A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Supplement 2017

Volume II

Thin Layer Chromatographic Tests

A charitable organisation
maintained exclusively
by Merck

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A Concise Quality Control Guide on Essential Drugs and other Medicines

Supplement 2017 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, 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 750 Minilabs have been supplied to over 90 countries across the African, Asian-Pacific and Latin American region already. The range of drug compounds is gradually extended aiming also for medicines to treat non-communicable diseases and mother and child health.

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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6.91 Amlodipine

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. Whether presented as salt made from benzenesulfonic or methanesulfonic acid, each tablet or capsule usually contains 5 or 10 mg of amlodipine per free base. Other dosage strengths are known to exist. Frequently, amlodipine is co-formulated with other cardiovascular medicines.

II. DISINTEGRATION TEST

All quick release amlodipine 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 test.

Verification of Drug Identity and Content via Thin Layer Chromatography

I. PRINCIPLE

Whether or not combined with other medicines, amlodipine besylate and amlodipine mesylate salt are extracted from tablets and capsules with methanol and determined by TLC with reference to an appropriate secondary standard. The method is also fit for use even when amlodipine is combined with atenolol, perindopril arginine, lisinopril, enalapril and hydrochlorothiazide.

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 pre-coated 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) Iodine chamber
19) Water
20) Methanol
21) Toluene
22) Glacial acetic acid
23) Reference standard, for example amlodipine 5 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 5 mg of amlodipine. 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 25-ml laboratory glass bottle and wash down all residual solids with 16.5 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 0.3 mg of total amlodipine per ml and be labelled as *'Amlodipine 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 0.3 mg of total amlodipine 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 amlodipine.

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 0.24 mg of total drug per ml and be labelled as *'Amlodipine Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of amlodipine 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 2.5 MG OF AMLODIPINE 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 25-ml 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 8.25 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.

5 MG OF AMLODIPINE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 16.5 ml of methanol using a straight pipette and a 25-ml laboratory glass bottle. Continue to work as above.

10 MG OF AMLODIPINE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 33 ml of methanol using a straight pipette and a 40-ml laboratory glass bottle. Continue to work as above.

Whether or not combined with other drugs, all stock sample solutions produced should finally contain 0.3 mg of total amlodipine per ml and be labelled as *'Amlodipine 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

Amlodipine stock sample solutions require no further dilution. They already represent the final working concentration of 0.3 mg of amlodipine per ml. If prepared from a high quality product, the sample solution should match the concentration of amlodipine of the higher working standard solution produced above. Just for more convenient handling, some of the supernatant liquid may want to be transferred into a 10-ml vial.

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 tablet and capsule excipients, different drug concentrations or combinations 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. Finally, gently dry the spots.

IX. DEVELOPMENT

Pipette 13 ml of methanol, 3 ml of toluene, 2 ml of glacial acetic acid and 2 ml of water 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 30 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 until the smell of acetic acid completely disappears. Then, best in a dark room, expose the chromatoplate to UV light of 254 and 365 nm using the battery-driven lamps supplied. Use the readings obtained at 365 nm for both, amlodipine identification and quantification purposes. When the chromatoplate is exposed to UV light of 254 nm after iodine staining then all spots observed at 254 nm before the staining are becoming more pronounced now.

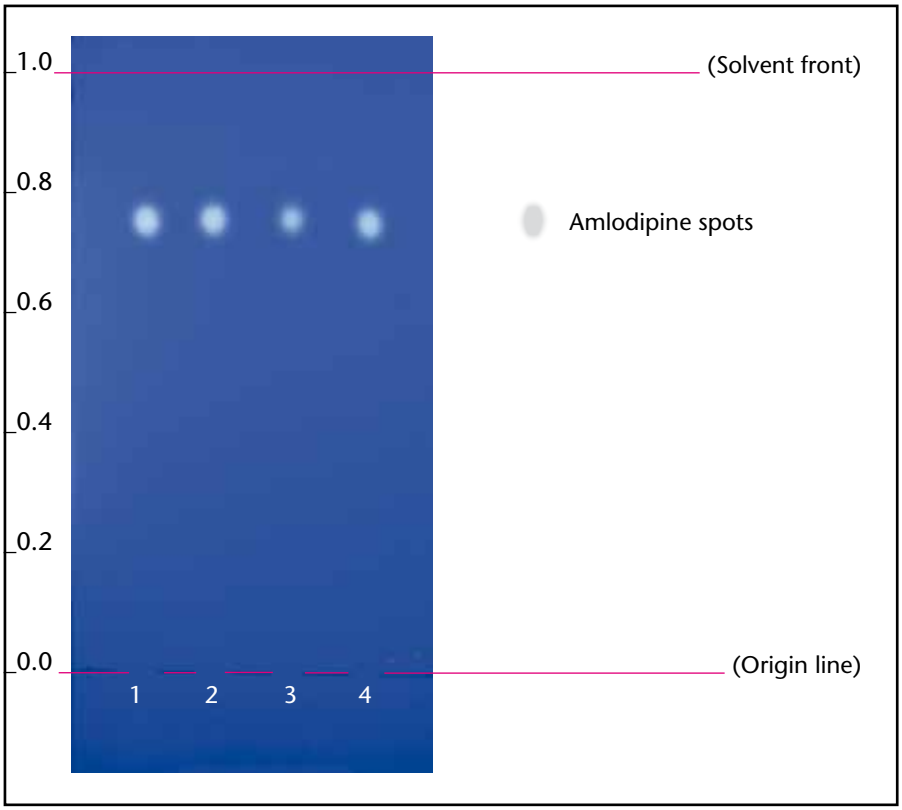
XI. CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 365 NM

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

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

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

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



XII. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.76 indicates the presence of amlodipine in the test solution. If combined with other cardiovascular medicines some more spots may become visible at different travel distances, the relative retention factor for atenolol being about 0.64, for hydrochlorothiazide about 0.84, for lisinopril about 0.29, for enalapril and perindopril about 0.59 and for arginine about 0.14, respectively. However, due to poor solubility in methanol, overall low concentration in the test solution and low sensitivity to UV light of 254 nm, many of amlodipine's partner drugs in fixed-dose combination products are falling below their limit of detection here and are requiring specific staining to make them visible. This is valid also for benzenesulfonic acid forming the anion in the amlodipine besylate salt settling as free base at a travel distance of about 0.82.

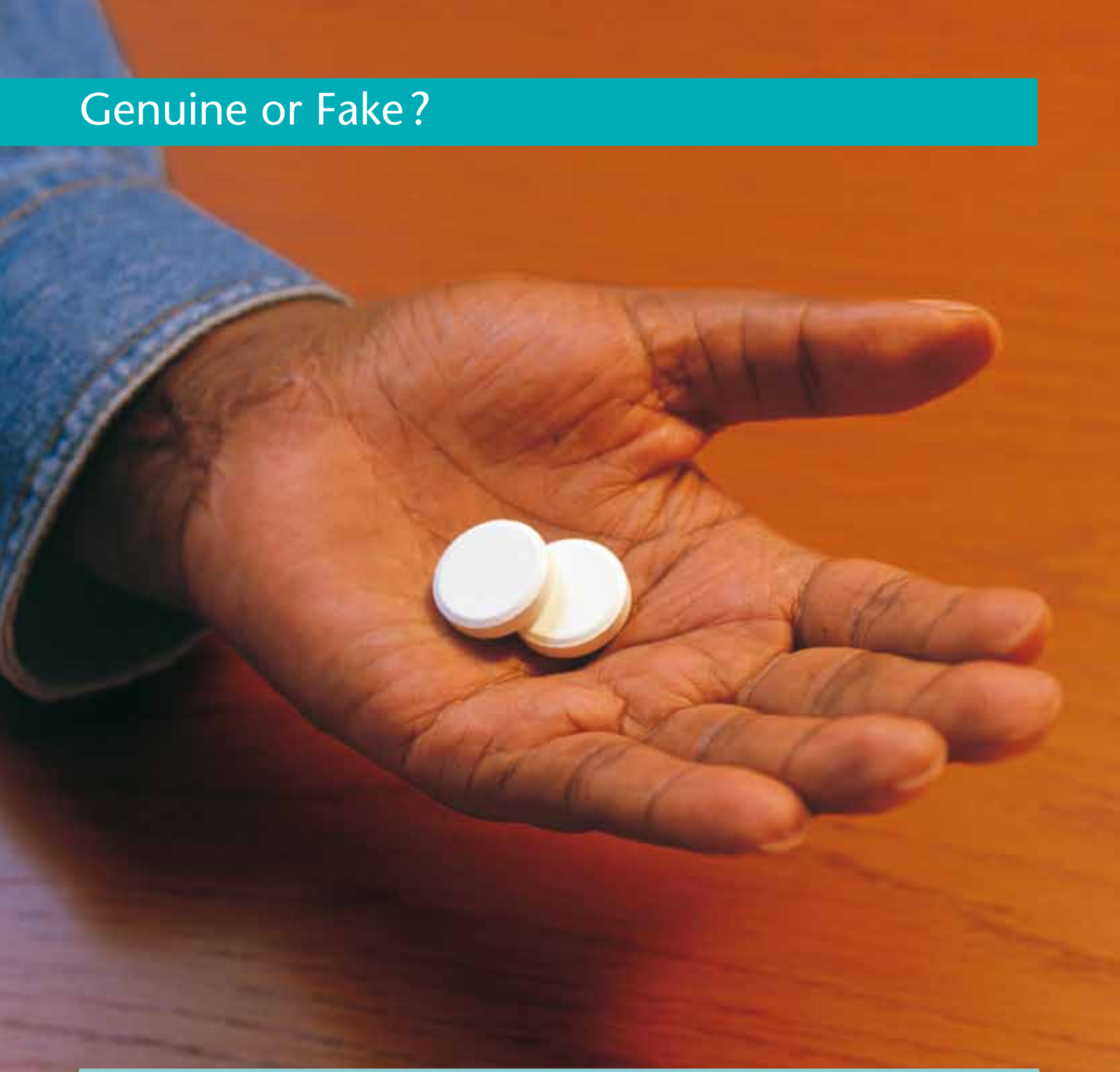
XIII. OBSERVATIONS MADE AT 365 NM

When exposing the chromatoplate to UV light of 365 nm in a dark room, all amlodipine spots already observed at 254 nm must now show a very intense white fluorescence. All other active agents potentially combined with amlodipine in the tablet or capsule formulation will show no fluorescence whatsoever here. Hence, readings for amlodipine taken at 365 nm are most specific. A smaller amlodipine spot from the test solution would indicate a poor drug content and no spot at all a complete absence of amlodipine.

XIV. RESULTS & ACTIONS TO BE TAKEN

The amlodipine 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 a picture of the reading with a digital camera turning off the flash first.

Genuine or Fake?



Fighting Counterfeit Medicines · Protecting People's Life

GPHF
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PQM Promoting the Quality of Medicines

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