

www.pharmaerudition.org

ISSN: 2249-3875



International Journal of Pharmaceutical Erudition

Research for Present and Next Generation

AUG 2026

Vol: 16 Issue:02
(53-57)





Received: 01.9.26

Revised: 16.9.26

Accepted: 30.9.26

Research Paper**ANALYTICAL METHOD VALIDATION FOR THE GRANISETRON IN API AND FINISHED FORMULATIONS BY RP UPLC METHOD****Vinay Sharma*, Dilip Agrawal, Rakesh Goyal**Dept. of Pharmaceutical Quality Assurance, Mahatma Gandhi College of Pharmaceutical Sciences,
Jaipur – 302022

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 μ m, 100 mm $\times$ 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 4minutes 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 μ L. 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 μ g/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 μ g/ml for Granisetron. The proposed method was found to be suitable and accurate for the determination of Granisetron in bulk and finished formulations.

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-HT_{1A}; 5-HT_{1B/C}; 5-HT₂; for α ₁-, α ₂-, or beta-adrenoceptors; for dopamine-D₂; or for histamine-H₁; benzodiazepine; picrotoxin and 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.¹⁻²

Literature survey reveals that HPLC-UV, LCMS/MS, UPLC/MS-MS methods have been reported for the estimation of Granisetron in bulk, finished formulations and in biological samples.³ 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.⁴ The objective of the present work is to develop a stability indicating UPLC method and validated as per ICH and USP validation guidelines 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.⁵⁻⁶ 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 $C_{18}H_{24}N_4O \cdot HCl$. 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.⁷ Granisetron is marketed under the trade name KYTRIL.⁸

EXPERIMENTAL

Chemicals and Reagents:

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).

Instrument:

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 www.pharmaerudition.org Aug 2026, 16(2), 53-57

quaternary pump and photodiode array detector.

Drug 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 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).

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.

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

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.

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.

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.

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 there solution between the neighbouring peak was greater than 2.0.

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 runtime 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.

REFERENCE

1. Valagaleti,R, Burns,P.K, Michael,G. Analytical Support for Drug Manufacturing in the United States From Active Pharmaceutical Ingredient Synthesis to Drug Product Shelf Life, Drug Information Journal.2025; 37- 407-38.
2. Pajander, J, Haugshoj, K.B, Bjorneboe, K, Wahlberg, P, Rantanen, J. Foreign matter identification from solid dosage forms, Journal of Pharmaceutical and Biomedical Analysis.2025; 80, 116 – 25.
3. M. Tswett. Adsorption analysis and chromatographic method. Application to the chemistry of chlorophyll, Berichte der Deutschen botanischenGesellschaft. 2025; 24, 384–93.
4. Goodman, L.S. and Gilman, A.G., The Pharmacological Basis of Therapeutics, 9th Edn. By Hardman, J.G., Limbard, L.E., Editors in Chief, McGraw - Hill, 2024.
5. Wolff, M.E., Edt, Burger's Medicinal Chemistry, Part IV, 4th Edn., Wiley Interscience, New York, 2021.
6. Deorge, R.F., Edt., Wilson and Gisvolds's Text book of Organic and Medicinal and Pharmaceutical Chemistry, 8th Edn., Lippincott Company, 2020.
7. Korolkovas, A., Essentials of Medicinal Chemistry, 2nd Edn., Wiley Interscience, New York, 2023.
8. Topliss, J.G..Edt.. Quantitative Structure - activity relationships of Drugs, Vol 19, Academic press, London, 2018.

Conflict of Interest

The authors declare that they have no conflict of interest