A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab®

Supplement 2011

Volume II

Thin Layer Chromatographic Tests

GPHF
GLOBAL PHARMA HEALTH FUND E.V.

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USP

PROMOTING THE QUALITY OF MEDICINES

A Concise Quality Control Guide on Essential Drugs and other Medicines

Supplement 2011 to Volume II on Thin Layer Chromatographic Tests

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About the GPHF-Minilab® Project

Counterfeit medicines proliferation constitutes serious health hazards. The World Health Organization (WHO) estimates that a disturbing proportion of ten to thirty percent of all drugs offered in developing countries are either counterfeit or of deficient quality already. Fighting fakes will ensure that decades of investments in healthcare are not undone through lack of vigilance.

To prevent counterfeit and substandard anti-infective medicines infiltrating drug supply organisations and priority disease programmes in malaria, TB and HIV/AIDS endemic countries, the Global Pharma Health Fund (GPHF) in Frankfurt, a charity maintained exclusively by Merck Darmstadt · Germany, set out to develop and supply at low cost the GPHF-Minilab®, a mini-laboratory for rapid drug quality verification and counterfeit medicines detection.

Since twelve years, GPHF-Minilabs are acting as a first-line defence against counterfeit and substandard quality medicines threatening the health of millions of people living in developing nations. Overall, more than 400 Minilabs have been supplied to over 70 countries across the African, Asian-Pacific and Latin American region already.

Main implementation partners are national health and medicines regulatory authorities together with the World Health Organization and the U.S. Pharmacopeia's Promoting the Quality of Medicines programme. Joint drug quality monitoring projects run by Interpol in South East Asia and East Africa triggered off the seizure of millions of counterfeit antimalarial pills without any active principles in the recent years.

The unchanged need for non-sophisticated and affordable drug quality monitoring in low-income countries forms the driving force behind the development of new GPHF-Minilab® test protocols today. The need for more testing emphasises the important collaboration with our US based implementing partners. For better health in developing countries, other parties are invited to join in.

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6.54 Dihydroartemisinin (incl. piperaquine phosphate co-formulations)

Primary Screening via Physical Inspection and Disintegration Test

I. PHYSICAL 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 20, 60 or 80 mg of dihydroartemisinin. More recently, single drug formulations for monotherapy are replaced by fixed-dose combination medicines consisting of a 40 mg of dihydroartemisinin and 320 mg of piperaquine tetraphosphate.

II. DISINTEGRATION TEST

All quick release dihydroartemisinin single dose and 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's a major defect if a drug product doesn't 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 test.

Verification of Drug Identity and Content via Thin Layer Chromatography

I. PRINCIPLE

Dihydroartemisinin is extracted from tablets and capsules with methanol and determined by TLC with reference to an authentic secondary standard. For a verification of piperaquine phosphate in fixed-dose combination medicines go to page 28 of this supplement.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Funnel
4) Label tape
5) Marker pen
6) Pencil and ruler
7) 10-ml vials
8) Set of straight pipettes (1 to 25 ml)
9) Set of laboratory glass bottles (25 to 100 ml)
10) Merck TLC aluminium plates precoated with silica gel 60 F_{254}, size 5x10 cm
11) Glass microcapillaries (2-µl filling capacity)
12) TLC developing chamber (500-ml jar)
13) Hot plate
14) Filter paper
15) Pair of scissors
16) Pair of tweezers
17) UV light of 254 nm
18) TLC dipping chamber (250-ml beaker)
19) Sulphuric acid solution 96%
20) Ethyl acetate
21) Methanol
22) Ammonia solution 25%
23) Authentic reference standard, for example, fixed-dose combination tablets containing 40 mg of dihydroartemisinin and 320 mg of piperaquine phosphate

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic drug product for reference purposes, for example, tablets containing 40 mg of dihydroartemisinin combined with 320 mg of piperaquine phosphate. Wrap up one reference tablet into aluminium foil and crush it down to a fine powder using a pestle. Carefully empty the aluminium foil over a 40-ml laboratory glass bottle and wash down all residual solids with 20 ml of methanol using a straight pipette. Close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to sit for an additional five minutes until undissolved residues settle below the supernatant liquid. The solution obtained should contain 2 mg of total dihydroartemisinin per ml and be labelled as *'Dihydroartemisinin Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

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

The stock standard solution requires no further dilution. It already represents the final working concentration of 2 mg of total dihydroartemisinin per ml. Just for more convenient handling, some of the supernatant liquid may want to be transferred into a 10-ml vial.

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

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 1.6 mg of total dihydroartemisinin per ml and be labelled as *'Dihydroartemisinin Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of dihydroartemisinin 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 PRODUCT CLAIMING TO CONTAIN 20 MG OF DIHYDROARTEMISININ PER UNIT

Take one whole tablet or capsule from an appropriate drug product sampled in the field. As usual, tablets are wrapped up into aluminium foil and crushed down to a fine powder. Transfer all the powder obtained into an appropriate laboratory glass bottle. Powder obtained from a sample capsule should be transferred directly into the bottle adding the cap and body shells last. For extraction, add 10 ml of methanol using a straight pipette, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to sit for an additional five minutes until undissolved residues settle below the supernatant liquid.

40 MG OF DIHYDROARTEMISININ PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 20 ml of methanol following the procedure described above.

60 MG OF DIHYDROARTEMISININ PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 30 ml of methanol following the procedure described above.

80 MG OF DIHYDROARTEMISININ PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 40 ml of methanol following the procedure described above.

All stock sample solutions produced should finally contain 2 mg of total dihydroartemisinin per ml and be labelled as *'Dihydroartemisinin Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue to work with the clear or hazy supernatant liquids.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Dihydroartemisinin stock sample solutions require no further dilution. They already represent the final working concentration of 2 mg of total dihydroartemisinin per ml. If prepared from a high quality product, the sample solution should match the concentration of dihydroartemisinin 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 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 dihydroartemisinin itself stays invisible, excipients and other drug compounds will show up to facilitate verification. All spots should be circular in shape and equally spaced across the origin line. Although their intensities might differ, their diameters 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 homogeneous spotting is not achieved first time.

IX. DEVELOPMENT

Pipette 16 ml of ethyl acetate, 4 ml of methanol and 3 ml of concentrated ammonia solution 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

When working on fixed-dose combination medicines, it is best to check the presence of piperaquine phosphate before that of dihydroartemisinin. For this, expose the dried chromatoplate first to UV light of 254 nm using the battery-driven lamp supplied.

After the presence or absence of piperaquine has been verified, the chromatoplate can be exposed to sulphuric acid staining for the detection of dihydroartemisinin. For this, fill the 250-ml plastic beaker supplied with a 190 ml of methanol followed by 10 ml of concentrated sulphuric acid solution and mix gently. Allow the mix to cool down and submerge the chromatoplate upside down into the staining solution using a pair of tweezers. Instantly remove the plate and let all surplus solution run down onto paper tissue. Wipe off residual liquid from the back of the plate and continue to dry off all staining solution on the hot plate supplied. During heating, all dihydroartemisinin spots are gradually becoming visible at daylight. Use this method of detection for both, dihydroartemisinin identification and quantification purposes.

After staining with sulphuric acid and heat, a further detection of dihydroartemisinin and piperaquine is possible when exposing the dyed chromatoplate to UV light of 366 nm in a dark room.

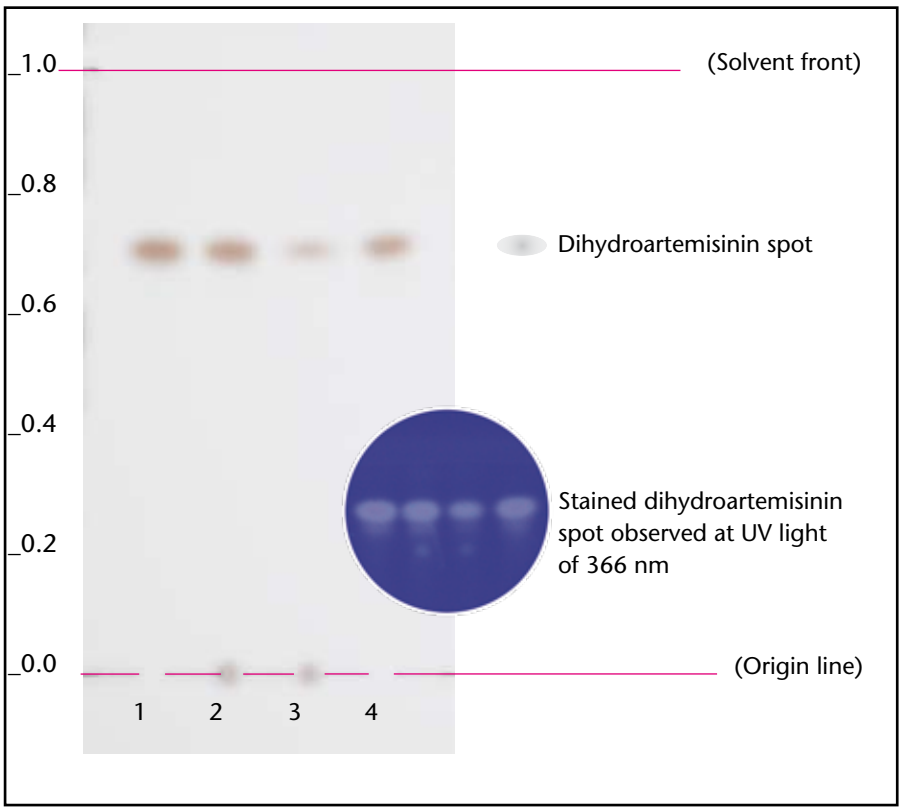
XI. CHROMATOPLATE OBSERVED AT DAYLIGHT AND UNDER UV LIGHT OF 366 NM AFTER SULPHURIC ACID STAINING

Run No.1:
Upper working standard representing 100% of total dihydroartemisinin

Run No.2:
A drug product of good quality with acceptable drug content

Run No.3:
A drug product of poor quality with unacceptable low drug content

Run No.4:
Lower working standard representing 80% of total dihydroartemisinin



XII. OBSERVATIONS MADE AT 254 NM BEFORE STAINING

Dihydroartemisinin stays invisible and no other spots should be detected unless the sample under investigation comes as fixed-dose combination medicines containing also piperaquine phosphate. In case of co-formulations, a spot representing piperaquine will become visible at a travel distance of about 0.54. Weak spots observed here are due to the low solubility of piperaquine phosphate in methanol used for sample extraction. Other spots generated by the test solution would point to other drugs.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER SULPHURIC ACID STAINING

A brown spot at a travel distance of about 0.69 indicates the presence of dihydroartemisinin in the test solution. No other spots should be visible even if dihydroartemisinin is combined with piperaquine phosphate. Additional strong spots generated by the test solution would point at other drugs or dihydroartemisinin degradation, the latter case being more likely when associated with a smaller principal spot. Auxiliary agents incorporated in the different tablet or capsule formulations might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XIV. OBSERVATIONS MADE AT 366 NM AFTER SULPHURIC ACID STAINING

Both spots, the one for dihydroartemisinin and piperaquine, previously observed step by step with different detection methods are now becoming visible simultaneously; the spot for dihydroartemisinin showing an off-white and the one for piperaquine a light blue fluorescence.

XV. RESULTS & ACTIONS TO BE TAKEN

The dihydroartemisinin 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 from scratch with a second sample. Reject the batch if the drug content cannot be verified in a third run. For a second opinion, refer additional samples to a fully-fledged drug quality control laboratory. Retain samples and put the batch on quarantine until a final decision on rejection or release has been taken. For documentation purposes, take pictures of all the readings with a digital camera turning off the flash first.

Genuine or Counterfeit?

Combating Counterfeit Medicines · Protecting People's Life

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