The Role of Planar Chromatography in Column Purification

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Introduction

Planar chromatography or thin layer chromatography (TLC) is commonly used as a simple but efficient tool for pre-purification, preliminary separation and monitoring of organic synthesis. This paper addresses the use of planar chromatography to optimize column purification of organic compounds. In this study, isocratic and gradient elution are evaluated for column separation of different type of organic compounds based on their retardation behavior on TLC. The interdependence between retardation factors (R_f) of TLC and column retention volume (CV) of both isocratic and gradient elution are analyzed. The results show that R_f and CV have a nonlinear relationship. This phenomenon translates into more difficult separation of weakly retarded solutes of complex sample when column is eluted under similar conditions. Gradient elution offers more flexibility when the depth of gradient is properly adjusted based on the retardation factor to achieve better separation.

Materials and Methods

A Biotage SP4™ purification system was used for column purification. The system was configured with a dual variable UV detector allowing flexible tuning of absorbance for high sample loading. Column used for the purification was FLASH 12+™M Si cartridges.

A series of compound was used for the study. R_f values were generated by using Biotage KP sil TLC plate for the compounds.

Results and Discussion

To illustrate the relationship between TLC R_f values and column retention behavior, different types of compounds were evaluated. These compounds show R_f values in the range from 0.05 to 0.53 when 9:1 hexane-ethylacetate was used as the developing solvent. Corresponding retention factors, in the number of column volumes (N), are obtained with the same solvent using a liquid chromatography system.

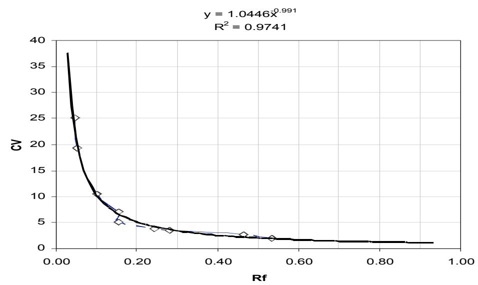

Figure 1. The plot of R_f values vs. the numbers of column volume.

Regression analysis shows that R_f values are high correlated to the numbers of the column volumes (N) as shown in Figure 1. The relationship is best described as $N = 1/R_f$. It indicates that R_f is a predictor of column retention of a given compound. On the other hand, the curve also implies that the prediction may become less reliable for column purification when the R_f value is very small (near 0) or very large (close to 1) R_f values under isocratic conditions.

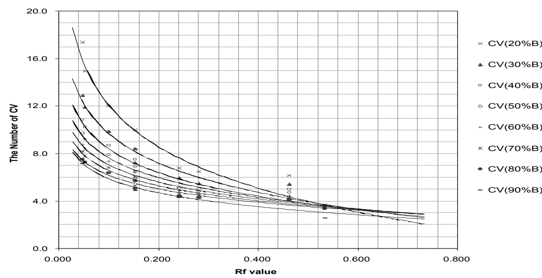

Figure 2. The plot of R_f values vs. the numbers of column volume under different gradient profiles.

Under different gradient depth, the relationship between R_f and N is shown in Figure 2. The relationship is logarithmic for each gradient depth studied. The dependence of R_f and N demonstrate is described with formula:

$N = A * \ln(R_f) + B$. The correlation coefficients R-square are greater than 0.94. As indicated in Figure 2, the elution of a more retained compound can accelerated under gradient condition while the resolution of little retained compounds can be still resolved by adjusting the depth of a gradient.

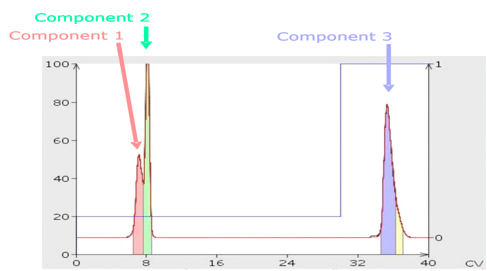

Figure 3. The isocratic elution profile of the separation of a 3-component sample. Column: Biotage F12M + Si; Flow rate: 15mL/min; Solvent: 99:1 Dichloromethane-methanol.

Over-retention always occurs to a component with very low R_f when directly transfer TLC conditions to column purification as shown by Component 3 (R_f = 0.05) in Figure 3. Closely adjacent peaks, Components 1 and 2 (R_f = 0.40 and 0.32 respectively), are not well resolved using isocratic elution.

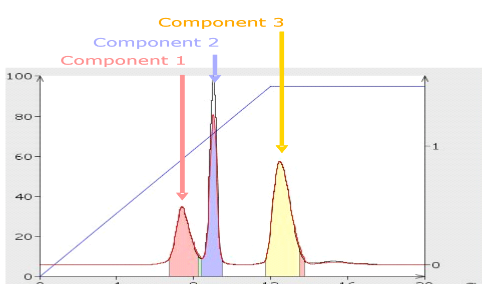

Figure 4. Gradient elution based on Component 3 (R_f value = 0.05). Column: Biotage F12M + Si; Flow rate: 15mL/min; Gradient: 0% B to 95% B in 12 CV with holding of 95% B at the end of the gradient.

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Based on the R_f of TLC profile, column purification of multiple-component samples are optimized using gradient elution. The magnitude of R_f depends on the solvent strength used for TLC. For example, a larger R_f will be observed when the portion of methanol is increased in the dichloromethane-methanol mixture that is the eluting solvent for TLC. On the other hand, the difference of (1- R_f) is complimentary to R_f. It may reflect the change of solvent strength required to move a component faster or slower on a TLC plate. The residual may be used as a reference to set the ending condition of a gradient elution in column purification.

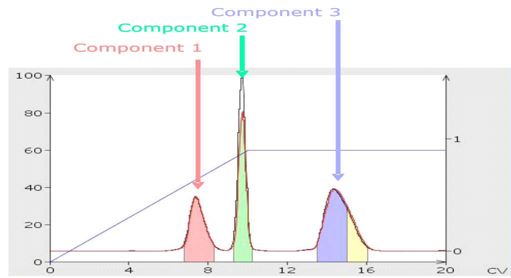

Figure 5. Gradient elution based on Component 2 (R_f value = 0.32). Column: Biotage F12M + Si; Flow rate: 15mL/min; Gradient: 0% B to 68% B in 10 CV with holding of 68% B at the end of the gradient.

Purification of a 3-component sample is shown in Figures 4, 5 and 6 under different gradation conditions based on their R_f values. In each case, adjacent peaks (Components 1 and 2) are fully resolved while the deeply retained component is eluted out without excessive use of solvent as compared to the isocratic separation illustrated in Figure 3.

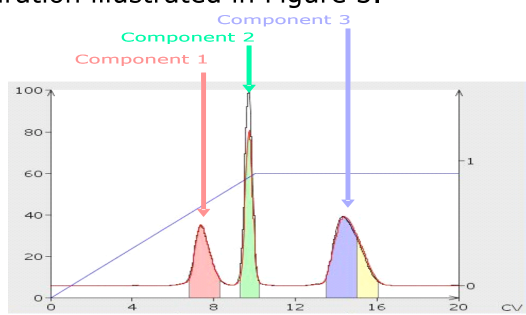

Figure 6. Optimized gradient elution based on Component 1 (R_f value = 0.40). Column: Biotage F12M + Si; Flow rate: 15mL/min; Gradient: 0% B to 60% B in 10 CV with holding of 60% B for 8 CV.

Figure 7 shows another example of gradient purification to separate a 7-component sample (R_f ranges from 0.07 to 71) using hexane-ethylacetate as solvents in Figure 7. The gradient elution from 3 to 93% ethylacetate allows the 7 components are based-line resolved.

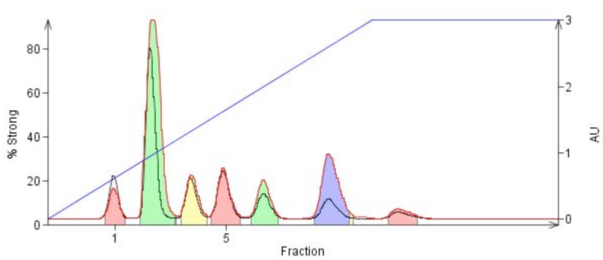

Figure 7. Separation of a 7-component sample using hexane and ethylacetate. Column: Biotage F12M + Si; Flow rate: 15mL/min; Gradient: 3% B to 93% B in 10 CV with holding of 60% B for 5 CV.

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Conclusion

R_f of TLC is highly correlated to the sample retention in column chromatography and it is a predictor of retention volumes. The derivative of R_f such as (1- R_f) reflects solvents stength required in gradient elution to optimize column purification. Biotage SPx automated flash purification system has integrated feature to optimize the gradient separation that will reduce chemists' work load and ehnace sample throughput.

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