Faster Method Development by Calibration of Analytical LC to ACCQ*Prep*™ Systems

Chromatography Technical Note
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Preparative LC (liquid 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 ISCO has developed techniques for significant reduction of method development time and resources based on calibration of analytical HPLC/UHPLC systems to match the preparative LC 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 ISCO's published papers on the subject. [1, 2, 3]

Purpose

This document defines the procedure for establishing a correlation between an analytical LC system and a Teledyne ISCO preparative LC system in order to reduce the time and resources required to develop focused gradients on the preparative LC system.

Scope

This procedure applies to Teledyne ISCO ACCQ*Prep*™ Models HP125 and HP150 running PeakTrak software version 4.2.68 or higher. For assistance with upgrading an existing ACCQ*Prep* system to the latest software version, contact Teledyne ISCO technical support.

Calibration Procedure Overview

The calibration is accomplished by using analytical and preparative columns with matching chemistries. A standard test mixture is prepared, and run with a pre-defined analytical scouting gradient. The same test mix 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 HPLC and the retention time of a given compound can be used to directly and reliably calculate an ACCQ*Prep* focused gradient method.

Materials Required

This calibration procedure requires the following materials.

- Analytical chromatography system—HPLC or UHPLC.
- Analytical column with chemistry matched to prep column (see note below).
- ACCQ*Prep* HP150 or HP125 with PeakTrak version 4.2.68 or higher.
- Preparative column with chemistry matched to analytical column (see note below).
- Mobile phase for both systems: Solvent A = water; Solvent B = methanol or acetonitrile.
- Note: a modifier such as trifluoroacetic acid may be used but, if so, the same modifier must be used in both the analytical scouting run and the focused gradient run.
- Teledyne ISCO Universal Test Mix (part number 605234835).

Note: Column chemistry matching is defined as both columns (analytical and preparative) coming from the same manufacturer, within the same brand/family, and ideally with the same pore size. The stationary phase particle sizes may be different.

Example: The chemistry of Teledyne ISCO RediSep® Prep column type C18AQ 2 x 50 mm matches the chemistry of RediSep Prep column type C18AQ 20 x 150 mm, but does not match the chemistry of RediSep Prep column type C18, as the non-AQ product has a different selectivity.

Step 1: Test Mix Preparation

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

1. Parent vial preparation
   a) Obtain test mix vial from Teledyne ISCO Universal Text Mix kit (605234835).
   b) Determine whether Solvent B will be methanol or acetonitrile.
   c) Add 4 mL Solvent B to the vial, replace cap, and shake to dissolve the powder.
   d) Add 1 mL water to the vial, shake briefly to ensure it is mixed, and set aside. This vial will be used in Step 2 of the calibration process and again in Step 3.
2. HPLC 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.
   b) Dilute the contents of the sample vial with 1 mL Solvent B.

Step 2: Analytical Scouting Gradient Calibration—HPLC or UHPLC

The parameters for the scouting run on the analytical system are different for HPLC versus UHPLC columns. 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.

A. HPLC system with 4.6 x 150 mm column

On an analytical system with 4.6 x 150 mm column, 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 1.0 mL/min.
2. Set the gradient to run from 5% Solvent B to 100 % Solvent B, and optionally 0% Solvent B to 100 % Solvent B for AQ type columns.

Note: Do not use an isocratic hold prior to the gradient.

3. Set the gradient length to 6 minutes, with a 6-minute isocratic hold at 100% Solvent B after the gradient.
4. Injection volume of the prepared HPLC sample will range from 1 µL to ~10 µL; this varies with your LC system.
5. Run the scouting method and note the retention times. A useful result for calibration purposes will have the following elution times (see Figure 1):
   a) With Solvent B = Methanol, peak 1 (Phenacetin) appears at < 8.2 minutes
   b) With Solvent B = Acetonitrile, peak 2 (N-Benzylbenzamide) appears at < 8.2 minutes.
   c) Note: peak intensity is not important so long as both peaks are clearly seen.

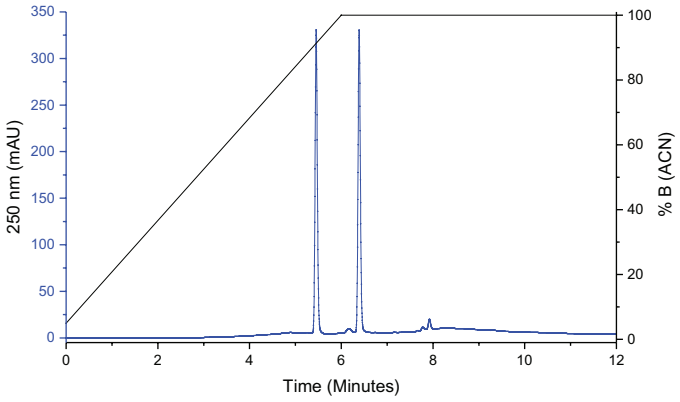

Figure 1: *Scouting run with 4.6 x 150 mm column and universal test mix in water/acetonitrile. Gradient 5% Solvent B to 100 % Solvent B over 6 minutes followed by 6-minute isocratic hold. Due to differences in LC systems, retention times may vary from those shown here.*

6. If the relevant peak elutes later than 8.2 minutes, increase the flow rate of the method while staying below the column pressure limit and repeat the scouting run with all other parameters unchanged.

B. UHPLC system with 2 x 50 mm column

For an analytical UHPLC system with 2 x 50 mm column, set up and run the analysis as follows:

1. Configure the scouting run for your 2 x 50 mm column at a flow rate of 0.4 mL/min.
2. Set the gradient to run from 5% Solvent B to 100% Solvent B or optionally, for AQ type columns, from 0% Solvent B to 100% Solvent B.

Note: Do not use an isocratic hold prior to the gradient.

3. Set the gradient length to 5 minutes with a 3-minute isocratic hold at 100% Solvent B after the gradient.
4. Injection volume of the prepared HPLC sample will range from 1 µL to ~10 µL; this varies with your LC system.
5. Run the scouting method and note the retention times. Note: peak intensity is not important so long as both peaks are clearly seen. See Figure 2.

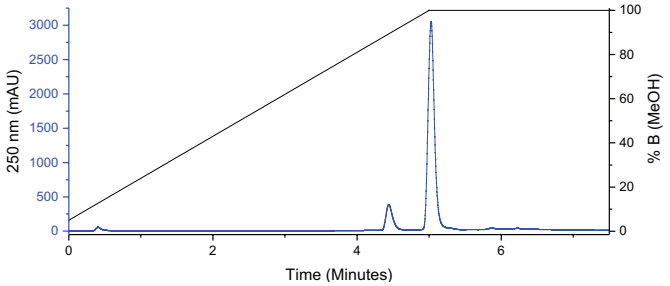

Figure 2: *Scouting run on 2 x 50 mm column; test mix in water/methanol. Gradient is 5% Solvent B to 100% Solvent B over 5 minutes with a 3-minute isocratic hold at end of gradient Due to differences in LC systems, retention times may vary from those shown here.*

Step 3: ACCQ*Prep* System Calibration

ACCQ*Prep* calibration is accomplished by adjusting the solvent composition of the prep method such that the relevant test compound from the universal test mix elutes at the same time as observed in the analytical scouting run. The procedure for calibrating the ACCQ*Prep* is different for Redi*Sep* columns versus non-Redi*Sep* columns. Proceed accordingly with Section A or B below. Table 4 is provided to summarize all calibration data for entering into PeakTrak.

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.

A. Setting the solvent composition when using Redi*Sep* columns

When using Teledyne ISCO Redi*Sep* Prep columns, the solvent composition needed to elute the relevant test mix compound at the correct retention time has already been determined. In this case use the values listed in Table 1 for the calibration and enter it into Table 4.

Column	Peak 1 Methanol	Peak 2 Acetonitrile
C18	50%	50%
C18AQ	50%	50%
C8	40%	40%

Table 1– Solvent composition to elute Universal Text Mix compounds on Redi*Sep* Prep columns. Note that for these columns, using modifiers such as trifluoroacetic acid or formic acid do not alter the required solvent composition.

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B. Setting the solvent composition when using non-Redi*Sep* columns

For non-Redi*Sep* columns, the solvent composition that will produce the proper retention time is most efficiently determined by using the focused gradient generator built into PeakTrak.

Proceed as follows.

1. Prepare the same HPLC sample as described in "Step 1: Test Mix Preparation."
2. Determine your injection volume using the values suggested in Table 2 below. *Note: the injection volume is not critical so long as the peaks are visible.*

Column ID (mm)	Suggested injection volume (mL)
4.6	0.02
10	0.05
20	0.2
21.2	0.2
30	0.5
50	1.0

Table 2– Suggested test mix injection volumes for various column diameters

3. Determine the target elution time for your method using the stated or calculated values in Table 3 below (± 0.5 min).

Column Length	Peak 1, Methanol	Peak 2, Acetonitrile
100	4 (±0.5) minutes	4 (±0.5) minutes
150	6 (±0.5) minutes	6 (±0.5) minutes
250	10 (±1) minutes	10 (±1) minutes
Other lengths	For column lengths not listed above: Retention time = 0.04 * Column Length (in mm), while using default flow rates from PeakTrak.	

Table 3– Elution times for various column lengths

4. Set up a scouting method for your column on the ACCQ*Prep* (if not already done).
5. Run the method.
6. View the results in PeakTrak and select the relevant peak. Use the first peak if Solvent B = methanol, or the second peak if Solvent B = acetonitrile.
7. Generate a focused gradient for that peak.
8. Open the METHOD EDITOR, click on EDIT GRADIENT and make the following changes:
   a) For a 20 mm or larger ID column, add 1.2 to the % Solvent B value in the initial gradient point. Enter the same value into the second gradient point. This will create an isocratic method with a column wash at the end.
   b) For a 10 mm ID column, subtract 8.4 from the % Solvent B value in the initial gradient point. Enter the same value into the second gradient point. This will create an isocratic method with a column wash at the end.
9. Run the test mix sample with this isocratic method.
10. Inject the sample and run the method.
11. When the run is completed, note the retention time.
    a) If the peak elutes earlier than the time shown in Table 3, lower the concentration of Solvent B and repeat the run.
    b) If the peak elutes later than the time listed in Table 3, increase the concentration of Solvent B and repeat the run.

The calibration for non-Redi*Sep* columns is now complete and the solvent composition causing the desired elution time can be entered into Table 4.

Step 4: Entering the calibration data into the ACCQ*Prep*

1. Go to TOOLS, CONFIGURATION, and PREP HPLC tab.
2. Click on CALIBRATE EXTERNAL HPLC
3. Click the "Enabled" button.
4. Enter the parameters from Table 4 into the CALIBRATE EXTERNAL HPLC window.
5. Click OK to save the calibration; click OK again to exit the CONFIGURATION screen

HPLC Parameter	Value to be entered to ACCQ*Prep*
Gradient starting %B	
Gradient Ending %B	
Gradient Length	
Retention time of calibration compound	
ACCQ*Prep* Parameter	**Value to be entered to ACCQ*Prep***
%B Causing elution at time listed in Table 2	

Table 4

Option: Post-calibration Preparative Runs Using a Different Flow Rate

After completing the ACCQ*Prep* calibration, preparative runs using a different flow rate than that determined by PeakTrak can be performed.

To scale the flow rate, proceed as follows.

1. Determine the values to be entered into PeakTrak, using Table 4 below to organize the data as follows:
   a) The HPLC Parameter values are from the earlier analytical scouting run.
   b) For Redi*Sep* columns, the ACCQ*Prep* Parameter is taken from Step 3 Table 1.
   c) For non-Redi*Sep* columns, the ACCQ*Prep* Parameter is the % Solvent B determined during Step 3 for that column.
2. Develop the focused gradient method in PeakTrak as follows:
   a) Go to TOOLS, CONFIGURATION, PREP HPLC tab.
   b) In the COLUMN NAME dropdown, select the column you wish to configure.
   c) Click the DEFINE METHODS button. The METHOD NAME showing should be "Default."
   d) Click the EDIT button.
   e) Change the FLOW RATE to the desired value.
   f) Hit the EXIT button and when prompted to save changes, choose YES, and hit OK.

The method with the scaled flow rate is now created and saved as the default method.

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 ISCO web page]

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