Stability-Indicating RP-HPLC Method for Determination of Guanfacine Hydrochloride in Bulk Drugs and in Pharmaceutical Dosage Form

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Abstract

A novel stability-indicating RP-HPLC method was developed and validated for quantitative determination of guanfacine hydrochloride in bulk drug and in pharmaceutical dosage form. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using Apollo, C18 (250mm x 4.6mm, 5µm) column with mobile phase of 50mM Ammonium acetate (volatile buffer) and acetonitrile (65:35, v/v). UV detection has been done at wavelength 220 nm. The guanfacine hydrochloride was subjected to the stress conditions of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. The stressed samples were analyzed by the proposed method. The analyte peak shape was excellent. The described method shows excellent linearity over a range of 30 – 450 µg/mL. The correlation coefficient for guanfacine hydrochloride was 0.999. The limit of detection for Guanfacine hydrochloride is 0.011 µg/mL and the limit of quantification is 0.038 µg/mL respectively.

Degradation was observed for guanfacine hydrochloride in base, thermal and in 30% H\textsubscript{2}O\textsubscript{2} conditions. The drug was found to be stable in the other stress conditions attempted. The degradation products were well resolved from main peak. The percentage recovery of guanfacine hydrochloride was ranged from (99.2% to 100.5%) in pharmaceutical dosage form. The developed method was validated with respect to the linearity, accuracy (recovery), precision, specificity and robustness. The forced degradation studies prove the stability indicating power of the method.

Keywords: Guanfacine hydrochloride, Liquid chromatography, Method validation, Pharmaceutical preparation

1. Introduction

Guanfacine hydrochloride (Fig. 1) is chemically N-(diaminomethylidene)-2-(2,6-dichlorophenyl) acetamide [1]. It has the cardiovascular effect of lowering blood pressure [2]. It is an agonist of the \( \alpha_{2A} \) subtype of nor epinephrine receptors. These receptors are concentrated heavily in the prefrontal cortex and the locus coeruleus, with the potential to improve attention abilities via modulating post-synaptic \( \alpha-2a \) receptors in the prefrontal cortex [3,4]. Drug lowered both systolic and diastolic blood pressure by activating the central nervous system \( \alpha-2a \) nor epinephrine auto receptors, which results in reduced peripheral sympathetic outflow and thus causes a reduction in peripheral sympathetic
It is also effective in treating the symptoms of attention-deficit hyperactivity disorder (ADHD) and also used in conjunction with stimulants to reduce, rebound, as well as induce, sleep [6].

![Chemical structure of Guanfacine hydrochloride.](image)

Stability testing forms is an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and enables recommendation of storage conditions, retest periods and shelf life to be established. The two main aspects of stability study of drug product plays an important role in shelf life determinations are assay of active drug and degradation products generated during stability study. The assay of drug product in stability test sample needs to be determined using stability-indicating method, as recommended by the international Conference on Harmonization (ICH) [7].

The objective of this work was to develop a simple, precise and rapid analytical LC procedure, which would be serve as stability-indicating assay method for dosage form of Guanfacine hydrochloride.

A literature survey reveals that only one spectrofluorometric assay method was reported for guanfacine hydrochloride presents in other drugs[8]. A GC-MS method was reported to determine guanfacine in Urine [9]. One scintillation-proximity assay method were also reported for guanfacine hydrochloride [10]. A spectrophotometric analysis of guanfacine by acid-dye and charge transfer complexation method were also reported [11].

None of the reported analytical procedures describe a method for the determination of guanfacine hydrochloride in pharmaceutical dosage form in presence of their degradation products.

In the present study attempts were made to develop a rapid, economical, precise and accurate method for the estimation of the guanfacine hydrochloride in presence of their degradation products. This paper mainly deals with the forced degradation of guanfacine hydrochloride under stress conditions like acid hydrolysis, base hydrolysis, oxidation, heat, light and validation of the developed method for the accurate quantification of guanfacine hydrochloride in bulk drug and solid dosage form.

### 2. Experimental

#### 2.1. Chemicals

99.7 % purity standard of Guanfacine hydrochloride was supplied by Mylan pharmaceutical (Hyderabad, India) and tablet Tenex (2 mg) were purchased from market. Acetonitrile (HPLC grade) were purchased from Merck Fine Chemicals (Mumbai, India). Ammonium acetate, Sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H₂O₂) were purchased from Qualigens fine chemicals (Glaxo Ltd, Mumbai, India). The 0.45 -Pump nylon filter was obtained from Advanced Micro devices Pvt. Ltd., (Ambala Cantt, India). Double distilled water was used throughout the experiment. Other chemicals used were of analytical or HPLC grade.

#### 2.2. HPLC Instrumentation conditions

HPLC system used was an Agilent Technology (1100 series, Germany), system equipped with auto sampler, quaternary pump,
degasser and a UV Detector. The out-put signal was monitored and processed using Agilent Chemstation software. The column used was C18, 5µm (4.6 x 250 mm, i.d., Apollo technology, Japan). Column temperature was maintained to 30 °C using column oven; the flow rate of mobile phase was kept 1.0 mL/min. The mobile phase consisted of 50 mM ammonium acetate buffer-acetonitrile (65:35 v/v). The buffer used in mobile phase contains 50 mM ammonium acetate filtered through 0.45µm nylon filter and degassed in ultrasonic bath prior to use. Injection volume 5 µL and ultraviolet (UV) detection at 220 nm. The photodiode array detector was used for analysis of forced degradation samples in scan mode with a range of 200 – 400 nm. The peak homogeneity was expressed in terms of peak purity and was obtained directly from the spectral analysis report using above mentioned software.

2. 3. Standard Solutions
The standard stock solution (3.0mg/mL) of guanfacine hydrochloride was prepared by dissolving 75mg of working standard in diluent (Water : Acetonitrile 70:30) and diluting with the same solvent upto 25 mL.

Standard solution was prepared from this stock solution by transferring 10 mL standard stock solution to 100 mL volumetric flask and diluted upto mark with diluent. These solutions contained 300 µg/mL Guanfacine hydrochloride.

2. 4. Sample Preparation of Dosage form
Twenty tablets of mean weight were crushed in mortar. An amount of powdered mass equivalent to 30 mg of guanfacine hydrochloride is transferred to a 100 mL volumetric flask. The drug from powder was dissolved and extracted with the diluent. To ensure complete extraction of drug it was sonicated for 25 min. and diluted to 100mL. This solution was filtered through the 0.45 mm pump nylon filter.

2. 5. Procedure for forced degradation
2. 5. 1. Acidic Degradation
15 mg of guanfacine hydrochloride was accurately weighed and dissolved in 5 mL diluent. 5 mL of 1.0 N HCl added and kept at room temperatue about 3 h, then solution was neutralized by 1.0 N NaOH to pH 7 and volume made up to 50 mL with diluent.

2. 5. 2. Alkali Degradation
15 mg of guanfacine hydrochloride was accurately weighed and dissolved in 5 mL diluent. 5 mL of 0.1 N NaOH were added and kept at 60 °C under reflux about 1 h , then solution was neutralized by 0.1N hydrochloride to pH 7 and volume made up to 50 mL with diluent.

2. 5. 3. Oxidative Degradation
15 mg of guanfacine hydrochloride was accurately weighed and dissolved in 5 mL diluent. 5 mL of 30% H2O2 solution added and kept at 60 °C under reflux about 1h, the solution was allowed to attended ambient temperature. The volume make up to 50 mL with diluent.

2. 5. 4. Thermal Degradation
75 mg of drug substance was kept at 80 °C for 24 h. The solution was prepared to achieve final concentration 300 µg/mL.

2. 5. 5. UV Degradation at 254 nm
75 mg of drug substance was exposed to UV short (254 nm) light for 48 h. The solution was prepared to achieve final concentration 300 µg/mL.

2. 5. 6. UV Degradation at 366 nm
75 mg of drug substance was exposed to UV long (366 nm) light for 48 h. The solution was prepared to achieve final concentration 300 µg/mL.

3. Results and Discussion
3. 1. Optimization of chromatographic conditions

The primary target is the development of stability-indicating HPLC method to achieve the resolution between guanfacine hydrochloride and its degradation products. To achieve the separation of degradation products, stationary phases of C-18 and combination of mobile phase 50mM ammonium acetate (volatile buffer) with acetonitrile was used. The separation of degradation product and guanfacine hydrochloride was achieved on Apollo ODS (Octadecyl silane) C18 stationary phase column and 50mM ammonium acetate : acetonitrile (65:35 v/v) as a mobile phase at the column temperature 30°C. The tailing factor obtained was less than 2 and retention time was also about 7.0 min for main peak which would reduce the total run time and ultimately increase productivity and reduce the cost of analysis as per sample. Forced degradation study showed that the method is highly specific and the entire degradation products were well resolved from the main peak. The developed method was found to be specific and valid as per ICH guidelines. System Suitability Parameters of Proposed RP-HPLC Method shown in Table 1.

Table 1. System Suitability Parameters of Proposed RP-HPLC Method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Guanfacine Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>7.2</td>
</tr>
<tr>
<td>Tailing Factor</td>
<td>1.36</td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>11291</td>
</tr>
</tbody>
</table>

3. 2. Result of forced degradation study

Singh and Bakshi [12] suggested a target degradation of 20-80% for establishing the stability-indicating nature of the assay method, as even intermediate degradation products should not interfere with any stage of drug analysis. Though conditions used for forced degradation were attenuated to achieve degradation in the range of 20-80%, this could not be achieved in other degradation conditions even after the exposure for prolonged duration except base, thermal and oxidative degradation condition.

In basic conditions, analyte undergoes the degradation for the formation of both polar and non polar impurities. The percentage (%) base degradation was found to be 42.1% with analyte peak having the peak purity 999.325.

In oxidative degradation analyte undergoes degradation for the formation of both polar and non polar impurities within short time period. The percentage (%) degradation was found to be 59.2% with analyte peak having the peak purity 999.293.

In thermal degradation analyte undergoes degradation for the formation of polar impurities. The thermal degradation percentage (%) was found to be 8.9% with analyte peak having the peak purity 999.655.

The Peak purity test results confirm analyte peak is homogeneous in all the stress conditions tested. The mass balance of guanfacine hydrochloride in stress samples was close to 100% and moreover, the unaffected assay of guanfacine hydrochloride in the tablets confirms the stability-indicating power of the method. The summary of forced degradation studies is given in Table 2.

Chromatographic peak purity data was obtained from the spectral analysis report and a peak purity value greater than 990 indicates a homogeneous peak. The peak purity values for analyte peaks was in the range of 999-1000 indicating homogeneous peaks and thus establishing the specificity of assay method. Fig. 2 shows the chromatogram of guanfacine hydrochloride tablet solution. Fig. 4 shows chromatogram of guanfacine hydrochloride standard solution. Fig. 6 shows chromatograms of base hydrolysis degradation. Fig. 8 shows the chromatogram of oxidative degradation and Fig. 9 shows chromatograms of thermal degradation. No other co-eluting peaks were found with the mean peaks suggested the specificity of the method for the estimation of
guanfacine hydrochloride in presence of degradation products.

4. Method Validation
The described method was validated with respect to specificity, linearity, system suitability, accuracy, robustness, LOD, LOQ and intermediate precision. Characteristics parameters of system suitability are given in Table 1.

4.1. Specificity
Photodiode array detection was used as an evidence for the specificity of the method and to evaluate the homogeneity of the drug peak. The peak purity values are determined. The peak purity were more than 999 for drug substance and drug product at 220 nm, which shows that the peaks of analyte were pure and also formulation excipients, diluent peaks and degradation peaks were not interfering with analyte peak.

<table>
<thead>
<tr>
<th>Stress conditions/duration</th>
<th>Degradation (%)</th>
<th>Peak purity data*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic/1.0N hydrochloride/ RT/3h</td>
<td>0</td>
<td>999.756</td>
</tr>
<tr>
<td>Basic/0.1N NaOH/ 60°C/1h</td>
<td>42.1</td>
<td>999.325</td>
</tr>
<tr>
<td>Oxidative/ 30% H₂O₂/ 60°C/1h</td>
<td>59.2</td>
<td>999.293</td>
</tr>
<tr>
<td>Thermal/80 °C/24h</td>
<td>8.9</td>
<td>999.655</td>
</tr>
<tr>
<td>Photo/UV 254nm/48h</td>
<td>0</td>
<td>999.788</td>
</tr>
<tr>
<td>366nm/48h</td>
<td>0</td>
<td>999.771</td>
</tr>
</tbody>
</table>

*Peak purity values in the range of 990-1000 indicate a homogeneous peak.
Fig. 4. Chromatogram of Standard Solution

Fig. 5. Chromatogram of Blank for Base hydrolysis

Fig. 6. Chromatogram of Sample for Base hydrolysis
Fig. 7. Chromatogram of Blank for Oxidative degradation

Fig. 8. Chromatogram of Oxidative degradation

Fig. 9. Chromatogram of Thermal degradation
4.2 Precision (Repeatability)

Six replicate analysis of tablets by the proposed method were done. The area % RSD for guanfacine hydrochloride was 0.4. The results of the precision study indicate that the method is reliable (RSD % < 2). The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts in different days in same laboratory. Results are shown in Table 3.

4.3 Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100% and 120% of the label claim of the tablet (2mg of guanfacine hydrochloride). Placebo equivalent to fifteen tablet was transferred into a 100 mL volumetric flask, and the amount of guanfacine hydrochloride at 80%, 100% and 120% of the label claim of the tablet was added to it. The recovery samples were prepared as per the procedure mentioned in sample preparation of dosage form. Three samples were prepared for each recovery level. The solutions were further analyzed, and the percentage recoveries were calculated. The results are shown in Table 4.

### Table 3. Result of precision of test method of Guanfacine Hydrochloride.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Analyst-I (Intra-day precision)</th>
<th>Analyst-II (Inter-day precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.36</td>
<td>99.73</td>
</tr>
<tr>
<td>2</td>
<td>99.11</td>
<td>99.47</td>
</tr>
<tr>
<td>3</td>
<td>99.45</td>
<td>98.65</td>
</tr>
<tr>
<td>4</td>
<td>98.89</td>
<td>98.99</td>
</tr>
<tr>
<td>5</td>
<td>99.65</td>
<td>99.25</td>
</tr>
<tr>
<td>6</td>
<td>98.71</td>
<td>99.69</td>
</tr>
<tr>
<td>Mean</td>
<td>99.02</td>
<td>99.12</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.36</td>
<td>0.42</td>
</tr>
</tbody>
</table>

4.4 Linearity

Linearity solutions for the method were prepared from stock guanfacine hydrochloride solution (3000 µg/mL) at six different concentrations levels tested from 30 µg/mL to 450 µg/mL (10 to 150% of the assay concentration of guanfacine hydrochloride, 300 µg/mL). Each linearity level solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area verses the concentration of the analyte. The equation of the calibration curve is y = 13.917x + 1.8702. The calibration graph was plotted and found to be linear in the aforementioned concentrations with correlation coefficient 0.999.

4.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ).

The LOD and LOQ for guanfacine hydrochloride were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute standard solutions with known concentrations (12). The LOD for guanfacine hydrochloride 0.011 µg/mL, and the LOQ was 0.038 µg/mL, respectively.

4.6 Robustness

To determine the robustness of the developed method experimental condition were purposely altered and the resolution between the peaks of guanfacine hydrochloride and adjacent base degradation product were evaluated.
The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 mL/min. The effect of percent organic strength on resolution was studied by varying acetonitrile from –10 to +10%. The effect of column temperature on resolution was studied at 25 °C and 35 °C instead of 30 °C while the other mobile phase components were held constant stated in chromatographic condition. The robustness results are shown in Table 5.

**Table 5.** Results of robustness study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Variations</th>
<th>Resolution*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.8 mL/min</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>1.2 mL/min</td>
<td>2.7</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>31.5 mL of Acetonitrile</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>38.5 mL of Acetonitrile</td>
<td>2.5</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>35°C</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Resolution between analyte peak and adjacent base degradant.

4. 7. Solution Stability

The stability of the guanfacine hydrochloride bulk drug solution and tablet assay solutions were measured at the time intervals of 24 and 48 hours. The stability of standard solutions was determined by comparing results of area% and peak purity of analyte. The area% values were within 0.5 % after 48 hours. The results indicate that the solution was stable for 48 hours at ambient temperature as there was no formation of any unknown peak and solution remains stable. The Peak purity value were 999.721. Assay of tablet sample solution was determined at both intervals which is within 0.5% of the initial value.

5. Conclusion

The method developed for quantitative determination of guanfacine hydrochloride is rapid, precise, accurate and selective. The method was completely validated showing satisfactory data for all method-validated parameters tested. The developed method is stability-indicating as all degradants were resolved betterly and can be used for assessing the stability of guanfacine hydrochloride as a bulk drug. The developed method can be conveniently used for the assay determination of guanfacine hydrochloride in bulk drugs and pharmaceutical dosage form in quality control department.

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**References**