

## Thin Layer Chromatography in the Purification of Lipids

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The purification of lipids has become simple and convenient. Some twenty years ago HPLC replaced preparative chromatography as the method of choice for the analysis of lipids. But these days commercially prepared pre-coated TLC plates do just as good a job as HPLC and are much more convenient to use. Additionally thin layer chromatography is frankly and inexpensive analytical tool, a feature having great merit in times of budgetary constraint. High performance TLC plates manufactured using silica gel or alumina prepared with uniform small particle size material for the stationary phase permits excellent separations with short elution times. As explained below, the analysis of complex lipids by TLC it is simply a matter of using specific spray reagents to detect particular functional groups in lipids: this simplicity in performance is not possible with HPLC. TLC is incredibly flexible in that it can be used in adsorption, reversed phase or complexation modes, such as for the separation of complex lipid components integrated into mammalian cell membranes or in vegetable oils. When performing a quantitative analysis of these lipid separations it is recommended that the thickness of the adsorbent placed on the prepared plate be greater than for qualitative analysis.

There is no lower cost high resolution technology available for the detection of lipids. Indeed, there are reports that some researchers substitute institutional size mayonnaise jars as TLC chambers which are then covered by a flat plate in which to perform experiments. Talk about being able to keep the costs of research down! Standard TLC plates are 20 cm tall with varying widths. The width of a TLC plate depends upon the number of samples to be chromatographed. We recommend use of a standard size commercially prepared TLC plate, which is 20 x 20 cm.

Thin layer chromatography (TLC) is currently used for two different methods of lipid analysis. In the first approach different classes of lipids are separately extracted and then each class of lipid is analyzed via unique TLC method. In the second approach complex mixtures of lipids are separated on TLC plates and then further characterized. The lipid classes are divided into neutral lipids such as triglycerides (which is formed from one molecule of glycerol and three fatty acids), polar lipids such as phospholipids, and cholesterol. Ideally lipids are chromatographed on a silica or alumina TLC plate using sequential solvent systems running in the same dimension. Relatively nonpolar lipids such as neutral lipids, fatty acids and cholesterol migrate to unique positions in the upper half of the chromatogram, whereas relatively polar lipids like phospholipids and sphingolipids are separated on the lower half of the chromatogram.

In the adsorption mode (either silica gel or alumina is an excellent sorbent agent) one principal application is for separation of different lipid classes from animal and plant tissues. Through the use of a mobile phase consisting of hexane and diethyl ether it is simple to resolve simple lipids such as cholesterol esters, triglycerides, free fatty acids, cholesterol and diacylglycerols. When performing a separation using reverse phase TLC plates everything except the flow of the solvent is backwards. Using the reverse phase technique polar compounds move faster than non-polar. The more polar the compound the less things move up the plate.

In a standard adsorption run complex lipids such as phospholipids and glycosphingolipids remain at the origin, and these can be quantified as if they are a single lipid class. This offers benefits over lipid purification using HPLC. If two dimensional TLC procedures are used with complex lipids better resolution may be possible than can be achieved in a single HPLC run. When performing two dimensional TLC excellent resolution can be achieved using either alumina or glass backed plates.

For routine analytical work ten or more samples can be applied to a 20 x 20 cm plate and then the amount of lipid present can be quantitated by charring-densitometry, which consists of spraying the plate with an oxidant and

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heating to carbonize the lipid. Prior to use it is recommended that the plates be heat activated at a temperature 110 F for an hour. The lipids migrate along the plate forming unique and separate spots on the plate. Then for the detection of spots after a run, in which the selected solvent front is able to migrate half way up the plate (approximately 30 minutes) the plates are air dried in a chemical hood at room temperature for 20 minutes. All can be easily visualized with a single water soluble dye such as amido black 10B. This dye preferentially interacts relatively nonpolar entities and when present in a 1 M sodium chloride solution will associate with lipid spots on the chromatogram.

The use of this amido black dye is generalized and does not help to differentiate the separate lipid compounds. To aid in the identification of separate lipid classes the TLC plates can be treated with a variety of specific reagents for different lipid types. The following aids are currently recommended:

1. Ninhydrin reagent shows up lipids which contain amino acids, useful for identification of phosphatidylethanolamine and phosphatidylserine.
2. Iodine vapor, or ammonium molybdate-perchloric acid spray or sulphuric acid spray can all be used to reveal the presence of all lipid materials
3. Dragendorff reagents are specific for choline
4. Dinitrophenylhydrazine and the Schiff reagent are specific for plasmalogens (any of a group of glycerol-based phospholipids in which a fatty acid group is replaced by a fatty aldehyde).
5. Hydroxylamine ferric acid chloride spray are specific for esterified fatty acids

The power of TLC technology for quantitative lipid detection is impressive. It is possible to detect as little as 25 ng of phospholipids, 25 ng of cholesterol, and 50 ng of neutral lipids and fatty acids.

Using these simple tools it is possible to identify lipid and lipid components quickly, and with high specificity.

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