



ARTESUNATE TABLETS

Draft revision for *The International Pharmacopoeia*
(September 2009)

DRAFT FOR COMMENT

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/09.341

Revision of International Pharmacopoeia monograph on Artesunate tablets

	Date
Principles of general revision of published monographs on artemisinin derivatives agreed by WHO Expert Committee on Specifications for Pharmaceutical Preparations	13-17 October 2008
Progress of general revision of published monographs on artemisinin derivatives discussed in Consultation on Specifications for Medicines and Quality Control Laboratory Issues	23-26 June 2009
Preliminary draft revised monograph prepared by contract laboratory	August 2009
Mailing of draft monograph for comments	September 2009
Collation of comments received	October 2009
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations for discussion	12-16 October 2009
Further follow-up action as required	November 2009 - ...

Explanatory note:

Suggested text for deletion in “~~strikethrough~~”.

Suggested text to be added or changed is underlined.

Proposed revision

[*Note from Secretariat: The proposed revision deals mainly with the HPLC tests for related substances and assay.*]

Artesunati compressi **Artesunate tablets**

Category. Antimalarial ~~drug~~.

Storage. Artesunate tablets should be kept in a well-closed container.

Additional information. Strength in the current WHO Model list of essential medicines: 50 mg. Strength in the current WHO Model list of essential medicines for children: 50 mg.

Requirements

Comply with the monograph for "Tablets".

Artesunate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of artesunate ($C_{19}H_{28}O_8$) stated on the label.

Identity tests

- Either test A alone or tests B, C, and D may be applied.
- A. To a quantity of the powdered tablets containing 0.050 g of Artesunate add 25 ml of acetone R, shake ~~to dissolve~~, and filter. Evaporate the filtrate at low temperature and dry overnight over desiccant silica gel R. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from artesunate RS or with the *reference spectrum* of artesunate.
- 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 5 volumes of ethyl acetate R and 95 volumes of toluene R as the mobile phase. Apply separately to the plate 2 μ l of the following 2 solutions in toluene R. For solution (A) shake a

~~quantity of the powdered tablets containing about 0.10 mg of Artesunate in dehydrated ethanol R, filter, and evaporate. Dissolve the residue in 1.0 ml of toluene R. 0.5 mg of Artesunate with 5 ml, filter and use the clear filtrate. For solution (B) use 0.10 mg of artesunate RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with anisaldehyde/methanol TS, and heat the plate to 120°C for 5 minutes. Examine the chromatogram in ultraviolet light (254 nm).~~

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

- C. To a quantity of the powdered tablets containing 0.1 g of Artesunate add 40 ml of dehydrated ethanol R, shake ~~to dissolve~~ for a few minutes, and filter. To half of the filtrate (keep the remaining filtrate for test D) 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 2 drops of hydrochloric acid (~70 g/l) TS and 2 drops of ferric chloride (50 g/l) TS; a light red-violet colour is produced.
- D. Evaporate the remaining filtrate from test C on a water-bath to a volume of about 5 ml. Place a few drops of the mixture on a white porcelain dish, add 1 drop of vanillin/sulfuric acid TS1, a reddish-brown colour is produced.

Related substances

[*Note from the Secretariat:*

- *It is proposed that the TLC method be omitted*
- *HPLC chromatographic system has been changed to allow separation of the β -artenimol peak*
- *limits for related substances have been modified (3 related substances are now specified, and a separate limit for the unknowns is now given).]*

~~• 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 below under Assay method A.~~

~~Inject alternately 20 μ l each of solutions A and C.~~

~~Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, 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 C (1.0%). Not more than one peak is greater than half the area of the principal peak obtained with solution C (0.5%). The sum of the areas of all the peaks, other than the principal peak, is not greater than twice the area of the principal peak obtained with solution C (2.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.~~

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the conditions given below under Assay method A.

Use solutions (1) and (3) as described below under Assay method A. For solution (4) dilute 1 ml of solution (1) to 100 ml with acetonitrile R. For solution (5) shake or sonicate a mixture of suitable amounts of each of the excipients stated on the label for 15 minutes with 10 ml acetonitrile, filter through a 0.45- μ m filter and use the filtrate.

Operate with a flow rate of 1.0 ml per minute. Maintain the column temperature at 30°C and use as detector an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject separately 20 μ l each of solutions (1), (3), (4) and (5). Record the chromatograms for about 4 times the retention time of artesunate. In the chromatogram obtained with solution (3), the following peaks are eluted at the following relative retention with reference to artesunate (retention time about 9 minutes): α -artemimol about 0.58, β -artemimol about 0.91 and impurity B (artemisinin) about 1.30. The assay is not valid unless the resolution factor between the peaks due to β -artemimol and artesunate is at least 1. The chromatogram obtained with solution (1) may show a peak due to impurity C eluting at a relative retention of about 2.7 with reference to artesunate.

In the chromatogram obtained with solution (1)

- the combined areas of any peaks corresponding to α -artemimol and β -artemimol (impurity A) are not greater than 3 times the area of the principal peak obtained with solution (4) (3.0%);

- the area of any peak corresponding to impurity B (artemisinin) is not greater than 0.5 times the area of the principal peak obtained with solution (4) (0.5%);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 0.07, is not greater than 0.3 times the area of the principal peak obtained with solution (4) (0.3%);
- the area of any other peak, other than the principal peak, is not greater than 0.3 times the area of the principal peak in the chromatogram obtained with solution (4) (0.3%);
- The sum of the corrected area of any peak corresponding to impurity C and the areas of all other peaks, other than the principal peak, is not greater than 4 times the area of the principal peak obtained with solution (4) (4.0%). Disregard any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution (4) (0.1%), and any peak eluting before acetonitrile, and, if information concerning the excipients used in manufacturing of the tablets is available, disregard any peak with the same retention time as that of any of the peaks in the chromatogram obtained with solution (5).

~~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 48 volumes of light petroleum R1, 36 volumes of ethyl acetate R and 1 volume of glacial acetic acid R as the mobile phase. Apply separately to the plate 10µl of each of the following 3 solutions in dichloromethane R. For solution (A) shake a quantity of the powdered tablets equivalent to about 10mg of Artesunate with 2 ml of dichloromethane R, filter, and use the filtrate. Prepare similarly solution (B) with the equivalent of about 0.05 mg of Artesunate per ml, and solution (C) with the equivalent of about 0.025mg of Artesunate per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, and spray with vanillin/sulfuric acid TS1. 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 (1.0%). Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%).~~

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 (12.5cm × 3.5mm) packed with particles of silica gel, the surface of which has been modified with chemically bonded octadecylsilyl groups (5µm). As the mobile phase, use a mixture of equal volumes of acetonitrile R and buffer pH 3.0 (dissolve 1.36 g of potassium dihydrogen phosphate R in 1000ml of water and adjust the pH to 3.0 with phosphoric acid (~1440 g/l) TS).~~

~~Prepare the following solutions in acetonitrile R. For solution (A) weigh and powder 20 tablets, shake a quantity of the powder equivalent to about 4.0 mg of Artesunate, accurately weighed, with 2ml of acetone R, and filter. Evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml. For solution (B) use 4.0 mg of artesunate RS per ml, and for solution (C) dilute solution A to obtain a concentration equivalent to 0.04 mg of Artesunate per ml.~~

~~Operate with a flow rate of 0.6 ml per minute. Maintain the column temperature at 30 °C and use an ultraviolet spectrophotometer set at a wavelength of about 216nm.~~

~~Inject alternately 20µl each of solutions A and B.~~

~~Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the percentage content of $C_{19}H_{28}O_8$.~~

A. 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 octadecylsilyl groups (3 µm)¹. As the mobile phase, use a mixture of 44 volumes of acetonitrile R and 56 volumes of buffer pH 3.0.

Prepare the buffer pH 3.0 by dissolving 1.36 g of potassium dihydrogen phosphate R in 900 ml of water R, adjust the pH to 3.0 with phosphoric acid (~1440 g/l) TS and dilute to 1000 ml with water R.

Prepare the following solutions in acetonitrile R. For solution (1) weigh and powder 20 tablets. Shake or sonicate a quantity of the powder containing about 40 mg of Artesunate, accurately weighed, for 15 minutes with 10 ml of acetonitrile R. Filter the resulting solution through a 0.45-µm filter, discarding the first few ml of the filtrate. For solution (2) dissolve 40 mg of artesunate RS, accurately weighed, and dilute to 10 ml. For solution (3) dissolve about 1 mg of

¹ Luna[®] is suitable.

artemimol RS, about 1 mg of artemisinin RS and about 10 mg of artesunate RS in 10 ml.

Operate with a flow rate of 1.0 ml per minute. Maintain the column temperature at 30°C and use as detector an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Inject separately 20 µl each of solutions (1), (2) and (3). Record the chromatograms for about 4 times the retention time of artesunate. In the chromatogram obtained with solution (3), the following peaks are eluted at the following relative retention with reference to artesunate (retention time about 9 minutes): α-artemimol about 0.58, β-artemimol about 0.91 and impurity B (artemisinin) about 1.30. The assay is not valid unless the resolution factor between the peaks due to β-artemimol and artesunate is at least 1. The chromatogram obtained with solution (1) may show a peak due to impurity C eluting at a relative retention of about 2.7 with reference to artesunate.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1) and (2), and calculate the content of artesunate (C₁₉H₂₈O₈).

- B. Weigh and powder 20 tablets. To a quantity of the powder containing about 0.5 g of Artesunate, accurately weighed, add 50 ml of neutralized ethanol TS, shake thoroughly, filter, and discard about 10 ml of the initial filtrate. Titrate 25 ml of the filtrate with sodium hydroxide (0.05 mol/l) VS, using 2 drops of phenolphthalein/ethanol TS as indicator.

Each ml of sodium hydroxide (0.05 mol/l) VS is equivalent to 19.22 mg of C₁₉H₂₈O₈.

Dissolution. Analyse the dissolution samples without delay.

Carry out the test as described under 5.5 Dissolution test for solid oral dosage forms, using as the dissolution medium, 900 ml of dissolution buffer, pH 6.8, TS and rotating the paddle at 75 revolutions per minute. At 45 minutes withdraw a sample of 10 ml of the medium through an inline filter. Allow the filtered sample to cool to room temperature [solution (1)].

Determine the concentration in solution (1) by carrying 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 μ m)². As the mobile phase, use a mixture of equal volumes of acetonitrile R and buffer pH 3.0 (prepare the buffer as described under Assay method A).

For solution (2) dissolve 25 mg of artesunate RS, accurately weighed, in acetonitrile R and dilute to 20 ml with the same solvent. Dilute 2 ml of the resulting solution to 50 ml with acetonitrile R.

Operate with a flow rate of 1.5 ml per minute. Maintain the column temperature at 30°C and use as detector an ultraviolet spectrophotometer set at a wavelength of about 210 nm.

Inject alternately 100 μ l each of solutions (1) and (2).

For each of the six tablets tested, calculate the total amount of artesunate (C₁₉H₂₈O₈) in the medium from the results obtained. The amount in solution for each tablet is not less than 80% of the amount stated on the label. If the amount obtained for one of the six tablets is less than 80%, repeat the test using a further six tablets; the average amount for all 12 tablets tested is not less than 75% and the amount obtained for no tablet is less than 60%.

Impurities. The impurities limited by the requirements of this monograph include those listed in the monograph for Artesunate.

² Luna[®] has been found suitable.