

## Ampicillin capsules (Ampicillini capsulae)

**Category.** Antibacterial drug.

**Storage.** Ampicillin capsules should be kept in a tightly closed container and stored at a temperature not exceeding 25 °C.

**Labelling.** The label should state whether the active ingredient is in the anhydrous form or is the trihydrate and the quantity should be indicated in terms of the equivalent amount of ampicillin. Expiry date.

Requirements

Comply with the monograph for [Capsules](#).

Ampicillin capsules contain not less than 90.0% and not more than 110.0% of the amount of  $C_{16}H_{19}N_3O_4S$  stated on the label.

### Identity tests

-Either tests A and B or tests B and C may be applied.

A. Carry out the test as described under [1.14.1 Thin-layer chromatography](#), using silica gel R1 as the coating substance and a mixture of 65 volumes of acetone R, 10 volumes of water, 10 volumes of toluene R and 2.5 volumes of glacial acetic acid R as the mobile phase. Apply separately to the plate 2 µl of each of the following two solutions. For solution (A) shake a quantity of the contents of the capsules equivalent to 50 mg of Ampicillin with 10 mL of a mixture of 4 volumes of acetone R and 1 volume of hydrochloric acid (0.1 mol/l) TS, filter and use the clear filtrate. For solution (B) dissolve 25 mg of ampicillin RS in 5 mL of the same mixture of solvents. After removing the plate from the chromatographic chamber allow it to dry in air, spray lightly with triketohydrindene/ethanol TS, dry at 90 °C for 15 minutes and examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

B. Shake a quantity of the contents of the capsules equivalent to 10 mg of Ampicillin with 3 mL of water and filter. To the filtrate add 0.1 g of hydroxylamine hydrochloride R and about 0.4 mL of sodium hydroxide (~80 g/l) TS and allow to stand for 5 minutes. Add 1.3 mL of hydrochloric acid (~70 g/l) TS and 0.5 mL of ferric chloride (25 g/l) TS; a violet-red to violet-brown colour is produced.

C. Shake a quantity of the contents of the capsules equivalent to 0.5 g of Ampicillin with 5 mL of water for 5 minutes, filter, wash the residue with ethanol (~50 g/l) TS and dry it under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 1 hour. Place 2 mg of the residue in a test-tube, add 1 drop of water followed by 2 mL of sulfuric acid (~1760 g/l) TS and mix; the solution is colourless. Immerse the test-tube for 1 minute in a water-bath; the solution remains colourless. Place 2 mg in a second test-tube, add 1 drop of water and 2 mL of formaldehyde/sulfuric acid TS and mix; the solution is colourless to slightly pink. Immerse the test-tube for 1 minute in a water-bath; an orange-yellow colour is produced.

### Loss on drying

Dry a quantity of the contents of the capsules equivalent to 0.1 g of the active ingredient at 60 °C under reduced pressure (not exceeding 0.6 kPa or 5 mm of mercury) for 3 hours. For capsules containing anhydrous Ampicillin the loss is not more than 40 mg/g, and for capsules containing Ampicillin trihydrate the loss is not less than 100 mg/g and not more than 150 mg/g of the initial quantity taken.

### Assay

To a quantity of the mixed contents of 20 capsules equivalent to about 0.12 g of Ampicillin, accurately weighed, add 400 mL of water and shake for 30 minutes. Dilute to 500 mL with water and filter. Transfer 10 mL of the filtrate to a 100 mL volumetric flask, add 10 mL of buffer borate, pH 9.0, TS and 1 mL of acetic anhydride/dioxan TS, allow to stand for 5 minutes at room temperature and dilute to volume with water. Transfer two 2 mL aliquots of this solution into separate stoppered tubes. To one tube add 10 mL of imidazole/mercuric chloride TS, mix, stopper the tube and place in a water-bath at 60 °C for exactly 25 minutes. Cool the tube rapidly to 20 °C (*solution A*). To the second tube add 10 mL of water and mix (*solution B*).

Without delay measure the absorbance of a 1 cm layer at the maximum at about 325 nm against a solvent cell containing a mixture of 2 mL of water and 10 mL of imidazole/mercuric chloride TS for *solution A* and water for *solution B*.

From the difference between the absorbance of *solution A* and that of *solution B* calculate the amount of  $C_{16}H_{19}N_3O_4S$  in the substance being examined by comparison with ampicillin RS. In an adequately calibrated spectrophotometer the absorbance of the *reference solution* should be  $0.29 \pm 0.02$ .