A Concise Quality Control Guide On Essential Drugs

Manual

Accompanying The GPHF-Minilab®

Volume II
Thin Layer Chromatography

GPHF
German Pharma Health Fund e.V.

An Initiative of Research Based Pharmaceutical Companies in Germany

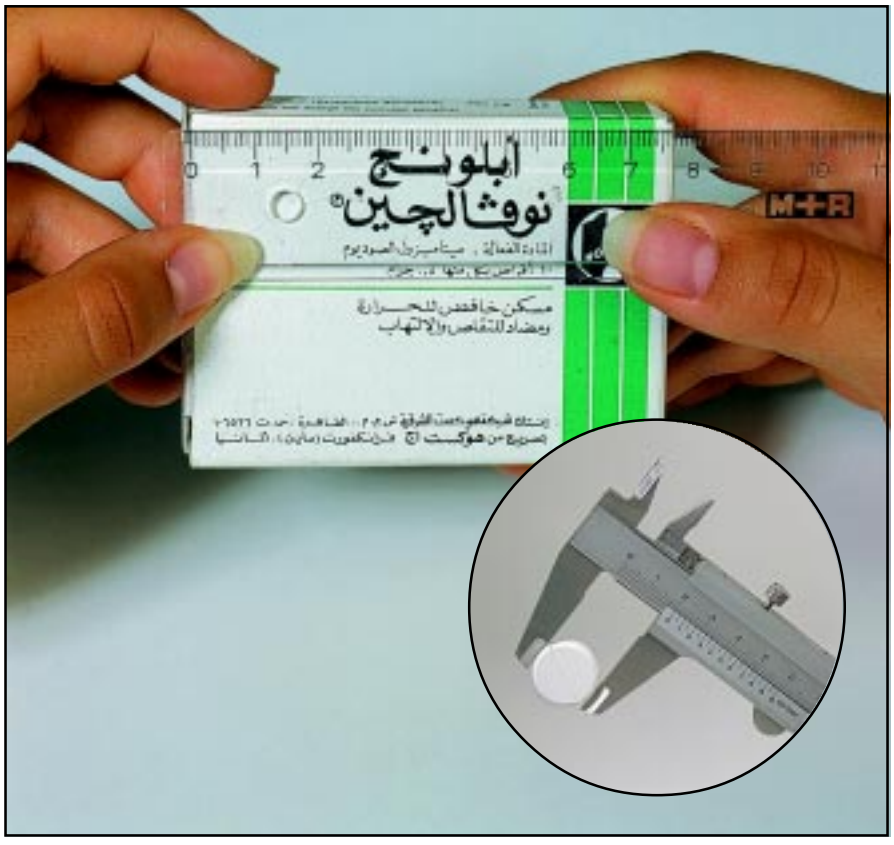
in cooperation with the

MEDICAL MISSION INSTITUTE

6 The Minilab's Operation Procedures at a Glance

The protector case contains a lot of bits and pieces which may not be familiar to you. Confused? Nothing to worry about. Soon you will have a clear picture of what you can do and of what you should not do. Unpack the lot and make your first few steps into the world of pharmaceutical quality control. Generations of pharmaceutical technicians have mastered this before. We are sure you will do as well. Good luck!

I. Visual Inspection



A caliper rule is added for a precise description of tablet and capsule sizes during visual inspection. The size of blister cards and folding boxes is measured using a graduated ruler.

II. Disintegration Test

Disintegration testing on tablets and capsules is performed using a 100-ml wide neck bottle filled up with warm water (37ºC). Disintegration should be completed when the alarm of the pre-set timer sounds after 30 minutes.

III. TLC: 1. Sample Preparation

The preparation of a stock standard and stock test solution requires one whole unit of an oral solid dosage form.

The Minilab supplies an appropriate collection of authentic secondary standards capable of supporting at least 300 TLC assays on suspicious drug products. They are packed in a set of fifteen tamper evident sealed plastic tubes each containing 20 - 100 reference tablets or capsules respectively. They can be ordered as full set or individually via Technologie Transfer Marburg (TTM) in Germany.

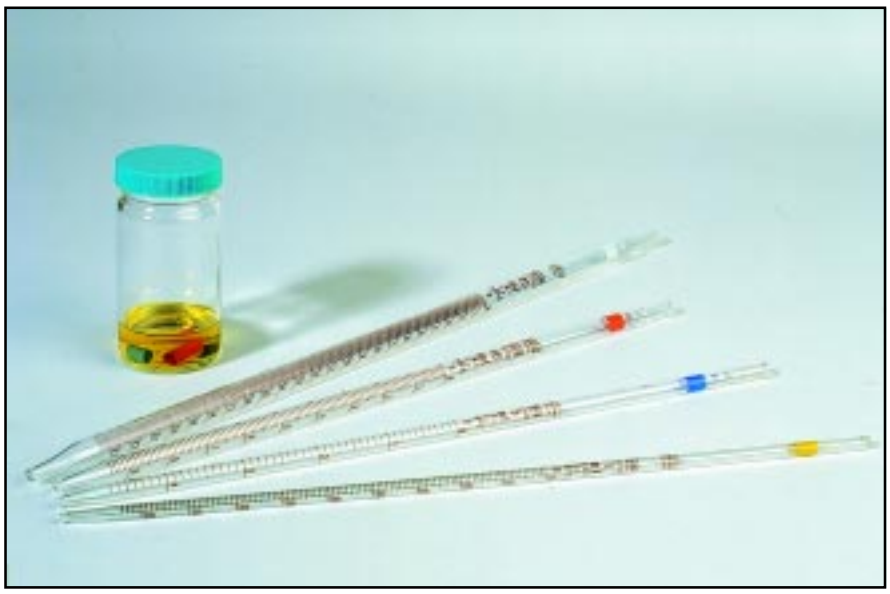
Note: After having removed a reference tablet or capsule, tightly close the container to ensure that the other reference samples do not reach their expiry date before time.

Using a funnel, sachets are emptied directly over a laboratoy glass bottle making sure that all residual solids are finally washed down into that bottle with a known volume of solvent.

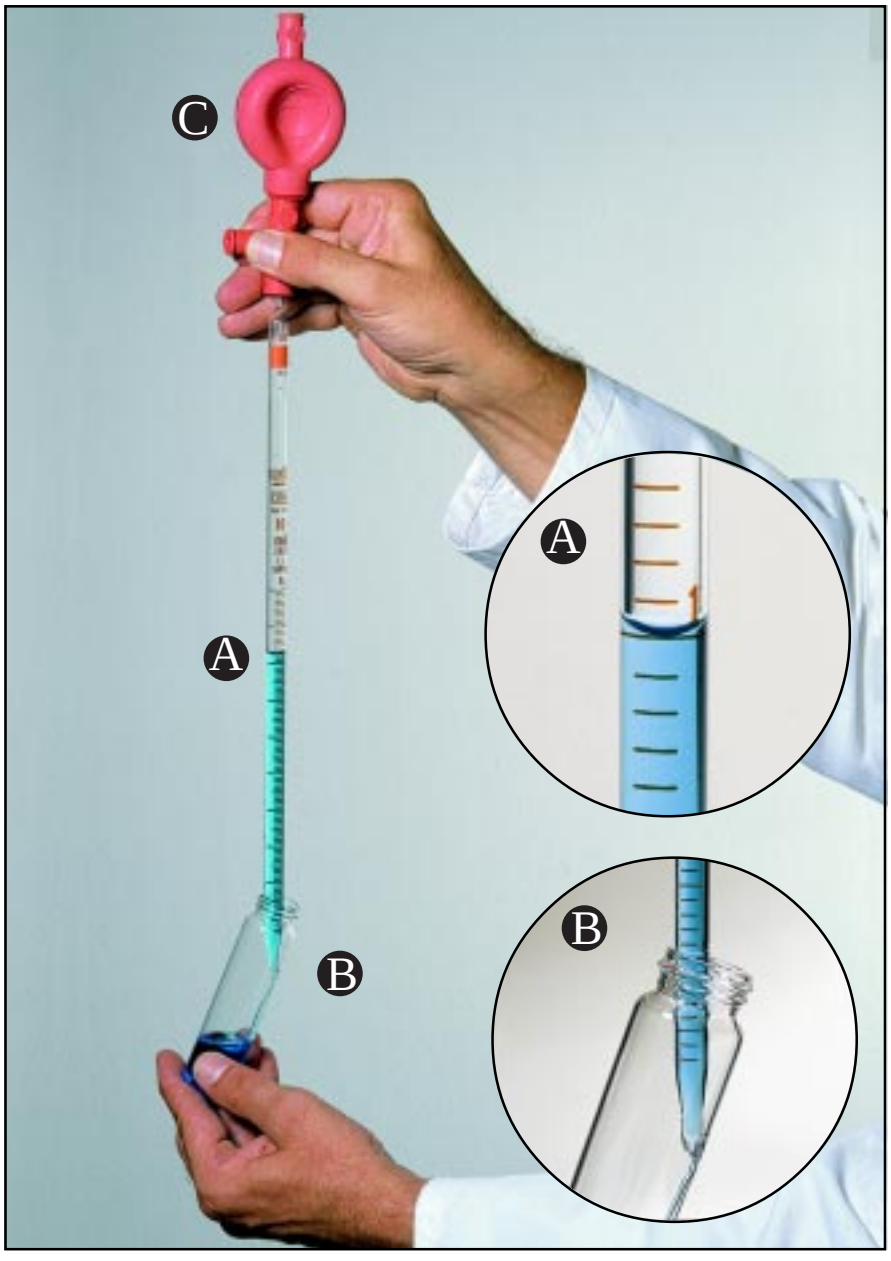
As for sachets, hard gelatin capsules are opened and emptied directly over a laboratory glass bottle, the cap and body shell are added to the bottle as well.

Soft gelatin capsules are cut into pieces using a scalpel, blade or pair of scissors. All pieces are completely transfered into a laboratory glass bottle ensuring that the scissors' or scalpel's blade are finally rinsed with a known volume of the appropriate solvent as directed in the individual monograph.

Tablets are crushed with a pestle prior to extraction, the precise procedure being as follows: Wrap up a tablet into aluminium foil and crush it down to a fine powder. Empty the aluminium foil over a laboratory glass bottle. Finally rinse the foil with the appropriate extraction solvent thus ensuring that nothing of the broken tablet gets lost.



All solids are dissolved in a known volume of extraction solvent using a set of various straight pipettes capable of delivering an accurate volume of 0.01 to 25.0 ml of solvent. The solution obtained will be either the stock standard or stock sample solution requiring further dilution down to its final concentration, the working standard or sample solution.

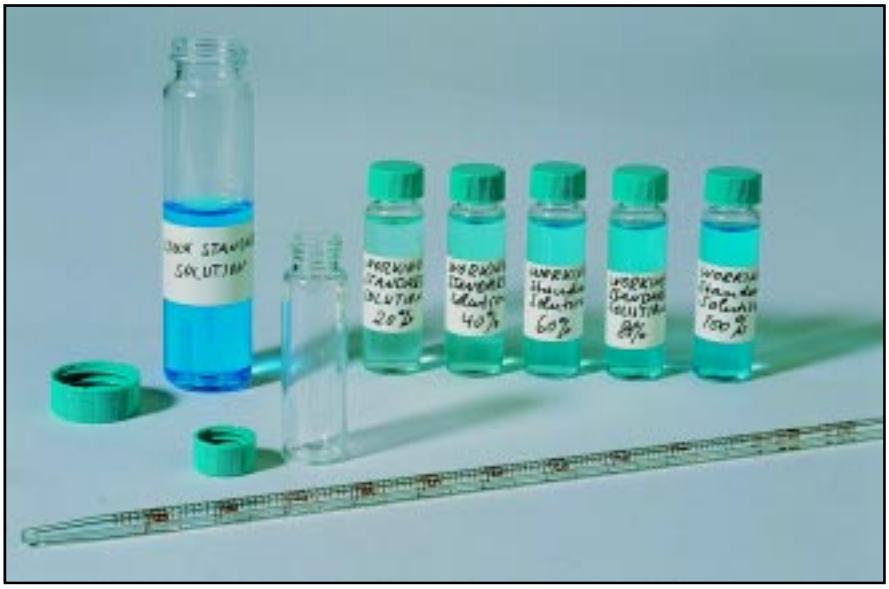


Pipetting: Hold the pipette in a vertical position to check that the solvent reaches the desired graduation mark (A). The mark should be in line with the bottom of the meniscus formed by the solvent.

The tip of the pipette (B) should be held against the side of the bottle.

A Peleus Ball (C) is added as a pipetting aid, thus eliminating the need for dangerous mouth pipetting.

Warning! All organic solvents are easily inflammable. Hence, do not work with naked flames or smoke when working with them! All solvents are toxic. Avoid dangerous mouth pipetting. Use the pumpette supplied. Do not risk your health!



Use the set of straight pipettes supplied in order to dilute the stock solutions down to their final working concentration.

A set of 10-ml vials (small glass bottles) accommodates the working solutions.

A label tape and marker pen for the permanent identification of the stock and working solutions. The label tape works well with all glass and plastic labware. The marker pen is water-resistant.

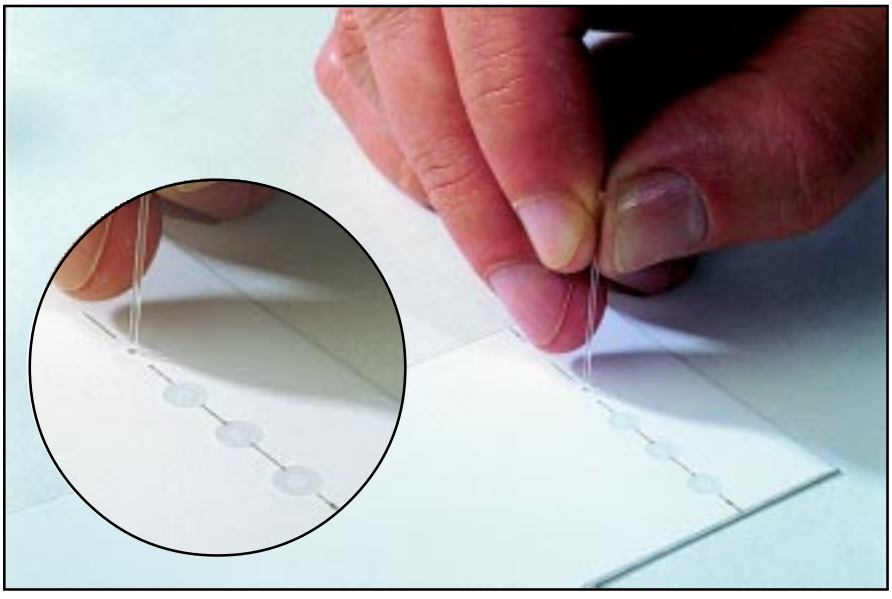
III. TLC: 2. Sample Application

A set of thin layer chromatographic plates further called tlc or chromatoplates. These are coated aluminium plates, the coat consisting of an adsorbent called silica gel 60 F254. They arrive in packs of fifty and are perfectly sized (5 x 10 cm) in order to fit into the TLC chamber supplied.

Note: Open the pack just before use. Remove one plate only. Return the package into the waterproofed protector case. As far as possible, avoid contact with moisture. Store in a dry place. Do not touch the plate's surface.

A pencil of soft grade and a ruler are supplied allowing to mark the origin and front line on a chromatoplate as well as the spot size after visualization with an ultraviolet light detector.

Disposable glass microcapillaries are supplied for sample application. They deliver an accurate volume of 2 microlitres (µl) when filled perfectly from end to end.



Apply the sample at the origin line of the chromatoplate using a microcapillary filled with the appropriate working solution. Rinse the capillary three times with extraction solvent (Filling with solvent and evacuating via a piece of filter paper or any other paper tissue) before spotting the next sample onto the plate. Dispose of the capillary when one chromatoplate has been fully loaded thus avoiding the risk of cross contamination between samples containing different drug products.

Check the uniformity of all spots using UV light of 254 nm. The spots will become visible and all of them should be circular in shape and equally spaced across the origin line. Repeat this step if a homogeneous spotting is not achieved.

Note: Spotting is precision work and it might need some training to become perfect. It is important not to scratch the absorbent layer as marks cause the mobile phase to elute unevenly an some spots might become distorted.

III. TLC: 3.Chromatoplate Development

A jar with a lid will serve as developing tank. All sides of the jar are to be lined with filter paper ensuring saturation of the tank's atmosphere with solvent vapour prior to adding the loaded TLC plates for development. Cut the filter paper down to size for a nice fit in the jar. Use the pair of scissors supplied.

Use the set of straight and transfer pipettes supplied for the preparation of the mobile phase; the actual vehicle which drives the sample spots along the TLC plate. Pipette all different solvents into the developing tank as directed in the individual monograph and mix. Close the tank and wait for about 15 minutes thus ensuring saturation of the TLC chamber with solvent vapour. Finally place a loaded TLC plate into the tank and wait till the solvent front has moved about three-quarters of the length of the plate.

Note: Freshly prepare the mobile phase for each day's work. Never ever return used solvents into their storage container!

Warning! All solvents are toxic. Avoid dangerous mouth pipetting. Use the pumpette supplied. Do not risk your health!

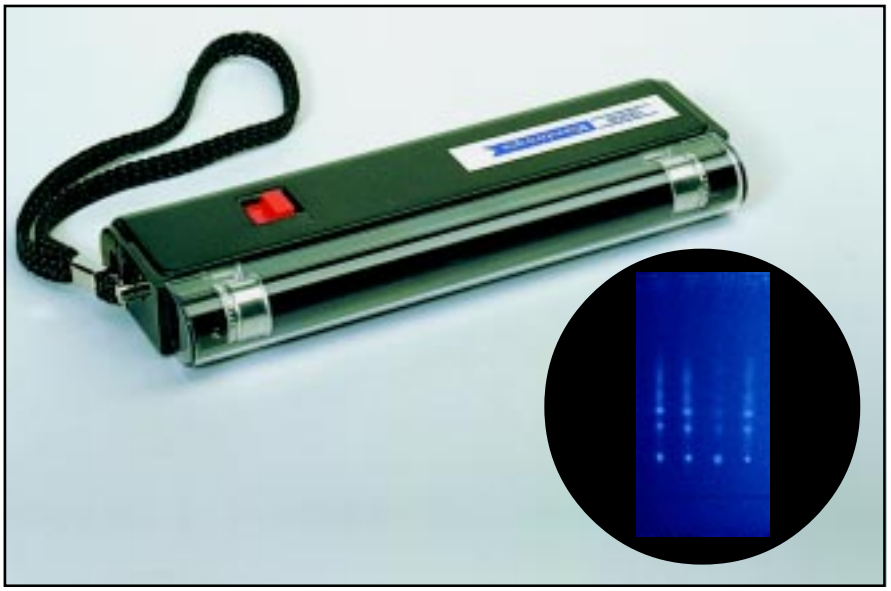
Once the chromatoplate has been developed, remove it from the TLC tank and allow any excess solvent to evaporate. A hot plate might be used in order to cut down the time for drying. The latter procedure is prefered if the mobile phase consists of low volatile solvents, for example ethylacetate, toluene or water.

In our case, a Philips World Travel Iron, placed upside down, serves as a hot plate. The iron can safely be used from all electrical circuits, and is provided with a voltage selector (110 - 240 V). As electrical sockets are not the same everywhere, an adaptor plug is added to the Minilab.

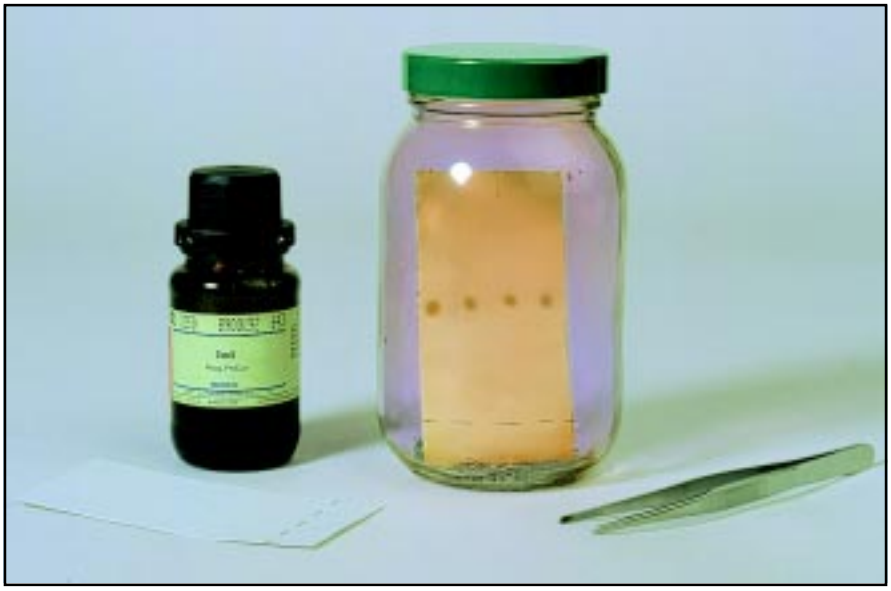
Warning! Avoid direct contact with the hot plate. Switch off the plate after use.

A battery driven ultra-violet lamp capable of radiating short waves of 254 nanometres in length is supplied for the detection of invisible spots on the chromatoplate. Move the developed plate to a dark room or place (below the table, in a drawer, box or cabinet) and expose it to the UV light source. Now, all spots sensitive to this light will become visible. Mark them for further evaluation using the pencil supplied.

Warning! Do not look directly into the UV light source when in use thus avoiding potential eye injuries.



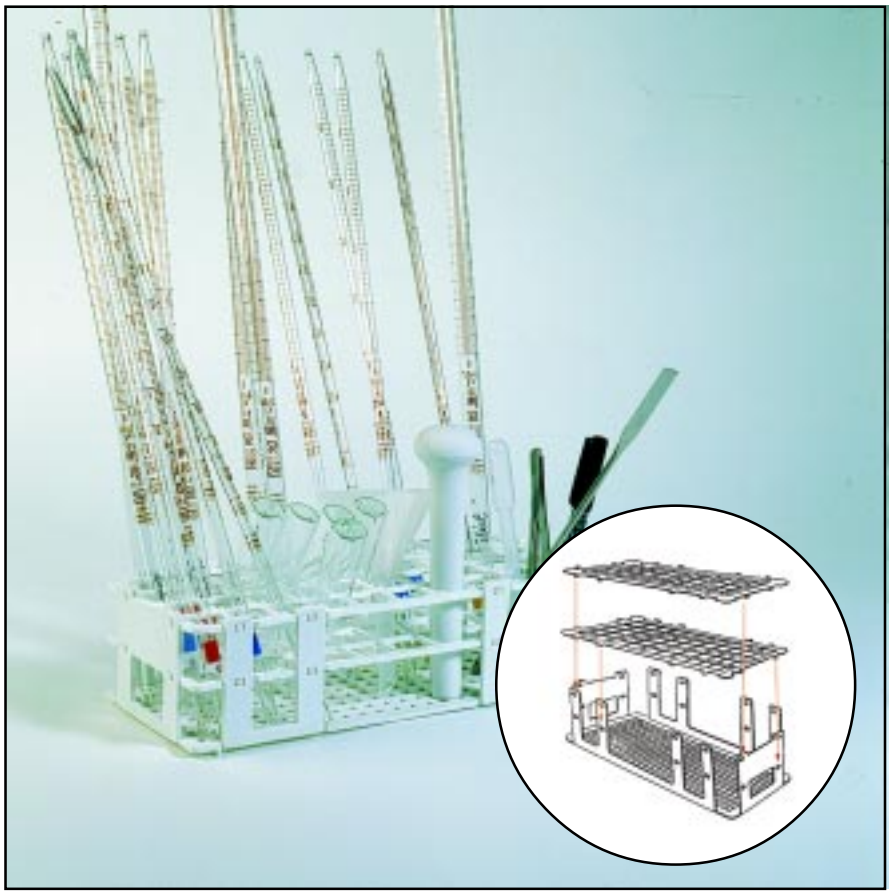
A second battery driven ultra-violet lamp capable of radiating long waves of 366 nanometres in length for further detection of invisible spots. Again, move the chromatoplate to a dark room or place (below the table, in a drawer, box or cabinet) and expose it to the UV light source. Now, all spots sensitive to this light will become visible. Mark them for further evaluation using the pencil supplied.



A second jar is supplied in order to establish a tank for iodine staining of invisible spots failing UV detection. Add some iodine crystals to the jar, close and heat it on a hot plate for about thirty seconds. Alternatively, expose the closed jar to direct sunlight for about five minutes. The iodine starts to sublime gradually saturating the jar's atmosphere with iodine vapour indicated by a deep violet mist.

Place the chromatoplate into the tank and most invisible spots are gradually turning yellowish brown. The developing time is completed after about one minute.

IV. Cleaning

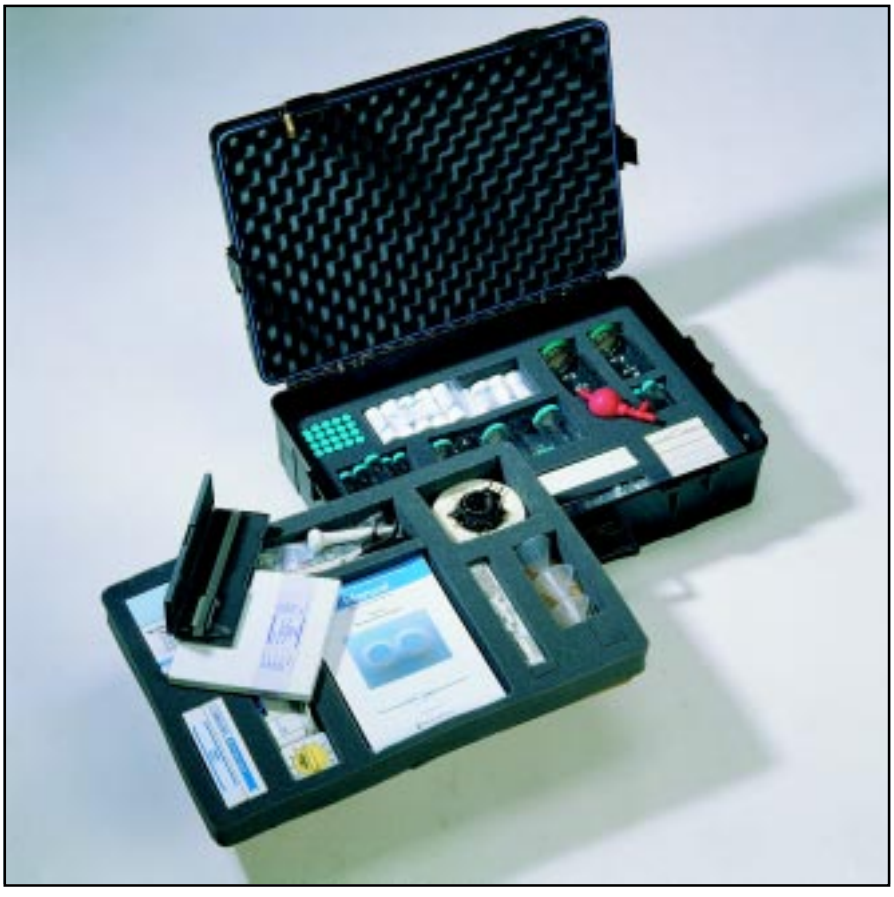


Thoroughly clean all straight pipettes, all the glassware and other laboratory equipment after use. Dispose of all sample solutions and rinse the empty bottles with water employing sufficient detergent. Finally, if available, rinse all glassware with deionized water prior to drying thus avoiding scums and stains. Place all pipettes and the other glass containers upside down into the rack supplied thus facilitating the drying process.

Very dirty or blocked straight pipettes are either soaked in appropriate extraction solvent, hot detergent solution, acetic acid 50% or hydrogen peroxide 10% prior to their final cleaning.

Note: Use a dedicated waste liquid container preferably made of polypropylene for the disposal of used reagents and test solutions. For further disposal, follow the rules of your local area.

V. Restoring the Minilab to its Former Condition



After cleaning and drying, return all material used into the protective case. Restore as seen on the pictures. Close the case and store it at a safe place. The case has an airtight and watertight fitting system so as to ensure perfect protection against moisture.

Final assembly of the Minilab.

7.7 Co-Trimoxazole

Primary Screening via Visual Inspection & Disintegration Test

I. VISUAL INSPECTION

Search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on general methods and operations. Write down all product particulars using the *Reporting Form* as a guide. Co-trimoxazole is an antibiotic combination of trimethoprim and sulfamethoxazole at a fixed ratio of 1:5. Each tablet usually contains 480 mg of co-trimoxazole (80 mg trimethoprim + 400 mg sulpha-methoxazole). Tablets containing only 120 mg of co-trimoxazole (20 mg trimethoprim + 100 mg sulphamethoxazole) are for paediatric use.

II. DISINTEGRATION TEST

All quick release co-trimoxazole tablets must pass the disintegration test as described in the opening chapters on general methods and operations. They should disintegrate in water at 37 °C in less than 30 minutes. It's a major defect if a drug product doesn't pass this test.

III. RESULTS & ACTIONS TO BE TAKEN

Drug products from unusually cheap sources, drug products with missing or incorrect accompanying documents and drug products with defective dosage forms, packaging or with incomplete, damaged or missing labels or with labels written in a foreign language should be subjected to a thin layer chromatographic assay.

Verification of Identity and Drug Content via Thin Layer Chromatography

I. PRINCIPLE

Trimethoprim and sulfamethoxazole are extracted from tablets with methanol and determined by TLC with reference to an authentic secondary standard.

II. EQUIPMENT AND REAGENTS

1) Pestle
2) Aluminium foil
3) Laboratory glass bottles with a filling capacity of 25 to 100 ml
4) Set of straight pipettes (1 to 25 ml)
5) 10-ml vials
6) Label tape
7) Marker pen
8) Pencil
9) Merck TLC aluminium plates pre-coated with silica gel 60 F 254, size 5x10 cm
10) Glass microcapillaries of 2 µl capacity
11) Hot plate (Philips Travel Iron)
12) TLC developing chamber (jar)
13) Filter paper
14) Pair of scissors
15) UV light of 254 nm
16) Iodine chamber
17) Pair of tweezers
18) Methanol
19) Ethylacetate
20) Co-trimoxazole 120 mg reference tablets

III. PREPARATION OF THE STOCK STANDARD SOLUTION

The preparation of a stock standard solution requires a whole reference tablet containing 120 mg of co-trimoxazole which is crushed prior to extraction, the precise procedure being as follows: Wrap up a tablet in aluminium foil and crush it down to a fine powder using a pestle. Empty the aluminium foil over a 25-ml laboratory glass bottle and wash down all residual solid with 10.0 ml of *methanol* using a straight pipette. Close the bottle and shake for about three minutes till most of the solid is dissolved. Allow the solution to stand for a further five minutes until the undissolved residue settles below the clear supernatant liquid. The solution should contain 12 mg of total drug per ml and be labelled as *'Co-trimoxazole Stock Standard Solution'*. Freshly prepare this solution for each test.

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

Pipette 2 ml of the clear stock standard solution into a 10-ml vial and add 2 ml of *methanol*. Close and shake the vial. This solution should contain 6 mg of total drug/ml and be labelled as *'Co-trimoxazole Working Standard Solution 100%'*.
The working standard solution represents a drug product of good quality containing 100 % of co-trimoxazole.

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

Pipette 2 ml of the clear stock standard solution into a 10-ml vial and add 3 ml of *methanol*. Close and shake the vial. This solution should contain 4.8 mg of total drug/ml and be labelled as *'Co-trimoxazole Working Standard Solution 80%'*.
The lower working standard solution represents a drug product of poor quality containing just 80% of the amount of co-trimoxazole as stated on the product's label. In the current investigation, this drug level represents the lower acceptable limit for a given product.

VI. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A DRUG PRODUCT CLAIMING A POTENCY OF 120 MG CO-TRIMOXAZOLE PER UNIT

The preparation of a stock sample solution requires a whole tablet from an appropriate drug product sampled in the field, the extraction procedure being the same as for the co-trimoxazole reference tablet: Wrap up a sample tablet in aluminium foil and crush it down to a fine powder. Quantitatively transfer the powder into a 25-ml laboratory glass bottle. Add 10 ml of *methanol*, close the bottle and shake for about three minutes till most of the solid is dissolved. Allow the solution to stand for a further five minutes until the undissolved residue settles below the clear supernatant liquid. Label the bottle as *'Co-trimoxazole Stock Sample Solution*'. The solution should contain 12 mg of total drug/ml. Freshly prepare this solution for each test.

VII. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A DRUG PRODUCT CLAIMING A POTENCY OF 480 MG CO-TRIMOXAZOLE PER UNIT

A) The preparation of a stock sample solution requires a whole tablet from an appropriate drug product sampled in the field, the precise extraction procedure being as follows: The tablet is crushed down to a fine powder and quantitatively transfered into a 25-ml laboratory glass bottle. Add 20 ml of *methanol*, close the bottle and shake for about three minutes till most of the solid is dissolved. Allow the solution to stand for a further five minutes until the undissolved residue settles below the clear supernatant liquid.

B) For further dilution, mix 2 ml of the clear liquid with 2 ml of *methanol* in a 10-ml glass vial. Close and shake the vial and label it as *'Co-trimoxazole Stock Sample Solution'*. The solution should contain 12 mg of total drug/ml. Freshly prepare this solution for each test.

VIII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Pipette 2 ml of the clear stock sample solution into a 10-ml vial and add 2 ml of *methanol*. Close the vial and label it as *'Co-trimoxazole Working Sample Solution'*.
The expected concentration of trimethoprim in the working sample solution is 1.0 mg/ml and that of sulfamethoxazole 5.0 mg/ml.
Both concentrations should match the drug level of trimethoprim & sulfamethoxazole in the higher working standard solution.

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 on the next page using microcapillary pipettes.

Up to four spots can be placed onto 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 intensity might differ, their diameter never should. Different intensities are due to residual amounts of tablet and capsule excipients or different drug concentrations in the sample solutions. A difference in spot size, however, relates to poor spotting. Repeat this step if a homogeneous spotting is not achieved.

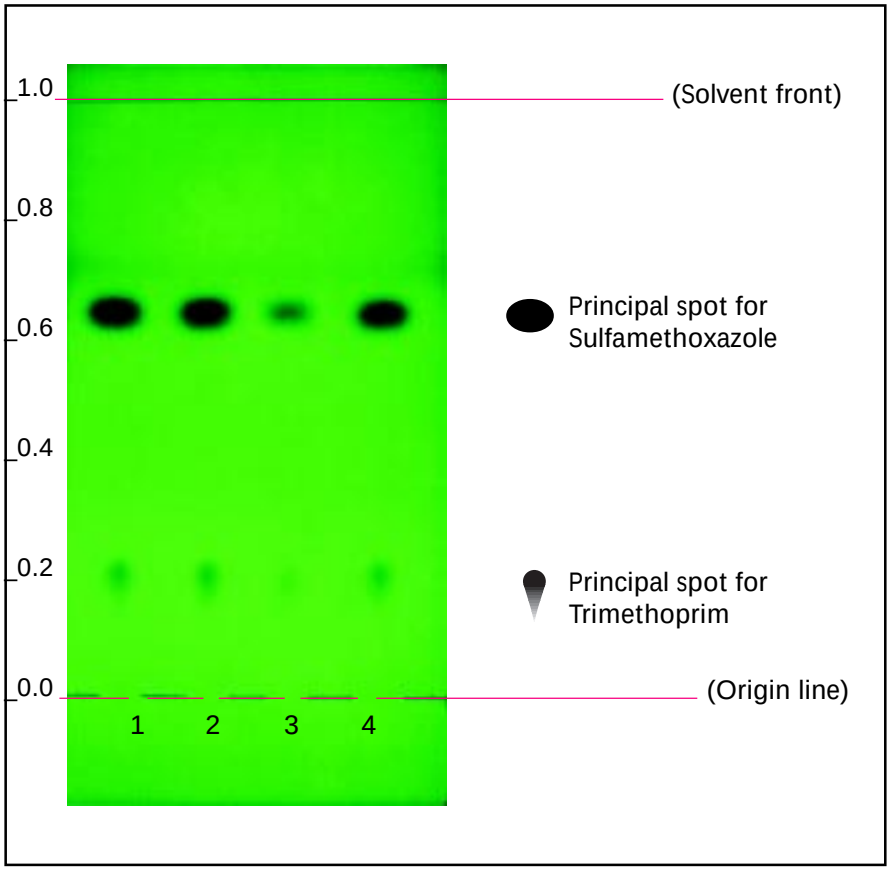
X. DEVELOPMENT

Pipette 15 ml of *ethylacetate* and 5 ml of *methanol* into the jar being used as TLC developing chamber. Close the chamber and mix thoroughly. Line the chamber's wall with filter paper and wait for about 15 minutes thus ensuring saturation of the chamber with solvent vapour. Carefully place the loaded TLC plate into the jar. Close the jar and develop the chromatoplate until the solvent front has moved about three-quarters of the length of the plate, the developing time being about 20 minutes. Remove the plate from the chamber, mark the solvent front and allow any excess solvent to evaporate using a hot plate if necessary.

XI. DETECTION

Dry off all residual solvent and observe the chromatoplate with UV light of 254 nm. Also inspect the plate after iodine staining.

XII. CHROMATOPLATE OBSERVED AT 254 NM



Run No.1:
Co-trimoxazole's upper working limit representing 100 % of total drug.

Run No.2:
A drug product of good quality.

Run No.3:
A drug product of poor quality.

Run No.4:
Co-trimoxazole's lower working limit representing 80 % of total drug.

XIII. OBSERVATIONS MADE AT 254 NM

The presence of co-trimoxazole is indicated by two principal spots, the one representing sulfamethoxazole being in the front with a travel distance of about 0.65 followed by a second spot representing trimethoprim at a distance of about 0.24. Additional strong spots generated by the test solution indicate drug degradation especially when associated with smaller principal spots. However, much fainter spots might emerge in the lower region of the chromatogram normally caused by auxiliary agents incorporated in the different tablet and capsule formulations.

XIV. OBSERVATIONS MADE DURING IODINE STAINING

Two brown spots are generated matching the spots already observed with UV light of 254 nm.The spot representing sulfamethoxazole easily attracts iodine and becomes instantly orange-brown whereas the one representing trimethoprim is a poor performer in terms of iodine staining. It might take some time that trimethoprim becomes visible especially if the iodine chamber is not properly activited.

XV. RESULTS & ACTIONS TO BE TAKEN

The principal spots in the chromatogram obtained with the test solution must correspond in terms of colour, size, 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 with a second sample from scratch. Reject the batch if the drug content can't be verified in a third run. For a second opinion, refer additional samples to a fully equipped drug control laboratory. Retain samples and put the batch on quarantine till a final decision on rejection or release has been taken.