ANALYTICAL METHOD VALIDATION COMMITTEE FOR NON PHARMACOPOEIAL PRODUCT

DEPARTMENT OF DRUG ADMINISTRATION

Metformin (Sustained Release) & Linagliptin Tablets

Metformin & Linagliptin Tablets contain not less than 90% and not more than 110% of the stated amount of Metformin & Linagliptin.

1. Identification:

1.1. Metformin HCl:

In the assay, the principle peak in the chromatogram obtained with the sample solution should correspond to the peak in the chromatogram obtained with the reference standard solution of Metformin HCl.

1.2. Linagliptin:

In the assay, the principle peak in the chromatogram obtained with the sample solution corresponds to the peak in the chromatogram obtained with the reference standard solution of Linagliptin.

2. Assay:

2.1 Reagents Required:

1. Acetonitrile (HPLC Grade)
2. Potassium dihydrogen orthophosphate (HPLC Grade)
3. Orthophosphoric acid HPLC Grade
4. HPLC Grade water

2.2 Preparation of reagent solution:

2.2.1 Buffer (0.02M Phosphate buffer): Weigh 0.675gm KH_2PO_4 in 250ml volumetric flask and sonicate in 200ml of water. Make volume up to 250ml with water and adjust pH to 4.0 with 10% phosphoric acid.

2.2.2 Mobile phase: Buffer: Acetonitrile (70:30)

2.3 Chromatographic system

Column: Octyldecylsilane (C18), (150*4.6 mm), 5 µm

Flow rate: 1.0 ml/min

Detector: PDA Detector

Wavelength: 265 nm (Metformin) & 295 nm (Linagliptin)

Injection volume: 20 µl

Column Oven temperature: 25 °C

2.4 Standard Solution

2.4.1 Metformin HCl Standard solution: Weigh accurately about 42.5 mg of working standard of Metformin HCl and transfer into 20 ml volumetric flask and sonicate to dissolve.

2.4.2 Linagliptin Standard solution: Weigh accurately about 12.5 mg Linagliptin working standard into separate 100 ml volumetric flask. Add about 70 ml of mobile phase and sonicate for about 10 minutes and make up the volume to 100 ml with mobile phase.

2.4.3 Standard combination: Pipette 2 ml of Linagliptin standard solution to 20 ml with mobile phase in a volumetric flask of Metformin HCl and make up to mark with mobile phase.

2.5 Sample Preparation: Weigh individually 20 tablets & crush the tablet into fine powder. Weigh accurately the powder eq. to 500 mg of Metformin HCl in 100 ml flask, add 70 ml of mobile phase & sonicate for 15 minutes to dissolve. After sonication, cool to room temperature and make volume to 100 ml with mobile phase. Filter the final solution through 0.2 µm membrane filter.

Note: Adjust the concentration of the standard preparation and sample preparation depending upon the strength of Metformin and Linagliptin Tablet.

2.6 Chromatographic Procedure:

Inject 20 µl of standard and sample solution separately and obtain the respective chromatogram. Measure the peak responses.

2.7 Calculations:

Content of Metformin Hydrochloride per Tablet:

$$\frac{\textbf{Metformin Spl Peak Area}}{\textbf{Metformin Std Peak Area}} \textbf{ X } \frac{\textbf{Std wt}}{\textbf{Std Dil.}} \textbf{ X } \frac{\textbf{Spl Dil.}}{\textbf{Spl wt}} \textbf{ X Average wt X Std Potency } (\%) \textbf{X} \frac{(\textbf{100} - \textbf{Std LOD})}{\textbf{100}}$$

Content of Linagliptin per Tablet:

$$\frac{\textbf{Linagliptin Spl Peak Area}}{\textbf{Linagliptin Std Peak Area}} \textbf{ X } \frac{\textbf{Std wt}}{\textbf{Std Dil.}} \textbf{ X } \frac{\textbf{Spl Dil.}}{\textbf{Spl wt}} \textbf{ X Average wt X Std Potency } (\%) \textbf{X} \frac{(\textbf{100} - \textbf{Std LOD})}{\textbf{100}}$$

3.0 Uniformity of the content (Linagliptin)

3.1 Reagents required: Same as Assay

3.2 Preparation of reagent solution: Same as Assay

3.3 Chromatographic system: Same as Assay

3.4 Standard Preparation:

Weigh accurately about 12.5 mg Linagliptin working standard in 50 ml volumetric flask and add 35 ml mobile phase. Dissolve by sonication and diluted to 50 ml with mobile phase. Dilute 5 ml resulting solution to 50 ml with mobile phase. Filter the resulting standard solution through 0.2 µm membrane filter. (25ppm)

3.5 Test Solution:

Take 10 tablets and transfer individually to 100ml volumetric flask. Add 60ml of mobile phase and sonicate for 15 min to disperse the tablet. Make up the volume with mobile phase. Filter the final solution through 0.2 µm membrane filter. (25ppm)

3.6 Chromatographic Procedure:

Inject 20 µl of standard and sample solution separately and obtain the respective chromatogram. Measure the peak responses and calculate the content of Linagliptin in each tablet by using the following formula:

3.7 Calculations:

Content of Linagliptin per Tablet:

$$\frac{\textbf{Linagliptin Spl Peak Area}}{\textbf{Linagliptin Std Peak Area}} \textbf{ X } \frac{\textbf{Std wt}}{\textbf{Std Dil.}} \textbf{ X } \frac{\textbf{Spl Dil.}}{\textbf{Spl wt}} \textbf{ X Average wt X Std Potency } (\%) \textbf{X} \frac{(\textbf{100} - \textbf{Std LOD})}{\textbf{100}}$$

4.0 Dissolution

4.1 Dissolution (Linagliptin):

4.1.1 Dissolution Medium: 0.1N HCl

4.1.2 Dissolution test condition

Volume : 900 ml

Apparatus: IP II (Basket)

RPM : 50

Time : 45 minutes

4.1.3 Standard Solution:

Weigh accurately about 13.5 mg of working standard of Linagliptin and transfer into 100 ml volumetric flask. Add about 60 ml of 0.1 N HCl, sonicate for 15 minutes, cool and make up the volume to 100 ml with 0.1 N HCl. Dilute 2 ml of the resulting solution to 100 ml with 0.1 N HCl. Filter the resulting standard solution through a membrane filter of 0.2 µm.

4.1.4 Sample Solution:

Place the stated volume of dissolution medium, free from dissolved air, into the vessel of the apparatus. Assemble the apparatus and warm the dissolution medium to 36.5 º to 37.5 º. Operate the apparatus immediately at the time and speed as specified. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the dissolution medium and top of the rotating blade. Filter the sample solution promptly through a membrane filter of 0.2 µm. Discard the first few ml of the filtrate.

4.1.5 Procedure: Separately inject 20 µl of standard and sample solution and blank solution (dissolution medium) and obtain the respective chromatograms. Measure the peak responses and calculate the % release of the drug by using following formula:

4.1.6 Calculation:

$$\%\ \boldsymbol{release\ of\ Linagliptin} = \frac{\boldsymbol{Spl\ peak\ area}}{\boldsymbol{Std\ peak\ area}}\ \boldsymbol{X}\ \frac{\boldsymbol{Std\ wt}}{\boldsymbol{Std\ Dil}}\ \boldsymbol{X}\ \frac{\mathbf{900}}{\boldsymbol{Label\ Claim}}\ \boldsymbol{X}\ \boldsymbol{Std\ Potency}\ (\%)\boldsymbol{X}\ \frac{\mathbf{100} - \boldsymbol{LOD}}{\mathbf{100}}\ \boldsymbol{X}\ \mathbf{100}\ \%$$

4.1.7 Tolerence Limit: NLT 80 % D of the stated amount

4.2 Dissolution (Metformin Sustained release tablet):

4.2.1 Dissolution Medium: 1000 ml phosphate buffer pH 6.8

Weigh 6.805 g of Potassium dihydrogen phosphate and 0.896 g of sodium hydroxide in 1000 ml volumetric flask. Add water and sonicate to dissolve. Make up the volume to 1000 ml with water. Adjust pH to 6.8 with dilute HCl or dilute sodium hydroxide.

4.2.2 Dissolution test condition

Apparatus: IP II (Basket)

Speed: 100 rpm

Time: 1 hour, 3 hours and 10 hours

4.2.3 Standard solution:

Weigh accurately about 25 mg of working standard of metformin hydrochloride and transfer into 100 ml volumetric flask. Dissolve with water and make up the volume to 100 ml with water. Dilute 2 ml of the standard solution to 100 ml with dissolution medium.

4.2.4 Sample solution:

Place the stated volume of dissolution medium, free from dissolved air, into the vessel of the apparatus. Assemble the apparatus and warm the dissolution medium to 36.5 º to 37.5 º. Operate

the apparatus immediately at the time and speed as specified in the individual monograph. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the dissolution medium and top of the rotating blade. Filter the sample solution promptly through a membrane filter of 0.2 µm. Discard the first few ml of the filtrate.

4.2.5 Procedure: Dilute 1 ml of the filtrate to 100 ml with dissolution medium. Measure the absorbance of the standard and sample solution at about 232 nm. Calculate the percentage of drug release in the tablet by using the following formula:

4.2.6 Calculation:

$$\%\ \boldsymbol{release\ of\ Metformin} = \frac{\boldsymbol{Spl\ Abs.}}{\boldsymbol{Std\ Abs.}}\ \boldsymbol{X}\ \frac{\boldsymbol{Std\ wt}}{\boldsymbol{Std\ Dil}}\ \boldsymbol{X}\ \frac{\boldsymbol{1000}}{\boldsymbol{Label\ Claim}}\ \boldsymbol{X}\ \frac{\boldsymbol{100}}{\boldsymbol{1}}\ \boldsymbol{xStd.Potency}\ (\%)\boldsymbol{X}\ \frac{\boldsymbol{100 - LOD}}{\boldsymbol{100}}\ \boldsymbol{X}\ \boldsymbol{100}\ \%$$

4.2.7 Tolerence Limit: 1 hr (25 % to 50 % of the stated amount)

3^{rd} hr (45% to 70 % of the stated amount)

8^{th} hr (NLT 80 % of the stated amount)