Revision of the monograph on Amoxicillin trihydrate

(AMOXICILLINUM TRIHYDRICUM)

Draft proposal for The International Pharmacopoeia

(February 2018)

DRAFT FOR COMMENT

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/16.680:

AMOXICILLIN TRIHYDRATE

(AMOXICILLINUM TRIHYDRICUM)

| Revision drafted based on provisions found in other pharmacopoeias and laboratory investigations | July 2016 to September 2017 |
| Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations | October 2016 |
| Discussion at informal consultation on quality control laboratory tools and specifications for medicines | 2–4 May 2017 |
| Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations | October 2017 |
| Draft revision sent out for public consultation | February to April 2018 |
| Further follow-up action as required | |
AMOXICILLIN TRIHYDRATE
(AMOXICILLINUM TRIHYDRICUM)

[Note from the Secretariat. It is proposed to revise the monograph on Amoxicillin trihydrate of The International Pharmacopoeia. The revision is based on laboratory investigations and on information found in the European Pharmacopoeia and the United States Pharmacopoeia. Comments are in particular sought regarding the nature of the impurities listed on the transparency list, i.e. whether they are synthesis-related impurities, degradation products or both. Changes for the current monograph are indicated in the text by insert or delete.]

C₁₆H₁₉N₃O₅S.3H₂O

Relative molecular mass. 419.5

Chemical name. (2S,5R,6R)-6-[[2(R)]-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; (−)-6-[2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; (2S,5R,6R)-6-[(R)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; 6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; CAS Reg. No. 61336-70-7.

Description. A white or almost white, crystalline powder; odourless.

Solubility. Slightly soluble in water and methanol R; very slightly soluble in ethanol (~750 g/L) TS, ether R, and practically insoluble in fatty oils; soluble in dilute acids and dilute solutions of alkali hydroxides.

Category. Antibacterial drug.

Storage. Amoxicillin trihydrate should be kept in a tightly closed container.

Additional information. Amoxicillin trihydrate is a semi-synthetic product derived from a fermentation product.

Requirements

Definition. Amoxicillin trihydrate contains not less than 95.0% and not more than the equivalent 102.0% of C₁₆H₁₉N₃O₅S, calculated with reference to the anhydrous substance.
Manufacture. The method of production is validated to demonstrate that the substance, if tested, would comply with a limit of not more than 20 µg/g of \(N,N\)-dimethylaniline using a suitable method.

Identity tests

• Either test A alone or tests B and C or test C and D may be applied.

A. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region.
   The infrared absorption spectrum is concordant with the spectrum obtained from amoxicillin trihydrate RS or with the reference spectrum of amoxicillin trihydrate.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silanized silica gel R3 as the coating substance and a mixture of 9 volumes of a solution containing 15.4 g of ammonium acetate R in 100 mL, the pH of which has been adjusted to 5.0 with glacial acetic acid R, and 1 volume of acetone R as the mobile phase. Apply separately to the plate 1 µL of each of 3 solutions in sodium hydrogen carbonate (40 g/L) TS containing (A) 2.5 mg of the test substance per mL, (B) 2.5 mg of amoxicillin trihydrate RS per mL and (C) a mixture of 2.5 mg of amoxicillin trihydrate RS and 2.5 mg of ampicillin trihydrate RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air until the solvents have evaporated. Expose the plate to iodine vapours until the spots appear 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). The test is valid only if the chromatogram obtained with solution (C) shows two clearly separated spots.

C. Place about 2 mg of the test substance in a test-tube (150 mm × 15 mm), moisten with 1 drop of water and add about 2 mL of sulfuric acid (~1760 g/L) TS. Mix the contents of the tube by swirling; the solution remains practically colourless. Place the tube in a water-bath for 1 minute; a dark yellow colour develops.

D Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”. The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

Specific optical rotation (1.4). Use a 2.0 mg/mL of the test substance solution in carbon-dioxide-free water R and calculate with reference to the anhydrous substance; \([\alpha]_D^0 = +290\) to +315.

Solution in hydrochloric acid and ammonia. Prepare a solution of 1.0 g in 10 mL of hydrochloric acid (0.5 mol/l) VS. Prepare a second solution of 1.0 g in 10 mL of ammonia (~100 g/L) TS. Examine both solutions immediately.

Neither of these solutions are more opalescent than opalescence standard TS3.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to method A; not more than 20 µg/g.

Sulfated ash (2.3). Not more than 10 mg/g.
**Water.** Determine as described under 2.8 *Determination of water by the Karl Fischer method*, Method A, using about 0.1 g of the test substance; the water content is not less than 0.115 g/g and not more than 0.145 g/g.

**pH value (1.13).** pH of a 2mg/mL solution in carbon-dioxide-free water R, 3.5–5.5.

**Related substances.** Carry out the test as described under 1.14.4 *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 (5μm). Prepare the following pH 5 buffer solution to be used in the mobile phases: to 250 mL of potassium dihydrogen phosphate (27.2 g/l) TS add sodium hydroxide (~80 g/l) TS until a pH of 5.0 is reached, and dilute the solution with sufficient water to produce 1000 mL. As mobile phase A use a mixture of 99 volumes of buffer solution pH 5.0 and 1 volume of acetonitrile R. As mobile phase B use a mixture of 8 volumes of buffer solution pH 5.0 and 2 volumes of acetonitrile R.

Prepare the following solutions in mobile phase A: solution (A) 1.5mg of Amoxicillin trihydrate per mL; solution (B) 0.015 mg of amoxicillin trihydrate RS per mL; and solution (C) 0.15 μg of amoxicillin trihydrate RS per mL.

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

Using a 50μl loop injector, inject solution B. Start the elution isocratically with the mobile phase mixture used for the equilibration. Immediately after elution of the amoxicillin peak start a linear gradient elution to reach a ratio of mobile phase A: B of 0:100 over a period of 25 minutes. Adjust the sensitivity of the system so that the height of the principal peak is at least 50% of the full scale of the recorder. Continue the chromatography with mobile phase B for 15 minutes, then equilibrate the column for 15 minutes with the mobile phase originally used for the equilibration. The mass distribution ratio for the first peak (amoxicillin) is 1.3–2.5. Inject mobile phase A using the 50μl loop injector and use the same elution gradient to obtain a blank. Inject solution C using the 50μl loop injector. Adjust the system to obtain a peak with a signal-to-noise ratio of at least 3.

Using the 50μl loop injector, inject solution A. Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak and any peak obtained in the blank chromatogram, is not greater than that of the principal peak obtained with solution B (1%).

Carry out the test as described under 1.14.4 *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 octadecysilyl groups (5μm).

Prepare the following buffer solution pH 5.0. Adjust the pH of 250 mL of potassium dihydrogen phosphate (27.2 g/L) TS with sodium hydroxide (~80 g/L) TS to 5.0 and dilute to 1000 mL with water R.

Use the following conditions for gradient elution:

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1 Agilent® Xbridge C18 column (4.6×250 mm, 5 μm) was found suitable.
mobile phase A: 1 volumes of acetonitrile R and 99 volumes of buffer solution pH 5.0;

mobile phase B: 20 volumes of acetonitrile R and 80 volumes of buffer solution pH 5.0.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_R$</td>
<td>92</td>
<td>8</td>
<td>Isocratic</td>
</tr>
<tr>
<td>$t_R$–$(t_R+25)$</td>
<td>92 to 0</td>
<td>8 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>$(t_R+25)$–$t_R$–$(t_R+40)$</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>$(t_R+40)$–$t_R$–$(t_R+55)$</td>
<td>0 to 92</td>
<td>100 to 8</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>$(t_R+55)$–$t_R$–$(t_R+70)$</td>
<td>92</td>
<td>8</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

$t_R$ = retention time of amoxicillin determined with solution (2)

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.

Prepare the following solutions immediately before use in mobile phase A. For solution (1) dissolve about 30 mg of the test substance and dilute to 20.0 mL. For solution (2) dilute 2.0 mL of solution (1) to 20.0 mL. Dilute 2.0 mL of this solution to 20.0 mL. For solution (3) dissolve 4.0 mg of cefadroxil R and dilute to 50.0 mL. To 5.0 mL of this solution add 2.0 mL of solution (1) and dilute to 100.0 mL.

Inject 50 μL of solution (3). The test assay is not valid unless in the chromatogram the resolution between the peaks due to amoxicillin and cefadroxil is at least 2.0.

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

In the chromatogram obtained with solution (1):
- the area of any impurity peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (1.0%).

Assay. Dissolve about 0.06 g, accurately weighed, in sufficient water to produce 500 mL.

Simultaneously, prepare a reference solution containing 0.06 g of amoxicillin trihydrate RS. Transfer 10.0 mL of one solution to a 100-mL volumetric flask and 10.0 mL of the other solution to a second 100-mL volumetric flask. To each add 10 mL of buffer borate, pH 9.0, TS, and 1 mL of acetic anhydride/dioxan TS, mix, allow to stand for 5 minutes at room temperature, and dilute to volume with water. Transfer two 2.0 mL aliquots of each solution to separate stoppered test tubes. To one tube containing the test solution, and to the other, containing the reference solution, add 10 mL of imidazole/mercuric chloride TS, mix, stopper the tubes, and place them in a water bath at 60°C for
exactly 25 minutes. Cool the tubes rapidly to 20 °C (solution A). To the remaining tubes add 10 mL of water and mix (solution B). Without delay, measure the absorbances of a 1 cm layer at the maximum at about 325 nm of both solutions A, using as a blank a mixture of 2.0 mL of water and 10 mL of imidazole/mercuric chloride TS placed in the solvent cell. For solutions B use water as a blank placed in the solvent cell.

From the difference between the absorbances of solutions A and solutions B, calculate the percentage content of C_{16}H_{19}N_{3}O_{5}S by comparison with amoxicillin trihydrate RS, with reference to the anhydrous substance.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given below under “Related substances”, with the following modifications:

Use as the mobile phase for isocratic elution the initial composition of the mixture of mobile phases A and B, adjusted where applicable.

Prepare the following solutions immediately before use in mobile phase. For solution (1) dissolve about 30 mg of the test substance, accurately weighed, and dilute to 50.0 mL. For solution (2) dissolve 30.0 mg of amoxicillin trihydrate RS and dilute to 50.0 mL.

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

Measure the areas of the peaks corresponding to amoxicillin obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of amoxicillin (C_{16}H_{19}N_{3}O_{5}S), using the declared content of amoxicillin (C_{16}H_{19}N_{3}O_{5}S) in amoxicillin trihydrate RS.

**Impurities**

![Chemical Structure](image)

A. (2S,5R,6R)-6-αmino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-aminopenicillanic acid).

![Chemical Structure](image)

B. (2S,5R,6R)-6-[(2S)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (1-amoxicillin),
C. (4S)-2-{5-(4-hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid (amoxicillin diketopiperazines).

D. (4S)-2-[[2R]-2-amino-2-(4-hydroxyphenyl)acetyl]amino[methyl]]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin).

E. (2R,4S)-2-[[2R]-2-amino-2-(4-hydroxyphenyl)acetyl]amino[methyl]]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin).

F. 3-(4-hydroxyphenyl)pyrazin-2-ol.

G. (2S,5R,6R)-6-[[2R]-2-[[2R]-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (d-(4-hydroxyphenyl)glycylamoxicillin).
H.(2R)-2-[(2,2-dimethylpropanoyl)amino]-2-(4-hydroxyphenyl)acetic acid.

I.(2R)-2-amino-2-(4-hydroxyphenyl)acetic acid.

J.co-oligomers of amoxicillin and of penicilloic acids of amoxicillin.

K. oligomers of penicilloic acids of amoxicillin.
L-(2S,5R,6R)-6-[(2S,5R,6R)-6-[[2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (6-APA amoxicillin amide).

Reagents to be added or to be revised:

Silica gel R3
Silica gel H.

Description. A white, homogeneous powder.

Particle size. 10–40 μm.

It may or may not be silanized.