Strategies for Improving Flash Chromatography Efficiency

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Introduction

For most organic and natural product chemists flash chromatography is a necessary part of their research. As such, many chemists need quick isolation of at least one desired component from a crude mixture in relatively high yield and purity. This need for speed, purity, and yield pits these desires against each other as you can typically optimize on only two of the three goals.

In this poster, we will describe some techniques that help chemists optimize flash purification and maximize speed, yield, and purity.

Experimental Protocol

Reagents and Materials

Reagents used in the study included: Hexane, ethyl acetate, and acetone were obtained from Reagents, Inc., Charlotte, NC. 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).

A Biotage Isolera™ was used for purification.

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 of acetone.

Goal Setting

Though a quick purification is ideal for a speedy conclusion to the synthesis, haste often leads to waste. A more efficient technique is to consider what you need the purification to accomplish.

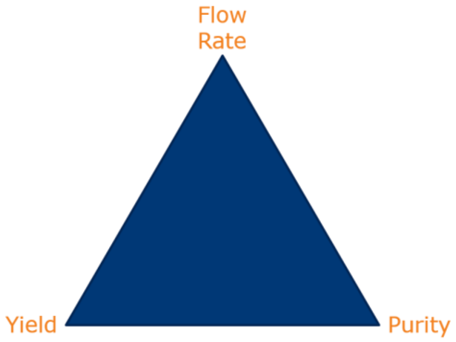


***Figure 1**. The Chromtographer's Triangle, which shows that flow rate, compound purity, and compound yield goals impact each other.*

There are three possible goals with purification –

1. Purity
2. Yield
3. Speed

Unfortunately, optimization on all three is not possible to the extent most chemists would like so, as a chemist, you typically need to prioritize whether you need high purity with potentially less yield, more compound with potentially less purity, or lower purity and yield but a fast purification, Figure 1.

Optimization Options

Using some technical innovation it is now possible to come closer to full method optimization where loading capacity increases without sacrificing yield or purification speed. To accomplish this you need…

1. TLC (2 runs, same solvents, different ratios)
2. High performance, high surface area silica cartridges
3. An optimized step gradient (based on the TLC data above)

Step gradients have major benefits over linear gradients including reduced purification time and increased loading capacity, Figure 2. Step gradients can also be optimized for the purification of several compounds rather than a single compound, which is how most linear gradients are based.

Results and Discussion

Isolera® systems have optimization capability built-in, simplifying its use. Both TLC to linear gradient and TLC to step gradient capability is included and these are compared.

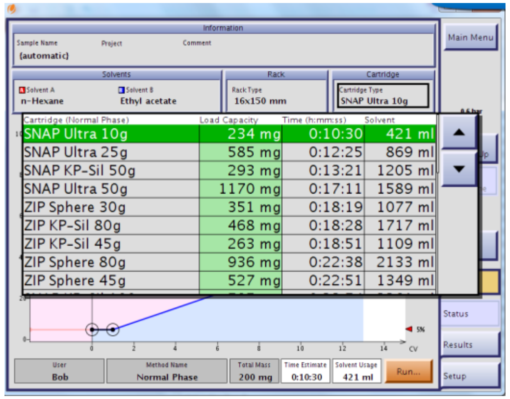
TLC to Gradient

While many chemists estimate the elution gradient or go with a default 0-100% and assume a 1% loading capacity (1% crude mixture load of the amount of silica in the cartridge), these strategies are not necessarily efficient. TLC data will assist with creating a gradient profile to elute all compounds with an Rf 0.1 or greater and provide a load estimate based on how well your targeted compound is separated.

For this poster, the separation of the 5-component mix was evident using both 20% EtOAc and 30% EtOAc in hexanes. Rf data from the TLC plate run in 20% EtOAc generated a linear gradient wheras the Rf data input from the two TLC plates (eluted in both 20% and 30% EtOAc, respectively) generated a step gradient alternatively.

Linear Gradient

The 20% EtOAc TLC data produced a gradient (5-40% EtOAC) with sample load of 234 mg, a run time of 10.5 minutes, and solvent consumption estimate of 421 mL, Figure 2.



Information
Sample Name: {automatic}
Project
Comment

Solvents
Solvent A: n-Hexane
Solvent B: Ethyl acetate

Rack
Rack Type: 16x150 mm

Cartridge
Cartridge Type: SNAP Ultra 10g

Cartridge (Normal Phase)	Load Capacity	Time (h:mm:ss)	Solvent
SNAP Ultra 10g	234 mg	0:10:30	421 ml
SNAP Ultra 25g	585 mg	0:12:25	869 ml
SNAP KP-Sil 50g	293 mg	0:13:21	1205 ml
SNAP Ultra 50g	1170 mg	0:17:11	1589 ml
ZIP Sphere 30g	351 mg	0:18:19	1077 ml
ZIP KP-Sil 80g	468 mg	0:18:28	1717 ml
ZIP KP-Sil 45g	263 mg	0:18:51	1109 ml
ZIP Sphere 80g	936 mg	0:22:38	2133 ml
ZIP Sphere 45g	527 mg	0:22:51	1349 ml

User: Bob
Method Name: Normal Phase
Total Mass: 200 mg
Time Estimate: 0:10:30
Solvent Usage: 421 ml

***Figure 2**. TLC to linear gradient software predicts a load of 234 mg with a 10.5-minute purification time for a 10-gram cartridge.*

Put into practice, the results yield a decent separation with a 200 mg load utilizing a 10 gram Biotage® SNAP Ultra cartridge, Figure 3. The fourth eluting peak is the targeted compound and it is mostly separated from its nearest neighbors.

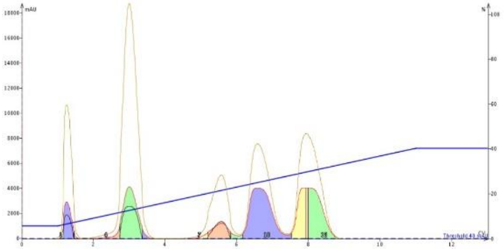
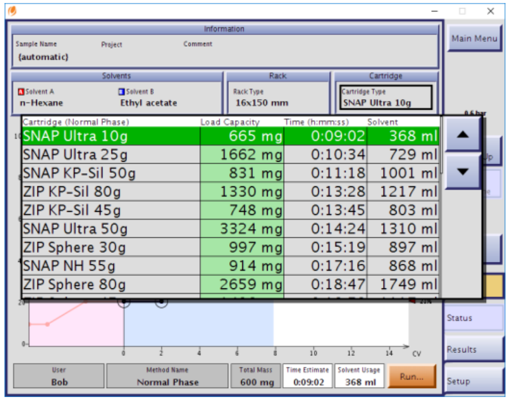


***Figure 3**. Linear gradient purification based on predictive TLC software. 200 mg was loaded and separated in 10.5 minutes.*

Using the Rf data from two TLC plates (eluting in 20% and 30% EtOAc), the Isolera TLC-to-step gradient capability determined a loading capacity of 665 mg for the same cartridge – nearly a 3-fold load increase, Figure 4.



Information
Sample Name: {automatic}
Project
Comment

Solvents
Solvent A: n-Hexane
Solvent B: Ethyl acetate

Rack
Rack Type: 16x150 mm

Cartridge
Cartridge Type: SNAP Ultra 10g

Cartridge (Normal Phase)	Load Capacity	Time (h:mm:ss)	Solvent
SNAP Ultra 10g	665 mg	0:09:02	368 ml
SNAP Ultra 25g	1662 mg	0:10:34	729 ml
SNAP KP-Sil 50g	831 mg	0:11:18	1001 ml
ZIP KP-Sil 80g	1330 mg	0:13:28	1217 ml
ZIP KP-Sil 45g	748 mg	0:13:45	803 ml
SNAP Ultra 50g	3324 mg	0:14:24	1310 ml
ZIP Sphere 30g	997 mg	0:15:19	897 ml
SNAP NH 55g	914 mg	0:17:16	868 ml
ZIP Sphere 80g	2659 mg	0:18:47	1749 ml

User: Bob
Method Name: Normal Phase
Total Mass: 600 mg
Time Estimate: 0:09:02
Solvent Usage: 368 ml

***Figure 4**. TLC to step gradient predicts a load of 665 mg with a 9-minute run time using the same 10-gram cartridge. The step gradient provides purification of nearly three times the material in less time than the linear gradient.*

Though the software predicted a load of 665 mg was possible and still be well-separated, only 400 mg were available to purify. Implementing the step-gradient resulted in a complete separation with improved resolution in less time compared to the linear gradient, even with a 2-fold load increase, Figure 5.

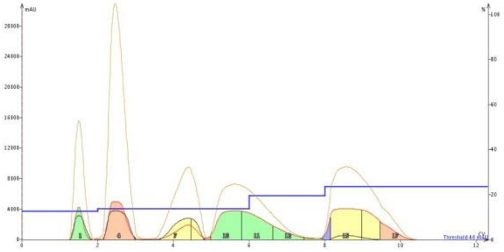


***Figure 5**. Step gradient purification with a 400 mg load shows complete separation of the five compounds.*

Conclusion

To maximize flash purification efficiency, consider using step gradients, especially with high performance cartridges, which allow for high loads of crude mixtures. This technique saves both time and solvent without sacrificing purity or yield, a great way to improve workflow efficiency.

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