

# Manual

Accompanying The GPHF-Minilab®

**Extension 2004  
More Antimalarials**

Fourth Supplement To Volume II  
Thin Layer Chromatography



An Initiative of Research Based Pharmaceutical Companies in Germany

# **A Concise Quality Control Guide On Essential Drugs And Other Medicines**

## **Fourth Supplement To Volume II On Thin Layer Chromatography**

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# Contents

Chapter

Page

## Foreword

<b>1</b>	Individual Standard Operation Procedures ..... 5 <i>Supplement to Volume II, Chapter 7</i>	5
7.37	Amodiaquine ..... 6	6
7.38	Lumefantrine/Artemether Fixed-Dose Combinations ..... 10	10
7.39	Primaquine ..... 14	14
<b>2</b>	Synopsis of Chromatographic Working Conditions ..... 18 <i>Supplement to Volume II, Chapter 8</i>	18
<b>3</b>	The Minilab's Updated List of Inventory Items ..... 19 <i>Supplement to Volume II, Chapter 10</i>	19
<b>4</b>	Health & Safety Instructions ..... 23	23

## 7.38 Lumefantrine/Artemether Fixed-Dose Combinations

### 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 a 120 mg of lumefantrine combined with 20 mg of artemether.

#### II. DISINTEGRATION TEST

All quick release lumefantrine/artemether fixed-dose combination 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

Lumefantrine/artemether fixed-dose combination tablets or capsules are extracted with acetone and determined by TLC with reference to an authentic secondary standard.

#### II. EQUIPMENT AND REAGENTS

- |   |  |
|---|--|
| 1) Pestle   | 14) Filter paper   |
| 2) Aluminium foil   | 15) Pair of scissors   |
| 3) Laboratory glass bottles with a filling capacity of 25 to 100 ml             | 16) Pair of tweezers   |
| 4) Funnel   | 17) UV light of 254 nm   |
| 5) Set of straight pipettes (1-25 ml)   | 18) Iodine chamber   |
| 6) 10-ml vials  | 19) TLC dipping chamber (Petri dish)   |
| 7) Label tape   | 20) Sulfuric acid 96%  |
| 8) Marker pen   | 21) Methanol   |
| 9) Pencil   | 22) Acetone  |
| 10) Merck TLC aluminium plates pre-coated with silica gel 60 F254, size 5x10 cm | 23) Ethylacetate   |
| 11) Glass microcapillaries of 2-µl filling capacity                             | 24) Glacial acetic acid  |
| 12) Hot plate   | 25) Toluene  |
| 13) TLC developing chamber (jar)  | 26) Authentic reference standard, for example lumefantrine/artemether 120/20 mg fixed-dose combination tablets |

#### III. PREPARATION OF THE LUMEFANTRINE STOCK STANDARD SOLUTION

The preparation of a stock standard solution requires an authentic product for reference purposes, for example a tablet containing 120 mg of lumefantrine combined with 20 mg of artemether. Wrap up the tablet in aluminium foil and crush it down to a fine powder using a pestle. Empty the foil into a 100-ml glass bottle and wash down residual solids with 50 ml of acetone using a straight pipette. Close the bottle and shake for about three minutes till most of the solids are dissolved. Leave the solution to sit for a further five minutes and allow the undissolved residues to settle at the bottom. In terms of lumefantrine, the solution obtained should contain 2.4 mg of total drug per ml and be labelled as '*Lumefantrine Stock Standard Solution*'. Freshly prepare this solution for each test. Continue to work with the hazy supernatant liquid or clear dilution obtained.

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

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 2 ml of acetone. Close and shake the vial. The solution obtained should contain 0.8 mg of total drug per ml and be labelled as '*Lumefantrine Working Standard Solution 100%*'.

In terms of lumefantrine, this higher working standard solution represents a product of good quality containing 100 % of total drug.

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

Pipette 4 ml of the stock standard solution into a 25-ml glass bottle and add 11 ml of acetone. Close and shake the vial. The solution obtained should contain 0.64 mg of total drug per ml and be labelled as '*Lumefantrine Working Standard Solution 80%*'.

In terms of lumefantrine, this lower working standard solution represents a medicine of poor quality containing just 80% of the amount of drug 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 LUMEFANTRINE  
STOCK SAMPLE SOLUTIONS FROM  
DRUG PRODUCTS CLAIMING A  
POTENCY OF 120 MG OF  
LUMEFANTRINE PER UNIT

Take one whole tablet or capsule from an appropriate drug product sampled in the field. Tablets are wrapped up into aluminium foil and crushed down to a fine powder prior to transfer into a 100-ml laboratory glass bottle. Powder obtained from a capsule should be put directly into the laboratory glass bottle adding finally the empty cap and body shells as well. Then, add 50 ml of acetone 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. In terms of lumefantrine, the solution obtained should contain 2.4 mg of total drug per ml and be labelled as '*Lumefantrine Stock Sample Solution*'. Freshly prepare this solution for each test. Continue to work with the hazy supernatant liquid or clear dilution obtained.

VII. PREPARATION OF LUMEFANTRINE  
WORKING SAMPLE SOLUTIONS

Pipette 1 ml of the stock sample solution into a 10-ml vial and add 2 ml of acetone. Close and shake the vial and label it as '*Lumefantrine Working Sample Solution*'.

The expected concentration of lumefantrine in this solution is 0.8 mg per ml and should match the concentration of lumefantrine of the higher working standard solution produced above.

VIII. 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 on the next page 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. 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 4 ml of ethylacetate, 2 ml of glacial acetic acid and 18 ml of toluene 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 observe the chromatoplate with UV light of 254 nm using the battery-driven fluorescent lamp supplied. Use this method of detection for the quantification of lumefantrine. Further verification of lumefantrine's identity and content can be achieved when observing the same plate in daylight after iodine staining.

For the detection of the artemether portion, dip the plate into methanolic sulfuric acid solution 5% and dry on a hot plate. For more details on this, consult the operation procedure for artesunate detection on page eight of the second TLC supplement supplied along all other manuals. Note that this method of detection will make it impossible to further observe the lumefantrine spots with UV light of 254 nm.

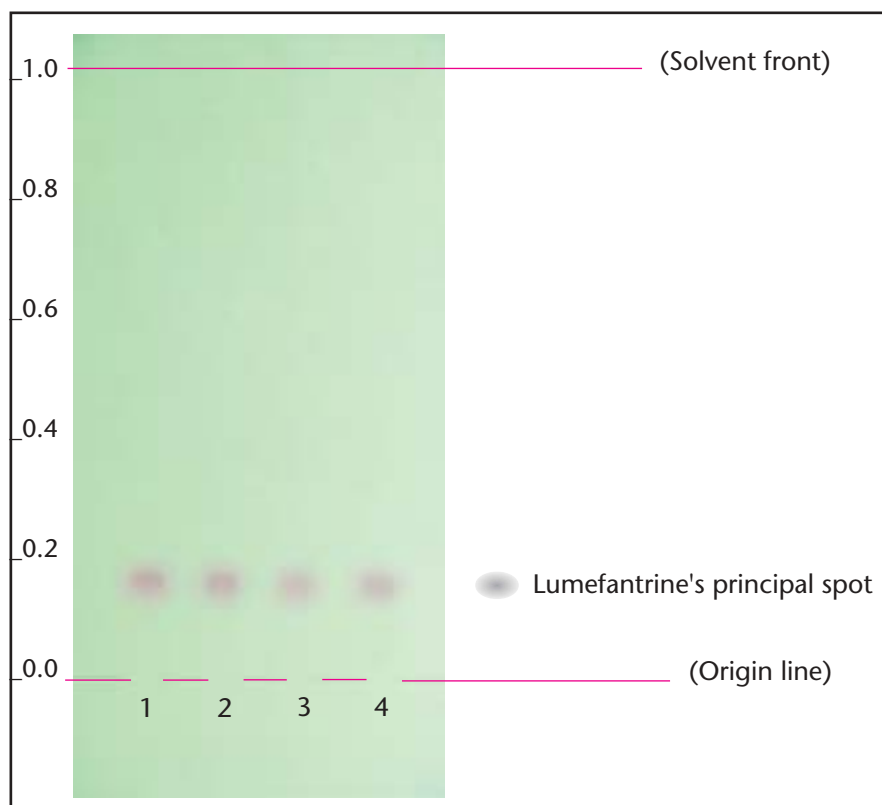
## XI. VIEWING LUMEFANTRINE ON A CHROMATOPLATE EXPOSED TO UV LIGHT OF 254 NM

Run No.1:  
Lumefantrine'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:  
Lumefantrine's lower working limit representing 80% of total drug.



## XII. OBSERVATIONS

The presence of lumefantrine is indicated by a strong blue-violet spot at a travel distance of about 0.16 when the chromatoplate is observed at 254 nm with the UV lamp supplied. In preparations where lumefantrine is presented in a fixed combination with artemether, a second principal spot can be observed at a travel distance of about 0.56 after the plate has been exposed to sulfuric acid and heat. As artemether's dosage strength is six

times lower than that of lumefantrine, these spots are looking weak but are not allowed to be missing. Additional strong spots generated by the lumefantrine test solution may 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 finished product formulations being in the market.

### XIII. ARTEMETHER CONTENT VERIFICATION

For artemether content verification, the upper working standard solution should contain 2 mg of artemether per ml. For this, extract one fixed-dose combination tablet or capsule with 10 ml of acetone. Adjust the concentration to 1.6 mg of artemether per ml when preparing the lower working standard solution (mix 4 ml of upper working standard solution with 1 ml of acetone). Spot 2  $\mu$ l of each working standard and sample solution and develop the chromatoplate as for lumefantrine. Artemether's principal spots are showing a relative retention factor of about 0.56 and are best detected with methanolic sulfuric acid solution on a hot plate as for artesunate (see page eight of the second TLC supplement).

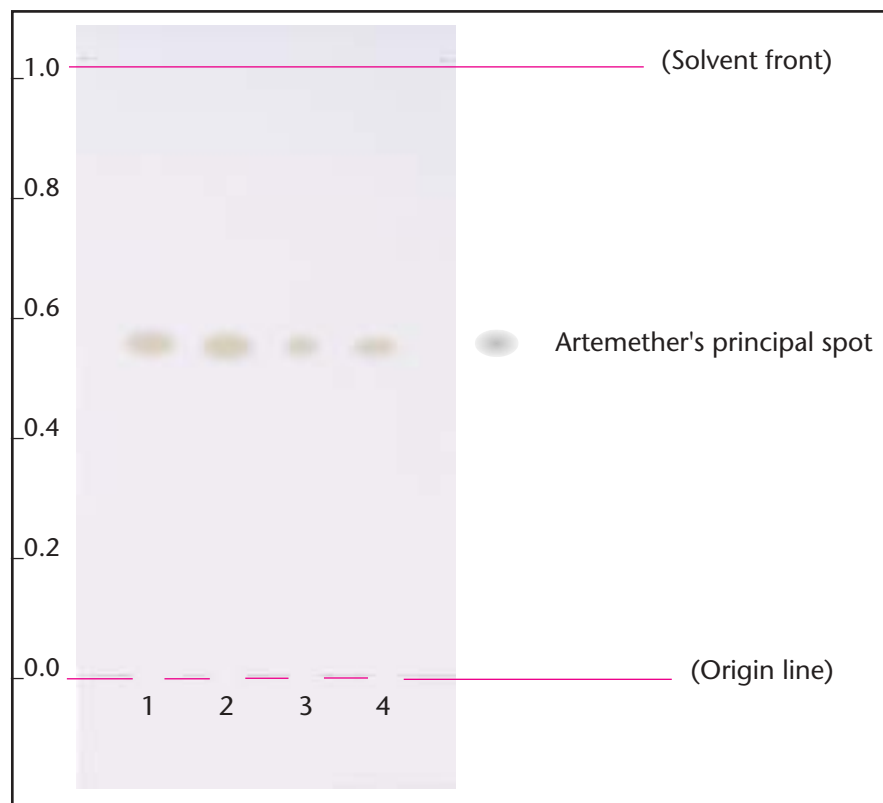
### XIV. VIEWING ARTEMETHER ON A CHROMATOPLATE EXPOSED TO SULFURIC ACID AND HEAT

Run No.1:  
Artemether'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:  
Artemether's lower working limit representing 80% of total drug.



### XV. RESULTS & ACTIONS TO BE TAKEN

The principal spot(s) 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.