PEPTIDE MAPPING: BEST PRACTICES FOR GENERATING RELIABLE AND ROBUST LIQUID CHROMATOGRAPHY METHODS

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INTRODUCTION

One of the goals of liquid chromatography method development is to develop a method which provides reliable and reproducible results. There are many factors that will affect the reproducibility of a method, including but not limited to mobile phase composition, mobile phase pH, column packing material, gradient conditions, etc.

The robustness of a method can also be impacted by design elements of the LC system itself. For example, methods which require the use of long and shallow gradients, or those that impart a small change in solvent composition per column volume, can be challenging. For these types of methods, a binary high-pressure mixing system where each solvent is delivered by a dedicated pump can deliver more reproducible gradients than ternary or quaternary low-pressure mixing systems which employ a gradient proportioning valve to generate the gradient.

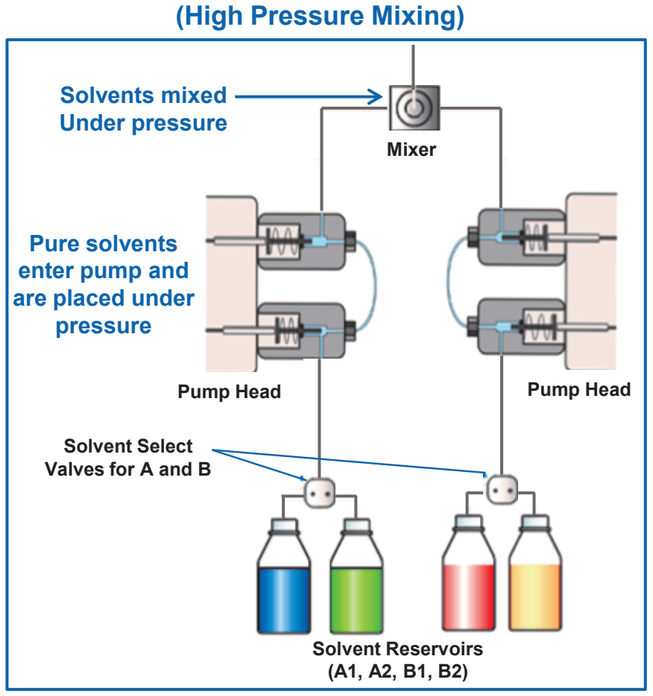
METHODS

Mobile phase A: 0.1% TFA in Water
Mobile phase B: 0.1% TFA in ACN
Column Temperature: 65 °C
Gradient: 1—50%B over 85 minutes
@ 0.200 mL/min
Detection at 214 nm
Sample: Waters MassPREP Enolase Digestion Standard
To remove potential for solvent/additive evaporation, mobile phase bottles were equipped with ACQUITY APC Reservoir Caps

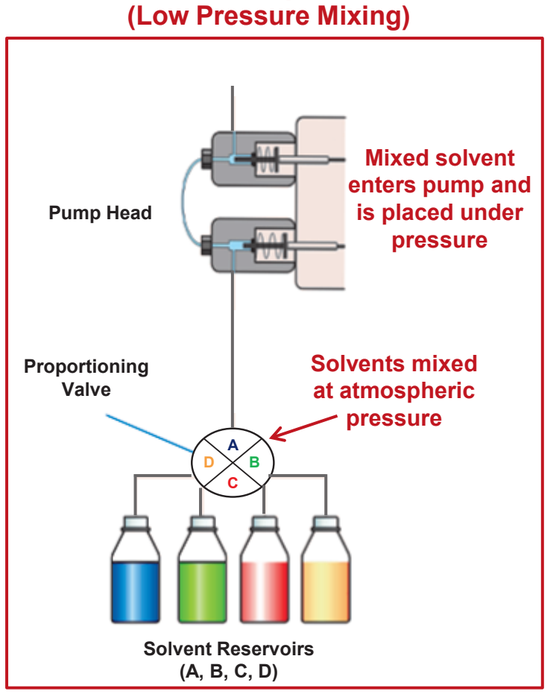
ACQUITY UPLC I-Class PLUS

Waters ACQUITY UPLC Peptide BEH C18, 130Å 1.7 µm, 2.1 x 100 mm

Binary Pump (High Pressure Mixing)



Quaternary Pump (Low Pressure Mixing)



RESULTS AND DISCUSSION

The ability of an LC system to provide precise and reproducible results over a number of injections may be important for both identification and quantitative purposes. For example, if gradient delivery varies slightly from injection to injection, small retention and selectivity differences may appear. Peptide mapping is one example of an application area that requires separation and resolution of compounds with varying chemical properties. The retention of peptides can be greatly affected by a small change in % organic, and any compositional inconsistency of the pump between injections will be seen in the resulting retention time reproducibility.

The same method conditions were used to evaluate the Waters MassPREP Enolase Digest Standard on both low pressure and high pressure mixing systems. The data in Figure 1 displays the retention time (RT) standard deviations (SD) acquired on both systems, with n = 8. The binary, high-pressure mixing system provides more stable retention times, indicating consistent and reproducible gradient delivery for each injection.

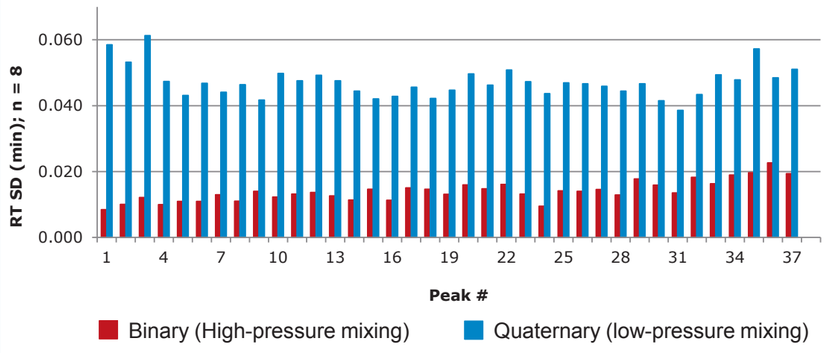
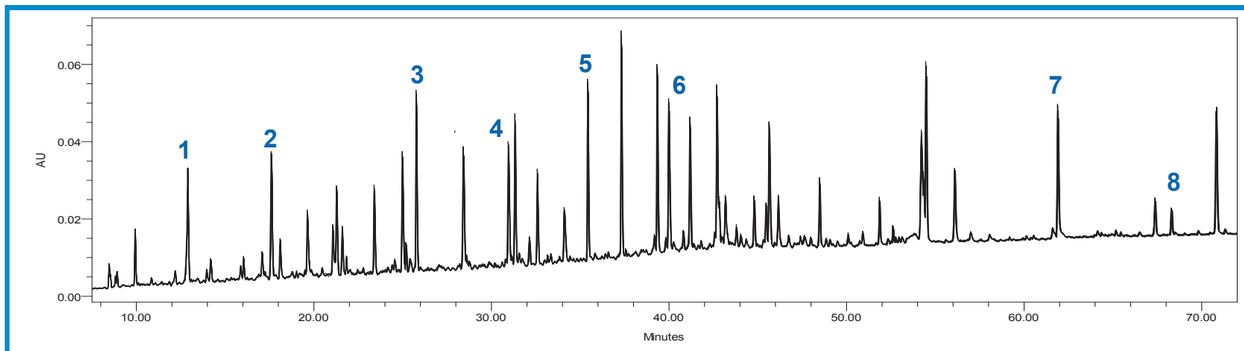


Figure 1. Comparison of retention time standard deviation seen using a high-pressure mixing binary pump vs a low-pressure mixing quaternary pump. The RT standard deviation (SD) seen on the binary pump are approximately 3x lower than on the quaternary pump due to the specific solvent delivery characteristics.

The figure below depicts the 8 overlaid injections run on the binary, high-pressure mixing system. This method was run at various time points over 136 days on the high-pressure mixing binary system. Due to potential variability in mobile phase preparation day-to-day, the relative retention times (RRT) were used for comparison over the extended time period. For easier visualization of the data, a small subset of peaks were tracked.



	Relative Retention Times (min)			
Peak	Day 1	Day 17	Day 136	St Dev
1	0.348	0.344	0.344	0.001
2	0.693	0.690	0.691	0.001
3	0.839	0.838	0.838	0.001
4	0.950	0.949	0.950	0.001
5	1.000	1.000	1.000	0.000
6	1.458	1.462	1.462	0.002
7	1.656	1.661	1.661	0.002
8	1.827	1.831	1.831	0.002

Figure 2. Top chromatogram is an overlay of 8 replicate injections collected on one day of analysis. In addition, the same method and standard sample was run at various time points (7 time-points in total, only a subset of data is shown) over the course of 136 days.

The table highlights the relative retention times of 8 select peaks observed on the various days, along with the resulting standard deviation of retention time.

The very low RT standard deviation across days highlights the reproducible gradient delivered by the ACQUITY UPLC I-Class PLUS system for methods which employ long, shallow gradients.

CONCLUSION

- **When developing methods, the instrument design may impact the reliability and robustness of the developed method and should be considered**
- **Separations which require the use of long, shallow gradients may be challenging for a pump to deliver reliably, which may negatively impact retention time reproducibility**
- **The binary high-pressure mixing ACQUITY UPLC I-Class PLUS system generated consistent retention times both within a single run (intra-day) and across 136 days (inter-day) when used to analyze an enolase digest standard**

References

1. Simeone, J, Hong, P, Performance of the ACQUITY UPLC I-Class PLUS System for Methods which Employ Long, Shallow Gradients. Waters Application Note 720006290EN. May 2018.

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