



WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

**THE INTERNATIONAL PHARMACOPOEIA
THIRD EDITION**

**PHARMACOPOEA INTERNATIONALIS
EDITIO TERTIA**

DRAFT MONOGRAPHS FOR

ANTIMALARIAL

SUBSTANCES AND DOSAGE FORMS

Kindly address your proposals to M. Schmid, Quality Assurance, Drug Management and Policies, World Health Organization, 1211 Geneva 27, Switzerland, Fax: (0041 22) 791 47 30, or e-mail: schmidm@who.ch, to reach us at the latest by the end of March 1999.

Contents

	page
1. Introduction	2
2. Monographs	2
2.1 List of substances and dosage forms	2
2.2 Quality specifications	3
3. Reagents	28

© World Health Organization (1998)

This document is not issued to the general public, and all rights are reserved by the World Health Organization (WHO). The document may not be reviewed, abstracted, quoted, reproduced or translated, in part or in whole, without the prior written permission of WHO. No part of this document may be stored in a retrieval system or transmitted in any form or by any means – electronic, mechanical or other – without the prior written permission of WHO.

The views expressed in documents by named authors are solely the responsibility of those authors.

1. Introduction

The selection of monographs for *The International Pharmacopoeia* is determined by the WHO Model List of Essential Drugs, which is updated periodically. Additional drugs of vital importance as identified by WHO disease programmes are also included.

The first drafts of these pharmacopoeial monographs (PHARM/98.380 and PHARM/98.380/Add. 1) were circulated and discussed with a number of persons specialized in the development of antimalarial drugs and pharmacopoeial monographs. In the revised monographs the specifications were adjusted to have a more uniform approach. Some of the new data still require confirmation as noted in the text *in bold and italic*. We would appreciate receiving your comments, suggestions and possible provision of additional methods for testing with appropriate quality limits. When formulating your comments, we would be grateful if you would refer to the "Guidance for those preparing or commenting on monographs of *The International Pharmacopoeia*", as described in the two WHO Technical Report Series, Twenty-ninth and Thirty-first Reports of the WHO Expert Committee on Specifications for Pharmaceutical Preparations, No. 704, 1984, Annex 5 and No. 790, 1990, Annex 3.

The final text of the monographs will be included in volume 5 of *The International Pharmacopoeia*. References to certain tests *Extractable volume for parenteral preparations*, *Test for bacterial endotoxins* and *Visual inspection of particulate matter in injectable preparations* comply to the texts in volume 5.

Graphic formulae will be included only in the final version of the monographs.

The procedure for dissolution testing for tablets and capsules is not yet supported by specific requirements, which are under development. In the view of the Secretariat of *The International Pharmacopoeia* dissolution requirements for individual monographs should preferably be established with regard to actual biopharmaceutical characteristics of representative products on the world market. The development of such requirements is a lengthy process, calling for intense international collaboration. Until their publication it is recommended to follow limits provided by the world's leading compendia.

Additional reagents not previously mentioned in Volumes 1-4 or in the draft volume 5 of the third edition of *The International Pharmacopoeia* are given in section 3.

Acknowledgments are due to the following specialists who participated both in person and by correspondence in the preparation of these monographs: Mrs AB Moraes da Silva, Brazil; Professor Tu Guoshi, China; Mr Wang Cun Zhi, China; Professor Xiao-Yu Li, China; Mrs K. Sinivuo, Finland; M. J. P. Helenport, France; Dr JHM^cB. Miller, France; Mrs A. Sulistiowati, Jakarta, Indonesia; Dr CB. Lugt, Netherlands; Dr Ng Tju Lik, Singapore; Dr M. Krummen, Switzerland; Dr W. Scheiwe, Switzerland; Ms A. Poompanich, Thailand; Mr G. Williams, UK; Dr S. Sur, Ukraine; Dr RR. Engle, USA; Mr P. Lim, USA; Dr Pham Hoang Ngoc, Viet Nam

2. Monographs

2.1 List of substances and dosage forms

	<i>page</i>
Arteether	3
Arteether injection	5
Artemether	6
Artemether capsules	8
Artemether tablets	10
Artemether injection	12
Artemisinin	14
Artemisinin capsules	16
Artemisinin tablets	18
Artesunate	20
Artesunate tablets	22
Dihydroartemisinin	24
Dihydroartemisinin tablets	26

2.2 Quality specifications

Arteetherum

Arteether

C₁₇H₂₈O₅

Relative molecular mass. 312.4

Chemical name. [3*R*-(3*R*,5*aS*,6*S*,8*aS*,9*R*,10*R*,12*S*,12*aR*)]-Decahydro-10-ethoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin; CAS Reg. No. 75887-54-6.

Description. A white or almost white, crystalline powder.

Solubility. Practically insoluble in water; sparingly soluble in dichloromethane R, ethanol (-750 g/l) TS and methanol R; soluble in arachis oil R.

Category. Antimalarial drug.

Storage. Arteether should be kept in a well-closed container, protected from light.

Labelling. The designation on the container should state if it is intended to be used for parenteral administration.

Additional information. The title of the monograph is provisional, it is pending selection of the International Nonproprietary Name.

The parenteral form is normally intended for intramuscular administration.

Requirements

Arteether contains not less than **97.0%** and not more than the equivalent of **102.0%** of C₁₇H₂₈O₅, calculated with reference to the dried substance.

Identity tests

• *Either tests A and B, or tests B, C and D may be applied.*

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from arteether RS or with the *reference spectrum* of arteether.

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

C. To 30 mg add about 1 ml of dehydrated ethanol R, and about 0.1 g of potassium iodide R. Heat the mixture on a water-bath; a yellow colour is produced.

D. Dissolve 30 mg in 6.0 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Melting range. 81.0–84.0 °C.

→ **Confirmation of this melting range for several batches would be appreciated.**

Specific optical rotation. Dissolve 2.0 g in about 90 ml of chloroform R while holding it in a water-bath at 25 °C for 10 minutes, dilute to 100 ml with chloroform R and measure the rotation; $[\alpha]_{20}^{20} = +154^\circ$ to $+156^\circ$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); 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 "Thin-layer chromatography" (Vol. 1, p. 83), 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 Arteether per ml, (B) 0.05 mg of Arteether per ml, (C) 0.025 mg of Arteether per ml, (D) 0.10 mg of Arteether per ml, and (E) 0.10 mg of arteether 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

→ **Confirmation of this test would be appreciated.**

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using the conditions given below under Assay.

Inject separately 20 µl of solutions A and C.

Disregard any peak obtained with an area less than 0.1 time the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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 (0.5%). 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 C (1.0%).

Assay. Determine by "High performance liquid chromatography" as described under p...., using a stainless steel column (25 cm x 4 mm) packed with *stationary phase A* (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following 3 solutions in the mobile phase. For solution (A) use 10 mg of Arteether per ml, for solution (B) use 10 mg of arteether RS per ml, and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Arteether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.5 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of C₁₇H₂₈O₅, taking into account the declared content in arteether RS.

Additional requirements for Arteether for parenteral use

Complies with the monographs for "Parenteral preparations" (Vol. 4, page 36), Extractable volume for parenteral preparations (page ...), Test for bacterial endotoxins (page ...) and Visual inspection of particulate matter in injectable preparations (p....).

Arteetheri injectio

Arteether injection

Composition. Arteether injection is a sterile solution of arteether in a suitable oil for injection.

Description. A clear, colourless to slightly yellowish, oily solution.

Category. Antimalarial drug.

Storage. Arteether injection should be kept protected from light.

Labelling. The oil used in the formulation should be indicated.

Additional information. Recommended strengths: 50 mg/ml (paediatric formulation); 75-150 mg/ml (adult formulation).

Arteether injection is usually prepared for intramuscular injection.

The solution is sterilized by a suitable method (see "Methods of sterilization", Vol. 4, page 18).

Requirements

Complies with the monographs for "Parenteral preparations" (Vol. 4, page 36), Extractable volume for parenteral preparations (page ...), Test for bacterial endotoxins (page ...) and Visual inspection of particulate matter in injectable preparations (p....).

Arteether injection contains not less than **95.0%** and not more than **105.0%** of the amount of $C_{17}H_{28}O_5$ stated on the label.

Identity tests

- *Either tests A and B, or tests B, C and D may be applied.*

A. To a volume of Arteether injection equivalent to 0.050 g add 25 ml of acetone R, mix and filter. Evaporate the filtrate at low temperature, and dry overnight over silica gel, desiccant, R. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from arteether RS or with the *reference spectrum* of arteether.

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

C. *Note:* This test cannot be performed if arachis oil is present in the formulation.

To a volume of Arteether injection equivalent to about 30 mg of Arteether add about 1 ml of dehydrated ethanol R, and about 0.1 g of potassium iodide R. Heat the mixture on a water-bath; a yellow colour is produced.

D. To a volume of Arteether injection equivalent to about 30 mg of Arteether add 6 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

- *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), 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 μ l of each of the following 5 solutions in toluene R. For solution (A) dilute a volume of Arteether injection with toluene R to obtain a concentration equivalent to 10 mg of Arteether per ml. Prepare similarly solution (B) with the equivalent to about 0.05 mg of Arteether per ml, solution (C) with the equivalent to about 0.025 mg of Arteether per ml, solution (D) with the equivalent to 0.10 mg of Arteether per ml and for solution (E) use 0.10 mg of arteether 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using the conditions given below under Assay.

Inject separately 20 µl of solutions A and C.

Disregard any peak obtained with an area less than 0.1 time the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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 (0.5%). 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 C (1.0%).

Assay. Determine by "High performance liquid chromatography" as described under p. ..., using a stainless steel column (25 cm x 4 mm) packed with *stationary phase A* (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following 3 solutions in the mobile phase. For solution (A) dilute a volume of Arteether injection to obtain a concentration equivalent to 10 mg of Arteether per ml, for solution (B) use 10 mg of arteether RS per ml, and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Arteether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.5 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of C₁₇H₂₈O₅, taking into account the declared content in arteether RS.

Artemetherum

Artemether

C₁₆H₂₆O₅

Relative molecular mass. 298.4

Chemical name. [3*R*-(3*R*,5*aS*,6*S*,8*aS*,9*R*,10*R*,12*S*,12*aR***)]-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*f*]-1,2-benzodioxepin; CAS Reg. No. 71963-77-4.

Description. White crystals or a white, crystalline powder.

Solubility. Practically insoluble in water; very soluble in dichloromethane R and acetone R; freely soluble in ethyl acetate R and dehydrated ethanol R.

Category. Antimalarial drug.

Storage. Artemether should be kept in a tightly closed container, protected from light and stored in a cool place.

Labelling. The designation on the container should state if it is intended to be used for parenteral administration.

Additional information. The parenteral form is normally intended for intramuscular administration.

Requirements

Artemether contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{16}H_{26}O_5$ using assay method A and not less than **97.0%** and not more than the equivalent of **102.0%** of $C_{16}H_{26}O_5$ using assay method B, both calculated with reference to the dried substance.

Identity tests

- *Either tests A and B or tests B, C and D may be applied.*

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the *reference spectrum* of artemether.

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

C. To 30 mg add about 1 ml of dehydrated ethanol R, and about 0.1 g of potassium iodide R. Heat the mixture on a water-bath; a yellow colour is produced.

D. Dissolve 30 mg in 6.0 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Melting range. 86.0–90.0 °C.

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R; $[\alpha]_{20}^{20} = +168^\circ$ to $+173^\circ$.

Chlorides. Shake 25 mg in 25 ml of water and filter. Proceed with the filtrate as described under "Limit test for chlorides" (Vol. 1, p. 116); the chloride content is not more than 0.1 mg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); 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 "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 μ l of each of the following 5 solutions in acetone R containing (A) 10 mg of Artemether per ml, (B) 0.05 mg of Artemether per ml, (C) 0.025 mg of Artemether per ml, (D) 0.10 mg of Artemether per ml and (E) 0.10 mg of artemether RS 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

→ **Confirmation of this test would be appreciated.**

B. Carry out the test as described under "High performance liquid chromatography" 5, p. ..., using the conditions given below under Assay.

Inject separately 20 μ l of solutions A and C.

Disregard any peak obtained with an area less than 0.1 time the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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 (0.5%). 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 C (1.0%).

Assay

- *Either Method A or Method B may be applied.*

A. Dissolve about 0.050 g of Artemether, accurately weighed, in sufficient dehydrated ethanol R to produce 100 ml. Dilute 2 ml of this solution to 100 ml with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at $55 \pm 1^\circ\text{C}$ for 5 hours. Allow to cool to room temperature.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 254 nm and calculate the amount of $\text{C}_{16}\text{H}_{26}\text{O}_5$ by comparison with artemether RS, similarly and concurrently examined.

B. Determine by "High performance liquid chromatography" p. ..., using a stainless steel column (25 cm x 4 mm) packed with *stationary phase A* (5 μm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following 3 solutions in the mobile phase. For solution (A) use 10 mg of Artemether per ml, for solution (B) use 10 mg of artemether RS per ml, for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.5 ml per minute for about 30 minutes.

Inject separately 20 μl of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of $\text{C}_{16}\text{H}_{26}\text{O}_5$, taking into account the declared content in artemether RS.

Additional requirements for Arteether for parenteral use

Complies with the monographs for "Parenteral preparations" (Vol. 4, page 36), Extractable volume for parenteral preparations (page ...), Test for bacterial endotoxins (page ...) and Visual inspection of particulate matter in injectable preparations (p....).

Artemetheri capsulae

Artemether capsules

Category. Antimalarial drug.

Storage. Artemether capsules should be kept in a hermetically closed container and stored in a cool place.

Labelling. Expiry date.

Additional information. Recommended strength: 40 - 100 mg.

Requirements

Complies with the monograph for "Capsules" (see Vol. 4, page 32).

Artemether capsules contain not less than 90.0% and not more than 110.0% of the amount of $C_{16}H_{26}O_5$ stated on the label.

Identity tests

- Either tests A and B, or tests B, C, and D may be applied.

A. To a quantity of the contents of the capsules equivalent to 0.040 g of Artemether add 40 ml of acetone R, shake to dissolve and filter. Evaporate the filtrate at low temperature, and dry overnight over silica gel, desiccant, R. Carry out the examination with the residue as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the reference spectrum of artemether.

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

C. To a quantity of the contents of the capsules equivalent to 0.08 g of Artemether add 40 ml of dehydrated ethanol R, shake to dissolve and filter. To half of the filtrate (keep the remaining filtrate for test D) add 10 mg of potassium iodide R, shake and, if necessary, warm gently; a light yellow colour is produced.

→ Confirmation of this test would be appreciated.

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 and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

- Either test A or test B may be applied.

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 μ l of each of the following 5 solutions in acetone R. For solution (A) take a quantity of the contents of the capsules equivalent to about 20 mg of Artemether, add 2 ml of acetone R, shake and filter, and use the filtrate. Prepare similarly solution (B) with the equivalent to about 0.05 mg of Artemether per ml, solution (C) with the equivalent to about 0.025 mg of Artemether per ml, solution (D) with the equivalent to about 0.10 mg of Artemether per ml and for solution (E) use 0.10 mg of artemether RS 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using the conditions given below under Assay B.

Inject separately 20 μ l of solutions A and C.

Disregard any peak obtained with an area less than 0.1 time the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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 (0.5%). 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 C (1.0%).

Assay

- Either Method A or Method B may be applied.

A. Mix the contents of 20 capsules and transfer a quantity equivalent to about 13 mg of Artemether, accurately weighed, to a 100 ml volumetric flask and dilute to volume with dehydrated ethanol R. Shake the flask for 15

minutes and filter, discarding the first 10 ml of the filtrate. Accurately measure 5 ml of the clear filtrate into a 50 ml volumetric flask, and dilute to volume with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at $55 \pm 1^\circ\text{C}$ for 5 hours. Allow to cool to room temperature. For the blank use 5 ml of dehydrated ethanol R diluted with sufficient hydrochloric acid /ethanol (1 mol/l) VS to produce 50 ml.

Measure the absorbance of a 1-cm layer at the maximum at about 254 nm against a solvent cell containing the blank and calculate the content of $\text{C}_{16}\text{H}_{26}\text{O}_5$ in the capsules being examined by comparison with artemether RS, similarly and concurrently examined.

B. Determine by "High performance liquid chromatography" p. ..., using a stainless steel column (25 cm x 4 mm) packed with *stationary phase A* (5 μm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following 3 solutions in the mobile phase. For solution (A) mix the contents of 20 capsules, take a quantity equivalent to about 0.05 g of Artemether, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness, and dissolve the residue in 5 ml of the mobile phase. For solution (B) use 10 mg of artemether RS per ml, and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.5 ml per minute for about 30 minutes.

Inject separately 20 μl of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of $\text{C}_{16}\text{H}_{26}\text{O}_5$, taking into account the declared content in artemether RS.

Dissolution. (See introduction).

Artemetheri compressi

Artemether tablets

Category. Antimalarial drug.

Additional information. Recommended strength: 40-50 mg.

Requirements

Complies with the monograph for "Tablets" (see Vol. 4, page 26).

Artemether tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $\text{C}_{16}\text{H}_{26}\text{O}_5$ stated on the label.

Identity tests

• *Either tests A and B, or tests B, C, and D may be applied.*

A. To a quantity of the powdered tablets equivalent to 0.040 g of Artemether add 40 ml of acetone R, shake to dissolve and filter. Evaporate the filtrate at low temperature, and dry overnight over silica gel, desiccant, R. Carry out the examination with the residue as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the reference spectrum of artemether.

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

C. To a quantity of the powdered tablets equivalent to 0.08 g of Artemether add 40 ml of dehydrated ethanol R, shake to dissolve and filter. To half of the filtrate (keep the remaining filtrate for test D) add 10 mg of potassium iodide R, shake and, if necessary, warm gently; a light yellow colour is produced.

→ **Confirmation of this test would be appreciated.**

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 and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

• *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 µl of each of the following 5 solutions in acetone R. For solution (A) take a quantity of the powdered tablets equivalent to about 20 mg of Artemether, add 2 ml of acetone R, shake and filter, and use the filtrate. Prepare similarly solution (B) with the equivalent to about 0.05 mg of Artemether per ml, solution (C) with the equivalent to about 0.025 mg of Artemether per ml and solution (D) with the equivalent to about 0.10 mg of Artemether, and for solution (E) use 0.10 mg of artemether RS 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using the conditions given below under Assay.

Inject separately 20 µl of solutions A and C.

Disregard any peak obtained with an area less than 0.1 time the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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 (0.5%). 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 C (1.0%).

Assay

• *Either Method A or Method B may be applied.*

A. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 13 mg of Artemether, accurately weighed, to a 100 ml volumetric flask and dilute to volume with dehydrated ethanol R. Shake the flask for 15 minutes and filter, discarding the first 10 ml of the filtrate. Accurately measure 5 ml of the clear filtrate into a 50 ml volumetric flask, and dilute to volume with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at $55 \pm 1^\circ\text{C}$ for 5 hours. Allow to cool to room temperature. For the blank use 5 ml of dehydrated ethanol R diluted with sufficient hydrochloric acid/ethanol (1 mol/l) VS to produce 50 ml.

Measure the absorbance of a 1-cm layer at the maximum at about 254 nm against a solvent cell containing the blank and calculate the content of $\text{C}_{16}\text{H}_{26}\text{O}_5$ in the tablets being examined by comparison with artemether RS, similarly and concurrently examined.

B. Determine by "High performance liquid chromatography" p. ..., using a stainless steel column (25 cm x 4 mm) packed with *stationary phase A* (5 µm). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following 3 solutions in the mobile phase. For solution (A) weigh and powder 20 tablets, take a quantity of the powder equivalent to about 0.05 g of Artemether, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness, and dissolve the residue in 5 ml of the mobile phase. For

solution (B) use 10 mg of artemether RS per ml, and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.5 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of C₁₆H₂₆O₅, taking into account the declared content in artemether RS.

Dissolution test. (See introduction).

Artemetheri injectio

Artemether injection

Composition. Artemether injection is a sterile solution of artemether in a suitable oil for injection.

Description. A clear, colourless or almost colourless, oily solution.

Category. Antimalarial drug.

Storage. Artemether injection should be kept protected from light and stored in a cool place.

Labelling. The oil used in the formulation should be indicated.

Additional information. Recommended strength: 80 mg/ml.

Artemether injection is usually prepared for intramuscular injection.

The solution is sterilized by a suitable method (see "Methods of sterilization", Vol. 4, page 18).

Requirements

Complies with the monographs for "Parenteral preparations" (Vol. 4, page 36), Extractable volume for parenteral preparations (page ...), Test for bacterial endotoxins (page ...) and Visual inspection of particulate matter in injectable preparations (p....).

Artemether injection contains not less than **95.0%** and not more than **105.0%** of the amount of C₁₆H₂₆O₅ stated on the label.

Identity tests

- *Either tests A and B or tests B, C and D may be applied.*

A. To a volume of Artemether injection equivalent to 0.050 g add 25 ml of acetone R, mix and filter. Evaporate the filtrate at low temperature, and dry overnight over silica gel, desiccant, R. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from artemether RS or with the *reference spectrum* of artemether.

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

C. *Note:* This test cannot be performed if arachis oil is present in the formulation.

To a volume of Artemether injection equivalent to about 30 mg of Artemether add about 1 ml of dehydrated ethanol R, and about 1 g of potassium iodide R. Heat the mixture gently on a water-bath; an intense yellow colour is produced.

D. To a volume of Artemether injection equivalent to about 30 mg of Artemether add 6 ml of dehydrated ethanol R. Place a few drops of the mixture on a white porcelain dish and add 1 drop of vanillin/sulfuric acid TS1; a pink colour is produced.

Related substances

- *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of 7 volumes of light petroleum R1 and 3 volumes of ethyl acetate R as the mobile phase. Apply separately to the plate 10 μ l of each of the following 5 solutions in acetone R. For solution (A) dilute a volume of Artemether injection with acetone R to obtain a concentration equivalent to 10 mg of Artemether per ml. Prepare similarly solution (B) with the equivalent to about 0.05 mg of Artemether per ml, solution (C) with the equivalent to about 0.025 mg of Artemether per ml, solution (D) with the equivalent to about 0.10 mg of Artemether per ml, and for solution (E) use 0.10 mg of artemether RS 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. C. *Note:* This test cannot be performed if arachis oil is present in the formulation.

Carry out the test as described under "High performance liquid chromatography" p. ..., using the conditions given below under Assay.

Inject separately 20 μ l of solutions A and C.

Disregard any peak obtained with an area less than 0.1 time the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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 (0.5%). 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 C (1.0%).

➔ **Confirmation of this test would be appreciated and to confirm that this test cannot be performed if arachis oil is present.**

Assay

- *Either Method A or Method B may be applied.*

A. Dilute an accurately measured volume of the injection equivalent to about 0.08 g of with sufficient dehydrated ethanol R to produce 100 ml. Dilute 5 ml of this solution with the same solvent to 50 ml and mix. Transfer a further 5 ml of the diluted solution to a 50 ml volumetric flask and dilute to volume with hydrochloric acid/ethanol (1 mol/l) VS. Stopper the flask and place it in a water-bath at 55 ± 1 °C for 5 hours. Allow to cool to room temperature.

Measure the absorbance of this solution in a 1-cm layer at the maximum at about 254 nm. (*Note.* If arachis oil is used in the formulation of Artemether injection subtract the value of 0.25 from the absorbance value determined; the correction in absorbance value for other oils would have to be established), and calculate the content of C₁₆H₂₆O₅ in the injection being examined by comparison with artemether RS, similarly and concurrently examined.

B. Determine by "High performance liquid chromatography" p. ..., using a stainless steel column (25 cm x 4 mm) packed with *stationary phase A* (5 μ m). As the mobile phase, use a mixture of 62 volumes of acetonitrile R and 38 volumes of water.

Prepare the following 3 solutions in the mobile phase. For solution (A) dilute a volume of Artemether injection to obtain a concentration equivalent to 10 mg of Artemether per ml, for solution (B) use 10 mg of artemether RS per ml and for solution (C) dilute a suitable volume of solution A to obtain a concentration equivalent to 0.05 mg of Artemether per ml.

Operate with a flow rate of 1.5 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 216 nm.

Equilibrate the column with the mobile phase at a flow rate of 1.5 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B, and calculate the content of C₁₅H₂₂O₅, taking into account the declared content in artemether RS.

Artemisinin

Artemisinin

C₁₅H₂₂O₅

Relative molecular mass. 282.3

Chemical name. (3*R*,5*aS*,6*R*,8*aS*,9*R*,12*S*,12*aR*)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4.3-*j*]-1,2-benzodioxepin-10(3*H*)-one; CAS Reg. No. 63968-64-9.

Description. Colourless needles or a 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, protected from light and stored in a cool place.

Requirements

Artemisinin contains not less than **98.0%** and not more than the equivalent of **102.0%** of C₁₅H₂₂O₅ using assay method A and not less than **97.0%** 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 tests A and B, or tests B, C and D may be applied.*

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). 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". The principal spot obtained with solution D, corresponds in position, appearance, and intensity with that obtained with solution E.

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 2 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 and 4 drops of starch TS; a violet colour is immediately produced.

Melting range. 150–153 °C.

Specific optical rotation. Use a 10 mg/ml solution in dehydrated ethanol R; $[\alpha]^{20}_{\text{D}} = +75^{\circ}$ to $+78^{\circ}$.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry to constant mass at 105 °C; it loses not more than 5 mg/g.

Related substances

- *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), using silica gel R1 as the coating substance and a mixture of equal volumes of light petrolcum 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 a solution of 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 µm). As the mobile phase for gradient elution, use the first 17 minutes a mixture of 6 volumes of acetonitrile R and 4 volumes of water which within 13 minutes should reach 100% acetonitrile.

Prepare the following 2 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 10 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 a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS in a mixture of 8 volumes of acetonitrile R and 2 volumes of water.

→ **Confirmation of the nomenclature used for the enantiomers is desired.**

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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Disregard any peak obtained with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of related substances in %. 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 (0.1%). 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 (0.2%).

Assay

- *Either Method A or Method B may be applied.*

A. Dissolve about 0.050 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, and dilute to volume with sodium hydroxide (0.05 mol/l) VS. Mix thoroughly and warm to 50±1 °C in a water-bath for 30 minutes, and cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank determination performed with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml, and calculate the amount of C₁₅H₂₂O₅ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined.

B. Determine by "High performance liquid chromatography" p...., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 μ m). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following 2 solutions in the mobile phase. For solution (A) use 1.0 mg of Artemisinin per ml, and for solution (B) use 1.0 mg of Artemisinin RS per ml.

For the system suitability test prepare a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 μ l of solutions A and B.

The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of $C_{15}H_{22}O_5$, taking into account the declared content in artemisinin RS.

Artemisinini capsulae

Artemisinin capsules

Category. Antimalarial drug.

Storage. Artemisinin capsules should be kept in a well-closed container and stored in a cool place.

Labelling. Expiry date.

Additional information. Recommended strength: 250 mg.

→ **Confirmation of the strength of the capsules available would be appreciated.**

Requirements

Complies with the monograph for "Capsules" (see Vol. 4, page 32).

Artemisinin capsules contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{22}O_5$ stated on the label.

Identity tests

• *Either tests A and B, or tests B, C and D may be applied.*

A. To a quantity of the contents of the capsules equivalent to 0.040 g of Artemisinin add 40 ml of acetone R, shake to dissolve and filter. Evaporate the filtrate at low temperature, and dry overnight over silica gel, desiccant, R. Carry out the examination with the residue as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). 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". The principal spot obtained with solution D, corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a quantity of the contents of the capsules equivalent to 10 mg of Artemisinin add 20 ml of dehydrated ethanol R, shake to dissolve, filter and evaporate to dryness. To half of the residue (keep the remaining residue

for test D) add 0.5 ml of dehydrated ethanol R, 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS and 4 drops of starch TS; a violet colour is immediately produced.

D. Evaporate the remaining filtrate from test C on a water-bath to dryness. Dissolve the residue in 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 2 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.

Related substances

- *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), 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. For solution (A) take a quantity of the contents of the capsules equivalent to about 20 mg of Artemisinin, add 2 ml of acetone R, shake and filter, and use the filtrate. Prepare similarly solution (B) with the equivalent to about 0.05 mg of Artemisinin per ml, solution (C) with the equivalent to about 0.025 mg of Artemisinin per ml, solution (D) the equivalent to about 0.10 mg of Artemisinin per ml, and for solution (E) use 0.10 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with a solution of 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 µm). As the mobile phase for gradient elution, use the first 17 minutes a mixture of 6 volumes of acetonitrile R and 4 volumes of water which within 13 minutes should reach 100% acetonitrile.

Prepare the following 2 solutions. For solution (A) mix the contents of 20 capsules, take a quantity equivalent to about 10 mg of Artemisinin, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml of a mixture of 8 volumes of acetonitrile R and 2 volumes of water, and for solution (B) use 10 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 a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Disregard any peak obtained with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of related substances in %. 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 (0.1%). 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 (0.2%).

Assay

- *Either Method A or Method B may be applied.*

A. Mix the contents of 20 capsules and transfer a quantity equivalent to about 0.50 g of Artemisinin, accurately weighed, to a 100 ml volumetric flask and dilute to volume with ethanol (~750 g/l) TS. Shake the flask and

filter. Dilute 10 ml of the filtrate to 100 ml with the same solvent. Accurately transfer 10 ml to a 50 ml volumetric flask, and dilute to volume with sodium hydroxide (0.05 mol/l) VS. Mix thoroughly and warm to 50 ± 1 °C in a water-bath for 30 minutes, and cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank determination performed with 10 ml of ethanol (~750 g/l) TS diluted with sufficient sodium hydroxide (0.05 mol/l) VS to produce 50 ml, and calculate the amount of $C_{15}H_{22}O_5$ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined.

B. Determine by "High performance liquid chromatography" p..., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 μ m). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following 2 solutions in the mobile phase. For solution (A) mix the contents of 20 capsules, take a quantity equivalent to about 1.0 mg of Artemisinin, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml, and for solution (B) use 1.0 mg of Artemisinin RS per ml.

For the system suitability test prepare a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 μ l of solutions A and B.

The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of $C_{15}H_{22}O_5$, taking into account the declared content in artemisinin RS.

Dissolution test. (See introduction).

Artemisinini compressi

Artemisinin tablets

Category. Antimalarial drug.

Storage. Artemisinin tablets should be kept in a cool place.

Additional information. Recommended strength: 250 mg.

Requirements

Complies with the monograph for "Tablets" (see Vol. 4, page 26).

Artemisinin tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{22}O_5$ stated on the label.

Identity tests

- *Either tests A and B, or tests B, C and D may be applied.*

A. To a quantity of the powdered tablets equivalent to 0.040 g of Artemisinin add 40 ml of acetone R, shake to dissolve and filter. Evaporate the filtrate at low temperature, and dry overnight over silica gel, desiccant, R. Carry out the examination with the residue as described under "Spectrophotometry in the infrared region"

(Vol. 1, p. 40). 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". The principal spot obtained with solution D, corresponds in position, appearance, and intensity with that obtained with solution E.

C. To a quantity of the powdered tablets equivalent to 10 mg of Artemisinin add 20 ml of dehydrated ethanol R, shake to dissolve, filter and evaporate to dryness. To half of the residue (keep the remaining residue for test D) add 0.5 ml of dehydrated ethanol R, 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS and 4 drops of starch TS; a violet colour is immediately produced.

D. Evaporate the remaining filtrate from test C on a water-bath to dryness. Dissolve the residue in 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 2 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.

Related substances

• *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), 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. For solution (A) take a quantity of the powdered tablets equivalent to about 20 mg of Artemisinin, add 2 ml of acetone R, shake and filter, and use the filtrate. Prepare similarly solution (B) with the equivalent to about 0.05 mg of Artemisinin per ml, solution (C) with the equivalent to about 0.025 mg of Artemisinin per ml, solution (D) the equivalent to about 0.10 mg of Artemisinin per ml, and for solution (E) use 0.10 mg of artemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with a solution of 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 µm). As the mobile phase for gradient elution, use the first 17 minutes a mixture of 6 volumes of acetonitrile R and 4 volumes of water which within 13 minutes should reach 100% acetonitrile.

Prepare the following 2 solutions. For solution (A) weigh and powder 20 tablets, take a quantity of the powder equivalent to about 10 mg of Artemisinin, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml of a mixture of 8 volumes of acetonitrile R and 2 volumes of water, and for solution (B) use 10 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 a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Disregard any peak obtained with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of related substances in %. 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 (0.1%). 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 (0.2%).

Assay

• *Either Method A or Method B may be applied.*

A. Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to about 0.50 g of Artemisinin, accurately weighed, to a 100 ml volumetric flask and dilute to volume with ethanol (~750 g/l) TS. Shake the flask and filter. Dilute 10 ml of the filtrate to 100 ml with the same solvent. Accurately transfer 10 ml to a 50 ml volumetric flask, and dilute to volume with sodium hydroxide (0.05 mol/l) VS. Mix thoroughly and warm to 50±1 °C in a water-bath for 30 minutes, and cool to room temperature.

Measure the absorbance of a 1-cm layer at the maximum at about 292 nm against a solvent cell containing a blank determination performed 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 amount of C₁₅H₂₂O₅ in the substance being tested by comparison with artemisinin RS, similarly and concurrently examined.

B. Determine by "High performance liquid chromatography" p..., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 µm). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following 2 solutions in the mobile phase. For solution (A) weigh and powder 20 tablets, take a quantity of the powder equivalent to about 1.0 mg of Artemisinin, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml, and for solution (B) use 1.0 mg of Artemisinin RS per ml.

For the system suitability test prepare a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

The test is not valid unless the relative retention times for α-dihydroartemisinin are about 0.6, for β-dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of C₁₅H₂₂O₅, taking into account the declared content in artemisinin RS.

Dissolution test. (See introduction).

Artesunatum

Artesunate

C₁₉H₂₈O₈

Relative molecular mass. 384.4

Chemical name. (3*R*,5*aS*,6*R*,8*aS*,9*R*,10*S*,12*R*,12*aR*)-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ol, hydrogen succinate; CAS Reg. No. 88495-63-0.

Description. A white, fine crystalline powder.

Solubility. Very slightly soluble in water; very soluble in dichloromethane R; freely soluble in ethanol (~750 g/l) TS and acetone R.

Category. Antimalarial drug.

Storage. Artesunate should be kept in a well-closed container, protected from light and stored in a cool place.

Requirements

Artesunate contains not less than **98.0%** and not more than the equivalent of **102.0%** of $C_{19}H_{28}O_8$ using assay method A and not less than **96.0%** and not more than the equivalent of **102.0%** of $C_{19}H_{28}O_8$ using assay method B, both calculated with reference to the anhydrous substance.

Identity tests

- *Either tests A and B or tests B, C, and D may be applied.*

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). 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 "Thin-layer chromatography" (Vol. 1, p. 83), 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 containing (A) 0.10 mg of Artesunate per ml and (B) 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. Dissolve 0.1 g of Artesunate in 40 ml of dehydrated ethanol R, shake 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 TS2 and allow to stand for 30 minutes; a red colour is produced.

Melting range. 132–135 °C.

Specific optical rotation. Use a 0.040 g/ml solution in dichloromethane R; $[\alpha]^{20}_{D} = +10^{\circ}$ to $+14^{\circ}$.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 μ g/g.

Sulfated ash. Not more than 1.0 mg/g.

Water. Determine as described under "Determination of water by the Karl Fischer method", Method A (Vol. 1, p. 135), using 2 g of Artesunate; the water content is not more than 5 mg/g.

pH value. pH of an aqueous suspension containing 10 mg/g, 3.5–4.5.

Related substances. Carry out the test as described under "High performance liquid chromatography" p. ... using the conditions given below under Assay.

Inject separately 20 μ l of solutions A and C.

Disregard any peak obtained with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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%). 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 C (2%).

Assay

- *Either Method A or Method B may be applied.*

A. Dissolve about 0.19 g, accurately weighed, in 30 ml of dehydrated ethanol R, and titrate with tetrabutylammonium hydroxide/2-propanol (0.1 mol/l) VS determining the endpoint potentiometrically as described under "Non-aqueous titration", Method B (Vol. 1, p. 132).

Each ml of tetrabutylammonium hydroxide/2-propanol (0.1 mol/l) VS is equivalent to 38.44 mg of C₁₉H₂₈O₈.

B. Determine by "High performance liquid chromatography" p., using a stainless steel column (12.5 cm x 3.5 mm), packed with *stationary phase A* (5 µm). 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 1000 ml of water, and adjust the pH to 3.0 with phosphoric acid (~1440 g/l) TS).

Prepare the following 3 solutions in acetonitrile R. For solution (A) use 4.0 mg of Artesunate per 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 a refractive index detector.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and B and calculate the content of C₁₉H₂₈O₈, using the declared content in artesunate RS.

Artesunati compressi

Artesunate tablets

Category. Antimalarial drug.

Storage. Artesunate tablets should be kept in a cool place.

Additional information. Recommended strength: 50-200 mg.

Requirements

Complies with the monograph for "Tablets" (see Vol. 4, page 26).

Artesunate tablets contain not less than **90.0%** and not more than **110.0%** of the amount of C₁₉H₂₈O₈ stated on the label.

Identity tests

- *Either tests A and B, or tests B, C, and D may be applied.*

A. To a quantity of the powdered tablets equivalent to 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 silica gel, desiccant, R. Carry out the examination with the residue as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). 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 "Thin-layer chromatography" (Vol. 1, p. 83), 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 µl of the following 2 solutions in toluene R. For solution (A) shake a quantity of the powdered tablets equivalent to about 0.10 mg of Artesunate in dehydrated ethanol R, filter and evaporate. Dissolve the residue in 1.0 ml of toluene R. 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 equivalent to 0.1 g of Artesunate add 40 ml of dehydrated ethanol R, shake to dissolve 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 TS2 and allow to stand for 30 minutes; a red colour is produced.

Related substances. Carry out the test as described under "High performance liquid chromatography" p. ... using the conditions given below under Assay.

Inject separately 20 µl of solutions A and C.

Disregard any peak obtained with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with solution C.

Measure the areas of the peak responses obtained in the chromatograms from solutions A and C, and calculate the content of related substances in %. 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%). 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 C (2%).

Assay

• *Either Method A or Method B may be applied.*

A. Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 0.20 g, accurately weighed, add 50 ml of dehydrated ethanol R, shake and filter. Titrate 25 ml with tetrabutylammonium hydroxide/2-propanol (0.1 mol/l) VS determining the endpoint potentiometrically as described under "Non-aqueous titration", Method B (Vol. 1, p. 132).

Each ml of tetrabutylammonium hydroxide/2-propanol (0.1 mol/l) VS is equivalent to 38.44 mg of C₁₉H₂₈O₈.

B. Determine by "High performance liquid chromatography" p. ..., using a stainless steel column (12.5 cm x 3.5 mm), packed with *stationary phase A* (5 µm). 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 1000 ml of water, and adjust the pH to 3.0 with phosphoric acid (~1440 g/l) TS).

Prepare the following 3 solutions in acetonitrile R. For solution (A) weigh and powder 20 tablets, take a quantity of the powder equivalent to about 4.0 mg of Artesunate, accurately weighed, add 2 ml of acetone R, shake 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 a refractive index detector.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 µl of solutions A and B.

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

Dissolution test. (See introduction).

Dihydroartemisinin

Dihydroartemisinin

$C_{15}H_{24}O_5$

Relative molecular mass. 284.4

Chemical name. (3*R*,5*aS*,6*R*,8*aS*,9*R*,10*S*,12*R*,12*aR*)]-Decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10-ol; CAS Reg. No. 81496-81-3.

Description. Colourless needles or a white or almost white, crystalline powder.

Solubility. Practically insoluble in water; slightly soluble in acetonitrile R, ethanol (~750 g/l) TS and dichloromethane R.

Category. Antimalarial.

Storage. Dihydroartemisinin should be kept in a well-closed container, protected from light and stored in a cool place.

Additional information. The title of the monograph is provisional, it is pending selection of the International Nonproprietary Name.

Melting temperature, about 137 °C with decomposition.

Requirements

Dihydroartemisinin contains not less than **97.0%** and not more than the equivalent of **102.0%** of $C_{15}H_{24}O_5$, calculated with reference to the dried substance.

Identity tests

• *Either tests A and B, or tests B, C and D may be applied.*

A. Carry out the examination as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from dihydroartemisinin RS or with the *reference spectrum* of dihydroartemisinin.

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

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 2 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 and 4 drops of starch TS; a violet colour is immediately produced.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry over phosphorus pentoxide R under reduced pressure (not exceeding 2.67 kPa or 20 mm of mercury); it loses not more than 10.0 mg/g.

Related substances

- *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), 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 μ l of each of the following 5 solutions in toluene R containing (A) 10 mg of Dihydroartemisinin per ml, (B) 0.05 mg of Dihydroartemisinin per ml, (C) 0.025 mg of Dihydroartemisinin per ml, (D) 0.10 mg of Dihydroartemisinin per ml, and (E) 0.10 mg of dihydroartemisinin RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with a solution of 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. ..., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 μ m). As the mobile phase for gradient elution, use the first 17 minutes a mixture of 6 volumes of acetonitrile R and 4 volumes of water which within 13 minutes should reach 100% acetonitrile.

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

For the system suitability test prepare a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 μ l of solutions A and B.

Disregard any peak obtained with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of related substances in %. 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 (0.1%). 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 (0.2%).

Assay. Determine by "High performance liquid chromatography" p., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 μ m). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following 2 solutions in the mobile phase. For solution (A) use 1.0 mg of Dihydroartemisinin per ml, and for solution (B) use 1.0 mg of Dihydroartemisinin RS per ml.

For the system suitability test prepare a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 μ l of solutions A and B.

The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of $C_{15}H_{22}O_5$, taking into account the declared content in artemisinin RS.

Dihydroartemisinini compressi

Dihydroartemisinin tablets

Category. Antimalarial drug.

Storage. Dihydroartemisinin tablets should be kept in a cool place.

Additional information. Recommended strength: 20-80 mg.

Requirements

Complies with the monograph for "Tablets" (see Vol. 4, page 26).

Dihydroartemisinin tablets contain not less than **90.0%** and not more than **110.0%** of the amount of $C_{15}H_{24}O_5$ stated on the label.

Identity tests

• *Either tests A and B, or tests B, C and D may be applied.*

A. To a quantity of the powdered tablets equivalent to 0.040 g of Dihydroartemisinin add 40 ml of acetone R, shake to dissolve and filter. Evaporate the filtrate at low temperature, and dry overnight over silica gel, desiccant, R. Carry out the examination with the residue as described under "Spectrophotometry in the infrared region" (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from dihydroartemisinin RS or with the reference spectrum of dihydroartemisinin.

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

C. To a quantity of the powdered tablets equivalent to 10 mg of Dihydroartemisinin add 20 ml of dehydrated ethanol R, shake to dissolve, filter and evaporate to dryness. To half of the residue (keep the remaining residue for test D) add 0.5 ml of dehydrated ethanol R, 1.0 ml of potassium iodide (80 g/l) TS, 2.5 ml of sulfuric acid (~100 g/l) TS and 4 drops of starch TS; a violet colour is immediately produced.

D. Evaporate the remaining filtrate from test C on a water-bath to dryness. Dissolve the residue in 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 2 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.

Related substances

• *Either test A or test B may be applied.*

A. Carry out the test as described under "Thin-layer chromatography" (Vol. 1, p. 83), 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 μ l of each of the following 5 solutions in toluene R. For solution (A) take a quantity of the powdered tablets equivalent to about 20 mg of Dihydroartemisinin, add 2 ml of acetone R, shake and filter, and use the filtrate. Prepare similarly solution (B) with the equivalent to about 0.05 mg of Dihydroartemisinin per ml, solution (C) with the equivalent to about 0.025 mg of Dihydroartemisinin per ml and solution (D) the equivalent to about 0.10 mg of dihydroartemisinin, and for solution (E) use 0.10 mg of artemether RS per ml. After removing the plate from the chromatographic chamber, allow it to dry in air, spray with a solution of 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. Furthermore, not more than one such spot is more intense than that obtained with solution C (0.5%, 0.25%, respectively).

B. Carry out the test as described under "High performance liquid chromatography" p. using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 μ m). As the mobile phase for gradient elution, use the first 17 minutes a mixture of 6 volumes of acetonitrile R and 4 volumes of water which within 13 minutes should reach 100% acetonitrile.

Prepare the following 2 solutions. For solution (A) weigh and powder 20 tablets, take a quantity of the powder equivalent to about 10 mg of Dihydroartemisinin, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness and dissolve the residue in 1.0 ml of a mixture of 8 volumes of acetonitrile R and 2 volumes of water, and for solution (B) use 10 μ g of Dihydroartemisinin per ml in a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

For the system suitability test prepare a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 μ l of solutions A and B.

Disregard any peak obtained with an area less than 0.2 times the area of the principal peak in the chromatogram obtained with solution B. The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of related substances in %. 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 (0.1%). 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 (0.2%).

Assay. Determine by "High performance liquid chromatography" p...., using a stainless steel column (10 cm x 4.6 mm) packed with *stationary phase A* (3 μ m). As the mobile phase, use a mixture of 6 volumes of acetonitrile R and 4 volumes of water.

Prepare the following 2 solutions in the mobile phase. For solution (A) weigh and powder 20 tablets, take a quantity of the powder equivalent to about 1.0 mg of Dihydroartemisinin, accurately weighed, add 2 ml of acetone R, shake and filter. Evaporate the filtrate to dryness, and dissolve the residue in 1.0 ml, and for solution (B) use 1.0 mg of Dihydroartemisinin RS per ml.

For the system suitability test prepare a solution containing 1.0 mg of artemisinin RS and 1.0 mg of dihydroartemisinin RS 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.

Equilibrate the column with the mobile phase at a flow rate of 0.6 ml per minute for about 30 minutes.

Inject separately 20 μ l of solutions A and B.

The test is not valid unless the relative retention times for α -dihydroartemisinin are about 0.6, for β -dihydroartemisinin about 1.0 and for artemisinin about 1.0, 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 content of $C_{15}H_{22}O_5$, taking into account the declared content in artemisinin RS.

Dissolution test. (See introduction).

3. Reagents

Anisaldehyde R. 4-Methoxybenzaldehyde; $C_8H_8O_2$.

Description. An oily liquid.

Solubility. Very slightly soluble in water, miscible with ethanol (~750 g/l) TS and ether R.

Boiling point. About 248 °C.

Anisaldehyde/methanol TS

Procedure. Slowly add 10 ml of glacial acetic acid R and 5 ml of sulfuric acid (~1760 g/l) TS to 55 ml of methanol R, and cool to room temperature. Separately add 0.5 ml of anisaldehyde R to 30 ml of methanol R. Mix the two solutions thoroughly.

Storage. Keep anisaldehyde/methanol TS protected from light.

Note: Anisaldehyde/sulfuric acid TS should be freshly prepared.

Anisaldehyde/sulfuric acid TS

Procedure. Add 5 ml of anisaldehyde R to 10 ml of sulfuric acid (~1760 g/l) TS.

Arteether RS. International Chemical Reference Substance.

Artemether RS. International Chemical Reference Substance.

Artemisinin RS. International Chemical Reference Substance.

Artesunate RS. International Chemical Reference Substance.

Bromophenol blue (1 g/l) TS. A solution of bromophenol blue R containing about 1.0 g of $C_{19}H_{10}Br_4O_5S$ per litre.

Dihydroartemisinin RS. International Chemical Reference Substance.

Hydrochloric acid/ethanol (1 mol/l) VS. Hydrochloric acid (~250 g/l) TS, diluted with dehydrated ethanol R to contain 36.47 g of HCl in 1000 ml of dehydrated ethanol R.

Method of standardization. Ascertain the exact concentration of the solution following the method described under hydrochloric acid (1 mol/l) VS, Volume 1, p. 184.

Hydroxylamine hydrochloride TS2

Procedure. Dissolve 3.5 g of hydroxylamine hydrochloride R in 95 ml of ethanol (~535 g/l) TS, add 0.5 ml of bromophenol blue (1 g/l) TS and sufficient potassium hydroxide/ethanol (0.5 mol/l) TS until a greenish tint is developed. Dilute the solution to 100 ml with ethanol (~535 g/l) TS.

Stationary phases for Liquid Chromatography

A. Particles of silica gel, the surface of which has been modified with chemically bonded octadecyl silyl groups.

B. Particles of silica gel, the surface of which has been modified with chemically bonded octyl silyl groups.

C. Particles of porous silica.

D. Particles of styrene-divinylbenzene copolymer.

Tetrabutylammonium hydroxide/propanol (0.1 mol/l) VS

Procedure. Prepare as described in Volume 2, page 321 for Tetrabutylammonium hydroxide (0.1 mol/l) VS using toluene R instead of benzene R.

Vanillin/sulfuric acid TS1

Procedure. Dissolve 5 g of vanillin R in 100 ml of sulfuric acid (~1760 g/l) TS.

Note: Vanillin/sulfuric acid TS1 should be freshly prepared.

Vanillin/sulfuric acid TS2

Procedure. Dissolve 1 g of vanillin R in sufficient ethanol (~750 g/l) TS to produce 100 ml. Carefully add to it drop by drop 2 ml of sulfuric acid (~1760 g/l) TS.

Note: Vanillin/sulfuric acid TS2 must be used within 48 hours.

* * *