1 Introduction

An ever increasing quantity of counterfeit and sub-standard drug products are flooding the markets of many of the developing countries. According to the World Health Organisation, counterfeits account for up to 7% of sales of pharmaceuticals in the world. Almost half of them relate to antibiotic and antiparasitic preparations.

For countless patients counterfeit drug products bring additional risk and at its worst death. Urgently needed drugs are often of poor quality. Products might contain the wrong active ingredient or might be contaminated with hazardous impurities. In the majority of cases, products contain no active ingredient whatsoever or are substantially deficient in content.

Both, the Minilab and the accompanying manual will serve the interest of all partners in the public health service intending to combat counterfeit and sub-standard drug products, especially those involved in the purchase, storage and distribution of a large amount of pharmaceuticals in their community. For example central hospitals additionally supplying own branches and other affiliations.

The reason for consistently checking the quality of pharmaceutical products is not only to identify sub-standard or counterfeit drugs but also to create a data file with which to compare the quality of the products still to come. With time, this data base of comparator drugs will allow the selection of reliable suppliers. With this shortlist in hand, the workload on the screening of counterfeit drugs will be considerably reduced.

The visual inspection of drug products should precede any testing which is in our case combined with a simplified disintegration test. Both tests will allow the identification of rough counterfeits for timely rejection prior to entering the major examination via thin layer chromatography. This procedure will save resources on reference samples, reagents and solvents.

Compared to Volume I, thin layer chromatography has now been introduced to verify the identity of all products in general and especially of suspicious products from unknown sources. It can also be used to decide on the presence or absence of impurities or degradation products. It is all of these but it has also been made quantitative in order to obtain confirmation whether the amount of drug stated on the label is actually in the product. The results obtained by a simple visual inspection of the chromatoplates can be accurate to 10% if great care is taken and skill executed. In order to achieve this accuracy additional training of staff might be required.

General information on each test method is obtained in the chapters 'Visual Inspection', 'Disintegration Test' and 'Thin Layer Chromatography'. A closer look on sample preparation and other basic laboratory operations is taken in the chapter 'The Minilab's Operation Procedures at a Glance'. More detailed information on the actual drug compound is obtained in the individual monographs describing standardized operation procedures on the identification of each active ingredient listed in this book.
All operation procedures are also provided in a summarized version on laminated sheets for routine work. Finally, overall laboratory management will be facilitated using the Reporting, Stock Record and Order Forms supplied.

The list of drugs included in this manual comprises a wide variety of antibiotics and antiparasitics as well as drugs for pain and inflammation management, for example acetylsalicylic acid (aspirin), amoxicillin, ampicillin, chloramphenicol, chloroquine, cloxacillin, co-trimoxazole erythromycin, mebendazole, metamizole, metronidazole, paracetamol, penicillin V, prednisolone and tetracycline. The Minilab does supply an appropriate collection of reference samples.

The tests described in this manual are intended only to verify the identity and potency of pharmaceutical preparations. They should not be used to replace pharmacopoeial monographs. All samples which are potentially counterfeit should be referred to a fully equipped laboratory for further investigations prior to taking legal actions.

Bear in mind that good analytical results of high reproducibility are achieved only by using reagents and solvents of high purity, so use the best available Analar Grade if possible.

Important Notice

Counterfeit drugs represent a risk for the health and life of patients. In order to avoid erroneous test results, which could turn out to be potentially dangerous for the patients, it is essential that the instructions and tests given in this manual are carefully studied and closely followed when working with the GPHF-Minilab®.

2 Health & Safety

Important Notice

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

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 which, 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.

3 Visual Inspection

Unfortunately, we will never be in the ideal situation to check all drug products we want to. Resources, for example reference standards, will always be limited. The establishment of screening procedures will help to get around this problem. This chapter will give guidance on how to visually inspect a large number of drug products in order to identify suspicious products for further examinations.
The visual inspection of pharmaceutical products covers the description of the packaging and labels as well as the description of the dosage forms. For the time being, we will only be concerned with single solid dosage forms and their containers.

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 an identity test. Drug content and gross degradation may be determined via thin layer chromatography.

3.1 Visual inspection of labels and packaging material

'Tablets and capsules should be kept in a container which affords protection against breakage or crushing, access of moisture, contamination, and, as far as possible deterioration' (British Pharmacopoeia 1980).

The container can be anything from a glass bottle to a blister pack or a tube made of metal and a securitainer made of plastic. These primary or immediate containers are very often protected by a folding carton normally containing a leaflet explaining the dosing regime and the drug's action or side effects. Tablets and capsules can be presented in a single or multi unit dose container, for example in a blister pack or bottle respectively. Patient packs may contain just ten or twenty tablets dispensed into a reclosable plastic bag whereas bulk packs may contain a thousand or more tablets dispensed into a sealed aluminium pouch. Indeed, there is a sense that there are as many different types of packaging used in the pharmaceutical industry as the products they contain.

Both, the immediate container and the folding carton should have a durable label fixed on their bodies. Labels can be replaced by print. The print on the label or packaging must be in a legible and indelible manner. The labels on the package and container of every official pharmaceutical product should ideally state (1) the name of the drug, (2) the drug's strength, potency or concentration, (3) the number of unit doses in the container, (4) the route of administration, (5) the manufacturer's or responsible distributor's name and full address, (6) the batch or lot number, (7) the expiry date (8) the storage conditions and (9) any other safety measures for example 'Keep away from Children'.

In case a pharmaceutical product is a branded product and the drug's name is not fully disclosed by its trade name (for example Aspirin) the label should also provide the scientific name of the active ingredient (for example acetylsalicylic acid).

A batch number is a unique number which consists either of figures or letters, or a combination of figures and letters by which the history of the product may be traced back to its origin.

Additional label requirements exist for coated tablets and capsules intended to resist the gastric fluid. They should be identified on the label by the addition "enteric-coated" tablets or capsules respectively.

Finally, should a label state that the product complies with all pharmacopoeial requirements it is not sufficient to state the pharmacopoeia itself (for example British Pharmacopoeia) but also its effective date (BP 1993) bearing in mind that quality will progress with time and that only products referred to in the latest pharmacopoeial edition will represent the latest advance in knowledge and technology.

Packaging operations such as labelling are very complicated processes. Just consider the infinite

number of different pack specifications in terms of product and product variant, dosage, pack size and type or market destination. Whatever label is on the product - the pharmacist, the doctor, the patient - will believe it. However, mislabelling, or the mis-use, misapplication or mix-up of other printed material is the most frequent cause for the recall of substandard or counterfeit pharmaceutical products.

For example: consider a situation where a product is repackaged for several export markets. The name of the product can be the same (or very similar), as well as the formulation and expiry date. Even the cartons may look the same. But, if any mix-up did occur the batch number might be different. It may not be noticeable at a glance but the batch number on the container and carton will almost certainly be different. The consequences of such a mix-up could be very serious indeed.

The packaging of pharmaceutical products, where it is necessary to assemble all bits and pieces at one time, is a very complex and expensive business. This complex process and the quality of the packaging material itself is very difficult to copy for people intending to launch counterfeit products. This is why it is appropriate to start with the packaging material for a prescreening of suspicious drug products.

But how is it possible to identify spurious products? The following questionnaires will give you a guide on how to separate the good from the fake ones. Only the suspicious ones should then be further scrutinized using colour reactions and thin layer chromatography. This description aid can also be used to fill in the Reporting Form correctly.

3.1.1 Description aid for the visual inspection of accompanying documents

Are there any delivery notes or other accompanying documents for example a Certificate of Analysis? Are they missing, incorrect or even manipulated as possibly indicated by a change in the style of writing or a different stroke from a different pen?

Drug products with missing, incorrect or spurious documents should be subjected to an identity test.

3.1.2 Description aid for the visual inspection of labels

If the shipment covers several palette loads: Are all palettes properly labelled and identifiable? Are the shippers properly labelled and identifiable? Is the drug product itself properly labelled describing at least (1) the name of the drug, (2) the drug's strength, (3) the pack size, (4) the manufacturer's name and address, (5) the batch number and (6) the expiry date? Does the label and other packaging material have its usual appearance/layout such as colour and format? Are the text and letters formatted as usual? Is the spelling correct? Is there any deviation from the usual company logo? Is the company stated on the label known as a reliable supplier of reputable drug products? Does the number of tablets or capsules stated on the label match the amount of units counted?

Drug products with incomplete, damaged or missing labels or with labels written in an unknown foreign language should be subjected to an identity test.

A label of an acceptable standard. This picture may give you an idea of how a label should look.

3.1.3 Description aid for the visual inspection of multi unit dose containers

Is the immediate container a multi unit dose container for example a bottle, tube or securitainer? Are these containers made of glass, plastic or metal? Which colour do they have? For example: Is it a white or a brown glass bottle? Is it a white, grey or brown plastic bottle? Do the screw caps have a different colour? Are they black, red or blue? Are the seals or caps tamper evident? Have any of those tamper evident seals been violated? Any use of child-proof closures? Is it a patient or a bulk pack? Patient packs may contain just ten or twenty tablets whereas bulk packs may contain a thousand or more tablets. The number of tablets stated on the label should match the number of tablets counted. Is the bottle protected by a carton? Does the label on the carton match the label on the bottle?

Bottles of good quality as multi unit dose containers for pharmaceutical products. This picture may give you an idea of how different they might look.

3.1.4 Description aid for the visual inspection of single unit dose containers

Is the immediate container a single unit dose container, for example a strip or blister pack? Is it an all aluminium blister card or a card with a different top and backing? Is the silver foil (backing) itself coloured? For example pink, green or blue? Does the silver foil show any printed text? If yes. What does the print describe? The drug's name? The manufacturer's name? The batch number or expiry date ? Is the print black or coloured? Is it a registered or continuous print? Is it a reversed print? Is the silver foil printed only on one or on both sides?

Is the packaging containing the tablets or capsules in moulded cavities made of clear and colourless or white and opaque plastic foil? Is the plastic foil coloured? Clear and brown? Clear and green? Green opaque? Brown opaque? How many pockets are on one blister card? Ten? Twenty? Even more? Blister cards are normally rectangular in shape. How long is each side? Use a ruler in order to check it.

Are the blister card's edges acute or rounded? Is the card perforated? Is there an embossed batch number on the card? Are the blister cards protected by a folding carton? Does the batch number on the folding carton match the embossed number on the card? How many cards are in that carton? Are all cards identical?

Strip and blister packs (push-through packs) of good quality as single unit dose containers for pharmaceutical products. The thermoformed blister card holds the tablets or capsules in its single pockets or cavities. The card is then sealed with the backing foil usually made of aluminium. Strip packs are entirely made of plastic-lined aluminium foil. The aluminium foil may be printed and show a different colour. This picture may give you an idea of how different blister packs or cards might look.

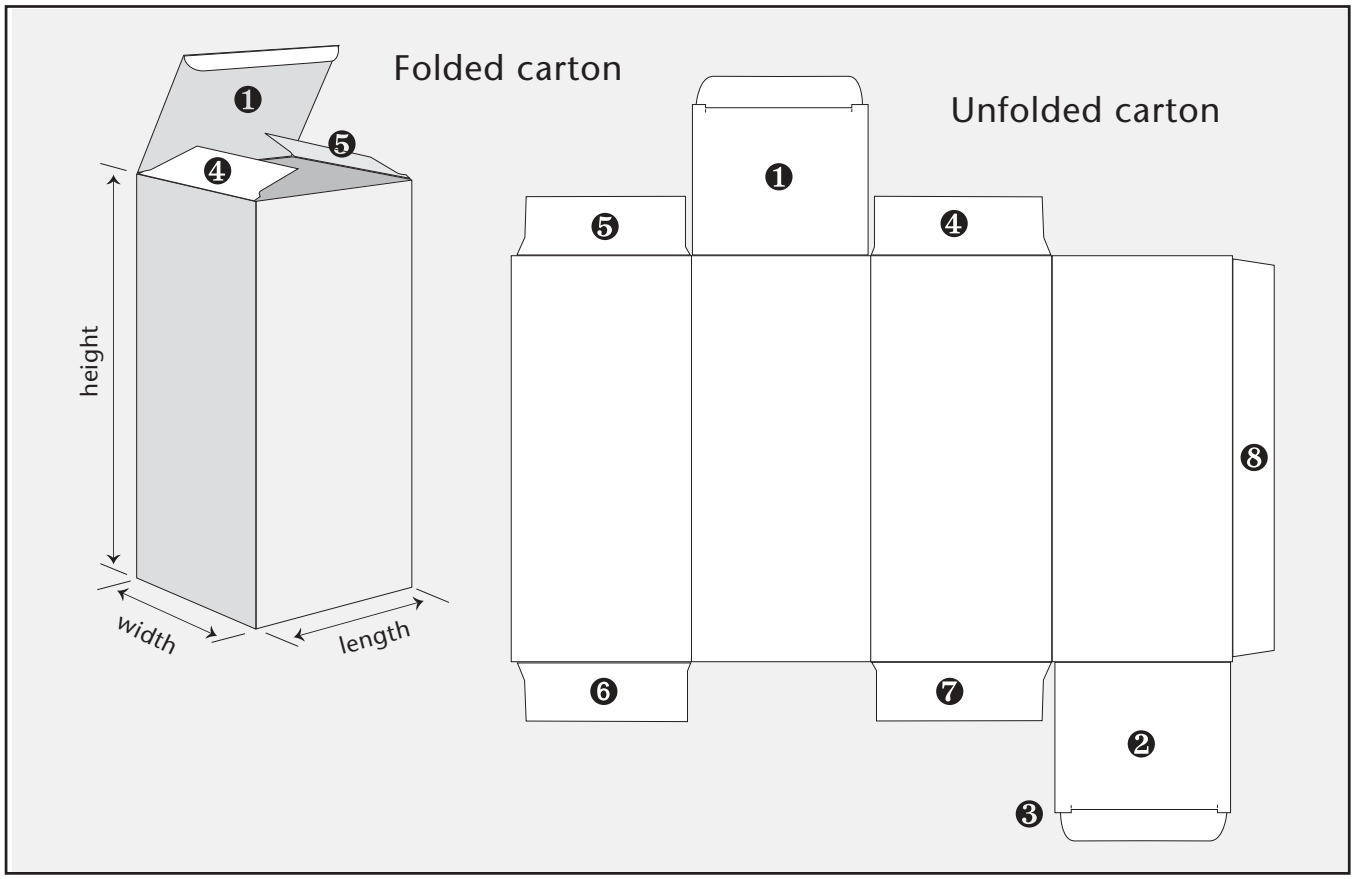
Common (A) and reversed print (B) observed on strip packs.

A common continuous (A) and a special registered print (B) observed on the back of blister packs. A dedicated label over each pocket of the blister card indicates a registered print which is more expensive to produce.

3.1.5 Description aid for the visual inspection of folding cartons

How is the quality of the carton's cardboard? Unfold a box: Does the unprinted interior look grey? This indicates recycled paper. Does the interior look yellowish? This indicates unbleached paper. Does the interior look white? This is bleached high quality paper. Does the size and shape of the framework match the size and shape of the original product? Draw a copy or make a xerox (1:1 scale) from both sides of the unfolded carton and tuck the copy beyond the Reporting Form for future examinations. How are the sizes and shapes from the top and bottom flaps? Do they comply with the original shape? Do the tuck-in flaps and side lids have straight or bevelled edges? Do the flaps show special cuts for a good friction fit? All of these require expensive production tools which forgers try to avoid.

Poor copies of comparator drugs are launched which are easily detectable via these tiny deviations from the original packaging.



Framework of a folding box most frequently used by the pharmaceutical industry. Experts call it a folding box with reverse tuck-in flaps (1 & 2) and friction fit (3): Both end flaps are tucked into their locking slots in order to close the carton. Special cuts (3) in the tuck-in flaps avoid their release after closure (friction fit). Tiny bevelled lids (4; 5; 6; 7) support the locking system in keeping the dust off the product. A glued joint (8) fixes the folding box.

A selection of folding cartons as seen in a chemist's shop. All of them are variations of the one principle 'Folding box with reverse tuck-in flaps and friction fit'. Do the flaps show special cuts for a good friction fit? Poor copies of originators might be easily detectable via such tiny deviations. Any discolouration observed due to an extensive exposure to sunlight?

3.2 Visual inspection of tablets

'Tablets are solid preparations each containing a unit dose of one or more medicaments and are obtained by compressing uniform volumes of particles. They are intended for oral use. Some are swallowed whole or after being chewed, some are dissolved or dispersed in water before administration, and some are retained in the mouth where the medicament is liberated. The particles to be compressed consist of one or more medicaments, with or without added substances such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the medicament in the digestive tract, authorised colouring matter and, when necessary, flavouring agents' (British Pharmacopoeia 1980).

Tablets are normally presented in a circular shape for easy swallowing. The surfaces of tablets are flat or convex and may be coated with a filmcoat to avoid dust formation during handling or with a coat resistant to gastric juice. Their hardness is well controlled. On the one hand, they are sufficiently hard to withstand handling without crumbling or breaking but on the other hand , they are sufficiently soft for easy disintegration during their passage through the gastro-intestinal tract.

Tablets come coated or uncoated, in a soluble, dispersible, effervescent or slow-release formulation and as lozenges for use in the mouth only. Uncoated tablets include single-layer and multi-layer tablets. When a broken section of an uncoated tablet is examined under a lens, either a relatively uniform texture (single-layer tablets) or a stratified structure (multi-layer tablets) is seen; there are no signs of coating.

Coated Tablets are tablets covered with one or more layers of mixtures of various substances such as natural or synthetic resins, gums and sugars frequently containing authorised dyes and sometimes flavouring agents. When the coating is thin, the tablets may be described as 'film-coated'. Coated tablets have a smooth, usually polished, and often coloured, surface; a broken section examined under a lens shows a core surrounded by a continuous layer of a different texture.

Enteric-coated Tablets are tablets covered with one or more layers of coatings intended to resist the gastric fluid but permit disintegration in the intestinal fluid. These properties may be achieved using special purpose polymers. Those tablets should be identified on the label as Enteric-coated tablets.

Sustained-release Tablets are coated or uncoated tablets containing special additives or prepared by special procedures which are intended to sustain the drug's rate of release in the gastro-intestinal tract and thus prolonging the drug's action. Such products should be indentified on the label as sustained-release or slow-release Tablets.

Tablets for release in the mouth are usually uncoated tablets. They are formulated to effect a slow release and local action of the medicament (lozenges and pastilles) or the release and absorption of the active ingredient under the tongue (sublingual tablets) or in other parts of the mouth. Lozenges are solid preparations, each containing one or more medicaments, usually in a flavoured base, which are intended to dissolve or disintegrate slowly in the mouth.

Effervescent Tablets are uncoated tablets generally containing acid substances and carbonates or bicarbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water for administration. Soluble Tablets are uncoated tablets that dissolve in water. The solution produced may be slightly hazy due to excipients used in the manufacture of the tablets. Dispersible Tablets are uncoated tablets that produce a uniform and smooth dispersion in water. Effervescent, soluble and dispersible tablets should disintegrate within five minutes in water of 25 °C.

Tablets of an acceptable standard: uncoated, film- and sugar-coated, coloured or uncoloured.

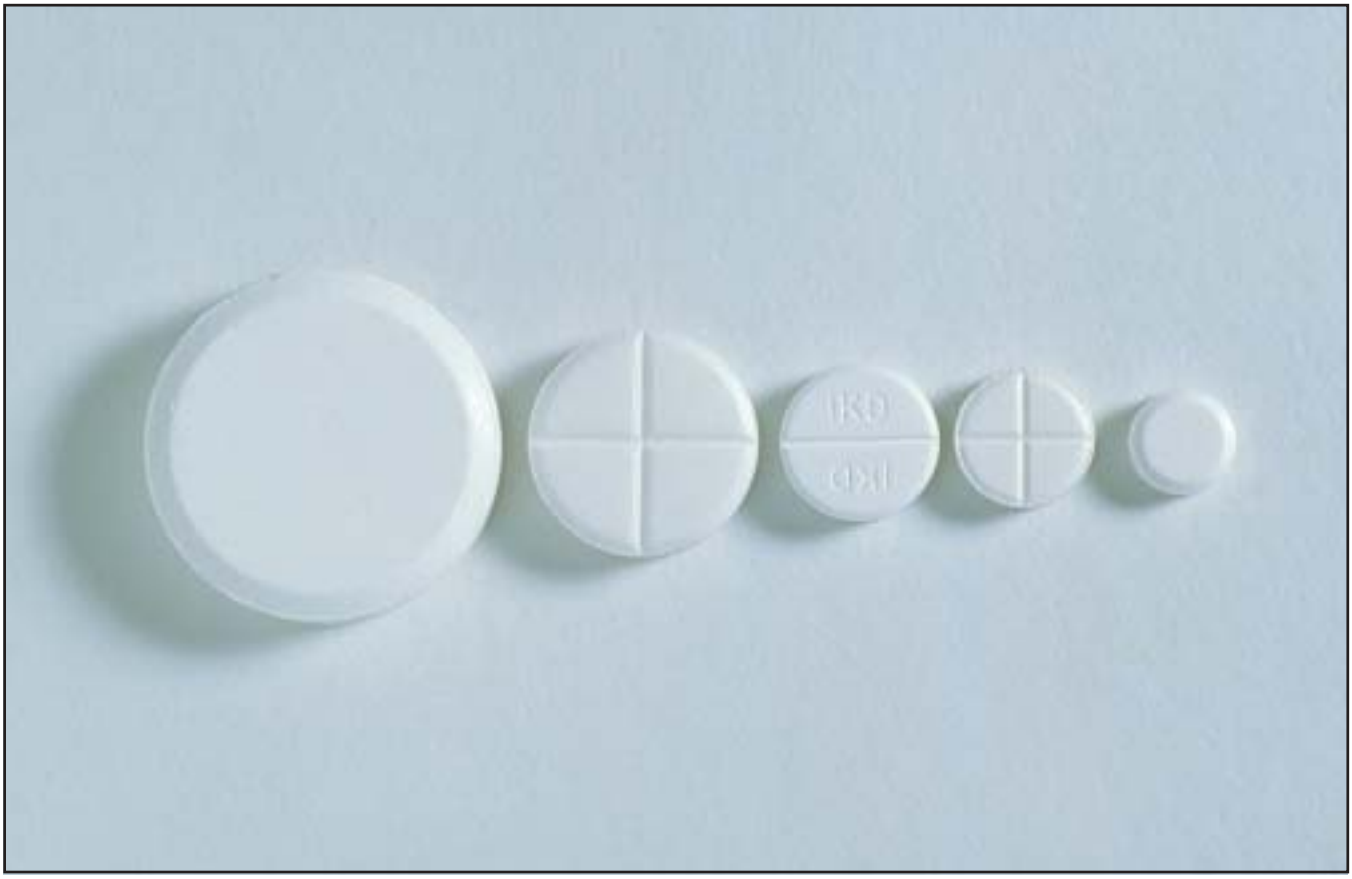
3.2.1 Description aid for the visual inspection of tablets

Tablets are normally presented in a circular or oval shape although triangular or pentagonal shaped tablets are known to exist. If presented in a circular shape their diameter can vary between 5.0 and 15.0 mm depending on the particular product being examined. All tablets which must not be swallowed, for example effervescent, soluble and dispersible tablets or lozenges, may show a much bigger diameter of up to 25 mm. Although the tablet thickness might vary their diameter never does. Hence, the tablet diameter is a unique property for a certain single product and a helpful means for identification purposes.

The tablet's surface can be flat or convex and may have bevelled or acute edges, lines or break-marks, engraved or embossed symbols or other markings. Tablets may be white or coloured. The colouring agent may have been added during the granulation process indicated by a coloured tablet core or added later during the coating process indicated by a white tablet core and a coloured coat only.

Tablets show no signs of blemishes such as dirty marks or spots, abrasion or erosion, cracks or chips or any other defects such as fusion or swelling. Drug products with such defects should be subjected to an identity test and, if passing, further scrutinized by thin layer chromatography for the determination of drug content and gross degradation.

Various tablet shapes and marks. Tablet marks are often embossed or engraved for easy identification. For documentation, draw a copy of them into the Reporting Form supplied.



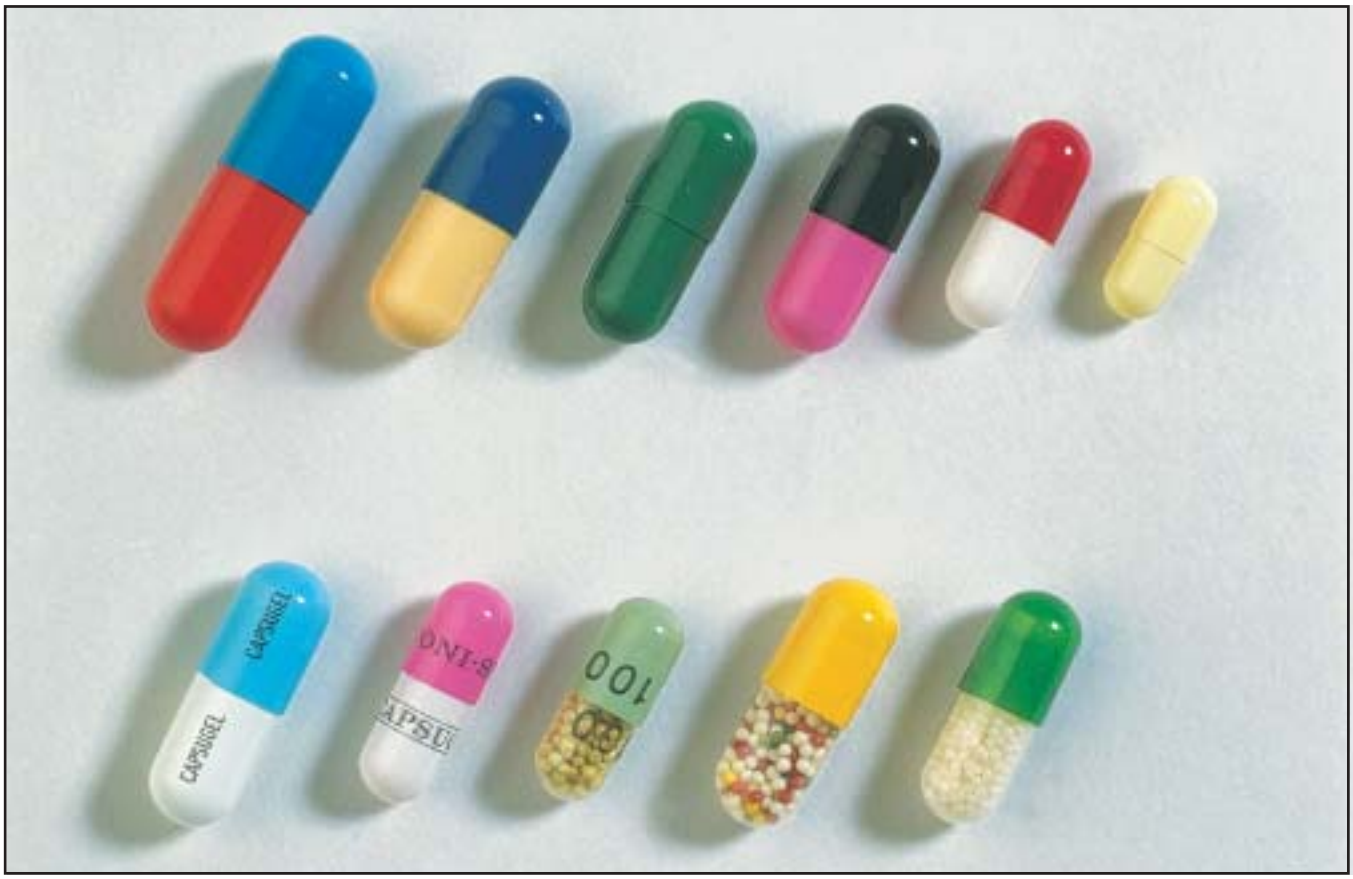
Circular shaped tablets in various sizes. For identification purposes, circular shaped tablets are best described by measuring their thickness and diameter and oval shaped tablets by measuring their thickness and length using a caliper rule. Write down the dimensions into the Reporting Form supplied.

3.3 Visual inspection of capsules

'Capsules are preparations with hard or soft shells, of various shapes and capacities, usually containing a single dose of medicament. They are intended for oral administration.

The capsule shells are composed of gelatin or starch , the consistency of which may be adjusted by the addition of substances such as glycerol and sorbitol or a mixture of these. Excipients such as surface-active agents, opaque fillers, anti-microbial preservatives, sweetening agents, flavouring agents and authorised colouring agents may be added. Capsules may bear surface markings. The contents of capsules may be of solid, liquid or paste-like consistency. They consist of one or more medicaments, with or without excipients such as solvents, diluents, lubricants, or disintegrating agents. The contents do not cause deterioration of the shells' (British Pharmacopoeia 1980).

As for tablets, capsules might be coated in such a manner that the shell resists the action of the gastric fluid but are attacked by the intestinal fluid to release the contents. Those capsules should be identified on the label as Enteric-coated Capsules. Capsules might also be designed in such a manner that they sustain the drug's rate of release in the gastro-intestinal tract and thus prolong the drug's action. Such products should be identified on the label as Sustained- or Slow-release Capsules.



Good quality hard gelatin capsules: The combination of capsule size and colour is almost unique for any one product and is a very good means for identification purposes.

Capsules are divided into two major categories called hard and soft gelatin capsules. The shells of hard gelatin capsules consist of two prefrabicated cylindrical sections, one end of which is rounded and sealed, the other being open. The contents of the capsule (drug with or without excipients) are filled into one of the sections, usually in solid form (powder or granules), which is then closed by slipping the other section over it. The security of the closure may be strengthened by suitable means.

The shells of soft gelatin capsules are thicker than those of hard gelatin capsules. They consist of a single part and may be of various shapes. The shells are formed, filled and sealed in a combined operation. Liquids may be enclosed directly; solids are usually dissolved or dispersed in a suitable excipient to give a paste-like consistency. Partial migration of the capsule contents and shell constituents may occur because of the nature of the materials and surfaces in contact.

Good quality soft gelatin capsules: The unique combination of size, shape and colours facilitates the identification and recognition of a given drug product.

3.3.1 Description aid for the visual inspection of capsules

All hard gelatin capsules show a common cylindrical shape and differ only in their size. However, the dimension of a particular capsule size is more or less constant with hardly any margin. Nine capsule sizes are available. They are known as triple zero, double zero, zero, zero elongated, one, two, three, four and five. In practice the capsule sizes from zero to three are the most common ones in use. Their precise dimensions are listed below and can be determined by using a template, a caliper rule or maybe an ordinary ruler.

In contrast to the capsule size, the number of capsule colours is unlimited. Cap and body shells may have the same or different colour. The shells may be clear and colourless or white and opaque or be of any other colour. Sometimes the shells are overprinted with an additional code or symbol. However, the combination of capsule size and colour is almost unique for any one product and is a very good means for identification purposes.

Compared to hard gelatin capsules, soft gelatin capsules show a longitudinal sealing line by which both sorts of capsules are easily differentiated. Unfortunately, the shapes and dimensions of soft gels vary as much as their colours and are not as easily described as hard gelatin capsules. Some are perfectly spherical others are oval or bottle-shaped. Preferably, the shells are clear and colourless allowing the capsule contents to be seen. However, white opaque or coloured opaque soft gelatin capsules are frequently in use allowing the protection of the drug substance against the effects of light. As for hard gelatin capsules, the unique combination of size, shape and colours facilitates the identification and recognition of a given drug product.

Capsules show no signs of blemishes such as fusion or swelling and the capsule shells is properly sealed and free of dents, cracks or loose powder. Additionally, no softening of the shells is observed. Drug products with such defects should be subjected to an identity test and, if passing, further scrutinized by thin layer chromatography for the determination of drug content and gross degradation.

Capsule size	Diameter of the body shell [mm]	Diameter of the cap shell [mm]	Length of closed capsules [mm]	Net volume [ml]
000	9.5	9.9	26.0	1.37
00	8.2	8.5	23.5	0.95
0 elong.	7.3	7.6	24.0	0.76
0	7.3	7.6	21.5	0.68
1	6.6	6.9	19.5	0.50
2	6.1	6.4	18.0	0.37
3	5.6	5.8	16.0	0.30
4	5.1	5.3	14.5	0.21
5	4.7	4.9	11.5	0.13

Sizes of hard gelatin capsules. A triple zero capsule with a fill weight of about 200 mg (about 0.3 ml) indicates a capsule underfill and a sub-standard drug product. Use a caliper rule in order to differentiate between capsule sizes. Note the capsule size of a given drug product in the Reporting Form supplied.

3.4 Quick beginner's summary

For documentation purposes, write down all product particulars using the *Reporting Form* as a guide during the visual inspection of pharmaceutical products.

General screening:

Drug products from particularly 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 disintegration and an identity test and, if passing, further scrutinized by thin layer chromatography for the determination of drug content and gross degradation. Repeat the examination with two other samples thus eliminating anomalous results. Should a product not pass all tests ask for a second opinion and refer an additional sample to a fully equipped drug control laboratory. Keep some retained samples in a safe place for future investigations.

Inspection of packaging material:

Tablets and capsules can be presented in single or multi unit dose containers such as blister packs or bottles. Patient packs may contain just ten or twenty tablets whereas bulk packs may contain a thousand or more tablets.
On reception, the container should be properly sealed and labelled and be without defects and damage. Seals should not have been violated. On opening of the container, a strong smell often indicates drug degradation. Exceptions are allowed for products containing flavours or drug substances with a characteristic odour for example penicillins. Excessive powder or pieces of tablets at the bottom of the container indicates the presence of abraded and broken tablets or crushed and opened capsules.
Other deleterious effects might be caused by excessive moisture uptake and are indicated by fused or sticky tablets and capsules or are shown by recrystallized drug substance on the solid formulation itself or on the container's wall.

Inspection of labels:

Both, the immediate container and the carton should have a durable label fixed on them. Labels can be replaced by print. The print on the label or packaging must be legible and indelible. The label should provide at least (1) the name of the drug, (2) the drug's strength, (3) the number of unit doses in the container, (4) the manufacturer's or distributor's name and full address, (5) the batch or lot number, (6) the expiry date and (7) the storage conditions.

Inspection of dosage forms:

Solid oral dosage forms are normally presented as uncoated tablets and capsules. Sometimes they are specially coated and should then be labelled as such; for example as enteric-coated capsules. Tablets might be designed as dispersible or effervescent tablets for dissolving in water prior to swallowing.

Tablets and capsules show no signs of blemishes such as dirty marks or spots, abrasion or erosion, cracks or chips or any other defects such as fusion or swelling. In addition, hard gelatin capsules show no signs of softening and the capsule shells are properly sealed and free of cracks and dents. The texture of a broken tablet or the content of an opened capsule must be white with no signs of any mottling or discolouration. Allowances must be made for products where the drug substance or any excipient itself consists of coloured powder or crystals.

Pharmaceuticals can not be blended with useless counterfeits if protected by tamper-evident seals. Any violated package should be subjected to further testing.

4 Disintegration Test

Tablets should be sufficiently hard to withstand handling without crumbling or breaking but they should also be sufficiently soft for easy disintegration in the stomach or intestine in order to make the drug available to the body. Due to poor drug processing or wrong storage tablets and capsules may harden and fail the disintegration test. The test determines whether tablets or capsules disintegrate in water within thirty minutes.

All uncoated tablets and capsules and all soluble, dispersible, effervescent and film-coated tablets, hence all quick release formulations, have to comply with this time of complete disintegration. Sugar-coated tablets may meet this specification but it is not a requirement. Only modified-release and enteric-coated tablets and capsules are allowed to deviate from this time of complete disintegration. These tablets and capsules should be labelled as such and must not be subjected to this test. Those products require a more sophisticated disintegration test which we do not yet perform.

However, simple counterfeit preparations such as capsules containing just sand or ground ceramics, or tablets made only of neat flour are easily spotted by their disintegration behaviour. Ground ceramics or sand settle straight at the bottom of the flask whereas the supernatant liquid stays clear or almost clear. Tablets and capsules containing only neat flour never really disintegrate. They just soak up water and form a sticky mass or just disintegrate in a couple of sticky lumps slowly settling at the bottom of a beaker. State of the art tablets and capsules containing modern disintegrants behave completely differently. For example, uncoated tablets of a good quality will normally completely disintegrate in water at 37 °C within 15 minutes.

Disintegration is defined as that state in which no residue of the tablets and capsules, except fragments of undissolved coating, remains in the test solution. It is a major defect if a drug product doesn't pass this test. The product can be rejected at this stage already. No further thin layer chromatographic assay or any other tests are required. This will save organic solvent and reference samples.

4.1. Quick beginner's summary

Place one tablet or capsule into a 100-ml wide neck bottle containing 100 ml of water. The temperature of the water should be close to body temperature (37 °C). Stir or shake the liquid from time to time and continue to operate like this for thirty minutes. You may stop earlier if the tablet or capsule disintegrates much faster. The tablet or capsule passes the test if no residue remains in the liquid or, if a residue remains, it consists of fragments of coating or is a soft mass with no palpable core.

Repeat the test on a further five tablets or capsules. The batch passes the test if all six tablets or capsules disintegrate. Repeat the entire test cycle should one tablet or capsule fail to disintegrate. Reject the batch should a further tablet or capsule fail again in the second run.

5 Thin Layer Chromatography

'TLC, a difficult name for a simple method'

The name chromatography was first used at the onset of this century by a botanist called Tswett who worked to separate coloured plant pigments. He used two Greek words, 'Chromatos' for colour and 'Grapha' for drawing, as a short description of his newly invented separation technique. A solution of mixed pigments were allowed to pass through a column of crushed chalk which on further passage of solvent finally separated the constituents of the mixture. The name chromatography has stuck since then, although most of the compounds to be separated nowadays are not necessarily coloured anymore. However, methods are now available for the visualization of uncoloured compounds for example the detection on exposure to ultraviolet radiation.

It was a chemist called Stahl who changed the experimental technique during the nineteen-fifties. Chalk and other adsorbents were now put on thin plates, for example onto microscope slides, and the name **t**hin **l**ayer **c**hromatography or in short **TLC** was introduced.

TLC has primarily been developed as a cheap and easy to use separation and identification technique for a mixture of unknown compounds for example vitamin preparations. It can also be used to decide on the presence or absence of impurities or degradation products.

It can be all of these but it can also be made quantitative in order to determine how much of each constituent is present in that mixture. The results obtained by a simple visual inspection of the chromatoplates can be accurate to 10% if great care is taken and skill executed. For our purposes and for many analyses in industry, this semi-quantitative technique is accurate enough.

In order to achieve this accuracy closely follow the operation procedures written down for each drug product in the individual monograph of this compendium. Also follow all advice given during additional training lessons and you will soon become an expert in thin layer chromatography.

5.1 How does a TLC system work?

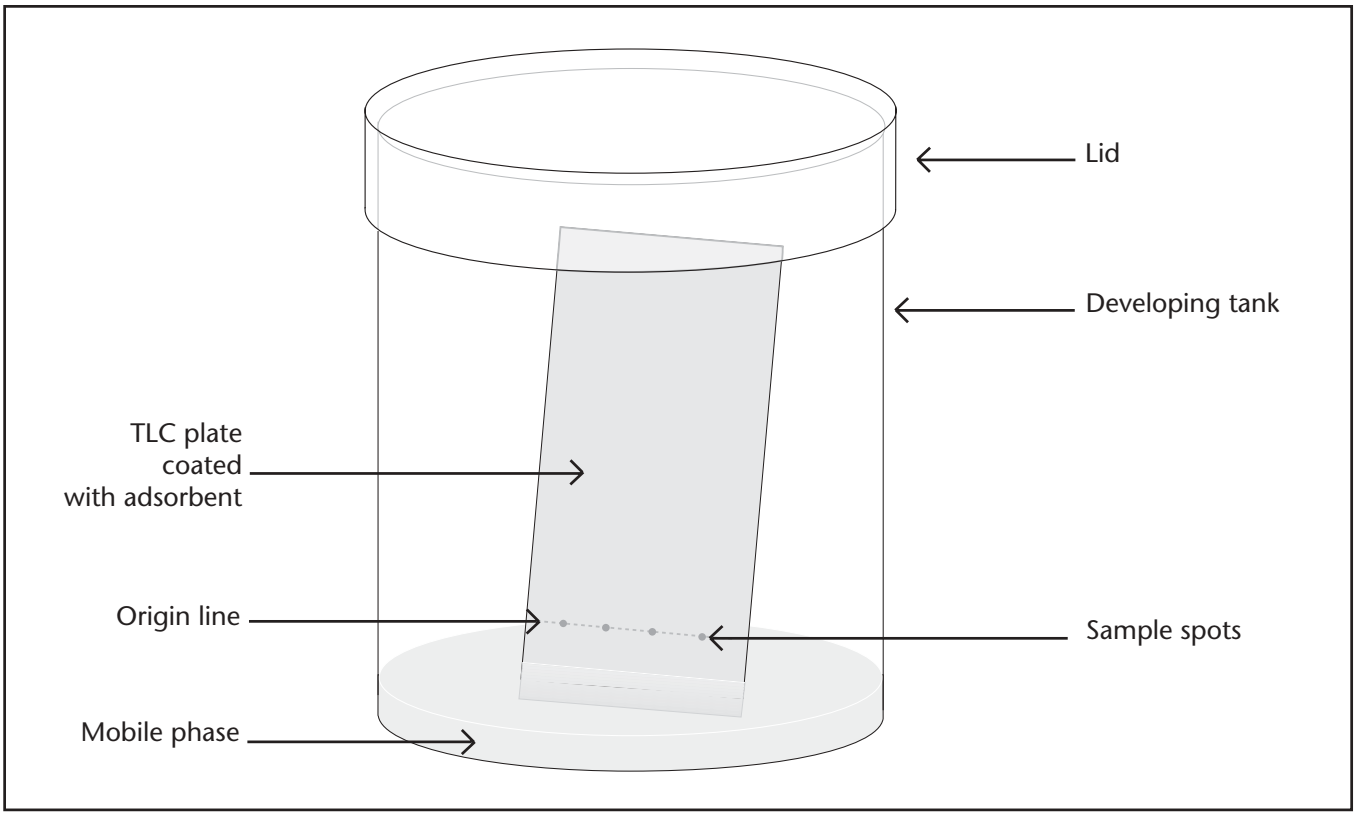
Thin layer chromatography, like all other analytical techniques, has its specialist vocabulary which you need to learn before you can understand how a TLC system is actually working.

The mixture to be separated is called the *sample* and the individual constiuents are called *components* or *solutes*. In our case all solutes are drugs. The sample, once in solution, is *applied* or *spotted* onto a *TLC* or *chromatoplate*. The plate itself consists of a solid support, for example glass, plastic or aluminium, *coated* with an *adsorbent layer* or *stationary phase* specially chosen to effect the separation.

The solute must be applied at a measured distance from the bottom of the plate called the *origin.* The loaded plate is then placed in a *tank* or *developing chamber* containing an *eluting solvent* or *mobile phase* which will flow over the plate and drive the solute away from its origin across the chromatogram. The run stops on removing the plate from the tank just before the *solvent front* reaches the plate's top end. The front line represents the finish and has to be marked. The distance between the origin and front line is the solute's or drug's 'racing distance' measured in centimetres.

Some of the drugs are eluted easily and get carried away alongside the mobile phase straight to the finish. Some others will never make it and stay at the origin and the rest will stop somewhere midway between the start and front line. Surprisingly, those drugs deposited midway between both lines will be the winner of the game. Only they can be identified using a comparator drug as a *standard* or *reference sample*. There is strong evidence of identical drugs in the test and standard solutions if the principal *spots* of both samples show the same travel distance. Additional confirmation can be derived from similarities in spot size, shape and colour or accompanying satellite spots visualized by common *detection* techniques for example exposure to *ultraviolet light* or *iodine vapour*.

The three major factors influencing a successful run in thin layer chromatography are the stationary phase, the mobile phase and the sample preparation.The secret of success is determined by the fine tuning of this magic triangle.



General description of a TLC system.

5.2 Stationary phase

The solid layer on a TLC plate is generally called the adsorbent. The nature and properties of the adsorbent are of crucial importance to the technique.

Adsorbents come with a wide variety of chemical structures consisting of very fine particles. Those particles are far from being perfectly round like billiard balls. In fact, they show porous surfaces whereby all pores provide additional surface area for adsorption. The surface area of a strong adsorbent will have an area of up to 500 square metres per gram. A microscope slide coated with eight grams of such an adsorbent will easily match the size of a football pitch which is truly a huge 'racing course' for such small things like molecules.

Silica gel is the most popular adsorbent. It comes in different particle sizes and with or without a binder denoted by the abbreviation G or H respectively. Additional coating with a fluorescent indicator is indicated by the abbreviation F 254. This does allow an easy detection of uncoloured spots with an ultraviolet light source of 254 nanometres. A few drugs need other adsorbents. For example, all tetracycline type of drugs need TLC plates coated with cellulose.

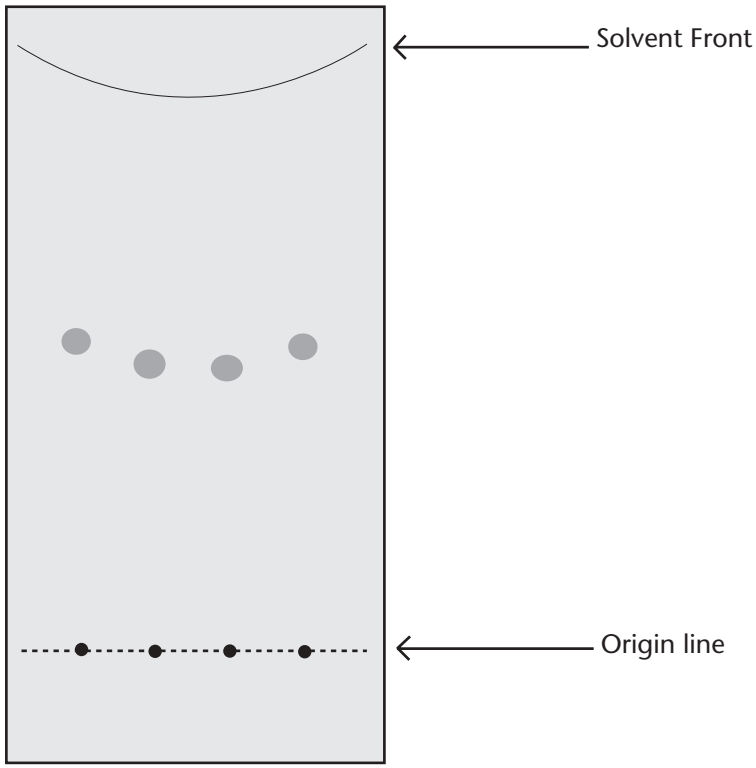
Bear in mind that adsorbents made of silica gel are quickly de-activated by moist atmosphere. However, the commercial plates supplied by Merck work perfectly well without prior drying when carefully stored in their original packaging. Merck aluminium or glass plates coated with silica gel 60 F254 have been found most satisfactory. Plates of 5 X 10 cm in size are required for our developing chamber.

5.3 Mobile phase

The mobile phase has two vital jobs to do: First, it must displace the drug from the adsorbent so that it can be carried in the mobile phase across the plate. Secondly, it must help to separate a mixture of drugs, excipients and impurities so that they can be deposited in different places of the chromatoplate and subsequently be identified. The effectiveness of a solvent in displacing solutes from an adsorbent is called *eluting power*. This power can be adjusted by using a mixture of different solvents further called the *solving system*. All solvent mixtures have already individually been designed for each drug listed in this compendium.

The major solvents used here are methanol, acetone, toluene and ethylacetate sometimes spiked with glacial acetic acid or ammonia in order to enhance the eluting power for a specific drug which might otherwise keep staying at the origin of the chromatoplate. It's like automobiles. Some run on unleaded petrol, others only on petrol spiked with lead and some others just won't run.

In order to obtain reproducible results you must ensure that the atmosphere in the TLC tank is saturated with respect to the solvent vapour. Pour all the solvent required into the tank and line all sides with filter paper leaving just a small slot for observation purposes. Close the tank and wait for about 15 minutes prior adding the loaded TLC plate. With mixed solvents, saturation of the air becomes even more important. Those mixtures must be prepared immediately before use and be rejected after the run. Otherwise evaporation of one or more compounds will occur and results can't be reproduced from one day to the other.



A TLC plate with identical samples developed in a non-closed tank: A concave solvent front develops caused by disturbing air ventilation and poor chamber saturation and even identical drug samples show different travel distances. Such a chromatogram cannot be used for identification purposes!

5.4 Sample

The sample can be anything from an aqueous solution to a mixture of oils or be a semi-solid product such as creams and ointments or it can be tablets or capsules containing a solid active ingredient. Each type of sample needs different treatment prior to application on a TLC plate. Currently, we are concentrating on oral solid dosage forms only.

5.4.1 Sample preparation

Solid samples such as drugs embedded in tablets or capsules cannot be applied directly to the TLC plate so they must be extracted and dissolved in a suitable solvent. All samples are diluted in two or three steps. This does save solvent. First, a *stock solution* is prepared which is then further diluted down to its final concentration called the *working solution* using, in all cases, a set of straight pipettes capable of delivering a known volume of 0.01 to 25 ml. Both, type and amount of solvent have already been determined and are described in each monograph.

5.4.2 Sample application

The sample should be spotted in one place and the spot should have the smallest diameter possible. Samples consisting of low volatile solvents, for example acetic acid and water, produce very small spot areas. Contrarily, highly volatile solvents form very big spots and methanol forms the biggest spots of all, especially when working under tropical climate conditions. Here, room temperatures of 30°C are easily exceeded and a maximum spreading of the spots can be observed. The best way to ensure that there is minimum spreading of the spot is to dry effectively between applications to the plate so as to remove the solvent.

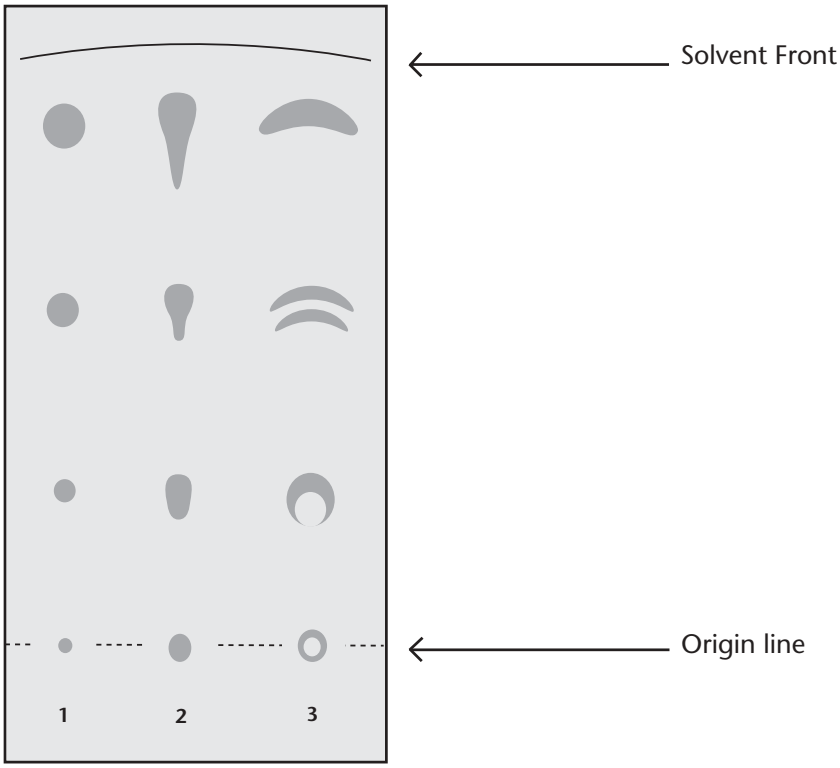
Applications are made with disposable microcapillary pipettes capable of delivering a known volume of 2 microlitres (µl) when filled from end to end. Prior to spotting, draw a line across the origin about 1.5 cm above the bottom end of the TLC plate thus ensuring that the spots are evenly spaced across the plate. You want to use a pencil of soft grade and a ruler to draw this line. Interrupt the line where samples shall be spotted.

Up to five spots can be placed on a plate of 5 x 10 cm at a time. 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 diameter never should. Different intensities are due to different amounts of tablet and capsule excipients or different drug concentrations in the sample solutions. A difference in spot size, however, relates to poor spotting. Spotting is precision work and might need some training. Repeat this step if a homogeneous spotting is not achieved.

It is important not to scratch the adsorbent layer when the drug solution is applied as marks cause the mobile phase to elute unevenly and the spots can become distorted.

For semiquantitative analysis, a sequence of standard solutions with known concentrations of the drug is spotted on the plate alongside the spot of the test solution; the same volumes of test and standard solution being used each time. The spot area should be kept as small as possible. It is best to place the chromatoplate onto a hot plate operated on a low level and spot the samples drop by drop thus ensuring a quick evaporation of solvent between applications. This procedure is especially important for solutions consisting of low volatile solvents; for example toluene, ethylacetate or water.

Finally, place the chromatoplate in the developing tank ensuring that the level of mobile phase does not reach where the samples have been spotted.



Sample application and its effect on spot size and shape. Run No. 1: Properly applied sample causing perfectly round spots. Run No. 2: Applying too much sample causing 'tear drops' or 'tailing'. Run No. 3: Applying the sample without drying off the solvent between drop by drop applications causing twisted or even twin spots.

5.4.3 Sample detection

Most compounds and drugs to be identified are not necessarily coloured and you want to visualize them after separation. Fortunately, methods are now available for the visualization of uncoloured compounds for example the detection of invisible spots on exposure to ultra-violet radiation or iodine vapour.

After development, you want to observe the chromatoplate in daylight first. Sometimes you will be lucky and a coloured spot shows up as seen regularly for all tetracycline type of drugs. Observe the size and intensity of both spots, the one from the reference as well as the one from the test sample. The colour might disappear so make sure that you mark the spot's size and shape for documentation purposes. Prior to any further spot detection, make sure that the chromatoplate is completely dried and free of solvents.

After inspection of the chromatoplate in daylight and after drying off all solvent, an examination of the plate with ultra-violet radiation follows in the dark. Two different wavelengths are used for detection: Short waves of 254 nanometres and long waves of 366 nanometres in length. The white adsorbent layer of a chromatoplate exposed to uv-light of 254 nm will turn light green due to its coating with a fluorescent agent. All spots loaded with drugs will cover up the adsorbent layer and extinguish the fluorescence previously observed. Most of the invisible spots are now turning dark green or violet. On exposure to uv-light of 366 nm, the chromatoplate stays pitch-black and only spots sensitive to this wavelength will be stimulated returning visible light of various colours, the colour depending on the actual drug under scrutiny. Observe the size and intensity of all visible spots and mark their sizes and shapes with the pencil supplied prior to leaving the dark room.

After inspection with an ultra-violet lamp, further detection of the plates takes place via iodine staining. Add a chromatoplate free of solvent to a jar containing iodine crystals and an atmosphere full of iodine vapour indicated by a deep violet mist. Allow to stand for about three minutes. Uncoloured spots attracting iodine vapour gradually turn brown and are made visible for further evaluation. Again, observe the size and intensity of the spots in daylight and with UV and mark them with a soft pencil for documentation. Sometimes you may want to apply heat additionally to the stained plates in order to make the spots last longer.

5.4.4 Sample identification

There is strong evidence that the drugs in the test and standard solution are identical if the principal spots of both samples show the same travel distance. Additional confirmation can be derived from similarities in spot size, shape and colour or accompanying satellite spots as frequently observed for penicillin preparations.

The spot's travel distance is described more accurately by quoting the relative retention factor or R_f value for short. Consult the picture on the opposite page and see how the measurements and calculations are precisely done: Instantly mark the solvent front when removing the chromatoplate from the developing tank. Dry off all residual solvent and detect the spots. Mark the center of the spots and measure both travel distances, the one from the solvent front and the one from the spots representing your drug product, using a graduated ruler. Starting point, in each case, is the origin line. The travel distance for the drug product is then divided by the one from the solvent front. The result obtained is the R_f value which may be expressed in percentage if multiplied by 100.

5.5 Quick beginner's summary

For all drug substances included in this manual the following procedure is applicable for confirming the identity and quantity of a given drug substance in its pharmaceutical dosage form via thin layer chromatography.

For drug identification, prepare one test and one standard solution both in the same solvents and at the same concentration as directed in the individual monograph. For drug content determination, prepare one test and various standard solutions all in the same solvents but in different concentrations as indicated in the individual monograph.

On a line parallel to and about 1.5 cm from the bottom edge of the chromatoplate , apply 2 µl of the test and 2 µl of each standard solution. Again, any deviation from this application volume is indicated in the individual monograph. Allow the spots to dry between each application.

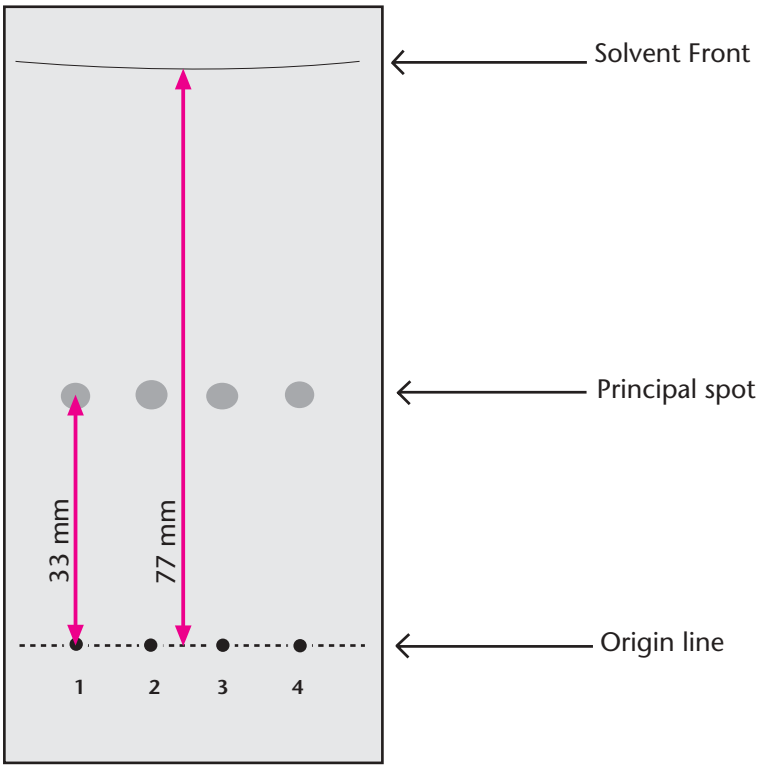
Develop the chromatogram in the solvent system as directed in the individual monograph until the solvent front has moved about three-quarters of the length of the plate. Remove the plate from the developing chamber, mark the solvent front using a pencil of soft grade, and allow any excess solvent to evaporate. Locate the spots on the plate by examination under ultraviolet light at 254 and 366 nanometer. Mark the size and shape of the spots.

The principal spot 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 standard solution. Further detection might be required by exposing the chromatoplates to iodine vapour.

$$R_f = \frac{\text{Distance moved by spot}}{\text{Distance moved by solvent front}}$$

$$R_f = \frac{33\text{mm}}{77\text{mm}}$$

$$R_f = 0{,}43$$



Sample identification via R_f value: The standard solutions are represented by run No.1 and 4 and the test solutions by run No.2 and 3. There is strong evidence that the drugs in the standard and test solution are identical if the principal spots of both samples show the same travel distance or R_f value.

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.

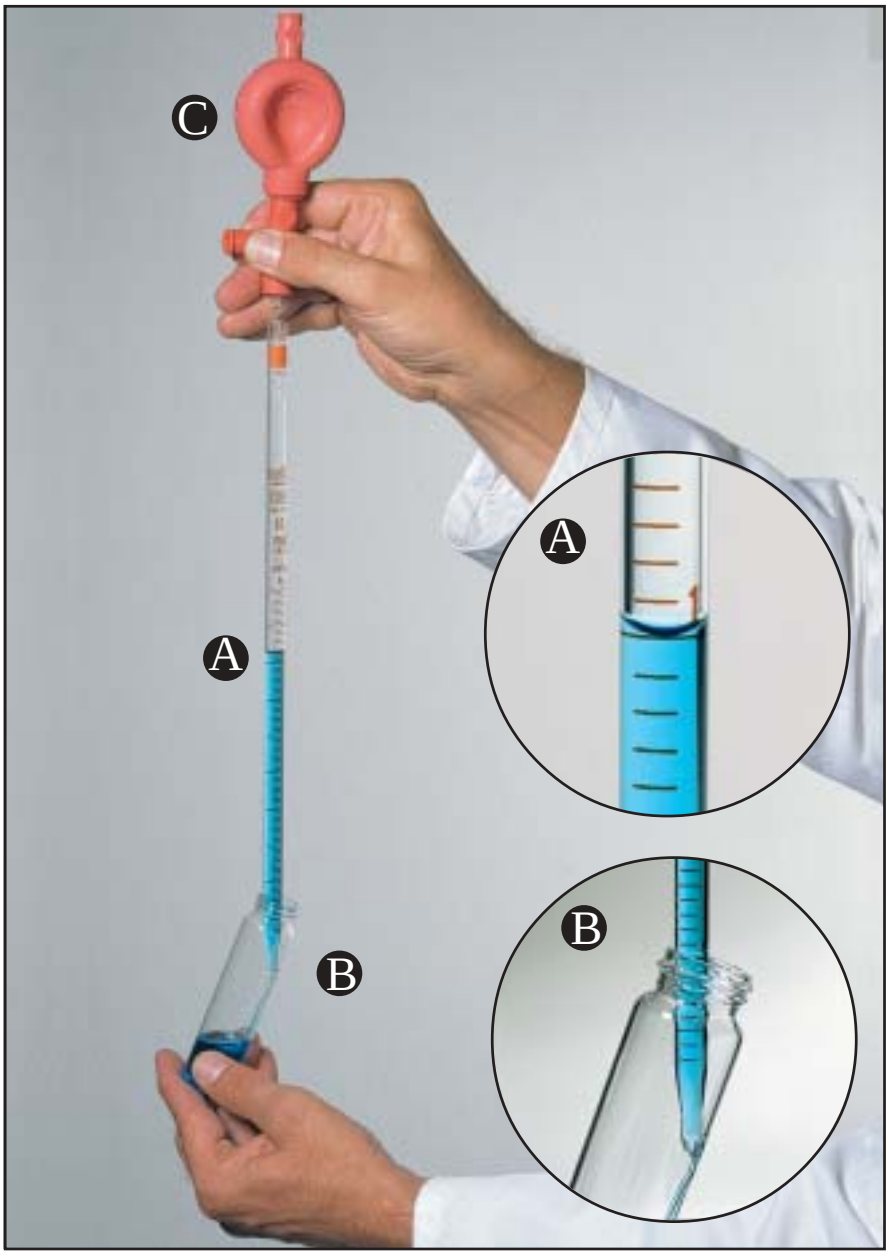
Using a funnel, sachets are emptied directly over a laboratoy glass bottle making sure that all residual solids are finally washed down into that bottle with a known volume of solvent.

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.

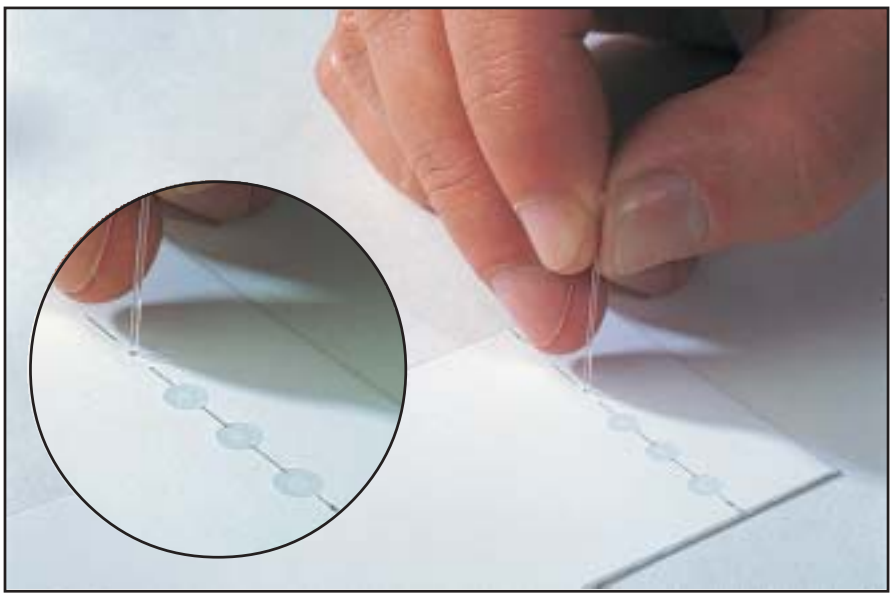
III. TLC: 2. Sample Application

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 F 254. 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.