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SFC Focused Gradient from Analytical SFC

keywords: ACCQ*Prep*, method development, SFC

Abstract

Preparative SFC (supercritical fluid chromatography) is widely used to purify synthesized compounds. One bottleneck in the purification process is method development. Significant time can be required to produce an efficient preparative purification method that resolves the desired compound from impurities and minimizes both time and solvent usage. Teledyne LABS has developed techniques for significant reduction of method development time and resources based on calibration of analytical HPLC/UHPLC systems to match the preparative SFC system using the existing scouting gradients typically employed by a research group. Once this calibration is complete, the user can easily calculate a preparative method simply by correlating the retention time of the desired compound from the analytical LC scouting run. For further details on this technology, see Teledyne LABS's published papers on the subject.[1,2,3]

If using silica, use new silica columns on both the analytical and preparative system, as these adsorb moisture from solvents that absorbed it from the air, changing retention time and selectivity.

Purpose

This document defines the procedure for establishing a correlation between an analytical SFC system scouting method and a Teledyne LABS preparative SFC system to reduce the time and resources required to develop focused gradients or isocratic methods on the preparative SFC system.

Scope

This procedure applies to Teledyne LABS ACCQ*Prep*™ SFC running PeakTrak software version 6.1.2 or higher. For assistance with upgrading an existing ACCQ*Prep* system to the latest software version, contact Teledyne LABS technical support.

Calibration Procedure Overview

The calibration is accomplished by using analytical and preparative columns with matching chemistries. A standard test sample is prepared and run with a predefined analytical scouting gradient. The same sample is used to set a retention time using the preparative column, and the solvent composition from this column is used to calibrate the analytical scouting run. Once the calibration is complete, a mixture can be run on an analytical SFC, and the retention time of a given compound can be used to directly and reliably calculate an ACCQ*Prep* SFC focused gradient method.

Materials Required

This calibration procedure requires the following materials:

- Analytical SFC chromatography system.
- Analytical column with chemistry matched to prep column (see note below).
- ACCQ*Prep* SFC with PeakTrak version 6.1.2 or higher.
- Preparative column with chemistry matched to analytical column (see note below).
- Mobile phase for both systems: co-solvent = methanol, 2-propanol, ethanol, or acetonitrile

> **Note**
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> *A modifier such as trifluoroacetic acid may be used, but in that case the same modifier must be used in both the analytical scouting run and the focused gradient run.*

- Teledyne ISCO Universal Test Mix (part number 60-5234-835). Depending on the columns to be used, another test compound may be used if it elutes isocratically using more than 15% co-solvent.

> **Note**
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> *Column chemistry matches when both columns (analytical and preparative) come from the same manufacturer, are within the same brand/family, and—ideally—have the same pore size. The stationary phase particle sizes may be different.*

Step 1: Test Mix Preparation

Prepare the test mix to be used in the calibration process as follows:

1. Parent vial preparation:

    a) Obtain a test mix vial from the Teledyne ISCO Universal Text Mix kit (60-5234-835).

    b) Add 5 mL co-solvent to be used to the vial, replace cap, and shake to dissolve the powder. This vial will be used in Step 2 of the calibration process and again in Step 3.

2. Analytical sample vial preparation:

    a) Transfer 20 µL of solution from the above parent vial and dispense into a second vial, such as an HPLC sample vial used on the analytical system.

    b) Dilute the contents of the sample vial with 1 mL co-solvent.

Step 2: Analytical Scouting Gradient

Set up and run the gradient for the applicable analytical column as follows: after performing the analytical scouting calibration, enter the run parameters and retention time into Table 4.

Set up the analytical SFC system with 4.6 x 150 mm column, then set up and run the analysis as follows:

1. Configure the scouting run for the 4.6 x 150 mm column at a flow rate of 4.00 mL/min.

2. Set the gradient to run from 5% to 50% co-solvent. A higher co-solvent percentage may be used—up to 70% B—if the analytical system can reliably deliver such a solvent composition.

Note

Do not use an isocratic hold prior to the gradient.

3. Set the gradient length to 6 minutes, with a 3-minute isocratic hold at 50% Solvent B after the gradient.

4. The injection volume of the prepared SFC sample will range from 1 µL to ~10 µL; this varies with your analytical system.

5. Run the scouting method and note the retention time of the model compound. A useful result for calibration purposes will have an elution time of more than 1.8 minutes using the suggested gradient method.

Note

Peak intensity is not important so long as the model compound peak is clearly seen. Silica columns should have the same "history"—silica columns have solvent "memories" and may show changes in peak retention due to water adsorption. Teledyne recommends that the silica purifications are run entirely on the ACCQPrep SFC for best results, both scout and preparative runs.
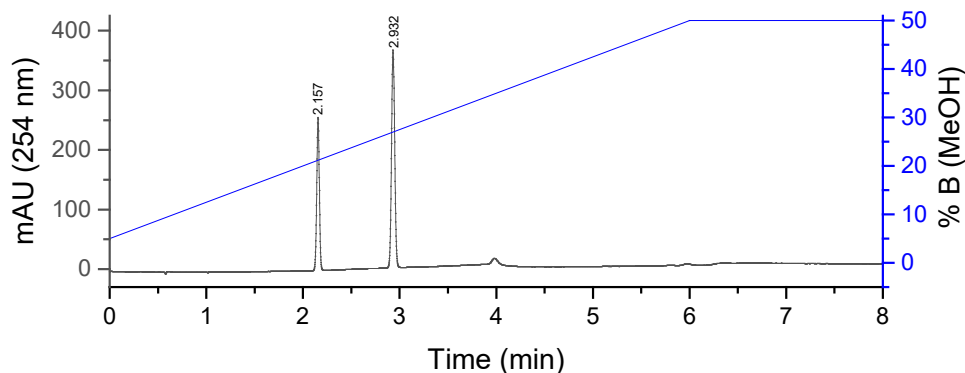

Figure 1. Example scouting run on a 4.6 x 150 mm Redi*Sep* diol column with gradient method using an Agilent SFC. The column was run at 4.00 mL/min with 2-propanol. The sample is Teledyne ISCO Universal Test Mix; peak 2 was used for the calibration to match Figure 2.

Step 3: ACCQPrep System Calibration

ACCQ*Prep* calibration is accomplished by adjusting the solvent composition of the prep method so that the relevant test compound from the universal test mix elutes in the middle of the isocratic portion of the calculated gradient (see Figure 2) Table 1 is provided to summarize all calibration data to enter into PeakTrak. Use the same solvent and column type as used in step 2.

Note

For systems having multiple column sizes of the same chemistry, only one column needs to be calibrated; PeakTrak software in the ACCQPrep will automatically scale the prep methods for the other columns, including the flow rates, according to column dimensions.

Running a scouting gradient on the ACCQ*Prep* SFC followed by calculating an isocratic method is the easiest way to set the solvent composition to cause elution at 3.5 minutes for a 20 x 150 mm column at 60 mL/min. See the *ACCQPrep SFC Operation Guide*, section 2.6 to see how to create an isocratic method. Run the sample using the calculated isocratic method, and, if needed, adjust the solvent composition to cause elution at 3.5 minutes on a 150 mm long column (5.8 minutes on a 250 mm column) at 60 mL/min.
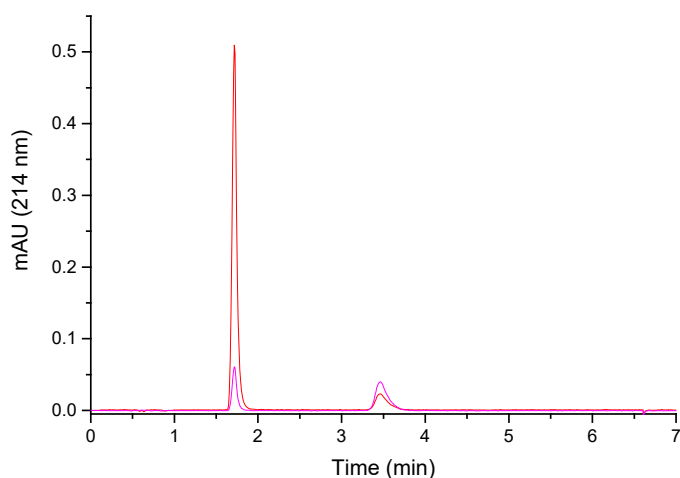

Figure 2. Example isocratic run using a 20 x 150 mm Redi*Sep* diol with 2-propanol (17%). The run was 7 minutes. The second eluting peak is used to set the solvent composition.

Step 4: Entering the calibration data into the ACCQPrep

1. Go to **Tools** > **Configuration** > **Prep SFC** tab.

2. Click on the **Calibrate External SFC** button

3. Click the **Enabled** button.

4. Enter the parameters from Table 1 into the **Calibrate External SFC** window. Select the desired focused gradient type-this can be changed for individual runs.

5. Click **OK** to save the calibration; click **OK** again to exit the **Configuration** screen

SFC Parameter	Value to be entered into ACCQPrep
Gradient starting % B	
Gradient ending % B	
Gradient Length	
Calibration compound retention time	
ACCQPrep Parameter	**Value to be entered into ACCQPrep**
%B causing elution of calibration compound	

Table 1

References

1. Silver, J.E. Calibration of Analytical HPLC to Generate Preparative LC Gradients for Peptide Purification. Peptides 2018, Proceedings of the 35th European Peptide Symposium, Dublin, Ireland, Aug 26–31, 2018; Timmons, P. B., Hewage, C. M., Lebl, M., Eds.; European Peptide Society & PSP, 2018.

2. Silver, J. Overview of Analytical to Preparative Liquid Chromatography Method Development. *ACS Comb. Sci.* **2019**, *21*, 609–613

3. Silver, J.E. Time on Target: Efficient Preparative LC Gradients for Purification of Natural Products from Calibration of Analytical Systems. Presented at the 19th Annual Oxford ICSB April 8-11, 2019. Poster PA24 [Available on Teledyne LABS web page]