Global Calibration Function of Analytical to Different Column Chemistries on the ACCQ*Prep*®

TELEDYNE ISCO
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Chromatography Technical Note TN54

Abstract

Chemists often evaluate different column chemistries for compound purification during analytical scouting runs. For example, it is common for chiral purification, for several column types to be screened on an analytical HPLC. Despite very different types of columns, with widely varying chemistry, the ACCQ*Prep* can calculate focused gradients from them with only a single calibration on one column type. Although the ACCQ*Prep* uses a global calibration for all columns installed on the system, that calibration is not limited to a single column chemistry. The global calibration can also be used for different column chemistries, so long as the analytical columns have the same dimensions and use the same gradient method table and flow rate, allowing fast and easy column and method screening.

Background

Many labs, such as those performing chiral purifications, do method development using many types of columns and solvents. The method development starts with a scouting run which allows an initial evaluation of retention and resolution. The scouting runs can then be used to create focused gradients for the ACCQ*Prep* preparative HPLC system. The ACCQ*Prep* preparative HPLC has a built-in calibration for the scouting method calculated by PeakTrak® software when columns are installed. This built-in calibration works for all columns, regardless of manufacturer. The external HPLC calibration in the ACCQ*Prep* can likewise be used for a variety of column chemistries provided the analytical columns are the same dimensions, and the same gradient table is used for all columns.

Experimental and Results

C18 runs with a C8 calibration

Universal Test Mix (PN 60-5234-835) was run on a Redi*Sep*® Prep C8 column (20x150 mm, 5 µ, 200 Å, PN 69-2203-858) to get a retention of 6 minutes for the first eluting peak using a methanol/water solvent system, as described in Technical Note 52 (TN52), Calibration of Analytical LC systems available on the Teledyne LABS web site.

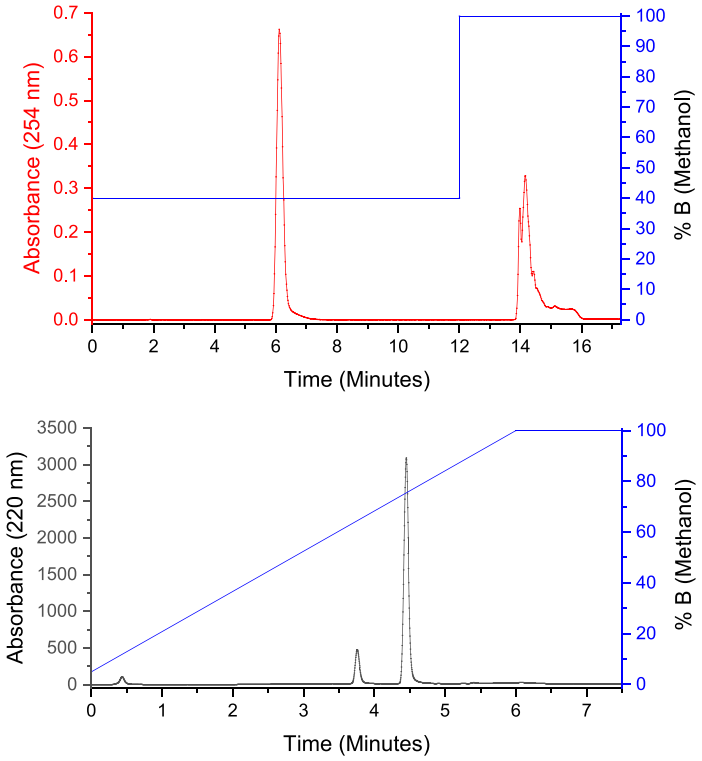

Figure 1—Calibration of Teledyne ISCO C8 columns on the ACCQ*Prep* (top), and UHPLC (bottom).

A matching C8 UHPLC column (2x50 mm, PN 69-2203-853) was run in a water/methanol gradient (5 to 100% B over 5 minutes, with an isocratic hold for 2.5 min at 100% methanol). The peak eluting at 3.757 minutes, corresponding to the peak eluting at 6 minutes on the ACCQ*Prep*, was used for the calibration into PeakTrak in the ACCQ*Prep*.

Preparative runs

The C8 analytical column was replaced with an analytical Redi*Sep* Prep UHPLC C18 column (2x50 mm, PN 69-2203-854) and test mix was run in a water/methanol gradient using the same gradient as used for the C8 column. As expected, the compounds eluted later in the gradient due to the increased retention of the C18 column, with 100 Å pores.

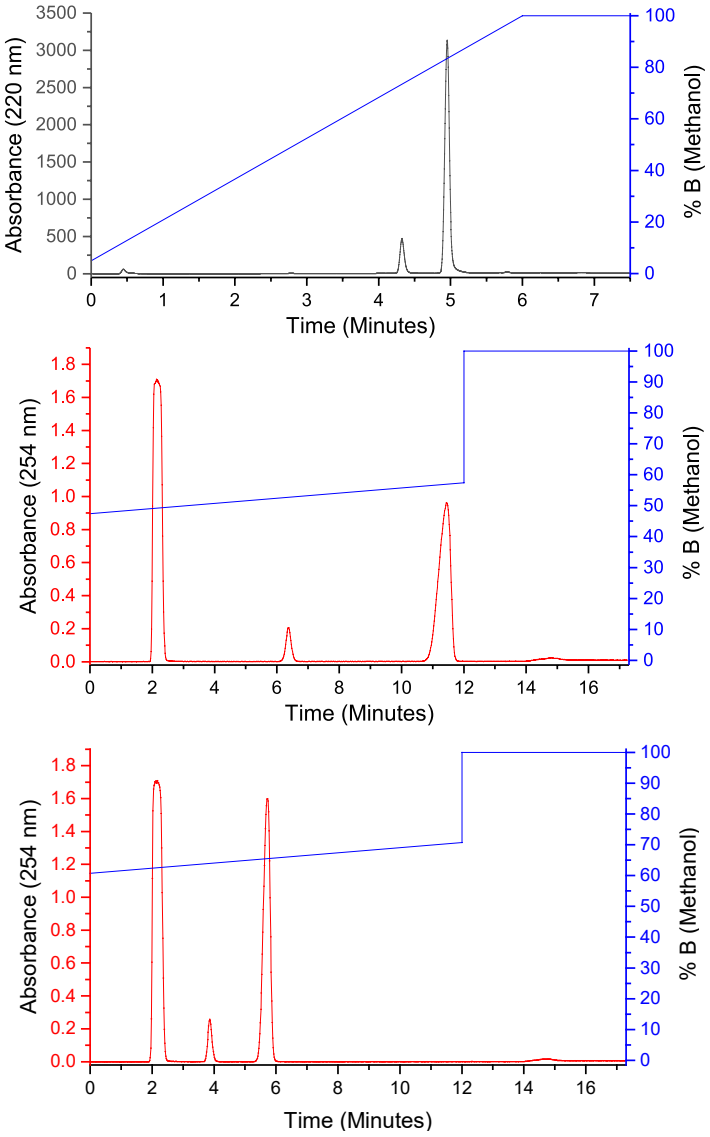

Figure 2—Analytical scouting runs and focused gradients on a C18 column, using the C8 column calibration. The first preparative run was focused on the compound eluting at 4.324 minutes in the analytical run, while the peak at 4.953 minutes was used to calculate the second preparative gradient.

The data from the C18 analytical run was used to calculate the preparative gradients in Figure two while using the calibration from the C8 columns in Figure 1. The preparative purifications utilized a Redi*Sep* Prep C18 column (20x150 mm, 5 µ, 100 Å, PN 69-2203-810) In both cases, the compounds eluted close to the expected 6-minute elution time.

The test mix was also run on the C8 preparative HPLC column with the C8 calibration; the analytical data in Figure 1 was used to calculate the gradient for the second eluting peak at 4.451 minutes.

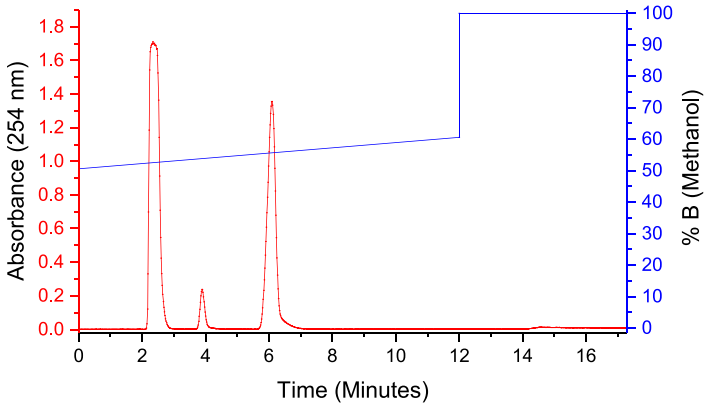

Figure 3—Preparative run on a Redi*Sep* Prep C8 column using the second compound eluting from the analytical run in Figure 1.

The chromatogram appears almost the same as the C18 column, the only difference is the solvent composition at the start and end of the focused gradient due to the differences in retention between the two columns.

Silica runs with a C18 calibration

A 4.6x150 mm Redi*Sep* Prep C18 column was calibrated on an Agilent analytical HPLC system as described in TN52 using a gradient from 5 to 100% methanol over 6 minutes, followed by 100% methanol for 6 minutes at 1.0 mL/min. The solvent was changed to hexane/ethyl acetate and the column replaced with a 4.6x150 mm Redi*Sep* Prep silica column. The same gradient method was used for the new solvent and column, 5 to 100% ethyl acetate over 6 minutes. A sample of methyl paraben was injected, and the retention time used for the focused gradient shown in Figure 4 on a 20x150 mm Redi*Sep* Prep silica column run in hexane/ethyl acetate. The sample eluted near the middle of the calculated gradient.

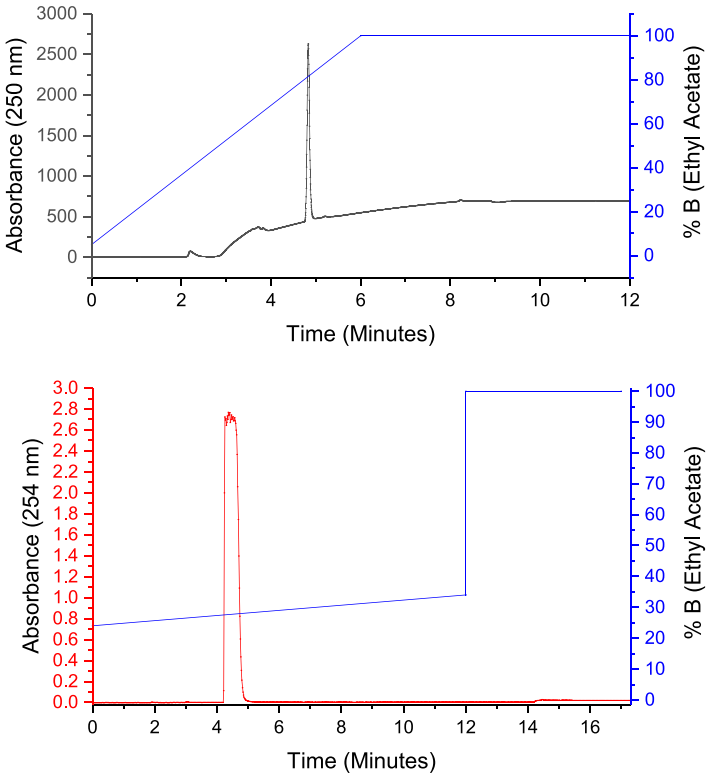

Figure 4—Scouting run and calculated focused gradient of a silica column using a calibration for a C18 column.

Conclusion

The calibration used for one column chemistry can be used for another column chemistry in the PeakTrak Focus Gradient Generator. This allows greater flexibility to choosing columns for the analytical and preparative systems and ensuring proper scalability. Follow the simple rule where the analytical columns used with the external HPLC need to be the same size with respect to length and inner diameters and run with the same gradient table and flow rate. This allows a user to use analytical scouting runs with different columns and solvent systems to determine the best purification method with higher loading capacity.

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