

BRIEFING

Lumefantrine and Artemether Tablets. This monograph was initially posted on the USP SALMOUS Standards Web page for review and public comment as Draft 1. A Draft 2 version was posted on September 26, 2008 to include a test for *Dissolution*. No comments were received. Therefore, the monograph was approved by the MD-AA and BPC expert committees to be an Authorized USP SALMOUS Standard. More *Dissolution* tests are expected to be added to the monograph. The chromatographic procedure in the *Dissolution* test was validated with a Nucleosil C18 brand of packing L1.

The HPLC procedure used in the test for *Related compounds* for lumefantrine is based on analysis performed with the Nucleosil C18 5- μ m brand of L1 column. The typical retention time for lumefantrine is 11 minutes. The HPLC procedure used in the *Assay* is based on analysis performed with the Symmetry C18 5- μ m brand of L1 column. The typical retention times for lumefantrine and artemether are 34 and 19 minutes, respectively.

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C50811; C68763

Lumefantrine and Artemether Tablets

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» Lumefantrine and Artemether Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of lumefantrine (C₃₀H₃₂Cl₃NO) and of artemether (C₁₆H₂₆O₅).

Packaging and storage—Preserve in well-closed, light-resistant containers. Store at room temperature.

USP Reference standards (11)—*USP Artemether RS*. *USP Artemether Related Compound A RS*. *USP Artemether Related Compound B RS*. *USP Lumefantrine RS*. *USP Lumefantrine Related Compound A RS*.

Identification—

A: *Thin-Layer Chromatographic Identification Test* (201)—
Solvent—Prepare a mixture of chloroform, methanol, ethyl acetate, and water (11 : 10 : 2 : 2).

Test solution—Transfer a portion of powdered Tablets, equivalent to 20 mg of artemether and 120 mg of lumefantrine, to a suitable vessel. Add 2 mL of water, 2 mL of ethyl acetate, 10 mL of methanol, and 11 mL of chloroform. Sonicate for 15 minutes, centrifuge at 4000 rpm, and use the clear supernatant.

Artemether standard solution—Dissolve an accurately weighed quantity of USP Artemether RS in *Solvent* to obtain a solution having a known concentration of about 0.8 mg per mL.

Lumefantrine standard solution—Dissolve an accurately weighed quantity of USP Lumefantrine RS in *Solvent* to obtain a solution having a known concentration of about 4.8 mg per mL.

Application volume: 20 μ L.

Developing solvent solution—Prepare a mixture of solvent hexane, ethyl acetate, and glacial acetic acid (20 : 5 : 2.5).

Procedure—Proceed as directed in the chapter, and then air-dry. Spray the plate with 20% sulfuric acid in methanol, place in an oven at 140° for about 10 minutes, and examine the plate. Evaluate the spots under daylight and under UV light at 366 nm against the *Artemether standard solution* and the *Lumefantrine standard solution*. The artemether appears as a greyish-purple spot on a white background under daylight and as a light yellow fluorescent spot on a blue background under UV light at 366 nm. The lumefantrine appears as a grayish-yellow spot on a white background under daylight and as a dark spot on a blue fluorescent background under UV light at 366 nm. The *R_F* values of artemether

and lumefantrine obtained from the *Test solution* correspond to those exhibited by the corresponding *Artemether standard solution* or *Lumefantrine standard solution*.

B: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

Dissolution (711)

DISSOLUTION OF ARTEMETHER—

Medium: water, partially degassed (6.5 to 7 mg of O₂/L); 1000 mL.

Apparatus 2: 100 rpm.

Time: 1 and 3 hours.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile, water, 1-propanol, and trifluoroacetic acid (500 : 400 : 100 : 1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Diluent: a mixture of water and acetonitrile (1 : 1).

Standard stock solution—Prepare a solution in *Diluent* containing about 0.2 mg per mL of USP Artemether RS.

Standard solution—Prepare a dilution of the *Standard stock solution* in *Medium* having a final concentration of about 0.02 mg per mL.

Test solution—Pass a portion of the solution under test through a suitable 0.45- μ m filter. Dilute quantitatively, and stepwise, if necessary with *Medium*.

Chromatographic system—The liquid chromatograph is equipped with a 210-nm detector and a 4-mm \times 12.5-cm column that contains packing 5- μ m L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 2.5%.

Procedure—Separately inject equal volumes (about 100 μ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of artemether dissolved by the formula:

$$\frac{r_U \times C_S \times 1000 \times 100}{r_S \times L}$$

in which *r_U* and *r_S* are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively; *C_S* is the concentration, in mg per mL, of artemether in the *Standard solution*; 1000 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and *L* is the Tablet label claim for artemether, in mg.

Tolerances—Not less than 45% (*Q*) of the labeled amount of artemether is dissolved in 1 hour, and not less than 65% (*Q*) of the labeled amount of artemether is dissolved in 3 hours.

DISSOLUTION OF LUMEFANTRINE—

Medium: 0.1 M hydrochloric acid containing 1% of benzalkonium chloride; 1000 mL.

Apparatus 2: 100 rpm.

Time: 45 minutes.

Standard solution—Dissolve an accurately weighed quantity of USP Lumefantrine RS in *Medium*, and dilute quantitatively, and stepwise, if necessary, with *Medium* to obtain a solution having a concentration of about 0.72 mg per mL.

Test solution—Pass a portion of the solution under test through a suitable 0.5- μ m filter. Quantitatively dilute with *Medium* to obtain a final concentration of about 0.72 mg per mL, considering complete dissolution of the Tablet label claim.

Procedure—Determine the percentage of lumefantrine dissolved by employing UV absorption at the wavelength at about 342 nm, on portions of the *Test solution* in comparison with the *Standard solution*, using 0.2-cm cells and *Medium* as the blank. Calculate the percentage of lumefantrine dissolved by the formula:

$$\frac{A_U \times C_S \times 1000 \times 100}{A_S \times L}$$

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in which A_U and A_S are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively; C_S is the concentration, in mg per mL, of the *Standard solution*; 1000 is the volume, in mL, of the *Medium*; 100 is the conversion factor to percentage; and L is the Tablet label claim for lumefantrine, in mg.

Tolerances—Not less than 60% (Q) of the labeled amount of lumefantrine is dissolved in 45 minutes.

Uniformity of dosage units (905): meet the requirements.

Related compounds—

TEST FOR ARTEMETHER—

Adsorbent: 0.25-mm layer of chromatographic silica gel.

Solvent—Prepare a mixture of acetonitrile and water (1:1).

Test solution—Disintegrate a number of Tablets, equivalent to 60 mg of artemether, in 6 mL of water. Add 6 mL of acetonitrile, sonicate for 15 minutes, centrifuge at about 4000 rpm, and pass through a membrane filter having a 0.5- μ m porosity.

Standard stock solution—Dissolve accurately weighed quantities of USP Artemether RS, USP Artemether Related Compound A RS, and USP Artemether Related Compound B RS in *Solvent* to obtain a solution having a known concentration of about 0.1 mg per mL of each Standard.

Standard solutions—Dilute an aliquot of *Standard stock solution* to obtain five *Standard solutions* having the following concentrations: 0.005 mg of each Standard per mL, 0.015 mg of each Standard per mL, 0.025 mg of each Standard per mL, 0.050 mg of each Standard per mL, and 0.075 mg of each Standard per mL.

Application volume: 20 μ L.

Developing solvent system—Prepare a mixture of solvent hexane, ethyl acetate, and glacial acetic acid (20:5:2.5).

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Expose the plate to UV light at 254 nm for 60 minutes. Spray the plate with 20% sulfuric acid in methanol, heat at 140° for about 10 minutes in a drying oven, and examine the plate under daylight. Estimate the percentage of all secondary spots observed in the chromatograms obtained from the *Test solution* by comparing each spot with the principal spots obtained from the chromatograms of the *Standard solutions*. The R_F values and limits of impurities are given in *Table 1*.

Table 1

Compound	Approximate R_F	Limit (%)
Impurity 1	0.25	1.5
Artemether related compound A ¹ (Dihydroartemisinin)	0.30	1.0
Impurity 2	0.35	0.5
Artemether related compound B ² (α -Artemether)	0.40	0.3
Artemether	0.55	—
Individual unknown impurity	—	0.2
Total unknown impurities	—	0.5

¹ (3R,5aS,6R,8aS,10S,12R,12aR)-Decahydro-10-hydroxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4.3-j]-1,2-benzodioxepin.

² (3R,5aS,6R,8aS,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano[4.3-j]-1,2-benzodioxepin.

TEST FOR LUMEFANTRINE—

Ion-pairing solution and **Solvent**—Prepare as directed in the *Assay*.

Solution A—Prepare a mixture of water, acetonitrile, *Ion-pairing solution*, and 1-propanol (50:25:20:5).

Solution B—Prepare a mixture of acetonitrile, *Ion-pairing solution*, water, and 1-propanol (65:20:10:5).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

System suitability solution—Dissolve an accurately weighed quantity of USP Lumefantrine RS and USP Lumefantrine Related Compound A RS in *Solvent* to obtain a solution having known concentrations of about 1.2 mg per mL and 0.02 mg per mL, respectively.

Test solution—Disintegrate 10 Tablets, equivalent to about 1200 mg of lumefantrine, with 60 mL of water. Add 200 mL of 1-propanol, and sonicate for about 15 minutes. Then add 200 mL of *Ion-pairing solution* and 400 mL of acetonitrile, sonicate again for 30 minutes, and dilute with acetonitrile to 1000 mL. Withdraw about 10 mL of the suspension, centrifuge for about 5 minutes at about 4000 rpm, and use the clear supernatant.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with a 300-nm UV detector and a 4.0-mm \times 12.5-cm column that contains 5- μ m packing L1. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–14	25	75	isocratic
14–19	25→0	75→100	linear gradient
19–25	0	100	isocratic
25–26	0→25	100→75	linear gradient
26–35	25	75	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, R , between lumefantrine and lumefantrine related compound A is not less than 0.5; the tailing factor is between 0.8 and 5.0; and the relative standard deviation for replicate injections is not more than 2.0% for the lumefantrine peak.

Procedure—Separately inject equal volumes (about 5 μ L) of the *System suitability solution* and the *Test solution* into the chromatograph, record the chromatograms, and identify the impurities using the relative retention times specified in *Table 2*.

Table 2

Compound	Relative Retention Time	Limit (%)
Lumefantrine related compound A*	0.9	0.1
Lumefantrine	1.0	—
Any other individual impurity	—	0.1
Total impurities	—	0.3

* (RS,Z)-2-(Dibutylamino)-2-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl)ethanol.

Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100(r_i/r_s)$$

in which r_i is the peak response for any individual impurity obtained from the *Test solution*; and r_s is the sum of the responses of all of the peaks. Disregard any peak less than 0.05%.

Assay—

Ion-pairing solution—Prepare a mixture of 5.65 g of sodium 1-hexanesulfonate and 2.75 g of monobasic sodium phosphate in 800 mL of water. Adjust the pH to 2.3 using phosphoric acid, dilute with water to 1000.0 mL, and filter.

Solvent—Prepare a mixture of 100 mL of *Ion-pairing solution*, 100 mL of 1-propanol, and 30 mL of water, and dilute with acetonitrile to 500.0 mL.

Solution A—Prepare a mixture of *Ion-pairing solution* and acetonitrile (7:3).

Solution B—Prepare a mixture of acetonitrile and *Ion-pairing solution* (7:3).

Mobile phase—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

Standard preparation—Dissolve an accurately weighed quantity of USP Artemether RS and USP Lumefantrine RS in *Solvent* to obtain a solution having known concentrations of about 0.2 mg per mL and 1.2 mg per mL, respectively.

Assay preparation—Disintegrate 10 Tablets, equivalent to about 200 mg of artemether and 1200 mg of lumefantrine, with 60 mL of water. Add 200 mL of 1-propanol, and sonicate for about 15

minutes. Then add 200 mL of *Ion-pairing solution* and 400 mL of acetonitrile, sonicate again for 30 minutes, and dilute with acetonitrile to 1000.0 mL. Withdraw about 10 mL of the suspension, centrifuge for about 5 minutes at about 4000 rpm, and use the clear supernatant.

Chromatographic system (see *Chromatography* (621))—The liquid chromatograph is equipped with either a programmable variable UV wavelength detector or two separate UV detectors capable of monitoring at 210 nm and 380 nm and with a 3.9-mm × 15-cm column that contains 5-μm packing L1. [NOTE—Set the detector for the first 30 minutes to 210 nm, then switch to 380 nm.] The flow rate is about 1.3 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–28	60	40	isocratic
28–29	60→0	40→100	linear gradient
29–45	0	100	isocratic
45–46	0→60	100→40	linear gradient
46–55	60	40	re-equilibration

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not less than 0.8 and not more than 4.5; and the relative standard deviation for replicate injections is not more than 2.0% for each peak.

Procedure—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the artemether and lumefantrine peaks. Calculate the quantities, in mg, of artemether (C₁₆H₂₆O₅) and lumefantrine (C₃₀H₃₂Cl₃NO) in the portion of Tablets taken by the formula:

$$CV(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard preparation*; *V* is the volume, in mL, of the *Assay preparation*; and *r_U* and *r_S* are the peak responses of the corresponding analyte peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.