

Manual

Accompanying The GPHF-Minilab®

**Extension 2002
Ten New Drugs**

Second Supplement To Volume II
Thin Layer Chromatography



An Initiative of Research Based Pharmaceutical Companies in Germany

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7.21 Artesunate

Primary Screening via Visual Inspection & Disintegration Test

I. VISUAL INSPECTION

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual. Write down all product particulars using the Reporting Form as a guide. Each tablet or capsule usually contains 50 or 200 mg of artesunate.

II. DISINTEGRATION TEST

All quick release artesunate tablets and capsules must pass the disintegration test as described in the opening chapters on general methods and operations of the main manual. They should disintegrate in water at 37 °C in less than 30 minutes. It is a major defect if a drug product does not pass this test.

III. RESULTS & ACTIONS TO BE TAKEN

Drug products from unusually cheap sources, drug products with missing or incorrect accompanying documents and drug products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels written in a foreign language should be subjected to a thin layer chromatographic assay.

Verification of Identity and Drug Content via Thin Layer Chromatography

I. PRINCIPLE

Artesunate is extracted from tablets and capsules with methanol and determined by TLC with reference to an authentic secondary standard.

II. EQUIPMENT AND REAGENTS

- 1) Pestle
- 2) Aluminium foil
- 3) Laboratory glass bottles with a filling capacity of 25 to 100 ml
- 4) Funnel
- 5) Set of straight pipettes (1 to 25 ml)
- 6) 10-ml vials
- 7) Label tape
- 8) Marker pen
- 9) Pencil
- 10) Merck TLC aluminium plates pre-coated with silica gel 60 F 254, size 5x10 cm
- 11) Glass microcapillaries of 2- μ l filling capacity
- 12) Hot plate
- 13) TLC developing chamber (jar)
- 14) Filter paper
- 15) Pair of scissors
- 16) Pair of tweezers
- 17) UV light of 254 nm
- 18) Iodine chamber
- 19) TLC dipping chamber (Petri dish)
- 20) Methanol
- 21) Sulfuric acid 96%
- 22) Glacial acetic acid
- 23) Ethylacetate
- 24) Acetone
- 25) Artesunate 50 mg reference tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of a stock standard solution requires a whole reference tablet containing 50 mg of artesunate which is crushed prior to extraction, the precise procedure being as follows: Wrap up a tablet into aluminium foil and crush it down to a fine powder using a pestle. Empty the aluminium foil over a 25-ml laboratory glass bottle and wash down all residual solid with 10.0 ml of methanol using a straight pipette. Close the bottle and shake for about three minutes till most of the solid is dissolved. Allow the solution to sit for a further five minutes until the undissolved residue settles below the hazy supernatant liquid. This solution should contain 5.0 mg of total drug per ml and be labelled as *‘Artesunate Stock Standard Solution’*. Freshly prepare this solution for each test.

IV. PREPARATION OF THE WORKING STANDARD SOLUTION 100% (UPPER WORKING LIMIT)

The artesunate stock standard solution requires no further dilution. It already represents the final working concentration of 5.0 mg of total drug per ml.

This working standard solution represents a drug product of good quality containing 100 % of artesunate.

V. PREPARATION OF THE WORKING STANDARD SOLUTION 80% (LOWER WORKING LIMIT)

Pipette 4 ml of the stock standard solution into a 10-ml vial and add 1 ml of methanol. Close and shake the vial. The solution obtained should contain 4.0 mg of total drug per ml and be labelled as *‘Artesunate Working Standard Solution 80%’*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of artesunate as stated on the product’s label. In the current investigation, this drug level represents the lower acceptable limit for a given product.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A DRUG PRODUCT CLAIMING A POTENCY OF 50 MG ARTESUNATE PER UNIT

The preparation of a stock sample solution requires a whole tablet or capsule from an appropriate drug product sampled in the field. Artesunate is extracted completely from the sample using the same procedure as for the authentic reference standard: Tablets are wrapped up into aluminium foil and crushed down to a fine powder prior to transfer into a 25-ml laboratory glass bottle. Powder obtained from a capsule should be transferred directly into the laboratory glass bottle putting finally the empty cap and body shells into the bottle as well. Add 10 ml of methanol using a straight pipette, close the bottle, and shake for about three minutes till most of the solids are dissolved. Allow the solution to sit for a further five minutes until the undissolved residue settles below the hazy supernatant liquid.

200 MG OF ARTESUNATE PER UNIT

Transfer the powder obtained into a 50-ml bottle, add 40 ml of methanol using a straight pipette, close the bottle, and shake for about three minutes till most of the solids are dissolved. Allow the solution to sit for a further five minutes until the undissolved residue settles below the hazy supernatant liquid.

Both solutions obtained should contain 5.0 mg of total drug per ml and be labelled as *‘Artesunate Stock Sample Solution’*. Freshly prepare these solutions for each test.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Artesunate stock sample solutions, prepared either from a 50 or 200 mg unit dosage form, require no further dilution. They already represent the final working concentration of 5.0 mg of total drug per ml.

VIII. SPOTTING

Mark an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate and apply 2 μ l of each test and standard solution prepared as shown in the picture opposite using the microcapillary pipettes supplied.

Up to five spots can be placed on a plate. Check the uniformity of all spots using UV light of 254 nm. Even if the active agent is not visible by UV residual auxiliary agents from the capsule or tablet matrix might well be. All spots should be circular in shape and equally spaced across the origin line. Although their intensities might differ, their diameter never should. Different intensities are due to residual amounts of tablet and capsule excipients or different drug concentrations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if a homogeneous spotting is not achieved first time.

IX. DEVELOPMENT

Pipette 18 ml of ethylacetate, 4 ml of acetone, and precisely 0.1 ml of glacial acetic acid into the jar being used as TLC developing chamber. Close the chamber and mix thoroughly. Line the chamber's wall with filter paper and wait for about 15 minutes thus ensuring saturation of the chamber with solvent vapour. Carefully place the loaded TLC plate into the jar. Close the jar and develop the chromatoplate until the solvent front has moved about three-quarters of the length of the plate, the developing time being about 15 minutes. Remove the plate from the chamber, mark the solvent front and allow any excess solvent to evaporate using a hot plate if necessary.

X. DETECTION

Dry off all residual solvent and expose the TLC plate to methanolic sulfuric acid solution 5%. For this, mix 19 ml of methanol with 1 ml of sulfuric acid 96% in the Petri dish supplied and get the chromatoplate soaked with developer by quickly dipping the plate into the sulfuric acid solution using a pair of tweezers. Instantly remove the plate from the solution and dry the back of the plate with paper tissue. Continue to dry off all developer solution on a hot plate and observe how the principal spots are gradually becoming visible in daylight. Allow not more than 15 seconds to finish all operations from soaking to drying. Use this method of detection for quantification purposes.

Alternatively, dry off all residual solvent and expose the TLC plate to iodine vapour for about one minute. Observe the chromatoplate in daylight during and after iodine staining. Note: The principal spots will become visible only if the iodine chamber has well been activated.

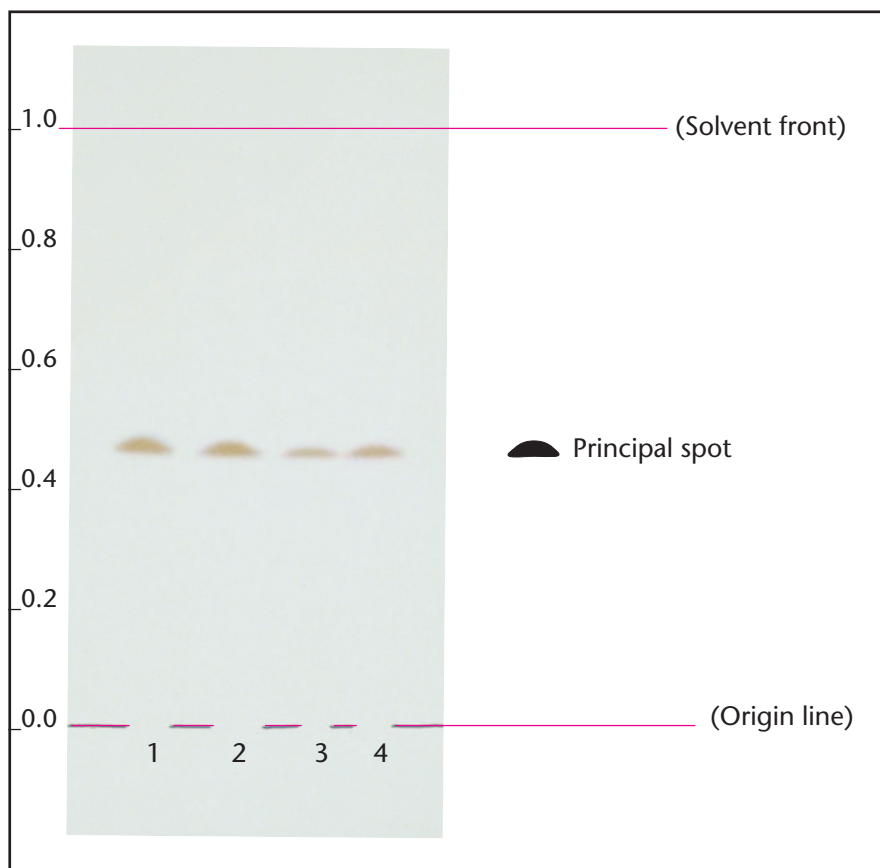
XI. CHROMATOPLATE OBSERVED AFTER EXPOSURE TO SULFURIC ACID

Run No.1:
Artesunate's upper working limit representing 100% of total drug.

Run No.2:
A drug product of good quality.

Run No.3:
A drug product of poor quality.

Run No.4:
Artesunate's lower working limit representing 80% of total drug.



XII. OBSERVATIONS MADE IN DAYLIGHT AFTER STAINING WITH SULFURIC ACID

The presence of artesunate is indicated by a strong dark spot at a travel distance of about 0.45. Additional strong spots generated by the test solution indicate drug degradation especially when associated with a smaller principal spot. Some fainter spots emerging near or on the origin line of the chromatoplate are normally caused by auxiliary agents incorporated in the different tablet and capsule formulations.

XIII. OBSERVATIONS MADE IN DAYLIGHT AFTER STAINING WITH IODINE

When exposing the chromatoplate to iodine vapour, several orange-brown spots are generated matching the pattern of spots already observed on the plate exposed to sulfuric acid. It is an alternative method in order to evaluate the quantities of artesunate present.

XIV. RESULTS & ACTIONS TO BE TAKEN

The principal spot in the chromatogram obtained with the test solution must correspond in terms of colour, size, intensity, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solution. This result must be obtained for each method of detection. If this is not achieved repeat the run with a second sample from scratch. Reject the batch if the drug content can't be verified in a third run. For a second opinion, refer additional samples to a fully equipped drug control laboratory. Retain samples and put the batch on quarantine till a final decision on rejection or release has been taken.