

## Correlating TLC to Isocratic Separation R<sub>f</sub> to CV Conversion

### Application Overview

When using Isco's CombiFlash® RETRIEVE™ or when purifying compounds that have no chromophore, it is possible to use thin layer chromatography (TLC) to locate purified compounds in the collected fractions.

If the retention factor (R<sub>f</sub>) of the compound is known, the fraction collection tube containing the desired substance can be estimated in terms of column volume. Column volume is the interstitial (void) volume of a packed column. This specification is listed for all Isco RediSep® columns in Application Note 3.

Knowing the R<sub>f</sub> and column volume gives you an idea of the range of fraction collection tubes that a compound may be located in.

### General Method

The compound to be separated is loaded onto the TLC plate and separated into several species.

#### Controlled Parameters

Several parameters of the TLC must be consistent to get a good correlation.

- The TLC solvent should be measured out in a volume-to-volume ratio so the mixture will remain consistent when it is transferred to a gradient-former system.
- Equilibration of the chamber and plate is recommended for best results.
- The plate should be run to within 1–4 cm of the top of the plate to achieve a good separation.
- The sample spot location and the solvent level should be marked before the plate is run.
- The solvent elution height and sample spot location should be marked at the end of the run.
- While performing TLC, the solvent level in the chamber should never touch the samples and the chamber should be covered to maintain an equilibria in the vapor phase.

Under these parameters, the solvent will visibly move up the plate in about 1 hour.

#### Assumptions

There are several assumptions made:

- The column and TLC plates use the same type of stationary phase.
- The solvent used in the TLC method is the same as the solvent used in the liquid separation. Therefore, the liquid separation must be isocratic.

- The R<sub>f</sub> of the TLC should be less than 0.5 and greater than 0.1, if this is not, then a different solvent system may be needed.

#### R<sub>f</sub> Values

**R<sub>f</sub>** — Retention factor. The distance the sample spot has traveled relative to the solvent front. R<sub>f</sub> is calculated from the formula:

$$R_f = \frac{Z_x}{(Z_f - Z_0)}$$

**Z<sub>x</sub>** — The distance the spot has traveled from its origin (sample line). Refer to Figures 1 and 2.

**Z<sub>f</sub>** — The distance from the original solvent line to the final solvent front.

**Z<sub>0</sub>** — The distance from the solvent line to the sample line.

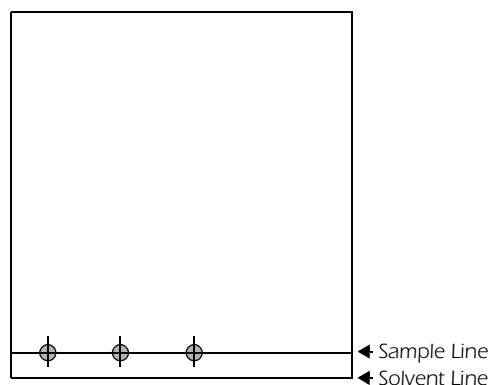


Figure 1: TLC Start

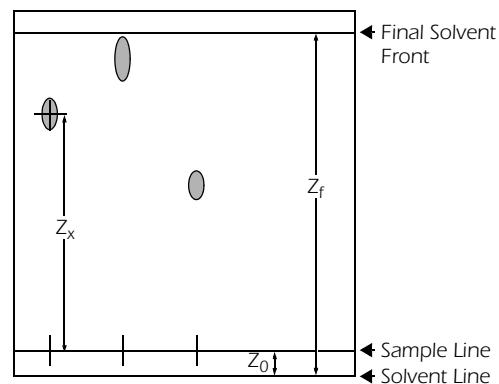


Figure 2: TLC Finish

## Column Volume Correlation

Once the  $R_f$  of a certain component in a mixture is known, then a correlation to column volumes (CV) can be made.

If the  $R_f$  value is between 0.1 and 0.5, it can be entered into the following formulas to give a range of collection tubes.

$$\text{First Tube} = \frac{R_f^{-1} \cdot 2 \cdot \text{Vol}_c}{\text{Vol}_f}$$

$$\text{Last Tube} = \frac{R_f^{-1} \cdot 3 \cdot \text{Vol}_c}{\text{Vol}_f}$$

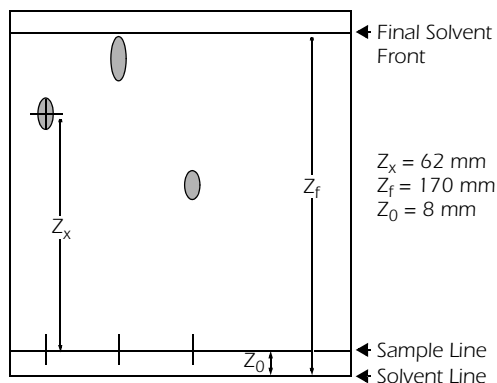
Where:

$\text{Vol}_c$  — The void volume of the column.

$\text{Vol}_f$  — The fraction volume collected in each tube.

### Example

Refer to Figure 3.



**Figure 3: Example TLC**

A spot of acetophenone elutes 62 mm ( $Z_x = 62$  mm), the distance from the solvent level to the horizontal sample line is 8 mm ( $Z_0 = 8$  mm), and the distance traveled by the solvent front is 170 mm ( $Z_f = 170$  mm).  $R_f$  is dimensionless so the units are dropped and the calculation is performed:

$$R_f = \frac{Z_x}{(Z_f - Z_0)} \quad \text{or} \quad \frac{62}{(170 - 8)} = 0.38$$

To correlate this to a purification through a 12 gram RediSep column with a column volume of 16.8 mL and collected as 10 mL fractions, apply the  $R_f$  to the following formulas:

$$\text{First Tube} = \frac{R_f^{-1} \cdot 2 \cdot \text{Vol}_c}{\text{Vol}_f} \quad \text{or} \quad \frac{0.38^{-1} \cdot 2 \cdot 16.8}{10} = 8$$

$$\text{Last Tube} = \frac{R_f^{-1} \cdot 3 \cdot \text{Vol}_c}{\text{Vol}_f} \quad \text{or} \quad \frac{0.38^{-1} \cdot 3 \cdot 16.8}{10} = 13$$

## Calculator

If you are viewing this application note in Adobe's Acrobat Reader, version 5 or later, you can use the  $R_f$  and Tube Correlation calculators below.

R <sub>f</sub> Calculator	
Z <sub>x</sub>	<input type="text"/>
Z <sub>f</sub>	<input type="text"/>
Z <sub>0</sub>	<input type="text"/>
R <sub>f</sub>	<input type="text"/>
<input type="button" value="Calculate"/>	

Tube Correlation Calculator		
<b>RediSep Size</b>		
4 g	Void Volume (Vol <sub>c</sub> ) <input type="text"/> mL	
12 g	Fraction Size (Vol <sub>f</sub> ) <input type="text"/> mL	
40 g	R <sub>f</sub> <input type="text"/>	
120 g	<b>Tube Range:</b>	
330 g	<input type="text"/> to <input type="text"/>	
Other	First Tube      Last Tube	
<input type="button" value="Calculate"/>		
<input type="button" value="Clear"/>		

## References

1. Colin F. Poole and Salwa K. Poole, "Chromatography Today", Elsevier Science Publishers B.V., 649-667, (1991).

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