A Compendium of Unofficial Methods

for

Rapid Screening of Pharmaceuticals

by

Thin-Layer Chromatography

by

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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.

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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).

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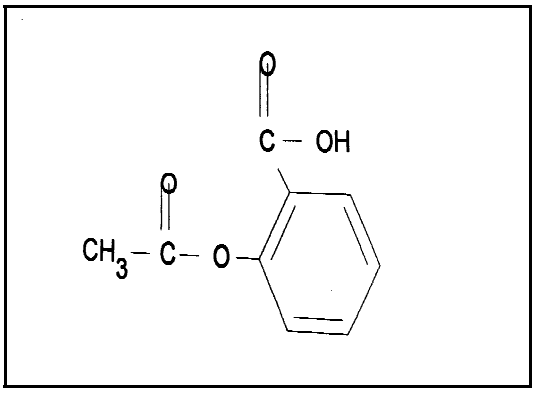
Acetylsalicylic acid
300 mg tablet

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:

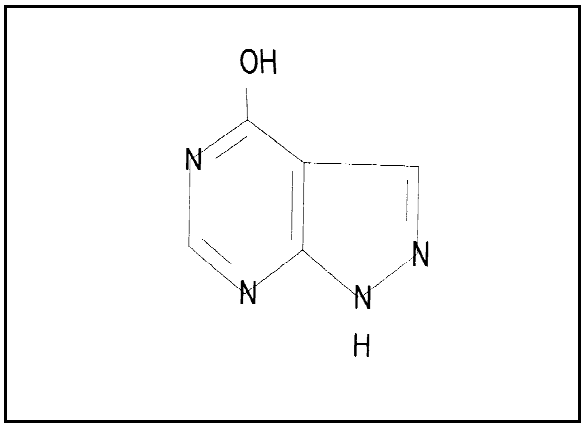
Dry the plate and observe under UV light (254 nm).

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:

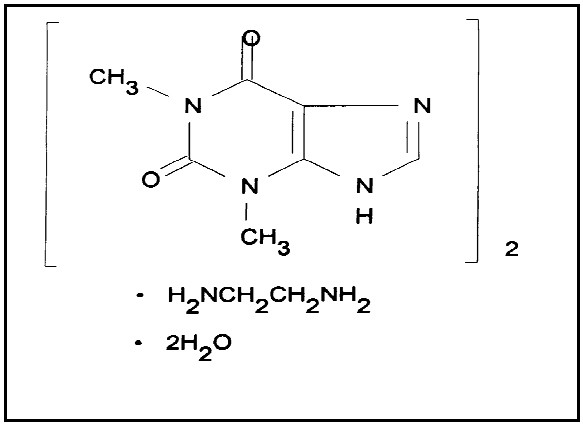
Dry the plate and observe under UV light (254 nm). Observe the intensity and the size of the spots.

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:

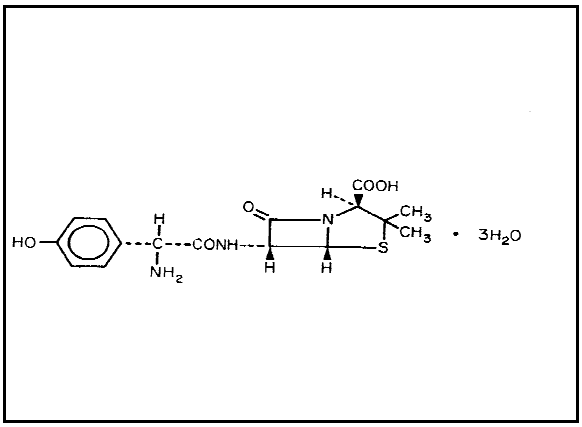
Dry the plate and observe under UV light. The maximum is at 270 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

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:

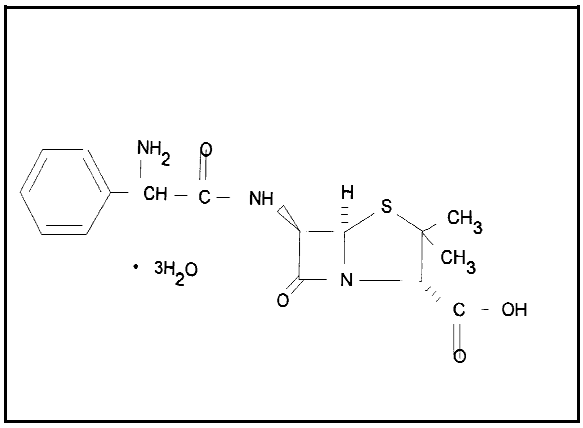
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

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:

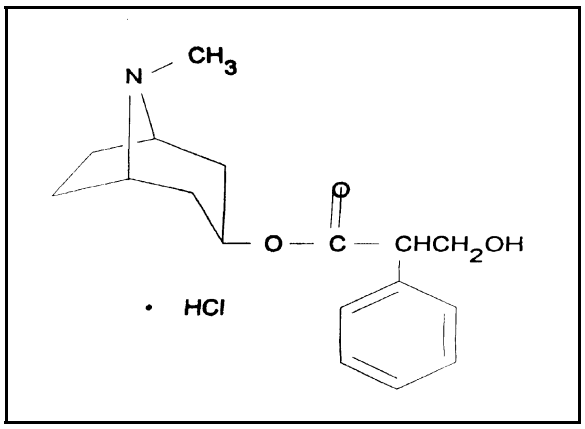
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

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:

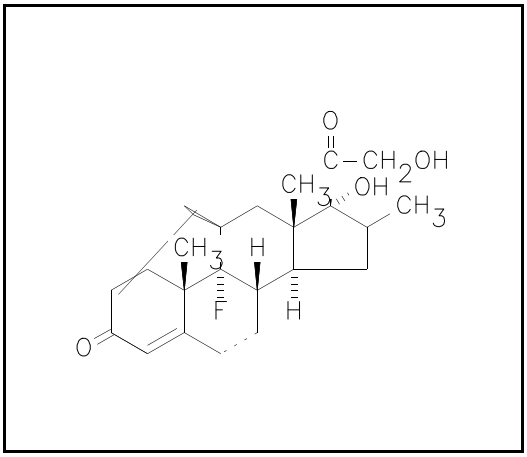
Dip the plate into the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

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:

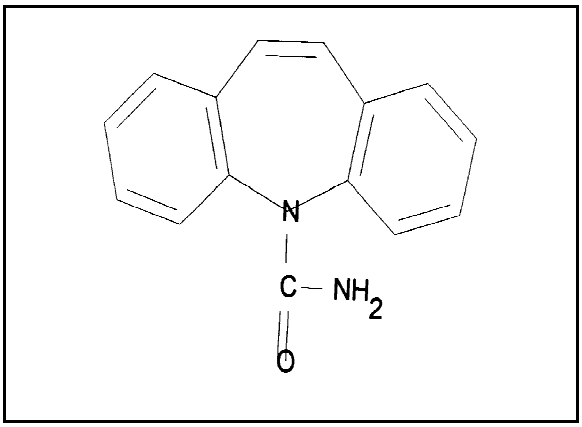
Dry the plate and observe under UV light. The maximum is at 240 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

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:

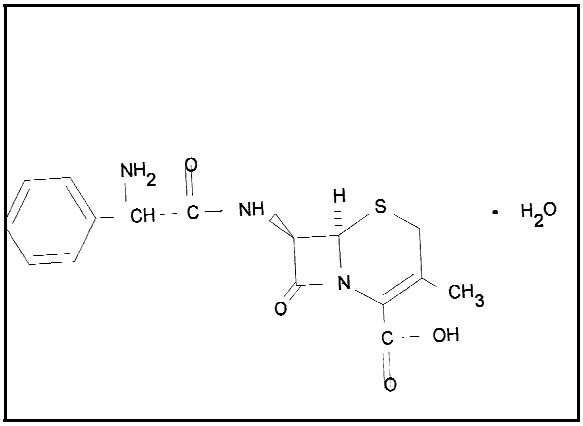
Dry the plate and observe under UV light. The maximum is at 235 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

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:

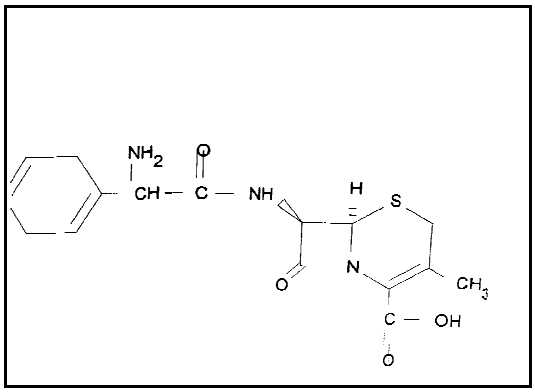
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spots.

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.

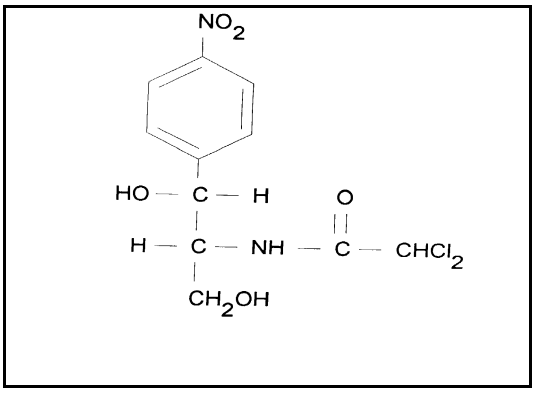
Note:

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:

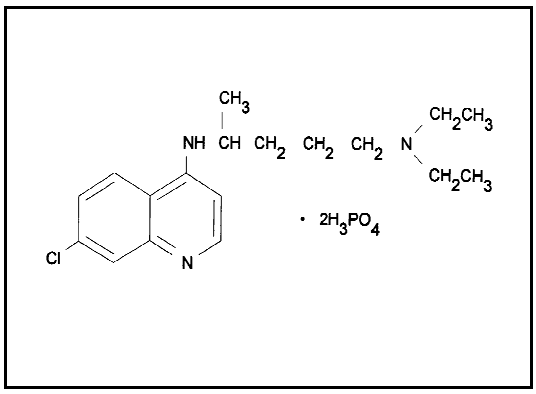
The 10.0 mg/mL concentration is too high to allow for quantitation. Therefore, dilute the sample and standard solutions 1:10 by adding 9 mL of anhydrous ethanol to 1 mL of the sample and 9 mL of anhydrous ethanol to 1 mL of each of the standards. Dry the plate and observe under UV light. The maximum 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:

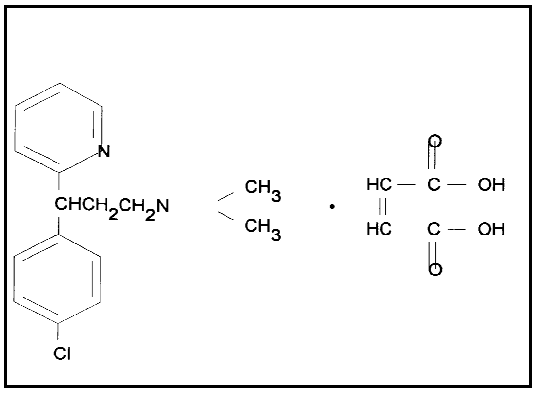
Dry the plate and observe under UV light. The maximum is at 333 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

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>:

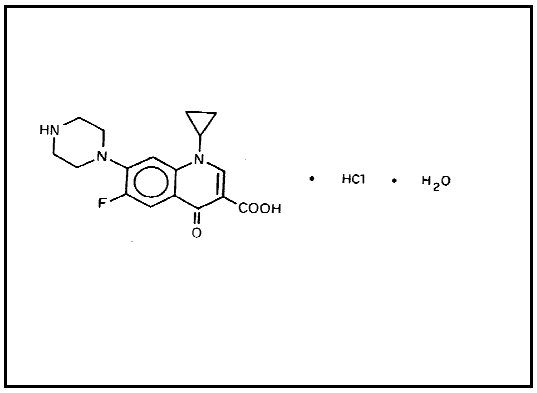
Dry the plate and observe under UV light. The maximum is at 265 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

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:

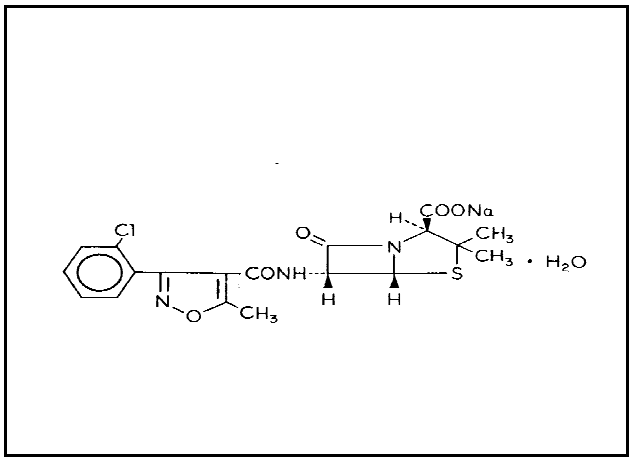
Dry the plate and observe under UV light (254 nm). Observe the size and intensity of the spots.

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.