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A Method to Save Time and Solvents Generating Purification Methods from Scouting Gradients

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Abstract

Preparative chromatography is an integral part of producing pure compounds in synthesis labs. Efficient preparative chromatography is fast, purifying many compounds within a short time period, while also reducing solvent usage and waste generation. Creating an efficient purification method from an analytical scouting gradient is difficult because the solvent composition eluting the compound of interest isn't obvious due to delays in the compound elution compared to the scouting method programmed gradient. Users resort to inefficient default gradients that will not separate the desired compound from impurities. This results in the need for several runs to optimize a gradient method, requiring more time and solvent. A method is presented that calculates an efficient focused gradient from a single scouting run after "calibrating" the scouting gradient to the preparative chromatography system. The procedure uses a scouting run which may be run on a UHPLC and generates a method on preparative system which can be run without further optimizations. Time and solvent savings using this technique are described. The procedure works for both normal and reverse phase chromatography.

Background

It is difficult to determine the solvent system that elutes a compound based solely on the peak retention time from a scouting run because the eluting solvent composition is delayed from the programmed gradient used for the scouting run. The gradient slope, mobile phase flow rate, internal column dimensions, and the analytical system dwell volume all play a role in the apparent gradient delay. Due to the difficulty in determining the actual solvent composition that elutes the compound, users often use one of the following techniques to purify their compounds:

- Iterative runs using a small amount of sample until a reasonable purification is obtained[1] which uses sample and solvent for each test run
- A default generic gradient (Figure 3) is useful for purifying a mixture of compounds with a wide range of structures. Such gradients need to be run sufficiently "flat" to allow resolution between compounds, requiring more time and solvent than an optimized method.

Time-on-Target

Time-on-Target [3] (ToT) is similar to the Accelerated Retention Window[2] algorithm, but the method to determine the apparent gradient delay (D_a) is different.

1. Set the desired elution time on the preparative system with a model compound using an isocratic method and note the solvent composition which gives the desired retention time (Figure 1). Also measure the system dwell and column volumes.
2. On the analytical system, matching column chemistry, run a scouting gradient with the same compound (Figure 2).

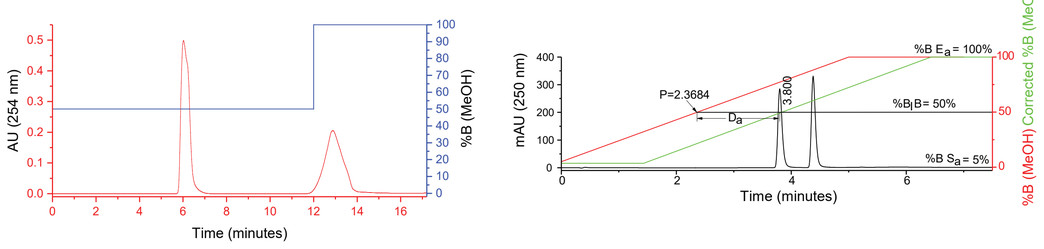

Figure 1—Phenacetin eluting in 50% methanol in water on C18 at 6 minutes (left), analytical scout calibration (right).

A compound requiring purification is run with the same scouting gradient (Figure 3). After application of the gradient delay, a solvent composition is determine which elutes the compound on the preparative system. A focused gradient is run (±5 %), which is corrected for the preparative dwell and column volume.

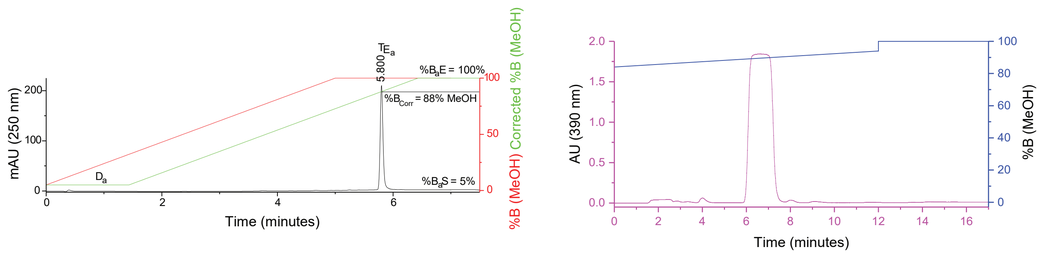

Figure 2—Scouting run of dimethyl yellow (left), purification of dimethyl yellow using focusing gradient calculated from the scouting run (right).

Comparison of methods

An ACCQ*Prep* HP150 was used for all runs. All runs assumed a 20 x 150 mm column running at 18.9 mL/min. Solvent usage includes equilibration (150 mL). The ACCQ*Prep* HP150 Focused Gradient Generator (based on ToT) was used to create gradients from a 4.6 x 150 mm column run on the same instrument prior to the preparative run. The default method was that configured for any column when the column is installed on the system.

Method type	Time	Solvent (L) per Run	Solvent Savings per Run (Compared to Iterative)	Solvent Savings per Run (Compared to Default)
Default	36.5	0.8	1.2	0
Iterative	87.5	2	0	-1.2
Focused Gradient †	47.5	0.62	1.38	0.18
Focused, no wash †	42.5	0.42	1.58	0.38
Focused, no scouting run	26	0.6	1.4	0.2
Focused, Terminate-on-Target †	36.5	0.32	1.68	0.48
Focused, no wash‡	20.5	0.42		
Focused, Terminate-on-Target‡	14.5	0.32		

† *Includes scouting run*

‡ *Multiple injections of same sample, no scouting run*

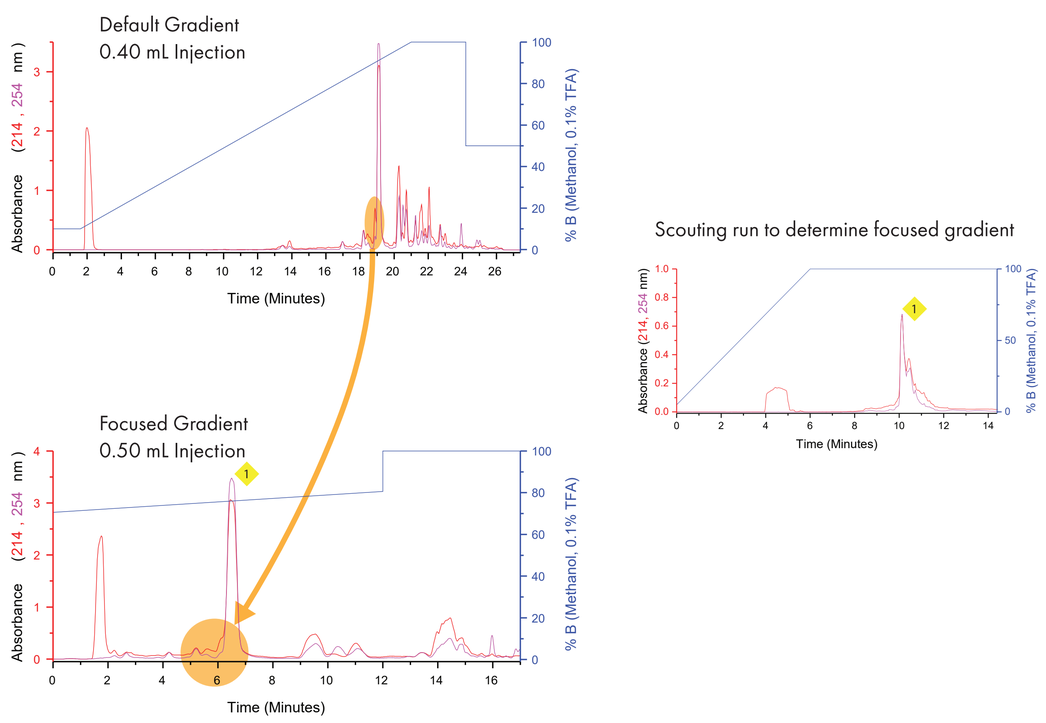

Figure 3—Crude piperine extract run with default gradient and optimized focused gradient. Shoulder on default run was resolved into 3 peaks!

Conclusion

Although the Default method requires less time, it does use nearly 200 mL more solvent for each run. The default method gradient is a compromise between speed and resolution, so some peaks are not fully resolved and require a second purification. The iterative method can provide the same results, but it takes much more time and solvent. The PurIon mass spectrometer allows mass-directed fractionation and allows the reduction of the focused gradient time from 12 minutes to ~6 minutes with the Terminate-on-Target feature. Terminate-on-Target utilizes mass-directed fractionation to end a run when the desired mass(es) have eluted from the column. The use of Terminate-on-Target results in same total purification time as the default gradient when including a scouting run and further reduces solvent usage. Time and solvent savings increase further when a sample requires multiple injections. The increased resolution provided by the focused gradient allowed 25% increased loading over the default gradient in the example purification; the increased loading reduces the number of runs required while still providing improved purity.

ACCQ*Prep*™ HP 150

with PurIon Mass Spectrometer & AutoSampler AS2x2

References

[1] https://www.chromacademy.com/lms/sco8/Theory_Of_HPLC_Gradient_HPLC.pdf Retrieved 27 May 2019

[2] Yan, B.; Collins, N.; Wheatley, J.; Irving, M.; Leopold, K.; Chan, C.; Shornisov, A.; Fang, L.; Lee, A.; Stocs, M.; Zhao, J. *J. Comb. Chem.* **2004**, 6(2), 255–261

[3] Silver, J.E. Proceedings of the 35th European Peptide Symposium Patrics B. Timmons, Chandralal M. Hewage, Michal Lebl (Editors) European Peptide Society & PSP, 2018