STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF LINAGLIPTIN IN BULK AND TABLET DOSAGE FORM.

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Abstract:
The Reverse Phase High Performance Liquid Chromatographic method was developed for the determination of linagliptin with its stability studies in its bulk and pharmaceutical dosage form which was precise, accurate and sensitive. The separation has been done by using the chromatographic conditions on an Inertsil ODS column (250 x 4.6 mm, 5µ particle size), the mobile phase of potassium dihydrogen phosphate buffer pH 6 and acetonitrile in the ratio 60:40 v/v, at a flow rate of 1 mL/min at an ambient temperature at a wavelength 246 nm at retention time of 3.67 minutes. The method developed was validated for specificity, precision, linearity, accuracy and forced degradation stability studies. The average recoveries of linagliptin were 98.0 – 102.0% and thereof a good linear relationship (r²=0.999) was observed between the concentration range of linearity in the range of 25-150% with respect to test concentration (12.5-75.0 µg/mL) and the above proposed method was validated for various parameters as per ICH guidelines and all the results obtained were satisfactory and passed all the forced degradation stability studies.

Keywords: Forced Degradation Stability studies, Linagliptin, Separation.

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1. INTRODUCTION:
Linagliptin is a DPP-4 inhibitor developed by Boehringer Ingelheim for treatment of type II diabetes. It is an inhibitor of DPP-4\textsuperscript{1,2}, an enzyme that degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintrotropic polypeptide (GIP). Linagliptin chemically known as 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3- methyl-1-[(4-methylquinazolin-2-yl) methyl]-3, 7-dihydro-1H-purine-2, 6-dione (Fig 1).

Fig 1: Structure of Linagliptin

The literature survey ensures that the developed analytical methods which include the UV spectrophotometric methods\textsuperscript{3,4}, some liquid chromatographic methods\textsuperscript{5,9}, simultaneous UV spectrophotometric methods with hplc\textsuperscript{10} and simultaneous determination of two drugs by using hplc\textsuperscript{11,12}. The above proposed method developed is the stability indicating studies - high performance liquid chromatography method for determination of linagliptin in presence of its degradation products for assurance of the purity of the bulk drug and stability of pharmaceutical dosage form which is validated, accurate, precise and sensitive.

2. EXPERIMENTAL:
2.1 Materials
Linagliptin was supplied as a gift sample from Emcure pharmaceuticals Ltd. All HPLC grade methanol, acetonitrile and orthophosphoric acids and potassium di hydrogen phosphate were obtained from S.D. Fine Chemicals Ltd., India, Qualigens Fine Chemicals Ltd., Mumbai, India.

2.2 Instrumentation
A HPLC used with the chromatographic system made of Waters consists of binary pump, auto sampler with PDA detector. Data acquisition was done by using empower 2 software. The column used was C\textsubscript{18}, (250 X 4.6 mm, 5μ) with mobile phase KH\textsubscript{2}PO\textsubscript{4} buffer: ACN (60: 40) v/v at a flow rate of 1 ml/min at a wavelength of 246 nm.

2.3 Preparation of solutions
2.3.1 Preparation of buffer
A 0.01 M solution of potassium dihydrogen phosphate was prepared by dissolving 0.680 g of potassium dihydrogen phosphate in 800 ml water and diluting to 1000 ml with water.

2.3.2 Preparation of stock solution
Weigh and transfer 5 mg of Linagliptin working standard into 10 mL volumetric flask, add 5 mL of diluent and sonicate to dissolve and dilute the remaining volume with diluent.

2.3.3 Preparation of sample solution
Finely grind pre weighed 20 tablets. Transfer grinded sample quantitatively equivalent to 5 mg of Linagliptin in to 10 mL volumetric flask add 5 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 1 mL of filtrate to 10 mL with mobile phase.

2.4 Method Validation
The developed method will be validated by the determination of the parameters like system suitability, linearity, accuracy and precision, specificity, ruggedness and robustness as per ICH guidelines.

2.5 Forced Degradation Studies
To perform the forced degradation study 10 mL of the tablet sample solution (50 μg/mL concentration) drug was subjected to acidic, alkaline, thermal and UV radiation conditions. For acidic degradation the drug was taken to it 10 mL of 0.1N HCl was added and was exposed to heat at a temperature of 60\degree C for 30 mins. For alkaline degradation the drug was treated with 10 mL of 1N NaOH and was exposed to heat at a temperature of 60\degree C for 30 mins. For thermal degradation the powered drug was exposed to heat at a temperature of 100\degree C in oven drier exposure for about 5 hours. For UV degradation, powdered drug was exposed to UV chamber for about 12 hrs and later the above solutions were made up to 100 mL using water.

3. RESULTS AND DISCUSSION:
3.1 Method Development
The method is developed by using the suitable chromatographic system conditions by using the mobile phase KH\textsubscript{2}PO\textsubscript{4} buffer: ACN (60: 40) v/v at a flow rate of 1 ml/min at a wavelength of 246 nm by using the column C\textsubscript{18}. The standard chromatogram (Fig 2) is developed by using the standard stock solution and further the validation and their parameters are achieved as per ICH guidelines.
3.1.1 System Suitability Studies
For system suitability, six replicate injections of the working standard or sample solution were injected and the system suitability parameters were evaluated. The results of system suitability were shown in Table 1.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
<th>Plate Count (N)</th>
<th>Tailing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.62</td>
<td>3147697</td>
<td>5670</td>
<td>1.1</td>
</tr>
<tr>
<td>2</td>
<td>3.615</td>
<td>3178410</td>
<td>5703</td>
<td>1.13</td>
</tr>
<tr>
<td>3</td>
<td>3.614</td>
<td>3171498</td>
<td>5730</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>3.616</td>
<td>3161334</td>
<td>5703</td>
<td>1.14</td>
</tr>
<tr>
<td>5</td>
<td>3.615</td>
<td>3172960</td>
<td>5713</td>
<td>1.13</td>
</tr>
<tr>
<td>6</td>
<td>3.618</td>
<td>3207718</td>
<td>5484</td>
<td>1.15</td>
</tr>
<tr>
<td>Avg</td>
<td>3.6163</td>
<td>3173269.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Std.dev</td>
<td>0.002250926</td>
<td>20063.73251</td>
<td></td>
<td>0.63</td>
</tr>
</tbody>
</table>

3.1.2 Specificity
The specificity of the developed method was determined by injecting blank, placebo solution, individual standards, mixed standard and sample solution separately.

3.1.3 Linearity
The linearity study was performed for the concentration range of 12.5-75 µg/mL for Linagliptin. Each level was injected into chromatographic system and calibration curve was plotted and the graph shown at Fig 3.
3.1.4 Accuracy
The accuracy of the developed method was evaluated by injecting triplicates of solutions of concentration 50%, 100% and 150%. The individual recovery and mean recovery values were calculated from the amount added and amount found. The results obtained are shown in Table 2.

Table 2: Results of Accuracy of Linagliptin

<table>
<thead>
<tr>
<th>Category</th>
<th>Standard</th>
<th>50% Solution</th>
<th>100% Solution</th>
<th>150% Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inj1 (Peak Area)</td>
<td>3152468</td>
<td>1568452</td>
<td>3186541</td>
<td>4765851</td>
</tr>
<tr>
<td>Inj2 (Peak Area)</td>
<td>3186542</td>
<td>1578655</td>
<td>3152486</td>
<td>4658725</td>
</tr>
<tr>
<td>Inj3 (Peak Area)</td>
<td>3175584</td>
<td>1572140</td>
<td>3155524</td>
<td>4721543</td>
</tr>
<tr>
<td>AVG</td>
<td>3171531</td>
<td>1573082</td>
<td>3164850</td>
<td>4715373</td>
</tr>
<tr>
<td>Std.dev</td>
<td>17394.75</td>
<td>5166.362</td>
<td>18845.98</td>
<td>5382.88</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.548465</td>
<td>0.328423</td>
<td>0.595478</td>
<td>1.141561</td>
</tr>
<tr>
<td>Amount Recovered</td>
<td>49.60</td>
<td>99.79</td>
<td>148.68</td>
<td></td>
</tr>
<tr>
<td>%Recovery</td>
<td>99.20</td>
<td>99.79</td>
<td>99.12</td>
<td></td>
</tr>
</tbody>
</table>

3.1.5 Precision
The precision of the method was ascertained separately from the peak area obtained by actual determination of six replicas of a fixed amount of the drug and formulation. The precision of this method was checked by repeatability of injection, repeatability (intra-assay), intermediate precision (inter-assay) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (%RSD) for ten determinations of peak areas of Linagliptin performed on the same day for both intra- and inter-assay variation, standard solutions of linagliptin.

3.1.6 LOD and LOQ
The LOD and LOQ of linagliptin was estimated from signal to noise ratio and were found to be 1.5 µg/mL and 4 µg/mL respectively and the chromatograms were shown at Fig 4 & 5.
**Fig 4: Chromatogram of LOD**

Chromatogram of LOD

- LOD: 1.5 µg/mL
- Retention Time: 3.621 min

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>% Area</th>
<th>Symmetry Factor</th>
<th>USP Plate Count</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>LINAGLIPTIN</td>
<td>3.621</td>
<td>190481</td>
<td>27156</td>
<td>100.00</td>
<td>1.14</td>
<td>6158</td>
<td>2.865</td>
</tr>
</tbody>
</table>

**Fig 5: Chromatogram of LOQ**

Chromatogram of LOQ

- LOQ: 4 µg/mL
- Retention Time: 3.625 min

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
<th>% Area</th>
<th>Symmetry Factor</th>
<th>USP Plate Count</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>LINAGLIPTIN</td>
<td>3.625</td>
<td>571440</td>
<td>81460</td>
<td>100.00</td>
<td>1.12</td>
<td>6248</td>
<td>9.887</td>
</tr>
</tbody>
</table>
3.1.6 Robustness
Robustness of the method was determined by changing experimental conditions deliberately. The effect of change in flow rate and wavelength on retention time, theoretical plate number and peak asymmetry were studied and the results were shown in Table 3.

Table 3: Results of Robustness

<table>
<thead>
<tr>
<th>Condition</th>
<th>Theoretical Plates (NLT 2500)</th>
<th>Symmetry Factor (NMT 2.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer-1</td>
<td>5915</td>
<td>1.13</td>
</tr>
<tr>
<td>Buffer-2</td>
<td>5959</td>
<td>1.13</td>
</tr>
<tr>
<td>Flow rate 1 mL</td>
<td>5140</td>
<td>1.13</td>
</tr>
<tr>
<td>Flow rate 1.2 mL</td>
<td>6421</td>
<td>1.15</td>
</tr>
</tbody>
</table>

3.2 Forced Degradation Studies
3.2.1 Acid Degradation
10 mL of the sample solution (50 μg/mL concentration) was taken and to it 10 mL of 1N HCl was added and was exposed to heat at a temperature of 60°C for 30 min. Later this solution was made up to 100 mL using water. This solution was then chromatographed and the results were shown in Fig 6.

3.2.2 Alkali Degradation
10 mL of the sample solution (50 μg/mL concentration) was taken and to it 10 mL of 1N NaOH was added and was exposed to heat at a temperature of 60°C for 30 min. Later this solution was made up to 100 mL using water. This solution was then chromatographed and the results were shown in Fig 7.
3.2.3 Thermal Degradation
The thermal degraded sample has the R Value of 3.55 and degradation was not observed in standard that was exposed to temperature of 105°C for 30 min.

3.5.4. UV radiation degradation
The samples was not degraded under uv radiation for about 2 hrs and the dry heat conditions and showed peak at R-value of 3.66.
This indicates that the drug is susceptible to acid / base hydrolysis, uv radiation and thermal degradation.
The results of accelerated degradation studies are listed in Table 4.

There are many spectrophotometric and liquid chromatographic methods for determination of Linagliptin but the present study of stability indicating high performance liquid chromatography for the determination of linagliptin in its bulk and pharmaceutical dosage form is linear, accurate, precise, rapid, selective, reproducible and sensitive for the estimation of the drug in quality control department in the pharmaceutical industry.

5. ACKNOWLEDGEMENTS:
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6. REFERENCES: