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IV. Preparative LC Solvent Systems

A. Ethyl Acetate or Less Polar Solvents

Ethyl acetate provides good solubility for many samples, while having a moderate affinity for the silica gel. It is widely used to remove polar contaminants from the silica and may extend the lifetime of the cartridge. The column can be rapidly equilibrated with the next mobile phase, and ethyl acetate is also easily distilled from collected fractions.

When the TLC solvent system contains ethyl acetate, the mobile phase is adjusted to provide an $R_f = 0.5$. The R_f for the compound can be quickly readjusted to 0.3-0.25 by utilizing a solvent system containing only 1/3 the ethyl acetate.

Example: If the initial solvent contained 60% EtOAc/40% MeCl₂, the optimized preparative solvent would be 20% EtOAc/80% MeCl₂. This one solvent system can be rapidly checked by TLC before performing the preparative separation.

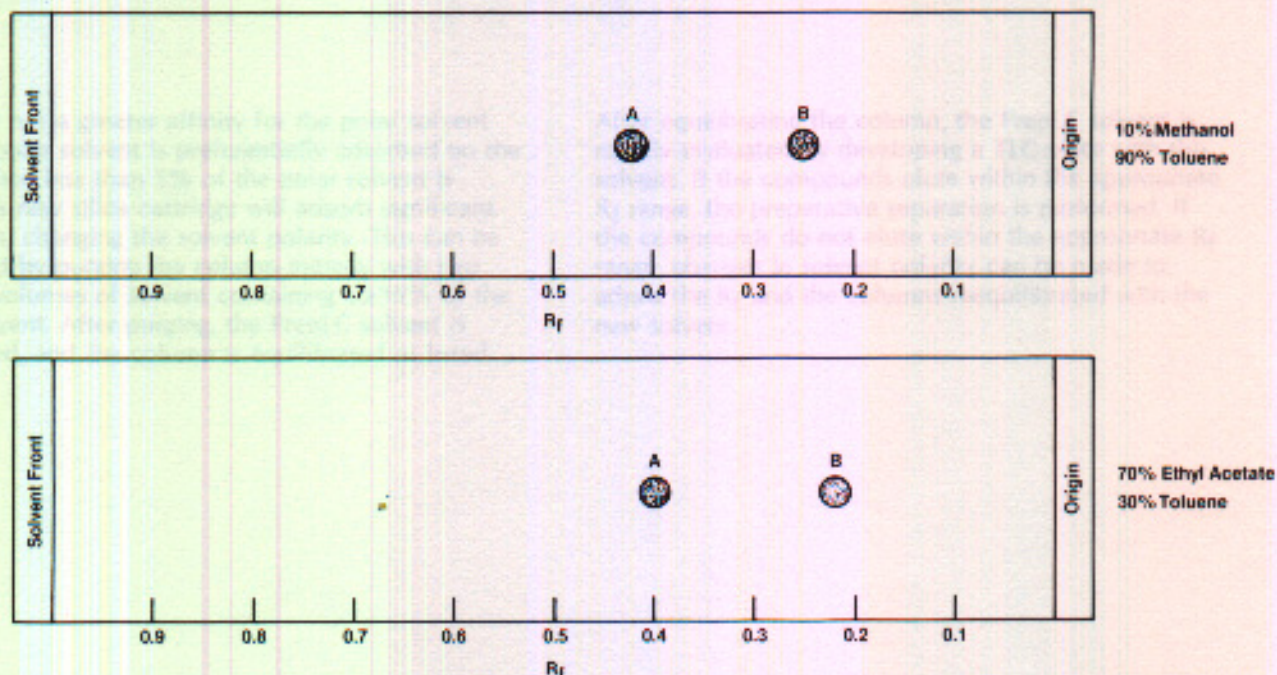
Table 5 provides a convenient way to alter solvent composition to utilize ethyl acetate while maintaining the solvent polarity. CAUTION: While finding a more appropriate solvent for preparative purposes, a change in either solvent may alter the resolution.

Table 5 Relationship of Ethyl Acetate to Other Polar Solvents

Ethyl Acetate	1 to 1
Methylethyl Ketone	1.7 to 1
Acetone	2.7 to 1
n-Butanol	3-4 to 1
n-Propanol	4.5 to 1
Ethanol	5-6 to 1
Methanol	7 to 1

Example: Table 5 explains that the amount of ethyl acetate in toluene required to produce an equivalent polarity is 7 times the amount of methanol required for the same results (Fig. 10).

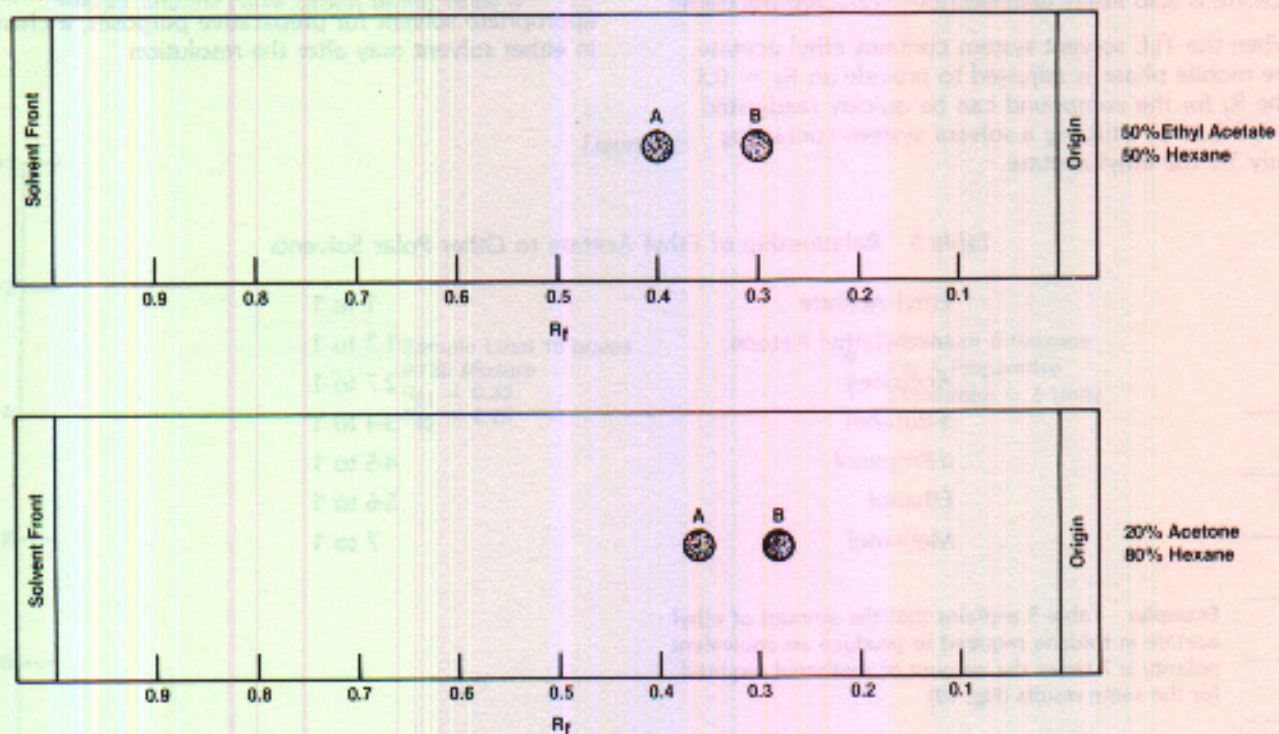
Figure 10 Conversion of Solvent Systems Based on Relative Polarity



The original TLC separation is 10% methanol 90% toluene.

The same solvent polarity is achieved with 70% ethylacetate 30% toluene.

Figure 10 (continued)



The original TLC separation is 50% ethyl acetate, 50% hexane.

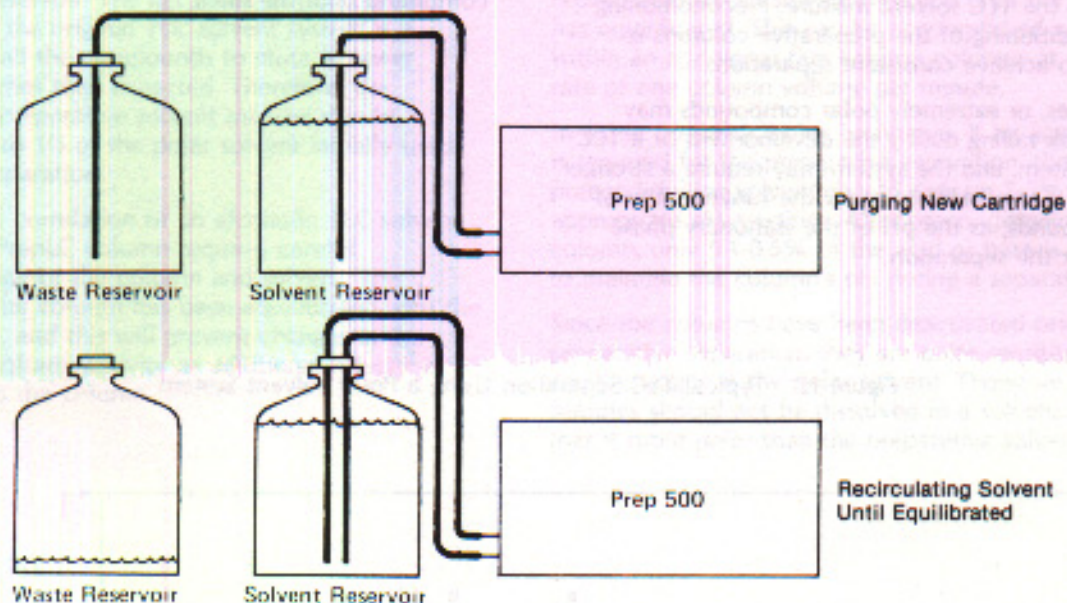
The same solvent polarity is achieved with 20% acetone, 80% hexane.

B. Equilibration of the Preparative LC Columns

When the preparative solvent mixture contains solvents no more polar than ethyl acetate (Table 1) and contains greater than 5% of the polar solvent, the preparative columns are equilibrated rapidly. Two column volumes of the preparative solvent are passed through the cartridge and discarded (Fig. 11). The

waste line is then diverted to the solvent reservoir and the solvent is recirculated through the column. Continuous stirring of the solvent mixture ensures rapid and continuous equilibration between the solvent and column. Generally, the solvent is equilibrated when the refractive index detector is stable on the 20X sensitivity setting.

Figure 11 Equilibrating a Preparative Cartridge



Silica gel has a greater affinity for the polar solvent and the polar solvent is preferentially adsorbed on the silica. When less than 5% of the polar solvent is present, a new silica cartridge will adsorb significant quantities, changing the solvent polarity. This can be prevented by purging the column initially with two column volumes of solvent containing 10-15% of the polar solvent. After purging, the PrepLC solvent is introduced, and the column is equilibrated as listed above.

After equilibrating the column, the PrepLC solvent is rapidly evaluated by developing a TLC plate with this solvent. If the compounds elute within the appropriate R_f range, the preparative separation is performed. If the compounds do not elute within the appropriate R_f range, changes in solvent polarity can be made to adjust the R_f and the columns reequilibrated with the new solvent.

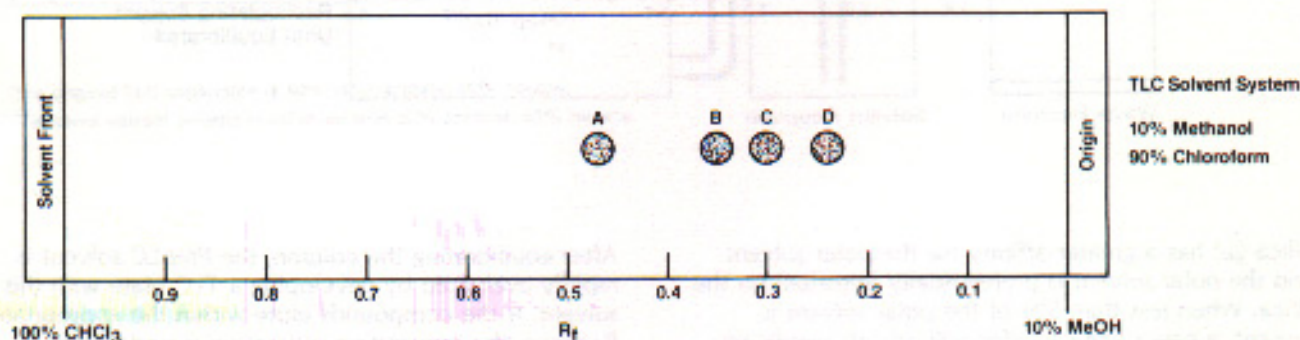
C. Solvent Systems More Polar Than Ethyl Acetate

When the mobile phase contains solvents equal to or less polar than ethyl acetate, correlation from TLC to PrepLC is good. As the affinity of the solvent for the packing material increases, correlation from TLC to PrepLC becomes less precise due to the TLC silica gel bed preferentially absorbing the more-polar solvent. Thus, during TLC development the solvent composition is not constant up the TLC plate (Fig. 12). To compensate for this change in solvent composition on TLC plates, the LC solvent mixture must be less polar than the TLC solvent mixture. Preconditioning and reconditioning of the preparative columns is required to achieve consistent separations.

Acids, bases, or extremely polar compounds may exhibit peak tailing during the development of a TLC solvent system, and the system may require a stronger solvent. It is important to know the functionality of the compounds, as the pH of the stationary phase may affect the separation.

To test the effect pH has on the separation, a small container of ammonium hydroxide can be placed within the TLC chamber. The ammonia vapors alter the pH on the silica surface without changing the TLC solvent composition. If the component's R_f did not change significantly with the ammonium hydroxide present, the compound is not basic and a solvent mixture containing a neutral or acidic solvent will be needed to elute the compounds from the PrepLC. If the R_f changed significantly or tailing was eliminated, a base must be incorporated in the preparative solvent mixture to prevent adsorption of the compound onto the silica.

Figure 12 Typical TLC Separation Using a Polar Solvent System



As shown in Fig. 12, the solvent near the origin contains 10% methanol, while the solvent at the solvent front has been depleted of methanol. This ongoing change (gradient) in the solvent composition across the plate also occurs on a preparative column. If a dry silica gel column is conditioned with a solvent containing alcohol, the solvent which initially elutes from the column is depleted of methanol. As the column continues to be purged with additional solvent, the stationary phase becomes saturated with methanol. The polarity of the stationary phase is decreased as the polarity of the eluent approaches that of the reservoir. The net result of purging the column with the original TLC solvent system is a tendency of all the compounds to elute in fewer column volumes than expected. Therefore, the equilibrated preparative solvent mixture should contain 1/10 to 1/5 of the polar solvent initially used in the TLC separation.

Reproducible correlation of an alcoholic TLC solvent system to a PrepLC column requires careful preconditioning of the column and solvent. This insures that the column has been equilibrated with the polar solvent, and this will prevent changes in the preparative column activity as additional solvent is passed across the column.

The preparative column is purged with 100% methanol, which simultaneously deactivates the column and removes any alcohol soluble silica residues. The flow rate should be no greater than one-half column volume per minute during the initial purge. To protect the detector from these residues, and reduce plugging of filters, the outlet tube from the detector is disconnected and two column volumes of the alcohol are purged through the preparative column. The tubing is reconnected, and the system purged with two column volumes of the equilibrated preparative solvent. Then, the preparative solvent is recirculated to the solvent reservoir until the column has equilibrated. This can be accomplished usually within an additional five column volumes at a flow rate of one column volume per minute.

In either preconditioning technique, if an acid or base is required for the preparative separation, the preconditioning solvent should contain 2-5% of the appropriate pH modifier. After preconditioning the column, only 0.1-0.5% of the acid or base is required to maintain the column's pH during a separation.

Since the columns have been deactivated prior to the preparative separation, they are quite sensitive to small changes in the polar solvent. Therefore, the samples should not be dissolved in a solvent system that is more polar than the preparative solvent system.