How Can Analytical Scouting Gradients Help Us Do Prep HPLC?

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Abstract

Analytical scouting gradients provide useful information prior to preparative HPLC while using very little sample. Scouting runs provide an estimate of compound purity. By observation of whether peaks show fronting or tailing, one may determine the best solvent systems and modifiers to purify compounds. Analytical scouting runs also allow calculation of a focused gradient for the preparative purification[1]. The potential sample loading on the preparative column can now be determined with little more information than the retention times of the desired compound and nearest impurity, the preparative gradient slope, and dimensions of the preparative column. The calculation determines the resolution between peaks which then determines sample loading. If multiple peaks are of interest for collection, the calculation also suggests whether a gradient can reduce the purification run time.

Background

During the purification of natural products, analytical HPLC is run to determine the purity of the mixture, whether a purified fraction or a synthesized compound. To fully characterize the purity of the mixture, a gradient is usually run from 5 to 10% organic up to 95–100% organic solvent. However, this analytical run may provide more information than just purity.

We can:

- Calculate the elution parameters for a focused gradient once the delay for the scouting gradient has been determined[1].
- Determine whether an isocratic, or slight focused gradient, would be suitable to resolve the compounds of interest or whether a steeper gradient is required from the resolution between the peaks of interest[2,3].
- Estimate the sample load based on the resolution between the peaks of interest.

Results and Discussion

Capsaicin is used as a model for other natural products as it is purified as a mixture of related active compounds, as often occurs with many natural products. The analytical chromatogram in Figure 1 will be used as a basis for the calculations for the preparative runs.

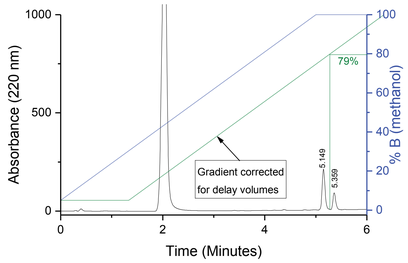

Figure 1–Analytical run of crude capsaicin in water/methanol

Steep Gradient or Focused Gradient?

Since there is interest in both compounds, the first question is whether the mixture could be purified with a flat focused gradient or whether a larger gradient would be needed. A gradient with a 10% range over 12 minutes is sufficiently close to isocratic that it acts as if it were isocratic. The advantage of a focused gradient is that it allows some variation due to differences in the analytical and preparative system pumping system, temperature, and other variances which may show retention time differences from the calculated isocratic solvent mixture.

Equations 1 and 2 allow determination about whether a compound could be purified with a nearly isocratic focused gradient or whether a gradient with a wider range is required.

$$\frac{\Delta t}{t_G} < 0.15$$

Equation 1–Methanol Gradients[2]

$$\frac{\Delta t}{t_G} < 0.25$$

Equation 2–Acetonitrile Gradients[3]

As the scouting gradient was run in methanol. Equation 1 applies since (5.359–5.149)/5 = 0.042, which is smaller than 0.15, so a focused gradient works well.

Something interesting to note is that a value of these compounds are barely resolved on flash chromatography, so if $\Delta t/t_G < 0.05$, prep HPLC is a better choice of purification technique.

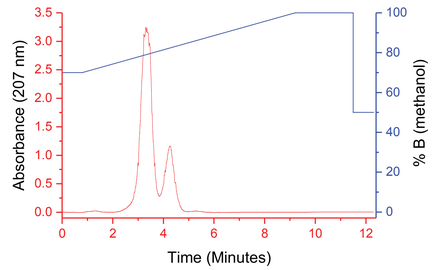

Figure 2–Purification of crude capsaicin with reverse phase flash chromatography

Determination of Elution Conditions

The elution conditions were calculated as described earlier[1]. Briefly, the dwell volume of the analytical UHPLC system and the column volume of the analytical column were measured. A "mixing volume" was empirically determined and added to determine the gradient delay on the UHPLC. The actual gradient was determined from the delay. After the gradient delay was applied, the actual elution solvent composition was determined from the average elution time of the two compounds (Figure 1).

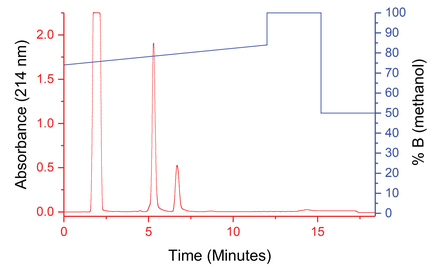

Figure 3–Purification of crude capsaicin with reverse phase preparative chromatography

Loading Capacity

The loading capacity depends on the resolution of the two compounds. Ideally, this is measured on the preparative system with a scouting run, since the solvent used to inject the sample affects resolution[4]. In addition, the sample isn't often weighed during analytical method development due to the small sample sizes needed. The difference in retention in a scouting gradient still provides some guidelines.

A mixture of parabens is used to determine sample loading. It was determined that a mixture of methyl and ethyl paraben could be loaded (1:1 mixture) to 156 mg (total mass of both compounds) within a focused gradient in methanol:water (Figures 4,5)

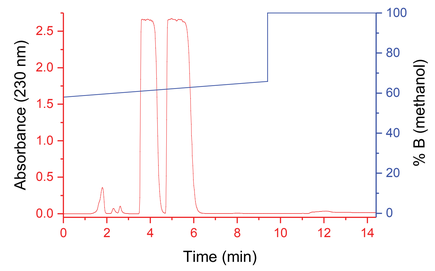

Figure 4–Maximum loading of methyl and ethyl parabens in a preparative purification

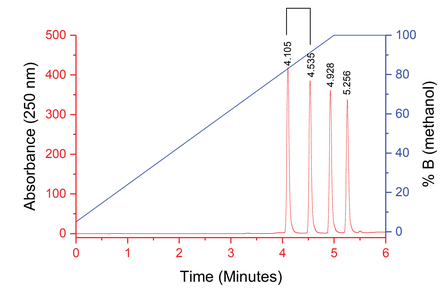

Figure 5–Analytical run of a mixture of parabens

In the analytical run, the parabens had a difference of 0.43 minutes. The capsaicins in Figure 1 had a time difference of 0.21 minutes. As a linear gradient is being run, the difference in time relates to the resolution of the compounds. To determine the capsaicin loading, the relationship 0.21/0.43 * 156 = 76 mg. This is close to 100 mg that could actually be loaded (Figure 6). Some of the difference arises because the capsaicin compounds are not present in equal quantities. The second eluting peak is present in a smaller quantity and doesn't exhibit the same broadening that a larger quantity of material would. If this peak were larger than the first eluting peak, the loading would be smaller than 75 mg, so this calculation is only a rough approximation at best.

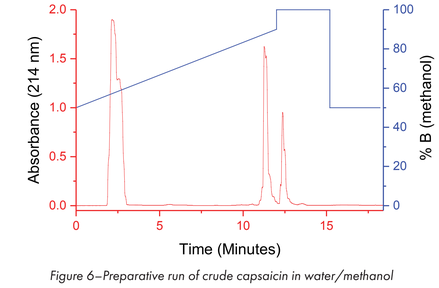

Figure 6–Preparative run of crude capsaicin in water/methanol

Conclusions

Analytical scouting gradients are run to determine the purity of extract fractions and synthesized compounds. These gradients can further be used to determine gradient conditions and whether isocratic conditions, or a flat focused gradient close to isoratic conditions, will purify the compounds of interest. The scouting gradient can also suggest column loading, but the loading is a rough approximation.

References

1 Preparative method development from analytical columns. Silver, J.E. Presented at the ICSB meeting, Oxford MS, April 2017.

2 Alcázar, A.; Jurado J.M.; González, A.G. Gradient Scouting in Reversed-Phase HPLC Revisited. *J. Chem. Ed.*, **2001**, *(88)*1, 74-76.

3 Dolan, J.W. *LC-GC* **2000**, *18*, 478.

4 Prep LC: How much can I load? Silver, J.E.; Bailey, C.; Johnson, D.; Paeschke, P.; Lewis, R.L Presented at the 255th ACS National Meeting, New Orleans, LA, March 18, 2018. Paper MEDI 154.

Experimental

Preparative HPLC was run on a ACCQPrep (Teledyne ISCO, PN 68-523-0035) or an EZPrep Flash/Prep system (PN 68-5230-025) with a RediSep Prep 150x20 mm C18 column (PN 69-2203-810) run at 19.9 mL/min. Samples were injected with an AutoSampler (PN 68-5237-092) Flash chromatography was run on the CombiFlash EZPrep with a RediSep Rf Gold 15.5 g C18 column (PN 69-2203-334). Analytical UHPLC was run on an Agilent 1290 UHPLC with an experimental RediSep Prep UHPLC column (50x2.1 mm) run at 0.5. Solvents were from JT Baker.

ACCQPrep HP125
by Teledyne ISCO