A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Supplement 2025 on palliative medicines in self-care incl. video tutorials

Physical Testing & Thin-Layer Chromatography

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For better understanding, this supplement contains quick response (QR) codes to allow the access to tutorial videos. See example below.

Health & Safety

Important Notice

The chemicals travelling alongside the GPHF-Minilab™ as well as pharmaceuticals to be tested may contain hazardous substances. Hence, users of the Minilab and bystanders should closely follow all instructions given in this and the main manual in order to avoid potential health risks resulting from accidental contact with these chemical and pharmaceutical substances.

Care must be exercised in the handling of chemicals and pharmaceuticals in order to avoid generating excessive dust or vapours in the atmosphere. Extraction should be used at points of activity that, in more austere circumstances, might be replaced by simple but sufficient air ventilation.

Symptoms such as drowsiness, respiratory problems, nausea or skin rash must be reported to the supervisor especially after accidental spillage of large amounts of organic solvents.

In the event of accidental spillage or splashing of liquids affecting skin or eyes, wash with copious amounts of water, report to the supervisor and if necessary, to the local surgery for further attention. Use protective clothes and safety spectacles when handling aggressive test solutions, for example strong acids and caustic solutions.

Use protective clothing, for example an apron and safety spectacles, prior to starting any work on medicines quality testing. Wash hands and face thoroughly after work.

7.120 Diphenhydramine hydrochloride

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During visual inspection, search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 25 or 50 mg of diphenhydramine hydrochloride. Other dosage strengths and co-formulations with paracetamol are known to exist. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release diphenhydramine tablet and capsule formulations have to pass the disintegration test as described at the beginning of the main manual. They should disintegrate in water at 37 °C in less than 30 minutes. It is a major defect if an instant release formulation does not pass this test.

II. RESULTS & ACTIONS TO BE TAKEN

Medicinal products from unusually cheap sources, medicinal products with missing or incorrect accompanying documents and medicinal products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels in a foreign language or medicinal products held under poor storage conditions should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Diphenhydramine hydrochloride is extracted from tablets or capsules with a known volume of methanol and is subsequently checked for identity and content by thin-layer chromatography (TLC) in comparison with a suitable secondary standard.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Funnel
4) Spatula
5) Label tape
6) Marker pen
7) Pencil and ruler
8) 10-ml vials
9) Set of graduated pipettes (1 to 25 ml)
10) Set of laboratory glass bottles (25 to 100 ml)
11) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
12) Glass microcapillaries (2-µl filling capacity)
13) TLC developing chamber (500-ml jar)
14) Hot plate
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) Iodine chamber
20) Plastic beaker (250 ml)
21) Ninhydrin
22) Methanol
23) Toluene
24) Ethyl acetate
25) Ammonia solution 25%
26) Sulfuric acid solution 96%
27) Reference agent, for example, diphenhydramine hydrochloride 50 mg tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic medicinal product for reference purposes, for example, tablets containing 50 mg of diphenhydramine 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 25-ml laboratory glass bottle and wash down all residual solids with 10 ml of methanol using a graduated pipette. Close the lab bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes

until undissolved residues settle below the supernatant liquid. The solution obtained should contain 5 mg of total diphenhydramine hydrochloride per ml and be labelled as *'Diphenhydramine 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 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 2.5 mg of total diphenhydramine hydrochloride per ml and be labelled as *'Diphenhydramine Working Standard Solution 100%'*.

This higher working standard solution represents a drug product of good quality containing 100 % of diphenhydramine hydrochloride. Further information on pipetting can be found in the video via the QR code provided here.

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 using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 2 mg of total diphenhydramine hydrochloride per ml and be labelled as *'Diphenhydramine Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of diphenhydramine as stated on the product's label. In the current investigation, this level of diphenhydramine represents the lower acceptable limit for a given product. Pharmacopoeial limits do not apply in our context.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 25 MG OF DIPHENHYDRAMINE HCL PER UNIT

Take a whole tablet or capsule from a suitable medicinal product sampled in the field. As usual, tablets are wrapped up into aluminium foil and crushed down to a fine powder using a pestle. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from sample capsules should be placed directly in the bottle adding the empty cap and body shells last. For extraction, add 5 ml of methanol using a suitable graduated pipette. Then, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid.

50 MG OF DIPHENHYDRAMINE HCL PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 25-ml laboratory glass bottle, add 10 ml of methanol with a suitable graduated pipette and extract the diphenhydramine hydrochloride. Continue working as described above.

All stock sample solutions produced should finally contain 5 mg of total diphenhydramine hydrochloride per ml and be labelled as *'Diphenhydramine Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue working with the clear or hazy supernatant liquids. Further information on sample preparation can be found in the video via the QR code provided here.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Pipette 2 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 *'Diphenhydramine Working Sample Solution'*. The expected concentration of diphenhydramine hydrochloride in this working sample solution is 2.5 mg per ml and should match the concentration of diphenhydramine hydrochloride 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 or different drug concentrations or combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

Gently dry the spots. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about 10 seconds. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 10 ml of methanol, 5 ml of ethyl acetate, 5 ml of toluene and 1 ml of ammonia solution 25% to the jar serving 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 TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by gentle drying. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about two minutes. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second. Further information on the drying process can be found in the video via the QR code provided here.

X. DETECTION

After drying off all solvent residues, view the chromatographic plate under UV light at 254 nm with the battery driven lamp provided. For further identification and quantification of diphenhydramine, stain the fresh chromatographic plate with iodine and observe the plate at daylight and again under UV light of 254 nm. After evaporation of the iodine stain the plate with methanolic sulphuric acid solution and dry in the heat. Using a second freshly developed chromatoplate, sulphuric acid staining can be replaced by staining with ninhydrin.

For sulphuric acid staining, fill the 250 ml plastic beaker provided with 190 ml of methanol followed by 10 ml of 96% sulphuric acid, then mix thoroughly. After the mixture cools, immerse the chromatography plate top-side first, then remove it immediately. Let excess liquid drain onto a paper towel, wipe the back of the plate, and dry it on the hot plate at maximum heat for 30 to 60 seconds. After drying, view the stained plate in daylight.

For ninhydrin staining, dissolve 3 g of ninhydrin in 150 ml of methanol and 30 ml of 96% acetic acid in the 250 ml beaker provided. Dip the chromatography plate top-side first using tweezers, then remove it immediately and let excess liquid drip onto a paper towel. After one minute, wipe the back of the plate and dry it on the hot plate at full heat. Diphenhydramine spots will appear in daylight almost instantly. Refer to page 36 of the main manual for ninhydrin staining. Note: Ninhydrin can stain skin, but the purple stains will fade in 1 - 2 days and are not harmful. Further information on the detection processes can be found in the video via the QR code provided here.

XI. OBSERVATIONS MADE AT 254 NM AFTER IODINE STAINING

Weak diphenhydramine spots previously observed at a travel distance of about 0.77 without iodine staining are now becoming very strong. Additional strong spots from the test solution suggest the presence of other drugs or diphenhydramine degrada-

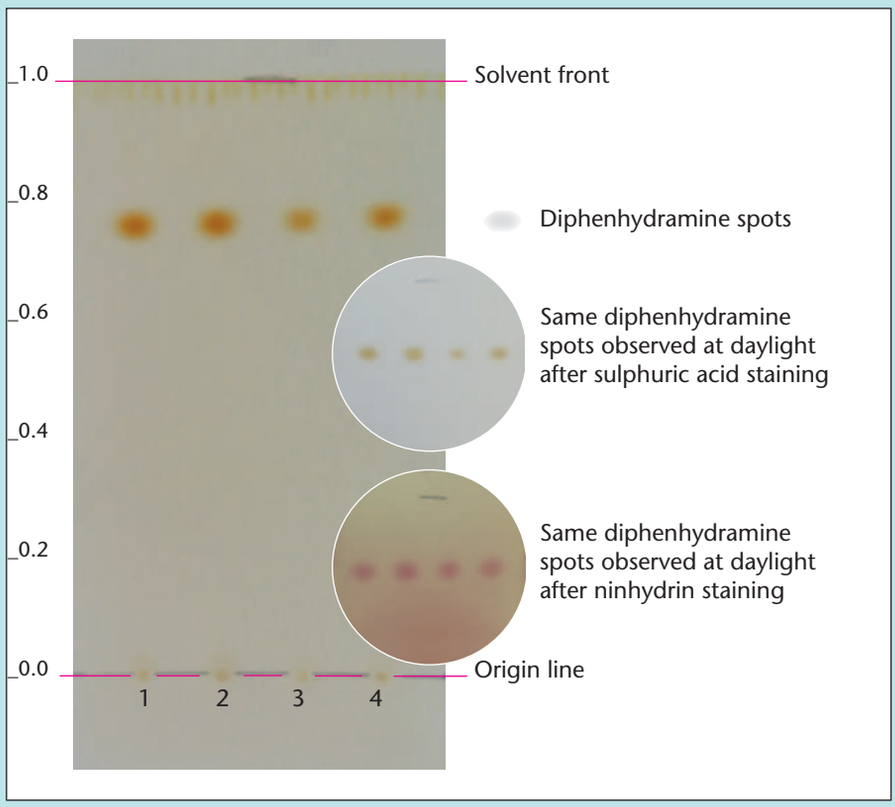
CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

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

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

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



tion, especially if the principal spot is smaller. A smaller principal spot may also indicate low diphenhydramine content, while no spot suggests its complete absence. Auxiliary agents in different finished products might produce faint spots, either near the solvent front or at the origin line.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

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

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER SULPHURIC ACID STAINING

When all the iodine has evaporated and the chromatography plate is stained with sulphuric acid, all the diphenhydramine spots turn yellowish-grey and become visible in daylight. Semi-quantitative readings are very good here.

XIV. OBSERVATIONS MADE AT DAYLIGHT AFTER NINHYDRIN STAINING

When all the iodine has evaporated and the chromatography plate is stained with ninhydrin, all the diphenhydramine spots turn pinkish-red and become visible in daylight. Semi-quantitative readings might become a challenge here.

XV. RESULTS & ACTIONS TO BE TAKEN

The diphenhydramine 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.

7.121 Hyoscine/Scopolamine butylbromide incl. solutions for injection

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

Hyoscine butylbromide is also known as scopolamine butylbromide. Both names are interchangeable. During visual inspection, search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 10 mg of hyoscine butylbromide and each injectable product usually 20 mg of hyoscine butylbromide per ml of solution. Co-formulations with paracetamol are known to exist. Verify the total weight of tablets using the electronic pocket balance provided. All quick release hyoscine tablet formulations have to pass the disintegration test as described at the beginning of the main manual. They should disintegrate in water at 37 °C in less than 30 minutes. It is a major defect if an instant release formulation does not pass this test. Concerning injectable solutions, it is also a major defect if a clear and colourless solution without haze and particulate matter is not presented. Sugar-coated tablets are excepted.

II. RESULTS & ACTIONS TO BE TAKEN

Medicinal products from unusually cheap sources, medicinal products with missing or incorrect accompanying documents and medicinal products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels in a foreign language or medicinal products held under poor storage conditions should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Hyoscine butylbromide is extracted from tablets and injectable solutions are diluted with a known volume of methanol and then the dissolved butylscopolamine cation is checked for identity and content by thin layer chromatography (TLC) in comparison to a suitable secondary standard.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Funnel
4) Spatula
5) Label tape
6) Marker pen
7) Pencil and ruler
8) 10-ml vials
9) Set of graduated pipettes (1 to 25 ml)
10) Set of laboratory glass bottles (25 to 100 ml)
11) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
12) Glass microcapillaries (2-µl filling capacity)
13) TLC developing chamber (500-ml jar)
14) Hot plate
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) Iodine chamber
20) Acetone
21) Methanol
22) Ethyl acetate
23) Sodium chloride salt
24) Ammonia solution 25%
25) Reference agent, for example, hyoscine butylbromide 10 mg tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic medicinal product for reference purposes, for example, tablets containing 10 mg of hyoscine butylbromide. Wrap up two reference tablets into aluminium foil and crush them down to a fine powder using a pestle. Carefully empty the aluminium foil over a 25ml laboratory glass bottle and wash down all residual solids with 5 ml of methanol using a graduated pipette. Close the lab bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid. The solution obtained should contain 4 mg of total hyoscine butylbromide per ml and be labelled as *'Hyoscine Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

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

The stock standard solution requires no further dilution. It already represents the final working concentration of 4 mg of total hyoscine butylbromide per ml. For more convenient handling, some of the supernatant liquid may be transferred to a 10-ml vial.

This higher working standard solution represents a medicinal product of good quality containing 100% of hyoscine butylbromide.

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 0.5 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 3.2 mg of total hyoscine butylbromide per ml and be labelled as *'Hyoscine Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of hyoscine butylbromide as stated on the product's label. In the current investigation, this level of hyoscine butylbromide represents the lower acceptable limit for a given product. Pharmacopoeial limits do not apply in our context.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM TABLETS CLAIMING TO CONTAIN 10 MG OF HYOSCINE BUTYLBROMIDE PER UNIT

Take one whole tablet from a suitable medicinal product sampled in the field. As usual, the tablet is wrapped up into aluminium foil and crushed down to a fine powder using a pestle. Transfer all the powder obtained into a 10-ml laboratory glass bottle. For extraction, add 2.5 ml of methanol using a suitable graduated pipette. Then, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid.

FROM INJECTABLE SOLUTIONS CLAIMING TO CONTAIN 20 MG OF HYOSCINE BUTYLBROMIDE PER ML

Open an ampoule and decant its contents into a 10-ml laboratory glass vial. Using a graduated pipette, transfer 0.5 ml of the fluid obtained into a second vial and add 2.0 ml of methanol. This will be the stock sample solution which should be clear and colourless.

All stock sample solutions produced should finally contain 4 mg of total hyoscine butylbromide per ml and be labelled as *'Hyoscine Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue working with the clear or hazy supernatant liquids.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

The stock sample solutions require no further dilution. They already represent the final working concentration of 4 mg of hyoscine butylbromide per ml. If prepared from a high quality product, the sample solutions should match the concentration of hyoscine butylbromide of the higher working standard solution produced above. For more convenient handling, some of the supernatant liquid may be transferred to a 10-ml vial.

VIII. SPOTTING

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

Up to five spots can be placed on a plate. Check the uniformity of all spots using UV light of 254 nm. All spots should be circular in shape and equally spaced across the origin line. Although their intensities might differ, their diameters never should. Different intensities are due to residual amounts of excipients or different drug concentrations or combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

Gently dry the spots. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about 15 seconds. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 7 ml of methanol, 7 ml of ethyl acetate, 7 ml of acetone and 3.5 ml of ammonia solution 25% to the jar serving as TLC developing chamber. Finally add 1 g of sodium chloride salt, close the chamber and mix thoroughly. Most of the salt remains insoluble. 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 18 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by gentle drying. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about two minutes. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

X. DETECTION

After drying off all solvent residues, the chromatography plate is viewed under UV light at 254 nm using the battery-powered lamp supplied. To identify and quantify hyoscine butyl bromide, colour the fresh chromatography plate with iodine and observe it first in daylight and then again under UV light at 254 nm.

XI. OBSERVATIONS MADE AT 254 NM

If present, hyoscine butyl bromide is barely detectable. If not combined with other medicines, no other major spots should be visible when analysing the test solution.

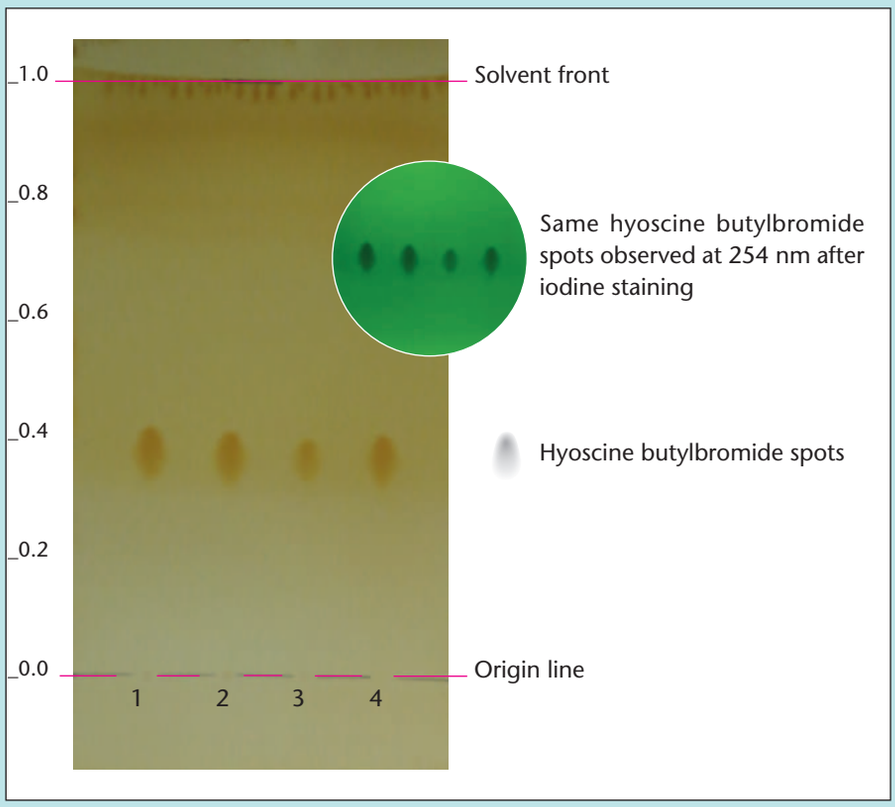
CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

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

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

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



XII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all spots representing hyoscine butylbromide are now turning yellowish-brown and become visible at a travel distance of about 0.40. 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 80 and 100 percent, respectively.

XIII. OBSERVATIONS MADE AT 254 NM AFTER IODINE STAINING

All hyoscine butylbromide spots previously observed at a travel distance of about 0.40 during iodine staining are now becoming even stronger. Additional strong spots from the test solution suggest the presence of other drugs or hyoscine butylbromide degradation, especially if the principal spot is smaller. A smaller principal spot may also indicate low hyoscine butylbromide content, while no spot suggests its complete absence. Auxiliary agents in different finished products might produce faint spots, either near the solvent front or at the origin line. The sugar from sugar-coated tablets is visible at a travel distance below 0.1 when the plate is stained with methanolic sulphuric acid solution.

XIV. RESULTS & ACTIONS TO BE TAKEN

The hyoscine butylbromide 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.

7.122 Loperamide hydrochloride incl. orodispersible tablets

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During visual inspection, search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual and report the findings. Consider to take pictures, for example, with a smartphone camera. Each classic tablet, orodispersible tablet or capsule usually contains 2 mg loperamide hydrochloride. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release loperamide tablet and capsule formulations have to pass the disintegration test as described at the beginning of the main manual. They should disintegrate in water at 37 °C in less than 30 minutes. It is a major defect if an instant release formulation does not pass this test.

II. RESULTS & ACTIONS TO BE TAKEN

Medicinal products from unusually cheap sources, medicinal products with missing or incorrect accompanying documents and medicinal products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels in a foreign language or medicinal products held under poor storage conditions should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Loperamide hydrochloride is extracted from tablets or capsules with a known volume of methanol and is subsequently checked for identity and content by thin-layer chromatography (TLC) in comparison with a suitable secondary standard.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Funnel
4) Spatula
5) Label tape
6) Marker pen
7) Pencil and ruler
8) 10-ml vials
9) Set of graduated pipettes (1 to 25 ml)
10) Set of laboratory glass bottles (25 to 100 ml)
11) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
12) Glass microcapillaries (2-µl filling capacity)
13) TLC developing chamber (500-ml jar)
14) Hot plate
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) Iodine chamber
20) Methanol
21) Ethyl acetate
22) Ammonium solution 25%
23) Reference agent, for example, loperamide hydrochloride 2 mg tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic medicinal product for reference purposes, for example, tablets containing 2 mg of loperamide hydrochloride. Wrap up two reference tablets into aluminium foil and crush them down to a fine powder using a pestle. Carefully empty the aluminium foil over a 10-ml laboratory glass bottle and wash down all residual solids with 4 ml of methanol using a graduated pipette. Close the lab bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid. The solution obtained should contain 1 mg of total loperamide hydrochloride per ml and be labelled as *'Loperamide Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

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

The stock standard solution requires no further dilution. It already represents the final working concentration of 1 mg of total loperamide hydrochloride per ml. For more convenient handling, some of the supernatant liquid may be transferred to a 10-ml vial.

This higher working standard solution represents a medicinal product of good quality containing 100% of loperamide 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 0.25 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.8 mg of total loperamide hydrochloride per ml and be labelled as *'Loperamide Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of loperamide as stated on the product's label. In the current investigation, this level of loperamide represents the lower acceptable limit for a given product. Pharmacopoeial limits do not apply in our context.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 2 MG OF LOPERAMIDE HCL PER UNIT

Take two whole classic tablets, orodispersible tablets or capsules from a suitable medicinal product sampled in the field. As usual, tablets are wrapped up into aluminium foil and crushed down to a fine powder using a pestle. Transfer all the powder obtained into a 10-ml laboratory glass bottle. Powder obtained from sample capsules should be placed directly in the bottle adding the empty cap and body shells last. For extraction, add 4 ml of methanol using a suitable graduated pipette. Then, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid.

All stock sample solutions produced should finally contain 1 mg of total loperamide hydrochloride per ml and be labelled as *'Loperamide Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue working with the clear or hazy supernatant liquids.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

The stock sample solutions require no further dilution. They already represent the final working concentration of 1 mg of loperamide hydrochloride per ml. If prepared from a high quality product, the sample solutions should match the concentration of loperamide of the higher working standard solution produced above. For more convenient handling, some of the supernatant liquid may be transferred to a 10-ml vial.

VIII. SPOTTING

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

Up to five spots can be placed on a plate. Check the uniformity of all spots using UV light of 254 nm. All spots should be circular in shape and equally spaced across the origin line. Although their intensities might differ, their diameters never should. Different intensities are due to residual amounts of 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. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about 15 seconds. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 17 ml of ethyl acetate, 2 ml of methanol and 0.5 ml of ammonia solution 25% to the jar serving 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 12 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by gentle drying. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about two minutes. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

X. DETECTION

After drying off all solvent residues, the chromatography plate is viewed under UV light at 254 nm using the battery-powered lamp supplied. To identify and quantify loperamide, colour the fresh chromatography plate with iodine and observe it first in daylight and then again under UV light at 254 nm.

XI. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When the chromatography plate is exposed to iodine vapour, any very faint loperamide spots that may have been previously observed at 254 nm at a distance of about 0.64 will turn yellowish-brown. Also observe the plate during iodine evaporation. The spots reflecting inferior products will gradually disappear first, followed by the reference spots representing 80 or 100 % drug content.

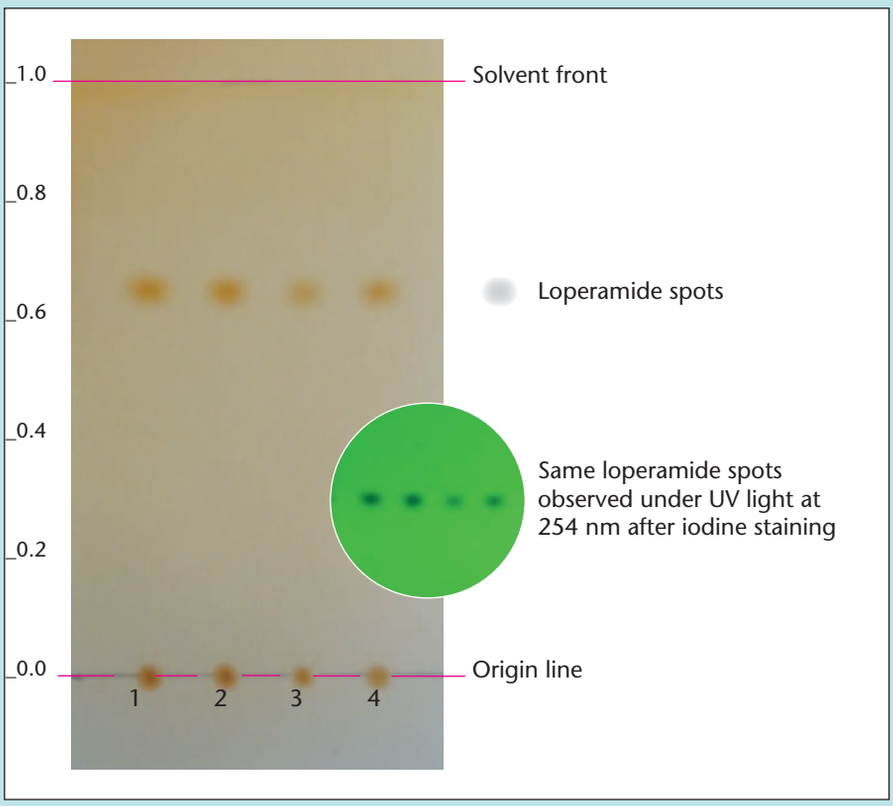
CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

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

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

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



XII. OBSERVATIONS MADE AT 254 NM AFTER IODINE STAINING

All weak loperamide spots previously observed at a travel distance of about 0.64 without iodine staining are now becoming very strong. Additional strong spots generated by the test solution would point at other drugs or loperamide 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 loperamide content and no spot at all a complete absence of loperamide. Auxiliary agents incorporated in different finished products might cause no or faint spots either travelling up to the solvent front or lingering near or on the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The loperamide 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.

7.123 Loratadine

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During visual inspection, search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 10 mg of loratadine per free base. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release loratadine tablet and capsule formulations have to pass the disintegration test as described at the beginning of the main manual. They should disintegrate in water at 37 °C in less than 30 minutes. It is a major defect if an instant release formulation does not pass this test.

II. RESULTS & ACTIONS TO BE TAKEN

Medicinal products from unusually cheap sources, medicinal products with missing or incorrect accompanying documents and medicinal products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels in a foreign language or medicinal products held under poor storage conditions should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Loratadine is extracted from tablets or capsules with a known volume of methanol and is subsequently checked for identity and content by thin-layer chromatography (TLC) in comparison with a suitable secondary standard.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Funnel
4) Spatula
5) Label tape
6) Marker pen
7) Pencil and ruler
8) 10-ml vials
9) Set of graduated pipettes (1 to 25 ml)
10) Set of laboratory glass bottles (25 to 100 ml)
11) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
12) Glass microcapillaries (2-µl filling capacity)
13) TLC developing chamber (500-ml jar)
14) Hot plate
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) Iodine chamber
20) Methanol
21) Ethyl acetate
22) Reference agent, for example, loratadine 10 mg tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic medicinal product for reference purposes, for example, tablets containing 10 mg of loratadine. Wrap up one reference tablet into aluminium foil and crush it down to a fine powder using a pestle. Carefully empty the aluminium foil over a 25-ml laboratory glass bottle and wash down all residual solids with 8 ml of methanol using a graduated pipette. Close the lab bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid. The solution obtained should contain 1.25 mg of total loratadine per ml and be labelled as *'Loratadine Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

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

The stock standard solution requires no further dilution. It already represents the final working concentration of 1.25 mg of total loratadine per ml. For more convenient handling, some of the supernatant liquid may be transferred to a 10-ml vial.

This higher working standard solution represents a medicinal product of good quality containing 100% of loratadine.

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 0.5 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 1 mg of total loratadine per ml and be labelled as *'Loratadine Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of loratadine as stated on the product's label. In the current investigation, this level of loratadine represents the lower acceptable limit for a given product. Pharmacopoeial limits do not apply in our context.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 10 MG OF LORATADINE PER UNIT

Take a whole tablet or capsule from a suitable medicinal product sampled in the field. As usual, tablets are wrapped up into aluminium foil and crushed down to a fine powder using a pestle. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from sample capsules should be placed directly in the bottle adding the empty cap and body shells last. For extraction, add 8 ml of methanol using a suitable graduated pipette. Then, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid.

All stock sample solutions produced should finally contain 1.25 mg of total loratadine per ml and be labelled as *'Loratadine Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue working with the clear or hazy supernatant liquids.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

The stock sample solutions require no further dilution. They already represent the final working concentration of 1.25 mg of loratadine per ml. If prepared from a high quality product, the sample solutions should match the concentration of loratadine of the higher working standard solution produced above. For more convenient handling, some of the supernatant liquid may be transferred to a 10-ml vial.

VIII. SPOTTING

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

Up to five spots can be placed on a plate. Check the uniformity of all spots using UV light of 254 nm. All spots should be circular in shape and equally spaced across the origin line. Although their intensities might differ, their diameters never should. Different intensities are due to residual amounts of 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. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about 15 seconds. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 10 ml of ethyl acetate and 10 ml of methanol to the jar serving 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 13 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by gentle drying. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about one minute. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

X. DETECTION

After drying off all solvent residues, view the chromatography plate under UV light at 254 nm with the battery driven lamp provided. For further identification and quantification of loratadine, the fresh chromatography plate is stained with iodine in the iodine chamber and the assays are read in daylight.

XI. OBSERVATIONS MADE AT UV LIGHT OF 254 NM

A dark spot at a travel distance of about 0.71 indicates the presence of loratadine in the test solution. Additional strong spots generated by the test solution would point at other drugs or loratadine 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 loratadine content and no spot at all a complete absence of loratadine. Auxiliary agents incorporated in different finished products might cause no or faint spots either travelling up to the solvent front or lingering near or on the origin line.

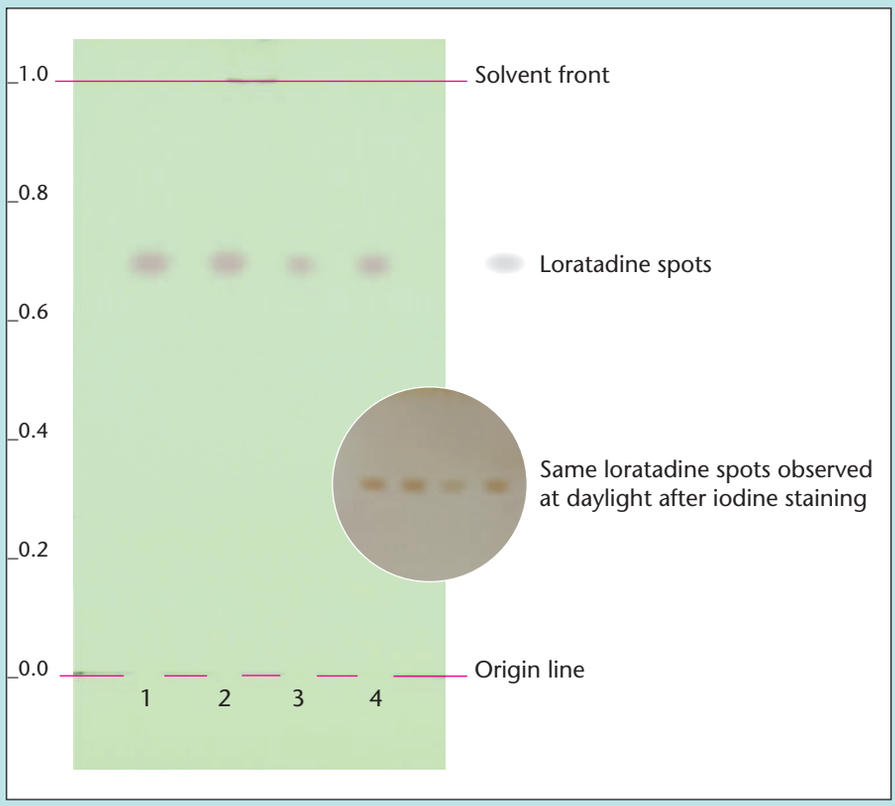
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

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

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



XII. OBSERVATIONS MADE IN DAYLIGHT AFTER IODINE STAINING

When exposing the chromatoplate to iodine vapour, all loratadine spots already observed at 254 nm are now turning yellowish brown. The loratadine spots coloured with iodine fade quickly. 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 80 and 100 percent, respectively.

XIII. RESULTS & ACTIONS TO BE TAKEN

The loratadine 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 or a smartphone turning off the flash first.

7.124 Norfloxacin incl. tinidazole co-formulations

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During visual inspection, search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 400 mg of norfloxacin per free base. Other dosage strengths and co-formulations with tinidazole are known to exist. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release norfloxacin tablet and capsule formulations have to pass the disintegration test as described at the beginning of the main manual. They should disintegrate in water at 37 °C in less than 30 minutes. It is a major defect if an instant release formulation does not pass this test.

II. RESULTS & ACTIONS TO BE TAKEN

Medicinal products from unusually cheap sources, medicinal products with missing or incorrect accompanying documents and medicinal products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels in a foreign language or medicinal products held under poor storage 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 tinidazole, norfloxacin is extracted from tablets or capsules with a known volume of acidified methanol and is subsequently checked for identity and content by thin-layer chromatography (TLC) in comparison with a suitable secondary standard. For a quick check on tinidazole quality, refer to the relevant protocol.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Funnel
4) Spatula
5) Label tape
6) Marker pen
7) Pencil and ruler
8) 10-ml vials
9) Set of graduated pipettes (1 to 25 ml)
10) Set of laboratory glass bottles (25 to 100 ml)
11) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
12) Glass microcapillaries (2-µl filling capacity)
13) TLC developing chamber (500-ml jar)
14) Hot plate
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) UV light of 366 nm
20) Iodine chamber
21) Plastic beaker (250 ml)
22) Ninhydrin
23) Toluene
24) Acetone
25) Methanol
26) n-Butanol
27) Ammonia solution 25%
28) Acetic acid solution 96%
29) Reference agent, for example norfloxacin 400 mg tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an authentic medicinal product for reference purposes, for example, tablets containing 400 mg of norfloxacin. Wrap up one reference tablet into aluminium foil and crush it down to a fine powder using a pestle. Carefully empty the aluminium foil over a 25-ml laboratory glass bottle and wash down all residual solids starting with 8 ml of acetic acid solution 96% and followed by 12 ml of methanol using appropriate graduated pipettes. Close the lab bottle and shake for about three minutes until most of the solids are dissolved. Allow the solu-

tion to stand for an additional five minutes until undissolved residues settle below the supernatant liquid. The solution obtained should contain 20 mg of total norfloxacin per ml and be labelled as *'Norfloxacin 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 0.5 ml of the stock standard solution into a 10-ml vial and add 9.5 ml of methanol. Close and shake the vial. The solution obtained should contain 1 mg of total norfloxacin per ml and be labelled as *'Norfloxacin Working Standard Solution 100%'*.

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

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

Pipette 0.5 ml of the stock standard solution into a 25-ml vial and add 12 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.8 mg of total norfloxacin per ml and be labelled as *'Norfloxacin Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of norfloxacin as stated on the product's label. In the current investigation, this level of norfloxacin represents the lower acceptable limit for a given product. Pharmacopoeial limits do not apply in our context.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 400 MG OF NORFLOXACIN PER UNIT

Take a whole tablet or capsule from a suitable medicinal product sampled in the field. As usual, tablets are wrapped up into aluminium foil and crushed down to a fine powder using a pestle. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from sample capsules should be placed directly in the bottle adding the empty cap and body shells last. For extraction, add 8 ml of acetic acid solution 96% followed by 12 ml of methanol using suitable graduated pipettes. Then, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid.

Whether or not combined with tinidazole, all stock sample solutions produced should finally contain 20 mg of total norfloxacin per ml and be labelled as *'Norfloxacin Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue working with the clear or hazy supernatant liquids.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Pipette 0.5 ml of the stock sample solution into a 10-ml vial and add 9.5 ml of methanol. Close and shake the vial and label as *'Norfloxacin Working Sample Solution'*. The expected concentration of norfloxacin free base in this working sample solution is 1 mg per ml and should match the concentration of norfloxacin 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 on the next page using the microcapillary pipettes supplied.

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

intensities are due to residual amounts of excipients or different drug concentrations or combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

Finally, dry the spots. To do this, place the chromatographic plate on the hot heating plate for about 15 seconds.

IX. DEVELOPMENT

A) Mobile phase for norfloxacin monopreparations and norfloxacin/tinidazole fixed-dose combinations: Using suitable graduated pipettes, add 16 ml of n-butanol, 2 ml of methanol and 7 ml of ammonia solution 25% to the jar serving 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 the half of the length of the plate, the developing time being about 30 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by drying the TLC plate on a hot heating plate for about one minute.

B) Mobile phase for the identification of norfloxacin next to other fluoroquinolones, for example ciprofloxacin, levofloxacin and moxifloxacin: Using suitable graduated pipettes, add 10 ml of methanol, 5 ml of acetone, 2.5 ml of toluene and 5 ml of ammonia solution 25% to the jar serving as TLC developing chamber. Load the chromatographic plate with extraction solutions of various quinolones prepared according to the corresponding protocols in the main manual. Then develop the plate as described above, with a development time of about 20 minutes, when the mobile phase runs through about three quarters of the length of the plate. Remove the TLC plate from the chamber, mark the solvent front and allow the excess solvent to evaporate by drying the TLC plate on a hot heating plate for about one minute. If the TLC plate is dried too little or not at all, not all solvent residues are removed, so that the background of the TLC plate becomes coloured and only pale-coloured quinolone spots are produced during subsequent staining with ninhydrin.

X. DETECTION

After drying the solvent residues, view the chromatographic plates from mobile phases A and B under UV light at 254 and 366 nm using the battery-powered lamps supplied. Ensure the workspace is dark when using the 366 nm UV lamp. For further identification of norfloxacin, stain the fresh plate with iodine in the iodine chamber, then observe it in daylight and under UV light of 254 nm.

To further distinguish between fluoroquinolones, observe the norfloxacin plate from mobile phase B under UV light of 366 nm and in daylight after evaporating iodine and staining with ninhydrin. For staining, dissolve 3 g of ninhydrin in 150 ml of methanol and 30 ml of 96% acetic acid in the 250 ml plastic beaker provided. Submerge the chromatoplate, lower side first, using tweezers, then immediately remove it, allowing excess liquid to drain onto a paper tissue. After one minute, wipe off any remaining liquid from the back of the plate and dry the plate on the hot plate until the fluoroquinolone spots become visible in different colors. The ninhydrin staining process is detailed on page 36 of the main manual. Stains on skin from ninhydrin are harmless and will fade in a day or two.

XI. OBSERVATIONS MADE AT 254 NM

Coming from mobile phase A, a strong blue spot at a travel distance of about 0.37 indicates the presence norfloxacin in the test solution. Additional strong spots suggest the presence of other drugs or norfloxacin degradation, especially if the main spot is smaller. A smaller main spot may indicate low norfloxacin content, while no spot suggests its absence. If combined with tinidazole, a second spot appears at about 0.77. Auxiliary agents may cause faint or no spots near the origin or solvent front.

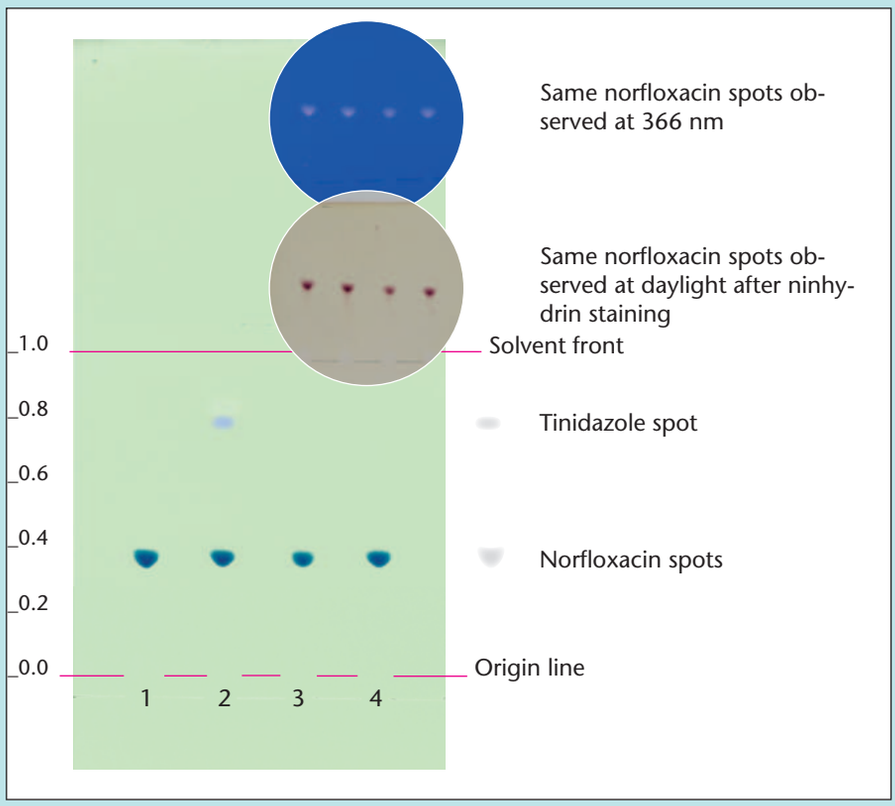
CHROMATOPLATE FROM MOBILE PHASE A OBSERVED UNDER UV LIGHT OF 254 NM

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

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

Run No.3:
A monopreparation of poor quality with unacceptable low norfloxacin content

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



Coming from mobile phase B, a stretched blue spot at a distance of about 0.36 indicates norfloxacin, while ciprofloxacin appears at about 0.44, levofloxacin at about 0.48, moxifloxacin at about 0.53, and tinidazole settles near the solvent front. These various travel distances help to distinguish norfloxacin from other fluoroquinolones.

XII. OBSERVATIONS MADE AT 366 NM

Regardless of their origin, mobile phase A or B, all norfloxacin and other fluoroquinolone spots, e.g. ciprofloxacin, levofloxacin, moxifloxacin exhibit varying shades of white fluorescence, with moxifloxacin being the brightest. These shades aid in identifying norfloxacin among the fluoroquinolones. While the acetic acid in the extraction fluid distorts some fluoroquinolone spots, the norfloxacin spot remains unaffected.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

When exposed to iodine vapor, chromatographic plates from mobile phase A or B show all norfloxacin and other fluoroquinolone spots previously observed at 254 nm and 366 nm now turning brown. Tinidazole may become visible but performs poorly.

XIV. OBSERVATIONS MADE AT DAYLIGHT AFTER NINHYDRIN STAINING

Starting from mobile phase B, staining the chromatoplate with ninhydrin reveals different colours for norfloxacin and other fluoroquinolones, aiding identification. Moxifloxacin spots turn blue, ciprofloxacin red, norfloxacin also red, and levofloxacin remains uncoloured and invisible.

XV. RESULTS & ACTIONS TO BE TAKEN

The norfloxacin 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.

7.125 Tinidazole incl. norfloxacin co-formulations and related metronidazole

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During visual inspection, search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 250, 300, 400, 500 or 600 mg of tinidazole per free base. Other dosage strengths and co-formulations with norfloxacin are known to exist. Tinidazole is often a component of so-called "pylo-kits" to combat Helicobacter pylori infections in the treatment of gastro-oesophageal reflux disease (gastric acid reflux, gastric ulcers, heartburn), for example the pylo-kit consisting of tinidazole 500 mg, clarithromycin 250 mg and lansoprazole 30 mg. However, these are single-dose compounded preparations contained in a kit and not fixed-dose combined preparations. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release tinidazole tablet and capsule formulations have to pass the disintegration test as described at the beginning of the main manual. They should disintegrate in water at 37 °C in less than 30 minutes. It is a major defect if an instant release formulation does not pass this test.

II. RESULTS & ACTIONS TO BE TAKEN

Medicinal products from unusually cheap sources, medicinal products with missing or incorrect accompanying documents and medicinal products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels in a foreign language or medicinal products held under poor storage 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 norfloxacin, tinidazole is extracted from tablets or capsules with a known volume of acetone and is subsequently checked for identity and content by thin-layer chromatography (TLC) in comparison with a suitable secondary standard. For a quick check on norfloxacin quality, refer to the relevant protocol.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Funnel
4) Spatula
5) Label tape
6) Marker pen
7) Pencil and ruler
8) 10-ml vials
9) Set of graduated pipettes (1 to 25 ml)
10) Set of laboratory glass bottles (25 to 100 ml)
11) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
12) Glass microcapillaries (2-µl filling capacity)
13) TLC developing chamber (500-ml jar)
14) Hot plate
15) Filter paper
16) Pair of scissors
17) Pair of tweezers
18) UV light of 254 nm
19) Iodine chamber
20) Acetone
21) Methanol
22) Ethyl acetate
23) Ammonia solution 25%
24) Electronic pocket balance
25) Reference agent, for example tinidazole as pure substance from commercial sources

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of the stock standard solution requires an agent for reference purposes, for example tinidazole as pure substance of high purity close to 100% obtained from commercial sources. Using the electronic pocket balance supplied, weigh in correctly about 0.3 g of neat tinidazole powder on a piece of aluminium foil formed as a weighing boat. Carefully empty the aluminium foil over a 25-ml laboratory glass bottle and wash down all residual solids with 15 ml of acetone using a straight pipette. Adjust the amount of acetone when the weighing result differs from the target weight. In order to overcome the balance's in-build dynamic inertia and to ensure correct read-

ings, 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. Close the lab bottle and shake until all tinidazole is dissolved. The solution obtained should contain 20 mg of total tinidazole per ml and be labelled as *'Tinidazole Stock Standard Solution'*. Freshly prepare this solution for each test. The final solution obtained should be without any observable residual solids. Further information on using the pocket scale can be found in the video via the QR code provided here.

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 4 ml of acetone. Close and shake the vial. The solution obtained should contain 4 mg of total tinidazole per ml and be labelled as *'Tinidazole Working Standard Solution 100%'*.

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

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

Pipette 2 ml of the stock standard solution into a 25-ml vial and add 10.5 ml of acetone using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 3.2 mg of total tinidazole per ml and be labelled as *'Tinidazole Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of tinidazole as stated on the product's label. In the current investigation, this level of tinidazole represents the lower acceptable limit for a given product. Pharmacopoeial limits do not apply in our context.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 250 MG OF TINIDAZOLE PER UNIT

Take a whole tablet or capsule from a suitable medicinal product sampled in the field. As usual, tablets are wrapped up into aluminium foil and crushed down to a fine powder using a pestle. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from sample capsules should be placed directly in the bottle adding the empty cap and body shells last. For extraction, add 12.5 ml of acetone using a suitable graduated pipette. Then, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to stand for an additional five minutes until undissolved residues settle below the supernatant liquid.

300 MG OF TINIDAZOLE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 25-ml laboratory glass bottle, add 15 ml of acetone with a suitable graduated pipette and extract the tinidazole. Continue working as described above.

400 MG OF TINIDAZOLE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 25-ml laboratory glass bottle, add 20 ml of acetone with a suitable graduated pipette and extract the tinidazole. Continue working as described above.

500 MG OF TINIDAZOLE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 50-ml laboratory glass bottle, add 25 ml of acetone with a suitable graduated pipette and extract the tinidazole. Continue working as described above.

600 MG OF TINIDAZOLE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 50-ml laboratory glass bottle, add 30 ml of acetone with a suitable graduated pipette and extract the tinidazole. Continue working as described above.

Whether or not combined with norfloxacin, all stock sample solutions produced should finally contain 20 mg of total tinidazole per ml and be labelled as *'Tinidazole Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue working 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 4 ml of acetone. Close and shake the vial and label as *'Tinidazole Working Sample Solution'*. The expected concentration of tinidazole free base in this working sample solution is 4 mg per ml and should match the concentration of tinidazole 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 or different drug concentrations or combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

Gently dry the spots. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about 10 seconds. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 15 ml of ethyl acetate, 5 ml of methanol and 0.5 ml of ammonia solution 25% to the jar serving 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 14 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by gentle drying. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about two minutes. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

X. DETECTION

After drying off all solvent residues, view the chromatographic plate under UV light at 254 nm with the battery driven lamp provided. For further identification and quantification of tinidazole, stain the fresh chromatoplate with iodine in the iodine chamber.

XI. OBSERVATIONS MADE AT 254 NM

A dark spot at a travel distance of about 0.61 indicates the presence of tinidazole in the test solution. Additional strong spots generated by the test solution would point at other drugs or tinidazole 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 tinidazole content and no spot at all a complete absence of tinidazole. The related metronidazole would show a relative retention factor of about

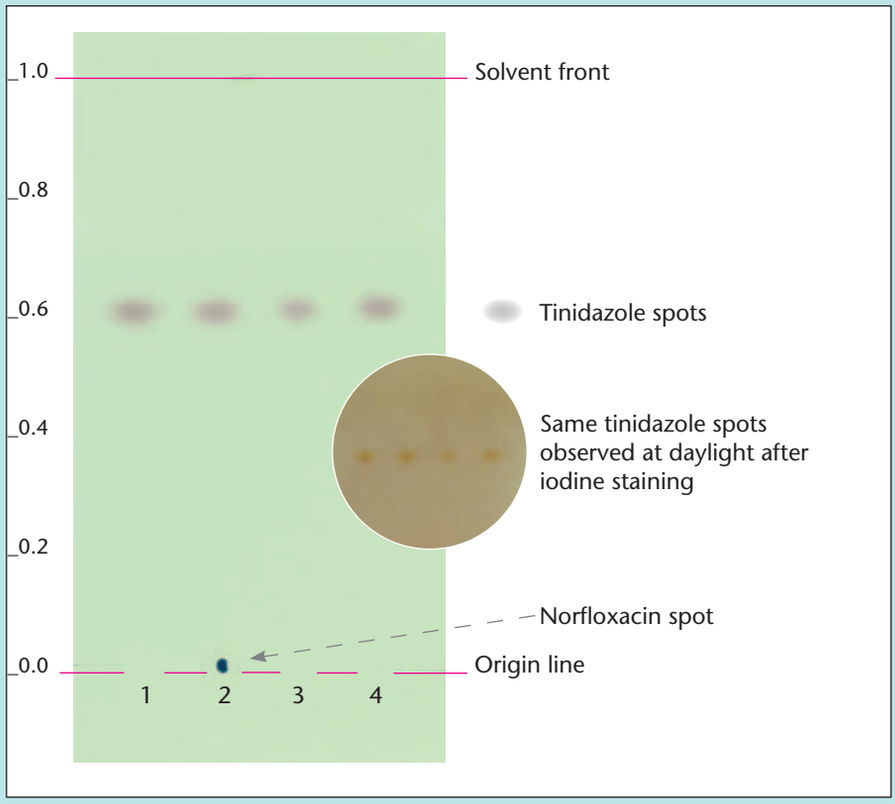
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

Run No.3:
A monopreparation of poor quality with unacceptable low tinidazole content

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



0.51. If tinidazole is combined with norfloxacin, norfloxacin does not move and settles on the origin line. Auxiliary agents incorporated in different finished products might cause no or faint spots either travelling up to the solvent front or lingering near or on the origin line.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER IODINE STAINING

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

XIII. RESULTS & ACTIONS TO BE TAKEN

The tinidazole 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.

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is joined by a thin-layer chromatographic test for the simple
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glycol and ethylene glycol, in syrups and other
liquid preparations for oral use.

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