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

# DEPARTMENT OF DRUG ADMINISTRATION

## Metformin & Sitagliptin Tablets

Metformin (Sustained Release) & Sitagliptin Tablets contain not less than 90 % and not more than 110 % of the stated amount of Metformin & Sitagliptin.

**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. Sitagliptin:**

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 Sitagliptin.

**2. Assay:**

**2.1 Reagents Required:**

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

**2.2 Preparation of reagent solution:**

**2.2.1 Buffer:** Weigh 1.36 gm $KH_2PO_4$, dissolve in 900 ml water and add 2.5 ml of triethylamine. Adjust to pH 3.5 with orthophosphate and dilute to 1000 ml with water.

**2.2.2 Mobile phase:** Buffer: Acetonitrile (75:25)

**2.2.3 Diluents:** 5 volume of Acetonitrile: 95 volume of 0.1% v/v orthophosphoric acid in water.

**2.3 Chromatographic system**

**Column:** Octyldecylsilane (C18), (250*4.6 mm), 5 µm

**Flow rate:** 1.0 ml/min

**Detector:** UV Detector

**Wavelength:** 205 nm

**Injection volume:** 20 µl

**Oven temperature:** 30 °C

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

**2.4.1 Metformin HCl Standard solution:** Weigh accurately about 25 mg of working standard of Metfornin HCl and transfer into 50 ml volumetric flask. Add about 35 ml of diluent and sonicate for about 10 minutes and make up the volume to 50 ml with diluents.

**2.4.2 Sitagliptin Standard solution:** Weigh accurately about 30 mg eq. of Sitagliptin from Sitagliptin Phosphate working standard into separate 100 ml volumetric flask. Add about 70 ml of diluent and sonicate for about 10 minutes and make up the volume to 100 ml with diluents. Dilute 5 ml of Sitagliptin standard solution to 50 ml with diluents.

**2.4.3 Standard combination:** Pipette 5 ml of Metformin HCl standard solution & Sitagliptin standard solution into 25 ml volumetric flask and make up the volume to 25 ml with diluent. Filter the final standard solution through 0.2 µm membrane filter.

**2.5 Sample Preparation:** Weigh individually 20 tablets & crush the tablet into fine powder. Weigh accurately the powder eq. to 50 mg of Metformin HCl in 100 ml flask, add 70 ml of diluents & sonicate for 15 minutes to dissolve. After sonication, dilute to 100 ml with diluents and stir for 20 minutes, filter through filter paper and dilute 5 ml filtrate to 25 ml with diluents. 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 Sitagliptin 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:**

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

**Content of Sitagliptin per Tablet:**

$$\frac{\boldsymbol{Sitagliptin\ Spl\ Peak\ Area}}{\boldsymbol{Sitagliptin\ Std\ Peak\ Area}}\ \boldsymbol{X}\ \frac{\boldsymbol{Std\ wt}}{\boldsymbol{Std\ Dil.}}\ \boldsymbol{X}\ \frac{\boldsymbol{Spl\ Dil.}}{\boldsymbol{Spl\ wt}}\ \boldsymbol{X\ Average\ wt\ X\ Std\ Potency}\ (\%)\boldsymbol{X}\frac{(\boldsymbol{100-Std\ LOD})}{\boldsymbol{100}}\boldsymbol{X}\ \frac{\boldsymbol{M.W.Sitagliptin}}{\boldsymbol{M.W.Sitaglipitin\ P}}$$

## 3.0 Uniformity of the content (Sitagliptin)

**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 33 mg of Sitagliptin Phosphate Monohydrate RS eq. to 25 mg Sitagliptin in 50 ml volumetric flask and add 35 ml diluents. Dissolve by sonication and diluted to 50 ml with diluents. Dilute 5 ml resulting solution to 50 ml with diluents. (50 ppm). Filter the resulting standard solution through 0.2 µm membrane filter.

**3.5 Test Solution:**

Take a single tablet and transfer to 100 ml volumetric flask with the help of 70 ml diluents. Sonicate for 30 min and diluted to 100 ml with diluents. Filter the solution and dilute 5 ml of filtrate to 50 ml with the diluent. Filter the final standard solution through 0.2 µm membrane filter. Prepare similarly for 9 more tablets.

**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 Sitagliptin in each tablet by using the following formula:

**3.7 Calculations:**

**Content of Sitagliptin per Tablet:**

$$\frac{\textbf{Sitagliptin Spl Peak Area}}{\textbf{Sitagliptin Std Peak Area}} X \frac{\textbf{Std wt}}{\textbf{Std Dil.}} X \frac{\textbf{Spl Dil.}}{\textbf{Spl wt}} X \textbf{ Average wt X Std Potency } (\%) X \frac{(\textbf{100} - \textbf{Std LOD})}{\textbf{100}} X \frac{\textbf{M.W. Sitagliptin}}{\textbf{M.W. Sitaglipitin P}}$$

## 4.0 Dissolution:

### 4.1 Dissolution (Sitagliptin):

**4.1.1 Dissolution Medium:** Water

**4.1.2 Dissolution test condition**

**Volume** : 900 ml

**Apparatus**: Basket

**RPM** : 100

**Time** : 30 minutes

**4.1.3 Standard Solution:**

Weigh accurately about 36.885 mg of Sitagliptin Phosphate Monohydrate RS eq. to 27.75 mg Sitagliptin and dissolve in water to produce 50 ml. Dilute 5 ml to 50 ml with water. (55.5 ppm)

**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 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.

**4.1.5 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:**

$$\%\ \boldsymbol{release\ of\ Sitagliptin} = \frac{\boldsymbol{Spl\ peak\ area}}{\boldsymbol{Std\ peak\ area}} \boldsymbol{X} \frac{\boldsymbol{Std\ wt}}{\boldsymbol{Std\ Dil}} \boldsymbol{X} \frac{\boldsymbol{900}}{\boldsymbol{Label\ Claim}} \boldsymbol{X}\ \boldsymbol{Std\ Potency}\ (\%) \boldsymbol{X} \frac{\boldsymbol{100 - LOD}}{\boldsymbol{100}} \boldsymbol{X}\ \boldsymbol{100}\ \%\boldsymbol{X} \frac{\boldsymbol{M.W.Sitagliptin}}{\boldsymbol{M.W.Sitaglipitin\ P}}$$

**4.1.7 Tolerence Limit:** NLT 75 % D of the stated amount

**4.2 Dissolution (Metformin Sustained release tablet):**

**4.2.1 Dissolution Medium:** 1000 ml phosphate buffer pH 6.8

27.22 g of monobasic potassium phosphate in 1000 ml water. Take 250 ml of the solution and add 112 ml of 0.2 M sodium hydroxide solution, then dilute to 1000 ml with water. Adjust the pH to 6.8.

**4.2.2 Dissolution test condition**

**Volume:** 1000 ml

**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:**

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

**4.2.7 Tolerence Limit:** 1 hr (25 % to 50 % of the stated amount)
3rd hr (45% to 70 % of the stated amount)
8th hr (NLT 80 % of the stated amount)