A Compendium of Unofficial Methods

for

Rapid Screening of Pharmaceuticals

by

Thin-Layer Chromatography

by

A. S. Kenyon and T. P. Layloff
Food and Drug Administration
Division of Testing and Applied Analytical Development
St. Louis, MO. 63101

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Introduction

Thin-layer chromatography provides a quick, economical, and reliable method for rapid screening of pharmaceuticals. The screening method can be used after little training, and in areas outside the laboratory. This compendium of drug analytical methods has been developed for rapid screening of drugs in such places as ports of entry, pharmacies, distribution centers, or areas lacking resources for other methods of analysis. The technique reduces the need for other analytical methods which are more costly and time consuming, and which require highly trained operators. The methods are based on a portable system using a plastic bag for development and are easy to use in field-type operations. None of the methods described are official in any compendium.

In working with any chemical, safety and disposal must be considered before performing an analysis. All chemicals are toxic, and should be handled accordingly. The analyst should not breathe or inhale vapors or dust from any of these chemicals, including the dust from the finely divided silica on the TLC plates. Plastic or rubber gloves should be used whenever contact with these chemicals is possible. In these methods, an effort has been made to reduce the risk of toxicity of the solvents by using small quantities to reduce exposure and by eliminating toxic chlorinated solvents. The toxicity of the chemicals used in these methods is similar to that of solvents used in applying paint. All analyses should be performed in areas with adequate ventilation. The rules of disposal for your local area should be followed. Chemicals used in TLC operations are flammable, and must be kept away from flames or ignition sources. Because iodine stains skin and clothing, protective clothing and rubber gloves should be worn when handling it.

This compendium describes the procedures for the analysis of the listed drugs in which rapid TLC is used as a screening method. These methods were developed in our laboratory and have not been collaboratively tested. If problems are encountered with any of these test methods, please notify us by FAX or by mail marked to the attention of the Director, Division of Drug Analysis, Food and Drug Administration.

FAX number: 314-539-2113. Address: 1114 Market St, Room 1002, St. Louis, MO 63101, USA.

Rapid Screening of Drugs by Thin-Layer Chromatography

The TLC method described here is semiquantitative. The method gives a good estimate of whether the drug is the same as that listed on the label, and if the content is the correct amount as specified. It is not intended to replace any official compendium method. The drugs selected for this compendium were taken from the list of essential drugs developed by the World Health Organization, essential drug lists from several countries in Africa, and reports of actual field use in developing countries. The methods do not cover the complete list of essential drugs, but represent many different classes of drugs. The drugs were selected on a priority basis, i.e., those needed for life-threatening diseases were given the highest priority.

The TLC method was designed to rapidly screen drugs by using a polyethylene bag as the chamber. Two reference concentrations representing the upper and lower concentration for the dosage limits (85% and 115 or 120%) are spotted on a plate along with the sample solution representing 100%. The sample solution is spotted between the reference solutions. The spots are examined visually either by ultraviolet light or by iodine staining. The drug is considered to be within specifications if the intensity of the sample spots lies between the intensity of the two reference solution spots. The sample should be further tested by an official method if the intensity of the sample spot lies near the lower limit. The screening method eliminates the need for further analysis of those drugs which show concentrations within the specification range.

Many drugs that are not listed in the compendium will need to be analyzed. Methods can be developed for a drug not listed in this compendium by following these simple steps:

1. Determine a suitable solvent system by studying the molecular structure of the drug; consult some reference book such as the Merck Index for a suitable solvent. Choose the solvent with the lowest polarity when more than one solvent is possible.

2. Prepare a solution of the standard drug at a concentration approximately equal to 1 mg/mL. Spot this solution on a TLC plate.

3. Prepare a developer solution mixture to have a middle range of polarity, such as equal volumes of toluene and methanol. If the drug is acidic, add a small amount of glacial acetic acid; if the drug is basic, add concentrated ammonium hydroxide. To select a developer mixture, dip the spotted plate into a beaker containing the developer, cover the beaker to prevent evaporation, and observe the movement of the spots.

4. Reduce the polarity of the developer if the spots follow the solvent front; increase the polarity of the developer if the spots do not move or move less than 1 cm.

5. After determining the solvent mixture for suitable separation, adjust the concentrations to show differences in spot intensities.

6. Add the new method to the compendium for later use.

The methods described in this compendium have been based on iodine staining as a satisfactory means of detection. In many cases the concentrations specified may be too high for suitable detection by ultraviolet light because of high absorbance at the 254 nm UV wavelength. The concentrations of the solutions are too high when no difference in intensity between solutions of different concentrations can be detected. In this case, the concentration should be reduced by diluting the prepared solutions of sample and reference. The concentrations specified for the sample and standards were determined experimentally to give suitable detection. Many drugs are supplied in dosages other than those listed in the compendium. The final concentration of the sample should be kept the same as the listed concentration when other dosages are used and may be prepared either by using larger volumes of solvent or by diluting a concentrated solution. Diluting the concentrated solution will use less solvent. The drug may also be supplied in different dosage forms, such as liquids. Drugs in liquid form are handled on a volume basis (mg/mL) and are diluted if necessary.

The availability of reference standards and their cost is a matter of concern to all who analyze drugs. These procedures have been written for standards supplied as primary or secondary standards. Primary standards are costly, but secondary standards can be used successfully. Secondary standards may be obtained from a previously analyzed sample or from reputable chemical suppliers. When either primary or secondary standards are used, the standards must be weighed on an analytical balance capable of weighing to 0.1 mg, and a large enough quantity must be weighed to minimize the error. The error can be further reduced by using a semi-micro balance (one that weighs to the 5th place or 0.01 mg). Another possibility for a reference material is a tablet containing a fixed quantity of the drug; it can be used by simply dissolving a reference tablet in the specified volume of solvent to produce the high and low concentrations needed for reference. No weighing is required when reference tablets are available, but at present, the availability of reference tablets is limited. Therefore weighing is usually necessary. A study is underway to develop reference tablets with the correct content to prepare the high and low reference solutions. It is noted that the procedures for both conditions are given for three of the drugs described here. These three drugs were used to establish the feasibility of the reference tablet concept.

The TLC procedures described are based on the use of a portable kit which is supplied with plastic bags, holders, and all the accessories required to perform the analysis. Volumes used in the compendium methods are those suitable for a flat plastic bag 8 cm wide. The kits have been supplied with plastic bags 10 cm wide which require 30 mL of the developer; therefore all volumes of the developer mixtures must be adjusted by increasing each volume by 50%. The flat 8 cm plastic tubing can be obtained in rolls (066 gage), and bags can be fabricated from the 8 cm tubing by using a bag sealer. It is recommended that a roll of flat plastic tubing and bag sealer be purchased to ensure an adequate supply over an extended period, and to reduce the cost of developer solvents.

TLC plates are available with many different coatings and supports. The methods developed in this compendium are based on plastic-backed silica plates containing a fluorescent material. Merck plastic-backed plates designated as 60 F254 have been found most satisfactory. TLC plates made by other manufacturers are also acceptable if they have the same specifications. Coated glass plates are suitable, but will increase the cost. A plate 5 X 10 cm is required for the apparatus. Cutting glass plates from larger plates is not recommended. Aluminum-backed plates have also been satisfactory when used with developers that are not too strongly acidic or basic. TLC plates without the fluorescent materials cannot be used for ultraviolet detection; the detection must be done by other means. If both kinds of plates are used, they must be kept separate to avoid mistakes. Plain silica-coated plates are more easily damaged. The 60 F254 plastic- backed plates give the best all-around performance.

The bag for iodine staining can be made as follows: Cut the development bag approximately 12 cm above the seal. Cut a slit in one side of the bag approximately 9 cm above the seal. Place some protective covering on a vertical surface to protect the surface from stain. The protective covering can be cardboard, plastic film, or any other type of material which can be discarded. Tape the bag (top of the bag above the slit) to a vertical surface on top of the protective covering. Tape the bottom of the bag to the vertical surface. Tape a small, flat, rigid object to the bag at the seal point of the bag so that the rigid object can act as a hinge to displace the iodine solution upwards.

The TLC analysis is based on the use of one dosage unit to prepare the needed concentration. The complete ground tablet must be placed into the vessel; it can be added by performing the grinding in a small plastic bag and then adding the bag and contents to the vessel. A bag approximately 3 X 5 cm is adequate. Bags of this size can be prepared from the flat 8 cm plastic tubing by sealing the bottom of the bag and then making two parallel vertical seals to make two small bags. These two bags will be approximately 3 X 5 cm each. Drugs in capsule form do not need to be ground.

The developers described do not include chloroform or other halogen compounds because of their toxicity. To eliminate chloroform, mixtures of solvents are required to achieve a polarity similar to that of chloroform as calculated from a series of polarities. The compositions of the developer solutions were selected so that they could be used safely where little or no laboratory facility exists. Other developers may be used for screening purposes to obtain different separations. Chloroform may be used by trained operators in well-equipped laboratories with proper hoods. Chloroform should not be used in open areas or by untrained personnel. Other developers produce different heights of the spots and different times for the solvent front to reach the migration limit. Spot positions should be kept between R_f values of 0.2 and 0.8.

When any drug is shown not to meet specifications, the analysis should be repeated to verify the result. The drug should be submitted for analysis by an

approved method when the result shows a marginal content near the 85% level. Most analyses will show a drug to be near the midpoint between the upper and lower reference solutions, thus eliminating the need for further analysis.

Detector Solutions

To detect the spots produced by the rapid TLC screening method, two solutions must be prepared to make the spots visible in white or ordinary light if they cannot be detected by UV. The necessary solutions are (a) a mixture of iodine and potassium iodide and (b) ninhydrin. These solutions are not necessary when ultraviolet and fluorescent plates are available. Most drugs are detectable by the iodine-KI staining method when UV is not available. The mycin drugs, known as aminoglycosides, are not visible either in UV light or by iodine stain, and must be stained with ninhydrin. These solutions are stable if kept in dark glass bottles, and can be prepared in sufficient quantity for long term usage.

Iodine-potassium iodide solution.

The following equipment is needed:

2 graduated cylinders with glass stoppers, 250 mL volume
1 actinic (brown) glass bottle with stopper, 500 mL
Plastic or rubber gloves and protective clothing (iodine can stain)

The following reagents are required for a single preparation:

8 g of potassium iodide
32 g of crystalline iodine
300 mL of 95% ethanol
25 mL of concentrated hydrochloric acid
81 mL of distilled water

Procedure: The iodine-KI solution is prepared by mixing 2 solutions.

Solution 1:

Dissolve 8 g (approximately one half teaspoon) of potassium iodide in a 250 mL graduated cylinder by adding 6 mL of water. After the potassium iodide has dissolved, add 200 mL of 95% ethanol. Dissolve 32 g (approximately one and one half teaspoons) of crystalline iodine to this solution.

Solution 2:

In another 250 mL graduated cylinder, place 75 mL of distilled water. Carefully add 25 mL of concentrated hydrochloric acid. Use caution when adding the acid slowly to the water. Use rubber gloves to prevent any burns. Add 100 mL of 95% ethanol to the acid solution. Mix this solution well.

Final solution:

To make the final iodine-KI solution, combine solutions 1 and 2 in a 500 mL brown glass bottle and cap the bottle tightly. This solution is stable and can be used over a period of several months if properly sealed. Replace the solution when excessive crystals of iodine form.

Ninhydrin solution:

The ninhydrin solution is needed to stain some drugs which are not visible by either UV or iodine staining. Because this solution will be used only for that one class of drugs (aminoglycosides), prepare the solution only when analyzing the mycins.

Prepare the ninhydrin solution in a 25 mL graduated cylinder with a stopper. When preparing the solution, take the precautions of using rubber gloves and protective clothing. Ninhydrin reacts immediately with the skin. If ninhydrin has touched the skin, wash those portions of the body with large amounts of water.

Add 25 mL of acetone to the graduated cylinder, and then add 0.1 g of ninhydrin. Stopper the graduated cylinder and shake well until all the ninhydrin is dissolved. The solution can be kept for a period of time. It is not necessary to prepare large amounts. A small bottle will last for many analyses.

References

1. Tape/slide presentation "Training for Rapid Screening of Drugs by TLC" by A. S. Kenyon, P. E. Flinn, and T. P. Layloff has been developed at the Division of Drug Analysis, Food and Drug Administration. Information on the availability of this presentation can be obtained through the Director, Division of Drug Analysis, FDA, St. Louis, MO.

2. "Rapid Screening of Pharmaceuticals by Thin-Layer Chromatography: Analysis of Essential Drugs by Visual Methods" by A. S. Kenyon, P. E. Flinn, and T. P. Layloff, Journal of AOAC International, 1995, 78, 41-49.

3. "A Simplified TLC System for Qualitative and Semi-Quantitative Analysis of Pharmaceuticals" by P. E. Flinn, A. S. Kenyon, and T. P. Layloff, Journal of Liquid Chromatography, 15(10), 1639 (1992).

Acknowledgments:

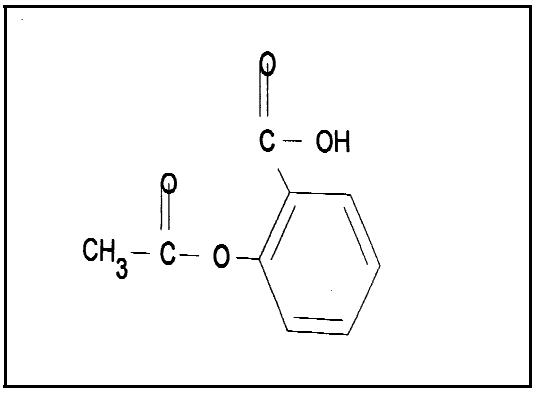
The following persons assisted in developing the experimental conditions:

Paul Flinn
Glenn Petrie
Helen Chiu
Stephanie Judge
Sandra Hick
Tan Le
Kim Story
Cris Beutler

Their work is gratefully acknowledged. Special appreciation is expressed to Helen Reynolds for editing this work.

Acetylsalicylic acid
300 mg tablet

Structure



Molecular formula and mass: $C_9H_8O_4$ - 180.15
Category: Analgesic
Sample:

Dissolve 1 tablet in 49 mL of methanol and 1 mL of glacial acetic acid. Concentration of the solution = 300 mg/50 mL = 6 mg/mL. The required concentration of the sample solution for analysis is 2 mg/mL. Dilute 1 mL of the 6 mg/mL solution to 3 mL by adding 2 mL of methanol. This solution will represent 100% sample.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (2 mg/mL) X 1.15 = 2.30 mg/mL. Weigh approximately 10 mg of the standard. If you weighed 8 mg of standard, dissolve it in: 8 mg X 2.30 mg/mL) = 18.4 mL of methanol. This makes the high standard solution concentration equal to 2.30 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (2 mg/mL) X 0.85 = 1.70 mg/mL. Dilute 1 mL of high standard solution to 1.35 mL by adding 0.35 mL of methanol (2.30/1.70 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 17 mL of toluene, 13 mL of ethyl acetate, and 1 mL of acetic acid. Add approximately 20 mL of this mixture to the TLC development bag. Develop until the

solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

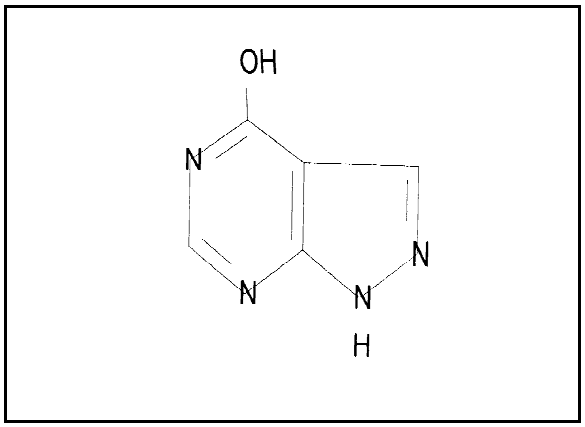
Dry the plate and observe under UV light (254 nm).

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Allopurinol
100 mg tablet

Structure:



Molecular Formula and Mass $C_5H_4N_4O$ - 136.11

Category : Uricosuric

Sample:

Grind 1 tablet and dissolve in 100 mL of 0.1 N NaOH. The concentration of the solution = (100 mg/100 mL) = 1 mg/mL. The required concentration of the sample solution representing 100% is 1 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 12 mg of standard. If you weighed 12.3 mg of standard, dissolve it in: 12.3 mg/1.15 mg/mL = 10.7 mL of 0.1 N NaOH.

Low standard:

The low limit is 85%; therefore the concentration of the high standard = (1 mg/mL) X 0.85 mg/mL. Dilute 1 mL of the 1.15 mg/mL solution to 1.35 mL by adding 0.35 mL to 1 mL of the high standard (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix together 17 mL of 95% ethanol, 8 mL of toluene, and 1.5 mL of concentrated ammonium hydroxide. Add approximately 20 mL of this mixture to the TLC development bag. Develop until the solvent front reaches to within 1 cm of the

top of the TLC plate.

Detection:

UV:

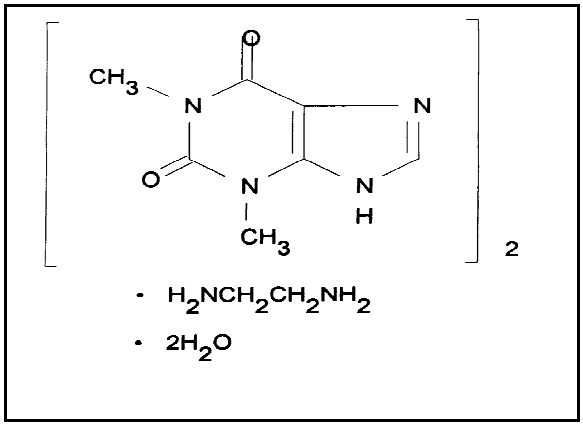
Dry the plate and observe under UV light (254 nm). Observe the intensity and the size of the spots.

Iodine stain:

The concentration of the solutions at 1 mg/mL is too low to be detectable by iodine staining. If you do not have a UV source, the concentration of the solutions must be increased to 5 mg/mL by dissolving 1 tablet in 20 mL of 0.1 N NaOH. Dip the plate in the iodine solution in the detection bag. Allow the plate to dry, and observe the intensity and size of the spots.

Aminophylline
100 mg capsule

Structure:



Molecular Formula & Mass: $(C_7H_8N_4O_2)_2.C_2H_4(NH_2)_2.2H_2O$ - 456.46

Category: Diuretic, cardiac stimulant

Sample:

Aminophylline contains two components, theophylline as the dihydrate and ethylene diamine. This means that the content of theophylline is only 79 mg as the anhydrous structure (100 mg X 360.46/456.46 = 79mg). The molecular weight correction is necessary because the standard is in the anhydrous form. The declared drug content of 100 mg per tablet is for the combined mixture. Grind 1 tablet and dissolve in 50 mL of distilled water. Shake at least 1 min. Concentration of the solution = 79 mg/50 mL = 1.58 mg/mL. The required concentration of the sample solution representing 100% is 0.50 mg/mL. Add 2.16 mL of distilled water to 1 mL of the 1.58 mg/mL solution to make the sample solution equal to 0.50 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard(anhydrous). If you weighed 4.6 mg of standard, dissolve it in: (4.6 mg)/(0.575 mg/mL) = 8.0 mL of distilled water. This makes the high standard solution concentration equal to 0.575 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of distilled water (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)

Center spot 100% sample

Right spot high standard (115%)

Development:

Mix 14 mL of acetone and 7 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

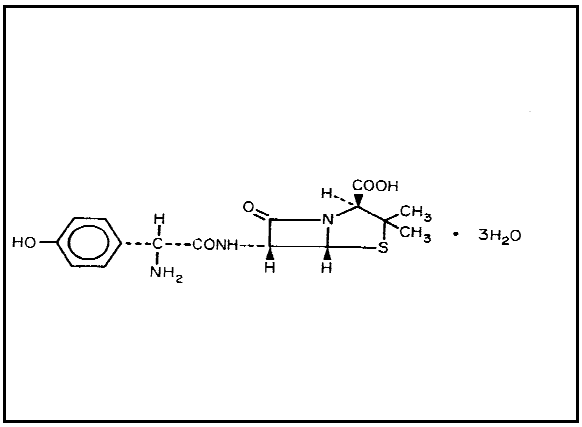
Dry the plate and observe under UV light. The maximum is at 270 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Amoxicillin
250 mg capsule

Structure:



Molecular Formula & Mass: $C_{16}H_{19}N_3O_5S$ **-** 365.40 (anhydrous)
419.45 (trihydrate)

Category: Antibacterial

Sample:

Dissolve the contents of 1 capsule in 10 mL of 0.1 N HCl. When completely dissolved, add 40 mL of acetone. The required concentration of the sample solution representing 100% is 5.0 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 120%; therefore the concentration of the high standard = (5.0 mg/mL) X 1.20 = 6 mg/mL. Weigh approximately 25 mg of standard. If you weighed 24 mg of standard, dissolve it in: (24 mg X 0.871 mg)/6 mg/mL = 3.48 mL of 4:1 acetone:0.1 N HCl.

Note: The ratio of the molecular weight of the anhydrous form to the trihydrate is: 365.4/419.45 = 0.871. This makes the high standard solution concentration equal to 6.00 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (5 mg/mL X 0.85) = 4.25 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of 4:1 acetone:0.1 N HCl (6.00/4.25 = 1.41).

Spotting:

Spot on the TLC plate as follows

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (120%)

Development:

Mix 26 mL of acetone, 4 mL of water, 4 mL of toluene, and 1 mL of glacial acetic acid. Add approximately 20 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

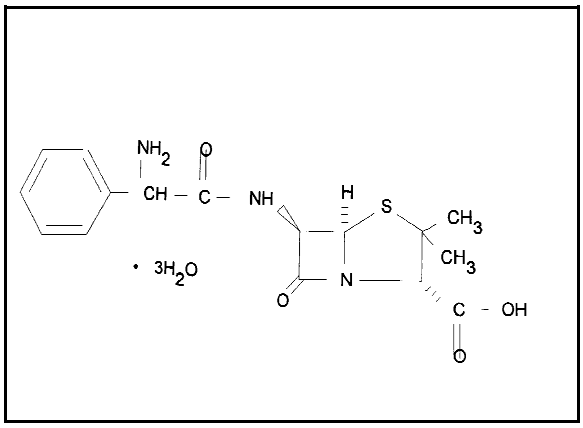
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Ampicillin
250 and 500 mg capsules

Structure:



Molecular Formula & Mass: $C_{16}H_{19}N_3O_4S$ - 349.42 (free acid);
$C_{16}H_{25}N_3O_7S$ - 403.42 (trihydrate).

Category: Antibacterial

Sample:

Ampicillin is supplied in different forms such as anhydrous free acid, the trihydrate, and the sodium salt. The most common formulation uses the trihydrate in capsule form. The drug content is listed on the label of the bottle as the equivalent of the free acid.

250 mg per capsule: Dissolve the contents of 1 capsule in 50 mL of a mixture of 10 mL of 0.1 N HCl and 40 mL of acetone. Shake at least 1 min. Concentration of the solution = 250 mg/50 mL = 5 mg/mL. The required concentration of the sample solution representing 100% is 5 mg/mL. This solution is required for iodine staining.

500 mg per capsule: Dissolve the contents of 1 capsule in 50 mL of the solvent to make a solution equivalent to 10 mg/mL. Add 1 mL of the solvent to 1 mL of this solution to make the final sample solution.

Standards:

High standard:

The high limit is 120%; therefore the concentration of the high standard = (5 mg/mL) X 1.20 = 6 mg/mL. Weigh approximately 60 mg of standard. If you weighed 54 mg of standard, dissolve it in: (54 mg)/(6 mg/mL) = 9.0 mL of 1:4 hydrochloric acid:acetone. This makes the high standard solution concentration equal to 6 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (5 mg/mL) X 0.85 = 4.25 mg/mL. Dilute 1 mL of the high standard to 1.4 mL by adding 0.4 mL of 1:4 hydrochloric acid:acetone (6.0/4.25 = 1.4).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (120%)

Development:

Mix 26 mL of acetone, 4 mL of water, 4 mL of toluene, and 1 mL of glacial acetic acid. Pour approximately 20 mL of this mixture into the TLC development bag. Develop until the solvent front reaches within 1 cm from the top of the TLC plate.

Detection:

UV:

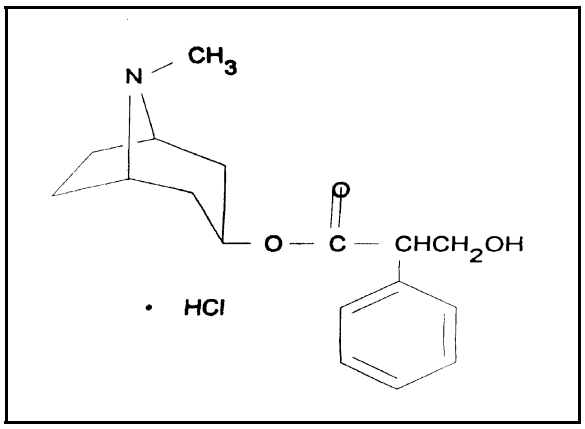
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detction bag. Allow the plate to dry and observe the size and intensity of the spots.

Atropine

Structure



Molecular Formula & Mass: $C_{17}H_{23}NO_3$ - 289.38

Category: Anticholinergic

Sample:

Standard:

High standard:

The high limit is 115%; therefore the concentration of high standard is (10 mg/mL) X 1.15 = 11.5 mg/mL. Weigh approximately 47 mg of standard. If you weighed 47.2.mg of standard, dissolve it in: (47.2 mg)/(11.5 mg/mL) = 4.1 mL of anhydrous ethanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (10 mg/mL) X 0.85 = 8.5 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (1.15/0.85 = 1.35).

Spotting:

Spot on TLC plate as follow:

Left spot low standard (115%)
Center spot 100% sample
Right spot high standard (85%)

Development:

Mix 22 mL of methanol and 0.25 mL of concentrated ammonium hydroxide. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 260 nm but a short

wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

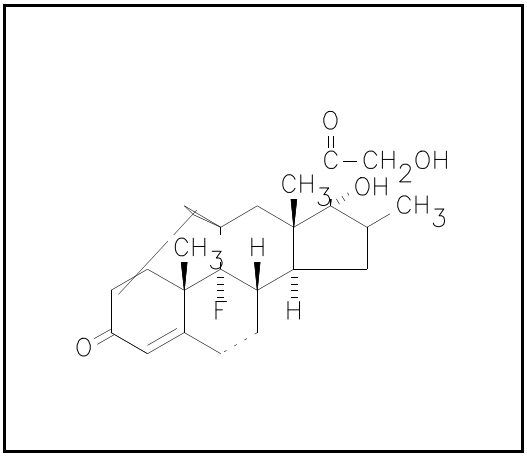
Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Note:

The UV absorption is weak but can be distinguished.

Betamethasone
4 mg tablet

Structure:



Molecular Formula & Mass: $C_{22}H_{29}FO_5$ - 392.45
Category: Glucocorticoid
Sample:

Grind 1 tablet and dissolve in 10 mL of 95% ethanol. Shake at least 1 min. Concentration of the solution = 4 mg/10 mL = 0.4 mg/mL. The required concentration of the sample solution representing 100% is 0.4 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.40 mg/mL) X 1.15 = 0.46 mg/mL. Weigh approximately 4 mg of standard. If you weighed 3.75 mg of standard, dissolve it in: (3.75 mg)/(0.46 mg/mL) = 8.05 mL of ethanol. This makes the concentration of the high standard solution equal to 0.46 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.40 mg/mL) X 0.85 = 0.34 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (0.46/0.34 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 14 mL of toluene and 7 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

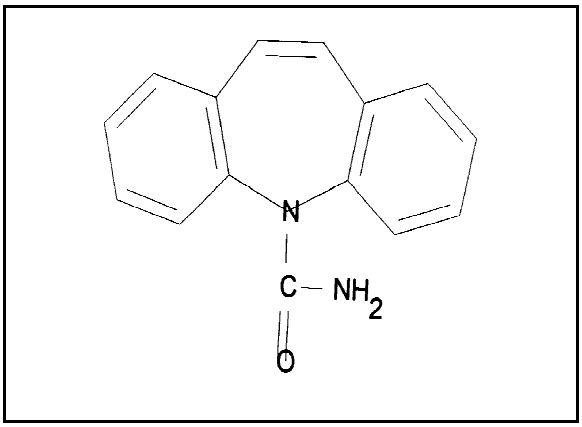
Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

The 0.40 mg/mL concentration is too low to allow for quantitation by iodine. The concentration needs to be 4 mg/mL. This can be accomplished by using 10 tablets rather than 1 tablet. Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Carbamazepine
100 mg tablet

Structure



Molecular Formula & Mass: $C_{15}H_{12}N_2O$ - 236.26
Category: Analgesic
Sample:
Grind 1 tablet and dissolve in 50 mL of anhydrous ethanol. Concentration of the solution = 100 mg/50 mL = 2 mg/mL. Dilute 1 mL of the 2 mg/mL solution with 1 mL of ethanol to make a final solution equal to 1 mg/mL.

Standard:
High standard:
The high limit is 115%; therefore the concentration of high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.98 mg of standard, dissolve it in: (4.98 mg)/(1.15 mg/mL) = 4.3 mL of anhydrous ethanol.
Low standard:
The low limit is 85%; therefore the concentration of low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (1.15/0.85 = 1.35).
Spotting:
Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)
Development:
Mix 22 mL of methanol and 0.25 mL of concentrated ammonium hydroxide. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.
Detection:

UV:

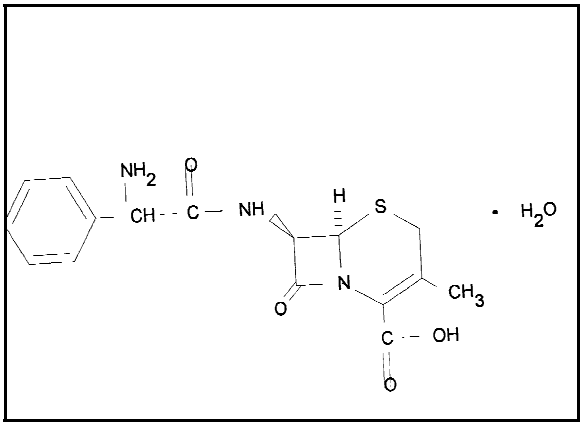
Dry the plate and observe under UV light. The maximum is at 235 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Cephalexin
250 mg capsule

Structure



Molecular Formula & Mass: $C_{16}H_{17}N_3O_4S$ - 347.40
Category: Antibacterial
Sample:

Dissolve the contents of 1 capsule in 50 mL of methanol. Concentration of the solution = 250 mg/50 mL = 5 mg/mL. The required concentration of sample solution representing 100% is 5 mg/mL.

Standard:

High standard:

The high limit is 115% therefore the concentration of high standard = (5 mg/mL) X 1.15 = 6 mg/mL. Weigh approximately 21 mg of standard. If you weighed 21.3 mg of standard, dissolve it in: (21.3 mg)/(6 mg/mL) = 3.55 mL of methanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (5 mg/mL) X 0.85 = 4.25 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 12.5 mL of ethyl acetate, 5 mL of acetone, 5 mL of glacial acetic acid, and 2.5 mL of distilled water. Add this mixture to the TLC development bag. Develop until the

solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 250 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

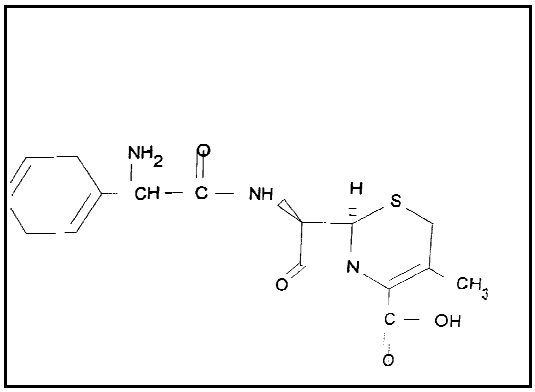
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Note:

The spots are not well defined in UV light; however, they are clearly distinguished in the iodine solution.

Cephradine
250 mg capsule

Structure:



Molecular Formula & Mass: $C_{16}H_{19}N_3O_4S$ - 349.41
Category: Antibacterial
Sample:

Dissolve the contents of 1 capsule in 50 mL of methanol. Shake at least 1 min. Concentration of the solution = 250 mg/50 mL = 5.0 mg/mL. The required concentration of sample solution representing 100% is 5.0 mg/mL.

Standards:

High standard:

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (5.0 mg/mL) X 1.20 = 6.0 mg/mL. Weigh approximately 30 mg of standard. If you weighed 28.75 mg of standard, dissolve it in: (28.75 mg)/(6.0 mg/mL) = 4.79 mL of methanol. This makes the high standard solution concentration equal to 6.0 mg/mL.

Low standard:

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (5.0 mg/mL) x 0.85 = 4.25 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of methanol (6.0/4.25 = 1.41).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (120%)

Development:

Mix 15 mL of ethyl acetate, 6 mL of acetone, 6 mL of glacial acetic acid, and 3 mL of water. Add 21 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 275 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

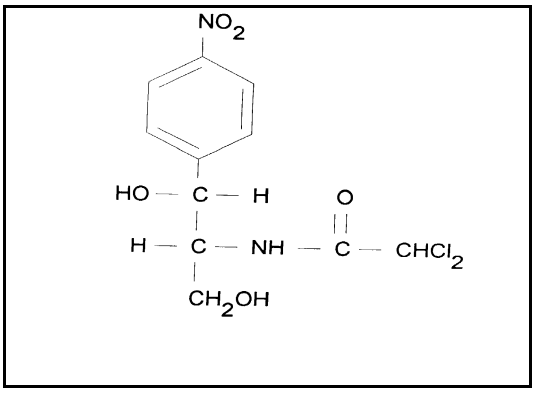
Note:

This procedure should work with any of the cephalosporins that have similar molecular masses and functional groups. Standards of cephradine, cephalexin, and cefuroxime were processed by this procedure, and R_f values were as follows:

Cephradine = 0.26
Cephalexin = 0.26
Cefuroxime = 0.47

Chloramphenicol
250 mg tablet

Structure:



Molecular Formula & Mass: $C_{11}H_{12}Cl_2N_2O_5$ - 323.14

Category: Antibacterial and antirickettsial

Sample:

Dissolve the contents of 1 capsule in 25 mL of anhydrous ethanol. Shake at least 1 min. Concentration of the solution = 250 mg/25 mL = 10.0 mg/mL. The required concentration of sample solution representing 100% is 10.0 mg/mL.

Standards:

High standard:

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (10.0 mg/mL) X 1.20 = 12.0 mg/mL. If you weighed 34.75 mg of standard, dissolve it in: (34.75 mg)/(12.0 mg/mL) = 2.90 mL of anhydrous ethanol. This makes the concentration of the high standard solution equal to 12.0 mg/mL.

Low standard:

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (10.0 mg/mL) X 0.85 = 8.50 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of ethanol (12/8.5 =1.41).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (120%)

Development:

Mix 14 mL of acetone and 7 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of

the top of the TLC plate.

Detection:

UV:

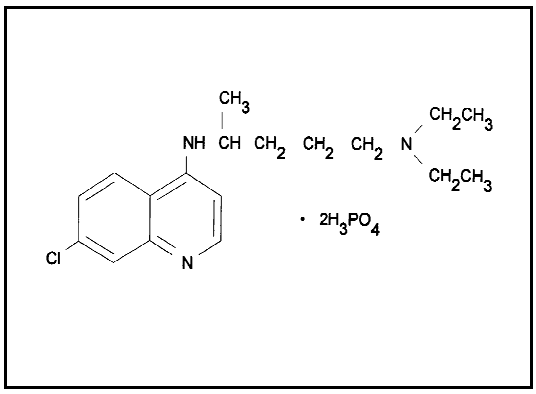
The 10.0 mg/mL concentration is too high to allow for quantitation. Therefore, dilute the sample and standard solutions 1:10 by adding 9 mL of anhydrous ethanol to 1 mL of the sample and 9 mL of anhydrous ethanol to 1 mL of each of the standards. Dry the plate and observe under UV light. The maximum is at 275 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Chloroquine diphosphate
250 mg tablet

Structure:



Molecular Formula & Mass: $C_{18}H_{26}ClN_3.2H_3PO_4$ - 515.86
Category: Antimalarial
Sample:

Grind 1 tablet and dissolve in 50 mL of distilled water. Shake at least 1 min. Concentration of the solution = 250 mg/50 mL = 5.0 mg/mL. The required concentration of the sample solution representing 100% is 0.50 mg/mL. Add 9 mL of distilled water to make a sample solution equal to 0.5 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL X 1.15 = 0.575 mg/mL.

When a reference tablet is available:

The reference tablet is specified to contain 2.3 mg of active drug. However, the content may vary. The concentration is shown on the label. If the tablet contains 2.19 mg of the drug, dissolve it in: 2.19 mg/0.575 mg/mL = 3.8 mL of distilled water.

When no reference tablet is available:

Weigh approximately 5 mg of standard. If you weighed 4.7 mg of standard, dissolve it in: (4.7 mg)/(0.575 mg/mL) = 8.17 mL of distilled water. This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of distilled water (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 21 mL of anhydrous ethanol, 3 mL of distilled water, and 1 mL of concentrated ammonium hydroxide. Add this mixture to the TLC development bag. Develop until the solvent front reaches to within 1 cm of the top of the TLC plate.

Detection:

UV:

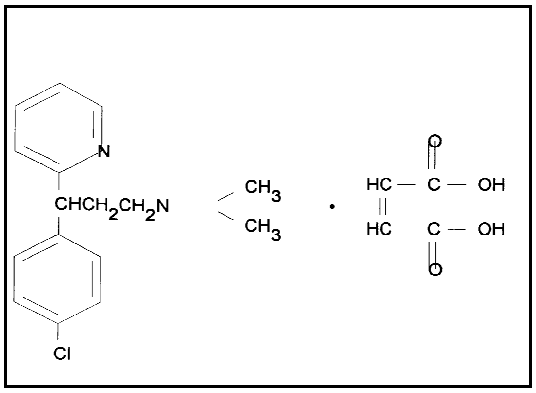
Dry the plate and observe under UV light. The maximum is at 333 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Chlorpheniramine maleate
2 mg tablet

Structure



Molecular formula & mass: $C_{20}H_{23}ClN_2O_4$ 390.88
Category: Antihistiminic
Sample:

Grind 4 tablets and dissolve in 8 mL of anhydrous ethanol. The required concentration of the sample solution representing 100% is 1 mg/mL. Because of the small volume required to make the proper concentration, multiple tablets are used so that sufficient volume of solvent is available for sampling.

Standard:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 9 mg of standard. If you weighed 8.9 mg of standard, dissolve it in: (8.9 mg)/(1.15 mg/mL) = 7.74 mL of anhydrous ethanol.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follow:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 22 mL of methanol and 1 mL of glacial acetic acid. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

<u>UV</u>:

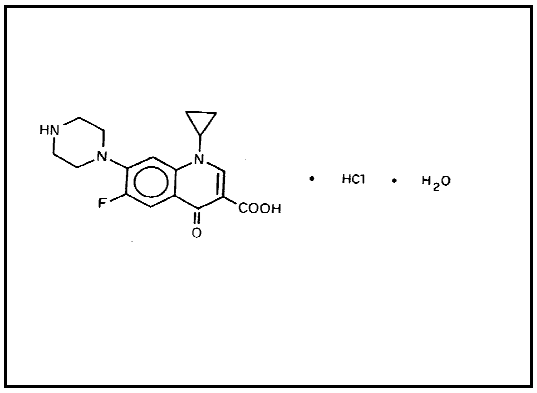
Dry the plate and observe under UV light. The maximum is at 265 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Ciprofloxacin hydrochloride
250 mg tablet

Structure:



Molecular Formula & Mass: $C_{17}H_{18}FN_3O_3.HCl.H_2O$ - 385.82

Category: Antibacterial

Sample:

Grind 1 tablet and dissolve in 50 mL of distilled water. Shake at least 2 min. Concentration of the solution = 250 mg/50 mL = 5 mg/mL. The required concentration of the sample solution representing 100% is 1 mg/mL. Add 4 mL of water to 1 mL of the 5 mg/mL solution to make the sample solution equal to 1 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of the standard. If you weighed 4.9 mg of the standard, dissolve it in: (4.9 mg/1.15 mg/mL) = 4.26 mL of distilled water. This makes the concentration of the high standard solution equal to 1.15 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of the high standard to 1.35 mL by adding 0.35 mL of distilled water (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)

Center spot 100% sample

Right spot high standard (115%)

Development:

Mix 2.5 mL of toluene, 5 mL of acetone, 10 mL of methanol, and 5 mL of concentrated ammonium hydroxide. Add this solution to the TLC development bag. Develop the plate until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

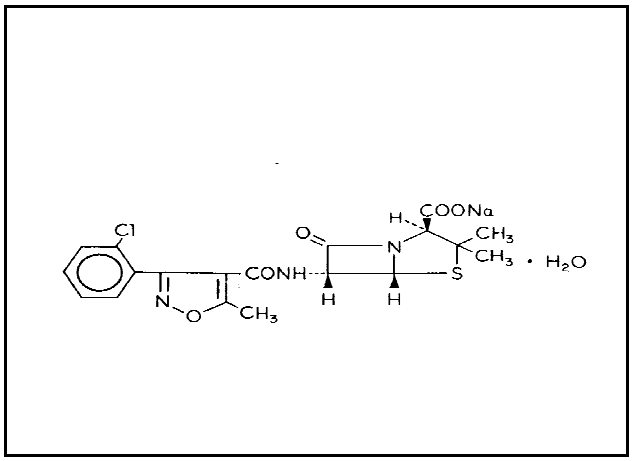
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Cloxacillin
250 and 500 mg capsules

Structure:



Molecular Formula and Mass: $C_{19}H_{17}ClN_3NaO_5S.H_2O$ -- 475.88 (salt,monohyd)
$C_{19}H_{18}ClN_3O_5S$ - 435.88 (free base)

Category: Antibacterial

Sample:

Dissolve the contents of 1 capsule(250 mg) in 50 mL of methanol to produce a solution with a concentration = 250 mg/ 50 mL = 5 mg/ml. The concentration required for the sample representing 100% = 5 mg/mL. No further dilution is needed. For the 500 mg capsule, dissolve the contents in 50 mL of methanol. Dilute 1 mL of this solution by adding 1 mL of methanol.

Dissolve the contents of 1 capsule(500mg) in 50 mL of methanol, and add 1mL of methanol to 1mL of the concentrated solution.

Standards:

The normal form of the drug is the sodium salt, monohydrate with a molecular mass of 475.88. The listed content of the drug is the free base with a mass of 435.88. A correction for the difference in mass must be made when the standard is weighed. The correction is the weight in mg multipled by (435.88/475.88 = 0.915)

<u>High standard:</u>

The high limit is 120%; therefore the concentration of the high standard is (5 mg/mL) X 1.2 = 6 mg/mL. Weigh approximately 25 mg of the standard as the salt monohydrate. If you weighed 24.5 mg, the corrected weight would be 24.5 X 0.916 = 22.44 mg as the free base. The volume of solvent needed is 22.44 mg/6 mg/mL = 3.74 mL of methanol.

Low standard:

The low limit is 85%; therefore the concentration of the low standard is 0.85 X 5 mg/mL = 4.25 mg/mL (6 mg/mL/4.25 mg/mL= 1.41). Add 0.41 mL of methanol to 1 mL of the high standard.

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot sample (100%)
Right spot high standard (120%)

Developer:

Mix 26 mL of acetone, 4 mL of water, 4 mL of toluene, and 1 mL of glacial acetic acid. Add approximately 20 mL of the developer mixture to the TLC developing bag. Develop until the solvent front reaches to within 1 cm from the top of the TLC plate.

Detection:

UV:

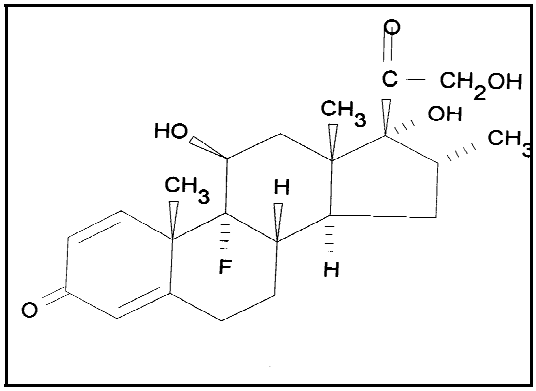
Dry the plate and observe under UV light at 254nm. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution. Allow the plate to dry until the stained spots become clearly visible. Observe the intensities of the spots.

Dexamethasone
4 mg tablet

Structure:



Molecular Formula & Mass: $C_{22}H_{29}FO_5$ - 392.45
Category: Glucocorticoid
Sample:

Grind 1 tablet and dissolve in 10 mL of 95% ethanol. Shake at least 1 min. Concentration of the solution = 4 mg/10 mL = 0.4 mg/mL. The required concentration of the sample solution representing 100% is 0.4 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.40 mg/mL) X 1.15 = 0.46 mg/mL. Weigh approximately 4 mg of standard. If you weighed 3.75 mg of standard, dissolve it in:
(3.75 mg)/(0.46 mg/mL) = 8.05 mL of 95% ethanol. This makes the high standard solution concentration equal to 0.46 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.40 mg/mL) X 0.85 = 0.34 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (0.46/0.34 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 14 mL of toluene and 7 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

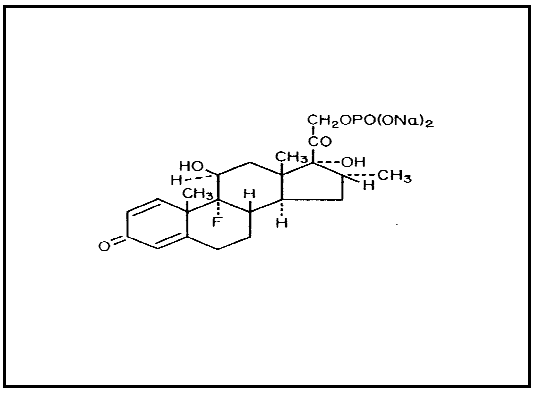
Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

The 0.40 mg/mL concentration is too low to allow for quantitation by iodine. The concentration needs to be 4 mg/mL. This can be accomplished by using 10 tablets rather than 1 tablet. Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Dexamethasone disodium phosphate
4 mg/mL injectionable

Structure:



Molecular Formula and Mass: $H_{29}FO_5.Na_2HPO_4$ - 534.45
Category: Glucocorticoid
Sample:

The sample concentration required is 4 mg/mL. Remove 1 mL of the sample from the vial with a 1 mL syringe. No further dilution is required.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (4.0 mg/mL) X 1.15 = 4.6 mg/mL. Weigh approximately 25 mg of the standard. If you weighed 22.05 mg of the standard, dissolve it in: (22.05 mg/4.6 mg/mL) = 4.79 mL of distilled water. This makes the high standard concentration equal to 4.6 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (4 mg/mL X 0.85) = 3.4 mg/mL. Dilute 1 mL of high standard to 1.35 by adding 0.35 mL of water (4.6/3.4 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 20 mL of methanol, 2 mL of toluene, 4 mL of acetic acid, and 2 mL of concentrated ammonium hydroxide. This solution will become warm as a result of the chemical reaction between the acid and base. Allow the developer solution to come to room temperature before starting the development. Add 20 mL of this developer to the TLC

development bag. Develop until the solvent front reaches 1 cm from the top of the plate.

Detection:

UV:

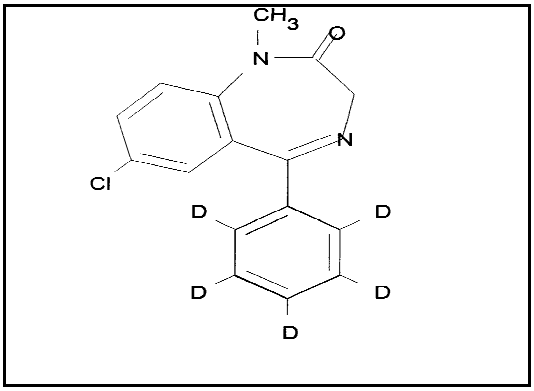
Dry the plate and examine the spots under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution. Allow the iodine to disappear, and observe the spots as soon as they become clear.

Diazepam
2 mg tablet

Structure:



Molecular Formula and Mass: $C_{16}H_{13}ClN_2O$ - 284.76 (the above structure shows deuterium atoms as D. In the normal structure the D is replaced by H atoms)

Category: Minor tranquilizer

Sample:

Grind 1 tablet and dissolve in 2 mL of 95% ethanol. Shake at least 1 min. Concentration of the solution = 2 mg/ 2 mL = 1 mg/mL. The required concentration of the sample solution representing 100% is 1 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg)/(1.15 mg/mL) = 4.1 mL of 95% ethanol. This makes the high standard solution concentration equal to 1.15 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 33 mL of methanol and 0.5 mL of concentrated ammonium hydroxide. Add 20 mL of this mixture to the TLC development bag. Develop until the solvent front reaches to

within 1 cm of the top of the TLC plate.

Detection:

UV:

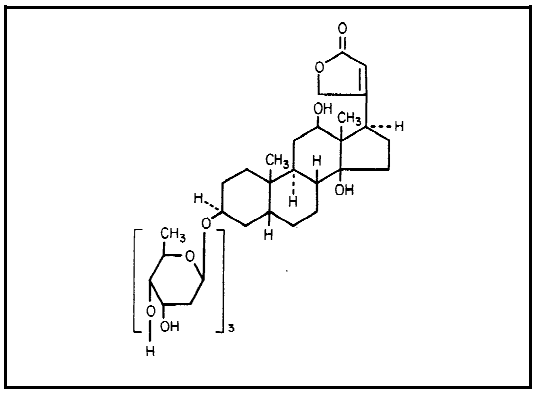
Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Digoxin
0.25 mg tablet

Structure:



Molecular formula and Mass: $C_{41}H_{64}O_{14}$ - 780.92
Category: Cardiotonic
Sample:

Because tablets of digoxin contain very small quantites of the active drug preparation of the solution is difficult. The usual content is 0.25 mg in a tablet which weighs over 100 mg. Therefore the tablets contain 99.75 mg of excipients. The small amount of liquid required to prepare the concentration needed for suitable TLC would be completely taken up by the powder, leaving no solution. The concentration needed for suitable TLC is 1 mg/mL; thus 4 tablets are required to obtain the quantity of drug necessary for analysis. It is not possible to add 1 mL of solvent to this quantity of powder. Sufficient liquid must be added to sample the solution.

Grind 4 tablets of digoxin and dissolve in 5 mL of anhydrous ethanol and 5 mL of water in a stoppered container. The concentration of this solution = (4 mg X 0.25)/10 mL = 0.1 mg/mL. The large volume is necessary to cover all the powder. Shake at least 2 min., and allow the undissolved material to settle. (The settling can be speeded up by a small centrifuge if available.) Transfer 4 mL of the supernatent liquid to a test tube (0.4 mg of digoxin). Evaporate the solution to dryness by placing the test tube in water heated to near boiling. When all the liquid has evaporated, add 0.4 mL of a mixture of anhydrous ethanol and water (50:50). The concentration of the sample solution is now 1 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of the standard. If you weighed 5.25 mg, dissolve it in: (5.25 mg/1.15 mg/mL) = 4.56 mL of the 50:50 water:anhydrous ethanol mixture.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1 mg/ml) X 0.85 = 0.85 mg/mL. Dilute 1 mL of the high standard to 1.35 mL by adding 0.35 mL of the 50:50 water:anhydrous ethanol mixture.

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Developer:

Mix 42 mL of ethyl acetate, 5 mL of methanol, and 2.5 mL of concentrated ammonium hydroxide. Add 20 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

Detection:

UV:

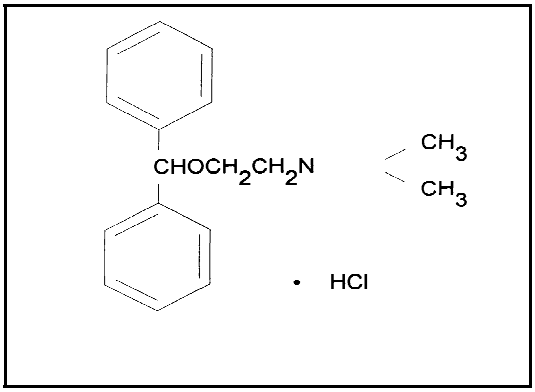
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution. Allow the iodine to fade until the spots become visible. Observe the size and intensity of the spots.

Diphenhydramine
25 mg tablet

Structure:



Molecular Formula & Mass: $C_{17}H_{21}NO$ - 255.4
Category: Antihistiminic
Sample:

Grind 1 tablet and dissolve in 5.0 mL of 95% ethanol. Shake at least 5 min. Concentration of the solution = 25 mg/5.0 mL = 5.0 mg/mL. The required concentration of the sample solution representing 100% is 5.0 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (5.0 mg/mL) X 1.15 = 5.75 mg/mL. Weigh approximately 25 mg of standard. If you weighed 24.75 mg of standard, dissolve it in: (24.75 mg)/(5.75 mg/mL) = 4.30 mL of anhydrous ethanol. This makes the high standard solution concentration equal to 5.0 mg/mL.

Low standard:

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (5.0 mg/mL) X 0.85 = 4.25 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (5.75/4.25 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 20 mL of methanol and 0.3 mL of concentrated ammonium hydroxide. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection :

UV.

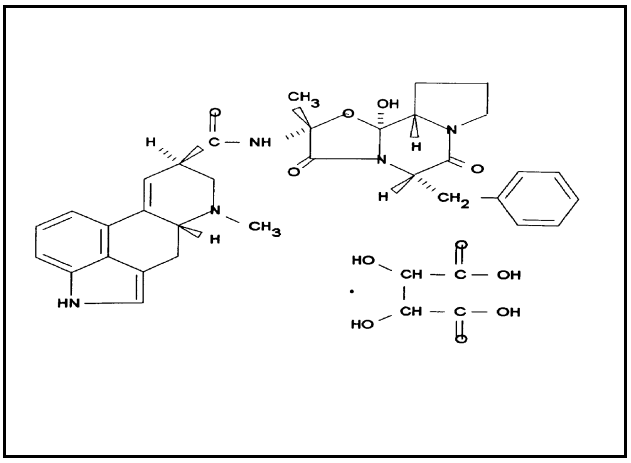
Dry the plate and observe under ultraviolet light at 254 nm. Observe the intensities and the sizes of the spots.

Iodine stain.

Dip the plate into the iodine solution. Allow the iodine to fade until the spots become visible. Observe the intensity and sizes of the spots.

Ergotamine tartrate
2 mg tablet

Structure:



Molecular Formula and Mass : $C_{70}H_{76}N_{10}O_{16}$ - 1312

Category: Vasoconstrictor

Sample:

Grind 4 tablets and dissolve in 8 mL of 95% ethanol. The drug content in each tablet is low. Enough liquid is needed to obtain a solution of the correct concentration. Shake at least 2 min. and allow the insolubles to settle. Withdraw approximately 1 mL of the supernatant liquid for the sample. The final concentration of the sample solution is 1 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of the standard. If you weighed 4.8 mg of the standard, dissolve it in: (4.8 mg/1.15 mg/mL) = 4.17 mL of 95% ethanol.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of 95% ethanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 24 mL of methanol and 0.4 mL of concentrated ammonium hydroxide. Add this solution to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

Detection:

UV:

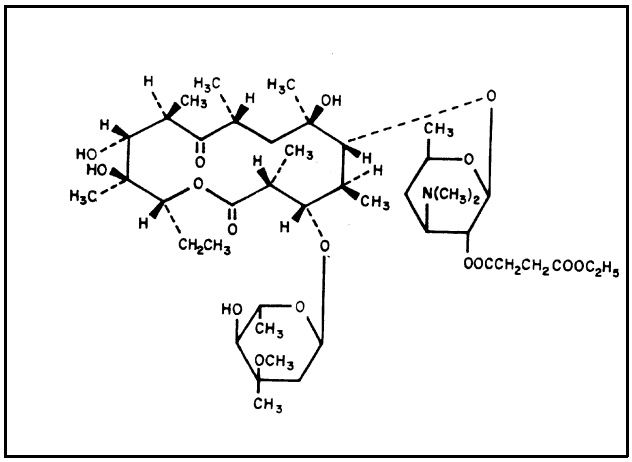
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution, and observe the size and intensity of the spots after they become visible.

Erythromycin ethyl succinate
250 mg tablet

Structure:



Molecular Formula & Mass: $C_{43}H_{75}NO_{16}$ - 862.08

Category: Antibacterial

Sample:

Many of the antibiotic drugs come in different forms. Erythromycin is available as the free base, salts, and esters. This assay is for erythromycin ethyl succinate. The drug content is quoted as the basic drug, erythromycin. Because the standard is in the form of the ethyl succinate, the drug content of the sample must be converted to the ethyl succinate, as follows:

Molecular weight of erythromycin ethyl succinate = 862.08

Molecular weight of erythromycin = 733.92

250 mg (as erythromycin) x 862.08/733.92 = 294 mg as the ethyl succinate. Each tablet contains 294 mg of erythromycin ethyl succinate.

Grind 1 tablet and dissolve in 50 mL of acetone. Shake at least 2 min. Concentration of the solution = 294 mg/50 mL = 5.87 mg/mL. The required concentration of sample solution representing 100% is 1.20 mg/mL. Add 3.89 mL of acetone to 1 mL of the 5.87 mg/mL solution to make the sample solution (5.87/4.89 = 1.20 mg/mL).

Standards:

<u>High standard:</u>

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (1.2 mg/mL) X 1.20 = 1.44 mg/mL. Weigh approximately 10 mg of standard. If you weighed 9.75 mg of standard, dissolve it in: (9.75 mg)/(1.44 mg/mL) = 6.77 mL of acetone. This makes the high standard solution concentration equal to 1.44 mg/mL.

<u>Low standard:</u>

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (1.2 mg/mL) x 0.85 = 1.02 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of methanol (1.44/1.02 = 1.41).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (120%)

Development:

Mix 16 mL of acetone and 8 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

The spots are not visible in the UV.

Iodine stain:

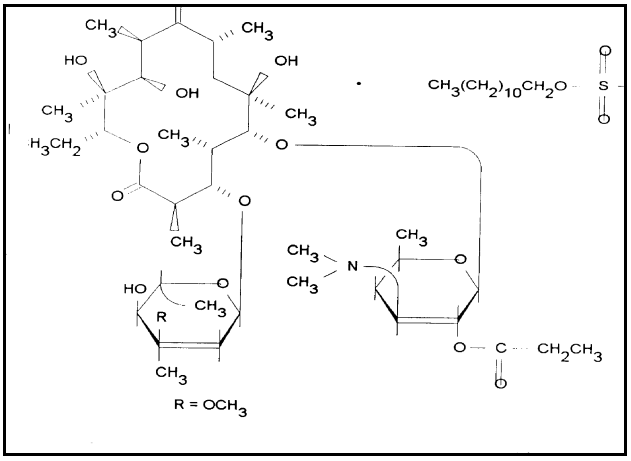
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots. To make the spots last longer, allow the plate to dry for 30 min. after it has been dipped in the iodine-KI solution. Heat the plate at 100EC for 5 min. The spots turn gray-green and are stable for 24 hr.

Notes:

The ethyl succinate ester is very easily hydrolyzed in the presence of water or methanol. Therefore the sample and standard must be prepared in acetone. The free base of erythromycin is visible as a spot near the spotting line (R_f = 0.04). If the content of erythromycin free base is to be determined, the sample must be dissolved in acetone and the TLC plate developed in the developing solvent for erythromycin free base (see erythromycin and erythromycin stearate) to prevent the hydrolysis of the ester to the free base during sample preparation.

Erythromycin estolate
250 mg tablet

Structure:



Molecular Formula & Mass: $C_{52}H_{97}NO_{18}S$ - 1056.43
Category: Antibacterial
Sample:

Many of the antibiotic drugs come in different forms. Erythromycin is available as the free base, salts, and esters. This assay is for erythromycin estolate (erythromycin propionate ester and lauryl sulfate salt). The drug content is quoted as the basic drug, erythromycin. Because the standard is in the form of the estolate, the drug sample content must be converted to the estolate, as follows:

Molecular weight of erythromycin ethyl succinate = 1056.43

Molecular weight of erythromycin = 733.92

250 mg (as erythromycin) X 1056.43/733.92 = 360 mg as the estolate.

Each tablet contains 360 mg of erythromycin estolate.

Grind 1 tablet and dissolve in 50 mL of acetone. Shake at least 2 min. Concentration of the solution = 360 mg/50 mL = 7.2 mg/mL. The required concentration of sample solution representing 100% is 1.50 mg/mL. Add 3.8 mL of acetone to 1 mL of the 7.2 mg/mL solution to make the sample solution (7.2/4.8 = 1.50 mg/mL).

Standards:

<u>High standard:</u>

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (1.5 mg/mL) X 1.20 = 1.80 mg/mL. Weigh approximately 10 mg of standard. If you weighed 9.75 mg of standard, dissolve it in: (9.75 mg)/(1.80 mg/mL) = 5.42 mL of acetone. This makes the high standard solution concentration equal to 1.80 mg/mL.

<u>Low standard:</u>

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (1.5 mg/mL) x 0.85 = 1.28 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding

0.41 mL of methanol (1.80/1.28 = 1.41).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (120%)

Development:

Mix 16 mL of acetone and 8 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

The spots are not visible in the UV.

Iodine stain:

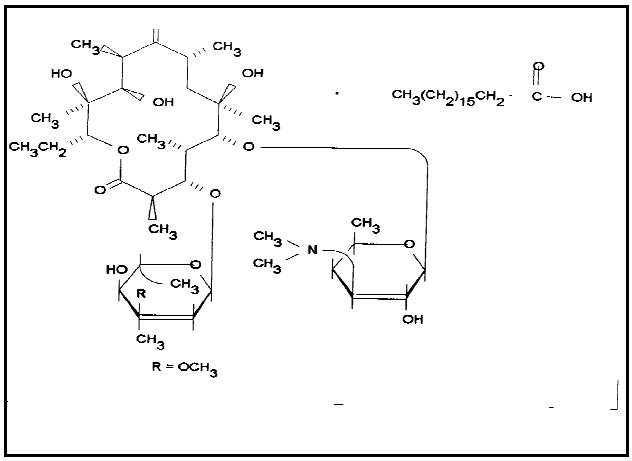
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots. To make the spots last longer, allow the plate to dry for 30 min. after it has been dipped in the iodine solution. Heat the plate at 100EC for 5 min. The spots turn gray-green and are stable for 24 hr.

Notes:

The estolate is the lauryl sulfate salt of erythromycin propionate. The erythromycin free base is visible if present as a spot near the spotting line (R_f = 0.04). If it is to be quantitatively determined, see the directions for erythromycin free base and erythromycin stearate.

Erythromycin and Erythromycin stearate
250 mg tablet

Structure:



Molecular Formula & Mass: $C_{37}H_{67}NO_{13}$ - 722.92

Category: Antibacterial

Sample:

Many of the antibiotic drugs come in different forms. Erythromycin is available as the free base, salts, and esters. This assay is for the free base and the salts. The drug content is quoted as the basic drug, erythromycin. Because the standard is in the form of the free base, the drug content can be calculated based on the activity of the standard.

Grind 1 tablet and dissolve in 50 mL of methanol. Shake at least 2 min. Concentration of the solution = 250 mg/50 mL = 5.0 mg/mL. The required concentration of sample solution representing 100% is 1.0 mg/mL. Add 4 mL of methanol to 1 mL of the 5.0 mg/mL solution to make the sample solution equal to 1.0 mg/mL.

Standards:

<u>High standard:</u>

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (1.0 mg/mL) X 1.20 = 1.20 mg/mL. Weigh approximately 10 mg of standard. If you weighed 9.75 mg of standard, dissolve it in: (9.75 mg)/(1.2 mg/mL) = 8.13 mL of methanol. This makes the high standard solution concentration equal to 1.2 mg/mL.

Low standard:

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (1.0 mg/mL) x 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of methanol (1.2/0.85 = 1.41).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample

Right spot high standard (120%)

Development:

Mix 21 mL of methanol, 2 mL of toluene, and 2 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

The spots are not visible in the UV.

Iodine stain:

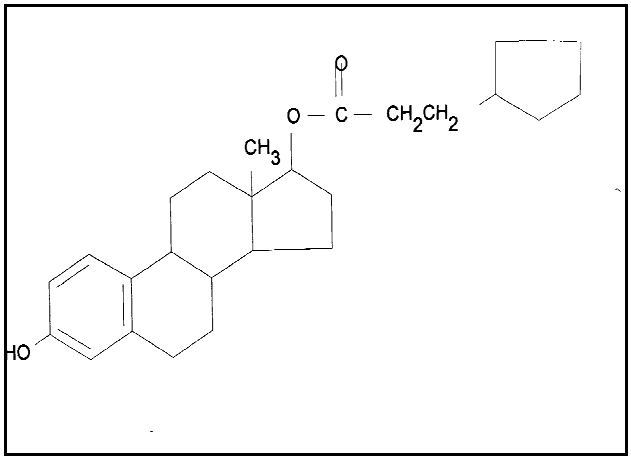
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots. To make the spots last longer, allow the plate to dry for 30 min. after it has been dipped in the iodine solution. Heat the plate at 100EC for 5 min. The spots turn gray-green and are stable for 24 hr.

Notes:

This procedure works for the free base and stearate form. It should also work with the lactobionate and gluconate forms. This solvent system can be used to separate the free base from the ethyl succinate and propionate (estolate) esters. The ethyl succinate hydrolyzes in methanol quickly (less than 24 hr) to form the free base and erythromycin. Thus, the esters should be quantitated by using the appropriate procedure (see erythromycin estolate and erythromycin ethyl succinate).

Estradiol cypionate
5 mg/mL injectable

Structure:



Molecular Formula & Mass: $C_{26}H_{36}O_3$ - 396.55

Category: Estrogen

Sample:

Dilute 0.5 mL of sample to 5 mL by adding 4.5 mL of acetone. Concentration of the solution = (0.5 mL x 5 mg/mL)/5 mL = 0.5 mg/mL. The required concentration of sample solution representing 100% is 0.50 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.5 mg/mL) x 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg)/(0.575 mg/mL) = 8.26 mL of acetone. This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.5 mg/mL) x 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of acetone (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 18 mL of toluene and 6 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

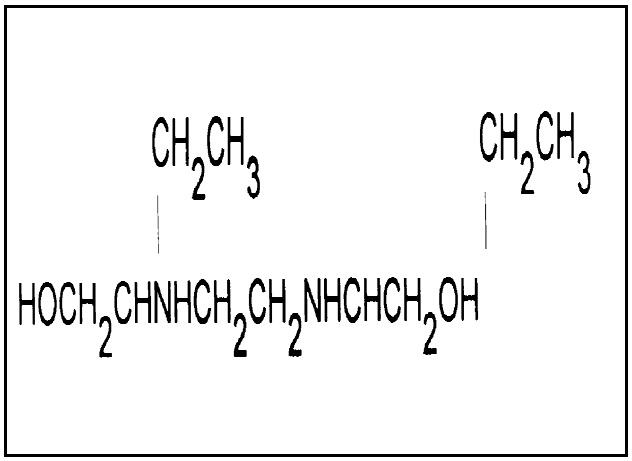
Iodine stain:

The 0.50 mg/mL concentration is too low to allow for quantitation by iodine. The concentration needs to be 5.0 mg/mL. This can be accomplished by using the liquid sample neat. Do not dilute the sample. Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Ethambutol HCl
100 and 400 mg tablets

Revised 8/18/1997
METHOD# 1

Structure



Molecular Formula & Mass: $C_{10}H_{24}N_2O_2$ - 204.31
Category: Antibacterial
Sample:
100 mg tablet:

Grind 1 tablet and dissolve in 10 mL of methanol which makes a solution having a concentration of 10 mg/mL. Concentration of the required solution = 2 mg/mL to represent a 100% solution. Take 1 mL of the 10 mg/mL solution and add 4 mL of methanol to make the required concentration of 2mg/mL.

400 mg tablet:

Grind 1 tablet and dissolve in 25 mL of methanol. Concentration of the solution =16 mg/mL. Add 7 mL of methanol to 1 mL of the 16 mg/mL solution to make a final concentration equal to 2 mg/mL.

Standard:
High standard:

The high limit is 115%; therefore the concentration of high standard = (2 mg/mL X 1.15) = 2.3 mg/mL. Weigh approximately 21 mg of standard. If you weighed 21.7 mg of standard, dissolve it in: (21.7 mg)/(2.3 mg/mL) = 9.4 mL of methanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (2 mg/mL) X 0.85 = 1.7 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol= 1.35).

Spotting:

Spot on the TLC plate as follow:

Left spot low standard (85%)

Center spot 100% sample

Right spot high standard (115%)

Development:

Mix 25 mL of methanol and 0.38 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

The spots are not visible under UV.

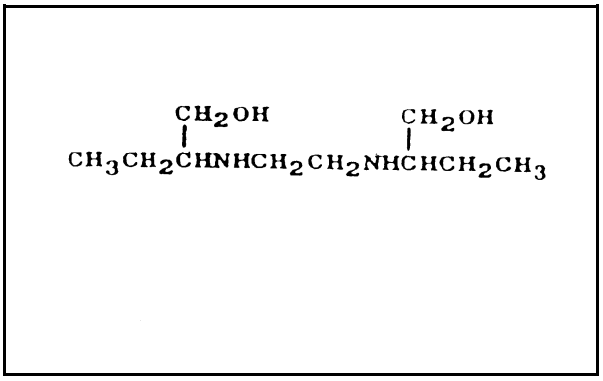
Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

METHOD#2
Revised March 16,1999

Ethambutol HCl
100 and 400 mg tablets

Structure



Molecular Formula & Mass: $C_{10}H_{26}Cl_2N_2O_2$ - 277
Category: Antibacterial (tuberculostatic)

Preparation of the sample solution:
Analytical balance available:

Prepare the sample solution by weighing an aliquot of the drug. Follow the procedure described in the previous section. Determine the weight of the drug and add solvent to produce a concentration of 2mg/mL. Measure the volume accurately using a combination of pipetts plus a 1 mL graduated tuberculin syringe for the fractional volumes. Pipetts are available in 1mL increments up to 10 mL. For example: You weighed 23 mg of the drug, then you would add 11.5mL of solvent to prepare a solution with a concentration of 2mg/mL. (Use a 10 and 1mL pipetts and the 0.5 mL is measured by a 1mL graduated syringe).

NOTE: The above procedure applies to all of the TB drugs using the aliquot method.

Analytical balance not available.

The tablets listed below are representative of different drug content. Adjust the volumes accordingly for different composition. Always use volumes in full mL so that fractional volumes is not required. Most drugs have their contents in multiples of 5 which give a whole number for the concentration. Dissolve the sample in volumes which do give you a whole number for the concentrated solution. An aliquot of the concentrated solution is diluted to obtain the proper concentration for the TLC.

100 mg tablet:

Grind 1 tablet and dissolve in 10 mL of methanol which makes a solution having a concentration of 10 mg/mL. Concentration of the required solution = 2 mg/mL to represent a 100% solution. Take 1 mL of the 10 mg/mL solution and add 4 mL of methanol to make the required concentration of 2mg/mL.

400 mg tablet:

Grind 1 tablet and dissolve in 25 mL of methanol. Concentration of the solution =16 mg/mL. Add 7 mL of methanol to 1 mL of the 16 mg/mL solution to make a final concentration equal to 2 mg/mL.

Preparation of standard or reference solutions:

Reference solutions are prepared depending on the availability of reference compounds. Reference tablets are available. The tablets contain a predetermined weight of the drug which when dissolved in 5 mL of the solvent will produce a solution concentration representing 115% of the sample solution. No weighing is required. Weighing is required when the reference compound is not available in tablet form. The reference solutions then must be prepared using either primary or secondary standards.

Preparation of the high standard when no reference tablets are available:

The high concentration limit is 115%; therefore the concentration of high standard = (2 mg/mL X 1.15) = 2.3 mg/mL. Weigh approximately 21 mg of standard. If you weighed 21.7 mg of standard, dissolve it in: (21.7 mg)/(2.3 mg/mL) = 9.4 mL of methanol.

Low standard:

The low limit is 85%; the concentration of low standard = (2 mg/mL) X 0.85 = 1.7 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol= 1.35). This low reference solution is always prepared by the same procedure regardless of the reference source.

Spotting:

Spot on the TLC plate as follows:

Sample each of the solutions with a 3µL capillary pipette and spot.

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 25 mL of methanol and 0.38 mL of concentrated ammonium hydroxide. Add 24 mL this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

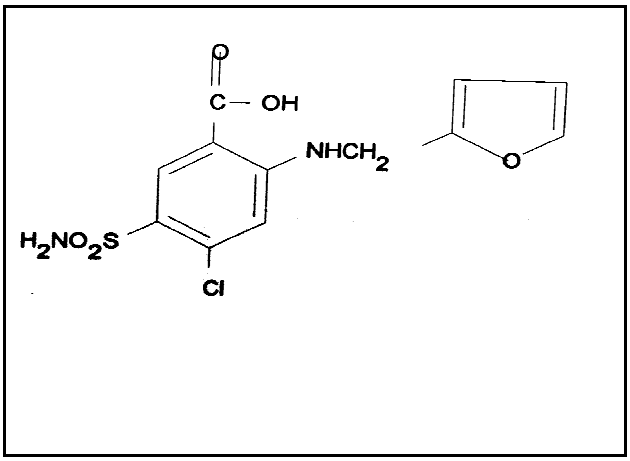
The spots are not visible under UV.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Furosemide
20, 40 and 80 mg tablets

Structure:



Molecular Formula and Mass: $C_{12}H_{11}ClN_2O_5S$ - 330.77
Category: Diuretic
Sample:

The sample solution representing 100% requires a concentration of 2.5 mg/mL.
20 mg tablet: Grind 1 tablet and dissolve in 8 mL of methanol.
40 mg tablet: Grind 1 tablet and dissolve in 16 mL of methanol.
80 mg tablet: Grind 1 tablet and dissolve in 32 mL of methanol.
Shake for at least 2 min.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (2.5 mg/mL) X 1.15 = 2.875 mg/mL. Weigh approximately 15 mg of standard. If you weighed 13.2 mg of standard, dissolve it in: (13.2 mg)/(2.875 mg/mL) = 4.59 mL of methanol. This makes the high standard solution concentration equal to 2.875 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (2.5 mg/mL) X 0.85 = 2.125 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (2.875/2.125 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 5 mL of toluene, 5 mL of acetone, 20 mL of methanol, and 1.5 mL of concentrated ammonium hydroxide. Pour 24 mL of the mixture into a TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

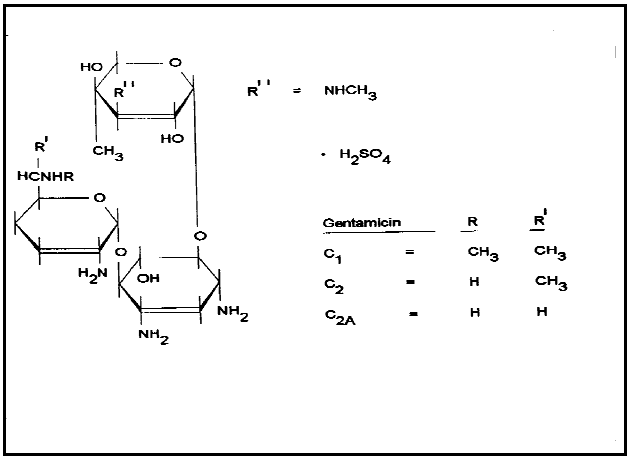
Dry the plate and observe under UV light. The maximum is at 275 nm but a short wavelength UV light (254 nm) works. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Gentamycin
40 mg/mL injectable

Structure:



Molecular Formula & Mass: C1;$C_{21}H_{43}N_5O_7.H_2SO4$ - 575.68
C1a;$C_{19}H_{39}N_5O_7.H_2SO4$ - 547.63
C2;$C_{20}H_{41}N_5O_7.H_2SO_4$ - 561.66

Category: Antibacterial

Gentamicin is one of the aminoglycoside antibiotics and is composed of more than one component. Injectable gentamicin sulfate is a mixture of three components: gentamicin C1, C1a, and C2 sulfates. The drug content is quoted as the basic drug, gentamicin. Because the standard is in the form of the sulfate, the potency is quoted as units of gentamicin activity. Thus the standards must be corrected for activity.

Sample:

Dilute 1 mL of the injectable solution to 8 mL by adding 7 mL of distilled water. Shake at least 1 min. Concentration of the solution = 5 mg/mL.

Standard:

<u>High standard:</u>

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (5.0 mg/mL) X 1.20 = 6.0 mg/mL. Weigh approximately 35 mg of standard. If you weighed 34.9 mg of standard with an activity of 648, dissolve it in:
(34.9 mg)(0.648)/(6.00 mg/mL) = 3.77 mL of distilled water. This makes the high standard solution concentration equal to 6.0 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (5.0 mg/mL) X 0.85 = 4.25 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of water (6.0/4.25 = 1.41).

Spotting:

Mix 20 mL of methanol, 4 mL of water, and 20 mL of concentrated ammonium

hydroxide. Add 22 mL to the TLC development bag and develop a blank TLC plate until the solvent front reaches 1 cm from the top of the plate. Remove the plate and allow it to dry until the odor of ammonia can no longer be detected. Remove the solvent from the developing bag.

Spot on the plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (120%)

Development:

Place 24 mL of the methanol-water-concentrated ammonium hydroxide mixture in the TLC development bag. Develop the plate until the solvent front reaches 1 cm from the top of the plate. Allow the plate to dry until the odor of ammonia cannot be detected.

Detection:

UV:

The spots are not visible in the UV.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the spots to dry and determine their size and intensity.

Visible:

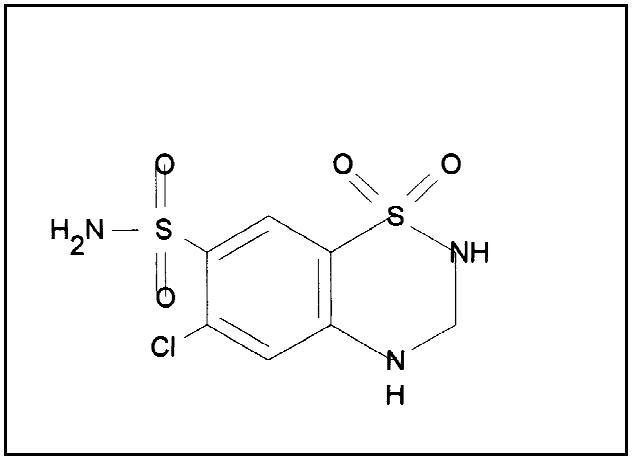
To make the spots visible in white light, dip the plate in a ninhydrin solution. Dissolve 100 mg of ninhydrin, 2,2-dihydroxy-1,3-indanedione (Sigma Chemical Co. No. N4876), in 25 mL of acetone. Place 7 mL of this solution in the detection bag instead of the iodine-KI solution. The TLC plate should be dry and completely free of any ammonia (it may be necessary to allow the plate to sit overnight). Dip the TLC plate in the ninhydrin solution. Allow the plate to dry for 60 min. A blue-violet color develops with gentamicin. Observe the size and intensity of the spots. If the TLC plate is not completely free of ammonia, the background color of the plate will change from pale pink to dark rose in about 1 hr.

Notes:

The use of ninhydrin is recommended because the iodine colors do not last very long. The color may become unreadable in 5 min. and yet may be readable for as long as 20 min. The ninhydrin color can be developed in less time if an oven or a hot plate is available. After the TLC plate air-dries for 30 min., the ammonia on the TLC plate can be removed by heating the plate at 100EC for 10 min. Dip the TLC plate in the ninhydrin solution and allow it to air-dry for 30 min. (The color usually develops in 30-60 min.) Heat the TLC plate for 2 min. at 100EC to fully develop the violet color. The TLC plate is pre-developed to remove the possibility of streaking or solvent front effects.

Hydrochlorothiazide
25 mg capsule

Structure



Molecular Formula & Mass: $C_7H_8ClN_3O_4S$ - 297.75
Category: Diuretic
Sample:

Dissolve the contents of 1 capsule in 50 mL of acetone. Shake at least 2 min. and then heat in a 50EC water bath for 60 min. Shake at least 2 min., allow the solution to settle, and cool to room temperature. Concentration of the solution = 25 mg/50 mL =0.50 mg/mL. The required concentration of sample solution representing 100% is 0.50 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.5 mg/mL) x 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg)/(0.575 mg/mL) = 8.26 mL of acetone. This makes the high standard solution concentration equal to 0.575 mg/mL. Dissolve the drug from 1 capsule in 50 mL of acetone. Shake for 2 min. then heat in a 50°C water bath for 60 min. Shake for 2 min., allow solution to settle and cool to room temperature. Concentration of solution = 25 mg/50 mL = 0.50 mg/mL. Required concentration of sample solution representing 100% is 0.50 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) x 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of acetone (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Add 24 mL of ethyl acetate to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

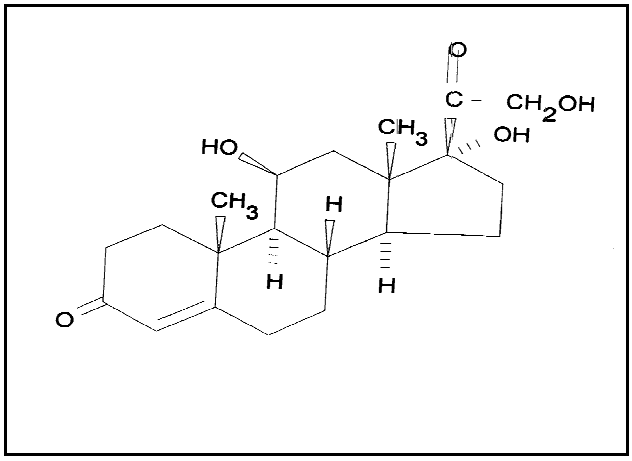
Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

The 0.50 mg/mL concentration is too low to allow for quantitation by iodine. The concentration must be 10.0 mg/mL. This can be accomplished by using 2.5 mL of acetone to dissolve 1 tablet. Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots. The spots take a long time to develop, and the plates can be read 24 hr later.

Hydrocortisone
10 mg tablet

Structure:



Molecular Formula & Mass: $C_{21}H_{30}O_5$ - 362.47
Category: Glucocoticoid, adrenocortical steroid
Sample:

Grind 1 tablet and dissolve in 20 mL of 95% ethanol. Shake at least 1 min. Concentration of the solution = 10 mg/20 mL = 0.50 mg/mL. The required concentration of the sample solution representing 100% is 0.5 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg)/(0.575 mg/mL) = 8.26 mL of 95% ethanol. This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of 95% ethanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 16 mL of toluene and 8 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

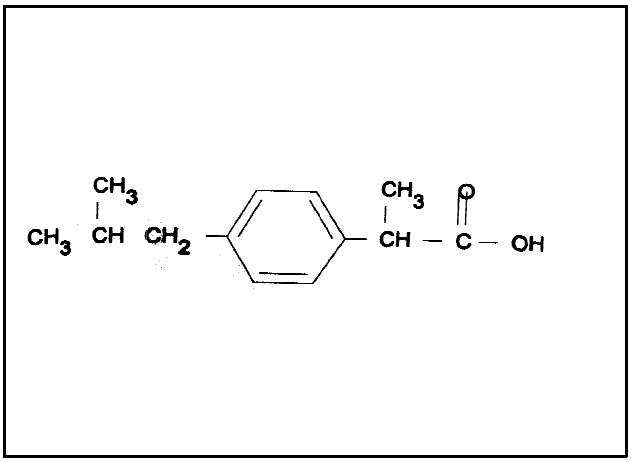
Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

The 0.50 mg/mL concentration is too low to allow for quantitation by iodine. The concentration needs to be 5 mg/mL. This can be accomplished by using 10 mL of 95% ethanol rather than 20 mL. Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Ibuprofen tablet **300mg**

Structure:



Molecular formula and weight: $C_{13}H_{18}O_2$ - 206.27

Category: Analgesic

Sample:

Grind 1 tablet of the drug, and dissolve in 50 mL of a mixture containing 1 mL of acetic acid and 49 mL of methanol (prepare a volume of the methanol/acid mixture greater than 50 mL before making the solution of the drug). This produces a solution having a concentration equal to 6 mg/mL. The concentration required for the analysis is 2 mg/mL. Take 1 mL of the 6 mg/mL solution and dilute to 3 mL by adding 2 mL of the acetic acid/methanol mixture to make the final concentration.

The drug is also supplied as a 400 mg tablet. In this case, grind and dissolve 1 tablet in 50 mL of the solvent mixture to make a concentration equal to 8 mg/mL. Take 1 mL of the 8 mg/mL solution and add 3 mL of the solvent mixture to make the final solution equal to 2 mg/mL.

Standards:

High standard:

The high standard solution concentration requires a solution equivalent to 115% of the sample concentration (1.15 X 2 mg/mL = 2.3 mg/mL). Weigh approximately 10 mg of the standard. Divide the weight of the standard by the high standard concentration to obtain the volume of solvent needed. For example, 9.4 mg was weighed, then the volume needed would be 9.4mg/2.3 = 4.09 mL of the acid/methanol mixture.

Low standard:

The low standard concentration is equivalent to 85% of the sample concentration. The concentration is 0.85 X 2 mg/mL= 1.7 mg/mL. This

concentration is prepared by taking 1 mL of the 2.3 mg/mL solution and adding 0.35 mL of the solvent mixture.

Spotting:

Spot the TLC plate as follows:
Left spot low standard(85%)
Center spot 100% sample
Right spot high standard(115%)

Development:

Prepare the developer by mixing 17 mL of toluene, 13 mL of ethyl acetate, and 1 mL of acetic acid. Add 24 mL of this solution to the TLC development bag. Develop until the solvent front reaches to within 1 cm from the top of the TLC plate.

Detection:

UV:

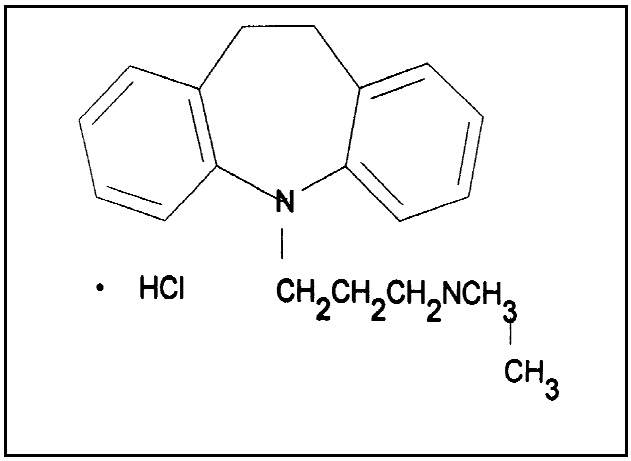
Dry the plate and observe under UV at 254 nm. Observe the sizes and intensities of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution. Remove and allow the iodine to sublime, and observe the spots as soon as they become distinct. Note the sizes and intensities.

Imipramine HCl
75mg capsule

Structure



Molecular Formula & Mass: $C_{19}H_{24}N_2$ - 280.40
Category: Antidepressant
Sample:

Dissolve the contents of 1 capsule in 75 mL of anhydrous ethanol. Concentration of the solution is 75 mg/75 mL = 1 mg/mL. The required concentration of sample solution representing 100% is 1 mg/mL.

Standard:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of standard. If you weighed 5.8 mg of standard, dissolve it in: (5.8 mg)/(1.15 mg/mL) = 5 mL of anhydrous ethanol.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 24 mL of methanol and 0.25 mL of concentrated ammonium hydroxide. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

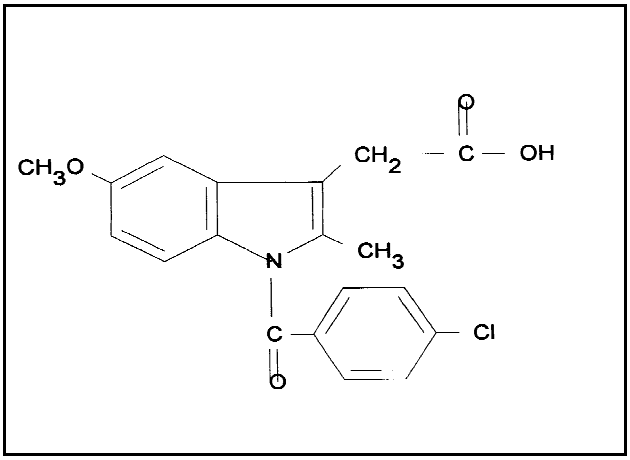
Dry the plate and observe under UV light. The maximum is at 270 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Indomethacin
25 mg capsule

Sample:



Molecular Formula & Mass: $C_{19}H_{16}ClNO_4$ - 357.81

Category: Anti-inflamatory, antipyretic,analgesic

Sample:

Dissolve the contents of 1 capsule in 5 mL of methanol. Shake at least 1 min. Concentration of the solution = 25 mg/5 mL = 5.00 mg/mL. The required concentration of the sample solution representing 100% is 5.00 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (5.00 mg/mL) x 1.15 = 5.75 mg/mL. Weigh approximately 25 mg of standard. If you weighed 25 mg of standard, dissolve it in: (25.0 mg)/(5.75 mg/mL) = 4.35 mL of methanol. This makes the high standard solution concentration equal to 5.75 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (5.00 mg/mL) x 0.85 = 4.25 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (5.75/4.25 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 25 mL of toluene, 12 mL of acetone, and 1 mL of glacial acetic acid. Add about 24 mL of this mixture to a TLC development bag. Develop until the solvent front reaches

within 1 cm of the top of the TLC plate.

Detection:

UV:

Allow the plate to dry and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

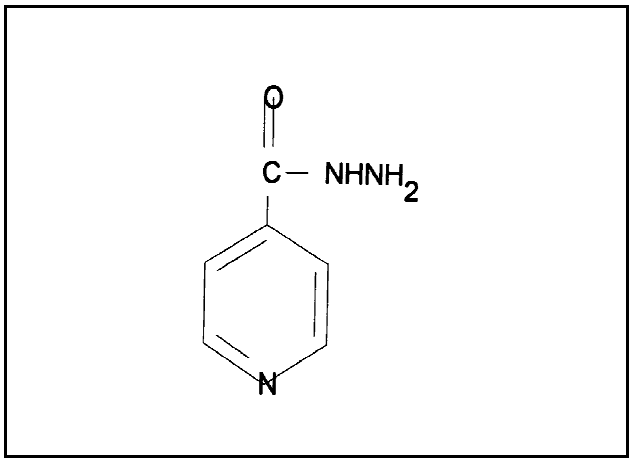
Dip the plate into the iodine-KI solution in the detection bag. Observe the size and intensity of the spots as the plate dries.

METHOD # 1 **Isoniazid**

300 and 100 mg tablets

Revised procedure(Aug.12,1997)

Structure:



Molecular Formula & Mass: $C_6H_7N_3O$ - 137.15

Category: Antibacterial (tuberculostatic)

Sample:

300 mg tablet

Crush 1 tablet and dissolve in 50 mL of methanol. Concentration of the solution = 300 mg/50 mL = 6 mg/mL. The required concentration of the sample solution representing 100% = 0.5 mg/mL. Take 1 mL of the 6 mg/mL solution and add 11 mL of methanol to make 12 mL of solution which makes a final concentration of 0.5mg/mL.

100 mg tablet

Crush 1 tablet and dissolve in 25 mL of methanol. Concentration of the solution = 100 mg/25 mL = 4 mg/mL. The concentration of the sample solution representing 100% = 0.5mg/mL. Take 1 mL of the 4mg/mL solution and add 7 mL of methanol to make 8 mL of solution with a concentration of 0.5mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.5 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 4-5 mg of standard. If you weighed 4.2 mg of standard, dissolve it in: (4.2mg)/(0.575 mg/mL) = 7.3 mL of methanol. This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.5 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Developer:

Mix 25 mL of methanol and 0.5 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

Detection:

UV:

Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

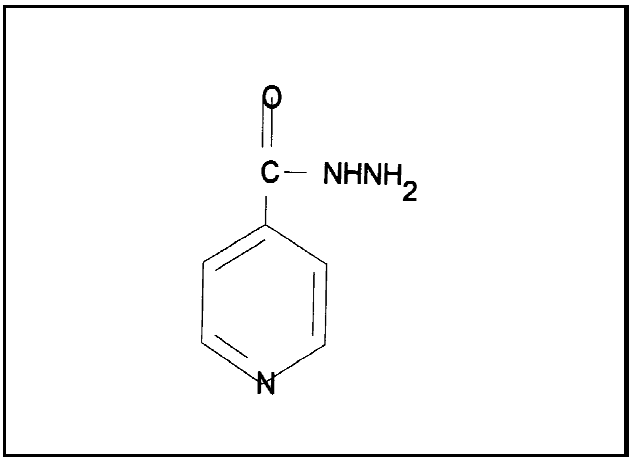
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spot.

METHOD #2

Isoniazid

300 and 100 mg tablets

Structure:



Molecular Formula & Mass: $C_6H_7N_3O$ - 137.15
Category: Antibacterial (tuberculostatic)

Preparation of sample solution:

Analytical balance available.

Prepare the sample solution by weighing an aliquot of the drug. Follow the procedure described in the previous sections. Determine the weight of the drug and add solvent to produce a concentration of 0.5mg/mL. The volumes are measured accurately by using a combination of pipetts plus a 1 mL graduated tuberculin syringe for the fractional volumes. Pipetts are available in 1mL increments up to 10 mL. For example: You weighed 5.25 mg of the drug, then you would add 10.5mL of solvent to prepare a solution with a concentration of 0.5mg/mL. (Use a 10 mL pipette and measure the 0.5 mL by a 1mL graduated tuberculin syringe).

Analytical balance not available.

The entire dosage form is used with the declared drug content taken as the weight of the sample. The tablet contents have many different dosages, and the ones described are representative. All volumes must be accurately measured by pipettes.

300 mg tablet

Grind to a fine powder 1 tablet in a polyethylene bag and dissolve in 50 mL of methanol. The solution concentration 300 mg/50 mL = 6 mg/mL. The required concentration of the sample solution representing 100% is 0.5 mg/mL. Take 1 mL of the 6 mg/mL solution and add 11 mL of methanol to make 12 mL of solution which makes a final concentration of 0.5mg/mL.

100 mg tablet

Grind to a fine powder 1 tablet and dissolve in 25 mL of methanol. The concentration of the solution is 100 mg/25 mL = 4 mg/mL. The required concentration of the sample solution representing 100% is 0.5 mg/mL. Take 1 mL of the 4 mg/mL solution and add 7 mL of methanol to make 8 mL of solution with a concentration of 0.5mg/mL.

Preparation of standards solutions:

Reference solutions are prepared depending on the availability of reference compounds. The reference materials may be either in the form of reference tablets or powders of primary/secondary standards. Reference tablets may be available containing a predetermined weight of the drug which when dissolved in 5 mL of the solvent produces a solution concentration representing 115% of the sample solution. No weighing is required.

Weighing is required when the reference compound is not available in tablet form. The reference solutions must be prepared using either primary or secondary standards.

<u>High standard solution:</u>

1. Reference tablet available.

The reference tablet contains 2.88 mg of isoniazid which when dissolved in 5 mL of methanol produces a solution having a concentration of 2.88mg/5 mL equal to 0.576 mg/mL which is 115% of the sample concentration. Drop one reference tablet into a vessel and add 5 mL of the solvent. No weighing is needed.

2. Reference material in the powder form (primary or secondary).

A reference solution having a concentration of 0.576 mg/mL is required. Weigh approximately 4-5 mg of powdered standard. For example: you weighed 4.2 mg of standard, dissolve it in: (4.2mg)/(0.575 mg/mL) = 7.3 mL of methanol. Measure the volumes by pipettes and a 1 mL graduated tuberculin syringe. This makes the high standard solution concentration equal to 0.575 mg/mL equal to 115% .

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.5 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (0.575/0.425 = 1.35). The low standard is always prepared by taking 1 mL of the high and adding 0.35 mL of the solvent when the high solution represents 115% of the sample.

Spotting:

Spot on the TLC plate as follows:

Sample each of the solutions with a 3µL capillary pipette and spot.

Left spot low standard (85%)
Center spot sample (100%)
Right spot high standard (115%)

Developer:

Mix 13 mL of methanol, 17 mL of acetone and 1 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

Detection:

<u>UV:</u>

Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots or stain with iodine when no UV available..

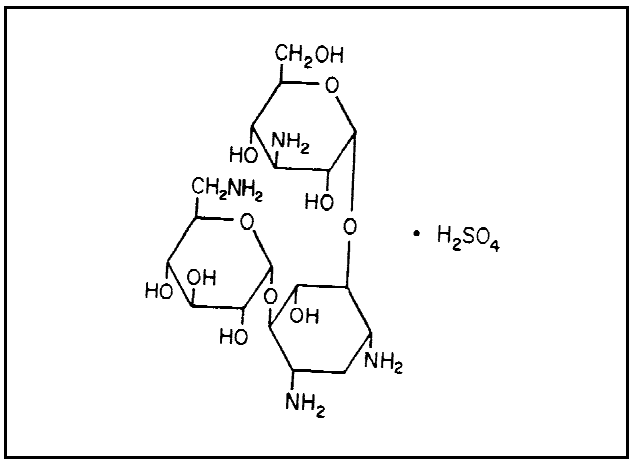
<u>Iodine stain:</u>

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe

the size and intensity of the spot.

Kanamycin sulfate
75 mg/2 mL injectable

Structure:



Molecular Formula & Mass: A & C; $C_{18}H_{36}N_4O_{11}$ - 386.43
B; $C_{18}H_{37}N_5O_{11}$ - 385.45

Category: Antimicrobial

Kanamycin is one of the aminoglycoside antibiotics and is composed of more than one component. Injectable kanamycin sulfate is a mixture of three components: kanamycin A, B, and C sulfates. The drug content is quoted as the basic drug, kanamycin. The standard is in the form of the sulfate and the potency is quoted as units of kanamycin activity. Thus the standards must be corrected for activity.

Sample:

Dilute 0.5 mL of the injectable solution to 15 mL by adding 14.5 mL of distilled water. Shake at least 1 min. Concentration of the solution = (0.5 mL x 75 mg)/(2 mL)/15 mL = 1.25 mg/mL. The required concentration of sample solution representing 100% is 1.25 mg/mL.

Standards:

<u>High standard:</u>

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (1.25 mg/mL) X 1.20 = 1.50 mg/mL. Weigh approximately 20 mg of standard. If you weighed 19.9 mg of standard with an activity of 648, dissolve it in: (19.9 mg)(0.648)/(1.50 mg/mL) = 8.60 mL of water. This makes the high standard solution concentration equal to 1.50 mg/mL.

<u>Low standard:</u>

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (1.25 mg/mL) x 0.85 = 1.06 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of water (1.50/1.06 =1.41).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (120%)

Development:

Mix 20 mL of methanol, 4 mL of water, and 20 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop a blank TLC plate until the solvent front reaches 1 cm from the top of the plate. Remove the plate and allow it to dry until the odor of ammonia can no longer be detected. Remove the solvent from the developing bag.

Detection:

UV:

The spots are not visible in the UV.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the spots to dry and determine their size and intensity.

Visible:

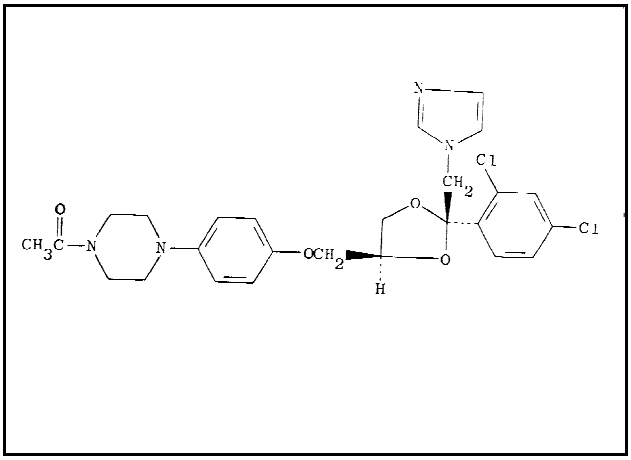
To make the spots visible in white light, dip the plate in a ninhydrin solution prepared by dissolving 100 mg of ninhydrin, 2,2-dihydroxy-1,3-indanedione (Sigma Chemical Co. No. N4876), in 25 mL of acetone. Place 7 mL of this solution in the detection bag instead of the iodine developing solution. The TLC plate should be dry and completely free of any ammonia (the plate may have to sit overnight). Dip the TLC plate in the ninhydrin solution. Allow the plate to dry for 60 min. A blue/violet color develops with kanamycin. Observe the size and intensity of the spots. If the TLC plate is not completely free of ammonia, the background color of the TLC plate changes from pale pink to dark rose in about 1 hr.

Notes:

The use of ninhydrin is recommended because the iodine intensities are variable and do not last very long. The ninhydrin color can be developed in less time if an oven or a hot plate is available. After the TLC plate air-dries for 30 min., the ammonia on the TLC plate can be removed by heating the plate at 100EC for 10 min. Dip the TLC plate in the ninhydrin solution and allow it to air-dry for 30 min. (The color usually develops in 30-60 min.) Heat the TLC plate for 2 min. at 100EC to fully develop the violet color. The TLC plate is pre-developed to remove the possibility of streaking or solvent front effects.

Ketoconazole
200 mg tablet

Structure:



Molecular Formula & Mass: $C_{26}H_{28}Cl_2N_4O_4$ - 531.44

Category: Antifungal

Sample:

Grind 1 tablet and dissolve in 50 mL of methanol. Concentration of the solution is 200mg/50 mL = 4 mg/mL. The solution concentration representing 100% is 1 mg/mL. Add 3 mL of methanol to 0 1 mL of the 4 mg/mL solution to make the final concentration of 1 mg/mL.

Standards:

High Standard:

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.6 mg of standard, dissolve it in: (4.6 mg)/(1.15 mg/mL) = 4 mL of methanol. This makes the high standard solution concentration equal to 1.15 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) x 0.85 = 0.85 mg/mL. Dilute 1 ml of high standard to 1.35 mL by adding 0.35 ml of methanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 10 mL of acetone, 9 mL of toluene, and 5 mL of methanol. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC bag.

Detection:

UV:

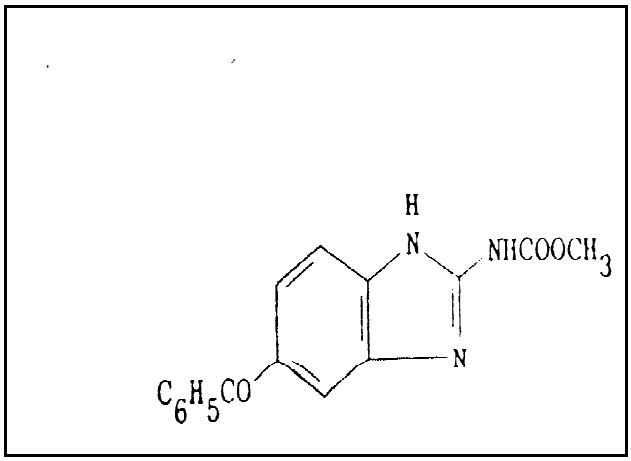
Dry the plate and observe under UV light. The maximum is at 225 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plates to dry and observe the size and intensity of the spots.

Mebendazole
100 mg tablet

Structure:



Molecular Formula & Mass: $C_{16}H_{13}N_3O_3$ - 295.30
Category: Anthelmintic
Sample:

Mebendazole is difficult to dissolve and requires the use of formic acid or acetic acid. Prepare a 1:1 solvent mixture of toluene and acetic acid by adding 30 mL of toluene to 30 mL of glacial acetic acid. Grind 1 tablet and suspend in 50 mL of the toluene-glacial acetic acid. Shake at least 2 min.; then heat in a 50EC water bath for 30 min. Shake at least 2 min., allow the solution to settle, and cool to room temperature. Concentration of the solution = 100 mg/50 mL = 2.0 mg/mL. The required concentration of sample solution representing 100% is 0.50 mg/mL. Add 3 mL of the toluene-glacial acetic acid to 1 mL of the 2.0 mg/mL solution to make the sample solution equal to 0.50 mg/mL.

Standard:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.5 mg of standard, dissolve it in: (4.5 mg)/(0.575 mg/mL) = 7.83 mL of the toluene-glacial acetic acid. This makes the high standard solution concentration equal to 0.575 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of the toluene-glacial acetic acid (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)

Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 18 mL of acetone and 6 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

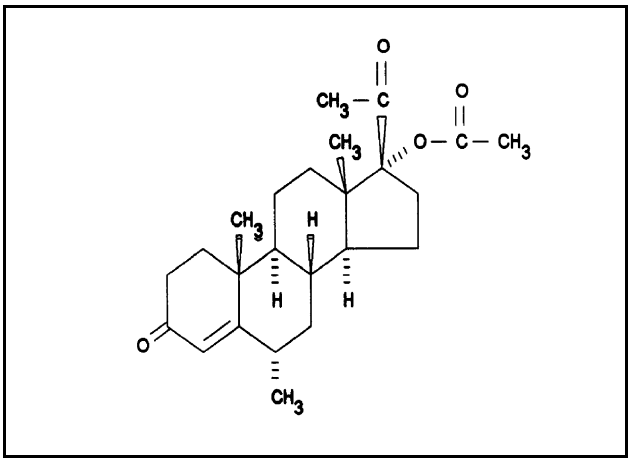
Dry the plate and observe under UV light. The maximum is at 245 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Medroxyprogesterone acetate
5 mg/mL inject.

Structure:



Molecular Formula & Mass: $C_{24}H_{34}O_4$ - 396.52
Category: Progesterone; with estradiol as oral contraceptive
Sample:

Dilute 1.0 mL to 10 mL by adding 9 mL of ethanol. Shake for 1 min. Concentration of solution = (5 mg/mL x 1 ml)/10 mL = 0.50 mg/mL. The required concentration of sample solution representing 100% is 0.50 mg/mL.

Standards:

High standard:

Since the sample concentration(100%) is 0.50 mg/mL and the high limit is 115%, the concentration of the high standard(115%) = 0.50 mg/mL X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg)/(0.575 mg/mL) = 8.26 mL of ethanol.
This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

Since the sample concentration (100%) is 0.50 mg/mL and the low limit is 85%, the concentration of the low standard (85%) = 0.50 mg/mL X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (0.575/0.425 = 1.35).

Spotting:

Spot on TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 16 mL of toluene and 8 mL of acetone. Add this mixture to the plastic TLC bag. Develop until the solvent front reaches to within 1 cm of the top of the TLC plate.

Detection:

UV:

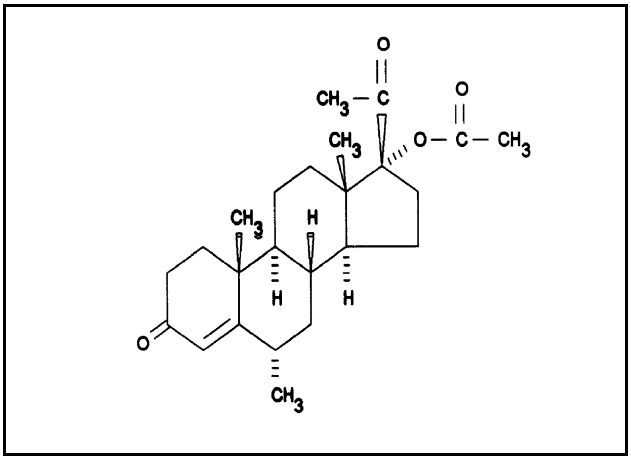
Dry the plate and observe under UV light. The maximum is at 241nm but a short wavelength UV(254 nm) light will work. Observe the size and intensity of the spots.

Iodine stain

Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the spot's size and intensity. This concentration is too low to allow quantitation by iodine. The neat solution of steroid can be used to spot a 5 mg/mL solution.

Medroxyprogesterone Acetate injection
100 mg/mL

Structure:



Molecular Formula & Mass: $C_{24}H_{34}O_4$ - 386
Category: Birth Control
Sample:

Dilute 1 ml of Medroxyprogesterone Acetate injection solution in 19 mL of methanol, making a concentration of 5 mg/mL. Concentration of sample solution for Iodine visualization is 5 mg/mL and represents 100%. For UV visualization, a concentration of 0.5 mg/mL is made by diluting 1 ml of the 5 mg/ml solution with 9 mL of Methanol.

Standards:

High standard:

Since the sample concentration (100%) is 0.50 mg/mL and the high limit is 115%, the concentration of the high standard = 0.50 mg/mL X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.6 mg of standard, dissolve it in: 4.6 mg / 0.575 mg/mL= 8.0 mL of methanol.This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

Since the sample concentration (100%) is 0.50 mg/mL and the low limit is 85%, the concentration of the low standard = 0.50 mg/mL X 0.85 = 0.425 mg/mL. Dilute 1 mL of the high standard to 1.35 mL by adding 0.35 mL of methanol, (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Developer:

Mix 18 mL of toluene and 6 mL of acetone. Add mixture to the plastic TLC bag.

Develop until solvent front reaches 1 cm from the top of the plate.

Detection:

UV:

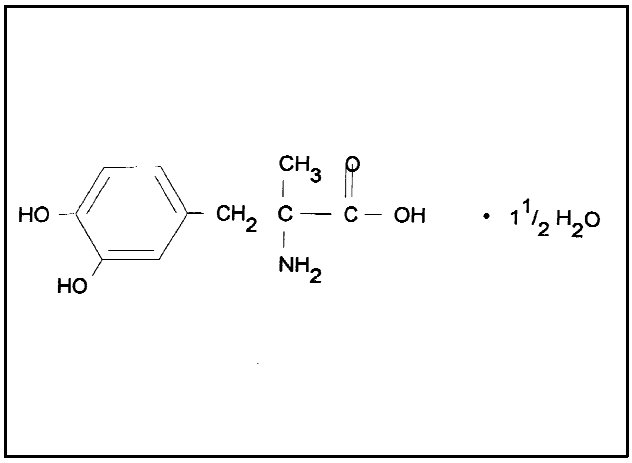
Observe spots under UV light for sample concentration = 5 mg/ml.

Iodine stain

Spots are well visualized in white light, if the sample concentration = 0.5 mg/ml. Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the spot's size and intensity.

Methyldopa
250 mg tablet

Structure:



Molecular Formula and Mass: $C_{10}H_{13}NO_4$ 211.21
Category: Antihypertensive
Sample:
Methyldopa must be dissolved in an acid media. Prepare the acid solution by mixing 98 mL of distilled water and 2 mL of concentrated hydrochloric acid. Use care when handling the strong acid because it is corrosive and will burn skin. Use rubber gloves and eye protection when handling the acid. The volume is required to prepare solutions Grind 1 tablet and dissolve in 50 mL of the acid solution. This volume of solvent produces a concentration of 5 mg/mL. The sample solution representing 100% is 5 mg/mL. No further dilution is necessary.
The drug also comes in 125 mg tablets. It is necessary to either use 2 tablets dissolved in 50 mL of the solvent or use 25 mL of the solvent to obtain the needed concentration of 5 mg/mL.
Standards:
<u>High standard:</u>
The high limit is 115%; therefore the concentration of the standard is(5 mg/mL) X 1.15 = 5.75 mg/mL. Weigh approximately 15 mg of the standard and add a volume of the acid solution to prepare the needed concentration. If you weighed 14.7 mg of the standard , then the volume of solvent needed would be (14.7 mg)/5.75 mg/mL = 2.55 mL of the acid solvent.
<u>Low standard:</u>
The low limit is equal to 85%; therefore the concentration of the low standard = (5 mg/mL) X 0.85. Add 0.35 mL of the acid solvent to 1 mL of the high concentration standard.l
Spotting:
Spot the solutions as follows:

Left spotlow standard (85%)
Center spot sample (100%)
Right spothigh standard (115%)

Developer:

Mix 25 mL of methanol and 0.4 mL of ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop the spots until the solvent front reaches within 1 cm from the top of the TLC plate.

Detection:

UV:

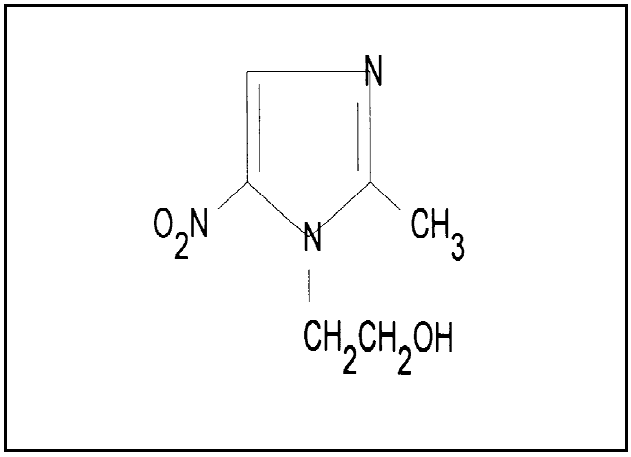
Allow the plates to dry and observe under UV light at 254 nm. Observe the size and the intensity of the spots.

Iodine stain:

Dip the plate into the solution of iodine-KI. Allow the plate to dry and observe the intensity of the spots as soon as they become clearly visible.

Metronidazole
250 and 500 mg tablets

Structure



Molecular formula & mass: $C_6H_9N_3O_3$ - 171.16
Category: Antiprotozoal (trichomonas)
Sample:
250 mg tablet:

Grind and dissolve 1 tablet in 50 mL water to prepare a solution having a concentration of 5 mg/mL. Dilute 1 mL of the 5mg/mL solution by adding 4 mL of water to the 1mL solution. The final concentration required is 1 mg/mL which represents 100%.

500 mg tablet:

Grind and dissolve 1 tablet in 50 mL of water making a solution having a concentration of 10 mg/mL. Dilute 1 mL of this solution by adding 9 mL of water.

Standard:
High standard

The high standard is 115%, the concentration of high standard is 1mg/mL X 1.15 = 1.15mg/mL. Weigh approximately 13mg of standard. If you weighed 13.15mg of standard, dissolve it in: (13.15mg)/(1.15mg/mL) = 11.4mL of water.

Low standard

The low limit is 85%, the concentration of low standard is 1mg/mL X 0.85 = 0.85mg/mL. Dilute 1mL of high standard to 1.35mL by adding 0.35mL of water (1.15/0.85=1.35).

Spotting:

Spot on TLC plate as follow:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 25mL methanol and 0.25mL ammonium hydroxide. Add 24 mL of this mixture to the plastic TLC bag. Develop until the solvent front reaches to within 1cm of the top of the TLC plate.

Detection:

UV;

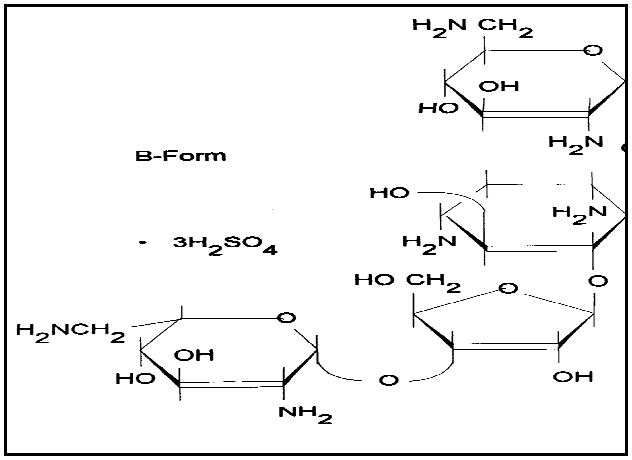
Dry the plate and observe under UV light. The maximum is at 305nm but at 254nm will work. Observe the size and intensity of the spots.

Iodine stain:

Dip plate in the iodine solution in the detection bag. Allow plate to dry and observe spot size and intensity.

Neomycin sulfate
500 mg tablets

Structure:



Molecular Formula & Mass: B & C; $C_{23}H_{46}N_6O_{13}$ - 614.67

Category: Antibacterial.

Neomycin is one of the aminoglycoside antibiotics and is composed of more than one component. Neomycin sulfate is a mixture of two components; neomycin B and C sulfates. The drug content is quoted as the basic drug, neomycin. The standard is in the form of the sulfate and has the potency quoted as units of neomycin activity. Thus the standards must be corrected for activity.

Sample:

Grind and dissolve the drug from 1 tablet in 50 mL of distilled water. Shake for 1 min. Concentration = 500 mg/50 mL = 10 mg/mL. Dilute the 10mg/mL solution to 5 mg/mL by adding 1 mL of distilled water to the 1 mL of the 10 mg/mL sample solution (10/2 = 5 mg/mL).

Standards:

<u>High standard:</u>

The high limit for antibiotics is 120%; therefore the concentration of the high standard = (5.0 mg/mL) X 1.20 = 6.0 mg/mL. Weigh approximately 35 mg of standard. If you weighed 34.9 mg of standard with an activity of 862, dissolve it in: (34.9 mg X 0.862)/(6.00 mg/mL) = 5.01 mL of distilled water. This makes the high standard solution concentration equal to 6.0 mg/mL.

<u>Low standard:</u>

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (5.0 mg/mL X 0.85) = 4.25 mg/mL. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of water (6.0/4.25 = 1.41).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (120%)

Development:

Mix 15 mL of methanol, 15 mL of acetone, 7.5 mL of distilled water, and 7.5 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop a blank TLC plate until the solvent front reaches 1 cm from the top of the plate. Remove the plate and allow it to dry until the odor of ammonia can no longer be detected. Remove the solvent from the developing bag. Prepare another mixture of the same solvent, and add 22 mL of the mixture to the TLC development bag. Develop the plate until the solvent front reaches 1 cm from the top of the plate. Allow the plate to dry until the odor of ammonia cannot be detected.

Detection:

UV:

The spots are not visible in the UV.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the spots to dry and determine their size and intensity.

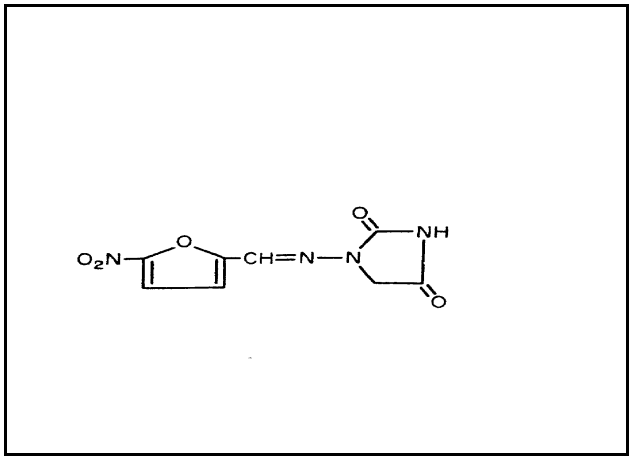
Visible:

To make the spots visible in white light, dip the plate in a ninhydrin solution, prepared by dissolving 100 mg of ninhydrin, 2,2-dihydroxy-1,3-indanedione (Sigma Chemical Co. No. N4876), in 25 mL of acetone. Place 7 mL of this solution in the detection bag instead of the iodine-KI developing solution. The TLC plate should be dry and completely free of any ammonia (this may require allowing the plate to sit overnight). Dip the TLC plate in the ninhydrin solution. Allow the plate to dry for at least 60 min. A blue-violet color develops with neomycin. Observe the size and intensity of the spots. If the TLC plate is not completely free of ammonia, the background color of the plate will change from pale pink to dark rose in about 1 hr. **Notes:**

The use of ninhydrin is recommended because the iodine intensities are variable and do not last very long. The ninhydrin color can be developed in less time if an oven or a hot plate is available. After the TLC plate air-dries for 30 min., the ammonia on the TLC plate can be removed by heating the plate at 100EC for 10 min. Dip the TLC plate in the ninhydrin solution and allow it to air dry at least 30 min. (The color usually develops in 30-60 min.) Heat the TLC plate at least 2 min. at 100EC to fully develop the violet color. The TLC plate is pre-developed to remove the possibility of streaking or solvent front effects.

Nitrofurantoin
25 mg capsule

Structure:



Molecular Formula & Mass: $C_8H_6N_4O_5$ - 238.16

Category: Antibacterial (urinary)

Sample:

Dissolve the contents of the capsule in 50 mL of acetone. Shake at least 2 min. and then heat in a 50EC water bath for 30 min. Shake at least 2 min., allow the solution to settle, and cool to room temperature. Concentration of the solution = 25 mg/50 mL = 0.50 mg/mL. The required concentration of the sample solution representing 100% is 0.50 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.5 mg of standard, dissolve it in: 4.5 mg/0.575 mg/mL = 7.83 mL of acetone. This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL x 0.85) = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of acetone (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 16 mL of acetone and 8 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

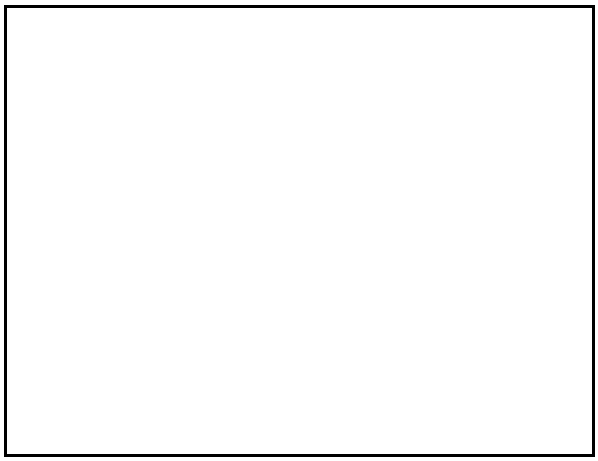
Dry the plate and observe under UV light. The maximum is at 360 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Norgestrel
0.5 mg tablet

Structure:



Molecular Formula & Mass: $C_{21}H_{28}O_2$ - 312.44

Category: Oral progesterone; with estradiol as oral contraceptive

Sample:

Grind 10 tablets and dissolve in 10 mL of anhydrous ethanol. Shake at least 5 min. Concentration of the solution = (10 x 0.5 mg)/10 mL = 0.50 mg/mL. The required concentration of the sample solution representing 100% is 0.50 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg)/(0.575 mg/mL) = 8.26 mL of anhydrous ethanol. This makes the high standard solution concentration equal to 0.575 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 16 mL of toluene and 8 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

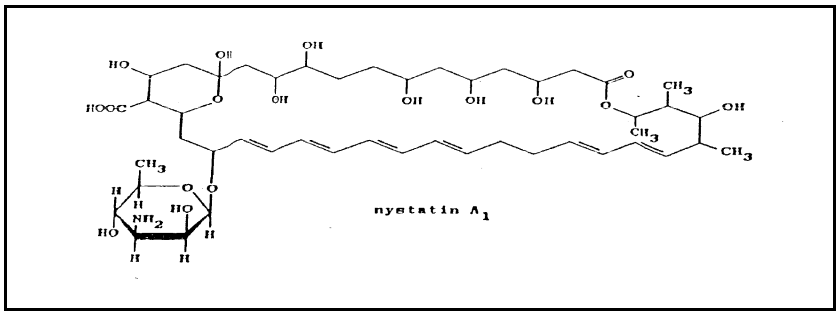
Dry the plate and observe under UV light. The maximum is at 241 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

This concentration is too low to allow quantitation by iodine-KI solution. A higher concentration of steroid can be prepared by using 1 mL of solvent and filtering the solution through glass wool in a small funnel. However, the ethanol tends to evaporate during this process and give high results.

Nystatin
100,000 units per gram powder

Structure



Molecular formula & mass: $C_{47}H_{75}NO_{17}$ - 926.22
Category: Antifungal
Sample:

The sample is in the form of a powder. To prepare a solution having a concentration equal to 2 mg/mL, weigh approximately 10 mg on an analytical balance, and add 5 mL of anhydrous ethanol.

Standard:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (2 mg/mL) X 1.15 = 2.3 mg/mL. Weigh approximately 11 mg of standard. If you weighed 10.9 mg of standard, dissolve it in: (10.9 mg)/(2.3 mg/mL) = 4.74 mL of anhydrous ethanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (2 mg/mL) X 0.85 = 1.7 mg/mL. Dilute 1 mL of the high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follow:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 1.5 mL of acetone, 7.5 mL of toluene, 15 mL of methanol, and 1.5 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

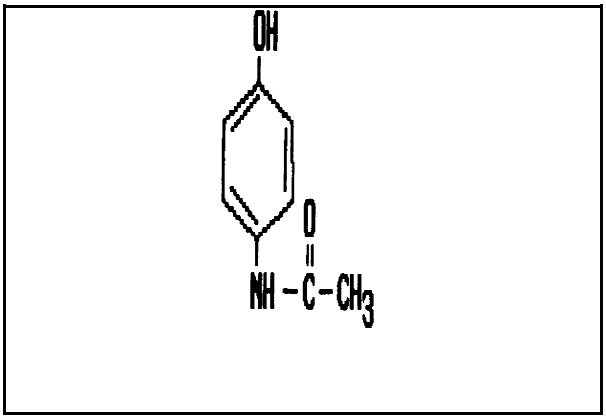
Dry the plate and observe under UV light. The maximum is at 300 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Paracetamol
(Acetaminophen)
325 mg tablet

Structure:



Molecular Formula & Mass: $C_8H_9O_2N$ - 151.16
Category: Analgesic, antipyretic
Sample:

Grind 1 tablet and dissolve in 50 mL of 95% ethanol. Shake at least 1 min. Concentration of the solution = 325 mg/50 mL = 6.5 mg/mL. The required concentration of the sample solution representing 100% is 5 mg/mL. To prepare the 5 mg/mL solution, dilute 1 mL of the 6.5 mg/mL solution with 0.3 mL of 95% ethanol. This solution is required for iodine staining. Paracetamol may be supplied in different contents. In this case, add 50 mL of the solvent to the ground tablet to make a master solution, and determine the concentration. Dilute an aliquot of the master solution to prepare a solution having a concentration equal to 5 mg/mL.

Reference solutions:

(a) When reference drug is available in tablet form.

<u>High reference solution:</u>

The high limit for the reference is equal to 115%; therefore the concentration of the high reference solution = (5 mg/mL) X 1.15 = 5.75 mg/mL. Add 95% ethanol to 1 tablet of the reference drug. To determine the volume of alcohol needed, divide the drug content of the reference tablet in mg by 5.75 mg/mL. If the reference tablet contains 46 mg: (46 mg/5.75 mg/mL) = 8 mL of 95% ethanol.

<u>Low reference solution:</u>

The low limit of the reference is 85%; therefore the concentration of the low reference solution = (5 mg/mL) X 0.85 = 4.25 mg/mL. Dilute 1 mL of the high reference solution to 1.35 mL by adding 0.35 mL of 95% ethanol (5.75/4.25 = 1.35).

(b) When no reference tablets are available.

When reference tablets are not available, a reference material must be weighed. The

reference material may be either a primary or a secondary standard. Weigh approximately 25 mg of the standard on an analytical balance. Divide the weight (mg) by 5.75 mg/mL to obtain the volume needed to make the proper concentration for the high solution.

The low concentration standard solution is prepared by diluting the high concentration reference as above.

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 24 mL of ethyl acetate, 3 mL of methanol, and 1 ml of concentrated ammonium hydroxide. Add 24m of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC sheet.

Detection:

Dry the TLC sheet until no odor can be detected. Dip the sheet into the iodine-KI solution. Allow the iodine to evaporate, and observe the difference between the intensities of the spots.

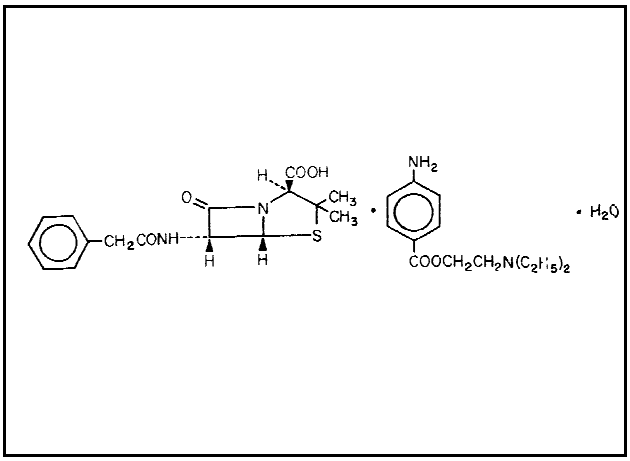
Note:

Follow this procedure for detection by iodine staining. Many drugs can be detected by UV light, and the concentrations may be different from the concentration needed for the iodine. Paracetamol can be detected by UV at a concentration of 1 mg/mL. To prepare the sample and reference solutions, dilute the above solutions by adding 4 mL of the solvent to 1 mL of the concentrated solutions.

Detection by UV is the simplest method and should be used whenever a UV source is available.

Penicillin G/Procaine
Suspension (injectable)
3000000 units/mL

Structure:



Molecular Formula and Mass: Penicillin G = $C_{16}H_{17}KN_2O_4S$ -372.47
Procaine = $C_{13}H_{20}N_2O_2$ - 236.30

Sample:

The drug is supplied as a suspension with 3000000 units in 10 mL of solutions, which equals 300000 units in a mL. The standards require 1000 units per mg; therefore 1 mL is equivalent to 300 mg of the active drug (the drug content is for the penicillin G based upon the free acid). Shake the suspension to obtain an uniform distribution. Remove 1 mL of the suspension and add 50 mL of methanol to make a solution concentration equivalent to 6 mg/mL. Add 0.5 mL of methanol to 1 mL of the 6 mg/mL solution to prepare a solution of 4.0 mg/mL representing 100%.

The procaine content may or may not be given. Many times the content of procaine will be approximately the same as the penicillin-G. The required concentration representing 100% is equal to 2.5 mg/mL**.**

Standards:

<u>High Standard:</u>

Weigh approximately 25 mg of the penicillin-G as the potassium salt. The drug content for this type antibiotic is based upon the free acid, so a correction must be made when the potassium salt is used. The molecular weight of the salt is 372.48 and the free acid is 334.47. The corrected weight of the active drug is the weight of the sample multiplied by 334.48 / 372.48; for example you weighed (24.6 mg X(334.48 / 372.48) = 22.09 mg is the actual weight of the free base.. The high limit is 120%; therefore the concentration of the high standard = 1.2 X 4 mg/mL = 4.8 mg/mL. The volume of solvent

needed will equal weight of the drug divided by the high concentration which in this case is 22.09 mg / (4.8 mg/mL) = 4.6 mL of methanol.

Low standard:

The low limit is 85%; therefore the concetration of the low standard = 0.85 X 4.0 mg/mL = 3.4 mg/mL. Add 0.41 mL of methanol to 1 mL of the high concentration standard (4.8 mg/mL).

Spotting:

Spot the solutions as follows:

Left spot low standard(85%)
Center spot sample(100%)
Right spot high standard(120%)

Development:

Mix 26 mL acetone, 4 mL toluene, 4 mL water, and 1 mL ammonium hydroxide. Pour 24 mL of this solution into the TLC development bag. Develop until the solvent front reaches to within 1 cm from the top of the TLC plate.

Detection:

UV:

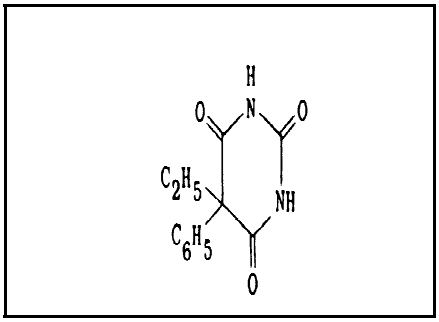
Dry the plate and observe under UV light at 254nm. Since this drug formulation has 2 compounds (penicilling-G and procaine), there will be 2 positions on the plate. The spots in the lower position are penicilline-G and the upper spots are procaine. Observe the size and the intensity of the spots. Concentrations may require adjustment for analysis of both drugs at the same time.

Iodine stain:

Dip the plate into the iodine-KI solution. Allow plate to dry and observe the spots' size and intensity.

Phenobarbital
15 mg tablet

Structure:



Molecular Formula & Mass: $C_{12}H_{12}N_2O_3$ - 232.23
Category: Anticonvulsant, hypnotic, sedative
Sample:

The sample contains 15 mg of the active drug, and a final solution concentration of 10 mg/mL is required to represent 100%. Grind 4 tablets and dissolve in 6 mL of anhydrous ethanol. Because of the high concentration needed for suitable visualization, it is necessary to use multiple tablets and a volume of liquid large enough to obtain a suitable sample. Shake at least 2-3 min.

Reference Materials:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (10 mg/mL) X 1.15 = 11.5 mg/mL. Weigh approximately 50 mg of standard. If you weighed 46 mg of standard, dissolve it in: (46 mg)/(11.5 mg/mL) = 4 mL of anhydrous ethanol. This makes the high standard solution concentration equal to 11.5 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (10 mg/mL) X 0.85 = 8.5 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (11.5/8.5 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 12 mL of acetone and 12 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

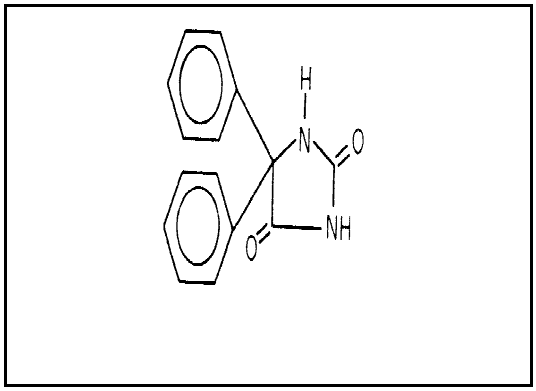
Dry the plate and observe under UV light. The maximum is at 250 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Phenytoin
100 mg capsules

Structure



Molecular Formula & Mass: $C_{15}H_{12}N_2O_2$ - 252.26
Category: Anticonvulsant, antiepileptic
Sample:

Dissolve the contents of 1 capsule in 10 mL of anhydrous ethanol. The required concentration of the sample solution representing 100% is 10 mg/mL.

Standard:

High standard:

The high limit is 115%; therefore the concentration of high standard = (10 mg/mL) X 1.15 = 11.5 mg/mL. Weigh approximately 41 mg of standard. If you weighed 41 mg of standard, dissolve it in: (41 mg)/(11.5 mg/mL) = 3.6 mL of anhydrous ethanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (10 mg/mL) X 0.85 = 8.5 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (11.5/8.5 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (1.15%)

Development:

Mix 15 mL of acetone and 10 mL of toluene. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 260 nm but a short

wavelength light (254 nm) will work. Observe the size and intensity of the spots.

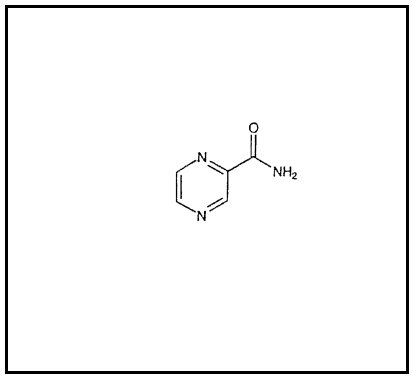
Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Pyrazinamide

400 mg tablet

Structure:



Molecular formula & mass: $C_5H_5ON_3$--123.11

Category: Antibacterial (tuberculostatic)

Preparation of the sample solution:

Analytical balance available.

Prepare the sample solution by weighing an aliquot of the drug. Follow the procedure described in the previous sections. Determine the weight of the drug and add the volume of solvent to produce a concentration such of 1mg/mL. The volumes must be measured accurately using a combination of pipetts plus a 1 mL graduated tuberculin syringe for the fractional volumes. Pipetts are available in 1mL increments up to 10 mL. For example: You weighed 10.5 mg of the drug, then you would add 10.5mL of solvent to prepare a solution with a concentration of 1.0 mg/mL. (use a 10 mL and a 1mL pipetts and the 0.5 mL is measured by a 1mL graduated syringe).

Analytical balance not available.

Grind 1 tablet to a fine powder in a small polyethylene bag and insert the bag and contents into a suitable vessel and add 50 mL of methanol. Shake vigorously for at least 1 minute to dissolve the powder. The concentration of this solution is = 400/50= 8 mg/mL. The required concentration for the sample solution representing 100 % solution is 1 mg/mL. The concentrated solution must be further diluted. Take 1 mL of the 8 mg/mL solution and add 7 mL of methanol which will prepare the concentration of 1 mg/mL. The volume of solvent needed must be adjusted to produce the proper concentration.

Preparation of standard solutions:

Reference solutions are prepared depending on the availability of reference compounds. The reference materials may be either in the form of reference tablets or powders of primary/secondary standards. Reference tablets may be available containing a predetermined weight of the drug which when dissolved in 5 mL of the solvent produces a solution concentration

representing 115% of the sample solution. No weighing is required.

Weighing is required when the reference compound is not available in tablet form. The reference solutions then must be prepared using either primary or secondary standards.

Reference solutions are prepared depending on the availability of reference compounds. Reference tablets are available in many cases. These tablets contain a predetermined weight of the drug which when dissolved in 5 mL of the solvent will produce the high reference solution concentration representing 115% of the sample solution. No weighing is required.

Weighing is required when the reference compound is not available in tablet form. The reference solutions then must be prepared using either primary or secondary standards.

Preparation of the High Standard:

1. Reference tablet available.

The reference tablet for pyrazinamide contains 5.75 mg. Add one reference tablet to a vessel and add 5 mL of methanol to prepare a solution having a concentration of 5.75mg/5mL equal to 1.15 mg/mL. This represents 115% of the sample concentration.

2. Reference material available as a powder.

Weigh approximately 10 mg of the standard. For example you weighed 9.7 mg, then the volume of solvent added would be 9.7 mg/1.15 mg/mL = 8.43 mL of methanol. This will make a final concentration of 1.15 mg/mL which will represent the 115% solution.

Preparation of the low standard:

Dilute 1 mL of the high standard to 1.35 mL by adding 0.35 mL of methanol.(1.15/0.85) = 1.35 which is the high concentration divided by the low concentration).

Spotting:

Spot on the TLC plate as follows:

Sample each of the solutions with a 3µL pipette and spot.

Left spot	low standard (85%)
Center spot	Sample (100%)
Right spot	high standard (115%)

Development:

Mix 13 mL of methanol, 17 mL of acetone and 1 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

Detection:

UV:

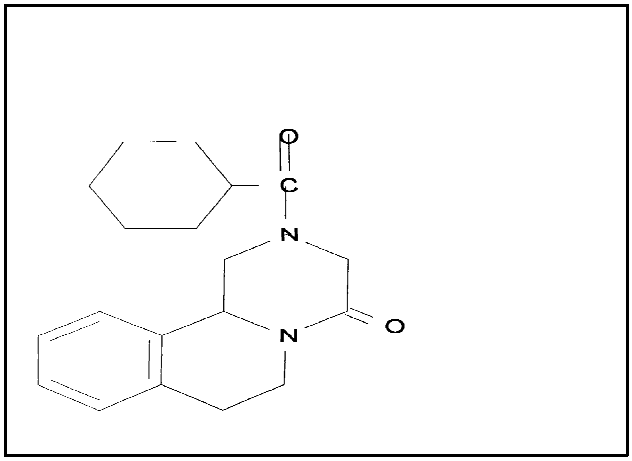
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots or stain with iodine when no UV available.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and

Praziquantel
600 mg tablet

Structure:



Molecular Formula & Mass: C19H24N2O2 - 312.41
Category: Anthelmintic
Sample:

Weigh 1 tablet. Break the weighed tablet into quarters by pressing on the scored marks with the thumbnail. Weigh one quarter of the tablet and calculate the quantity of praziquantel contained in the quartered sample weight as follows:
(600 mg x mass of tablet portion)/mass of whole tablet = mg of praziquantel.
Example: (600 mg x 0.2256 g)/0.9262 g = 160. mg of praziquantel when the whole tablet weighs 0.9262 mg and the fraction of the tablet weighs 0.2256 mg. Grind and suspend the weighed tablet in 50 mL of methanol. Shake at least 2 min.; then heat in a 50EC water bath for 30 min. Shake at least 2 min., allow the solution to settle, and cool to room temperature. Dilute 1 mL of this solution to a concentration of 1.0 mg/mL by using the following formula: (no. of mg of praziquantel) X 50 = 3.2 mL. Therefore, to prepare a 1.0 mg/mL solution add 2.2 mL of methanol to 1 mL of the sample solution: 160/(50 X 3.2) = 1.0 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (1.0 x 1.15) = 1.15 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg)/(1.15 mg/mL) = 4.13 mL of methanol. This makes the high standard solution concentration equal to 1.15 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1.0 x 0.85) = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 16 mL of acetone and 8 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

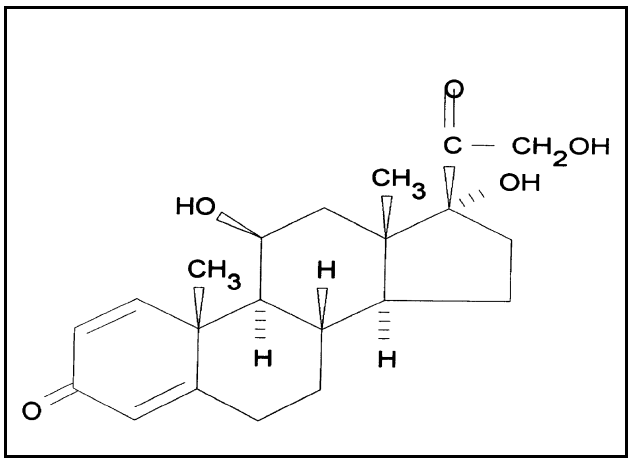
The UV maximum is at 205-210 nm; thus a short wavelength UV light (254 nm) will not detect praziquantel.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Prednisolone, 98%
5 mg tablet

Structure:



Molecular Formula & Mass $C_{21}H_{28}O_5$ (anhydrous) - 360.45
sesquihydrate - 387.48

Category: Glucocorticoid, adrenocortical steroid, anti- inflammatory

Sample:

Grind 1 tablet and dissolve in 5 mL of methanol. Shake at least 1 min. Concentration of this solution is equivalent to 1 mg/mL. This concentration will represent 100% of the sample.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.6 mg of standard, dissolve it in: (4.6 mg)/(1.15 mg/mL) = 4 mL of methanol.
This makes the high standard solution concentration equal to 1.15 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 ml of high standard to 1.35 mL by adding 0.35 ml of methanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 22.5 mL of acetone, 22.5 mL of toluene, and 1 mL of methanol. Add 24 mL of

this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC bag.

Detection:

UV:

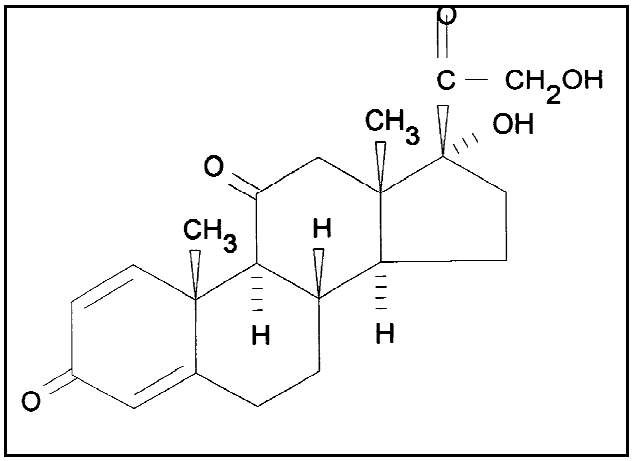
Dry the plate and observe under UV light. The maximum is at 250 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detection bag. Allow the plates to dry and observe the size and intensity of the spots.

Prednisone
10 mg tablet

Structure:



Molecular Formula & Mass: $C_{21}H_{26}O_5$ - 358.44
Category: Glucocorticoid, adrenocortical steroid
Sample:

Grind 1 tablet and dissolve in 20 mL of 95% ethanol. Shake at least 1 min. Concentration of the solution = 10 mg/20 mL = 0.50 mg/mL. The required concentration of the sample solution representing 100% is 0.5 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: 4.75 mg/0.575 mg/mL = 8.26 mL of anhydrous ethanol. This makes the high standard solution concentration equal to 0.575 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 16 mL of toluene and 8 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

<u>UV</u>:

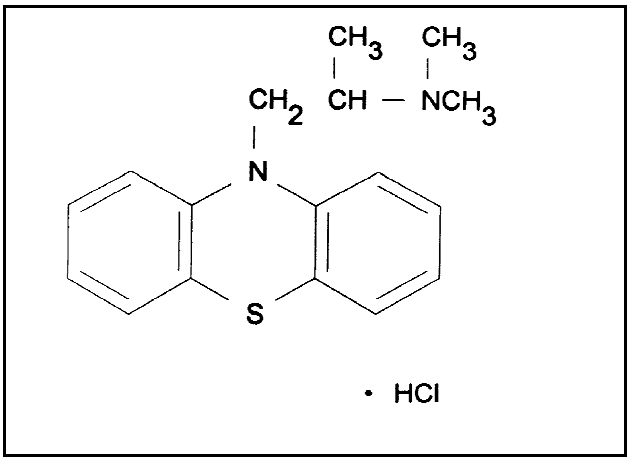
Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

The 0.50 mg/mL concentration is too low to allow for quantitation by iodine. The concentration needs to be 5 mg/mL. This can be accomplished by using 10 mL of anhydrous ethanol rather than 20 mL. Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Promethazine
25 mg tablet

Structure:



Molecular Formula & Mass: $C_{17}H_{20}N_2S$ - 284.41
Category: Antihistaminic
Sample:

Grind1 tablet and dissolve in 25 mL of anhydrous ethanol. Shake at least 1 min. Concentration of the solution = 25 mg/25 mL = 1.0 mg/mL. The required concentration of sample solution representing 100% is 0.25 mg/mL. Dilute 1 mL of the 1mg/mL sample solution to 4 mL by adding 3 mL of ethanol to obtain a 0.25 mg/mL sample solution(1/4 = 0.25 mg/mL).

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.25 mg/mL) x 1.15 = 0.288 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: (4.75 mg) /(0.288 mg/mL) = 16.5 mL of anhydrous ethanol. This makes the high standard solution concentration equal to 0.288 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.25 mg/mL) x 0.85 = 0.21 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (0.288/0.21 = 1.35).

Spotting:

Spot on TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 20 mL of acetone and 4 mL of water. Add this mixture to the TLC development

bag. Develop until the solvent front reaches to within 1 cm of the top of the TLC plate.

Detection:

UV:

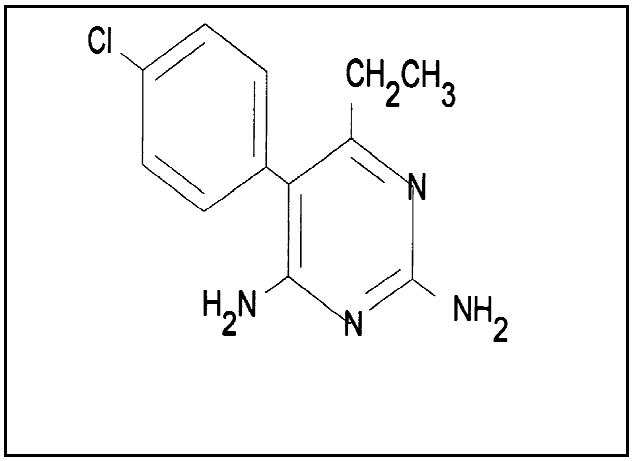
Dry the plate and observe under UV light. The maximum is at 250 nm but a short wavelength UV light(254 nm) will work. Observe the size and intensity of the spots.

Iodine stain

The 0.25 mg/mL concentration is too low to allow for quantitation by iodine. The concentration needs to be 1 mg/mL. This can be accomplished by using the undiluted sample solution. Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Pyrimethamine
25 mg tablet

Structure:



Molecular Formula & Mass: $C_{12}H_{13}ClN_4$ - 248.71
Category: Antimalarial
Sample:

Grind 1 tablet and dissolve in 12.5 mL of 95% ethanol. Concentration of the solution = 2 mg/mL. This concentration represents 100% of the sample.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (2 mg/mL) X 1.15 = 2.3 mg/mL. Weigh approximately 25 mg of standard. If you weighed 23 mg of standard, dissolve it in: (23 mg/2.3 mg/mL) = 10 mL of 95% ethanol. This makes the high standard solution concentration equal to 2.3 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (2 mg/mL) X 0.85 = 1.70 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of 95% ethanol (2.3/1.70 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 11 mL of acetone, 11 mL of toluene, and 2.5 mL of methanol. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

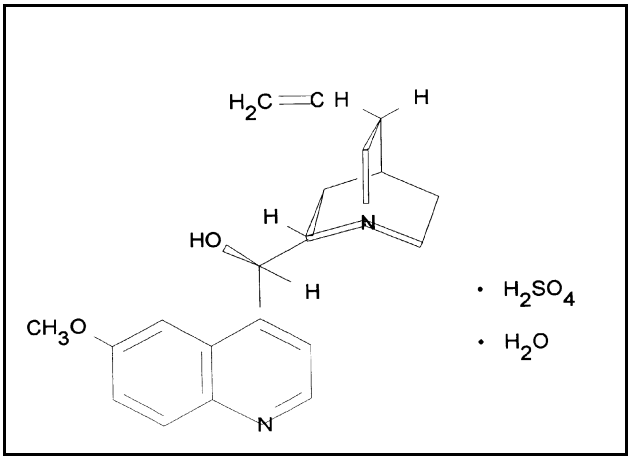
Dry the plate and observe under UV light. The maximum is at 280 nm but a short wavelength UV light (254) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Quinine sulfate
250 mg tablet

Structure:



Molecular Formula & Mass: $2C_{20}H_{24}N_2O_2.H_2SO_4.2H_2O$ - 782.97
Category: Antimalarial
Sample:

Grind 1 tablet and dissolve in 50 mL of distilled water. Shake at least 1 min. Concentration of the solution = 250 mg/50 mL = 5.0 mg/mL. The required concentration of sample solution representing 100% is 0.50 mg/mL. Add 9 mL of distilled water to 1 mL of the 5.0 mg/mL solution to make the sample solution equal to 0.50 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.7 mg of standard, dissolve it in: (4.7 mg/0.575 mg/mL) = 8.17 mL of distilled water. This makes the high standard solution concentration equal to 0.575 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of distilled water (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 21 mL of anhydrous ethanol, 3 mL of water, and 1 mL of concentrated

ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 235 nm but a short wavelength UV light (254 nm) will work.

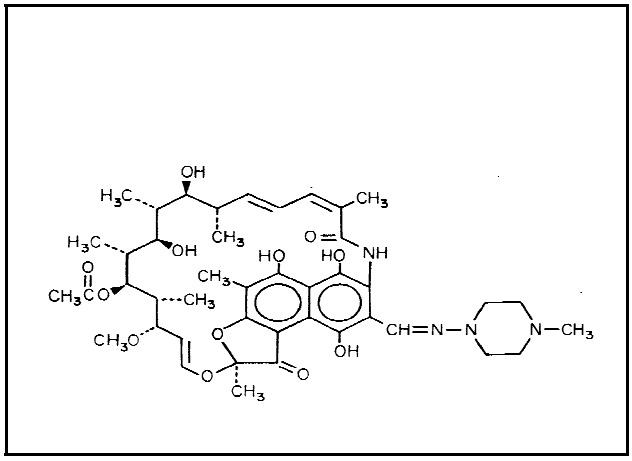
Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Method #1
Rifampicin
150 mg capsules

Structure:



Molecular Formula & Mass: $C_{48}H_{58}N_4O_{12}$ - 822.96
Category: Antibacterial, Antitubercular
Sample:

Dissolve the contents of 1 capsule in 50 mL of methanol. Shake at least 1 min. Concentration of the solution = 150 mg/50 mL = 3.0 mg/mL. The required concentration of sample solution representing 100% is 1.0 mg/mL. Add 2 mL of methanol to 1 mL of the 3.0 mg/mL solution to make the sample solution equal to 1.0 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (1.0 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 7-8 mg of standard. If you weighed 7.5 mg of standard, dissolve it in: (7.5 mg/1.15 mg/mL) = 6.52 mL of methanol. This makes the high standard solution concentration equal to 1.15 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1.0 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 21 mL of methanol, 2 mL of toluene, and 2 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

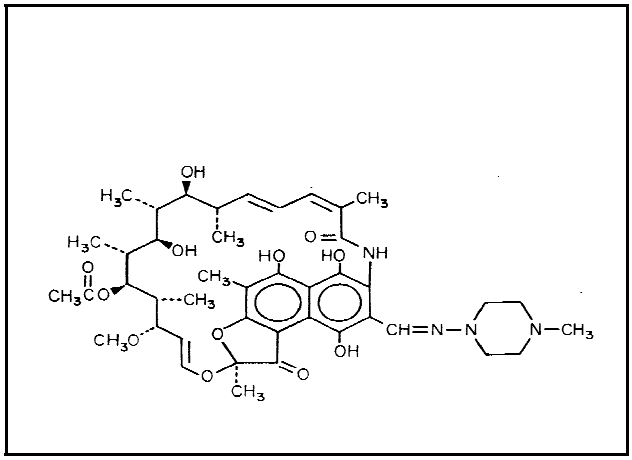
Visible:

Dry the plate and observe under white light. The maximum is at 445 nm. Observe the size and intensity of the spots.

METHOD #2

Rifampicin
150 mg capsules

Structure:



Molecular Formula & Mass: $C_{43}H_{58}N_4O_{12}$ - 822.96
Category: Antibacterial, (tuberculostatic)

Preparation of the sample solution:

The dosage forms of rifampin are supplied in many different quantities of the drug. The procedure described here is to serve as representative. Weights and volumes need to be adjusted for other dosages. Solutions are prepared by two different methods.
Analytical balance available.

Prepare the sample solution by weighing an aliquot of the drug. Follow the procedure described in the previous sections. Determine the weight of the drug in the aliquot and add solvent to produce a concentration such as 1mg/mL. The volumes must be measured accurately by using a combination of pipetts plus a 1 mL graduated tuberculin syringe for the fractional volumes. Pipetts are available in 1mL increments up to 10 mL. For example: You weighed 10.5 mg of the drug, then you would add 10.5mL of solvent to prepare a solution with a concentration of 1.0 mg/mL. (use a 10 mL pipette and the 0.5 mL is measured by a 1mL graduated tuberculin syringe).

Analytical balance not available.

Grind 1 tablet to a fine powder in a small polyethylene bag and insert the bag and contents into a suitable vessel and add 25 mL of methanol. Shake vigorously for at least 1 minute to dissolve the powder. The concentration of this solution is = 150/25= 6 mg/mL. The required concentration for the sample solution representing 100 % solution is 1 mg/mL. The concentrated solution must be further diluted. Take 1 mL of the 6 mg/mL solution and add 5 mL of methanol to prepare the proper concentration of 1 mg/mL.

Preparation of standard solutions:

Preparation of standard solutions:

Reference solutions are prepared depending on the availability of reference compounds. The reference materials may be either in the form of reference tablets or powders of primary/secondary standards. Reference tablets may be available containing a predetermined weight of the drug which when dissolved in 5 mL of the solvent produces a solution concentration representing 115% of the sample solution. No weighing is required. Weighing is required when the reference compound is not available in tablet form. The reference solutions then must be prepared using either primary or secondary standards.

Preparation of the High Standard:

1. Reference tablet available.

The reference tablet for rifampin contains 5.75 mg. Add one reference tablet to a vessel and add 5 mL of methanol to prepare a solution having a concentration of 5.75mg/5mL equal to 1.15 mg/mL. This represents 115% of the sample concentration.

2. Reference material available as a powder.

Weigh approximately 10 mg of the standard. For example you weighed 9.7 mg, then the volume of solvent added would be 9.7 mg/1.15 mg/mL = 8.43 mL of methanol. This will make a final concentration of 1.15 mg/mL which will represent the 115% solution.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (1.0 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Sample each of the solutions with a 3µL capillary pipette ans spot.

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 13 mL of methanol, 17 mL of acetone and 1 mL of concentrated ammonium hydroxide. Add 24 mL of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

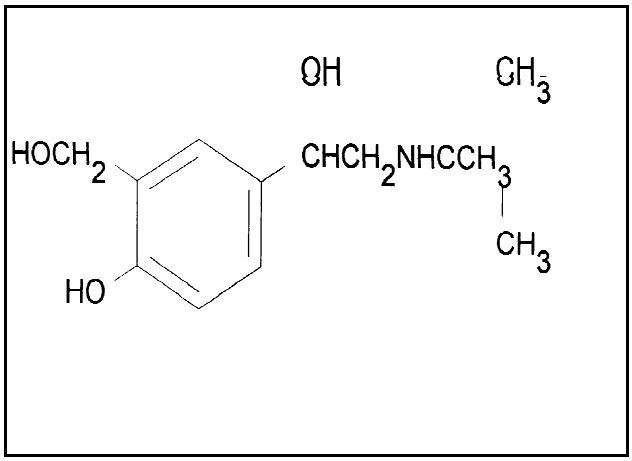
Detection:

Visible and UV:

Dry the plate and observe under white light. The maximum is at 445 nm. Observe the size and intensity of the spots. Rifampin has a strong absorption in the UV at 254 nm allowing excellent detection. The spots are better observed by UV when the stray lights of the room are eliminated.

Salbutamol (albuterol)
4 mg tablet

Structure:



Molecular Formula and Mass:$C_{13}H_{21}NO_3$ - 239.31
Category: Bronchodilator
Sample:

This drug contains a small quantity of the active drug, and must be dissolved in a small volume of solvent. Grind 1 tablet and dissolve in 4 mL of anhydrous ethanol. Shake at least 2 min. No further dilution is necessary. Concentration of the solution is equivalent to 1 mg/mL. The required concentration of sample solution representing 100% is 1 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of the standard. If you weighed 5.24 mg of the standard, dissolve it in: (5.24 mg/1.15 mg/mL) = 4.55 mL of anhydrous ethanol.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL X 0.85) = 0.85 mg/mL. Dilute 1 mL of the 1.15 mg/mL solution to 1.35 mL by adding 0.35 mL of anhydrous ethanol (1.15/0.85 = 1.35).

Spotting;

Spot on the TLC plate as follows:

Left spot	high standard (115%)
Center spot	100% sample
Right spot	low standard (85%)

Development:

Mix 50 mL of methanol and 0.75 mL of ammonium hydroxide. Add 24 mL of this solution to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

Detection:

UV

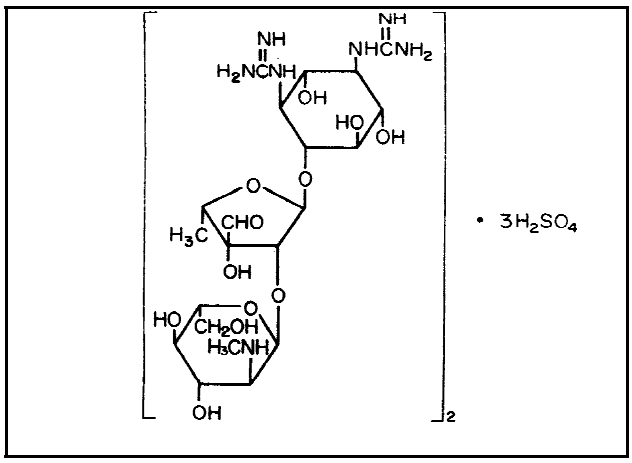
Allow the plate to dry. Observe under the ultraviolet at 254 nm. Visually examine the intensities and sizes of the spots.

Iodine stain

Dip the plate into the iodine solution. Remove from the solution and allow the iodine to disappear. Observe the spots as soon as the spots become clearly visible.

Streptomycin sulfate
200 mg/mL injectable

Structure:



Molecular Formula & Mass: $(C_{21}H_{39}N_7O_{12})_2.3\ H_2SO_4$ - 1457.38
Category: Antibacterial (tuberculostatic)
Sample:
NOTE: Streptomycin must be handled differently from the other TB drugs. The solvents , developer, and detection are changed.

Streptomycin is an aminoglycoside antibiotic. The standard is in the form of the sulfate and the potency is quoted as units of streptomycin activity. Thus the standards must be corrected for activity. The drug is supplied as a 1 gram of the free base in a vial which is reconstituted by adding 5 mL of water to make a solution having a concentration of 200 mg/mL.

Dilute 1 mL of the injectable solution to 40 mL by adding 39 mL of distilled water. Shake at least 1 min. The sample concentration = (1 mL x 200 mg/mL)/40 mL = 5 mg/mL.

Standard:

High standard:

The drug is normally supplied as the sulfate, but the content of the drug is based upon the free base which requires a correction to the weighed quantity. The mass of the sulfate is 1457.38, and the mass of the free base is 1163.14. The correction is the weight of standard multiplied by 1163.14/1457.38 = 0.798

The high limit for antibiotics is 120%; therefore the concentration of high standard = (5 mg/mL) X 1.20 = 6.0 mg/mL. Weigh approximately 30 mg of sulfate standard. If you weighed 29.8 mg of sulfate standard with an activity of 766 µg per mg, dissolve it in: (29.8 mg X 0.798)/6.0 mg/mL = 3.96 mL of distilled water. This makes the high standard solution concentration equal to 6 mg/mL.

Low standard:

The low limit for antibiotics is 85%; therefore the concentration of the low standard = (5 mg/mL) X 0.85 = 4.25 mg/mL. The concentration ratio between the high and low standard is 6/4.25 =1.41. Dilute 1 mL of high standard to 1.41 mL by adding 0.41 mL of water.

Spotting:

Spot on the plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (120%)

Development:

Mix 4 mL of concentrated ammonium hydroxide and 4 mL of distilled water in a container fitted with a stopper. Carefully add 12 mL of glacial acetic acid. The solution will become very hot and generate ammonia gas. Quickly stopper the container, shake well to dissolve the ammonia gas, and cool to room temperature. Add 12 mL of ethyl acetate to the cooled mixture. Add 24 mL of this mixture to the TLC development bag. Develop the plate until the solvent front reaches 1 cm from the top of the plate. Allow the plate to dry until the odor of acetic acid cannot be detected.

Detection:

UV:

The spots are not visible in the UV.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and determine the size and intensity of the spots.

Streptomycin is an aminoglycoside which can also be stained with ninhydrin.

Ninhydrin solution:

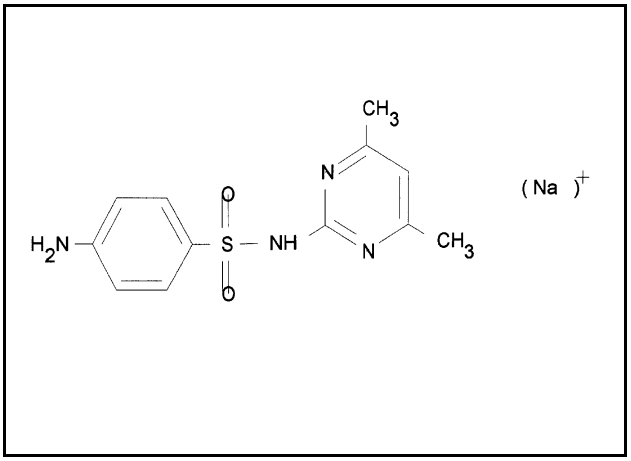
The ninhydrin solution is needed to stain some drugs which are not visible by either UV or iodine staining. Because this solution will be used only for that one class of drugs (aminoglycosides), prepare the solution only when analyzing the mycins.

Prepare the ninhydrin solution in a 25 mL graduated cylinder with a stopper. When preparing the solution, take the precautions of using rubber gloves and protective clothing. Ninhydrin reacts immediately with the skin. If ninhydrin has touched the skin, wash those portions of the body with large amounts of water.

Add 25 mL of acetone to the graduated cylinder, and then add 0.1 g of ninhydrin. Stopper the graduated cylinder and shake well until all the ninhydrin is dissolved. The solution can be kept for a period of time. It is not necessary to prepare large amounts. A small bottle will last for many analyses.

Sulfamethazine

Structure:



Formula and molecular weight: $C_{12}H_{14}N_4O_2S$ - 278.32
Category: Antibacterial
Sample:

This drug was not available .

Standards:

High standard

The high limit is 115%; therefore the concentration of the high standard = (2.5 mg/mL) X 1.15 = 2.875 mg/mL. Weigh approximately 10 mg of the standard drug. If you weighed 9.8 mg of the standard drug, dissolve it in: 9.8 mg/2.875 mg/mL = 3.4 mL of methanol. This makes the high standard solution concentration equal to 2.875 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (2.5 mg/mL) X 0.85 = 2.125 mg/mL. Dilute 1 mL of the high standard by adding 0.35 mL of methanol (2.875/2.125 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 15 mL of ethyl acetate and 35 mL of toluene. Add 24 mL of this solution to the TLC development bag. Develop the plate until the solvent front reaches 1 cm from the top of the plate.

Detection:

UV:

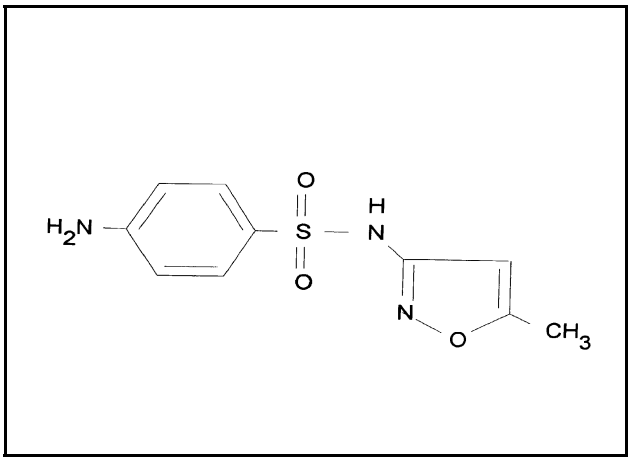
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow to dry until the spots are clearly visible. Observe the size and intensity of the spots.

Sulfamethoxazole
500 mg Tablet

Structure:



Molecular Formula and Mass: $C_{10}H_{11}N_3O_3S$ -253.31
Category: Antibacterial
Sample:

This drug is supplied both as a single component and in combination with other drugs; therefore the drug content will vary. The sample described here has a drug content of 500 mg. Some combinations have a drug content of 400 mg. The concentration of the sample, measured by ultraviolet light, is 0.5 mg/mL, but to be measured by iodine staining, the concentration must be 5 mg/mL. In all cases, grind 1 tablet and dissolve in 50 mL of methanol. Shake at least 5 min. The solution of the 500 mg tablet will have a concentration of 10 mg/mL. For the iodine staining, add 1 mL of methanol to 1 mL of the 10 mg/mL solution to prepare a 5 mg/mL solution. For UV detection, add 9 mL of methanol to 1 mL of the 5 mg/mL solution to prepare a 0.5 mg/mL solution. Both the 5 mg/mL solution (iodine staining) and the 0.5 mg/mL solution (UV detection) represent a sample concentration of 100%. Always use UV detection if it is available.

Standards:

<u>High standard</u>:

The high limit is 115%; therefore, the concentration of the high standard = (5 mg/mL X 1.15) or (0.5 mg/mL x 1.15). Weigh approximately 10 mg of the standard, and add sufficient methanol to prepare the 5 mg/mL solution. If you weighed 9.75 mg, dissolve it in: 9.75 mg/(5.0 X 1.15) = 1.7 mL of methanol for the 5 mg/mL solution. For the 0.5 mg/mL solution, add 9 mL of methanol to 1 mL of the 5.0 mg/mL solution.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (5 X 0.85) = 4.25 mg/mL. To prepare this concentration, add 0.35 mL of methanol to 1 mL of the

5.75 mg/mL solution (5.75/4.25 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 9 mL of ethyl acetate and 21 mL of toluene. Add 24 mL of this solution to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the plate.

Detection:

UV:

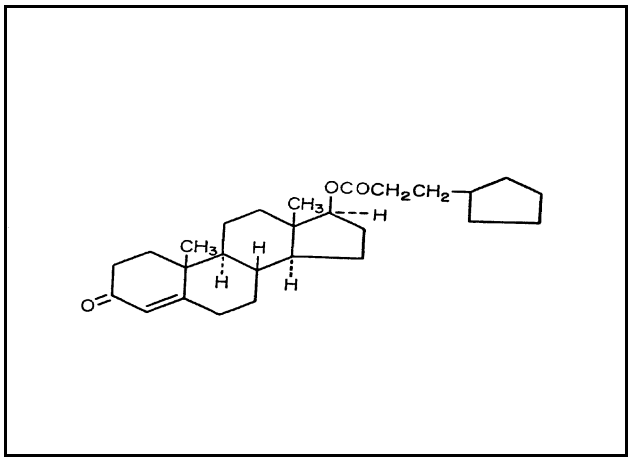
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution. Observe the spots as soon as they become clearly visible.

Testosterone Cypionate
200mg/mL injectable

Structure



Molecular Formula & Mass: $C_{27}H_{40}O_3$ - 412.59
Category: Androgen
Sample:

Dilute 0.1 mL of sample to 40 mL with methanol. Concentration of the solution = (0.1 mL X 200 mg/mL)/40 mL = 0.5 mg/mL. The required concentration of sample solution representing 100% is 0.5 mg/mL.

Standard:

High standard:

The high limit is 115%; therefore the concentration of high standard = (0.5 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.93 mg of standard, dissolve it in: (4.93 mg)/(0.575 mg/mL) = 8.6 mL of methanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (0.5 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follow:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 18 mL of toluene and 6 mL of acetone. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

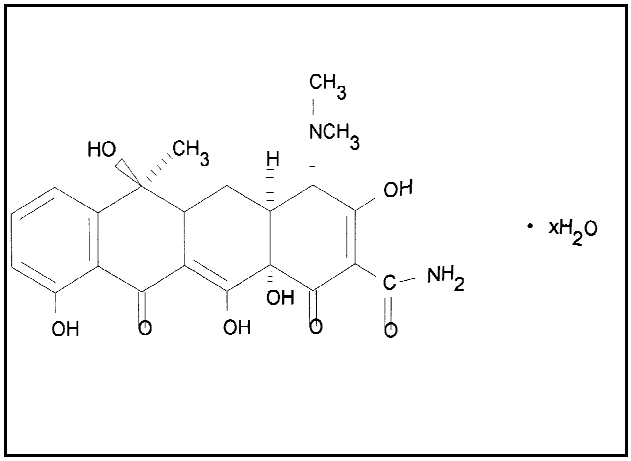
Dry the plate and observe under UV light. The maximum is at 250 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Tetracyline
250 mg and 500 mg tablets

Structure:



Molecular Formula & Mass: $C_{22}H_{24}N_2O_8$ - 444.43
Category: Antiamebic **,** antibacterial, antirickettsial
Sample:

250 mg tablet: Grind 1 tablet and dissolve in 50 mL of methanol to prepare a 5 mg/mL solution. The solution needed to represent 100% requires a concentration of 1 mg/mL. Dilute 1 mL of the 5 mg/mL solution with 4 mL of methanol for a final concentration of 1 mg/mL.

500 mg tablet: Grind 1 tablet and dissolve in 50 mL of methanol to prepare a 10 mg/mL solution. Dilute 1 mL of the 10 mg/mL solution with 9 mL of methanol for a final concentration of 1 mg/mL.

Standards:

<u>High standard:</u>

The high limit is 120%; therefore the concentration of the high standard = (1.0 mg/mL X 1.20) = 1.20 mg/mL. Weigh approximately 5.0 mg of standard. If you weighed 4.7 mg of standard, dissolve it in: (4.7 mg)/(1.20 mg/mL) = 3.91 mL of methanol. This makes the high standard solution concentration equal to 1.20 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (1.0 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of the high standard to 1.41 mL by adding 0.41 mL of methanol (1.20/0.85 = 1.41).

Spotting:

Spot on the TLC plate as follows:

Left spot low standard (85%)

Center spot	100% sample
Right spot	high standard (120%)

Development:

Mix together 12 mL of ethyl acetate, 12 mL of glacial acetic acid, 8 mL of methanol, and 2 mL of concentrated ammonium hydroxide. Add 24 mL of the mixture into a TLC development bag. Develop until the solvent front reaches 1 cm from the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

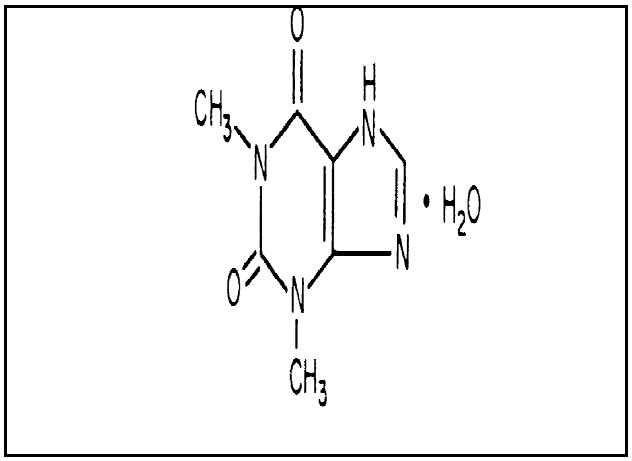
Dip the plate in the iodine-KI solution in the TLC detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Visual:

The plate can also be observed without iodine-KI solution by allowing it to sit overnight. The spots begin as faint yellow spots and turn a brownish color as the plate sits. Observe their size and intensity.

Theophylline
200 mg capsule

Structure:



Molecular Formula & Mass: $C_7H_8N_4O_2$ - 180.17
Category: Diuretic, cardiac stimulant
Sample:

Dissolve the contents of 1 capsule in 50 mL of distilled water. Shake at least 1 min. Concentration of the solution = 200 mg/50 mL = 4.0 mg/mL. The required concentration of sample solution representing 100% is 0.50 mg/mL. Add 7 mL of distilled water to 1 mL of the 4.0 mg/mL solution to make the sample
solution equal to 0.50 mg/mL.

Standards:

High standard:

The high limit is 115%; therefore the concentration of the high standard = (0.50 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.6 mg of standard, dissolve it in: (4.6 mg/0.575 mg/mL) = 8.0 mL of distilled water. This makes the high standard solution concentration equal to 0.50 mg/mL.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (0.50 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of distilled water (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 16 mL of acetone and 8 mL of toluene. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

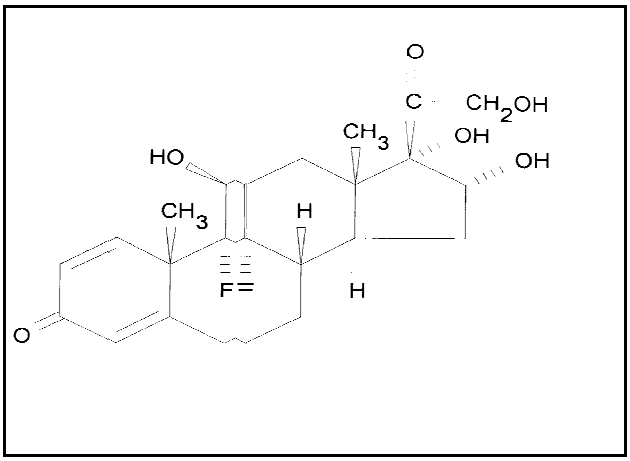
Dry the plate and observe under UV light. The maximum is at 270 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Triamcinolone
4 mg tablet

Structure:



Molecular Formula and Mass: $C_{21}H_{27}FO_6$ - 394.45
Category: Glucocorticoid
Sample:

This drug has a low dosage content of 4 mg. It is necessary to use multiple tablets in order to increase the volume of solvent so that a suitable sampling can be made. Grind and dissolve 3 tablets in 4 mL of ethanol to prepare a sample solution equivalent to 3 mg/mL. Shake well for 2 minutes. Allow the insolubles to settle before sampling, and remove approximately 1mL for the sample spotting.

Standards:

<u>High standard:</u>

The high standard concentration is 115% of the concentration of the sample which is equal to 1.15 X 3 = 3.45 mg/mL. Weigh approximately 10 mg and dissolve in the correct volume of ethanol. For example: You weighed 9.75 mg, then the volume needed is 9.75 mg/ 3.45 mg/mL = 2.82 mL of ethanol.

<u>Low standard:</u>

The concentration of the low standard is 85% of the concentration of the sample which is 0.85 X 3 = 2.55 mg/mL. This solution can be prepared by taking 1 mL of the 3.45mg/mL solution and adding 0.35 mL of ethanol.

Development:

Mix together 20 mL of toluene, 10 mL of acetone, and 3 mL of ethanol. Add 24 mL of this solution to the TLC development bag. Develop the plate until the solvent front reaches 1 cm from the top of the plate.

Detection:

<u>UV:</u>

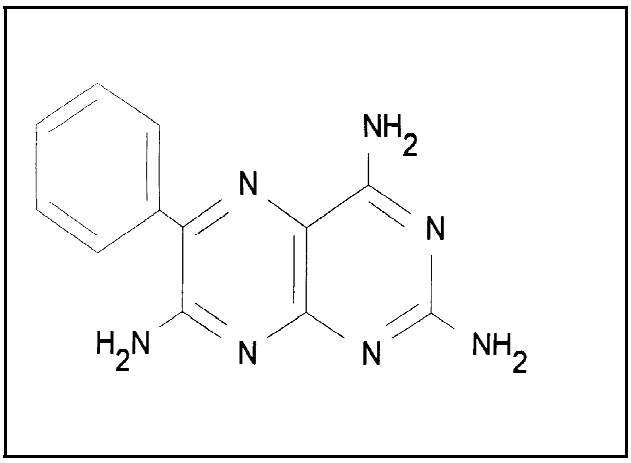
Dry the plate and observe at 254 nm ultraviolet light. Observe the intensities and the sizes of the spots.

Iodine stain:

Dip the plate into the iodine solution, and allow the iodine to fade until the spots are clearly visible.

Triamterene
50mg capsule

Structure



Molecular Formula & Mass: $C_{12}H_{11}N_7$ - 253.26
Category: Diuretic
Sample:

Dissolve the drug from 1 capsule in 100 mL of methanol. Concentration of the solution = 50 mg/100 mL = 0.5 mg/mL. The required concentration of sample solution representing 100% is 0.5 mg/mL.

Standard:

High standard:

The high limit is 115%; therefore the concentration of high standard = (0.5 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.7 mg of standard, dissolve it in: (4.7 mg)/(0.575 mg/mL) = 8.2 mL of methanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (0.5 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 24 mL of methanol and 0.25 mL of concentrated ammonium hydroxide. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

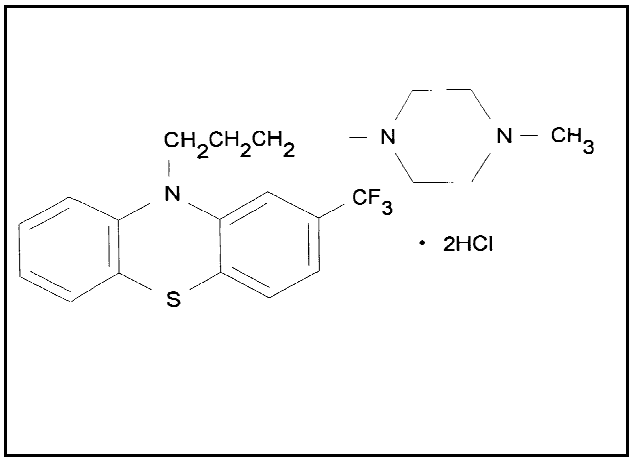
Dry the plate and observe under UV light. The maximum is at 270 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Trifluoperazine HCl
2, 5, or 10 mg tablet

Structure:



M o l e c u l a r
Formula and Mass: $C_{21}H_{24}F_3N_3S$ - 407.49
$C_{21}H_{26}Cl_2F_3N_3S$ (dihydrochloride)

Category: Antipsychotic, sedative

Sample:

A solution of 0.5 mg/mL is needed to make a 100% sample solution.
2 mg tablet: Grind 1 tablet and dissolve in 4 mL of anhydrous ethanol.
5 mg tablet: Grind 1 tablet and dissolve in 10 mL of anhydrous ethanol.
10 mg tablet: Grind a1 tablet and dissolve in 20 mL of anhydrous ethanol.

Standards:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.5 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 4.6 mg of standard, dissolve it in: 4.6 mg/0.575 mg/mL = 8 mL of anhydrous ethanol. This makes the high standard solution concentration equal to 0.575 mg/mL.

<u>Low standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.5 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of the high standard to 1.35 mL by adding 0.35 mL of anhydrous ethanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:
Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Mix 24 mL of acetone and 1 mL of concentrated ammonium hydroxide. Add 24 mL

of this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

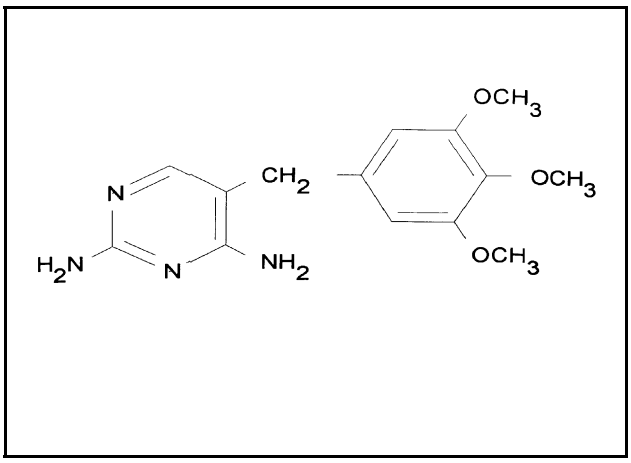
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Trimethoprim
80 mg tablet
(with 400 mg of sulfamethoxazole)

Structure



Molecular Formula & Mass: $C_{14}H_{18}N_4O_3$ - 290.32
Category: Antibacterial
Sample:

This drug is normally found as a component together with sulfamethoxazole. The single strength tablet contains 400 mg of sulfamethoxazole and 80 mg of trimethoprim. Double strength tablets are also available. Grind 1 tablet (80 mg of trimethoprim),and dissolve in 50 mL of methanol to prepare a solution equivalent to 1.6 mg/mL. Add 2.2 mL of methanol to 1 mL of the 1.6 mg/mL solution to make a final solution concentration equal to 0.5 mg/mL.

Standard:

<u>High standard:</u>

The high limit is 115%; therefore the concentration of high standard = (0.5 mg/mL) X 1.15 = 0.575 mg/mL. Weigh approximately 5 mg of standard. If you weighed 5.1 mg of standard, dissolve it in: (5.1 mg)/(0.575 mg/mL) = 8.9 mL of methanol.

<u>Low standard</u>:

The low limit is 85%; the concentration of the low standard = (0.5 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 11mL of acetone, 11 mL of toluene, and 2.5 mL of methanol. Add this mixture to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

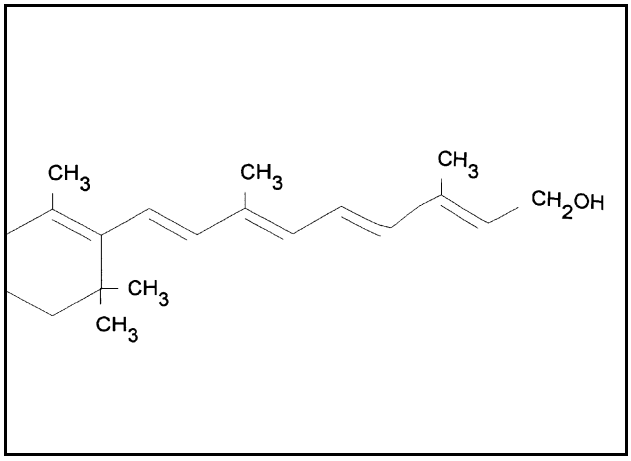
Dry the plate and observe under UV light. The maximum is at 278 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Vitamin A -Retinol
7.5 mg capsule
(in cottonseed oil)

Structure



Molecular Formula & Mass: $C_{20}H_{30}O$ - 286.44
Category: Vitamin
Sample:

Dissolve 1 capsule (alcohol base) in 15 mL of anhydrous ethanol. The concentration of solution = (7.5 mg/15 mL) = 0.5 mg/mL. The required concentration of sample solution representing 100% is 0.5 mg/mL.

Standard:

High standard:

The high limit is 115%; therefore the concentration of high standard = (0.5 mg/mL) X 1.15 = 0.575 mg/mL. The standard is Vitamin A acetate capsule (1 g of solution = 30.3 mg of retinol). Weigh approximately 229 mg of standard solution. If you weighed 229.74 mg of standard solution, calculate as follows: (30.3 mg of retinol X 229.74 g of solution)/1 g solution = 6.96 mg of retinol. Dissolve it in: (6.69 mg)/(0.575 mg/mL) = 11.63 mL of ethanol.

Low standard:

The low limit is 85%; therefore the concentration of low standard = (0.5 mg/mL) X 0.85 = 0.425 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (0.575/0.425 = 1.35).

Spotting:

Spot on the TLC plate as follows:

Left spot	low standard (85%)
Center spot	100% sample
Right spot	high standard (115%)

Development:

Mix 21 mL of toluene and 3 mL of ethyl acetate. Add this mixture to the TLC

development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 325 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

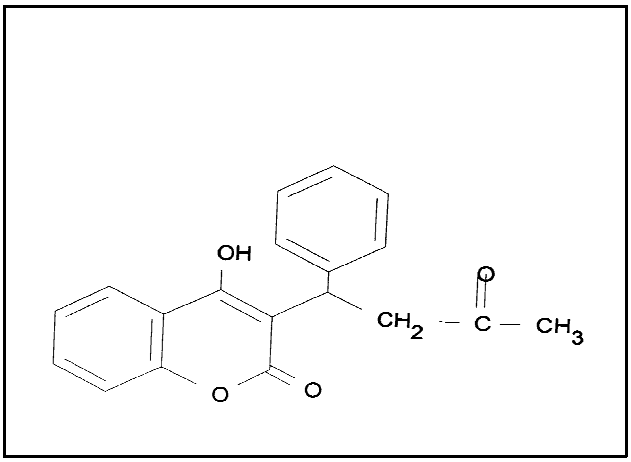
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Note:

The cottonseed oil in this drug lowers its solubility. It also makes spotting much more difficult. The R_f value for the sample is lower than that of the standard because the standard is acetate base but the sample is alcohol base and thus more polar.

Warfarin
2.5 mg tablet

Structure



Molecular formula & mass: $C_{19}H_{16}O_4$ - 308.32
Category: Anticoagulant
Sample:

Grind 4 tablets and dissolve in 10 mL of anhydrous ethanol. Concentration of the solution = (2.5 mg X 4)/10 mL = 1 mg/mL. The required concentration of the sample solution representing 100% is 1 mg/mL.

Standard:

High standard:

The high limit is 115%; therefore the concentration of high standard = (1 mg/mL) X 1.15 = 1.15 mg/mL. Weigh approximately 5 mg of standard. If you weighed 5.8 mg of standard, dissolve it in: (5.8 mg)/(1.15 mg/mL) = 5 mL of anhydrous ethanol.

Low standard:

The low limit is 85%; therefore the concentration of the low standard = (1 mg/mL) X 0.85 = 0.85 mg/mL. Dilute 1 mL of high standard to 1.35 mL by adding 0.35 mL of ethanol (1.15/0.85 = 1.35).

Spotting:

Spot on the TLC plate as follow:

Left spot low standard (85%)
Center spot 100% sample
Right spot high standard (115%)

Development:

Add 24 mL of 100% ethyl acetate to the TLC development bag. Develop until the solvent front reaches within 1 cm of the top of the TLC plate.

Detection:

UV:

Dry the plate and observe under UV light. The maximum is at 310 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

Iodine stain:

Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

Note:

The sample used was a sodium salt. If this sample is used, it must be taken into consideration. For example, if the percent concentration of sample is 94, it needs to be adjusted as follows:

(330/308) X 94% = 101%. The final concentration of the sample is 101%.

330 g/mol = molecular weight of the sodium salt sample

308 g/mol = molecular weight of the pure standard

Simple, At-Site Detection of Diethylene Glycol/Ethylene Glycol Contamination of Glycerin and Glycerin-based Raw Materials by Thin-Layer Chromatographic

Allen S. Kenyon[1], Shi Xiaoye[2], Wang Yan[2], and Ng Wai Har[3]

FDA Division of Testing and Applied Analytical Development

St. Louis, MO 63101

and

Robert Prestridge and Kirsten Sharp

Australian Therapeutic Goods Administration

Woden, ACT 2606 Australia

Reprint: Journal of AOAC International Vol.81,No.1,44-50,1998

1. Visiting Scientist, Food and Drug Administration, Division of Testing and Applied Analytical Development (DTAAD), 1114 Market Street, Room 1002, St. Louis, MO 63101. Communications should be addressed to Dr. Thomas Layloff, Director, at this address.

2. Shanghai Institute for Drug Control, Republic of China. On technical assignment to DTAAD, St. Louis, MO.

3. Senior Laboratory Officer, Institute of Science and Forensic Medicine, Singapore. On technical assignment to DTAAD, St. Louis, MO.

Abstract

This paper describes a rapid, inexpensive thin-layer chromatographic (TLC) method that separates diethylene glycol (DEG) from glycerin and other glycols. Studies with collaborating laboratories of the World Health Organization have shown that about 6% DEG in glycerin and about 2% DEG in acetaminophen (paracetamol) elixirs may be detected by direct visual inspection of the developed TLC sheets. Staining the sheet permits detection of DEG at less than 0.1%. The method costs less than $1.00 per test and takes 20 minutes with visual inspection, longer when staining is required. The visual method can be performed without laboratory facilities by personnel having little previous training. Samples testing positive by the visual method can be submitted to a laboratory for confirmation and quantitation of DEG.

Introduction

In 1937, 105 Americans died of renal failure when they ingested a sulfanilamide elixir containing 72% DEG used as a diluent (1). In spite of this famous incident, additional deaths attributed to DEG poisoning continue to occur (2). The fourth largest outbreak of DEG poisonings associated with pharmaceutical products occurred in Haiti in 1996, 59 years after the U.S. sulfanilamide elixir episode, and was linked to the deaths of many children(3,4).

Glycols such as glycerin are present in many formulations of medicinal, cosmetic, and food products. As a result of world trade, such commodities can become contaminated by DEG or ethylene glycol and may unwittingly be accepted for use in manufacture of pharmaceuticals. Investigations of DEG-related poisonings in various countries have repeatedly revealed lack of testing for contamination and other quality-control measures at ports of entry or during the pharmaceutical manufacturing process (2-4).

The minimum safe level for humans for ingested DEG is not known, but at a 1997 interagency workshop supported by the U.S. Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and the World Health Organization (WHO), a detection level of 0.1% DEG was selected as adequate for screening raw materials and glycerin-based elixirs.

Earlier we reported the development of a novel TLC system used for rapid screening analysis of many drugs(5-7). The purpose of this work was to adapt this method to detect the presence of DEG in glycerin in raw materials and liquid oral pharmaceuticals.

We required that the method (a) distinguish DEG from related components (e.g., glycerin, and ethylene and propylene glycols) often present in glycerin and glycerin-based materials and products; (b) be sufficiently sensitive and reliable to prevent future DEG-related fatalities; (c)

allow detection of gross DEG contamination (about 6% DEG in glycerin and about 2% DEG in elixirs) through direct visual inspection of the sheet without spraying and at locations without laboratory facilities, such as at ports of entry; and (d) allow detection of DEG at 0.1% through spraying or staining the sheet. (Throughout, we use percentage of DEG to refer to the percentage in the original sample before dilution.)

Separation of glycols by TLC has been reported previously (8), although with developing solvent systems not used here. Because glycols are not visible by the usual UV illumination methods, investigators have used a variety of staining sprays to make the spots visible (9).

Experimental

The chemicals and solvents used for the TLC developing solvent (toluene, acetone, ammonium hydroxide), as well as reference materials of DEG, glycerin, ethylene glycol, propylene glycol, and sorbitol, were all pure grade as obtained from commercial sources. The toluene and acetone were chromatographic grade, but reagent grade solvents are also satisfactory .

Samples of contaminated or uncontaminated glycerins and syrups were prepared or obtained from various sources. Some were representative samples that had been analyzed by the CDC, Atlanta, GA, and the FDA National Forensic Chemistry Center (NFCC) in Cincinnati, OH, by gas chromatography (GC) and GC/mass spectrometry (GC/MS).

The rapid screening TLC apparatus used in this work (Figure 1) and its operation have been previously described (5,6) (US Patent 5,350,510.). :The apparatus was produced by Granite Engineering. Inc., P.O. Box 1269, Granite City, Illinois 62040, USA. The TLC method described here is not specific for this apparatus, but was used because of the ease, reliability, cost, and portability.

Preparation of TLC sheets

Merck plastic-backed silica-coated 20 X 20-cm sheets or equivalent, either with or without fluorescent indicator, were used; at low DEG concentrations, best results were obtained with the fluorescent sheets.

Cut the sheet into eight equal parts, each 5 X 10 cm. To prevent edge effects, remove 1 mm of the coating layer from the sides and the bottom of the sheet by bearing down on a #2 pencil along a straight edge. Draw the spotting line 2.5 cm from the bottom of the sheet without cutting into the coating by very lightly applying a pencil tip. Mark the three spotting locations at 1.5, 2.5, and 3.5 cm from the left edge. Mark the development stopping line by placing a straight edge across the sheet 1 cm from its top and removing 1 mm of the silica coating by bearing down on a pencil.

Preparation of solutions

To reduce viscosity, dilute glycerin and other glycols threefold with methanol before spotting; typically, add 1 mL of methanol to 0.5 mL of the glycol in a 1.5-mL plastic centrifuge tube graduated at 0.5, 1.0, and 1.5 mL. Prepare lower concentrations by diluting aliquots from the initial solutions with methanol. Use solutions of the pure glycols as reference materials to assign the spot positions. The developing solvent is toluene:acetone:5M ammonium hydroxide (5:85:10); prepare daily.

Spotting of the solutions and TLC sheet development

This TLC system accommodates two sheets, with three samples or reference solutions spotted on each sheet. Spot the sample or reference solutions at the marked spotting locations with a 3-µL capillary pipette. Always apply a reference solution of DEG as one of the spots on each sheet. Allow the spots to dry for approximately 10 minutes.

Clamp the spotted sheets onto the aluminum trays in the system.(The developing solvent may be reused for additional analysis during the same day by replacing the volume lost in the saturation pads.) Add 24 mL of the developing solvent to the plastic bag, and insert the assembled sheets into the polyethylene bag until the large saturation pad just touches the developing solvent solution. Position the assembly in the bag with a wire rod (fabricated from a coat hanger), which also serves as a tool for removing the assembly from the bag after development. Clamp the bag containing the assembly in the system's metal support, which seals the top of the bag to maintain a uniform environment.

Migration and detecting the spots

Allow 2 minutes for the system to establish equilibrium. Grasp the bottom of the bag and gently pull it down, forcing the developing solvent solution up onto the TLC sheets to initiate migration. At this point, the bottoms of the TLC sheets are immersed in the developing solvent to a depth of 1 cm. Allow the developing solvent to migrate upward until the solvent front reaches the scored top line on the sheets (approximately 20 minutes). Remove the assembly containing the sheets from the bag with the positioning rod, and allow the sheets to air dry.

At high concentrations of DEG (6% or more in glycerin, 2% or more in elixirs), the spots may be visually detected by transmitted daylight; the spots appear dark relative to the sheet because of the difference in refractive index. At lower concentrations of DEG, the sheets must be stained to make the spots visible. The Appendix details two methods for staining the developed sheets.

Results and Discussion

Several developing solvents were considered in this study. We wished to avoid hazardous solvents such as chloroform or benzene that have been frequently used in TLC developing solvent

systems.

Three different developing solvent mixtures were evaluated: (a) n-butanol saturated with 1.5 M ammonium hydroxide; (b) chloroform:acetone:5M ammonium hydroxide (10:80:10); and (c) toluene:acetone:5M ammonium hydroxide (5:85:10). The development time for (a) was 75 minutes, whereas (b) and (c) each required 20 minutes. The WHO has banned use of chloroform in a TLC developing solvent because of its carcinogenic nature; therefore, the chloroform in developing solvent (b) was replaced by a 1:1 mixture of toluene and acetone to form developing solvent (c) to achieve approximately the same solubility behavior. Developing solvent (c) proved to be the most satisfactory because it reduced the migration time in (a) and eliminated toxic chloroform in (b). The retardation times for developing solvents (b) and (c) were identical.

To achieve detection at 0.1% DEG, we tried several different staining procedures; however, most required spraying with highly toxic (e.g., benzidine) or costly materials. These procedures require the use of a well-ventilated hood, a face mask for the operator, and a well-equipped laboratory, and essentially rule out performing the test in open, nonlaboratory environments.

Visual observation of the spots in bright transmitted white light was the most simple and effective detection method when the DEG content was greater than 2% in elixirs or 6% in glycerin. Figure 2 shows the appearance of the spots on the developed sheets. Circles were drawn around the spots to show their locations. Sample A was pure glycerin. Sample B contained 4.5% glycerin and 25.5% DEG; the DEG was readily detected but not glycerin. Sample C contained 18% glycerin and 18% DEG; both DEG and glycerin were easily seen Samples D and E were syrups containing no drug. Sample D contained 2.2% glycerin and 14% DEG; sample E contained 67% glycerin and no DEG.

Table 1 lists the retardation factors (R_fs) for the reference glycols using the three solvent mixtures (a,b andc) , and illustrates that glycerin, DEG, and other glycols can be detected by matching the R_f values of sample spots with those of authentic references. No study was made on possible variation of R_f with temperature or humidity.

The method can also be used to estimate the concentration of DEG contamination in glycerin or syrup samples. The concentration of DEG may be estimated by spotting solutions having different concentrations of DEG and directly comparing the intensities of the spots. In this procedure, the intensity of a sample spot (and hence the amount or concentration of DEG) can be visually estimated to be between certain limits, bracketed by two reference DEG spots of different concentration. Detection using only transmitted light worked well with the samples studied, since the concentration of DEG in the glycerin ranged between 18 and 25%.

Several stains that enhance the detection of glycols(8) have been reported, but most are toxic and costly, require operation in a hood to protect the analyst from the harmful sprays, and lack sensitivity. Many of the recommended stains are applicable only for high concentrations of glycol. Use of these stains was dismissed as impractical after several trials. As recently as 1996, the Pharmacopeia of India(10) described a staining procedure in which benzidine was used to detect DEG at the 0.1% level. This method worked well in our hands but is not recommended for use in open places or without suitable laboratory facilities. Two staining procedures were found feasible for use in open, nonlaboratory places, namely, (a) iodine vapor/starch spray and (b) oxidation of the glycols with potassium permanganate. Details of these staining methods are described in the Appendix.

Figure 3 shows the detection of DEG in glycerin at concentrations from 0.1 to 0.75% as measured by densitometry at 400 nm after the sheets were stained with iodine vapor and sprayed

with starch. The densitometer was used to obtain performance data and to show that the intensity of the spots were a function of concentration. The densitometer response for the 0.1% concentration was low and erratic, and that point is not shown; however, the spot was visible to the eye. The responses were linear with a correlation coefficient of >0.99, based on average values obtained in duplicate runs by three analysts. The responses from solutions of higher DEG concentrations were also linear. The intensities from different sheets could not be compared because of differences in staining and fading; standards and samples must be run on the same sheet. An estimate of the concentration can be determined visually without the need for any instrumentation by spotting solutions of bracketing concentrations of DEG along with the sample.

Children's acetaminophen elixir obtained from a local pharmacy was spiked with DEG. Samples of the laboratory-contaminated product were diluted to various concentrations down to 0.1% DEG. The developed spots were oxidized with potassium permanganate. An actual separation at the level of 0.25% DEG is shown in Figure 5 as yellow spots on a purple background, demonstrating the ability to separate the sugars (sucrose and sorbitol), the glycols, and the other components in a final dosage form. The listed major components of the children's acetaminophen elixir were citric acid, glycerin, propylene glycol, sucrose/sorbitol, and acetaminophen; the minor components did not interfere with the separation of the glycols.

[4] Since the size of the spot is proportional to the concentration, at lower concentrations the spots can be very small and difficult to detect. The detection limit can be lowered just by increasing the volume. If the volume of material spotted is increased from 3 to 15 μL, the detection limit for DEG can be lowered from 0.1% to 0.05%.

The detection levels for contamination of glycerin were different from those for the elixirs

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[4]Color photo of Fig 5 can be supplied upon request

or syrups. Glycerin, because of its high viscosity, required at least a threefold dilution for the contaminant to separate, which meant that the detection limit must be higher for pure glycerin than for samples of elixirs or syrups. With care, DEG could be detected in glycerin down to a level of 0.025% with iodine vapor/starch spray and down to 0.01% in elixirs or syrups with permanganate.

Staining by oxidation with permanganate is faster, easier to perform, and more sensitive, and it eliminates the need for spray equipment and a large glass developing tank, but the spots fade faster. Selection of the two staining methods was based on low toxicity, low cost, availability of reagents, and ease of operation. Permanent records of the developed TLC sheets can be made by photo copy.

A common practice for increasing the sensitivity of TLC is to multiply spots (11) by applying the spot, allowing it to dry, and spotting additional material on top of the dried material. By spotting three pipette loadings on a single position, the detection level has effectively been increased, making the developed spot more visible.

This method will not separate DEG and ethylene glycol, but if both are present in approximately equal amounts, the developed spots have the contour of a distorted figure eight. Since both DEG and ethylene glycol are toxic, it does not matter that the pair cannot be distinguished as long as they can be separated from the other nontoxic glycols.

Testing with even the simple visual method of detection presented here would have been adequate to detect the contaminated materials that have caused the large numbers of deaths among children and adults in the last 60 years.

Acknowledgments

The National Center for Environmental Health, Atlanta, GA, and the FDA NFCC kindly supplied samples and suggestions. The authors thank Karen Wolnik and Kevin Mulligan of the NFCC and Thomas P. Layloff of the FDA Division of Testing and Applied Analytical Development (DTAAD), St. Louis, for helpful discussions and review of this work, and Walter L. Zielinski and William B. Furman, also of DTAAD, for help in preparing technical revisions of this paper. This assistance and these contributions are gratefully acknowledged.

Special thanks and appreciation are expressed to the following Collaborating Laboratories of the World Health Organization for their assistance in evaluating the method and their helpful comments:

Aiache, J.M., Laboratoire de Biopharmacie, Clermont, France.

Török, I., National Institute of Pharmacy, Budapest, Hungary.

Howard, R.K., Therapeutic Goods Administration, Woden, Australia.

Wongpinairat, C., Ministry of Public Health, Thailand

Westermark, M., Centre for Chemical Reference Substances, Stockholm, Sweden.

Lasis,A., Health Protecton Branch, Ontario, Canada.

Lambert, M. J., Health Protection Branch, Quebec, Canada.

The method was independently validated by Virginia Kleekamp and Tom Lavelle, FDA, DTAAD, St. Louis, MO.

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Appendix

Staining Procedures for Detecting Low Levels of DEG on Developed TLC Sheets

Method 1. Iodine/Starch Stain

1. Place an amount (approximately 25 to 50g) of iodine crystals on the bottom of a glass TLC developing tank that can be sealed with a lid or cap. The tank must be deep enough that the sheet can hang vertically in the iodine vapor. Suspend the developed sheet in the tank, cover the tank with the lid, and allow the sheet to be exposed to the vapor for 5 minutes.

2. Remove the sheet and allow it to stand in the open air for 1 hour to remove all the iodine that has been absorbed by the silica coating on the sheet. If the iodine is not removed at this point, the detection of low concentrations of DEG will be masked by the background.

3. Prepare a 1% starch solution by adding 1 g of starch to 10 mL of water, making a slurry, adding 90 mL of water, and boiling until the solution becomes clear. (The starch solution will appear turbid when cooled, but this will not harm the solution.) Spray the sheet with the 1% starch solution and observe the spots. The method of detecting the spots will depend upon the concentration level of the DEG. Once the DEG is detected, it will not be necessary to proceed to another step. Three levels of detection are possible:

Level 1: For DEG levels of 2 to 6% or higher. Observe the sheets visually by transmitted light immediately after they are dried. The spots appear dark because of the difference in refractive index. No further staining is necessary. If no spots are detected, proceed to Level 2.

Level 2: For DEG levels of 0.5% or higher. Immerse the sheet in iodine vapor. The iodine-stained spots will be visible. If no spots are visible, proceed to level 3.

Level 3: For DEG levels from 0.1 to 0.5%. Spray the sheet with a 1% starch solution. If no spots are observed, the concentration of DEG is below 0.25%.

Method 2: Oxidation of Glycols by Potassium Permanganate

The method was developed by Robert Prestridge and Kirsten Sharp of Australian Therapeutic Goods Administration Laboratories

Woden, Australia

Develop sheets and observe the sheets in a bright transmitted light. If the concentration is above 6% in glycerin or 2% in elixirs, the spots are directly visible. If no spots are visible, proceed with the oxidation of the glycols by potassium permanganate.

1. Allow the developed sheets to air dry for approximately 10 minutes. The presence of a small amount of ammonium hydroxide remaining in the sheets speeds up the oxidation.

2. Prepare the staining solution *immediately* before use by adding 75 to 80 mg of solid potassium permanganate to 12 mL of the used or fresh developing solvent. The permanganate solution is robust enough to allow variation in the amount of permanganate used; it does not have to be weighed exactly. Shake vigorously in a closed container or stir rapidly in an open container until the potassium permanganate has dissolved. (The use of the developing solvent for the potassium permanganate prevents the spots from dissolving or being distorted.) Pour the potassium permanganate staining solution into a shallow vessel such as a watch glass or petri dish. Attach a clip to the developed sheet, and immerse the sheet far enough into the potassium permanganate to cover the surface. Allow the sheet to remain covered with the liquid for 2 to 3 seconds. The staining solution may be used to stain a number of sheets before it must be replaced.

3. Remove the stained sheet and allow it to dry. The oxidized spots of the glycols appear yellow on a purple background. They begin to form after short time, and form at different rates; DEG is the slowest. The first spot appears in approximately 5 minutes and formation is complete in 30

minutes. The difference in formation rate may be used to distinguish DEG and ethylene glycol if present individually.

4. Properly dispose of all used or unused staining solution after treating the samples, since it will not keep. Keep volumes to a minimum.

Glycols	Solvent A	solvents B & C
Glycerin	30	16
Dietylene glycol	52	39
Ethylene glycol	56	42
Propylene glycol	64	54
Sucrose	0	0
Sorbitol	0	0

Table 1. Retardation factors (R_f) for Glycols

LEGENDS FOR FIGURES:

Figure 1. Rapid screening TLC apparatus.

Figure 2. TLC separation of diols.

Figure 3. Detection of low DEG concentrations.

Figure.4. TLC separation of contaminated and noncontaminated

Rapid Screening of Pharmaceuticals by Thin Layer Chromatography:

Analysis of Essential Drugs by Visual Methods

A. S. KENYON, P. E. FLINN, and T. P. LAYLOFF

Food and Drug Administration, Center for Drug Evaluation and Research, Division of Testing and Applied Analytical Development, 1114 Market St., Room 1002, St. Louis, MO 63101-2045

Reference: Journal of AOAC International, 78(1),41-49,1995
NOTE: The original paper has been modified to change procedures.

Abstract

A method for rapidly screening pharmaceuticals by thin layer chromatography 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. The method is low-cost, maintenance-free, fast, and reliable, and it 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. The solvents, developers, concentrations, and procedures were developed for a list of essential drugs supplied by the World Health Organization. From this list, 3 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 by Saudi Arabia 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. If the analysis requires handling organic solvents, it should be possible to perform the procedure in open air with a minimum amount of solvent and with no hood available. 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) has been useful for rapidly screening drugs by comparing the sample solution with different concentrations of reference material. The success of this system, however, has required that the laboratory have electricity and special equipment. Even though this method is fast and economical, and the operator requires little training, it has not been able to meet the needs of locations without electricity and with only limited resources. The work described here 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 the drugs declared essential by the World Health Organization and to a few additional ones to show the general applicability of the method. It must be emphasized that the method described here is intended not as a replacement for other analytical 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 (and other drugs) 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 the rigid, fragile developing chambers were replaced by flexible polyethylene bags for developing the chromatogram, and

tested the analytical capability of the system with the drug theophylline. A new system was then 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 and prevents distortion of the spots. Reproducibility between analysts and laboratories is easily achieved.

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). Seal each tube with an impulse sealer at a point ca 6 cm from the bottom. (The development bags can also be supplied prefabricated.)

(**b**) *Saturation pads.*--Cut rectangular saturation pads from heavy filter paper (0.3 mm thick). (The pads are also available in 20 H 20 cm sheets.) Cut 1 large pad, 14.4 H 7.0 cm, and 2 small pads, each 10.0 H 5.7 cm, for each complete assembly. Adjust the pads as necessary if the polyethylene bag has different dimensions. 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 H 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 (6) insofar as possible.

(**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 ampicillin, paracetamol, 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 variance was found to be approximately 3%.

Preparation of Samples

All analyses described in this report use the declared content of the drug as the weight of 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 grinding 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! For rapidly screening pharmaceuticals semiquantitatively, visually compare the intensity of the spots of the sample with those obtained from solutions of references at different concentrations. 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%). The appropriate concentrations of the solutions for spotting 3 :L onto 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. Remove ca 1-2 mm of the silica coating from the side edges of the 5 H 10 cm film and ca 1-2 mm from the bottom edge. 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 (be careful not to inhale the dust), 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. 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 :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 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. 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 beg.

Allow the TLC sheets to completely dry. 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 essential, based upon frequency of usage and their effect on improving the quality of life:

1. Amoxicillin, all oral forms
2. Ampicillin, all oral and injectable forms
3. Benzylpenicillin (Penicillin-G), all injectable forms
4. Chloramphenicol, all oral and injectable forms
5. Chloroquine diphosphate
6. Mebendazole, all oral forms
7. Paracetamol (acetaminophen), 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. Cloxicillin, all forms
11. Estradiol cypionate
12. Sulfamethoxazole, all forms
13. Theobromine or theophylline
14. Trifluoperazine HCl

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. It will be noted that chloroform has been included even though it is carcinogenic; it was used in our well-equipped laboratory where suitable handling was available. All TLC can be performed with other development solvents so that 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.

These chemicals 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. A volume of 4 mL was selected because it could be handled easily with a 5 mL graduated syringe. 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+. 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 40EC 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 40EC 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 40EC 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: ampicillin, chloroquine phosphate, and paracetamol. 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 to the analyst, who could make a small adjustment in the volume of solvent needed to produce the specified concentration if the measured content was different from that prescribed. 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 ampicillin, chloroquine phosphate, and paracetamol 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 paracetamol with the USP standards and the reference tablets. Similar data were obtained with the 2 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 response of the intensity of the spots as a function of concentration was checked by densitometer in the UV at 254 nm. Correlations 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 allows an estimation of the drug content. The results for 100% of the sample 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_f 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 further 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 tablets 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 for visual methods is larger than that for instrumental analysis which makes the determination at or near the lower and upper concentration limits unreliable. Samples that show a content near the limits should be analyzed by an official method. The majority of samples will show a concentration near the middle of the limits, and as such will screen the drug.

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