

FUSION QbD® - CONSIDERING DWELL VOLUME

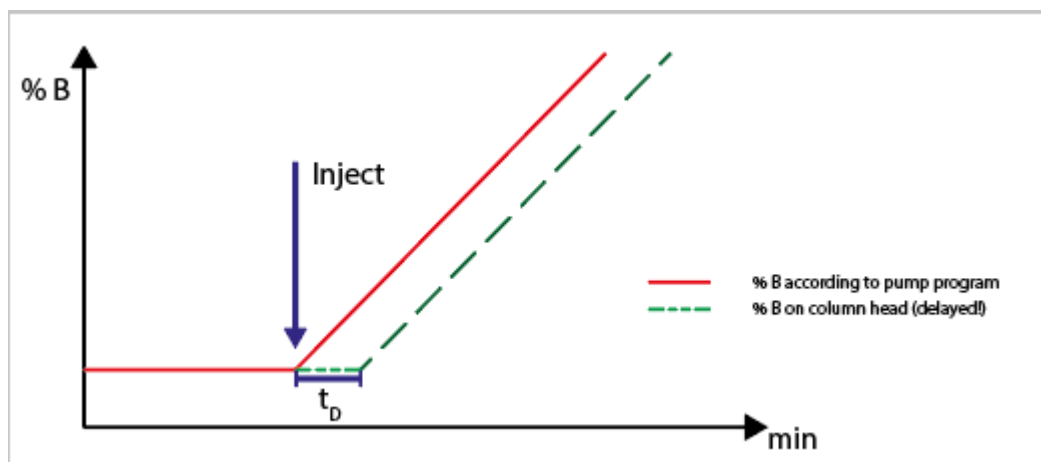
Developing Methods which are Robust to Dwell Volume Differences Across LC Systems on Transfer Using an AQbD Approach with Fusion QbD

Just to Get the Context:

What is the benefit of an initial isocratic hold time (IHT) in a chromatographic run?

Remember: all LC systems have a device specific Gradient Delay Time (GDT) – the time required for the mobile phase to travel from the mixing point (mixer or GPV*) to the head of the analytical column. Therefore, **there will always be an isocratic phase at the beginning of each run due to the GDT**, even if not defined in the pump program.

*GPV = Gradient Proportioning Valve



An IHT can be optimized to support successful transfer across different LC systems.

The Challenge to Solve:

Transferring an analytical method to an LC system with a different dwell volume will change the initial isocratic phase time before the gradient start time:

- This can affect the overall selectivity of the method – peaks that were previously baseline separated may now co-elute, and changes in elution order can also occur.
- The separation of weakly retained components, i.e., those that elute at the front of the chromatogram, can be strongly affected.
- However, the retention times of all peaks may be significantly different than expected.^[1]

Definitions:

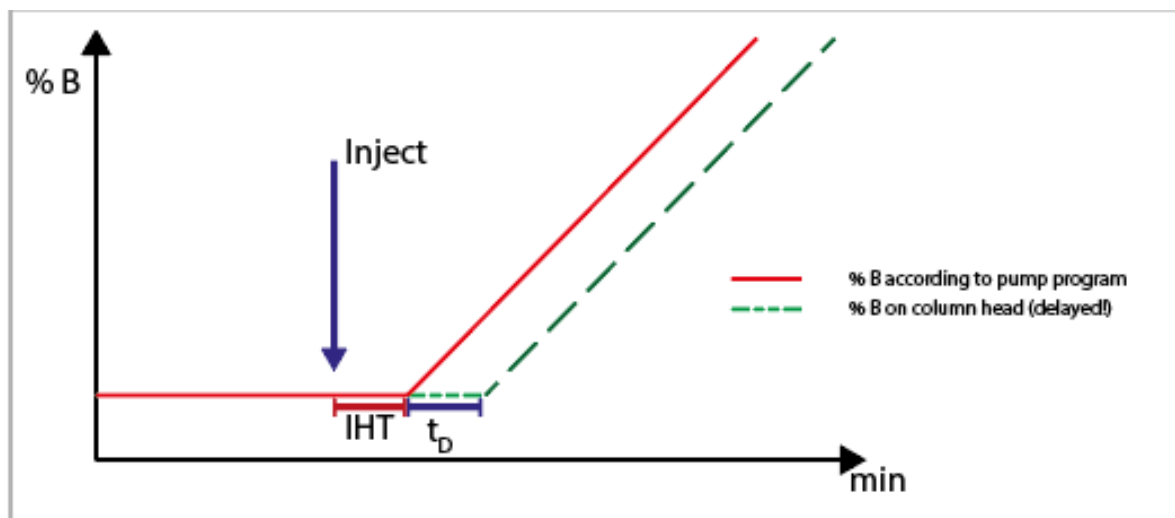
Dwell Volume (V_D): Volume of mixer + volume of flow path between mixer and column inlet (specific to each LC system)

Dwell Time (t_D): (V_D/F) – where F is the pump flow rate in mL/min

Isocratic Hold Time (IHT): Time between injection and start of gradient – this is specified in the pump program

Gradient Delay Time (GDT): Dwell Time + Isocratic Hold Time ($t_D + \text{IHT}$)

Gradient delay due to dwell volume and IHT in final method:



Section 1: Traditional Approach [2,3]

In this section, traditional solutions allowed according to USP<621>[5] are presented schematically. For the sake of simplicity, column dimension differences are not considered.

For this discussion, consider the following development LC system and final method:

Development System: $V_D = 0.770$ ml, $t_D = 1.54$ min

Final Method: $F = 0.500$ mL/min, IHT = 1.00 min

Section 1: Traditional Approach

In the traditional approach the method must be modified for each LC system on which it will be run to compensate for t_D differences between the development system and the “target” system, which is usually a QC system.

We want to consider the following two scenarios for target LC systems relative to the development LC system and final method.

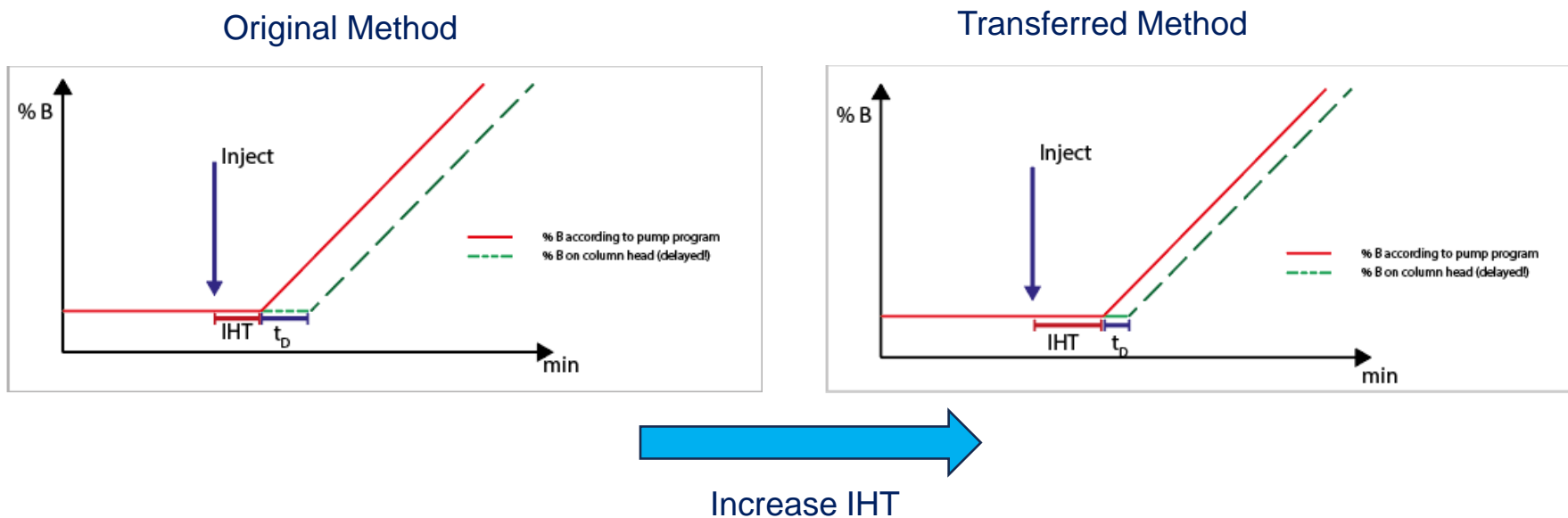
Scenario A: System A has a **smaller** dwell volume ($V_D = 0.375$ ml, $t_D = 0.75$ min)

Scenario B: System B has a **larger** dwell volume ($V_D = 1.150$ ml $t_D = 2.30$ min)

Section 1: Traditional Approach

Scenario A – transfer to a System with smaller V_D

1. Determine the t_D for the new LC system.
2. Adjust the current IHT in the method, as shown in the figure below, to compensate for the difference in t_D between the LC systems.



Section 1: Traditional Approach

Scenario A – transfer to a System with smaller V_D

Increase the IHT by 0.79 minutes (2.54 min – 1.75 min = 0.79 min)

- This adjustment maintains a constant Gradient Delay Time ($t_D + \text{IHT}$) between the two LC systems.
- To avoid changing the pump program of the method, in some instrument methods the required delay can also be set via other parameters (e.g., ***Initial Hold After Injection****).

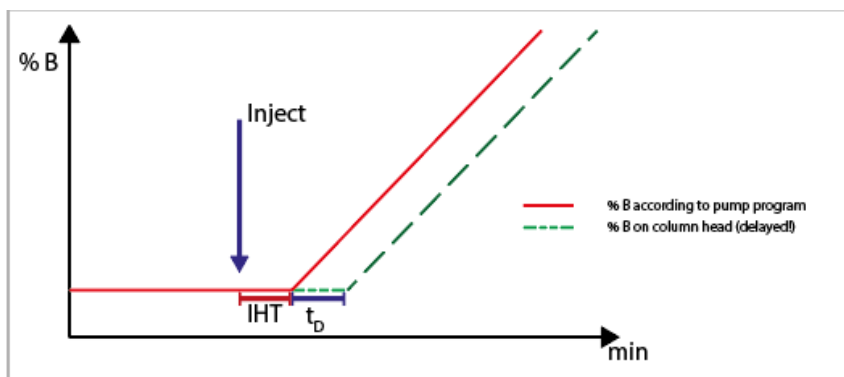
* – terminology is CDS specific.

Section 1: Traditional Approach

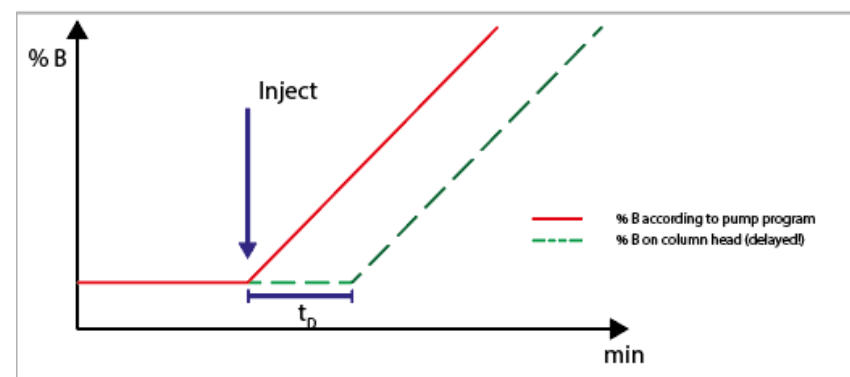
Scenario B – transfer to a System with larger V_D

Here the Gradient Delay Time difference of 0.76 min ($3.30 - 2.54$) must be compensated for by reducing the IHT to 0.24 min ($1.00 - 0.76$) to maintain a constant Gradient Delay Time of 2.54 minutes between the two LC systems.

Original Method



Transferred Method



Reduce/Remove IHT

Section 1: Traditional Approach

Scenario B – transfer to a System with larger V_D

Decrease the IHT by 0.24 minutes (1.00 min – 0.76 min)

- **CRITICAL** – this can only be done if the final method has a reasonable IHT.
- When the final method does not have an IHT, the only alternatives are:
 - Use a **Delay Injection*** function available in the CDS systems of many instrument manufacturers.
 - Do not run the method on that specific LC system.

* – terminology is CDS specific.

Summary:

What is the benefit of an Isocratic Hold Time (IHT) in an LC method?

- An IHT can be used to compensate for different dwell volumes across LC systems during method transfer – by adjusting the IHT, the GDT can be maintained constant.
- When transferring to a system with a *smaller* V_D , the IHT can be *increased* for operation on the target system.
- When transferring to a system with a *larger* V_D , the IHT can be *decreased* for operation on the target system.
- Supporting flexible method transfer to multiple LC systems more than compensates for the small amount of additional overall run time resulting from an IHT in the LC method.

But do we need to adjust the method for each LC – isn't there a better way



Section 2: Simulating the Delay Volume with Fusion QbD

Here the goal is to avoid the need to modify the method for each LC system on which it will be run to compensate for t_D differences between systems.

AQbD method development with Fusion QbD can provide a final method which:

- Is maximally robust to V_D differences across target LC systems for transfer.
- Specifies the operational V_D range of LC systems for which required method performance will be maintained without compensating adjustments.
- Critically, **this approach does not require changing the method** for successful transfer.

Section 2: Simulating the Delay Volume with Fusion QbD

Step 1: Define the IHT Study Range for a Method Optimization Study

Continuing with the example in Section 1:

- The method development system has a V_D of 0.770 mL, resulting in a t_D of 1.54 minutes.
- The Scenario A and Scenario B target LC systems have V_D values of 0.375 mL and 1.150 mL, respectively.

These values equate to a t_D range of 0.75 – 2.30 minutes.

- Therefore, to be robust to the Scenario A and Scenario B target LC systems presented in Section 1 the final method requires a minimum operational t_D range of **-0.79 – +0.76 min.**

Section 2: Simulating the Delay Volume with Fusion QbD

- Including flow rate (**F**) as a study variable in a multivariate optimization study enables establishing the robust MODR for **F** . Therefore, we will include a study range of 0.400 – 0.600 mL/min (0.500 ±0.100 mL/min) in this example.
- The following table GDT variation as a function of 1) the **F** study range, and 2) the V_D range defined by the development system and the Scenario 1 and 2 LC systems.

F (mL/min)	GDT ($V_D = 0.375$ mL)	GDT ($V_D = 0.770$ mL)	GDT ($V_D = 1.150$ mL)	IHT LB ($V_D = 0.770$ mL)	IHT UB ($V_D = 0.770$ mL)
0.400	0.94 min	1.93 min	2.88 min	-0.99 min	0.95 min
0.500	0.75 min	1.54 min	2.30 min	-0.79 min	0.76 min
0.600	0.63 min	1.28 min	1.92 min	-0.65 min	0.64 min

- As the table shows, the **F** of 0.400 mL/min defines the extremes of the GDT range.
- Given an **F** study range of 0.400 – 0.600 mL/min, this in turn defines an IHT study range of **0.00 – 2.00 min** rounded (Lower Bound = 1.00 – 0.99, Upper Bound = 1.00 + 0.95).

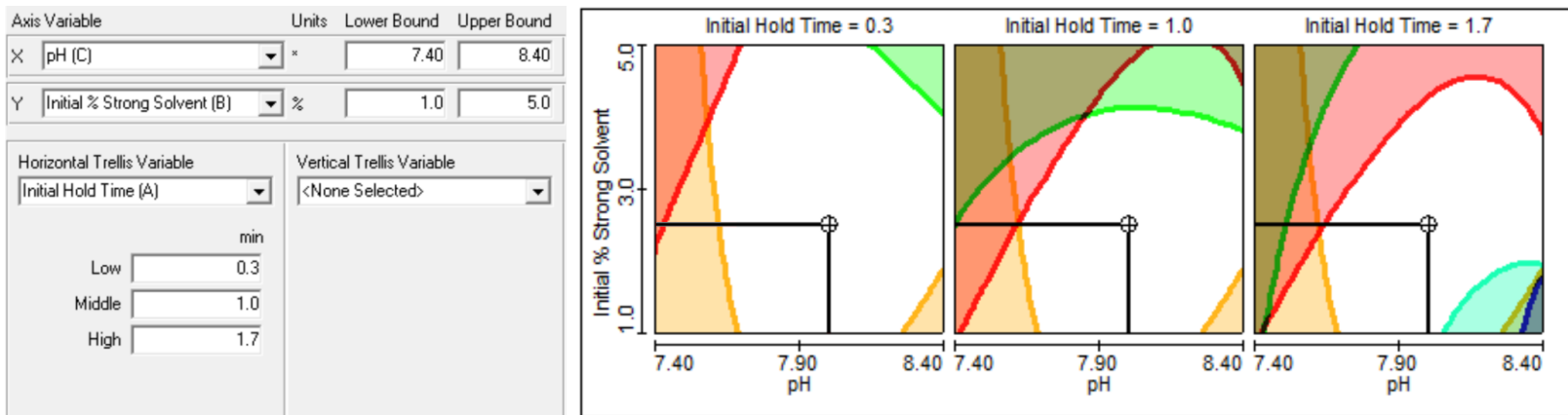
Section 2: Simulating the Delay Volume with Fusion QbD

Step 2: Determination of a workable region with respect to the dwell volume according to AQbD

- Once the experiment is run and the data are modeled, the models can then be used in a simulation mode to identify the operational V_D range within which the method's specified performance requirements are always met.
- This range can then be included in the Method Operable Design Region (MODR*) defined for the method according to AQbD best practices.

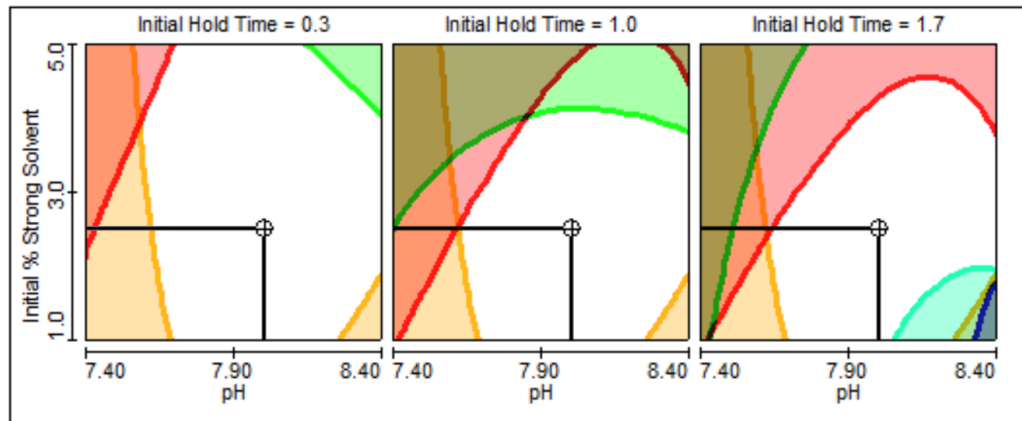
* ICH Q14: An MODR consists of combined ranges for two or more variables within which the analytical procedure is shown to be fit for the intended use. Parameter ranges (e.g., PAR or MODR) can be proposed by the applicant based on development data and are subject to regulatory approval. Moving within an established parameter range does not require regulatory notification. [4]

Section 2: Simulating the Delay Volume with Fusion QbD



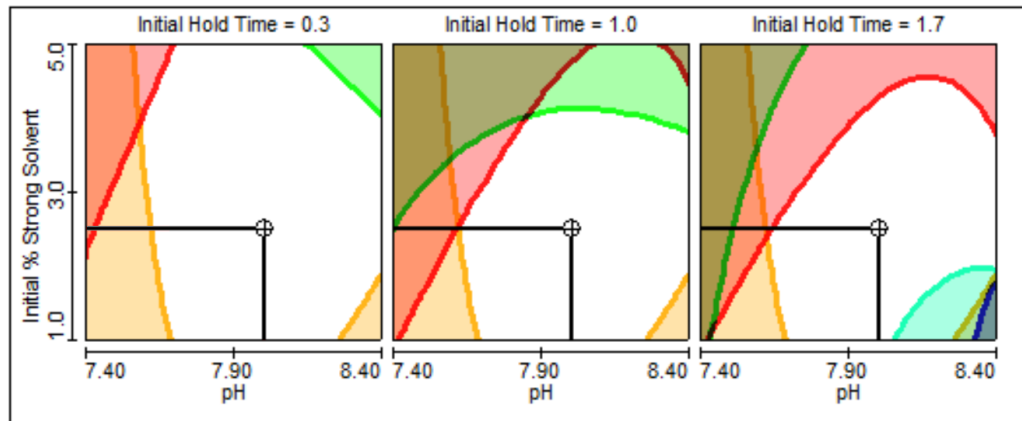
- Each of the three trellis graphs in the image above is constructed by assigning a color to each modeled method performance characteristic. The shaded region of a given color within each graph defines the method conditions which will result in an **underperforming** method for the performance characteristic assigned that color.
- The unshaded region therefore defines method conditions which **simultaneously meets ALL target performance requirements**.

Section 2: Simulating the Delay Volume with Fusion QbD



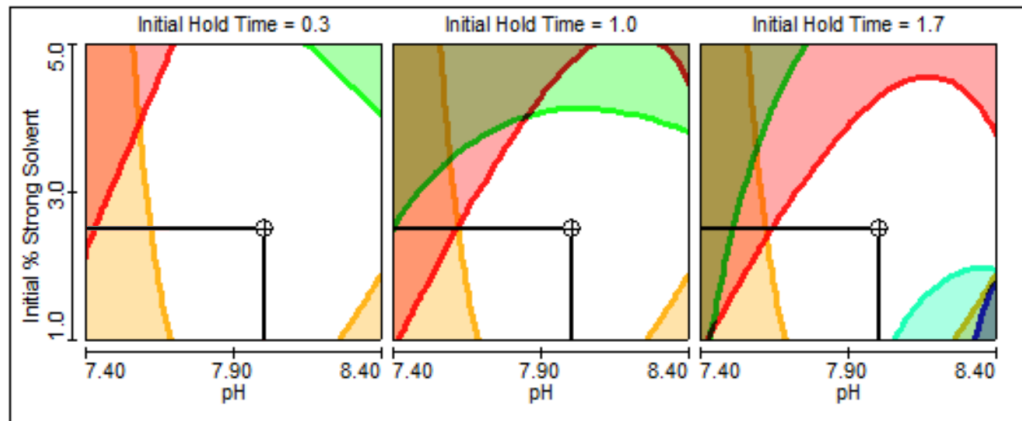
- Taken together, the three trellis graphs define a robust operating range of 0.30 – 1.70 minutes of IHT.
- Setting the IHT to 1.00 minutes in the final method, the midpoint of this range, results in an operational IHT range of ± 0.70 minutes. This defines an allowable V_D range for method transfer of ± 1.40 mL ($0.70/0.500$) around the method development system value of 0.770 mL.

Section 2: Simulating the Delay Volume with Fusion QbD



- In other words, the method can be successfully transferred to an LC system with a V_D range from the smallest possible value to an upper limit of 2.10 mL.
- Conversely, the method could fail if transferred to an LC system with a V_D value of >2.10 mL.

Section 2: Simulating the Delay Volume with Fusion QbD



According to ICH Q14, an MODR consists of combined ranges of two or more variables within which the analytical procedure is shown to be fit for the intended use.

In AQbD it is already common practice to specify a MODR for method parameters such as temperature, flow rate, pH, gradient duration, slope, etc. As this presentation clearly demonstrates, this approach can also be directly applied to dwell volume.

Conclusion:

- Using Fusion QbD to model dwell volume enables development of a robust final method which can be directly transferred to a wide, and quantitatively specified, range of LC systems without required edits to compensate for V_D differences.
- This AQbD approach is fundamentally different, as the traditional approach requires calculated adjustments to the method pump program on an LC-by-LC basis.
Critically, the manual approach assumes that any required changes will not adversely affect method performance due to interactions with other significant method parameters, and so cannot provide any V_D robustness estimation.
- Therefore, the AQbD approach provides much greater flexibility and much lower method transfer risk compared to the traditional approach.

References

- [1] Euerby, M. et al., Understanding How Dwell Volume Can Affect Selectivity in Reversed-Phase Gradient Chromatography, LCGCEurope, 2019, 32(6), 288-296
- [2] Paul, C. et al., Thermo Fisher Scientific White Paper 72711, An instrument parameter guide for successful (U)HPLC method transfer, 2018.
- [3] Hong, P. et al., Waters Corporation White Paper, Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer, 2016.
- [4] ICH HARMONISED GUIDELINE, ANALYTICAL PROCEDURE DEVELOPMENT Q14, Draft version, March 2022
- [5] USP General Chapter <621> CHROMATOGRAPHY, United States Pharmacopeia and National Formulary, usp.org.

Contact us for more Information



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