

Choose an Appropriate Re-Equilibration or Post Time for Gradient HPLC - Tech Information

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Choosing an Appropriate Re-Equilibration (Post Time) for Gradient HPLC & LC-MS

Introduction

During gradient HPLC or LC–MS analyses, achieving consistent retention times and reproducible chromatography depends heavily on proper re-equilibration, commonly referred to as Post Time. This is the period at the end of each run during which both the mobile phase and the column return to the method's initial conditions.

Using too little Post Time risks retention drift and poor reproducibility; using too much wastes solvent and reduces throughput. This article outlines how to determine an optimal Post Time and considerations specific to different chromatographic modes, including Cogent TYPE-C™ Silica columns.

Why Post Time Is Necessary

During a gradient run, the proportion of strong solvent (%B) increases, often dramatically affecting analyte retention and the stationary-phase environment. Before starting the next injection, the system must return to initial solvent composition to ensure that retention and selectivity match the method's validated conditions. Without adequate Post Time, chromatographers may observe shifting retention times, broadened peaks, or inconsistent quantification.

Determining the Optimal Post Time

A reliable approach for setting Post Time is to begin with a large, conservative value during the method-development phase. Then reduce it stepwise while monitoring retention time precision across multiple runs. When shortening Post Time causes retention precision to fall outside acceptance criteria, the appropriate Post Time is the next longer value that maintains performance. This process should be confirmed for robustness by testing on different instruments and on different days.

A Quick Screening Method

A fast, practical way to estimate the required Post Time is to observe system pressure. Once the backpressure returns to the value observed at the method's initial conditions and stabilizes, the system has likely re-equilibrated sufficiently. While this “quick and dirty” technique is not a substitute for method validation, it offers a rapid approximation useful during early development or troubleshooting.

How Many Column Volumes Are Needed?

The number of column volumes (CVs) required for full re-equilibration depends on:

- The magnitude of the gradient (difference between initial and final %B)
- The retention mechanism dominant in the method
- The physicochemical properties of the analytes

Ion-exchange phases often require up to 100 column volumes for proper re-equilibration. In contrast, many gradient methods using Cogent TYPE-C™ Silica columns show stable retention with as few as 3–5 column volumes. This difference reflects the unique adsorption and solvent-interaction characteristics of TYPE-C silica surfaces.

Conclusion

Determining the correct Post Time is essential for achieving reproducible retention and robust chromatography. Start with an intentionally long Post Time, reduce gradually until precision fails, and confirm robustness across instruments and days. Quick pressure-based assessments can provide rapid estimates, while actual CV requirements vary by separation mode.

Notably, Cogent TYPE-C™ Silica columns often require far fewer column volumes for full re-equilibration, increasing throughput and reducing solvent consumption.



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MicroSolv Technology Corporation

9158 Industrial Blvd. NE, Leland, NC 28451

Tel: (732) 380-8900

Fax: (910) 769-9435

Email: customers@mtc-usa.com

Website: www.mtc-usa.com