorded and plotted against standard concentration and the graph resulted in straight line. The correlation coefficient, slope, intercept and % Y-intercept values were calculated and tabulated for Granisetron and. From this data it was clearly indicated that the method is linear over the range of LOQ to 150%. More over the method is sensitive for detection of Granisetron and low levels in both bulk and finished formulations. The compiled results were tabulated below table-3

Accuracy

Recovery studies were performed to judge the accuracy of the developed method. The study was evaluated by spiking the known quantity of Granisetron at various levels on the placebo. From the amount of Granisetron found the % recovery was calculated. Recovery was performed at different levels ranging from 50% to 150 % of the specification level. The % recovery of each impurity was found to be within the acceptance criteria of 95% to 105%. So the method is accurate for the determination of Granisetron. The mean recovery values are for the drug Granisetron tabulated in table-2.

Table. 3: Results for validation parameters.

Parameter	Results
Correlation coefficient	0.999
Method precision (% RSD)	0.32
Intermediate precision (% RSD)	0.34
Precision at 50 (% RSD)	0.52
Precision at 150(% RSD)	0.23
% Accuracy (50%)	99.8
% Accuracy (100%)	100.0
% Accuracy (150%)	100.9
Solution stability after 48 hrs.	0.2% difference from initial
Mobile phase stability after 48 hrs	0.2% difference from initial

Specificity: The specificity of the method was evaluated by verifying the peak purity of the sample. The method was found to be specific as there was no interference from blank and placebo at the retention time of main peak. No degradant peaks were observed at the retention time of Granisetron during the degradation and stability study indicates that the method is stability indicating and also purity angle was found to be less than purity threshold indicates that there was no spectral co-elution for Granisetron peak in this method and also the resolution between the neighbouring peak was greater than 2.0.

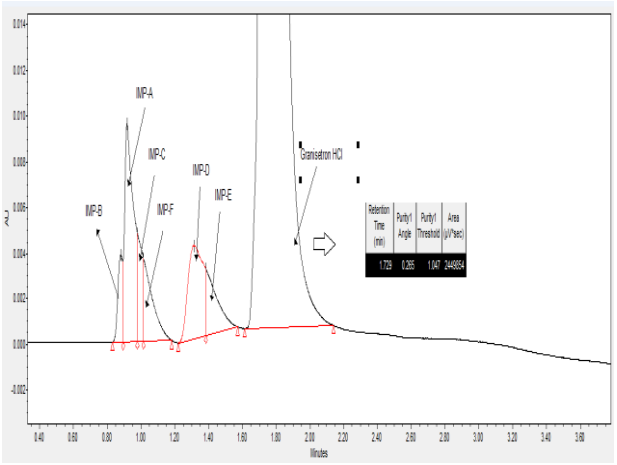


Fig. 3: Typical impurities blend Granisetron chromatogram.

Forced degradation studies

Forced degradation studies were performed to establish the stability indicating power of the method. In this study Granisetron raw material, finished product and placebo were subjected to acidic, basic, peroxide, thermal and photolytic stress studies on sample concentration of 0.2 mg/ml in diluent. Sample equivalent to 20 mg of Granisetron was placed into 50 ml volumetric flask added 30 ml of diluent and sonicated for 20 min with intermediate shaking at a temperature not more than 20

°C and then added respective degradant (Acid, Alkali, Oxidant) and performed the stress study. Samples were neutralised after degradation and then diluted to the volume with diluent and injected to verify the stability indicating power of the analytical method. Stress conditions under which the study was performed, the amount of Granisetron remains, % impurities generated and mass balance results were tabulated in table-3.

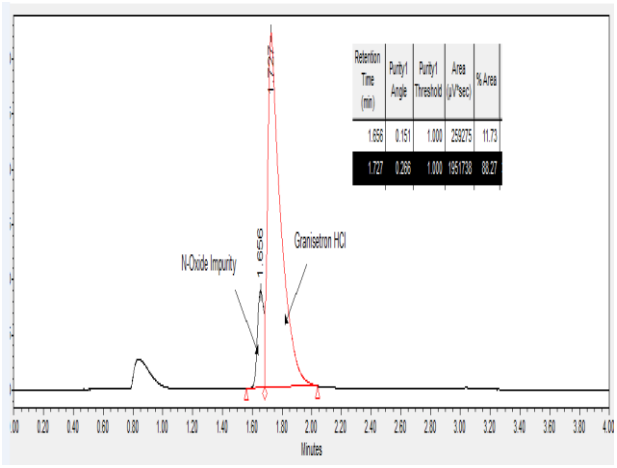


Fig. 4: Typical H2O2 degradation Granisetron chromatogram.

Table.4: Stress condition and its results.

Sl. No	Stress condition	% Drug remained	% impurities	Mass Balance
1	2N HCl_50°C_3 Hrs	98.9	1.1	97.6
2	1 N NaOH_50°C_3 Hrs	99.3	0.9	96.8
3	10 % H2O2_50°C_2 hrs	81.9	18.1	95.2
4	105 °C_48 Hrs	98.7	1.6	98.9
5	Photolytic stability	94.5	4.8	98.4

CONCLUSION

A novel, reverse phase liquid chromatographic method has been developed and validated for the estimation of Granisetron in presence of its known impurities. The developed method is LC-MS compatible. The short run time of the method is found to be simple, accurate, precise, linear, specific and robust and can also be used for quantifying the Granisetron during the residue analysis. Hence the validated method can be used for the determination of Granisetron in bulk and in finished formulations.

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CONFLICTS OF INTERESTS: The authors declare no conflict of interest.

NOTE: DRL-IPDO communication No.: IPDO IPM-00571 has been allotted for this research article.

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