A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Online Supplement 2022 on more Medicine to Treat Cardiovascular Disorders

Physical Testing & Thin-Layer Chromatography

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PQM Promoting the Quality of Medicines

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Table of Contents

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 2022 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.

7.103 Irbesartan incl. hydrochlorothiazide or amlodipine 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 issued 2022 and report the findings. Consider to take pictures, for example, with a smartphone camera. Independent from salt form and crystal water content, each tablet usually contains 75, 150 or 300 mg of irbesartan per free base. Other dosage strengths are known to exist. Tablets may be combined with 15 or 25 mg of hydrochlorothiazide or with 5 or 10 mg of amlodipine. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release irbesartan tablet or capsule formulations must also pass the disintegration test as described at the beginning of the 2022 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 other cardiac medicines, irbesartan is extracted from tablets or capsules with a known volume of ammoniacal methanol solution and then checked for identity and content by thin-layer chromatography (TLC) against a suitable secondary standard. For fixed-dose combinations, refer to the hydrochlorothiazide or amlodipine protocol in the 2022 main manual for additional testing.

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) Methanol
21) Acetone
22) Toluene
23) Acetic acid solution 96%
24) Ammonia solution 25%
25) Reference agent, for example, irbesartan 150 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 150 mg of irbesartan per 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 25-ml laboratory glass bottle and wash down all residual solids with 14 ml of methanol using a graduated pipette. Using a new pipette, add to the existing mix 1 ml of ammonia solution 25%, 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

10 mg of total irbesartan per ml and be labelled as *'Irbesartan 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 25-ml vial and add 11.5 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.8 mg of total drug per ml and be labelled as *'Irbesartan Working Standard Solution 100%'*.

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

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 14.6 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.64 mg of total drug per ml and be labelled as *'Irbesartan Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of irbesartan as stated on the product's label. In the current investigation, this level of irbesartan 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 75 MG OF IRBESARTAN PER UNIT

Take one 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. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from a sample capsule should be placed directly in the bottle adding the cap and body shells last. For extraction, add 7 ml of methanol followed by 0.5 ml of ammonia solution 25% 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.

150 MG OF IRBESARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 25-ml laboratory glass bottle, add 14 ml of methanol followed by 1 ml of ammonia solution 25% with suitable graduated pipettes and extract the irbesartan. Continue working as described above.

300 MG OF IRBESARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 50-ml laboratory glass bottle, add 28 ml of methanol followed by 2 ml of ammonia solution 25% with suitable graduated pipettes and extract the irbesartan. Continue working as described above.

Whether or not combined with other cardiovascular medicines, all stock sample solutions produced should finally contain 10 mg of total irbesartan per ml and be labelled as *'Irbesartan 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 25-ml vial and add 11.5 ml of methanol. Close and shake the vial and label as *'Irbesartan Working Sample Solution'*.

The expected concentration of irbesartan in the working sample solutions is 0.8 mg per ml and should correspond to the concentration of irbesartan in the higher working standard solution prepared 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 actives and 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 and shake the chromatography plate with the supplied tweezers in the hot air stream directly above the heating plate for about 90 seconds until the smell of the ammonia solution has almost disappeared. During shaking, the underside of the chromatography plate may directly touch the heating plate for a fraction of a second each time the TLC plate swings back and forth. Allow the chromatography plate to rest for a few minutes and allow residual ammonia solution to completely disappear before development. The latter step improves the resolution between the spots of the different sartans.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 17 ml of toluene, 4 ml of acetone, and 4 ml of acetic acid solution 96% to 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 12 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by placing the chromatographic plate on the heating plate provided. The heating plate is operated at the highest level and the chromatography plate is left there for a full minute before it is removed to cool down to ambient temperature.

X. DETECTION

After drying off all solvent residues, view the chromatography plate under UV light at 254 nm with the battery-powered lamp provided. Use this detection method for the identification and quantification of irbesartan. When combined with hydrochlorothiazide (HCT), this agent is barely visible due to its low concentration and may appear as a very faint shadow below the irbesartan spot when a high dose of HCT meets a low dose of irbesartan in a tablet and the measurements are taken in a completely dark room. When combined with amlodipine, the latter drug can be detected as a tiny spot near the line of origin, and when this spot is irradiated with UV light at 366 nm, a strong white fluorescence appears. For HCT identification, the stock solution can be used. For checking the HCT or amlodipine content, refer to the relevant assay protocols in the main manual issued 2022.

XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.35 indicates the presence of irbesartan in the test solution. Additional strong spots generated by the test solution would point at other drugs or irbesartan 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 irbesartan content and an absent spot may indicate

CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

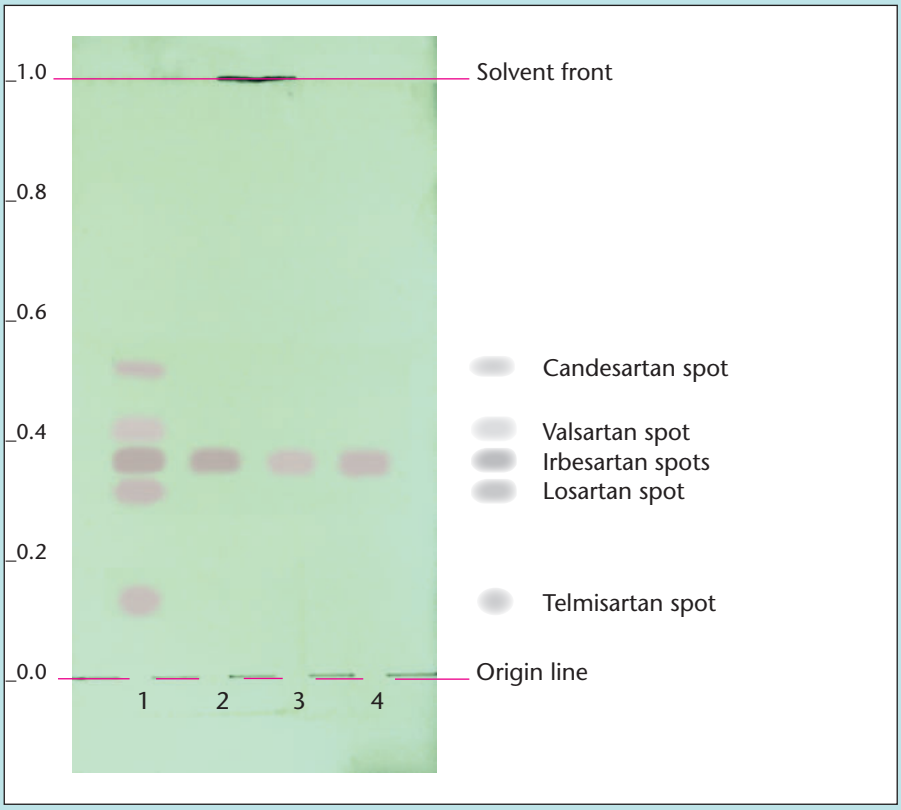
Run No.1:
Upper working standard representing 100% of total irbesartan

Also showing that the selected mobile phase is specific, other sartans were added to the standard solution

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

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

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



a complete absence of irbesartan. Excipients present in various finished products may cause fainter spots that either migrate up to the solvent front or linger near or on the line of origin. When hydrochlorothiazide (HCT) is combined with irbesartan in a favourable ratio, under ideal conditions a very faint HCT spot may appear at a travel distance of about 0.15 below the irbesartan spot. In reverse cases, due to strong dilutions, the HCT concentration will drop below its limit of detection. If combined with amlodipine, a tiny spot is visible at a travel distance of about 0.06 near the line of origin. In order to show that the selected mobile phase is specific for many sartans, candesartan (R_f = 0,53), valsartan (R_f = 0.41), losartan (R_f = 0.29) and, telmisartan (R_f = 0.12) were added to the reference solution as seen above on run number one.

XII. OBSERVATIONS MADE AT 366 NM

When irbesartan is combined with amlodipine, the presence of the latter compound is confirmed by a strong white fluorescence at a travel distance of about 0.06 very close to the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The irbesartan 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.

7.104 Losartan potassium incl. hydrochlorothiazide or amlodipine 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 issued 2022 and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 25, 50 or 100 mg of losartan potassium salt. Other dosage strengths are known to exist. Tablets may be combined with 15 or 25 mg of hydrochlorothiazide or with 5 or 10 mg of amlodipine. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release losartan tablet or capsule formulations must also pass the disintegration test as described at the beginning of the 2022 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 other cardiac medicines, losartan potassium salt is extracted from tablets or capsules with a known volume of methanol and then checked for identity and content by thin-layer chromatography (TLC) in comparison to a suitable secondary standard. For fixed-dose combinations, refer to the hydrochlorothiazide or amlodipine protocol in the 2022 main manual for additional testing.

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) Methanol
21) Acetone
22) Toluene
23) Acetic acid solution 96%
24) Reference agent, for example, losartan potassium 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 losartan potassium. 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 losartan potassium per ml and be labelled as *'Losartan 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 4 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 1 mg of total drug per ml and be labelled as *'Losartan Working Standard Solution 100%'*.

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

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 methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.8 mg of total drug per ml and be labelled as *'Losartan Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of losartan potassium salt as stated on the product's label. In the current investigation, this level of losartan potassium 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 LOSARTAN POTASSIUM PER UNIT

Take one 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. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from a sample capsule should be placed directly in the bottle adding the 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 LOSARTAN POTASSIUM PER UNIT

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

100 MG OF LOSARTAN POTASSIUM PER UNIT

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

Whether or not combined with other cardiovascular medicines, all stock sample solutions produced should finally contain 5 mg of total losartan potassium salt per ml and be labelled as *'Losartan 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 4 ml of methanol. Close and shake the vial and label as *'Losartan Working Sample Solution'*.

The expected concentration of losartan potassium in the working sample solutions is 1 mg per ml and should correspond to the concentration of losartan potassium in the higher working standard solution prepared 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 actives and 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.

Dry the spots by placing the chromatography plate on the heating plate provided. The heating plate should be operated at maximum level and the underside of the chromatographic plate should touch the heating plate for about 15 seconds. Allow the chromatography plate to rest for a few minutes and to cool to ambient temperature before developing. The latter step improves the resolution between the spots of the different sartans.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 17 ml of toluene, 4 ml of acetone, and 4 ml of acetic acid solution 96% to 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 12 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by placing the chromatographic plate on the heating plate provided. The heating plate is operated at the highest level and the chromatography plate is left there for a full minute before it is removed to cool down to ambient temperature.

X. DETECTION

After drying off all solvent residues, view the chromatography plate under UV light at 254 nm with the battery-powered lamp provided. Use this detection method for the identification and quantification of losartan. When combined with hydrochlorothiazide (HCT), this agent is barely visible due to its low concentration and may appear as a very faint shadow below the losartan spot when a high dose of HCT meets a low dose of losartan in a tablet and when the readings are taken in a completely dark room. When combined with amlodipine, the latter drug can be detected as a tiny spot near the line of origin, and when this spot is irradiated with UV light at 366 nm, a strong white fluorescence appears. For HCT identification, the stock solution can be used. For checking the HCT or amlodipine content, refer to the relevant assay protocols in the main manual issued 2022.

XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.29 indicates the presence of losartan in the test solution. Additional strong spots generated by the test solution would point at other drugs or losartan degradation, the latter case being more likely when associated with a smaller principal spot. A smaller principal spot from the test

CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

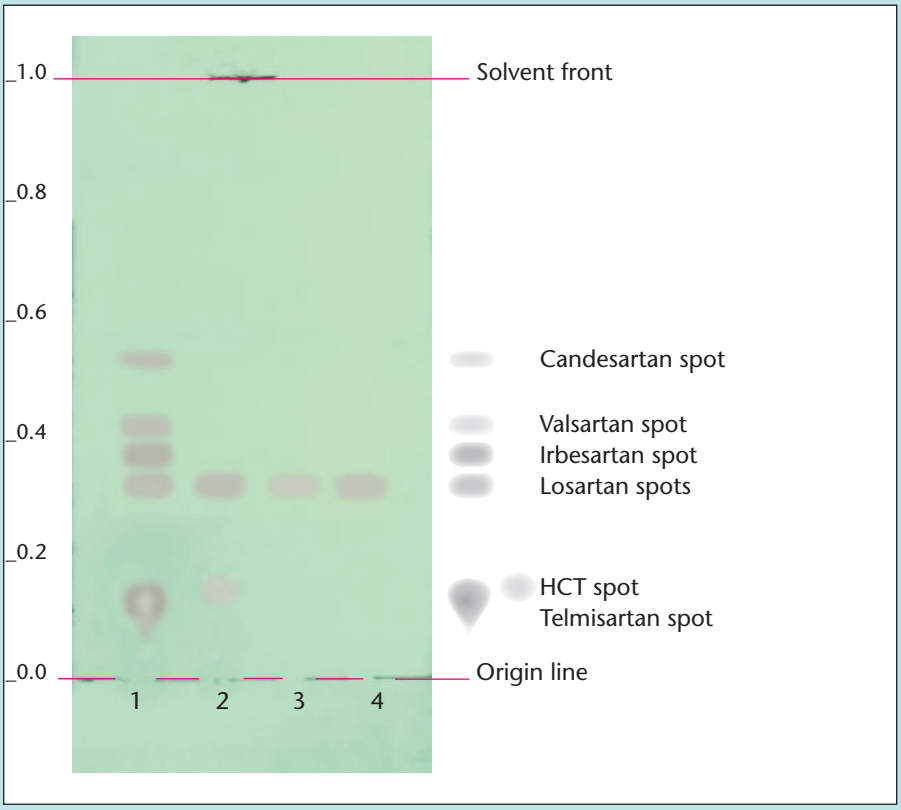
Run No.1:
Upper working standard representing 100% of total losartan

Also showing that the selected mobile phase is specific, other sartans were added to the standard solution

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

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

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



solution may also indicate a poor losartan content and an absent spot may indicate a complete absence of losartan. Excipients present in various finished products may cause fainter spots that either migrate up to the solvent front or linger near or on the line of origin. When hydrochlorothiazide (HCT) is combined with losartan in a favourable ratio, under ideal conditions a very faint HCT spot may appear at a travel distance of about 0.15 below the losartan spot. In reverse cases, due to strong dilutions, the HCT concentration will drop below its limit of detection. If combined with amlodipine, a tiny spot is visible at a travel distance of about 0.06 near the line of origin. In order to show that the selected mobile phase is specific for many sartans, candesartan (R_f = 0.53), valsartan (R_f = 0.41), irbesartan (R_f = 0.35) and, telmisartan (R_f = 0.12) were added to the reference solution as seen above on run number one.

XII. OBSERVATIONS MADE AT 366 NM

When losartan is combined with amlodipine, the presence of the latter compound is confirmed by a strong white fluorescence at a travel distance of about 0.06 very close to the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The losartan 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.

7.105 Methyldopa anhydrous/sesquihydrate incl. hydrochlorothiazide 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 issued 2022 and report the findings. Consider to take pictures, for example, with a smartphone camera. Methyldopa comes frequently in form of its sesquihydrate. Each tablet usually contains 125, 250 or 500 mg of anhydrous methyldopa. Tablets may be combined with 15 or 25 mg of hydrochlorothiazide. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release methyldopa tablet or capsule formulations must also pass the disintegration test as described at the beginning of the 2022 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

Anhydrous methyldopa and methyldopa sesquihydrate, also in combination with hydrochlorothiazide, are extracted from tablets or capsules with a known volume of acidified methanol and subsequently checked for identity and content by thin-layer chromatography (TLC) in comparison with a suitable secondary standard. For fixed-dose combinations, refer to the hydrochlorothiazide protocol in the 2022 main manual for additional testing.

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) Distilled or drinking water
21) Methanol
22) n-Butanol
23) Acetone
24) Acetic acid solution 96%
25) Hydrochloric acid solution 32%
26) Reference agent, for example, methyldopa 125 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 125 mg of methyldopa. 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 12 ml of methanol using a graduated pipette. Finally,

add to the existing mix 0.5 ml of hydrochloric acid solution 32%, 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 10 mg of total methyldopa per ml and be labelled as *'Methyldopa 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 using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.5 mg of total drug per ml and be labelled as *'Methyldopa Working Standard Solution 100%'*.

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

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

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of methyldopa as stated on the product's label. In the current investigation, this level of methyldopa 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 125 MG OF METHYLDOPA PER UNIT

Take one 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. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from a sample capsule should be placed directly in the bottle adding the cap and body shells last. For extraction, add 12 ml of methanol followed by 0.5 ml of hydrochloric acid solution 32% 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.

250 MG OF METHYLDOPA PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 50-ml laboratory glass bottle, add 24 ml of methanol followed by 1 ml of hydrochloric acid solution 32 % with suitable graduated pipettes and extract the methyldopa. Continue working as described above.

500 MG OF METHYLDOPA PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 100-ml laboratory glass bottle, add 48 ml of methanol followed by 2 ml of hydrochloric acid solution 32 % with suitable graduated pipettes and extract the methyldopa. Continue working as described above.

Whether or not combined with other medicines, all stock sample solutions produced should finally contain 10 mg of total methyldopa per ml and be labelled as *'Methyldopa 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 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 *'Methyldopa Working Sample Solution'*.

The expected concentration of methyldopa in the working sample solutions is 0.5 mg per ml and should correspond to the concentration of methyldopa of the higher working standard solution prepared 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 actives and 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.

Dry the spots by placing the chromatography plate on the heating plate provided. The heating plate should be operated at maximum level and the underside of the chromatographic plate should touch the heating plate for about 15 seconds.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 12 ml of n-butanol, 4 ml of acetone, 3.5 ml of water and 0.5 ml of acetic acid solution 96% to 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 TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by placing the chromatographic plate on the heating plate provided. The heating plate is operated at the highest level and the chromatography plate is left there for a full minute before it is removed to cool down to ambient temperature.

X. DETECTION

After drying off all solvent residues, view the chromatographic plate under UV light of 254 nm with the battery driven lamp provided. The performance is weak. For further identification and quantification, stain the chromatoplate with iodine vapours. First, view the stained chromatography plate again under UV light at 254 nm. All previously observed weak spots will now become much clearer. When the iodine plate is heated, all the methyldopa spots stained with iodine turn grey to black depending on the range of the working concentration. Note that this change in colour occurs not only with methyldopa but with all methyldopa-related agents, for example, with levodopa or carbidopa with the colour change being weakest with carbidopa. Use these black spots for the final reading and interpretation of the chromatographic plate. Again, readings may become clearer, when re-exposing the heated iodine plate to UV-light of 254 nm. If methyldopa is combined with hydrochlorothiazide (HCT), the latter compound will stay invisible due to its dilution below the limited of detection. However, the presence of HCT can best be confirmed if a suitable methyldopa stock solution is used for the TLC run. This is for rapid HCT identification; for further semi-quantification, refer to the HCT assay protocol in the main manual issued 2022.

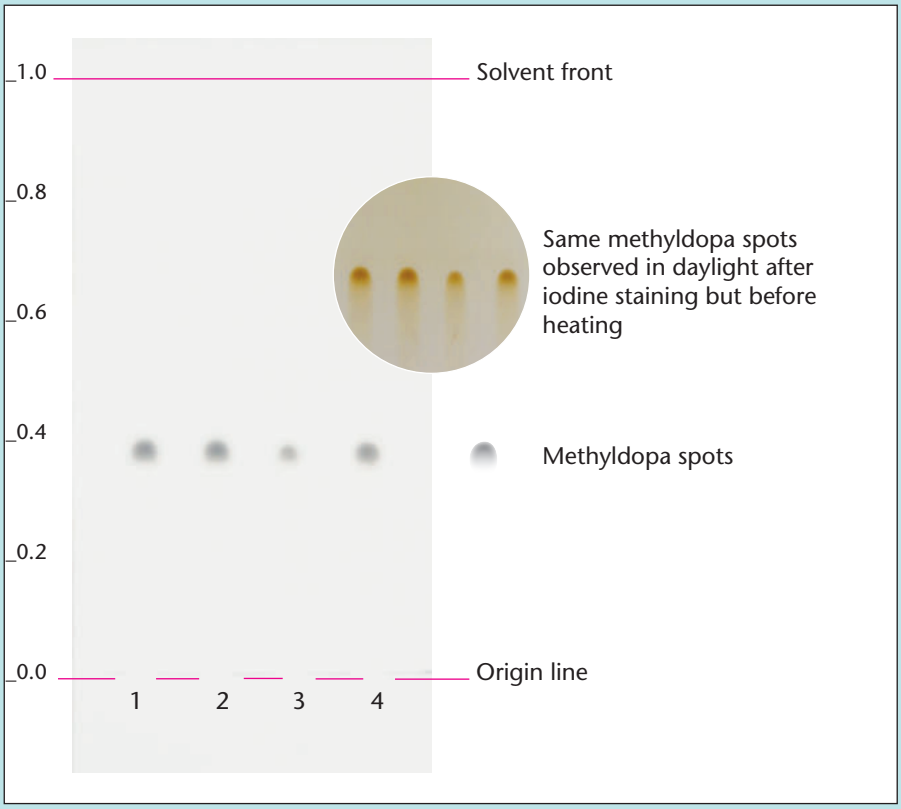
CHROMATOPLATE OBSERVED IN DAYLIGHT AFTER IODINE STAINING AND HEATING

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

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

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

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



XI. OBSERVATIONS MADE AT 254 NM AFTER IODINE STAINING

A strong dark spot at a travel distance of about 0.38 indicates the presence of methyldopa in the test solution. Additional strong spots generated by the test solution would point at other drugs or methyldopa 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 methyldopa content and no spot at all a complete absence of methyldopa. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling up to the solvent front or emerging near or on the origin line. Other methyldopa related agents are clearly separated from the methyldopa spot and their relative retention factors are as follows: about 0.31 for levodopa and about 0.78 for carbidopa. When hydrochlorothiazide (HCT) is combined with methyldopa in a favourable ratio, a HCT spot may become visible at a travel distance of about 0.87.

XII. OBSERVATIONS MADE IN DAYLIGHT AFTER IODINE STAINING AND HEATING

With further exposure of the iodine plate to heat, all methyldopa spots previously observed at 254 nm and in daylight now turn grey, with different shades and sizes indicating different methyldopa concentrations. Other related agents, e.g. levodopa and carbidopa, behave similarly here. However, this colour change is weakest with carbidopa.

XIII. RESULTS & ACTIONS TO BE TAKEN

The methyldopa 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.

7.106 Telmisartan incl. hydrochlorothiazide or amlodipine 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 issued 2022 and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 20, 40 or 80 mg of telmisartan. Other dosage strengths are known to exist. Tablets may be combined with 15 or 25 mg of hydrochlorothiazide or with 5 or 10 mg of amlodipine. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release telmisartan tablet or capsule formulations must also pass the disintegration test as described at the beginning of the 2022 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 other cardiac medicines, telmisartan is extracted from tablets or capsules with a known volume of an ammoniacal methanol solution and then checked for identity and content by thin-layer chromatography (TLC) in comparison to a suitable secondary standard. For fixed-dose combinations, refer to the hydrochlorothiazide or amlodipine protocol in the 2022 main manual for additional testing.

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) Methanol
21) Ethyl acetate
22) Acetic acid solution 96%
23) Ammonia solution 25%
24) Reference agent, for example, telmisartan 20 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 20 mg of telmisartan. 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 7.5 ml of methanol using a graduated pipette. Using a new pipette, add to the existing mix 0.5 ml of ammonia solution 25%, 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 2.5 mg of total telmisartan per ml and be labelled as *'Telmisartan 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 4 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.5 mg of total drug per ml and be labelled as *'Telmisartan Working Standard Solution 100%'*.

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

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 methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.4 mg of total drug per ml and be labelled as *'Telmisartan Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of telmisartan as stated on the product's label. In the current investigation, this level of telmisartan 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 20 MG OF TELMISARTAN PER UNIT

Take one 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. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from a sample capsule should be placed directly in the bottle adding the cap and body shells last. For extraction, add 7.5 ml of methanol followed by 0.5 ml of ammonia solution 25% 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.

30 MG OF TELMISARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 25-ml laboratory glass bottle, add 11.25 ml of methanol followed by 0.75 ml of ammonia solution 25 % with suitable graduated pipettes and extract the telmisartan. Continue working as described above.

40 MG OF TELMISARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 25-ml laboratory glass bottle, add 15 ml of methanol followed by 1 ml of ammonia solution 25 % with suitable graduated pipettes and extract the telmisartan. Continue working as described above.

60 MG OF TELMISARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 50-ml laboratory glass bottle, add 22.5 ml of methanol followed by 1.5 ml of ammonia solution 25 % with suitable graduated pipettes and extract the telmisartan. Continue working as described above.

80 MG OF TELMISARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 50-ml laboratory glass bottle, add 30 ml of methanol followed by 2 ml of ammonia solution 25 % with suitable graduated pipettes and extract the telmisartan. Continue working as described above.

Whether or not combined with other cardiovascular medicines, all stock sample solutions produced should finally contain 2.5 mg of total telmisartan per ml and be labelled as *'Telmisartan 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 4 ml of methanol. Close and shake the vial and label as *'Telmisartan Working Sample Solution'*.

The expected concentration of telmisartan in the working sample solutions is 0.5 mg per ml and should correspond to the concentration of telmisartan of the higher working standard solution prepared 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 actives and 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 and shake the chromatography plate with the supplied tweezers in the hot air stream directly above the heating plate for about two minutes until the smell of the ammonia solution has almost disappeared. During shaking, the underside of the chromatography plate may directly touch the heating plate for a fraction of a second each time the TLC plate swings back and forth.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 18 ml of ethyl acetate, 2 ml of methanol, and 1 ml of acetic acid solution 96% to 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 12 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by placing the chromatographic plate on the heating plate provided. The heating plate is operated at the highest level and the chromatography plate is left there for a full minute before it is removed to cool down to ambient temperature.

X. DETECTION

After drying off all solvent residues, view the chromatography plate under UV light of 254 nm with the battery-powered lamp provided. Use this detection method for the identification and quantification of telmisartan. If combined with hydrochlorothiazide (HCT), due to low concentration, this active agent is hardly visible and may appear as a very faint shadow above the telmisartan spot when a high dose of HCT meets a low dose of telmisartan in a tablet and the readings are taken in a completely dark room. In combination with amlodipine, the latter drug can be detected as a tiny spot near the origin line, and when this spot is irradiated with UV light at 366 nm, a strong white fluorescence appears. For HCT identification, the stock solution can be used. For checking the HCT or amlodipine content, refer to the relevant assay protocols in the main manual issued 2022.

XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.46 indicates the presence of telmisartan in the test solution. Additional strong spots generated by the test solution would point at other drugs or telmisartan degradation, the latter case being more likely

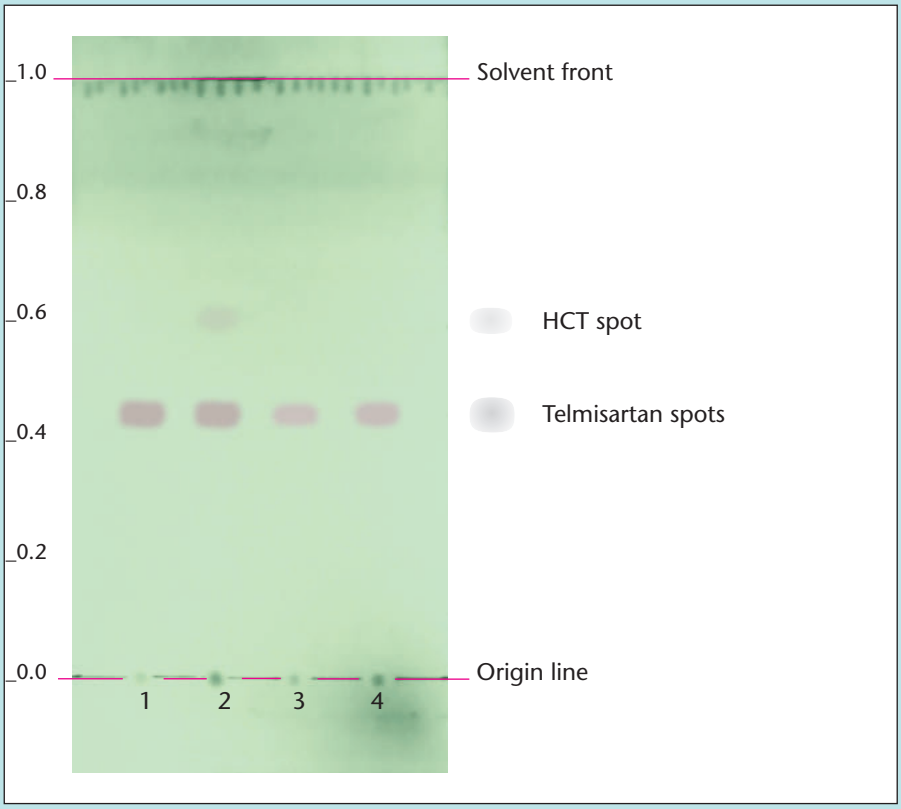
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

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

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



when associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor telmisartan content and no spot at all a complete telmisartan absence. Excipients present in various finished products may cause fainter spots that either migrate up to the solvent front or linger near or on the line of origin. When hydrochlorothiazide (HCT) is combined with telmisartan in a favourable ratio, under ideal conditions a very faint HCT spot may appear at a travel distance of about 0.59 above the telmisartan spot. In reverse cases, due to strong dilutions, the HCT concentration will drop below its limit of detection. If combined with amlodipine, a tiny spot is visible at a travel distance of about 0.03 near the origin line. Other sartans are well separated and would settle well above the telmisartan spot, for example candesartan at about $R_f = 0.68$, irbesartan at about $R_f = 0.62$, losartan at about $R_f = 0.61$ and valsartan at about $R_f = 0.64$.

XII. OBSERVATIONS MADE AT 366 NM

When telmisartan is combined with amlodipine, the presence of the latter compound is confirmed by a strong white fluorescence at a travel distance of about 0.03 very close to the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The telmisartan 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.

7.107 Valsartan incl. hydrochlorothiazide or/and amlodipine 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 issued 2022 and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 80, 160 or 320 mg of valsartan. Other dosage strengths are known to exist. Tablets may be combined with 15 or 25 mg of hydrochlorothiazide or with 5 or 10 mg of amlodipine and may come as double or even triple fixed-dose combination product. Verify the total weight of tablets or capsules using the electronic pocket balance provided. All quick release valsartan tablet or capsule formulations must also pass the disintegration test as described at the beginning of the 2022 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 other cardiac medicines, valsartan is extracted from tablets or capsules with a known volume of methanol and then checked for identity and content by thin-layer chromatography (TLC) in comparison to a suitable secondary standard. For fixed-dose combinations, refer to the hydrochlorothiazide or amlodipine protocol in the 2022 main manual for additional testing.

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) Methanol
21) Acetone
22) Toluene
23) Acetic acid solution 96%
24) Reference agent, for example, valsartan 80 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 80 mg of valsartan. 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 10 mg of total valsartan per ml and be labelled as *'Valsartan 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 9 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 1 mg of total drug per ml and be labelled as *'Valsartan Working Standard Solution 100%'*.

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

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 methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.8 mg of total drug per ml and be labelled as *'Valsartan Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of valsartan as stated on the product's label. In the current investigation, this level of valsartan 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 80 MG OF VALSARTAN PER UNIT

Take one 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. Transfer all the powder obtained into a 25-ml laboratory glass bottle. Powder obtained from a sample capsule should be placed directly in the bottle adding the 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.

160 MG OF VALSARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 25-ml laboratory glass bottle, add 16 ml of methanol with a suitable graduated pipette and extract the valsartan. Continue working as described above.

320 MG OF VALSARTAN PER UNIT

Place the powder obtained from a whole sample tablet or capsule in a 50-ml laboratory glass bottle, add 32 ml of methanol with a suitable graduated pipette and extract the valsartan. Continue working as described above.

Whether or not combined with other cardiovascular medicines, all stock sample solutions produced should finally contain 10 mg of total valsartan per ml and be labelled as *'Valsartan 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 9 ml of methanol. Close and shake the vial and label as *'Valsartan Working Sample Solution'*.

The expected concentration of valsartan in the working sample solutions is 1 mg per ml and should correspond to the concentration of valsartan in the higher working standard solution prepared 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 actives and 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.

Dry the spots by placing the chromatography plate on the heating plate provided. The heating plate should be operated at maximum level and the underside of the chromatographic plate should touch the heating plate for about 15 seconds. Allow the chromatography plate to rest for a few minutes and to cool to ambient temperature before developing. The latter step improves the resolution between the spots of the different sartans.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 17 ml of toluene, 4 ml of acetone, and 4 ml of acetic acid solution 96% to 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 12 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by placing the chromatographic plate on the heating plate provided. The heating plate is operated at the highest level and the chromatography plate is left there for a full minute before it is removed to cool down to ambient temperature.

X. DETECTION

After drying off all solvent residues, view the chromatography plate under UV light at 254 nm with the battery-powered lamp provided. Use this detection method for the identification and quantification of valsartan. In combination with hydrochlorothiazide (HCT), this agent is barely visible due to its low concentration and may appear as a very faint shadow under the valsartan spot when a high dose of HCT meets a low dose of valsartan in a tablet and the measurements are taken in a completely dark room. In combination with amlodipine, the latter drug can be detected as a tiny spot near the line of origin, and when this spot is irradiated with UV light at 366 nm, a strong white fluorescence appears. For HCT identification, the stock solution can be used. To check the HCT or amlodipine content, see the corresponding assay protocols in the main manual issued 2022.

XI. OBSERVATIONS MADE AT 254 NM

A strong blue-violet spot at a travel distance of about 0.41 indicates the presence of valsartan in the test solution. Additional strong spots generated by the test solution would point at other drugs or valsartan 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 valsartan content and an absent spot may indicate a complete absence of valsartan. Excipients present in various finished products may

CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

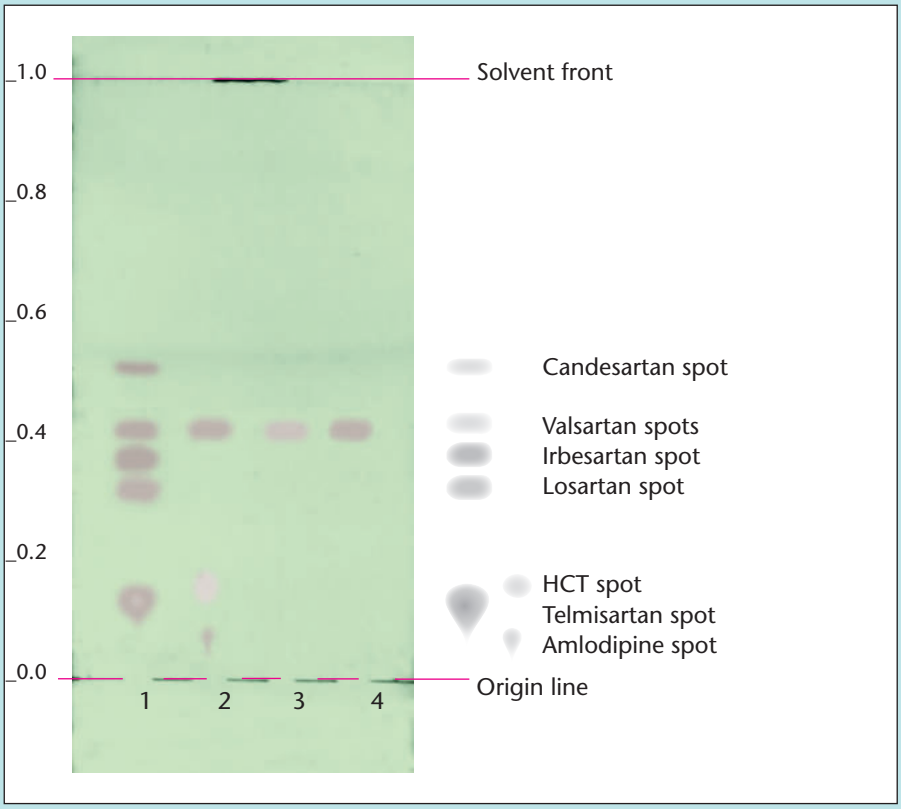
Run No.1:
Upper working standard representing 100% of total valsartan

Also showing that the selected mobile phase is specific, other sartans were added to the standard solution

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

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

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



cause fainter spots that either migrate up to the solvent front or linger near or on the line of origin. When hydrochlorothiazide (HCT) is combined with valsartan in a favourable ratio, under ideal conditions a very faint HCT spot may appear at a travel distance of about 0.15 below the valsartan spot. In reverse cases, due to strong dilutions, the HCT concentration will drop below its limit of detection. If combined with amlodipine, a tiny spot is visible at a travel distance of about 0.06 near the line of origin. For triple fixed-dose combination products, three spots for valsartan, HCT and amlodipine can be observed under ideal conditions. In order to show that the selected mobile phase is specific for many sartans, candesartan (R_f = 0.53), irbesartan (R_f = 0.35), losartan (R_f = 0.29) and, telmisartan (R_f = 0.12) were added to the reference solution as seen above on run number one.

XII. OBSERVATIONS MADE AT 366 NM

When valsartan is combined with amlodipine, the presence of the latter compound is confirmed by a strong white fluorescence at a travel distance of about 0.06 very close to the origin line.

XIII. RESULTS & ACTIONS TO BE TAKEN

The valsartan 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.

- 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.

This electronic supplement to the printed Minilab Manual 2022 expands the list of
cardiovascular medicines to overall fourteen active pharmaceutical ingredients
including their fixed-dose combinations to treat cardiovascular disorders.

The 2022 expanded print version of the method inventory of the
GPHF-Minilab™ manual now includes a collection of test
methods for 107 active pharmaceutical ingredients
for rapid quality verification of a wide range
of finished pharmaceutical products.

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