

Focused Gradients— What Do You Mean the Compound Eluted too Early or too Late?!

Abstract

The focused gradient generator calculates gradients for most compounds, however some compounds elute too early or too late to allow calculation of a gradient where the compound elutes at the expected ~4-5 column volumes. This document describes how to adjust columns and solvents to potentially allow the compound to elute in a focused gradient.

Overview

The focused gradient generator creates a good gradient for most compounds from scouting runs. The exceptions, when it is unable to calculate a good gradient, can generally be handled by applying basic chromatography theory. This document will not address the causes and solutions of poor peak shape, discussed here in this [Effects of Mobile Phase Solvents on Calculated Gradients](#) poster.

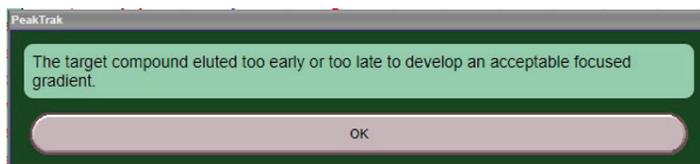


Figure 1- Message indicating the ACCQPrep® is unable to calculate a focused gradient because the compound eluted too early or too late.

The focused gradient generator assumes a elution time of between 4 to 5 column volumes (CV) for the preparative run. We will use column volumes because this measure is invariant to column width, length, and flow rate, ignoring dwell volume. When the program indicates it is unable to calculate a gradient as per Figure 1, it really means it is unable to create a gradient that causes elution at 4 to 5 column volumes, but the compound is often able to elute with a different retention time. The message in Figure 1 causes confusion because the user can often see the peak eluting during the scouting run, and the gradient reported by the system seems to be very high. The Apparent Gradient Delay (Da) is much larger than users anticipate, so they feel the system should have calculated a gradient.

Compound elutes too early

The most common problem is that the compound elutes too early for the system to calculate a gradient. The common causes for this could be that the compound doesn't adsorb on the column, or the user set the

starting solvent composition of the scouting gradient too high. If B solvent is added to the water (A solvent) to prevent bacterial growth, the scouting gradient should start at 0% B.

Does the compound elute at the void during the scouting run?

If the compound elutes at the void during the scouting run, this means the compound was not retained with the starting solvent composition of the scouting run. The user has several options:

1. If possible, use a lower %B in the scouting run. Many users start at 10% B to avoid "phase collapse". Most columns can actually run fine at 5% B, so try a lower initial solvent composition.
2. Try using an "AQ" type column, such as the RediSep® Prep C18AQ. When using this column, set the initial point of the scouting run to 0% B because AQ type columns resist phase collapse. This expands the range where a focused gradient can be calculated. In addition, many polar compounds show improved retention on an AQ type column.
3. Try a weaker B solvent. Users commonly use acetonitrile for a B solvent, and this is a stronger solvent than methanol. See the [Acetonitrile/Methanol Substitution in C18 Reverse Phase Application Note](#), for example: The compound may not elute using the technique employed for the scouting run. For example, if the scouting run was reverse phase, maybe a HILIC procedure would work better.

If the compound has retention during the scouting run

Sometimes, the compound elutes too early to calculate a focused gradient, while still being somewhat retained on the column. Part of the reason for not being able to calculate a gradient is that most algorithms assume the peak of interest doesn't move down the column until the solvent composition matches the composition that causes the desired retention. The Focused Gradient algorithm doesn't make that assumption, but neither does it know how the compound elutes in a given column and a given gradient- the retention model for a given compound, solvent, and column are all part of Da. This means

a gradient will lead to incorrect results, where the compound may elute later than expected in a calculated focused gradient!

To try to improve the retention, the user can try steps one through three listed above. Alternatively, since we know the compound will be retained, one can create a column method that can be used for early-eluting compounds. As shown in the table below.

Segment length (min)	% B Solvent	Notes
Start	S	S=starting % B for the scouting method used to create focused gradients. Use the default flow rates for the column.
12	S+20%	The segment length should be 8 min for 100 mm columns, 12 min for 150 mm long columns, and 20 min for 250 mm columns.
0	100	Step to 100% B for washing
5	100	Set this segment to 3.3 minutes for 100 mm long columns, and 8.3 minutes for 250 mm long columns

This gradient method can be saved as one of the standard methods for a column, so the method doesn't need to be entered into the system each time.

Compound elutes too late

If the system says it is unable to calculate a gradient, and the desired compound is visible during the isocratic hold at the end of the scouting run, the compound is strongly retained on the column, and it elutes too late for the system to calculate a gradient with an elution time of 4 to 5 CV. We know the compound elutes because we see it in the scouting run. Just do a purification at 100% B solvent and the compound will elute. Another possibility is to use a stronger solvent. Methanol can be replaced with acetonitrile or tetrahydrofuran, which is also stronger than acetonitrile, and the scouting gradient re-run.

Conclusion

The scouting run for a focused gradient can provide a lot of information, even it is unable to calculate a focused gradient. It still shows whether a reaction occurred, and the presence of impurities that are well resolved from the desired compound. It is still possible to create a method to elute the compound even if the system can't calculate a gradient that might purify the desired peak.

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