

**ANALYTICAL METHOD VALIDATION COMMITTEE FOR NON  
PHARMACOPOEIAL PRODUCT**

**DEPARTMENT OF DRUG ADMINISTRATION**

**Metformin (Immediate Release) & Linagliptin Tablets**

Metformin & Linagliptin Tablets contain not less than 90 % and not more than 110 % of the stated amount of Metformin and 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  $\text{KH}_2\text{PO}_4$  in 250ml volumetric flask and sonicate in 200ml of water. Make volume upto 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  $\mu\text{m}$

**Flow rate:** 1.0 ml/min

**Detector:** PDA Detector

**Wavelength:** 265 nm (Metformin), 295 nm (Linagliptin)

**Injection volume:** 20  $\mu\text{l}$

**Oven temperature:** 27  $^{\circ}\text{C}$

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**2.4 Standard Solution (850 mg Metformin+ 50 mg Sitagliptin Tablet):**

**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 20ml 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 make volume up to mark 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.

**2.8 Calculations:**

**Content of Metformin Hydrochloride per Tablet:**

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

**Content of Linagliptin per Tablet:**

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

**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:**

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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:**

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

**4.0 Dissolution (Metformin+Linagliptin):**

**4.1 Dissolution Medium: 0.1N HCl**

**4.2 Dissolution test condition**

**Volume** : 900 ml

**Apparatus:** Paddle

**RPM** : 50

**Time** : 45 minutes

**4.3 Standard Solution:**

**4.3.1 Linagliptin Standard solution**

Weigh accurately about 12.5 mg Linagliptin working standard in 100 ml volumetric flask and add 70 ml dissolution medium. Dissolve by sonication and diluted to 100 ml with dissolution medium. Dilute 2 ml resulting solution to 100 ml with dissolution medium. Filter the resulting standard solution through 0.2 µm membrane filter. (2.5ppm)

**4.3.2 Metformin Hydrochloride Standard solution**

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Weigh accurately about 10 mg Metformin Hydrochloride working standard in 50 ml volumetric flask and add 35 ml dissolution medium. Dissolve by sonication and diluted to 50 ml with dissolution medium. Dilute 5 ml resulting solution to 50 ml with dissolution medium. Filter the resulting standard solution through 0.2 µm membrane filter. (20 ppm)

**4.4 Sample Solution:**

Place 1 tablet in each dissolution vessel and run the apparatus as per above condition and collect the sample solution from each jar at specified time. After the completion of the dissolution, filter the resulting solution.

**4.5 Procedure:**

**4.5.1 Linagliptin**

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:

**4.5.2 Metformin Hydrochloride**

Dilute 2ml of sample solution to 50ml volumetric flask with dissolution medium. Measure the absorbance of the sample and standard solution in UV-Vis spectrophotometer at about 232 nm and calculate the percentage release of each tablet with respect to claim amount using the formula for Metformin Hydrochloride.

**4.6 Calculation:**

$$\begin{aligned} & \% \text{ release of Linagliptin} \\ &= \frac{\text{Spl peak area}}{\text{Std peak area}} \times \frac{\text{Std wt}}{\text{Std Dil}} \times \frac{900}{\text{Label Claim}} \times \text{Std Potency (\%)} \times \frac{100 - LOD}{100} \times 100 \% \end{aligned}$$

$$\begin{aligned} & \% \text{ release of Metformin} \\ &= \frac{\text{Spl Abs.}}{\text{Std Abs.}} \times \frac{\text{Std wt}}{\text{Std Dil}} \times \frac{1000}{\text{Label Claim}} \times \frac{100}{1} \times \text{Std. Potency (\%)} \times \frac{100 - LOD}{100} \times 100 \% \end{aligned}$$

**4.7 Tolerance Limit:**

Metformin: NLT 70 % D of the stated amount

Linagliptin: NLT 80 % D of the stated amount