Idaho State Police
Forensic Laboratory Training Manual
Thin Layer Chromatography

1.0.0 Backround

Thin layer chromatography (TLC) is an analytical technique that often offers a quick and easy separation of chemical compounds. The forensic chemist uses this technique primarily for screening drug samples and for isolating active constituents from solutions containing two or more compounds. TLC is especially applicable to drugs that cannot be analyzed by gas chromatography (GC). LSD, for example, may degrade on some GC columns; but is easily detected by TLC and sufficient quantities of the drug can be separated and isolated by TLC for further confirmatory techniques.

TLC is a physicochemical separation method. Since the technique is identical to the function of a micro column, the theoretical aspects of column adsorption chromatography are also applicable to TLC. The thin separatory layer (stationary phase) is usually placed on a support plate of glass. After dissolving a small portion of the sample mixture with an appropriate solvent, the solution is applied, as a spot, at the starting point of the plate, i.e., at the "origin." All necessary drug standards are spotted at the origin (and, occasionally, a combination of the sample and standard solutions applied as a single spot to verify chromatography resolution). After the "spots" have dried, the plate is placed into a TLC tank containing a suitable solvent (mobile phase or solvent system). The TLC tank is immediately covered and separation takes place as a result of capillary migration (development process). As the mobile phase moves over the adsorbed spot, the equilibrium is shifted and constituents present in the spot may be desorbed. The more tightly adsorbed compounds are desorbed to a lesser extent than the more loosely absorbed ones. A new equilibrium is established as the redissolved compounds are carried to the edge of the spot, where they come into contact with fresh adsorbent. Throughout this process, the composition of the mobile phase is continuously altered by the interchange of compounds between the adsorbent and the mobile phase. Whenever two compounds adsorb at the same site, the compound that is more strongly adsorbed, will displace the other. The displaced compound will then form a spot further away from the origin. The more similar the adsorptive properties of two compounds are, the more difficult it is to separate them. Compounds having nearly identical properties cannot be separated under most TLC conditions.

At the termination of the development process, the plate is removed from the TLC tank, air-dried and visualized (detection process). Under given conditions of temperature, solvent system and type of adsorbent, the chromatographic behavior of sample constituents is described in terms of "Rf" values. The Rf value is a characteristic of a particular substance and is described as the ratio of the distance traveled by the constituent to the distance traveled by the solvent. This can be expressed as follows:

$$Rf = \frac{\text{distance the (spot center) of the constituent traveled from origin}}{\text{distance the solvent front (mobile phase) traveled from origin}}$$

Distance for calculating Rf values are usually measured in centimeters. Since Rf values are a function of a number of variables, they should be considered only as guideline values.

2.0.0 Absorbents

Many adsorbents are used in TLC. These include silica gel, alumina, diatomaceous earth (kieselguhr), cellulose, magnesium silicate (florisil), ion exchange resins, and polyamide powder. These adsorbents may be purchased with or without either a binder (5-15% calcium sulfate, starch, or carboxymethylcellulose) and/or an inorganic fluorescent substance. The adsorbent is applied to a backing as a uniform coating. The most common support is a glass plate, but other supports such as plastic sheets and aluminum foil are also used.

Silica gel is the most popular adsorbent used by forensic chemists. It is slightly acidic in nature and works quite well for separating alkaloids.

Today, commercially available pre-coated TLC plates are widely used in forensic laboratories. Their popularity may be attributed to their high degree of coating uniformity, convenience, and moderate cost. Commercially available TLC plates come in a number of variations in order to facilitate their use for different drugs or different classes of drugs. Each variation is designated by the use of a specific suffix highlighted on the carton label. The suffixes "60", "90", or "150" indicate the mean pore diameter in angstroms. The suffix "G" indicates a calcium sulfate (gypsum) binder and "HL" a silicon dioxide/aluminum oxide binder. The designation "F" indicates a fluorescent indicator is present in the adsorbent, and the subscript number (i.e., F_{254}) gives the excitation wavelength for viewing the quenching of a fluorescent background. The letter "P" is the code designation for preparative thin layer chromatography, while "R" indicates a specially purified adsorbent. "RP" indicates a silanized gel for reverse-phase work. It is very important to select the proper type of pre-coated plate for a particular drug group.

3.0.0 Variables Which Affect TLC Rf Values and Separation

A number of factors can affect the reproducibility of Rf values and resolution so actual results may vary from literature values and may also vary from run to run. For this reason, it is preferable to run actual standards alongside unknown samples rather than make identifications based on calculated Rf values.

Some of the factors that can affect the reproducibility of Rf values and separation quality are:

1. The relative humidity and ambient temperature of the TLC system,
2. The degree of activation or the moisture content of the adsorbent,
3. The moisture content in the solvent system,
4. Accidental contamination of absorbent or solvent system,
5. Thickness of the adsorbent layer,
6. Presence of impurities (including undesirable commercial ingredients such as binders and preservatives) in the adsorbents or solvents used,
7. Possible reaction between the sample spot on the TLC plate and the solvent system, which may be further aggravated in the presence of the adsorbent material,
8. Sample degradation,
9. Variation in the properties between adsorbent batches,
10. The pH of the adsorbent and/or solvent system,
11. Chamber design or the degree to which equilibration is achieved and maintained within the chamber, and

12. The drying conditions of the TLC plate.

Of the above twelve factors, the most commonly encountered are contamination and extraneous water in the adsorbent. The finely divided adsorbent provides a large surface area, which can rapidly pick up any organic vapors in the atmosphere. These organic contaminants are often observed as a dark band at the solvent front, and are rarely serious enough to cause problems when monitoring the movement of different components with the solvent front. Another form of contamination that may be encountered is oil from the hands caused by careless handling of the plates before development. Oil from the hands can be transferred to the edges and back of the plates and dissolved by the solvent system. As the solvent moves up the plates, the oil is deposited throughout the adsorbent.

The amount of water present in the adsorbent, the sample, and/or the solvent system is probably the most important variable in TLC. The presence of excessive moisture can lead to distortion of the TLC separations, resulting in non-reproducible results. Moisture is present in the adsorbent in three forms: (1) water of constitution, (2) water of hydration, and (3) free water. The free water can be removed from the adsorbent by heating the thin layer plate to 105°C for one hour (preferably in a forced draft oven). Water in the free state is not necessarily detrimental to the chromatographic separation. It should be recognized, however, that the water level must be consistent from plate to plate. Water of hydration, if calcium sulfate is the binder, can be removed by drying at 180°C for one hour. The water of constitution can be removed by heating to 450°C - 500°C for several hours. In most cases, the water of hydration and the water of constitution are allowed to remain while the free water is removed by drying at 105°C for one hour. While always a potential problem, the moisture content of TLC plates is rarely found at levels that necessitate pre-drying of the plates prior to routine use.

Extraneous moisture can be introduced to the plate by blowing the dust from the plate, resulting in moisture from the breath being condensed onto the plate. Water may also be introduced at the point of sample application if the plate is not dried long enough to allow the water to evaporate after it has condensed from the atmosphere. The developing solvent may also contain water.

4.0.0 Solvents

Suggested Reading Reference (2) lists the most common TLC systems used for isolating numerous drugs. For each TLC system, all of the required conditions are described, e.g., the type of plate to use, sample preparation prior to spotting, solvent system, equilibration time, development distance, and the visualization method. Additional solvent systems applicable to drug analysis can be found elsewhere in the literature.

On infrequent occasions, external conditions, such as insufficient humidity, high temperature, etc., may lead to chromatography where component Rf values are consistently higher or lower. To a limited extent, this can be corrected by modifying the solvent system. For most drug applications, reducing the polarity of the solvent system will reduce the Rf values, and increasing the polarity of the solvent system will increase the Rf values. Care should be exercised when attempting to modify a solvent system since resolution may become adversely affected.

5.0.0 Visualization of the Developed Chromatogram

The first two steps in performing thin layer chromatography are spotting and development, but results cannot be evaluated without visualization. Spray reagents, which visualize certain chemical groups, are quite common in most analytical laboratories. They are suitable for detecting the drug in question, but since most of these reagents react with certain organic groups rather than with particular classes of drugs, considerable caution should be used in assigning specificity to their use. For example, ninhydrin, a general reagent for the detection of primary amines, is not specific for amphetamines, since positive reactions can occur from any primary amine. Therefore, it is important to establish the identification of a TLC spot on the basis of a sample/standard comparison (i.e., the color and Rf values with those of known drugs spotted on the same plated on which the unknowns were spotted.)

In addition to the chemical visualization method mentioned above, physical detection methods can also be used with many drugs. For example, quinine and LSD fluoresce naturally when they are exposed to long-wavelength UV light. Using their fluorescent properties, it is possible to detect them without chemically altering the sample. Some pre-coated plates are available with fluorescent indicators, which can be used to detect substances absorbing at particular wavelength. For example, sodium fluorescein fluoresces when exposed to UV light of 254 nm wavelength. Therefore, substances absorbing this wavelength will contrast sharply by appearing dark while quenching the greenish-yellow fluorescing background. After the spots have been visualized, they are compared with the proper reference standards and controls.

6.0.0 Elution of TLC Spots

A secondary advantage of TLC is that it permits elution of the migrated spots from the TLC plate for further analysis. Although this is now being superseded by preparative high performance liquid chromatography (HPLC), the method of TLC spot elution warrants mentioning because of its simplicity and because it can serve as an alternative.

As mentioned above, LSD can be isolated in sufficient quantities by streaking a sample extract onto a TLC plate and developing with the proper solvent system. The developed streak (conforming to the Rf for standard LSD) is scraped from the plate, transferred onto filter paper, and washed with a solvent to retrieve the LSD for further confirmation by a technique such as infrared spectrophotometry (IR). This identification can be accomplished within the time span of about an hour. (Because LSD free base is subject to slow degradation, elution and final identification should be performed immediately and without delay.)

7.0.0 Readings

1. Clark, Isolation and Identification of Drugs, 3rd edition, vol.1, pg 392-424.
2. Four articles dealing with TLC of LSD isomers, Microgram, Vol. VII, Dec 1974, pp. 149 - 155.
3. R. Tandon, "A New Solvent System For The Separation of Cocaine From Other Alkaloids by Thin Layer Chromatography, Microgram, Vol. XI, May 1978, pp. 82 - 87.
4. J. Delattre, B. Van Hoeck, "Thin Layer Chromatographic Separations of Heroin Mixtures, Microgram, Vol. XIII March 1980, pp. 36 - 39.

8.0.0 Exercises: These exercises may be omitted during marijuana training.

1. Compile a list of the TLC visualizing agents most commonly used in drug analysis. Also indicate the applications, advantages, and disadvantages, potential hazards, and safety considerations of each agent.
2. Spot LSD on a TLC plate. Irradiate the spot for 5 minutes with a UV light. Spot LSD again next to the irradiated spot and then develop the plate in a solution of 9 parts chloroform and 1 part methanol. Explain the results.
3. Evaluate by a TLC system suggested by literature:
   a) Mixture of cocaine and lidocaine
   b) Mixture of methamphetamine and amphetamine
   c) Mixture of methamphetamine and ephedrine
   d) Mixture of psilocyn and psilocybin
   e) Mixture of LAMPA (LSMP) and LSD

   Document your answers by photocopying the TLC plates and indicating approximate location of the spots. Also indicate the developing solvents used and the mode of visualization.

9.0.0 Questions:

1. Using non-technical terms, describe TLC as you would to a jury.
2. Why is spot size important when using plates that do not contain a pre-adsorbent area? How can spot size be minimized?
3. What effect, if any, will sample concentration have on TLC results? Explain.
4. What is the best way to preserve the results of a thin layer chromatogram?
5. Why do spots having a larger Rf value generally have a larger diameter than those with low Rf values?
6. What is the purpose of the binder on TLC plates?
7. What is silica gel?
8. Is TLC always a form of adsorption chromatography? Explain.
9. What is meant by "chamber saturation" and equilibration?
10. What is preparative TLC and how is it performed?
11. How can the chromatographic properties of TLC plates be modified?
12. When can TLC be used to identify a controlled substance?

10.0.0 History

Prior to revision 3 modules in the training manual did not have individual history pages.

Revision #	Issue or review date	History	Author or Reviewer
3	7/08/11	Updated reference 7.0.0, Added 10.0.0 Changed 8.0.0	David Sincerbeaux