



Waters Associates
Liquid Chromatography

Preparative Liquid Chromatography and Its Relationship to Thin Layer Chromatography

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Table of Contents

I. Introduction	1
II. Factors to be Considered When Choosing a Preparative Solvent	1
III. Developing the Preparative Solvent System	2
A. Optimizing TLC Separations	2
B. Estimation of Column Volumes	4
C. Estimation of Preparative Sample Load	8
D. Effect of Increased Sample Load	11
IV. Preparative LC Solvent Systems	17
A. Ethyl Acetate or Less Polar Solvents	17
B. Equilibration of the Preparative LC Columns	19
C. Solvent Systems More Polar than Ethyl Acetate	22
V. Gradient Techniques	24
A. Step Gradients	24
B. Continuous Gradients	31

I. Introduction

The first step in Preparative Liquid Chromatography separations is selection of the proper mobile phase. To achieve this, one of two popular methods can be applied: Thin Layer Chromatography (TLC) or High Performance Liquid Chromatography (HPLC). The direct correlation between HPLC and preparative liquid chromatography has been the subject of many papers, but the use of TLC has not been widely documented. The following information deals with developing appropriate silica gel thin layer chromatography separations to evaluate a mixture and successfully correlating the TLC separations to the preparative silica column.

Both TLC and column chromatography are liquid-solid adsorption techniques. In TLC, the liquid (mobile) phase ascends by capillary action on a thin layer of solid (stationary) phase coated onto a backing support. While in column chromatography, the solvent percolates down through the column's adsorbent. Because of the similar relationship to column technology, TLC separations often reproduce on a column. Consequently, TLC is a rapid method for determining the solvent required for preparative liquid chromatography separations.

II. Factors to Consider When Choosing a Preparative Solvent

Predicting a solvent combination that will facilitate the TLC separation and elute the sample from a silica bed with the desired separation is often the most challenging aspect of preparative chromatography. However, much of the necessary information is readily available to the chemist from a basic knowledge of the compounds. A range of solvents that are appropriate for a preparative separation can be chosen on the basis of three criteria:

1. Solubility
2. Affinity
3. Resolution

1. Solubility

Many TLC solvent systems provide minimal solubility of the sample, but to elute a sample from a column the solvent (mobile phase) must provide greater solubility of the sample because the sample concentration is relatively high. It is best to dissolve the sample completely in the mobile phase to prevent on-column crystallization during injection or unpredictable elution characteristics.

The first step in solvent selection is determining the relative solubility of the sample. Then, the solvents should be listed in order of decreasing solubility. The desired mobile phase would provide the greatest solubility, while providing affinity for the sample on the stationary phase.

2. Affinity

If you have selected a solvent system which provides good solubility of the sample, but the sample has a much higher affinity for the silica gel than the solvent, the sample will remain at the origin (R_f value will be too low. Conversely, if the affinity for the silica gel is lower the R_f will be higher). To achieve a separation, the sample must have relatively equal affinity for the solvent and the packing material.

Example: If the R_f for the compound is too high using toluene, a less polar solvent like hexane might be substituted for or added to the toluene. If the R_f values are too low, a more polar solvent like ethyl acetate should be substituted for or added to the toluene. (See Table I).

Table 1. Solvent Solubility Screening Table

Water
Methanol
Ethanol
Acetone
Diethyl Ether
Ethyl Acetate
Dichloromethane
Toluene
Chloroform
Cyclohexane
Petroleum Ether
Hexane

↑
Increasing Polarity
↓

Generally, a TLC separation is first done by adjusting the compound's R_f between 0.3-0.5 (Fig. 1a). For the preparative LC solvent, the TLC solvent system's polarity must be decreased to lower the R_f (Fig. 1b).

The most desirable R_f value for small sample loads on a preparative column is between 0.15-0.35. This R_f range is optimal for a preparative separation, in terms of sample load, resolution, residence time and solvent usage (Fig. 2).

Figure 1 Comparison of Optimized R_f Range for TLC and Preparative LC Separations

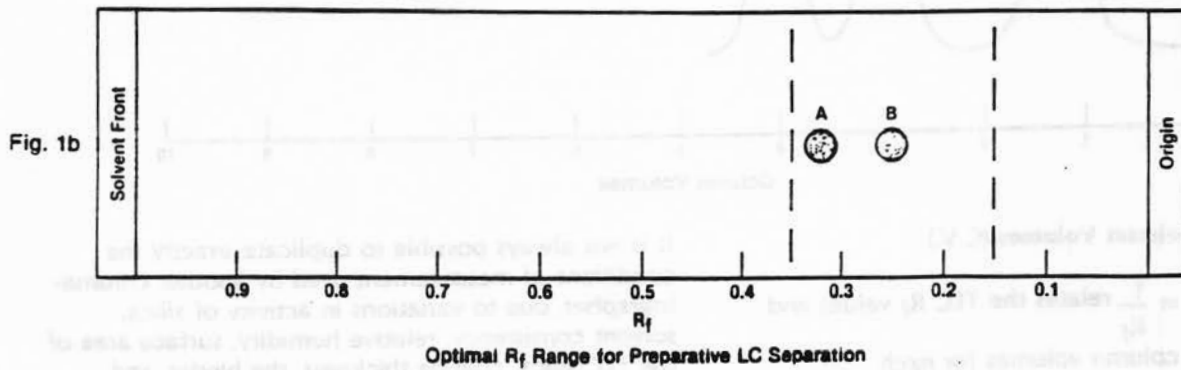
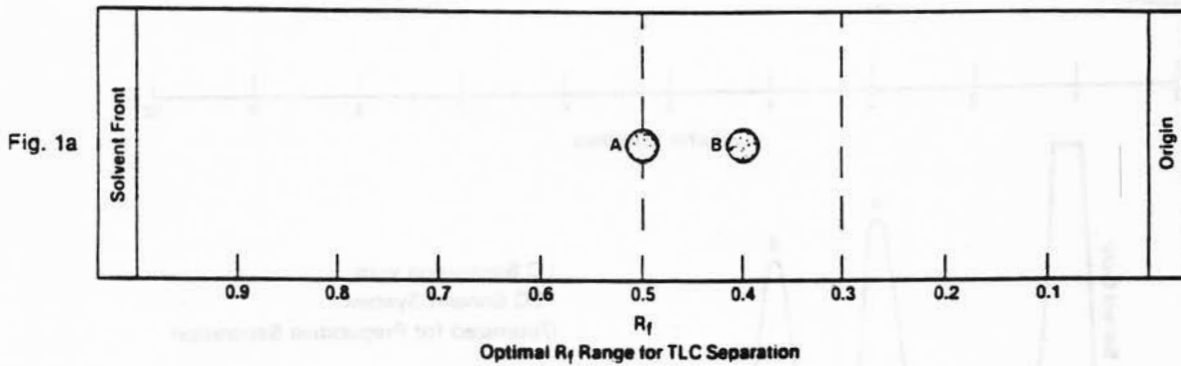
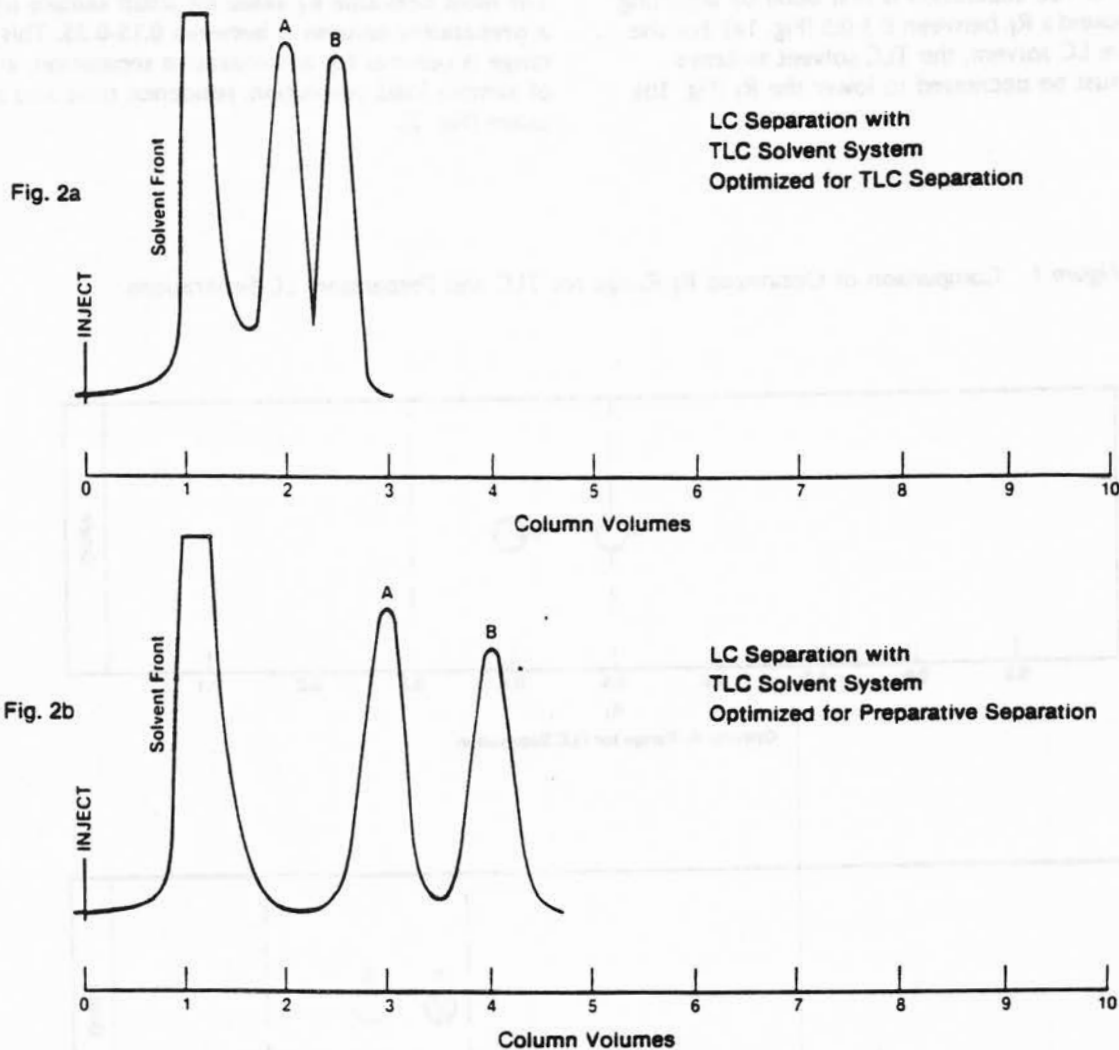


Figure 2 Comparison of LC Separation Using Optimized TLC and LC Solvent Systems



B. Estimation of Column Volumes (C.V.)

The equation $C.V. = \frac{1}{R_f}$ relates the TLC R_f values and

the preparative LC column volumes for each component, where a column volume is the volume of solvent required to elute a non-retained solute from the column. In Table 3 below values of C.V. have been calculated for various R_f 's, but it is important to remember that the equation is only a guideline (The optimum range for preparative LC is 3-8 column volumes).

Table 3.

Relationship of R_f and Column Volume, $C.V. = \frac{1}{R_f}$

R_f	C.V.
.15	6.7
.20	5.0
.25	4.0
.30	3.3
.35	2.8
.40	2.5

It is not always possible to duplicate exactly the conditions of measurement used by another chromatographer, due to variations in activity of silica, solvent consistency, relative humidity, surface area of the TLC silica, coating thickness, the binder, and general TLC techniques. Therefore, the relationship between the R_f values and the column volume will vary in use.

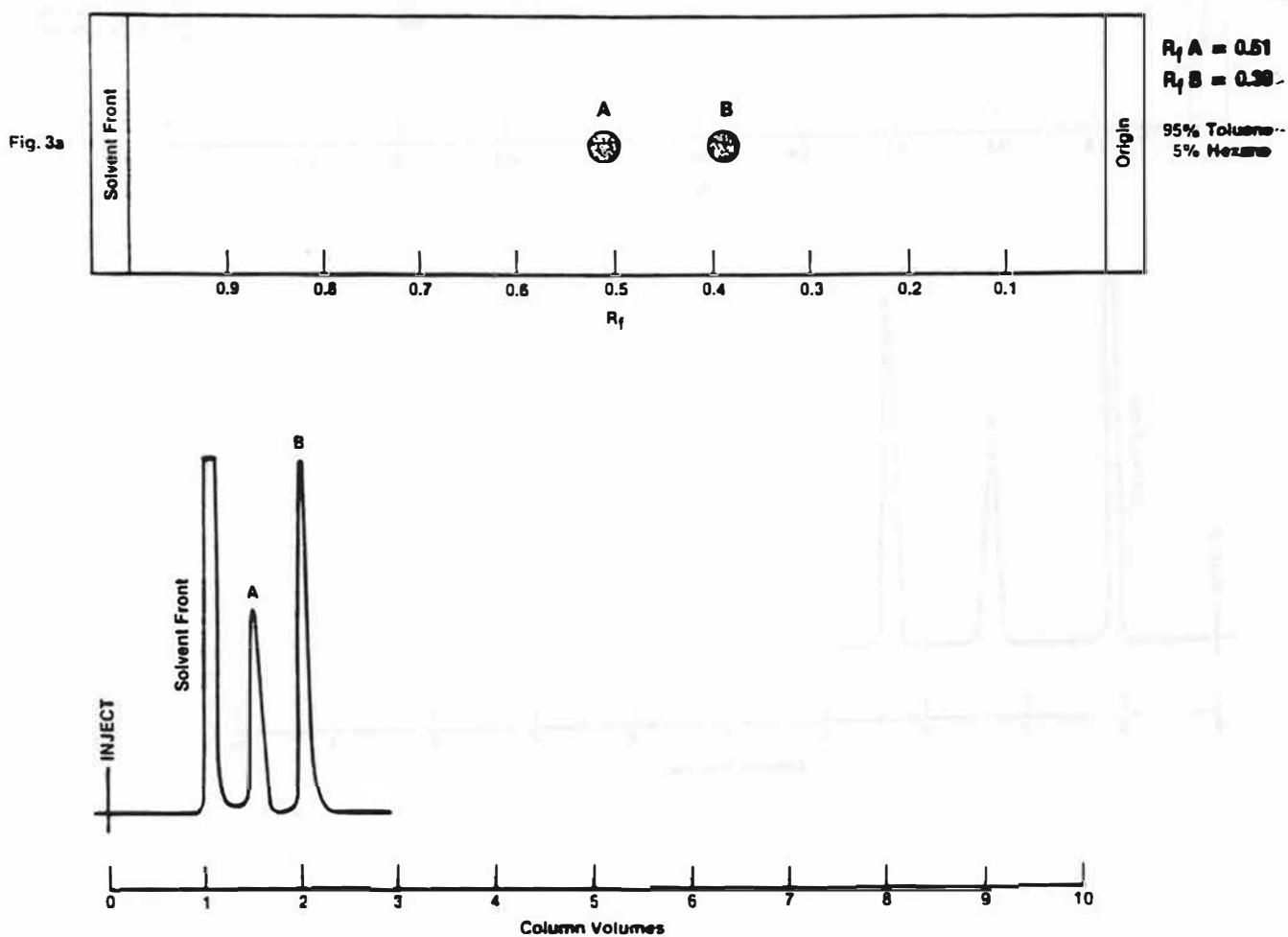
The equation $C.V. = \frac{1}{R_f}$ relates TLC data and LC data

sufficiently to complete a successful preparative separation. Generally, the LC column volumes will be equal to or less than the calculated values. The elution volume will also be dependent upon the sample load and solvent used to solubilize the sample.

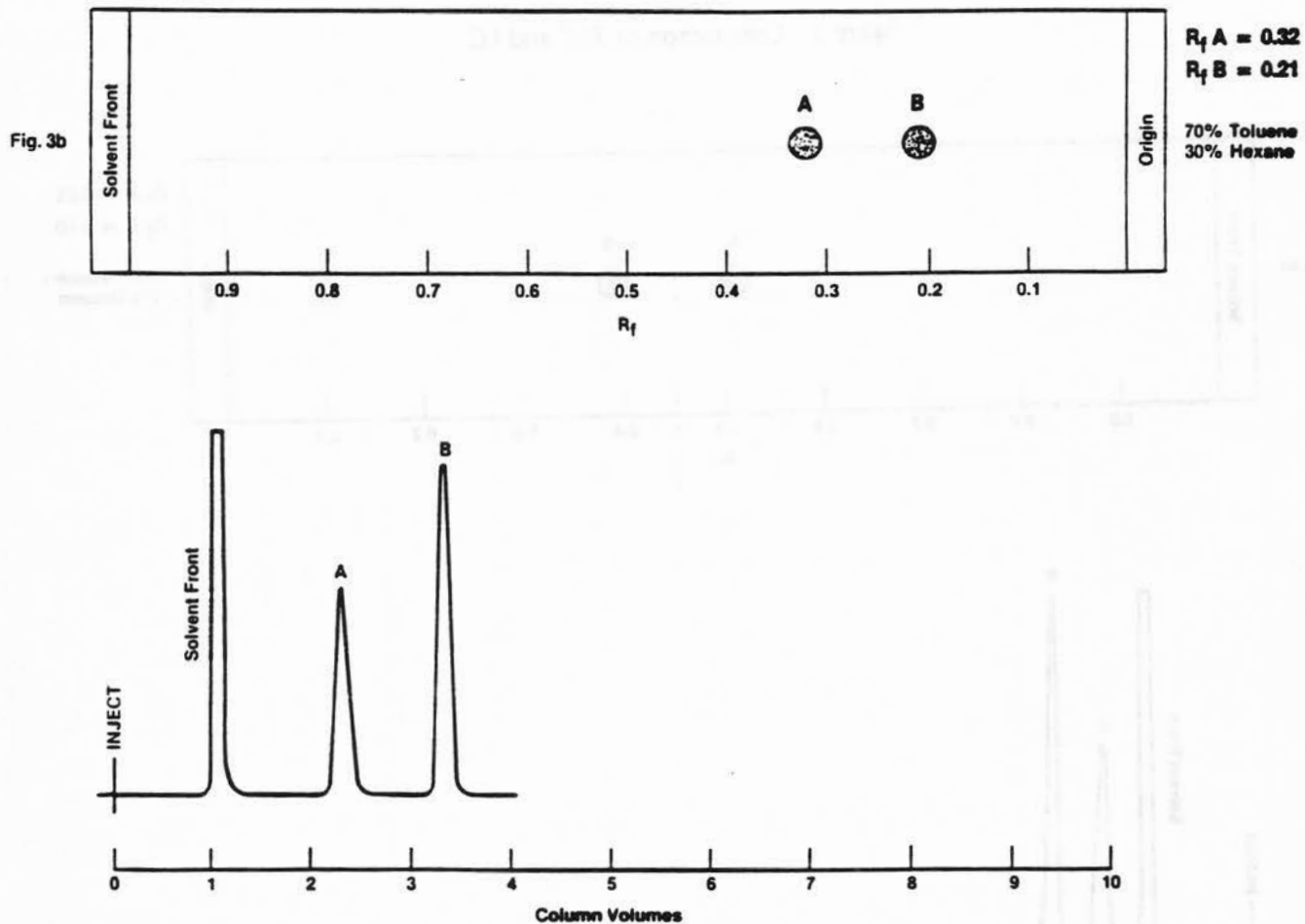
Example: The solvent scouting technique for a typical TLC separation of a *cis-trans* reaction mixture is best illustrated with an example in Fig. 3. This exhibit also compares TLC to ALC for the sample compound. The reaction mixture was separated by TLC with 95:5 toluene-hexane (Fig. 3a). This solvent system provided the best TLC separation with the compounds at R_f 0.5 and 0.39, respectively.

With the same solvent system on a μ PORASIL™ column (silica gel), the compounds eluted at 1.6 and 2 C.V.'s. Although the compounds are separated, this solvent is not the best choice for a preparative LC, since the compounds are not retained sufficiently on the column.

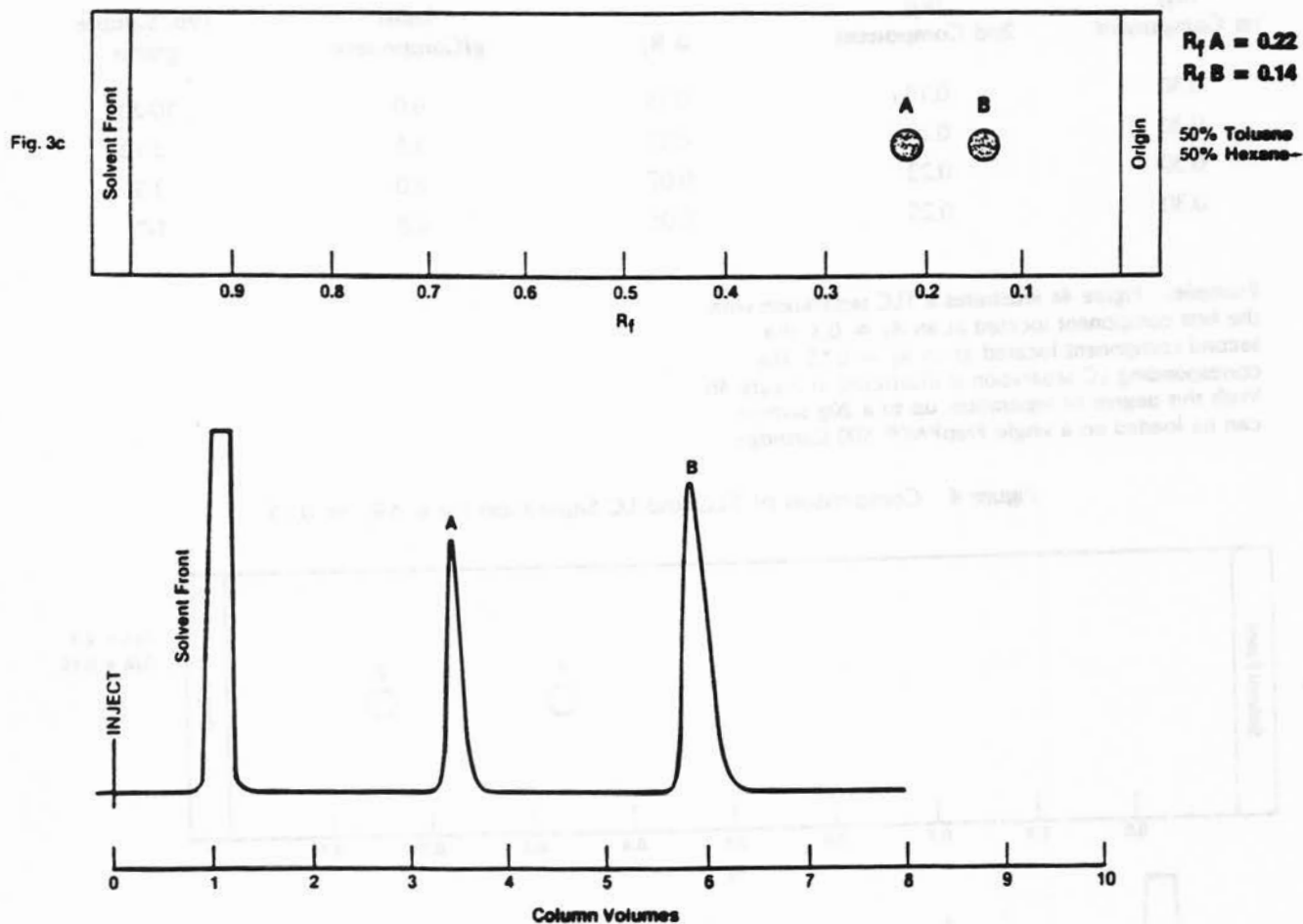
Figure 3 Comparison of TLC and LC



In Fig. 3b, the concentration of hexane has been increased to 30%. By lowering the solvent polarity, the TLC R_f values are lowered (0.32 & 0.21). On the LC, the compounds elute at 2.3 and 3.3 C.V.'s, which is satisfactory for a preparative LC separation.



By modifying the TLC solvent system to 50% hexane, the R_f 's for the compounds are changed to 0.22 and 0.14, respectively. Figure 3c compares the LC separation and the TLC separation for this solvent system.



Either the 30:70 or the 50:50 hexane solvent system would provide a satisfactory preparative separation under normal loading conditions. For larger loads, the 50:50 system would be preferable, as the optimum solvent system is determined by considering the quantity of material which would be separated. This loading factor will be discussed in the next section.

C. Estimation of Preparative Sample Load

The quantity of sample which can be loaded onto a preparative column is a function of the separation initially achieved on the TLC plate. Changing the TLC conditions to increase the separation will significantly

increase the amount of sample that can be loaded onto the preparative LC. Using the initial TLC conditions, a preparative separation can be performed following the guidelines listed in Table 4.

Table 4. Guidelines for Estimating Load

(R _f) 1st Component	(R _f) 2nd Component	Δ R _f	Load g/Component	Typ. Sample grams
0.30	0.15	0.15	6.0	10-20
0.30	0.20	0.10	3.5	5-10
0.30	0.23	0.07	2.0	3-5
0.30	0.25	0.05	0.8	1-2

Example: Figure 4a illustrates a TLC separation with the first component located at an R_f = 0.3, the second component located at an R_f = 0.15. The corresponding LC separation is illustrated in Figure 4b. With this degree of separation, up to a 20g sample can be loaded on a single PrepPAK® 500 Cartridge.

Figure 4 Comparison of TLC and LC Separation for a ΔR_f = 0.15

