

# Manual

Accompanying the GPHF-Minilab™

Supplement 2016

Volume II

## THIN LAYER CHROMATOGRAPHIC TESTS



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Merck Darmstadt · Germany



**USAID**  
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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

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

Main implementation partners are national health and medicines regulatory authorities together with the World Health Organization and the U.S. Pharmacopeia's Promoting the Quality of Medicines programme. Joint drug quality monitoring projects run by Interpol in South East Asia and East Africa triggered off the seizure of millions of counterfeit antimalarial pills without any active principles in the recent years.

The unchanged need for non-sophisticated and affordable drug quality monitoring in low-income countries forms the driving force behind the development of new GPHF-Minilab™ test protocols today. The need for more testing emphasises the important collaboration with our US based implementing partners. For more patient safety and better health in developing countries, other parties are invited to join in.

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## 6.86 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 benzylpenicillin sodium salt. Not working with the free base is down to the history of benzylpenicillin development and quite unusual. Many pharmacopoeias do not highlight this point.

#### II. EQUIPMENT AND REAGENTS

- |  |  |
|--|--|
| 1) Pocket balance  | 13) TLC developing chamber (500-ml jar)  |
| 2) Aluminium foil  | 14) Hot plate  |
| 3) Spatula   | 15) Filter paper   |
| 4) Funnel  | 16) Pair of scissors   |
| 5) Label tape  | 17) Pair of tweezers   |
| 6) Marker pen  | 18) UV light of 254 nm   |
| 7) Pencil and ruler  | 19) Iodine chamber   |
| 8) 10-ml vials   | 20) Water  |
| 9) Set of straight pipettes (1 to 25 ml)   | 21) Methanol   |
| 10) Set of laboratory glass bottles (25 to 100 ml)   | 22) Ethyl acetate  |
| 11) Merck TLC aluminium plates pre-coated with silica gel 60 F <sub>254</sub> , size 5x10 cm | 23) Glacial acetic acid  |
| 12) Glass microcapillaries (2-µl filling capacity)   | 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  $\mu$ l 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.

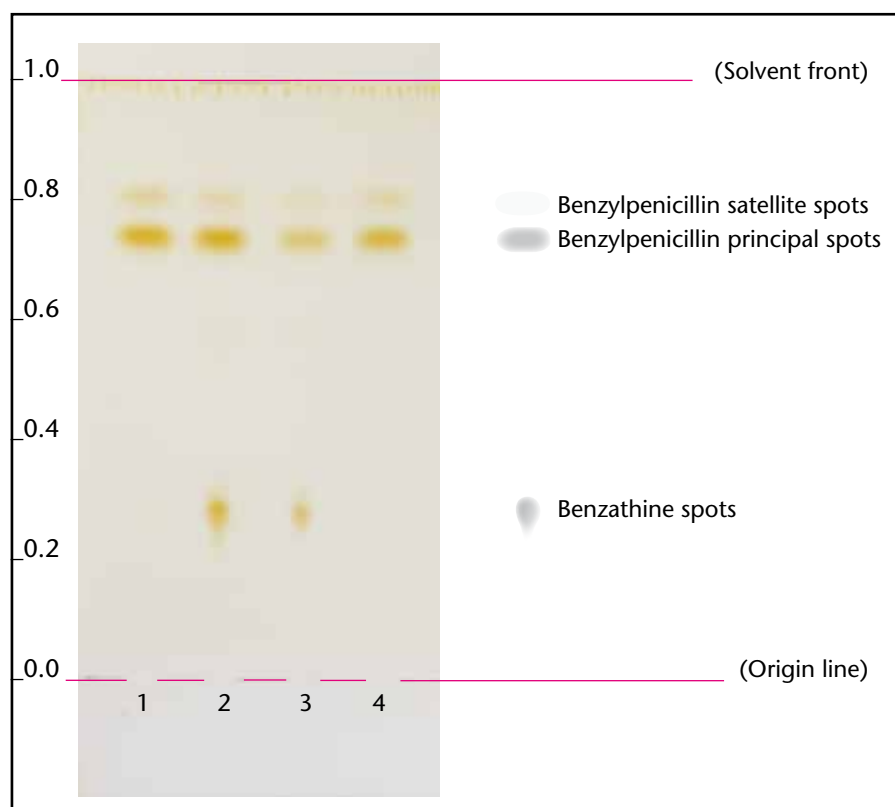
### XI. CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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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Merck Darmstadt · Germany

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



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**PROMOTING THE QUALITY OF MEDICINES**

**United States Agency for  
International Development**  
USAID/GH/OHS  
2231 Crystal Drive  
Arlington, VA 22202  
Phone +1-571-551-7207  
aboni@usaid.gov · www.usaid.gov

**United States  
Pharmacopeial Convention**  
Global Public Health  
12601 Twinbrook Parkway  
Rockville, MD 20852  
Phone +1-301-816-6370  
jin@usp.org · www.pqmusp.org