Eliminate your Application and Chromatography Challenges

LC Application Scientist Session

Information Contributed by USA HPLC Applications Scientist Team

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This Session will cover

Troubleshooting your Method, Application / Chromatography

- Sample prep considerations
- Troubleshooting by following the LC flow. Mobile Phase and System Hygiene
- Step through Method Setup to highlight parameters that are critical but often overlooked or misunderstood

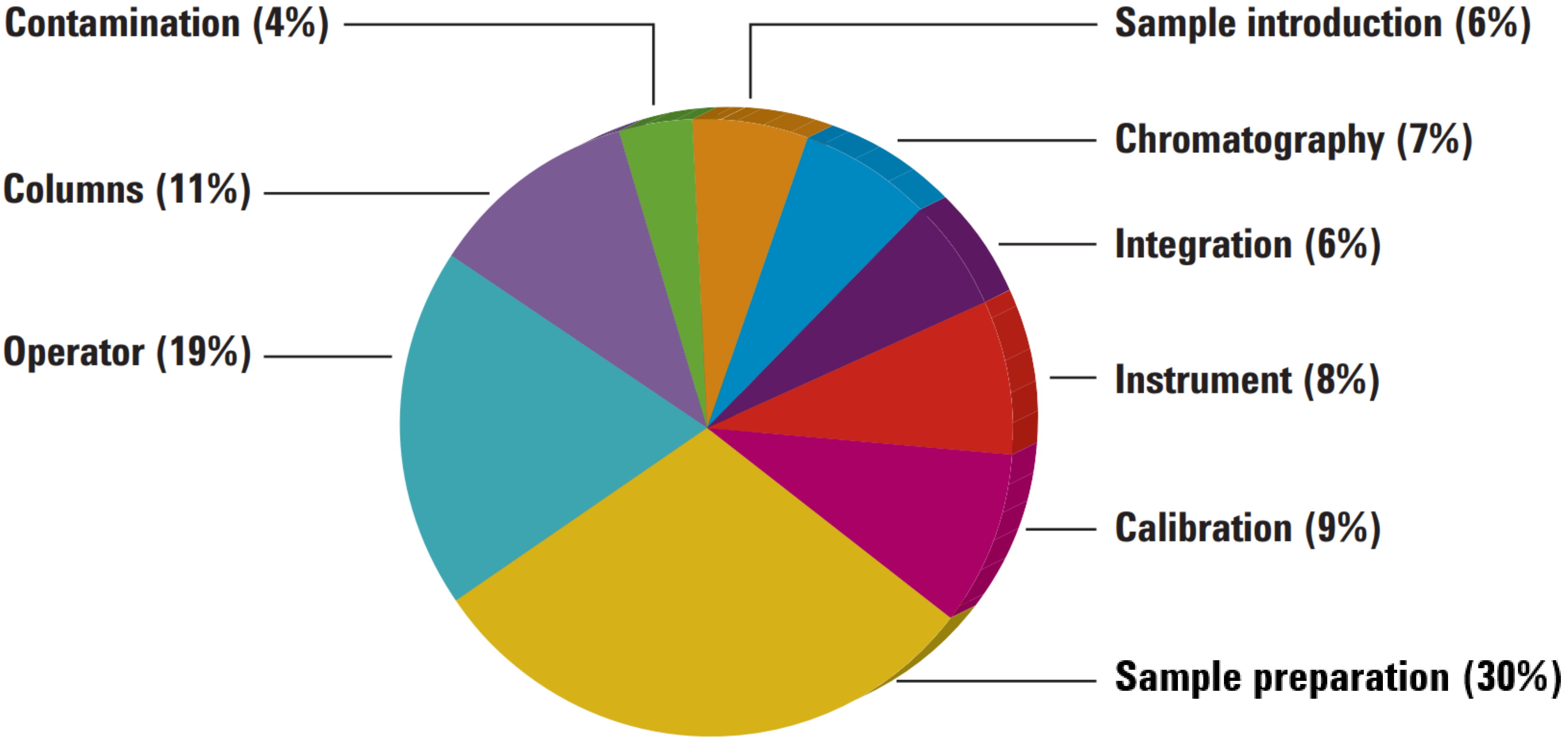
Optimizing your Application / Method Transfer considerations

- What needs to be considered when implementing App notes and transferring method between systems
- Delay volume, column void volumes
- Capillary selection and connections
- Column considerations (dimension and particle size)

Advancing your Application / Chromatography

- How to choose the appropriate LC system for the application that is going to be run.
- Where to find resources or info

Sources of Error Generated During Chromatographic Analysis

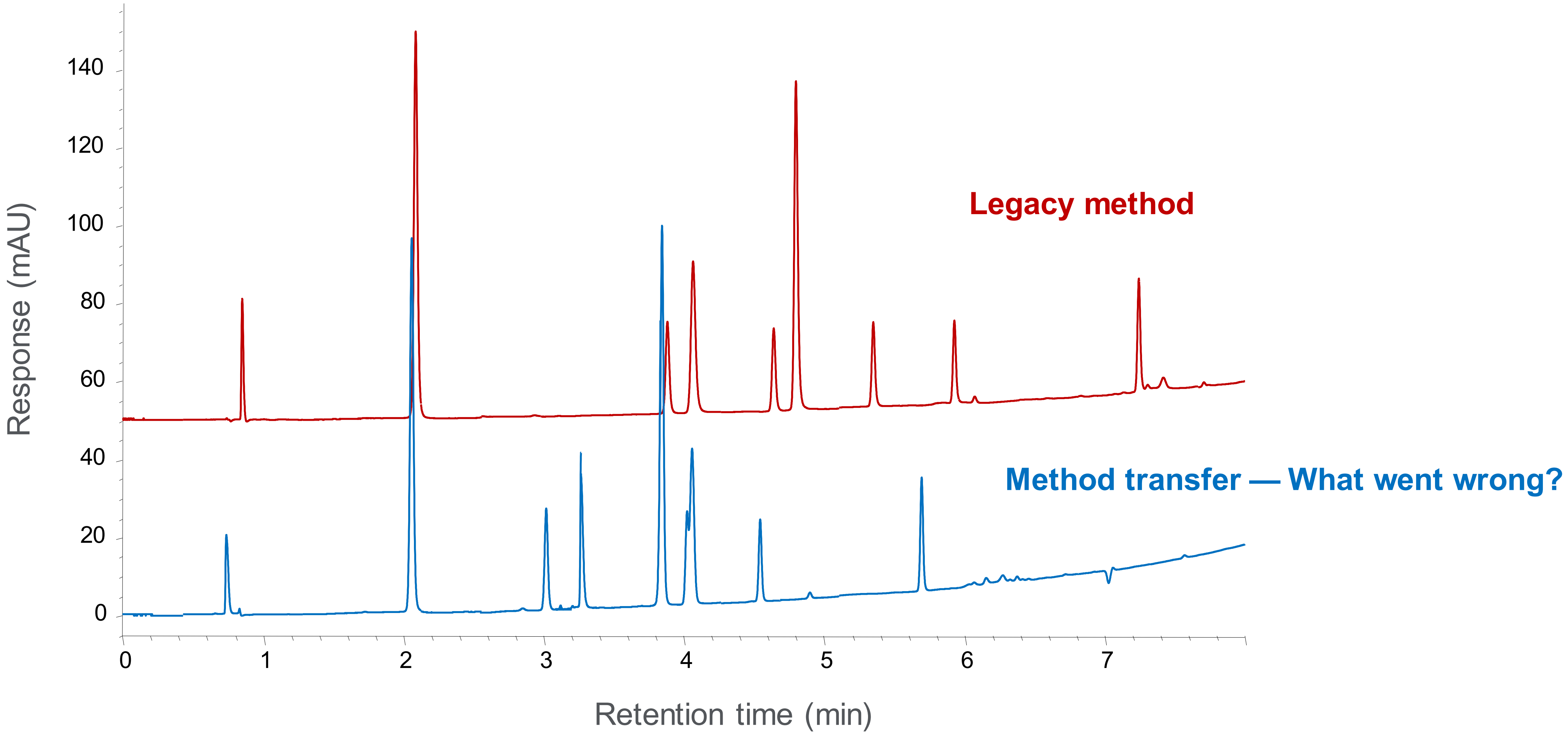


Data taken from Agilent Technologies survey

What is Method Transfer?

Different Aspects
From particle size to particle size (HPLC to UHPLC) ⇨ often associated with a change of instruments due to constraints in max. pressure, extra-column band broadening etc.. Some fine-tuning of method might be required due to frictional heating effects ⇨ available tools: Agilent Method translator, Third Party Method development SW (S-Matrix, ACD Labs, ChromSword, etc.)
From one eluent type or phase chemistry to an other ⇨ Method development ⇨ available tools: Method development SW (ACD Labs, ChromSword, S-Matrix etc.)
From column dimension to column dimension (e.g. 4.6 to 2.1 mm i.d., 50 mm to 100 mm) ⇨ recalculate flow rates, recalculate gradient times, adjust connection capillaries and flow cells, due to delay volume results may vary, method might need revalidation ⇨ available tools: Method Translator
From Instrument to Instrument ⇨ with method/instrument change: Isocratic Hold / Pre-injection, Vol. Modification ⇨ available tools w/o method or instrument change: none until ISET

Method Transfer Considerations & Application Optimization



Design Differences (U)HPLC Systems

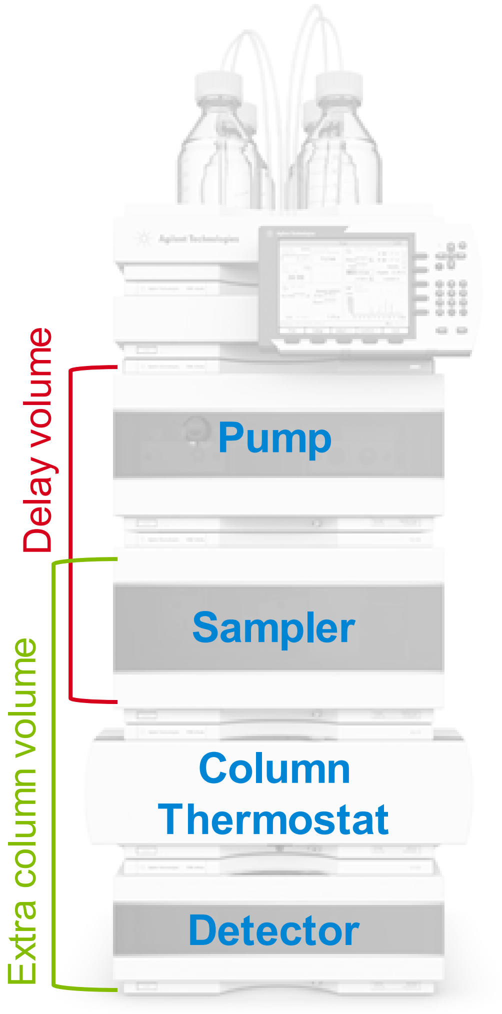
- Delay volumes (gradient formation)
- Power range (flow x pressure)
- Extra column volume
- Temperature
- Data rates
- Sensitivity

Problem:
Instrument to Instrument Method Transferability
- Not possible
- Requires Re-Development
- Requires Re-Validation

Resulting in huge additional cost factor

Instrument to Instrument Method Transferability

- Important Parameters

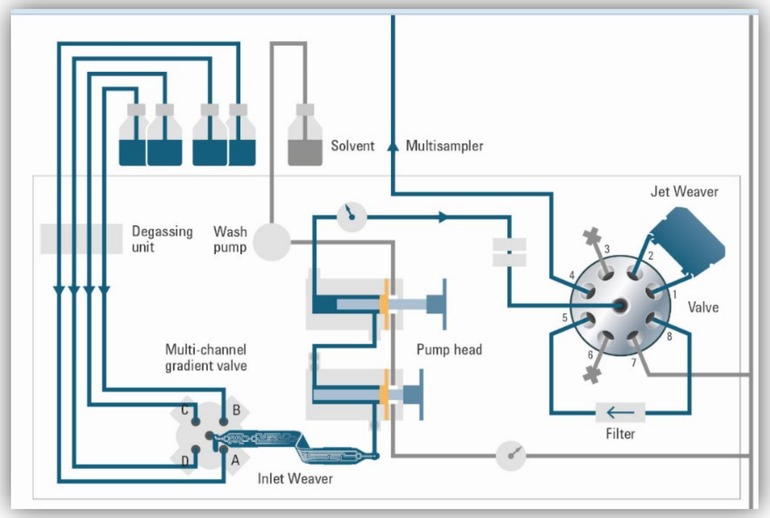


Delay volume Gradient mixing behavior Pressure x flow rate	Retention Time Resolution
Delay volume Extra column volume Injection volume	Retention Time Resolution Sensitivity
Temperature profile Extra column volume	Retention Time Resolution
Data rate Extra column volume Path-length	Resolution Retention Time Sensitivity

Comparison of Gradient Delay Volume (Dwell Volume)

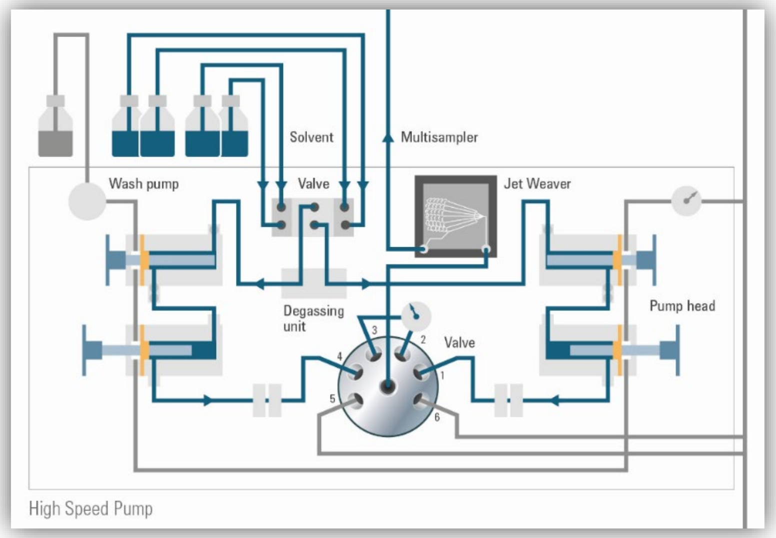
1290 Infinity II Flexible Pump (Quaternary)

- Integrated Degasser
- 4 solvent channels with concurrent mixing of all 4 channels
- Lower in price, typically, than binary pump



1290 Infinity II High Speed Pump (Binary)

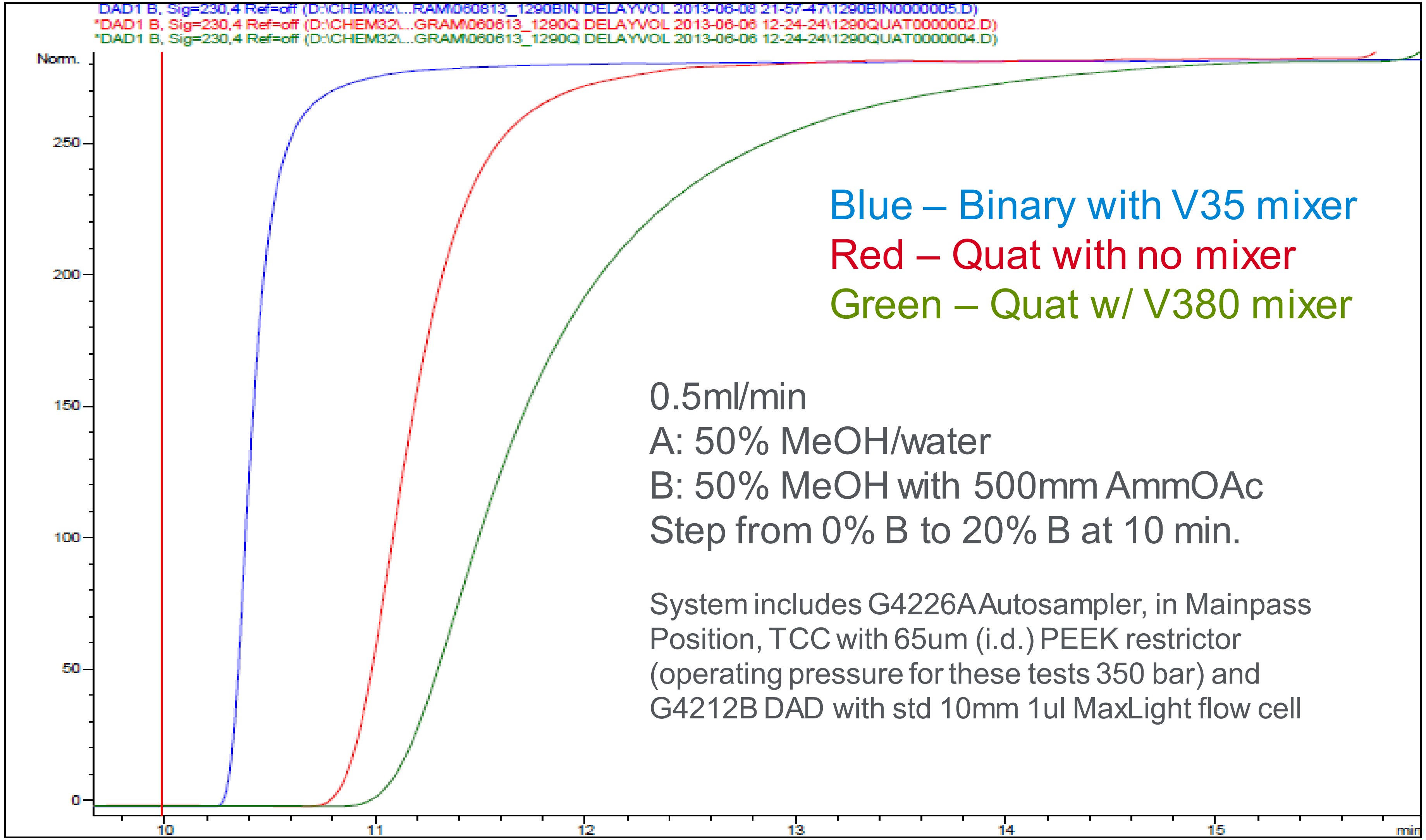
- Integrated Degasser
- 4 solvent channels available, mixing of 2 channels possible
- Better performance concept is widely accepted
- Greater control over dwell volume vs. Quaternary pump



*Incl. Degasser for both pumps
**with solvent selection valve

8 June 22, 2020

1290 Family Delay Volume Profiles

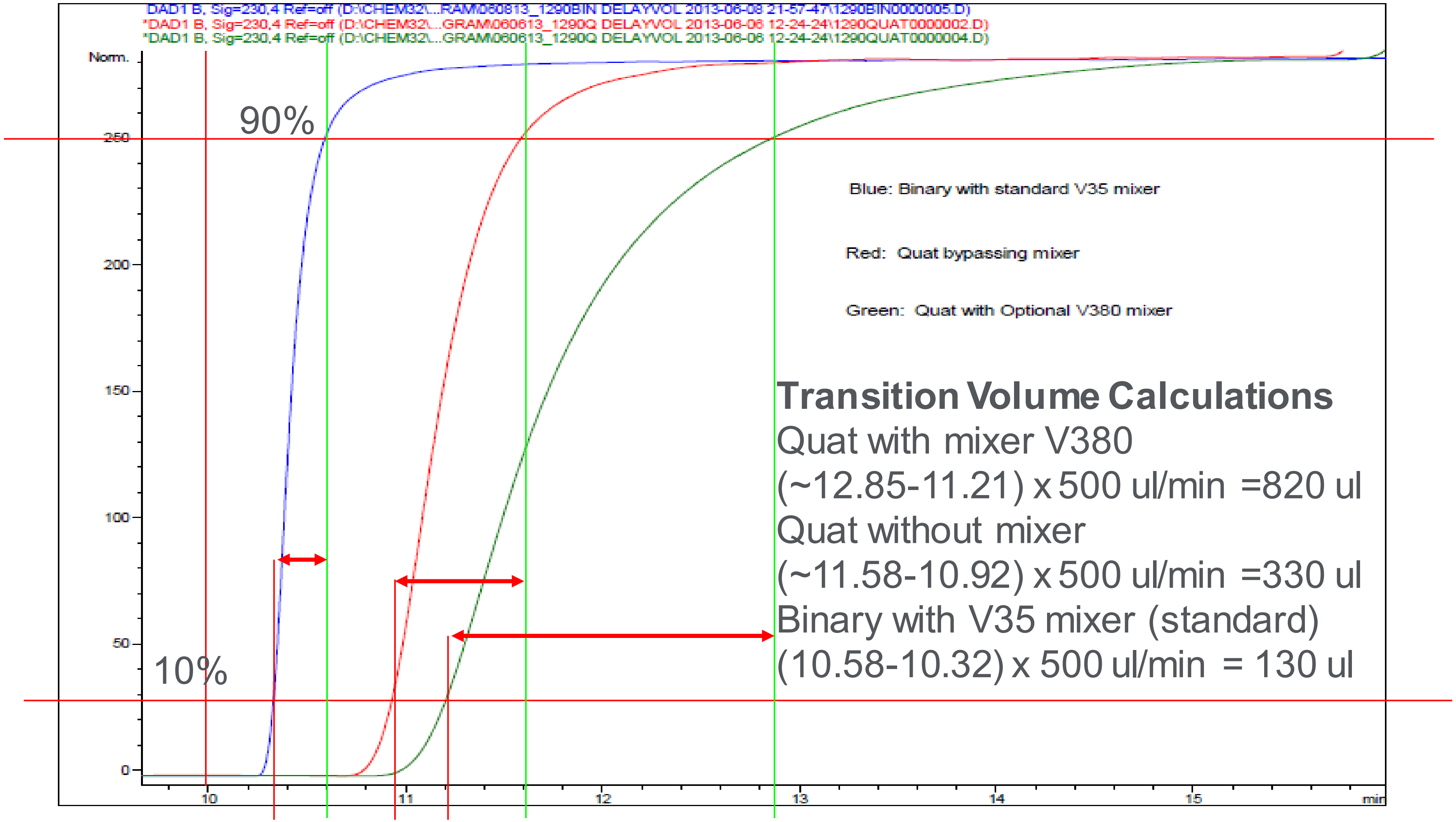


Blue – Binary with V35 mixer
Red – Quat with no mixer
Green – Quat w/ V380 mixer

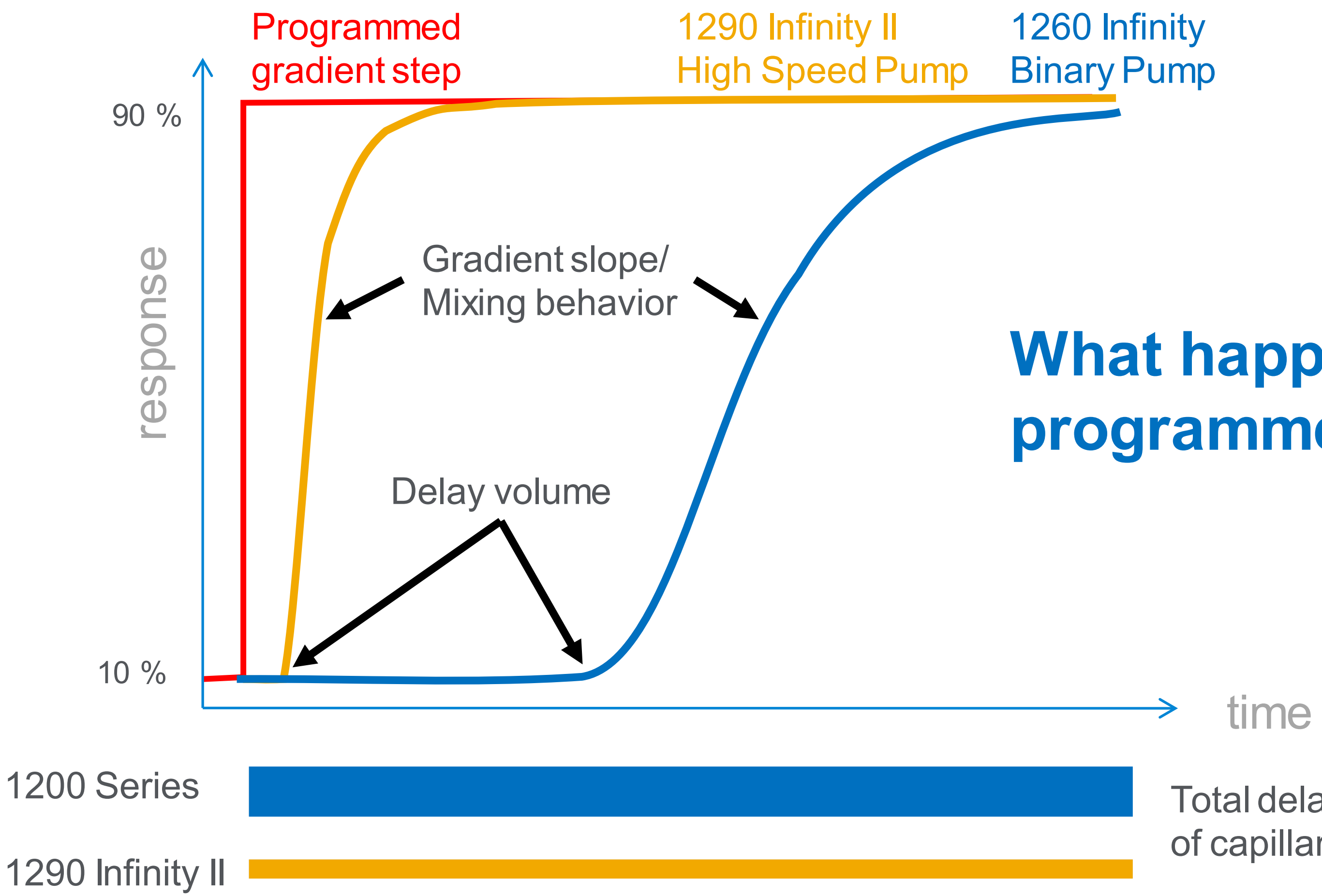
0.5ml/min
A: 50% MeOH/water
B: 50% MeOH with 500mm AmmOAc
Step from 0% B to 20% B at 10 min.

System includes G4226A Autosampler, in Mainpass Position, TCC with 65um (i.d.) PEEK restrictor (operating pressure for these tests 350 bar) and G4212B DAD with std 10mm 1ul MaxLight flow cell

1290 Delay Volume – 10-90% Transition



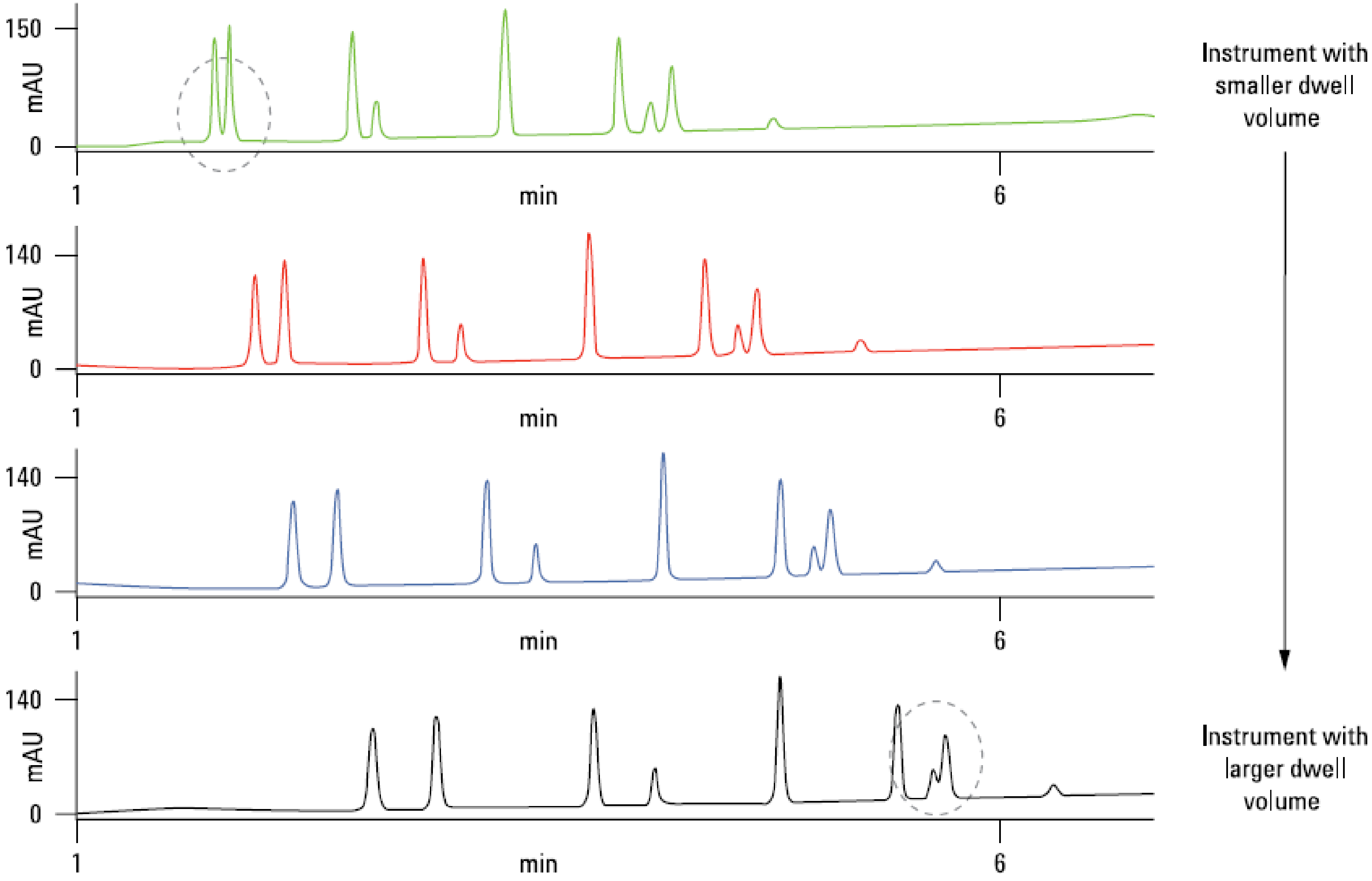
Method Transfer to UHPLC instruments
Impact of delay volume and mixing behavior...



What happens with a programmed gradient?

Total delay volume of the system (sum of capillaries, mixer, cells, valves..)

Chromatographic Test Result; Different Delay Volumes



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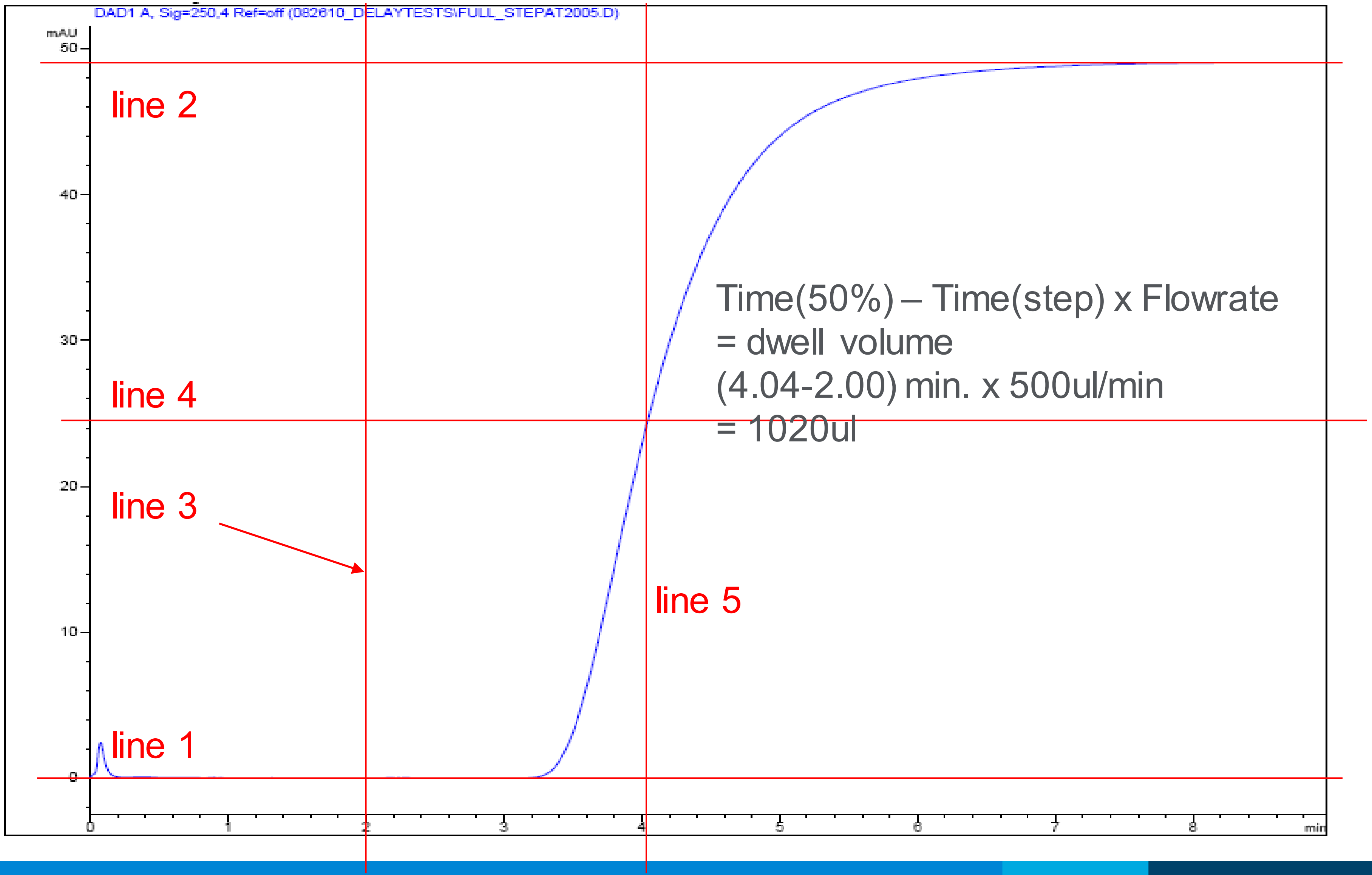
Example -- How to Calculate Delay Volume

5990-7595EN
The LC Handbook



Summary of Available Data

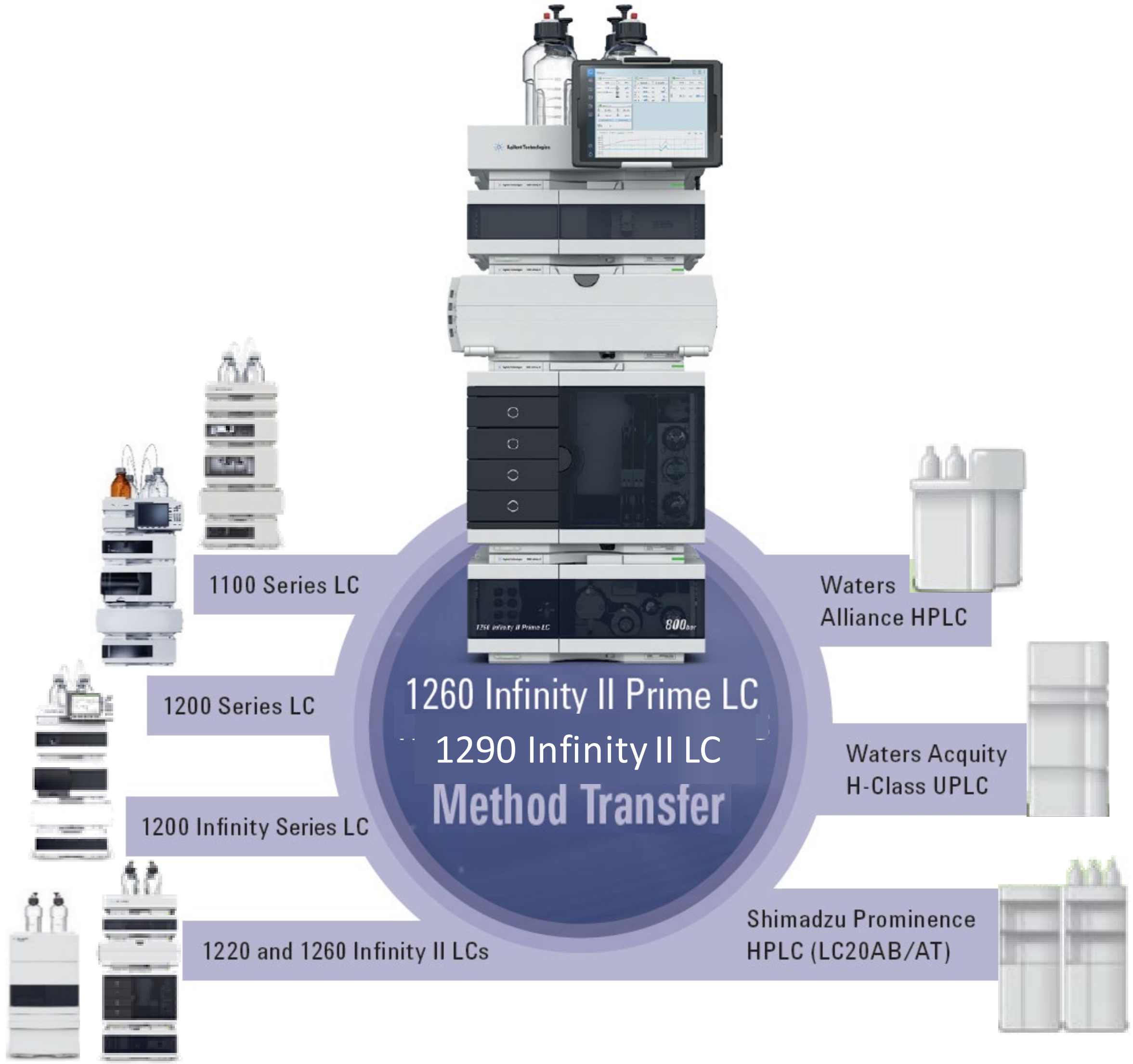
Delay Volume Summary for Agilent Infinity 1290 Systems					
Pump	Configuration	Sampler	Liftoff	Midpoint	Transition
1290 bin @ 350 Bar	35ul JetWeaver	G4226A	125	225	130
1290 quat @ 350 Bar	no mixer	G4226A	375	570	330
1290 quat @ 350 Bar	380ul JetWeaver	G4226A	450	820	820
For Reference…					
1260 bin @ 350 Bar	as shipped	G1367E	720	1105	545

Summary of Available Data

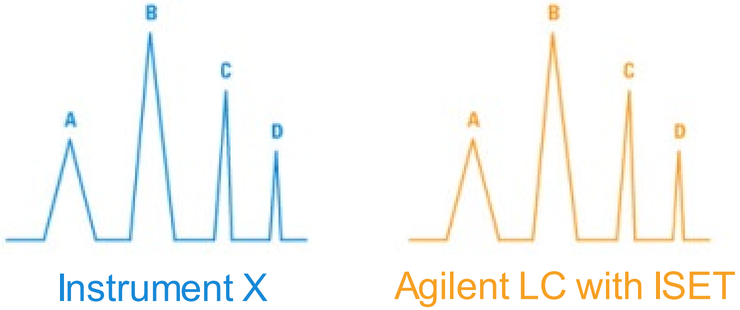
Delay Volume Summary for Binary 1260 Systems					
Pump	Configuration	Sampler	Liftoff	Midpoint	Transition
1260 bin @ 350 Bar	as shipped	G1367E	720	1105	545
1260 bin @ 350 Bar	minus damper	G1367E	635	955	460
1260 bin @ 350 Bar	minus damper with purge valve in channel A	G1367E	550	835	425
1260 bin @ 350 Bar	minus mixer/damper with purge valve in channel A	G1367E	270	375	175
1260 bin @ 350 Bar	minus mixer/damper with purge valve in channel A	G1367E with bypass (ADVR)		110	
For Reference					
1290 bin @ 350 Bar	35ul JetWeaver	G4226A	125	225	130

Intelligent System Emulation Technology (ISET)

One-Click LC System Emulation

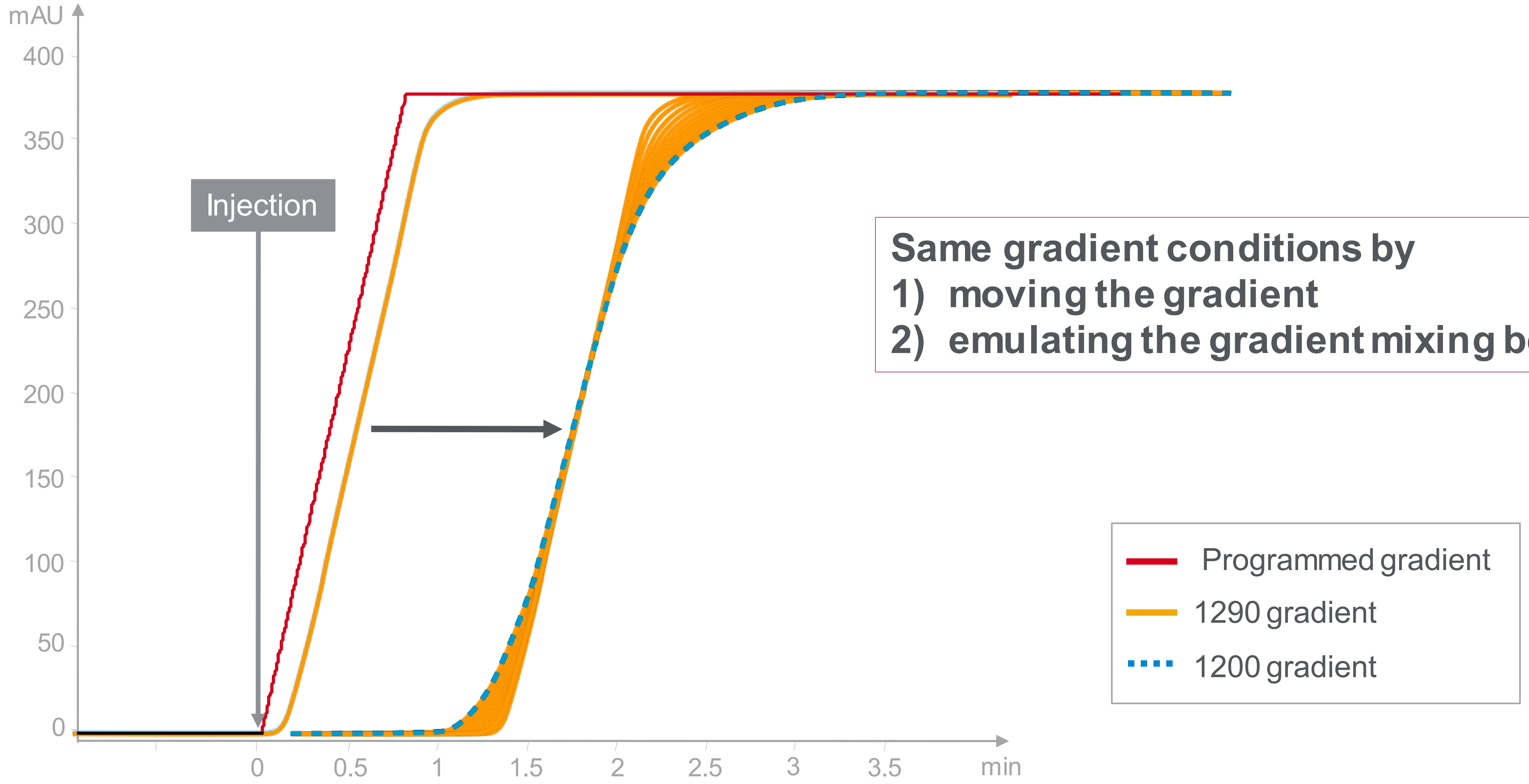


- Emulates other (U)HPLC instruments – by a simple mouse click
- Runs existing (U)HPLC methods – without modifying method or system
- Delivers same retention times and peak resolution – for infinitely better method transfer



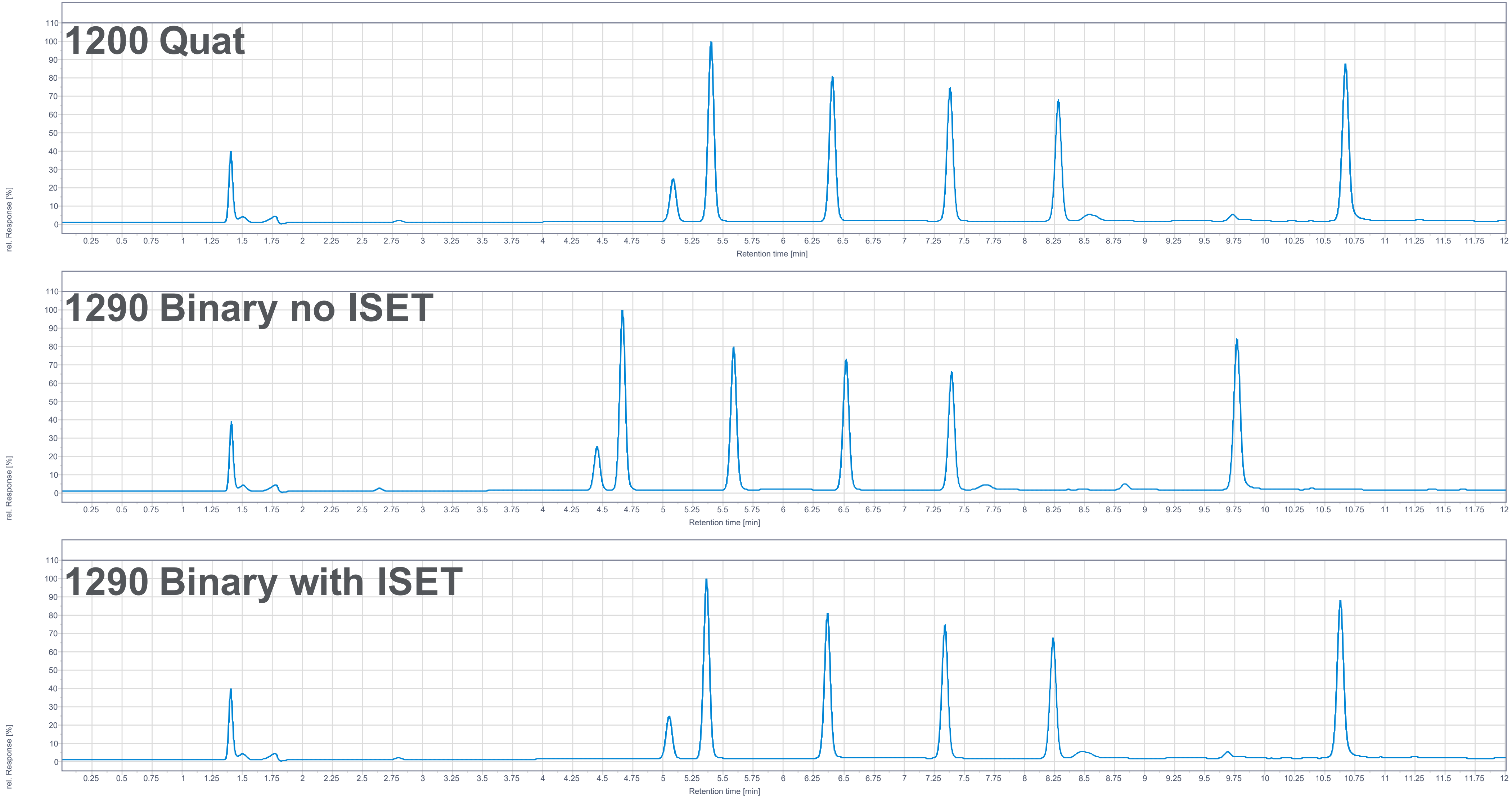
Intelligent System Emulation Technology (ISET)

How does it work?

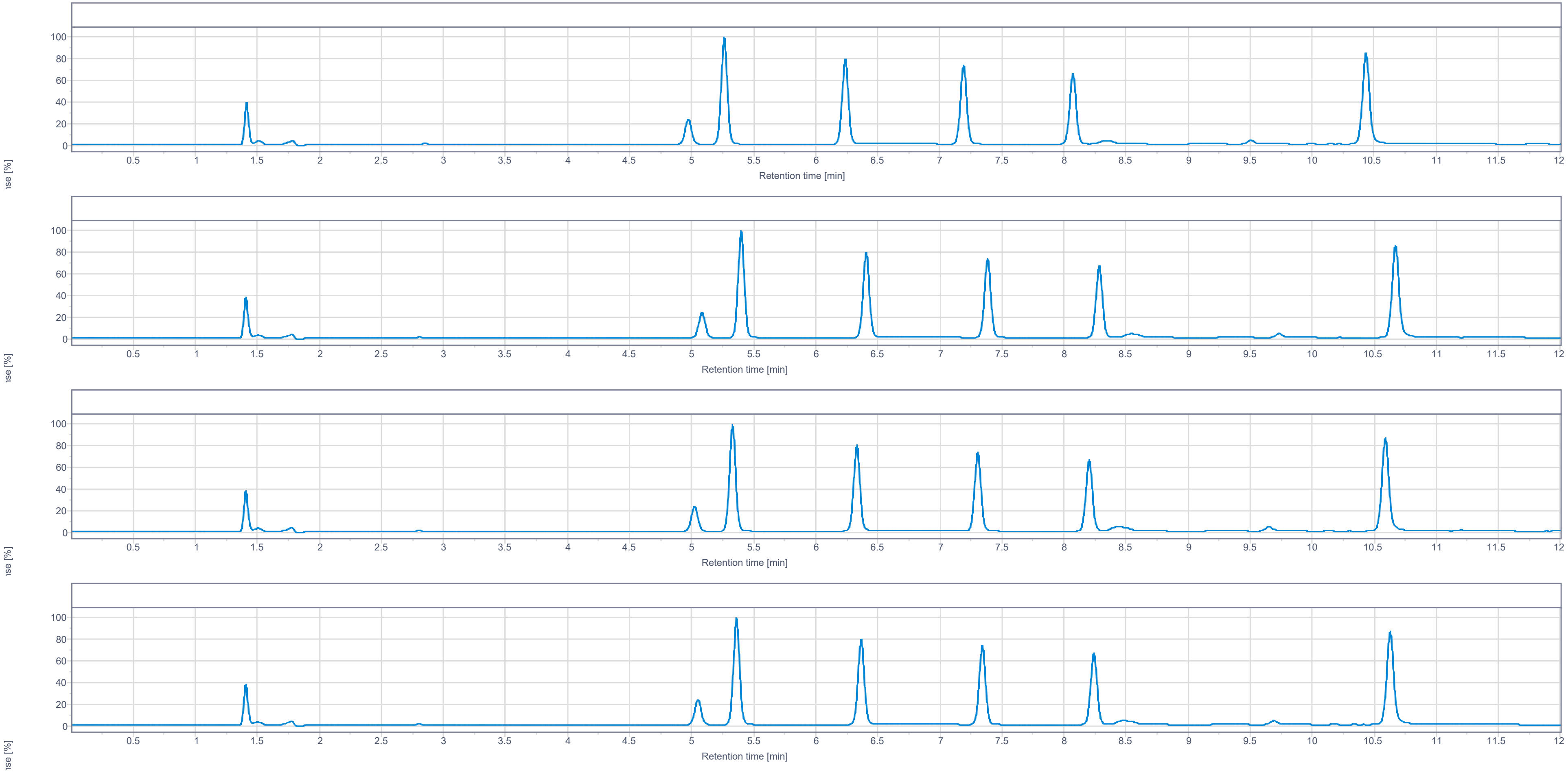


Same gradient conditions by
1) **moving the gradient**
2) **emulating the gradient mixing behavior**

Intelligent System Emulation Technology (ISET)



ISET



Can you tell which is which?
You can run the fastest UHPLC methods, and still run your legacy methods

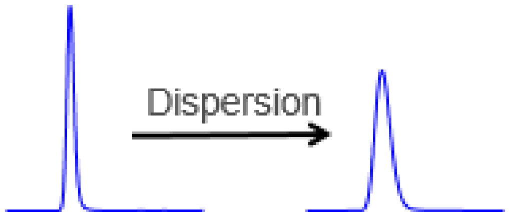
Lowest dispersion for highest resolution

Agilent **A-Line Quick Connect UHPLC column fittings** for truly dead-volume-free fluidic connections

- Tool-free connection up to 1300 bar
- Spring loaded design for easy and **truly zero-dead volume** connection
- Removable and reusable for all column types

System dispersion

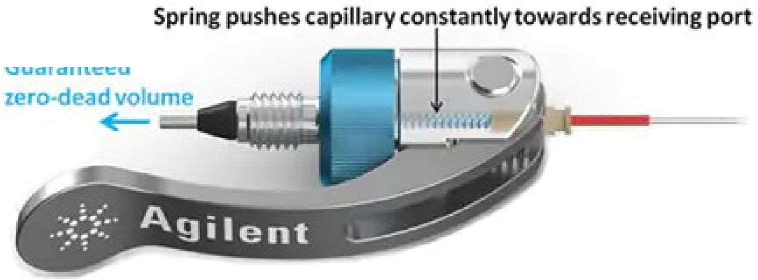
- *"Dispersion is the sample bandspreading or dilution which occurs in connecting tubing, sample valves, flow cells and in column end-fittings."*



Peak height: Loss of sensitivity

Peak width: Loss of resolution

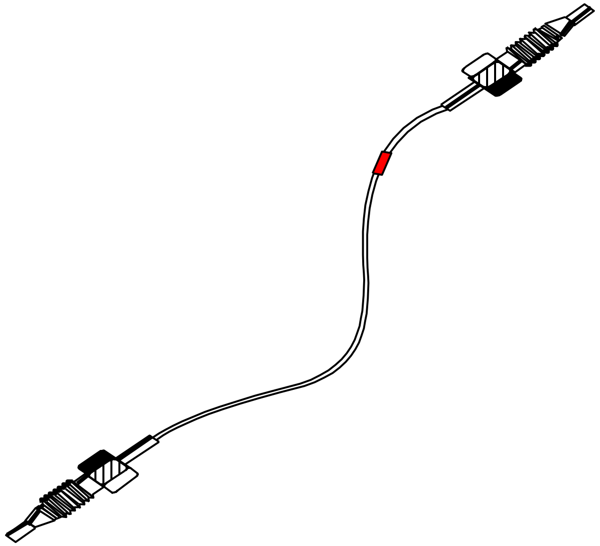
- Capillaries (Inner diameter, length)



Tubing Pressure and Internal Volume

Parameter	Black	Red	Green	Blue
Tubing Description	**1**	**2**	**3**	**4**
i.d. (mm)	0.075	0.127	0.178	0.254
Total Length (mm)	500	500	500	500
Flow rate (ml/min)	1.000	1.00	1.00	1.00
Solvent Name	ACN/wa	ACN/wa	ACN/wa	ACN/wa
Viscosity (cP)	1.2	1.2	1.2	1.2
Internal Volume (ul)	**2.209**	**6.334**	**12.4**	**25.3**
Expected pressure (bar)	**129.1**	**15.7**	**4.1**	**1.0**

Color	i.d.
Black	0.075 mm (0.003 inches)
Red	0.12 mm (0.005 inches)
Green	0.17 mm (0.007 inches)
Blue	0.25 mm (0.01 inches)
Clear	0.50 mm (0.02 inches)



500mm – typical sample flow path with UV detn. LC/MS -- near 1000mm

Tubing Dimensions and System Dispersion

	HPLC 1260	UHPLC 1290
Column to Detector	18-30cm 0.17 18-30cm 0.12	18-30cm 0.12 25cm 0.075
TCC outlet to column	10cm 0.17 10cm 0.12	10cm 0.12 10cm 0.075
ALS to TCC inlet	18-30cm 0.17 18-30cm 0.12	18-30cm 0.12 45cm 0.075
Needle Seat	Green 0.17 - 2.3uL Red 0.12 - 1.7uL	Red 0.12 - 1.7uL Black 0.075 - 0.9uL
Pump to ALS	20-40cm 0.17 20-40cm 0.12	20-40cm 0.12 20cm 0.075

https://www.agilent.com/cs/library/usermanuals/public/Copy%20of%20UltraLowDispersionKit_TN_EN.pdf

Optimize Dispersion for Small Columns
2.1 x 50 mm Poroshell 120 1.9um

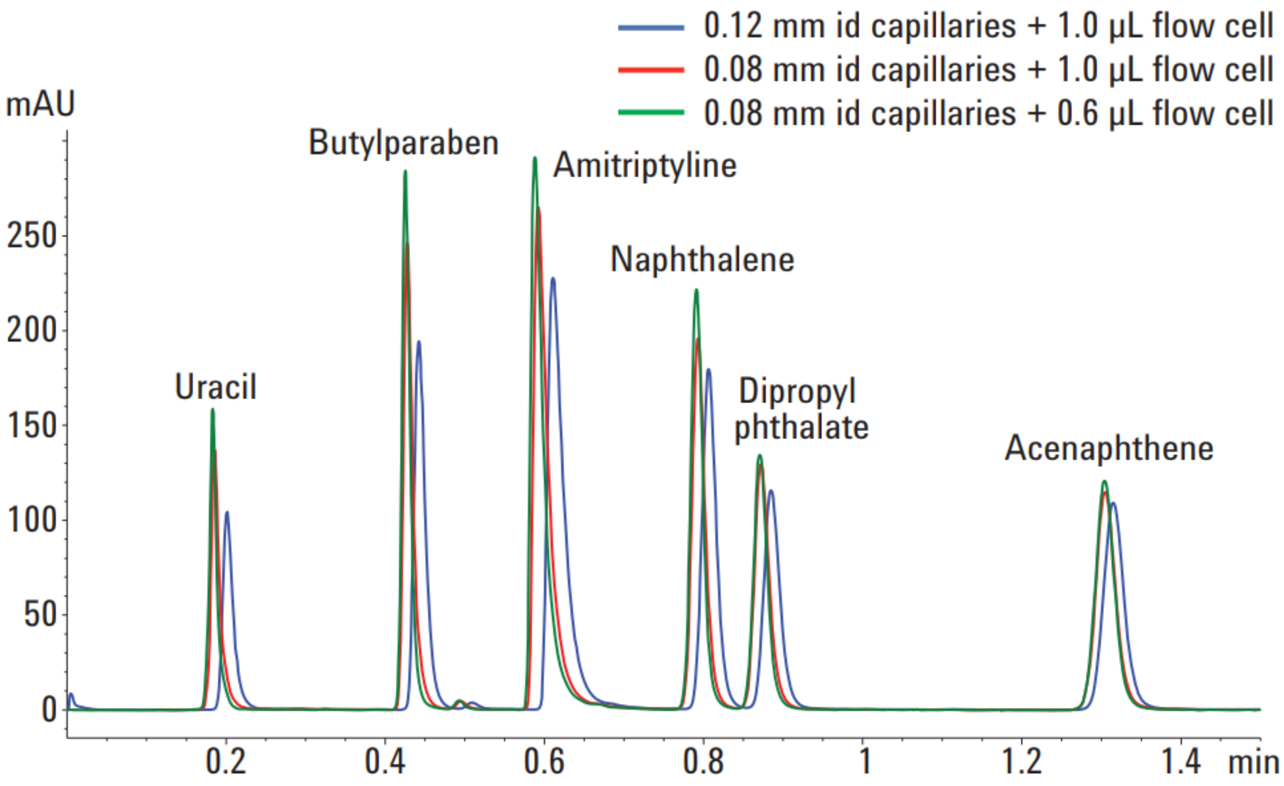


Figure 1A. The performance of an Agilent InfinityLab Poroshell 1.9 µm column is improved when LC system volume is reduced by using smaller internal diameter capillaries and a smaller volume detector flow cell.

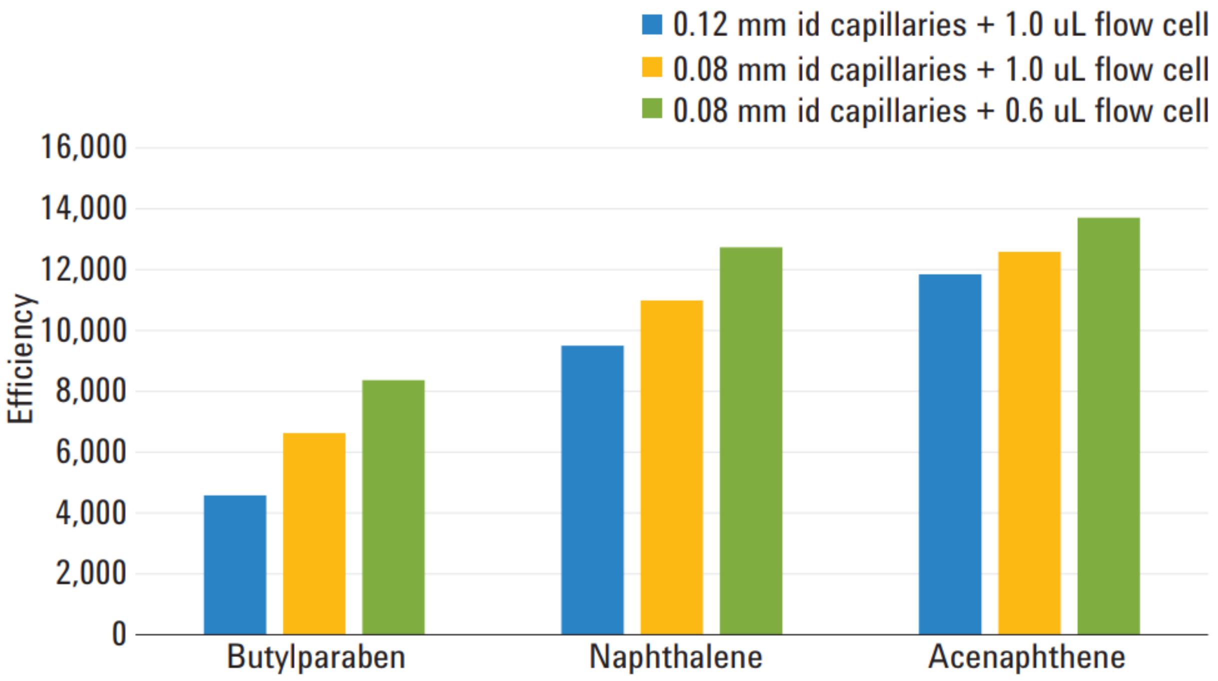
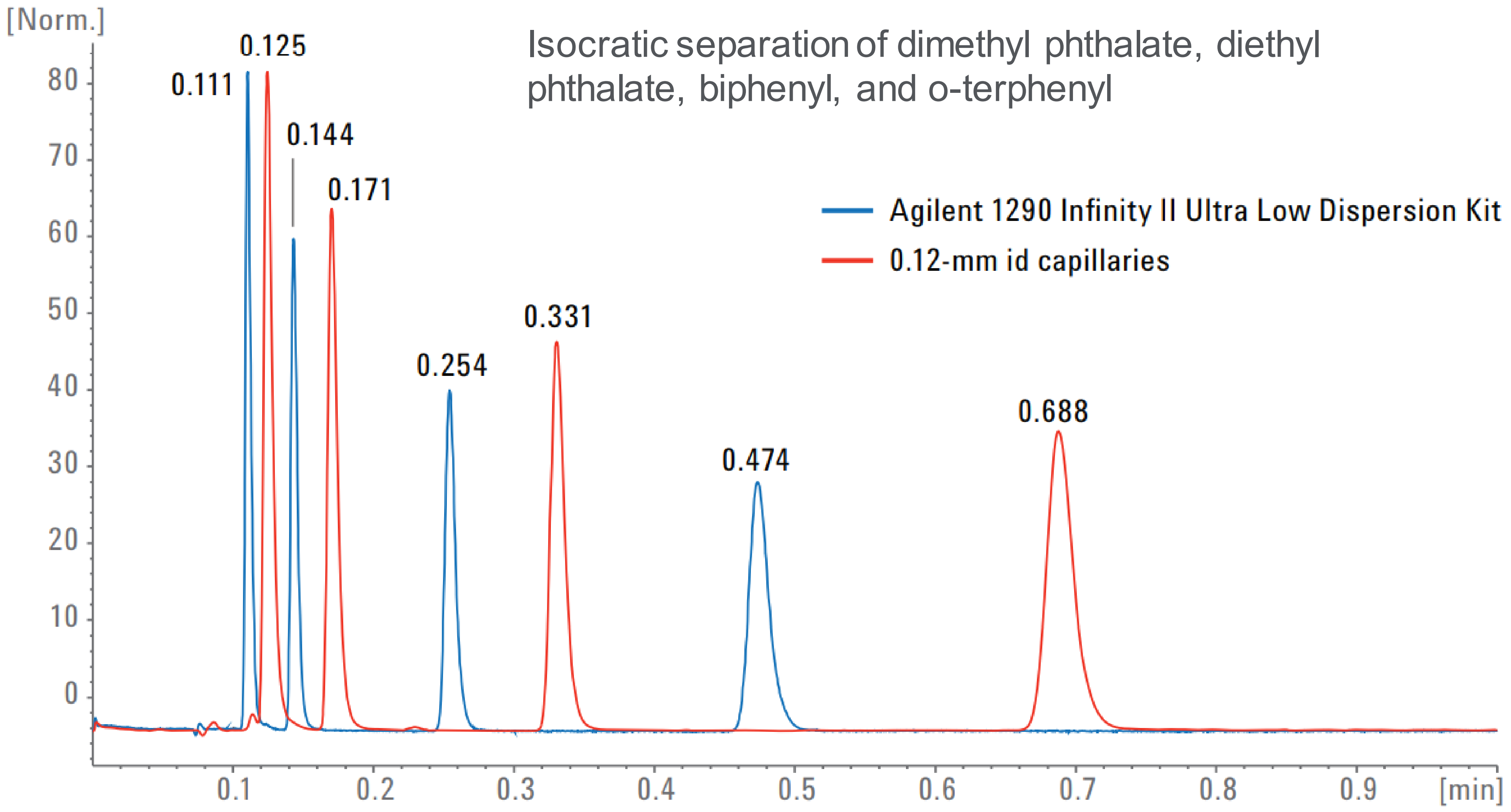


Figure 1B. The performance of an Agilent InfinityLab Poroshell 1.9 µm column is improved when LC system volume is reduced by using smaller internal diameter capillaries and a smaller volume detector flow cell.

App Note: 5991-7560EN

Optimizing System Dispersion on the Agilent 1290 Infinity II LC



App Note: 5991-5984EN

Increased Pressure Requirements (column particle size)

d_p (µm)	ΔP (bar)	N
5.0	14.5	25,000
3.0	66.9	41,000
1.5	531	83,000
1.0	1800	125,000
0.75	4270	166,000
0.50	14400	250,000

For a 30 cm column, 5.0 to 0.50 µm particle size reduction:

- N (theoretical plates) increases 10-fold
- ΔP (change in pressure) increases 1000-fold

Column Length and Particle Size

Column Length (mm)	Column Efficiency N(5 µm)	Column Efficiency N(3.5 µm)	Column Efficiency N(1.8 µm)
150	12,500	21,000	35,000
100	8,500	14,000	23,250
75	6000	10,500	17,500
50	4,200	7,000	12,000
30	N.A.	4,200	6,500
15	N.A.	2,100	2,500

Efficiency (N)

Pressure

Analysis Time

Peak Volume

Solvent Usage

Column Length (mm)	Analysis Time*
150	-
100	-33%
75	-50%
50	-67%
30	-80%
15	-90%

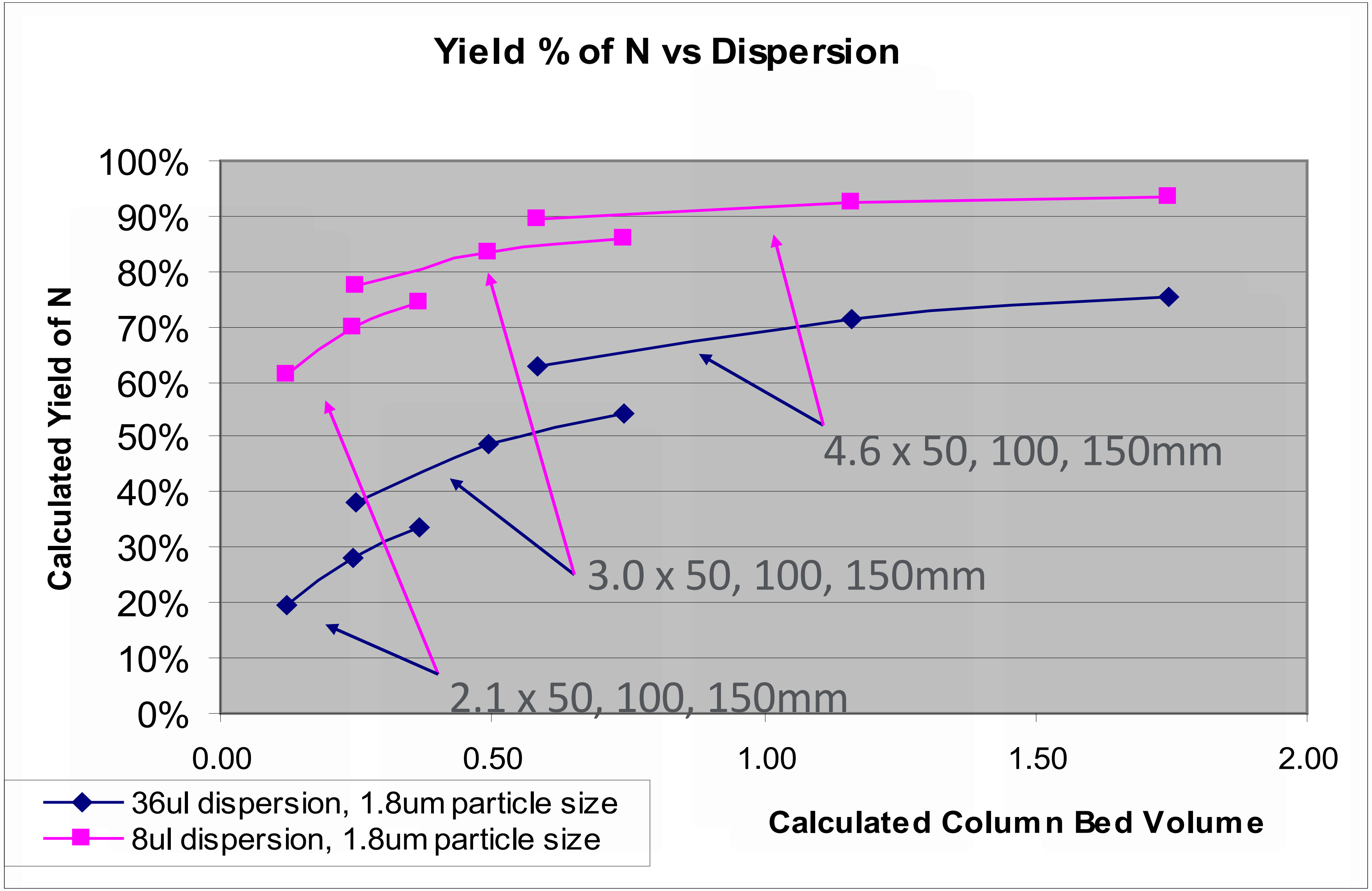
* Reduction in analysis time compared to 150 mm column; all columns 4.6-mm i.d.

Predicting Column Pressure and Performance

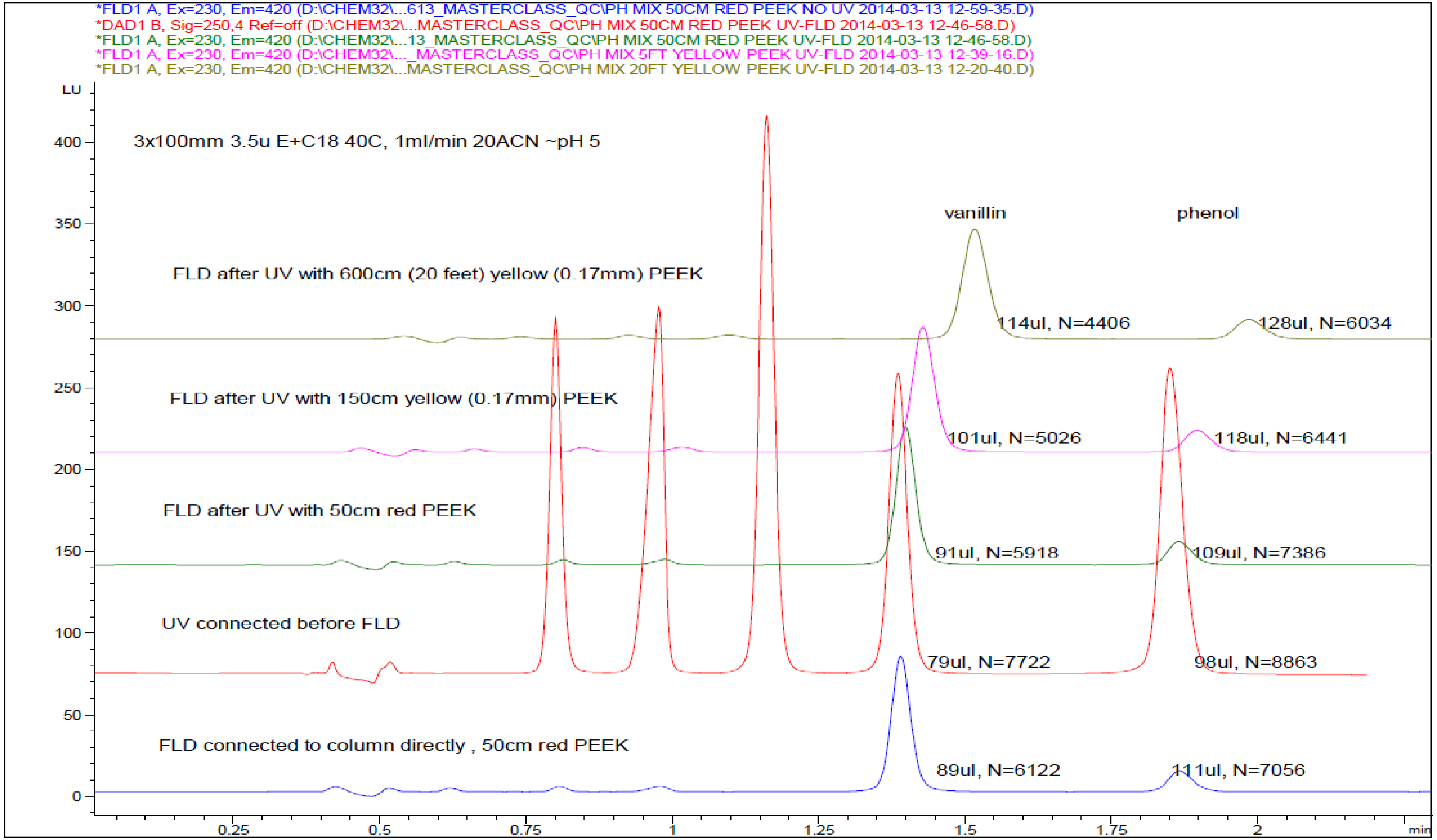
Parameter	Media	ZORBAX			Poroshell 120	
i.d. (mm)		2.100	3.000	3.000	3.000	3.000
Length (mm)		50.0	50.0	100.0	100.0	100.0
Particle Size (uM)		1.8	1.8	1.8	1.9	2.7
Column void fraction		0.60	0.60	0.60	0.60	0.60
Permeability (PF)		900	900	900	900	900
Reduced plate height value		2.30	2.30	2.30	1.70	1.70
Flow rate (ml/min)		0.5000	1.0000	1.000	1.000	1.000
Linear Velocity (mm/sec)		4.01	3.93	3.93	3.93	3.93
Viscosity (cP) 25C ACN/Water		1.2	1.2	1.2	1.2	1.2
Column Volume (ml)		**0.104**	**0.212**	**0.424**	**0.424**	**0.424**
Expected pressure (bar)		**401.0**	**393.0**	**785.9**	**705.4**	**349.3**
Estimated Efficiency** (Neue)		**12077**	**12077**	**24155**	**30960**	**21786**

**Estimates do not consider system dispersion effects. Actual results will vary.

Yield estimates – 1.8um particles 2.1-4.6mm i.d.



Multi-Detector Connections Bandspreading



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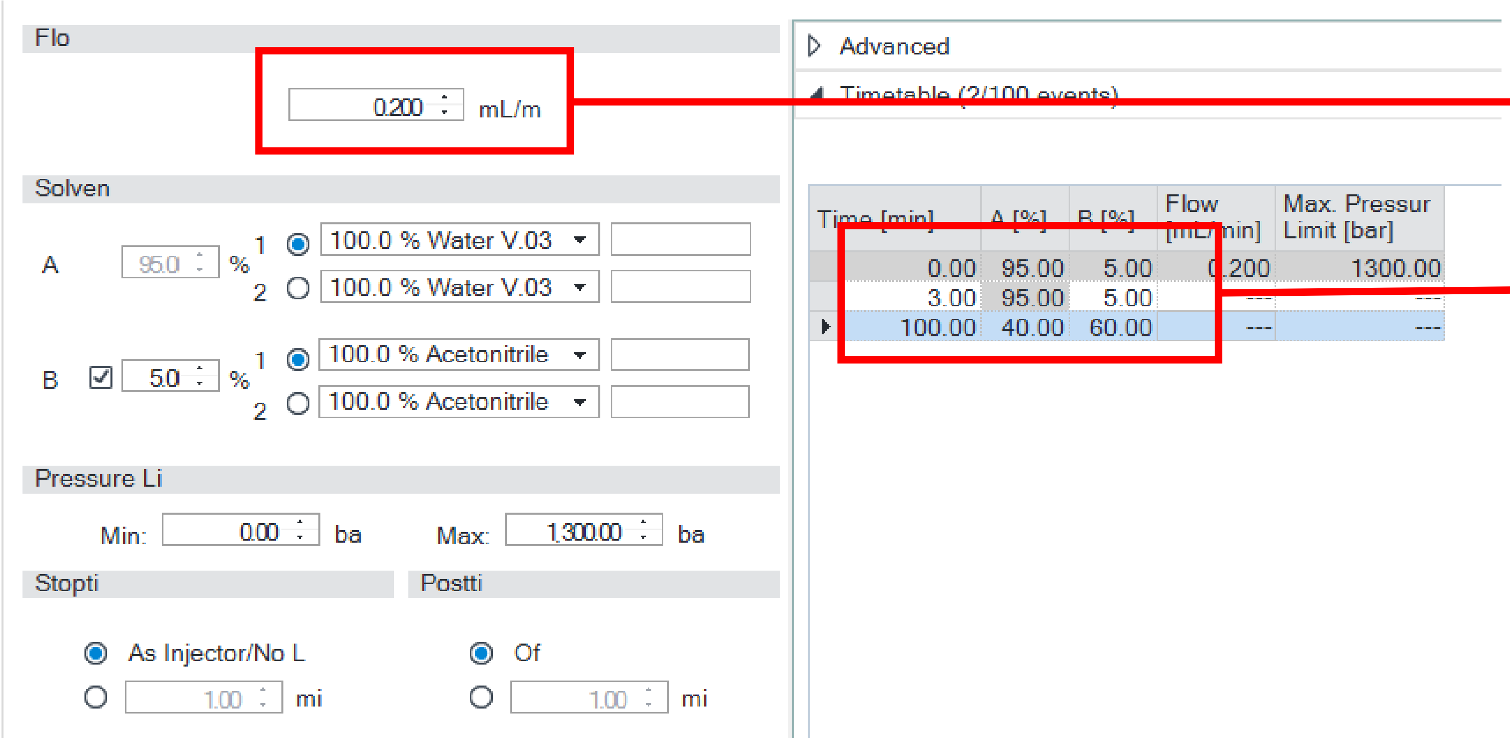
Use The Right Tool for the Job

We might get this to work, but the results may not be what we needed

(U)HPLC Systems

- Each component of the system has settable values, however not all settable values can be achieved
- Before using or purchasing a system, the user must be certain that the desired parameters can be run accurately and reproducibly
- Instrument specifications should be obtained from the appropriate manuals
- From these specifications, it can be determined if that component may be used

Pump Parameters

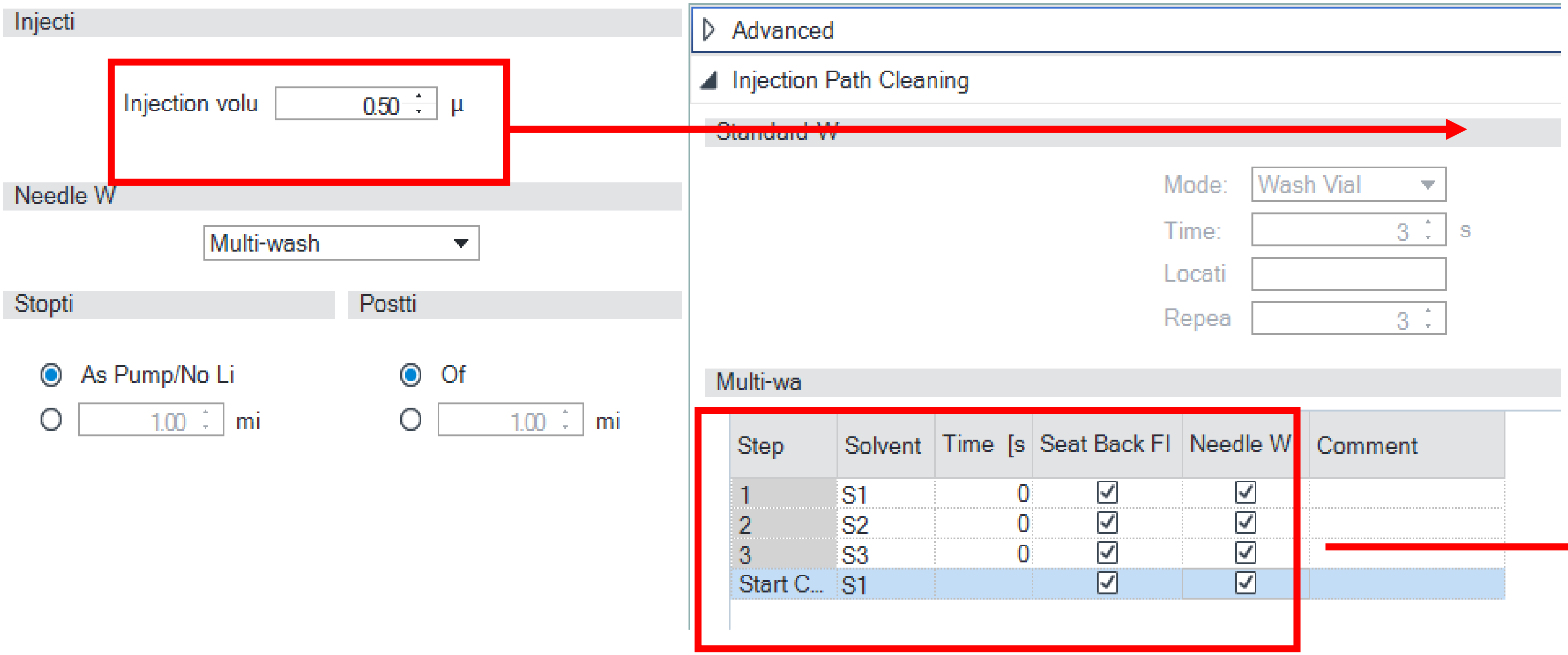


Note flow rate 0.2 ml/min

Note gradient change (%B/min)

- Low flow rate (<0.5 ml/min) start to move to binary pump due lower delay volumes
- Shallow gradient (<1%B/min) requires binary pump due to lower delay volume and more precise changes

AutoSampler Parameters

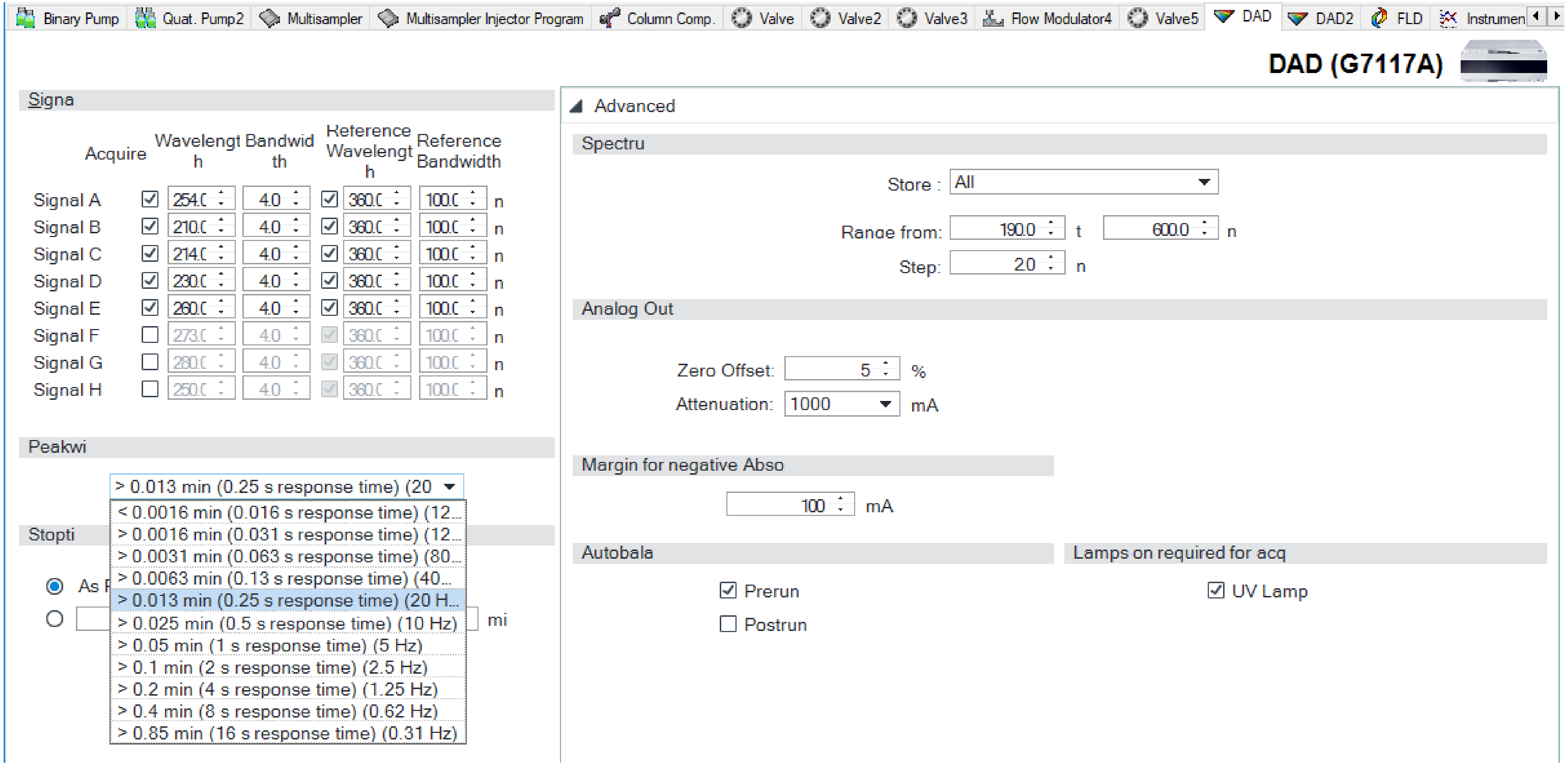


Newer, UHPLC methods may have lower injection volumes

'Sticky samples or more sensitive detectors may require aggressive cleaning

- Shorter (<150mm) and narrower columns (<3mm) may have lower injection volumes. Make sure the sampler precision and accuracy are correct for low volumes
- More sensitive detectors (or higher salt concentrations in the buffers) may require additional wash steps for both the needle and needle seat

Detector Parameters



As columns become shorter peaks elute more quickly requiring detector data rates to increase.

Resources — Primers

[5990-7595EN](#)
The LC Handbook
Guide to LC Columns and Method Development

[5991-2359EN](#)
Two Dimensional Liquid Chromatography

[5990-3777EN](#)
High Performance Capillary Electrophoresis

[5991-5509EN](#)
Supercritical Fluid Chromatography

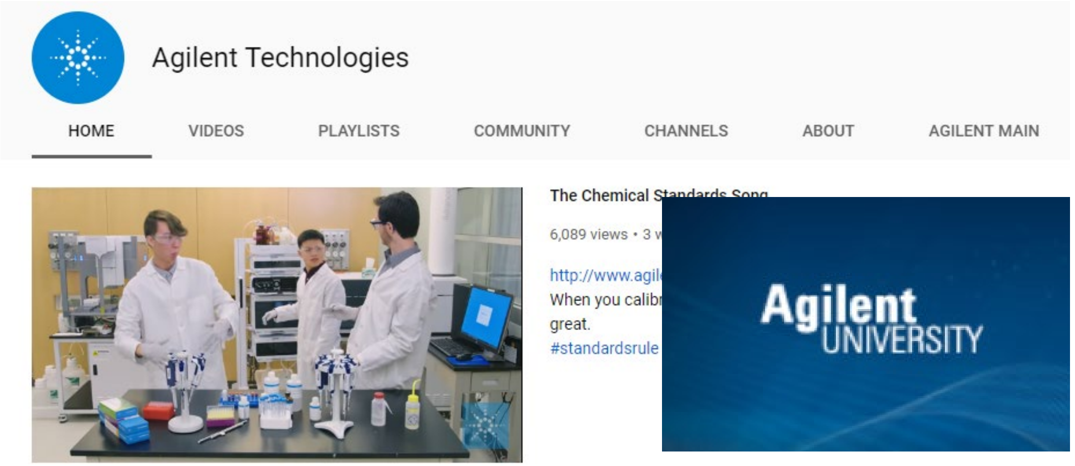
[5989-6639EN](#)
Principles in Preparative HPLC

[5991-3326EN](#)
Sample Preparation Fundamentals for Chromatography

[5980-1397EN](#)
Fundamentals of UV-visible Spectroscopy

Resources for Support

- Collection of LC resources: https://community.agilent.com/docs/DOC-1852-lc-insights-to-go#jive_content_id_LC_Troubleshooting
- Agilent support resources: https://community.agilent.com/community/resources
- Agilent University: http://www.agilent.com/crosslab/university
- Agilent resource center: http://www.agilent.com/chem/agilentresources
- InfinityLab Supplies Catalog (5991-8031EN)
- Your local FSE and Specialists
- Youtube – Agilent Channel

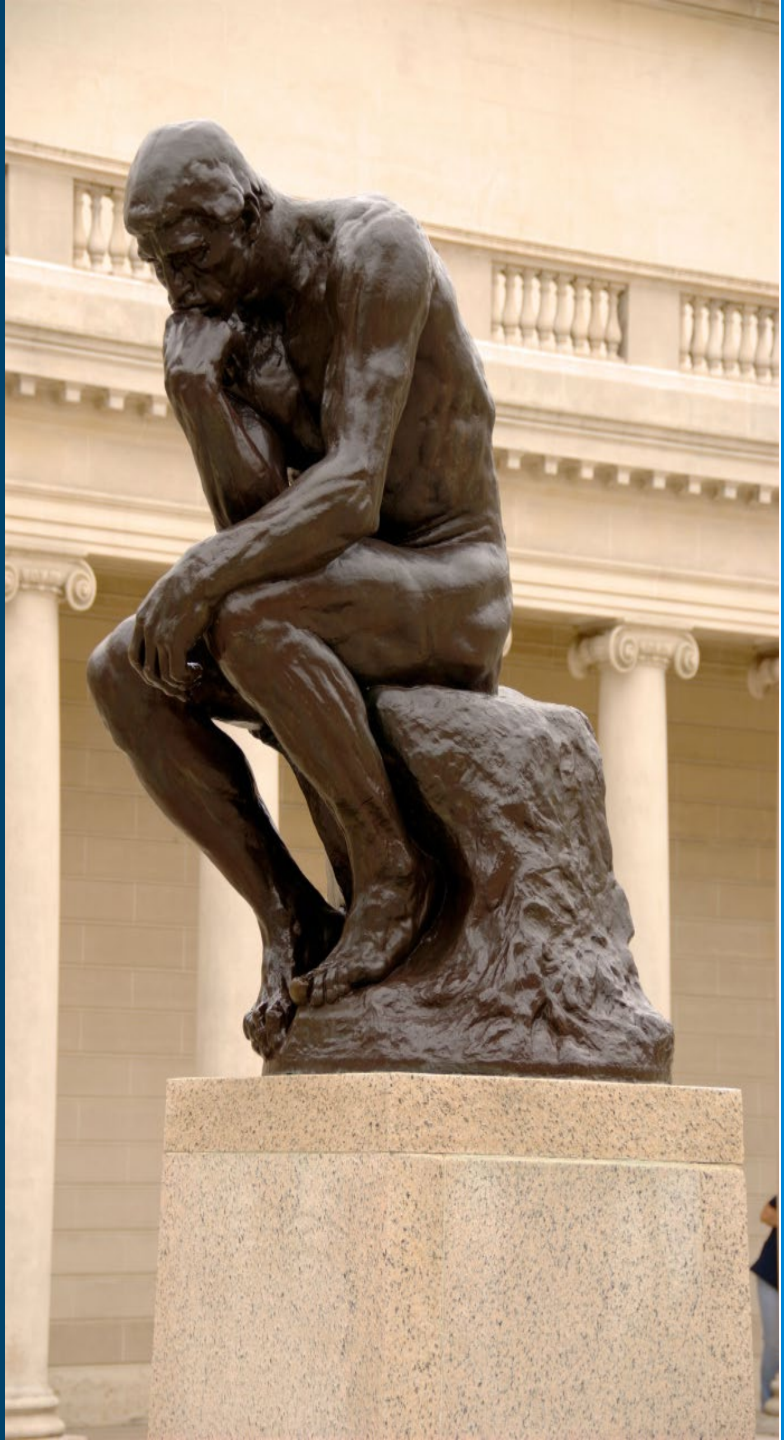


- Sales and support phone assistance (US and Canada):

1-800-227-9770 Phone Tree Navigation Assistance

gc-column-support@agilent.com
lc-column-support@agilent.com
spp-support@agilent.com
spectro-supplies-support@agilent.com

Thanks for your attention!



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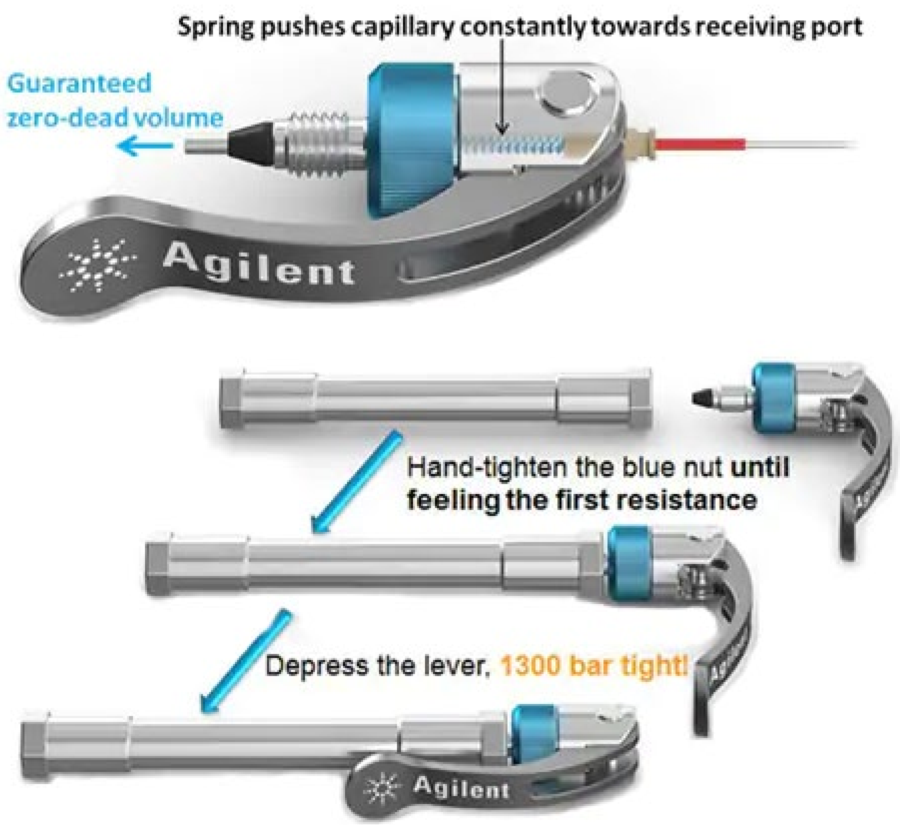
Universal Low Dispersion Fittings

Quick Connect



Quick Turn

Agilent application note 5991-5525EN