# Automated Step-gradient Methods Improve Flash Purification Throughput and Provide Cost Reductions

Bob Bickler

Biotage, 10430 Harris Oaks Blvd., Suite C, Charlotte North Carolina 28269, USA.

## Introduction

For many years flash chromatography has been performed using isocratic elution, linear gradients, or a combination thereof. Often the elution methods are based on previous experience, gut-feel, or generic default gradients while sample loading usually follows the 1% rule (1% of the cartridge media mass) resulting in less than optimal purifications.

Several years ago Biotage developed and patented a TLC to linear gradient algorithm that uses TLC data to create a linear gradient and estimate sample load for up to three compounds. New software recently developed by Biotage for its Isolera™ Spektra flash chromatography systems now provides the option of optimizing the purification for up to six compounds using as few as two TLC separations to create a step gradient.

## Experimental Protocol

### Reagents and materials

Reagents used in the study included: Hexane, Ethyl acetate, Naphthalene, 1-Nitronaphthalene, 3,5-Dibenzyloxy acetophenone, Butyl paraben, and Methyl paraben, all from Sigma Aldrich (Milwaukee, WI).

TLC plates and flash cartridges were from Biotage (Charlotte, NC).

### Sample Preparation

A 5-component crude mix of Naphthalene, 1-Nitronaphthalene, 3,5-Dibenzyloxyacetophenone, Butyl paraben, and Methyl paraben were combined and dissolved in Ethyl acetate at ~1 g each/5 mL.

### Chromatography Method Development

Prior to flash purification each compound was chromatographed using TLC at two Hexane/Ethyl acetate ratios (90:10 and 70:30). Two different gradients were used with the sample, a generic 5% to 40% linear gradient and a TLC data based step gradient using the Isolera Spektra gradient optimization feature (figure 1).

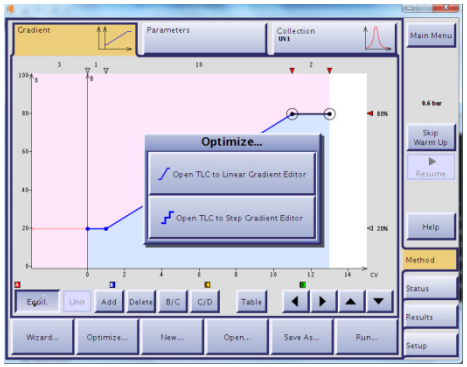


*Figure 1. The Isolera™ Spektra gradient optimization feature provides a choice of TLC to linear gradient or TLC to step gradient*

To create the step gradient the strong solvent percentage and Rf data from a minimum of two TLC runs is entered into a table (figure 2). The Isolera Spektra then uses that data to create a step gradient optimized to separate each compound (figure 3).

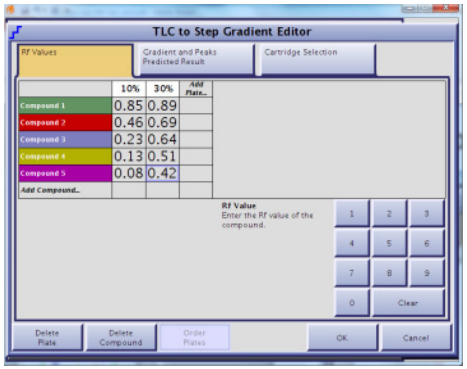


*Figure 2. The TLC to step gradient method creator uses TLC solvent % and Rf data (left) to generate and display the expected gradient for the sample.*

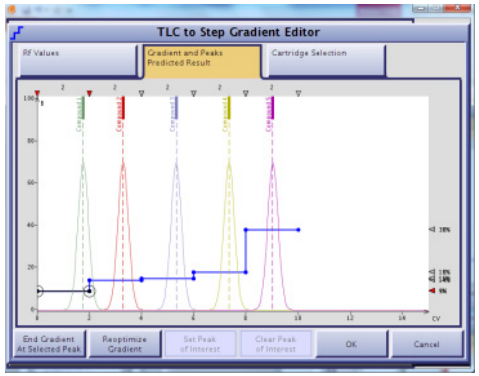


*Figure 3. The TLC solvent and Rf data are converted into a multiple step gradient designed to optimally separate each compound and maximize sample load.*

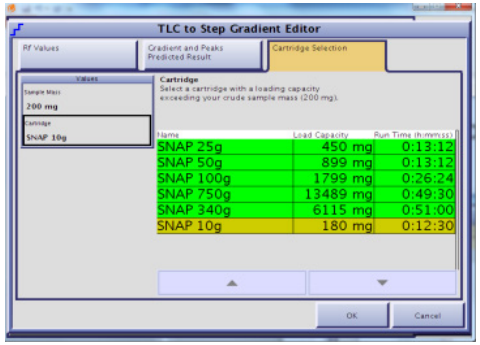


*Figure 4. From the TLC data, Isolera™ Spektra will also suggest cartridges based on crude sample size, the cartridge's sample capacity for the gradient conditions, and the estimated run time at default flow rates. Green highlighted cartridges have more than enough capacity, yellow highlighted cartridges have just enough capacity.*

## Results and Discussion

The results from the TLC to step gradient optimization estimate a Biotage® SNAP KP-Sil 10g cartridge will be able to purify 180 mg in 12.5 minutes at the default flow rate (12 mL/min). The 1% sample load rule was used for the generic linear gradient using the same cartridge size which equated to a 100 mg load.

For all purifications a flow rate of 20 mL/min was used. The step gradient purification sample load was 200 mg.

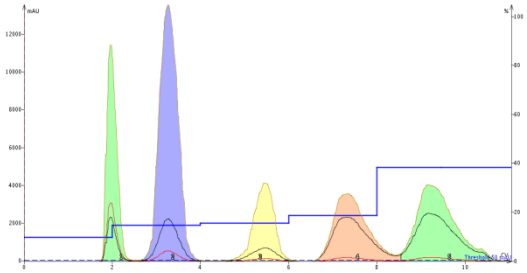

*Figure 5. Actual step gradient purification of 200 mg of the 5-component crude sample. The separation required only 11 column volumes or 8 minutes to complete with better overall separation than the generic linear gradient.*

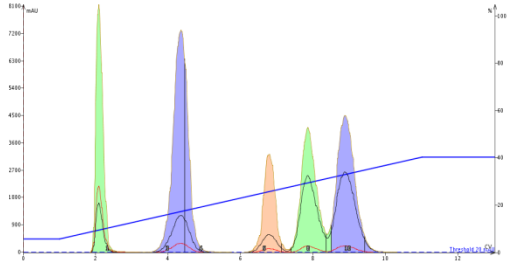

*Figure 6. TLC to linear gradient of 100 mg of the 5-component crude sample requiring 13 column volumes to complete (10 min). Sample load was 100mg and the last three compounds are not fully resolved*

## Conclusion

The results clearly show the Isolera Spektra automated TLC to step gradient reduces purification costs in several ways:

- Increased sample loading per cartridge reduces the number of required cartridges or cartridge size required
- Less time needed to purify therefore more productivity
- Less solvent consumed reducing purification costs

Using the TLC to step gradient is simple and fast requiring only a minute or so to enter the data into the Isolera and create the method. The methods are scalable to larger cartridges increasing purification savings even more.



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