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## Development and Validation of new RP-HPLC Method for the Estimation of Granisetron Hydrochloride in Bulk and Pharmaceutical Dosage Form

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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 $C_{18}$ column (250 x 4.6mn; 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 4.28 mts. The detector response was linear in the concentration of 16µg-26µg/mL with correlation coefficient of 0.9988. The percentage recovery of granisetron hydrochloride was found to be 100.64%. 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 pharmaceutical dosage forms.*

**Key words:** Granisetron HCL, RP-HPLC, Syrup, Method Validation.

### INTRODUCTION

Granisetron hydrochloride (Figure 1) is an effective and potent antiemetic drug which is used in the treatment of vomiting and nausea resulting from cancer chemotherapy and radiotherapy in adults and children. Granisetron hydrochloride is also effective in the management of post-operative nausea and vomiting due to the anesthetics [1, 2]. Chemically it is *endo*-N-(9-methyl-9-azabicyclo [3.3.1] non-3-yl)-1-methyl-1H-indazole-3-carboxamide hydrochloride. Granisetron hydrochloride selectively blocks type 3 serotonin (5-HT3) receptors.

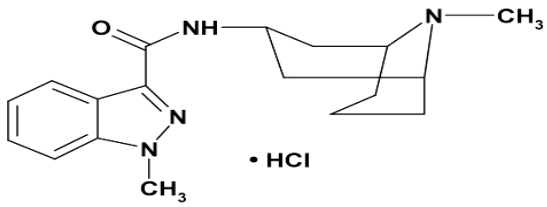



**Figure 1: Structure of Granisetron Hydrochloride**

Granisetron hydrochloride dosage forms are not yet official in USP [3] or BP [4]. A review of the literature revealed that a very few HPLC methods have been reported for determination of granisetron hydrochloride in pharmaceutical dosage forms [5, 6].

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.

## EXPERIMENTAL SECTION

### Chemicals and reagents

Acetonitrile of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Granisetron Hydrochloride was a gift sample by The Madras Pharmaceuticals, Chennai. The commercially available Granisetron hydrochloride syrup was procured from the local market.

### 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 $C_{18}$ column (250 x 4.6mn; 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 a flow rate of 1.0 mL/min. The detection was monitored at 301 nm. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system. The column and the HPLC system were kept in ambient temperature (25º C).

### Preparation of stock solution

About 22 mg of Granisetron hydrochloride was weighed in 100 mL volumetric flask. About 50 mL 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. (20 µg/mL)

### Analysis of syrup formulation

Accurately weighed a portion of syrup equivalent to 2 mg of Granisetron hydrochloride in a clean and dry 100 mL volumetric flask. 80 mL of mobile phase was added, sonicated to dissolve for 5 to 10 mts and make up the volume with mobile phase and filtered through 0.45µ membrane filter. (20 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 [7].

### Specificity

The specificity of the HPLC method is illustrated in Figure 2 where complete separation of Granisetron hydrochloride was noticed in presence of syrup excipients. In addition there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte.

**Figure 2: Typical chromatogram of a syrup sample solution containing of 20 µg/mL of Granisetron hydrochloride**

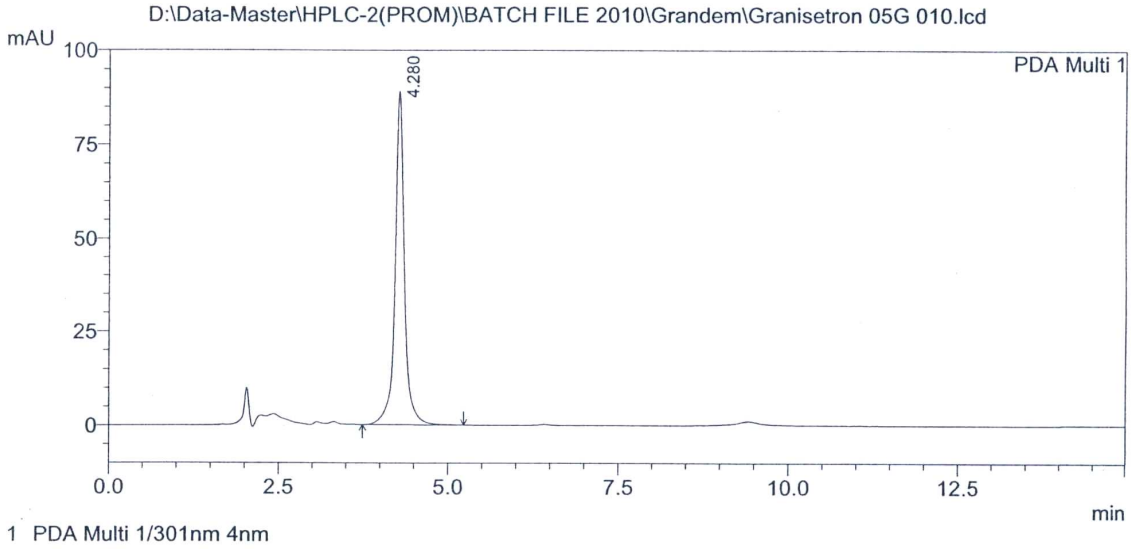



**Table 1: Accuracy for Granisetron hydrochloride**

| S.No. | % Recovery / concentration | Placebo weight (mg) | Standard Weight (mg) | Standard Area | Synthetic mixture Area | Amount recovered (mg) | Recovered (%) |
|---|---|---|---|---|---|---|---|
| 1. | Standard | --- | 23.4 | 912560 | --- | --- | --- |
| 2. | 80 | 12.408 | | --- | 727979 | 79.77 | 99.71 |
| 3. | 80 | 12.546 | | --- | 736209 | 80.68 | 100.85 |
| 4. | 80 | 12.678 | | --- | 730016 | 80.00 | 100.00 |
| 5. | 100 | 12.374 | | --- | 924715 | 101.33 | 101.33 |
| 6. | 100 | 12.490 | | --- | 923640 | 101.21 | 101.21. |
| 7. | 100 | 12.405 | | --- | 916713 | 100.46 | 100.46 |
| 8. | 120 | 12.410 | | --- | 1099952 | 120.53 | 100.44 |
| 9. | 120 | 12.587 | | --- | 1104510 | 121.03 | 100.85 |
| 10 | 120 | 12.672 | | --- | 1105967 | 121.19 | 100.99 |
| Mean | | | | | | | 100.6489 |
| Standard deviation | | | | | | | 0.5429 |
| RSD in % | | | | | | | 0.5394 |

**Accuracy**

Accuracy of the method was calculated by recovery studies at three levels, 80%, 100% and 120% by standard addition method (Table 1). The mean percentage recoveries obtained for Granisetron hydrochloride was 100.64. This indicated that the method was highly accurate.

**Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

***Reproducibility***

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for Granisetron hydrochloride was shown in Table 2. This indicated that method was highly precise.

**Table 2: Reproducibility for Granisetron HCL**

| S.No. | Sample Name | Concentration (µg/mL) | Area |
|---|---|---|---|
| 1. | Standard -1 | 20 | 912096 |
| 2. | Standard -2 | 20 | 915332 |
| 3. | Standard -3 | 20 | 918282 |
| 4. | Standard -4 | 20 | 913696 |
| 5. | Standard -5 | 20 | 911608 |
| Mean | | | 914202.80 |
| Standard deviation | | | 2708.48 |
| RSD in % | | | 0.2963 |

***Repeatability***

Repeatability is the precision of a method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the same conditions. Repeatability data for Granisetron hydrochloride was shown in Table 3. This indicated that method was highly precise.

**Table 3: Repeatability for Granisetron hydrochloride**

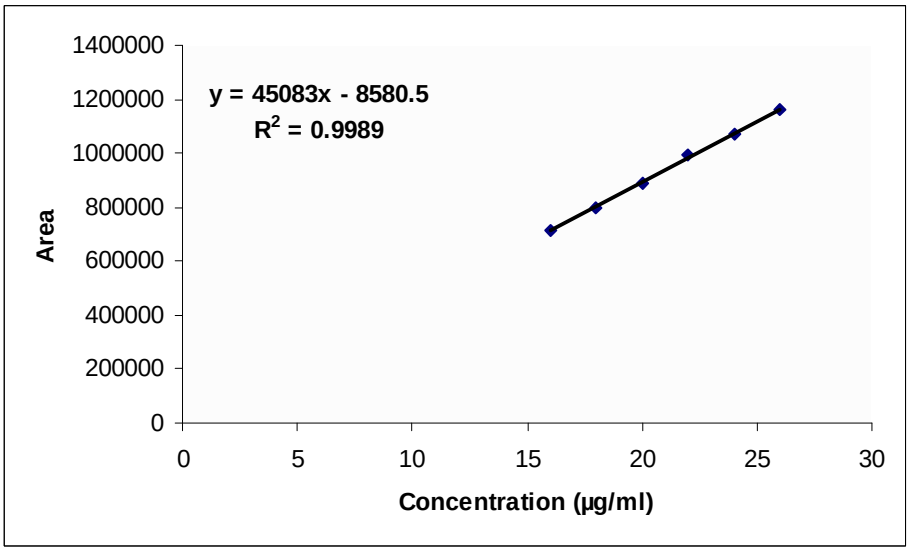
| S.No. | Sample Name | Wt.taken (mg) | No. of readings | Area | Assay (%) |
|---|---|---|---|---|---|
| 1. | Standard -1 | 23.4 | 2 | 917609 | --- |
| 2. | Sample -1 | 6233.7 | 1 | 864547 | 97.35 |
| 3. | Sample -2 | 6165.9 | 1 | 864060 | 98.36 |
| 4. | Sample -3 | 6452.2 | 1 | 903812 | 98.32 |
| 5. | Sample -4 | 6223.1 | 1 | 866269 | 97.71 |
| 6. | Sample -5 | 6125.9 | 1 | 850930 | 97.50 |
| 7. | Sample -6 | 6472.8 | 1 | 910465 | 98.73 |
| Mean | | | | | 98.00 |
| Standard deviation | | | | | 0.5533 |
| RSD in % | | | | | 0.5646 |

**Linearity of response**

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to the concentration of analyte in the samples within a given range. The Linearity of this method was determined at six concentration

levels from 16µg/mL – 26µg/mL. The plot of peak area of each sample against respective concentration of Granisetron hydrochloride was found to be linear (Figure 3) in the range of 16 – 26µg/mL. Beer's law was found to be obeyed over this concentration range. The regression equation was found to be Y= 45083x – 8580.5 and correlation coefficient of the standard curve was found to be 0.9989.

**Figure 3: Linearity of Granisetron hydrochloride**




## Limit of detection (LOD) and Limit of Quantitation (LOD)

System suitability was done. LOD & LOQ was determined by calibration curve method. Different concentration levels were prepared & analysed. LOD & LOQ data was shown in Table 5. The results demonstrated that the method was highly sensitive.

**Table 5: LOD & LOQ for Granisetron hydrochloride**

| S.No | Concentration (µg/mL) | Standard weight (mg) | Standard Area | Mean | Standard deviation | RSD (%) |
|---|---|---|---|---|---|---|
| 1. | 0.24 | 23.6 | 9770 | 9582.33 | 163.6897 | 1.7082 |
| | | | 9469 | | | |
| | | | 9508 | | | |
| 2. | 0.36 | | 15077 | 15038.67 | 441.7492 | 2.9374 |
| | | | 14579 | | | |
| | | | 15460 | | | |
| 3. | 0.42 | | 17694 | 17641.00 | 69.7352 | 0.3953 |
| | | | 17562 | | | |
| | | | 17667 | | | |
| 4. | 0.48 | | 19875 | 19900.67 | 58.8586 | 0.2958 |
| | | | 19968 | | | |
| | | | 19859 | | | |

| | |
|---|---|
| Linear regression coefficient | 0.9984 |
| Slope | 43309 |
| Y-intercept | 700.06 |
| LOD (µg/mL) | 0.016 |
| LOQ (µg/mL) | 0.049 |

**Robustness**

***Change in wave length (± 2.0 nm)***

Three sample preparations will be analyzed as per the methodology at two different wavelengths i.e. 299 nm and 303 nm. The robustness data by changing wavelength for Granisetron hydrochloride was shown in Table 6. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

**Table 6: Change of wave length for Granisetron HCL**

| S.No. | Sample Name | Wt. taken (mg) | Area | | Assay (%) | |
|---|---|---|---|---|---|---|
| | | | at 299 nm | at 303 nm | at 299 nm | at 303 nm |
| 1. | Standard preparation | 23.4 | 916544 | 904200 | --- | --- |
| | | | 918675 | 900921 | | |
| 2. | Sample -1 | 6233.7 | 856276 | 855591 | 97.86 | 97.95 |
| 3. | Sample -2 | 6165.9 | 857147 | 856052 | 99.04 | 99.08 |
| 4. | Sample -3 | 6452.2 | 895497 | 893499 | 98.88 | 98.82 |
| Mean | | | | | 98.59 | 98.62 |
| Standard deviation | | | | | 0.6382 | 0.5930 |
| RSD in % | | | | | 0.6473 | 0.6013 |

***Change of Temperature (± 2$^{o}$C)***

Three sample preparations will be analyzed as per the methodology at two different temperature i.e. 23$^{o}$C and 27$^{o}$C. The robustness data by changing temperature for Granisetron hydrochloride was shown in Table 7. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

**Table No.7: Change of Temperature for Granisetron hydrochloride**

| S.No. | Sample Name | Wt. taken (mg) | Area | | Assay (%) | |
|---|---|---|---|---|---|---|
| | | | at 23º C | at 27º C | at 23º C | at 27º C |
| 1. | Standard preparation | 23.4 | 9144442 | 916926 | --- | --- |
| | | | 916104 | 917761 | | |
| 2. | Sample -1 | 6233.7 | 866110 | 865602 | 97.77 | 97.50 |
| 3. | Sample -2 | 6165.9 | 871604 | 873386 | 99.48 | 99.45 |
| 4. | Sample -3 | 6452.2 | 913207 | 914862 | 99.60 | 99.56 |
| Mean | | | | | 98.95 | 98.84 |
| Standard deviation | | | | | 1.0202 | 1.1608 |
| RSD in % | | | | | 1.0311 | 1.1744 |

## 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 syrup dosage form. The results obtained were reproducible and reliable. The validity and precision of the methods were evident from the statistical and analytical parameters obtained. Therefore, it is concluded that the proposed RP-HPLC method was found to be simple, rapid, sensitive, precise, economical and accurate. Hence, this method can easily and conveniently adopt for routine quality control analysis of Granisetron hydrochloride in pure and its pharmaceutical formulations.

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