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Tübingen, June 16th, 2017

To
Mr. Rich Whitworth, Texere Publishing
Judging Panel, Humanity in Science Award

The Analytical Scientist
in partnership with
KNAUER Wissenschaftliche Geräte GmbH.

Letter of recommendation for Dr. Richard W.O. Jähnke, Global Pharma Health Fund e.V., Frankfurt, Germany, for the Humanity in Science Award

Dear Mr. Whitworth,
for the "Humanity in Science Award" I would like to nominate:

Dr. Richard W.O. Jähnke
Global Pharma Health Fund e.V. (GPHF)
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60389 Frankfurt, GERMANY
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F +49-69-46939-852
M +49-171-161-4577
richard.jaehnke@gphf.org
www.facebook.com/minilab, www.twitter.com/gphf, www.gphf.org

Dr. Richard W.O. Jähnke has developed and continuously improved the **"GPHF Minilab™"** (www.gphf.org) which represents a breakthrough **for the rapid and inexpensive identification of substandard and falsified medicines in low- and middle income countries** in Africa, Asia and Latin America.

The GPHF Minilab is unique as a well-designed, complete field test kit for medicine quality analysis. No expensive laboratory is required for its use, and most procedures can even be carried out in the absence of electricity and running water. It is routinely used by the Promoting the Quality of Medicines Program of the United States Pharmacopeial Convention [1, 2], and has recently been described as "a key component of drug quality surveillance systems" in developing countries [3]. More than 800 Minilabs have been supplied across 95 countries, and Cambodia, Laos, Vietnam, Madagascar, Nigeria and Tanzania have adopted the technology for post-marketing antimalarial drug quality monitoring. I myself have used the Minilab in a recent study on substandard and falsified medicines in Malawi [4]. From my experience, I would like to emphasize the excellent quality of the manuals which accompany the Minilab, prepared under scientific leadership of Dr. Jähnke. Every year, Dr. Jähnke prepares a new supplement to these manuals, expanding the range of essential medicines which can be investigated by the Minilab. As director of one of the largest academic schools of pharmacy in Germany, I know how much scientific competence and effort is required to produce manuals of such clarity, precision and practical applicability. I had the privilege of teaching African health staff in the use of the Minilab, based on these manuals, and found this technology an outstanding break-through for medicine quality analysis in developing countries.

Dr. Jähnke has not only developed this technology, but also ensured its application in countless training seminars in Africa, Asia and Latin America. The high international appreciation for the Minilab is also shown by the highly-reputed partner organizations which have supported the work of Dr. Jähnke and the GPHF, listed under www.gphf.org/en/minilab/index.htm

The United Nations have declared access to “safe, effective, quality, and affordable essential medicines” as one of the Sustainable Development Goals in their 2030 Agenda. Yet, substandard and falsified medical products continue to present a serious problem for public health. Low- and middle-income countries (LMICs) are especially affected by this problem since they often lack resources, infrastructure and trained personnel to assure the quality of medicines.

The gold standard methods for drug quality analysis are defined in the leading pharmacopeias, such as the United States Pharmacopeia. The equipment required for pharmacopeial analysis, especially for HPLC, is expensive and delicate. Therefore, in LMICs only few laboratories exist which can carry out such analyses. In most cases, no capacity exists in such countries for the regular surveillance of drug quality on the various levels of the drug supply chain, thereby opening the possibility for substandard and falsified medicines to enter the market. The GPHF Minilab now offers a well-tested, appropriate technology to rapidly identify falsified medicines and to protect the population from their harmful effects.

In my opinion, it is more than justified that the excellent scientific and humanitarian work of Dr. Richard W.O. Jähnke receives recognition by a prize. For the “Humanity in Science Award”, with its emphasis on analytical chemistry and humanitarian science, Dr. Jähnke’s work appears to be ideally suited.

If desired by the organizers, I would be happy to introduce Dr. Jähnke and his work to a broader audience at the special jubilee reception at KNAUER's headquarters in Berlin, Germany on 2 October, 2017.

Yours sincerely

(Prof. Dr. Lutz Heide)
Director, Pharmaceutical Institute

1. Hajjou M, Krech L, Lane-Barlow C, Roth L, Pribluda VS, Phanouvong S, et al. Monitoring the quality of medicines: results from Africa, Asia, and South America. Am J Trop Med Hyg. 2015;92(6 Suppl):68-74. Epub 2015/04/22. doi: 10.4269/ajtmh.14-0535. PubMed PMID: 25897073; PubMed Central PMCID: PMC4455073.
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CURICULUM VITAE

Dr. Richard W. O. Jähnke,

Born on the 17th of May 1958

in Neheim-Hüsten, Germany,

Married, two sons

Career Development

1996	-	today	International public health project and information management incl. specialist counselling on rapid drug quality verification and Minilab use for the Global Pharma Health Fund (GPHF) in Frankfurt (Germany), a charitable organisation established as CSR project by the life science company Merck, Darmstadt (Germany)
1993	-	1996	Business Development Manager for pharmaceutical products and pharmaceutical contract manufacturing services at the German subsidiaries of Packaging Coordinator Inc. in Philadelphia (USA) and Laboratoires Sérolam in Paris (France)
1989	-	1992	Principal Scientist at Beecham Pharmaceuticals Plc Epsom/London
1985	-	1989	PhD research work in the field of pharmaceutical formulation at the school of pharmacy of Frankfurt University
1979	-	1984	Starting and completing pharmacy studies at Bonn University

Direct Contact

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Full citations of papers published by the nominee that describe the work:

A Concise Quality Control Guide on Essential Drugs and other Medicines: Supplement 2017 to Volume II on Thin Layer Chromatographic Tests.
By Richard W. O. Jähnke and Kornelia Dwornik,
2017, 32 pages, 5 new test protocols and one update on more vital medicines to treat infectious and cardiovascular diseases

A Concise Quality Control Guide on Essential Drugs and other Medicines: Supplement 2016 to Volume II on Thin Layer Chromatographic Tests.
By Richard W. O. Jähnke, Kornelia Dwornik and Ram Pathak,
2016, 32 pages, 5 new test protocols and one update on more vital medicines to treat infectious diseases

A Concise Quality Control Guide on Essential Drugs and other Medicines: Supplement 2015 to Volume II on Thin Layer Chromatographic Tests
By Richard W. O. Jähnke and Kornelia Dwornik,
2015, 28 pages, 5 new test protocols on medicines to treat cardiovascular diseases and for reproductive health

(For these three, and for previous manuals, see www.gphf.org/en/minilab/manuals.htm)

Use of thin-layer chromatography to detect counterfeit sulfadoxine/pyrimethamine tablets with the wrong active ingredient in Malawi.
By Felix Khuluza, Stephen Kigera, Richard W. O. Jähnke and Lutz Heide
Malaria J (2016) 15: 215. doi 10.1186/s12936-016-1259-9.

For further information, see www.gphf.org

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

The Promoting the Quality of Medicines (PQM) program, funded by the U.S. Agency for International Development (USAID), is implemented by the U. S. Pharmacopeial Convention (USP).

A Concise Quality Control Guide on Essential Drugs and other Medicines

Supplement 2017 to Volume II on Thin Layer Chromatographic Tests

Written by
Richard W. O. Jähnke and Kornelia Dwornik

* * *

Reviewed by
Sanford Bradby, Yanga Dijiba, Latifa El Hadri, Mustapha Hajjou, Victor Pribluda, Lukas Roth, and Souly Phanouvong

* * *

Published by
the Global Pharma Health Fund (GPHF), a charity initiated and maintained by Merck (Germany), and the Promoting the Quality of Medicines (PQM) programme implemented by the United States Pharmacopeial Convention (USP).

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Acknowledgements
This supplement was made possible by the generous financial support of the American people through the United States Agency for International Development (USAID). The contents are the responsibility of GPHF and PQM and do not necessarily reflect the views of USAID or the United States Government.

* * *

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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GPHF-Minilab™ assembled and supplied by Technologie Transfer Marburg, Cölbe, Germany

Table of Contents

Chapter **Page**

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.

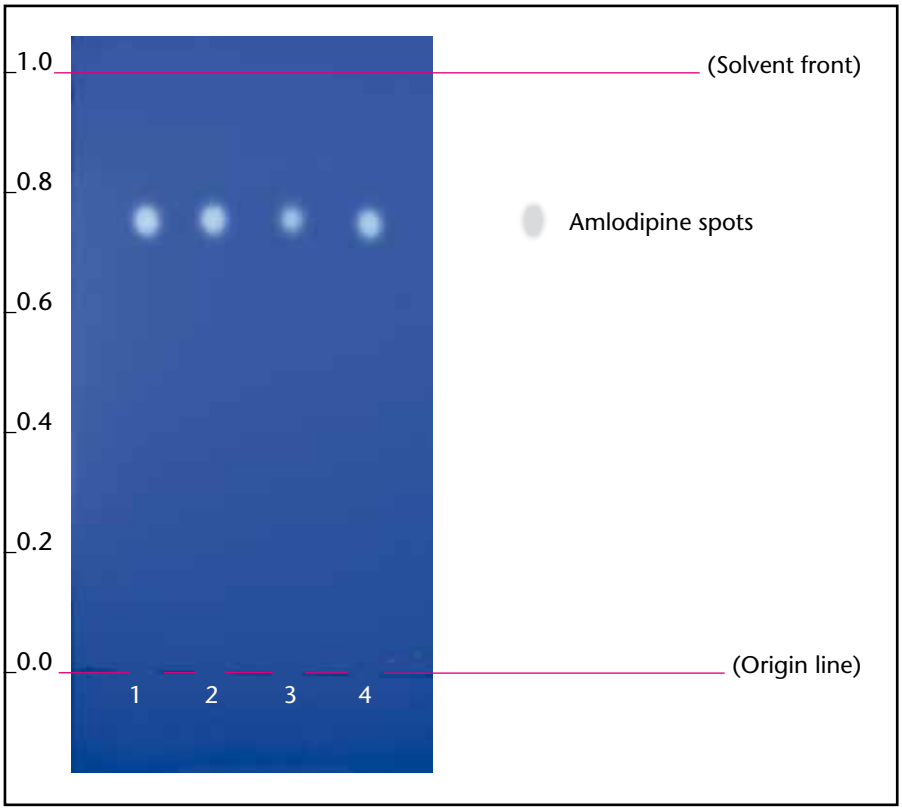
XI. CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 365 NM

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.

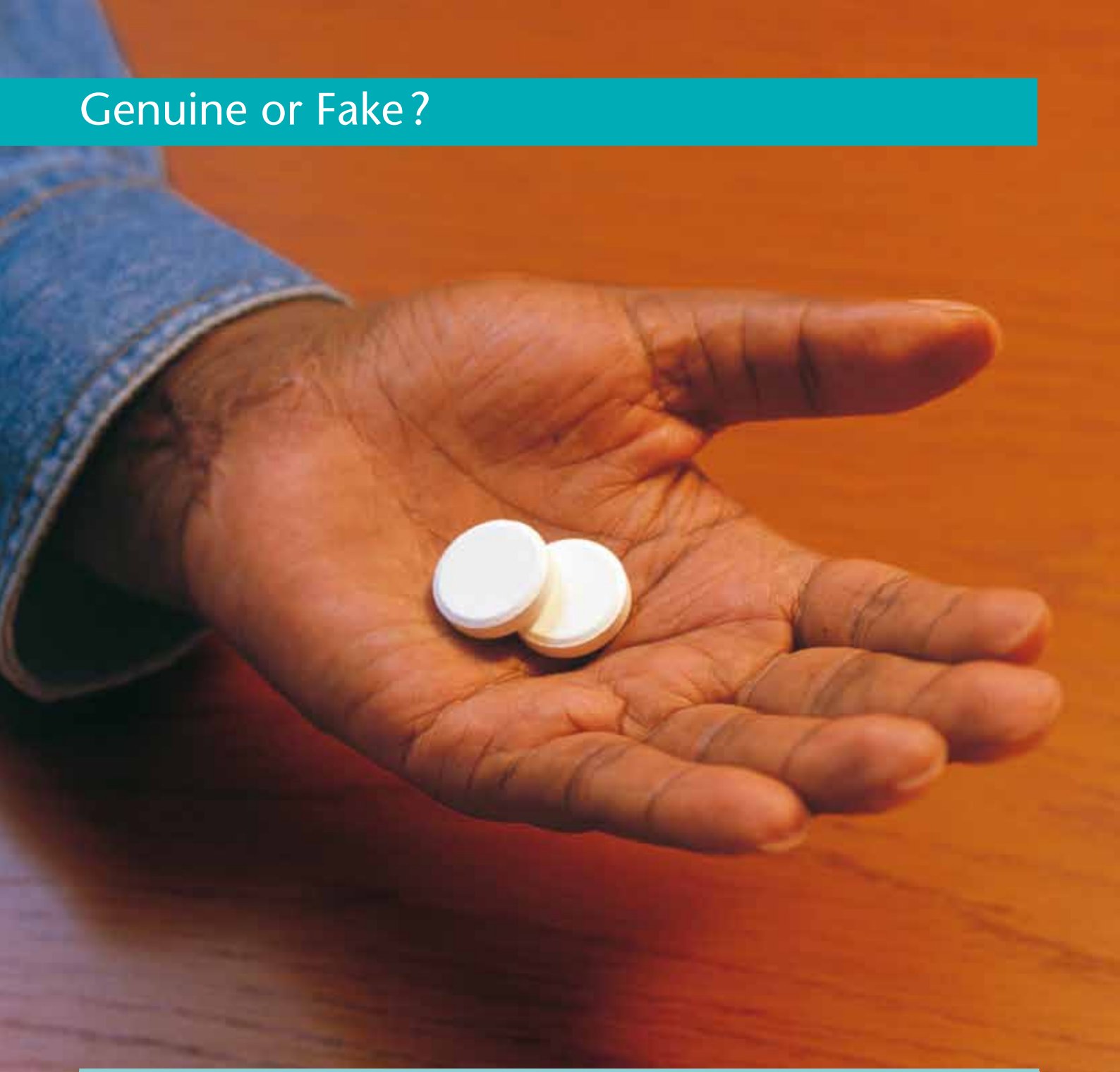
XIII. OBSERVATIONS MADE AT 365 NM

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
GLOBAL PHARMA HEALTH FUND E.V.

A charitable organisation maintained exclusively by Merck

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PQM Promoting the Quality of Medicines

The Promoting the Quality of Medicines (PQM) program, funded by the U.S. Agency for International Development (USAID), is implemented by the U. S. Pharmacopeial Convention (USP).

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A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

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Supplement 2016

Volume II

Thin Layer Chromatographic Tests

GPHF
GLOBAL PHARMA HEALTH FUND E.V.

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USAID
FROM THE AMERICAN PEOPLE

USP

PROMOTING THE QUALITY OF MEDICINES

A Concise Quality Control Guide on Essential Drugs and other Medicines

Supplement 2016 to Volume II on Thin Layer Chromatographic Tests

Written by
Richard W. O. Jähnke, Kornelia Dwornik and Ram Pathak

* * *

Reviewed by
Daniel Bempong, Sanford Bradby, Yanga Dijiba, Latifa El Hadri, Mustapha Hajjou, Lukas Roth, Patrick Lukulay and Souly Phanouvong

* * *

Published by
the Global Pharma Health Fund (GPHF), a charity initiated and maintained by Merck Darmstadt · Germany, and the U.S. Pharmacopeia's Promotion the Quality of Medicines programme (USP PQM)

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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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GPHF-Minilab™ assembled and supplied by Technologie Transfer Marburg, Cölbe, Germany

Table of Contents

Chapter **Page**

6.86 Benzathine benzylpenicillin (Penicillin G benzathine)

Primary Screening via Physical Inspection

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. The benzathine benzylpenicillin complex consists of two parts of benzylpenicillin per one part of benzathine. It is presented as powder for injection coming in vials usually containing 1.2 or 2.4 million units of the historic penicillin G sodium standard of 0.0006 mg per unit. This translates into 0.72 and 1.44 g of benzylpenicillin sodium equivalents and 0.92 and 1.84 g of anhydrous benzathine benzylpenicillin, respectively. The contents of vials may be expressed in gram, international units or both. A million units are sometimes replaced by the metric prefix "mega", hence, in this case by 1.2 and 2.4 mega units of the underlying benzylpenicillin sodium equivalent. Other dosage strengths are known to exist. Due to a variable quantity of water, product purity and the addition of dispersing agents, the total powder content of one vial may exceed the theoretical values of 0.92 and 1.84 g for neat benzathine benzylpenicillin by about 10%. The names benzathine benzylpenicillin and penicillin G benzathine can be used interchangeably.

II. 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

The total content from a benzathine benzylpenicillin vial is first suspended in a small quantity of water and then mixed with methanol till complete dissolution. Afterwards, the presence and content of the active principle in the test solution is verified by TLC against benzylpenicillin potassium as control. Compared to benzylpenicillin sodium, the potassium salt requires no cold storage. Nevertheless, all stoichiometric calculations are related to the benzylpencillin sodium salt. Not working with the free base is down to the history of benzylpencillin development and quite unusual. Many pharmacopoeias do not highlight this point.

II. EQUIPMENT AND REAGENTS

1) Pocket balance
2) Aluminium foil
3) Spatula
4) Funnel
5) Label tape
6) Marker pen
7) Pencil and ruler
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) Iodine chamber
20) Water
21) Methanol
22) Ethyl acetate
23) Glacial acetic acid
24) Reference standard, for example benzylpenicillin potassium as analytical reagent grade of commerce

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires benzylpenicillin potassium as analytical reagent grade of commerce or appropriate finished products or raw material of good quality (>85%) 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 0.3 g of benzylpenicillin potassium using a spatula. Carefully empty

the aluminium foil over a 10-ml laboratory glass bottle and wash down all the powder obtained with 5.7 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 5.5 ml of methanol when 0.29 g or 6.1 ml of methanol when 0.32 g of reference standard have been collected from the bulk container, respectively. Close the laboratory bottle and shake until all solids are dissolved. The final solution obtained should contain 50 mg of total benzylpenicillin sodium equivalents per ml and be labelled as *'Penicillin G 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 too 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 0.3 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 vial and add 19 ml of methanol. Close and shake the vial. The solution obtained should contain 2.5 mg of total benzylpenicillin sodium equivalents per ml and be labelled as *'Penicillin G Working Standard Solution 100%'*.

This higher working standard solution represents a drug product of good quality containing 100 % of total benzylpenicillin sodium equivalents.

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

Pipette 1 ml of the stock standard solution into a 25-ml vial and add 24 ml of methanol. Close and shake the vial. The solution obtained should contain 2 mg of total benzylpenicillin sodium equivalents per ml and be labelled as *'Penicillin G Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the total benzylpenicillin sodium equivalents 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 A STOCK SAMPLE SUSPENSION FROM A PRODUCT CLAIMING TO CONTAIN 920 MG OF BENZATHINE BENZYLPENICILLIN OR 720 MG (1.2 MILLION UNITS, 1.2 MEGA) OF BENZYLPENICILLIN SODIUM EQUIVALENTS PER VIAL

Take a sealed vial from a corresponding drug product sampled in the field. Use appropriate straight pipettes for each dissolution step. Open the vial, add 1.9 ml of water, close with the rubber stopper and shake. Open again, mix the aqueous content with 2.5 ml of methanol and completely transfer the suspension obtained into a 25-ml laboratory glass bottle. Rinse the empty vial two times each with 5 ml of methanol and combine the rinsing solutions with the penicillin suspension; the overall quantity of solvent used finally being 14.4 ml.

1840 MG OF BENZATHINE BENZYLPENICILLIN OR 1440 MG (2.4 MILLION UNITS, 2.4 MEGA) OF BENZYLPENICILLIN SODIUM EQUIVALENTS PER VIAL

Take a sealed vial from a corresponding drug product sampled in the field. Use appropriate straight pipettes for each dissolution step. Open the vial, add 3.8 ml of water, close with the rubber stopper and shake. Open again, mix the aqueous content with 4 ml of methanol and completely transfer the suspension obtained into a 40-ml laboratory glass bottle. Rinse the empty vial three times each with 7 ml of methanol and combine the rinsing solutions with the penicillin suspension. The overall quantity of solvent used finally being 28.8 ml.

Next to benzathine, all suspensions produced should finally contain 50 mg of total benzylpenicillin sodium equivalents per ml and be labelled as *'Penicillin G Stock Sample Suspension'*. Freshly prepare these suspensions for each test. Continue to work with the benzathine benzylpenicillin suspensions obtained.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

In order to obtain a uniform suspension, thoroughly shake the stock sample container. Instantly pipette 1 ml of the benzathine benzylpenicillin suspension into a 25-ml vial and add 19 ml of methanol. Close the vial, shake till complete dissolution and label as *'Penicillin G Working Sample Solution'*.

The expected amount of total benzylpenicillin sodium equivalents in this working sample solution is 2.5 mg per ml and should match the amount of benzylpenicillin sodium equivalents 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. 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.

As residual water may cause blurred spots and tailing, completely dry off all solvent from the sample spots before chromatoplate development. For this, just move the plate back and forward through the air. At this stage, the use of a hot plate will lead to instant penicillin degradation and should be avoided at all times.

IX. DEVELOPMENT

Pipette 17 ml of ethyl acetate, 5 ml of glacial acetic acid and 3 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 20 minutes. Remove the plate from the chamber, mark the solvent front and allow any excess solvent to evaporate now even using a hot plate till the smell of acetic acid almost disappears.

X. DETECTION

Dry off all residual solvent and expose the chromatoplate to UV-light of 254 nm before and after iodine staining using the battery-driven lamp supplied. Staining with iodine vapour will take a few seconds only. Use these methods of detection for both, benzylpenicillin identification and quantification purposes.

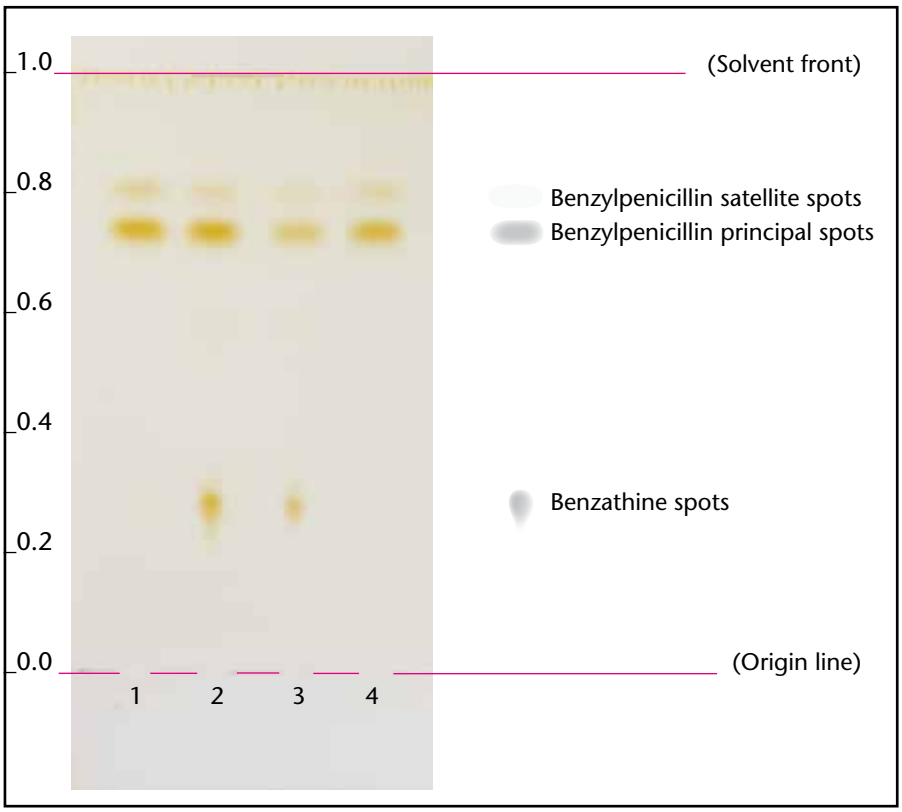
XI. CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

Run No.2:
A product of good quality with an acceptable content of benzylpenicillin and benzathine

Run No.3:
A product of poor quality with an unacceptable low content of benzylpenicillin and benzathine

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



XII. OBSERVATIONS MADE AT 254 NM BEFORE IODINE STAINING

A blue-violet spot at a travel distance of about 0.74 indicates the presence of benzylpenicillin in the test solution. Due to the low concentration, penicillin G performs weak and the benzathine fraction with a relative retention factor of about 0.28 stays almost invisible.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

A strong brown principal spot at a travel distance of about 0.74 combined with a much weaker satellite spot at about 0.81 indicates the presence of benzylpenicillin in the test solution. A spot with a relative retention factor of about 0.28 clearly shows the benzathine fraction now. Additional strong spots generated by the test solution would point at other drugs or benzylpenicillin degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor penicillin content due to low concentration or under fill, and no spot at all a complete absence of benzylpenicillin. 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.

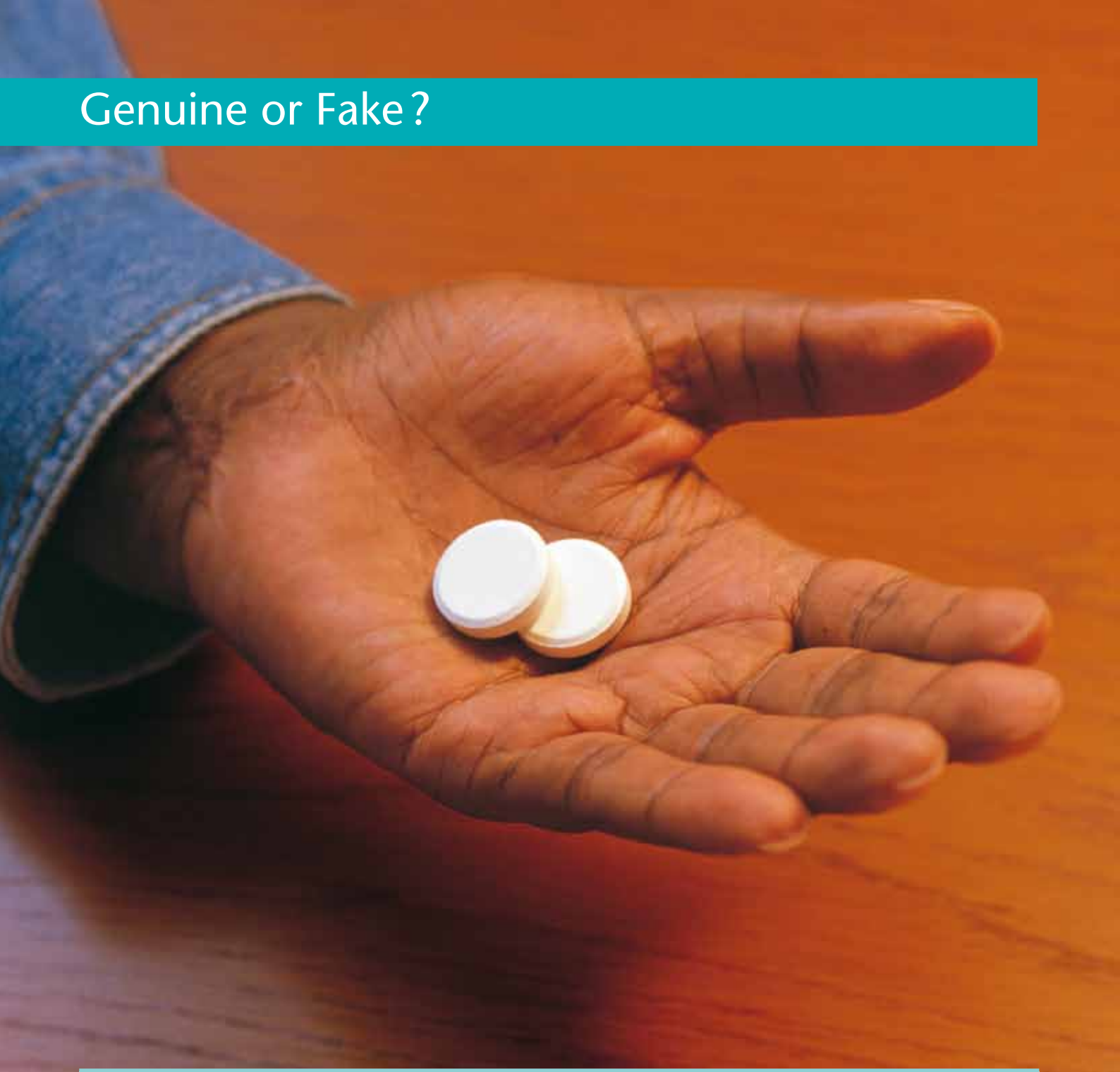
XIV. OBSERVATIONS MADE AT 254 NM AFTER IODINE STAINING

When exposing the iodine plate to UV light of 254 nm, all benzylpenicillin and benzathine spots already observed during the iodine staining at daylight and before the staining at UV-light of 254 nm are becoming much more pronounced now. This will facilitate further assay reading and interpretation.

XV. RESULTS & ACTIONS TO BE TAKEN

The principal benzylpenicillin 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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A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Supplement 2015

Volume II

Thin Layer Chromatographic Tests

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PROMOTING THE QUALITY OF MEDICINES

A Concise Quality Control Guide on Essential Drugs and other Medicines

Supplement 2015 to Volume II on Thin Layer Chromatographic Tests

Written by
Richard W. O. Jähnke and Kornelia Dwornik

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* * *

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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 680 Minilabs have been supplied to 90 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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GPHF-Minilab™ assembled and supplied by Technologie Transfer Marburg, Cölbe, Germany

Table of Contents

Chapter **Page**

6.80 Atenolol

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 25, 50 or 100 mg of atenolol.

II. DISINTEGRATION TEST

All quick release atenolol 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

Atenolol is extracted from tablets and capsules with methanol and determined by TLC with reference to an appropriate secondary standard.

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) TLC dipping chamber (250-ml beaker)
20) Ninhydrin
21) Methanol
22) Ammonia solution 25%
23) Reference standard, for example atenolol 50 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 50 mg of atenolol. 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 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. The solution obtained should contain 5 mg of total atenolol per ml and be labelled as *'Atenolol 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 5 mg of total atenolol 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 atenolol.

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

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of atenolol 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 25 MG OF ATENOLOL 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 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.

50 MG OF ATENOLOL PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 10 ml of methanol using a straight pipette and a 25-ml laboratory glass bottle. Continue to work as above.

100 MG OF ATENOLOL PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 20 ml of methanol using a straight pipette and a 40-ml laboratory glass bottle. Continue to work as above.

All stock sample solutions produced should finally contain 5 mg of total atenolol per ml and be labelled as *'Atenolol 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

Atenolol stock sample solutions require no further dilution. They already represent the final working concentration of 5 mg of atenolol per ml. If prepared from a high quality product, the sample solution should match the concentration of atenolol 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 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 20 ml of methanol and 0.2 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

Dry off all residual solvent and observe the chromatoplate first under UV light of 254 nm using the battery-driven lamp supplied. Then, expose the plate to iodine vapour for about one minute. Use the iodine staining for both, atenolol identification and quantification purposes.

Further verification of drug identity and content can be achieved when immersing the iodine plate in ninhydrin staining solution. However, the staining result will be more pronounced when using a freshly developed plate without prior contact to iodine. For the staining, weigh in 3 g of ninhydrin (about 10 times a well-filled spatula) and dissolve in a mix of a 150 ml of methanol and 30 ml of glacial acetic acid. Submerge the iodine plate into the staining solution using a pair of tweezers. Instantly remove the plate again from the solution and let all surplus liquid run down onto paper tissue. Wipe off residual liquid from the back of the plate and continue to dry off all staining solution at full level of the hot plate supplied. During heating, all atenolol spots are gradually becoming visible at daylight after about one minute. Again, use this method of detection for both, atenolol identification and quantification purposes. The ninhydrin staining process is illustrated on page 26 of the main manual issued 2008. Note that skin contaminated with ninhydrin solution will be stained as well. However, this is not dangerous to health and the violet spots will disappear after about a day or two.

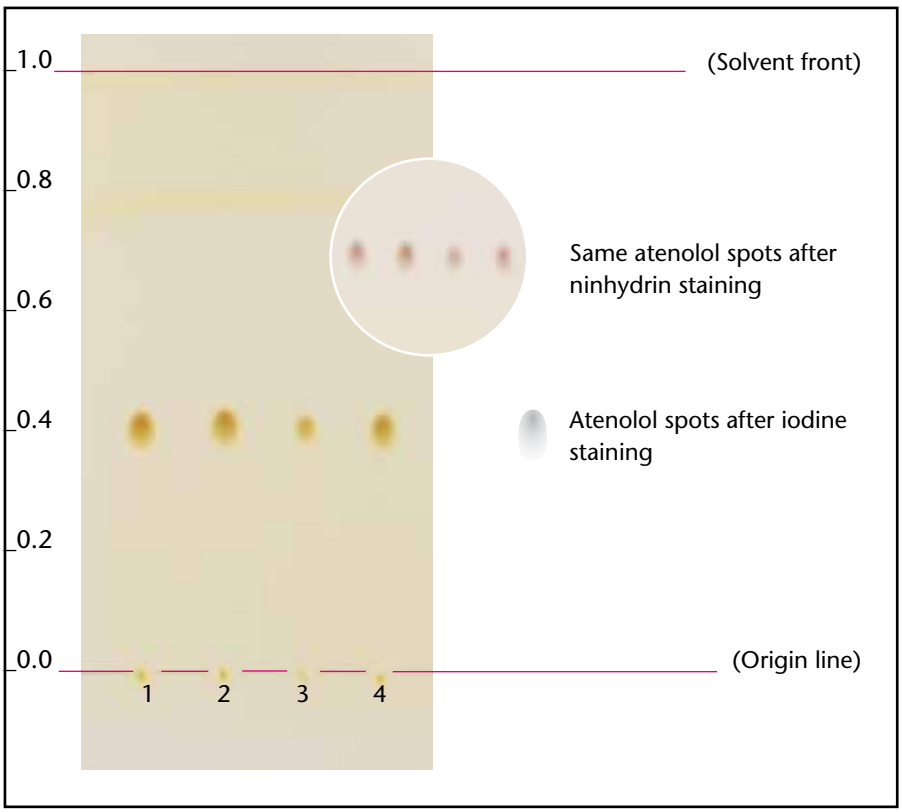
XI. CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

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

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

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



XII. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.41 indicates the presence of atenolol in the test solution. However, atenolol performs weak here.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all atenolol spots already observed at UV-light of 254 nm are now turning deep orange brown. Atenolol performs strong here. Additional strong spots generated by the test solution would point at other drugs or atenolol 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 atenolol content and no spot at all complete atenolol absence. 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. 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 DAYLIGHT AFTER NINHYDRIN STAINING

When exposing the iodine plate to ninhydrin and heat, all atenolol spots already observed during iodine staining are now turning reddish brown or even deep purple if a freshly developed plate without previous contact to iodine is used. Again, a smaller principle spot from the test solution will indicate a poor atenolol content and no spot complete atenolol absence.

XV. RESULTS & ACTIONS TO BE TAKEN

The atenolol 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.

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Malaria Journal

METHODOLOGY

Open Access

Use of thin-layer chromatography to detect counterfeit sulfadoxine/pyrimethamine tablets with the wrong active ingredient in Malawi

Felix Khuluza[1], Stephen Kigera[2], Richard W. O. Jähnke[3] and Lutz Heide[1,4*]

Abstract

Background: Substandard and falsified anti-malarial medicines pose a serious threat to public health, especially in low-income countries. Appropriate technologies for drug quality analysis in resource-limited settings are important for the surveillance of the formal and informal drug market. The feasibility of thin-layer chromatography (TLC) with different solvent systems was tested using the GPHF Minilab in a study of the quality of sulfadoxine/pyrimethamine tablets in Malawi.

Methods: Twenty eight samples of sulfadoxine/pyrimethamine tablets were collected from randomly selected health facilities of four districts of southern Malawi. A mystery shopper approach was used when collecting samples from illegal street vendors, and an overt approach for the other facilities. Samples were subjected to visual inspection, disintegration testing and TLC analysis. 10 samples were further investigated according to the methods of the US Pharmacopeia using high performance liquid chromatography (HPLC).

Results: One sample was found to be falsified, containing a mixture of paracetamol tablets and co-trimoxazole tablets. These had been repackaged into paper strip packs labelled as a brand of sulfadoxine/pyrimethamine. TLC with different solvent systems readily proved that these tablets did not comply with their declaration, and provided strong evidence for the active pharmaceutical ingredients which were actually contained. Full pharmacopeial analysis by HPLC confirmed the results suggested by TLC for this sample, and showed two further samples to be of substandard quality.

Conclusions: Due to the absence of the declared anti-malarial ingredients and due to the presence of other pharmaceutical ingredients, the identified falsified medicine represents a serious health risk for the population. Thin-layer chromatography (TLC) using different solvent systems proved to be a powerful method for the identification of this type of counterfeiting, presenting a simple and affordable technology for use in resource-limited settings.

Keywords: Falsified medicines, SSFFC medicinal products, Sulfadoxine/pyrimethamine, Thin-layer chromatography, GPHF Minilab

Background

Substandard and falsified antimalarial medicines pose a serious threat to public health. The worldwide spread of falsified medicines has been addressed as a "global pandemic", and in this context it has been correctly emphasized that "diagnostics are at the heart of any successful epidemic response effort" [1]. Therefore, analytical methods to identify falsified medicines are essential in order to fight this specific pandemic.

The gold standard methods for drug quality analysis are defined in the leading pharmacopeias, such as the

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International Pharmacopeia, the United States Pharmacopeia, the British Pharmacopeia, the Pharmacopeia of Japan, and others. They rely primarily on high performance liquid chromatography (HPLC) for analysis of the content of the active ingredient, of dissolution, and of the presence of related substances. The required instruments cost 50,000–100,000 $ (USA) for standard equipment, and more for advanced equipment [2]. They are complicated and delicate mechanical and electronic tools, requiring careful handling by trained professionals as well as an infrastructure including an electricity supply of constant voltage, very pure organic solvents, and regular maintenance by skilled technicians. For low-income countries, the full pharmacopeial analysis of medicines is a formidable challenge and can usually be achieved only for a limited number of samples in the national drug quality control laboratories. In most cases, no capacity exists in such countries for the regular surveillance of drug quality on the various levels of the drug supply chain, thereby opening the possibility for substandard and falsified medicines to enter the market [3].

Appropriate technologies for drug analysis in resource-limited settings would allow a more regular surveillance of the formal and informal drug market, aiding in the rapid detection of falsified medicines and potentially deterring criminal counterfeiters from bringing their products into the market. A number of such appropriate analytical technologies have been reported [2–4]. Few of them are ready for wide-spread application. The best-established one is thin-layer chromatography (TLC). For drug quality analysis in resource-limited settings, TLC is mostly employed in form of the Minilab™ supplied by the Global Pharma Health Fund (GPHF), a charity supported by the Merck pharmaceutical company [5]. The Minilab is a pre-assembled kit containing all analytical tools for the qualitative and semi-quantitative TLC analysis of about 100 essential medicines, and does not require electricity, running water or any sophisticated infrastructure. It is supplied with a manual describing the analytical procedure for each drug, and only very limited training is required for its use. TLC analysis using the Minilab has been used in many studies in Africa, Asia and South America [6, 7]. However, also limitations of this technology in comparison to full pharmacopeial analysis have been pointed out [8].

Before embarking on a larger study on the quality of antimalarial drugs in Malawi, the feasibility of TLC analysis was tested using samples of the anti-malarial drug sulfadoxine/pyrimethamine (SP) as example. In governmental and church health facilities of Malawi, SP is used for intermittent preventive malaria treatment in pregnancy. Private vendors frequently sell SP also to other patients as a single-dose malaria therapy, despite the fact that artemisinin-based combination therapies are now recommended as first-line therapy for malaria. In the course of this pilot study, a falsified SP sample was identified which contained active ingredients different from the declared ones. TLC analysis using different solvent systems allowed not only to prove that this sample did not conform to its declaration, but also provided strong evidence which active ingredients were actually contained. This demonstrates a power and versatility of TLC analysis which should be considered when the relative merits of different analytical techniques for drug quality analysis are discussed. This study highlights the usefulness of TLC analysis especially in the case of falsified drugs which contain active ingredients different from the declared ones.

Methods

Sample collection

Out of the 13 districts of southern Malawi, four were randomly selected. From each of these districts, a list of government health centers was obtained. From each of three districts, two health centres were selected randomly, and samples were collected from these health centres as well as from the respective district hospital. The fourth district comprised one of Malawi's larger cities. From this district two urban and two rural health centres were randomly selected, and samples were collected from there, from the district health office and from the central hospital. If church-affiliated health facilities, private pharmacy shops, drug stores, or illegal street vendors could be identified nearby the selected government health facilities, then drugs samples were also collected from there. Samples were collected by members of the Pharmacy Department, University of Malawi. A mystery shopper approach was used for the illegal street vendors, and an overt approach for the other facilities. The mystery shopper stated that he had been asked by friends in his village to buy this medicine for them. Samples of 150 tablets were collected if available, otherwise smaller numbers. If medicines with generic and brand name were available, the brand name medicine was sampled. If several brand name medicines were available, the most expensive brand name medicine was sampled. In most sites, however, only a single type of sulfadoxine/pyrimethamine tablets was available. Samples were transported to the Pharmacy Department, College of Medicine, Blantyre, within 48 h, and stored below 25 °C until analysis.

Visual inspection, disintegration testing and testing for uniformity of mass of dosage units

The external packaging, primary packaging and (if available) package leaflets were inspected, including batch number and expiry dates. The tablets were visually

inspected, especially for undamaged, unaltered surfaces and colour uniformity. Disintegration testing for instant-release oral dosage forms was carried out according to the manual of the GPHF Minilab [9]; in short, six tablets were kept in water at 37 °C under occasional shaking or stirring, and complete disintegration within 30 min was confirmed. For uniformity of mass of dosage unit, the exact weight of 20 tablets was determined; acceptable deviations were: up to ±5 % in at least 18 tablets, and up to ±10 % in no more than two tablets.

Thin-layer chromatographic (TLC) testing

TLC testing was done according to the procedure given by the manual of the GPHF Minilab for sulfadoxine (including SP formulations) [9]. From each sample, three tablets were analysed individually. In short, each tablet was crushed to a fine powder and extracted with 20 ml methanol by vigorous shaking for 3 min. After sedimentation of undissolved residues, 1 ml of the supernatant was removed and diluted with 3 ml methanol. Using a microcapillary, 2 µl of this solution were applied to a TLC plate (Merck silica gel 60 F254, 0.2 mm thickness, 5 × 10 cm). Authentic standard solutions of sulfadoxine/pyrimethamine with known concentrations were applied as comparison. The plate was developed in a solvent system of ethyl acetate:methanol 15:5 for approximately 15 min. After drying off the residual solvent, the active pharmaceutical ingredients were visualized first under UV light (254 nm), and subsequently by iodine vapour. The results were documented using an inexpensive digital camera (Canon PowerShot SX600 HS).

For comparison to authentic paracetamol and co-trimoxazole samples, the solvent systems given by the manual of the GPHF Minilab for analysis of paracetamol[9], pyrimethamine [10] and co-trimoxazole [9] were used, i.e. acetone:toluene:acetic acid 10:10:0.5 (for the experiment depicted in Fig. 2b); ethyl acetate:methanol:acetone:conc. aqueous ammonia 12:6:2:0.5 (Fig. 2c); ethyl acetate:methanol 15:5 (Fig. 2d).

HPLC analysis according to the United States Pharmacopeia (USP)

Following the methods specified in USP38-NF33, identification of the active ingredients by TLC and HPLC, HPLC analysis (=assay) for the content of sulfadoxine and pyrimethamine, analysis for uniformity of dosage units with respect to the content of the active ingredients, and analysis of their dissolution was carried out in the WHO-prequalified drug quality control laboratory of the Mission for Essential Drugs and Supplies, Nairobi, Kenya. HPLC analysis for sulfadoxine and pyrimethamine was carried out using a Gemini 5 µm C6-Phenyl 110Å HPLC column 250 × 4.6 mm (Phenomenex, USA) and an isocratic solvent system of 0.1 % aqueous phosphoric acid:acetonitrile 83:17, flow rate 1.2 ml/min. The wavelength for UV detection was 230 nm. For the identification of paracetamol and co-trimoxazole, the respective methods of USP38-NF33 for those drugs were used.

Ethical approval

This study was approved by the College of Medicine Research and Ethics Committee, University of Malawi.

Results

Sample collection

Twenty eight samples of sulfadoxine 500 mg/pyrimethamine 25 mg tablets were collected in four districts in southern Malawi. 15 were collected from government health facilities, seven from church-affiliated health facilities, four from private pharmacies and drug stores, and two from illegal street vendors. 21 of the samples were found to be distributed under the generic name "sulfadoxine/pyrimethamine", and seven under brand names. According to the information on the packaging, the samples had been produced by six different manufacturers, with 18 samples produced in in India (by two different manufactures), three in China, three in Tanzania, two in Cyprus and two in Malawi. Comparison with the records of the Pharmacy, Medicines and Poisons Board, i.e. the national drug regulatory agency of Malawi, showed that the SP tablets from five of the manufacturers were registered in Malawi, but the tablets from one of the manufactures were not. The non-registered type represented the most common SP preparation collected in government and church-affiliated health facilities, accounting for 17 of the 28 samples.

Visual inspection

Only a single sample clearly failed visual inspection. It was purchased from an illegal street vendor and was sold in an opened, apparently genuine cardboard box labelled "Novidar (SP)", a brand name of SP manufactured and sold by the Malawian manufacturer Pharmanova Ltd. The cardboard box contained paper strip packs labelled "Novidar (SP)" which, however, were found to be of two different kinds (Fig. 1). One (hereafter called type N) was stamped with the same batch number and expiry date as given on the outer packaging (i.e. the cardboard box). The other one was stamped with two dates ("27/04/2010" and "20/11/2015"), different from those given on the outer package. These paper strip packs were made from a thinner type of paper than those of type N. A part of the tablets in these strip packs had apparently adsorbed moisture. Upon opening of the strip packs, some tablets were found to stick to the paper, and to break easily.

Fifteen paper strips of this kind were contained in this sample. Although they were all uniform in their

Fig. 1 Primary packaging and tablets of Novidar (SP)™ and of the falsified samples type X and type Y

appearance and stamp, they were found to contain two different kinds of tablets (Fig. 1). One (hereafter called type X) did not carry an imprint on its front side. The other one (hereafter called type Y) was imprinted with the letters "UCL". In contrast, the tablets with the paper strips of type N were imprinted with "Novidar SP" on the front side, which is consistent with the genuine product of Pharmanova Ltd.

Thin-layer chromatographic analysis

The tablets of types N, X and Y were subjected to thin-layer chromatographic (TLC) analysis according to the procedure given in the manual of the GPHF Minilab for sulfadoxine/pyrimethamine tablets (see Methods section). An authentic standard of sulfadoxine 500 mg/pyrimethamine 25 mg was used for comparison. Detection was carried out first with UV light (254 nm), then with iodine staining. The result is shown in Fig. 2a. The complete analytical procedure was repeated again, starting from different tablets. The results were identical to those shown in Fig. 2a.

Both in the first and the second analysis, the tablets of type N showed spots identical in Rf value and intensity to those of sulfadoxine and pyrimethamine in the authentic standard. This strongly indicates that this product contains the declared active ingredients in the declared amounts, and most likely represents the original product "Novidar (SP)" of the Malawian manufacturer Pharmanova Ltd. Type X showed no spots identical in Rf value to those of sulfadoxine and pyrimethamine. This proves the absence of relevant quantities of both active principles in this product. Instead, TLC analysis showed another compound, giving a spot of an Rf value slightly lower than sulfadoxine. The different Rf value, and the different response to iodine staining (Fig. 2a), prove that this compound is different from sulfadoxine.

Type Y showed no spot identical in Rf value to that of pyrimethamine from the authentic standard. This proves the absence of relevant quantities of pyrimethamine in this product. However, TLC analysis did show a spot similar in Rf value and intensity to that of sulfadoxine, indicating the presence either of sulfadoxine or of a compound with similar chromatographic behaviour. Furthermore, TLC analysis showed a spot of a further compound, with an Rf value clearly lower than pyrimethamine.

The imprint "UCL" is used by the Kenyan pharmaceutical manufacturer Universal Corporations Ltd. Therefore, tablets of type Y were compared the with drugs from this manufacturer. And indeed, Sulfran™ tablets (co-trimoxazole 480 mg) marketed by UCL in Malawi were found to be perfectly identical in shape, size and imprint to the tablets of type Y. It was furthermore speculated that type X may represent paracetamol tablets.

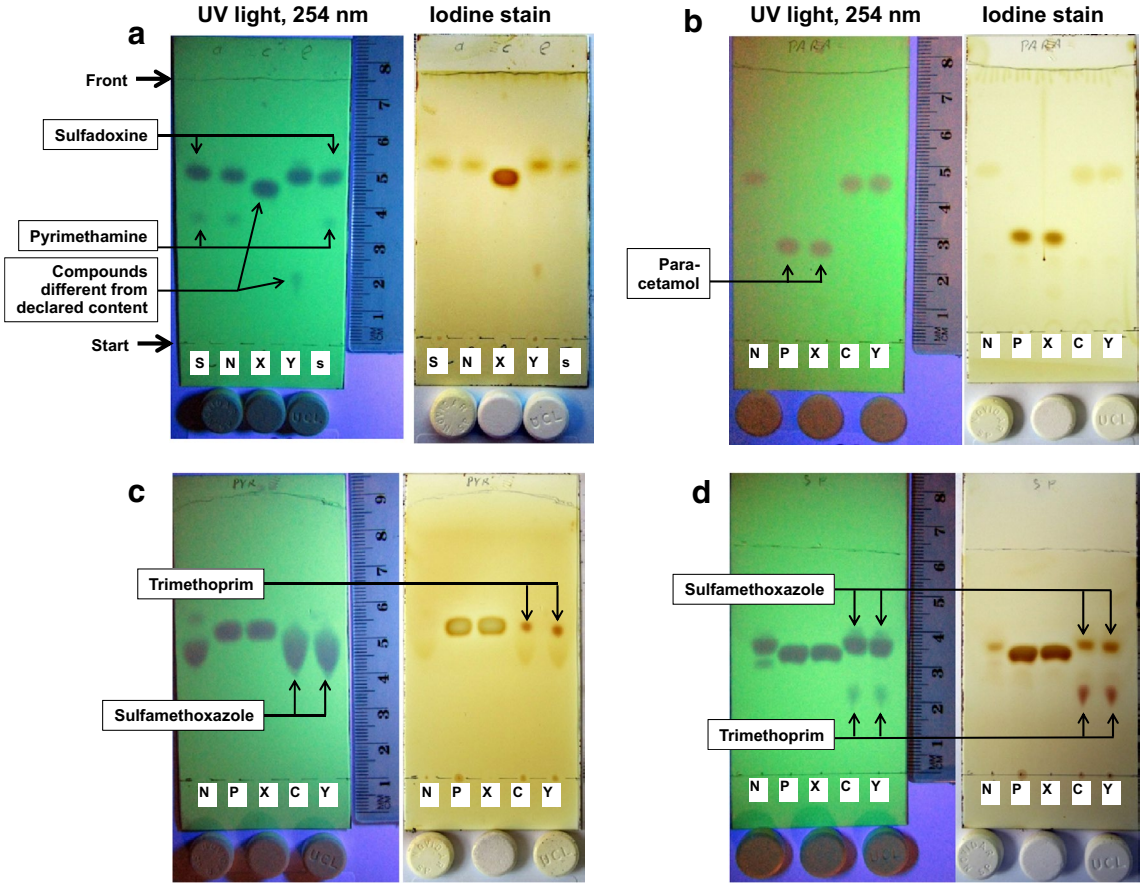

Fig. 2 TLC analysis of Novidar (SP)™ tablets (labled as N) and of the falsified samples type X and type Y (labeled as X and Y). **a** Comparison to an authentic standard of sulfadoxine 500 mg/pyrimethamine 25 mg (*S*) and to an authentic standard containing only 80 % of these amounts (*s*). **b**–**d** Comparison to paracetamol 500 mg (*P*) and co-trimoxaxole 480 mg (*C*), using different TLC solvent systems

To test these hypotheses, the tablets were further analysed in comparison to authentic paracetamol 500 mg tablets and to co-trimoxazole 480 mg tablets using the TLC solvent systems given in the manual of the GPHF Minilab for paracetamol (Fig. 2b) for pyrimethamine (Fig. 2c) and for sulfamethoxazole and co-trimoxazole (Fig. 2d). In all three solvent systems, type X showed identical results as paracetamol 500 mg tablets, and type Y showed identical results as co-trimoxazole 480 mg. Both type X and type Y proved to be clearly different from their declared content, i.e. sulfadoxine/pyrimethamine.

Visual inspection, TLC analysis and disintegration testing of further samples

Of the 27 further SP samples collected in this study, one showed chippings upon visual inspection (hereafter called sample Z). Otherwise, all samples passed visual inspection, as well as TLC analysis and disintegration testing, performed according to the Minilab manual, and also testing of the uniformity of mass of dosage units.

Full pharmacopeial analysis including High-Performance Liquid Chromatography (HPLC)

Ten samples were subjected to a full pharmacopeial analysis according to the methods of the United States Pharmacopeia (USP) in the WHO-prequalified drug quality control laboratory of the Mission for Essential Drugs and Supplies (MEDS) in Nairobi, Kenya. These included the sample containing a mixture of tablets of types X and Y, the sample Z showing chippings, and eight further, randomly selected samples. While authentic sulfadoxine and pyrimethamine standards showed HPLC retention times of 8.24 and 3.26 min, respectively, tablets of type X showed a peak at 3.43 min (paracetamol), and tablets of type Y showed peaks at 9.07 min (sulfamethoxazole) and 2.11 min (trimethoprim), proving that these tablets did not contain the declared active ingredients. Using authentic standards for paracetamol and co-trimoxazole and the appropriated USP methods for these drugs, the tablets of type X and type Y were confirmed to represent

paracetamol 500 mg tablets and co-trimoxazole 480 mg tablets.

The sample with chippings (sample Z) failed pharmacopeial analysis both for sulfadoxine content (71.8 % of declared content) and for dissolution of sulfadoxine and pyrimethamine (55.5 % and 52 % dissolution in 30 min). One further sample failed dissolution for pyrimethamine (37.6 % dissolution in 30 min). Of the total of 10 samples subjected to analysis according to the USP, therefore seven passed the analysis in all aspects.

Discussion

This study identified a sample labeled as sulfadoxine 500 mg/pyrimethamine 25 mg tablets which was sold by an illegal street vendor in Malawi and which contained, instead of the declared content, a mixture of paracetamol 500 mg tablets and co-trimoxazole 480 mg tablets. Apparently, paracetamol and co-trimoxazole tablets had been intentionally mislabelled for reasons of profit. In the International Drug Price Indicator Guide 2014 [11], the prices of one tablet of paracetamol 500 mg and co-trimoxazole 480 mg are given as 0.48 and 1.26 US cents, respectively, in international bulk procurement. In contrast, the price of sulfadoxine 500 mg/pyrimethamine 25 mg is given as 7.17 US cents. A similar difference exists in the retail prices of these medications in Malawi. In the price list of the Medical Aid Society of Malawi (MASM), they are given as 15, 20 and 80 Malawi Kwacha, corresponding to 2.60, 3.47 and 13.9 US cents per tablet, respectively (John Mponda, MASM, personal communication).

Due to the absence of the declared anti-malarial ingredients, and due to the presence of other pharmaceutical ingredients with their own potential risks and adverse effects, these falsified medicines represent a serious health risk for the population. The national drug regulatory agency, i.e. the Pharmacy, Medicines and Poisons Board of Malawi (PMPB) was informed about this finding.

For poor-quality and falsified medicines there is yet no universally accepted terminology. The World Health Organization (WHO) summarily addresses them as "substandard/spurious/falsely-labelled/falsified/counterfeit (SSFFC)" medicinal products. Several authors classify them into three main categories [1]: (1) falsified medicines, resulting from intentional fraudulent manufacturing; (2) substandard medicines, resulting from unintentional errors caused in manufacturing; and (3) degraded medicines which become of poor quality due to poor storage or transport conditions, or to poor handling. Two samples in this study failed pharmacopeial analysis, both due to insufficient dissolution and one also for insufficient content of an active ingredient. They may belong to the second or third category mentioned above. Only one of them (sample Z) showed defects already in visual inspection, but both passed TLC analysis and disintegration testing following the procedures of the GPHF Minilab manual [9]. This is consistent with earlier results that full pharmacopeial analysis is required for reliable detection of substandard or degraded medicines [8].

Falsified medicines, the first category mentioned above, may be further subdivided according to their composition and the resulting risk which they pose for public health: (a) falsified medicines which contain the declared active ingredients and are of acceptable quality; (b) falsified medicines which contain insufficient amounts of active ingredient or are of insufficient quality; (c) falsified medicines which contain no active ingredient; (d) falsified medicines which contain other active ingredient than the declared ones. The latter category presents the highest threat to public health. The present finding of an SP sample in Malawi which contains not the declared active pharmaceutical ingredients but different ones falls into this category. For small gangs of criminals the procedure of misappropriating drugs, relabelling them as more expensive medicines and selling them with higher profit may become increasingly attractive: since there are no production costs other than for the repackaging, this procedure probably offers a higher profit margin than any other method of drug counterfeiting. Therefore, widespread surveillance for that kind of counterfeiting may be desirable, especially in poor countries where this type is most likely to occur.

The TLC experiments using different solvent systems shown in Fig. 2 show the power and versatility of thin-layer chromatography in the identification of falsified medicines which contain the wrong active ingredient. Using only very simple equipment and inexpensive chemicals, these experiments not only proved that the investigated samples of type X and Y did not conform to their declaration of sulfadoxine/pyrimethamine, but also provided strong evidence that they actually represented paracetamol and co-trimoxazole tablets, respectively. To the best of the knowledge of the authors, no other readily available analytical technology could have given this result with comparable cost, speed and ease.

Obviously, the simple and inexpensive TLC technology has limitations. Figure 2 shows that TLC analysis could not differentiate between the chemically quite similar compounds sulfadoxine and sulfamethoxazole. In contrast, the higher resolution power of HPLC was able to differentiate between these compounds, showing retention times of 8.24 and 9.07 min, respectively.

Conclusions

Out of 28 samples of sulfadoxine/pyrimethamine tablets collected in Malawi, one was found not to contain the declared active ingredients but to represent a mixture of paracetamol and co-trimoxazole tablets. This type of counterfeiting represents a serious risk to public health. Thin-layer chromatography (TLC) using different solvent systems proved to be a powerful, affordable and simple method for the identification of this sample, presenting an appropriate technology for drug analysis in resource-limited settings.

Authors' contributions

FK and LH jointly designed the study. FK collected the medicines, carried out the chemical analysis, analysed the data and drafted the manuscript. SK participated in the analysis according the procedures of the US Pharmacopeia. RJ advised on the design of the study, the TLC analysis procedures and the interpretation of the data. LH participated in the collection of medicines, chemical analysis, data analysis, and manuscript preparation. All the authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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