ARTEMISININ

Draft for proposed revision for *The International Pharmacopoeia*  
(July 2012)

*DRAFT FOR COMMENT*

Should you have any comments on the attached proposed revision, please send these to Dr Herbert Schmidt, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mail: schmidt@who.int (with a copy to gaspardm@who.int) by 7 September 2012.

We are now sending out our working documents electronically and they are also placed on the Medicines web site for comment. If you do not already receive our draft monographs please let us have your e-mail address (to bonnyw@who.int) and we will add it to our electronic mailing list.
**SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/12.493**

*Proposed revision of monograph on Artemisinin*

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<th>Event</th>
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<tr>
<td>Draft revision prepared by expert</td>
<td>April 2012</td>
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<tr>
<td>Discussion of revision at consultation on specifications for <em>The International Pharmacopoeia</em> and quality control issues</td>
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<tr>
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<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for discussion</td>
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<td>Further follow-up action as required</td>
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</table>
PROPOSED REVISION

[Note from the secretariat: The proposed revision deals primarily with the high-performance liquid chromatography (HPLC) tests for related substances and assay. The HPLC tests conditions are changed according to the adopted “Tests and specifications for artemisinin starting material” in the “Recommendations for quality requirements when artemisinin is used as a starting material in the production of antimalarial active pharmaceutical ingredients”.

Comments are sought in particular as to whether reference to acetonitrile for chromatography and water for chromatography grade reagents are necessary or whether the general statement is sufficient in chapter 1.14.4 High-performance liquid chromatography: “If an ultraviolet detector is employed, the solvents used for the preparation of the mobile phase should be free of stabilizers and transparent at the wavelength of detection.”.

Proposed modifications indicated by text deleted and text inserted.]

Artemisininum - Artemisinin

C_{15}H_{22}O_{5}

Relative molecular mass. 282.3

Chemical name. (3R,5aS,6R,8aS,9R,12S,12aR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyran[4,3-j]-1,2-benzodioxepin-10(3H)-one; CAS Reg. No. 63968-64-9.

[Note from the secretariat: The chemical name to be confirmed.]

Description. Colourless needles or white crystalline powder.

Solubility. Practically insoluble in water; very soluble in dichloromethane R; freely soluble in acetone R and ethyl acetate R; soluble in glacial acetic acid R, methanol R and ethanol (~750 g/l) TS.

Category. Antimalarial drug.

Storage. Artemisinin should be kept in a well-closed container and protected from light.

Requirements

Artemisinin contains not less than 97.0% and not more than the equivalent of 102.0% of C_{15}H_{22}O_{5}, using Assay method A, and not less than 98.0% and not more than the
equivalent of 102.0% of C₁₅H₂₂O₅ using Assay method B, both calculated with reference to the dried substance.

Identity tests

• Either test A alone 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 artemisinin RS or with the reference spectrum of artemisinin.

B. See the test described below under "Related substances test B". The principal spot obtained with solution D corresponds in position, appearance, and intensity with that obtained with solution E.

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following two solutions in dichloromethane R. For solution (A) use 0.1 mg of the test substance per ml. For solution (B) use 0.1 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air or in a current of cool air. Spray with anisaldehyde/sulfuric acid TS, and heat the plate to 105 °C for about 7 minutes. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance and intensity with that obtained with solution B.

[Note from the secretariat: The procedure for test B is the same as the thin-layer chromatography (TLC) test for related substances in the current monograph. Since it is proposed to delete the latter test in this monograph revision, a full description under Identity tests is required. The test needs to be confirmed.]

C. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add about 0.5 ml of hydroxylamine hydrochloride TS2 and 0.25 ml of sodium hydroxide (~80 g/l) TS. Heat the mixture in a water-bath to boiling, cool, add 5 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a deep violet colour is immediately produced.

D. Dissolve 5 mg in about 0.5 ml of dehydrated ethanol R, add 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS, allow to stand for one minute and add 4 drops of starch TS; a violet colour is immediately produced.

Melting range. 151 – 154°C.

[Note from the secretariat: It is proposed that the melting range test be omitted, since it is not adding value.]

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R and calculate with reference to the dried substance:
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$[^{o}D]_{20^oC} = +75^o$ to $+78^o$

**Sulfated ash.** Not more than 1.0 mg/g.

**Loss on drying.** Dry to constant mass at 80 °C; it loses not more than 5.0 mg/g.

**Related substances**

- 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 a stainless steel column (10 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (µm). The mobile phases for gradient elution consist of a mixture of acetonitrile and water, using the conditions shown in the following table:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v of acetonitrile)</th>
<th>Mobile phase B (% v/v of water)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 17</td>
<td>60</td>
<td>40</td>
<td>Isocratic</td>
</tr>
<tr>
<td>17 – 30</td>
<td>60 → 100</td>
<td>40 → 0</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30 – 35</td>
<td>100 → 60</td>
<td>0 → 40</td>
<td>Return to initial conditions</td>
</tr>
<tr>
<td>35 – 45</td>
<td>60</td>
<td>40</td>
<td>Isocratic – re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions. For solution (A) use 10 mg of Artemisinin per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water, and for solution (B) use 50 µg of Artemisinin per ml in a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

For the system suitability test prepare solution (C) containing 1 mg of artemisinin RS per ml and 1 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of the related substances as a percentage. In the chromatogram obtained with solution A, the area of any peak, other than the principal peak, is not greater than that obtained with solution B (0.5%). Not more than one peak is greater than half the area of the principal peak obtained with solution B (0.25%). The sum of the areas of all peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution B (1.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.
Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given below under Assay.

Use solutions (1) and (2) as described below under Assay. For solution (3) dilute 1 ml of solution (1) to 100 ml with the mobile phase.

Inject separately 20 µl of solutions (1), (2) and (3). Record the chromatograms for about 1.5 times the retention time of artemisinin. In the chromatogram obtained with solution (2), impurity A (artemisitene) is eluted at the relative retention of about 0.79 with reference to artemisinin (retention time about 10 minutes). The test is not valid unless the resolution factor between the peaks due to impurity A and artemisinin is at least 4. The chromatogram obtained with solution (1) may show a peak due to impurity B eluting at a relative retention of about 0.85 with reference to artemisinin.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 0.027 is not greater than 0.15 times the area of the principal peak in the chromatogram obtained with solution (3) (0.15%);

- the area of any peak corresponding to impurity B is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (3) (0.3%);

- the area of any other peak, other than the principal peak, is not greater than 0.15 times the area of the principal peak in the chromatogram obtained with solution (3) (0.15%);

- the sum of the corrected area of any peak corresponding to impurity A and the areas of all other peaks, other than the principal peak, is not greater than the area of the principal peak obtained with solution (3) (1.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (3) (0.05%).

B. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R1 as the coating substance and a mixture of equal volumes of light petroleum R1 and ether R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in toluene R containing (A) 10 mg of Artemisinin per ml, (B) 0.05 mg of Artemisinin per ml, (C) 0.025 mg of Artemisinin per ml, (D) 0.10 mg of Artemisinin per ml, and (E) 0.10 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/ sulfuric acid TS, and heat the plate to 105 °C for 7 minutes. Examine the chromatogram in daylight.

Any spot obtained with solution A, other than the principal spot, is not more intense than that obtained with solution B (0.5%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.25%).

Assay

- Either method A or method B may be applied.
A. Determine by 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 octadecylsilyl groups (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following solutions in the mobile phase: solution (A) 1.0 mg of Artemisinin per ml, and solution (B) 1.0 mg of artemisinin RS per ml.

For the system suitability test prepare solution (C) containing 1 mg of artemisinin RS per ml and 1 mg of artenimol RS per ml in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

Operate with a flow rate of 0.6 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject alternately 20 µl each of solutions A, B, and C.

The test is not valid unless the relative retention of α-artenimol compared with artemisinin is about 0.6, and the resolution between the peaks is not less than 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of \( C_{15}H_{22}O_5 \) with reference to the dried substance.

Carry out the test as described under 1.14.4 “High performance liquid chromatography”, using a stainless steel column (15 cm × 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm). As the mobile phase use a mixture of equal volumes of acetonitrile for chromatography R and water for chromatography R.

Prepare the following solutions in the mobile phase. For solution (1) use 5.0 mg of the test substance per ml. For solution (2) use 5.0 mg of artemisinin RS per ml.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 210 nm.

Inject separately 20 µl of solutions (1) and (2). Record the chromatograms for about 1.5 times the retention time of artemisinin. In the chromatogram obtained with solution (2), impurity A (artemisitene) is eluted at the relative retention of about 0.79 with reference to artemisinin (retention time about 10 minutes). The test is not valid unless the resolution factor between the peaks due to impurity A and artemisinin is at least 4. The chromatogram obtained with solution (1) may show a peak due to impurity B eluting at a relative retention of about 0.85 with reference to artemisinin.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the percentage content of \( C_{15}H_{22}O_5 \) with reference to the dried substance.

B. Dissolve about 0.05 g of Artemisinin, accurately weighed, in sufficient ethanol (~750 g/l) TS to produce 100 ml, and dilute 10 ml to 100 ml with the same solvent. Accurately transfer 10 ml to a 50-ml volumetric flask, dilute to volume with sodium hydroxide (0.05 mol/l) VS, mix thoroughly, and warm to 50 °C in a water-bath for 30 minutes. Cool to room temperature.
Measure the absorbance of a 1-cm layer at the maximum at about 292nm against a solvent cell containing a blank prepared with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml. Calculate the percentage content of $\text{C}_{15}\text{H}_{22}\text{O}_{5}$ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined, and with reference to the dried substance.

**Impurities**

The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information.

A. 

(3$R$.5$a$S,6$R$,8$a$S,12$S$,12$a$R)-3,6-dimethyl-9-methylideneoctahydro-3,12-epoxypyrano[4,3-$j$]-1,2-benzodioxepin-10(3$H$)-one (artemisitene)

B. 

(3$R$.5$a$S,6$R$,8$a$S,9$S$,12$S$,12$a$R)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12$H$-pyrano[4,3-$j$]-1,2-benzodioxepin-10(3$H$)-one (9-$e$pi-artemisinin)

*[Note from the secretariat: The chemical names of related substances A and B to be confirmed.]*

New reagents to be added:

**Acetonitrile for chromatography R**

Chromatographic grade of acetonitrile R containing at least 99.8% of $\text{C}_{2}\text{H}_{3}\text{N}$ that complies with the following test

**Transmittance.** At least 98% at 240 nm using water R in the reference cell.

**Water for chromatography R**

Deionised water R with a resistivity of at least 0.18 Mohm.

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