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TECHNICAL ARTICLE

# STANDARD OPERATING PROCEDURE (SOP) FOR THIN LAYER CHROMATOGRAPHY (TLC)

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**ABSTRACT**: Thin layer chromatography (TLC) system plays very important role for analysis of several chemical compounds. TLC is a separation technique which is used for separating compounds that are dissolved in solution. It is a technique for identification and purification of mixtures of several components for analytical purposes. Applications are found in diverse fields such as in Forensic Science, Pharmaceuticals, Agriculture, Food and Flavor, Clinical tests and in many others. Standard Operating Procedure (SOP) of Thin layer chromatography has been developed. The different steps involved for operating Thin layer chromatography system have been successfully explained.

**KEYWORD**: SOP, Thin layer chromatography, TLC, mobile-phase

## INTRODUCTION:

Chromatography is the modern and versatile method used for the separation and purification of several compounds. The method was first discovered by T swett, a Russian botanist, in 1906, for the separation of colored substances into individual components. In chromatography, separation is achieved by the differential movement of individual components over a stationary phase under the influence of a mobile phase[1-4]. Thin layer chromatography is another type of adsorption chromatography. This involves separation of a mixture over a thin layer of an adsorbent such as silica, alumina etc. A thin layer of an adsorbent is spread over a glass plate of suitable size[5-11]. The solution of a mixture to be separated is applied as a small spot about 10–20 mm above one end of the TLC plate. The TLC plate is then placed in a closed jar containing the solvent. As the solvents moves up the plate, the components of the mixture move up along the plate to different distances, depending on their degree of adsorption, and separation takesplace[12-14].

### Purpose

To describe the standard procedure of TLC to ensure compliance with provision of Good Laboratory Practice Regulations .

### Scope

Describes the finest details of the steps to be followed in the one of the simplest but precision requiring analytical technique of TLC.

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All the scientific staff members carrying out the TLC are responsible for strictly adhering to the procedures given in this text.

**DIFFERENT STEPS OF STANDARD OPERATING PROCEDURE (SOP )**

**1. Preparation of thin-layer chromatography (TLC) plates**

1. Select the type of plate such as metal (aluminum) ,plastic or glass plate according to application.
2. Select the suitable size of TLC plate. The usual size of the plate is 20cm × 20 cm , although smaller sizes may be used such as 20cm x 10cm,10cm x 10cm etc.

3. The plate must be cleaned with a detergent followed by water in order to make it clear and completely free from any impurities

4. The cleaned plate should be dried in a hot air oven.

5. Select suitable adsorbent materials such as silica gel, alumina, aluminum silicate, bauxite etc.

6. The adsorbent silica gel is prepared by mixing silica gel and water in the ratio of 1:2 (1 part of silica gel two parts of water),

7.Mixture is continuously stirred in order to prevent the formation of lumps.

8. Once homogeneous slurry is formed, it is immediately poured on TLC plates and spread uniformly by tilting the plate or by help of TLC applicator.

9. All precaution should be taken that the slurry is spread uniformly over the plate as a thin film.

10. The coated TLC plate is then air dried for twenty minutes.

11. The TLC plate is then placed in the oven at $100^0$C for 30 minutes for activation.

12. After the plate is prepared, it should be kept in TLC Plate holder.

**Note**

1. Generally Home-made plates are less reproducible than commercially available plates.

2. The quality of 'home-made' TLC plates should be carefully monitored. Activation i.e. heating at 100 $^0$C for 30 min before use may be helpful in maintaining performance.

3.Preparing TLC plates by dipping glass plates into a slurry of silica with subsequent drying gives very variable results and is not to be recommended.

4. It is advised to use an applicator to apply the stationary phase, on the plate so as to get a uniform and thin layer on the plate. Lack of uniformity and thinness can badly affect the success of the experiment.

5. Experience suggests that it is best to standardize on a particular brand of commercially available plates, such as Silica gel 60 $F_{254}$. However, even with commercial plates batch-to-batch variations in retention time, and also in sensitivity may be encountered.

**2. Spotting of sample and standard on TLC Plate**

1. The TLC plate should be prepared by marking the origin by drawing a light pencilline at least 2 to 2.5 cm from the bottom of the plate without disturbing the silica surface in any way.

2. Another line should then be drawn on the plate 10 cm above the origin to indicate the position of the solvent front.

3. The samples and standards should be applied at the line with sufficient distance (Figure.1). Loading of Sample and standard should be performed using a micropipette or syringe or capillary.

4. Size of spot should be normal neither small nor large. If larger spots are produced then resolution will be impaired.

5. The volume of solvent applied should be kept between 5–10 µL of solution (containing about 10 µg of analyte).

6.Spot should be air dried.Donot force dry the spots by blowing air.

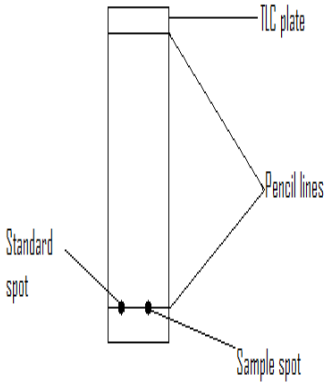



**Figure.1: Marking the line and loading of sample and standard on TLC plate**

### 3.Developing the chromatogram

1. Suitable amount of solvent is added in chromatography chamber.

2. The solvent should be added at least 20-30 minutes before the chromatogram is to be developed to saturate the atmosphere with solvent vapors.

3. The chromatogram is developed by placing the loaded plate in pre-saturated chromatography chamber.

4. It is very important to ensure that the level of the solvent is above the bottom edge of the silica layer on the plate but below the level of the spots applied to the plate(Figure 2).

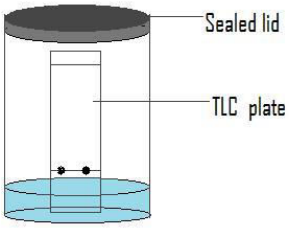



**Figure. 2: Development of chromatogram**

5. The developing of chromatogram should be observed to ensure that the solvent front is being drawn up uniformly.

6. The mobile phase movement is primarily due to capillary forces and, as the stationary phase is dry.

7. Set up must not be disturbed in order to obtain effective result.

### 4. Visualizing the chromatogram

1. Take out the plate from chromatography chamber after development and air dried.

2. The chromatogram should be examined under UV light under short and long wavelength (254 and 366 nm) in a suitable TLC viewing chamber.

3. If a fluorescent marker has been added to the silica, many substances present appear as dark areas against a fluorescent background.

4. The plate is sprayed with chromogenic/spraying reagent by using TLC sprayer.

5. In clinical toxicology the use of chromogenic chemical/reagents generally gives more useful information.

6.Plates can be dipped in reagent/chemical with special precautions otherwise silica tends to be lost and the chromatogram destroyed.

**5. Calculation of Retention factor ($R_f$)**

1. Retention factor or retardation factor can be calculated by using following formula. It is represented by symbol $R_f$.

$$R_f = Ls/Le$$

Where Ls is distance travelled by analyte and Le is distance travelled by solvent (Fig3)

2. $R_f$ value for sample as well as standard should be calculated.

3. Similar $R_f$ value of sample and standard qualitatively confirm the presence of compound.

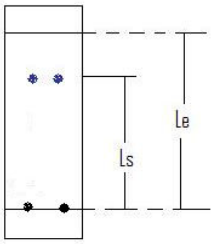
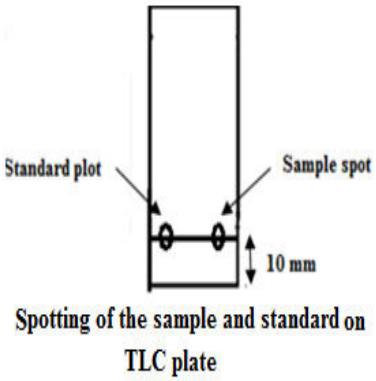



**Figure. 3: Calculation of $R_f$ value by measuring Ls and Le**

Whole process of TLC is summarized in Fig 4



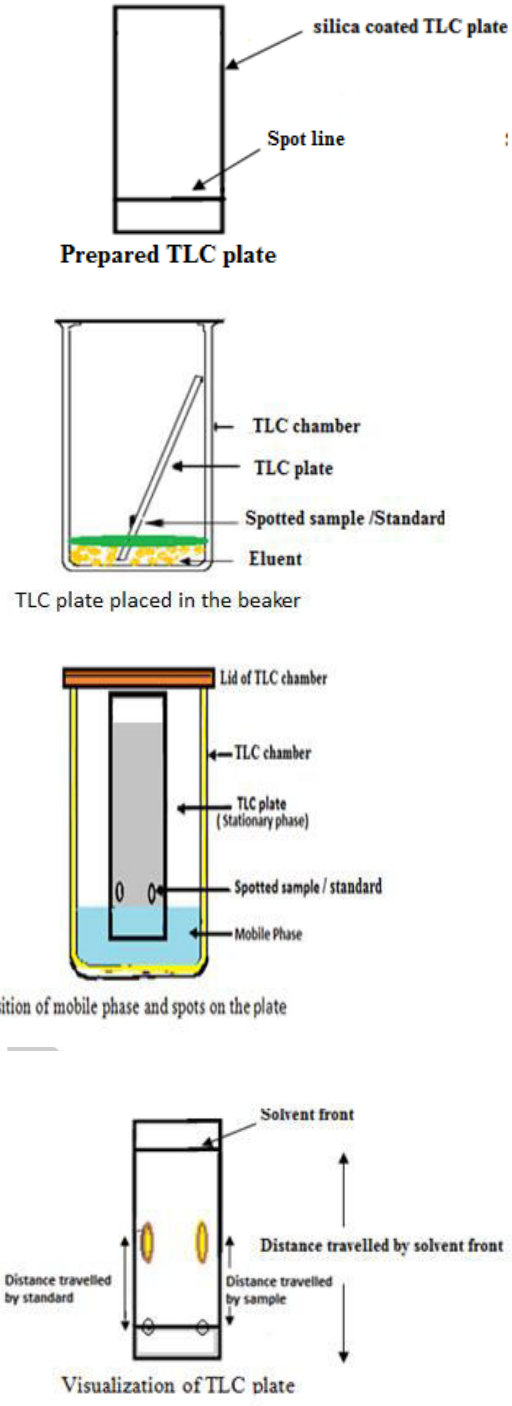




**Figure. 4: Different Steps involved while performing TLC(Complete Process)**

**SAFETY PRECAUTIONS FOR TLC**

1. For carrying out TLC of toxin/toxic chemicals, an area in the laboratory should be reserved for this purpose and all the TLC work must be restricted to that area only.

2. Surface on which TLC is carried out should be a non – absorbent.

3. This region must be protected from direct – sunlight.

4. Solvents used in TLC are highly flammable and highly combustible. Thus care should be taken to keep apparatus such as heaters, burners away from the TLC area.

5. The solvents used for TLC must be stored in safety cabinets.

6. Warning signs must be put up in regions where TLC of toxin/toxic chemicals is being carried out.

7. Spotting must be carried out in shallow trays that can contain the spillage of the standard solution.

8. In case of any spillage of standard, it must be cleared with filter paper and it must be disposed. The area can then be sprayed with a 4% solution of sodium hypochlorite or a detergent to clear it.

9. After experiment, all glass wares and TLC plates must be soaked is 1 % sodium hypochlorite solution for two hours in order to decontaminate them.

**DO'S AND DON'T'S WHILE PERFORMING TLC**

**Do's**

1. Sample preparation for TLCmust always be carried out in fume hoods.
2. Personal hygiene must be maintained (nails cut) and protective clothes and masks must be used.
3. Solvents used for TLC are highly toxic, volatile and carcinogenic hence gloves and surgical masks which covers mouth and nose must be used.
4. Safety spectacles must be work throughout the entire process.
5. A laboratory coat must be worn as several chemicals, dyes and spraying reagents are used which can stain clothes.
6. Spraying of TLC plates must be carried out in fume hoods or spray cabinets.
7. When viewing the plates under U.V light, the eyes should be protected by wearing spectacles or should be viewed through U.V. filters.

**Don'ts**

1. Do not eat, drink around areas where TLC is being carried out.

2. The used organic solvents must never be dumped in the sink.

3. The used organic solvents must be disposed off into an appropriate waste bottle.

3. Organic solvents such as acetone can be disposed of by keeping it on a water bath in a fume hood.

4. Do not force dry the spots with drier on the plates.

5. Do not keep plates in air, in order to protect them from moisture.

5. Do not disturb the TLC jarThe used organic solvents once the solvent start to run the plate.

6. Do not mix all organic solvents in a waste bottle as they can form an explosive mixture.

## REFERENCES:

[1] Berezkin VG, Mardanov RG, Maliovska I, Rozylo JK. Thin layer chromato- graphy with an isolated support and forced flow of the mobile phase. J Planar Chromatography. 2002;15:377-9.
[2] Campbell A, Chejlana MJ, Sharma J. Use of a modified flatbed scanner for documentation and quantification of thin layer chromatography detected by fluorescence quenching. J Planar Chromatography. 2003;16:244-6.
[3] Carpinella MC, Giorda LM, Ferrayoli CG, Palicos SM. Antifungal effects of different organic extracts from Meliaaze darachL. on phytopathogenic fungi and their isolated active components. JAgric FOOD Chromatography. 2003;51:2506- 11.
[4] Laboratory procedure manual, Forensic Toxicology. Selective and Scientific books Publisher and Distributors;2005.
[5] Marutoiu C, Filip M, Tigae C, Coman V, Gresu R, Marcu G. Synthesis and characterization of alumina R chemically modified with n-octyl for use as a stationary phase in TLC. J Planar Chromatography.2003;16:183-5.
[6] Rack KD,Coats JR. Comparative degradation of organophosphorus insecticide in soil: Specificity of enhanced microbial degradation. J Food Chem. 1990; 36:193-9.
[7] Rack KD, Coats JR. Enhanced Biodegradation of Pesticides in the environment. ACS Symposium series 426, American Chemical Society, Washington DC; 1990.
[8] Reich E, Schibli A. High performance Thin Layer Chromatography for the analysis of medicinal plants. Thieme Medicinal Publishers, Inc. New York; 2006.
[9] Reiffova K,Vicova VP, Orinak A, Florida K, Gondova T. Preliminary TLC analysis of fructoligo saccharides as feed additives. J Planar Chromatography. 2003;16: 52-7.
[10] Sanganalmath PU, Yogaraje CV, Gowtham MD, Nayak VG, Mohan BM. Quantitative Densitometric determination of Quinolphosin Postmortem Blood by HPTLC. IJMT LM.2007;9,2:30-3.
[11] Sethi PD. High Performance Thin Layer Chromatography: Quantitative analysis of pharmaceutical formulation, CBS Publishers and Distributors.
[12] Sharma BK. Instrumental Methods of Chemical Analysis. Krishna Prakashan Media (P) Ltd, Meerut, 16th Edition;1997.
[13] WillardHH.InstrumentalMethodsofAnalysis.6thedition,CBS Publisher sand Distributors, Delhi;1986.
[14] Yagiie C, et al. Multiresidue determination of organochlorine pesticides and polychlorinated biphenyls in milk by Gas Chromatography with electron capture detector after extraction by matrix solid-phase dispersion.AOAC.2001; 84,5:1561-8.

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