CLAVULANATE POTASSIUM

(KALII CLAVULANAS)

Revised draft proposed monograph for The International Pharmacopoeia

(February 2018)

DRAFT FOR COMMENT

Should you have any comments on this draft, please send these to Dr Herbert Schmidt, Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or email: schmidt@who.int by 13 April 2018.

In order to speed up the process for receiving draft monographs and for sending comments, please let us have your email address (to bonnyw@who.int) and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/16.681:

**Clavulanate potassium**
*(Kalii clavulanas)*

<table>
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<tr>
<td>Revision drafted taking into consideration specifications and tests published other pharmacopoeias and the scientific literature</td>
<td>July 2016</td>
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<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
<td>October 2016</td>
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<tr>
<td>Laboratory investigations to validate and verify the laboratory investigations</td>
<td>July 2016 to September 2017</td>
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<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
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<tr>
<td>Draft revision sent out for public consultation</td>
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<td>Further follow-up action as required</td>
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Clavulanate potassium

(Kalii clavulanas)

[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

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

Description. A white or almost white, crystalline powder.

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

Category. β-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; $[\alpha]_{D}^{0} = +53$ 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

¹ A Waters Atlantis T3 column was found suitable.
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.

<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>
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<tbody>
<tr>
<td>0–4</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>4–15</td>
<td>100 to 50</td>
<td>0 to 50</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>15–18</td>
<td>50</td>
<td>50</td>
<td>Isocratic</td>
</tr>
<tr>
<td>18–19</td>
<td>50 to 100</td>
<td>50 to 0</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>19–30</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

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-methylpentan-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; tetramethylethylene diamine R; 1,1,3,3-tetramethylbutylamine R; N,N'-diisopropylethylenediamine 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-methylpentan-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 octadecysilyl groups (10 µm).²

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

**Impurities**

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

B. 3-[3,6-bis(2-hydroxyethyl)pyrazin-2-yl]proanoic 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, \(N, N', N', N'\) -
tetramethylethylenediamine)

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

L. \(N, N'\)-diisopropylenethane-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

\[ \text{C}_9\text{H}_{16}\text{O}_2 \]

*Molecular weight.* 156.2.

*Description.* Clear liquid.

*Relative density* \( \rho_{20} \). About 0.998.

*Boiling point.* About 130 °C.

\[ \text{N,N'-Diisopropylethylene diamine R} \]

\[ \text{C}_8\text{H}_{20}\text{N}_2 \]

*Molecular weight.* 144.3.

*Other name.* N,N’-Bis(1-methylethyl)-1,2-ethanediameine.

*Description.* Colourless or yellowish, hygroscopic liquid, corrosive, flammable.

*Relative density* \( \rho_{20} \). About 0.798.

*Boiling point.* About 170 °C.

\[ \text{1,1-Dimethylethylamine R} \]

\[ \text{C}_4\text{H}_{11}\text{N} \]

*Molecular weight.* 73.1.

*Other names.* 2-Amino-2-methylpropane, tert-Butylamine.

*Description.* Liquid, miscible with ethanol (~710 g/L) TS.

*Relative density* \( \rho_{20} \). About 0.694.

*Boiling point.* About 46 °C.

\[ \text{2-Ethylhexanoic acid R} \]

\[ \text{C}_8\text{H}_{16}\text{O}_2 \]

*Molecular weight.* 144.2.
Description. Colourless liquid.

Relative density $d_{20}^{20}$. 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-nitroterephthalate R

Macrogol 20000 R modified by treating with 2-nitroterephthalate acid.

Description. A hard, white or almost white, waxy solid.

Solubility. Soluble in acetone R.

3-Methylpentan-2-one R

C$_6$H$_{12}$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$_4$H$_{10}$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

$C_8H_{16}N_2$
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 $\rho_{20}^{20}$. About 0.78.

Boiling point. About 121 °C.