Flash Chromatography Method Development in a Flash!

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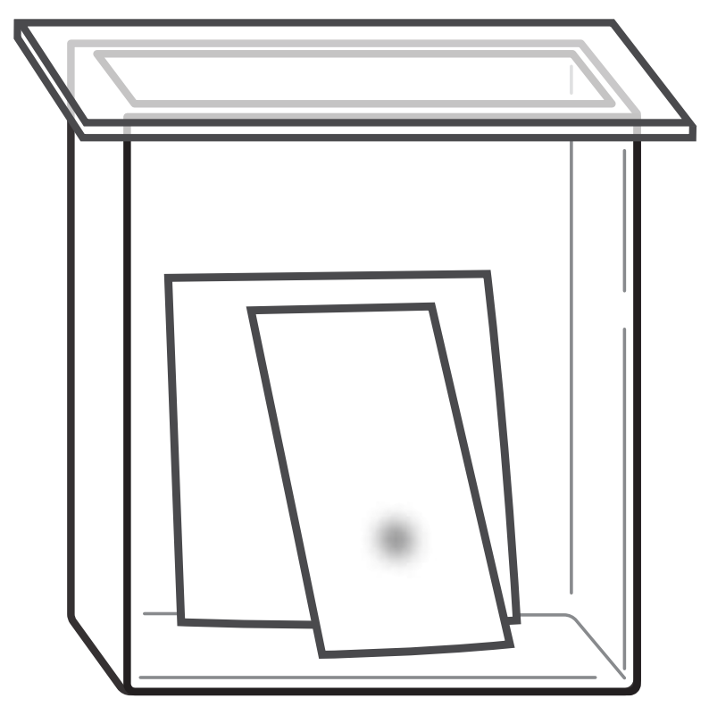
Abstract

Flash chromatography is intended as a rapid purification method;[1] however, automated flash chromatography instruments allow the use of alumina, various reverse phase, and amine columns in addition to silica used by Still et al. Method development for silica columns can be done with thin layer chromatography (TLC) plates; however, TLC for other media can be slow to develop, and the TLC plates for those media aren't commonly found in many labs. Furthermore, creating a TLC method may require several trials.

The use of the Time-on-Target algorithm[2] allows rapid method development using scouting gradients commonly used for high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) using any column chemistry. Scout runs need the same amount of time as a single TLC plate and can be run on the flash system or analytical HPLC. The result is a focused gradient that is typically completed in a few minutes, thus returning flash chromatography to its roots.

Background

Flash chromatography method development has traditionally been run with silica thin layer chromatography (TLC) because it is easy to run, inexpensive, and because the column elution time in column volumes is the inverse of the TLC plate retention factor allowing prediction of elution with a given solvent system. However, modern automated flash chromatography uses other columns than silica gel such as alumina, amine, and various types of reverse phase media. TLC for these media may be slow to run, or not readily available in a lab. Reverse phase methods are sometimes developed on HPLC systems, yet these may still take many trials to develop a method, and the results may not be as expected because most analytical columns don't match the flash media*. A newer technique, the Time-on-Target (ToT) algorithm developed, for preparative HPLC, works very well for flash chromatography. The infographic below compares the advantages of each method.

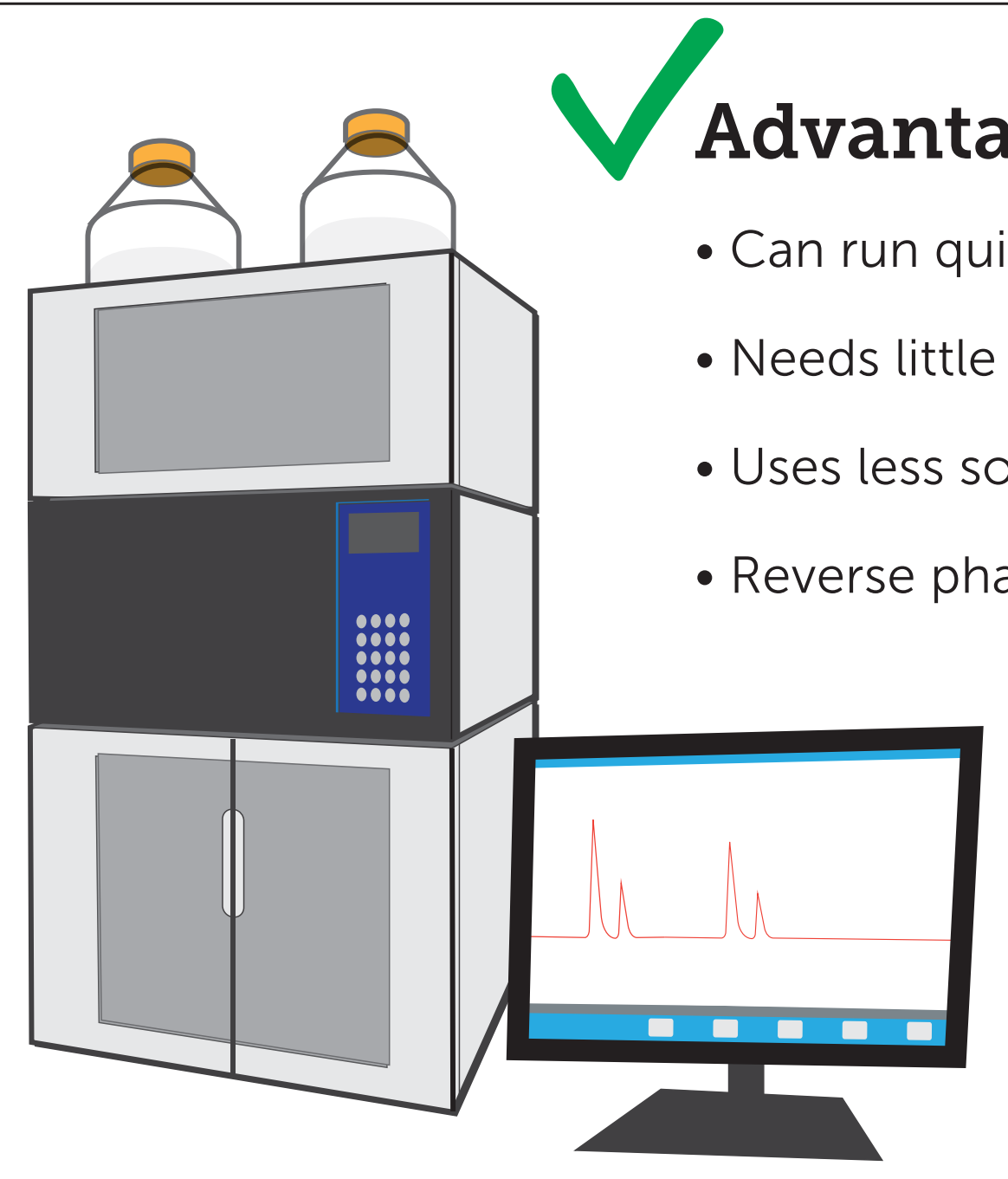


✓ Advantages of TLC:

- Inexpensive.
- Uses little solvent.
- Many plates can be run at the same time.

✗ Disadvantages of TLC:

- Must be run correctly.[3]
- Many plates and solvent compositions are often needed to develop a method.
- Plates often don't match column media, so selectivity and retention doesn't match the flash column.
- Reverse phase TLC plates are slow to run.
- Some TLC plates aren't commonly used (amine, for example.)
- Spots are sometimes hard to visualize.

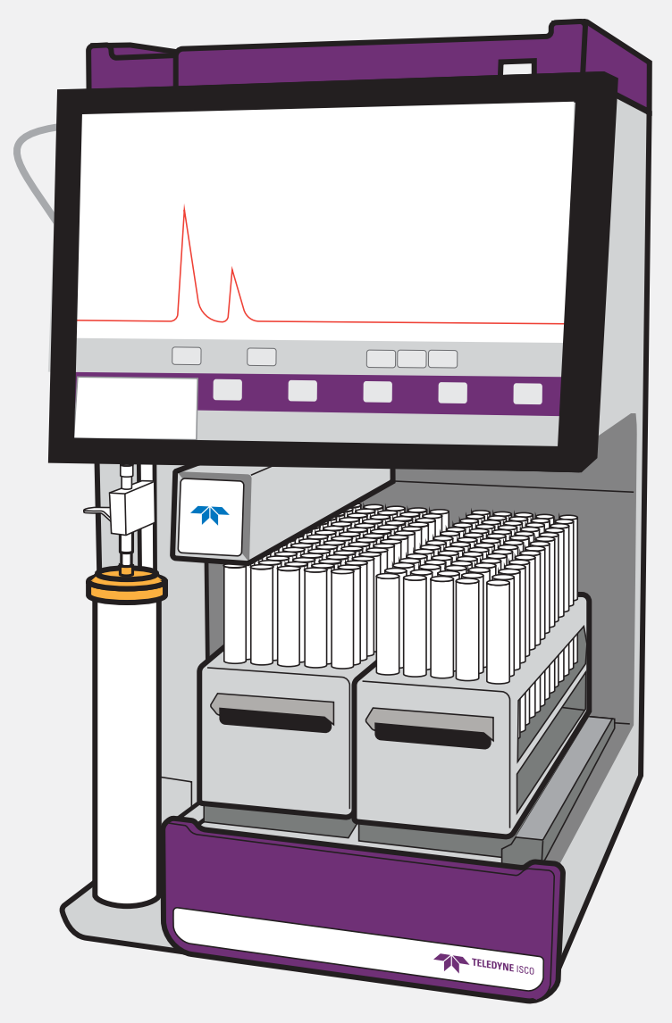


✓ Advantages of HPLC:

- Can run quickly.
- Needs little sample.
- Uses less solvent than flash system.
- Reverse phase runs faster than TLC.

✗ Disadvantages of HPLC:

- Several trials are needed to create a method (unless Time-on-Target algorithm used).
- Analytical columns don't match the flash column media.*
- Comparable analytical column is not available (for example, alumina).
- Need to calculate scale-up such as gradient segment lengths.



✓ Advantages of ToT:

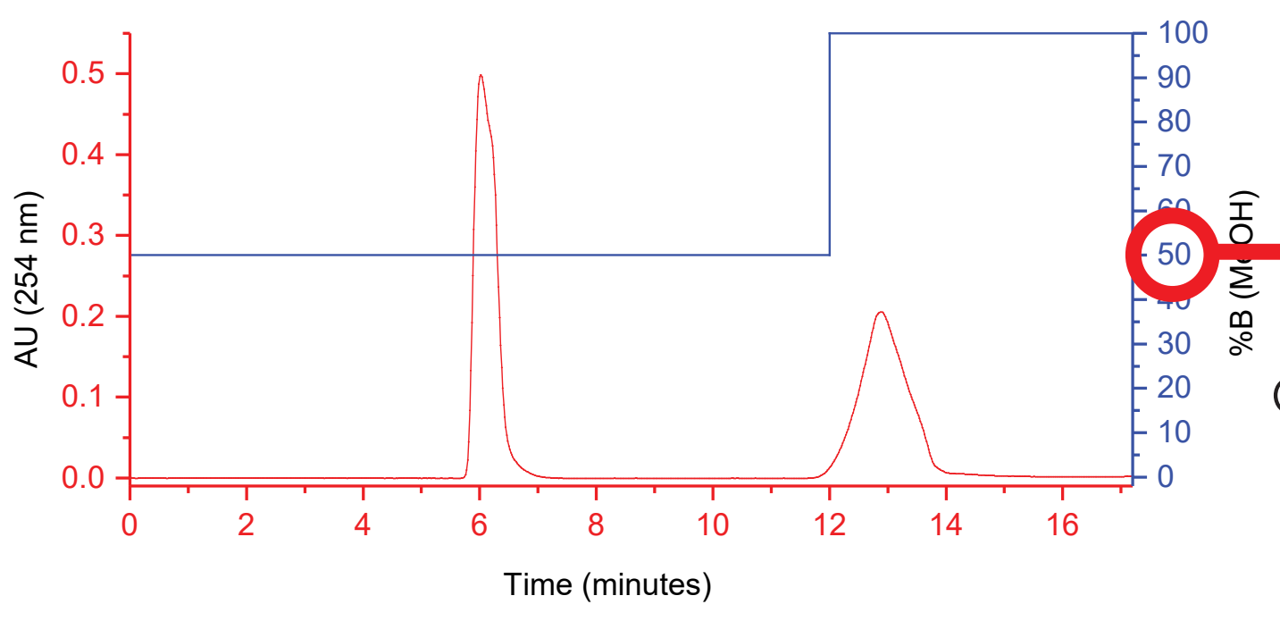
- Provides a method in the same time as a single TLC plate.
- Needs little sample.
- Uses the same column chemistries for method development as prep, so the elution is more predictable.
- Easy to scale to larger columns.
- Easy to see if the chromatography works.
- Will the compound elute?
- Peak shape suggests good elution.
- Can use HPLC for the scouting run.*
- Can use detectors (ELSD, Mass spectrometer) to see compound.
- Runs on the flash system.

✗ Disadvantages of ToT:

- Uses more solvent than TLC

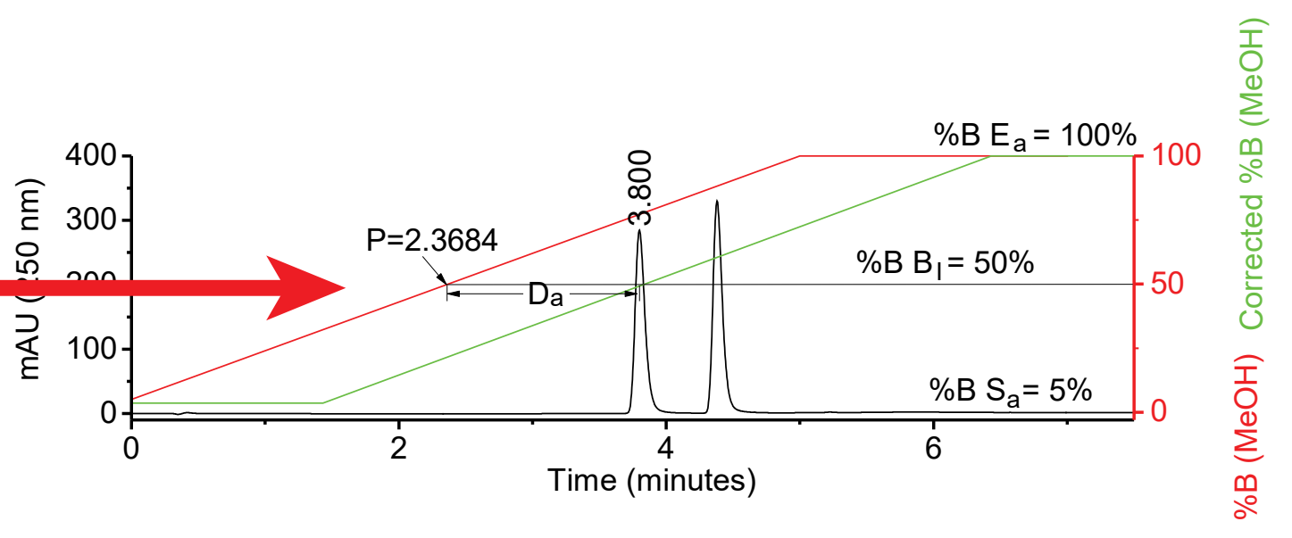
The "Time-on-Target" algorithm: explanation and examples

The ToT algorithm determines the apparent gradient delay needed to cause elution of a compound at a desired time in a focused gradient. The desired retention time generally resolves the active compound from impurities. An isocratic run of a model compound is used to determine the desired retention time. The solvent composition used to elute this compound is used to determine the apparent gradient delay for a given scouting run. Compounds to be purified are run with the same scouting gradient. The apparent gradient delay is applied to the retention of those compounds to calculate a focused gradient for purification.

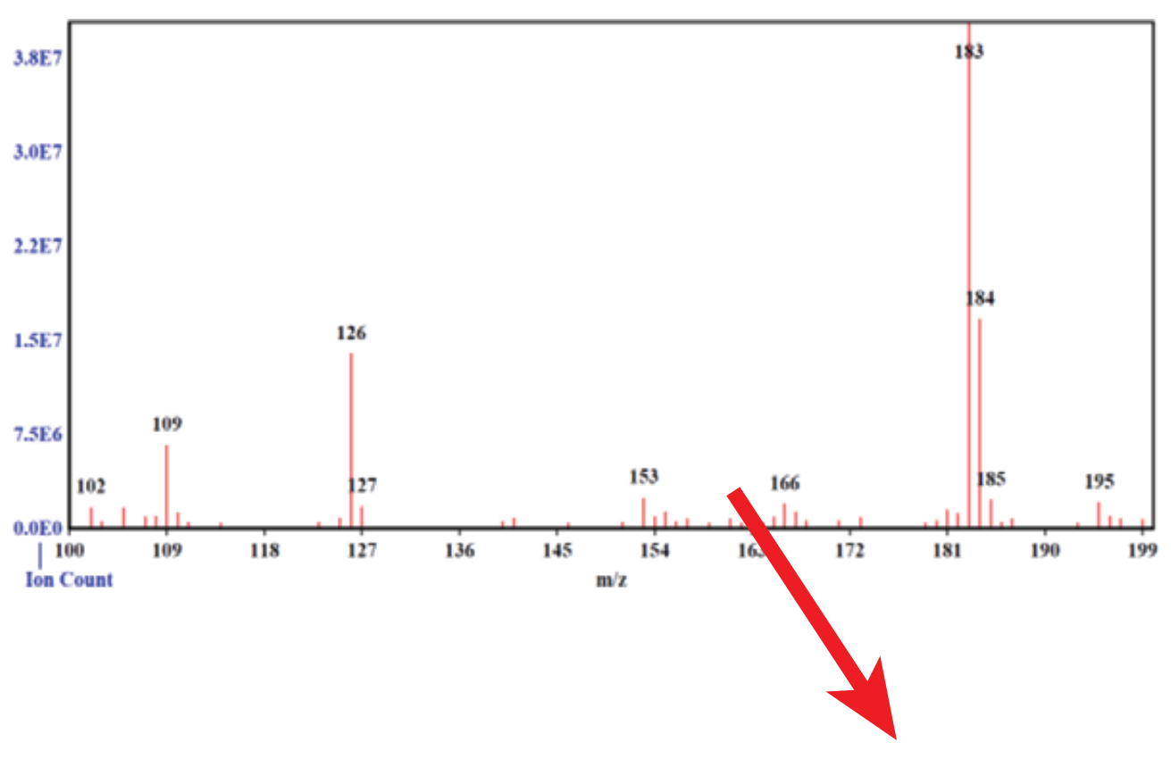


50% B in isocratic method.

Calibration compound retention time sets 50% B in scout run.

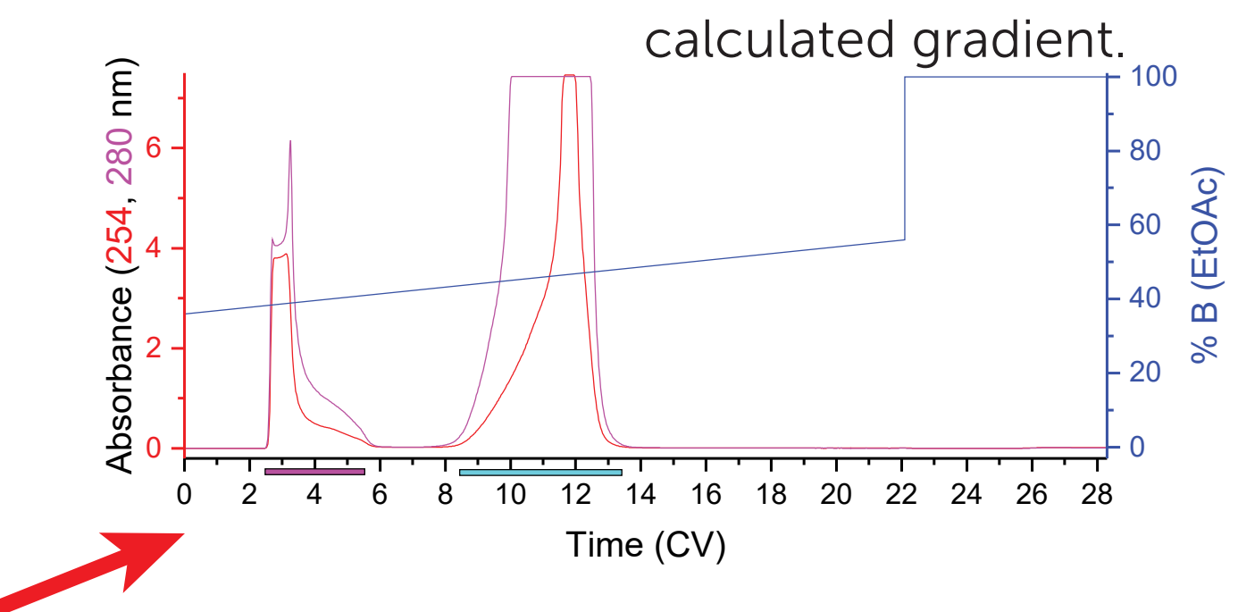


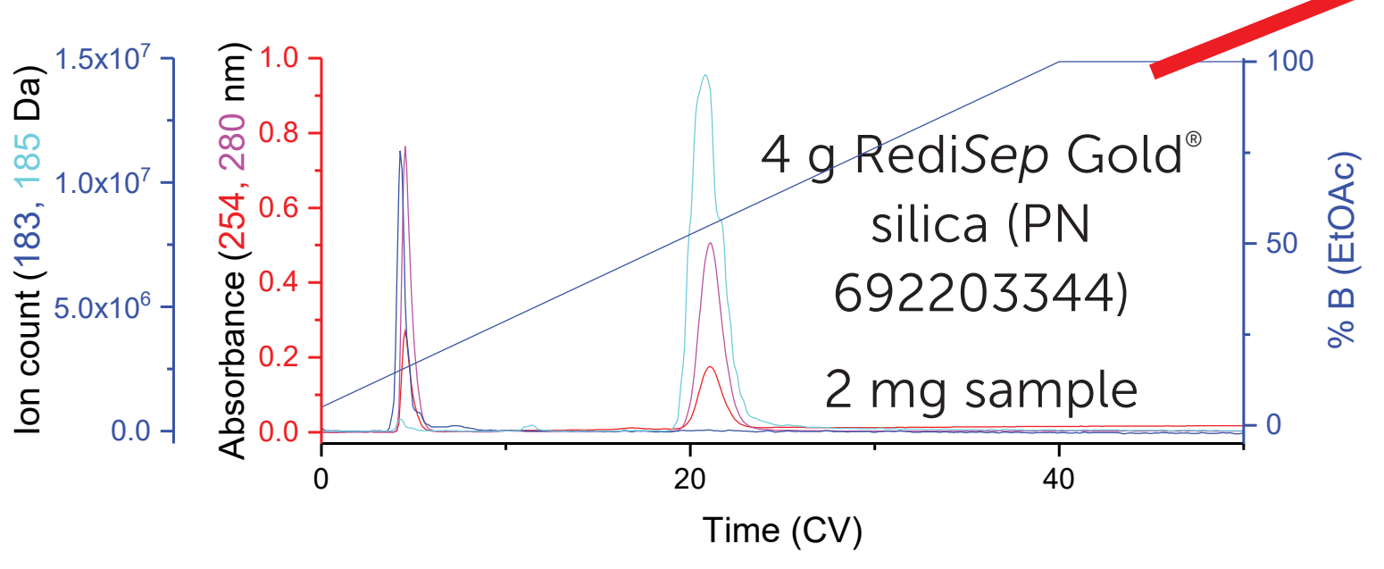
Silica Example



Crude benzidine mixture show the desired product at 185 Da and an impurity at 183 Da, likely azobenzene.

Prep run using calculated gradient.

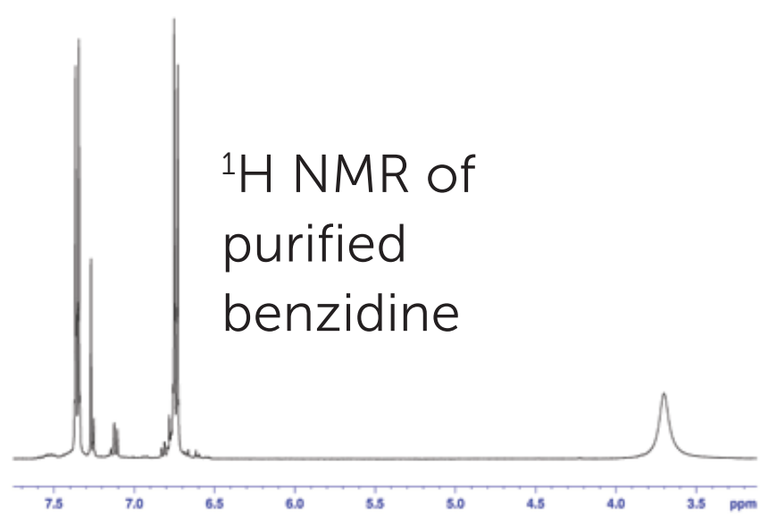




4 g Redi*Sep* Gold® silica (PN 692203344)

2 mg sample

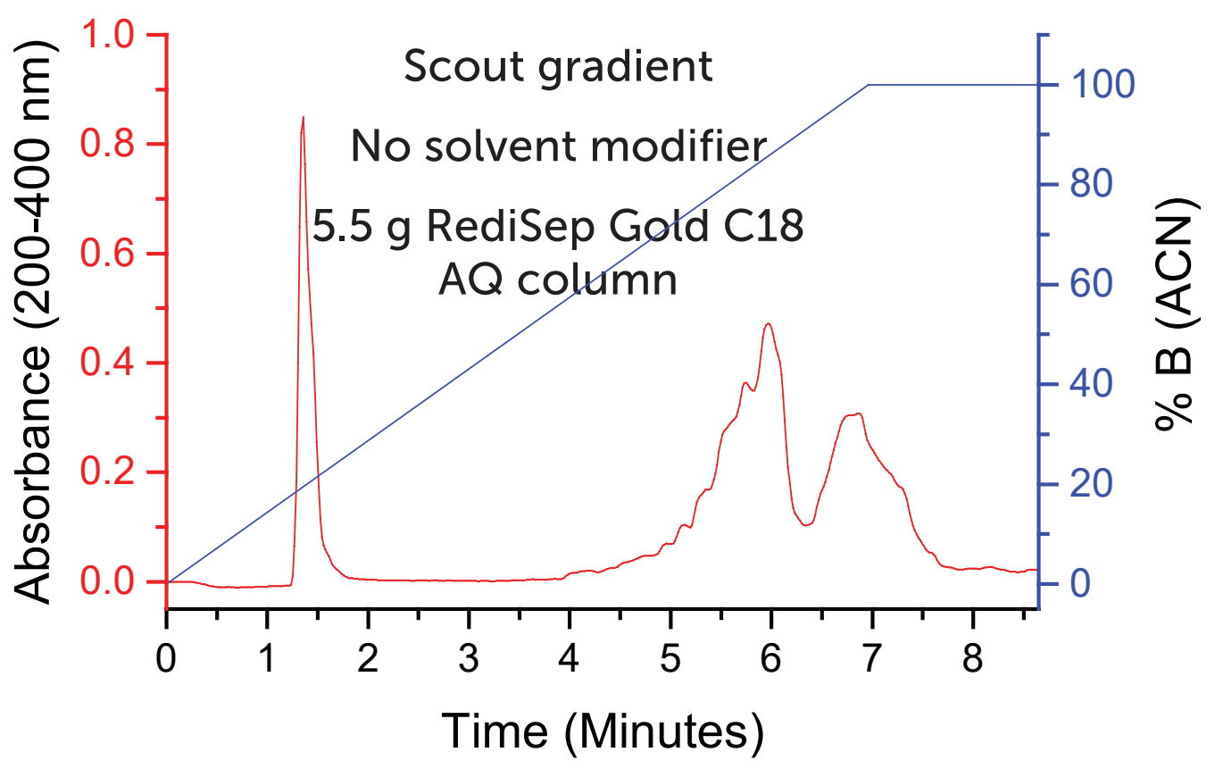
Scouting run.



^{1}H NMR of purified benzidine

Benzidine (0.5 g) run on a 12 g Redi*Sep* Gold® silica column (PN 692203345) adsorbed on 3 g silica and placed in a solid load cartridge (PN 693873235)

C18 Example

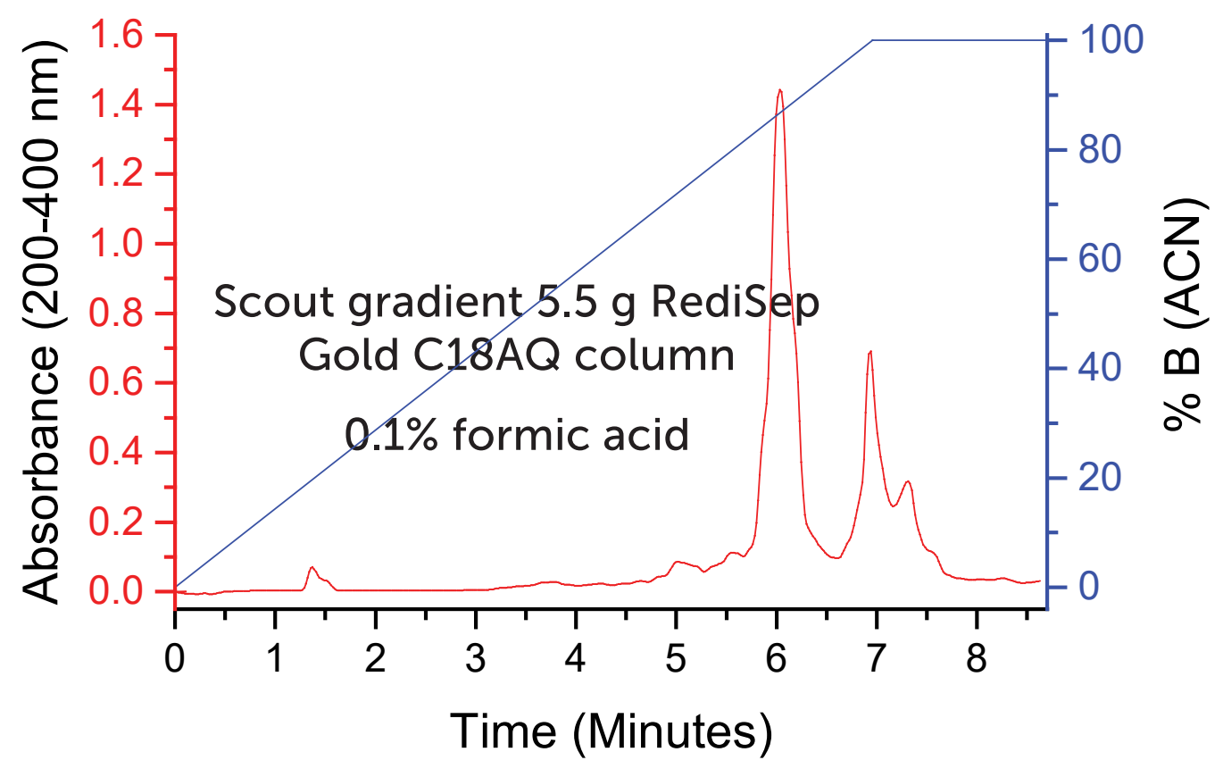


Scout gradient

No solvent modifier

5.5 g RediSep Gold C18 AQ column

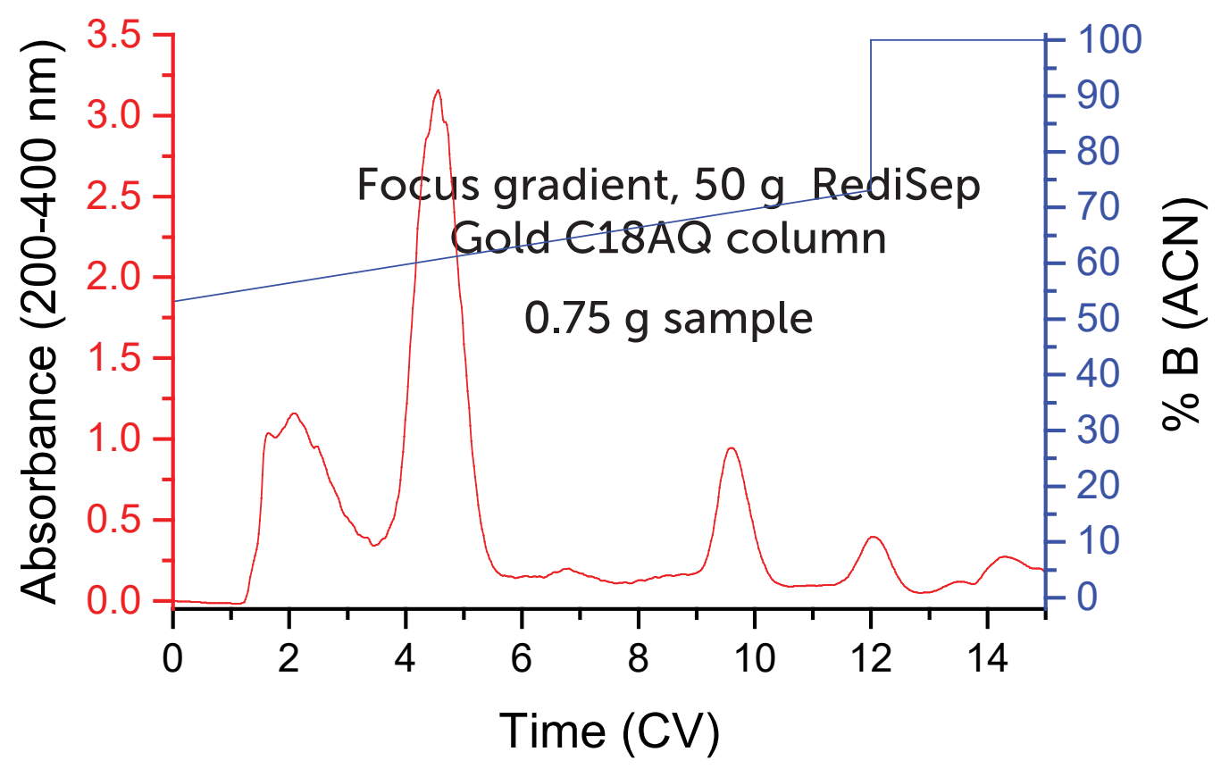
Crude curcumin extracts elutes without a solvent modifier, but the peaks have poor shape. The scouting gradient indicates a solvent modifier is needed.



Scout gradient 5.5 g RediSep Gold C18AQ column

0.1% formic acid

Adding 0.1 % formic acid greatly improves peak shape. The elution time incorrectly suggests that 90% B solvent elutes the compound.



Focus gradient, 50 g RediSep Gold C18AQ column

0.75 g sample

Focused gradient of curcumin extract.

Note elution at ~60% B as determined by the apparent gradient delay applied to the scout run.

Conclusion

The ToT algorithm, although originating as an aid to making efficient preparative HPLC purification methods, works very well for flash chromatography. The scouting run provides information about how well the sample will run in a solvent system, and it does so within the same time as it takes to run a single TLC plate. It works on all adsorbent flash media. If desired, reverse phase methods can be developed using RediSep® Prep analytical columns. The ToT algorithm, built into PeakTrak® software, allows fast method development with a single scouting run.

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[1] Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *The Journal of Organic Chemistry* **1978**, *43 (14)*, 2923–2925. https://doi.org/10.1021/jo00408a041.

[2] Silver, J. "Overview of Analytical-to-Preparative Liquid Chromatography Method Development."*ACS Combinatorial Science*, **2019**, *21*(9), 609–613. DOI: 10.1021/acscombsci.8b00187

[3] Silver, J. Let Us Teach Proper Thin Layer Chromatography Technique! *J. Chem. Educ.* **2020**, *97, 12*, 4217–4219. https://pubs.acs.org/doi/10.1021/acs.jchemed.0c00437

* Teledyne ISCO RediSep Prep columns match reverse phase RediSep flash columns

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