Government of Nepal
Ministry of Health and Population
Department of Drug Administration
National Medicines Laboratory
Quality and Method Validation Section

Analytical profile of Azelastine Hydrochloride and Fluticasone Propionate Nasal Spray

Analytical Profile No.: Aze Fluti 080/81/AP 136

Azelastine Hydrochloride and Fluticasone Propionate Nasal Spray contains not less than 90.0% and not more than 110.0% of the stated amount of Azelastine Hydrochloride and Fluticasone Propionate.

Usual Strength: Each spray contains

Azelastine Hydrochloride 137 mcg (0.10% w/w)

Fluticasone Propionate 50 mcg (0.0365 % w/w)

1. Identification:

In the Assay, the principle peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the certified reference solution.

2. Assay: *Determine by liquid chromatography*

2.1 Preparation of Buffer solution:

Weigh about 1.15 gm. of Ammonium dihydrogen orthophosphate, dissolve and dilute to 1000 ml with water, mix and filter.

2.2 Preparation of Mobile phase:

Mix 450 volumes of methanol, 400 volumes of buffer and 150 volumes of acetonitrile and add 1 ml of triethylamine. Adjust the pH to 5.0 ± 0.2 with orthophosphoric acid and degas.

2.3 Preparation of Diluent:

Mix 350 volumes of buffer, 350 volumes of acetonitrile and 300 volumes of methanol and degas.

2.4 Preparation of diluent for S methyl Fluticasone impurity:

Mix 70 volumes of acetonitrile and 30 volumes of water and degas.

2.5 Preparation of Azelastine Hydrochloride stock solution:

Weigh accurately about 30 mg of azelastine hydrochloride working standard and transfer into a 250 ml volumetric flask.Add about 170 ml of diluent sonicate to dissolve equilibrate to room temperature and dilute to volume with diluent and mix.

2.6 Preparation of Fluticasone propionate stock solution:

Weigh accurately about 25 mg of Fluticasone propionate working standard and transfer into a 250 ml volumetric flask. Add about 170 ml of diluent sonicate to dissolve equilibrate to room temperature and dilute to volume with diluent and mix.

2.7 Preparation of Standard solution:

Pipette out 10 ml of Azelastine Hydrochloride stock solution and 5 ml of Fluticasone propionate stock solution into a 100 ml of volumetric flask and dilute to volume with diluent and mix.

2.8 Preparation of N Oxide-A impurity stock solution:

Weigh accurately about 2.5 mg of N-oxide A and transfer into a 50 ml volumetric flask. Add about 35 ml of diluent, sonicate to dissolve, equilibrate to room temperature and dilute to volume with diluent and mix.

2.9 Preparation of S-methyl fluticasone impurity stock solution:

Weigh accurately about 5 mg of S -methyl Fluticasone and transfer into a 50 ml volumetric flask. Dissolve and dilute to volume with diluent for S- methyl fluticasone impurity and mix.

2.10 Preparation of Resolution solution:

Pipette 2ml of Azelastine Hydrochloride stock solution, 1 ml of Fluticasone propionate stock solution, 5ml of N-oxide A impurity stock solution and 1 ml of S-methyl Fluticasone impurity stock solution into a 20 ml volumetric flask and dilute to volume with diluent and mix.

2.11 Preparation of composite sample:

Shake the bottle gently by tilting it upward and downward for 5 seconds and pool the content of 10 bottles into a stoppered glass bottle.

2.12 Preparation of Sample solution:

Weigh accurately about 3.0 gm. of composite sample (Equivalent to 3 mg of Azelastine Hydrochloride and 1.095 mg of Fluticasone Propionate) into a 250 ml volumetric flask. Add about 170 ml of diluent; sonicate for 15-20 minutes, equilibrate to room temperature and dilute to volume with diluent and mix.Filter through 0.45µm syringe filter.

2.13 Chromatographic conditions:

Column: C18, 15 cm*4.6 mm, 5µm column or equivalent.

Detector: UV

Detector wavelength: 239 nm for Fluticasone Propionate and **290 nm** for Azelastine

Flow rate: 1.2 ml per minute

Injection volume: 50 µL

Column temperature: 45 °C

Blank: Diluent

2.14 Procedure: Separately inject equal volumes of Blank, Resolution solution, Standard solution and sample solution and record the peak area responses for the principal peaks and check for the system suitability requirements.

2.15 System suitability requirements:

The test is not valid unless:

1. The resolution between the peaks corresponding to Azelastine and N-oxide A obtained in the chromatograms of resolution solution is not less than 1.5
2. The resolution between the peaks corresponding to Fluticasone propionate and S- methyl Fluticasone obtained in the chromatograms of resolution solution is not less than 1.5.
3. The % Relative standard deviation for the peak area response of Azelastine and Fluticasone propionate for injection of standard solution is not more than 2.0%.

3. **Other tests:** As per Pharmacopoeial Requirement