DEPARTMENT OF DRUG ADMINISTRATION
National Medicines Laboratory
ANALYTICAL METHOD VALIDATION COMMITTEE

Metformin Sustained Release & Sitagliptin Tablets

Analytical Profile No: Met Sit 073/074/AP 016

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

1. Identification:

1.1. Metformin HCl: In the assay, the principle peak in the chromatogram obtained with the test solution should correspond to the peak in the chromatogram obtained with the reference solution of Metformin HCl.

1.2. Sitagliptin: In the assay, the principle peak in the chromatogram obtained with the test solution should correspond to the peak in the chromatogram obtained with the reference solution of Sitagliptin.

Tests:

2. Dissolution:

2.1 Metformin: *Determine by UV Spectroscopy*

2.1.1 Dissolution Parameters:

Apparatus:	Basket
Medium:	1000 ml phosphate buffer pH 6.8
Speed and Time:	100 rpm & 1 hour, 3 hours and 10 hours
Temperature:	37±0.5℃

Withdraw a suitable volume of the medium and filter through 0.2 µm membrane filter.

2.1.2 Test Solution: Use the filtrate, dilute if necessary with dissolution medium to obtain a solution having similar concentration to that of reference solution.

2.1.3 Reference Solution: Weigh accurately about 25 mg metformin hydrochloride RS 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.

2.1.4 Procedure: Measure the absorbance of the standard and sample solution at about 232 nm. Calculate the percentage release of metformin hydrochloride in each tablet.

2.1.5 Limit:

Not less than 25 % to 50 % of the stated amount in 1st hour

Not less than 45% to 70 % of the stated amount in 3rd hour

Not less than 80 % of the stated amount in 10th hour

2.2 Sitagliptin: *Determine by liquid chromatography*

2.2.1 Dissolution Parameters:

Apparatus: Basket

Medium: 900 ml water

Speed and Time: 100 rpm & 30 minutes

Temperature: 37 ± 0.5 °C

Withdraw a suitable volume of the medium and filter through 0.2 µm membrane filter.

2.2.2 Test Solution: Use the filtrate.

2.2.3 Reference Solution: Weigh accurately about 36.885 mg of Sitagliptin Phosphate Monohydrate reference standard eq. to 27.75 mg Sitagliptin and dissolve in water to produce 50 ml. Dilute 5 ml to 50 ml with water. Filter the resulting solution through 0.2 µm membrane filter paper. (55.5 ppm)

2.2.4 Chromatographic System: Proceed as directed under the Assay.

2.2.5 Procedure: Using chromatographic parameter and system suitability parameter as that of Assay inject replicate standard solution. Inject test solutions. Measure the peak responses.

Calculate the content of Sitagliptin per tablet.

2.2.6 Limit: Not less than 75 % (D) of the stated amount.

3. Uniformity of Content (Sitagliptin): *Determine by liquid chromatography*

3.1 Test Solution: Weigh individually 10 tablets and transfer individually into ten 100 ml volumetric flask. Add about 75 ml of diluent and sonicate for 30 minutes with intermittent shaking. Allow the sample to cool to room temperature and make up the volume to 100 ml with diluent. Filter the solution and dilute 5 ml of filtrate to 50 ml with the diluent. Filter the final solution through 0.2 µm membrane filter.

3.2 Reference Solution: Weigh accurately about 33 mg of Sitagliptin Phosphate Monohydrate reference standard eq. to 25 mg Sitagliptin in 50 ml volumetric flask and add 35 ml diluents. Dissolve by sonication and dilute to 50 ml with diluents. Dilute 5 ml resulting solution to 50 ml with diluents. (50 ppm). Filter the resulting solution through 0.2 µm membrane filter.

3.3 Chromatographic Condition: Proceed as directed under Assay

3.4 Procedure: Using chromatographic parameter and system suitability parameter as that of Assay inject replicate standard solution. Inject test solutions. Measure the peak responses.

Calculate the content of Sitagliptin in each tablet.

3.5 Limit: 85-115 % of the stated amount of Sitagliptin.

4. Assay: *Determine by liquid chromatography*

4.1 Test Solution: Weigh individually 20 tablets & crush them into fine powder. Weigh accurately the powder eq. to 50 mg of Metformin HCl and transfer it into 100 ml flask, add 70 ml of diluents, dissolve with the aid of sonicator for 15 minutes to dissolve. Cool to room temperature and make up the volume to 100 ml with diluents and stir for 20 minutes. Filter through filter paper and dilute 5 ml of filtrate to 25 ml with diluents. Filter the final solution through 0.2 µm membrane filter.

4.2 Reference Solution:

4.2.1 Metformin Hydrochloride Reference Solution: Weigh accurately about 25 mg of Metformin hydrochloride reference standard and transfer into 50 ml volumetric flask. Add about 35 ml of the diluent and dissolve with the aid of sonicator for 10 minutes and make up the volume to 50 ml with diluent.

4.2.2 Sitagliptin Reference Solution: Weigh accurately about 30 mg equivalent of Sitagliptin from Sitagliptin Phosphate reference 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.

4.2.3 Combined Reference Solution: Pipette 5 ml of Metformin HCl reference solution & Sitagliptin reference solution into 25 ml volumetric flask and make up the volume to 25 ml with diluent. Filter the final solution through 0.2 µm membrane filter.

4.3 Chromatographic system

Column:	C 18 (4.6 X 250 cm), 5 µm
Flow rate:	1.0 ml/min
Wave length:	205 nm
Injection volume:	20 µl
Column temperature:	30 ºC
Detector:	UV Detector

Mobile Phase: Buffer: Acetonitrile (75: 25)

Buffer: Weigh 1.36 g 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.

Diluent: 5 volume of Acetonitrile: 95 volume of 0.1% v/v orthophosphoric acid in water.

4.4 Procedure: Inject the reference solution. The test is not valid unless the resolution between Metformin and Sitagliptin is not less than 2, the column efficiency is not less than 2000 theoretical plates and tailing factor is not more than 2.0 and the relative standard deviation for replicate injections in not more than 2.0%. Inject the test solution. Measure the peak responses.

Calculate the content of Metformin hydrochloride and Sitagliptin in the tablets.

5. Other tests: As per pharmacopoeial requirement.