Consultation documents

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The International Pharmacopoeia

Moxifloxacin hydrochloride
(Moxifloxacini hydrochloridum)

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/16.651, January 2018). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. The proposed monograph is based on information found in Ph.Eur. 8.0, Pharmeuropa 29.3, USP 39 and the Indian Pharmacopoeia 2014, in the scientific literature, submitted by pharmaceutical manufacturers, and on laboratory investigations performed by a WHO Collaborating Centre and a collaborating laboratory. The monograph is proposed for inclusion in The International Pharmacopoeia.]

Molecular formula. $C_{21}H_{25}ClFN_3O_4 \cdot H_2O$

Relative molecular mass. 455.9.

Graphic formula

Chemical name. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride monohydrate; CAS Reg. No. 192927-63-2 (monohydrate).

Description. A light yellow or yellow powder or crystals.

Solubility. Sparingly soluble in water R, slightly soluble in ethanol (~760 g/L) TS, practically insoluble in acetone R.
Category. Antibacterial, antituberculosis.

Storage. Moxifloxacin hydrochloride should be kept in tightly closed containers, protected from light.

Labelling. The designation on the container of Moxifloxacin hydrochloride should state the substance is in the form of the monohydrate.

Additional information. Moxifloxacin hydrochloride may exhibit polymorphism.

Requirements

Definition. Moxifloxacin hydrochloride contains not less than 98.0% and not more than 102.0% (“Assay”, method A) or not less than 99.0% and not more than 101.0% (“Assay”, method B) of \( \text{C}_{21}\text{H}_{25}\text{ClFN}_{3}\text{O}_{4} \), calculated with reference to the anhydrous substance.

Manufacture. The production method is validated to demonstrate the satisfactory enantiomeric purity of the final product.

Identity tests

- Either tests A, C and D or tests B, 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 moxifloxacin hydrochloride RS or the reference spectrum of moxifloxacin hydrochloride.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and moxifloxacin hydrochloride RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from moxifloxacin hydrochloride RS.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 4 volumes of 1-butanol R, 4 volumes of methanol R and 2 volumes of ammonia (~100 g/L) TS as the mobile phase. Apply separately to the plate 10 µL of each of the following two solutions. For solution (A) dissolve a quantity of the test substance in methanol R to give a solution containing 1 mg of the test substance per mL. Dilute a portion of the solution with methanol R to give a solution containing 0.05 mg of the test substance per mL. For solution (B) use an approximately 0.05 mg/mL solution of moxifloxacin hydrochloride RS in methanol R. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber allow it to dry in air or in a current of air. Examine the chromatogram under ultraviolet light (366 nm). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to moxifloxacin in the chromatogram obtained with solution (B).

C. Prepare a solution of 50 mg of the test substance in 5 mL of water R, add 1 mL of nitric acid (~130 g/L) TS, mix, allow to stand for 5 minutes and filter. The filtrate yields reaction A described under 2.1 General identification tests as characteristic of chlorides.
Moxifloxacin hydrochloride (Ph. Int.)

D. Determine the specific optical rotation (1.4) using a solution of 0.200 g in 20.0 mL of a mixture of equal volumes of acetonitrile R and water R. Calculate with reference to the anhydrous substance; the specific optical rotation is between -125 to -138.

Clarity and colour of solution. Dissolve 1.0 g of the test substance in 20 mL of sodium hydroxide (~85 g/L) TS. The solution is not more intensely coloured than reference solution GY₂ (1.11.2, Method II).

pH value (1.13). pH of a 2 mg/mL solution in carbon-dioxide-free water R, 3.9 to 4.6.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 0.2 g of the substance, 30 mL anhydrous methanol and 3 minutes stirring before titration starts; the water content is not more than 45 mg/g.

Sulfated ash (2.3). Not more than 1.0 mg/g, determined on 1.0 g in a platinum crucible.

Related substances. Perform the test in subdued light, preferably using low-actinic glassware.

Carry out the tests as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm x 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded phenylsilyl groups (5 µm).¹

Use the following mobile phase: Mix 28 volumes of methanol R and 72 volumes of a solution containing 0.5 g/L of tetrabutylammonium hydrogen sulfate R, 1.0 g/L of potassium dihydrogen phosphate R and 3.4 g/L of phosphoric acid (~1440 g/L) TS.

Operate with a flow rate of 1.3 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 293 nm. Maintain the column temperature at 45°C.

Prepare solvent (A) by dissolving 0.50 g of tetrabutylammonium hydrogen sulfate R and 1.0 g of potassium dihydrogen phosphate R in about 500 mL of water R. Add 2 mL of phosphoric acid (~1440 g/L) TS and 0.050 g of anhydrous sodium sulfite R, then dilute to 1000.0 mL with water R.

Prepare the following solutions in solvent (A). For solution (1) dissolve about 50.0 mg of the test substance and dilute to 50.0 mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 1.0 mL of this solution to 10.0 mL. For solution (3) use a solution containing about 1 mg of moxifloxacin for peak identification RS (containing moxifloxacin and the impurities A, B, E and F) per mL.

Inject alternately 10 µL of solution (1), (2) and (3). Record the chromatograms for about 2.5 times the retention time of moxifloxacin.

Use the chromatogram supplied with moxifloxacin for peak identification RS and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B, E and F in the chromatogram obtained with solution (1). The impurities, if present, are eluted at the following relative retention with reference to moxifloxacin (retention time about 11 to 14 minutes): impurity F about 0.9; impurity A about 1.1; impurity B about 1.3; and impurity E about 1.7.

¹ An Inertsil Ph and a Zorbac Eclipse XDB-Phenyl column were found suitable.
The test is not valid unless in the chromatogram obtained with solution (3) the resolution between the peak due to moxifloxacin and the peak due to impurity A is at least 1.5 and the chromatogram obtained is similar to the chromatogram supplied with moxifloxacin for peak identification RS.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.4, is not greater than the area of the peak due to moxifloxacin in the chromatogram obtained with solution (2) (0.1%);
- the area of any peak corresponding to impurity E, when multiplied by a correction factor of 3.5, is not greater than the area of the peak due to moxifloxacin in the chromatogram obtained with solution (2) (0.1%);
- the area of any peak corresponding to impurity F is not greater than the area of the peak due to moxifloxacin in the chromatogram obtained with solution (2) (0.1%);
- the area of any other impurity peak is not greater than the area of the peak due to moxifloxacin in the chromatogram obtained with solution (2) (0.10%);
- the sum of the corrected areas of any peak corresponding to impurity B and E and the areas of all other impurity peaks is not greater than 3 times the area of the peak due to moxifloxacin obtained with solution (2) (0.3%). Disregard any peak with an area less than 0.5 times the area of the peak due to moxifloxacin in the chromatogram obtained with solution (2) (0.05%).

Assay

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under "Related substances".

Prepare the following solutions in solvent (A). For solution (1) dissolve 50.0 mg of the substance to be examined and dilute to 50.0 mL. Dilute 2.0 mL of this solution to 20.0 mL. For solution (2) dissolve 50.0 mg of moxifloxacin hydrochloride RS and dilute to 50.0 mL. Dilute 2.0 mL of this solution to 20.0 mL.

Inject alternately 10 µL of solution (1) and (2).

Measure the areas of the peaks corresponding to moxifloxacin obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of \(C_{21}H_{25}ClFN_3O_4\), using the declared content of \(C_{21}H_{25}ClFN_3O_4\) in moxifloxacin hydrochloride RS.

B. Dissolve about 0.320 g, accurately weighed, in 50 mL of water R. Titrate with sodium hydroxide (0.1 mol/L) VS, determining the end-point potentiometrically. Read the volume added to reach the first point of inflection. Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 43.79 mg of \(C_{21}H_{25}ClFN_3O_4\).
Impurities

A. 1-cyclopropyl-6,8-difluoro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [synthesis-related impurity]

B. 1-cyclopropyl-6,8-dimethoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [synthesis-related impurity]

C. 1-cyclopropyl-8-ethoxy-6-fluoro-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [synthesis-related impurity]

D. 1-cyclopropyl-8-fluoro-6-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [synthesis-related impurity]
E. 1-cyclopropyl-6-fluoro-8-hydroxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [synthesis-related impurity]

F. 1-cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS)-1-methyloctahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid [synthesis-related impurity]

G. methyl 1-cyclopropyl-6-fluoro-8-methoxy-7-[(4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylate [synthesis-related impurity]

Reagent to be established

Sodium hydroxide (~85 g/L) TS
A solution of sodium hydroxide R in water R containing about 85 g/L of NaOH.

Sodium sulfite, anhydrous R
Anhydrous sodium sulfite of a suitable quality should be used.
Moxifloxacin tablets  
(Moxifloxacini compressi)

This is a draft proposal of a monograph for The International Pharmacopoeia (Working document QAS/16.650, January 2018). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. The proposed monograph is based on information provided by the British Pharmacopoeia and pharmaceutical manufacturers, found in the scientific literature, and on laboratory investigations. The monograph is proposed for inclusion in The International Pharmacopoeia.]

Category. Antibacterial, antituberculosis.

Labelling. The designation on the container of moxifloxacin tablets should state that the active ingredient is Moxifloxacin hydrochloride and the quantity should be indicated in terms of equivalent amount of moxifloxacin.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML) 400 mg per tablet. Strength in the current WHO EML for children: 400 mg per tablet.

Requirements

Comply with the monograph for Tablets.

Definition. Moxifloxacin tablets contain Moxifloxacin hydrochloride. They contain not less than 90.0% and not more than 110.0% of the amount of moxifloxacin \((C_{21}H_{24}FN_{3}O_{4})\) stated on the label.

Identity tests

- Either test A or test B may be applied.

A. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R6 as the coating substance and a mixture of 4 volumes of 1-butanol R, 2 volumes of methanol R and 2 volumes of ammonia \((\sim 100 \text{ g/L})\) TS as the mobile phase. Apply separately to the plate 10 µL of each of the following two solutions. For solution (A) shake a quantity of the powdered tablets, equivalent to about 20 mg of moxifloxacin, with 20 mL of methanol R and filter. Dilute 1 mL of the filtrate to 20 mL with methanol. For solution (B) use an approximately 0.055 mg/mL solution of moxifloxacin hydrochloride RS in methanol R. Develop the plate for a distance of 15 cm. After removing the plate from the chromatographic chamber allow it to dry in air or in a current of air. Examine the chromatogram under ultraviolet light \((366 \text{ nm})\). The principal spot in the chromatogram obtained with solution (A) corresponds in position, appearance and intensity with the spot due to moxifloxacin in the chromatogram obtained with solution (B).

B. 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 the retention time of the peak due to moxifloxacin in the chromatogram obtained with solution (2).

**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 hydrochloric acid (~3.65 g/L) TS and rotating the paddle at 50 revolutions per minute. At 30 minutes withdraw a sample of 10.0 mL of the medium through an in-line filter. Allow the filtered sample to cool to room temperature. Measure the absorbance (1.6) of a 1 cm layer of the resulting solution, suitably diluted with the dissolution medium, if necessary, at the maximum at about 295 nm, using the dissolution medium as the blank. Measure at the same time and under the same conditions the absorbance of a suitable solution of moxifloxacin hydrochloride RS in the dissolution medium.

For each of the tablets calculate the total amount of moxifloxacin (C$_{21}$H$_{24}$FN$_3$O$_4$) in the medium. Each mg of moxifloxacin hydrochloride (C$_{21}$H$_{25}$ClFN$_3$O$_4$) is equivalent to 0.917 mg of moxifloxacin (C$_{21}$H$_{24}$FN$_3$O$_4$).

Evaluate the results as described under 5.5 Dissolution test for solid oral dosage forms, Acceptance criteria. The amount of moxifloxacin in solution for each tablet is not less than 75% (Q) of the amount declared on the label.

*[Note from the Secretariat. It is intended to determine the absorptivity value of moxifloxacin during the establishment of moxifloxacin hydrochloride RS. The value will then be included in the test description.]*

**Related substances.** Perform the test in subdued light, preferably using low-actinic glassware.

Carry out the tests as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (25 cm × 4.6 mm) packed with end-capped particles of silica gel, the surface of which has been modified with chemically-bonded phenylsilyl groups (5 µm).

Use the following mobile phase: mix 28 volumes of methanol R and 72 volumes of a solution containing 0.5 g/L of tetrabutylammonium hydrogen sulfate R, 1.0 g/L of potassium dihydrogen phosphate R and 3.4 g/L of phosphoric acid (~1440 g/L) TS.

Operate with a flow rate of 1.3 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 293 nm. Maintain the column temperature at 45°C.

Prepare solvent by dissolving 0.50 g of tetrabutylammonium hydrogen sulfate R and 1.0 g of potassium dihydrogen phosphate R in about 500 mL of water R. Add 2 mL of phosphoric acid (~1440 g/L) TS and 0.050 g of anhydrous sodium sulfite R, then dilute to 1000.0 mL with water R.

Prepare the following solutions in solvent (A). For solution (1) dissolve a quantity of the powdered tablets, equivalent to about 100 mg of moxifloxacin in 100 mL of solvent (A) with sonication and filter. For solution (2) dilute 1 volume of solution (1) to 100 volumes. Dilute 1 volume of this solution to 10 volumes. For solution (3) use a solution containing about 1 mg of moxifloxacin for peak identification RS (containing moxifloxacin and the impurities A, B, E and F) per mL.

Inject alternately 10 µL of solution (1), (2) and (3). Record the chromatograms for about 2.5 times the retention time of moxifloxacin.

1 A Zorbax Eclipse XDB-Phenyl column was found suitable.
Use the chromatogram supplied with moxifloxacin for peak identification RS and the chromatogram obtained with solution (3) to identify the peaks due to impurities A, B and E in the chromatogram obtained with solution (1). The impurities, if present, are eluted at the following relative retention with reference to moxifloxacin (retention time about 11 to 14 minutes): impurity A about 1.1; impurity B about 1.3; impurity E about 1.7.

The test is not valid unless in the chromatogram obtained with solution (3) the resolution between the peak due to moxifloxacin and the peak due to impurity A is at least 1.5 and the chromatogram obtained is similar to the chromatogram supplied with moxifloxacin for peak identification RS.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity B, when multiplied by a correction factor of 1.4, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
- the area of any peak corresponding to impurity E, when multiplied by a correction factor of 3.5, is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
- the area of any other impurity peak is not greater than 2 times the area of the principal peak in the chromatogram obtained with solution (2) (0.2%);
- the sum of the areas of all impurity peaks is not greater than 10 times the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained with solution (2) (0.1%).

**Assay.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Related substances”.

Prepare the following solutions in solvent (A). For solution (1) weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, accurately weighed, containing the equivalent of about 500 mg of moxifloxacin into a 500 mL volumetric flask. Add 400 mL of solvent (A), sonicate for 30 minutes, dilute to volume and filter. Dilute 1 volume of the filtrate to 10 volumes. For solution (2) use a solution containing about 0.11 mg moxifloxacin hydrochloride RS, accurately measured, per mL.

Inject alternately 10 µL of solution (1) and (2).

Measure the areas of the peaks corresponding to moxifloxacin obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of C_{21}H_{24}FN_{3}O_{4} in the tablets using the declared content of C_{21}H_{25}ClFN_{3}O_{4} in moxifloxacin hydrochloride RS. Each mg of C_{21}H_{25}ClFN_{3}O_{4} is equivalent to 0.917 mg of moxifloxacin (C_{21}H_{24}FN_{3}O_{4}).

**Impurities**

The impurities limited by the requirements of this monograph include the impurities listed in the monograph for Moxifloxacin hydrochloride.
Clindamycin palmitate hydrochloride
(*Clindamycini palmitas hydrochloridum*)

This is a draft proposal of a monograph for *The International Pharmacopoeia*
with line numbers is available for comment at www.who.int/medicines/areas/
quality_safety/quality_assurance/projects.

**Molecular formula.** $C_{34}H_{63}ClN_2O_6S\cdot HCl$

**Relative molecular mass.** 699.85

**Graphic formula**

![Graphic formula](image)

**Chemical name.** L-threo-α-D-galacto-Octopyranoside, methyl 7-chloro-6,7,8- trideoxy-6-[(1-methyl-4-propyl-2-pyrrolidinyl)-carbonyl]amino]-1-thio-2- hexadecanoate, monohydrochloride, (2S-trans)-; Methyl 7-chloro- 6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L- threo-α-D-galacto-octopyranoside 2-palmitate monohydrochloride; CAS Reg. No. 25507-04-4.

**Description.** A white or almost white powder.

**Solubility.** Freely soluble in ethanol and in dichloromethane; soluble in water.

**Category.** Antibacterial.

**Storage.** Clindamycin palmitate hydrochloride should be preserved in a tightly closed container.

**Additional information.** Clindamycin palmitate hydrochloride is a semi-synthetic product derived from a fermentation product.

**Requirements**

**Definition.** Clindamycin palmitate hydrochloride contains not less than 91.0% and not more than 102.0% of $C_{34}H_{63}ClN_2O_6S\cdot HCl$, calculated with reference to the anhydrous substance.

**Identity tests**

Either test A alone or tests B and C may be applied
Clindamycin palmitate hydrochloride (Ph. Int.)

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 clindamycin palmitate hydrochloride RS or with the reference spectrum of clindamycin palmitate hydrochloride.

B. 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).

C. A 10 mg/mL solution yields reaction B described under 2.1 General identification tests as characteristic of chlorides.

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. Use 0.1 g of the test substance. The water content is not more than 30 mg/g.

**pH value** (1.13). pH of a 10 mg/mL solution in carbon-dioxide-free water R, 2.8–3.8.

**Sulfated ash** (2.3). Not more than 5 mg/g.

**Related substances.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using a column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octylsilyl groups (5 μm).1

Use the following conditions for gradient elution:

- mobile phase A: Ammonium acetate (~0.40 g/L) TS - acetonitrile R (50:50);
- mobile phase B: Acetonitrile R.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (%) v/v</th>
<th>Mobile phase B (%) v/v</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–30</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–80</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>80–81</td>
<td>0 to 100</td>
<td>100 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>81–90</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions in methanol R. For solution (1) dissolve about 100 mg of clindamycin palmitate hydrochloride and dilute to 10.0 mL. For solution (2) dilute 2.0 mL of solution (1) to 100 mL. For solution (3) dissolve about 75 mg clindamycin palmitate hydrochloride RS (containing clindamycin palmitate hydrochloride and impurity A) and dilute to 10.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Inject alternately 20 μL each of solution (1), (2) and (3).

In the chromatogram obtained with solution (3) the retention time of clindamycin palmitate is about 37 minutes. The test is not valid unless the resolution between the peaks due to clindamycin palmitate and impurity A (relative retention about 0.9) is at least 3.0.

1 Agilent® Zorbax Elipse XDB-C8 has been found suitable.
In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to clindamycin palmitate: impurity B about 0.8 and impurity A about 0.9.

In the chromatogram obtained with solution (1):

- the area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);
- the sum of the areas of all impurity peaks is not more than 3.5 times the area of the principal peak in the chromatogram obtained with solution (2) (7.0%). Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

**Assay.** 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 octylsilyl groups (5 μm).

As the mobile phase, use a mixture of 10 volumes of ammonium acetate (~0.40 g/L) TS and 90 volumes of acetonitrile R.

Prepare the following solutions in mobile phase. For solution (1) transfer about 50 mg of the test substance, accurately weighed, into a 50 mL volumetric flask, dissolve and dilute to volume. For solution (2) dissolve about 50 mg of clindamycin palmitate hydrochloride RS and dilute to 50.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Inject alternately 20 μL each of solutions (1) and (2) and record the chromatograms for about 40 minutes.

Measure the areas of the peak responses corresponding to clindamycin palmitate obtained in the chromatograms from solutions (1) and (2) and calculate the percentage content of clindamycin palmitate hydrochloride ($C_{34}H_{63}ClIN_2O_6S.HCl$), using the declared content of clindamycin palmitate hydrochloride ($C_{34}H_{63}ClIN_2O_6S.HCl$) in clindamycin palmitate hydrochloride RS.

\[ \text{Clindamycin palmitate hydrochloride (Ph. Int.)} \]

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\[ ^2 \text{ Waters Symmetry C8 column has been found suitable.} \]
Impurities

A. L-threo-α-D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-\([(1\text{-methyl-}4\text{-ethyl-2-pyrrolidinyl\)-carbonyl\]amino\]-1-thio-2-hexadecanoate, (2S-trans); Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-ethyl-L-2-pyrrolidine carboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside 2-palmitate (clindamycin B palmitate) (synthesis-related impurity)

B. L-threo-α-D-galacto-Octopyranoside, methyl-epimer-7-chloro-6,7,8-trideoxy-6-\([(1\text{-methyl-}4\text{-propyl-2-pyrrolidinyl\)-carbonyl\]amino\]-1-thio-2-hexadecanoate, (2S-trans); Methyl-epimer-7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside 2-palmitate; (7-epiclindamycin 2-palmitate) (synthesis-related impurity)

Reagents to be established

Ammonium acetate (~0.40 g/L) TS
A solution of ammonium acetate R containing about 0.385 g of C\textsubscript{2}H\textsubscript{7}NO\textsubscript{2} per litre (approximately 0.005 mol/L).

Docusate sodium R
C\textsubscript{20}H\textsubscript{37}NaO\textsubscript{7}S
A commercially available reagent of suitable grade.

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Clindamycin palmitate powder for oral solution
(\textit{Clindamycini palmitas pulvis pro solutione perorali})

This is a draft proposal of a monograph for \textit{The International Pharmacopoeia} (Working document QAS/16.655, February 2018). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

\textbf{Category.} Antibacterial.

\textbf{Storage.} Clindamycin palmitate powder for oral solution should be kept in a tightly closed container.

\textbf{Additional information.} Strength in the current WHO Model List of Essential Medicines (EML): 75 mg/5 mL (as palmitate). Strengths in the current WHO EML for Children: 75 mg/5 mL (as palmitate).

Clindamycin palmitate powder for oral solution may contain excipients that are suspended in the reconstituted solution. Shake until the solution is uniform.

\textbf{Labelling.} The designation on the container of clindamycin palmitate powder for oral solution should state that the active ingredient is clindamycin palmitate hydrochloride and the quantity should be indicated in terms of equivalent amount of clindamycin.

\textbf{Requirements}

Complies with the monograph for \textit{Liquid preparations for oral use}; the powder complies with the section of the monograph entitled \textit{Powders for oral solutions, oral suspensions and oral drops}.

\textbf{Definition.} Clindamycin palmitate powder for oral solution is a solution of Clindamycin palmitate hydrochloride in a suitable vehicle, which may be flavoured. It is prepared from the powder as stated on the label before use. When freshly constituted the oral solution contains not less than 90.0\% and not more than 110.0\% of the labelled amount of clindamycin (C_{18}H_{33}ClN_{2}O_{5}S).

\textbf{Identity test}

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).

\textbf{pH value} (1.13). pH of a solution constituted as directed in the labelling, 2.5–5.0.

\textbf{Loss on drying.} Dry the powder for oral solution to constant mass at 60°C under reduced pressure; it loses not more than 20 mg/g.
Clindamycin palmitate powder for oral solution (Ph. Int.)

Related substances

Use the oral solution immediately after preparation.

Carry out the test as described under 1.14.4 High-performance liquid chromatography using a column (25 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octysilyl groups (5 μm).¹

Use the following conditions for gradient elution:
- mobile phase A: Ammonium acetate (~0.40 g/L) TS – acetonitrile R (50:50);
- mobile phase B: Acetonitrile R.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–30</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–80</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>80–81</td>
<td>0 to 100</td>
<td>100 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>81–90</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions in methanol R. For solution (1) transfer a quantity of the oral solution, equivalent of about 57 mg of clindamycin to a 10 mL volumetric flask and dilute to volume. For solution (2) dilute 2.0 mL of solution (1) to 100.0 mL. For solution (3) dissolve about 75 mg clindamycin palmitate hydrochloride RS (containing clindamycin palmitate hydrochloride and impurity A) and dilute to 10.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Inject alternately 20 μL each of solution (1), (2) and (3).

In the chromatogram obtained with solution (3) the retention time of clindamycin palmitate is about 37 minutes. The test is not valid unless the resolution between the peaks due to clindamycin palmitate and impurity A (relative retention about 0.9) is at least 3.0.

In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to clindamycin palmitate: impurity B about 0.8 and impurity A about 0.9.

In the chromatogram obtained with solution (1):
- the area of any impurity peak is not more than the area of the principal peak in the chromatogram obtained with solution (2) (2.0%);  
- the sum of the areas of all impurity peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with solution (2) (8.0%). Disregard any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with solution (2) (0.05%).

¹ Agilent® Zorbax Elipse XDB-C8 (4.6 × 250 mm, 5 μm) has been found suitable.
Assay. Use the oral solution immediately after preparation.

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 (5 μm).²

As the mobile phase, use a mixture of 10 volumes of ammonium acetate (~0.40 g/L) TS and 90 volumes of acetonitrile R.

Prepare the following solutions in mobile phase. For solution (1) dissolve a quantity of the oral solution, equivalent to about 30 mg of clindamycin, accurately weighed, and dilute to 50.0 mL, filter and use the filtrate. For solution (2) dissolve about 50 mg clindamycin palmitate hydrochloride RS and dilute to 50.0 mL.

Operate with a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Inject alternately 20 μL each of solutions (1) and (2) and record the chromatograms for about 40 minutes.

Measure the areas of the peak responses corresponding to clindamycin palmitate obtained in the chromatograms from solutions (1) and (2). Determine the weight per mL (1.3.1) of the oral solution and calculate the percentage content of clindamycin (C₁₈H₃₃ClN₂O₅S) in the oral solution, using the declared content of clindamycin palmitate hydrochloride (C₃₄H₆₃ClN₂O₆S·HCl) in clindamycin palmitate hydrochloride RS. Each mg of clindamycin palmitate hydrochloride (C₃₄H₆₃ClN₂O₆S·HCl) is equivalent to 0.607 mg clindamycin (C₁₈H₃₃ClN₂O₅S).

Impurities

The impurities limited by the requirements of this monograph include those listed in the monograph for Clindamycin palmitate hydrochloride.

² Waters Symmetry C8 has been found suitable.
Amoxicillin trihydrate
(\textit{Amoxicillium trihydricum})

This is a draft proposal of a revised monograph for \textit{The International Pharmacopoeia} (Working document QAS/16.680, February 2018). The working document with line numbers and tracked changes is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

\textbf{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 Pharmacopeia. 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 monograph available on the above-mentioned website are indicated in the text by \textit{insert} or \textit{delete}.

\begin{center}
\includegraphics[width=0.5\textwidth]{amoxicillin_structure.png}
\end{center}

C_{16}H_{19}N_{3}O_{5}S\cdot3H_{2}O

\textbf{Relative molecular mass.} 419.5

\textbf{Chemical name.} (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-carboxylic acid trihydrate; (-)-6-[2-Amino-2-(p-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.

\textbf{Description.} A white or almost white, crystalline powder.

\textbf{Solubility.} Slightly soluble in water and methanol R; very slightly soluble in ethanol (~750 g/L) TS, practically insoluble in fatty oils.

\textbf{Category.} Antibacterial.

\textbf{Storage.} Amoxicillin trihydrate should be kept in a tightly closed container.

\textbf{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 102.0% of C_{16}H_{19}N_{3}O_{5}S, 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; [α]_{D}^{20} = +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 10ml of ammonia (~100 g/l) TS. Examine both solutions immediately.

Neither of these solutions are more opalescent than opalescence standard TS3.
Amoxicillin trihydrate (Ph. Int.)

**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 (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 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:

<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>0–t&lt;sub&gt;R&lt;/sub&gt;</td>
<td>92</td>
<td>8</td>
<td>Isocratic</td>
</tr>
<tr>
<td>t&lt;sub&gt;R&lt;/sub&gt;–(t&lt;sub&gt;R&lt;/sub&gt;+25)</td>
<td>92 to 0</td>
<td>8 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>(t&lt;sub&gt;R&lt;/sub&gt;+25)–t&lt;sub&gt;R&lt;/sub&gt;–(t&lt;sub&gt;R&lt;/sub&gt;+40)</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>(t&lt;sub&gt;R&lt;/sub&gt;+40)–t&lt;sub&gt;R&lt;/sub&gt;–(t&lt;sub&gt;R&lt;/sub&gt;+55)</td>
<td>0 to 92</td>
<td>100 to 8</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>(t&lt;sub&gt;R&lt;/sub&gt;+55)–t&lt;sub&gt;R&lt;/sub&gt;–(t&lt;sub&gt;R&lt;/sub&gt;+70)</td>
<td>92</td>
<td>8</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

<sub>t<sub>R</sub> = retention time of amoxicillin determined with solution (2)</sub>

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).

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1 Agilent® Xbridge C18 column (4.6×250 mm, 5 μm) was found suitable.
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.** 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 (C₁₆H₁₉N₃O₅S), using the declared content of amoxicillin (C₁₆H₁₉N₃O₅S) in amoxicillin trihydrate RS.

**Impurities**

![Chemical structure](image)

A. (2S,5R,6R)-6-amino-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 (l-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]carboxymethyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penicilloic acids of amoxicillin),

and epimer at C*

E. (2RS,4S)-2-[[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid (penilloic 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).
Clavulanate potassium
(Kalii clavulanas)

This is a draft proposal of a revised monograph for The International Pharmacopoeia (Working document QAS/16.681, February 2018). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. It is proposed to include the monograph on Clavulanate potassium in The International Pharmacopoeia. The monograph is based on laboratory investigations and on information found in the Chinese Pharmacopoeia, the European Pharmacopoeia and the United States Pharmacopeia. 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.]

Molecular formula. \( \text{C}_8\text{H}_8\text{KNO}_5 \).

Relative molecular mass. 237.3.

Graphic formula

![Chemical structure of Clavulanate potassium](image.png)

Chemical name. Potassium \((2R,3Z,5R)-3-(2\text{-hydroxyethylidene})-7\text{-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate, CAS Reg. No.61177-45-5.}

Description. A white or almost white, crystalline powder.

Solubility. Freely soluble in water R, slightly soluble in ethanol (\(~710 \text{ g/L} \)) TS, very slightly soluble in acetone R.

Category. \(\beta\)-Lactamase inhibitor.

Storage. Potassium clavulanate should be kept in tightly closed containers, protected from light, at a temperature of 2°C to 8°C.

Additional information. Potassium clavulanate is hygroscopic.

Requirements

Definition. Potassium clavulanate contains not less than 96.5% and not more than 102.0% of \( \text{C}_8\text{H}_8\text{KNO}_5 \), calculated with reference to the anhydrous substance.

Manufacture. The method of production is validated to demonstrate that the substance, if tested, would comply with the limit of not more than 0.01% for clavam-2-carboxylate using a suitable method.
Identity tests
Either tests A and D or tests B, 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 reference spectrum of potassium clavulanate.

B. 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 from solution (1) is similar to that obtained from solution (2).

C. [Note from the Secretariat. It is intended to add a TLC test specific for clavulanic acid and amoxicilline.]

D. Ignite a small quantity, dissolve the residue in water and filter. Add 2 mL of sodium hydroxide (~80 g/L) TS to the filtrate. It yields the reaction described under 2.1 General identification tests as characteristic of potassium.

Solution S. Dissolve 0.400 g of the test substance in carbon-dioxide-free water R and dilute to 20.0 mL with the same solvent.

pH value (1.13). Dilute 5 mL of solution S to 10 mL with carbon dioxide-free water R; the value lies between 5.5 to 8.0.

Specific optical rotation (1.4). Use solution S; \([\text{d}^20] = +53 \text{ to } +63\) with reference to the anhydrous substance.

Polymeric impurities and other impurities absorbing at 278 nm
Prepare fresh solutions and perform the test without delay.

Dissolve 50.0 mg of the test substance in phosphate buffer, pH 7.0 (0.1 mol/L) TS and dilute to 50.0 mL with the same buffer solution. Measure the absorbance immediately. The absorbance (1.6) of the solution determined at 278 nm is not greater than 0.40.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, method A, using about 0.50 g of the substance; the water content is not more than 5 mg/g.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (10 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecysilyl groups (5 µm).¹

Prepare the following phosphate buffer, pH 4.0. Dissolve 7.8 g of sodium dihydrogen phosphate R in about 800 mL of water R, adjust to pH 4.0 with phosphoric acid (~105 g/L) TS and dilute to 1000.0 mL with the same solvent.

Use the following conditions for gradient elution:

- mobile phase A: phosphate buffer, pH 4.0;
- mobile phase B: a mixture of equal volumes of methanol R and mobile phase A.

¹ A Waters Atlantis T3 column was found suitable.
Operate with a flow rate of 1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm. Maintain the column temperature at 40°C.

Prepare the following solutions immediately before use in mobile phase A. For solution (1) dissolve about 25 mg of the test substance and dilute to 25.0 mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3) dissolve 10 mg of lithium clavulanate R and 10 mg of amoxicillin trihydrate R and dilute to 100 mL.

Inject 20 µL of solution (3). The test is not valid unless in the chromatogram obtained the resolution between the peaks due to clavulanate (retention time about 3 minutes) and the peak due to amoxicillin (with a relative retention of about [value to be determined]) is at least 13.

Inject alternately 20 µL each of solution (1) and (2).

In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to clavulanate (retention time about 3 minutes): impurity E about 2.3; impurity G about 3.6.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to either impurity E or impurity G is not greater than the area of the peak due to clavulanic acid in the chromatogram obtained with solution (2) (1.0%);
- the area of any other impurity peak is not greater than 0.2 times the area of the peak due to clavulanic acid in the chromatogram obtained with solution (2) (0.2%);
- the sum of the areas of all impurity peaks is not greater than 2 times the area of the peak due to clavulanic acid in the chromatogram obtained with solution (2) (2.0%). Disregard any peak with an area less than 0.05 times the area of the peak due to clavulanic acid in the chromatogram obtained with solution (2) (0.05%).

Aliphatic amines. The method can be used to determine the following aliphatic amines: 1,1-dimethylethylamine (impurity H); N,N,N’,N’-tetramethylethylenediamine (impurity J); 1,1,3,3-tetramethylbutylamine (impurity K); N,N’-diisopropylethane-1,2-diamine (impurity L); 2,2’-oxybis(N,N-)dimethylethylamine (impurity M).

Carry out the test as described under 1.14.5 Gas chromatography. Use a fused-silica capillary column, 50 m long and 0.53 mm in internal diameter, coated with poly(dimethyl)(diphenyl) siloxane R (film thickness: 5 µm).

As an internal standard use a solution containing 0.5 µL of 3-methylpentane-2-one R per mL of water R. For solution (1) transfer 1.00 g of the test substance to a centrifuge tube. Add 5.0 mL of the internal standard solution, 5.0 mL of sodium hydroxide (~8.5 g/L) TS, 10.0 mL
of water R, 5.0 mL of 2-methylpropanol R and 5 g of sodium chloride R. Shake vigorously for 1 minute. Centrifuge to separate the layers and use the upper layer. For solution (2) dissolve 80.0 mg of each of the following amines: 1,1-dimethylethylamine R; tetramethylethylenediamine R; 1,1,3,3-tetramethylbutylamine R; N,N’-diisopropylethylendiamine R and 2,2’-oxybis(N,N-dimethylethylamine) R in hydrochloric acid (~70 g/L) TS and dilute to 200.0 mL with the same acid. Transfer 5.0 mL of this solution into a centrifuge tube. Add 5.0 mL of the internal standard solution, 10.0 mL of sodium hydroxide (~8.5 g/L) TS, 5.0 mL of 2-methylpropanol R and 5 g of sodium chloride R. Shake vigorously for 1 minute. Centrifuge to separate the layers and use the upper layer.

As a detector use a flame ionization detector.

Use nitrogen R as the carrier gas at an appropriate pressure and a split ratio 1:10 with a flow rate of about 6 mL/min.

Maintain the temperature of the column at 35°C for 7 minutes, then raise the temperature at a rate of 30°C per minutes to 150°C and maintain for 15 minutes. Keep the temperature of the injection port at 200°C and that of the flame ionization detector at 250°C.

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

In the chromatogram obtained with solution (1) the following impurities, if present, are eluted at the following relative retention with reference to 3-methylpentane-2-one (internal standard, retention time about 11.4 minutes): impurity H about 0.55; impurity J about 1.07; impurity K about 1.13; impurity L about 1.33; impurity M about 1.57.

Measure the peak responses of the aliphatic amines and of the internal standard. Calculate the percentage content of each impurity using the ratios of the responses of the each aliphatic amine to the responses of the internal standard. Use the ratios of the peak responses of the corresponding reagents as a reference. The sum of the percentage contents of all aliphatic amines is less than 0.2%.

2-Ethylhexanoic acid. Carry out the test as described under 1.14.5 Gas chromatography.

Use a fused-silica capillary column 10 m long and 0.53 mm in internal diameter coated with macrogol 20000 2-nitrotetraphthalate R (film thickness: 1.0 µm).

As an internal standard use a solution containing 1.0 mg 3-cyclohexylpropionic acid R per mL of cyclohexane R. For solution (1) transfer 1.00 g of the test substance to a centrifuge tube. Add 4.0 mL of a 33% (V/V) solution of hydrochloric acid R. Shake vigorously for 1 minute with 1.0 mL of the internal standard solution. Allow the phases to separate (if necessary, centrifuge for a better separation). Use the upper layer. For solution (2) dissolve 75.0 mg of 2-ethylhexanoic acid R in the internal standard solution and dilute to 50.0 mL with the same solution. To 1.0 mL of the solution add 4.0 mL of a 33% (V/V) solution of hydrochloric acid R. Shake vigorously for 1 minute. Allow the phases to separate (if necessary, centrifuge for a better separation). Use the upper layer.

As a detector use a flame ionization detector.

Use nitrogen as the carrier gas at an appropriate pressure with a flow rate of about 6 mL/minute.
Maintain the temperature of the column at 40°C for 2 minutes, then raise the temperature at a rate of 30°C per minutes to 200°C and maintain for 3 minutes. Keep the temperature of the injection port at 200°C and that of the flame ionization detector at 300°C.

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

The test is not valid unless the resolution between the peaks due to 2-ethylhexanoic acid (first peak) and due to the internal standard is at least 2.0.

Measure the peak responses of 2-ethylhexanoic acid and of the internal standard. Calculate the percentage content of 2-ethylhexanoic acid in the test substance using the ratios of the responses of 2-ethylhexanoic acid to the responses of the internal standard; the content is not more than 0.8%.

**Assay.** Carry out the test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (40 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (10 µm).^2^ Prepare the following phosphate buffer, pH 4.0. Dissolve 15 g of sodium dihydrogen phosphate R in about 800 mL of water R, adjust to pH 4.0 with phosphoric acid (~105 g/L) TS and dilute to 1000.0 mL with the same solvent.

As the mobile phase use a mixture of 5 volumes of methanol R and 95 volumes of phosphate buffer, pH 4.0.

Operate with a flow rate of 1 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 230 nm.

Prepare the following acetate buffer, pH 6.0. Dissolve 4.1 g of sodium acetate R in about 800 mL of water R, adjust to pH 6.0 with glacial acetic acid R and dilute to 1000.0 mL with the same solvent.

Prepare the following solutions immediately before use, using acetate buffer, pH 6.0 as the solvent. For solution (1) dissolve 50.0 mg of the test substance and dilute to 50.0 mL. For solution (2) dissolve 50.0 mg of lithium clavulanate RS and dilute to 50.0 mL. For solution (3) dissolve 10 mg of amoxicillin trihydrate R in 10 mL of solution (2).

Inject 10 µL of solution (3). The assay is not valid unless the resolution between the peaks due to clavulanate (retention time about 5 minutes) and the peak due to amoxicillin (with a relative retention of about [value to be determined]) is at least 3.5.

Measure the areas of the peaks corresponding to clavulanate obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of C₈H₈KNO₅, using the declared content of clavulanic acid (C₈H₉NO₅) in lithium clavulanate RS. 1 mg of clavulanic acid (C₈H₉NO₅) is equivalent to 1.191 mg of potassium clavulanate C₈H₈KNO₅.

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^2^ A Zorbax eclipse XDB-C18 column was found suitable.
**Impurities**

A. 2,2'--(pyrazine-2,5-diyl)diethanol

B. 3-[3,6-bis(2-hydroxyethyl)pyrazin-2-yl]propanoic acid

C. 2,2'-((3-ethylpyrazine-2,5-diyl)diethanol

D. 4-(2-hydroxyethyl)-1H-pyrrole-3-carboxylic acid


F. 4-[[4-(2-hydroxyethyl)-1H-pyrrol-3-yl]carbonyl]oxy)methyl]-1H-pyrrole-3-carboxylic acid
G. 4-[[[(1S)-1-carboxy-2-(4-hydroxyphenyl)ethyl]amino]-4-oxobutanoic acid (N-(hydrogensuccinyl)tyrosine)

H. 2-methylpropan-2-amine (2-amino-2-methylpropane, tert-butylamine, 1,1-dimethylethylamine)

J. N,N,N',N'-tetramethylethane-1,2-diamine (1,2-bis(dimethylamino)ethane, N,N,N',N'-tetramethylethylene diamine)

K. 2,4,4-trimethylpentane-2-amine (2-amino-2,4,4-trimethylpentane, 1,1,3,3-tetramethylbutylamine)

L. N,N'-diisopropylethane-1,2-diamine (N,N'-bis(1-methylethyl)ethane-1,2-diamine)

M. 2,2'-oxybis(N,N-dimethylethanamine), bis[2-(dimethylamino)ethyl] ether, N,N,N',N'-tetramethyl(oxydiethylene) diamine

Reagents to be established

**Amoxicillin trihydrate R**
Amoxicillin trihydrate of a suitable quality should be used.

**3-Cyclohexylpropionic acid R**
\[ C_9 H_{16} O_2 \]
Molecular weight. 156.2.
Description. Clear liquid.
Relative density $\rho_{20}^{20}$. About 0.998.
Boiling point. About 130°C.

$\text{N,N'}$-Diisopropylethylenediamine R
$\text{C}_8\text{H}_{20}\text{N}_2$
Molecular weight. 144.3.
Other name. $\text{N,N'}$-Bis(1-methylethyl)-1,2-ethanediameine.
Description. Colourless or yellowish, hygroscopic liquid, corrosive, flammable.
Relative density $\rho_{20}^{20}$. About 0.798.
Boiling point. About 170°C.

1,1-Dimethylethylamine R
$\text{C}_4\text{H}_{11}\text{N}$
Molecular weight. 73.1.
Other names. 2-Amino-2-methylpropane, $\text{tert}$-Butylamine.
Description. Liquid, miscible with ethanol (~710 g/L) TS.
Relative density $\rho_{20}^{20}$. About 0.694.
Boiling point. About 46°C.

2-Ethylhexanoic acid R
$\text{C}_8\text{H}_{16}\text{O}_2$
Molecular weight. 144.2.
Description. Colourless liquid.
Relative density $\rho_{20}^{20}$. About 0.91.
Related substances. Carry out the test as described under 1.14.5 Gas chromatography using the conditions given in the test for 2-ethylhexanoic acid in the monograph on Potassium clavulanate. Prepare the following solution: suspend 0.2 g of 2-ethylhexanoic acid in 5 mL of water R, add 3 mL of 33% (V/V) solution of hydrochloric acid R and 5 mL of hexane R, shake for 1 minute, allow the layers to separate and use the upper layer. Inject 1 µL of this solution. The sum of the area of any peaks, other than the principal peak and the peak due to the solvent, is not greater than 2.5% of the area of the principal peak.

Lithium clavulanate R
Lithium clavulanate of a suitable quality should be used.

Macrogol 20000 R
Description. White or almost white solid with a waxy or paraffin-like appearance.
Solubility. Very soluble in water, soluble in methylene chloride, practically insoluble in alcohol, in fatty oils and in mineral oils.

Macrogol 20000 2-nitrotetraphthalate R
Macrogol 20000 R modified by treating with 2-nitrotetraphthalate acid.
Description. A hard, white or almost white, waxy solid.
Solubility. Soluble in acetone R.

3-Methylpentane-2-one R
$\text{C}_6\text{H}_{12}\text{O}$
**Molecular weight.** 100.2.
**Description.** Colourless, flammable liquid.
**Relative density** $d_{20}^{20}$. About 0.815.
**Boiling point.** About 118°C.

**2-Methylpropanol R**

$C_4H_{10}O$

**Molecular weight.** 74.1.
**Other names.** Isobutyl alcohol, 2-Methylpropan-1-ol.
**Description** Clear colourless liquid.
**Solubility.** Soluble in water, miscible with ethanol (~710 g/L) TS.
**Relative density** $d_{20}^{20}$. About 0.80.
**Boiling point.** About 107°C.

**2,2'-Oxybis(N,N-dimethylethylamine) R**

$C_8H_{20}N_2O$

**Molecular weight.** 160.3.
**Other name.** Bis(2-dimethylaminoethyl) ether.
**Description.** Colourless, corrosive liquid.
**Relative density** $d_{20}^{20}$. About 0.85.

**Phosphate buffer, pH 7.0 (0.1 mol/L) TS**

**Procedure.** Dissolve 1.361 g of potassium dihydrogen phosphate R in 100.0 mL of water. Adjust the pH using a 14,20 g/L solution of anhydrous disodium hydrogen phosphate R.

**Poly(dimethyl)(diphenyl)siloxane R**
Stationary phase for gas chromatography. Contains 95% of methyl groups and 5% of phenyl groups.

**Sodium hydroxide (~8.5 g/L) TS**
A solution of sodium hydroxide R containing about 8.5 g/L of NaOH.

**1,1,3,3-Tetramethylbutylamine R**

$C_8H_{19}N$

**Molecular weight.** 129.3.
**Other name.** 2-Amino-2,4,4-trimethylpentane.
**Description.** Clear, colourless liquid.
**Relative density** $d_{20}^{20}$. About 0.805.
**Boiling point.** About 140°C.

**Tetramethylethylenediamine R**

$C_6H_{16}N_2$

**Molecular weight.** 116.2.
**Other name.** N,N,N',N’-Tetramethylethylenediamine.
**Description.** Colourless liquid, miscible with water and with ethanol (~710 g/L) TS.
**Relative density** $d_{20}^{20}$. About 0.78.
**Boiling point.** About 121°C.

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

This is a draft proposal of a monograph for The International Pharmacopoeia
(Working document QAS/16.660, February 2018). The working document with
line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

[Note from the Secretariat. It is proposed to include the monograph on Amoxicillin and clavulanic acid tablets in The International Pharmacopoeia. The monograph is based on laboratory investigations and on information found in the British Pharmacopoeia, the Chinese Pharmacopoeia, the European Pharmacopoeia and the United States Pharmacopeia.]

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 (C₁₆H₁₉N₃O₆S) and clavulanic acid (C₈H₉NO₅) stated on the label.

Identity test
Carry out the test as described under 1.14.4 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 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 45 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 1.14.4 High-performance liquid chromatography using the conditions as described under “Assay”.

For each of the tablets tested, calculate the total amount of amoxicillin \((\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S})\) and clavulanic acid \((\text{C}_8\text{H}_9\text{N}_2\text{O}_5\text{S})\) in the medium using the declared content of amoxicillin \((\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S})\) in amoxicillin trihydrate RS and the declared content of clavulanic acid \((\text{C}_8\text{H}_9\text{N}_2\text{O}_5\text{S})\) in lithium clavulanate RS.

The amount of amoxicillin and clavulanic acid in solution for each tablet is not less than 75% (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 1.14.4 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 (5 µm).\(^1\)

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.

\(^{1}\) Shim-pack CL-ODS C\(_{18}\) has been found suitable.
Amoxicillin and clavulanic acid tablets (Ph. Int.)

Use the following conditions for gradient elution:

- mobile phase A: mix 10 volumes of acetonitrile R with 990 volumes of the buffer solution;
- mobile phase B: mix 200 volumes of acetonitrile R with 800 volumes of the buffer solution.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – tr</td>
<td>92</td>
<td>8</td>
<td>Isocratic</td>
</tr>
<tr>
<td>tr–(tr + 25)</td>
<td>92 to 0</td>
<td>8 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>(tr + 25)–(tr + 40)</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>(tr + 40)–(tr + 41)</td>
<td>92</td>
<td>8</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>(tr + 41)–(tr + 55)</td>
<td>92</td>
<td>8</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

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 RS per mL mobile phase A. For solution (4) use a solution containing 0.75 mg of lithium clavulanate RS per mL 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 retention 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 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 1.14.4 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 (5 μm).1

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 RS and 0.2 mg of lithium clavulanate RS per mL 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 and the symmetry factor of the peak due to clavulanic acid in the chromatogram obtained with solution (2) is less than 1.5.

Measure the areas of the peak responding to amoxicillin and clavulanic acid and calculate the content of amoxicillin (C\textsubscript{16}H\textsubscript{19}N\textsubscript{3}O\textsubscript{5}S) and clavulanic acid (C\textsubscript{8}H\textsubscript{9}NO\textsubscript{5}) in the tablets using the declared content of amoxicillin (C\textsubscript{16}H\textsubscript{19}N\textsubscript{3}O\textsubscript{5}S) in amoxicillin trihydrate RS and the declared content of clavulanic acid (C\textsubscript{8}H\textsubscript{9}NO\textsubscript{5}) in lithium clavulanate RS.

**Impurities**

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

**Reagents and test solutions to be established**

**Phosphoric acid (~7.8 g/L) TS**

*Procedure.* Dilute 9.2 g of phosphoric acid (~1440 g/L) TS with sufficient water to produce 1000 mL.

**Sulfuric acid (~50 g/L) TS**

*Procedure.* Mix 500 mL of sulfuric acid (~100 g/L) TS with sufficient water to produce 1000 mL.

**Quinine sulfate R**

Quinine sulfate of a suitable quality should be used.

**Cefadroxil R**

Cefadroxil of a suitable quality should be used.

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Dissolution test for solid oral dosage forms

This is a draft proposal of a revised monograph for *The International Pharmacopoeia* (Working document QAS/18.756, February 2018). The working document with line numbers is available for comment at www.who.int/medicines/areas/quality_safety/quality_assurance/projects.

**[Note from the Secretariat. Following the publication of the document Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability it is proposed to add an acetate buffer pH 4.5 to chapter 5.5 Dissolution test for solid oral dosage forms to assist the guidelines recommendation that pharmacopoeial buffers should be used to determine the in vitro dissolution characteristics of multisource products. It is further proposed to revise the requirements for the qualification of dissolution testers by introducing the concept of an “enhanced mechanical calibration”.

The text prescribes that the pH of buffers used as dissolution media has to be adjusted to within 0.05 units of the specified value. Advice is sought whether this adjustment has to be made at the temperature at which the dissolution test is usually performed, i.e. 37 ± 0.5°C (see line 144 of the working document available at www.who.int/medicines/areas/quality_safety/quality_assurance/projects).

In the online document, changes from the current chapter are indicated in the text by insert or delete.]**

### 5.5 Dissolution test for solid oral dosage forms

This text is based on the internationally-harmonized texts developed by the Pharmacopoeial Discussion Group (PDG). It has been developed in line with the style and requirements used in *The International Pharmacopoeia*. The additional section on monographs of *The International Pharmacopoeia* is not part of the PDG text.

For further guidance, see also the chapter Dissolution testing of tablets and capsules in the Supplementary Information section.

This test determines the amount of active ingredient(s) released from a solid oral dosage form, such as a tablet or a capsule, under controlled conditions using a known volume of dissolution medium within a predetermined length of time.

**Basket apparatus.** The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material, which should not sorb, react or interfere with the dosage form, the active ingredient or the dissolution medium; a motor; a drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water-bath of any convenient size or heated by a suitable device such as a heating jacket to maintain the temperature inside the vessel at 37 ± 0.5°C during the test and to keep the dissolution medium in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation or vibration beyond that due to the smoothly rotating stirring element. Apparatus that permits observation of the preparation and stirring element during the test is preferable. The vessel is cylindrical with a hemispherical bottom and a capacity of 1 litre. Its height is 160–210 mm and its inside diameter is 98–106 mm. Its sides are flanged at the top. A fitted cover may be used to retard evaporation. If a cover is used it provides sufficient openings to allow ready insertion of the thermometer.
and withdrawal of samples. The shaft is positioned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. A speed-regulating device is used that allows the shaft rotation speed to be selected and maintained at a specified rate within ± 4%.

Shaft and basket components of the stirring element are fabricated of stainless steel, type 316 or equivalent, to the specifications shown in Figure 1. A basket having a gold coating of about 2.5 µm (0.0001 inch) thick may be used. The dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at 25 ± 2 mm during the test.

Figure 1. **Basket stirring element.**
Dimensions in millimetres

1. Screen with welded seam: 0.22–0.31 mm wire diameter with wire opening of 0.36–0.44 mm. After welding the screen may be slightly altered.
2. Maximum allowable runout at “A” is 1.0 mm when the part is rotated on centre line axis with basket mounted.
Dissolution test for solid oral dosage forms (Ph. Int.)

**Figure 2.** Paddle stirring element.
Dimensions in millimetres

A and B dimensions do not vary more than 0.5 mm when part is rotated on centre line axis. Tolerances are ± 1.0 mm unless otherwise stated.

**Paddle apparatus.** Use the assembly from the basket apparatus except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel at any point and rotates smoothly without significant wobble that could affect the results. The vertical centre line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom
of the shaft. The paddle conforms to the specifications shown in Figure 2. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. A small, loose piece of non-reactive material, such as not more than a few turns of wire helix, may be attached to dosage units that would otherwise float. An alternative sinker device is shown in Figure 3. Other validated sinker devices may be used.

![Figure 3. Alternative sinker. Dimensions in millimeters.](image)

**Recommended procedure**

**Conventional-release (or immediate-release) dosage forms**

*Procedure.* Place the stated volume of the dissolution medium (± 1%) in the vessel of the specified apparatus. Assemble the apparatus, equilibrate the dissolution medium to 37 ± 0.5°C and remove the thermometer. The test may also be carried out with the thermometer in place, provided it is shown that results equivalent to those obtained without the thermometer are obtained. Place one dosage unit in the apparatus taking care to exclude air bubbles from the surface of the dosage unit. Operate the apparatus at the specified rate. Within the time interval specified, or at each of the times stated, withdraw a sample from a zone midway between the surface of the dissolution medium and the top of the rotating basket or blade not less than 1 cm from the vessel wall. Where multiple sampling times are specified replace the samples withdrawn for analysis with equal volumes of fresh dissolution medium at 37°C or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test and verify the temperature (37 ± 0.5°C) of the medium at suitable times. Perform the analysis as directed in the individual monograph using a suitable assay method. Test samples are filtered immediately upon sampling using in-line filtration unless filtration is demonstrated to be unnecessary. Use an inert filter that does not cause adsorption of the active substance or contain extractable
Consultation documents WHO Drug Information Vol. 32, No. 1, 2018

Dissolution test for solid oral dosage forms (Ph. Int.)

substances that would interfere with the analysis. Centrifugation is not recommended unless validated for the specific test. The test is to be conducted with six dosage form units in parallel.

If automated equipment is used for sampling or the apparatus is otherwise modified verification is necessary that the modified apparatus will produce results equivalent to those obtained with the apparatus described in this chapter.

Dissolution medium. A suitable dissolution medium is used. The volume specified refers to measurements made between 20°C and 25°C. If the dissolution medium is a buffered solution allow the medium to equilibrate to a temperature of 37 ± 0.5°C and adjust the solution so that its pH is within 0.05 units of the specified pH. Dissolved gases can cause bubbles to form which may change the results of the test. In such cases dissolved gases must be removed prior to testing.\(^1\)

Time. Where a single time specification is given the test may be concluded in a shorter period if the requirement for minimum amount dissolved is met. Samples are to be withdrawn only at the stated times, within a tolerance of ± 2%.

Determine the quantity of active ingredient dissolved at the specified time(s) indicated in the individual monograph. The result should be expressed as a percentage of the content stated on the label of the dosage form.

Sustained-release solid dosage forms

Procedure. Proceed as described for conventional-release dosage forms.

Dissolution medium. Proceed as described for conventional-release dosage forms.

Time. The test-time points, generally three, are expressed in hours.

Delayed-release, solid dosage forms

Procedure. Use method A or B.

Method A

• Acid stage. Place 750 mL hydrochloric acid (0.1 mol/L) VS in the vessel and assemble the apparatus. Allow the medium to equilibrate to a temperature of 37 ± 0.5°C. Place one dosage unit in the apparatus, cover the vessel and operate the apparatus at the specified rate. After 2 hours of operation in hydrochloric acid (0.1 mol/L) VS withdraw a sample of the fluid and proceed immediately as directed under buffer stage. Perform an analysis of the sample using a suitable assay method.

• Buffer stage. Complete the operations of adding and adjusting the pH within 5 minutes. With the apparatus operating at the rate specified add to the fluid in the vessel 250 mL of a 0.2 M solution of trisodium orthophosphate R that has been equilibrated to 37 ± 0.5°C. Adjust, if necessary, with hydrochloric acid (~70 g/L) TS or sodium hydroxide (~80 g/L)

\(^1\) One appropriate method of deaeration is as follows: heat the medium, while stirring gently, to about 41°C, immediately filter under vacuum using a filter having a pore size of 0.45 µm or less, with vigorous stirring and continue stirring under vacuum for at least 5 minutes, preferably 15 minutes, until no more bubbles are observed. Other validated deaeration techniques for removal of dissolved gases may be used.
Dissolution test for solid oral dosage forms (Ph. Int.)

TS to a pH of 6.8 ± 0.05. Continue to operate the apparatus for 45 minutes or for the specified time. At the end of the time period withdraw a sample of the fluid and perform the analysis using a suitable assay method.

Method B

• **Acid Stage.** Place 1000 mL of hydrochloric acid (0.1 mol/L) VS in the vessel and assemble the apparatus. Allow the medium to equilibrate to a temperature of 37 ± 0.5°C. Place one dosage unit in the apparatus, cover the vessel and operate the apparatus at the specified rate. After 2 hours of operation in hydrochloric acid (0.1 mol/L) VS withdraw a sample of the fluid and proceed immediately as directed under buffer stage. Perform an analysis of the sample using a suitable assay method.

• **Buffer stage.** For this stage of the procedure use buffer that has previously been equilibrated to a temperature of 37 ± 0.5°C. Drain the acid from the vessel and add 1000 mL of pH 6.8 phosphate buffer, prepared by mixing three volumes of hydrochloric acid (0.1 mol/L) VS with one volume of a 0.20 M solution of trisodium orthophosphate R and adjusting, if necessary, with hydrochloric acid (~70 g/L) TS or sodium hydroxide (~80 g/L) TS to a pH of 6.8 ± 0.05. This may also be accomplished by removing from the apparatus the vessel containing the acid and replacing it with another vessel containing the buffer and transferring the dosage unit to the vessel containing the buffer. Continue to operate the apparatus for 45 minutes or for the specified time. At the end of the time period withdraw a sample of the fluid and perform the analysis using a suitable assay method.

**Time.** All test times stated are to be observed within a tolerance of ± 2%, unless otherwise specified.

**Acceptance criteria**

**Conventional-release (or immediate-release) dosage forms**

Unless otherwise specified in the individual monograph the requirements are met if the quantities of active ingredient(s) dissolved from the dosage forms tested conform to Table 1. Continue testing through the three levels unless the results conform at either S₁ or S₂. The quantity, Q, is the specified amount of dissolved active ingredient expressed as a percentage of the labelled content; the 5%, 15% and 25% values in the acceptance table are percentages of the labelled content so that these values and Q are in the same terms.

<table>
<thead>
<tr>
<th>Level</th>
<th>Samples tested</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁</td>
<td>6</td>
<td>Each value is not less than Q + 5%</td>
</tr>
<tr>
<td>S₂</td>
<td>6</td>
<td>Average value of the 12 dosage units (S₁ + S₂) is equal to or greater than Q and no unit is less than Q - 15%</td>
</tr>
<tr>
<td>S₃</td>
<td>12</td>
<td>Average value of 24 dosage units (S₁ + S₂ + S₃) is equal to or greater than Q; not more than 2 units are less than Q - 15%; no unit is less than Q - 25%.</td>
</tr>
</tbody>
</table>
Dissolution test for solid oral dosage forms (Ph. Int.)

**Sustained release dosage forms**

Unless otherwise specified in the individual monograph the requirements are met if the quantities of active ingredient(s) dissolved from the dosage forms tested conform to Table 2. Continue testing through the three levels unless the results conform at either L1 or L2. Limits on the amounts of active ingredient(s) dissolved are expressed in terms of the labelled content. The limits embrace each value of Qj, the amount dissolved at each specified fractional dosing interval. Where more than one range is specified the acceptance criteria apply individually to each range.

Table 2

<table>
<thead>
<tr>
<th>Level</th>
<th>Samples tested</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>6</td>
<td>No individual value lies outside each of the stated ranges and no individual value is less than the stated amount at the final test time</td>
</tr>
<tr>
<td>L2</td>
<td>6</td>
<td>The average value of the 12 dosage units (L1 + L2) lies within each of the stated ranges and is not less than the stated amount at the final test time; none is more than 10% of the labelled content outside each of the stated ranges; and none is more than 10% of labelled content below the stated amount at the final test time</td>
</tr>
<tr>
<td>L3</td>
<td>12</td>
<td>The average value of the 24 dosage units (L1 + L2 + L3) lies within the stated ranges and is not less than the stated amount at the final test time; not more than 2 of the 24 dosage units are more than 10% of labelled content outside each of the stated ranges; not more than 2 of the 24 dosage units are more than 10% of labelled content below the stated amount at the final test time; and none of the 24 dosage units is more than 20% of labelled content below the stated content at the final test time; none of the units are more than 20% of labelled content outside each of the stated ranges or more than 20% of labelled content below the stated amount at the final test time</td>
</tr>
</tbody>
</table>

**Delayed-release dosage forms**

**Acid stage.** Unless otherwise stated in the individual monograph the requirements of this part of the test are met if the quantities, based on the percentage of the labelled content of active ingredient(s) dissolved from the dosage units tested conform to Table 3. Continue testing through the three levels unless the results of both acid and buffer stages conform at an earlier level.

Table 3

<table>
<thead>
<tr>
<th>Level</th>
<th>Samples tested</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>No individual value exceeds 10% dissolved</td>
</tr>
<tr>
<td>A2</td>
<td>6</td>
<td>Average value of the 12 dosage units (A1 + A2) is not more than 10% dissolved, and no individual value is greater than 25% dissolved</td>
</tr>
<tr>
<td>A3</td>
<td>12</td>
<td>Average value of 24 dosage units (A1 + A2 + A3) is not more than 10% dissolved, and no individual value is greater than 25% dissolved.</td>
</tr>
</tbody>
</table>

**Buffer stage.** Unless otherwise specified in the individual monograph the requirements are met if the quantities of active ingredients dissolved from the units tested conform to Table 4. Continue testing through the three levels unless the results of both stages conform at an earlier level. The value of Q in Table 4 is the specified total amount of API dissolved in both the acid
and buffer stages, expressed as a percentage of the labelled content. The 5%, 15% and 25% values in the table are percentages of the labelled content so that these values and Q are in the same terms.

**Table 4**

<table>
<thead>
<tr>
<th>Level</th>
<th>Samples tested</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>6</td>
<td>No value is less than Q + 5%</td>
</tr>
<tr>
<td>B₂</td>
<td>6</td>
<td>Average value of the 12 dosage units (B₁ + B₂) is equal to or greater than Q, and no unit is less than Q - 15%</td>
</tr>
<tr>
<td>B₃</td>
<td>12</td>
<td>Average value of the 24 dosage units (B₁ + B₂ + B₃) is equal to or greater than Q; not more than 2 units are less than Q - 15%, and no unit is less than Q - 25%</td>
</tr>
</tbody>
</table>

**Monographs of *The International Pharmacopoeia***

The following additional statements apply to the individual monographs of *The International Pharmacopoeia*.

**Performance of dissolution test equipment**

Periodically qualify the equipment utilizing an “enhanced mechanical calibration” such as the procedure described in the international standard procedure ASTM 2503-07 or a combination of a mechanical calibration to determine conformance of the dissolution tester to the dimensions and tolerances as given above and the analysis of suitable reference tablets to verify the performance of the test assembly.

**Test conditions**

The following specifications are given in the individual monographs:

- the apparatus to be used;
- the composition and volume of the dissolution medium;
- the rotation speed of the paddle or basket;
- the preparation of the test and reference solutions;
- the time, the method and the amount for sampling of the test solution or the conditions for continuous monitoring;
- the method of analysis;
- the limits of the quantity or quantities of active pharmaceutical ingredient(s) required to dissolve within a prescribed time.

**Dissolution media**

If a buffer is added to the dissolution medium adjust its pH to within ± 0.05 units of the prescribed value.

In specific cases, and subject to approval by the relevant regional or national authority, dissolution media may contain enzymes and/or surfactants. The addition of enzymes may be considered, for example, for formulations containing gelatin in the outer layer when dissolution failures can be ascribed to the cross-linking of this excipient (e.g. hard and soft
gelatin capsules). For the testing of preparations containing poorly aqueous-soluble active substances modification of the medium may be necessary. In such circumstances a low concentration of surfactant may be prescribed.

Below are some examples of dissolution media.

- **Dissolution buffer pH 1.3, TS**
  Dissolve 2 g of sodium chloride R in 800 mL of water R, adjust the pH to 1.3 with hydrochloric acid (~70 g/L) TS and dilute to 1000 mL with water R.

- **Dissolution buffer pH 2.5, TS**
  Dissolve 2 g of sodium chloride R in 800 mL of water R, adjust the pH to 2.5 with hydrochloric acid (~70 g/L) TS and dilute to 1000 mL with water R.

- **Dissolution buffer pH 3.5, TS**
  Dissolve 7.507 g of glycine R and 5.844 g of sodium chloride R in 800 mL of water R, adjust the pH to 3.5 with hydrochloric acid (~70 g/L) TS and dilute to 1000 mL with water R.

- **Dissolution buffer pH 4.5, TS1**
  Dissolve 2.99 g of sodium acetate R in 900 mL of water R, adjust the pH to 4.5 by adding about 14 mL of acetic acid (~120 g/L) TS and dilute to 1000 mL with water R.

- **Dissolution buffer pH 4.5, TS2**
  Dissolve 6.8 g of potassium dihydrogen phosphate R in 900 mL of water R, adjust the pH to 4.5 either with hydrochloric acid (~70 g/L) TS or sodium hydroxide (~80 g/L) TS and dilute to 1000 mL with water R.

- **Dissolution buffer, pH 6.8, TS**
  Dissolve 6.9 g of sodium dihydrogen phosphate R, 0.9 g of sodium hydroxide R and 2.5 g of sodium dodecyl sulfate R in 800 mL of water R, adjust the pH to 6.8 with sodium hydroxide (~80g/L) TS and dilute to 1000 mL with water R.

- **Dissolution buffer, pH 6.8, 0.25% SDS TS**
  Dissolve 6.9 g of sodium dihydrogen phosphate R, 0.9 g of sodium hydroxide R and 2.5 g of sodium dodecyl sulfate R in 800 mL of water R, adjust the pH to 6.8 with sodium hydroxide (~80g/L) TS and dilute to 1000 mL with water R.

- **Dissolution buffer pH 7.2, TS**
  Dissolve 9.075 g of potassium dihydrogen phosphate R in water R to produce 1000 mL (solution A). Dissolve 11.87 g of disodium hydrogen phosphate R in sufficient water R to produce 1000 mL (solution B). Mix 300 mL of solution A with 700 mL of solution B.

- **Gastric fluid, simulated, TS**
  Dissolve 2.0 g of sodium chloride R and 3.2 g of pepsin R in 7.0 mL of hydrochloric acid (~420 g/L) TS and sufficient water R to produce 1000 mL. This test solution has a pH of about 1.2.

- **Intestinal fluid pH 6.8, simulated, TS**
  Mix 77.0 mL of sodium hydroxide (0.2 mol/L) VS, 250.0 mL of a solution containing 6.8 g potassium dihydrogen phosphate R and 500 mL of water R. Add 10.0 g pancreatin R, mix and adjust pH to 6.8 ± 0.1. Dilute to 1000 mL with water R.

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