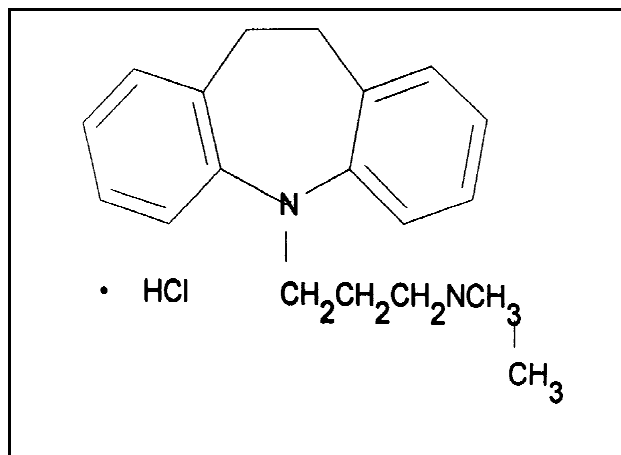


Imipramine HCl 75mg capsule

Structure



Molecular Formula & Mass: C₁₉H₂₄N₂ - 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:

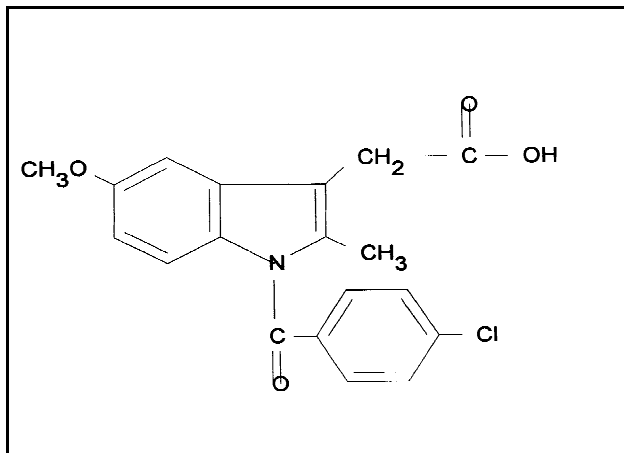
Dry the plate and observe under UV light. The maximum is at 270 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

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: C₁₉H₁₆ClNO₄ - 357.81

Category: Anti-inflammatory, 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:

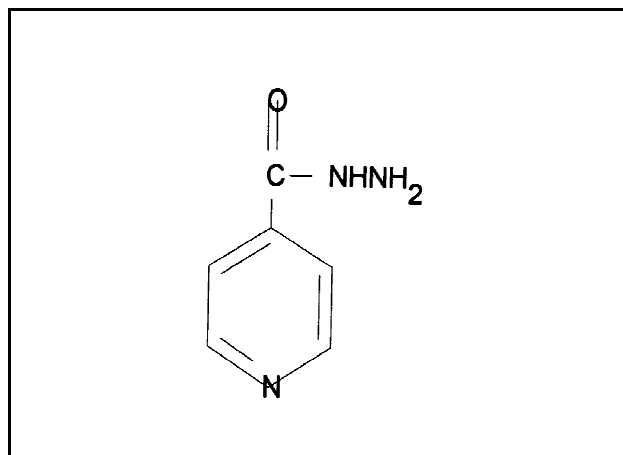
Dip the plate into the iodine-KI solution in the detection bag. Observe the size and intensity of the spots as the plate dries.

METHOD # 1

Isoniazid 300 and 100 mg tablets

Revised procedure(Aug.12,1997)

Structure:



Molecular Formula & Mass: C₆H₇N₃O - 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:

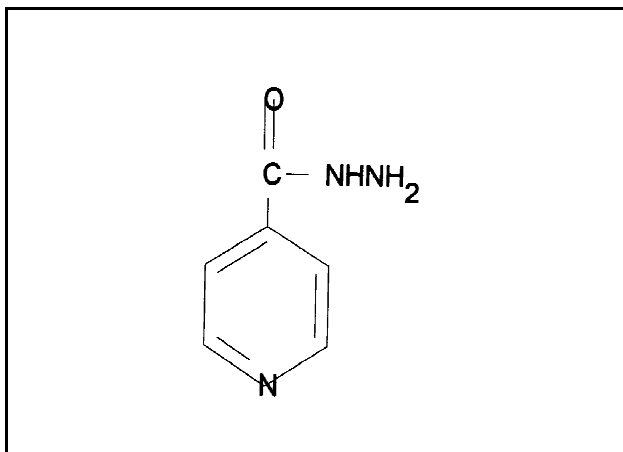
Dip the plate in the iodine-KI solution in the detection bag. Allow the plate to dry and observe the size and intensity of the spot.

METHOD #2

Isoniazid

300 and 100 mg tablets

Structure:



Molecular Formula & Mass: C₆H₇N₃O - 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 $100 \text{ mg}/25 \text{ mL} = 4 \text{ mg/mL}$. 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 0.5 mg/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.

High standard solution:

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 $2.88 \text{ mg}/5 \text{ mL}$ 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: $(4.2 \text{ mg})/(0.575 \text{ mg/mL}) = 7.3 \text{ mL}$ 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% .

Low standard:

The low limit is 85%; therefore the concentration of the low standard = $(0.5 \text{ mg/mL}) \times 0.85 = 0.425 \text{ mg/mL}$. 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 $3 \mu\text{L}$ 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:

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..

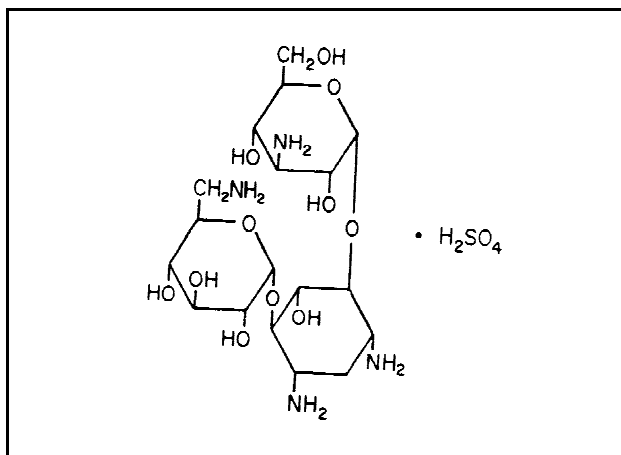
Iodine stain:

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

the size and intensity of the spot.

Kanamycin sulfate 75 mg/2 mL injectable

Structure:



Molecular Formula & Mass: A & C; $\text{C}_{18}\text{H}_{36}\text{N}_4\text{O}_{11}$ - 386.43
B; $\text{C}_{18}\text{H}_{37}\text{N}_5\text{O}_{11}$ - 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 = $(0.5 \text{ mL} \times 75 \text{ mg}) / (2 \text{ mL}) / 15 \text{ mL} = 1.25 \text{ mg/mL}$. The required concentration of sample solution representing 100% is 1.25 mg/mL.

Standards:

High standard:

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

Low standard:

The low limit for antibiotics is 85%; therefore the concentration of the low standard = $(1.25 \text{ mg/mL}) \times 0.85 = 1.06 \text{ mg/mL}$. 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:

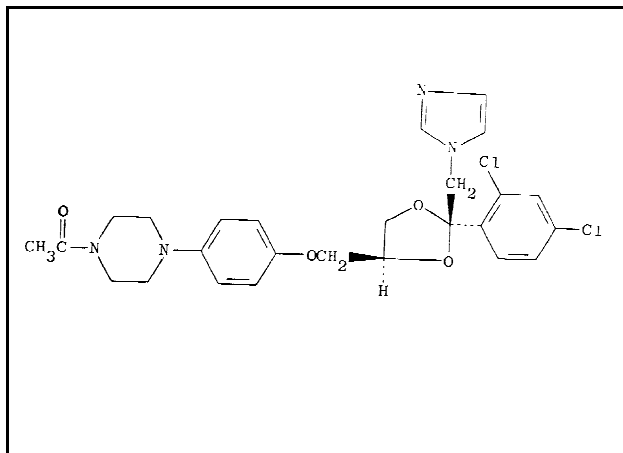
To make the spots visible in white light, dip the plate in a ninhydrin solution prepared by dissolving 100 mg of ninhydrin, 2,2-dihydroxy-1,3-indanedione (Sigma Chemical Co. No. N4876), in 25 mL of acetone. Place 7 mL of this solution in the detection bag instead of the iodine developing solution. The 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 100°C 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 100°C 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: C₂₆H₂₈Cl₂N₄O₄ - 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:

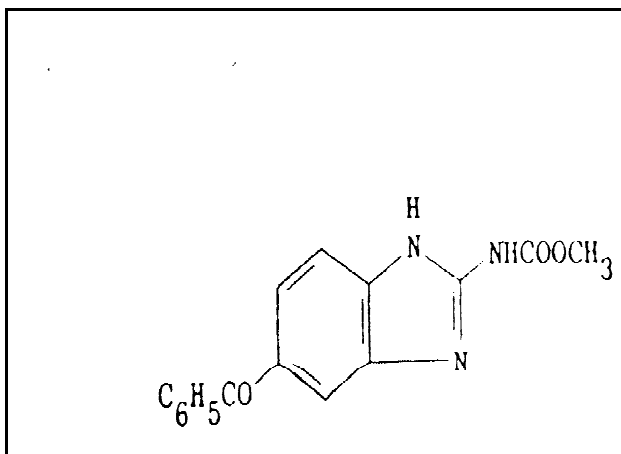
Dry the plate and observe under UV light. The maximum is at 225 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

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: C₁₆H₁₃N₃O₃ - 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 50°C 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:

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 the toluene-glacial acetic acid. 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 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:

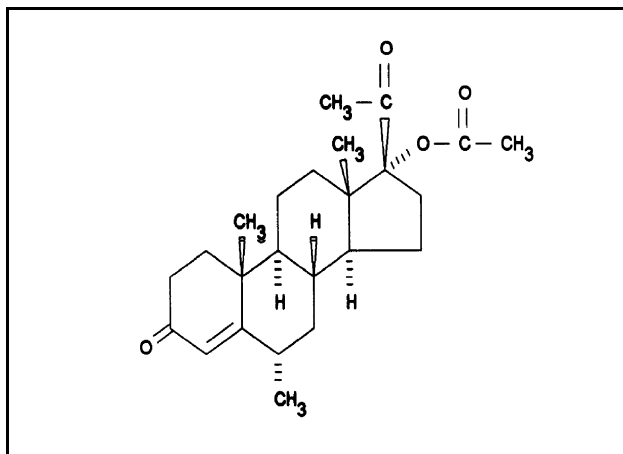
Dry the plate and observe under UV light. The maximum is at 245 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

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 = $(5 \text{ mg/mL} \times 1 \text{ ml})/10 \text{ mL} = 0.50 \text{ mg/mL}$. 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%) = $0.50 \text{ mg/mL} \times 1.15 = 0.575 \text{ mg/mL}$. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: $(4.75 \text{ mg})/(0.575 \text{ mg/mL}) = 8.26 \text{ mL}$ 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%) = $0.50 \text{ mg/mL} \times 0.85 = 0.425 \text{ mg/mL}$. 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.

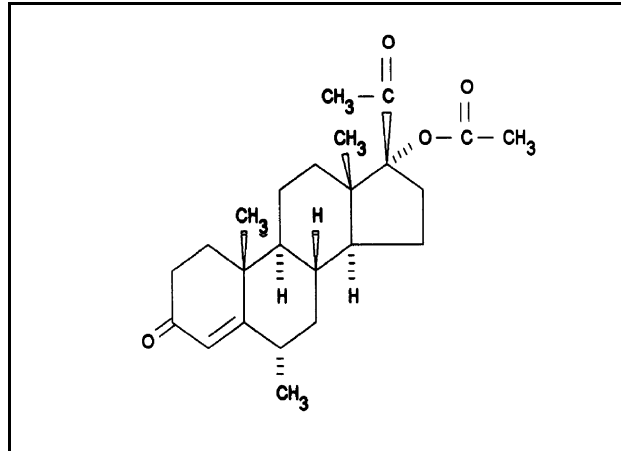
Iodine stain

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 = $0.50 \text{ mg/mL} \times 1.15 = 0.575 \text{ mg/mL}$. Weigh approximately 5 mg of standard. If you weighed 4.6 mg of standard, dissolve it in: $4.6 \text{ mg} / 0.575 \text{ mg/mL} = 8.0 \text{ mL}$ 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 = $0.50 \text{ mg/mL} \times 0.85 = 0.425 \text{ mg/mL}$. 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:

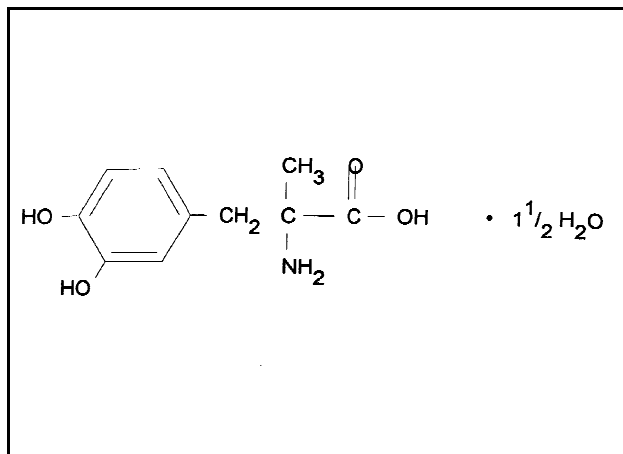
Observe spots under UV light for sample concentration = 5 mg/ml.

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: C₁₀H₁₃NO₄ 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:

High standard:

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.

Low standard:

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.

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:

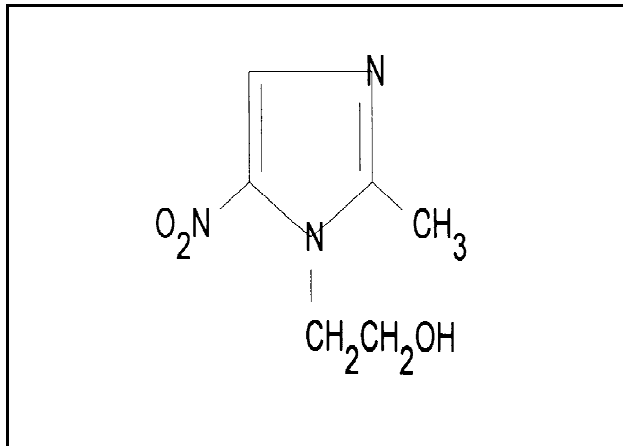
Allow the plates to dry and observe under UV light at 254 nm. Observe the size and the intensity of the spots.

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: C₆H₉N₃O₃ - 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;

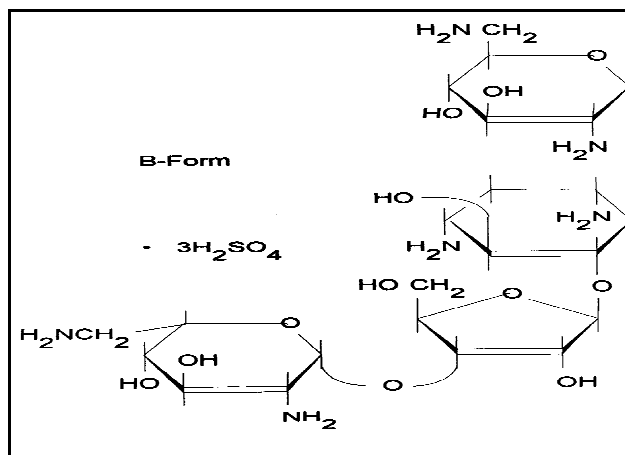
Dry the plate and observe under UV light. The maximum is at 305nm but at 254nm will work. Observe the size and intensity of the spots.

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; C₂₃H₄₆N₆O₁₃ - 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:

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 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.

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 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.

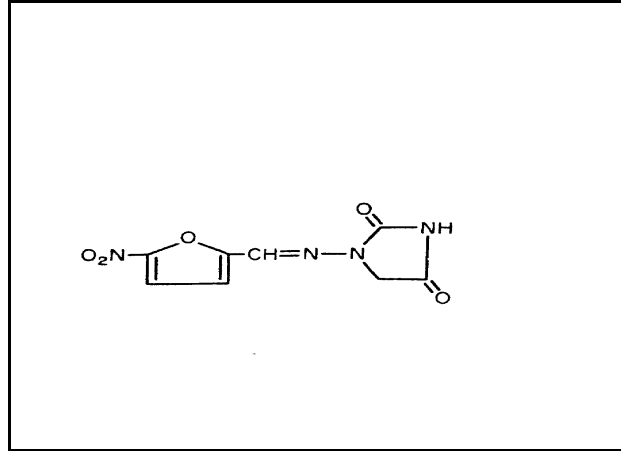
Visible:

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: C₈H₆N₄O₅ - 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 50°C 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:

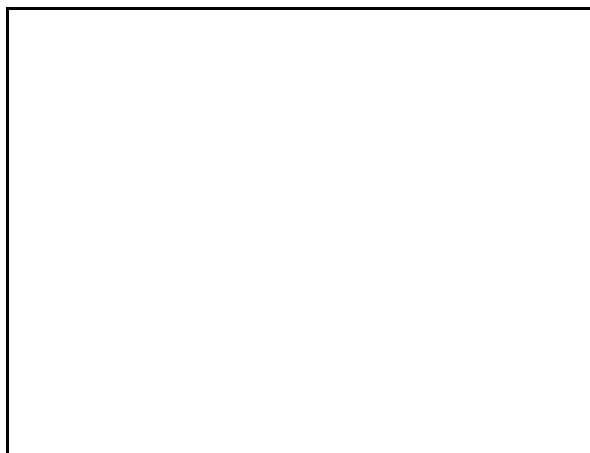
Dry the plate and observe under UV light. The maximum is at 360 nm but a short wavelength UV light (254 nm) will work. Observe the size and intensity of the spots.

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 = $(10 \times 0.5 \text{ mg})/10 \text{ mL} = 0.50 \text{ mg/mL}$. 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 = $(0.50 \text{ mg/mL}) \times 1.15 = 0.575 \text{ mg/mL}$. Weigh approximately 5 mg of standard. If you weighed 4.75 mg of standard, dissolve it in: $(4.75 \text{ mg})/(0.575 \text{ mg/mL}) = 8.26 \text{ mL}$ 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 = $(0.50 \text{ mg/mL}) \times 0.85 = 0.425 \text{ mg/mL}$. 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.

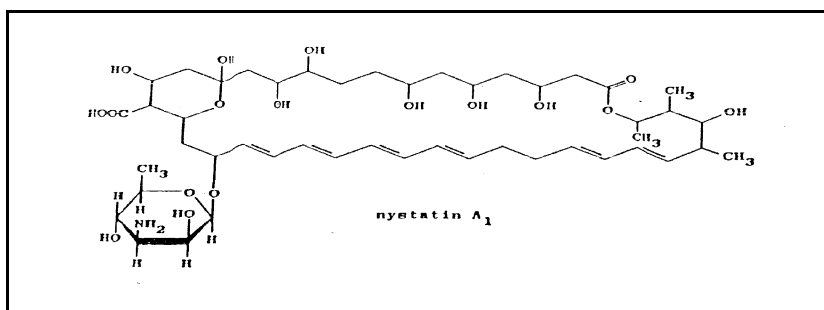
Iodine stain:

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: C₄₇H₇₅NO₁₇ - 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:

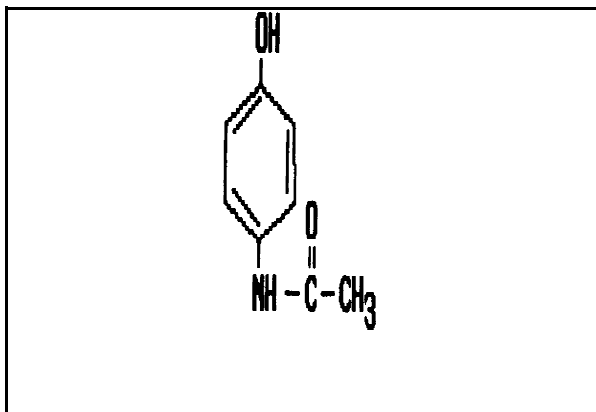
Dry the plate and observe under UV light. The maximum is at 300 nm but a short wavelength light (254 nm) will work. Observe the size and intensity of the spots.

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 = $325 \text{ mg}/50 \text{ mL} = 6.5 \text{ mg/mL}$. 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.

High reference solution:

The high limit for the reference is equal to 115%; therefore the concentration of the high reference solution = $(5 \text{ mg/mL}) \times 1.15 = 5.75 \text{ mg/mL}$. 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: $(46 \text{ mg}/5.75 \text{ mg/mL}) = 8 \text{ mL}$ of 95% ethanol.

Low reference solution:

The low limit of the reference is 85%; therefore the concentration of the low reference solution = $(5 \text{ mg/mL}) \times 0.85 = 4.25 \text{ mg/mL}$. 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.