A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Supplement 2024 on more Medicine to Treat Diabetic Disorders et al.

Physical Testing & Thin-Layer Chromatography

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PQM Promoting the Quality of Medicines

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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.

7.114 Carbamazepine

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 soluble, chewable or sustained release tablet usually contains 200, 300, 400 or 600 mg of carbamazepine per free base.

Other dosage strengths are known to exist. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release carbamazepine 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

Carbamazepine 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) UV light of 366 nm
20) Toluene
21) Methanol
22) Ethyl acetate
23) Sulphuric acid solution 96%
24) Reference agent, for example, carbamazepine 200 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 200 mg of carbamazepine. 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 20 mg of total carbamazepine per ml and be labelled as *'Carbamazepine 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 drug per ml and be labelled as *'Carbamazepine Working Standard Solution 100%'*.

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

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 carbamazepine per ml and be labelled as *'Carbamazepine Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of carbamazepine as stated on the product's label. In the current investigation, this level of carbamazepine 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 200 MG OF CARBAMAZEPINE 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 10 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.

300 MG OF CARBAMAZEPINE PER UNIT

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

400 MG OF CARBAMAZEPINE PER UNIT

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

600 MG OF CARBAMAZEPINE PER UNIT

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

All stock sample solutions produced should finally contain 20 mg of total carbamazepine per ml and be labelled as *'Carbamazepine 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 *'Carbamazepine Working Sample Solution'*.

The expected concentration of carbamazepine in this working sample solution is 1 mg per ml and should match the concentration of carbamazepine 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 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, 8 ml of toluene and 2 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 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, view the chromatographic plate under UV light at 254 nm with the battery driven lamp provided. For further identification of carbamazepine, stain the fresh chromatoplate with sulphuric acid in the heat. To do this, fill the 250 ml plastic beaker provided with 190 ml of methanol, followed by 10 ml of sulphuric acid solution 96%, and mix thoroughly. Allow the mixture to cool and immerse the chromatography plate in the staining solution with the bottom side first. Immediately remove the plate from the solution and allow excess liquid to drain onto a paper towel. Wipe the remaining liquid from the back of the plate and dry the entire staining solution for about 30 to 60 seconds at maximum heat setting on the hot plate provided. After removing the chromatographic plate from the heating plate, view the stained plate under UV light at 254 and 366 nm in the dark.

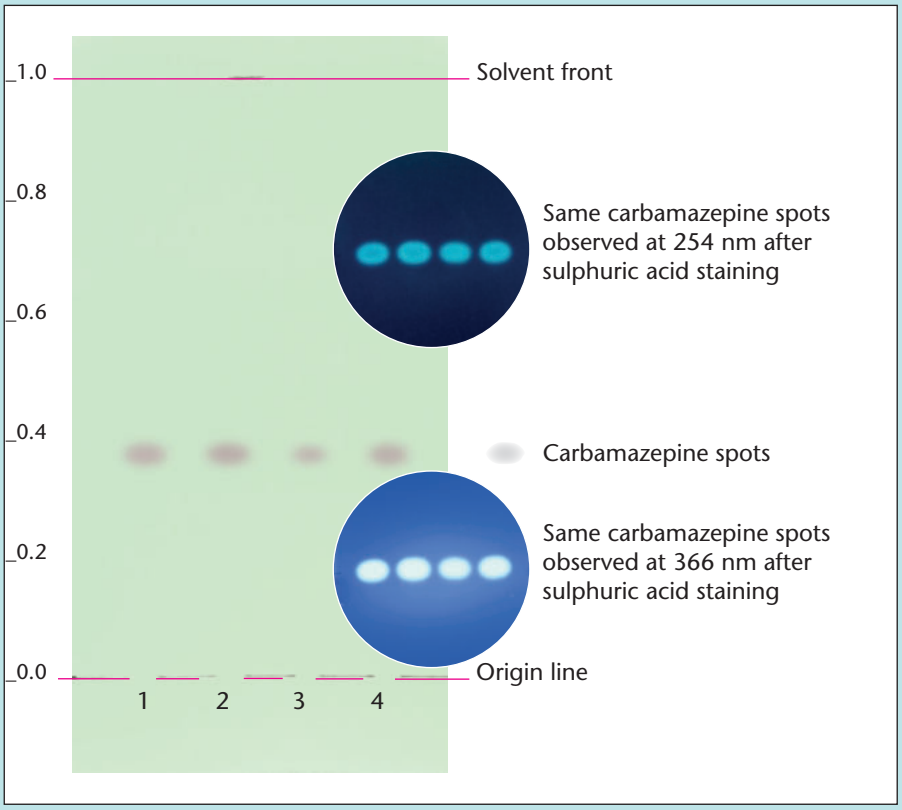
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

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

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



XI. OBSERVATIONS MADE AT 254 NM

A dark spot at a travel distance of about 0.37 indicates the presence of carbamazepine in the test solution. Additional strong spots generated by the test solution would point at other drugs or carbamazepine 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 carbamazepine content and no spot at all a complete absence of carbamazepine. 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 254 AND 366 NM AFTER SULPHURIC ACID STAINING

When the chromatographic plate is stained with sulphuric acid in the heat, no carbamazepine spots are visible in daylight. However, if the stained plate is irradiated with UV light of 254 and 366 nm in a dark room, all carbamazepine spots now show an extremely strong fluorescence, which is hardly ever observed with any other active pharmaceutical ingredient.

XIII. RESULTS & ACTIONS TO BE TAKEN

The carbamazepine 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.

7.115 Empaglifozin incl. linagliptin co-formulations

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During the visual inspection, look for deficiencies in labelling, packaging and dosage forms as described in the introductory chapters on general methods and operations of the main manual and report the results. Consider taking photographs, for example, with a smartphone camera. Each tablet usually contains 10 or 25 mg of empaglifozin. When combined with linagliptin, the dosage strength is reduced to 5 or 12.5 mg of empaglifozin.

Fixed-dose metformin combinations are not approved in the European Union. Check the total weight of the tablets and capsules using the electronic pocket scale provided. All rapid-release empaglifozin tablet and capsule formulations must 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 serious deficiency if a rapid-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 linagliptin, empaglifozin 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) Toluene
20) Methanol
21) n-Butanol
22) Ethyl acetate
23) Ammonia solution 25%
24) Acetic acid solution 96%
25) Sulphuric acid solution 96%
26) Distilled/tap/bottled water
27) Reference agent, for example, empaglifozin 25 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 25 mg of empaglifozin. 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 2.5 mg of total empaglifozin per ml and be labelled as *'Empaglifozin 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 2.5 mg of total empaglifozin 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 empaglifozin.

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 2 mg of total empaglifozin per ml and be labelled as *'Empaglifozin Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of empaglifozin as stated on the product's label. In the current investigation, this level of empaglifozin 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 5 MG OF EMPAGLIFOZIN PER UNIT

Take two whole 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 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 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.

10 MG OF EMPAGLIFOZIN PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 25-ml laboratory glass bottle, add 4 ml of methanol with a suitable graduated pipette and extract the empaglifozin. Continue working as described above.

12.5 MG OF EMPAGLIFOZIN PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 25-ml laboratory glass bottle, add 5 ml of methanol with a suitable graduated pipette and extract the empaglifozin. Continue working as described above.

25 MG OF EMPAGLIFOZIN 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 empaglifozin. Continue working as described above.

Whether or not combined with linagliptin, all stock sample solutions produced should finally contain 2.5 mg of total empaglifozin per ml and be labelled as *'Empaglifozin 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 2.5 mg of empaglifozin per ml. If prepared from a high quality product, the sample solutions should match the concentration of empaglifozin 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 and combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

Finally, dry the spots by placing the TLC plate on the hot heating plate for about 15 seconds.

IX. DEVELOPMENT

Using suitable graduated pipettes, add 11 ml of ethyl acetate, 7 ml of methanol, 1 ml of toluene and 1 ml of ammonia solution 25% to the jar serving as TLC developing chamber. This mobile phase «A» runs fast. An alternative mobile phase «B» consists of 12 ml of n-butanol, 6 ml of water and 3 ml of acetic acid solution 96% runs slower and needs about double time for TLC plate development. But it will help in generating more information on ID and content. After the preparation of the mobile phase, close the chamber each time 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 for mobile phase «A» and about 40 minutes for mobile phase «B». 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 empaglifozin, stain the fresh chromatoplate with sulphuric acid in the heat. To do this, fill the 250 ml plastic beaker provided with 190 ml of methanol, followed by 10 ml of sulphuric acid solution 96%, and mix thoroughly. Allow the mixture to cool and immerse the chromatography plate in the staining solution with the bottom side first. Immediately remove the plate from the solution and allow excess liquid to drain onto a paper towel. Wipe the remaining liquid from the back of the plate and dry the entire staining solution for about 30 to 60 seconds at maximum heat setting on the hot plate provided. After removing the chromatographic plate from the heating plate, view the stained plate at daylight.

XI. OBSERVATIONS MADE AT 254 NM

Mobile phase «A»: A very faint spot at a travel distance of about 0.43 indicates the presence of empaglifozin in the test solution. When combined with linagliptin an additional strong spot appears above empaglifozin with a relative retention factor of about 0.63. Lactose will stay at the origin line and mannitol stays invisible at a travel distance of about 0.10 or below.

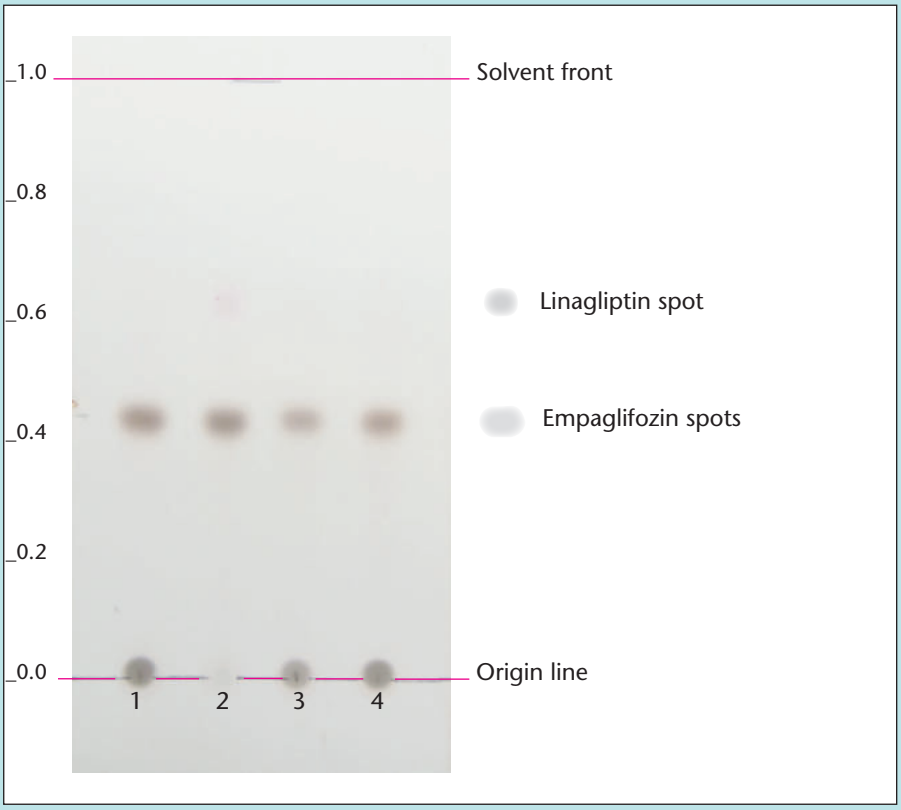
CHROMATOPLATE FROM MOBILE PHASE «A» OBSERVED AT DAYLIGHT AFTER SULPHURIC ACID STAINING

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

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

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

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



Mobile phase «B»: A very faint spot at a travel distance of about 0.69 indicates the presence of empaglifozin in the test solution. When combined with linagliptin an additional strong spot appears below empaglifozin with a relative retention factor of about 0.41. With this mobile phase, lactose will show a retention factor of about 0.14 and mannitol of about 0.25 when detected differently.

XII. OBSERVATIONS MADE AT DAYLIGHT AFTER SULPHURIC ACID STAINING

When the chromatographic plates of both mobile phases are heat-stained with sulphuric acid, all empaglifozin spots turn grey-black and become visible in daylight. Linagliptin spots remain invisible or turn slightly pink. Additional strong spots generated by the test solution would point at other drugs or empaglifozin 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 empaglifozin content and no spot at all complete empaglifozin absence. Auxiliary agents incorporated in different finished products might cause no, faint or partly strong spots either travelling up to the solvent front or lingering near or on the origin line. For example, lactose is a very strong colour former here and also becomes visible. In contrast to lactose, mannitol is hardly visible here but shows an extremely weak fluorescence when the stained TLC plate is additionally exposed to UV of 366 nm.

XIII. RESULTS & ACTIONS TO BE TAKEN

The emaglifozin 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 mobile phase and 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.

7.116 Gliclazide incl. metformin 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 soluble or controlled release tablet usually contains 30, 60 or 80 mg of gliclazide. Other dosage strengths and metformin co-formulations are known to exist. Verify the total weight of tablets and capsules using the electronic pocket balance provided. All quick release gliclazide 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 metformin hydrochloride, gliclazide 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 metformin quality, please 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) Ninhydrin
21) Acetone
22) Methanol
23) Ethyl acetate
24) Ammonia solution 25%
25) Acetic acid solution 96%
26) Reference agent, for example, gliclazide 60 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, single drug tablets containing 60 mg of gliclazide. 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 6 ml of acetone 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 gliclazide per ml and be labelled as *'Gliclazide Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

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

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 3 ml of acetone. Close and shake the vial. The solution obtained should contain 2.5 mg of total drug per ml and be labelled as *'Gliclazide Working Standard Solution 100%'*.

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

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

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 4 ml of acetone using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 2 mg of total gliclazide per ml and be labelled as *'Gliclazide Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of gliclazide as stated on the product's label. In the current investigation, this level of gliclazide 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 30 MG OF GLICLAZIDE 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 with a pestle. Transfer all the powder obtained into a 10- or 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 3 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.

60 MG OF GLICLAZIDE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 25-ml laboratory glass bottle, add 6 ml of acetone with a suitable graduated pipette and extract the gliclazide. Continue working as described above.

80 MG OF GLICLAZIDE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 25-ml laboratory glass bottle, add 8 ml of acetone with a suitable graduated pipette and extract the gliclazide. Continue working as described above.

Whether or not combined with metformin hydrochloride, all stock sample solutions produced should finally contain 10 mg of total gliclazide per ml and be labelled as *'Gliclazide 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 3 ml of acetone. Close and shake the vial and label as *'Gliclazide Working Sample Solution'*.

The expected concentration of gliclazide in this working sample solution is 2.5 mg per ml and should match the concentration of gliclazide 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 page 15 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 and combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

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 15 ml of ethyl acetate, 5 ml of methanol 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 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 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 gliclazide, stain the chromatographic plate with iodine in the iodine chamber and ninhydrin in the heat.

If there is only minimal iodine residue on the plate, the iodine plate can be further used for ninhydrin staining. If there is a lot of iodine on the chromatographic plate, the iodine can be removed by slightly heating the TLC plate. For the subsequent staining, weigh out 3 g of ninhydrin (about 10 times a well-filled spatula) and dissolve it in a mixture of 150 ml of methanol and 30 ml of acetic acid solution 96% in the 250 ml beaker provided. Dip the chromatographic plate, downside first, into the staining solution using tweezers. Immediately remove the plate from the solution and allow excess liquid to drip off onto a paper towel. Wait another minute, wipe off any residual liquid from the back of the plate and then proceed to dry all the staining solution at full heat on the heating plate provided. During heating, gliclacide spots will gradually become visible in daylight after about one minute. The ninhydrin staining procedure is shown on page 36 of the main manual. Note that skin contaminated with ninhydrin solution will also be stained. However, this is not hazardous to health and the purple stains disappear after about one to two days.

XI. OBSERVATIONS MADE AT 254 NM

A weak spot at a travel distance of about 0.29 indicates the presence of gliclazide in the test solution. As metformin hydrochloride is insoluble in acetone, no second spot is visible in test solutions coming from metformin co-formulations. Any residual metformin would stay at the line of origin. Additional strong spots generated by the test solution would point at other drugs or gliclazide degradation, the latter case be-

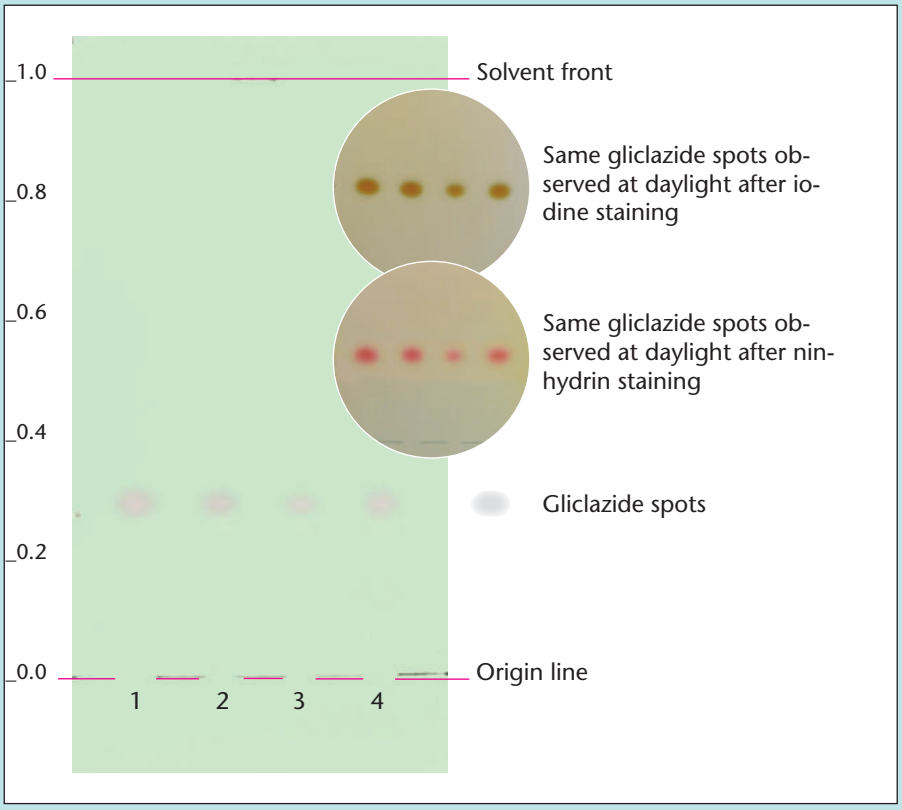
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

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

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



ing more likely when associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor gliclazide content and no spot at all a complete absence of gliclazide. 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 the chromatography plate is stained with iodine, all gliclazide spots previously observed at 254 nm turn orange-brown and become visible in daylight. The staining with iodine is already strong in daylight and the performance becomes even stronger when the TLC plate is again irradiated with UV light of 254 nm.

XIII. OBSERVATIONS MADE AT DAYLIGHT AFTER STAINING WITH NINHYDRIN

After the remaining iodine has been removed from the TLC plate by heating, further staining with ninhydrin can begin. All gliclazide spots turn now pink-red in the heat and so does the background of the chromatography plate, although fortunately both colourations have different shades and intensities.

XIV. RESULTS & ACTIONS TO BE TAKEN

The gliclazide 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.

7.117 Glimepiride

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During the visual inspection, look for deficiencies in labelling, packaging and dosage forms as described in the introductory chapters on general methods and operations of the main manual and report the results. Consider taking photographs, for example, with a smartphone camera. Each tablet or capsule usually contains 1, 2, 3, 4 or 6 mg of glimepiride. Glimepiride may be combined with metformin by adding 500 or even a 1000 mg of metformin hydrochloride salt to the tablet or capsule formulation. Co-formulations with other antidiabetic agents, for example, pioglitazone are known to exist. Check the total weight of the tablets and capsules using the electronic pocket scale provided. All rapid-release glimepiride tablet and capsule formulations must 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 serious deficiency if a rapid-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

Glimepiride 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) in comparison with a suitable secondary standard. It should be noted that due to the unfavourable ratio of glimepiride and metformin hydrochloride (often 1:500 to 1:1000), corresponding co-formulations cannot be processed with this method. For practical reasons alone, the small amount of liquid needed for the extraction of glimepiride is often completely absorbed by the powder obtained from heavy combination tablets. In addition, due to slight spot deformation, quantification of glimepiride from fixed-dose metformin combinations is more than difficult. However, sample preparation and perfect TLC assay results from glimepiride monoformulations are not a problem. For a quick check on metformin quality, please 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) Toluene
20) Methanol
21) Ethyl acetate
22) Ammonia solution 25%
23) Reference agent, for example, glimepiride 6 mg tablets

III. PREPARATION OF THE EXTRACTION SOLVENT

To obtain the ammoniacal methanol solution for glimepiride extraction, work with a mixture of one part of ammonia solution 25 % and 39 parts of methanol. When working with two samples only, the total amount of ammoniacal methanol solution needed to

prepare the control and test solutions does not exceed 20 ml. To do this, mix 0.5 ml of ammonia solution 25 % with 19.5 ml of methanol. For each additional sample, prepare 2 to 6 ml more extraction liquid.

IV. 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 6 mg of glimepiride. 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 10- or 25-ml laboratory glass bottle and wash down all residual solids with 6 ml of ammoniacal methanol solution 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 glimepiride per ml and be labelled as *'Glimepiride Stock Standard Solution'*. Freshly prepare this solution for each test. Continue to work with the clear or hazy supernatant liquid.

V. 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 glimepiride 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 glimepiride.

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

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

VII. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 1 MG OF GLIMEPIRIDE PER UNIT

Take two whole 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- or 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 2 ml of ammoniacal methanol solution 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.

2 MG OF GLIMEPIRIDE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 10- or 25-ml laboratory glass bottle, add 2 ml of ammoniacal methanol solution with a suitable graduated pipette and extract the glimepiride. Continue working as described above.

3 MG OF GLIMEPIRIDE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 10- or 25-ml laboratory glass bottle, add 3 ml of ammoniacal methanol solution with a suitable graduated pipette and extract the glimepiride. Continue working as described above.

4 MG OF GLIMEPIRIDE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 10- or 25-ml laboratory glass bottle, add 4 ml of ammoniacal methanol solution with a suitable graduated pipette and extract the glimepiride. Continue working as described above.

6 MG OF GLIMEPIRIDE PER UNIT

Place the powder obtained from a whole sample tablet or capsule into a 10- or 25-ml laboratory glass bottle, add 6 ml of ammoniacal methanol solution with a suitable graduated pipette and extract the glimepiride. Continue working as described above.

Whether or not combined with other antidiabetic agents, all stock sample solutions produced should finally contain 1 mg of total glimepiride per ml and be labelled as *'Glimepiride Stock Sample Solution'*. Freshly prepare these solutions for each test. Continue working with the clear or hazy supernatant liquids.

VIII. 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 glimepiride per ml. If prepared from a high quality product, the sample solutions should match the concentration of glimepiride 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.

IX. 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 and combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

Dry the spots. To do this, place the chromatoplate onto the hot heating plate for about 10 seconds.

X. DEVELOPMENT

Using suitable graduated pipettes, add 12 ml of toluene, 8 ml of ethyl acetate, 4 ml of methanol and 0.05 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.

XI. DETECTION

After drying off all solvent residues, view the chromatographic plate under UV light at 254 nm with the battery driven lamp provided. This detection method will be fit for the identification and quantification of glimepiride.

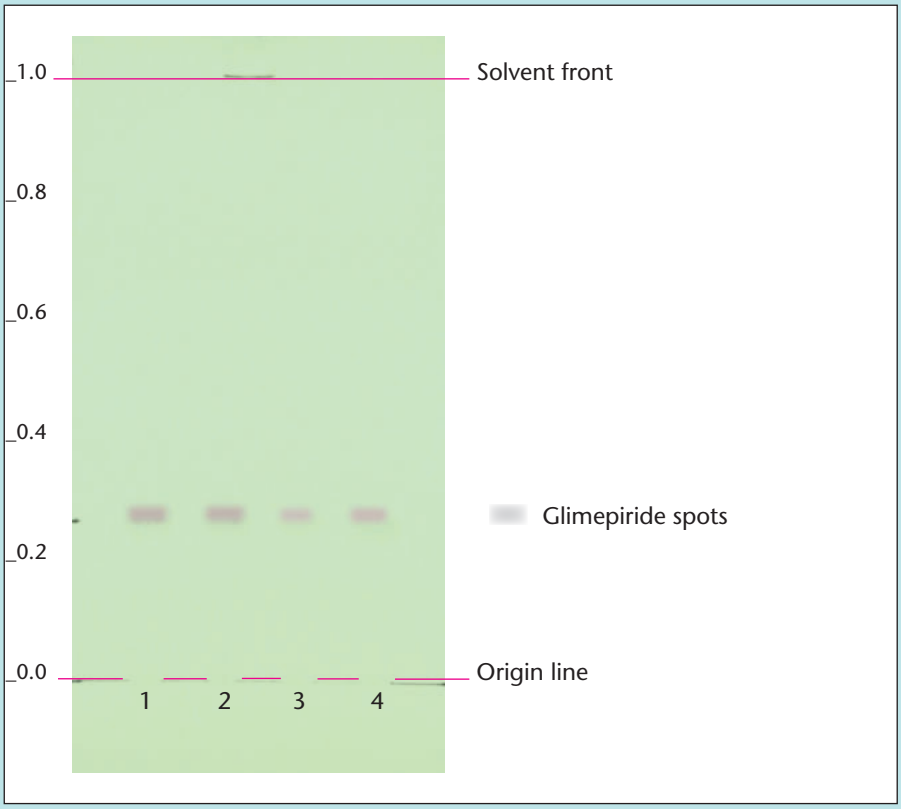
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

Run No.2:
A glimepiride single drug product of good quality with acceptable drug content

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

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



XII. OBSERVATIONS MADE AT 254 NM

A dark spot at a travel distance of about 0.28 indicates the presence of glimepiride in the test solution. When combined with metformin hydrochloride, a bold spot of metformin appears on the line of origin, and when additionally combined with pioglitazone, a third spot with a small tail would appear at a distance of about 0.38 directly above the glimepiride spot. The mobile phase consisting of toluene:ethyl acetate:acetic acid solution 96% (12:8:1 v/v), in which pioglitazone settles below glimepiride and where metformin settles again at the line of origin, may be considered slightly better for separation. Additional strong spots produced by the test solution would indicate other drugs or glimepiride degradation, the latter case being more likely if associated with a smaller principal spot. A smaller principal spot from the test solution may also indicate a poor glimepiride content and an absent spot may indicate a complete absence of glimepiride. Excipients present in various finished products may cause no or faint spots that either migrate to the solvent front or linger near or on the line of origin.

XIII. RESULTS & ACTIONS TO BE TAKEN

The glimepiride 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.

7.118 Sitagliptin hydrochloride/phosphate/tartrate/malate incl. their hydrates, with or without metformin

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During the visual inspection, look for deficiencies in labelling, packaging and dosage forms as described in the introductory chapters on general methods and operations of the main manual and report the results. Consider taking photographs, for example, with a smartphone camera. Whether sitagliptin comes as a hydrochloride, phosphate, tartrate, fumarate or malate salt, each tablet usually contains 25, 50 or 100 mg of sitagliptin per free base.

Sitagliptin may be combined with metformin by adding 500, 850 or 1000 mg of metformin hydrochloride salt to the tablet or capsule formulation. Check the total weight of the tablets and capsules using the electronic pocket scale provided. All rapid-release sitagliptin tablet and capsule formulations must 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 serious deficiency if a rapid-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 metformin hydrochloride, sitagliptin hydrochloride/phosphate/tartrate/ malate 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. For a quick check on metformin quality, please 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) Toluene
21) Methanol
22) Ethyl acetate
23) Ammonia solution 25%
24) Reference agent, for example, sitagliptin 50 mg tablets presented as phosphate monohydrate salt

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 sitagliptin 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 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 sitagliptin per ml and be labelled as *'Sitagliptin 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 5 mg of total sitagliptin 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 sitagliptin.

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 4 mg of total sitagliptin per ml and be labelled as *'Sitagliptin Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of sitagliptin as stated on the product's label. In the current investigation, this level of sitagliptin 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 SITAGLIPTIN 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 SITAGLIPTIN 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 sitagliptin. Continue working as described above.

100 MG OF SITAGLIPTIN PER UNIT

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

Whether or not combined with metformin hydrochloride, all stock sample solutions produced should finally contain 5 mg of total sitagliptin per ml and be labelled as *'Sitagliptin 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 5 mg of sitagliptin per ml. If prepared from a high quality product, the sample solutions should match the concentration of sitagliptin 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 and combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

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 11 ml of ethyl acetate, 7 ml of methanol, 1 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.

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 sitagliptin, stain the chromatoplate with iodine in the iodine chamber.

XI. OBSERVATIONS MADE AT 254 NM

A dark spot at a travel distance of about 0.68 indicates the presence of sitagliptin in the test solution. When combined with metformin hydrochloride a broad track of metformin appears below sitagliptin with a "relative retention factor" starting from zero and ending at about 0.38. Additional strong spots generated by the test solution

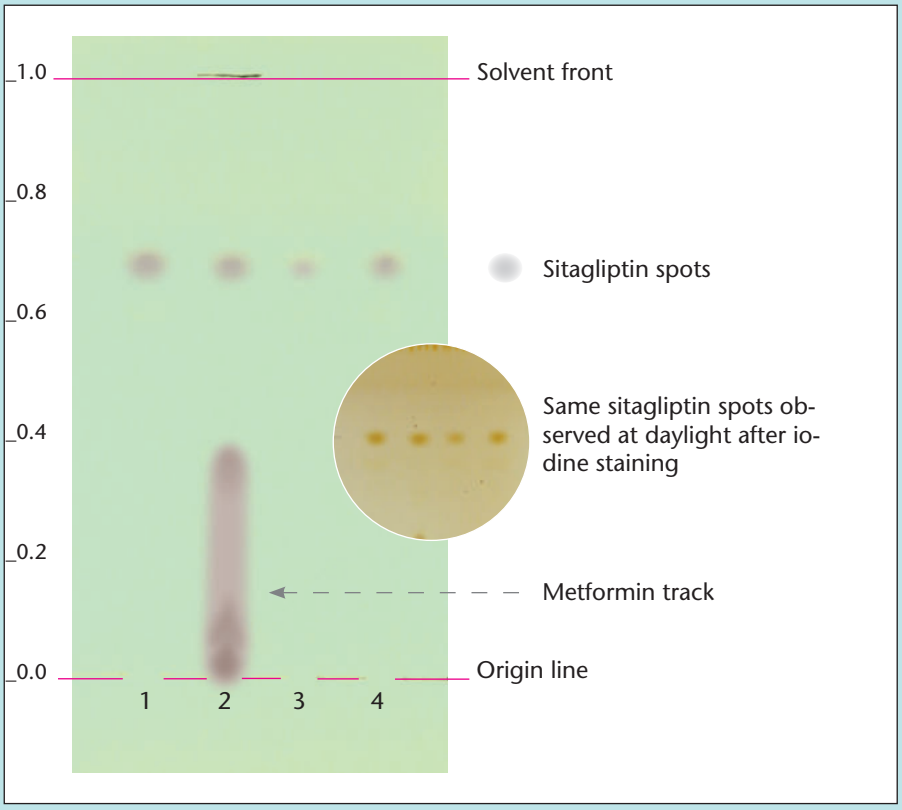
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

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

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



would point at other drugs or sitagliptin 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 sitagliptin content and no spot at all a complete sitagliptin absence. 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 sitagliptin 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 sitagliptin 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.

7.119 Vildagliptin incl. metformin co-formulations and related linagliptin and sitagliptin

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During the visual inspection, look for deficiencies in labelling, packaging and dosage forms as described in the introductory chapters on general methods and operations of the main manual and report the findings. Consider taking photographs, for example, with a smartphone camera. Each tablet usually contains 50 mg of vildagliptin per free base. Vildagliptin may be combined with metformin by adding 850 or a 1000 mg of metformin hydrochloride salt to the tablet or capsule formulation. Other dosage strengths are known to exist. Check the total weight of the tablets and capsules using the electronic pocket scale provided. All rapid-release vildagliptin tablet and capsule formulations must 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 serious deficiency if a rapid-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 metformin, vildagliptin is extracted from tablets or capsules with a known volume of acetone and then checked for identity and content by thin layer chromatography (TLC) in comparison with a suitable secondary standard. However, with some formulations matrix problems occur especially when containing hydroxypropyl cellulose. This and other polymers may nullify the assay. For a quick check of metformin quality, please 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) Acetone
22) Toluene
23) Methanol
24) n-Butanol
25) Ethyl acetate
26) Ammonia solution 25%
27) Acetic acid solution 96%
28) Sulphuric acid solution 96%
29) Distilled/tap/bottled water
30) Reference agent, for example, vildagliptin 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 vildagliptin. 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 5 ml of acetone 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 vildagliptin per ml and be labelled as *'Vildagliptin 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 10 mg of total vildagliptin 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 vildagliptin.

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 acetone using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 8 mg of total vildagliptin per ml and be labelled as *'Vildagliptin Working Standard Solution 80%'*.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of vildagliptin as stated on the product's label. In the current investigation, this level of vildagliptin 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 50 MG OF VILDAGLIPTIN 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 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.

Whether or not combined with metformin, all stock sample solutions produced should finally contain 10 mg of total vildagliptin per ml and be labelled as *'Vildagliptin 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 10 mg of vildagliptin per ml. If prepared from a high quality product, the sample solutions should match the concentration of vildagliptin 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 and combinations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if homogeneous spotting is not achieved first time.

Dry the spots. To do this, place the chromatoplate onto the hot heating plate for about 15 seconds.

IX. DEVELOPMENT

Mobile phase «A» for vildagliptin quantification: Using suitable graduated pipettes, add 11 ml of ethyl acetate, 7 ml of methanol, 1 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.

Mobile phase «B» for the separation of vildaglipitin, linagliptin and sitagliptin after their extraction with methanol (VIL 10 mg/ml, LIN 1.25 mg/ml, SIT 5 mg/ml): Add 12 ml of n-butanol, 3 ml of methanol, 3 ml of water and 3 ml of acetic acid solution 96% to the jar serving as TLC developing chamber and mix. Wait 15 minutes for chamber saturation. Then place the loaded TLC plate into the jar, close it and develop the chromatoplate for about 40 minutes. Remove the TLC plate from the chamber, mark the solvent front and carefully dry the plate in the hot air stream over the heating plate for about two minutes.

X. DETECTION

After drying of all solvent residues, the chromatographic plate is viewed under UV light of 254 nm with the battery operated lamp provided. For initial identification and quantification of vildagliptin, stain the chromatography plate with iodine in the iodine chamber and view in daylight and again under UV light at 254 nm.

Also stain the iodine plate with sulphuric acid in the heat and view the obtained plate under UV light at 366 nm. To do this, fill the 250 ml plastic beaker provided with 190 ml of methanol, followed by 10 ml of sulphuric acid solution 96%, and mix thoroughly. Allow the mixture to cool and immerse the chromatography plate bottom first into the staining solution, at the same time ensuring by deep immersion that the vildagliptin spot located on the upper part of the chromatography plate is captured, too. Instantly remove the plate again from the solution and allow excess liquid to drain onto a paper towel. Wipe the remaining liquid from the back of the plate and dry the entire staining solution for about 30 to 60 seconds at maximum heat setting on the hot plate provided. After removing the chromatographic plate from the heating plate, view the stained plate at UV light of 366 nm.

XI. OBSERVATIONS MADE AT 254 NM BEFORE IODINE STAINING

If the TLC plate is from mobile phase «A», no spots should be visible. Vildagliptin is not detectable at 254 nm and when combined with metformin hydrochloride, metformin is cut out of the system by using acetone for extraction, in which metformin HCl is not soluble. Any residual metformin would remain at the line of origin. However, using mobile phase «B» to separate the gliptins reveals a strong spot for linagliptin and a weaker spot for sitagliptin.

XII. OBSERVATIONS MADE IN DAYLIGHT AFTER IODINE STAINING

When the chromatoplate from mobile phase «A» is exposed to iodine vapour, all vildagliptin spots turn yellowish-brown and become visible at a distance of about 0.59. Vildagliptin spots from formulations containing highly dispersed silica migrate very slightly less. Additional strong spots in the test solution would indicate other drugs or degradation of vildagliptin, the latter case being more likely when associated with a smaller main spot. A smaller main spot in the test solution may also indicate low vildagliptin content, and an absent spot may indicate a complete absence of vildagliptin.

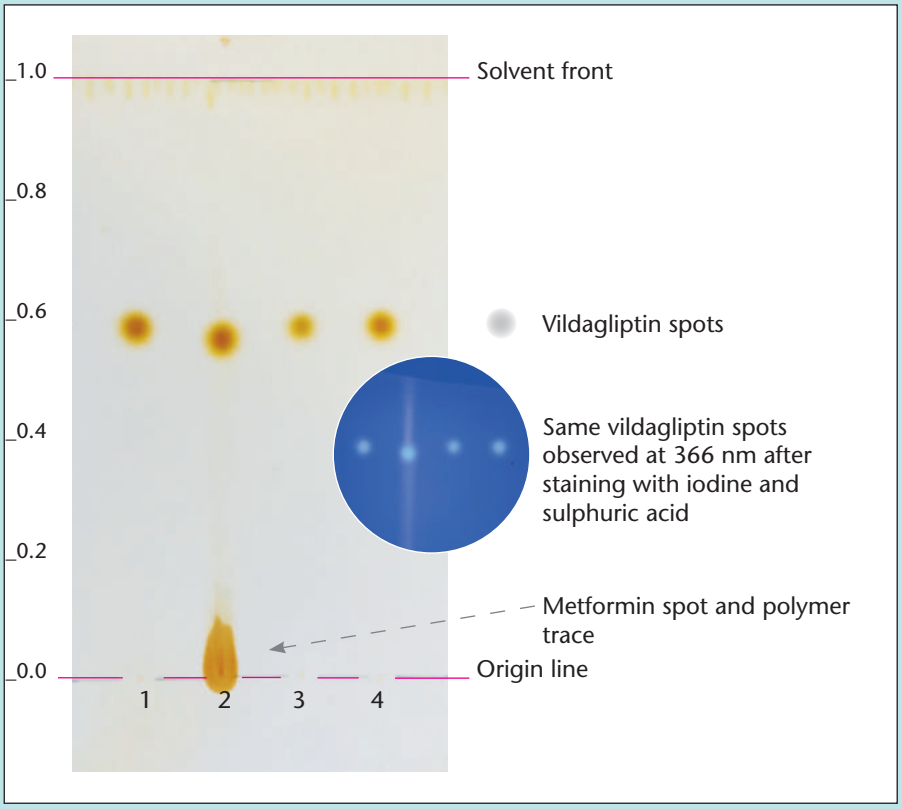
CHROMATOPLATE FROM THE MOBILE PHASE «A» OBSERVED AT DAYLIGHT AFTER IODINE STAINING

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

Run No.2:
A fixed-dose metformin combination of good quality with acceptable vildagliptin content and povidone polymer and silica in the matrix

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

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



Excipients present in different finished products may cause no or faint spots that either migrate to the solvent front or linger near or on the line of origin. Polymers may cause complete tracks from the origin to the front line instead of spots. Now, vildagliptin is also visible on the TLC plate from the separation of gliptins, this time next to linagliptin. The staining of sitagliptin is only faint or not pronounced at all.

XIII. OBSERVATIONS MADE AT 254 NM AFTER IODINE STAINING

All spots previously stained with iodine now turn very dark. This also applies to sitagliptin, so that for the first time all three gliptins can be observed simultaneously on the chromatoplate from mobile phase «B». Vildagliptin having a relative retention factor of about 0.37, linagliptin of about 0.48 and sitagliptin of about 0.58.

XIV. OBSERVATIONS MADE AT 366 NM AFTER IODINE AND SULFURIC ACID STAINING

With additional staining of the iodine plate with sulphuric acid and heat, all vildagliptin spots produce clear white fluorescence. The presence of iodine and heat is important. On cooling, this fluorescence disappears but may eventually be reactivated by heating the TLC plate again. The assays work best and interference from the matrix can be minimised if the standing time is extended during sample preparation to allow the majority of the very fine silica particles to sink to the bottom of the vessel.

XV. RESULTS & ACTIONS TO BE TAKEN

The vildagliptin 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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