Correlating TLC to Isocratic Separation

R_f to CV Conversion

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Chromatography Application Note AN09

Application Overview

When using Isco's Combi*Flash*® 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_f) 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 Redi*Sep*® columns in Application Note 3.

Knowing the R_f 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_f 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_f Values

R_f — Retention factor. The distance the sample spot has traveled. Rf is calculated from the formula:

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

Z_x — The distance the spot has traveled from its origin (sample line). Refer to Figures 1 and 2.

Z_f — The distance from the original solvent line to the final solvent front.

Z_0 — The distance from the solvent line to the sample line.

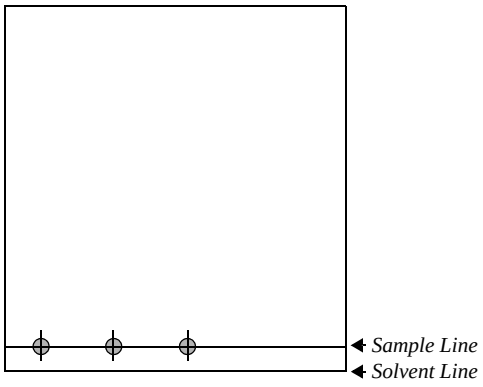

Figure 1: TLC start

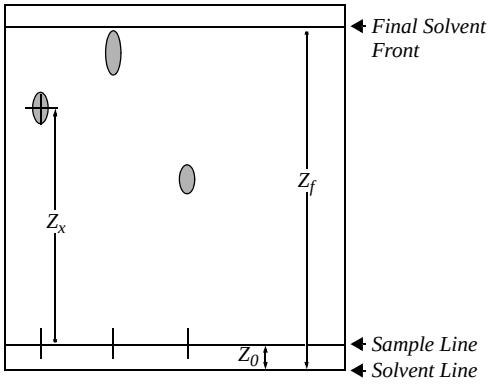

Figure 2: TLC finish

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.

$$First \frac{R_f^{-1} \cdot 2 \cdot Vol_c}{Vol_f}$$

$$Last\ Tube = \frac{R_f^{-1} \cdot 3 \cdot Vol_c}{Vol_f}$$

Where:

Vol_c — The void volume of the column.

Vol_f — The fraction volume collected in each tube.

Example

Refer to Figure 3.

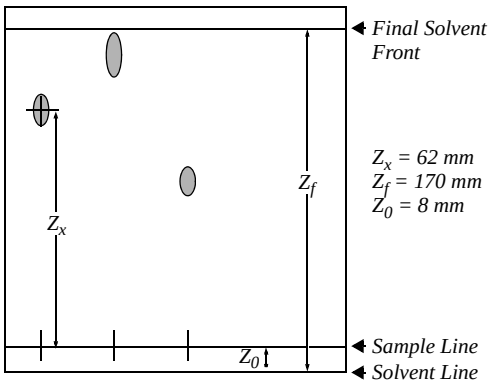

Figure 3: Example TLC

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=8mm), 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:

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

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:

$$First \frac{R_f^{-1} \cdot 2 \cdot Vol_c}{Vol_f} \quad or \quad \frac{0.38^{-1} \cdot 2 \cdot 16.8}{10} = 8$$

$$Last\ Tube = \frac{R_f^{-1} \cdot 3 \cdot Vol_c}{Vol_f} \quad or \quad \frac{0.38^{-1} \cdot 3 \cdot 16.8}{10} = 13$$

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.

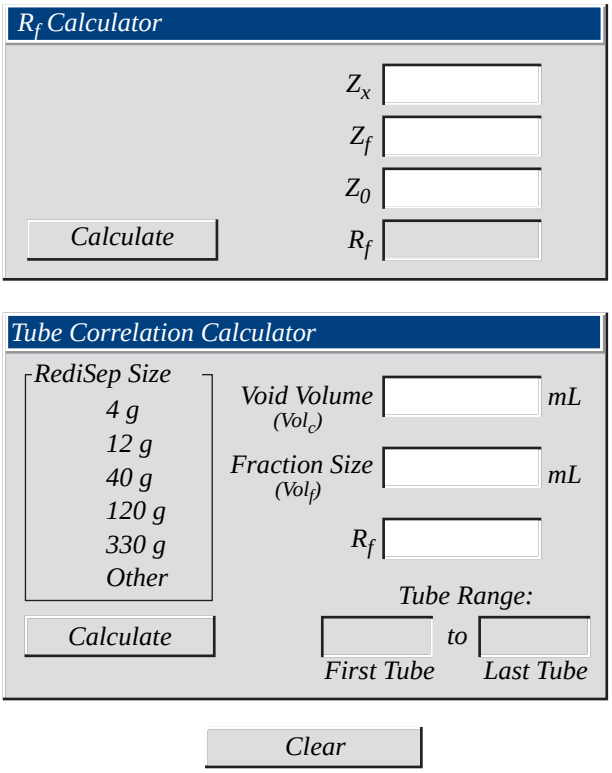

Figure 4: R_f and Tube Correlation Calculator

References

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

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