A SIMPLE AND SENSITIVE METHOD FOR THE DETERMINATION OF ALISKIREN HEMIFUMARATE USING HPLC-UV DETECTION

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ABSTRACT

A simple and easy to use reverse phase HPLC method for the determination of Aliskiren hemifumarate in tablet dosage forms was developed. Liquid chromatographic separation of aliskiren was achieved on a Waters Xbridge C18 (150 X 4.6mm, 5µM particle size) column employing a mobile phase of 0.03 Trifluoroacetic acid (TFA) in water and 0.03%TFA in Acetonitrile and water (95:5) at a flow rate of 0.8mL/min. Aliskiren was monitored using a dual mode wavelength detector at 230/254nm. The method was linear in the concentration of 1-100 µg/mL and the limit of detection and limit of quantification of the method was 0.2µg/mL and 0.6µg/mL. The method developed had also been validated for its accuracy, robustness, specificity and repeatability and successfully applied to the determination of aliskiren in pharmaceutical dosage forms like tablets. The method offers good sensitivity and reliable accuracy to be used for routine analysis.

Keywords: HPLC, Aliskiren hemifumarate, RSD values.

INTRODUCTION

Hypertension, one among other associated disorders like diabetes, is the leading cause of morbidity and mortality, if left uncontrolled. The main cause of hypertension is still yet unknown but from the understandings of the body physiological mechanism for maintaining blood pressure, it was known that the renin angiotensin system (RAS) play a major and crucial role in maintaining a constant blood pressure. Apart from maintaining the blood pressure, the RAS system also helps in maintaining body electrolyte balance and had been thus recognized as an important target in treating hypertension and its associated disorders that result from untreated hypertension.

Many drugs like the so called Angiotensin converting enzyme inhibitors eg: captopril, enalapril and Angiotensin receptor blockers like losartan, valsartan etc; are under current use and are the first choice drugs for treating hypertension. However, one of these drugs suffers from adverse effects. So, long ago renin enzyme was a target for drug discovery companies. As a result of several efforts, aliskiren available as the hemifumarate salt resulted as a first in class drug for effective management of hypertension. It is metabolized slowly in the body resulting in stronger half lives which restrict it once a day dosing. The cytochrome P450 susceptibility is also less and a major proportion of the drug is eliminated unchanged via faces.

Previous methods have been developed using either fluorescence detection or mass spectrophotometric measurements. But these methods employ either solid phase extraction or radiochemical based measurements. Our objective in the present study is to develop and validate a selective and sensitive analytical method employing high performance liquid chromatography with ultraviolet detection and its application for the measurement of aliskiren hemifumarate in pharmaceutical dosage forms offering higher degree of precision and quality in accordance to standard regulatory requirements. The method posse’s higher sensitivity than the earlier existing methods.
EXPERIMENTAL

Chemicals and reagents
Aliskiren hemifumarate was obtained as a gift sample (Morepen laboratories, India). All other solvents are of the analytical grade and used without any further purification. Acetonitrile (Merck, Mumbai, India). Ultra pure water (Ranbaxy, India) and Trifluoroacetic acid (Merck, India) were used.

Equipment
HPLC from Shimadzu (version LC-2010C), equipped with a quaternary pump and a thermostat column jacket for maintaining ambient temperature during chromatographic separation was used for analysis. Integration was performed with Class-VP software (version 6.14).

Chromatographic conditions
The mobile phase consists of 0.03% trifluoroacetic acid (TFA) in water and 0.03% TFA in acetonitrile and water (95:5) and separation was achieved on a waters Xbridge reverse phase C18 column (150 x 4.6 mm i.d, particle size 5 µM) with 10% B from 0 to 2.5 min, linearly increased to 95% B from 2.5 min to 8.50 min and was held until 10.50 min before returning to starting condition within remaining 4.5 min (Solvent A: 0.03% TFA in water, Solvent B: 0.03% TFA in acetonitrile and water (95:5); flow rate 0.8mL/min.). The eluent was monitored at the detection wavelength of 230/254 nm with a dual wavelength ultra violet detector. Total runtime was 15 min and the retention time of aliskiren was 9.64 min. Water: Methanol (50:50) was used as diluent. A linear relationship was found for aliskiren over the range of 1.0 µg/mL to 100 µg/mL with a correlation coefficient of 0.999. During validation, the with-in day and between-day coefficient of variation (CV) values were within the range of 0.8% to 0.6%. In accordance with the guidelines for analytical methods validation, this sensitive HPLC method can thus be and so, used for quantification of aliskiren.

Preparation of aliskiren reference solution
A stock solution of aliskiren hemifumarate was prepared by accurately weighing 10 mg of aliskiren hemifumarate into a 10 mL volumetric flask to produce a stock solution of 1 mg/mL in diluent. The stock solution was stored at 2–8°C, protected from light and suitable dilutions were made and used appropriately during method development.

Procedure for tablets
Rasilez tablets (Novartis, Brazil) tablets containing 150 mg of aliskiren hemifumarate were obtained commercially and a stock solution of aliskiren hemifumarate was prepared by accurately weighing a suitable quantity of tablet powder equivalent to 10 mg of aliskiren base into a volumetric flask to produce 1 mg/mL solution in diluent. Thereafter, suitable dilutions were prepared to produce a stock solution of 20µg/mL solution and 50 µL of solution was injected into HPLC system and a chromatogram was recorded at a flow rate of 0.8 mL/min. The injections were repeated six times and the peak areas were recorded.

The peak area for each of the drug concentrations was calculated. The plot of peak area vs the respective drug concentration gives the calibration curve. The recovery studies were carried out by adding a known amount of aliskiren hydro bromide to the pre analyzed samples and subjecting them to proposed HPLC method.

RESULTS AND DISCUSSION
The typical chromatogram for the proposed method is shown in Fig. 1.

Linearity
A good linear relationship (r=0.999) was observed with a concentration range of 1-100 µg/mL. The regression equation was constructed by linear regression fitting. It was found that correlation coefficient and regression analysis are within the limits. The peak areas of different concentrations are shown in Table 1. The peak areas of aliskiren hemifumarate were reproducible, as indicated by a low coefficient of variation.

Specificity
In order to determine the specificity of the method, a placebo solution (in-house mixture of all the tablet excipients) was prepared and was analyzed to evaluate the absence of interference from the formulation excipients on the aliskiren peak, indicating that there was no interference with or suppression of the peak at the retention time of aliskiren due to the commonly used tablet excipients.

**Robustness**

Robustness of a method is its ability to remain unaffected by small deliberate variations in the method parameters. The following changes in the optimum parameter values were examined, the flow rate of the mobile phase (adjusted by ± 0.02 mL/min) and the detection wave length (adjusted by ± 3 nm).

**System suitability test**

System suitability test to evaluate the resolution and reproducibility of the proposed method was performed by injecting six replicates of a reference solution containing 20µg/mL. The parameters measured were peak area, retention time etc. The RSD values calculated in the system suitability test were within the limit (<2.0%) as in table 2 indicating the system is suitable for the intended method of analysis.

**Accuracy**

The accuracy of the proposed method was calculated by injecting known concentrations of drug solution of aliskiren in the dosage formulation at three different concentration levels (50, 100 and 150 %) with reference to label claim of tablet. Recoveries ranged from 98 to 99%. The accuracies are generally calculated as the percentage of drug recovered from the tablet as given in table 3.

**Limit of detection and limit of quantification**

Limit of quantification, the lowest concentration which can be quantified with a greater degree of precision with the proposed method was 0.5µg/mL and limit of detection of the following method was 0.2µg/mL.

**Intraday and inter day precision**

The precision of the proposed method was determined in terms of intra-day and inter-day. For intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and RSD was 0.8 % (limit RSD < 2.0 %). In addition, the day-to-day (inter-day) precision was studied by injecting the same concentration of standard solution on consecutive days and the RSD was 0.6 % (limit RSD < 2.0 %).

Table 1: Standard graph for the estimation of aliskiren hemifumarate

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Area of the analyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32139</td>
</tr>
<tr>
<td>5</td>
<td>166205</td>
</tr>
<tr>
<td>10</td>
<td>360389</td>
</tr>
<tr>
<td>20</td>
<td>757021</td>
</tr>
<tr>
<td>40</td>
<td>1603347</td>
</tr>
<tr>
<td>60</td>
<td>2547844</td>
</tr>
<tr>
<td>80</td>
<td>3380888</td>
</tr>
<tr>
<td>100</td>
<td>4228081</td>
</tr>
</tbody>
</table>

Table 2: Results of system suitability test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>RSD (%)</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td>Retention time</td>
<td>9.607</td>
<td>9.617</td>
<td>0.476</td>
<td>Pass</td>
</tr>
<tr>
<td>Area</td>
<td>751233</td>
<td>764071</td>
<td>0.873</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Mean of six replicates

RSD-Relative standard deviation.
CONCLUSION
To conclude, the following developed method offers several methods than the existing methodology in being the simpler preparation of mobile phase, good precision and accuracy. The method offers good sensitivity than the current existing methods for determination of aliskiren in pharmaceutical dosage forms. The method can be further extended for the determination of aliskiren in various biological matrices, if desired.

Table-3: Results of accuracy test

<table>
<thead>
<tr>
<th>Nominal conc (µg/mL)</th>
<th>Mean conc found (µg/mL)</th>
<th>RSD (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>360388</td>
<td>0.57</td>
<td>98.36</td>
</tr>
<tr>
<td>20</td>
<td>757024</td>
<td>1.15</td>
<td>98.77</td>
</tr>
<tr>
<td>40</td>
<td>1603349</td>
<td>0.68</td>
<td>99.12</td>
</tr>
</tbody>
</table>

Mean of three replicates
RSD-Relative standard deviation.

Fig.-1: Chromatogram of aliskiren hemifumarate at 20µg/mL concentration in diluent.

REFERENCES

[RJC-758/2011]