Bridging the Gap between UHPLC and HPLC:
Easy Method Transfer Using Fused-Core® Columns

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Abstract

Many chromatographers are now exploiting the speed and efficiency advantages of UHPLC as part of their method development scheme. The use of low dispersion UHPLC instrumentation and highly efficient columns allows them to screen different column stationary phases and analysis conditions (pH, organic modifier, temperature, etc.) much more quickly than ever before. Moreover, with this approach the resulting high speed and/or high resolution method will be more robust and efficient, and will be able to generate analytical results that enable faster and better decisions with high productivity.

However, when it is necessary to transfer such methods to a quality control or production environment, method developers often must cope with the limited availability of UHPLC instrumentation and operator expertise in such laboratories. Often, it is necessary to transfer the method to a longer and larger ID column with a much larger particle size to be able to transfer the method to conventional instrumentation (300–400 bar pressure limit).

Fused-Core® UHPLC columns with 2.7 µm particle size can deliver performance comparable to sub-2-µm columns at 40–50% of the back pressure. This benefit makes method transfer much easier between UHPLC and HPLC systems. It is only necessary to make modifications to the extracolumn volume and dispersion of the HPLC system, with some additional adjustments to the analysis conditions.

We will discuss the parameters that must be considered and adjusted when transferring isocratic and gradient methods from UHPLC instruments to conventional HPLC systems. Isocratic and gradient examples will be shown in which Fused-Core UHPLC separations are transferred to different HPLC instruments having different extracolumn dispersion and different delay volumes. Guidance will be offered to make such method transfers more successful.

Objectives

- Discuss important parameters that affect method transfer for isocratic and gradient methods.
- Demonstrate transfer of an isocratic and gradient method from UHPLC to HPLC, and summarize results.
- Show importance of injection delay for gradient method transfer.

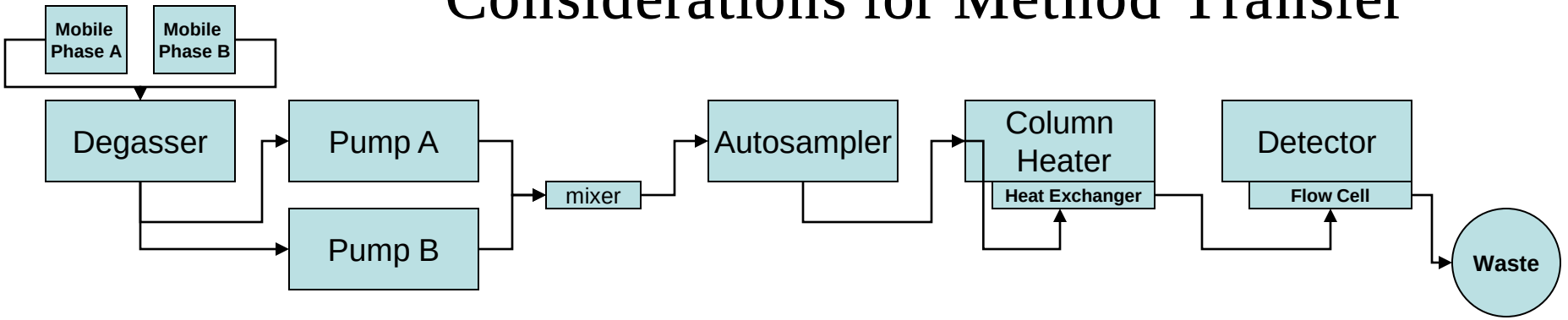
General (U)HPLC Method Transfer Concerns

- Column length and diameter
- Instrument brand and model
- Instrument pressure limit
- Pumping system type: low pressure mixing vs. high pressure mixing
- Gradient delay volume and flow rate / pressure dependence
- Mixer volume
- Flow Rate
- Column Oven type and Column Temperature
- Detector flow cell and volume
- Detector data rate and response time
- Injector type and injection volume

ACN/Water						
	ACN viscosities	1.01	0.91	0.75	0.62	0.52
ID (mm)	Length (mm)	25°C	30°C	40°C	50°C	60°C
3.0	20	3.12	3.46	4.20	5.00	5.00
3.0	30	2.26	2.51	3.05	3.69	4.40
3.0	50	1.46	1.62	1.97	2.38	2.84
3.0	75	1.01	1.12	1.36	1.65	2.00
3.0	100	0.78	0.86	1.04	1.26	1.50
3.0	150	0.53	0.59	0.71	0.86	1.00

ACN/Water						
	ACN viscosities	1.01	0.91	0.75	0.62	0.52
ID (mm)	Length (mm)	25°C	30°C	40°C	50°C	60°C
2.1	20	1.53	1.70	2.06	2.45	2.45
2.1	30	1.11	1.23	1.49	1.81	2.16
2.1	50	0.72	0.79	0.97	1.17	1.39
2.1	75	0.49	0.55	0.67	0.81	0.98
2.1	100	0.38	0.42	0.51	0.62	0.74
2.1	150	0.26	0.29	0.35	0.42	0.49

Considerations for Method Transfer



Parameter	I	G	Impact on Method Transfer
Pumping System • High Pressure Mixing • Low Pressure Mixing	N	Y	• High pressure mixing systems typically have lower delay volumes, with less "rounding" of gradient profiles • Some systems have pressure-dependent delay volume.
Mixer Volume	N	Y	• Affects delay volume • Affects gradient shape and especially baseline noise with some solvents and additives (e.g., TFA)
Tubing Volume	Y	Y	• Affects Extracolumn Dispersion (aka Peak Variance) and delay volume
Delay Volume	N	Y	• Differences can change selectivity • Affects retention times in gradients
Flow Rate	Y	Y	• Can affect extracolumn dispersion, esp. for different instruments
Injector Type • Autosampler (Fixed loop, flow through needle) • Manual injector	Y	Y	• Can affect extracolumn dispersion, esp. for different instruments • Fixed loop has lower dispersion than flow through needle type • Manual injectors often least dispersion

Parameter	I	G	Impact on Method Transfer
Column Heater • Forced air • Block heater (contact)	Y	Y	• Actual measured temperatures will vary among instruments. • Measured temperature may not match method set point.
Detector Type • VWD • Diode Array (DAD)	Y	Y	• Each detector brand and model can vary in performance within model and within brand. • Performance among brands can vary for noise, sensitivity, linearity
Flow Cell • Design • Volume • Temperature	Y	Y	• Flow cell design can affect dispersion significantly • Flow cell pathlength affects noise and signal. • Mismatch of column and detector temperatures increases noise.
Detector Settings • Data rate (pts/sec) • Response Time • Detection wavelength bandwidth • Reference wavelength	Y	Y	• Inadequate data rate causes poor peak fidelity, decreases observed efficiency, and increases tailing. • Bandwidth mismatch or use/absence of reference wavelength for DAD can cause peak area ratio changes and RI anomalies, respectively.

Guidance for Maximum Flow Rates for UHPLC Methods to be Transferred to HPLC with HALO

ACN/Water						
	ACN viscosities	1.01	0.91	0.75	0.62	0.52
ID (mm)	Length (mm)	25°C	30°C	40°C	50°C	60°C
3.0	20	3.12	3.46	4.20	5.00	5.00
3.0	30	2.26	2.51	3.05	3.69	4.40
3.0	50	1.46	1.62	1.97	2.38	2.84
3.0	75	1.01	1.12	1.36	1.65	2.00
3.0	100	0.78	0.86	1.04	1.26	1.50
3.0	150	0.53	0.59	0.71	0.86	1.00

MeOH/Water						
	MeOH viscosities	1.62	1.47	1.21	1.00	0.83
ID (mm)	Length (mm)	25°C	30°C	40°C	50°C	60°C
3.0	20	1.93	2.15	2.60	3.10	3.10
3.0	30	1.40	1.56	1.89	2.29	2.73
3.0	50	0.91	1.00	1.22	1.48	1.76
3.0	75	0.63	0.69	0.85	1.02	1.24
3.0	100	0.48	0.53	0.64	0.78	0.93
3.0	150	0.33	0.37	0.44	0.53	0.62

ACN/Water						
	ACN viscosities	1.01	0.91	0.75	0.62	0.52
ID (mm)	Length (mm)	25°C	30°C	40°C	50°C	60°C
2.1	20	1.53	1.70	2.06	2.45	2.45
2.1	30	1.11	1.23	1.49	1.81	2.16
2.1	50	0.72	0.79	0.97	1.17	1.39
2.1	75	0.49	0.55	0.67	0.81	0.98
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2.1	150	0.26	0.29	0.35	0.42	0.49

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ID (mm)	Length (mm)	25°C	30°C	40°C	50°C	60°C
2.1	20	0.95	1.05	1.28	1.52	1.52
2.1	30	0.69	0.76	0.93	1.12	1.34
2.1	50	0.44	0.49	0.60	0.72	0.86
2.1	75	0.31	0.34	0.41	0.50	0.61
2.1	100	0.24	0.26	0.32	0.38	0.46
2.1	150	0.16	0.18	0.22	0.26	0.30

- Calculations are approximate.
- Pressure for maximum flow rate set for 80% of 400 bar.
- Estimates made with 0.005” ID tubing in typical length (~60-70 cm) to account for system pressure (not including flow cell and autosampler)

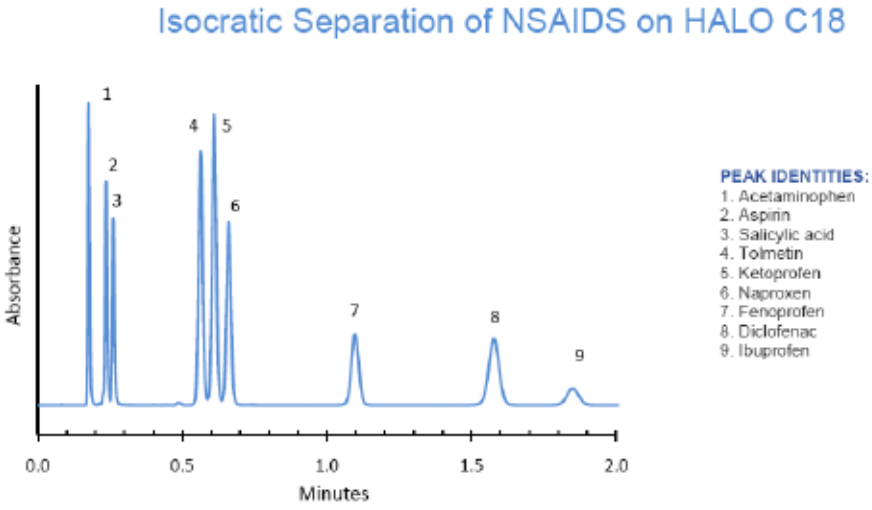
Isocratic Method Transfer Example

Transfer method to Agilent 1100 and 1200SL Systems

Original Shimadzu Inst. Method

Column:	4.6 x 50 mm, HALO C18
Mobile Phase:	43:57 A / B
Mobile Phase A:	0.020M sodium phosphate (pH=2.5)
Mobile Phase B:	50:50 MeOH / ACN premix
Flow Rate:	3.0 mL / min.
Pressure:	338 Bar
Temperature:	35°C
Detection:	UV 254 nm, VWD
Injection Volume:	2.0 µL
Sample Solvent:	50:50 MeOH / water
Data Rate:	50 Hz.
Response Time:	0.02 sec.
Flow Cell:	2.5 µL semi-micro
LC System:	Shimadzu Prominence UFLC XR
Pressure maximum:	600 bar

Application Note: 13-NS



For isocratic separation, flow rate and injection volume are scaled with ratio of column IDs squared and by volume ratio, respectively

$$F_{col2} = F_{col1} \cdot \left(\frac{ID_{col2}}{ID_{col1}} \right)^2$$

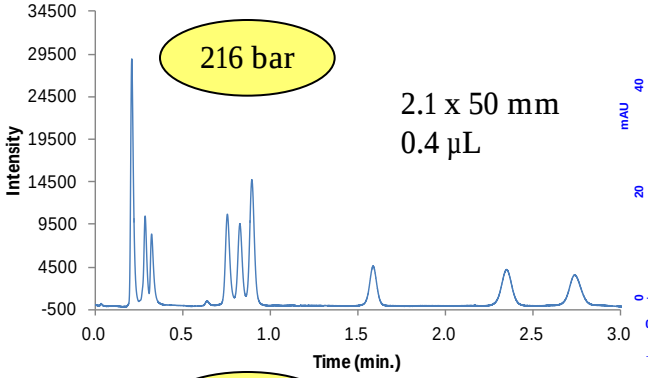
$$\left(V_{inj}\right)_{col2} = \left(V_{inj}\right)_{col1} \cdot \left(\frac{ID_{col2}}{ID_{col1}} \right)^2 \cdot \frac{L_{col2}}{L_{col1}}$$

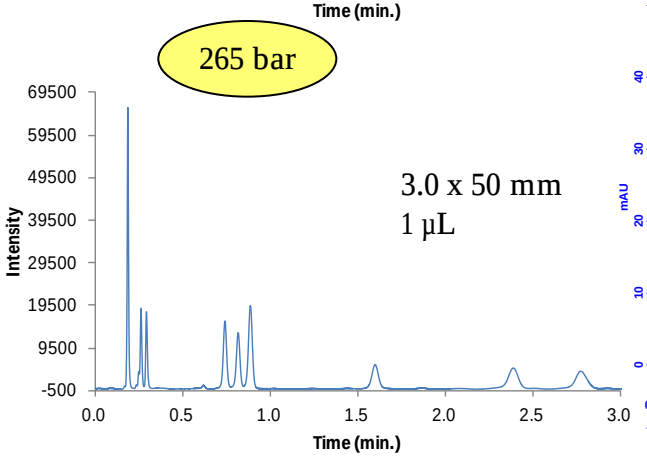
Instruments, Configurations and Method Parameter Settings

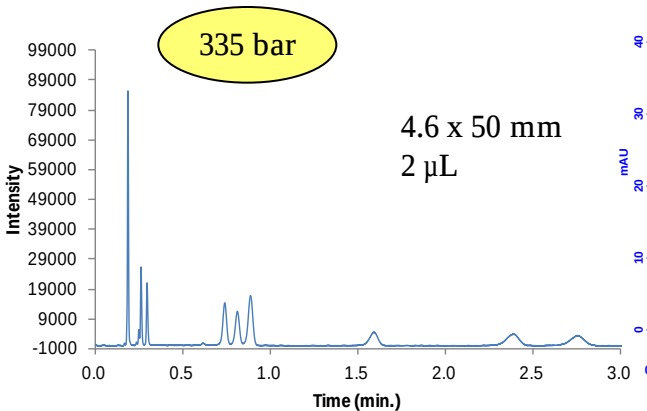
Parameter	Agilent 1100	Agilent 1200	Shimadzu Prominence
Pressure Limit (bar)	400	600	600
Column Heater Type	Block	Block	Forced Air
Detector Type	VWD	DAD	VWD
Flow Cell Volume (µL)	5	2	2.5
Flow Cell Path length (mm)	10	6	5
Min. Response Time (sec.)	0.062	0.02	0.02
Maximum Data Rate	13.7 Hz	80 Hz (40 Hz used)	50 Hz

Isocratic Method Transfer from Shimadzu to 1100 and 1200

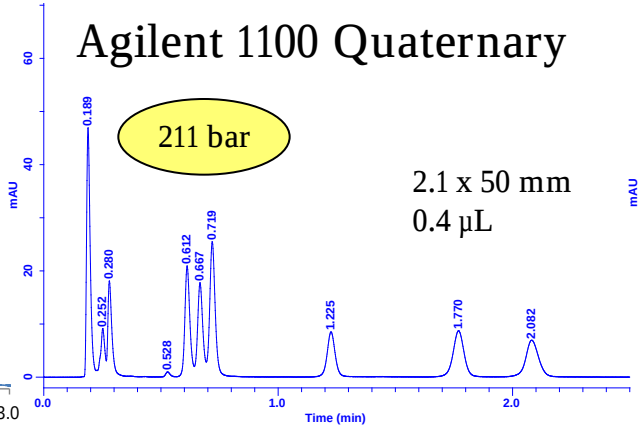
Shimadzu Prominence

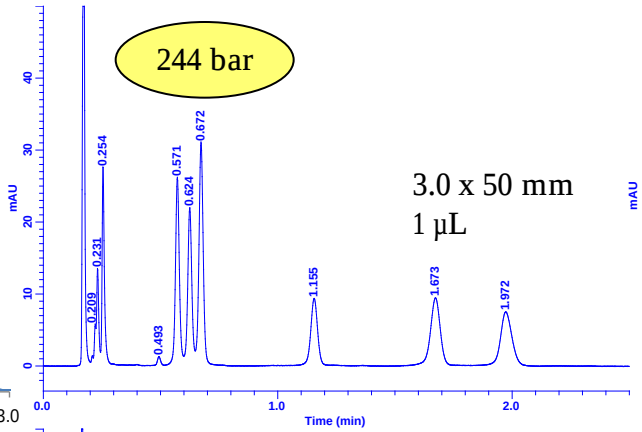


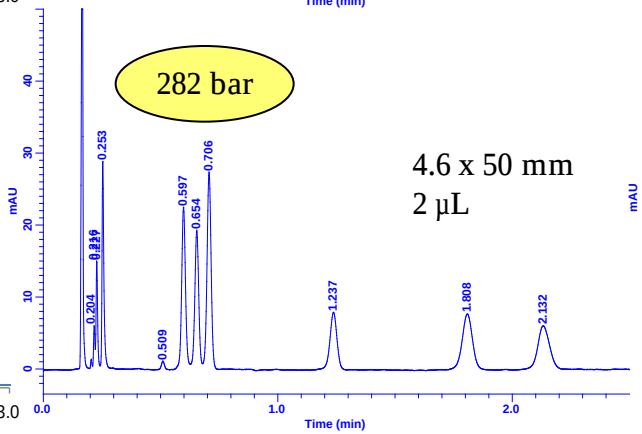




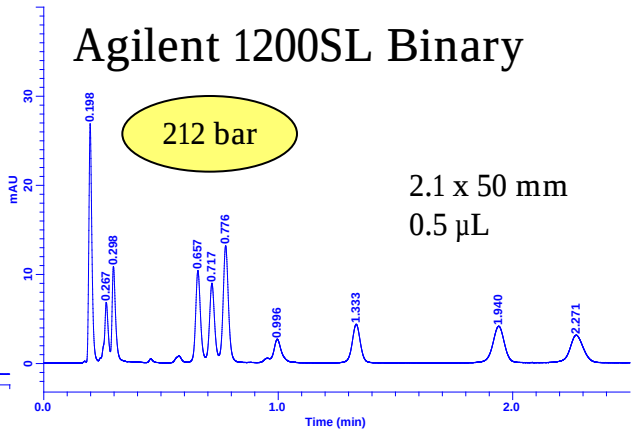
Agilent 1100 Quaternary

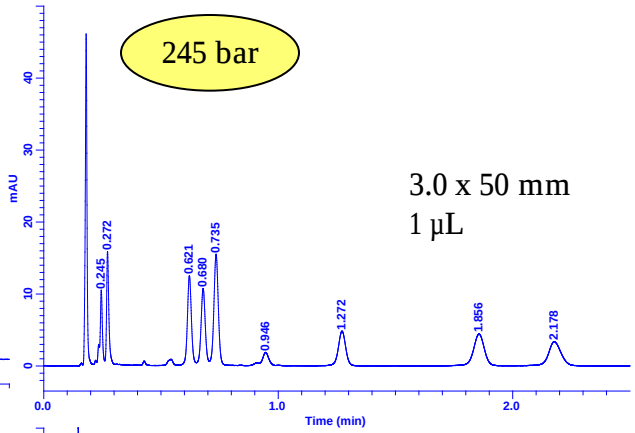


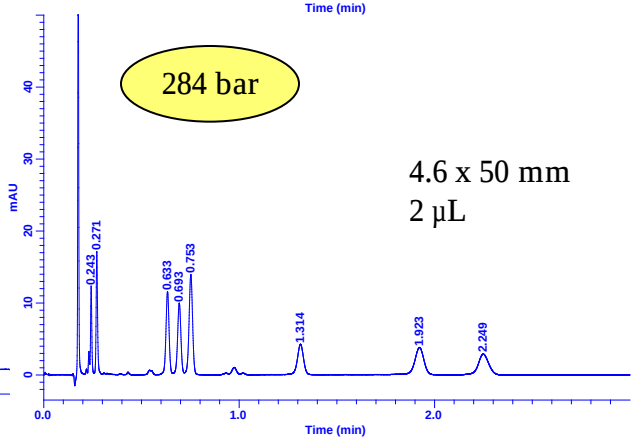




Agilent 1200SL Binary







Results for Isocratic Method Transfer from Shimadzu to Agilent 1100 and 1200

Results

- Resolution results for respective peak pairs ranged from 91-117% for the Agilent 1100, and from 95-107% for the Agilent 1200.
- Theoretical plate counts increased with k for respective analytes as expected, up to ~k = 8-9
- Plate counts increased slightly with increasing ID (4.6 > 3.0 > 2.1).

Observations

- DAD reference signal had to be turned off to avoid RI disturbance near start of run.
- Data rate for Agilent 1200 was set at 40 Hz to more closely match 50 Hz rate of Shimadzu.
- Flow cell path length differences among instruments caused peak areas and heights to vary.
- Analytical wavelength bandwidth and slit width on Agilent 1200 DAD had to be adjusted lower to give comparable peak areas.

Gradient Method Transfer Example

Transfer method to Agilent 1100 and Shimadzu Prominence Systems

Original Agilent 1200 Inst. Method

Column:	2.1 x 50 mm, HALO C18
Mobile Phase A:	water with 0.1% HCOOH
Mobile Phase B:	ACN with 0.1% HCOOH
Gradient:	3% ACN to 70% ACN in 2.7min.
Flow Rate:	0.42 mL / min.
Max. Pressure:	116 Bar
Temperature:	45°C
Detection:	DAD 275 nm, Bandwidth, 8
Injection Volume:	2.0 µL
Sample Solvent:	50:50 MeOH / water
Data Rate:	40 Hz.
Response Time:	0.02 sec.
Flow Cell:	2 µL micro flow cell
Pressure max.:	600 bar

- Standard mixer was removed
- All tubing was minimal length and 0.005” ID
- Automatic delay volume reduction was turned ON.

Flow rates and injection volumes are scaled as with isocratic methods.

$$F_{col2} = F_{col1} \cdot \left(\frac{ID_{col2}}{ID_{col1}} \right)^2$$

$$\left(V_{inj}\right)_{col2} = \left(V_{inj}\right)_{col1} \cdot \left(\frac{ID_{col2}}{ID_{col1}} \right)^2 \cdot \frac{L_{col2}}{L_{col1}}$$

Delay volumes of each instrument should be measured, and, if available, injector delay should be used to correct the effective delay volume to that of the original method.

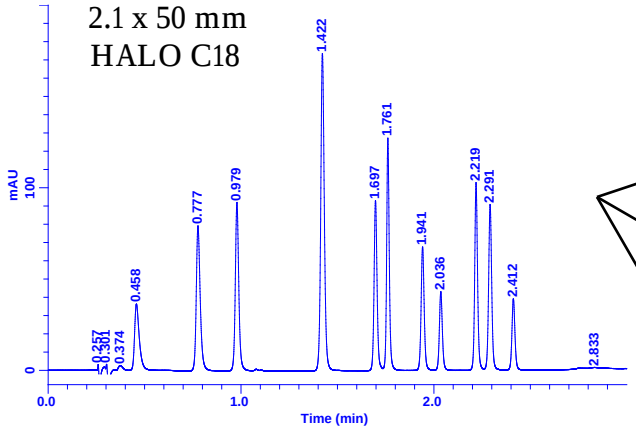
$$T_{injdelay} = \frac{V_{D(HPLC)} - V_{D(UHPLC)}}{F}$$

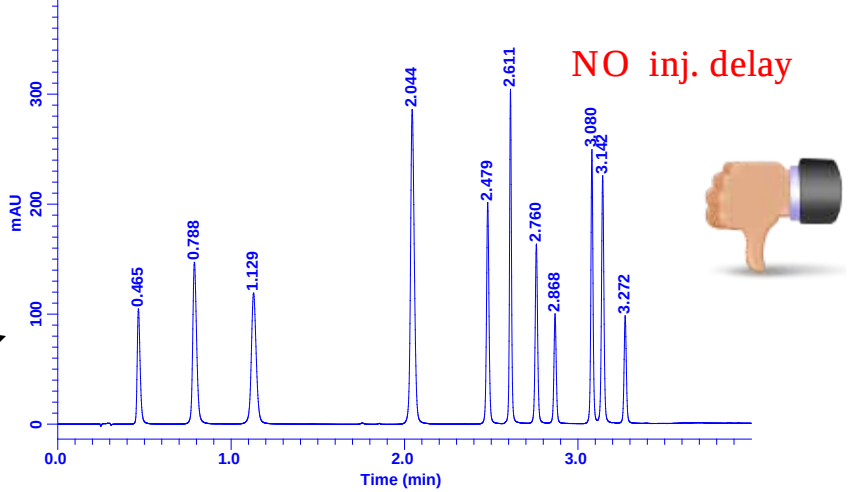
An injector program can be set on some instruments so that the gradient starts, and the sample is injected after a defined time delay, $T_{injdelay}$

Advantages of Injection Delay

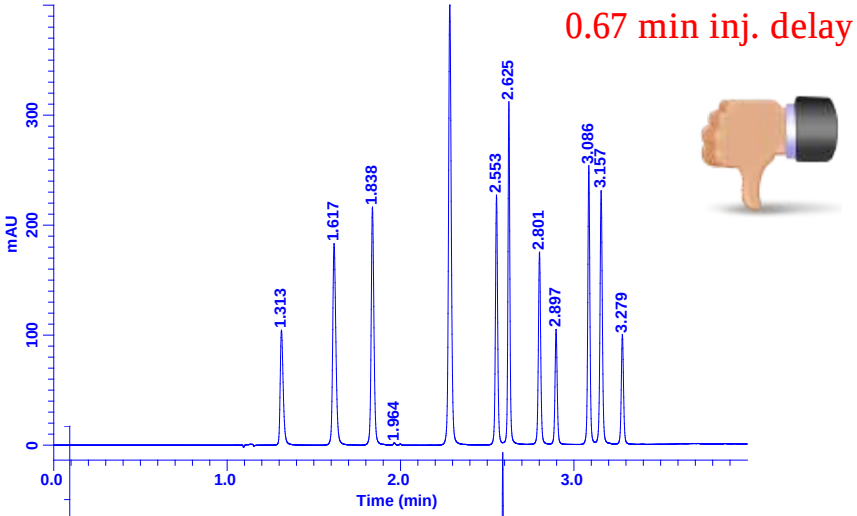
Agilent 1200SL Binary

2.1 x 50 mm
HALO C18



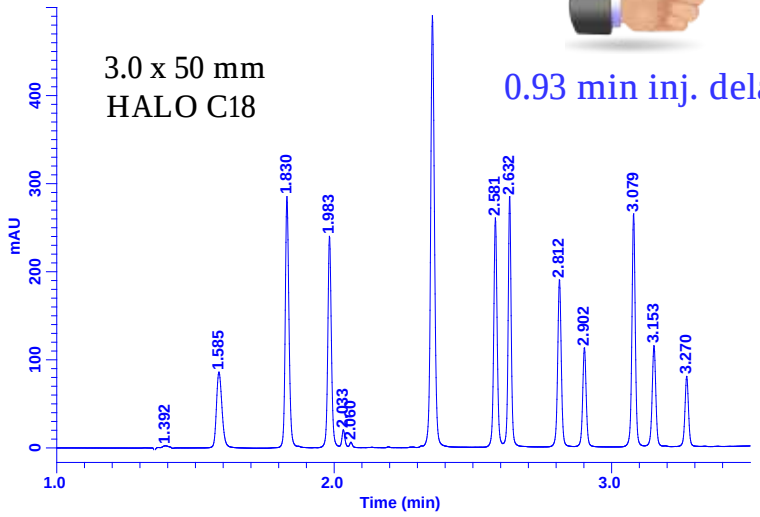


NO inj. delay

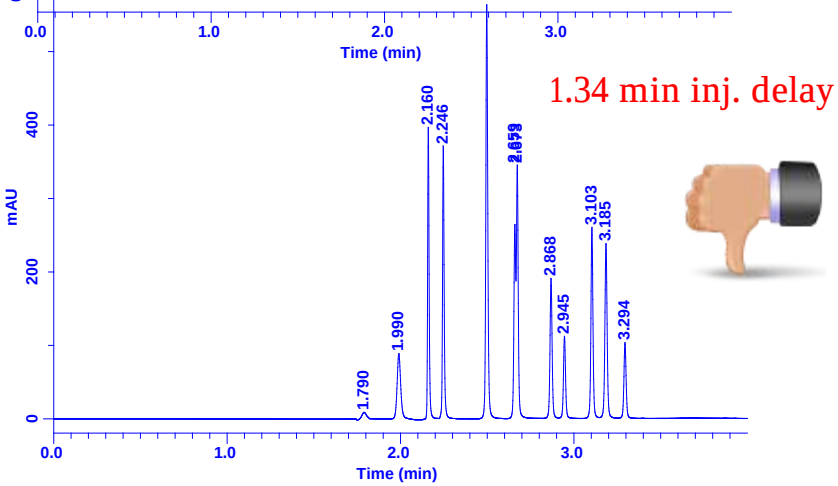


0.67 min inj. delay

Agilent 1100 Quaternary

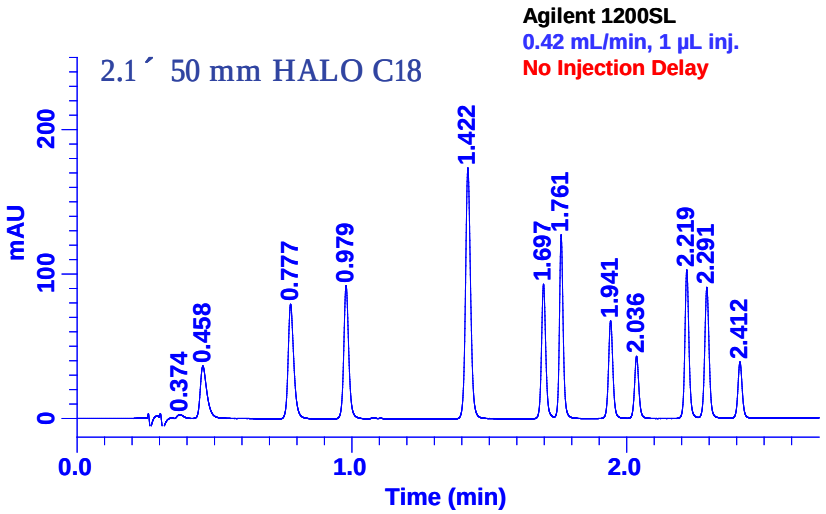


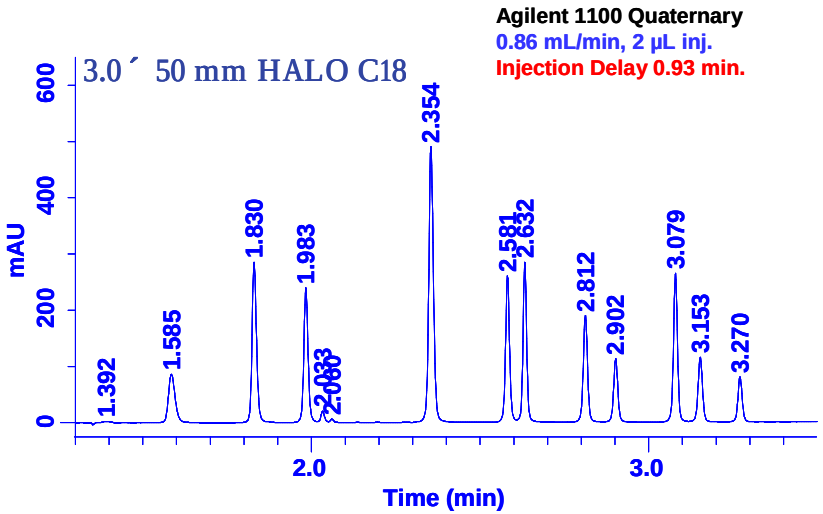
0.93 min inj. delay

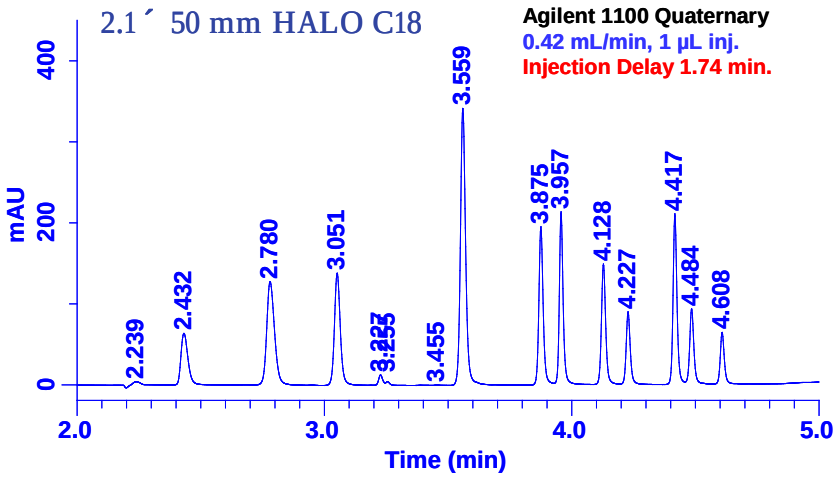


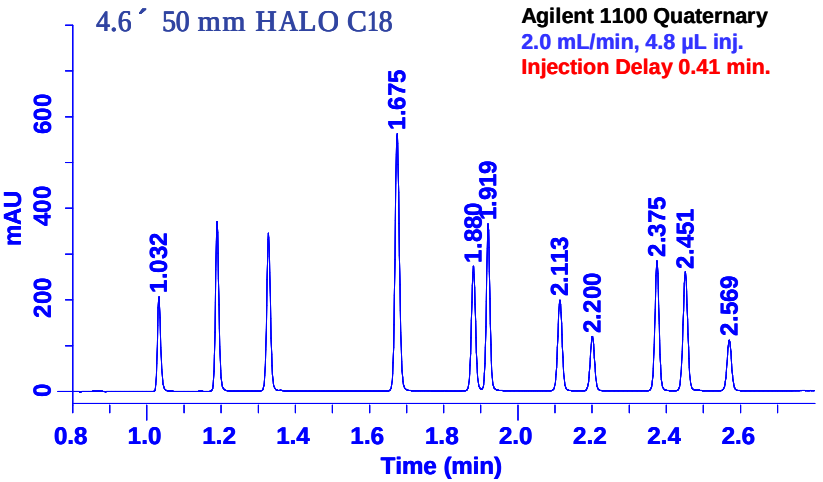
1.34 min inj. delay

Gradient Transfer to Agilent 1100 with Different HALO Column IDs



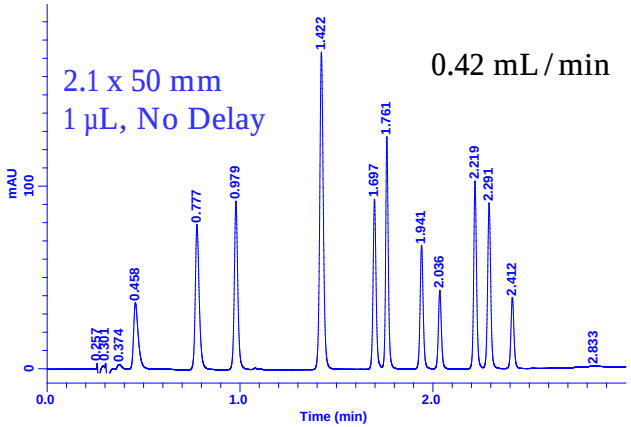




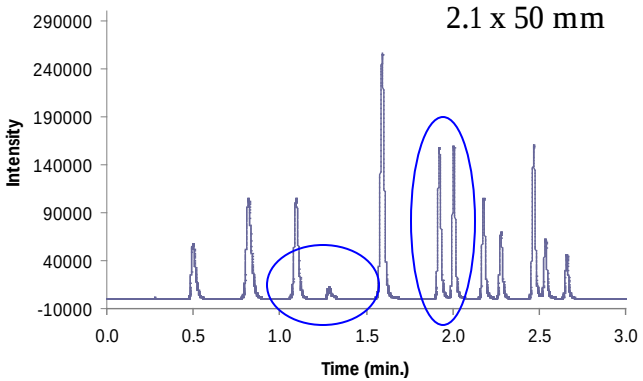


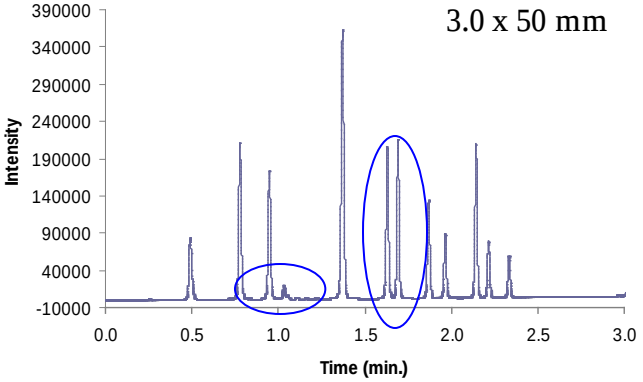
Peak identities (in order): hydroquinone, resorcinol, catechol, phenol, 4-nitrophenol, 4,4'-biphenol, 2-chlorophenol, 4-chlorophenol, 2,2'-biphenol, 2,6 –dichlorophenol, 2,4-dichlorophenol

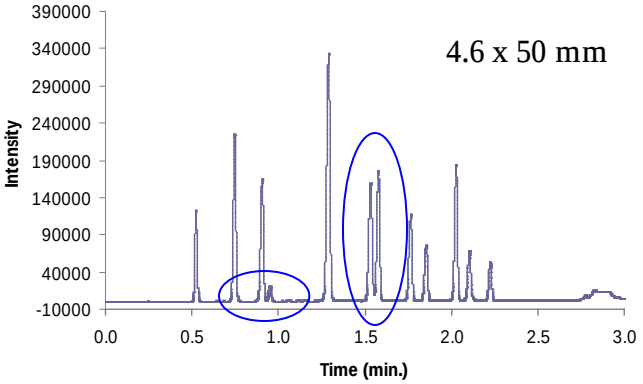
Gradient Method Comparison Chromatograms
Agilent 1200 vs. Shimadzu Prominence



Without use of injection delay on Shimadzu system changes in resolution occur.







Results for Gradient Method Transfer from Agilent 1200 to Agilent 1100 and Shimadzu

Results

- Resolution results for respective peak pairs ranged from 97-120% for the Agilent 1100, and from 81-108% for the Shimadzu Prominence.
- Injector program feature of Agilent 1100 system and software allowed for adjustment of effective delay volume using injection delay.
- Demonstrated changes in selectivity and resolution on Agilent 1100 with different injection delay times.
- Gradients run on Shimadzu system w/o injection delay showed major changes in selectivity.

Observations

- Flow cell volume and path length differences among instruments caused peak areas and heights to vary.
- Analytical wavelength bandwidth and slit width on Agilent 1200 DAD had to be adjusted lower to give comparable peak areas.

How Should One Measure Method Transfer Success?

- Comparable peak shape, efficiency, selectivity, and resolution
- Comparable injection-to-injection repeatability
- Comparable S/N Ratio (impact on LOD and LOQ, linearity, UV spectral quality, etc.)
- Comparable accuracy and precision and other figures of merit
- Not necessarily identical retention times!

Recommendations for Method Transfer from UHPLC to HPLC with HALO Columns:

UHPLC		HPLC	
Max. Pressure:	600-1200 bar	Max. Pressure:	400 bar
Extracolumn Volume:	~7–15 µL	Extracolumn Volume:	~10–20 µL optimized, 35–55 µL "as-is"
Band Spreading:	~5–10 μL^2	Band Spreading:	~5–10 μL^2 optimized, 40–100 μL^2 "as-is"

HALO 2.1 mm × L	→	HALO 2.1 mm × L
HALO 2.1 mm × L	→	HALO 3.0 mm × L
HALO 3.0 mm × L	→	HALO 3.0 mm × L
HALO 3.0 mm × L	→	HALO 4.6 mm × L

- Minimize ECV by using minimal length of 0.005" ID tubing in sample flow path.
- Use Flow Cell with volume of 1–5 µL
- Set Detector Time Constant or Response Time[1] to fastest setting
- Set Data Rate[3] 10 Hz
- For gradient separations, adjust column equilibration time considering larger column volume, larger HPLC delay volume, and higher flow rate with larger ID column.
- If necessary, for gradient methods, program an injection delay to reduce "effective delay volume" of the HPLC system to match the delay volume of the UHPLC system.

Note: Response Time = 2.2 × Time Constant

Summary and Conclusions

- Discussed important parameters for transfer of an isocratic and a gradient method using HALO columns with 3 different IDs in same length
- Presented results from method transfer experiments, and discussed issues that can arise.
- Provided guidance for successful transfer of methods from UHPLC to HPLC using HALO Fused-Core columns.