LC Method Translation: Will Switching Column Dimensions Speed Up Sample Analyses?

Answer that question in seconds with Restek's online Pro *EZ*LC method translator.

Introduction

Saving time, solvent, and sample through method translation is a very popular way to make improvements in liquid chromatography (LC) methods. For LC method translation, you need to keep the same particle type (e.g., Raptor) and stationary phase (e.g., Biphenyl), but it is possible that using a shorter, narrower column with a smaller particle size can offer big opportunities for speeding up your analysis while maintaining acceptable results. Those opportunities might mean higher sample throughput, more time for routine maintenance, or any number of other benefits that come from quicker methods using less mobile phase.

But changing a column alone is not going to do the trick, especially in cases where separation and resolution of critical pairs is essential. You need to change the method conditions to match the new column so you don't lose critical resolutions, and that's where LC method translation calculators come into play.

To achieve the goal of having very nearly the same separation in less time, you need to translate your column to one that has smaller dimensions, but that still maintains the same efficiency (typically expressed as a nearly equivalent total number of theoretical plates). However, it is not always practical or even possible to find a new column dimension that maintains the same number of plates as the original column (plus or minus 10%). That doesn't mean that a successful translation isn't possible; it just means that there will be some changes to your current separations. The new column and method might still help you meet your analytical goals in less time, so it is worth investing a little time with method translation software to explore your options.

Let's walk through how you could use Restek's Pro *EZ*LC method translator (www.restek.com/ezlc-mt) to determine if switching a method to a new column will save time while still giving quality results.

LC Method Translation Scenario

In this example, our lab has successfully developed a method using a 100 mm x 3.0 mm, 2.7 µm Raptor Biphenyl column. We also have a 50 mm x 2.1 mm, 2.7 µm column in house and—knowing that moving to a column with smaller dimensions may decrease the analysis time—we want to know whether the current method on the larger column could be successfully translated to the shorter, narrower column.

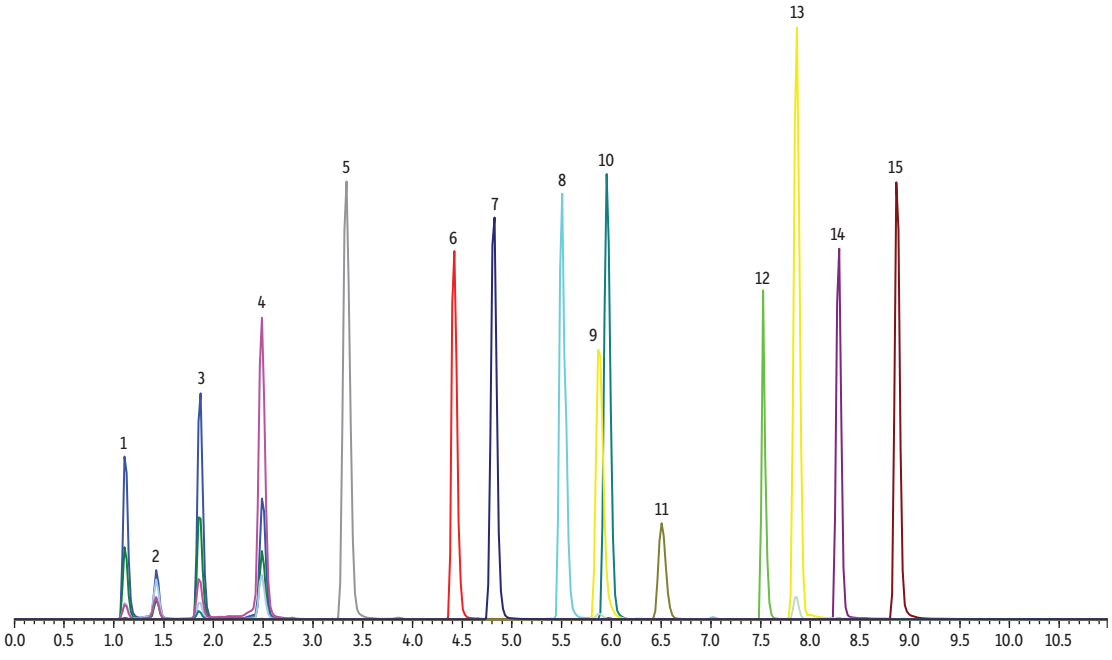
In this particular case, the smaller column represents a significant loss in total theoretical plates. That means we should not expect the exact same separation in less time with a properly translated method, so we need to be careful that we do not lose critical resolutions as a result of LC method translation. But, even though we know we won't get the same separation, will we still get acceptable chromatographic results?

Calculating new method conditions and predicting the new results is exactly what a method translation calculator is designed to do. So, let's see how it works.

Method Translation Example

We want to translate the analysis of certain drugs of abuse to a smaller dimension column to see if we can further decrease our analysis time without sacrificing performance even though we're happy with the chromatographic performance provided by the original, longer column. Of the 15 drugs of abuse, there are five pairs of isobars that require chromatographic separation because the MS/MS would not be able to differentiate them. The original method does that, so we are particularly interested in whether the translated method will as well. Figure 1 below shows the results of the original method on the 100 mm x 3.0 mm column with 2.7 µm particles.

Figure 1: Chromatographic results using the original column dimensions and method.



	Peaks	t_R (min)	Precursor Ion	Product Ion
1.	Morphine	1.11	286.2	152.1
2.	Hydromorphone	1.43	286.2	184.9
3.	Norcodeine	1.86	286.1	151.9
4.	Norhydrocodone	2.49	286.1	199.0
5.	6β-Naltrexol	3.32	344.3	326.1
6.	Tramadol	4.42	264.2	58.0
7.	Normeperidine	4.82	234.1	160.2
8.	Mirtazapine	5.50	266.1	195.1
9.	Clozapine	5.87	328.2	271.1
10.	Pentazocine	5.95	286.2	218.1
11.	7-Aminoflunitrazepam	6.50	284.1	135.0
12.	Fluoxetine	7.53	310.1	148.0
13.	Loxapine	7.86	328.1	271.1
14.	EMDP	8.28	264.2	235.2
15.	Thioridazine	8.87	371.2	126.1

Column Raptor Biphenyl (cat.# 9309A1E)
Dimensions: 100 mm x 3.0 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Temp.: 40 °C
Sample
Diluent: Water
Conc.: 0.5-10 µg/mL
Inj. Vol.: 5 µL
Mobile Phase
A: Water, 0.1% formic acid
B: Methanol, 0.1% formic acid

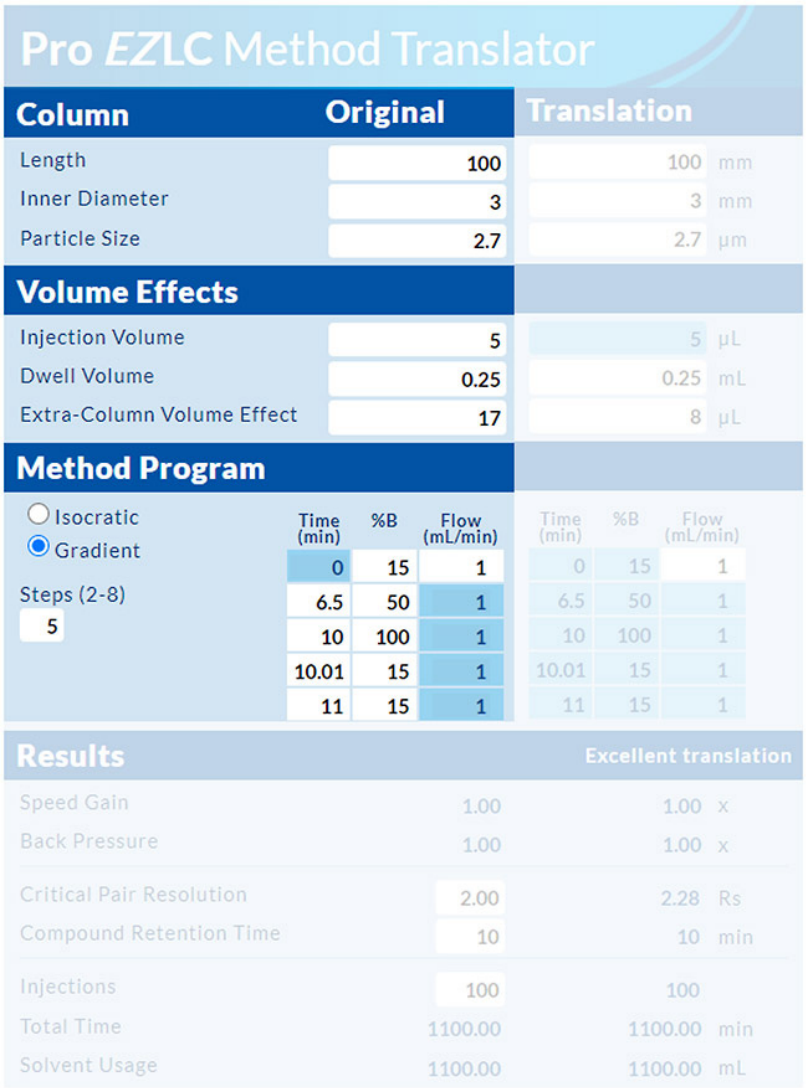
Time (min)	Flow (mL/min)	%A	%B
0.00	1.0	85	15
6.50	1.0	50	50
10.00	1.0	0	100

Detector MS/MS
Ion Source: Electrospray
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Notes A standard mix with 15 drugs of abuse was prepared in concentrations ranging from 500-10,000 ng/mL in water. The solution was vortexed at 3000 rpm for 10 seconds to mix, and the supernatant was injected for LC-MS/MS analysis.

Our next step is to provide the LC method translation calculator with information about our current column and method so it can provide the best translated method conditions for the new column dimensions. Note that when using this calculator, you must be transferring between columns of the same particle type, for example FPP to FPP (fully porous particle) or SPP to SPP (superficially porous particle). Figure 2 illustrates entering the original column dimensions, injection volume, dwell volume, "extra-column volume effect" value, and the mobile phase conditions, which is a gradient analysis in this case.

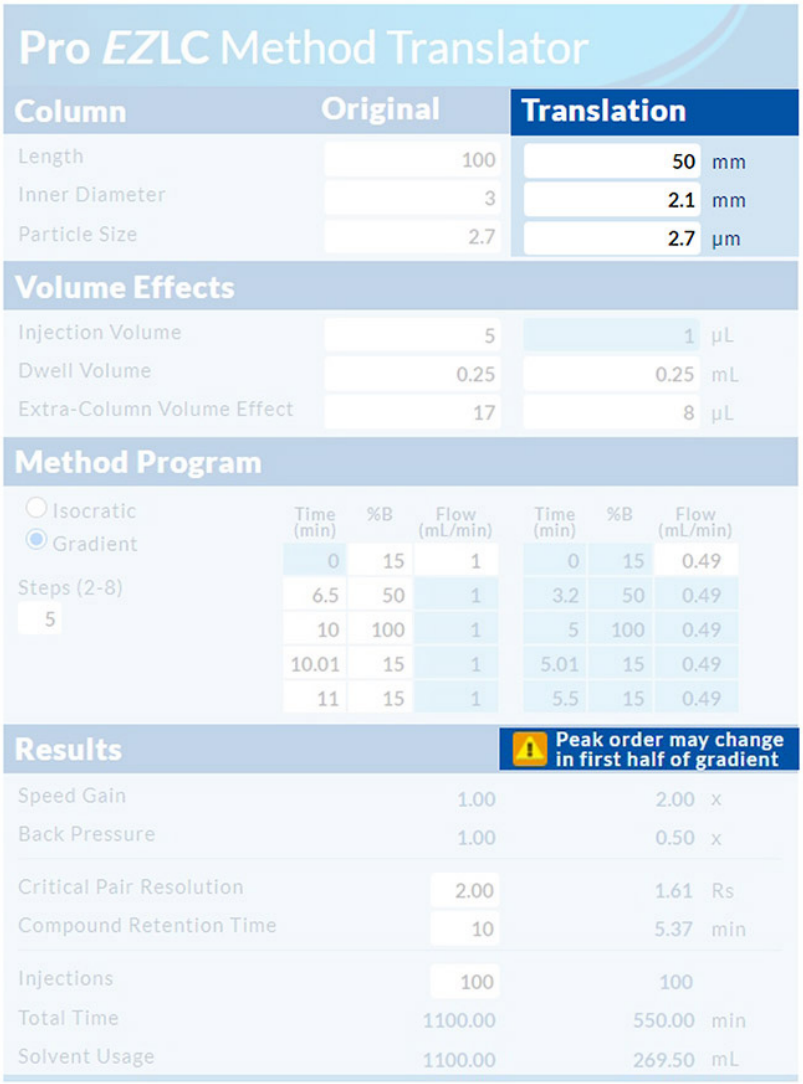
Take special note of the default dwell volume and extra-column volume effect values because, while they may not be your instrument's values, they are particularly important because the translator will take them into account when determining values like critical pair resolutions and compound retention times under the translated run conditions. If you don't know these values for your particular instruments, look up this information in your instrument documentation, contact the instrument manufacturer, or research ways to determine these values empirically. You can find out more about these values in the translator's glossary, which you can access by clicking the "?" button in the upper right corner of the translator.

Figure 2: The first step of using an LC method translator: entering your current column, instrument, and method details.



The next step is to supply the new column dimensions and then let the method translator do its job! Figure 3 shows the new column we want to explore in this example. Once the new column information is entered, a warning message might be displayed, as it is in our example. The warning is there to alert you to possible changes in chromatographic selectivity under the new method conditions (later in our example we'll determine if this warning applies or not). To achieve similar selectivity, it is important for the translated method to match the mobile phase conditions under which compounds eluted in the original method. The method translator attempts to create new mobile phase gradient conditions that allow compounds to elute under the same mobile phase composition as the original method, but sometimes the new column dimensions won't allow that. If the translated column's volume is significantly different than the original column, it may result in compounds eluting before or after the original mobile phase composition is achieved in the column. In those cases, early eluting compounds in particular may elute under different %B conditions, and that may affect your selectivity. For more information about this, explore the program's glossary. There is also a downloadable version of the Pro *EZ*LC method translator that includes a special chart for visualizing this effect.

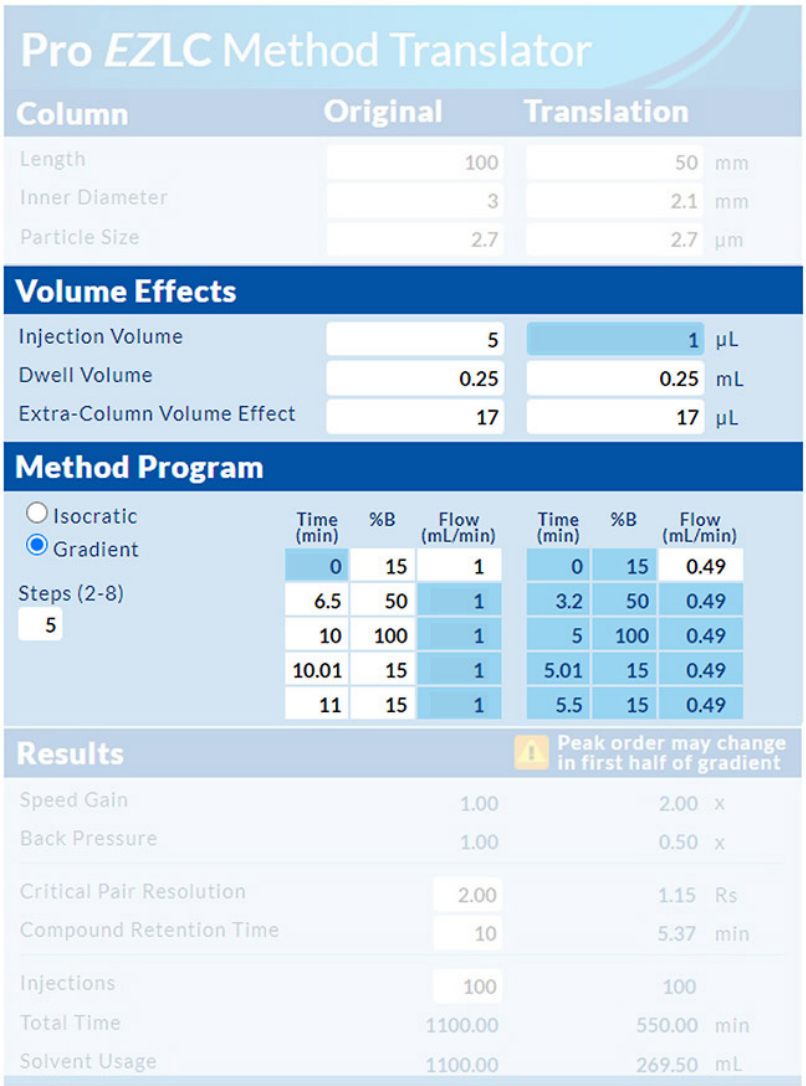
Figure 3: Manually enter the dimensions of the new column.



Using Volume Effects

Figure 4 shows the new, scaled injection volume and method conditions that the Pro *EZ*LC method translator calculates. Keep the dwell volume and extra-column volume fields the same if you will be running the new method on the same instrument without making other changes. However, if you are transferring the method to a different instrument or making other changes, you will need to take the new values into account.

Figure 4: Translated injection volume scaled to the new column dimensions and new method program conditions to try to match the elution profile of the original method on the new column.



Note that you can translate gradient as well as isocratic methods, and that the translated flow rate is an editable field. You can change the translated flow rate and see how it affects the retention times and resolutions of your analytes as shown in the Results section below.

The Pro *EZ*LC method translator also attempts to only return flow values that are within an ideal range for a given set of column dimensions, so if the actual calculated translated flow falls outside those values, Pro *EZ*LC method translator will adjust the flow to be within a desirable range for the new column. For more information on this feature, see the glossary.

Interpreting Results

This section of the LC method translation software will show the gains in analysis speed and changes in back pressure to be expected under the translated method with the new column. It also allows you to calculate time and solvent savings over a user-specific number of runs. In this example, we can see the new method generates results in half the time, under half the pressure, and using only a quarter of the solvent required by the old method. But, will we still have adequate resolution between our critical compounds? The "critical pair resolution" and "compound retention time" fields are where we'll be able to determine if the new column and method will still provide acceptable results.

If we review the results from the original method again, we can see that the critical pair with the smallest resolution value is morphine (peak 1) and hydromorphone (peak 2), which have an original resolution of 4.88. Those two peaks are fairly well resolved, so we can predict that the overall loss of efficiency between the original and the new columns may not sacrifice resolution, but we can check by simply putting the original resolution of 4.88 into the appropriate field and seeing the predicted resolution (Figure 5). We also input the retention time for morphine to get an idea of how its retention time will change under the new conditions.

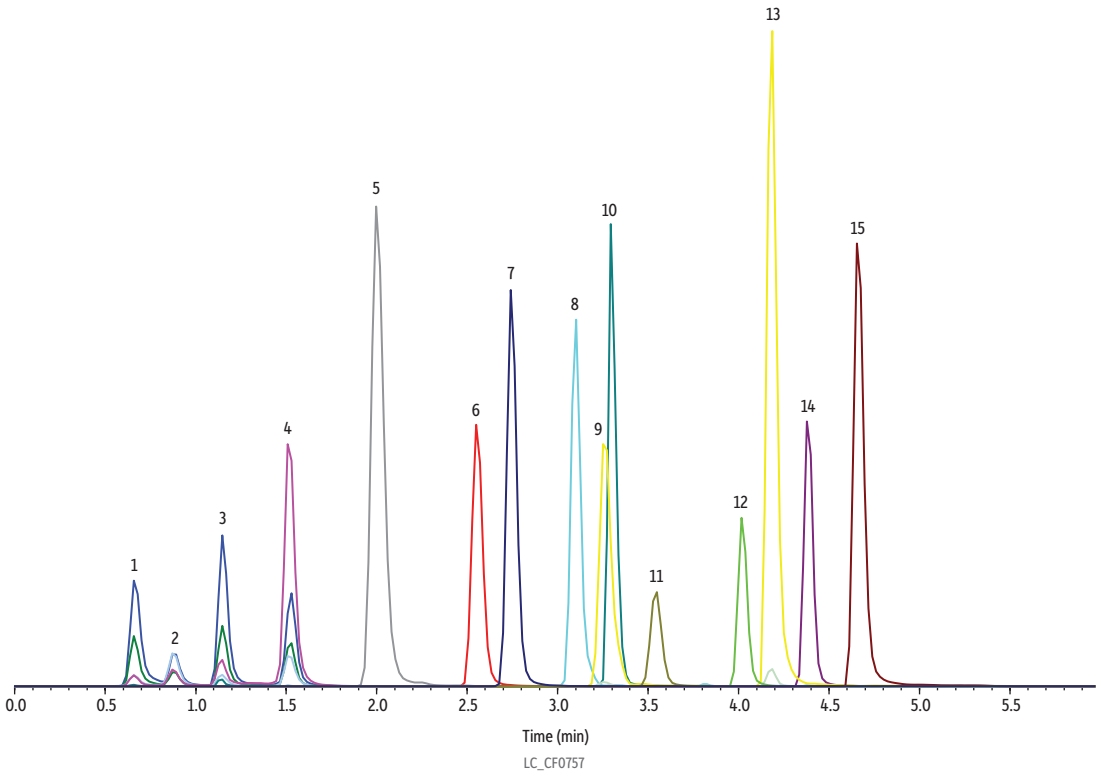
Figure 5: Using the Results section to check critical resolutions and retention times under the new column and method conditions.

Results		Peak order may change in first half of gradient	
Speed Gain	1.00	2.00	x
Back Pressure	1.00	0.50	x
Critical Pair Resolution	4.88	2.80	Rs
Compound Retention Time	1.11	0.65	min
Injections	100	100	
Total Time	1100.00	550.00	min
Solvent Usage	1100.00	269.50	mL

As we would have predicted by the loss of total plate count, we do see a reduction in the resolution between these two critical pairs, but we also can see that the new resolution is predicted to be well beyond what is required for baseline separation (R = 1.5). Based on this, we could conclude that if our earliest and closest eluting critical pair of compounds are still resolved using the new column with the translated conditions, then the rest of our chromatography is also likely going to be okay, but let's not leave it theoretical.

Figure 6 shows the empirical results of the same set of compounds analyzed using the new column dimensions and the translated method conditions.

Figure 6: Empirical results generated using the new column and the translated method conditions.



Peaks	t_R (min)	Precursor Ion	Product Ion
1. Morphine	0.66	286.2	152.1
2. Hydromorphone	0.88	286.2	184.9
3. Norcodeine	1.15	286.1	151.9
4. Norhydrocodone	1.51	286.1	199.0
5. 6β-Naltrexol	2.0	344.3	326.1
6. Tramadol	2.55	264.2	58.0
7. Normeperidine	2.74	234.1	160.2
8. Mirtazapine	3.1	266.1	195.1
9. Clozapine	3.26	328.2	271.1
10. Pentazocine	3.3	286.2	218.1
11. 7-Aminoflunitrazepam	3.54	284.1	135.0
12. Fluoxetine	4.02	310.1	148.0
13. Loxapine	4.18	328.1	271.1
14. EMDP	4.38	264.2	235.2
15. Thioridazine	4.66	371.2	126.1

Column Raptor Biphenyl (cat.# 9309A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Temp.: 40 °C
Sample
Diluent: Water
Conc.: 0.5-10 µg/mL
Inj. Vol.: 1 µL
Mobile Phase
A: Water, 0.1% formic acid
B: Methanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	85	15
3.20	0.5	50	50
4.90	0.5	0	100

Detector MS/MS
Ion Source: Electrospray
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC
Notes A standard mix with 15 drugs of abuse was prepared in the concentrations ranging from 500-10,000 ng/mL in water. The solution was vortexed at 3000 rpm for 10 seconds to mix, and the supernatant was injected for LC-MS/MS analysis.

In the actual analysis using the new column and method, morphine's retention time was 0.66 minutes, which is just 0.01 minutes higher than the LC method translation software's predicted retention time. The resolution between morphine and hydromorphone was empirically determined to be R = 3.07. The predicted resolution was R = 2.80, roughly a 10% difference, but erring on the conservative side when it comes to critical pair resolutions. So, with the empirical results demonstrating an even better resolution than the prediction, we can see that, in this example, it would be safe to move from the original column and adopt the new column and translated method conditions without fear of losing critical pair resolution.

If, on the other hand, a critical pair had a resolution of R = 2 under the original conditions, it is likely that this particular translation (100 mm x 3.0 mm, 2.7 µm to a 50 mm x 2.1 mm, 2.7 µm) would not have enough resolving power under the translated conditions to avoid a coelution (method translator predicts R = 1.15 under these conditions).

Conclusion

There are lots of reasons why a lab may be interested in translating a method from one column to another, and a free, online LC method translation tool like Restek's Pro *EZ*LC method translator can make exploring those options a matter of a few minutes at your computer rather than hours or days in the lab. And while many instances of LC method translation deal with labs trying to save time, sample, and/or solvent, like the example presented here, it can work the other way, too. If you have to translate a successful method developed on a state-of-the-art UHPLC instrument to a production facility with traditional HPLC instruments, a method translator can help make sure the same results can be achieved using columns with larger dimensions than the original.

So, in our example, the lab could make the method translation with confidence, cutting their analytical run time in half, operating under half the back pressure (which is easier on your instrument), using just a quarter of the solvent they would otherwise have consumed, and likely experiencing gains in sensitivity as well. Sounds like a great deal to us.

Does your lab have opportunities for method translation? If so, check out the Restek Pro *EZ*LC method translator at www.restek.com/ezlc-mt or contact us at if you have any questions. Happy translating!

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Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

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