

Optimized Flash Chromatography Purification: From TLC to large scale in three steps

Veronica Thomason

Teledyne Isco, Inc.
P.O. Box 82531
Lincoln, NE 68501



Abstract

Method development, necessary for optimizing conditions before flash chromatography, is often perceived as a time consuming step for synthetic organic chemists. A quick approach for developing optimal chromatographic purification using PeakTrak 2.0 software's time saving features, such as the R_f -to-gradient calculator and the auto scale-up, developed by Teledyne Isco for use with their CombiFlash Companion automated purification systems, will be discussed.

Background

Chemists will perform thin layer chromatography (TLC) of crude reaction mixtures to determine conditions for purification by flash chromatography. Several runs by TLC are needed to determine ideal parameters for flash chromatography. These include the stationary and mobile phases that provide R_f values within the range of 0.25 to 0.35. With PeakTrak's R_f -to-Gradient feature, chemists simply perform only two TLCs of different solvent composition that provide R_f values within the range of 0.2 to 0.8.

R_f to Gradient Calculator Feature

The solvent composition and corresponding R_f values of the target compound and its closest impurity are entered into the R_f -to-Gradient Calculator, as shown in figure 1. The software suggests a gradient profile specific to the sample for separation between the target compound and the nearest impurity.



Figure 1. Optimized gradient calculated from R_f values

Scale-up Feature

When it is determined that the purification must be scaled up to accommodate several grams of material, the same successful purification protocol can be scaled up to accommodate larger amounts of material by utilizing the auto Scale-up feature. Typically a few milligrams of sample are sufficient for an initial separation assessment on a 4g RediSep flash column. To scale up a method, simply select the desired column size to be scaled to. The software saves the new method incorporating parameters optimal for the selected column (figure 2).

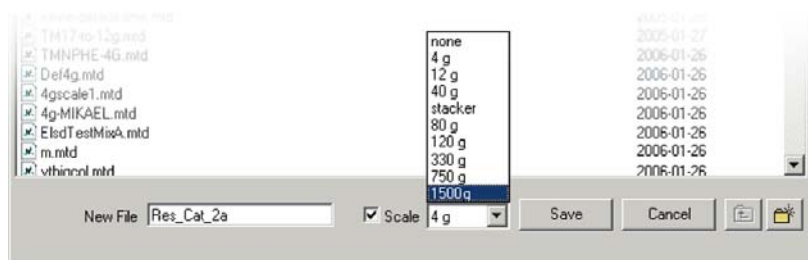


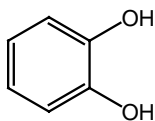
Figure 2. Scale-up Feature

Experiment

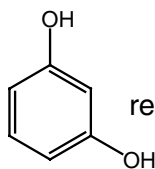
The *CombiFlash* Companion system's PeakTrak 2.0 software was used to develop methods for the separation of two regioisomers on a scale for purification of milligrams to several grams. *RediSep* normal phase silica gel TLC plates were used to establish the optimal mobile phase that provided an R_f within the range of 0.2 to 0.8 for two diols. For a mixture of resorcinol and catechol, a TLC plate was run with a mobile phase mixture of ethyl acetate in hexane.

1. Separate resorcinol and catechol on RediSep silica TLC plates

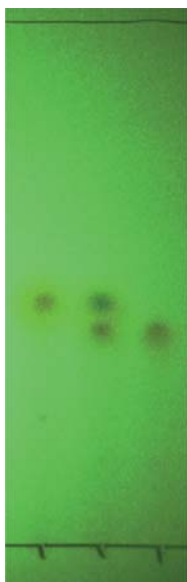
catechol



resorcinol



50% ethyl acetate



catechol $R_f=0.48$
resorcinol $R_f=0.40$

30% ethyl acetate



catechol $R_f=0.34$
resorcinol $R_f=0.21$

Figure 3. Left spot: catechol, middle spot: resorcinol and catechol, right spot: resorcinol.

2. Convert R_f values to an optimized method

Shown in figure 4, the R_f values for both the target compound, resorcinol, and nearest impurity, catechol, for both TLC plates are entered into Peak *Trak* software. The software constructs a linear gradient to sharpen peaks while inserting an isocratic hold to further separate the two compounds.

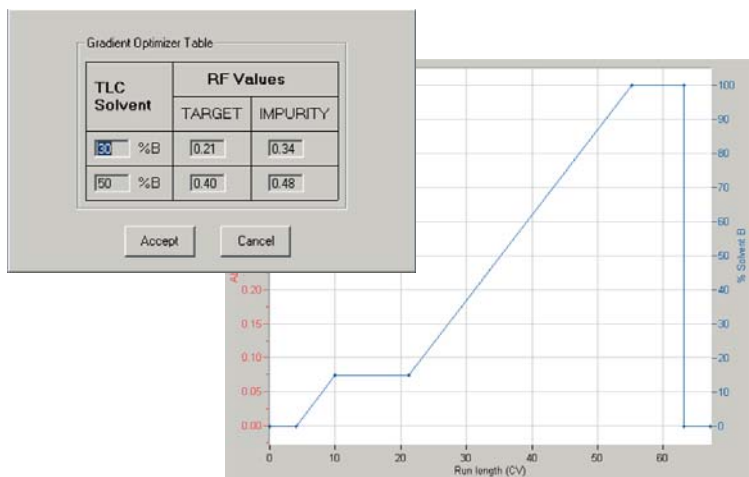
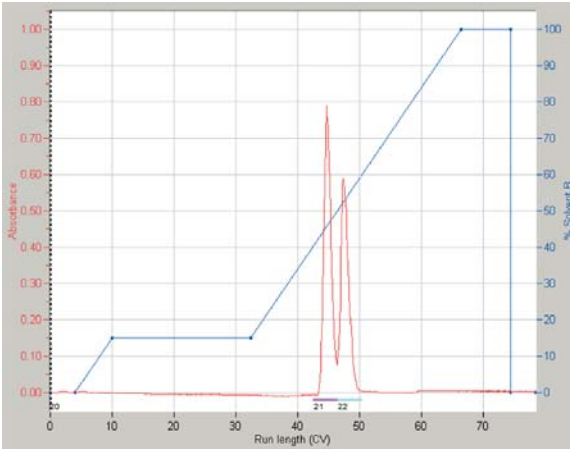


Figure 4. R_f to Gradient Calculator

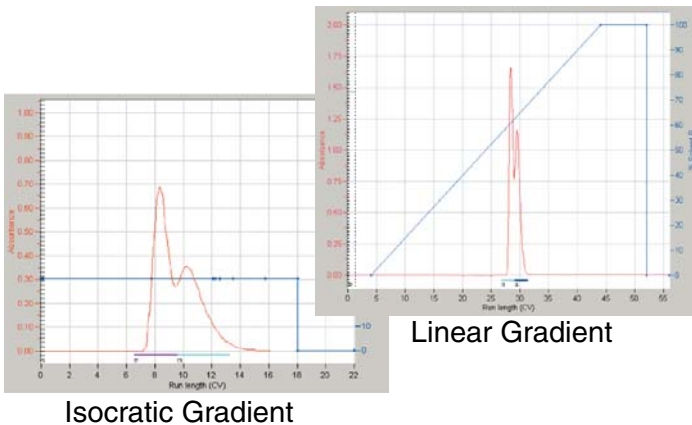
The separation on a 4g RediSep flash column is performed as an initial assessment on the Companion (figure 5). During the course of the run, the software determines the isocratic hold length that achieves higher resolution while maintaining high sample concentrations in collected fractions.



Optimized Gradient

Figure 5. Chromatograms of 40 mg of 1:1 mixture of catechol (right peak) and resorcinol (left peak)

The increase in purity is noticed when compared with separations using a full isocratic or linear gradient separation (figure 6).



Linear Gradient

Isocratic Gradient

Figure 6. Chromatograms of attempts to purify same mixture using isocratic and linear gradients.

3. Scale up from milligrams to grams

Separation of the two diols can easily be scaled from 40mg to 7.5g by using the same method automatically scaled to the appropriate column size. Maintaining the same 1.0 wt.% sample load, the separation on a 750 gram RediSep flash column is performed on the Companion XL which separated the compounds with the same purity (figure 7).

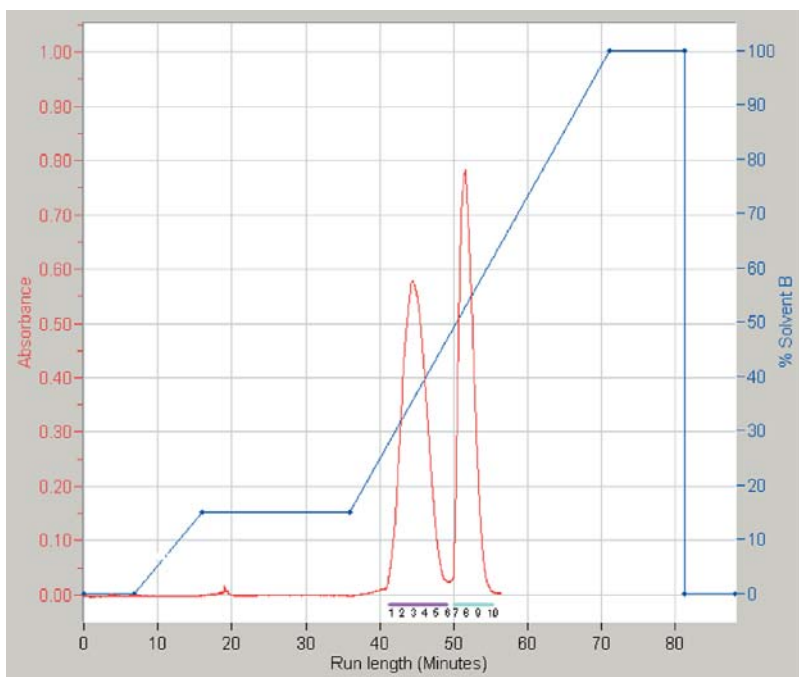


Figure 7. Chromatogram of a 7.5 gram mixture (1:1) of catechol (right peak) and resorcinol (left peak) on a RediSep 750g Normal Phase column.

Consistent Purity

Relative retention times and resolution are maintained with PeakTrak's auto scale-up feature when scaling from one sample size to another as seen in the series of chromatograms in figure 8.

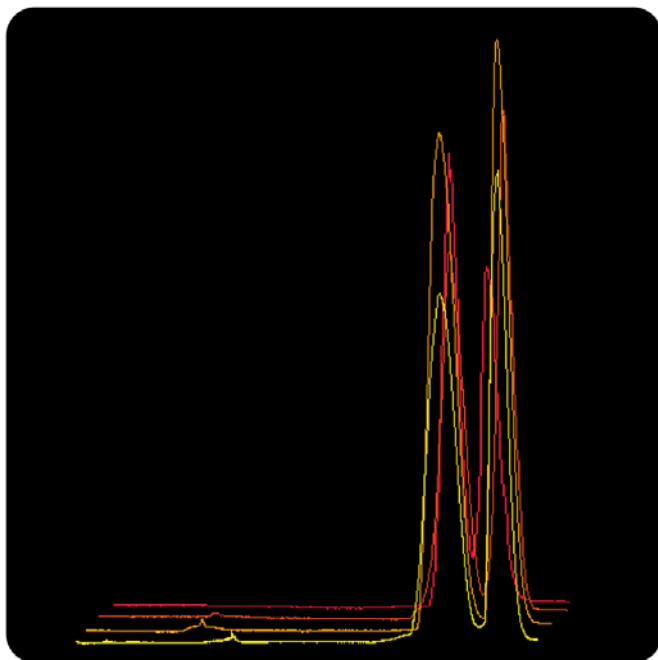


Figure 8. Chromatograms of catechol and resorcinol using hexane/ethyl acetate as the mobile phase at 1.0 wt% sample load on 4, 12, 120, and 750 gram RediSep columns.

Summary

The increased number of compounds that can be produced when automated flash chromatography is used is well documented among synthetic organic chemists. Additional productivity gains can be realized by using software features that suggests run conditions for high products purity and allows quick and simple scale-up for purifications from milligrams to several grams or even hundreds of grams of compound.

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TELEDYNE ISCO

A Teledyne Technologies Company

P.O. Box 82531, Lincoln, Nebraska, 68501 USA

Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091

E-mail: IscoInfo@teledyne.com