BENZATHINE BENZYLPCNILLIN FOR INJECTION
(BENZATHINI BENZYLPCNILLINI AD INJECTIONEM)

Draft proposal for inclusion in The International Pharmacopoeia
(October 2021)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to Dr Herbert Schmidt, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidt@who.int), with a copy to Ms Sinéad Jones (jonessi@who.int) by 4 December 2021.

Our working documents are sent out electronically and they will be placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects) for comments under the “Working documents in public consultation” link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

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Please send any request for permission to: Ms Sinéad Jones, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications, Department of Health Products Policy and Standards, World Health Organization, CH-1211 Geneva 27, Switzerland, email: jonessi@who.int.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.880:

BENZATHINE BENZYLПENICILLIN FOR INJECTION
(BENZATHINI BENZYLПENICILLINI AD INJECTIONEM)

<table>
<thead>
<tr>
<th>Description</th>
<th>Date</th>
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<tbody>
<tr>
<td>Monograph drafted based on information found in the scientific literature, submitted by manufacturers and on laboratory investigations.</td>
<td>March 2021</td>
</tr>
<tr>
<td>Discussion at the Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications.</td>
<td>May 2021</td>
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<tr>
<td>Monograph sent out for public consultation.</td>
<td>October – December 2021</td>
</tr>
<tr>
<td>Further follow-up action as required.</td>
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</tbody>
</table>

[Note from WHO Secretariat. It is proposed to include the monograph on Benzathine benzylpenicillin for injection in The International Pharmacopoeia.]

The monograph is based on information found in the scientific literature, submitted by manufacturers and on laboratory investigations.

The monograph is expected to play an important role in ensuring access to safe, effective and quality assured BENZATHINE BENZYLПENICILLIN FOR INJECTION. Manufacturers of this product, regulatory authorities, procurement agencies and other stakeholders are therefore invited to provide their feedback to the Secretariat of The International Pharmacopoeia.

If not already done, manufacturers are also invited to submit information and samples of their products. With their support, manufacturers will help ensure that the proposed monograph adequately controls the quality of the products they
manufacture. For further information, please contact Dr Herbert Schmidt at

schmidt@who.int.
BENZATHINE BENZYLПENICILLIN FOR INJECTION
(BENZATHINI BENZYLПENICILLINI AD INJECTIONEM)


Storage. Benzathine benzylpenicillin should be kept in a hermetically closed container, protected from light, and stored at a temperature not exceeding 30 °C.

Labelling. The designation on the container of Benzathine benzylpenicillin for injection should indicate the amount of Benzathine benzylpenicillin both in mass and activity units.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 900 mg benzylpenicillin (about 1.2 million IU) in 5 mL vial and 1.44 g benzylpenicillin (about 2.4 million IU) in 5 mL vial. Additional strength mentioned on the 9th Invitation to Manufacturers of Reproductive Health Products to Submit an Expression of Interest (EOI) for Product Evaluation to the WHO Prequalification Team: medicines: benzathine benzylpenicillin 150,000 units in vial.

Requirements

The powder for injection and the reconstituted injection comply with the monograph for Parenteral preparations.

Definition. Benzathine benzylpenicillin for injection contains not less than 90.0% and not more than 110.0% of benzathine benzylpenicillin ((C₁₆H₁₈N₂O₄S)₂,C₁₆H₂₀N₂).

Manufacture. In the manufacture of preparations containing dispersed particles of Benzathine benzylpenicillin tetrahydrate for intramuscular injection, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.
Identity tests

- Either test A or test B may be applied.

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 two principal peaks in the chromatogram obtained with solution (1) corresponds to the retention times of the peak due to benzathine and benzylpenicillin obtained with solution (2).

C. Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R5 as the coating substance and a freshly prepared mixture of acetone R and a solution of 154 g/L of ammonium acetate R, adjusted to pH 7.0 with ammonia R, (30:70 V/V) as the mobile phase. Apply separately to the plate 1 µL of each of the following two solutions in methanol R: For solution (A), dissolve a quantity of the mixed contents nominally containing 25.0 mg of the Benzathine benzylpenicillin in 5 mL. For solution (B), dissolve 25.0 mg of benzathine benzylpenicillin RS in 5 mL. Develop the plate for 2/3 of its length. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of air. Expose the plate to iodine vapour until the spots appear and examine in daylight. The test is not valid unless the chromatogram obtained with solution (B) shows two clearly separated spots. The two principal spots in the chromatogram obtained with solution (A) correspond in position, appearance and intensity with those in the chromatogram obtained with solution (B).

Related substances. Prepare the solutions immediately before use and by diluting to volume immediately after dissolution of the test and reference substance. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (4.6 mm x 15 cm) packed with end-capped particles of silica gel,
the surface of which has been modified with chemically-bonded octadecylsilyl groups (3 µm).\(^1\)

Use the following conditions for gradient elution:

- mobile phase A: 10 volumes phosphate buffer pH 3.3, 30 volumes of methanol R, and 60 volumes of water R;
- mobile phase B: 5 volumes phosphate buffer pH 3.3, 70 volumes of methanol R, and 25 volumes of water R.

Prepare the phosphate buffer pH 3.3 by dissolving 34 g of potassium dihydrogen phosphate R in 900 mL of water R. Adjust to pH 3.3 with phosphoric acid (~1440 g/L) TS and dilute to 1000 mL with water R.

<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>0–2</td>
<td>85</td>
<td>15</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2–16</td>
<td>85 to 0</td>
<td>15 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>16–26</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>26–27</td>
<td>0 to 85</td>
<td>100 to 15</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>27–40</td>
<td>85</td>
<td>15</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 220 nm. Maintain the column temperature at 50 °C.

Prepare as a diluent a solution containing 1.3 g/L of disodium hydrogen phosphate R and 6.8 g/L of potassium dihydrogen phosphate R in water R.

\(^1\) A YMC-Pack ODS-A and a Waters Atlantis T3 column were found suitable.
Prepare the following solutions:

For solution (1), transfer a quantity of the mixed contents nominally containing 70.0 mg of the Benzathine benzylpenicillin to a 50.0 mL flask, add 25 mL of methanol R and shake to dissolve. Dilute to volume with the diluent and filter. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL with a mixture of equal volumes of methanol R and the diluent. For solution (3), dilute 1.0 mL of solution (2) to 10.0 mL with a mixture of equal volumes of methanol R and the diluent. For solution (4), dissolve 3 mg of benzathine benzylpenicillin for peak identification RS (containing benzathine benzylpenicillin and the impurities A, B, C, D, E, F, G, H, I, J and K) in 1 mL of methanol R and dilute to 2 mL with the diluent.

Inject 20 µL each of solutions (1), (2), (3) and (4).

Use the chromatogram supplied with benzathine benzylpenicillin for peak identification RS and the chromatogram obtained with solution (4) to identify the peaks due to benzylpenicillin, benzathine and the impurities C, E, F, J and K in the chromatogram obtained with solution (1). Benzathine and the impurities, if present, are eluted at the following relative retentions with reference to benzylpenicillin (retention time about 7 minutes): impurity A about 0.18; benzathine about 0.30; impurity D about 0.36; impurity G about 0.38; impurity J about 0.44; impurity E about 0.51 and 0.60; impurity B about 0.69; impurity F about 0.84 and 0.88; impurity H about 1.22; impurity I about 1.42; impurity C about 1.75; and impurity K about 2.90.

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution between the peaks due to the epimers of impurity F is at least 1.0 and between the peaks due to the impurities D and G at least 1.5. Also, the test is not valid unless, in the chromatogram obtained with solution (3), the peak due to benzylpenicillin is obtained with a signal-to-noise ratio of at least 20.

In the chromatogram obtained with solution (1):
the sum of the areas of any peaks corresponding to impurity E (the sum of the isomers), when multiplied with a correction factor of 1.9, is not greater than 3 times the sum of the areas of the peaks due to benzathine and benzylpenicillin in the chromatogram obtained with solution (3) (0.3 %);

- the sum of the areas of any peaks corresponding to impurity F (the sum of the epimers), when multiplied with a correction factor of 1.5, is not greater than 3 times the sum of the area of the peaks due to benzathine and benzylpenicillin in the chromatogram obtained with solution (3) (0.3 %);

- the area of any peak corresponding to impurity C is not greater than 2 times the sum of the areas of the peaks due to benzathine and benzylpenicillin in the chromatogram obtained with solution (2) (2.0 %);

- the area of any peak corresponding to impurity K is not greater than the sum of the areas of the peaks due to benzathine and benzylpenicillin in the chromatogram obtained with solution (2) (1.0 %);

- the area of any peak corresponding to impurity J is not greater than 0.5 times the sum of the areas of the peaks due to benzathine and benzylpenicillin in the chromatogram obtained with solution (2) (0.5 %);

- the area of any other impurity peak is not greater than twice the sum of the areas of the peaks due to benzathine and benzylpenicillin in the chromatogram obtained with solution (3) (0.2 %).

- The sum of the areas of all impurity peaks, including the corrected areas of any peaks corresponding to impurities E and F, is not greater than 3.5 times the sum of the areas of the peaks due to benzathine and benzylpenicillin in the chromatogram obtained with solution (2) (3.5%). Disregard all peaks with an area of less than 0.5 times the sum of the areas of the peaks due to benzathine benzylpenicillin in the chromatogram obtained with solution (3) (0.05%). Disregard also the peak due to benzathine.

Assay. Prepare the solutions immediately before use and by diluting to volume immediately after dissolution of the test and reference substance. Carry out the test as
described under 1.14.4 High-performance liquid chromatography, using the conditions given above under “Related substances” with the following modifications:

As the mobile phase, use a mixture of 15 volumes of mobile phase B and 85 volumes of mobile phase A.

Prepare the following solutions: for solution (1), determine the weight of the contents of 20 containers. Transfer a quantity of the mixed contents nominally containing 40.0 mg of the Benzathine benzylpenicillin to a 100.0 mL flask, add 50 mL of methanol R and shake to dissolve. Dilute to volume with the diluent and filter. For solution (2), dissolve 40.0 mg of benzathine benzylpenicillin RS in 50 mL of methanol R, dilute to 100.0 mL with the diluent.

Inject 20 µL each of solutions (1) and (2) and record the chromatogram for 30 minutes. The substances are eluted in the order: benzathine, benzylpenicillin. Measure the areas of the peaks corresponding to benzathine (C₁₆H₂₀N₂) and to benzylpenicillin (C₁₆H₁₈N₂O₄S) obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of benzathine benzylpenicillin ((C₁₆H₁₈N₂O₄S)_2,C₁₆H₂₀N₂) per sealed container, using the declared contents of benzathine benzylpenicillin ((C₁₆H₁₈N₂O₄S)_2,C₁₆H₂₀N₂) in benzathine benzylpenicillin RS.

**Bacterial endotoxins.** Carry out the test as described under 3.4 Test for bacterial endotoxins; contains not more than 0.01 IU of endotoxin RS per mg of benzathine benzylpenicillin.

**Impurities**

The impurities limited by the requirements of this monograph include those listed in the monograph on Benzathine benzylpenicillin.

**Reference substances evoked in the monograph**

**Benzathine benzylpenicillin for peak identification RS**
It is intended to refer to the corresponding reference substance established by the European Pharmacopoeia.

**Benzathine benzylpenicillin RS**

ICRS to be established.

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