

Manual

Accompanying the GPHF-Minilab®

Supplement 2010

Volume II

THIN LAYER CHROMATOGRAPHIC TESTS



A Charity Initiated and Maintained
by Merck, Darmstadt · Germany



U.S. PHARMACOPEIA
DRUG QUALITY AND
INFORMATION PROGRAM

A Concise Quality Control Guide on Essential Drugs and other Medicines

SUPPLEMENT 2010 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.

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 ten years, GPHF-Minilabs are acting as a first-line defence against counterfeit and substandard medicines threatening the health of millions of people living in developing nations. Overall, more than 330 Minilabs have been supplied across 70 countries in Africa, Asia and Latin America already.

Main implementation partners are national health and medicines regulatory authorities together with the World Health Organization and the U.S. Pharmacopeia Drug Quality and Information Program. Joint drug quality monitoring projects run in South East Asia and East Africa triggered off the seizure of millions of counterfeit antimalarial pills without any active principles by Interpol 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 is also the starting point for an intensified collaboration with our US based implementing partners. For better health in developing countries, other parties are invited to join in.

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Table of Contents

	Page
New TLC Test Protocols	4
<i>Supplement to Volume II, Chapter 6</i> <i>More Antimalarial, Antibacterial, Antituberculosis and Anthelmintic Medicines</i>	
6.42 Albendazole	4
6.43 Atovaquone (incl. proguanil co-formulations)	8
6.44 Cefixime	12
6.45 Cefuroxime axetil	16
6.46 Halofantrine	20
6.47 Levofloxacin	24
6.48 Moxifloxacin	28
6.49 Proguanil	32
6.50 Prothionamide	36
Summary Table of Chromatographic Working Conditions	40
<i>Supplement to Volume II, Chapter 7</i>	
Updated List of GPHF-Minilab® Reference Standards	41
<i>Supplement to Volume II, Chapter 10</i>	
Health & Safety Instructions	43

6.50 Prothionamide

Primary Screening via Visual Inspection and Disintegration Test

I. VISUAL & 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 250 mg of prothionamide.

II. DISINTEGRATION TEST

All quick release prothionamide 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

Prothionamide 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) Funnel
- 4) Label tape
- 5) Marker pen
- 6) Pencil
- 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 pre-coated with silica gel 60 F 254, size 5x10 cm
- 11) Glass microcapillaries (2- μ l 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) Iodine chamber
- 19) Ethyl acetate
- 20) Methanol
- 21) Secondary reference standard, for example, prothionamide 250 mg tablets

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 250 mg of prothionamide. 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 25 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 10 mg of total drug per ml and be labelled as '*Prothionamide 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)

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 3 ml of methanol. Close and shake the vial. The solution obtained should contain 2.5 mg of total drug per ml and be labelled as '*Prothionamide Working Standard Solution 100%*'.

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

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

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 4 ml of methanol. Close and shake the vial. The solution obtained should contain 2 mg of total drug per ml and be labelled as '*Prothionamide Working Standard Solution 80%*'.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of prothionamide 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 250 MG OF PROTHIONAMIDE 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 a 40-ml laboratory glass bottle. Powder obtained from sample capsules should be transferred directly into the bottle adding the cap and body shells last. For extraction, add 25 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.

All stock sample solutions obtained should finally contain 10 mg of total drug per ml and be labelled as '*Prothionamide 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

Pipette 1 ml of the stock sample solution into a 10-ml vial and add 3 ml of methanol. Close and shake the vial and label as '*Prothionamide Working Sample Solution*'.

The expected concentration of prothionamide in the working sample solution is 2.5 mg per ml and should match the concentration of prothionamide 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 μ l of each test and standard solution 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. 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 18 ml of ethyl acetate and 2 ml of methanol 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 10 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 under UV light of 254 nm using the battery-driven lamp supplied. Use this method of detection for both, identification and quantification purposes. Further verification of drug identity and content can be achieved when observing the plate at daylight after iodine staining.

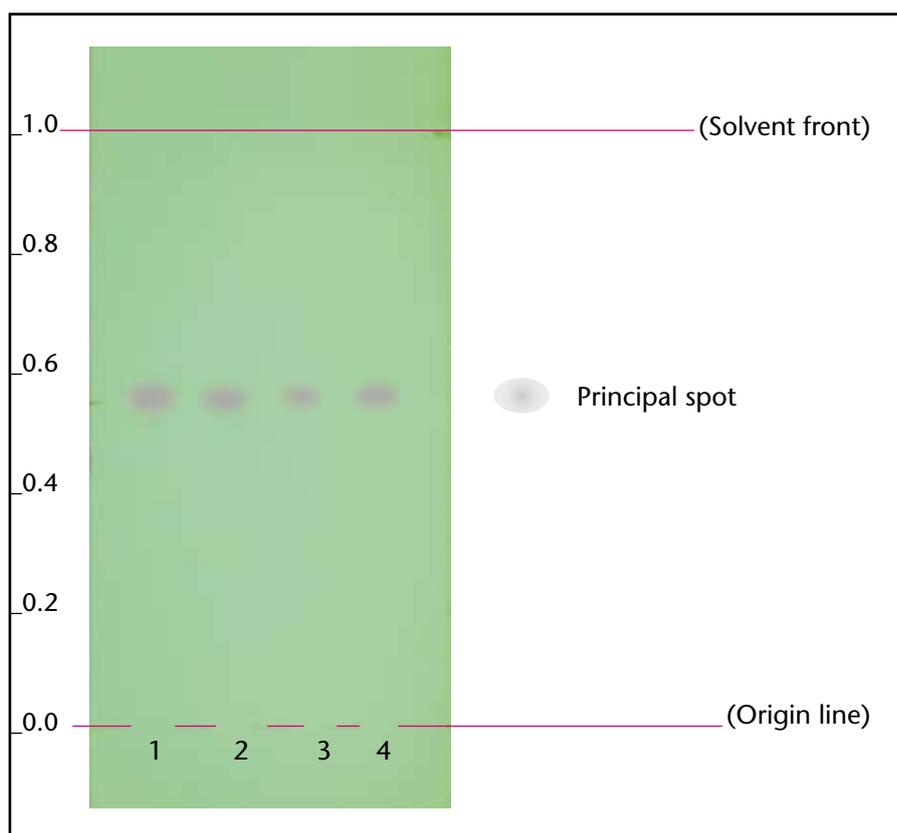
XI. CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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 prothionamide



XII. OBSERVATIONS MADE AT 254 NM

A grey-violet spot at a travel distance of about 0.52 indicates the presence of prothionamide in the test solution. Additional strong spots generated by the test solution would point at other drugs or prothionamide 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 emerging near or on the origin line.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all prothionamide spots already observed at 254 nm are now turning yellowish brown. Still observe the plate when iodine evaporates already. Spots reflecting poor quality products will disappear first gradually followed by the reference spots representing a drug content of 80 and 100 percent, respectively.

XIV. RESULTS & ACTIONS TO BE TAKEN

The prothionamide 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 precise drug content determination, 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.

Genuine or Counterfeit?



Fighting Counterfeit Medicines · Protecting People's Life

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