Edition: BP 2025 (Ph. Eur. 11.6 update)

Nitrazepam Oral Suspension

General Notices

Action and use

Benzodiazepine.

DEFINITION

Nitrazepam Oral Suspension is a suspension of Nitrazepam in a suitable flavoured vehicle.

The oral suspension complies with the requirements stated under Oral Liquids and with the following requirements.

Content of nitrazepam, $C_{15}H_{11}N_3O_3$

90.0 to 105.0% of the stated amount.

IDENTIFICATION

A. Carry out the method for *thin-layer chromatography*, Appendix III A, using the following solutions.
(1) Shake a quantity of the oral suspension containing 2.5 mg of Nitrazepam with 10 mL of *acetonitrile*, centrifuge and use the supernatant liquid.
(2) 0.025% w/v of *nitrazepam BPCRS in methanol*.
(3) 0.025% w/v each of *nitrazepam BPCRS* and *diazepam BPCRS* in *methanol*.

CHROMATOGRAPHIC CONDITIONS

(a) Use as the coating *silica gel*.
(b) Use the mobile phase as described below.
(c) Apply 10 µL of each solution.
(d) Develop the plate to 15 cm.
(e) After removal of the plate, dry in air, spray with *dilute potassium iodobismuthate solution* and examine in daylight.

MOBILE PHASE

5 volumes of *concentrated ammonia*, 10 volumes of *methanol* and 85 volumes of *ethyl acetate*.

SYSTEM SUITABILITY

The test is not valid unless the chromatogram obtained with solution (3) shows two clearly separated spots.

CONFIRMATION

The principal spot in the chromatogram obtained with solution (1) corresponds in position and colour to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) shows a peak with the same retention time as the principal peak in the chromatogram obtained with solution (2).

TESTS

Carry out the following tests protected from light.

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Shake a quantity of the oral suspension containing 5 mg of Nitrazepam with 60 mL of *acetonitrile*, add sufficient *acetonitrile* to produce 100 mL and filter.
(2) Dilute 1 volume of solution (1) to 100 volumes with *acetonitrile*.
(3) 0.01% w/v of *nitrazepam BPCRS* and 0.0002% w/v of *clonazepam BPCRS* in *acetonitrile*.
(4) Dilute 1 volume of solution (2) to 10 volumes with *acetonitrile*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 4.0 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Licrospher RP8 is suitable) and a stainless steel guard column (4 mm × 2 mm) packed with *octylsilyl silica gel for chromatography* (5 µm) (Phenomenex C8 is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 270 nm.
(f) Inject 10 µL of each solution.

MOBILE PHASE

Mobile phase A 0.05M *sodium dihydrogen orthophosphate*, adjusted to pH 3.0 with *orthophosphoric acid*.

Mobile phase B 20 volumes of mobile phase A and 80 volumes of *acetonitrile*.

When the chromatograms are recorded under the prescribed conditions the retention times relative to nitrazepam (retention time about 9 minutes) are: impurity A, about 1.3 and impurity B, about 1.6.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0→3	55	45	isocratic
3→10	55→37	45→63	linear gradient
10→20	37	63	isocratic
20→22	37→55	63→45	linear gradient
22→30	55	45	re-equilibration

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the peaks due to nitrazepam and clonazepam is at least 1.3.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity A is not greater than 1.8 times the area of the principal peak in the chromatogram obtained with solution (2) (1.8%);

the area of any peak corresponding to impurity B is not greater than 3.2 times the area of the principal peak in the chromatogram obtained with solution (2) (3.2%);

the area of any other *secondary peak* is not greater than half of the area of the principal peak in the chromatogram obtained with solution (2) (0.5%);

the sum of the areas of all *secondary peaks* is not greater than 6 times the area of the principal peak in the chromatogram obtained with solution (2) (6%).

Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (4) (0.1%).

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Add a weighed quantity of the oral suspension containing 12.5 mg of Nitrazepam to 25 mL of *water*, extract with three 50-mL quantities of *chloroform*, filtering each extract through phase-separating paper and add sufficient *chloroform* to produce 200 mL.
(2) 0.00625% w/v of *nitrazepam BPCRS* in *chloroform*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (20 cm × 4.6 mm) packed with *silica gel for chromatography* (5 µm) (Lichrosorb or Partisil is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 2 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 254 nm.
(f) Inject 20 µL of each solution.

MOBILE PHASE

10 volumes of *absolute ethanol* and 90 volumes of *n-hexane*.

Under the prescribed conditions the retention time of the peak due to nitrazepam is about 8 minutes.

DETERMINATION OF CONTENT

Determine the *weight per mL* of the oral suspension, Appendix V G, and calculate the content of $C_{15}H_{11}N_3O_3$, weight in volume, using the declared content of $C_{15}H_{11}N_3O_3$ in *nitrazepam BPCRS*.

STORAGE

Nitrazepam Oral Suspension should be protected from light.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Nitrazepam.