DETERMINATION OF GABAPENTIN IN BULK DRUG AND IN PHARMACEUTICAL DOSAGE FORM BY HPLC METHOD

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ABSTRACT

This paper describes validation of the selective, precise and accurate isocratic HPLC method for the assay of Gabapentin as bulk and pharmaceutical dosage forms. The mobile phase composition was ammonium dihydrogen orthophosphate buffer and methanol in 60:40(v/v) proportions Column used was strong cation exchange column bonded with phenyl sulphonic acid. Quantitation was achieved by UV detection at 200 nm. A linear response \( (r^2 \geq 0.999) \) was observed in the range of 2.5 mg/mL - 7.5 mg/mL. The method shows good recoveries (Average 101.2%) and the relative standard deviation intra and inter-day were found to be less than 0.5%. Validation parameters were also determined. The method can be used for quality control assay of Gabapentin as bulk and in finished dosage form and for the stability studies as the method separates Gabapentin from its degradation products and excipients.

Key words: Gabapentin HPLC Dosage form Method Validation.

INTRODUCTION

Gabapentin is L-(aminomethyl)-cyclohexaneacetic acid (Fig 1) used in the treatment of epilepsy. Presently Gabapentin is widely used as a medication to relieve pain, especially neuropathic pain. Gabapentin increases GABA levels in the brain clinically. Even though exact mechanism of action is unknown, but its therapeutic action on neuropathic pain is thought that Gabapentin inhibits calcium influx by inhibiting calcium channels in presynaptic terminals. Literature survey reveals that several methods have been reported for quantitative determination of Gabapentin in human plasma or serum. Many of the proposed methods have some limitations such as long run time or using special reaction condition which may not be suitable for routine analysis. Few methods are reported for direct HPLC analysis method with UV detection. The stability indicating HPLC method with UV detection at 210 nm was reported using special reaction condition which may not be suitable for routine analysis. Many of the proposed methods have some limitations such as long run time or using special reaction condition which may not be suitable for routine analysis. Few methods are reported for direct HPLC analysis method with UV detection. The method requires no extraction, no pre column derivetization step or no programmed linear gradient HPLC method. The method can be determined on simple HPLC with isocratic method. The method was used successfully applied for determination of assay of Gabapentin capsule and tablets.

Figure 1. Chemical structure of L-(aminomethyl)-cyclohexane acetic acid (Gabapentin)

EXPERIMENTAL

Materials and Methods
A sample of Gabapentin assigned purity 99.53 % of pharmaceutical grade is received from Biocon Pharmaceuticals, Bangalore, India. Gabapentin capsule of strength 300 mg and tablet of strength 100 mg procured from the market. Ammonium dihydrogen orthophosphate of analytical grade purchased from Merck (Mumbai, India) HPLC grade methanol were purchased from Qualigens (Mumbai India). High purity water was prepared by Millipore millii Q plus purification system. (France)

Equipment
The M/S Shimadzu–Japan HPLC system with a photodiode array detector system (SPD –M20A) was used for the method development and forced degradation studies. The out put signal was monitored and processed using LC-solution (Shimadzu-Japan). The LC system used for method validation was Shimadzu HPLC LC- 2010 CHT with quaternary gradient pumps. The out put signal was monitored and processed using LC-solution (Shimadzu-Japan) on Pentium computer (Compaq-Mumbai).

Chromatographic conditions
Separation was achieved by using a strong cation exchange column, 250 mm x 4.6 mm dia column with 10 μL particle sizes. (Whatman-Maidstone, England). The isocratic mobile phase pumped at the rate of 1.0 mL min⁻¹ comprised of 0.15 molar ammonium dihydrogen orthophosphate buffer in milli Q water adjusted the pH to 3.0 with orthophosphoric acid and methanol 60:40(v/v). The diluent used was methanol prepared daily and degassed by passing through 0.45 μm Millipore filter and ultrasonication for 10 min. The column temperature was maintained at 25 °C with detection at 200 nm. The injection volume was 10 μL. Prior to the injection of the drug solution, the column was equilibrated for 60 minutes.

Preparation of standard solution
The stock standard solution of drug substance 5 mg/mL was prepared using methanol as solvent.

Preparation of sample solution
To determine the content of Gabapentin, twenty capsules content were weighed and transferred into a clean and dry mortar. Then crushed and mixed well to prepare homogeneous mixture. A sample equivalent to 125 mg of Gabapentin was taken in 25 mL volumetric flask with the aid of 15 mL methanol and sonicated for 10 minutes diluted to 25 mL (5 mg/mL). All the experiment was conducted in triplicate. The overlay chromatogram of placebo and drug substance are shown in fig.2.
conditions were purposely altered. One factor at a time was changed to
level as described in the sample preparation. The percentage of recovery was
equivalent to about 50, 80, 100, 120 and 150% of the assay nominal sample
linearity, precision and accuracy were obtained. Calibration standard solution
between the highest and lowest concentration. of analyte where acceptable
were treated by linear least square regression analysis. The standard curves
different HPLC systems and different HPLC columns in different days in the
day precision) of the method was also evaluated using two different analysts,
six assay values obtained was calculated. The intermediate precision (inter-
mg/mL against qualified reference standard. The percentage of R.S.D. of
out six independent assays of test sample of Gabapentin. (concentration 5

Method Validation
Precision
Assay of method precision (intra-day precision) was evaluated by carrying
six independent assays of test sample of Gabapentin. (concentration 5
mg/mL) against qualified reference standard. The percentage of R.S.D. of
six assay values obtained was calculated. The intermediate precision (inter-
day precision) of the method was also evaluated using two different analysts,
different HPLC systems and different HPLC columns in different days in the
same laboratory.

Linearity
The standard calibration curves were prepared with five calibration over
a concentration range of 2.5 mg/mL -7.5 mg/mL (2.5, 4.0, 5.0, 6.0 and 7.5
mg/mL) for Gabapentin. The data of peak area versus drug concentration
were treated by linear least square regression analysis. The standard curves
are evaluated intra- day and inter-day linearity. The range was the interval
between the highest and lowest concentration. of analyte where acceptable
linearity, precision and accuracy were obtained. Calibration standard solution
of five levels was prepared daily by the stock solution 25 mg/mL in methanol
to concentrations of 2.5, 4.0, 5.0, 6.0 and 7.5 mg/mL in methanol.

Accuracy
A study of recovery of Gabapentin from spiked placebo was conducted.
Samples were prepared by mixing placebo with Gabapentin raw material
equivalent to about 50, 80, 100, 120 and 150% of the assay nominal sample
concentration. Sample solutions were prepared in triplicate for each spike
level as described in the sample preparation. The percentage of recovery was
calculated.

Robustness
To determine the robustness of the developed method, experimental
conditions were purposely altered. One factor at a time was changed to
estimate the effect. Thus, five replicate injections of standard solution were
injected under each parameter and observed the change on the tailing factor
for Gabapentin peak and the R.S.D. for peak area of Gabapentin. The flow rate
of mobile phase was changed by ± 10% i.e. 0.9 to 1.1 mL min⁻¹. The effect
of pH of buffer in mobile phase was studied by varying ± 0.2 pH units. The
effect of column temperature was studied at 20 and 30°C instead of 25°C. The
effect of ±10% of the organic phase composition (methanol) in mobile phase
was studied by changing buffer and methanol composition by 64:36 (v/v) and
56:44 (v/v).

Solution Stability and Mobile Phase Stability
Stability solution of Gabapentin in the assay method (concentration 5 mg/
ML) was carried out by leaving both the test solutions of sample and reference
standard in tightly capped volumetric flasks at room temperature in a dark
chamber for 72 h. The same sample solution was assayed for 24 h interval
up to the study period. The mobile phase stability was also carried out by
assaying prepared sample (concentration 5 mg/mL) solution against freshly
prepared reference standard solution for 24 h interval for 72 h. Mobile phase
prepared was kept constant during the study period. The % R.S.D. of assay of
Gabapentin was calculated for the study period during mobile phase stability
and solution stability experiments.

RESULTS AND DISCUSSION

Method Development and Optimization
The HPLC procedure was optimized with a view to develop a stability
indicating assay method. In the first place the drug has low absorbitivity
at normal UV range 220 to 380 nm. It was found that the drug is having
significant absorbance at 200-215 nm. Hence 200 nm wavelength was selected.
The Gabapentin is small highly polar molecule which can exist as cation, anion
and zwitterions due to its acid. pKₐ of 3.7 and base pKₐ of 10.7. Initially we
tried with ion pairing agent like heptane sulphanic acid and octane sulphanic
acid using buffer and organic modifier on C18 column. As the desired result
is not achieved on C18 column, strong cation exchange column bonded with
phenyl sulphanic acid was used. Buffer ammonium dihydrogen phosphate
improved the peak shape of the Gabapentin. When the mobile phase consisting
0.15 molar ammonium dihydrogen ortho phosphate buffer and methanol in
ratio 60:40 v/v eluted the Gabapentin and retention time was about 6.5
min.[Fig 2] and well separated between Gabapentin and its degradants with a
flow rate of 1.0 mL min⁻¹. As the related impurity was not used in the method,
the Gabapentin in dosage form degraded up to 97% wherever possible and
run on the system. All the degradants were well separated and no interference
observed from any of the degradants under all stress condition.

Method Validation
Precision
Method repeatability (intra-day precision) was evaluated by assaying
six samples, prepared as described in the sample preparation. The mean %
assay and percentage R.S.D. for assay values were found to be 99.7 and 0.2
%, respectively, which is well within the acceptance criteria i.e. assay value
should be between 97.0 and 103.0% and R.S.D. should be not more than 2.0%.
The intermediate precision (inter-day precision) was performed by assaying
six samples prepared by different analyst, different HPLC system and different
HPLC column in different days as described in the sample preparation. The
mean % assay and percentage R.S.D. for assay values were found to be 99.4
and 0.1 %, respectively. The results of intra-day precision and inter-day
precision were evaluated with respect to student’s t- test and found that t-test
was passed. The result shows that good precision of the method

Linearity
Linearity of the method confirmed by preparing Gabapentin standard
curves for the analytical range of 2.5-7.5 mg/mL. Excellent correlation between
analyte peak area and concentration of the drug was observed with r²≥0.999 for
all standard curves. Precision and accuracy were established for drug substance
from 2.5 to 7.5 mg/mL. Therefore range for the method is 2.5-7.5 mg/mL.

Accuracy
The percentage recovery of Gabapentin in pharmaceutical dosage forms
ranged from 97 to 105% (Table I).
Table I. Recovery results of Gabapentin in pharmaceutical dosage form.

<table>
<thead>
<tr>
<th>Spike Level (%)</th>
<th>Average 'mg' added</th>
<th>Average 'mg' found</th>
<th>Mean % recovery</th>
<th>Percentage R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>49.76</td>
<td>52.17</td>
<td>104.3</td>
<td>0.23</td>
</tr>
<tr>
<td>80</td>
<td>80.0</td>
<td>81.69</td>
<td>102.1</td>
<td>0.08</td>
</tr>
<tr>
<td>100</td>
<td>100.0</td>
<td>102.56</td>
<td>102.6</td>
<td>0.33</td>
</tr>
<tr>
<td>120</td>
<td>120.0</td>
<td>117.11</td>
<td>97.6</td>
<td>0.27</td>
</tr>
<tr>
<td>150</td>
<td>150.0</td>
<td>148.88</td>
<td>99.3</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Robustness
In all the deliberate varied chromatographic condition carried out i.e. flow rate, column temperature, pH of the buffer in mobile phase and organic phase composition in mobile phase, the tailing factor and the % R.S.D. for the Gabapentin peak area from the five replicate injections of standard was found to be with in the acceptable limits, illustrating the robustness of the method (Table II).

Table II. Results of robustness study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>observed value</th>
<th>Variation</th>
<th>Tailing factor</th>
<th>RSD for five injection of standard (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>0.9 mL, 1.1 mL</td>
<td>0.9 mL</td>
<td>1.2</td>
<td>0.63</td>
</tr>
<tr>
<td>Column temperature</td>
<td>20°C, 30°C</td>
<td>20°C</td>
<td>1.1</td>
<td>0.62</td>
</tr>
<tr>
<td>pH (±0.2 units of the set pH)</td>
<td>2.8, 3.2</td>
<td>2.8</td>
<td>1.2</td>
<td>0.43</td>
</tr>
<tr>
<td>Mobile phase composition</td>
<td>Buffer: Methanol(54:46), Buffer: Methanol(66:34)</td>
<td>1.1</td>
<td>1.1</td>
<td>0.28, 0.97</td>
</tr>
</tbody>
</table>

Solution Stability and Mobile Phase Stability
The R.S.D. of assay of Gabapentin during solution stability and mobile phase stability experiments was within 1%. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay determination was stable up to 72 h.

Results of Forced Degradation Studies
All the stressed samples prepared were injected into the HPLC system with photodiode array detector as per the described chromatographic conditions. Degradation was not observed in heat. But almost completely degraded in acid hydrolysis, base hydrolysis and oxidative conditions. Degradation was found to be partial in UV and light. All degradants peaks were resolved from Gabapentin. All the chromatograms of the stressed samples were evaluated for peak purity of Gabapentin using Shimadzu LC solution Software. In all the forced degradation samples impurity was not detected in the Gabapentin peak. The spectral homogeneity (Peak purity) 200-400nm was determined in the forced degraded samples. The threshold was set at ≥0.990. The peak purity and percent degradation (Table-III) for Gabapentin in capsule were demonstrated that the proposed LC method was able to separate Gabapentin from degradants generated during forced degradation studies. The chromatogram of oxidation condition was shown in figure 3.

Analysis of marketed products:
The validated method was used in the analysis of two Gabapentin drug products from two different manufacturer. One is Gabapentin tablet of strength 100 mg and another one is Gabapentin capsule of strength 300 mg. In both cases assay obtained is more than 98% and no interference of impurity peak observed in Gabapentin peak.
CONCLUSION

The developed LC method is sensitive, accurate, rugged and selective for the determination of Gabapentin in a capsule formulation. Application of this method for the analysis in capsule shows that neither the degradation products nor the excipients, including the preservatives interfere with the analytical determination. This indicates that the proposed method could be used as a stability indicating method for the determination of Gabapentin either in bulk powder or in pharmaceutical formulations.

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