Amoxicillin and clavulanic acid tablets (Amoxicillini et acidi clavulanici compressi)

2018-01

Category. Antibacterial, β-Lactamase inhibitor.

Storage. Amoxicillin and clavulanic acid tablets should be kept in a tightly closed container and protected from light.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 500 mg amoxicillin (as trihydrate) and 125 mg clavulanic acid (as potassium salt). Strength in the current EML for Children: 500 mg amoxicillin (as trihydrate) and 125 mg clavulanic acid (as potassium salt).

Labelling. The designation on the container should state that the active ingredients are amoxicillin trihydrate and clavulanate potassium and that the quantities should be indicated in terms of equivalent amounts of amoxicillin and clavulanic acid.

Requirements

Comply with the monograph for *Tablets*.

Definition. Amoxicillin and clavulanic acid tablets contain amoxicillin trihydrate and clavulanate potassium. They contain not less than 90.0% and not more than 120.0% of the amounts of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanic acid ($C_8H_9NO_5$) stated on the label.

Identity test

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions given under “Assay”. The retention times of the two principal peaks in the chromatogram obtained with solution (1) correspond to the retention times of the peaks due to amoxicillin and clavulanic acid in the chromatogram obtained with solution (2).

Water. Determine as described under *2.8 Determination of water by the Karl Fischer method*, Method A, using a quantity of the powdered tablets; the water content is not more than 100 mg/g. The limit is applicable for tablets containing 500 mg amoxicillin (as trihydrate).

Dissolution. Carry out the test as described under *5.5 Dissolution test for solid oral dosage forms* using as the dissolution medium 900 mL of water R and rotating the paddle at 75 revolutions per minute. At 30 minutes withdraw a sample of 10 mL of the medium through an in-line filter and use the filtrate, dilute with water if necessary, to obtain a solution containing the equivalent of about 0.25 mg of amoxicillin per mL (solution (1)). For solution (2) dissolve a suitable amount of amoxicillin trihydrate RS and lithium clavulanate RS in a suitable volume of water R to obtain a solution containing about 0.25 mg of amoxicillin and about 0.0625 mg of clavulanic acid per mL.

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using the conditions as described under “Assay”.

For each of the tablets tested, calculate the total amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) and clavulanic acid ($C_8H_9NO_5$) in the medium using the declared content of amoxicillin ($C_{16}H_{19}N_3O_5S$) in amoxicillin trihydrate RS and the declared content of clavulanic acid ($C_8H_9NO_5$) in lithium clavulanate RS.

The amount of amoxicillin in solution for each tablet is not less than 85% (Q) of the amount declared on the label and the amount of clavulanic acid is not less than 80% (Q) of the amount declared on the label.

Clavulanate polymer and other fluorescent impurities. Carry out the test as described under *1.9 Fluorescence spectrophotometry*.

Prepare the following buffer solution. Dissolve 15.6 g of sodium dihydrogen phosphate R in 800 mL of water R, adjust the pH to 5.0 using sodium hydroxide (~40 g/L) TS and add sufficient water R to produce 1000 mL.

Prepare the following solutions freshly. For solution (1) add to a quantity of the powdered tablets, containing the equivalent of 0.1 g of clavulanic acid, 50 mL of the buffer solution. Stir the sample until it is evenly dispersed and add sufficient buffer solution to produce 100.0 mL. Shake the solution vigorously for 1 minute, shake mechanically for 5 minutes, sonicate for 5 minutes and filter. For solution (2) prepare a solution containing 0.42 µg per mL of quinine sulfate R in sulfuric acid (~50 g/L) TS.

Measure the fluorescence of the solutions (1) and (2) with an excitation wavelength of 360 nm and an emission wavelength of 440 nm, using the phosphate buffer solution in the reference cell. The fluorescence obtained with solution (1) is not more intense than that obtained with solution (2) (5% w/w, calculated with respect to the content of clavulanic acid). [*Note*: The fluorescence of quinine sulfate is 118 times more intense than that of an equivalent concentration of clavulanate polymer.].

Related substances. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm×4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

Prepare the following buffer solution, pH 5.0: Adjust the pH of 250 mL of potassium dihydrogen phosphate (27.2 g/L) TS to 5.0

with sodium hydroxide (~80 g/L) TS and dilute to 1000 mL with water R.

Use the following conditions for gradient elution:

mobile phase A: mix 10 volumes of acetonitrile R with 990 volumes of buffer solution,

mobile phase B: mix 200 volumes of acetonitrile R with 800 volumes of the buffer solution.

Time(min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	comments
0 =tr	92	8	Isocratic
tr=(tr + 25)	92 to 0	8 to 100	Linear gradient
(tr + 25) =(tr + 40)	0	100	Isocratic
(tr + 40)= (tr + 41)	92	8	Return to initial composition
(tr + 41) =(tr + 55)	92	8	Re-equilibration

tr = retention time of amoxicillin determined with solution (1).

Prepare the following solutions immediately before use. For solution (1) transfer a quantity of the powdered tablets containing the equivalent of about 30 mg of amoxicillin into a 20 mL volumetric flask, add 15 mL of mobile phase A and sonicate for 20 minutes, with occasional shaking. Allow to cool to room temperature, make up to volume with mobile phase A and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes with mobile phase A. For solution (3) use a solution containing 4 µg of cefadroxil R and 30 µg of amoxicillin trihydrate RS per mL of mobile phase A. For solution (4) use a solution containing 0.75 mg of lithium clavulanate RS per mL of mobile phase A. For solution (5) add 1.0 mL of water R to 0.20 g of amoxicillin trihydrate R. Shake and add dropwise sodium hydroxide (~80 g/L) TS to obtain a solution. The pH of the solution is about 8.5. Store the solution at room temperature for 4 h. Dilute 0.5 mL of this solution to 50.0 mL with mobile phase A.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 254 nm.

Inject 50 µL of solution (3) with isocratic elution at the initial mobile phase composition. The test is not valid unless the resolution factor between the peaks due to amoxicillin and cefadroxil is at least 2.0.

Inject alternately 50 µL each of solution (4) and (5). Use the chromatogram obtained with solution (4) to identify the peak corresponding to clavulanic acid. Use the chromatogram obtained with solution (5) to identify the peaks corresponding to amoxicillin dimer (impurity J; n = 1) and amoxicillin trimer (impurity J; n = 2). The following impurities and substances are eluted at the relative retentions with reference to amoxicillin (retention time about 10 minutes): clavulanic acid about 0.3; amoxicillin dimer (impurity J; n = 1) about 4.1; amoxicillin trimer (impurity J; n = 2) about 4.5.

Inject alternately 50 µL of each of solution (1) and (2).

In the chromatogram obtained with solution (1):

- -the area of any peak corresponding to amoxicillin dimer (impurity J; n = 1) is not greater than twice the area of the principal peak in the chromatogram obtained with solution (2) (2%);
- -the area of any other impurity peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1%).

Assay. Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (5 µm).

As the mobile phase use a mixture of 5 volumes of methanol R and 95 volumes of sodium dihydrogen phosphate (~7.8 g/L) TS, adjusted to pH 4.4 with phosphoric acid (~1440 g/L) TS.

Operate with a flow rate of 2.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 220 nm.

Prepare the following solutions. For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets containing the equivalent of about 0.25 g of amoxicillin, accurately weighed, into a 500 mL volumetric flask, add 400 mL of water R and shake for 10 minutes. Make up to volume with water R and filter. For solution (2) use 0.5 mg of amoxicillin trihydrate RS and 0.2 mg of lithium clavulanate RS per ml of water R.

Inject alternately 20 µL of solution (1) and (2). The assay is not valid unless the resolution factor between the peaks due to amoxicillin and clavulanic acid is at least 3.5.

Measure the areas of the peak responding to amoxicillin and clavulanic acid and calculate the content of amoxicillin ($C_{16}H_{19}N_3O_5$

S) and clavulanic acid ($C_8H_9NO_5$) in the tablets using the declared content of amoxicillin ($C_{16}H_{19}N_3O_5S$) in amoxicillin trihydrate RS and the declared content of clavulanic acid ($C_8H_9NO_5$) in lithium clavulanate RS.

Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph on Amoxicillin trihydrate.