A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Supplement 2018

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 2018 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).

* * *

* * *

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 800 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.

* * *

Made by Grimm Graphic Design, Ochsenfurt, Germany

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

Table of Contents

Chapter **Page**

New TLC Test Protocols 4

Supplement to Volume II, Chapter 6
High volume over-the-counter analgesic and antiallergic medicines

6.97 Cetirizine 4
As dihydrochloride salt in tablet and capsule formulations

6.98 Chlorphenamine (Chlorpheniramine) 8
As maleate salt in tablets and capsules incl. co-formulations with paracetamol, phenylephrine et al.

6.99 Diclofenac 12
As sodium or potassium salt in tablets and capsules incl. co-formulations with paracetamol

6.100 Mefenamic acid 16
As free base in tablet and capsule formulations

6.101 Naproxen 20
As free base or sodium salt in tablet and capsule formulations

Summary Table of Chromatographic Working Conditions 24

Supplement to Volume II, Chapter 7

Updated List of GPHF-Minilab™ Reference Standards 25

Supplement to Volume II, Chapter 10

Health & Safety 27

6.98 Chlorphenamine maleate incl. usual co-formulations

Primary Screening via Visual 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 2 or 4 mg of chlorphenamine maleate. Other dosage strengths are known to exist. Note that the strengths shown on the labels relate to the maleate salt and not to the concentration of the chlorphenamine free base. The names chlorphenamine and chlorpheniramine are used interchangeably. In addition, the maleate fraction is sometimes expressed as hydrogen maleate thus making a point on the existence of maleic acid anhydride. Frequently, in cold remedies, chlorphenamine is combined with paracetamol, phenylephrine hydrochloride, caffeine and ascorbic acid.

II. DISINTEGRATION TEST

All quick release chlorphenamine tablets and capsules including appropriate co-formulations 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 an instant release formulation 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, or stored in poor conditions, should be subjected to a thin layer chromatographic test.

Verification of Drug Identity and Content via Thin Layer Chromatography

I. PRINCIPLE

When combined with paracetamol, phenylephrine hydrochloride, caffeine or ascorbic acid, chlorphenamine maleate is extracted from tablets and capsules with a ready mix of acetone and ethyl acetate and determined by TLC with reference to an appropriate secondary standard. When working on chlorphenamine mono preparations, the extraction mix can be replaced by methanol. When facing a set of mixed samples, then fall back to the acetone/ethyl acetate extraction fluid. For the verification of paracetamol's identity and content, prepare appropriate sample and control solutions in methanol as per paracetamol protocol on page 140 of the main manual. Then, continue to work on this protocol.

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
20) Ninhydrin
21) Water
22) Toluene
23) Acetone
24) Methanol
25) n-Butanol
26) Ethyl acetate
27) Acetic acid solution 96%
28) Reference standard, for example chlorphenamine maleate 6 mg capsules

III. PREPARATION OF THE EXTRACTION MEDIUM FOR FIXED-DOSE COMBINATION PRODUCTS

When combined with other medicines, the extraction of low-dose chlorphenamine maleate next to high doses of other drugs is a challenge. Using methanol, all partner drugs are being extracted simultaneously and an overload of the chromatoplate

will be observed. A chromatoplate overload will hamper the free migration of chlorphenamine and an unusual low retention factor will be observed. Avoiding this, work with a mix consisting of one part of acetone and one part of ethyl acetate. This mix will have the power to extract chlorphenamine maleate perfectly well but keeps the unwanted compounds out or at lower non-disturbing levels. Working on one sample only, the total amount of extraction fluid needed for the preparation of control and test solutions will be about 22 ml. For this, mix 11 ml of acetone with 11 ml of ethyl acetate. For each additional 2- and 4-mg sample, prepare 4 or 8 ml more of extraction solvent, respectively.

IV. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic drug product for reference purposes, for example, capsules containing 6 mg of chlorphenamine maleate. Open one capsule by carefully separating the cap from the bottom shell and transfer all the powder into a 25-ml laboratory glass bottle adding the empty capsule shells last. For extraction, suspend the powder in 12 ml of methanol when working on mono preparations or a mix of acetone and ethyl acetate when working on fixed-dose combination products using each time a straight pipette. When facing a set of mixed samples, then fall back to the extraction fluid consisting of acetone and ethyl acetate or prepare two different standards, one in methanol for the mono preparations and another one in the acetone/ethyl acetate mix for the fixed-dose preparations. 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 0.5 mg of total chlorphenamine maleate per ml and be labelled as *'Chlorphenamine Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

Note that both, control and sample solutions, must arrive from the same extraction solvent each time. As different extraction solvents have an impact on spot size and shape, a crossover comparison of results obtained with drug in methanol and results obtained with drug in a mixture of acetone and ethyl acetate is not possible.

V. 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 0.5 mg of total chlorphenamine maleate 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 chlorphenamine.

VI. 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 the extraction fluid selected. Close and shake the vial. The solution obtained should contain 0.4 mg of total chlorphenamine maleate per ml and be labelled as *'Chlorphenamine Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of chlorphenamine maleate as stated on the product's label. In the current investigation, this drug level represents the lower acceptable limit for a given product.

VII. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 2 MG OF CHLORPHENAMINE MALEATE 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 10-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 4 ml of the solvent selected 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.

4 MG OF CHLORPHENAMINE MALEATE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 8 ml of the solvent selected using a straight pipette and a 25-ml laboratory glass bottle for collection. Continue to work as above.

Whether or not combined with other medicines, all stock sample solutions produced should finally contain 0.5 mg of total chlorphenamine maleate per ml and be labelled as *'Chlorphenamine Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue to work with the clear or hazy supernatant liquids.

VIII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Chlorphenamine stock sample solutions require no further dilution. They already represent the final working concentration of 0.5 mg of total chlorphenamine maleate per ml. If prepared from a high quality product, the sample solution should match the concentration of chlorphenamine of the higher working standard solution produced above.

IX. SPOTTING

Mark an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate and apply 2 µl of each test and standard solution as shown in the picture opposite using the microcapillary pipettes supplied.

Up to five spots can be placed on a plate. Check the uniformity of all spots using UV light of 254 nm. All spots should be circular in shape and equally spaced across the origin line. Although their intensities might differ, their diameters never should. Different intensities are due to residual amounts of excipients, 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.

Gently dry the spots. For this, hold the chromatoplate with the pair of tweezers supplied into the stream of hot air just above the hot plate for about 30 seconds.

X. DEVELOPMENT

Pipette 8 ml of methanol, 6 ml of n-butanol, 4 ml of toluene, 2 ml of water and 0.2 ml of acetic acid solution 96% 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.

XI. 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 about one minute. Use these methods of detection for both, chlorphenamine identification and quantification purposes.

A further verification of chlorphenamine identity and content can be achieved when immersing the iodine plate in ninhydrin staining solution. 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 acetic acid solution 96%. Submerge the iodine plate into the staining solution using a pair of tweezers. Instantly remove the plate again from the solution and let 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 chlorphenamine spots are gradually becoming visible at daylight after about one minute. 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.

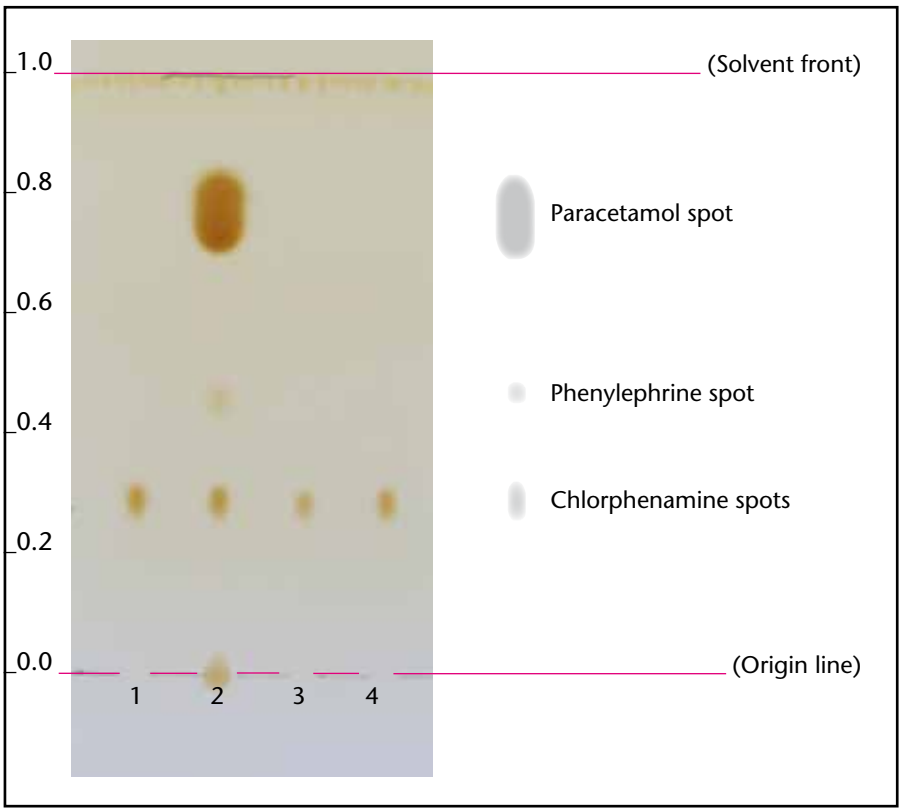
XII. CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

Run No.2:
A fixed-dose combination product of good quality with acceptable chlorphenamine content

Run No.3:
A mono-dose formulation of poor quality with unacceptable low chlorphenamine content

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



XIII. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.28 indicates the presence of chlorphenamine in the test solution. If combined with other medicines, a spot with a relative retention factor of about 0.43 would further indicate the presence of ascorbic acid, a spot at about 0.64 the presence of caffeine and a spot at about 0.77 the presence of paracetamol. Due to high dilution and weak performance, phenylephrine and maleic acid will not become visible at their expected travel distances of about 0.45 and 0.57, respectively.

XIV. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

Additional spots generated by the test solution would point to more compounds or degradation products. For example, traces of phenylephrine could now emerge. Apart from degradation, a smaller chlorphenamine spot could also be due to a poor chlorphenamine content and no spot at all due to a complete chlorphenamine absence. 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.

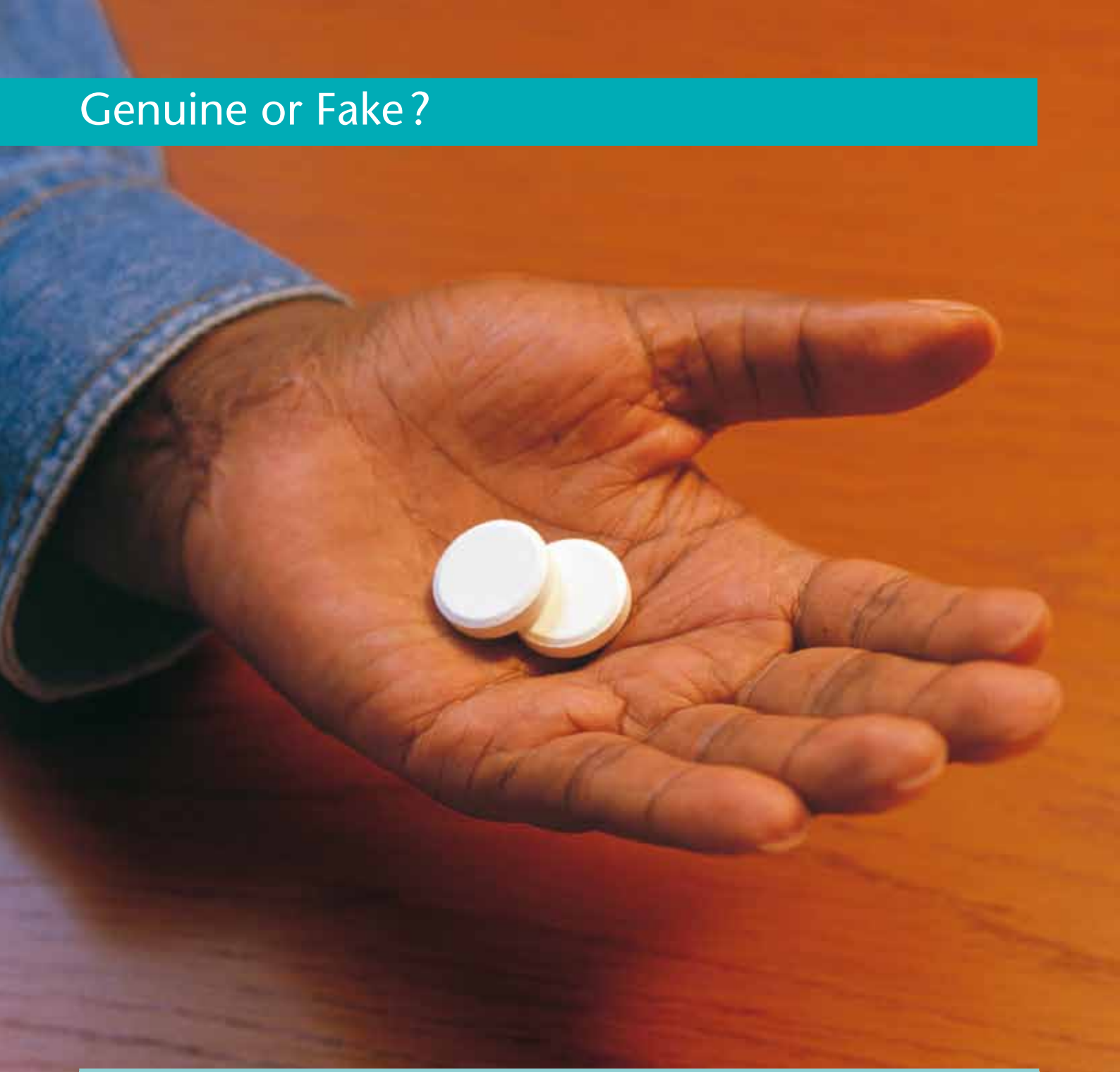
XV. OBSERVATIONS MADE AT DAYLIGHT AFTER NINHYDRIN STAINING

When exposing the iodine plate to ninhydrin and heat, all chlorphenamine spots already observed during iodine staining are turning pink and any ascorbic acid yellowish brown. Plates without prior contact to iodine will perform better.

XVI. RESULTS & ACTIONS TO BE TAKEN

The chlorphenamine 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

Global Pharma Health Fund
Frankfurt am Main, Germany
Phone +49-69-46939-662
Fax +49-69-46939-852
info@gphf.org · www.gphf.org

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).

Promoting the Quality of Medicines (PQM)
USP Global Public Health Programs
12601 Twinbrook Parkway
Rockville, MD 20852, USA
Phone +1-301-816-6370
jin@usp.org · www.usp.org/global