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## Toxicology using thin layer chromatography

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TOXICOLOGY USING THIN LAYER CHROMATOGRAPHY

By Arnold C. Fellman

A Thesis

Presented to the Faculty of  
The College of Medicine of the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree of Doctor of Medicine

Under the Supervision of

Milton Simons, M.D.

Omaha, Nebraska

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This is an era of chemicals, medicines, and drugs --- an era of poisons. And people, unfortunately, have the fallibilities of people. We arrive at the unavoidable consequence of poisoning of people. It thus becomes incumbent upon the physician to establish the nature of a particular "poison" from which a patient might become toxic, for as circumstances could have it, a person might be unable or unwilling to disclose the source of his intoxication. An obligation of the physician and the hospital is, therefore, established. Forensic medicine is an evergrowing responsibility, and ways to facilitate its efficiency must ever be sought.

The basic intent of this project, then, was to attempt a pilot study to determine the feasibility of establishing a hospital laboratory method for toxicology using Thin Layer Chromatography. Consideration was given to the elements of time, expense, procedure, accuracy, reproducibility, and predictability. The design of the project was such as to establish a method, and experiment with the variables concerned with the accuracy and predictability of controlled Thin Layer Chromatography procedures; and thus, to define certain limits as to the use of TLC (hereafter, the term Thin Layer Chromatography will be abbreviated as TLC) in toxicology and forensic pathology.

Information was drawn upon from numerous sources <sup>4-12</sup> dealing with the methods of TLC. It should be noted that by no means does this project attempt to exhaust the many and varied methods of utilizing TLC. Rather, a single derived method was established in detail and the factors involved in the feasibility of this method are dis-

cussed, with minute attention given to the simplest of procedures. Near the termination of the project several experiments were attempted utilizing the outlined method in solving practical toxicology problems. These attempts were undertaken solely to judge whether the results of the experimental method would be "in the ball park." Furthermore, the limits of the proposed method are in no way exhausted. Rather, because of the nature of this project, the conclusions drawn are those of inference and extrapolation based upon generally observed trends within the framework of this pilot study.

This writing, then, includes within its context the following:

- 1) a discussion of the need for a practical hospital laboratory method in toxicology, 2) a proposed method using the application of Thin Layer Chromatography including some of the principles involved; materials needed; procedures to be done; and notes on accuracy, sources of error, and hints for increased efficiency; as well as a proposed drug list for toxicological investigation, 3) a discussion of the variables inherent within each major aspect of the proposed method with regards to accuracy and predictability utilizing TLC in this manner, 5) a conclusion as to the usefulness of TLC in the general hospital laboratory, and 6) several case reports illustrating the use of TLC in toxicological investigation.

#### The Need

The desirability, if not, necessity for the physician to know what exogenous chemicals a given patient has in his system is probably too obvious to dwell upon, be they medical or legal reasons. For every

physician has probably been called upon to diagnose the etiology of symptoms and signs presented by a highly irritable or severely depressed or perhaps unconscious patient, and the answers are not always available by history! Furthermore, this patient may have ingested many more than one variety of "poison." The problem is ever present, indeed, increasingly present. A few examples deserve citing:

The leading cause of accidental poisoning by drugs in this country<sup>3</sup> is acetylsalicylic acid. Its consumption amounts to tons per day to relieve the discomforts of a painridden public who assumes that aspirin is a completely benign substance. Narcotics and analgesic drugs are of great therapeutic value resulting in large quantities being prescribed, and frequently overprescribed, each year, and each year they account for an increasing number of both accidental and suicidal poisonings. The situation is not unique to the United States alone. In Australia,<sup>1</sup> suicidal death occurs more than twice as frequently as accidental death, and the total rate of death from poisoning doubled in the period of 1961 to 1966.

Drug dependence is a leading factor in drug abuse. Better than one hundred substances have been listed which may result in drug dependence, action on the central nervous system apparently being the common denominator. In the United Kingdom in 1959 there were 23,000 known dependents on barbiturates and about 90,000 dependents on amphetamines, that is, more than one in 400 of that population were known drug dependents! The Federal Bureau of Narcotics of the United States figures show that one person in 4000 is dependent on a narcotic drug.

The problem of deliberate self-poisoning or attempted suicide by ingestion of a drug or chemical is especially common among teen-agers and adults. A study in 1964 at Cook County Hospital in Chicago<sup>4</sup> of 468 emergency room self-poisoning cases revealed that these patients used 102 different drugs and chemicals in the act. Table 1. is a rough breakdown of that group. Indeed, there are many unhappy people living

Table 1.— Cook County Study of Poisoned Teen-agers and Adults

Drug or Chemical Used	Number of Patients
Barbiturates	98
Aspirin	81
Tranquilizers	40
Rubbing Alcohol	13
Bleach	12
Rat Poison	9
Iodine	7
Lysol <sup>®</sup>	3
Ammonia	3
Other Drugs	171
Other Chemicals	31

in intolerable circumstances, and the seeds of, as well as overt, serious depression and psychiatric illness are present. The drugs for comfort and escape are quite available!

And, of course, the accidental poisoning of children is a continual threat. The incidence is highest among children from one to five years of age. The causes: careless housekeeping, poor heating facilities,

pesticides, insecticides, pica, negativism, imitation of parents taking pills, confusion with food (those good tasting "chewable" children's medications), chance, and so forth.

The mode of poisoning of 1,645 children admitted to Cook County Children's Hospital from January 1964 to June 1965 is listed in Table 2.

Table 2.-- Cook County Study of Poisoned Children

Agent	Number of Patients	Per Cent
Internal Medicaments		35
Aspirin	318	
Unknown Pills	127	
Phenobarbital	38	
Ferrous Sulfate	36	
Ex-Lax <sup>®</sup>	22	
Dilantin <sup>®</sup>	21	
Reserpine	17	
Corrosives		25
Bleach	284	
Lye	101	
Ammonia	24	
Lead Paint or Plaster	363	20
Hydrocarbons	149	10
Others	145	10

Then there are the Psychodelic drugs, the Hallucinogens: Peyote, mescaline, STP, lysergic acid diethylamide....! In brief, the need for

a practical hospital laboratory method in toxicology reflects the necessity of knowledge for the physician.

On the pages that follow, then, is a proposed method using the application of Thin Layer Chromatography. In addition to the steps involved in the method, certain principles involved are discussed, and noted on accuracy, sources of error, and hints for increased efficiency are included. Following the proposed method is a discussion of the variables inherent within each major aspect.



A Method

Preparation of TLC Plates

Materials.--

Silica Gel G with gypsum binder (Warner-Chilcott No. 8076 in 500 gm jar

Glass Plates - 20 cm X 20 cm and/or 20 cm X 5 cm

Spreader and aligning tray

200ml beaker and stirring rod

Sterile water (room temperature)

0.1 N NaOH and/or 0.1 N KHSO<sub>4</sub>

Plate Preparation.--

- 1) Wash with soap and water to remove all spots, streaks, and grease.
- 2) Place dried plates on aligning tray.
- 3) Wipe plate surfaces with methanol soaked towel, then dry.
- 4) Use immediately.

Procedure.--

- 1) Weigh out 15 gm Silica Gel into 200 ml beaker.
- 2) Add slowly 45 ml sterile water.
- 3) Stir solution for approximately one minute to dissolve all gel from walls and bottom.
- 4) Pour within one minute into applicator (which rests on right hand side of tray with arrow pointing to left).
- 5) Move applicator at even speed across plates and off left end (should require three seconds).
- 6) Allow to air dry until pure white.
- 7) Store as free from humidity and dust as possible.

8) When ready to use, heat activate at  $110^{\circ}$  C for 10 min, then use immediately.

Alternate Long-Term Procedure.--

- 1) Weigh out 200 gm Silica Gel into 1000 ml flask.
- 2) Add 500 ml either 0.1 N NaOH or 0.1 N  $\text{KHSO}_4$ .
- 3) Shake vigorously for 30 min, use immediately at 50 ml coating per five 20 x 20 cm plates (250 microns thick layer).
- 4) May be used during a three month period if shaken well before each use.
- 5) Use prepared plates within two weeks.

Notes.--

- 1) If Fluorescent plates are desired, add 1 ml 2',7'-dichlorofluorescein solution to silica-water mixture and mix in well.
- 2) Sources of error and trouble:
  - a) When dry, if plates are streaky or spotty, it may be due to spoiled Silica gel, dirty plates, or roughened spreader bottom.
  - b) Gel inadequately dissolved --- use a little warmer water.

Preparation of Specimen for TLC

Materials.--

10-15 ml of fresh or refrigerated whole blood specimen to be analyzed

Or 100 or more ml of fresh or refrigerated urine specimen

Centrifuge machine

Stable Tungstic Acid digestion solution (Hycel<sup>®</sup>)

100 ml volumetric flask

Small funnel and filter paper; and 200 ml beaker

250 ml separatory funnel

Three 200 ml beakers labeled: "basic", "acid-neutral", "inorganic"

Reagent chloroform, ethanal, and ethyl acetate

Water bath or hot plate

Supply of small (3-4 in) test tubes with labeling pencil

Conc  $\text{NH}_4\text{OH}$

Reflux apparatus

Activated Charcoal

0.1 N  $\text{H}_2\text{SO}_4$

Procedure.--

I) Blood

A) Digestion

- 1) Centrifuge 10 or more ml whole blood for 10 min at 1400 rpm.
- 2) Draw off serum (up to 5 ml), add to 100 ml volumetric 3/4 filled with Tungstate digestion solution. Make to 100 ml with Tungstate solution. Shake well.
- 3) Filter into 200 ml beaker.

B) Extraction

- 1) Using separatory funnel, extract three times with 50 to 100 ml aliquates of chloroform. Collect chloroform (bottom) layer in 200 ml beaker marked "acid-neutral."
- 2) Alkalinize fraction remaining in separatory funnel with conc  $\text{NH}_4\text{OH}$  to pH 9-10.
- 3) Extract three times with 50 to 100 ml aliquates of chloroform:alcohol:ethyl acetate - 4:0.5:0.5. Collect organic (bottom) layer in

200 ml beaker marked "basic". Retain inorganic (top) layer as precaution.

4) Evaporate chloroform-organic layers of basic and acid-neutral beakers using water bath or hot plate such that gentle boiling of evaporant ensues. Continue process until 1-2 ml of concentrate remains (watch carefully!). Allow this to evaporate, off of heat, until about  $\frac{1}{2}$  ml of concentrate remains in bottom of beaker.

5) Add  $\frac{1}{2}$  ml methanol by rinsing walls of beaker.

6) Pipette this 1 ml specimen into a small test tube and label.

7) Add 1 ml methanol to beaker again, working it around bottom. Pipette this into a second small test tube and label.

8) Allow material in these test tubes to evaporate to about  $\frac{1}{2}$  ml each. This, then, is the spotting material.

## II) Urine and other Fluids

### A) Digestion - using strong hydrolysis

1) Add 10 ml of 1.0 N HCl to every 100 ml of specimen.

2) Reflux gently for 1 hr.

3) Add activated charcoal to remove any abnormal color if present.

4) Cool. Filter through paper. Check pH 4-5, adjust accordingly.

5) Using about 150 ml proceed to extraction.

### B) Extraction (same as above)

## III) Standard Drug Solutions (Controls)

1) Dissolve 0.04 gm of commercially available drug in 20 ml absolute ethanol.

2) If drug is insoluble in ethanol, dissolve 0.04 gm in small volume of 0.1 N  $H_2SO_4$  and dilute to 20 ml with ethanol.

## Notes.--

- 1) If amphetamines are suspected, these are very volatile and evaporation must be carried out so as not to lose them. A suggested method is evaporation in an oven overnight.
- 2) Sources of error and trouble:
  - a) Extraction must be performed vigorously, then layers must be allowed to separate completely.
  - b) Evaporation must be gentle enough to prevent loss of drugs.
  - c) Specimen must be allowed to evaporate down to  $\frac{1}{2}$  ml or less so that it will be concentrated enough.

## Spotting Plates

## Materials.--

110° C oven

Micro-capillary tubes

Marker template

Pointed Marker

## Procedure.--

- 1) Heat activate prepared plate vertically at 110° C for 10 min. Use immediately thereafter.
- 2) Mark plate with pointed marker using guide marks of template, i.e. dot every 1 cm on a horizontal line  $1\frac{1}{2}$  cm from plate bottom.
- 3) Scratch a horizontal line where solvent front run end is desired (eg. 10 cm, 12 cm, or 15 cm).
- 4) Scratch vertical lines on both sides of plate to cut off thin edges.
- 5) Spot specimens on plates, acid-neutral on one plate (label) and basic

on the other, using spot sizes that remain under  $\frac{1}{2}$  cm in diameter. Allow spots to dry before succeeding applications. Apply spot 3 to 4 times placing two adjacent spots of the same specimen at 4 or 5 different areas across plate so that average Rf values can be determined, and so that different detecting sprays can be used on same plate. Allow all spots to dry thoroughly before developing.

Notes.--

- 1) For smaller spots use graduated micro-pipettes.
- 2) For quicker drying of spots, maintain warm plate by setting on warm hot plate.
- 3) If specimen appears too dilute, apply 5 to 10 times at a given spot.
- 4) Stahl<sup>12</sup> recommends use of regular dotted line across front line end instead of solid line. The most accurate method is to mark front end after development while plate remains in tank. This will provide exact solvent front for more accurate Rf determinations.
- 5) Sources of error and trouble:
  - a) Spot sizes should be kept small enough so as not to over-lap.
  - b) Spots must be centered on starting line.
  - c) Crystalline suspensions (instead of proper transparent solutions) of specimen may cause excessive tail formation in development.

Determining Proper Solvent System

- 1) Adjustments needed to effect TLC separation are generally more readily accomplished by altering solvent system components.
- 2) Since adsorption chromatography relies on electrostatic attraction, a solvent's eluting power correlates with its dielectric constant. Rf

values are inversely proportional to the attractive force between the sorbent (eg, Silica gel) and the material sorbed (eg, drug specimen), the weaker the force the higher the compound can climb on the plate, that is, the greater the Rf value. This principle works with adsorption chromatography such that the higher the dielectric constant of the solvent system, the higher the Rf value. Other concurrent chromatographic mechanisms such as partitioning and ion exchange will negate this principle. Blending solvents yields a solution with a dielectric constant satisfactory for separation of given specimens' components. Lists of tried solvent systems are available in several of the references cited in the bibliography. The method proposed herein utilizes varying combinations of chloroform, methanol, and acetone.

#### Quick Screening Method for Determining Proper Solvent System

- 1) Make up small quantities of several different solvent systems.
- 2) Place several specimen spots on separated areas of a heat activated plate.
- 3) Drop one or two drops of a given solvent system on to the center of the specimen spot.
- 4) Spray with the predetermined spraying detection reagent.
- 5) A given drug in a specimen will migrate with the spreading drop of solvent a given distance. A satisfactory solvent system is one in which the drug forms a ring half way between the original spot and the solvent front.

## Preparation and Use of Developing Chamber

## Ascending Development

## Materials.--

Two developing chambers (tanks) with lids (must be even and large enough to receive plate size used); eg, 21 X 21 X 9 cm rectangular tanks.

Sealing gel (eg, Silicon grease)

Supply of Whatmann paper and tape

Supply of chloroform, methanol, and acetone

## Procedure.--

- 1) Line smaller sides of clean, dry tank with exact duplicate sizes of Whatmann paper such that  $3/4$  to  $4/5$  of each of both sides is covered. Secure with small piece of tape at top edge.
- 2) Fill tank labeled "acid-neutral" with 100 ml of established solvent (eg, chloroform: acetone - 9 : 1). Fill tank labeled "basic" with 100 ml of established solvent (eg, chloroform:methanol - 85:15).
- 3) Saturate Whatmann paper by tilting tank, keeping lids on tank.
- 4) Equilibrate tank with solvent prior to its use by sealing lid on with petroleum jelly or Silicon grease to insure airtight seal, then allow tank to stand for at least one hour at room temperature in draft-free location. Before each use, the liner strips are re-saturated with solvent and the tank again equilibrated.
- 5) Set spotted plate in appropriate tank resting starting edge evenly on bottom and tilting plate at  $10-15^{\circ}$  slant; cover with lid immediately using jelly seal.



6) Allow to run until solvent front travels desired distance. If etched horizontal line is used as end point allow front to reach line over entire length. If estimated end point is used, remove lid and mark solvent front at various points along solvent front while plate remains in tank, then remove plate and let dry. With the above solvent systems, runs require about 20 - 30 minutes at room temperature.

Notes.--

1) For more accuracy:

a) Line all four walls evenly with Whatmann paper.

b) Use blocking apparatus on tank bottom to maintain all plates at same angle, or use metal plate rack inside tank to hold plates at constant angle; in this case, about 300 ml of solvent is required in tank.

2) Sources of error and trouble:

a) Keep temperature around tank even on all sides. For more rapid development, incubate entire tank.

b) Protect chambers from direct sunlight.

c) When placing plate in tank, be sure not to joggle tank or plate such that the solvent splashes unevenly.

d) Make certain silica gel contacts solvent at all areas of plate bottom, and that spots are above solvent such that solvent must climb to reach them.

e) Oxidizable compounds may decompose during application. (See Stahl <sup>12</sup> p 17).

f) Utilize a given solvent mixture for no more than five runs before

replacing with fresh solutions. Remember that the more volatile components of the solvent mixture will evaporate faster thus changing the component ratio and, therefore, the Rf values!

#### Principles Affecting Rf Values

- 1) Increased temperature and time of activation produce uniform nearly linear increase in adsorption power of silica; therefore, Rf values decrease. Therefore, use uniform temperature and time for activation and use plates at uniform time after removal from oven as plates deactivate quickly when allowed to stand unprotected.
- 2) Increased solvent dielectric constant produces increased Rf values.
- 3) Increased solvent polarization produces increased Rf values.

#### Detection by Spraying

##### Materials.--

Exhaust hood

Chromatography spray assemblies, preferably:

2 spray cans with guns, and 10 amber spray jars with lids

Glass plates for blocking: 3 5 X 20 cm plates and 2 20 X 20 cm plates

Short wave U.V. light assembly (Wood's lamp)

Chemicals for sprays (see Reagent list)

Developing tank with lid

##### Procedures.--

#### A) General

- 1) Set developed dried plate at an angle so that glass plates can be used to block off all but the desired spot-set's column.

- 2) Hold all sprays vertical to plate about one foot away and spray with a wavy motion so that all areas are covered with an adequate, even amount of spray.
- 3) Allow area to dry about 30 seconds before covering to move to next area.

#### B) Acid-neutral plates

- 1) Florescene spray: spray first column, allow to develop for at least one minute. Examine under U.V. light in darkened room. Barbiturates, glutemide, caffeine, and phenacetin are some drugs that will appear as dark absorbing spots.
- 2) Iodine spray: spray on first column (over florescene) first with sol A and then with sol B. Caffeine and phenacetin show up as brown spots within three minutes. Glutemide may also stain, as will lipids running near the front.
- 3) Mercurous Nitrate spray: spray second column. Barbiturates will appear within 30 seconds as gray spots.
- 4) Mercuric Sulfate spray: spray third column, first with sol A, let stand 5 minutes. Often barbiturates appear as white spots. Counter spray with sol B. Barbiturates and glutemide appear blue to purple as blue background fades. These spots may fade rapidly, therefore, record soon.
- 5) Furfural stain: spray fourth column with Furfural spray, let stand 5 minutes. Then stand in developing tank containing a thin layer of conc HCl. Cover. Meprobamate and etkinimate appear as dense black spots within 5 minutes.

## C) Basic plates

- 1) Florescene spray: spray first column, allow to develop at least one minute. Examine under U.V. light in darkened room. Phenothiazines appear as dark absorbing spots. Quinine appears as luminous blue spot.
- 2) Iodoplatinate spray: spray on first column (over florescene). Alkaloids (all basic drugs) appear as blue, violet, purple or gray black spots within 5 to 10 minutes.
- 3) Dragendorff spray: spray second column with the mixed solution. Alkaloids appear as orange to red spots within 30 to 60 minutes.
- 4) FPN reagent: spray third column. Phenothiazines appear as multicolored spots. Some fade soon afterwards.
- 5) Ninhydrin spray: spray fourth column, then irradiate for 15 minutes with U.V. light. Amphetamines appear as red spots.

## Notes.--

- 1) Sources of error and troubles:
  - a) Blocking plates must be closely applied to plate so that sprays won't diffuse into adjacent columns.
  - b) Sprays must be held far enough away to prevent dripping.
  - c) Spray apparatus must produce a fine mist so that drops aren't thrown at plate.
  - d) Spray reagents must be fresh, and spray gun must be cleansed of preceeding spray.
  - e) Adequate time must be allowed for some reactions to develop, but quick fading reactions must be recorded at once.

- 2) Aids to speed development of reactions:
  - a) Heat in low oven (60-70° C), but guard against baking which will discolor plate.
  - b) Hot or cold air may be used to aid drying..

#### Quick Screening Method for General Drug Classification

- 1) Heat activate one plate.
- 2) Using 3 to 4 applications per spot, apply spots across the plates in a line leaving about 2 cm between spots.
- 3) Using glass plate blocks, spray a sequence of reagents, one per spot.
- 4) Specimens containing adequate concentrations of drugs will react with the identifying color of a given group, eg, barbiturates will produce a bluish spot when sprayed with Mercuric Sulfate reagent; alkaloids will show as a gray spot with Iodoplatinate reagent, etc.

#### Note.--

- 1) This method may also work if spots are applied to a pre-sprayed section of the plate for some reagents.

## Reagent List

I) Solvents for Ascending TLC:

- A) Acid-Neutral Group - chloroform:acetone 9:1
- B) Alkaloid Group - chloroform:methanol 85:15

II) Detecting Reagents for Acid-Neutral Group:

- A) Florescein spray - prepare 2 gm% solution of 2',7'-dichloro-fluorescein in 95% ethanol.
- B) Iodine spray - sol A: weigh 2 gm of iodine, 1 gm of KI and make to 100 ml with 95% ethanol.  
- sol B: mix 1 part 25% HCl v/v with 1 part 95% ethanol.
- C) Mercurous Nitrate spray - prepare 1% aqueous solution of mercurous nitrate.
- D) Mercuric Sulfate spray - sol A: suspend 5 gm of red mercuric oxide in 20 ml conc  $H_2SO_4$ , cool, and dilute to 250 ml with water.  
- sol B: prepare 50 mg% soln of diphenylcarbazone in chloroform.
- E) Furfural stain - prepare 10% soln of redistilled furfural (fresh) in ethanol. Use conc HCl for developing tank.

## III) Detecting Reagents for Basic (Alkaloid) Groups:

- A) Fluorescein spray - (same as for Acid-Neutral).
- B) Iodoplatinate spray - dissolve 1 gm of platinic chloride ( $PtCl_4$ ) in 50 ml water. Dissolve 10 gm of KI in 250 ml water. Mix both solutions and make to 500 ml with water. Store in dark bottle!
- C) Dragendorff spray - sol A: prepare bismuth nitrate 17% in

20% acetic acid.

- sol B; prepare 40% KI in water. Mix

sol A ; sol B ; glacial acetic acid ; H<sub>2</sub>O at 7 : 3 : 20 : 70.

Stable for one week in a dark bottle! Refrigerate.

D) FPN reagent - mix 5 ml of 5% FeCl<sub>3</sub> w/v, 45 ml of 20% HClO<sub>4</sub> v/v, and 50 ml of 50% HNO<sub>3</sub> v/v.

E) Ninhydrin spray - use prepared canned spray if available; or prepare fresh 0.4% ninhydrin in acetone.

## Drug List

Brand	Generic
<b>Tranquilizers</b>	
Stelazine	trifluoperazine
Compazine	prochlorperazine
Thorazine	chlorpromazine
Triavil	w/ perphenazine
Sparine	promazine
Mellaril	thioridazine
Permitil	fluphenazine
Equinil	meprobamate
Serax	oxazepam
Valium	diazepam
Librium	chlordiazepoxide

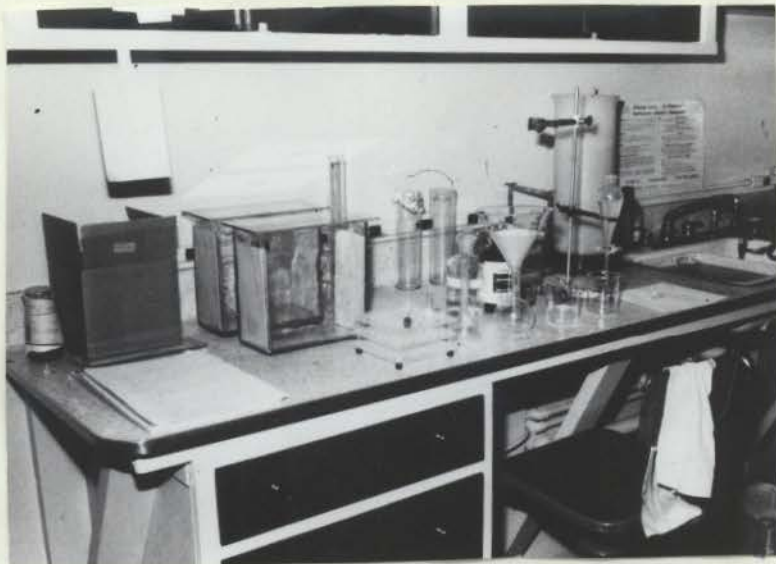
**Sedatives - Hypnotics**

Atarax	hydroxyzine
Nembutal	pentobarbital-Na
Luminal	phenobarbital-Na
Amytal	amobarbital-Na
Seconal	secobarbital-Na
Butisol	butobarbital-Na
Doriden	gluthemide



Brand	Generic
<b>Antihypertensives</b>	
Serpasil	reserpine
Harmonyl	deserpidine
Singoserp	syrosengopine
Aldomet	methyldopa
<b>Anticonvulsives</b>	
Dilantin	w/ diphenylhydantoin
Mysoline	primidone
<b>Antidepressants - Stimulants</b>	
Parnate	tranylcypromaine
Dexedrine	dextroamphetamine-SO <sub>4</sub>
Benzedrine	amphetamine
Elavil	amitriptyline
Perofrane	desipramine
Tofranil	imipramine
Ritalin	methylphenidate
Aventyl	nortriptyline
Caffeine	
<b>Anticholinergics</b>	
Belladenal	w/ atropine

Brand	Generics
<b>Antiarrhythmics</b>	
	papaverine
<b>Analgesics</b>	
Demerol	meperidine
Dolophine	methadone
Darvon	w/ propoxyphen
A.S.A.	phenacetin, aspirin, salicylamide
	morphine
	codeine
	heroin
	dihydromorphinone
	narcotine
<b>Anti-inflammatories</b>	
Butazolidin	phenylbutazone



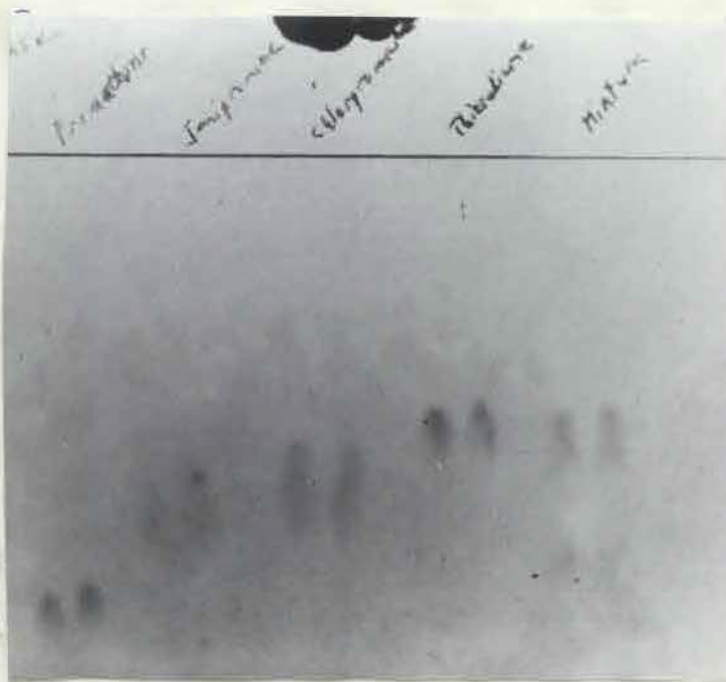
1. General Equipment



2. Spotting the Plate



3. Spraying the Plate



4. Markers Showing on the  
Developed Plate

## Variables within Aspects of the Method

## Preparation of TLC Plates.--

Uniform TLC plates are necessary for uniform, predictable results, and the achieving of such uniformity can be quite a problem. In my experience, factory prepared plates left some to be desired. The gel coating tended to be quite brittle and would easily break off the glass plate. Uniformity of thickness throughout the individual plates was not constant. It must be mentioned that the particular plates tried may have been defective samples and not representative of what is available now. Also, only one brand was tried! Suffice it to say that self-prepared plates are quite a bit less expensive than pre-prepared plates. But the act of their preparation requires ample practice and experimentation before consistent products are achieved. Also, the variables of storage time, humidity, temperature, and cleanliness may be very important factors to control.

## Preparation of Specimen for TLC.--

This aspect is the time consuming one. Also, since many of the "poisons" to be detected will be in relatively small quantities, careful and complete extraction steps must be followed, and evaporation must be carried to a point which will neither produce too little specimen to work with or burning of specimen, nor too great an amount and thus too dilute a specimen to show up on the plate.

## Spotting Plates.--

This aspect is fairly straight forward; however, it should be noted that uniformity of number of spots is necessary if any type of quanti-

tative work is to be attempted.

#### Preparation and Use of Developing Chamber.--

The reproducibility of Rf values is highly dependent on this aspect of the procedure. The major sources of error are enumerated in the method outline, and attention given to precision here will greatly aid in producing accurate, predictable results. It is most important to be aware of the different rates of evaporation of the different solvents.

#### Detection by Spraying.--

This aspect involves chemical reactions which will result in the appearance of the tell-tale markers which by their color and/or Rf value will betray the offending "poison". It must be remembered that chemical reactions require proper ingredients and therefore old reagents may give negative or else false results. The order of spraying may also be important, for some reagents will be effective when sprayed over others, and some will not. Also recall that some markers fade quite quickly and some require many minutes to show up. Above all it is important to have enough spots so that all necessary reagents may be used, and so that several spots of the same agent to be detected are available for control.

#### General Accuracy and Predictability Using TLC.--

As is readily apparent from the above two sections, there are numerous variables to be reconciled with in the use of TLC in the prescribed manner. If each little aspect is not carried out with careful attention payed to minute details, reproducibility is greatly

hampered. Indeed, TLC is a most sensitive medium for drug detection, in that its accuracy is easily disturbed. Predictable results demand exacting standards of both materials and procedural methods. In the short time I experimented with the method developed above, I was unable to produce consistent results time after time, even utilizing sample controls of known composition. When working with a few unknown specimens of blood, I was able to detect only a few of the drugs being used by the patients involved. However, these patients were not using toxic quantities. Later, the method was used on toxic patients with encouraging results. It became evident to me that the method, if used to its most careful extreme of precision, would produce useful, predictable and accurate results a fair percentage of the time. It will by no means detect all "poisons" within the specimens analyzed. Also, because of the closeness of Rf values of one drug to that of another drug, certainty of results in many instances will be in doubt.

#### Usefulness of TLC in the Hospital Laboratory.--

There are many "tools" utilized in the laboratory for analyzing the constituents of body fluids and tissues. Thin Layer Chromatography is one which, I have concluded, has a definite place in the field of toxicology. Its advantages include its sensitivity to minute quantities of substances, its several ways of differentiating between similar substances, its ability of testing for numerous different substances at the same time, its ability to retain a semipermanent record of results, its relatively simple and inexpensive component parts, its availability as a general screening procedure, fairly specific procedure, or if

desired, a quantitative procedure. Its disadvantages include its demand of exacting procedural methods and materials (ei, its numerous potential sources of error), the time requirements for its complete process, its inherent incapability of certain, positive identification. TLC used in the prescribed manner would serve only as providing a reasonable clue for the physician, but then all diagnostic procedures are thusly limited. For the perplexing problems involved in toxicology, any useful suppliment to the physician's armamentarium is a desired entity.

#### Illustrative Case Reports

##### Case No. 1.--

R.D., a local druggist, was brought into a local hospital with symptoms resembling an acute withdrawal reaction, possibly due to drugs. Upon questioning, he denied any drug use or abuse. Two samples of blood were received by the laboratory and evaluated by TLC. Both showed negative results for detection of blood levels of drugs. An eight hour urine sample was collected and evaluated by TLC. The results were weakly positive for a phenothiazine, and quite suggestive for amphetamines with an Rf value resembling a bi-amphetamine control. When confronted with these results, the patient admitted to his abuse and addiction to Seconal<sup>®</sup> and amphetamines. He was managed accordingly.

##### Case No. 2.--

D.N. was admitted by advice of his physician to a local psychiatric hospital for management of acute depressive neurotic behavior. A drug etiology was sought by history with none obtained. Blood



samples were drawn and analyzed by TLC for the presence of barbiturates and **Boriden**<sup>®</sup> which were high on the physician's index of suspicion. Results were negative for these as well as other possible depressant drugs. The physician considered these results in the management of his patient.

The above case examples demonstrate two of the uses of TLC in laboratory toxicology. The first case illustrates the use of positive results. The patient, when confronted by "scientific evidence" admitted to his drug abuse, and in this way could better be treated. The second case illustrates the use of negative results, which the doctor considered in his management. In neither case did TLC prove the use or disuse of drugs, but rather, it served as a useful tool in providing clues to aid the physician. This, then, is the value of Thin Layer Chromatography in toxicology.

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