A Concise Quality Control Guide On Essential Drugs And Other Medicines

Manual

Accompanying The GPHF-Minilab®

Artesunate

Special Issue On Artesunate
Colour Reaction And Thin Layer Chromatography

GPHF
German Pharma Health Fund e.V.

An Initiative of Research Based Pharmaceutical Companies in Germany

Colour Reaction

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 an identity test.

Verification of Identity via Colour Reaction

I. EQUIPMENT AND REAGENTS

1) Circular filter paper
2) Pestle
3) Spatula
4) Microspoon
5) Graduated test-tube
6) Transfer pipette
7) Fast Red TR diazonium salt with a dye content of 15% from Sigma-Aldrich Fine Chemicals
8) Sodium hydroxide 4%
9) Glacial acetic acid
10) Water

II. PREPARATION OF TEST SOLUTION

Fill the tip of the spatula (50 to 100 mg) with Fast Red TR salt and transfer the powder into one of the graduated test-tubes supplied. Add 5 ml of water, one drop of glacial acetic acid and shake till all solids are dissolved. This will be your test solution which needs to be prepared freshly before use.

III. PREPARATION OF SAMPLE

Put one tablet on a circular filter paper. Break down the tablet into small bits and pieces using a pestle. Grind till a fine powder is produced. Seperate the coating from the powder if a colour-coated tablet has been used. If the drug has been formulated as a capsule just open one by carefully separating the cap from the bottom shell and use the powder content directly.

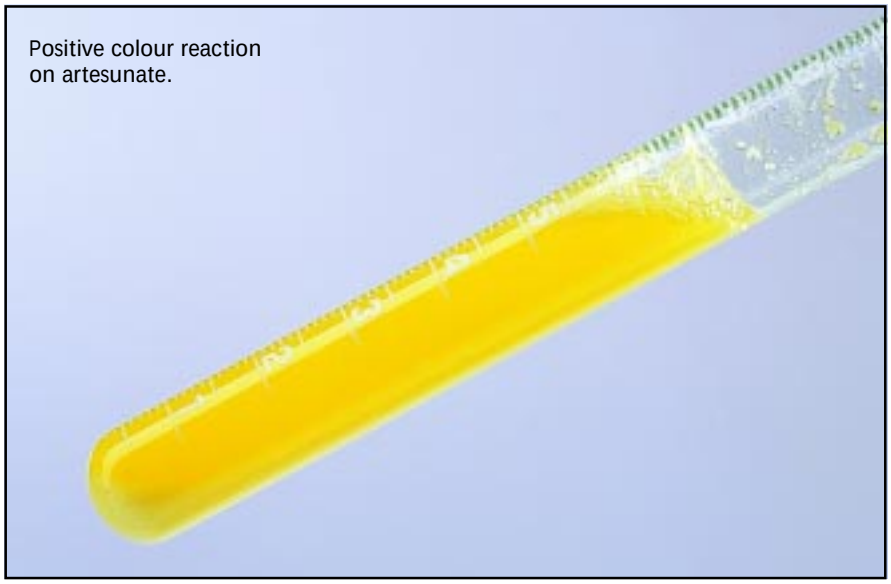
Place the entire powder obtained from a 50 mg tablet/capsule or one fourth of the powder obtained from a 200 mg tablet/capsule (about equivalent to 10 microspoons each) into the test tube. This will be your sample.

IV. COLOUR REACTION

Add to the sample 5 ml of *sodium hydroxide 4%*, shake thoroughly, and allow the solution to sit for 15 minutes at room temperature. Using a transfer pipette, add 30 drops (0.6 ml) of *glacial acetic acid* and thoroughly shake the test-tube again. To this mixture, add 0.5 ml of *Fast Red TR test solution* and shake again; a vivid lemon colour is instantly produced. Repeat the examination with two other samples thus eliminating anomalous results.

Note: Alternatively to shaking, a glass rod can be used for mixing. However mixing is achieved, it must be done thoroughly.

V. COLOUR OBSERVED



Positive colour reaction on artesunate.

VI. IMPORTANT NOTE

The colour reaction described here is specific to artesunate only in a slightly acidic reaction mix. Thus verification of acidity/pH is important. Litmus blue test paper must turn into red and litmus red must stay red. Universal pH indicator test paper shall show a pH of 4. In caustic solutions a yellow or orange colour indicates also the presence of other drugs, for example of artemisinin, primaquine and sulfa-doxine. However, no colour observed here clearly indicates that there is no drug present at all.

VII. RESULTS & ACTIONS TO BE TAKEN

Confirm the drug's identity and verify its potency via a thin layer chromatographic assay if the product shows a positive respond on the colour reaction performed. Do not spoil valuable resources from the TLC kit on batches definitely failing to produce the colour requested. In this case, reject the batch and retain some samples. Refer to a fully equipped drug quality control laboratory for further investigation. Put the batch on quarantine till a final decision on rejection or release has been taken.

Thin Layer Chromatography

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 chromatograpic assay.

Verification of Identity and Drug Content via Thin Layer Chromatograpy

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 F254, size 5x10 cm
11) Glass microcapillaries of 2-µl filling capacity
12) Hot plate
13) TLC developing chamber (jar)
14) Filter paper
15) Pair of scissors
16) Iodine chamber
17) Petri dish
18) Methanol
19) Sulfuric acid 96%
20) Glacial acetic acid
21) Ethylacetate
22) Acetone
23) 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. Just transfer the undiluted supernatant liquid of the stock standard solution into a 10-ml vial and label it as *'Artesunate Working Standard Solution 100%'*. 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. This solution should contain 5.0 mg of total drug/ml and be labelled as *'Artesunate Stock Sample Solution'*. Freshly prepare the sample solution for each test.
VII.	PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A DRUG PRODUCT CLAIMING A POTENCY OF 200 MG ARTESUNATE PER UNIT	As usual, wrap up a sample tablet into aluminium foil and crush it 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 20 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. For further dilution, mix 4 ml of the hazy supernatant liquid with 4 ml of methanol in a 10-ml vial. Close and shake the vial and label it as *'Artesunate Stock Sample Solution'*. The sample solution should now contain 5.0 mg of total drug/ml. Freshly prepare this solution for each test.

VIII. 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.

IX. SPOTTING

Mark an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate and apply 2 µl 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 auxialliary agents from the capsule or tablet matrix as well as residual extraction medium will 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.

X. 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.

XI. DETECTION

Chromatoplate A: 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 a petri dish and soak the chromatoplate 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 by using a hot plate and observe how the principal spots are gradually becoming visible in daylight. Allow not more than 15 seconds to finish all operation procedures from dipping to drying. Use this method of detection for quantification purposes.

Chromatoplate B: Replace the methanolic sulfuric acid solution by a mixture of 18 ml of methanol, 2 ml of anisaldehyde and 2 ml of sulfric acid 96% (to be mixed in the same order as listed) and repeat the operation procedure on detection mentioned above with this alternative reagent solution. Carefully dry the plate at moderate heat eventually using the hot plate, from time to time, in order to support and accelerate air drying and spot detection.

Chromatoplate C: 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 been well activated.

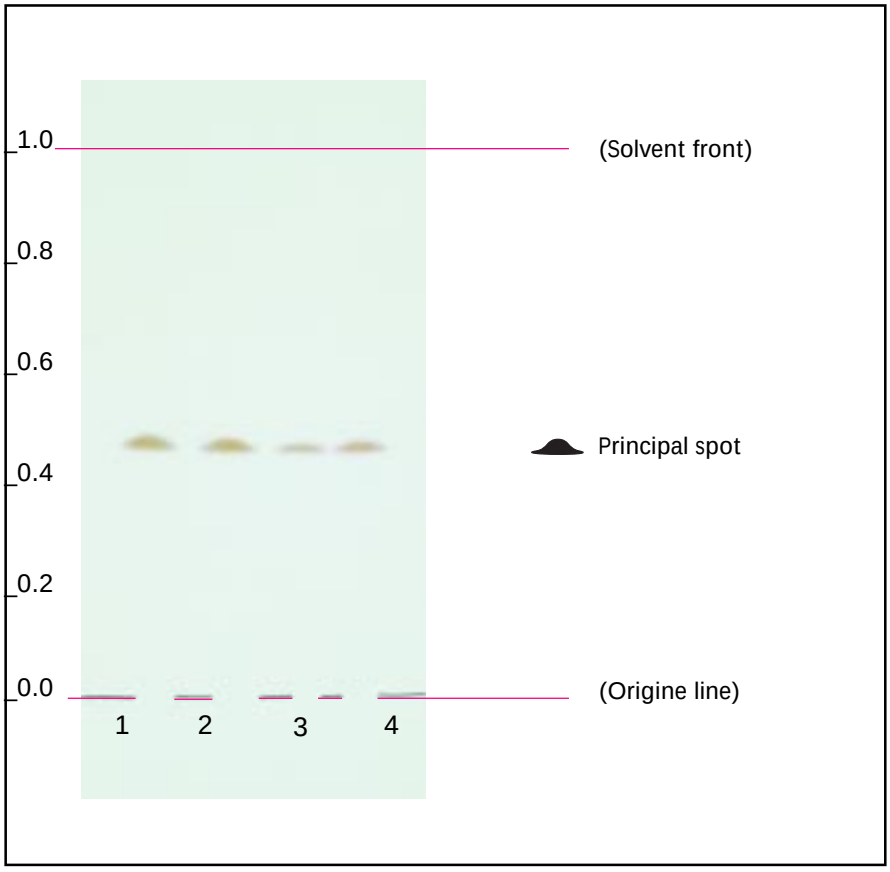
XII. CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER EXPOSURE TO SULFURIC ACID SOLUTION

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.



XIII. OBSERVATIONS MADE AFTER EXPOSURE TO SULFURIC ACID SOLUTION

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.

XIV. OBSERVATIONS MADE AFTER EXPOSURE TO ANISALDEHYDE SOLUTION

Several pinkish spots are generated matching the pattern of spots already observed on the plate exposed to sulfuric acid solution. With time, the spots' colour might change from pinkish red to violet blue. This is an alternative method of detection in order to evaluate the quantities of artesunate present.

XV. OBSERVATIONS MADE AFTER EXPOSURE TO IODINE VAPOUR

Several orange-brown spots are generated matching the pattern of spots already observed on the plate exposed to sulfuric acid solution. It is another alternative to evaluate the quantities of artesunate present.

XVI. 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.

Detecting Counterfeit and Substandard Drugs

The GPHF-Minilab®: Simple Test Methods for the Quality Assurance of Pharmaceuticals

Visual Inspection

Disintegration Test

Colour Reactions

Thin Layer Chromatography

- rapid drug quality control at low cost
- for the top range of essential drugs
- for hospitals, retail pharmacies ...
- physical and chemical testing on the spot
- a laboratory assembled in two cases
- self-contained for in- and outdoor use
- tropics-compatible
- ready-packed for world-wide delivery by air
- comes with easy-to-read manuals and a full set of starter kit chemicals

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