

Manual

Accompanying the GPHF-Minilab™

COVID-19 Special Issue
- Dexamethasone tablets
- Dexamethasone injections

Physical Testing & Thin-Layer Chromatography



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A charity voluntarily supported by Merck KGaA, Darmstadt, Germany



USAID
FROM THE AMERICAN PEOPLE



A Concise Quality Control Guide on Essential Drugs and other Medicines

COVID-19 SPECIAL ISSUE

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About the GPHF-Minilab™ Project

Substandard and falsified medicines proliferation poses a serious health threat. The World Health Organization (WHO) estimates that a worrying proportion of ten percent of all medicines available in developing countries are already either falsified or of poor quality. Fighting falsified medicines will ensure that decades of investments in healthcare are not undone through lack of vigilance.

Given the prevalence of counterfeit and poor quality medicines around the world, with the highest burden in developing countries, the Global Pharma Health Fund (GPHF), a non-profit organization solely supported by Merck KGaA, has developed and cost-effectively provides the GPHF-Minilab™, a mini-laboratory for the detection of substandard and falsified medicines.

For many years, the GPHF-Minilabs have served as a first line of defence against fake and low-quality medicines that threaten the health of millions of people in developing countries. In total, more than 900 Minilabs have been delivered to nearly 100 countries in the African, Asia-Pacific and Latin American regions.

GPHF-Minilabs are typically used for medical quality screening for risk-based post-market surveillance, at border controls for inspection, within the supply chain and at the point of care/dispense for due diligence, etc. Minilabs are portable, easy to use, cost-effective, require minimal training and provide rapid results for identification, quantification and disintegration. GPHF-Minilabs do not replace full compendium laboratory testing. Rather, they use their technology to identify defective medicines for full laboratory testing, which has become established best practice for screening technologies.

The Minilabs' method inventory currently includes 100 active pharmaceutical ingredients in their respective finished products for the treatment of communicable and non-communicable diseases, for example antibiotics, antituberculostatics, antimalarials, antivirals, antidiabetics, anthiasthmatics, analgesics, and cardiovascular and gastrointestinal medicines. These are now joined by dexamethasone used to alleviate some symptoms seen in severe COVID-19 cases.

Main implementation partners are national health and medicines regulatory authorities together with the World Health Organization (WHO) and USAID's Promoting the Quality of Medicines Plus program. The data generated with the GPHF-Minilab™ efficiently pointed out fake antimalarial and antibacterial medicines without active ingredients and triggered global medical product alerts from the WHO several times. Minilabs save lives.

COVID-19 increases the unmet need for non-sophisticated and affordable drug quality monitoring in low-income countries. This is the driving force behind the development of new GPHF-Minilab™ testing protocols for dexamethasone tablets and injections today. The need for more testing also underlines the importance of collaboration, for example with our US based implementing partners.

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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 2020 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.

7.101 Dexamethasone in tablets

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During visual inspection, search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual issued 2020 and report the findings. Consider to take pictures, for example, with a smartphone camera. Each tablet usually contains 2, 4 or 8 mg of dexamethasone per free base. Other dosage strengths are known to exist. Verify the total weight of tablets using the electronic pocket balance supplied. All quick release dexamethasone tablet

formulations must also pass the disintegration test as described in the 2020 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 written in a foreign language, or stored in poor conditions, should be subjected to a thin-layer chromatographic test.

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Dexamethasone is extracted from tablets with a known volume of methanol and then checked for identity and content by TLC with reference to a suitable secondary standard.

II. EQUIPMENT AND REAGENTS

- 1) Pestle
- 2) Aluminium foil
- 3) Funnel
- 4) 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 F₂₅₄ 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) TLC dipping chamber (250-ml beaker)
- 21) Distilled or drinking water
- 22) Methanol
- 23) Acetone
- 24) Ethyl acetate
- 25) Sulphuric acid solution 96%
- 26) Reference agent, for example, dexamethasone 8 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 8 mg of dexamethasone: 1) Wrap up one reference tablet into aluminium foil and crush it down to a fine powder using a pestle. 2) 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. 3) Close the bottle and shake for about three minutes until most of the solids are dissolved. 4) Allow the solution to sit for an additional five minutes until undissolved residues settle below the supernatant liquid. The solution obtained should contain 1 mg of total dexamethasone per ml and be labelled as '*Dexamethasone Stock Standard Solution*'. Freshly prepare this solution for each test. 5) Continue to work with the clear or hazy supernatant liquid.

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

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

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

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

Pipette 4 ml of the stock standard solution into a 10-ml vial and add 1 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.8 mg of total dexamethasone per ml and be labelled as '*Dexamethasone Working Standard Solution 80%*'.

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

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A PRODUCT CLAIMING TO CONTAIN 2 MG OF DEXAMETHASONE PER TABLET

Take two whole tablets from an appropriate drug product sampled in the field. As usual, wrap them up into aluminium foil and crush them down to a fine powder. Transfer all the powder obtained into a 25-ml laboratory glass bottle. For extraction, add 4 ml of methanol using a graduated pipette, close the bottle and shake for about three minutes until most of the solids are dissolved. Allow the solution to sit for an additional five minutes until undissolved residues settle below the supernatant liquid.

4 MG OF DEXAMETHASONE PER TABLET

Transfer the powder obtained from one whole sample tablet into a 25-ml laboratory glass bottle, add 4 ml of methanol using a graduated pipette and extract the dexamethasone. Continue to work as above.

8 MG OF DEXAMETHASONE PER TABLET

Transfer the powder obtained from one whole sample tablet into a 25-ml laboratory glass bottle, add 8 ml of methanol using a graduated pipette and extract the dexamethasone. Continue to work as above.

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

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

VIII. SPOTTING

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

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

Gently dry the spots. For this, hold the chromatoplate with the tweezers supplied for approx. 30 seconds in the stream of hot air just above the heating plate. Shake the TLC plate constantly and each moment the chromatography plate flips down, it may touch the surface of the heating plate for fractions of a second.

IX. DEVELOPMENT

Pipette 20 ml of ethyl acetate, 2 ml of acetone and 0.2 ml of water into the jar being used as TLC developing chamber. Close the chamber and mix thoroughly. Line the chamber's wall with filter paper and wait for about 15 minutes thus ensuring saturation of the chamber with solvent vapour. Carefully place the loaded TLC plate into the jar. Close the jar and develop the chromatoplate until the solvent front has moved about three-quarters of the length of the plate, the developing time being about 10 minutes. Remove the plate from the chamber, mark the solvent front and allow any excess solvent to evaporate. For this, hold the chromatoplate with the tweezers supplied for approx. two minutes in the stream of hot air just above the heating plate. Shake the TLC plate constantly and each moment the chromatography plate flips down, it may touch the surface of the heating plate for fractions of a second.

X. DETECTION

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

For further identification and semi-quantification, stain the chromatoplate with sulphuric acid in the heat. For this, fill the 250-ml plastic beaker supplied with a 190 ml of methanol followed by 10 ml of concentrated sulphuric acid solution 96% and mix gently. Let the mixture cool down and dip the chromatographic plate into the staining solution using tweezers. Immediately remove the plate from the solution and drain off all excess liquid onto a paper towel. Wipe the remaining liquid from the back of the plate and continue to dry all the staining solution on the heating plate provided. During heating, any dexamethasone spots and spots from other related corticosteroids will gradually become visible in daylight. Note that the staining procedure with sulphuric acid solution is very similar to that described with ninhydrin on page 36 in the 2020 main manual. After reading the chromatoplate in daylight, further verification of the identity and content of dexamethasone can be done if the chromatoplate is exposed to UV light of 366 nm in a dark room.

XI. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.44 indicates the presence of dexamethasone in the test solution. Additional strong spots generated by the test solution would point at other drugs or dexamethasone 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 dexamethasone content and no spot at all a

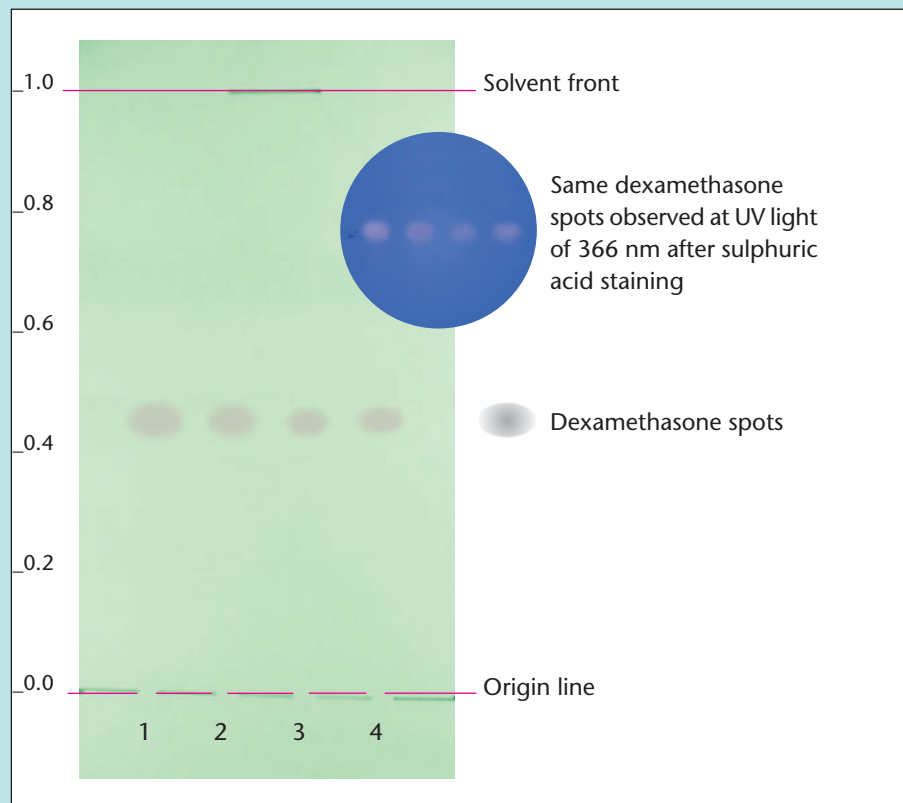
CHROMATOPLATE OBSERVED UNDER
UV LIGHT OF 254 NM

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

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

Run No.3:
A product of poor quality with unaccept-
able low dexamethasone content

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



complete dexamethasone absence. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line. Other related corticosteroids are clearly separated from the dexamethasone spot and their relative retention factors are as follows: about 0.36 for prednisone, about 0.32 for prednisolone, about 0.34 for methylprednisolone and about 0.37 for hydrocortisone. Note that non-pharmaceutical grade filling agents may contain methyl or propyl paraben as preservatives, which may appear at a relative retention factor of about 0.64.

XII. OBSERVATIONS MADE AT DAY-
LIGHT AFTER SULPHURIC ACID
STAINING

With further exposure of the chromatography plate to sulphuric acid and heat, the dexamethasone spots previously observed at 254 nm now turn grey, with different intensities indicating different drug concentrations. Other related corticosteroids behave very similarly, with the exception of hydrocortisone, which turns dirty yellow here.

XIII. OBSERVATIONS MADE AT 366 NM
AFTER SULPHURIC ACID STAINING

When the stained plate is exposed to UV light of 366 nm in a dark room, dexamethasone and each spot of other related corticosteroids now show variations of a very weak dark pink to dark blue colour with the exception of hydrocortisone, which shows a bright white fluorescence here. In any case, dexamethasone, with its prominent relative retention factor of about 0.44, always moves to the front of the pack, setting it apart from all other corticosteroids.

XIV. RESULTS & ACTIONS TO BE TAKEN

The dexamethasone 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.

7.102 Dexamethasone phosphate disodium salt in solutions for injection

Primary Screening on Product Deficiencies by Physical Testing

I. PHYSICAL TESTING

During visual inspection, look for deficiencies in labelling, packaging and dosage form as described in the introductory chapters on general methods and operations of the main manual issued in 2020 and report the findings. Consider taking pictures, for example, with a smartphone camera. Each ml of sterile solution usually contains about 3.3 mg of dexamethasone (as disodium phosphate) which is equivalent to about 4 mg of dexamethasone 21-phosphate

or about 4.37 mg of dexamethasone 21-phosphate disodium salt. Other dosage strengths are known to exist, for example, the strength in the current WHO list of essential medicines for adults or children is 4 mg of dexamethasone per free base (presented as disodium phosphate salt) per ml. The aqueous content of dexamethasone phosphate ampoules and vials looks clear and colourless and shows no foreign matters/particles. If this is not the case, it is a significant deficiency.

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

Verification of Drug Identity and Content by Thin-Layer Chromatography

I. PRINCIPLE

Injectable solutions containing dexamethasone sodium phosphate are diluted with a known volume of water and the identity and content of dexamethasone phosphate are then checked by TLC with reference to a suitable control solution.

Important Note: Dexamethasone sodium phosphate salt is hygroscopic and very unstable at ambient temperature and therefore already difficult to handle in the fully-fledged laboratory and almost impossible to handle in the field as a reference agent. When comparing finished pharmaceutical products against each other to bypass the stability problems observed with solid reference standards, great care must be taken that both the reference and sample solutions have exactly the same dexamethasone phosphate content, for example, product samples containing 4 mg of dexamethasone phosphate (equivalent to about 4.37 mg of dexamethasone sodium phosphate) per ml of injectable solution can only be compared with reference solutions also containing 4 mg of dexamethasone phosphate per ml of injection fluid. Ready-to-use dexamethasone injection solutions can usually be stored in their original packaging protected from light at 25 °C for about two to three years. The actual storage instructions and expiration date on the label apply only.

II. EQUIPMENT AND REAGENTS

- 1) Marker pen
- 2) Label tape
- 3) Pencil and ruler
- 4) 10-ml vials
- 5) Set of graduated pipettes (1 to 25 ml)
- 6) Set of laboratory glass bottles (25 to 100 ml)
- 7) Merck TLC aluminium plates pre-coated with silica gel 60 F₂₅₄ size 5x10 cm
- 8) Glass microcapillaries (2-µl filling capacity)
- 9) TLC developing chamber (500-ml jar)
- 10) Hot plate
- 11) Filter paper
- 12) Pair of scissors
- 13) Pair of tweezers
- 14) UV light of 254 nm
- 15) UV light of 366 nm
- 16) TLC dipping chamber (250-ml beaker)
- 17) Distilled or drinking water
- 18) Methanol
- 19) n-Butanol
- 20) Acetic acid solution 96%
- 21) Sulphuric acid solution 96%
- 22) Reference solution, for example, 8 mg of dexamethasone phosphate in 2-ml ampoules each ml containing 4 mg of dexamethasone phosphate or 4.37 mg of dexamethasone phosphate disodium salt

III. PREPARATION OF THE STOCK STANDARD SOLUTION

For the preparation of the stock standard solution, an authentic medicinal product is required as a reference, for example, dexamethasone phosphate 8 mg ampoules from reputable sources containing 2 ml of injection solution, each ml containing 4 mg of dexamethasone phosphate: 1) Before opening, shake and tap the ampoule vigorously several times onto a soft surface, e.g. a book, so that the liquid in the ampoule head runs down and combines with the liquid in the ampoule body. 2) Then break off the empty ampoule head and transfer the entire solution from the ampoule body into a 10 ml laboratory glass bottle. 3) To force the solution through the narrow neck of the ampoule, it may be useful to loosely connect the open neck of the ampoule to the open mouth of the laboratory glass bottle and shake this construction, with the ampoule sitting upside down on the laboratory bottle, vigorously from top to bottom. 4) Close the laboratory bottle after a full transfer of the solution is completed. Unfortunately, simple decanting does not work. 5) Label as '*Dexamethasone phosphate/DP stock standard solution*'. No further dilution is necessary.

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

Pipette 1 ml of the stock standard solution into a 10-ml vial and add 7 ml of water using appropriate graduated pipettes. Close and shake the vial. The solution obtained should contain 0.5 mg of total dexamethasone phosphate per ml and be labelled as '*DP Working Standard Solution 100%*'. Freshly prepare this solution for each test. Continue to work with the clear solution diluted.

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

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

Pipette 0.5 ml of the stock standard solution into a 10-ml vial and add 4.5 ml of water using appropriate graduated pipettes. Close and shake the vial. The solution obtained should contain 0.4 mg of total dexamethasone phosphate per ml and be labelled as '*DP Working Standard Solution 80%*'.

This lower working standard solution represents a medicinal product of poor quality containing just 80% of the amount of dexamethasone phosphate as stated on the product's label. In the current investigation, this level of dexamethasone phosphate 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 4 MG OF DEXAMETHASONE PHOSPHATE PER 1-ML VIAL/AMPOULE

Take a 4 mg ampoule and first check that the dexamethasone phosphate content indicated on the label is the same between the sample and the control solution. Then transfer the entire liquid content into a 10 ml laboratory glass bottle in the same way as described above for the reference ampoule. Obtain 0.5 ml of injection solution from the laboratory bottle using a suitable graduated pipette and combine with 3.5 ml of water in a second 10 ml laboratory bottle. Close, shake and label. Note that handling vials is much easier than handling ampoules, as pipetting can be done directly through the opening from them without having to use a laboratory vial.

8 MG OF DEXAMETHASONE PHOSPHATE PER 2-ML VIAL/AMPOULE

Take an 8 mg ampoule and first check that the dexamethasone phosphate content indicated on the label is a perfect match between the sample and the control solution. Then transfer the entire liquid content into a 10-ml laboratory glass bottle in the same way as described above for the reference ampoule. Obtain 1 ml of injection solution from the laboratory bottle using a suitable graduated pipette and combine with 7 ml of water in a second 10-ml laboratory bottle. Close, shake and label. Note that handling of vials is much easier than handling ampoules as pipetting can be done directly through the opening from them without having to use a laboratory vial.

All stock sample solutions produced should finally contain 0.5 mg of total dexamethasone phosphate per ml and be labelled as '*DP Stock Sample Solution*'. Freshly prepare these solutions for each test. Continue to work with the clear solutions diluted.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Dexamethasone phosphate stock sample solutions require no further dilution. They already represent the final working concentration of 0.5 mg of dexamethasone phosphate per ml. If prepared from a high quality product, the sample solution should match the concentration of dexamethasone phosphate 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 tweezers supplied for approx. 2 minutes in the stream of hot air just above the heating plate. Shake the TLC plate constantly and each moment the chromatography plate flips down, it may touch the surface of the heating plate for fractions of a second.

IX. DEVELOPMENT

Pipette 12 ml of n-butanol, 4 ml of water and 4 ml of acetic acid solution 96% into the jar used as TLC development chamber. The mobile phase taken is the same as in the European Pharmacopoeia edition 10.0. Close the chamber and mix thoroughly. Line the chamber wall with filter paper and wait about 15 minutes to ensure saturation of the chamber with solvent vapour. Carefully place the loaded TLC plate into the jar. Close the jar and **develop the chromatographic plate until the solvent front has moved about fifty percent (!) of the total plate length, with a development time of about 30 minutes.** When extending the time for development to 60 minutes, molecular diffusion will start to affect the shape of the dexamethasone phosphate spots. Remove the TLC plate from the chamber, mark the solvent front and let excess solvents to evaporate from the chromatographic plate. Acetic acid and n-butanol are low volatility solvents where the hot plate is normally used for evaporation from the TLC plate over a longer period of time. On the other hand, the dexamethasone phosphate spots are very sensitive to heat and the TLC plate must not be overheated. Therefore, allow residual mobile phase to evaporate for approx. 30 seconds putting the TLC plate directly on the heating plate provided and then continue to dry the chromatographic plate for approx. four minutes in the stream of hot air directly above the heating plate using the tweezers provided. To do this, shake the chromatography plate constantly and each time the TLC plate flips down, it should also touch the surface of the heating plate for fractions of a second. Remember that any overheating will cause the dexamethasone phosphate spots to disappear. Alternatively, the TLC plate can be dried with a hair dryer.

X. DETECTION

Dry off any residual solvent and observe the chromatoplate under UV light of 254 nm with the battery-powered lamp provided preferably in a dark room. Use this detection method for both identification and quantification.

For further identification and semi-quantification, heat stain the chromatoplate with sulphuric acid. To do this, fill the 250 ml plastic beaker provided with 190 ml of methanol followed by 10 ml of concentrated 96% sulphuric acid solution and mix gently. Let the mixture cool down and dip the chromatography plate into the staining solution using tweezers. Immediately remove the plate from the solution and allow all excess liquid to drip off onto a paper towel. Wipe the remaining liquid from the back of the plate and proceed to dry all the staining solution on the heating plate provided. During heating, all dexamethasone phosphate spots will gradually become visible in daylight after about one minute. After reading the chromatographic plate in daylight, further verification of the identity and content of dexamethasone phosphate can be done by exposing the TLC plate to UV light of 366 nm in a dark room.

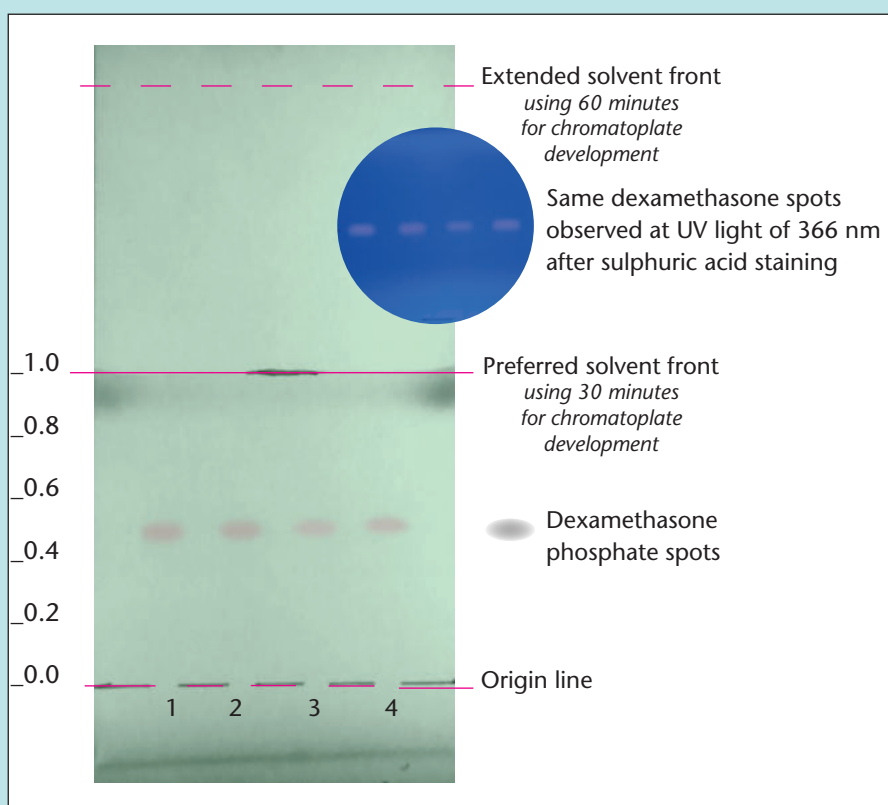
CHROMATOPLATE OBSERVED UNDER UV LIGHT OF 254 NM

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

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

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

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



XI. OBSERVATIONS MADE AT 254 NM

A blue-violet spot at a travel distance of about 0.49 indicates the presence of dexamethasone phosphate in the test solution. Additional strong spots generated by the test solution would point at other drugs or dexamethasone phosphate 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 dexamethasone content and no spot at all a complete dexamethasone phosphate absence. Auxiliary agents incorporated in different finished products might cause some fainter spots either travelling alongside the solvent front or emerging near or on the origin line. Just for information: If dexamethasone were present in the form of its free base, it would travel a distance of about 0.87.

XII. OBSERVATIONS MADE AT DAY-LIGHT AFTER SULPHURIC ACID STAINING

Upon further exposure of the chromatographic plate to sulphuric acid and heat, the dexamethasone phosphate spots previously observed at 254 nm now turn grey, with different intensities indicating different drug concentrations.

XIII. OBSERVATIONS MADE AT 366 NM AFTER SULPHURIC ACID STAINING

Upon further exposure of the stained chromatographic plate to UV light of 366 nm in a dark room, dexamethasone phosphate spots show a weak reddish-grey fluorescence.

XIV. RESULTS & ACTIONS TO BE TAKEN

The dexamethasone phosphate 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 nearly 100 countries.

This supplement is a special issue on priority dexamethasone medication for symptom relief in severe COVID-19 cases in hospital.

The method inventory of the GPHF-Minilab main manual, published in 2020, consists of a collection of test procedures for more than 100 active pharmaceutical ingredients for the rapid verification of medical quality for a wide range of finished pharmaceutical products.



Global Pharma Health Fund
www.gphf.org