



THE MULTI-PREP® THIN LAYER CHROMATOGRAPHY DRUG IDENTIFICATION AND TROUBLESHOOTING MANUAL

PRECAUTIONS:

1. For In Vitro Diagnostic Use only
2. Please read the entire manual before using this procedure!
3. Steps involving organic solvents or toxic spray reagents should be carried out in an operational fume hood. Do not pipet reagents by mouth

LIMITATIONS: The Multi-Prep Drug Identification System is a screening procedure designed to provide preliminary test results only. All positive test results should be confirmed by an independent and more specific method. Gas chromatography/mass spectrometry (GC/MS) is the confirmatory method of choice. Any reliance on positive findings from the Multi-Prep Drug Identification System for employment purposes or any other purpose is not advised without confirmatory testing.

1. WORKSTATION SETUP:

- a. A flexible rubber tubing with check valve) Nalgene 3 3/16" length x 1/4" i.d.) or pinch clamp is placed on the vacuum box hose nipple and run to a waste receptacle or sink.
- b. A vacuum hose is attached to the hose nipple on the column mounting plate vacuum control assembly and run to a vacuum pump. Make sure that the vacuum control valve is in the open position (fully counterclockwise). We recommend using a Gast Pump (5.5 cfm / 1/3 H.P., Cat # 3500805-5) during the aspiration step. Water removal during the aspiration step is very important for achieving good results during spotting and visualization. For this reason we recommend that workstations be aspirated one at a time unless the vacuum pump is strong enough to achieve at least 7" of Mercury with a multiple workstation setup.

Note: *Gast pumps must be maintained according to the instructions included with each pump. Urine and/or wash solutions must not be pulled directly into these pumps. If this inadvertently happens, the pump must be immediately flushed as per the maintenance instructions. Failure to do so may cause the pump to freeze up. As an added safety precaution many laboratories position a properly shielded trap between the pump and the vacuum box.*

- c. Multi-Prep or Detectabuse 15 mL Reservoir Extraction Columns with synthetic cotton filters, are placed in the column mounting plate and secured with a slight twist of the column. Unused holes are blocked with masking tape or tapered plugs.

2. COLUMN CONDITIONING

Columns are conditioned with 2 mL of Methanol.

3. DRUG ADSORPTION AND PURIFICATION: *All solutions pass through the columns by gravity flow*

A. Urine methodology:

1. Three mL of bicarbonate buffer pH 8.4 is added to each column. After the buffer has passed through the column, urine is added to each column. 10 mL of urine is generally recommended although most labs will find that 5 mL of urine is satisfactory for routine drug screening when using a single TLC plate.
2. When the urine has passed through the column (2-4 mins for 10 mL) the upper cotton filter is removed with a long hemostat or wooden applicator stick and a 5.0 mL wash of 0.35M phosphate buffer, pH 8.4 or a solution of sodium bicarbonate. 2-4% is immediately added to each column.

Note: *Sodium bicarbonate slowly converts to sodium carbonate with a concurrent increase in pH. Prepare solution at least weekly to minimize this effect.*

B. Serum/Plasma Methodology:

1. Column Conditioning: The upper cotton filter is removed and columns are washed with 2 mL of Methanol.
2. Sample Preparation:
For general drug extractions 2.0 mL of serum and plasma are diluted 1:1 with physiological saline or 10% ammonium chloride. A more specific extraction may be achieved by substitution of an acidic or basic buffer as the diluent (i.e. Cannabuse Acetate Buffer, pH 4.0, Cat # 1600102-5 or Carbonate/Bicarbonate Buffer, pH 9.2, Cat. # 1400002-0).
3. Diluted samples are now added to the conditioned columns.
4. When the diluted serum/plasma has passed through the column a 5.0 mL wash of 0.625M Phosphate Buffer pH 9.1 is added to each column.

Note: *Prepare wash buffer by diluting 1 part 0.25M Phosphate Buffer, pH 9.1 with 3 parts deionized water*

4. COLUMN ASPIRATION:

- a. After the wash solution has passed through the columns (2-4 mins) each column mounting plate loaded with washed columns is transferred to a second vacuum box set up for aspiration.* The pump is turned on and the vacuum control valve is turned fully clockwise. At this point the initial vacuum should read at least 7" of Mercury. While the first set of columns are aspirating a second set of samples are set up on the first "adsorption" workstation(s). The "adsorption" and aspiration workstations should be emptied after each use. The idea is to keep a continuous flow of samples moving from adsorption to washing to aspiration and spotting. If only one set of samples is being run then of course adsorption and aspiration may be done with one hardware setup.
- b. After 5 minutes the vacuum release valve is rotated fully counterclockwise and the vacuum pump is turned off.

Note: *It is important to keep in mind that the cleanliness of the extract and a good drug recovery is partially dependent upon the efficiency of the aspiration step. Any urine or wash solution remaining on the column contains pigment which may show up on the TLC plate. In addition recovery of certain polar drugs such as benzoylecgonine is adversely affected by water remaining on the column during the elution step.*

While the columns are aspirating, TLC plates are placed on the Multi-Prep TLC spotter and heaters are adjusted to 80°C. Sample applicator racks are loaded with sample applicator tips. The tips should be inspected at this time for air gaps between the cotton and powder matrix. Gaps are eliminated by inserting a wooden applicator stick through the plastic retaining ring and gently pushing the cotton down against the powder bed. The loaded rack is put into position over the TLC plates. (See spotter manual for operation details).

5. ELUTION SOLVENT

Elution can be accomplished using our regular Multi-Prep solvent (Ethyl Acetate:Isopropanol 85:15 or n-Butylchloride:Ethyl Acetate (80:20). The latter mixture spots more quickly and gives a somewhat cleaner extract without sacrificing extraction efficiency. Elution must be done under well ventilated conditions to minimize worker exposure. (See Safety Data Sheet).

6. SPOTTING

a. Single Plate Spotting

For single plate spotting one of two techniques may be used. The TLC column racks are put into position over the loaded sample applicator racks, loaded with columns and the sample extracts are eluted and spotted onto one plate.

METHOD 1 – One Step direct Spotting (Fastest Technique)

Pipet 1.7 mL of Multi-Prep elution solvent into each column and allow the solvent to drain by gravity flow through the column and sample applicator tip onto the TLC Plate (approx. 10 min.). No TLC significant drug residue remains in the column or tip using this method.

Note: Recent studies in our laboratory indicate that pigment continues to be eluted off the column even after all of the drugs have been eluted (1.2 mL). The excess 0.5 mL solvent added in this one step elution is used to wash all of the eluted drugs off of the sample applicator tip. This method is suitable for most laboratories however laboratories using a dipping technique incorporating Mandelin's reagent may wish to use Method 2.

METHOD 2 – Two Step Direct Spotting (Cleanest Technique)

Pipet 1.2 mL of elution solvent into each column and allow to drain through the column as in Method 1. After the solvent has drained through the column and the sample applicator tip the column rack is removed from the spotter and 0.5 mL of elution solvent is added to each sample applicator tip. This method of adding less solvent to the column and washing the sample applicator tip result in cleaner extracts with drug recoveries comparable to Method 1.

b. Sample Splitting – Direct Spotting Method

Method 1: a. The TLC spotter racks are put into position on the TLC spotters as in Para. 5a. The front two heaters are used with glass plates (80°C) and the two back heaters are used with glass plates (80°C) or plastic sheets (70°C). Plastic sheets should be held down with a glass TLC plate (or other suitable weight) positioned just behind the spotting area to prevent the sheet from lifting off the heater.

b. (1) The first TLC plate is spotted by pipetting 1.1 mL of Multi-Prep Elution solvent into each column and allowing the solvent to drain by gravity through the column and sample applicator tip onto the TLC plate (3-5 min.). When this first aliquot of solvent has spotted on the first plate the racks are lifted off the plate and repositioned over a second TLC sheet or plate. In general recovery of basic drugs is slightly favored in this first elution.

(2) The second plate is spotted by pipetting a second 1.0 mL aliquot of Multi-Prep Elution Solvent into each column as with the basic plate. In general, recovery of acidic, neutral drugs and benzoylecgonine is slightly favored in this second elution. (No TLC significant residue remains in the column or tip using this technique).

c. The TLC spotter racks are lifted off the spotter when columns have completely drained and the TLC plates are removed from the spotter when spotting is completed (columns and sample applicator tips are fully drained) and allowed to cool prior to TLC development.

METHOD 2 – Sample collection for splitting confirmation or manual spotting

A. COLLECTION

Load elution rack with the proper number of 15 x 85 mm disposable tubes and place rack in clean vacuum box.

Place column mounting plate with aspirated columns onto the rack using the location pins for proper positioning. (Note: The small workstation does not use location pins).

Add 1.2 mL of elution solvent to each column and allow to drain by gravity into the glass tubes.

Additional solvent may be added to the tubes at this time to make splitting more convenient. Eluates will contain more pigment if the excess solvent is added directly to columns during the elution step. See Note under 5.a.1.

B. SPLITTING

The collected eluates are poured or pipetted directly into the sample applicator tips (i.e. ½ for acid plate and ½ for basic plate) and allowed to spot directly onto the TLC plates. Some drug residue (25-30%) remains in each tip using this spotting technique. 0.3 mL of elution solvent may be added to each tip after the initial eluate has finished spotting in order to wash this remaining drug residue from the tips. Unwashed tips will contain enough drug residue for analysis by GC, GC/MS, HPLC, etc. An alternate method is to drop a sample applicator tip into the eluate and allow it to soak up elution solvent by capillary action. When the first tip is fully saturated (about 2 min.) it is removed and placed into the sample applicator rack. A 0.5 mL aliquot of elution solvent is added to the applicator and allowed to spot directly onto the TLC plate. A second applicator is dropped into the eluate and the process is repeated. Each sample applicator will pick up approximately half of the total sample eluate. No TLC significant drugs remain on the sample applicator tip using this technique.

C. SAMPLE CONCENTRATION

The collected eluates can be dried down and used for HPLC, GC, GC/MS analysis or manual TLC spotting.

D. MANUAL SPOTTING

After all of the elution solvent has been collected the column mounting plate is removed and one or two drops of 10% HCl in methanol are added to each eluate to prevent loss of amphetamines. Solvent is evaporated at less than 60°C. Hand spotting is accomplished in the traditional manner with spotting capillaries after first adding approximately 50 microliters of elution solvent to each dried down eluate.

DEVELOPMENT – TLC SEPARATION

1. When the Davidow TLC Development System is used the following instructions apply: ethyl acetate, methanol, concentrated ammonium hydroxide solution and water (17:2:1:0.4), are individually added into the developing chamber. When a standard glass tank is used it should be lined with filter paper in order to provide maximum saturation and it should be equilibrated for ten minutes before the TLC plate is inserted. In a multiple developing tank the plates should be arranged so that the layer surface of each plate faces either a heavy sheet of filter paper (cut slightly shorter than the size of the plate so the layer does not touch the paper) or, when more than one plate is developed at a time, the layers should face each other.

2. It is important that the solvent level is approximately 0.5 cm from the bottom of the TLC plate when the plate is first immersed and that it does not come in contact with the spotted samples. It is best to calibrate each tank for its proper volume. When a standard glass tank is used approximately 95 mL of solvent is required.

In a multiple developing tank approximately 190 mL of developing solvent is required.

The solvents should be freshly prepared for each run and mixed well just prior to use because after standing a gradient may form within the mixture.

3. Place the spotted plates in the developing tank. Quickly cover tank. (Note: Developing tank should be kept in a draft-free location.) After the solvent front reaches the 10 cm finish line, development should be stopped by removing the plate from the tank and pre-drying it with a hot air dryer. After pre-drying, keep the plate at 75°C

For ten minutes in an appropriate drying oven or on the Multi-Prep Spotter hotplate to remove the ammonia.

PREPARATION OF TLC SPRAY REAGENTS

Ninhydrin – Part A: Ninhydrin, 100 mg x 4 vials
Part B: 2,4,6 – Collidine in Methanol, 200 mL

Precaution: In vitro diagnostic reagents – For Laboratory Use Only. Dust Hazardous: Avoid contact with skin, eyes or clothing

Stability: See expiration date on label. Store un-reconstituted in dark at 15-30°C. Stable for three weeks after reconstitution when stored in cool, dark location.

Reconstitution: 2 mg of Ninhydrin is required for each 1 mL of diluent used.

Diphenylcarbazone – Part A: Diphenylcarbazone, 47 mg.
Part B: Acetone:Water (1:1), 500 mL

Precaution: In vitro diagnostic reagent – For Laboratory Use Only. May be irritating to skin and mucous membranes. Light sensitive

Stability: See expiration date on label. Store un-reconstituted in dark at 15-30°C. Stable for at least one working day after reconstitution. The color of the solution is a good indicator of whether it still may be used. It is useable until the color fades significantly from its original intensity of orange.

Reconstitution: Approximately 0.2 mg of diphenylcarbazone is required for each 2 mL of diluent used.

Mercuric Sulfate Solution – 0.25% Mercuric Oxide in 10% sulfuric Acid, 16 oz.

Precaution: In vitro diagnostic reagent. For Laboratory Use Only. Poison. Do not pipet by mouth. If swallowed call a physician immediately. Irritating to skin and mucous membranes. Corrosive – causes burns to skin and eyes. For eyes get immediate medical attention.

Stability: See expiration date on label. Store tightly closed at 15-30°C

Iodoplatinate – Solution A: 0.79% Chloroplatinic Acid in H₂O, 240 mL
Solution B: 19.35% KI in H₂O, 240 mL

Precaution: In vitro diagnostic reagent. For Laboratory Use Only. May be irritating to skin and mucous membranes.

Stability: See expiration date on label. Stable for two weeks after mixing when refrigerated

Preparation: Mix equal parts of A and B.

Dragendorf Solution – Part A: 0.95% Bismuth Subnitrate in 30.6% Acetic Acid, 240 mL
Part B: 11.21% KI in H₂O, 240 mL

Precaution: In vitro Diagnostic reagent. For Laboratory Use Only. Corrosive reagent – causes burns to skin and eyes. For eyes get immediate medical attention.

Stability: See expiration date on label. Store tightly closed at room temperature. Light sensitive – store in dark. Stable for two weeks after mixing when kept refrigerated and protected from light.

Preparation: Mix equal parts of A and B.

OPTIONAL SPRAY REAGENTS

Ammoniacal Silver Nitrate – 50% AgNO₃:5N NH₄OH (1:1)

Precaution: Irritating to skin and mucous membranes. Poison – may be fatal if swallowed. Get immediate medical attention.

Preparation: 505 AgNO₃ is mixed with equal parts of 5N NH₄OH immediately prior to use. If cloudy add NH₄OH drop by drop until solution clears.

10% Sodium Borohydride in 1N NaOH

Precaution: Caustic reagent – causes burns to skin and eyes. For eyes get immediate medical attention.

Preparation: Prepare only enough reagent for one day's work.

Sodium Nitrite – 2% in 1.5N HCl

Precaution: corrosive – causes burns to skin and eyes. For eyes get immediate medical attention.

Stability: slowly oxidized to nitrate when exposed to air.

N-(1-Naphthyl) Ethylenediamine – 2% Aqueous Solution

Precaution: May be irritating to eyes and mucous membranes. Poisoning may occur by ingestion.

Stability: Stable for at least two months when stored in dark, cool location.

DRUG IDENTIFICATION

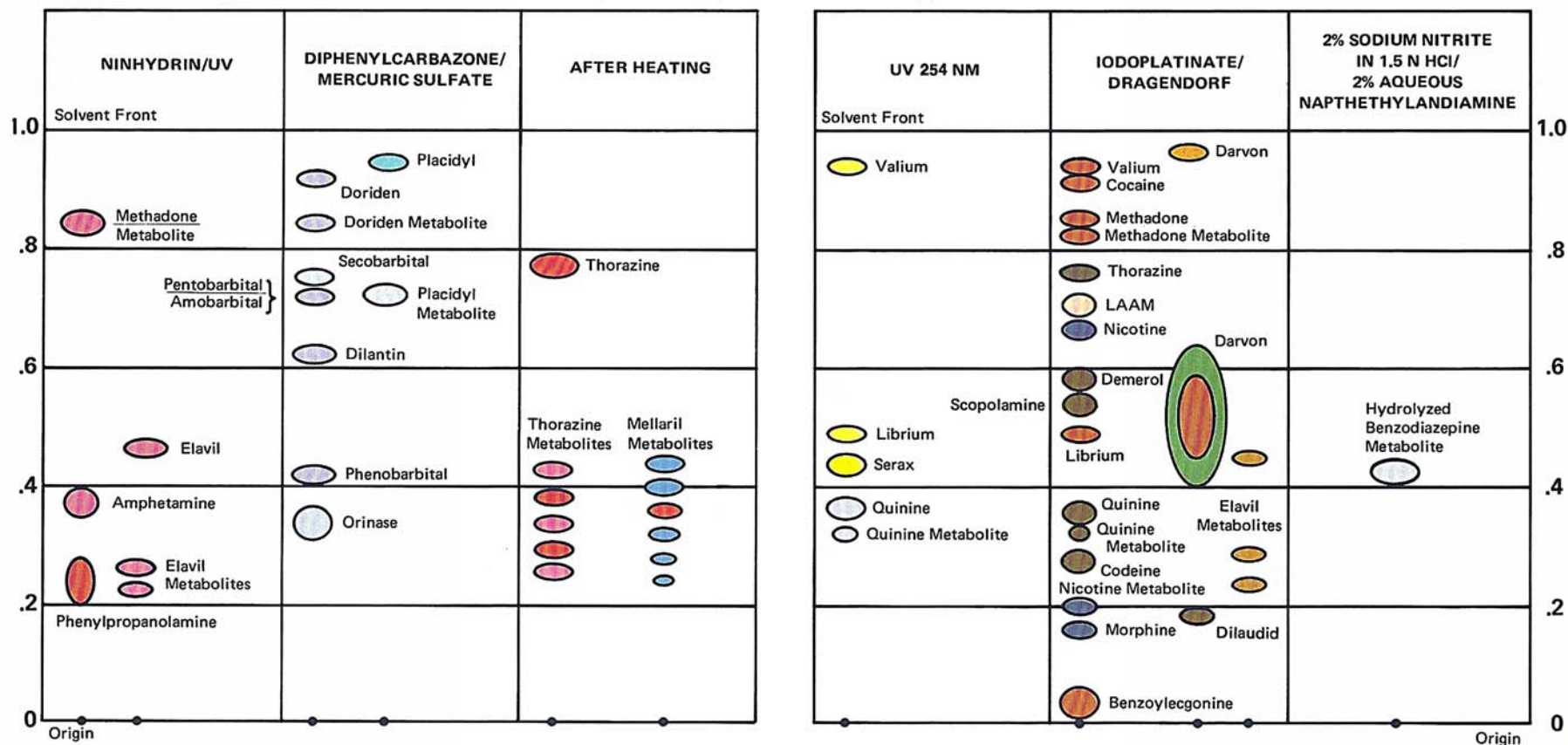
Rf values in TLC drug analysis vary depending on the solvent temperature and the degree of vapor saturation achieved in the developing chamber. It is recommended that drugs be identified by referring to the control standard on the same plate developed under identical conditions.

The spray sequence described will identify different drugs by consecutively spraying reagents specific for one or the other compound groups.

Since excessive applications of reagents will interfere with a successive test, over-spraying must be avoided. A yardstick for spraying is to stop application whenever the reference standard just begins to color. If only a single compound is to be detected higher sensitivities can be obtained by applying only those reagents specific for the compound, rather than a spray sequence.

Morphine in concentrations down to 0.2 ug/mL can be detected in this way. The presence of a drug is confirmed if the Rf value of the drug, as well as its specific color, are identical with one of the control standards. Specific colors are obtained by consecutively applying five different spray reagents.

TLC DRUG SEPARATION AND IDENTIFICATION CHART



TROUBLESHOOTING GUIDE

1. PROBLEM – Buffered urine does not pass through the column within five minutes

POSSIBLE CAUSES – Normally this problem is seen in a low percentage of samples (less than 5%). It occurs when old samples are used which have become viscous due to evaporation, microbial growth or sedimentation. An occasional fresh sample may also exhibit sedimentation following the addition of buffer. Any one of these conditions may contribute to the blockage of the resin bed during the urine adsorption step.

CORRECTION.

a. BEFORE URINE IS ADDED: Certain laboratories by virtue of their client mix must process a high percentage of “dirty” urines. Column beds used to process these urines should be loosened by inverting the columns and tapping them gently on the counter top before inserting them into the column mounting plate.

b. AFTER URINE IS ADDED:

1. The most stubborn samples may be helped along by removing the upper cotton filter and agitating the resin bed with a wooden applicator stick until flow resumes under vacuum.

-OR-

2. the sample can be repeated i.e. pour contents from plugged column into a fresh column.

-OR-

3. A new urine aliquot can be filtered or centrifuged prior to analysis with a fresh column.

2. PROBLEM – The column wash solution does not pass through the column within five minutes.

POSSIBLE CAUSES – Same as Problem 1.

CORRECTION – The remaining wash solution will usually pass quickly through the column during the column aspiration step. If this does not solve the problem then the resin bed must be agitated as in Method 1b.

3. PROBLEM – A high percentage (greater than 5%) of the columns do not drain by gravity during the first elution step.

POSSIBLE CAUSES – This condition occurs when the aspiration vacuum is too weak or not of long enough duration.

CORRECTION-

a. Make sure that the vacuum pump is large enough to create an initial vacuum level of at least 7” of Mercury. It is normal for the vacuum to drop below the initial reading as the columns begin to dry and more air is pulled through them due to the lowered resistance. If 7” of Mercury cannot be achieved then the aspiration time must be extended 3 minutes beyond the normal aspiration time for each inch of Mercury below 7”. Initial vacuum readings of less than 7” of Mercury may result in poorly draining columns and sample applicator tips even with the extended aspiration time.

b. Poorly draining columns can be helped along by forcing the first few drops of liquid (mostly water) through the column. The remainder of solvent should be allowed to flow by gravity in order to achieve proper sample splitting. The rationale behind this requirement is that a small amount of solvent with drugs remains on the column after the first elution is removed during the second elution.

4. PROBLEM – Liquid builds up in the sample applicator tips resulting in tiny spots or in extreme cases no spotting of the column eluate

POSSIBLE CAUSES –

a. There is an air gap in the powder bed blocking liquid flow.

b. The sample applicator tip is not making contact with the TLC plate.

c. There is excessive water in the applicator tip due to poor column aspiration.

d. The applicator tip is blocked with silica gel (usually accompanied by a hole in the silica gel beneath the sample applicator tip).

e. The plate temperature is too high

f. The solvent flow is blocked due to a defect in the sample applicator tip.

CORRECTION – After the elution solvent has drained through the columns, tips should be inspected for good solvent flow noting the relative solvent height in each tip. Solvent levels in poorly flowing tips will be distinctly higher than in normal flowing tips and the solvent spot will be too small or nonexistent. The poorly flowing tips should be lifted off the plate, tapped with the index finger to release trapped air and quickly dropped back into position on the surface of the TLC plate. If this does not work the sample applicator must be removed, pressure applied to the top of the tip to push out trapped silica gel by pressing with the index finger until a drop of liquid appears at the bottom of the sample applicator, and the tip quickly returned to the surface of the TLC plate. If all of the TLC spots are too small the temperature may be decreased until the desired spot size is achieved. If the applicator tip is blocked by a defect in the plastic the blockage may be removed by pushing a narrow pin or wire through the tip opening into the powder.

5. PROBLEM – TLC spots are too large.

POSSIBLE CAUSES

a. GLASS PLATES

1. The retaining spring clips on the Multi-Prep TLC Spotter heaters are loose allowing the plates to lift away from the theater (warping) forming an air gap which reduces heat transfer resulting in large TLC spots.
2. The heater temperature is too low. Glass plates should be spotted at 80°C. If the back heaters are being used check that the heater switch is on the “ALL” position. The back heaters are not activated when the heater switch is in the “FRONT” position.
3. The sample applicator tips are not touching the TLC plate allowing the solvent to drip onto the TLC plate.
4. The air intake on the underside of the Multi-Prep TLC Spotter chassis is obstructed, reducing the flow of air across each TLC plate.

CORRECTION –

1. Tighten the retaining spring clip screws.
2. Make sure that all heaters are at the proper temperature. Use caution over 90°C: plates may crack.
3. Make sure that all TLC applicator tips are touching the silica gel surface taking care not to push the tips into the silica gel which could block the tip causing reduced solvent flow.
4. Check the air intake for an obstruction – For Example: a plastic or paper sheet beneath the spotter will be pulled up towards the fan obstructing the air flow.

b. PLASTIC TLC SHEETS

POSSIBLE CAUSES

- 1-4. Same as with glass plates.
5. TLC plates are warping in the middle causing an insulating air gap.

CORRECTION –

- 1-4. Same as with glass plates.
5. a. The temperature should be reduced to 70°C to minimize warping.
 - b. A weight such as a glass TLC plate or weighted block the width of the TLC sheet should be placed on the TLC sheet approximately 1” behind the sample applicator tips to hold down the plastic sheet.