Rapid Screening of Pharmaceuticals by Thin Layer Chromatography: Analysis of Essential Drugs by Visual Methods

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# Abstract

A method for rapidly screening pharmaceuticals by Thin-Layer Chromatography (TLC) has been designed for use in areas with limited resources and by operators with limited training. An apparatus was designed to perform the analysis in a plastic bag under equilibrium conditions. Results can be reproduced by different operators and in different locations. The analysis can be performed without electricity or in a remote area away from a laboratory. It is especially suited for field use in developing countries. The method is low-cost, maintenance-free, fast, and reliable and also uses limited volumes of solvents.

The analyses can be performed without weighing if reference materials can be supplied in tablet form provided the drug content is listed and only 1 unit is required for each analysis.

All procedures were developed for the analysis of drugs from a partial list of essential drugs established by the World Health Organization. Three drugs were selected and prepared in the form of reference tablets. Comparisons with the analyses of the drugs in standard dosage forms were made by using reference tablets and primary USP standards. Comparable results were obtained, proving the concept that the screening process can be conducted by using reference tablets and without weighing either the sample or the reference. The method has been successfully demonstrated and used in Swaziland, in the U.S.A. by high school teachers, and in Saudi Arabia by personnel from the Ministry of Health. Personnel can be trained to perform screening analysis of drugs in a short time.

#### Introduction

A simple analytical method for rapid screening of drugs is needed for use in areas that do not have fully equipped laboratories. The method should fulfill the following criteria: It must be inexpensive, must require only limited facilities and minimum training, and must be capable of quickly analyzing the drugs. The analysis must be operable without electricity or a laboratory facility. It must be possible to prepare solutions without weighing either the sample or reference, provided reference tablets are available. The procedure must be performed in open air and with no hood available if it is to be suitable for screening in field type operations. No electronic measurements should be needed, and estimates should be made from visual inspections under daylight. The TLC method satisfies these requirements.

A previously developed TLC method (1) was useful for rapidly screening drugs by comparing the sample solution with different concentrations of reference material. The success of their system required the laboratory to have electricity and special equipment. Even though their method was fast and economical, and the operator required little training, it did not meet the needs of locations without electricity and with only limited resources. This work was directed toward the development of a rapid screening system which could be performed either in a well-equipped laboratory or in remote areas with or without electricity, and by persons having limited technical background. Another objective was to establish the necessary specifications for the reference tablets. The study was limited to a few drugs declared essential by the World Health Organization and to additional ones to show the general applicability of the method. The method described here is intended not as a replacement for official methods, but only as a rapid screening procedure for drugs in areas with limited facilities.

TLC as normally practiced uses thick glass tanks, beakers, or other glass containers for the development of the chromatograms. A general systematic procedure that uses inexpensive, portable, unbreakable equipment would be useful for the rapid screening of pharmaceuticals in a field environment. Some of the desirable features of such a system would include portability, low cost, and simplicity of operation with minimal training to achieve acceptable results. The apparatus should be designed so that it can also be used where laboratory facilities are unavailable and no instrument maintenance is required.

Flinn et al. (2) described a simple, inexpensive TLC method in which rigid and fragile developing chambers were replaced by flexible polyethylene bags for developing the chromatogram. They tested the analytical capability with theophylline in the experimental system. A prototype system was developed to provide a more efficient analysis under equilibrium conditions. The complete assembled apparatus (Figure 1), known as "SPEEDY TLC" (3), is commercially available. The apparatus consists of a rigid aluminum support, 2 aluminum trays for holding the TLC sheets, a polyethylene development bag, and saturator pads. The TLC apparatus allows the analyses to be performed under equilibrium conditions to prevent distortion of the spots. Reproducibility between analysts and laboratories using the device is good.

Other containers such as beakers and jars have been used, but none were as successful as the polyethylene bag. The solvent level can be controlled and the development stage started and stopped as desired by simply manipulating (raising and lowering) the bag while it is in the holder. The entire system can easily be supplied in kit form so that analyses can be carried out without delay.

#### Experimental

Apparatus

(a) Development bag.--Fabricate the development bag, 40 cm long, from large rolls of 0.006-gauge flat polyethylene tubing, 8 cm wide (commercially available from any plastic bag supplier). Seal each tube with an impulse sealer at a point ca 6 cm from the bottom. (The development bags can also be supplied prefabricated from Granite City Engineering(3).)

(b) Saturation pads.--Cut rectangular saturation pads from heavy filter paper (0.3 mm thick). (The pads are available in 20 H 20 cm sheets from

laboratory supply houses.) Cut 1 large pad, 13.1 H 7.1 cm, and 2 small pads, each 10.0 H 6.0 cm, for each complete assembly. Adjust the pads to fit the polyethylene bag. To establish and maintain equilibrium in the plastic bag chamber before and during development, clamp the larger pad between the 2 aluminum TLC support trays so that it extends below the holders and into the solvent. Fit the 2 smaller saturation pads between the aluminum tray and the TLC sheet. Insert 1 of these small pads in each tray between the clip and the tray first, and then insert the TLC sheet on top of each pad. (Some of the thin layer area that contacts the clip may be disturbed while the TLC sheet is slid between the clip and saturation pad, but this does not cause a problem because the development step is stopped before the solvent front reaches the clip area.)

(c) Thin layer support.--Use plastic sheets coated with a layer of Silica Gel 60 (E. Merck, F-254 or equivalent) 200 :m thick. (The plastic-backed TLC sheets, 20 x 20 cm, are the most desirable.) Cut each sheet into 8 equal parts to form smaller sheets each measuring 5  $\pm$  10 cm.

(d) Plastic bag for visualizing solution.--Prepare the bag used for the iodine dipping solution from the same roll of plastic tubing as the development bag, except that only a short piece is required. Cut off the front part of the top portion, leaving it only slightly longer than the TLC sheet. Replace this bag or thoroughly clean it each day to prevent build-up of the dried iodine solution, which can eventually damage the thin layer during the dipping process.

(e) Small plastic bag.--A small plastic bag(approximately 3 X 6 cm) is fabricated from a development bag using a heat sealer for crushing the sample tablets.

(f) Positioning rod.--From a portion of a wire coat hanger, prepare a small rod with a small S hook on one end to correctly insert and remove the saturators, TLC film, and their holders within the plastic developing assembly bag, remove the film holders from the bag, and manipulate the drying pad used to dry the bag between developments.

(g) Liquid Chromatograph.--With automatic injector, variable wavelength detector, and data module (Waters) and a 30 cm  $C_{18}$  column. The drugs were assayed by U.S. Pharmacopeia procedures when a method was described.

(h) Densitometer.--For measuring the intensity of the TLC spots at 254 nm (Shimadzu Dual-Wavelength TLC Scanner, Model CS-930).

Chemicals

All standards were either USP primary standards or secondary standards which had been previously compared to a USP primary standard.

Reference tablets of acetaminophen, ampicillin, and chloroquine phosphate were prepared by the Department of Pharmacy, University of Maryland, Baltimore, MD, according to the specifications developed in this work. A conformity study was made on each batch by high performance liquid chromatography, and the coefficient of variance was found to be approximately

## 3%.

### Preparation of Samples

All analyses described in this report use the declared content of the dosage form as the weight of the drug in the sample, since the entire unit of the formulated drug is used.

Tablets.--The sample tablet must be pulverized to ensure that it is completely dissolved within the small plastic bag. Instead of grinding the tablet, crush it inside the plastic bag before applying the solvent both to speed up and to ensure a thorough extraction. Crush with a smooth object, such as a pestle or small hammer, by pressing on the tablet with a rolling motion, rather than by striking. (Striking the film with the object punctures the film and causes some loss of the drug. If the dosage unit is hard-coated, it may be necessary to initiate the process by striking it once or twice to crack it, but care must be taken not to puncture the bag.) After crushing the dosage unit, open the bag so that the solvent will have easy access to the powder. Drop the bag and its contents into a flask. Perform all manipulations of the bag carefully while it contains powder, to ensure against loss.

Capsules.--Drugs in capsule form do not need to be crushed, since the contents are already in powder form. To speed the dissolving process, slit the end of the capsule with a sharp razor blade, making the slit large enough to prevent closure from swelling, which would slow the process when the solvent is added. Some capsules can be separated by gently pulling apart the 2 sections and dumping the contents into the flask.

#### TLC Analysis

WARNING! Take necessary precautions to avoid skin contact with liquids and solids required for this work and avoid breathing their vapors!

To avoid the necessity of weighing when the solutions of references are prepared for analysis, use individual preweighed references furnished prepackaged in tablet form and containing a predetermined amount of the drug. If a reference tablet is not available, weigh a primary or secondary standard.

Note: The quantity of sample is based on the quantity of drug declared for that particular dosage unit (tablet, capsule, etc.). The sample and reference solutions are prepared from 1 unit of each dosage form. The desired concentration is obtained by a simple volume adjustment. The sample solution from drugs having large dosages may be prepared from a fraction of the tablet (if the tablet can be broken into clean equal fractions); however, this could lead to error. An effort was made to keep constant the volumes used for preparing solutions to reduce the glassware needed. In all cases, the volumes have been kept low by first preparing a concentrated solution and diluting an aliquot as necessary.

Preparation of solutions.--Prepare solutions of the reference tablets and sample in the required solvent. (Some drugs require a lower or higher concentration, depending on the intensity of the visualized spot.) Prepare the

sample solution by adding 1 entire unit of the dosage to a 100 mL wide-mouthed bottle with cap, then add 50mL of the solvent. Dilute an aliquot of this solution if necessary to make the required concentration. Prepare the reference solutions by dissolving 1 reference tablet to produce the high concentration and diluting a portion of this solution to give the low concentration reference. The concentrations of the reference solutions should represent 85 and 115% of the expected sample concentration for most drugs (low and high concentration limits, respectively; for antibiotics, the respective limits are 85 and 120%). A volume of 4 mL was selected for preparation of the reference solutions because it could be handled easily with a 5 mL graduated syringe. The appropriate concentrations of the solutions of the TLC sheet have been experimentally determined.

Preparation of the TLC sheet.--Prepare the TLC sheet as shown in Figure 2. Gently mark a fine line across the bottom of the 5 H 10 cm sheet (the vertical position of the film is the long dimension) 2.5 cm from the bottom edge. Use a dull #2 pencil, and do not press hard enough to mar the silica surface. Mark another line across the sheet 1 cm down from the top edge. To limit the maximum migration point, remove the silica coating from this upper line in a zone ca 2 mm wide by applying pressure to the pencil as it is being drawn across the sheet. Mark 2 visible dots at the sheet edge 1 cm from the bottom for reference points as the limiting level of the liquid. Remove ca 1 mm of the silica coating from the side edges of the 5 H 10 cm film and ca 5 mm from the bottom edge (a fine-tooth hacksaw blade works well for this operation). Completely remove the coating around the edges, leaving no material, since any remaining coating or loose powder will transport liquid and cause edge effects which distort the spots. Do not breathe any of the finely divided silica. Remove loose powder, using a clean, soft brush or weak air jet, or by lightly tapping one edge of the TLC sheet on a hard solid object. Remove remaining loose powder by rubbing lightly with your finger, being careful not to remove any of the attached thin layer(do not breathe any of the finely divided silica). Mark 3 spotting positions on the bottom penciled line at 1.5, 2.5, and 3.5 cm from 1 edge of the sheet.

Spotting the film.--Spot 3  $\mu$ L aliquots of the solutions at positions on the line drawn at 2.5 cm from the bottom. Spot the sample solution, expected to be equivalent to 100%, in the center spot position. Spot the 85% reference at the left position and the 115% reference (120% reference for antibiotics) at the right position. Spot the solutions as shown in Figure 2. Allow the spots to dry for 5 min (some solvents may require longer time) before development to ensure that no solvent remains.

Development and visualization of spots.--The apparatus has 2 plate holders so that duplicate analyses can be performed under identical conditions if desired. Place 1 of the 2 spotted TLC sheets on top of each saturation pad contained in each of the 2 aluminum trays. Place another saturation pad slightly larger than the above pads between the bottoms of the 2 aluminum trays. Then clamp these trays together with a small clip. It is not necessary to remove the clip when changing saturation pads or TLC sheets. Slip the saturation pads and the TLC sheet under the clip, making sure that the TLC sheet is properly aligned and held in position.

Pour 17-20 mL of the developing solution into the plastic bag. Carefully lower the assembled aluminum holders into the bag without getting any solvent on the TLC sheet. The long saturator pad should dip into the solvent during this operation to saturate the developing chamber. Clamp the assembled bag in the rigid support straight up and down (some room is available on each side of the bag to make minor adjustments) with no crimping when the bag is properly positioned. Seal the bag with its contents by making 1 or 2 folds at the top of the bag and clamping.

Allow 10 min for the system to reach equilibrium, and slowly pull down the bag until the developing solvent reaches 1 cm from the bottom of the TLC sheet. The developing solution will begin to migrate and will continue until the upper line on the TLC sheet is reached. Watch the migration of the solvent, and keep the liquid level constant at the 1 cm mark (lower marking) on the TLC sheet by making minor adjustments of the bag during the development time. The solvent migration stops automatically when it reaches the upper marked (scored) line. Do not allow the TLC sheet to stand after the solvent has reached the automatic stop level. Remove the assembled aluminum holders with the TLC sheets from the polyethylene bag.

Allow the TLC sheets to completely dry. Visually compare the intensity of the spots of the sample with those obtained from solutions of references at different concentrations. The spots from most drugs will not be visible under white light, and will require special treatment to make them visible. If a source of ultraviolet radiation is available, always examine the plate under UV light at 254 nm(electric operated is better than battery) before using any other means to detect the spots. For drugs that produce light or invisible spots because they have weak absorbance in UV light, treat the spots by dipping the dried film into a visualization bag (similar to the development bag except that it is shorter) containing a solution of iodine and acidified potassium iodide (4) prepared as described by Senanayake and Wijesekera(5). The iodine stains the spots, making them visible in white light.

#### Discussion

The World Health Organization through UNICEF identified the following drugs as most essential, based upon frequency of usage and their effect on improving the quality of life:

- 1. Acetaminophen, all oral forms
- 2. Amoxicillin, all oral forms
- 3. Ampicillin, all oral and injectable forms
- 4. Benzylpenicillin (Penicillin-G), all injectable forms

5. Chloramphenicol, all oral and injectable forms

- 6. Chloroquine diphosphate
- 7. Mebendazole, all oral forms
- 8. Praziquantel, all oral forms
- 9. Quinine sulfate, all oral forms

The following drugs were added to the list to demonstrate the applicability of the method:

- 10. Cloxacillin, all forms
- 11. Estradiol cypionate
- 12. Sulfamethoxazole, all forms
- 13. Theobromine or theophylline
- 14. Trifluoperazine HCI

The above drugs were used to establish the suitability of the apparatus for rapid screening apparatus of pharmaceuticals. All analyses were performed either with USP primary standards or with secondary standards which had been compared previously to the primary standards. The objective of this portion of the work was to develop the complete method needed for visual observation by iodine staining when the reference drugs were supplied in tablet form. Another goal was to reduce the number of solvents needed for development.

The formulated drugs in normal dosage forms and contents were obtained from commercial pharmaceutical suppliers. The concentrations needed for the sample and standards were prepared by weighing and diluting aliquots. Table 1 shows the drug content of the reference tablets found suitable for viewing when stained with iodine.

The number of chemicals used as solvents and developers must be minimal if a method is to be successful in areas with limited supplies. Table 2 shows the minimum number of chemicals needed for the analyses of the above drugs. This list is not necessarily complete, but it is sufficient to begin such analyses. Chloroform has been used as a developer solvent even though it is carcinogenic; it was used in our well-equipped laboratory where suitable handling facilities were available. All TLC can be performed with other development solvents so chloroform can be eliminated. Studies are under way to find other solvents by using the polar series of chemicals to establish a system free of chloroform.

The chemicals listed here are the most widely used for TLC analyses; however, additional solvents may be necessary for other pharmaceuticals. All solvents have been found compatible with the polyethylene bag. Chemicals must be handled properly, and all analyses must be performed in areas with ventilation, preferably in a hood with a suitable air flow if one is available.

As emphasized above, the rapid screening of pharmaceuticals is intended to be used in areas where equipment and training are limited. If the analyses were performed in well-equipped laboratories with highly trained personnel, it would only be necessary to indicate the final concentration needed. The method was developed to use only 1 unit of a reference tablet and volume of 4 mL to prepare the highest desired concentration. The volume of solvents required was kept at a minimum to reduce cost, decrease exposure to chemicals, and decrease waste disposal. It was also found that measuring small volumes accurately is difficult with pipets or limited equipment. Experience showed that pipets are impractical for use by the unskilled analyst, whereas graduated syringes are easy to use. Although the graduated syringe is not as accurate as volumetric glassware, it has sufficient accuracy for this type of estimation.

Procedures were developed for preparing reference solutions based upon the use of weighed standards, since no reference tablets existed at that time. The specifications for a single dosage unit call for the drug content to fall between 85 and 115% of the declared content for most drugs and between 85 and 120% for the antibiotics. This criterion was used to establish the suitable conditions for the reference solutions.

The drug contents of the reference tablets were determined on the basis that a single tablet contained the quantity necessary to prepare a concentration equivalent to the highest allowable concentration of the sample (115 or 120%). The lower concentration of the reference (85% of the sample concentration in both cases) can always be obtained by diluting an aliquot of the high concentration solution with the same volume regardless of the concentration because the preparation is based on a percentage (1 mL diluted to 1.35 for 115% solutions and 1 mL diluted to 1.41 for 120% solutions).

Table 3 shows the suggested concentration for the sample, the reference tablet content, and the high concentration of the reference when 1 unit of a reference tablet is dissolved in 4 mL solvent. The volume to be added can be adjusted when the reference tablets contain a weight other than the suggested amount.

The sample solutions are prepared from 1 dosage unit dissolved in 50 mL; therefore the same volume of solvent is used in most cases except for those drugs with a small content. When necessary, an aliquot was used to prepare the suggested concentration from high dosage drugs. Table 4 shows the drugs, the content of a typical dosage unit, the solvent system, and any required dilution to make the desired final concentration. The volume needed will have to be modified when the declared content of the drug differs from that listed.

Table 5 lists the developers that have been found satisfactory. Other developers could have been used. Chloroform has been used in the developer for several of the drugs as a matter of convenience in developing TLC methods. However, because chloroform is carcinogenic, it may be desirable to substitute another solvent from the polar series. Any developing system may be used as long as the relative retention lies between 0.1 and 0.8.

The ability to analyze these drugs visually in white light due to a change in intensity of the spots with concentration was verified by measuring the intensity in the UV at 254 nm with a densitometer. Plots of concentration versus intensity

were found to be linear with a correlation of 0.99+ for all drugs tested. The densitometer measurements demonstrated that differences in intensity were sufficient for visual analysis. Because spots vary in size with concentration, size and intensity differences can readily be detected visually. All TLC sheets were dipped into a solution of iodine after the UV measurements, and the intensities were compared visually. Again the differences could be seen well enough to decide whether the drug was within specifications. The results showed that if reference tablets were available, drugs could be rapidly screened with the same confidence as a comparison with USP standards. The data established the quantity of drug required in each reference tablet and the conditions for analysis.

To be suitable, the reference tablets must be stable over a period of time and variation in temperature. In many areas of the world, daily temperatures range around 40°C during a large part of the year. All the drugs listed in Table 1 were tested for stability over a period of 1 year at 40°C under anhydrous conditions. Since no reference tablets existed at this stage of the investigation, formulated drugs in normal dosage forms were used. The formulated drugs were stored in sealed glass bottles and in a 40°C oven. Samples were removed at intervals and analyzed by liquid chromatography using high and low concentrations methods to detect possible degradation and assay. USP primary standards were used as references (6). The listed drugs showed no degradation when not exposed to moisture at this elevated temperature. Some drugs were in capsules and others were in tablet form. It would be expected that drugs would be more stable in tablet form than powder.

To test the concept of using reference tablets in rapid screening of drugs by TLC, the following 3 drugs were selected from the essential drug list: acetaminophen, ampicillin, and chloroquine phosphate. These drugs were selected because of the broad range of differences in concentration needed for suitable visual analysis. The reference tablets were prepared by the Department of Pharmacy, University of Maryland. If reference tablets of drugs were available, neither the sample nor reference would need to be weighed, and the complete analysis could be done in remote areas or away from a laboratory.

The total weight of each of the reference tablets was selected to be 100 mg for convenient handling. This meant that different reference tablets would contain a wide range of excipients. The excipient content ranged from slightly over 50% to 97+%. Table 6 shows the suggested weight for the active drug, the measured assay, the standard deviation, and the percent of the expected assay. The measured content of the active drug and standard deviation would be supplied. The volume of solvent needed for the high standard is determined by dividing the weight (mg) by the concentration needed for the high standard (mg/mL). The volume can be measured by a 5 mL graduated syringe which is accurate to within the overall accuracy of the analysis. All reference tablets were formulated to disintegrate quickly in the solvent system to eliminate grinding. The assays listed for the tablets were determined by liquid chromatography with

USP primary standards as the references, and enough samples were analyzed to establish a reliable standard deviation. The standard USP methods with slight modifications were used insofar as possible (6).

Samples of acetaminophen, ampicillin, and chloroquine phosphate in their standard dosage forms were analyzed by comparing the results with the USP primary standards and with the reference tablets. The analyzed content was used for the reference tablets, and the USP standards were weighed on an analytical balance. As an example, Figure 3 shows the comparison of acetaminophen (paracetamol) with the USP standards and the reference tablets. Similar data were obtained with the two other drugs. The least squares fit of the data is presented as the solid line, which shows that the data from the reference tablets agree with the data from USP standards. A variance is recognized in the assay content of each dosage form as well as a variance in the reference tablets. The linearity of the spot intensity as a function of concentration was checked by densitometer in the UV at 254 nm. It was possible in some cases to measure the intensity of the iodine spots in the visible range, but in most cases it was difficult because the spots and background changed with time. Correlations of the UV intensities by least squares fit of intensity versus concentration for the 3 drugs were in the range of 0.99+ for the reference tablets and USP standards. It was shown that concentration versus response is linear, which allowed an estimation of the drug content. The results for 100% of the samples shown in the plot has a variance; therefore if this spot is ignored and a calibration curve is developed with the USP standards, an estimate of the concentration can be made.

Table 7 shows the estimated content of these 3 drugs. All are within the specifications, even though the cloroquine phosphate content is low. The low value of chloroquine could be due to the extraction from the reference tablet, which contains only 2+% of the active ingredient. The R<sub>f</sub> values for the 3 drugs are shown for reference. The data show that reference tablets supplied with a known content could be used to rapidly screen pharmaceuticals without weighing with a confidence equal to weighing with USP standards. When the spots indicate that sample is out of specification, the sample must be submitted for an official analysis, since this method is intended only as a screening process.

Each reference tablet should be individually packaged in an aluminum or plastic wrap to prevent exposure to moisture. The packaging for the single tablet would be similar to that commonly found in many over-the-counter drugs.

Rapid screening of drugs by the plastic bag method has been tested successfully over a period greater than 1 year in Swaziland, Africa, under the direction of Project HOPE, and personnel were trained with minimal effort (7). Over 100 separate analyses were performed, and new methods were developed. The method has been further tested in high schools and incorporated into a teaching module tested by personnel from the Ministry of Health, Saudi Arabia. All have reported success with the method for screening.

## Conclusions

1. The rapid screening method by TLC has been demonstrated to be useful in remote areas where resources and training are limited. Operators with limited chemistry backgrounds can be trained quickly to perform analyses successfully. Teachers of high school chemistry have shown that the method can aid in improving the general chemistry curriculum.

2. Use of reference tablets containing a predetermined drug content makes it possible to analyze drugs without weighing or the use of electricity. Results obtained with reference tablets are equivalent to those from USP standards for screening purposes.

3. A system has been developed which is low-cost and free of maintenance, uses small quantities of solvent, requires limited laboratory equipment, and helps the environment.

4. The coefficient of variance (3 to 5%) for visual methods is larger than for instrumental analysis which makes the determination unreliable at or near the lower or upper concentration limits. Those samples which show a content near the limits of the specifications should be analyzed by an official method. Most samples will show a concentration near the middle of the limits, and the difference in intensities is easily detected.

# References

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Drug	Content, mg/tablet
Amoxicillin	24
Ampicillin	24
Benzylpenicill	lin 24
Chloramphen	nicol 48
Chloroquine p	phosphate 2.3
Cloxicillin	24
Estradiol cypi	ionate 23
Mebendazole	
Paracetamol	46
Praziquantel	23
Sulfamethoxa	azole 23
Theophylline	2.3
Trifluoperazin	

Table 1. Drugs and their contents used for reference tablets

Table 2. Chemicals needed for TLC analyses

Acetone (4 L) Ammonium hydroxide (500 mL) Chloroform (4 L) Ethanol, 95% Ethyl acetate (4 L) Formic acid (500 mL) Glacial acetic acid (500 mL) Hydrochloric acid (500 mL) lodine crystals (100 g) Methanol (4 L) Potassium iodide (100 g) Toluene (4 L) Distilled water

	sted concentration or visual analysis,	Tablet content,	Master concentration,
Drug	mg/mL	mg	mg/mL
Group A			
Amoxicillin	5.0	24.0	6.0
Ampicillin	5.0	24.0	6.0
Benzylpenicillin	5.0	24.0	6.0
Chloramphenicol	10.0	48.0	12.0
Cloxicillin	5.0	24.0	6.0
Group B			
Chloroquine diphosp	hate 0.5	2.3	0.575
Estradiol cypionate	5.0	23.0	5.75
Mebendazole	0.5	2.3	0.575
Paracetamol	10.0	46.0	11.5
Praziquantel	5.0	23.0	5.75
Sulfamethoxazole	5.0	23.0	5.75
Theophylline	0.5	2.3	0.575
Trifluoperazine HCI	0.5	2.3	0.575

Table 3. Preparation of solutions from the reference tablets<sup>a</sup>

 $\overline{a}$  USP limits of acceptability for individual tablets of drugs: Group A, 85-120%; Group B, 85-115%.

Drug Dilu-		Declared	Final concn, Solvent	., Co	oncn,
Dilu-	wt, mg	mg/ml	mL	mg/mL	tion
Amoxicillin	250	5.0	0.1N HCl, 10 + acetone, 40	5	no
Ampicillin (5)	250	5.0	0.1N HCl, 10 + acetone, 40	5	no
Benzylpenicillin*		5.0	methanol, 50	5	no
Chloramphenicol	250	10	acetone, 25	10	no
Chloroquine phosp	hate	50 0.5	water, 50	5	1 mL
to 10 Cloxicillin	0 250	5.0	mothanal 50	5	20
Estradiol cypionate		5.0 4	methanol, 50 methanol, 1	5 4	no no
Mebendazole	100	0.5	chloroform, 5 + HCOOH (19	2	1 mL to 4
Paracetamol	300	10	methanol, 30	<sup>′</sup> 10	no
Praziquantel	600	5.0	water, 50	12	1 mL
2.4 Sulfamethoxazole	500	5.0	methanol, 50	10	1 mL
to 4					
Trifluoroperazine H Theophylline	ICI 100	2 0.5 0.5	ethanol, 4 ethanol, 50	0.5 2	no 1 mL
to 4					

# Table 4. Preparation of sample solutions

Ampicillinacetone-water-toluene-acetic acid13 +Benzylpenicillinacetone-water-toluene-acetic acid13 +Chloramphenicolchloroform-methanol9 +Chloroquine phosphateethanol-ammonium hydroxide-water85 +Cloxicillinacetone-water-toluene-acetic acid13 +Estradiol cypionatechloroform-methanol9 +Mebendazolechloroform-toluene-ethyl acetate- methanol45 +Paracetamolethyl acetate-methanol-formic acid90 +Benzylpenicillinethyl acetate-methanol-ammonium hydroxide85 +	position
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Chloramphenicolchloroform-methanol9 +Chloroquine phosphateethanol-ammonium hydroxide-water85 +Cloxicillinacetone-water-toluene-acetic acid13 +Estradiol cypionatechloroform-toluene-ethyl acetate- methanol45 +Mebendazolechloroform-methanol-formic acid90 +Paracetamolethyl acetate-methanol-ammonium hydroxide85	2 + 2 + 0.5
Chloroquine phosphate Cloxicillinethanol-ammonium hydroxide-water acetone-water-toluene-acetic acid chloroform-toluene-ethyl acetate- methanol85 + 13 + 13 +Estradiol cypionatechloroform-toluene-acetic acid methanol45 - 45 - 46 - <b< td=""><td>2 + 2 + 0.5</td></b<>	2 + 2 + 0.5
Cloxicillinacetone-water-toluene-acetic acid13 +Estradiol cypionatechloroform-toluene-ethyl acetate- methanol45 -Mebendazolechloroform-methanol-formic acid90 +Paracetamolethyl acetate-methanol-ammonium hydroxide85	1
Estradiol cypionate chloroform-toluene-ethyl acetate- methanol 45 Mebendazole chloroform-methanol-formic acid 90 + Paracetamol ethyl acetate-methanol-ammonium hydroxide 85	4 + 11
methanol45Mebendazolechloroform-methanol-formic acid90 +Paracetamolethyl acetate-methanol-ammonium85	2 + 2 + 0.5
Mebendazolechloroform-methanol-formic acid90 +Paracetamolethyl acetate-methanol-ammonium85	
Paracetamol ethyl acetate-methanol-ammonium hydroxide 85	+ 36 + 6 + 1
hydroxide 85	+ 5 + 5
<b>j</b>	
	+ 10 + 5
Praziquantel chloroform-acetone 1 +	- 1
Quinine sulfate ethanol-ammonium hydroxide-water 85	+ 4 + 11
Sulfamethazine chloroform-acetone-methanol-ammonium	
hydroxide-water 60	)+40+20+1.5+1
Sulfamethoxazole chloroform-acetone-methanol-ammonium	
hydroxide-water 60	+40+20+1.5+1
Theobromine chloroform-acetone	1 + 1
Theophylline chloroform-acetone	1 + 1
Trifluoperazine acetone-ammonium hydroxide 2	24 + 1

# Table 5. Developers that have been found satisfactory<sup>a,b</sup>

(Footnotes to Table 5) <sup>a</sup> The developing solutions are expressed as parts, not percentages.

<sup>b</sup> Developer containing volatile materials such as ammonium hydroxide should be prepared just before use and not stored for future operations. Other developers may be stored in capped bottles; however, it is best to prepare only the quantity of developer needed for a single day's operation.

<sup>c</sup> The solvent and developing solutions for ampicillin have been described in USP XXII (6).

# Table 6. Assay of reference tablets

Drug	Tablet wt, mg	Content, mg Prescribed	Std Actual dev.	Percent of specified
Paracetamol	100	46	45.04±0.67	97.9
Chloroquine phosphate	e 100	2.3	2.19±0.04	95.2
Ampicillin	100	24	24.4±0.12	101.6

Table 7. Estimated content of 3 drugs

Drug	Estimated content in dosage form, %
Paracetamol	99
Chloroquine phosph	nate 85
Ampicillin	92