A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab®

Supplement 2013

Volume II

Thin Layer Chromatographic Tests

GPHF
GLOBAL PHARMA HEALTH FUND E.V.

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Merck Darmstadt · Germany

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USP

PROMOTING THE QUALITY OF MEDICINES

A Concise Quality Control Guide on Essential Drugs and other Medicines

Supplement 2013 to Volume II on Thin Layer Chromatographic Tests

Written by
Richard W. O. Jähnke, Kornelia Dwornik, Kai Fischer and Souly Phanouvong

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Reviewed by
Adrian Bajoras, Daniel Bempong, Sanford Bradby, Kennedy Chibwe, Yanga Dijiba, Latifa El Hadri, Mustapha Hajjou, Lukas Roth and Patrick Lukulay

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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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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 USP PQM and do not necessarily reflect the views of USAID or the United States Government.

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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 570 Minilabs have been supplied to over 80 countries across the African, Asian-Pacific and Latin American region already.

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

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

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Made by Grimm Graphic Design, Ochsenfurt, Germany

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

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6.72 Ofloxacin (as single formulation tablet and capsule)

Primary Screening via Visual Inspection and Disintegration Test

I. VISUAL & 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 a 100 to 400 mg of ofloxacin free base.

II. DISINTEGRATION TEST

All quick release ofloxacin 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's a major defect if a drug product doesn't 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

Ofloxacin tablets and capsules are extracted with aqueous acetic acid soution and determined by TLC with reference to an authentic 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) UV light of 366 nm
19) Iodine chamber
20) Glacial acetic acid
21) Methanol
22) Water
23) Ammonia solution 25%
24) Secondary reference standard, for example, tablets containing 200 mg of ofloxacin free base

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 200 mg of ofloxacin free base. 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 40-ml laboratory glass bottle and wash down all residual solids with 19.6 ml of water followed by 0.4 ml of glacial acetic acid using appropriate straight pipettes. 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 10 mg of total drug per ml and be labelled as *'Ofloxacin 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)

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 7 ml of methanol. Close and shake the vial. The solution obtained should contain 1.25 mg of total drug per ml and be labelled as *'Ofloxacin Working Standard Solution 100%'*.

This higher working standard solution represents a drug product of good quality containing 100 % of ofloxacin free base.

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

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 9 ml of methanol. Close and shake the vial. The solution obtained should contain 1 mg of total drug per ml and be labelled as *'Ofloxacin Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of ofloxacin free base 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 100 MG OF OFLOXACIN 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 9.8 ml of water followed by 0.2 ml of glacial acetic acid using appropriate straight pipettes, 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.

200 MG OF OFLOXACIN PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 19.6 ml of water followed by 0.4 ml of glacial acetic acid using appropriate straight pipettes and a 25-ml laboratory glass bottle as sample container. Continue to work as above.

400 MG OF OFLOXACIN PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 39.2 ml of water followed by 0.8 ml of glacial acetic acid using appropriate straight pipettes and a 40-ml laboratory glass bottle as sample container. Continue to work as above.

All stock sample solutions produced should finally contain 10 mg of total drug per ml and be labelled as *'Ofloxacin Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue to work with the clear or hazy supernatant liquids.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Pipette 1 ml of the stock sample solution into a 10-ml vial and add 7 ml of methanol. Close and shake the vial and label as *'Ofloxacin Working Sample Solution'*.

The expected concentration of ofloxacin free base in this working sample solution is 1.25 mg per ml and should match the concentration of ofloxacin 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.

Note that the filling of the microcapillary pipettes might take some time when handling aqueous sample solutions. As traces of water are causing blurred spots and tailing, completely dry off all extraction solvent before chromatoplate development using the hot plate supplied.

IX. DEVELOPMENT

Pipette 14 ml of methanol, 4 ml of concentrated ammonia solution 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 and observe the chromatoplate under UV light of 254 and 366 nm using the battery-driven lamps supplied. Use these methods of detection for both, identification and quantification purposes. Make sure that the work place is really dark with little or no ambient light when operating the UV lamp of 366 nm. Further verification of drug identity and content can be achieved when observing the plate at daylight after iodine staining.

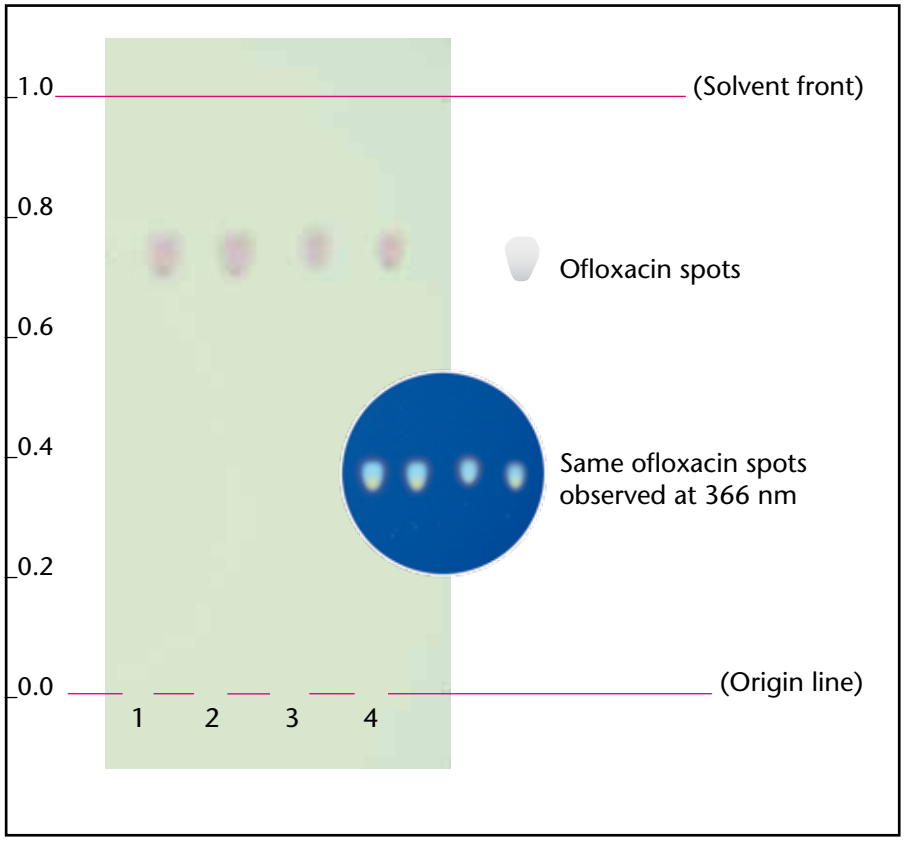
XI. CHROMATOPLATES OBSERVED UNDER UV LIGHT OF 254 NM

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

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

Run No.3:
A product of poor quality with un-acceptable low ofloxacin content

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



XII. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.71 indicates the presence of ofloxacin in the test solution. Additional strong spots generated by the test solution would point at other drugs or ofloxacin degradation, the latter case being more likely when associated with a smaller principal spot. Auxiliary agents incorporated in the different tablet or capsule formulations might cause some fainter spots emerging near or on the origin line.

XIII. OBSERVATIONS MADE AT 366 NM

When exposing the chromatoplate to UV light of 366 nm in a dark room, all ofloxacin spots already observed at 254 nm must now show an intense yellowish-blue fluorescence. Bear in mind that the colour shown here can be indicative only. The actual shade of the reference spot on the plate will be valid for decision making.

XIV. OBSERVATIONS MADE AT DAY-LIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all ofloxacin spots already observed at 254 and 366 nm are now turning yellowish brown. 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 80 and 100 percent, respectively.

XV. RESULTS & ACTIONS TO BE TAKEN

The ofloxacin 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 some 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

GPHF
GLOBAL PHARMA HEALTH FUND E.V.

A charitable organisation
maintained exclusively by
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

USAID
FROM THE AMERICAN PEOPLE

USP

PROMOTING THE QUALITY OF MEDICINES

U.S. Agency for International Development
Office of Health, Infectious Diseases and Nutrition, Ronald Reagan Bldg.,
1300 Pennsylvania Avenue NW
Washington, DC 20523-3700, USA
Phone +1-202-712-4789
Fax +1-202-216-3702
aboni@usaid.gov · www.usaid.gov

United States Pharmacopeia
Promoting the Quality of Medicines program
12601 Twinbrook Parkway
Rockville, MD 20852-1790, USA
Phone +1-301-816-8162
Fax +1-301-816-8374
pqm@usp.org · www.pqmusp.org