A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab®

Supplement 2014

Volume II

Thin Layer Chromatographic Tests

GPHF
GLOBAL PHARMA HEALTH FUND E.V.

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PROMOTING THE QUALITY OF MEDICINES

A Concise Quality Control Guide on Essential Drugs and other Medicines

Supplement 2014 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 international police organisation Interpol 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 falsified medicines will ensure that decades of investments in healthcare are not undone through lack of vigilance.

To prevent counterfeit and extreme poor 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 many 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 600 Minilabs have been supplied to over 80 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 more patient safety and better health in developing countries, other parties are invited to join in.

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6.74 Aminosalicylate (PAS) sodium and free base as modified release granules

Primary Screening via Physical Inspection and Disintegration Test

I. PHYSICAL INSPECTION

Search for deficiencies on labelling and packaging 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. Modified release granules usually contain an average content of 60% by weight of para-aminosalicylate sodium salt (PAS sodium) or para-aminosalicylic acid (PAS).

II. DISINTEGRATION TEST

Unlike tablets, aminosalicylate modified release granules are not tested for disintegration.

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

Both forms, para-aminosalicylate sodium salt (PAS sodium) and para-aminosalicylic acid (PAS), are extracted from modified release granules with methanol and the presence and content of the active principles in the test solution are then verified by TLC against an appropriate reference standard.

II. EQUIPMENT AND REAGENTS

1) Pocket balance
2) Pestle
3) Aluminium foil
4) Funnel
5) Label tape
6) Marker pen
7) Pencil
8) 10-ml vials
9) Set of straight pipettes (1 to 25 ml)
10) Set of laboratory glass bottles (25 to 100 ml)
11) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
12) Glass microcapillaries (2-µl filling capacity)
13) TLC developing chamber (500-ml jar)
14) Hot plate
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) UV light of 366 nm
20) Iodine chamber
21) Methanol
22) Acetone
23) Toluene
24) Glacial acetic acid
25) Reference standard, for example para-aminosalicylic acid anhydrate (4-amino-2-hydroxybenzoic acid) as analytical reagent grade of commerce

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires para-aminosalicylic acid anhydrate (4-amino-2-hydroxybenzoic acid) as analytical reagent grade of commerce or appropriate raw material of good quality for reference purposes. Put a piece of aluminium foil onto the weighing pan of the electronic pocket balance supplied, zero the balance and weigh in correctly about 0.30 g of para-aminosalicylic acid using a spatula. Carefully empty the aluminium foil over a 40-ml laboratory glass bottle and wash down all the powder obtained with 30 ml of methanol using a straight pipette. Write down each time the exact weighing result and adjust the amount of methanol

for dissolution appropriately, for example, using 29 ml of methanol when 0.29 g or 32 ml of methanol when 0.32 g of reference standard have been collected from the bulk container. Close the laboratory bottle and shake until all solids are dissolved. The final solution obtained should contain 10 mg of total para-aminosalicylic acid per ml and be labelled as *'PAS Stock Standard Solution'*. Freshly prepare this solution for each test.

Important note: The balance supplied cannot perfectly manage quantities below 0.25 g. The relative standard deviation of +/- 2% is considered to high. With higher quantities measured, the deviation drops to about +/- 1% only. Also, the balance will not easily pick up changes of a few milligrams added or removed when carefully approaching the target weight of 0.30 g step by step. Hence, lift the aluminium foil or tap the weighing pan with a pen or spatula each time after a few more milligrams have been added or removed thus overcoming any dynamic inertia and ensuring correct readings.

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

Pipette 1 ml of the stock standard solution into a 25-ml laboratory glass bottle and add 19 ml of methanol. Close and shake the vial. The solution obtained should contain 0.5 mg of total para-aminosalicylic acid per ml and be labelled as *'PAS Working Standard Solution 100%'*.

This higher working standard solution represents a drug product of good quality containing 100 % of para-aminosalicylic acid.

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

Pipette 1 ml of the stock standard solution into a 25-ml laboratory glass bottle and add 24 ml of methanol. Close and shake the vial. The solution obtained should contain 0.4 mg of total para-aminosalicylic acid per ml and be labelled as *'PAS Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of para-aminosalicylic acid 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 GRANULES CLAIMING TO CONTAIN IN AVERAGE 60% (W/W) OF PARA-AMINOSALICYLIC ACID

Take one sealed sachet or a bulk storage container from an appropriate drug product sampled in the field. Open and weigh in well above a 1000 mg of granules. Wrap up in aluminium foil and slowly grind the granules down to a fine powder. Take your time and ensure that the coating is cracked for each granule. From the final powder obtained, recover a 1000 mg or the equivalent of 600 mg of para-aminosalicylic acid following the same weighing steps as explained for the stock standard solution above. Transfer the powder into a 100-ml laboratory glass bottle. For extraction, add 60 ml of methanol using a straight pipette. Adjust the volume of methanol when the target weight of a 1000 mg of granules has not been met precisely. Close the lab 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 consisting mainly of empty coating material settle below the supernatant liquid.

GRANULES CLAIMING TO CONTAIN IN AVERAGE 60% (W/W) OF PARA-AMINOSALICYLATE SODIUM AS DIHYDRATE

From a sealed sachet or bulk storage container, recover enough granules to produce 1380 mg of powder being equivalent to 600 mg of para-aminosalicylic acid. Work as above for granules containing para-aminosalicylic acid using again 60 ml of methanol for extraction. Adjust the volume of methanol when the target weight of a 1380 mg of granules has not been met precisely.

All stock sample solutions obtained should finally contain 10 mg of total para-aminosalicylic acid per ml and be labelled as *'PAS 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 25-ml laboratory glass bottle and add 19 ml of methanol. Close and shake the vial and label as *'PAS Working Sample Solution'*.

The expected concentration of para-aminosalicylic acid in the working sample solution is 0.5 mg per ml and should match the concentration of PAS 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 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 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 10 ml of toluene, 10 ml of acetone and 2 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 observe the chromatoplate under UV light of 254 nm using the battery-driven lamp supplied. Use this method of detection for both, aminosalicylic acid identification and quantification purposes. Further verification of drug identity and content can be achieved when observing the plate under UV light of 366 nm in a dark chamber and at daylight after iodine staining. Iodine staining will be completed after about 30 seconds.

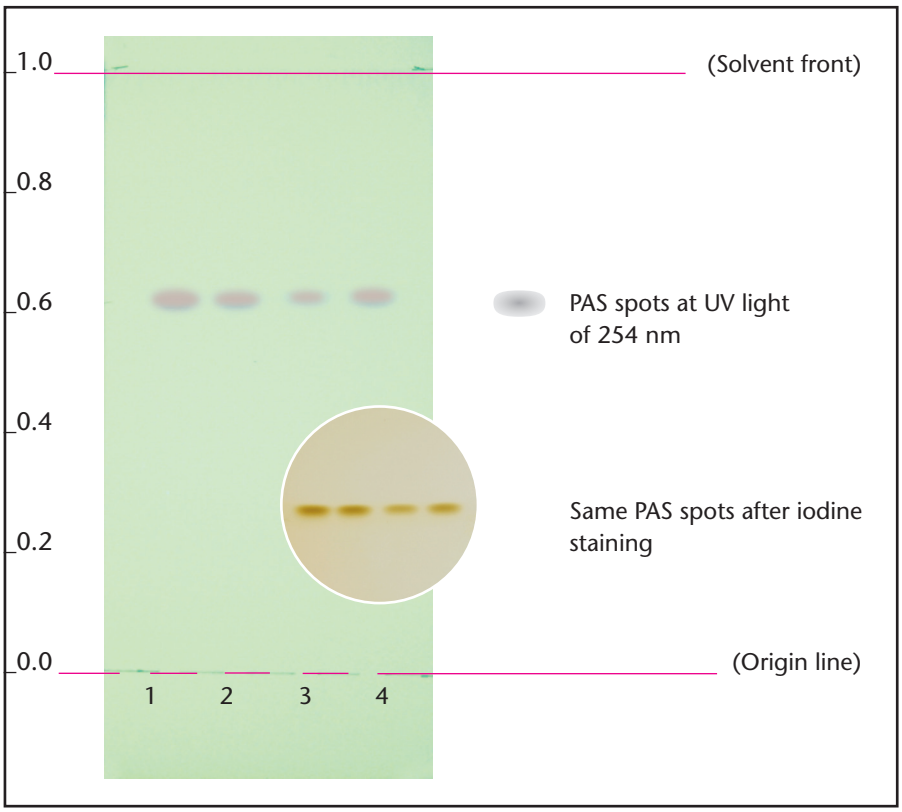
XI. CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

Run No.1:
Upper working standard representing 100% of total para-aminosalicylic acid (PAS)

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

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

Run No.4:
Lower working standard representing 80% of total para-aminosalicylic acid (PAS)



XII. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.62 indicates the presence of para-aminosalicylic acid (PAS) in the test solution. Additional strong spots generated by the test solution would point at other drugs or PAS degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principle spot from the test solution may also indicate a poor PAS content and no spot complete drug absence. Auxiliary agents incorporated in the different granule formulations might cause some fainter spot either travelling alongside the solvent front or emerging near or on the origin line.

XIII. OBSERVATIONS MADE AT 366 NM

When exposing the chromatoplate to UV light of 366 nm in a dark room, all PAS spots already observed at 254 nm are now showing a bluish fluorescence. However, PAS performs weak here.

XIV. OBSERVATIONS MADE AT DAY-LIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all PAS spots already observed at 254 nm are now turning orange brown. PAS performs strong here. 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 an 80 and 100 percent, respectively.

XV. RESULTS & ACTIONS TO BE TAKEN

The PAS 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 Fake?

Fighting Counterfeit Medicines · Protecting People's Life

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