A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Review and Extension
2022
now with 107 Test Protocols

Physical Testing & Thin-Layer Chromatography

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Introductory Remarks

Next to physical testing, this "Concise Quality Control Guide on Essential Drugs and other Medicines" contains a collection of 107 thin-layer chromatographic test protocols for 107 essential active pharmaceutical ingredients including a multitude of solid and liquid formulations, salt forms, dosage strengths and fixed-dose combination products. This method inventory reduces science to working practice. It forms the base of each GPHF-Minilab™, a portable mini-laboratory developed for health authorities and other healthcare providers for rapid medicines quality verification in low- and middle-income countries. The Global Pharma Health Fund (GPHF), a charitable organisation living on donations made by Merck KGaA Darmstadt (Germany), initiated and supported the development of both, Minilab and associated method inventory. Further contributions came from the Promoting the Quality of Medicines (PQM) programme implemented by the United States Pharmacopeial Convention (USP). Minilab testing provides quick, economical and reliable results for the detection of falsified medicines where the contents are different, much higher or lower than indicated on the label. Assay readings are semi-quantitative and fit for in-house use. For forensic action, samples should undergo fully-fledged testing.

The quality of medicines administered to patients currently represents one of the major concerns of many national and international health care organisations. Facing the circulation of fake and poor quality medicines globally with the highest burden being in developing countries, the Minilab and its method inventory aim to make basic, non-sophisticated drug quality monitoring and due diligence testing more widely available. Enhancing medicines testing capacity with GPHF-Minilabs™ will allow national authorities and other stakeholders performing post-marketing surveillance operations more often in order to detect falsified medicines, identify other drug quality issues and use the data obtained for patient protection. Hundreds of Minilabs have been supplied to well over ninety countries across the African, Asian-Pacific and Latin American region already. Data generated with the GPHF-Minilab™ pointed efficiently to fake antimalarial and antibacterial medicines without any active pharmaceutical ingredients and prompted several times global medical product alerts by the World Health Organization (WHO). Minilabs save lives.

On the ground, GPHF partners with public, private and faith based training facilities for better pharmaceutical advancement. These collaborations are helping in building local and regional capacity in pharmaceutical quality assurance. It helps in strengthening pharmaceutical systems and accessing quality-assured medicines, too. The GPHF also offers training courses to familiarize Minilab users with the fake medicines testing procedures outlined in this manual.

The Global Pharma Health Fund has published this manual since 1998 and updated it annually. It has been published with USP's Promoting the Quality of Medicines programme since 2010. We at GPHF take this opportunity to thank all those who have given inputs and guidance in all these years. Today's review and extension reflects and consolidates the experience made in the past twenty years. For questions about earlier editions and supplements, or anything related to the new manual, send an email to info@gphf.org. For the procurement of full Minilab sets, manuals or replacement items contact GPHF's licensed logistic facility Technologie Transfer Marburg at ttm@ttm-germany.de.

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and many others more

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

Minilab Test Protocols Sorted by Therapeutic Classes*

Transmissible Diseases

Antibacterials
Amoxicillin
Ampicillin
Azithromycin
Benzathine benzylpenicillin
Benzylpenicillin
Cefalexin
Cefazolin
Cefixime
Cefotaxime
Cefpodoxime
Ceftriaxone
Cefuroxime
Chloramphenicol
Chlorhexidine
Ciprofloxacin
Clarithromycin
Clavulanic acid
Clindamycin
Cloxacillin
Doxycycline
Erythromycin
Gentamicin
Levofloxacin
Metronidazole
Moxifloxacin
Ofloxacin
Phenoxymethylpenicillin
Procaine benzylpenicillin
Sulfamethoxazole/Trimethoprim
Tetracycline

Antimycobacterials
Amikacin
Capreomycin
Cycloserine
Dapsone
Ethambutol
Ethionamide
Isoniazid
Kanamycin
Levofloxacin
Moxifloxacin
Ofloxacin
P-Aminosalicylic acid
Protionamide
Pyrazinamide
Rifampicin
Streptomycin

Antimalarials
Amodiaquine
Artemether
Artesunate
Atovaquone
Chloroquine
Dihydroartemisinin
Doxycycline
Halofantrine
Lumefantrine
Mefloquine
Piperaquine
Primaquine
Proguanil
Pyrimethamine
Pyronaridine
Quinine
Sulfadoxine
Sulfamethoxypyrazine

Anti(retro)virals
Aciclovir
Didanosine
Efavirenz
Indinavir
Lamivudine
Nevirapine
Oseltamivir
Ritonavir
Stavudine
Zidovudine

Anthelminthics
Albendazole
Mebendazole
Praziquantel

Antifungals
Fluconazole
Griseofulvin

Non-Transmissible Diseases

Analgesics
Acetylsalicylic acid
Diclofenac
Mefenamic acid
Naproxen
Paracetamol

Antiallergics
Cetirizine
Chlorphenamine
Dexamethasone
Prednisolone

Antiasthmathics
Aminophylline
Salbutamol

Cardiovascular agents
Amlodipine
Atenolol
Bisoprolol
Captopril
Furosemide
Hydrochlorothiazide
Irbesartan
Lisinopril
Losartan
Methyldopa
Nifedipine
Simvastatin
Telmisartan
Valsartan

Endocrine agents
Clomifene
Glibenclamide
Metformin

Gastrointestinal agents
Metoclopramide
Omeprazole
Ranitidine

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*Usual fixed-dose combinations are included. For full detail on this, see alphabetical order in the table of contents.

Table of Contents

** Non-modified, instant soluble tablets and capsules containing the active pharmaceutical ingredient per free base as single agent are forming the baseline for each test protocol by default. Any further inclusion of salt forms, pharmaceutical formulations and fixed dose combination products is named separately. In addition, each protocol includes a number of common dosage strengths.*

7.10A Artemether in lumefantrine dispersible/traditional tablets and dry syrups

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each dispersible and traditional tablet usually contains 20 or 80 mg of artemether combined with a 120 or 480 mg of lumefantrine, respectively. Frequently, co-formulated products are also presented as dry powder for oral suspensions usually containing a 180 or 360 mg of artemether and a 1080 or 2160 mg of lumefantrine per bottle, respectively. Independent from the total amount of active ingredients in the dry powder, each 5 ml of the ready suspension should finally contain 15 mg of artemether and 90 mg of lumefantrine. Verify the total weight of tablets or the fill weight of capsules and dry syrups using the electronic pocket balance supplied. All quick release artemether/lumefantrine tablet and capsule formulations must also pass the disintegration test. 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.

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, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Artemether is extracted from lumefantrine fixed-dose combination tablets, dispersible tablets and powder for oral suspensions with a known volume of methanolic acetic acid solution and then checked for identity and content by TLC with reference to a suitable secondary standard. For a rapid drug quality verification of the lumefantrine fraction consult the appropriate protocol shown in this manual.

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) TLC dipping chamber (250-ml beaker)
20) Toluene
21) Methanol
22) Ethyl acetate
23) Acetic acid solution 96%
24) Sulphuric acid solution 96%
25) Electronic pocket balance
26) Reference agent, for example, fixed-dose combination tablets containing 20 mg of artemether and a 120 mg of lumefantrine or, alternatively, artemether neat substance from commercial sources

III. PREPARATION OF THE STOCK STANDARD SOLUTION

If the reference standard comes as a tablet containing 20 mg of artemether, may-be even combined with a 120 mg of lumefantrine, then wrap one 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 4.5 ml of methanol followed by 0.5 ml of acetic acid solution 96% 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. Next to lumefantrine, the solution obtained should contain 4 mg of total artemether per ml and be labelled as *'Artemether Stock Standard Solution'*. Freshly prepare

this solution for each test. Continue to work with the clear or hazy supernatant liquid.

If the artemether reference standard comes as powder of high purity close to 100%, then weigh in correctly about 0.3 g using the electronic pocket balance supplied. Further, dissolve the powder in 67.5 ml of methanol followed by 7.5 ml of acetic acid solution 96% thus obtaining again a solution containing 4 mg of total artemether per ml of extraction solvent. Adjust the amount of extraction solvent when the weighing result differs from the target weight. In order to overcome the balance's in-build dynamic inertia and to ensure correct readings, lift the weighing boat or tap the weighing pan with a pen or spatula each time after a few more milligrams have been added or removed. In order to ensure complete dissolution, observe the shaking and sitting times as mentioned above. Label as above. The final solution obtained should be clear without any observable residual solids. Freshly prepare this solution for each test.

IV. PREPARATION OF THE WORKING STANDARD SOLUTION 100% (UPPER WORKING LIMIT)

Pipette 2 ml of the stock standard solution into a 10-ml vial and add 2 ml of methanol. Close and shake the vial. The solution obtained should contain 2 mg of total artemether per ml and be labelled as *'Artemether Working Standard Solution 100%'*.

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

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

Pipette 2 ml of the stock standard solution into a 10-ml vial and add 3 ml of methanol. Close and shake the vial. The solution obtained should contain 1.6 mg of total artemether per ml and be labelled as *'Artemether Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of artemether 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 TABLET CLAIMING TO CONTAIN 20 MG OF ARTEMETHER

Take one whole tablet from an appropriate drug product sampled in the field. As usual, wrap up the tablet into aluminium foil and crush it down to a fine powder. Transfer all the powder obtained into a 25-ml laboratory glass bottle. For extraction, add 5 ml of methanol followed by 0.55 ml of acetic acid solution 96% using a set of appropriate straight pipettes. Close the lab 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.

TABLET CLAIMING TO CONTAIN 80 MG OF ARTEMETHER

Take one whole sample tablet and extract the powder obtained with 20 ml of methanol followed by 2.2 ml of acetic acid solution 96% using appropriate straight pipettes and a 25-ml laboratory glass bottle as sample container. Continue to work as above.

POWDER FOR ORAL SUSPENSION CLAIMING TO CONTAIN 180 MG OF ARTEMETHER PER BOTTLE

Take one whole bottle from a corresponding drug product sampled in the field. For extraction, add to the dry powder still sitting in the original container 45 ml of methanol followed by 5 ml of acetic acid solution 96% using a set of appropriate straight pipettes. Close the sample bottle and thoroughly shake for about three minutes. Allow the mix to sit for an additional five minutes until undissolved residues settle below the supernatant liquid.

POWDER FOR ORAL SUSPENSION CLAIMING TO CONTAIN 360 MG OF ARTEMETHER PER BOTTLE

Take one whole sample bottle and add to the dry powder still sitting in the original container 90 ml of methanol followed by 10 ml of acetic acid solution 96% using a set of appropriate straight pipettes. Close the sample container and thoroughly shake for about three minutes. Allow the mix to sit for an additional five minutes until undissolved residues settle below the supernatant liquid.

Next to lumefantrine, all stock sample solutions produced should finally contain 3.6 mg of total artemether per ml and be labelled as *'Artemether Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue to work with the clear or hazy supernatant liquids.

Note that all artemether stock sample solutions obtained can be used to produce both, the artemether and the lumefantrine working sample solutions following this and the lumefantrine test protocol in this manual.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Pipette 2.5 ml of the stock sample solution into a 10-ml vial and add 2 ml of methanol. Close and shake the vial and label as *'Artemether Working Sample Solution'*.

The expected concentration of artemether in this working sample solution is 2 mg per ml and should match the concentration of artemether 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. Even if artemether itself stays invisible, excipients and other drug compounds will show up to facilitate verification. 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.

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

IX. DEVELOPMENT

Pipette 18 ml of toluene, 4 ml of ethyl acetate and 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 10 minutes. Remove the plate from the chamber, mark the solvent front and allow any excess to evaporate. For this, hold the chromatoplate with the pair of tweezers supplied into the current of hot air just above the hot plate for about two minutes.

X. DETECTION

When working on fixed-dose combination medicines, it is best to check the presence of lumefantrine before that of artemether. For this, expose the dried chromatoplate first to UV light of 254 nm using the battery-driven lamp supplied.

For the detection of the artemether fraction, expose the chromatoplate to sulphuric acid staining. For this, fill the 250-ml plastic beaker supplied with a 190 ml of methanol followed by 10 ml of concentrated sulphuric acid solution and mix gently. Allow the mix to cool down and submerge the chromatoplate **upside down** 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 on the hot plate supplied. During heating, all artemether spots are gradually becoming visible at daylight. Use this method of detection for both, artemether identification and quantification purposes. Note that the staining operation performed with sulphuric acid solution is very similar to that with ninhydrin illustrated on page 36 of the main manual.

After sulphuric acid staining and chromatoplate reading at daylight, a further verification of artemether identity and content can be achieved when subjecting the chromatoplate to UV light of 366 nm in a dark room.

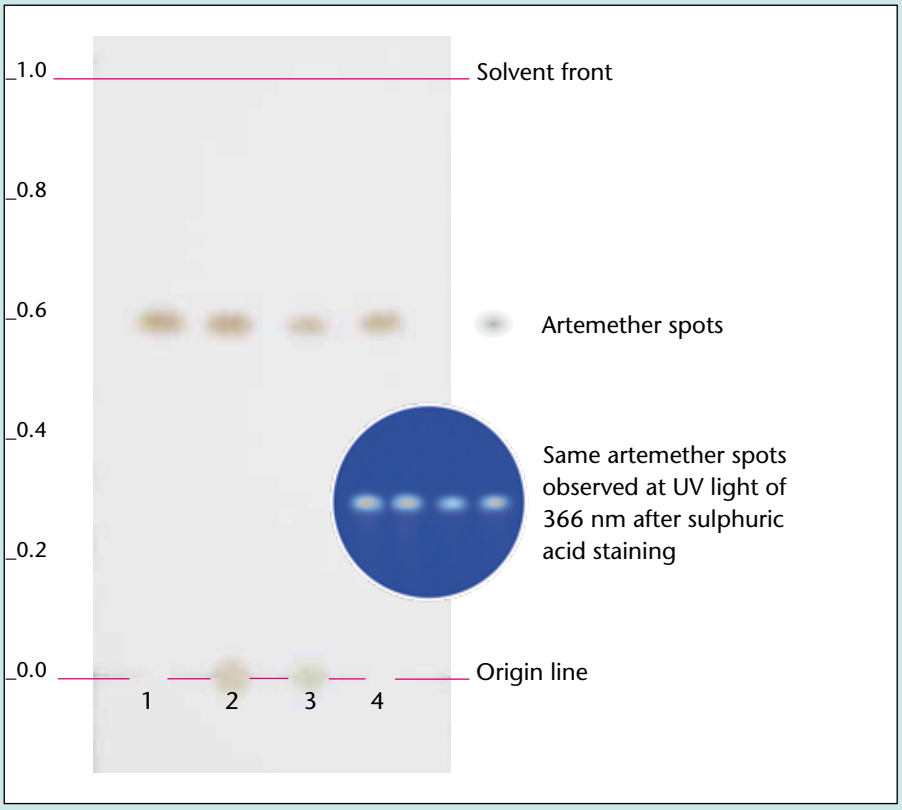
CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER SULPHURIC ACID STAINING

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

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

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

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



XI. OBSERVATIONS MADE AT 254 NM BEFORE STAINING

Artemether itself stays almost invisible and no other spots should be detected unless the medicine under investigation is presented as a co-formulated product. In the latter case, a strong violet spot at a travel distance of about 0.20 indicates the presence of lumefantrine and, in case of dry powders for oral suspensions, a second strong spot between a travel distance of 0.40 and 0.50 indicates the presence of a preservative either from the benzoate or paraben family. Saccharin sodium as sweetener in dispersible tablets would settle at about 0.20 but stays below its limit of detection due to strong dilutions during sample preparation. For a better identification of the lumefantrine fraction go to 286 of this manual.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER SULPHURIC ACID STAINING

A dark brown spot at a travel distance of about 0.58 indicates the presence of artemether in the test solution. Auxiliary agents incorporated in the different tablet and powder formulations may cause further spots near or on the origin line. Beyond this, no other spots should be visible even if artemether is combined with lumefantrine. Additional strong spots generated by the test solution would point at other drugs or artemether 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 artemether content and no spot at all a complete artemether absence.

XIII. OBSERVATIONS MADE AT 366 NM AFTER SULPHURIC ACID STAINING

When exposing the chromatoplate to UV light of 366 nm after heating with sulphuric acid, all brown artemether spots previously observed at daylight are now showing an off-white fluorescence.

XIV. RESULTS & ACTIONS TO BE TAKEN

The artemether 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.

7.34 Clavulanic acid as potassium salt in amoxicillin co-formulations

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet or capsule usually contains a 125 mg of clavulanic acid preferable presented as potassium clavulanate combined with 250, 500 or 875 mg of anhydrous amoxicillin. Lower dosage strengths, for example, 62.5 mg of clavulanic acid in oral dosage forms are known to exist. In addition, co-formulated clavulanic acid is frequently presented as powder for oral suspensions. Independent from the total amount of active ingredients in the dry powder, each 5 ml of the ready suspension usually contains a 125 or 250 mg of amoxicillin and a 31.25 or 62.5 mg of clavulanic acid, respectively. Verify the total weight of tablets or the fill weight of capsules and dry syrups using the electronic balance supplied. All quick release tablets and capsules containing clavulanic acid must also pass the disintegration test. 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.

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, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Potassium clavulanate is extracted from amoxicillin tablets and capsules with a known volume of water and then checked for identity and content by TLC with reference to a suitable secondary standard. For a rapid drug quality verification of the amoxicillin fraction consult the appropriate protocol shown in this manual.

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) TLC dipping chamber (250-ml beaker)
19) Iodine chamber
20) Water
21) Methanol
22) Ninhydrin
23) Ethyl acetate
24) Acetic acid solution 96%
25) Reference agent, for example, tablets containing the equivalent amount of a 125 mg of clavulanic acid combined with 500 mg of amoxicillin

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 125 mg of clavulanic acid combined with 500 mg of amoxicillin. 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 50-ml laboratory glass bottle and wash down all residual solids with 25 ml of water 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. Next to amoxicillin, the solution obtained should contain 5 mg of total clavulanic acid per ml and be labelled as *'Clavulanic Acid Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the 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 3 ml of methanol. Close and shake the vial. The solution obtained should contain 1.25 mg of total clavulanic acid per ml and be labelled as *'Clavulanic Acid Working Standard Solution 100%'*.

This higher working standard solution represents a drug product of good quality containing 100 % of clavulanic acid.

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

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 4 ml of methanol. Close and shake the vial. The solution obtained should contain 1 mg of total clavulanic acid per ml and be labelled as *'Clavulanic Acid Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of clavulanic acid 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 62.5 MG OF CLAVULANIC ACID PER TABLET OR CAPSULE

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 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 12.5 ml of water 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.

125 MG OF CLAVULANIC ACID PER TABLET OR CAPSULE

Take one whole sample tablet or capsule and extract the powder obtained with 25 ml of water. Continue to work as above.

375 MG OF CLAVULANIC ACID PER BOTTLE

Take the entire powder from one bottle of dry syrup and extract with 75 ml of water. Continue to work as above.

625 MG OF CLAVULANIC ACID PER BOTTLE

Take the entire powder from one bottle of dry syrup and extract with 125 ml of water. Continue to work as above.

750 MG OF CLAVULANIC ACID PER BOTTLE

Take the entire powder from one bottle of dry syrup and extract with 150 ml of water. Continue to work as above.

1250 MG OF CLAVULANIC ACID PER BOTTLE

Take the entire powder from one bottle of dry syrup and extract with 250 ml of water. Continue to work as above.

Next to amoxicillin, all stock sample solutions produced should finally contain 5 mg of clavulanic acid per ml and be labelled as *'Clavulanic Acid Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue to work with the 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 3 ml of methanol. Close and shake the vial and label as *'Clavulanic Acid Working Sample Solution'*.

The expected concentration of clavulanic acid in this working sample solution is 1.25 mg per ml and should match the concentration of clavulanic acid 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. Even if clavulanic acid itself stays invisible, excipients and some amoxicillin will show up to facilitate verification. 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 and 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.

Note that due to a higher viscosity, the filling and emptying of the microcapillary pipettes might take some time when handling aqueous sample solutions arriving from dry syrups. Still, filling and emptying micropipettes from end-to-end is essential for correct quantitative work. So, take time and stay precise. As traces of water are causing blurred spots and tailing, completely dry off all extraction solvent before chromatoplate development. In order to drive out all residual water fast and completely, it is normally good to dry the chromatoplate at maximum temperature. However, in this case, the test solution contains also the very heat sensitive clavulanic acid. Hence, spot drying has to switch from hot and fast to lukewarm and long. For this, hold and shake the chromatoplate in the current of hot air just above the hot plate for about two minutes. Intermittingly, make the chromatoplate touching the hot plate directly for a second. Keep in mind that the chromatoplate has to stay lukewarm at any time. When getting hot, clavulanic acid degrades readily!

IX. DEVELOPMENT

Pipette 15 ml of ethyl acetate, 5 ml of acetic acid solution 96% and 5 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 completely dry off all solvents from the mobile phase before spot detection keeping in mind that clavulanic acid degrades readily when the chromatoplate is getting too hot. Hence, hold and shake the chromatoplate in the current of hot air just above the hot plate for about three minutes. Intermittingly, make the chromatoplate touching the hot plate directly for a second. However, the chromatoplate has to stay lukewarm only. When getting hot, the clavulanic acid spots will gradually disappear.

X. DETECTION

Dry off all residual solvent and observe the chromatoplate under UV light of 254 nm using the battery-driven lamp supplied. After the absence or presence of any foreign drug compounds has been verified, the chromatoplate can then be exposed to iodine vapour. Use iodine staining for both, clavulanic acid identification and quantification purposes. A further verification of drug identity and content can be achieved when observing the plate at daylight after staining with ninhydrin. Ninhydrin detects also aspartame in situations where this non-saccharide sweetener has been used in dry syrups.

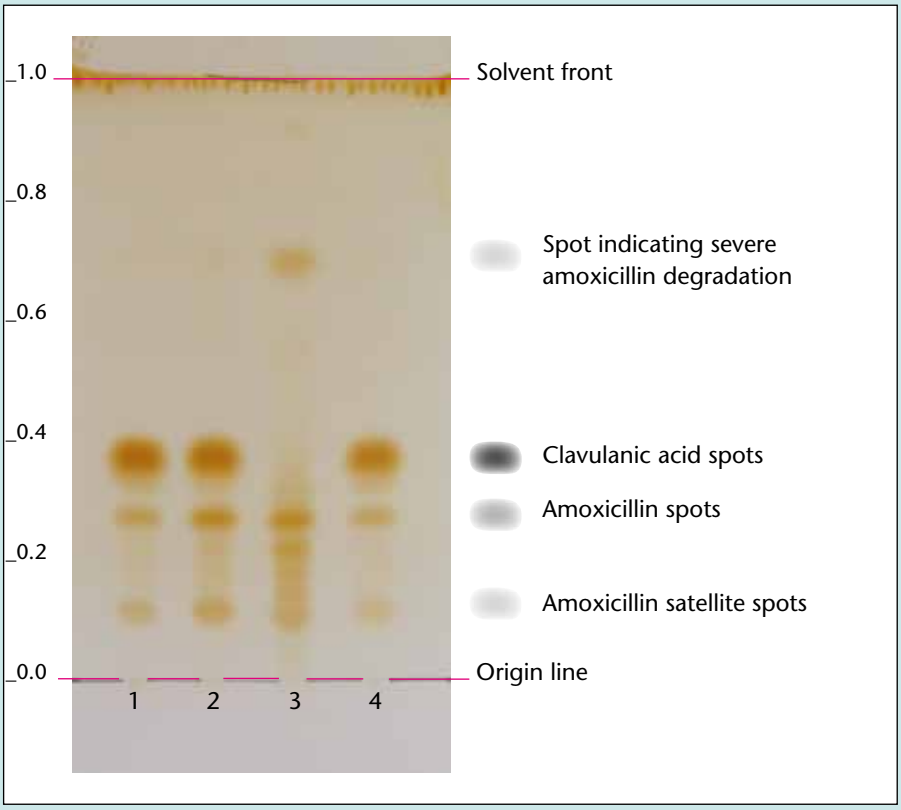
CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

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

Run No.3:
A fixed-dose combination product where clavulanic acid is missing and amoxicillin degrading

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



XI. OBSERVATIONS MADE AT 254 NM

Clavulanic acid stays invisible and spots at a travel distance of about 0.28 indicate the presence of amoxicillin in the test solution. Additional strong spots generated by the test solution would point at other drugs. For a further verification of amoxicillin identity and content follow the relevant protocol shown in this manual.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

A strong yellow-brown spot at a travel distance of about 0.38 indicates the presence of clavulanic acid in the test solution. Amoxicillin spots already observed at 254 nm are now turning yellowish brown, too. Additional strong spots generated by the test solution would point at other drugs or some degradation of clavulanic acid or amoxicillin, the latter case being more likely when each time associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor clavulanic acid content and no spot at all a complete absence of clavulanic acid. Still observe the plate when iodine evaporates. 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 different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The spot for clavulanic acid 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.

7.47 Erythromycin stearate

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet or capsule usually contains erythromycin stearate in quantities equivalent to 250 or 500 mg of erythromycin free base. Depending on the geographical region, dosage strengths of a 180 and 360 mg free base can be observed. Other strengths and salt forms are known to exist. Verify the total weight of tablets or the fill weight of capsules using the electronic pocket balance supplied. All quick release erythromycin tablet and capsule formulations must also pass the disintegration test. 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.

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, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Erythromycin stearate is extracted from tablets and capsules with a known volume of methanol and then checked for identity and content by TLC with reference to a suitable 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) Ethyl acetate
20) Methanol
21) Ammonia solution 25%
22) Acetic acid solution 96%
23) Reference agent, for example, tablets containing erythromycin stearate in a quantity equivalent to 250 or 500 mg mg of erythromycin 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 347 or 694 mg of erythromycin stearate equivalent to 250 or 500 mg of erythromycin 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 100-ml laboratory glass bottle and wash down all residual solids with 25 or 50 ml of methanol, for 250 mg or 500 mg of erythromycin free base per unit respectively, using each time an appropriate 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 10 mg of erythromycin free base per ml and be labelled as *'Erythromycin 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 erythromycine stock standard solution requires no further dilution. It already represents the final working concentration of 10 mg of erythromycin free base 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 erythromycin stearate.

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 8 mg of erythromycin free base per ml and be labelled as *'Erythromycin Working Standard Solution 80%'.*

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of erythromycin stearate 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 250 MG OF ERYTHROMYCIN FREE BASE 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 50-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 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.

360 MG OF ERYTHROMYCIN FREE BASE (500 MG OF ERYTHROMYCIN STEARATE) PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 36 ml of methanol using a straight pipette and a 50-ml laboratory glass bottle as sample container. Continue to work as above.

500 MG OF ERYTHROMYCIN FREE BASE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 50 ml of methanol using a straight pipette and a 100-ml laboratory glass bottle as sample container. Continue to work as above.

All stock sample solutions produced should finally contain 10 mg of erythromycin free base per ml and be labelled as *'Erythromycin 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

Erythromycin stock sample solutions require no further dilution. They already represent the final working concentration of 10 mg of erythromycin free base per ml. If prepared from high quality products, these sample solutions should match the concentration of erythromycin free base 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. Even if erythromycin itself stays invisible, some excipients will show up to facilitate verification. 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.

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

IX. DEVELOPMENT

A) Erythromycin stearate content verification: pipette 20 ml of methanol, 5 ml of ethyl acetate and 0.5 ml of concentrated ammonia solution 25% 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 for about two minutes on the hot plate supplied.

B) Erythromycin stearate versus azithromycin: in order to make the difference in travel distances between both drug compounds more pronounce, reverse the pH of the mobile phase from above with 1 ml of acetic acid solution 96% and perform a second run with a new plate. Use the pH indicator paper supplied to verify the acidity of the mobile phase after thorough shaking. The yellow pH indicator paper should turn to pale red at least and any smell of ammonia should have disappeared.

X. DETECTION

Dry off all residual solvent and expose the chromatoplate to iodine vapour for about one minute. Remove the plate from the iodine chamber and observe the plate at daylight. Use this method of detection for both, erythromycin and azithromycin identification and quantification purposes.

Further verification of erythromycin identity and content can be achieved when staining the iodine plate with sulphuric acid in the heat. For this, fill the 250-ml plastic beaker supplied with a 190 ml of methanol followed by 10 ml of concentrated sulphuric acid solution and mix gently. Allow the mix to cool down and submerge the chromatoplate into the staining solution using a pair of tweezers. Instantly remove the plate and let surplus solution run down onto paper tissue. Wipe off residual liquid from the back of the plate and continue to dry off all staining solution on the hot plate supplied. During heating, all erythromycin and azithromycin spots are gradually becoming visible at daylight.

XI. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

Mobile phase A: a strong orange-brown spot at a travel distance of about 0.62 indicates the presence of erythromycin stearate in the test solution. Additional strong spots generated by the test solution would point at other drugs or erythromycin 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 erythromycin stearate content and no spot at all a complete erythromycin absence. Auxiliary agents incorporated different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line. Still observe

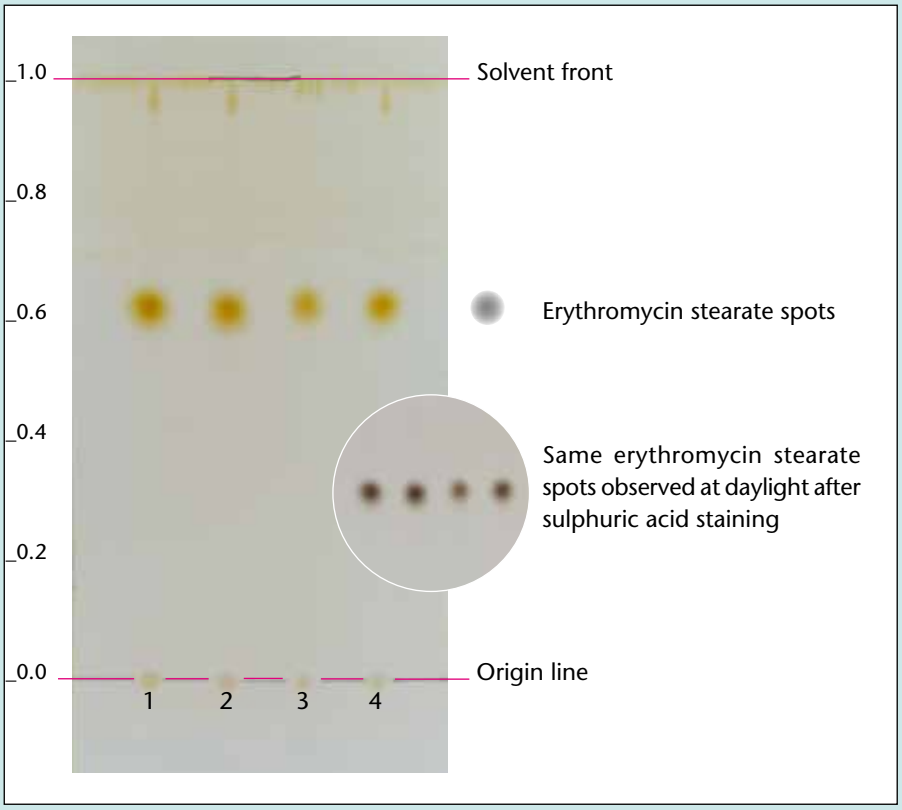
CHROMATOPLATE COMING FROM THE MOBILE PHASE «A» OBSERVED AT DAYLIGHT AFTER STAINING WITH IODINE

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

Run No.2:
A product of good quality with an acceptable content of erythromycin stearate

Run No.3:
A product of poor quality with an unacceptable low content of erythromycin stearate

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



the plate when iodine evaporates. 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.

Mobile phase B: erythromycin stearate spots are running in the front settling at a travel distance of about 0.38 followed by spots from azithromycin with a relative retention factor of about 0.28. Spots from both compounds are showing a distinct shape assisting in identification.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER SULPHURIC ACID STAINING

When exposing the iodine plate further to sulphuric acid and heat, then all erythromycin stearate spots already observed after iodine staining are now turning dark brown or even black. This is valid for both, mobile phase A and B. The same change in colour can also be observed for azithromycin. Both drug compounds perform very strong here. Hence, for better semi-quantitative readings after sulphuric acid staining, halve the appropriate drug concentrations in the working solutions.

XIII. RESULTS & ACTIONS TO BE TAKEN

The spot for erythromycin stearate 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.

7.48 Ethambutol hydrochloride incl. usual co-formulations

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet or capsule usually contains a 100 to 400 mg of ethambutol hydrochloride. Higher strengths, for example, 800 mg of ethambutol hydrochloride are known to exist. Verify the total weight of tablets or the fill weight of capsules using the electronic pocket balance supplied. Whether or not combined with other medicines, all quick release ethambutol tablet and capsule formulations must also pass the disintegration test. 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.

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, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Whether or not combined with other antituberculosis medicines, ethambutol hydrochloride is extracted from tablets and capsules with a known volume of methanol and then checked for identity and content by TLC with reference to a suitable secondary standard. When ethambutol is combined with rifampicin, isoniazid or pyrazinamide, then, for further testing, consult also the relevant protocols shown in this manual.

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) TLC dipping chamber (250-ml beaker)
19) Ninhydrin
20) Methanol
21) Toluene
22) Ammonia solution 25%
23) Acetic acid solution 96%
24) Reference agent, for example, ethambutol hydrochloride 400 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 400 mg of ethambutol hydrochloride. 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 50-ml laboratory glass bottle and wash down all residual solids with 40 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 10 mg of total drug per ml and be labelled as *'Ethambutol 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 *'Ethambutol Working Standard Solution 100%'.*

This higher working standard solution represents a drug product of good quality containing 100 % of ethambutol hydrochloride.

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

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of ethambutol hydrochloride 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 ETHAMBUTOL HYDROCHLORIDE 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 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.

275 MG OF ETHAMBUTOL HYDROCHLORIDE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 27.5 ml of methanol using a straight pipette and a 50-ml laboratory glass bottle as sample container. Continue to work as above.

400 MG OF ETHAMBUTOL HYDROCHLORIDE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 40 ml of methanol using a straight pipette and a 50-ml laboratory glass bottle as sample container. Continue to work as above.

Whether or not combined with other antituberculosis medicines, all stock sample solutions produced should finally contain 10 mg of total ethambutol per ml and be labelled as *'Ethambutol 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 *'Ethambutol Working Sample Solution'*. The expected concentration of ethambutol hydrochloride in this working sample solution is 1.25 mg per ml and should match the concentration of ethambutol 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. Even if ethambutol itself stays invisible, some excipients or drugs from combination products will show up to facilitate verification. 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.

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

IX. DEVELOPMENT

Pipette 12 ml of methanol, 10 ml of toluene and 0.5 ml of concentrated ammonia solution 25% 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. In case of fixed-dose combinations, just before placing the loaded TLC plate into the jar, dry the empty space above the sample spots of the origin line again for one minute directly on the hot plate thus ensuring to work with a highly activated plate free of moisture. After activation for perfect TB medicines separation, place the 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 on the hot plate supplied for about two minutes. No smell of ammonia solution should be left on the plate.

X. DETECTION

Dry off all residual solvent and observe the chromatoplate under UV light of 254 nm using the battery-driven lamp supplied. Use this method of detection first in case ethambutol is combined with other antituberculosis medicines.

For the detection of ethambutol itself, thoroughly dry the chromatoplate till the smell of ammonia solution completely disappears. Then, expose the TLC plate to ninhydrin staining as shown on page 36 of this manual. For this, dissolve 3 g of ninhydrin (about 10 times a well filled spatula) in a mixture of a 150 ml of methanol and 30 ml of acetic acid solution 96%. Use the plastic beaker supplied to accommodate the staining solution. This will allow dipping the chromatoplate into the solution using a pair of tweezers. Instantly remove the plate from the beaker and dry the back of the plate with paper tissue. Continue to dry off all staining solution on a hot plate and observe how the ethambutol spots are gradually becoming visible. Use this method of detection for ethambutol quantification purposes. Ninhydrin staining will make it impossible to further observe spots made of isoniazid or pyrazinamide under UV light of 254 nm.

Note that all skin contaminated with ninhydrin solution will be stained as well. However, this is not dangerous to health and the blue-violet spots will disappear after about two days.

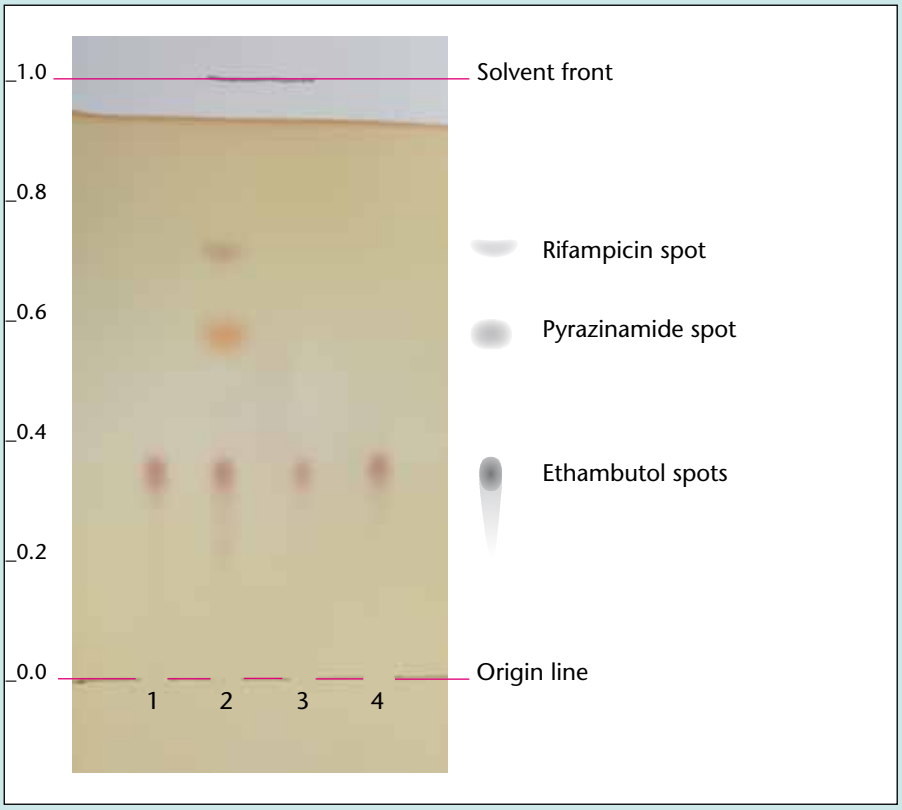
CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER NINHYDRIN STAINING

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

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

Run No.3:
A single drug product of poor quality with unacceptable low ethambutol content

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



XI. OBSERVATIONS MADE AT 254 NM

Ethambutol stays invisible and no other spots should be detected unless the medicine under investigation is presented as a fixed-dose combination product containing also other antituberculosis compounds. In the latter case, spots made of isoniazid will become visible at a travel distance of about 0.45 and spots made of pyrazinamide at a travel distance of about 0.57. Spots made of rifampicin will be visible at a travel distance of about 0.72 at daylight already. For all of this, consult also the picture shown on page 405.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER STAINING WITH NINHYDRIN

A red spot at a travel distance of about 0.34 indicates the presence of ethambutol in the test solution. Next to ethambutol, rifampicin and pyrazinamide will be become visible, too. Additional strong spots generated by the test solution would point at other drugs or ethambutol 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 ethambutol content and no spot at all a complete ethambutol absence. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The ethambutol 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.

7.61 Lamivudine incl. usual co-formulations

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet or capsule usually contains 30, 60, 150 or 300 mg of lamivudine. Appropriate solutions for oral administration are normally coming in a dosage strength of 10 mg of lamivudine per ml. Frequently, lamivudine is combined with other antiretroviral medicines. Verify the total weight of tablets or the fill weight of capsules using the electronic pocket balance supplied. Whether or not combined with other medicines, all quick release lamivudine tablet and capsule formulations must also pass the disintegration test. 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.

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, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Whether or not combined with other antiretrovial medicines, lamivudine solutions are diluted and tablets or capsules are extracted with a known volume of water and then checked for identity and content by TLC with reference to a suitable secondary standard. When lamivudine is combined with nevirapine, stavudine, zidovudine, efavirenz or tenofovir, then, for further testing, consult also the appropriate protocols shown in this manual.

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) Ethyl acetate
19) Methanol
20) Toluene
21) Water
22) Reference agent, for example, lamivudine 150 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 150 mg of lamivudine. 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 50-ml laboratory glass bottle and wash down all residual solids with 30 ml of water 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 drug per ml and be labelled as *'Lamivudine 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 3 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 *'Lamivudine Working Standard Solution 100%'*.

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

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 4 ml of methanol. Close and shake the vial. The solution obtained should contain 1.0 mg of total drug per ml and be labelled as *'Lamivudine Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of lamivudine 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 SOLID DOSAGE FORMS CLAIMING TO CONTAIN 30 MG OF LAMIVUDINE PER UNIT

Take two (!) whole tablets or capsules 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 sample capsules should be transferred directly into the bottle adding the cap and body shells last. For extraction, add 12 ml of water 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.

SOLID DOSAGE FORMS CLAIMING TO CONTAIN 60 MG OF LAMIVUDINE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 12 ml of water using a straight pipette and a 25-ml laboratory glass bottle as sample container. Continue to work as above.

SOLID DOSAGE FORMS CLAIMING TO CONTAIN 150 MG OF LAMIVUDINE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 30 ml of water using a straight pipette a 50-ml laboratory glass bottle as sample container. Continue to work as above.

SOLID DOSAGE FORMS CLAIMING TO CONTAIN 300 MG OF LAMIVUDINE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 60 ml of water using a straight pipette and a 100-ml laboratory glass bottle as sample container. Continue to work as above.

LIQUID DOSAGE FORMS CLAIMING TO CONTAIN 10 MG OF LAMIVUDINE PER ML

Transfer 2 ml of the solution presented into a 10-ml laboratory glass bottle and dilute with 2 ml of water using each time appropriate straight pipettes. Close the lab bottle and shake for mixing.

Whether or not combined with other medicines, all stock sample solutions produced should finally contain 5 mg of total lamivudine per ml and be labelled as *'Lamivudine 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 3 ml of methanol. Close and shake the vial and label as *'Lamivudine Working Sample Solution'*.

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

Note that the filling and emptying of the microcapillary pipettes might take some time when handling aqueous sample solutions. As traces of water are causing spot tailing, completely dry off all extraction solvent before chromatoplate development. For this, hold the chromatoplate with the pair of tweezers into the current of hot air just above the hot plate for about 30 seconds. When lamivudine is combined with tenofovir, gently dry the spots by moving the chromatoplate back and forward through the air. Any use of the hot plate will lead to instant degradation of the thermolabile tenofovir disoproxil fumarate complex and should be avoided at all times.

IX. DEVELOPMENT

Pipette 11 ml of ethyl acetate, 5 ml of methanol and 4 ml of toluene 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.

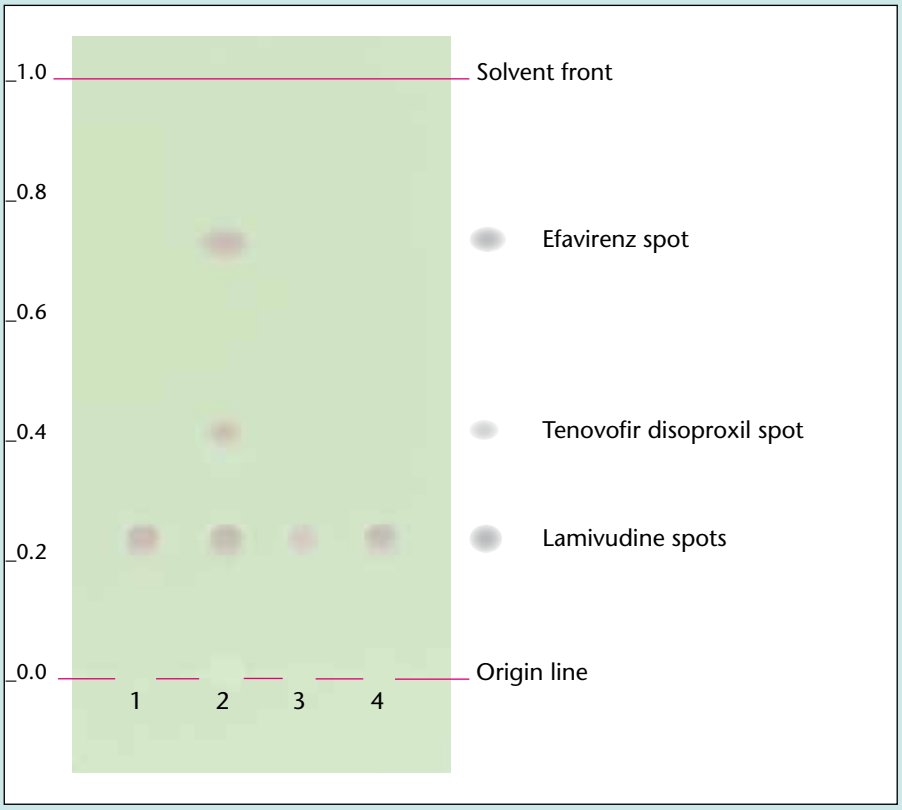
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

Run No.3:
A single drug product of poor quality with unacceptable low lamivudine content

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



X. DETECTION

Dry off all residual solvent and observe the chromatoplate under UV light of 254 nm using the battery-driven lamp supplied. Use this method of detection for both, identification and quantification purposes.

XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.23 indicates the presence of lamivudine in the test solution. If combined with other antiretroviral medicines, a spot with a relative retention factor of about 0.42 would further indicate the presence of tenofovir disoproxil, a spot at about 0.62 the presence of nevirapine or zidovudine and a spot at about 0.72 the presence of efavirenz. Additional strong spots generated by the test solution would point at other drugs or lamivudine degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot could also be due to a poor lamivudine content and no spot at all due to a complete lamivudine absence. Auxiliary agents incorporated in different finished products might cause some fainter spots emerging near or on the origin line.

XII. RESULTS & ACTIONS TO BE TAKEN

The lamivudine 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.

7.73 Moxifloxacin hydrochloride

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet or capsule usually contains 436.8 mg of moxifloxacin hydrochloride. This translates into the customarily expressed label claim of 400 mg of moxifloxacin free base. Confusing label claims of 400 mg of moxifloxacin hydrochloride are known to exist, the product then presenting about 367 mg of moxifloxacin free base only. Verify the total weight of tablets or the fill weight of capsules using the electronic pocket balance supplied. All quick release moxifloxacin tablet and capsule formulations must also pass the disintegration test. 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.

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 llanguage, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Moxifloxacin hydrochloride tablets and capsules are extracted with a known volume of methanol and then checked for identity and content by TLC with reference to a suitable 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) TLC dipping chamber (250-ml beaker)
20) Ninhydrin
21) Methanol
22) Water
23) Ammonia solution 25%
24) Acetic acid solution 96%
25) Reference agent, for example, tablets containing moxifloxacin hydrochloride in an amount equivalent to 400 mg of moxifloxacin per 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 an equivalent of 400 mg of moxifloxacin 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 50-ml laboratory glass bottle and wash down all residual solids with 40 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 10 mg of total drug per ml and be labelled as *'Moxifloxacin 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 *'Moxifloxacin Working Standard Solution 100%'*.

This higher working standard solution represents a drug product of good quality containing 100 % of moxifloxacin 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 *'Moxifloxacin Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of moxifloxacin 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 367 MG OF MOXIFLOXACIN FREE BASE 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 50-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 36.7 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.

400 MG OF MOXIFLOXACIN FREE BASE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 40 ml of methanol using a straight pipette and a 50-ml laboratory glass bottle as sample container. Continue to work as above.

All stock sample solutions produced should finally contain 10 mg of total moxifloxacin per ml and be labelled as *'Moxifloxacin 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 *'Moxifloxacin Working Sample Solution'*.

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

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

IX. DEVELOPMENT

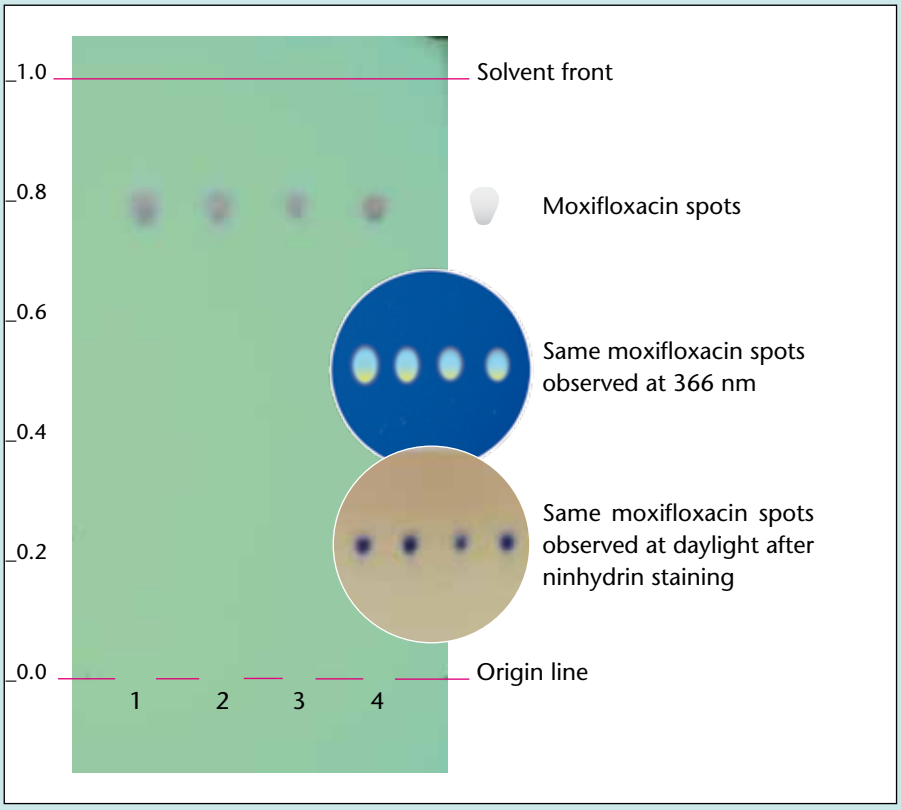
Pipette 12 ml of methanol, 4 ml of concentrated ammonia solution 25% and 4 ml of water into the jar being used as TLC developing chamber. Close the chamber and mix thoroughly. Line the chamber's wall with filter paper and wait for about 15 minutes thus ensuring saturation of the chamber with solvent 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 40 minutes. Remove the plate from the chamber, mark the solvent front and allow any excess solvent to evaporate on the hot plate supplied for about two minutes.

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 ninhydrin staining. For this, 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% using the 250-ml beaker supplied. Submerge the chromatoplate **upside down** 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. Wait a further minute, wipe off any residual liquid from the back of the plate and then continue to dry off all staining solution at full level of the hot plate supplied. During heating, all moxifloxacin spots are gradually becoming visible at daylight after about one minute. The ninhydrin staining process is illustrated on page 36 of this manual. 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.

CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM



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

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

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

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

XI. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.78 indicates the presence of moxifloxacin in the test solution. Additional strong spots generated by the test solution would point at other drugs or moxifloxacin 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 moxifloxacin content and no spot at all a complete moxifloxacin absence. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XII. OBSERVATIONS MADE AT 366 NM

When exposing the chromatoplate to UV light of 366 nm in a dark room, all moxifloxacin 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.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER STAINING WITH NINHYDRIN

When exposing the chromatoplate to ninhydrin and heat, all moxifloxacin spots already observed at 254 and 366 nm are now turning into a distinct blue colour. This will help in identification when moxifloxacin is directly compared to other fluoroquinolone antibiotics.

XIV. RESULTS & ACTIONS TO BE TAKEN

The moxifloxacin 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 precise drug content determination, 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.

7.86 Primaquine diphosphate

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet or capsule usually contains about 8.75, 13.15 or 26.3 mg of primaquine diphosphate. This translates into the customarily expressed label claims of 5, 7.5 or 15 mg of primaquine free base, respectively. The content of a capsule or the broken section of a tablet must be orange-brown. Verify the total weight of tablets or the fill weight of capsules using the electronic pocket balance supplied. All quick release primaquine tablet and capsule formulations must also pass the disintegration test. 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.

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, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Primaquine phosphate is extracted from tablets and capsules with a known volume of water and then checked for identity and content by TLC with reference to a suitable 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) Spatula
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) TLC dipping chamber (250-ml beaker)
20) Iodine chamber
21) Water
22) Methanol
23) Ninhydrin
24) Ethyl acetate
25) Ammonia solution 25%
26) Acetic acid solution 96%
27) Electronic pocket balance
28) Reference agent, for example, primaquine diphosphate neat substance from commercial sources

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires primaquine diphosphate as pure substance or appropriate raw material of high purity 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 primaquine diphosphate using a spatula. Carefully empty the aluminium foil over a 25-ml laboratory glass bottle and wash down all the powder obtained with 17.1 ml of water using a straight pipette. Write down each time the exact weighing result and adjust the amount of water for dissolution appropriately, for example using 16.5 ml of water when 0.29 g or 18.2 ml of water when 0.32 g of reference standard have been collected from the bulk container. Close the laboratory bottle and shake until all solids are dissolved. Provided that the purity of the reference standard is next to 100%, then the final solution obtained

should contain about 10 mg of total primaquine per free base per ml and be labelled as *'Primaquine 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. In addition, 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 10-ml vial and add 9 ml of water. Close and shake the vial. The solution obtained should contain 1 mg of total drug per ml and be labelled as *'Primaquine Working Standard Solution 100%'*.

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

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 11.5 ml of water. Close and shake the vial. The solution obtained should contain 0.8 mg of total drug per ml and be labelled as *'Primaquine Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of primaquine 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 5 MG OF PRIMAQUINE PER UNIT

Take one whole sample 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 sample capsules should be transferred directly into the bottle adding the cap and body shells last. For extraction, add 5 ml of water 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.

7.5 MG OF PRIMAQUINE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 7.5 ml of water using a straight pipette and a 25-ml laboratory glass bottle as sample container. Continue to work as above.

15 MG OF PRIMAQUINE PER UNIT

Take one whole sample tablet or capsule and extract the powder obtained with 15 ml of water using a straight pipette and a 25-ml laboratory glass bottle as sample container. Continue to work as above.

All stock sample solutions produced should finally contain 1 mg of total primaquine per ml and be labelled as *'Primaquine 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

Primaquine stock sample solutions require no further dilution. They already represent the final working concentration of 1 mg of total drug per ml. If prepared from a high quality product, the sample solution should match the concentration of primaquine 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 and emptying of the microcapillary pipettes might take some time when handling aqueous sample solutions. As traces of water are causing spot tailing, completely dry off all extraction solvent before chromatoplate development. For this, hold the chromatoplate with the pair of tweezers into the current of hot air just above the hot plate for about one minute.

IX. DEVELOPMENT

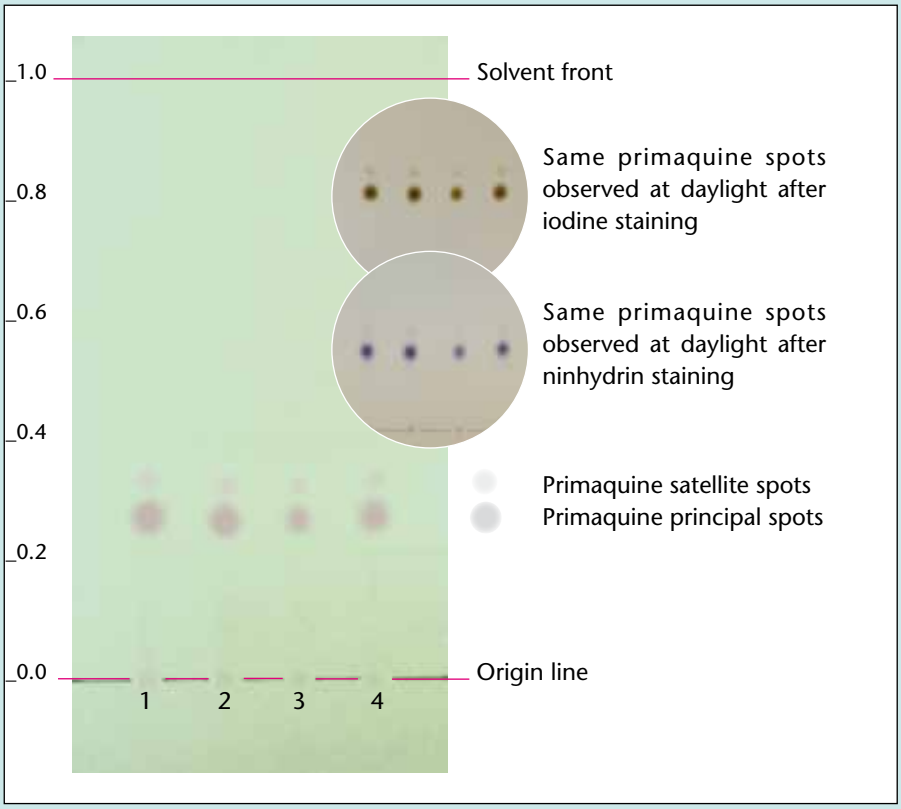
Pipette 20 ml of methanol, 5 ml of ethyl acetate and 0.5 ml of concentrated ammonia solution 25% into the jar being used as TLC developing chamber. Close the chamber and mix thoroughly. Line the chamber's wall with filter paper and wait for about 15 minutes thus ensuring saturation of the chamber with 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 for about two minutes on the hot plate supplied.

X. DETECTION

Dry off all residual solvent and expose the chromatoplate to UV-light of 254 nm using the battery-driven lamp supplied. Use this method of detection for both, primaquine identification and quantification purposes. Further verification of drug identity and content can be achieved when observing the plate at daylight after iodine staining.

Alternatively to iodine staining, spot colouration with ninhydrin is possible, too. For this, 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 chromatoplate 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 primaquine spots are gradually becoming visible at daylight after about one minute. The staining process is illustrated on page 36 of this manual. 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.

CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM



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

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

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

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

XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.27 combined with a smaller satellite spot just above the principal spot indicates the presence of primaquine in the test solution. Additional strong spots generated by the test solution would point at other drugs or even primaquine 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 primaquine content and no spot at all a complete primaquine absence.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all spots already observed at 254 nm are now turning greenish black. Primaquine performs strong here and the colour stays stable. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER NINHYDRIN STAINING

When exposing a second chromatoplate to ninhydrin and heat, then all primaquine spots previously observed at UV light of 254 nm are now turning lilac. This will facilitate further assay reading and interpretation.

XIV. RESULTS & ACTIONS TO BE TAKEN

The primaquine 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.

7.93 Quinine in most common salt forms for oral and parenteral use

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet or capsule usually contains 300 mg of quinine sulphate. Different dosage strengths of a 100, 200, 250 and 500 mg of quinine sulphate are known to exist. Various preparations may present quinine in different salt forms whereby the amount of a 100 mg of quinine free base equals about 121 mg of quinine sulphate, 122 mg of quinine dihydrochloride, 122 mg of quinine hydrochloride dihydrate and 170 mg of quinine bisulphate. The customarily expressed dosage strength on product labels usually refers to the quinine salt rather than the free base. Quinine injectable solutions should be clear and free of particulate matter. Verify the total weight of tablets or the fill weight of capsules using the electronic pocket balance supplied. All quick release quinine tablet and capsule formulations must also pass the disintegration test. 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.

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, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Quinine injection fluids are diluted and tablets or capsules are extracted with a known volume of methanol and the presence and content of quinine is then verified by TLC with reference to an appropriate secondary standard. Dilutions are the same for quinine sulphate or any form of hydrochloride salt as their quinine free base content is virtually the same each time. Adjustments must be made for bisulphate and other less common quinine salts. Those products are not part of 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) UV light of 366 nm
19) Iodine chamber
20) Methanol
21) Ammonia solution 25%
22) Electronic pocket balance
23) Reference agent, for example, quinine sulphate 300 mg tablets or, alternatively, quinine hemisulphate salt monohydrate as pure substance from commercial sources

III. PREPARATION OF THE STOCK STANDARD SOLUTION

If reference tablets containing 300 mg of quinine sulphate are supplied, then wrap one tablet into aluminium foil and crush it down to a fine powder using a pestle. Carefully empty the aluminium foil over a 50-ml laboratory glass bottle and wash down all residual solids with 30 ml of methanol using a straight pipette. Close the bottle and thoroughly 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 quinine sulphate or about 8.3 mg of quinine free base per ml and be labelled as *'Quinine Stock*

Standard Solution'. Freshly prepare this solution for each test. Continue to work with the hazy supernatant liquid.

If the reference tablets are replaced by quinine hemisulphate salt monohydrate of high purity close to 100%, then weigh in correctly about 0.3 g of pure substance using the electronic pocket balance supplied. Transfer all the powder into a 50-ml laboratory glass bottle and dissolve in 30 ml of methanol in order to obtain again a solution containing 10 mg of quinine sulphate per ml of extraction solvent. Shake thoroughly till all the powder is fully dissolved. Adjust the amount of methanol when the weighing result differs from the target weight. In order to overcome the balance's in-build dynamic inertia and to ensure correct readings, lift the weighing boat or tap the weighing pan with a pen or spatula each time after a few more milligrams have been added or removed. In order to ensure complete dissolution, observe the shaking and standing times as mentioned above. Label as above. Freshly prepare this solution for each test. The final solution obtained should be clear without any observable residual solids. Shake harder if this is not achieved first time.

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 quinine sulphate or about 1.0 mg of quinine free base per ml and be labelled as *'Quinine Working Standard Solution 100%'*.

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

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 1.0 mg of quinine sulphate or about 0.8 mg of quinine free base per ml and be labelled as *'Quinine Working Standard Solution 80%'*.

This lower working standard solution represents a drug product of poor quality containing just 80% of the amount of quinine 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 200 MG OF QUININE SULPHATE OR HYDROCHLORIDE PER TABLET OR CAPSULE

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 sample capsules should be transferred directly into the bottle adding the cap and body shells last. For extraction, add 20 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.

250 MG OF QUININE SULPHATE OR HYDROCHLORIDE PER TABLET OR CAPSULE

Take one whole sample tablet or capsule and extract the powder obtained with 25 ml of methanol using an appropriate straight pipette and a 50-ml laboratory glass bottle as sample container. Continue to work as above.

300 MG OF QUININE SULPHATE OR HYDROCHLORIDE PER TABLET OR CAPSULE

Take one whole sample tablet or capsule and extract the powder obtained with 30 ml of methanol using an appropriate straight pipette and a 50-ml laboratory glass bottle as sample container. Continue to work as above.

500 MG OF QUININE SULPHATE OR HYDROCHLORIDE PER TABLET OR CAPSULE

Take one whole sample tablet or capsule and extract the powder obtained with 50 ml of methanol using an appropriate straight pipette and a 100-ml laboratory glass bottle as sample container. Continue to work as above.

250 MG OF QUININE HYDROCHLORIDE PER ML OF FLUID

Dilute 1 ml of the injection fluid with 24 ml of methanol using appropriate straight pipettes and a 25-ml laboratory glass bottle.

300 MG OF QUININE HYDROCHLORIDE PER ML OF FLUID

Dilute 1 ml of the injection fluid with 29 ml of methanol using appropriate straight pipettes and a 50-ml laboratory glass bottle.

All stock sample solutions produced should finally contain 10 mg of quinine sulphate or 10 mg of quinine hydrochloride per ml being each time equivalent to about 8.3 mg of quinine free base. Freshly prepare these solutions for each test and label them as *'Quinine Stock Sample Solution'*. 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 *'Quinine Working Sample Solution'*.

The expected concentration of quinine sulphate or hydrochloride in this working sample solution is 1.25 mg per ml and should match the quinine salt and free base concentration 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.

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

IX. DEVELOPMENT

Pipette 20 ml of methanol and 0.5 ml of concentrated ammonia solution 25% 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. For this, place the developed chromatoplate for about one minute directly on the heating plate supplied.

X. DETECTION

Dry off all residual solvent and observe the chromatoplate under UV light of 254 nm using the battery-driven lamp supplied. Use this method of detection for both, identification and quantification purposes. Further verification of drug identity and content can be achieved when observing the plate a) under UV light of 366 nm in a dark room and b) at daylight after iodine staining. Complete staining may take up to one minute.

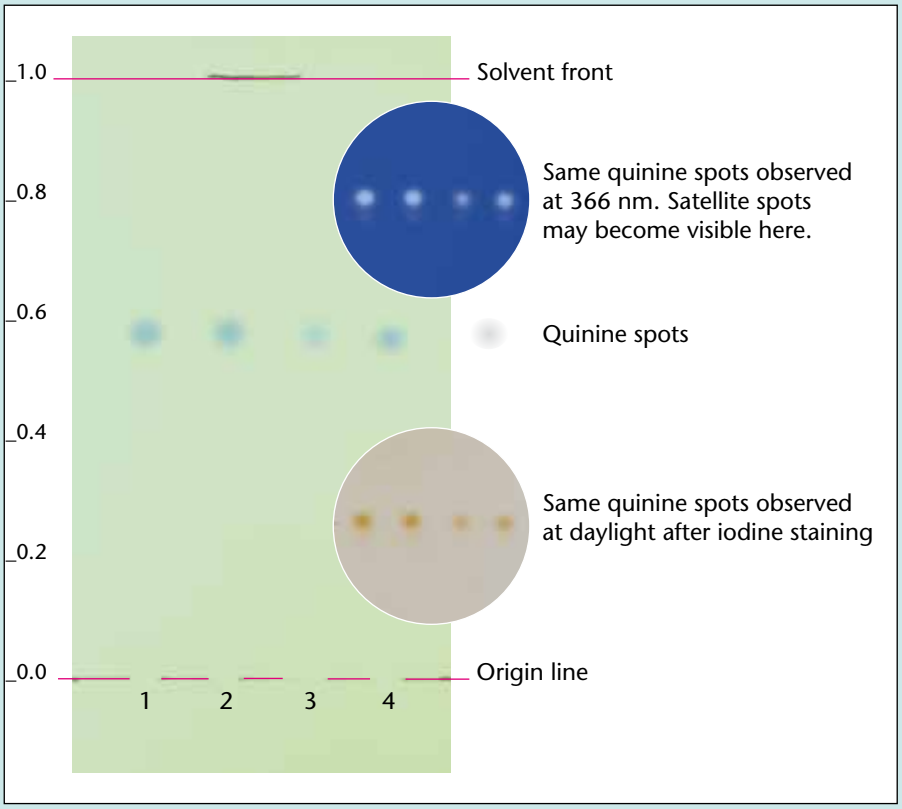
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

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

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



XI. OBSERVATIONS MADE AT 254 NM

A strong blue spot at a travel distance of about 0.59 indicates the presence of quinine in the test solution. Additional strong spots generated by the test solution would point at other drugs or quinine 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 quinine content and no spot at all a complete quinine absence. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line.

XII. OBSERVATIONS MADE AT 366 NM

On exposer to 366 nm in a dark room, the blue fluorescence observed for the quinine spots at 254 nm will now turn into an intense white fluorescence. In addition, under ideal detection conditions, a minor satellite spot probably arriving from dihydroquinine will now become visible just below each quinine spot. The latter observation will further emphasise the existence of quinine in the test solution.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all quinine spots already observed at 254 and 366 nm are now turning orange-brown. Still observe the plate when iodine evaporates. 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. RESULTS & ACTIONS TO BE TAKEN

The quinine 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 the readings with a digital camera turning off the flash first.

8 List of Minilab Inventory Items

The first, second and third list of inventory items below reflect the kind and quantities of equipment, chemicals and reference agents supplied when a regular GPHF-Minilab™, is ordered. The fourth list shows optional reference agents to be used only for expert projects or where specific regional preferences in drug therapy become applicable, for example, antiviral and antiretroviral reference agents for avian influenza and HIV/AIDS programmes. Optional reference agents include also items which are very expensive, require storage below ambient temperature or are difficult to obtain. Minilabs can also be tailored to cater for specific needs in Malaria and TB endemic countries. The Global Pharma Health Fund's logistic partner Technologie Transfer Marburg maintains an appropriate stock of inventory items ready for global shipment. There, the management of orders will be facilitated and errors be avoided when using the catalogue numbers shown in the table below. There is no minimum order value. The contact details for the procurement of replacement items or full Minilab sets are as follows:

Technologie Transfer Marburg
Industriestrasse 10
35091 Cölbe, Germany
ttm@ttm-germany.de
phone +49-6421-8737-30
fax +49-6421-8737-37

1. GPHF-Minilab Lab Ware		
Order No.	*Item*	*Qty*
	Teaching, Training, Guidance	
AG040069	Minilab Manual 2022 with test protocols for 107 active pharmaceutical ingredients	1
	Health & Safety	
AG020046	Safety spectacles incl. protection against UV light	1
	Physical Inspection	
AG020045	Vernier calliper, measure up to 190 mm	1
AG020025	Graduated ruler, 20 cm in length	1
AG020057	Electronic pocket balance incl. instruction booklet, precision 0.01 g, max. weight 60 g	1
AG020101	Calibration weight, 50 g	1
AG020102	Case for calibration weight	1
	Disintegration Testing	
AG020021	Laboratory glass bottle with closure, 125-ml filling capacity	5
AG020049	Thermometer, alcohol centigrade, 0 - 100 °C	1
AG020018	Pre-set timer, 60 min.	1
	Preparation of Stock and Working Solutions	
AG020008	Spatula, double ended, stainless steel, 210 mm in length	1
AG020038	Pestle, length 150 mm, head diameter 36 mm	1
AG020044	Pair of scissors, length 250 mm	1
AG020047	Cutting blade	1
AG020001	Aluminium foil with a thickness of 30 µm, 45 x 1000 cm	1
AG020050	Funnel made of polypropylene, 65/9 mm	3
AG020020	Laboratory glass bottle with closure, 10-ml filling capacity (vial)	20
AG020022	Laboratory glass bottle with closure, 25-ml filling capacity	6
AG020024	Laboratory glass bottle with closure, 50-ml filling capacity	15
**	Laboratory glass bottle with closure, 125-ml filling capacity (see disintegration testing)	
AG020026	Straight pipette, 1-ml filling capacity, graduated 0.01 ml	10
AG020028	Straight pipette, 2-ml filling capacity, graduated 0.01 ml	5
AG020030	Straight pipette, 5-ml filling capacity, graduated 0.1 ml	10
AG020027	Straight pipette, 10-ml filling capacity, graduated 0.1 ml	10
AG020029	Straight pipette, 25-ml filling capacity, graduated 0.1 ml	5
AG020037	Pipette filler/aid (Peleus Ball)	1
AG020041	Test tube rack accommodating straight pipettes etc., 40 holes, disassembled	1
AG020012	Universal pH indicator test paper	1
	Table continued on the next page	

Order No.	*Item*	*Qty*
	Labelling of Stock and Working Solutions	
AG020009	Label tape	1
AG020019	Marker pen, black, water-resistant	2
	Spotting of Control and Sample Solutions	
AG020005	Pencil, soft grade	1
AG020006	Pencil sharpener	1
**	Graduated ruler, 20 cm in length (see visual inspection above)	
AG020061	Glass micro-capillaries capable of delivering a known volume of 2 µl, pack of 10 x 100	1
AG020011	Hot plate (travel iron put upside down)	1
AG020054	Adaptor plug for the worldwide use of electrical appliances	1
	Preparation of the Mobile Phase	
AG020033	Transfer pipette made of polypropylene, 3-ml filling capacity, graduated 0.5 ml	10
**	Set of straight pipettes (see preparation of stock and working solutions above)	
	Thin Layer Chromatographic (TLC) Plates	
AG020007	Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, 5 x 10 cm, pack of 50	8
	TLC Plate Development	
AG020023	TLC developing chamber/laboratory glass bottle with closure, 500-ml filling capacity	1
AG020043	Circular filter paper, 150 mm in diameter, pack of 100	3
**	Pre-set timer (see disintegration testing above)	
	Detection of Spots	
AG020055	UV-lamp supplying light of 254 nm plus one pack of 4 batteries	1
AG020056	UV-lamp supplying light of 366 nm (black light) plus one pack of 4 batteries	1
AG020060	Replacement batteries for UV-lamps, pack of 4	2
**	Hot plate (see spotting of working standard and sample solution above)	
AG020059	TLC dipping chamber/250-ml beaker made of polypropylene, 65 mm open diameter	1
AG020023	Iodine staining chamber/laboratory glass bottle with closure, 500-ml filling capacity	1
AG020035	Pair of tweezers, stainless steel, length 130 mm	1
AG020010	Glass rod for stirring, ø 5mm, 200 mm in length	2
	Packaging	
AG020014	Heavy duty flight case	1
AG020100	Set of inner workings for the heavy duty flight case	1
AG020058	Padlock	2

2. GPHF-Minilab Solvents and Chemicals

Order No.	*Item*	*Qty*
	Chemicals of analytical reagent grade of commerce	
AG010001	Acetone, 1000 ml	4
AG010034	Ammonia solution 25 %, 1000 ml	1
AG010037	n-Butanol, 1000 ml (substitutable by isobutanol, but the relevant Rf-values will be lower)	1
AG010007	Ethyl acetate, 1000 ml	3
AG010006	Acetic acid solution 96%, 1000 ml	2
AG010035	Hydrochloric acid solution 32%, 1000 ml	1
AG010010	Iodine, 100 g	1
AG010013	Magnesium chloride hexahydrate, 250 g	1
AG010014	Methanol (Methyl alcohol), 1000 ml	15
AG010039	Ninhydrin, 50 g	1
AG010036	Sodium chloride, 500 g	1
AG010018	Sulphuric acid solution 96%, 1000 ml	1
AG010020	Toluene, 1000 ml	1

3. GPHF-Minilab Reference Agents (regular supply)		
Order No.	*Item*	*Qty*
	Reference Agents on Antibacterial Medicines	
AG030004	Amoxicillin 500 mg - tube of 20	1
AG030168	Ampicillin trihydrate - pack of 5 g	1
AG030086	Azithromycin 250 mg - tube of 20	1
AG030113	Benzylpenicillin potassium - pack of 25 g (serves all three: benzylpenicillin sodium/procaine/benzathine)	1
AG030182	Cefalexin 500 mg - tube of 20	1
AG030098	Cefazolin sodium - pack of 5 g	1
AG030120	Cefixime 400 mg - tube of 10 (supply pending)	
AG030134	Cefotaxime sodium - pack of 5 g	1
AG030123	Cefpodoxime 100 mg (as cefpodoxime proxetil) - tube of 10	1
AG030099	Ceftriaxone disodium hemiheptahydrate - pack of 5 g	1
AG030066	Cefuroxime 250 mg (as cefuroxime axetil) - tube of 10	1
AG030135	Chloramphenicol - pack of 25 g	1
AG030044	Ciprofloxacin 250 mg (as hydrochloride) - tube of 20	1
AG030091	Clarithromycin 250 mg - tube of 20	1
AG030092	Clavulanic acid/Amoxicillin 125/500 mg - tube of 10	1
AG030137	Clindamycin 300 mg (as hydrochloride) - tube of 20	1
AG030138	Cloxacillin sodium monohydrate - pack of 5 g	1
AG030112	Doxycycline 100 mg (as hyclate) - tube of 20	1
AG030174	Erythromycin 250 mg (as erythromycin stearate) - tube of 20 (supply pending)	
AG030114	Gentamicin sulphate - pack of 5 g	1
AG030067	Levofloxacin 250 mg - tube of 10	1
AG030026	Metronidazole 250 mg - tube of 20	1
AG030093	Ofloxacin 200 mg - tube of 10	1
AG030155	Phenoxymethylpenicillin 590 mg / 1,000,000 IU (as potassium) - tube of 20	1
AG030012	Sulfamethoxazole/Trimethoprim (Cotrimoxazole) 100/20 mg - tube of 20	1
AG030176	Tetracycline hydrochloride - pack of 25 g	1
	Reference Agents on Antimalarial Medicines	
AG030162	Amodiaquine dihydrochloride dihydrate - pack of 5 g	1
AG030157	Artemether - pack of 5 g	1
AG030158	Artesunate - pack of 5 g	1
AG030175	Atovaquone/Proguanil hydrochloride 62.5/25 mg - tube of 20	1
AG030171	Chloroquine diphosphate - pack of 25 g	1
AG030159	Dihydroartemisinin - pack of 5 g	1
AG030160	Lumefantrine - pack of 5 g	1
AG030087	Piperaquine tetraphosphate 320 mg - tube of 20 (supply pending)	
AG030139	Primaquine diphosphate - pack of 5 g	1
**	Proguanil hydrochloride - use atovaquone/proguanil HCl 62.5/25 mg co-formulated tablets from above	1
AG030140	Pyrimethamine 25 mg - tube of 20	1
AG030172	Quinine hemisulphate salt monohydrate - pack of 5 g	1
AG030167	Sulfadoxine - pack of 5 g	1
	Reference Agents on Antimycobaterial Medicines	
AG030088	Cycloserine 250 mg - tube of 10	1
AG030127	Dapsone 50 mg - tube of 20	1
AG030015	Ethambutol hydrochloride 400 mg - tube of 20	1
AG030089	Ethionamide 250 mg - tube of 10	1
	Table continued on the next page	

Order No.	*Item, cont'd*	*Qty*
AG030020	Isoniazid 100 mg - tube of 20	1
AG030094	Kanamycin monosulphate - pack of 5 g	1
AG030067	Levofloxacin 250 mg - tube of 10	1
AG030068	Moxifloxacin 400 mg - tube of 10	1
AG030093	Ofloxacin 200 mg - tube of 10	1
AG030096	P-Aminosalicylic acid (PAS, 4-amino-2-hydroxybenzoic acid) - pack of 5 g	1
AG030070	Protionamide 250 mg - tube of 10	1
AG030033	Pyrazinamide 500 mg - tube of 20	1
AG030035	Rifampicin 150 mg - tube of 20	1
AG030097	Streptomycin hemitrisulphate - pack of 5 g	1
	Reference Agents on Anthelminthic Medicines	
AG030166	Albendazole - pack of 5 g	1
AG030023	Mebendazole 100 mg - tube of 20	1
AG030045	Praziquantel 600 mg - tube of 20	1
	Reference Agents on Antifungal Medicines	
AG030142	Fluconazole 50 mg - tube of 10	1
AG030165	Griseofulvin - pack of 5 g	1
	Reference Agents on Cardiovascular Medicines	
AG030125	Amlodipine 5 mg - tube of 20	1
AG030109	Atenolol 50 mg - tube of 20	1
AG030110	Bisoprolol 5 mg (as fumarate) - tube of 40	1
AG030111	Captopril 50 mg - tube of 20	1
AG030016	Furosemide 40 mg - tube of 20	1
AG030107	Hydrochlorothiazide 25 mg - tube of 20	1
AG030183	Irbesartan 150 mg - tube of 20	1
AG030143	Lisinopril 10 mg - tube of 20	1
AG030184	Losartan potassium 50 mg - tube of 20	1
AG030185	Methyldopa 125 mg - tube of 20	1
AG030144	Nifedipine 20 mg - tube of 20	1
AG030145	Simvastatin 20 mg - tube of 20	1
AG030186	Telmisartan 20 mg - tube of 20	1
AG030187	Valsartan 80 mg - tube of 20	1
	Reference Agents on Analgesic Medicines	
AG030128	Acetylsalicylic acid 100 mg - tube of 20	1
AG030131	Diclofenac sodium 25 mg - tube of 20	1
AG030154	Mefenamic acid - pack of 25 g	1
AG030133	Naproxen 250 mg - tube of 20	1
AG030029	Paracetamol 500 mg - tube of 20	1
	Reference Agents on Antiallergic and Antiasthmatic Medicines	
AG030169	Aminophylline being served by theophylline - pack of 25 g	1
AG030129	Cetirizine dihydrochloride 10 mg - tube of 20	1
AG030136	Chlorphenamine maleate - pack of 25 g	1
AG030178	Dexamethasone 8 mg - tube of 20	1
AG030031	Prednisolone 5 mg - tube of 100	1
AG030146	Salbutamol hemisulphate - pack of 5 g	1
	Reference Agents on Endocrine Medicines	
AG030108	Clomifene citrate 50 mg - tube of 20	1

AG030017	Glibenclamide 5 mg - tube of 40	1
AG030102	Metformin hydrochloride 500 mg - tube of 20	1
	Reference Agents on Gastrointestinal Medicines	
AG030148	Metoclopramide hydrochloride 10 mg - tube of 20	1
AG030149	Omeprazole 20 mg - tube of 20	1
AG030173	Ranitidine hydrochloride - pack of 5 g	1

4. GPHF-Minilab Reference Agents (optional supply)		
Order No.	*Item*	
	Reference Agents on Antibacterial Medicines	
AG030126	Chlorhexidine (as diacetate) - pack of 5 g	
	Reference Agents on Antimalarial Medicines	
AG030071	Halofantrine hydrochloride 250 mg - tube of 10 (supply pending)	
AG030177	Mefloquine hydrochloride - pack of 1 g	
AG030090	Pyronaridine tetraphosphate/Artesunate 180/60 mg - tube of 20 (supply pending)	
AG030151	Sulfamethoxypyrazine - pack of 5 g (supply pending)	
	Reference Agents on Antimycobacterial Medicines	
AG030100	Amikacin disulphate - pack of 5 g	
AG030101	Capreomycin disulphate - pack of 5 g	
	Reference Agents on Anthelminthic Medicines	
AG030163	Praziquantel - pack of 5 g	
	Reference Agents on Anti(retro)viral Medicines	
AG030152	Aciclovir 200 mg - tube of 10	
AG030048	Didanosine 200 mg - tube of 10 (supply pending)	
AG030116	Efavirenz 50 mg - tube of 10	
AG030161	Efavirenz 600 mg - tube of 10	
AG030049	Indinavir 200 mg - tube of 10	
AG030050	Lamivudine 150 mg - tube of 10	
AG030051	Nevirapine 200 mg - tube of 10	
AG030041	Oseltamivir 75 mg - tube of 10	
AG030115	Ritonavir 100 mg - tube of 10	
AG030053	Stavudine 40 mg - tube of 10	
AG030103	Zidovudine 100 mg - tube of 10	
	Reference Agents on Antiallergic and Antiasthmatic Medicines	
AG030179	Dexamethasone phosphate 8 mg - reference solution in 10 x 2-ml ampoules à 4 mg of dexamethasone 21-phosphate per ml	

- Detecting falsified and substandard medicines in low and middle-income countries
- Protecting consumers and medicines supply chains
- Boosting medicines testing capacities for priority medicines
- Assisting in post-marketing medicines quality monitoring
- Complementing the work of existing medicines control laboratories

The GPHF-Minilab™
is a unique miniature laboratory which comes with
affordable test methods for a rapid and easy detection of
falsified and substandard medicines as entry-level technology for
resource limited health settings in low- and middle-income countries.

In more than twenty years of project work, the GPHF-Minilab™ has proven
its suitability in up to a 100 countries.

A comprehensive review of the Minilab's general methods and operations and
its test protocols drawn from the main manuals issued 1998, 2008 and 2020
including their many extensions issued each year.

Topped with testing protocols for more active pharmaceutical ingredients
listed as priority medicines for the treatment of communicable and
non-communicable diseases, this new manual now provides
simple testing procedures for 107 active pharmaceutical
ingredients for rapid and economical drug quality
verification of a wide range of finished
pharmaceutical products.

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