Practical UHPLC:

Selectivity and Rapid Method Development, Method Translations & Instrument Transfers

Gemma Lo

Contents

- How can UHPLC help us?
- Ultra resolution separations
- UHPLC practical hints and tips
- Maximising selectivity for UHPLC method development
- Translating methods to UHPLC
- Transferring UHPLC methods between instruments

How Can UHPLC Help Us?

- UHPLC with small particles (sub-2 micron) provide higher performance than HPLC (**but with higher backpressure**)

- Column efficiency is inversely proportional to particle size (d_P)

$$N \propto \frac{1}{d_P}$$

- Therefore, decreasing the particle size by 3x increases efficiency by 3x e.g. by changing from 5 µm to 1.7 µm

- We can also choose to reduce column length by 1/3 to achieve around the same efficiency (N α L)...this means run time will decrease by a factor of 3 (at constant mobile phase linear velocity).

150 mm, 5 µm	≈	50 mm, 1.7 µm
15 mins.	→	5 mins

How Can UHPLC Help Us?

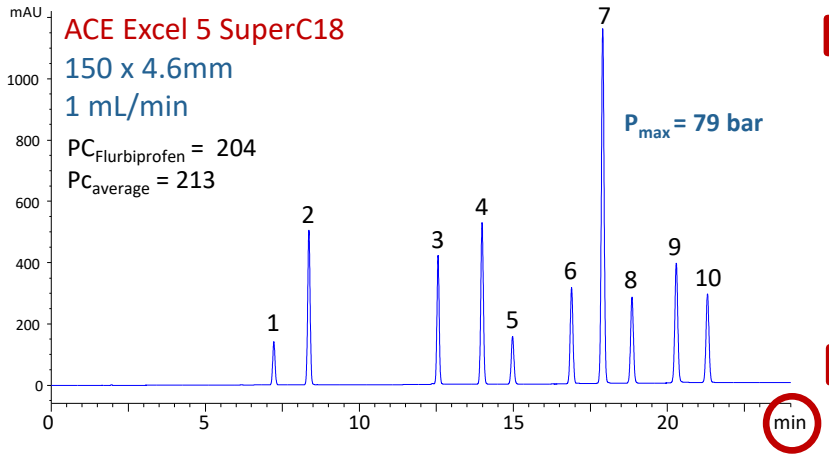
- UHPLC offers several key potential benefits over HPLC:
  - 1. Speed of analysis
  - 2. Higher resolution
  - 3. ‘Ultra-high’ resolution separations (long columns)
  - 4. Increased Productivity

1 & 2. Speed and Higher resolution

- Speed / Resolution

$$P_x = 1 + \frac{t_G}{W_x}$$

$$P_{Average} = 1 + \frac{t_G}{\frac{1}{10}\sum_1^{10} W_p}$$



ACE Excel 5 SuperC18
150 x 4.6mm
1 mL/min

$PC_{Flurbiprofen}$ = 204
$Pc_{average}$ = 213

P_{max} = 79 bar

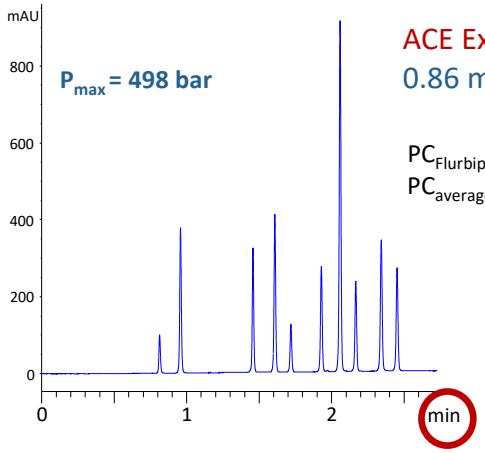
Resolution Values

Peak Pair	150 x 4.6	50 x 3.0	100 x 3.0
1,2	7.8	7.5	11.1
2,3	29.6	26.6	37.4
3,4	9.8	7.9	9.9
4,5	6.2	5.4	6.9
5,6	11.7	10.0	12.7
6,7	6.1	6.0	8.7
7,8	5.6	5.1	6.6
8,9	8.2	8.0	10.3
9,10	5.7	4.9	7.3

Speed

- >> speed
- ≈ resolution

50 x 3.0mm



P_{max} = 498 bar

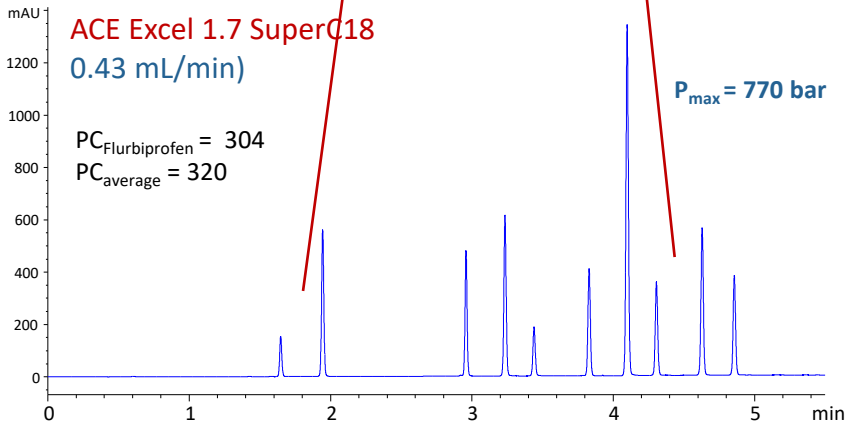
ACE Excel 1.7 SuperC18
0.86 mL/min)

$PC_{Flurbiprofen}$ = 183
$PC_{average}$ = 187

Resolution

- > speed
- >> resolution

100 x 3.0mm

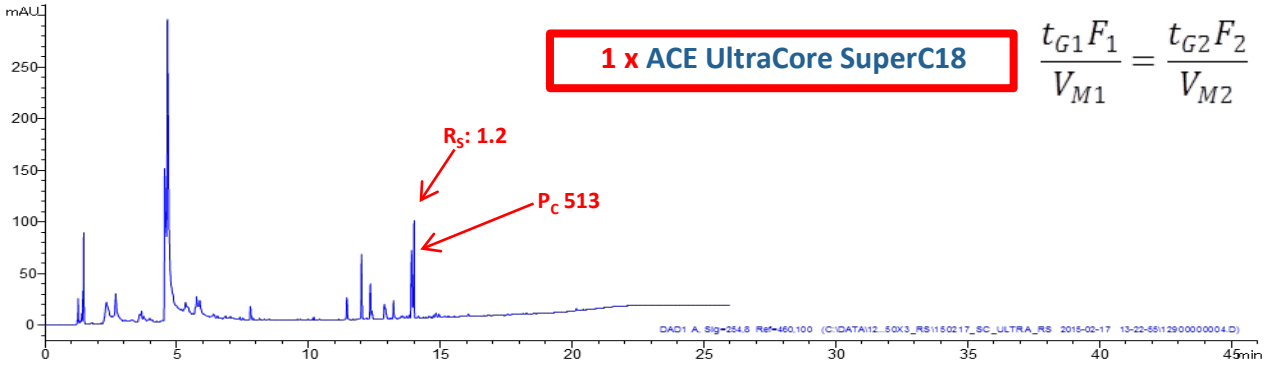


ACE Excel 1.7 SuperC18
0.43 mL/min)

$PC_{Flurbiprofen}$ = 304
$PC_{average}$ = 320

P_{max} = 770 bar

3. Ultra-High Resolution: Echinacea; Longer Column



1 x ACE UltraCore SuperC18

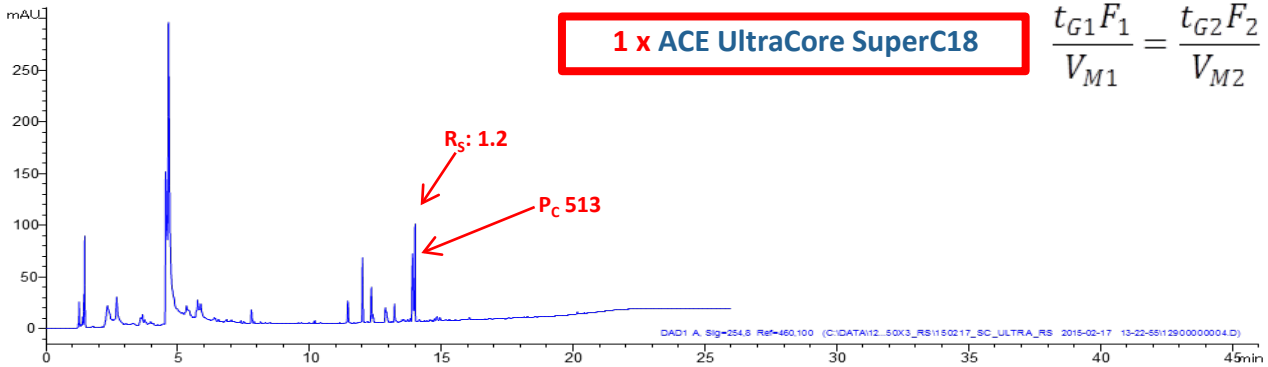
$$\frac{t_{G1}F_1}{V_{M1}} = \frac{t_{G2}F_2}{V_{M2}} = k^*$$

Column	150 x 3.0 mm
t_G	20 min
Flow:	0.43 mL/min
Inj. Vol.	5 µL
P_{MAX}	130 bar

3. Ultra-High Resolution: Echinacea; Longer Column

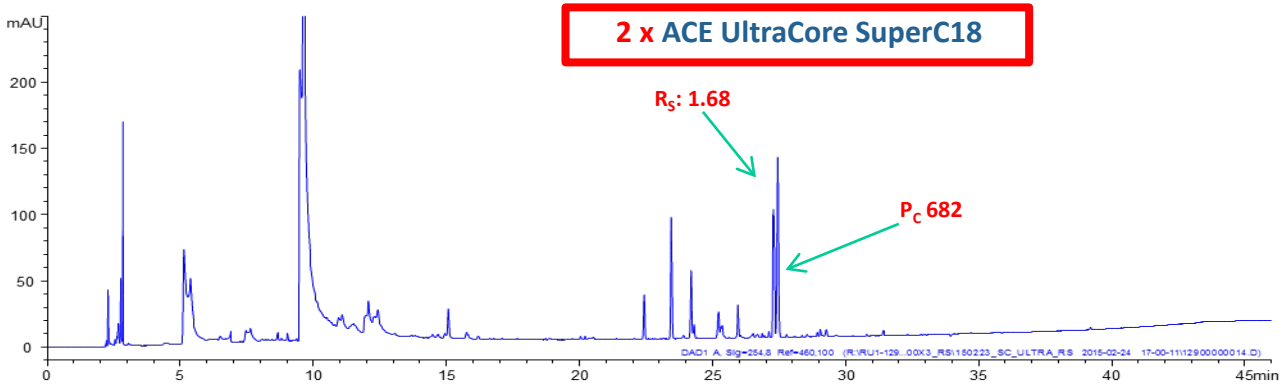
1 x ACE UltraCore SuperC18

$$\frac{t_{G1}F_1}{V_{M1}} = \frac{t_{G2}F_2}{V_{M2}} = k^*$$



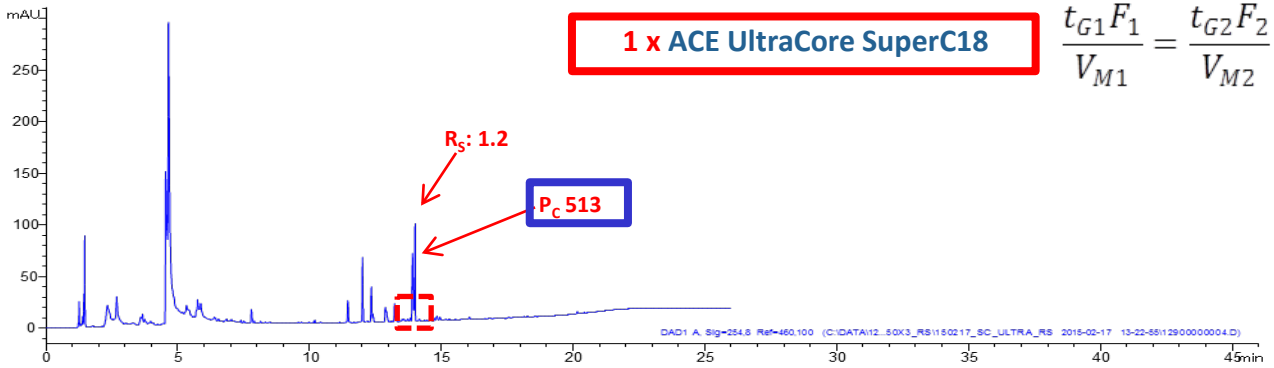
Column	150 x 3.0 mm
t_G	20 min
Flow:	0.43 mL/min
Inj. Vol.	5 µL
P_{MAX}	130 bar

2 x ACE UltraCore SuperC18



Column	300 x 3.0 mm
t_G	40 min
Flow:	0.43 mL/min
Inj. Vol.	10 µL
P_{MAX}	223 bar

3. Ultra-High Resolution: Echinacea; Longer Column



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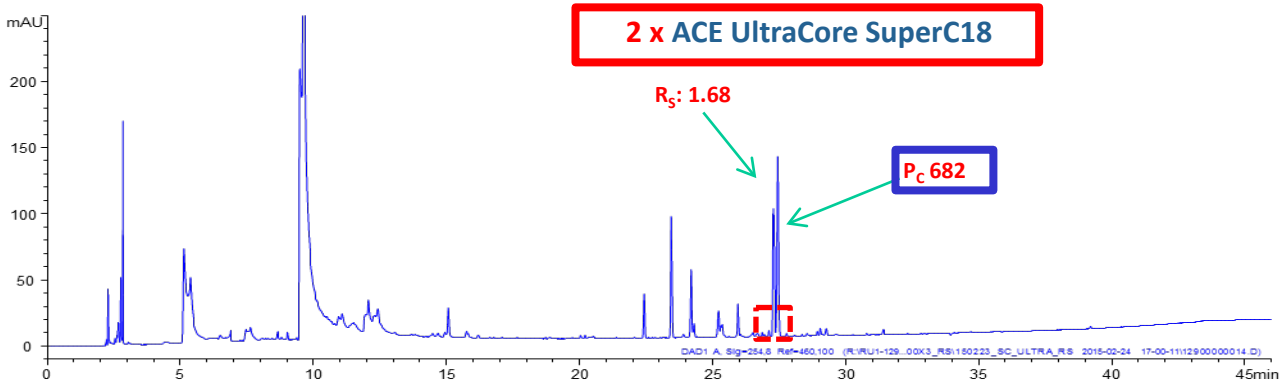
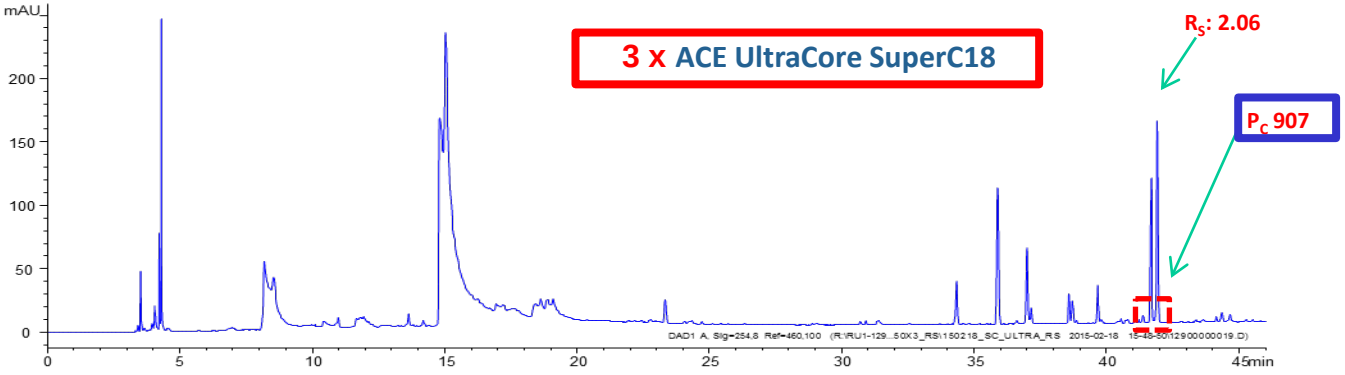
t_G	20 min
Flow:	0.43 mL/min
Inj. Vol.	5 µL
PMAX	130 bar

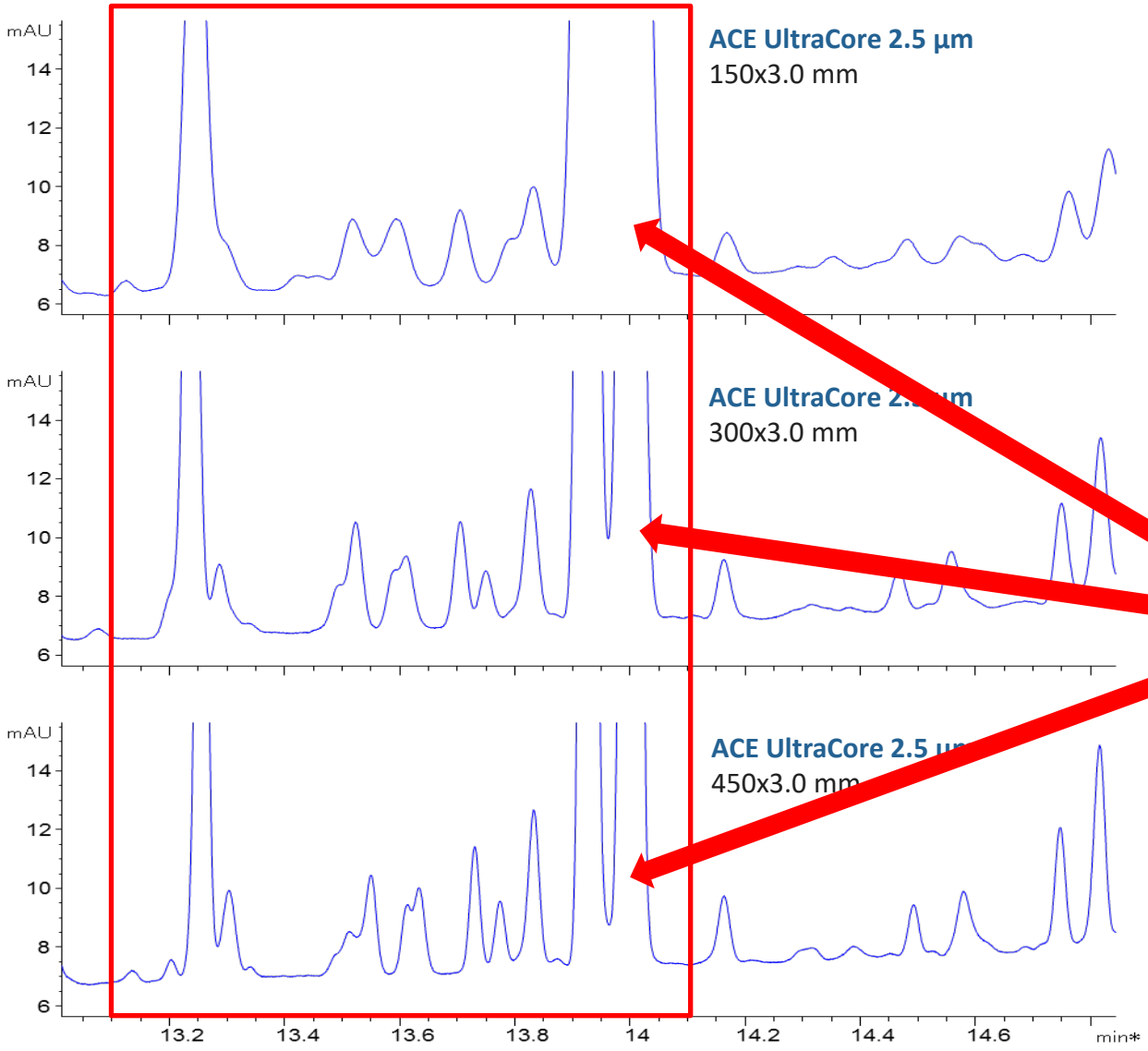


Column	300 x 3.0 mm
t_G	40 min
Flow:	0.43 mL/min
Inj. Vol.	10 µL
PMAX	223 bar



Column	450 x 3.0 mm
t_G	59.96 min
Flow:	0.43 mL/min
Inj. Vol.	15 µL
PMAX	338 bar

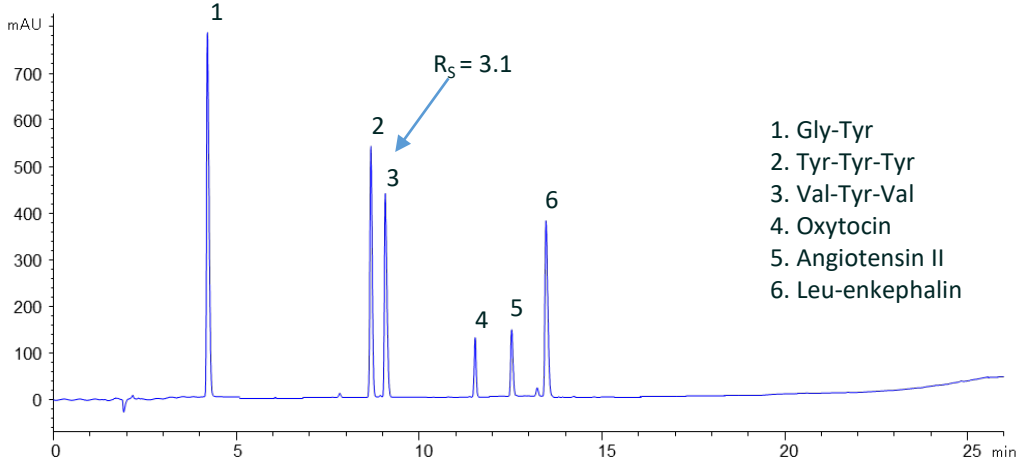
3. Ultra-High Resolution: Echinacea; Aligned Data (ZOOM IN)



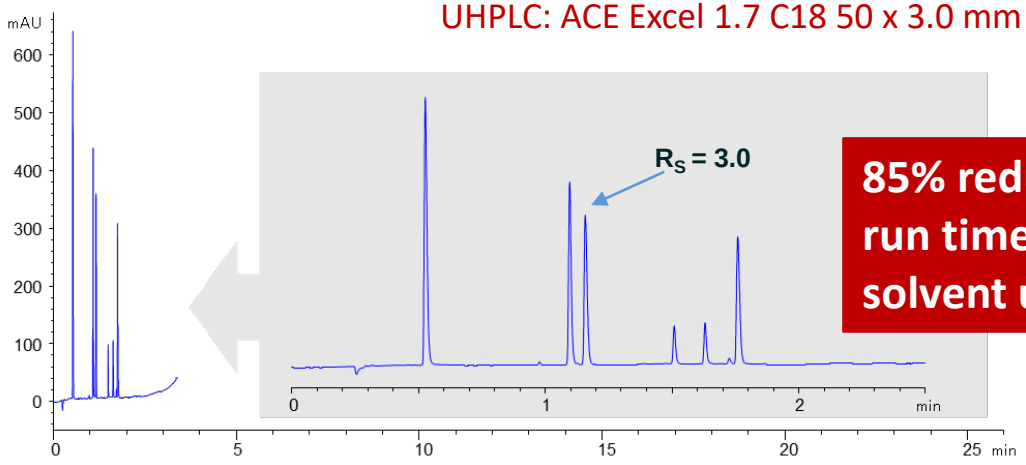
Improved resolution

4. Increased Productivity

HPLC: ACE Excel 5 C18 150 x 4.6 mm



UHPLC: ACE Excel 1.7 C18 50 x 3.0 mm



- Improve productivity by translating existing HPLC methods to UHPLC.
- The ACE LC Translator spreadsheet is available as a free download to help achieve method translation.

85% reduction in run time and solvent use!

Download free at: MAC-MOD.com

Mobile Phase: A: 0.05% TFA in H_2O, B: 0.05% TFA in MeCN, Temperature: 60 °C, Detection: UV, 220 nm
HPLC conditions: 1.00 mL/min, 4-40 %B in 17 minutes, then 40-90 %B in 6 minutes
UHPLC conditions: 1.00 mL/min, 4-40 %B in 2.41 minutes, then 40-90 %B in 0.85 minutes

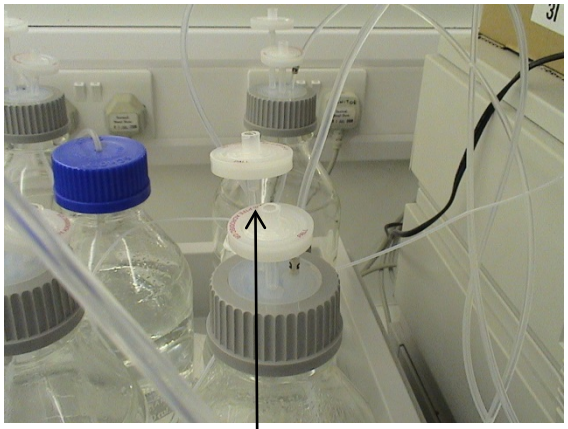
UHPLC Practical Hints & Tips

UHPLC Hints & Tips: Filtering Mobile phase

- General good practice: replace mobile phases regularly to prevent bacterial growth.
- Filter mobile phases through a 0.2 µm membrane filter prior to use
  - Removes damaging microparticulates and bacterial contaminants
- Nylon filters: aqueous solvents
- PTFE (Teflon): organic solvents
- Universal filters also available
- Use high quality solvents, particluarly for LC-MS.

Solvents / Buffer Solutions on Instruments

- Ensure you seal mobile phase reservoirs on the UHPLC instrument
- This prevents dirt / dust and microbial contamination



0.45 µm syringe filter fitted to solvent bottle cap

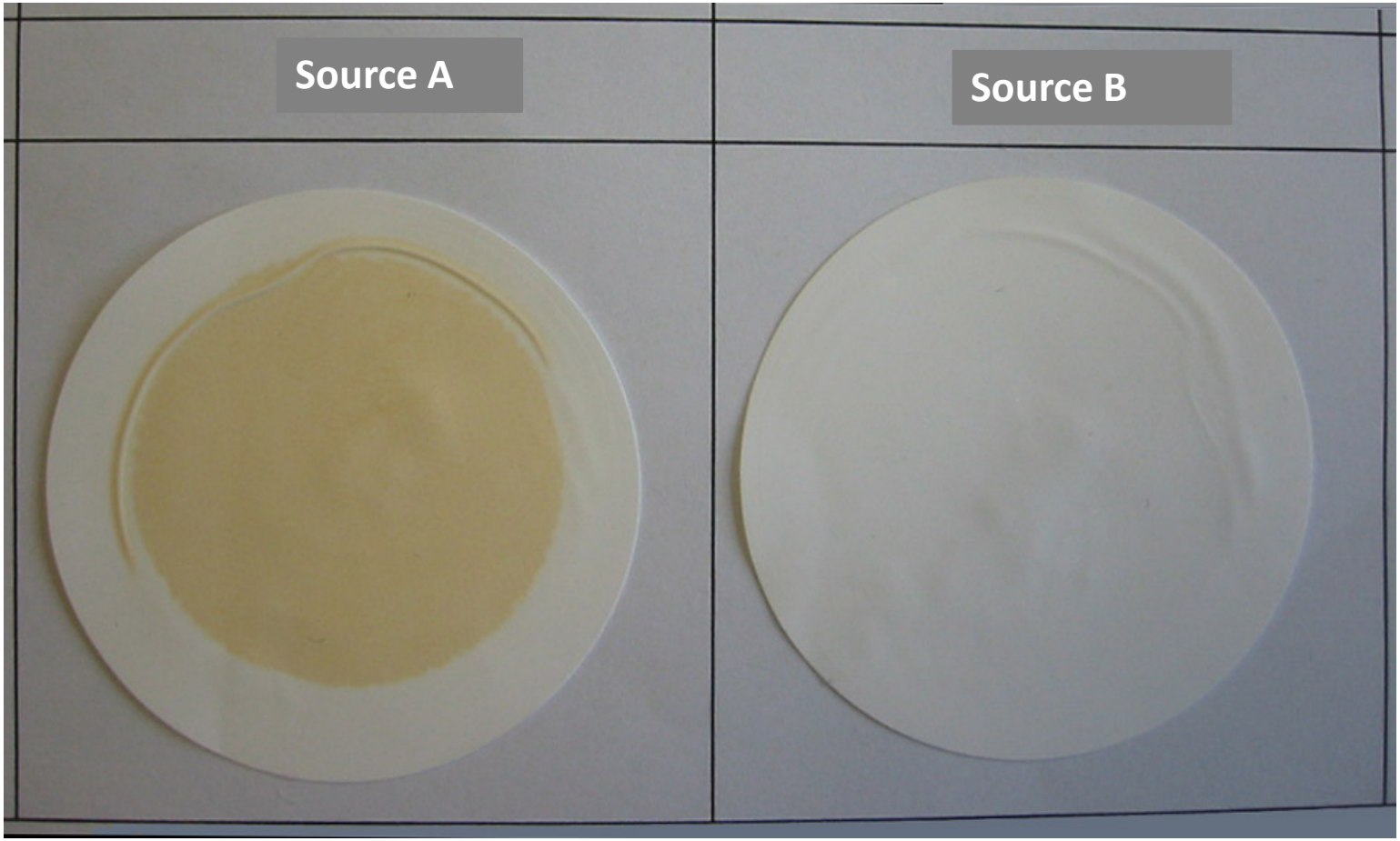
S.C.A.T. SafetyCaps on solvent bottles

S.C.A.T. SafetyWasteCaps on waste bottles

Particularly if 100% aqueous is used on a solvent line

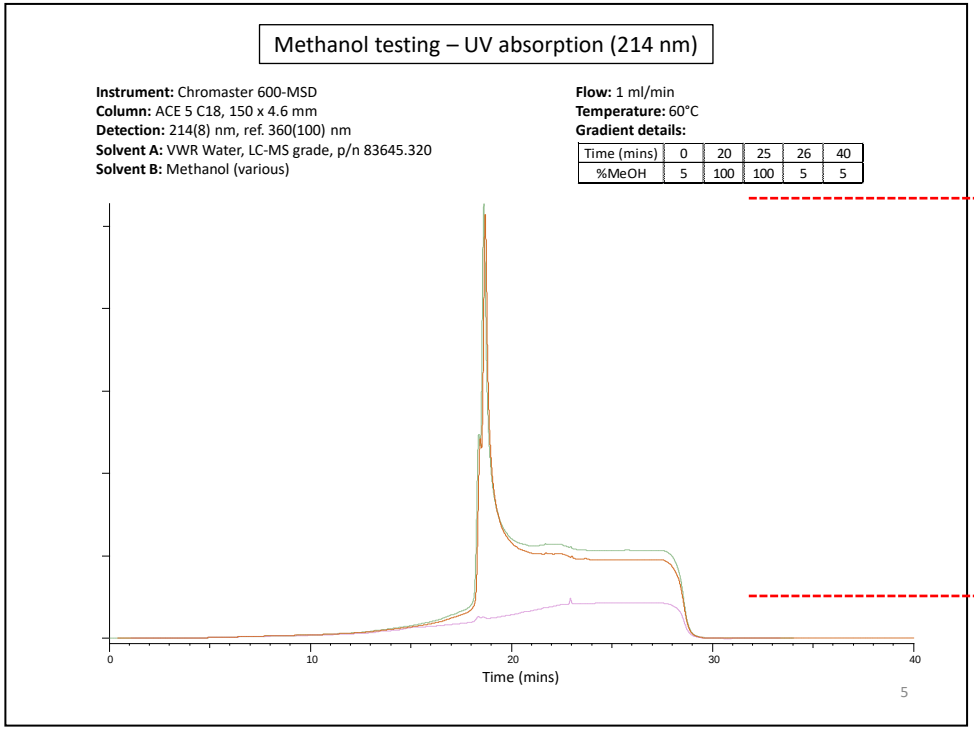
Not all HPLC water is good!

Bottled HPLC grade water!



Organic Solvents LC-UV

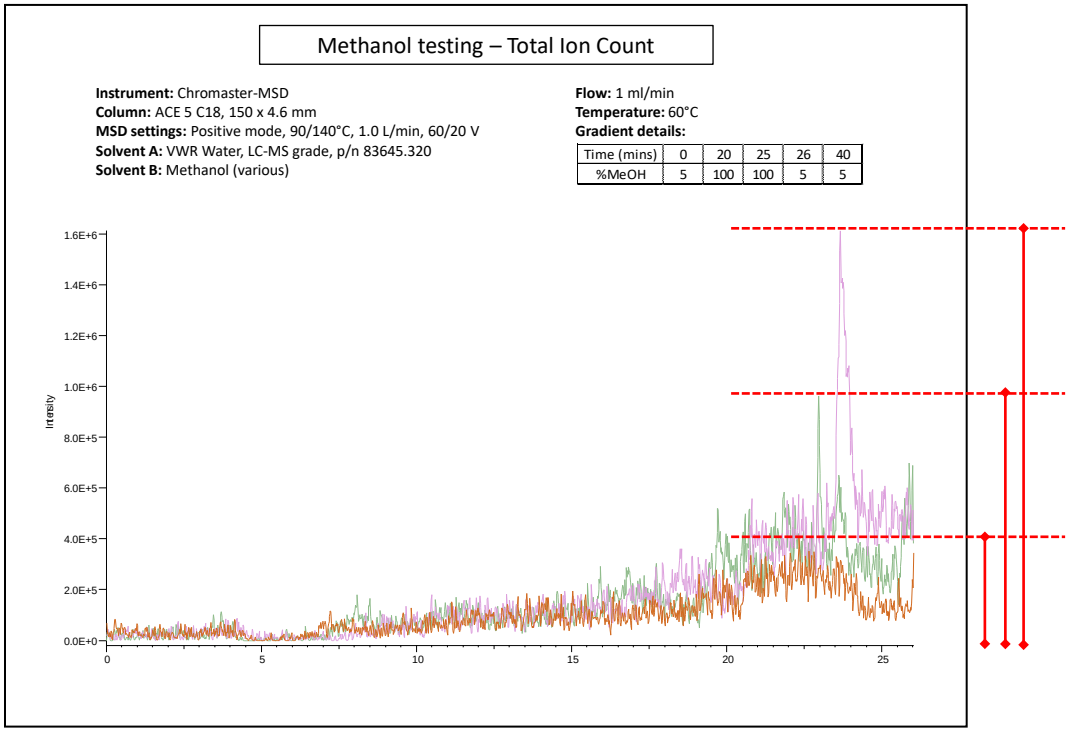
- Always use the highest quality organic solvents you can afford…



Especially for UHPLC, gradient analyses and LC-MS work

Organic Solvents LC-MS

- Always use the highest quality organic solvents you can afford…



Especially for UHPLC, gradient analyses and LC-MS work

UHPLC Method Development: Using Selectivity

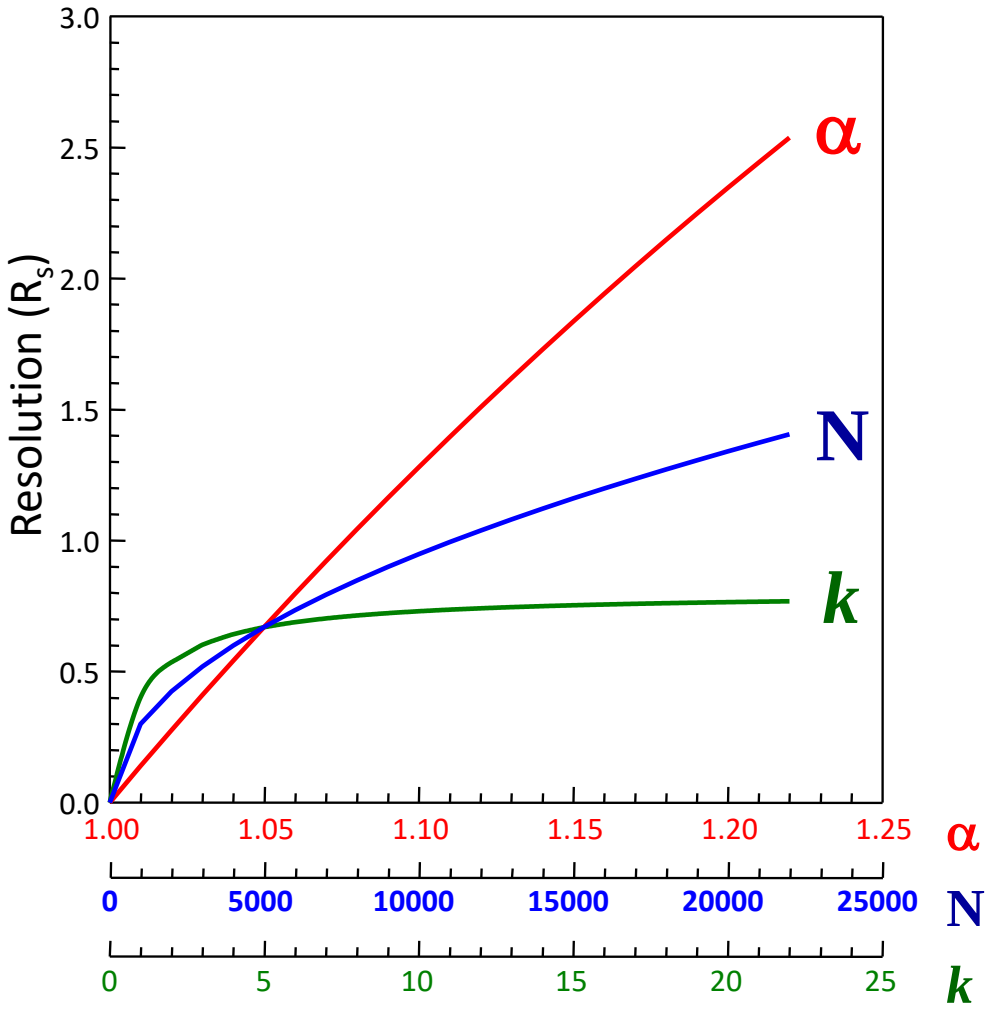
The Power of Selectivity

The Resolution Equation

Efficiency Selectivity Retention

$$R_s = \frac{\sqrt{N}}{4} \bullet \frac{\alpha\text{-}1}{\alpha} \bullet \frac{k}{1+k}$$

Selectivity is the key to analyte resolution with efficiency boosting performance.



Zhao, J.H. and P.W. Carr. Analytical Chemistry, (1999) 71, 2623-2632

Which Factors[a] Affect Selectivity?

Isocratic Separations

- Column stationary phase
- Organic Modifier
- pH (ionised analytes only)
- % Organic modifier
- Buffer selection
- Column temperature
- Buffer concentration

MOST Influence

LEAST Influence

Gradient Separations

- All parameters for isocratic
- Gradient steepness
- k^* (t_G, F, V_m, ΔΦ, Mw)

$$k^* = \frac{0.87 t_G F}{\Delta \Phi V_m M}$$

- Dwell volume
- Column dimensions

- Stationary phase chemistry: one of the most powerful parameters.
- ACE novel phases have been specifically designed to offer complementary selectivity – great for method development

a Adapted from 'Introduction to Modern Liquid Chromatography", 3rd Edition, Snyder, Kirkland, Dolan, 2010, p.29, Wiley & sons

ACE Novel Reversed Phase Columns

- The ACE range contains a wide range of stationary phases:
  - Traditional phases (C18, C8, C4, CN, Phenyl)
  - Novel phases (C18-AR, C18-PFP, C18-Amide, CN-ES, SuperC18)

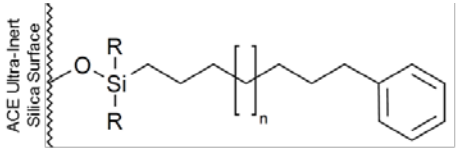
- The ACE novel stationary phases have been specifically designed to provide different retention mechanisms and therefore **different selectivity**.

- These phases are a powerful method development tool and can provide separations that may not be achievable using a traditional C18.

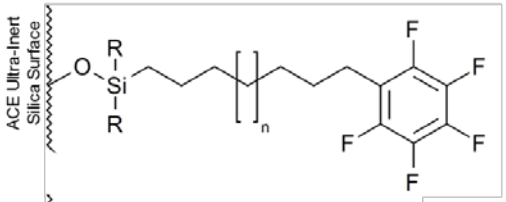
- Available in 1.7, 2, 3, 5 and 10 µm HPLC / UHPLC format.

ACE Novel Phases

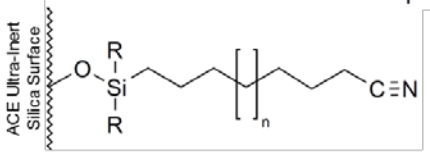
- ACE novel chemistries: C18 like retention, reproducibility and stability.



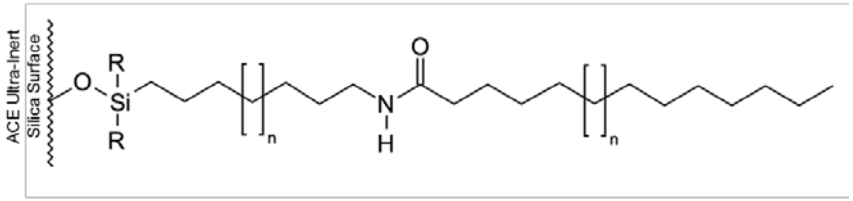
ACE C18-AR (hydrophobic and phenyl interactions)



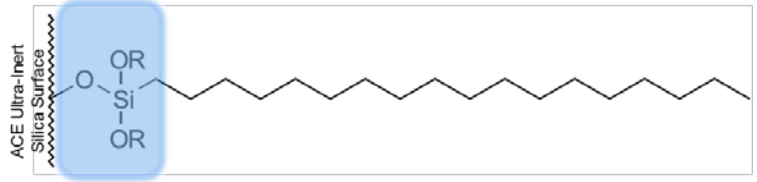
ACE C18-PFP (hydrophobic and PFP interactions)



ACE CN-ES (hydrophobic and polar interactions)



ACE C18-Amide (embedded amide group to increase retention of polar components and alternative selectivity).



Proprietary Encapsulated Bonding Technology

ACE SuperC18 (high retentivity with extended pH stability).

ACE® Stationary Phases: Key Mechanisms of Interactions

- Tanaka[1] characterisation can help understand mechanisms and weightings with different column chemistries

Bonded Phase	Separation Mechanism and Relative Strength[1]				
	Hydrophobic Binding	π-π Interaction	Dipole-Dipole	Hydrogen Bonding	Shape Selectivity
ACE C18	****	-	-	*	**
ACE C18-AR	****	*** (donor)	*	**	***
ACE C18-PFP	****	*** (acceptor)	****	***	****
ACE SuperC18	****	-	-	-	**
ACE C18-Amide	****	-	**	****	**/***
ACE CN-ES	***	*	***	**	*

Approximate value – determined by semi-quantitative mechanism weightings and/or by reference to other ACE phases using >100 characterising analytes.

[1] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, N. Tanaka, *J. Chromatogr. Sci.* 27 (1989) 721.

ACE® Stationary Phases: Key Mechanisms of Interactions

- Specifically designed phases to maximize selectivity

Bonded Phase	Separation Mechanism and Relative Strength[1]				
	Hydrophobic Binding	π-π Interaction	Dipole-Dipole	Hydrogen Bonding	Shape Selectivity
ACE C18	****	-	-	*	**
ACE C18-AR	****	*** (donor)	*	**	***
ACE C18-PFP	****	*** (acceptor)	****	***	****
ACE SuperC18	****	-	-	-	**
ACE C18-Amide	****	-	**	****	**/***
ACE CN-ES	***	*	***	**	*

Approximate value – determined by semi-quantitative mechanism weightings and/or by reference to other ACE phases using >100 characterising analytes.

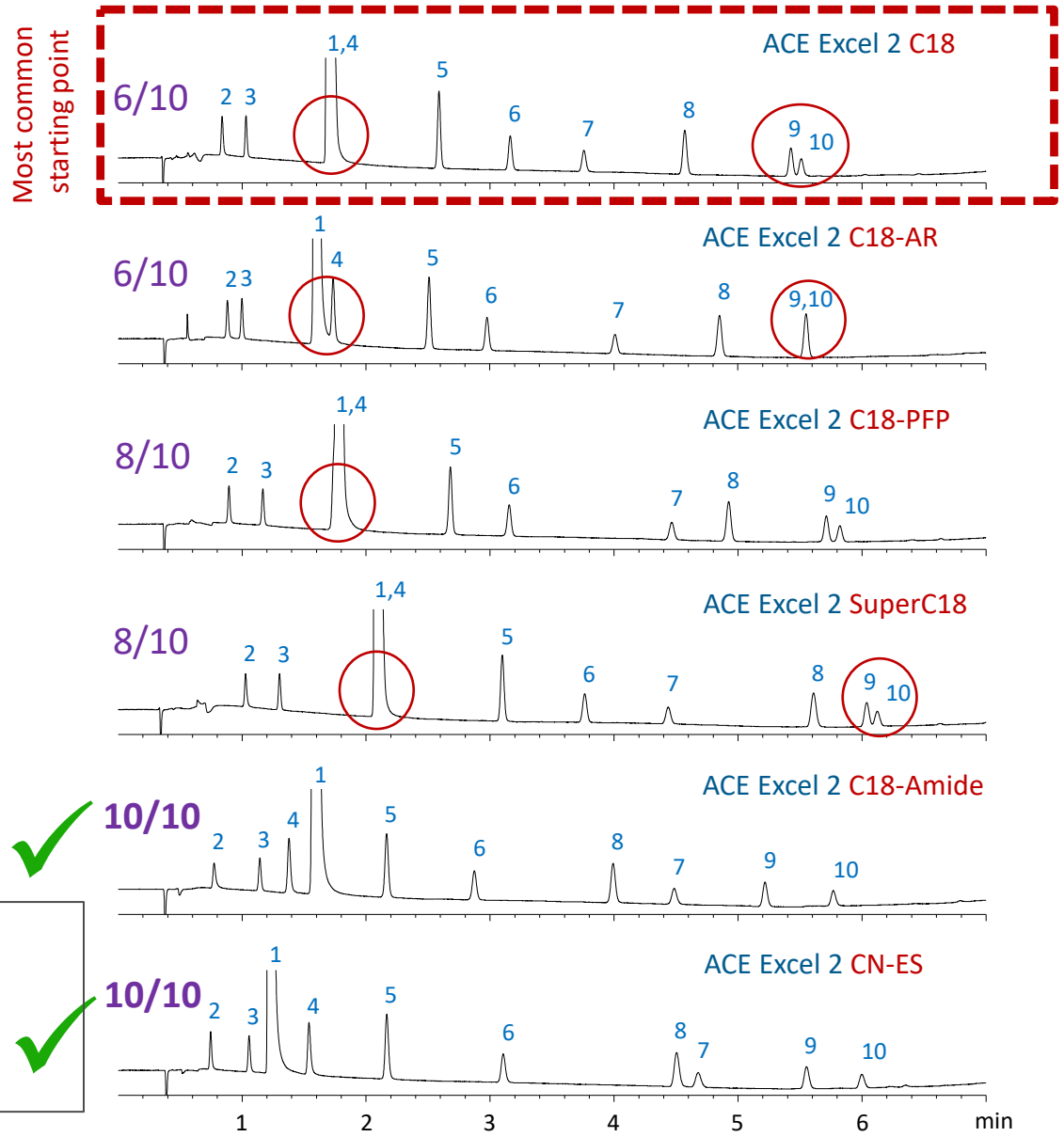
- Multiple mechanisms of interaction: ideal for method development

Screening combinations of column chemistries and solvents is systematic and helpful…plus can be semi-automated

The Complete Method Development Solution

MeOH

- Paracetamol and related compounds (10 analytes).
- Sample screened on six 100 x 3.0 mm ACE columns
- Methanol and MeCN used separately as the organic modifiers.



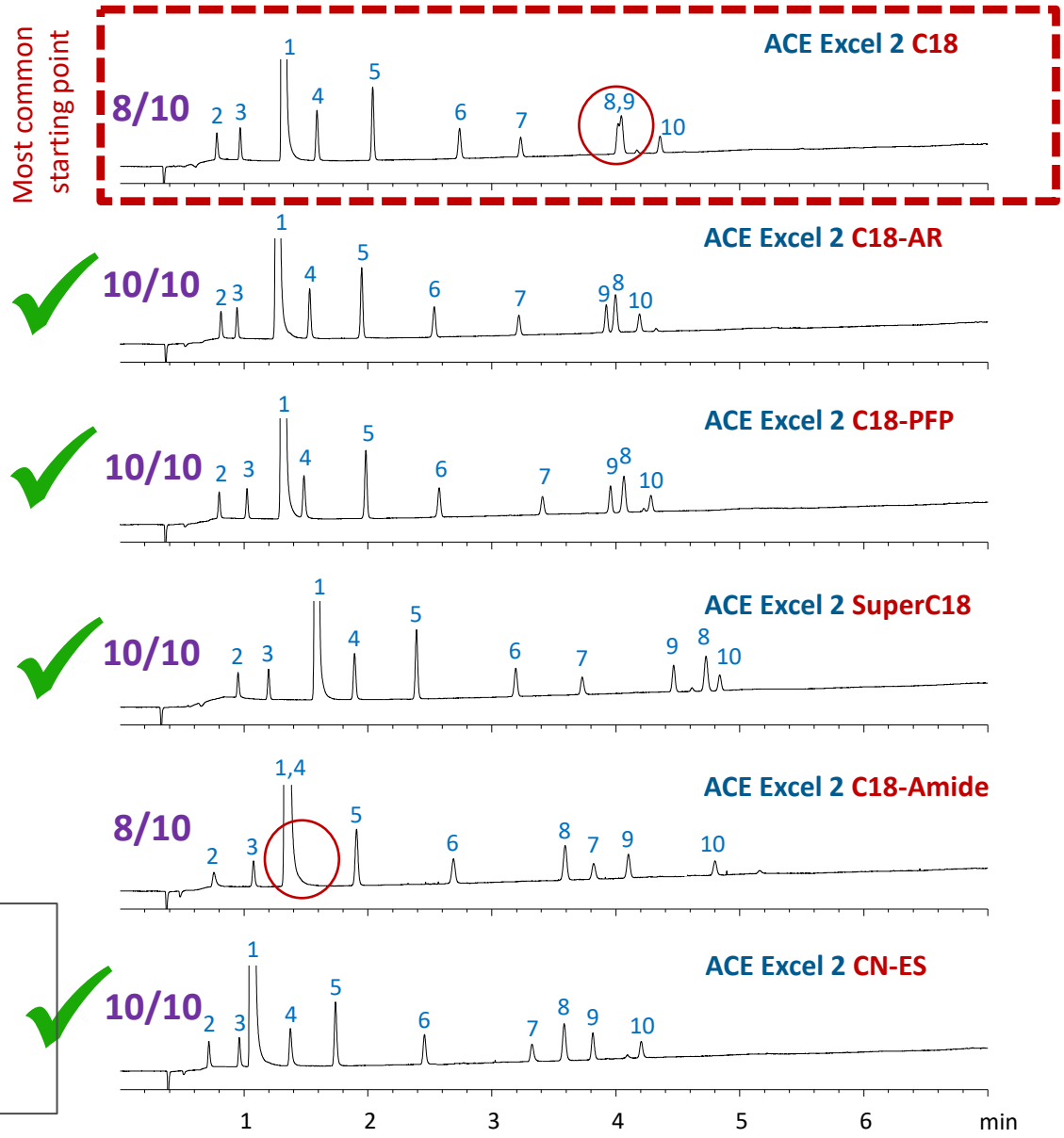
General scouting conditions:
100 x 3.0 mm columns
A: 20 mM Ammonium acetate pH 6.0
B: 20 mM Ammonium acetate pH 6.0 in MeOH or MeCN:H_2O (9:1 v/v)
Gradient: 5 to 95%B in 10 mins
Temp: 40°C, 2mL injection, Flow rate: 1.2 ml/min
Sample: Acetaminophen with rel subs at 0.5% w/w

The Complete Method Development Solution

MeCN

- Paracetamol and related compounds (10 analytes).
- Sample screened on six 100 x 3.0 mm ACE columns
- Methanol and MeCN used separately as the organic modifiers.

Total of 6 options identified from the screen where all 10 analytes separated



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Gradient: 5 to 95%B in 10 mins
Temp: 40°C, 2mL injection, Flow rate: 1.2 ml/min
Sample: Acetaminophen with rel subs at 0.5% w/w

Method Translation

HPLC to UHPLC

Isocratic Translations

Isocratic Method Translations: General Principles

- Maintain a constant length to particle size ratio, L/d_p (for the same phase type and phase vendor)

$$N = \frac{L}{HETP}$$

  - Will give ~ similar performance i.e. efficiency (selectivity, resolution)*

- Thus, 300 x 3.9 mm, 10 µm ≈ 150 x 4.6 mm, 5 µm = 30,000

Particle Size (µm) \ Column Length (mm)	50	75	100	150	250	300
1.7	29,412	44,118	58,824			
1.8	27,778	41,667	55,556			
1.9	26,316	39,474	52,632			
2	25,000	37,500	50,000	75,000		
2.5	20,000	30,000	40,000	60,000	100,000	
2.6	19,231	28,846	38,462	57,692	96,154	
2.7	18,519	27,778	37,037	55,556	92,593	
3	16,667	25,000	33,333	50,000	83,333	
5	10,000	15,000	20,000	30,000	50,000	
10	5,000	7,500	10,000	15,000	25,000	30,000

Columns meeting L/dp of 30,000

*** Neue et al., Stimuli to the revision process: USP <621>. *Pharm Forum*. 2009; 35(6): 1622-1626.**

Isocratic Method Translations: General Principles

- Once the new column format has been selected, isocratic translations are fairly straightforward.
  - Step 1: Scale injection volume (V_i) to new column dead volume (V_M)
  - Step 2: Scale flow rate (F)

$$V_{i2} = \frac{V_{i1} \times V_{M2}}{V_{M1}}$$

    - If no change in d_P

$$F_2 = \frac{F_1 \times d_{c2}^2}{d_{c1}^2}$$

    - If d_P changes can use

$$F_2 = \frac{F_1 \times d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}}$$

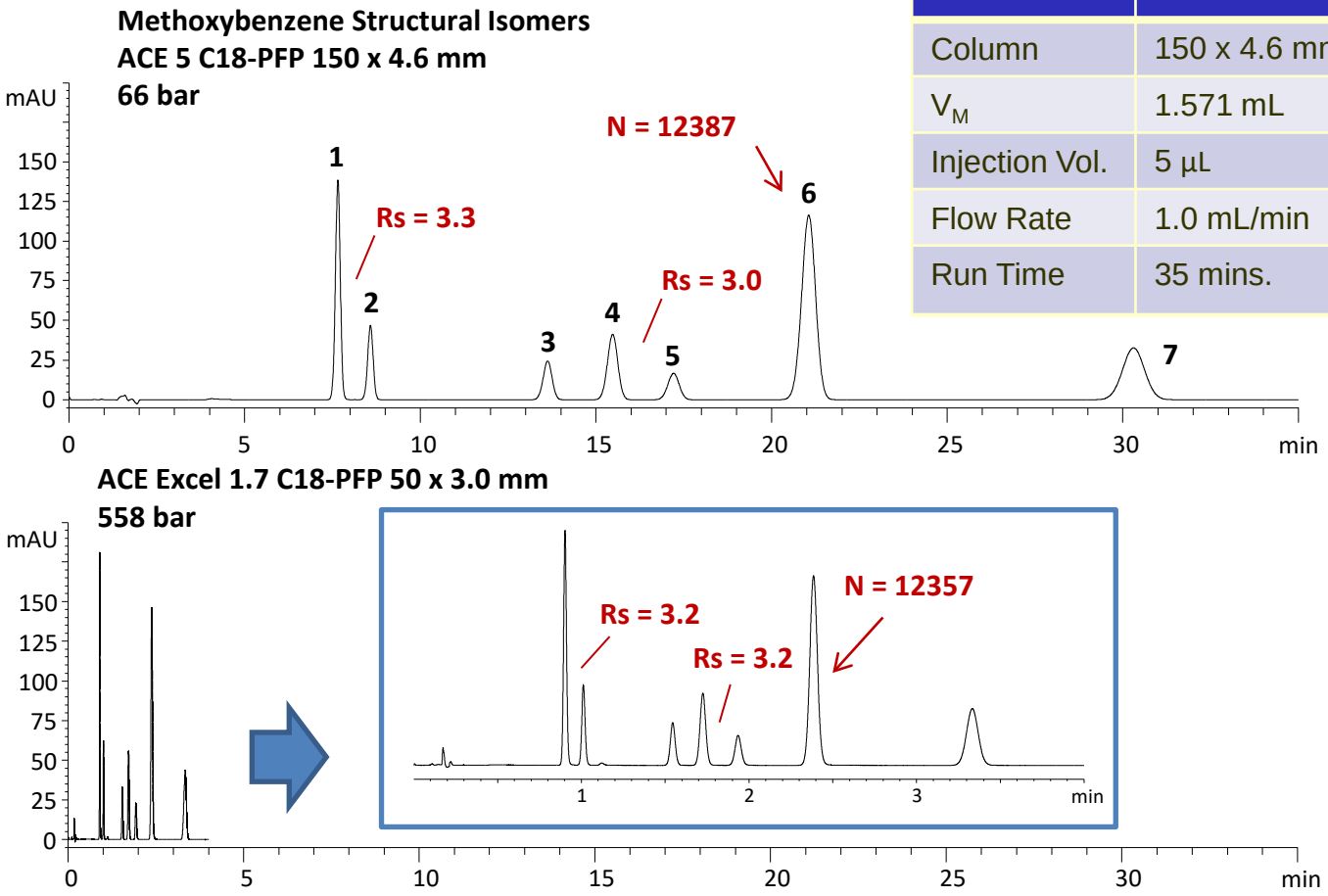
  - Step 3: Determine new run time

$$t_2 = \frac{t_1 F_1 V_{M2}}{F_2 V_{M1}}$$

  - Backpressure of new method can be estimated

$$P_2 = \frac{P_1 \times F_2 \times L_2 \times d_{c1}^2 \times d_{p1}^2}{F_1 \times L_1 \times d_{c2}^2 \times d_{p2}^2}$$

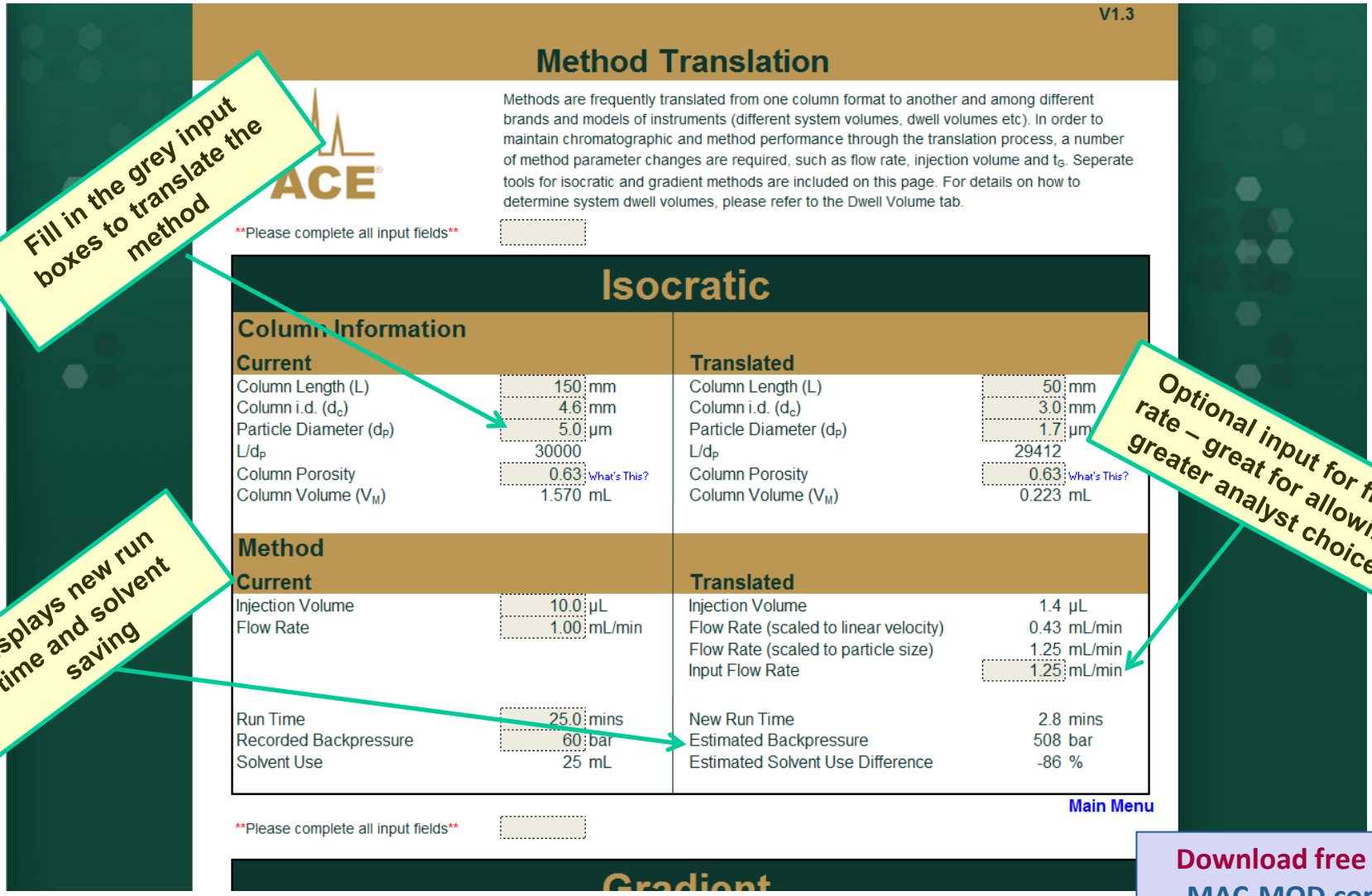
Isocratic Method Translations: HPLC to UHPLC



	HPLC Method	UHPLC Method
Column	150 x 4.6 mm, 5µm	50 x 3.0 mm, 1.7µm
V_M	1.571 mL	0.223 mL
Injection Vol.	5 µL	0.7 µL
Flow Rate	1.0 mL/min	1.25 mL/min
Run Time	35 mins.	4 mins

	Original method	Translated method	Difference
Run time	35 minutes	4 minutes	**-88.6%**
Solvent consumption	35.0 mL	5.0 mL	**-85.7%**

FREE ACE® Method Translation Tool: Isocratic Example



Download free at MAC-MOD.com

Gradient Translations

Translations of Gradient Methods – The Basics

1. ## Calculate column volumes
   - Better to experimentally determine porosity for accuracy

$$V_M \approx \pi \left(\frac{d}{2}\right)^2 L\varepsilon$$

2. ## Translate injection volume
   - To give similar response

$$Inj_2 = Inj_1 \times \left(\frac{V_{m2}}{V_{m1}}\right)$$

3. ## Translate flow rate
   - Constant linear velocity

$$F_2 = F_1 \times \frac{{d_{c_2}}^2}{{d_{c_1}}^2}$$

   - Scaled to new d_p

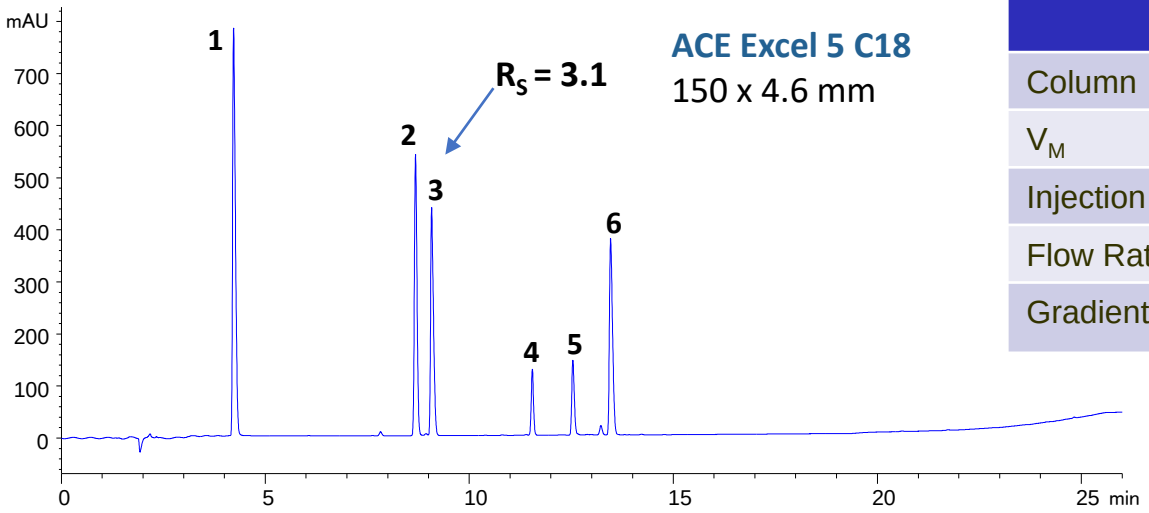
$$F_2 = \frac{F_1 \times d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}}$$

4. ## Translate gradient time
   - To maintain constant k*

$$t_{G2} = \frac{t_{G1} V_{M2} F_1}{V_{M1} F_2}$$

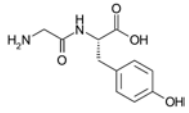
5. ## Consider V_D/V_M ratio

UHPLC Gradient Method Translations: Peptides

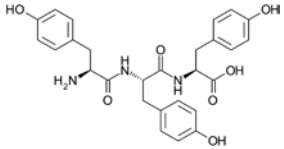


ACE Excel 5 C18
150 x 4.6 mm

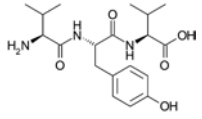
	Original Method
Column	150 x 4.6 mm, 5 µm
V_M	1.571 mL
Injection Vol.	10 µL
Flow Rate	1.0 mL/min
Gradient time	17 min.



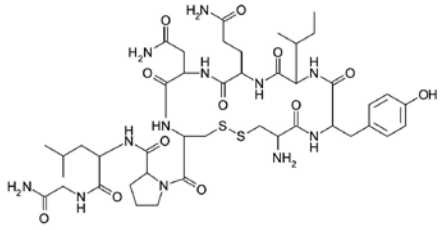
1. Gly-Tyr



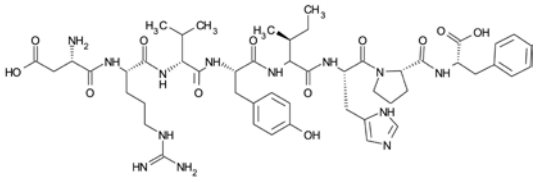
2. Tyr-Tyr-Tyr



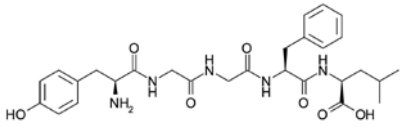
3. Val-Tyr-Val



4. Oxytocin



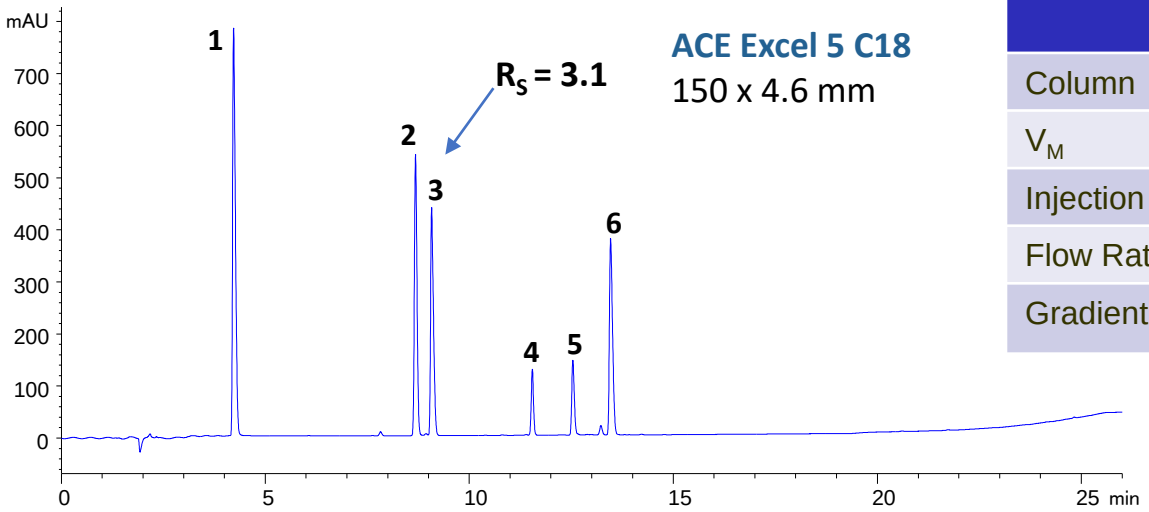
5. Angiotensin II



6. Leu-enkephalin

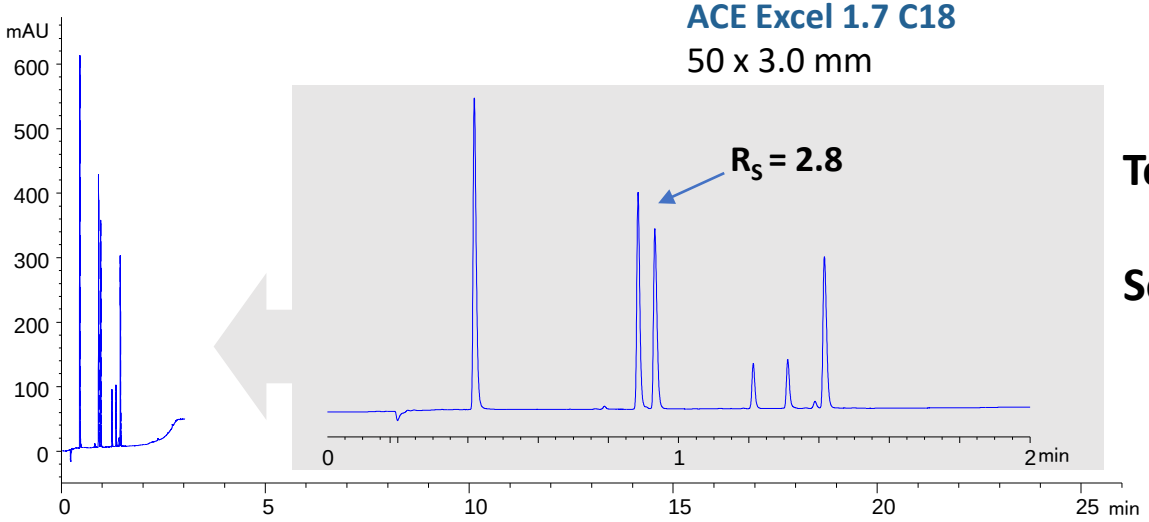
Mobile Phase A: 0.05% TFA (aq), B: 0.05% TFA in MeCN
Gradient: 5 to 40 %B in 17.0 mins, then 40 to 90 %B in 6.0 minutes and hold 90%B for 2.0 mins
Temp: 60 °C Flow Rate: 1.0 mL/min Detection: UV 220 nm.

UHPLC Gradient Method Translations: Peptides



ACE Excel 5 C18
150 x 4.6 mm

	Original Method	Translated Method
Column	150 x 4.6 mm, 5 µm	**50 x 3.0 mm, 1.7 µm**
V_M	1.571 mL	**0.223 mL**
Injection Vol.	10 µL	**1.4 µL**
Flow Rate	1.0 mL/min	**1.25 mL/min**
Gradient time	17 min.	**1.93 min.**



ACE Excel 1.7 C18
50 x 3.0 mm

Total run time: **-88%**

Solvent consumption: **-85%**

Gradient Method Translations: Correcting for V_D/V_M

- For maximum accuracy of gradient translations, it may be necessary to correct for the change in ratio of V_D/V_M between the original and translated method.

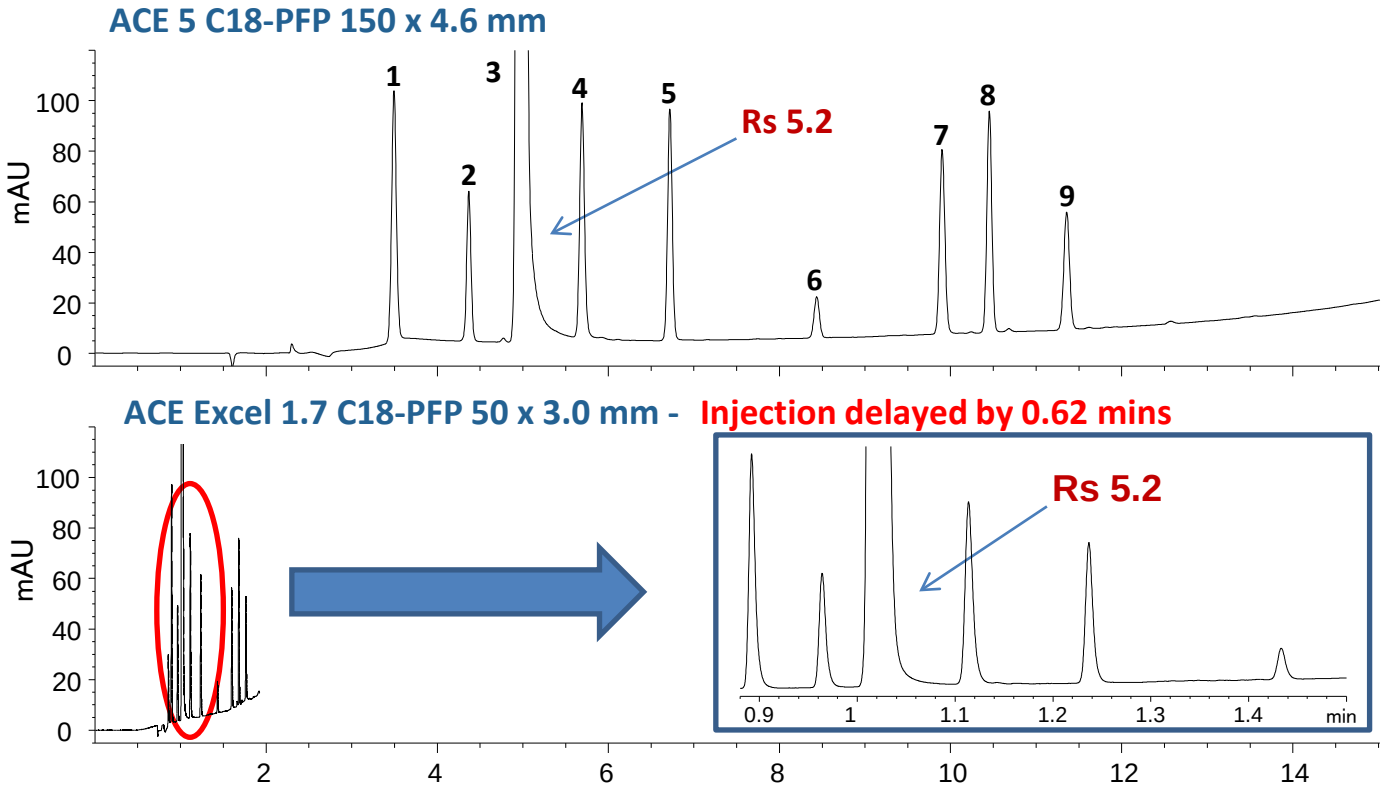
$$\Delta = \left(\frac{V_D}{V_M}\right)_{original} - \left(\frac{V_D}{V_M}\right)_{new}$$ *must approach zero*

- This is increasingly important when translating to small i.d. (therefore small V_M) UHPLC column formats.
- The correction can be made using

$$x = \left[\left(\frac{V_{D1}}{V_{M1}}\right) - \left(\frac{V_{D2}}{V_{M2}}\right)\right] \times \frac{V_{M2}}{F_2}$$

- **Negative value:** injection must be delayed *x* minutes after gradient starts.
- **Positive value:** a pre-gradient isocratic hold of *x* minutes should be added to gradient program.

Gradient Method Translations: Why correct for V_D/V_M?



t_G = 15 minutes
F = 1.0 mL/min

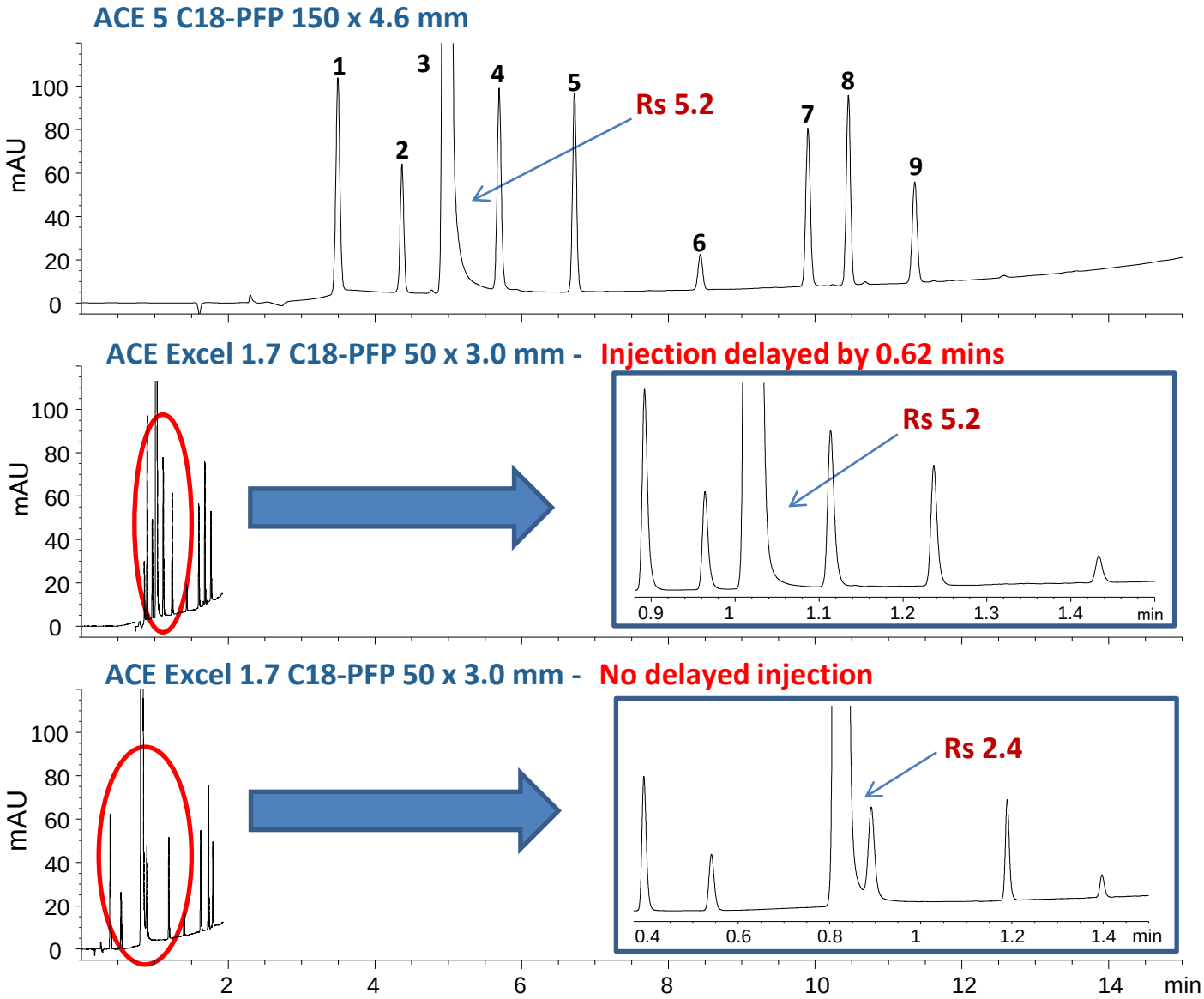
t_G = 1.70 minutes
F = 1.25 mL/min

- Calculations tell us to delay injection until 0.62 minutes after the gradient starts to correct for V_D/V_M

$$x = \left[\left(\frac{V_{D1}}{V_{M1}}\right) - \left(\frac{V_{D2}}{V_{M2}}\right)\right] \times \frac{V_{M2}}{F_2} = \left[\left(\frac{1.098}{1.570}\right) - \left(\frac{0.926}{0.223}\right)\right] \times \frac{0.223}{1.25} = \textbf{-0.62 minutes}$$

Gradient analysis, A= 20mM ammonium acetate pH 6.0 (aq), B= 20 mM ammonium acetate pH 6.0 in MeCN:water 80:20 v/v, 5-95%B in 15.0 mins, hold 95%B for 2.0 mins, 40°C, 1.0 mL/min, 230 nm.
1. 4-aminophenol, 2. hydroquinone, 3. 4-acetamidophenol (paracetamol), 4. 2-aminophenol, 5. 2-acetamidophenol, 6. phenol, 7. 4-nitrophenol, 8. 4-chloroacetanilide, 9. 2-nitrophenol. Impurities were spiked at 0.5% w/w.

Gradient Method Translations: Why correct for V_D/V_M?



t_G = 15 minutes
F = 1.0 mL/min

t_G = 1.70 minutes
F = 1.25 mL/min

t_G = 1.70 minutes
F = 1.25 mL/min

FREE ACE® Method Translator Tool: Gradient Example

Input data into the grey boxes

Input dwell volume of LC systems

Gradient

Column Information

Current			Translated		
Column Length (L)	150	mm	Column Length (L)	50	mm
Column i.d. (d_c)	4.6	mm	Column i.d. (d_c)	3.0	mm
Particle Diameter (d_P)	5.0	µm	Particle Diameter (d_P)	1.7	µm
L/d_P	30000		L/d_P	29412	
Column Porosity	0.63	What's This?	Column Porosity	0.63	What's This?
Column Volume (V_M)	1.570	mL	Column Volume (V_M)	0.223	mL

Method

Current			Translated		
Injection Volume	10.0	µL	Injection Volume	1.4	µL
Flow Rate	1.00	mL/min	Flow Rate (scaled to linear velocity)	0.43	mL/min
			Flow Rate (scaled to particle size)	1.25	mL/min
			Input Flow Rate	1.25	mL/min
LC Name	HPLC 1		LC Name	UHPLC 1	
Dwell Volume (V_D)	1.098	mL	Dwell Volume (V_D)	0.926	mL
Recorded Backpressure	67	bar	Estimated Backpressure	568	bar
			Estimated Run Time Difference	-87	%
			Estimated Solvent Use Difference	-83	%

Gradient	Time	%B
	0.00	5
	15.00	95
	17.00	95
	17.50	5

Suggested Re-equilibration Time: 16.8 mins

Gradient	Time	%B
	0.00	5
	1.70	95
	1.93	95
	1.98	5

Suggested Re-equilibration Time: 2.6 mins

Time to delay injection after the gradient begins: 0.62 mins (770 µL)

Correct translation of this method requires that the injection is delayed until after the gradient begins by this time. A delayed injection can be added to a method in many LC instrument software packages.

New Gradient

This section tells you if a delayed injection of X mins is required

Download free at MAC-MOD.com

Method Transfer

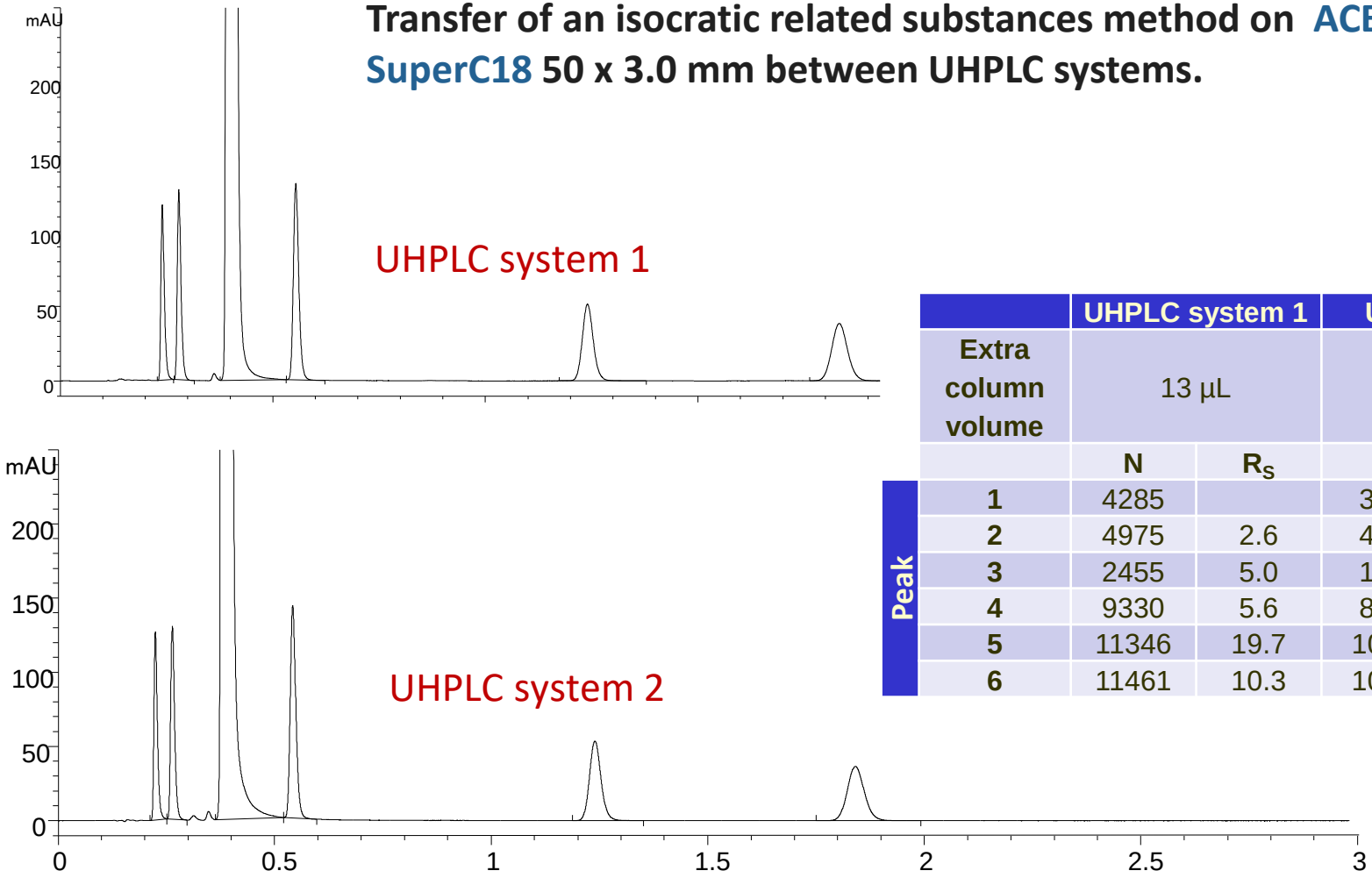
Between Different instruments

Instrument to Instrument Same Method Transfers

- Isocratic
  - If column format remains identical, no changes necessary.
  - (If column format changes, translation required.)
- Gradient
  - If column format remains identical, need to correct for influence of differing system dwell volumes only. Flow rate, injection volume and gradient times remain unchanged.
  - (If column format changes, translation plus differing system dwell effects need to be calculated.)

Instrument to instrument method transfer: Isocratic

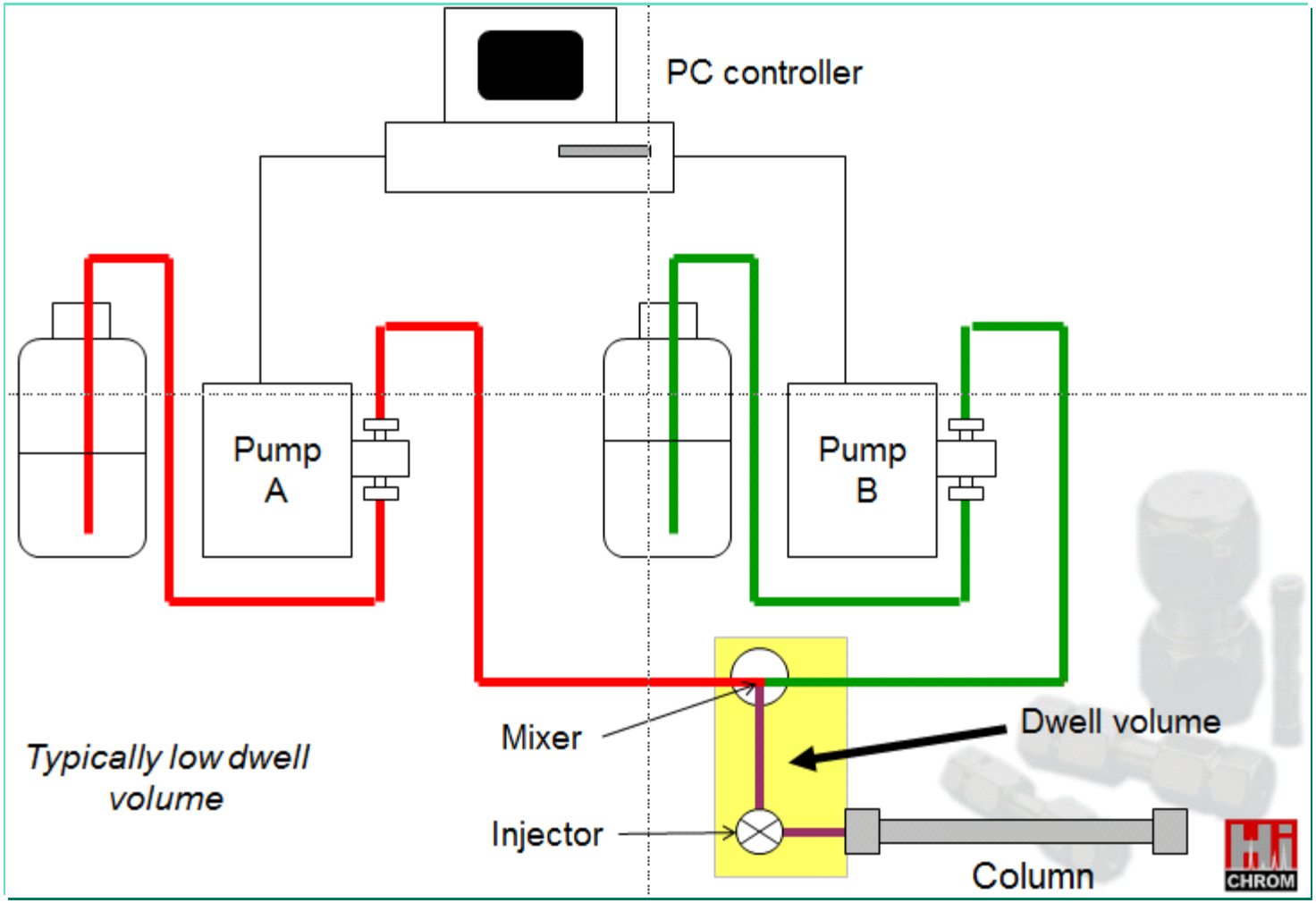
Transfer of an isocratic related substances method on ACE Excel 1.7 SuperC18 50 x 3.0 mm between UHPLC systems.



		UHPLC system 1		UHPLC system 2	
	Extra column volume	13 µL		15.5 µL	
		N	R_S	N	R_S
Peak	1	4285		3748	
	2	4975	2.6	4388	2.5
	3	2455	5.0	1831	4.8
	4	9330	5.6	8303	5.1
	5	11346	19.7	10561	19.4
	6	11461	10.3	10644	10.1

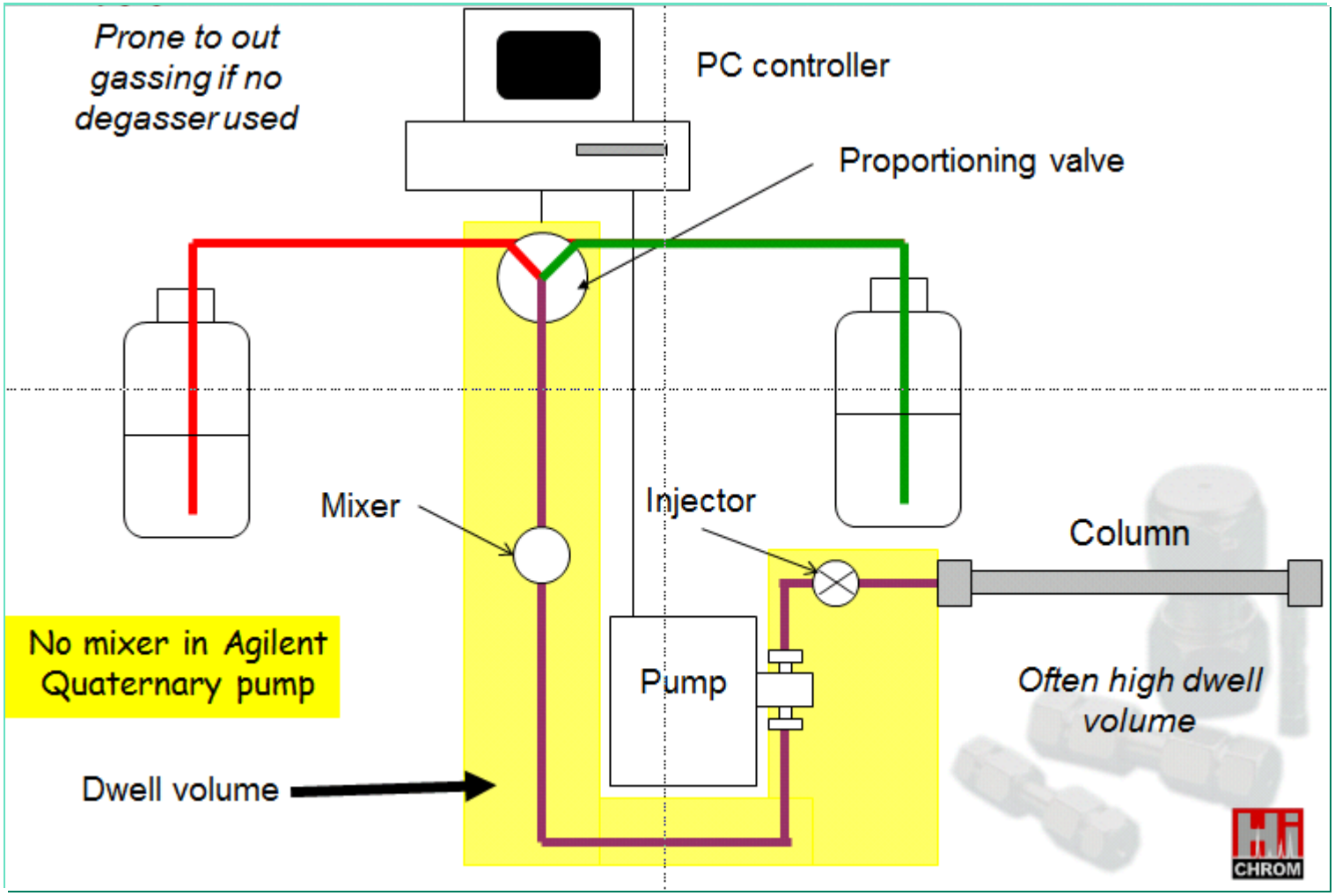
Isocratic analysis 0.2% H_3PO_4 in MeCN:MeOH:water 35:5:60 v/v/v, 40°C, 1.25 mL/min, 0.7 µL , Injection vol., 254 nm.
1. 4-hydroxybenzoic acid, 2. 4-hydroxyisophthalic acid, 3. acetylsalicylic acid (aspirin), 4. salicylic acid, 5. acetylsalicylsalicylic acid, 6. salsalate. Impurities were spiked at 0.5% w/w.

Binary Pump Configuration: High Pressure Mixing



Data courtesy of Euerby & Petersson

Quaternary Pump Configuration: Low Pressure Mixing



Data courtesy of Euerby & Petersson

Instrument to Instrument Same Method Transfers

- For gradient method transfer, t_G, F and V_M all remain identical.

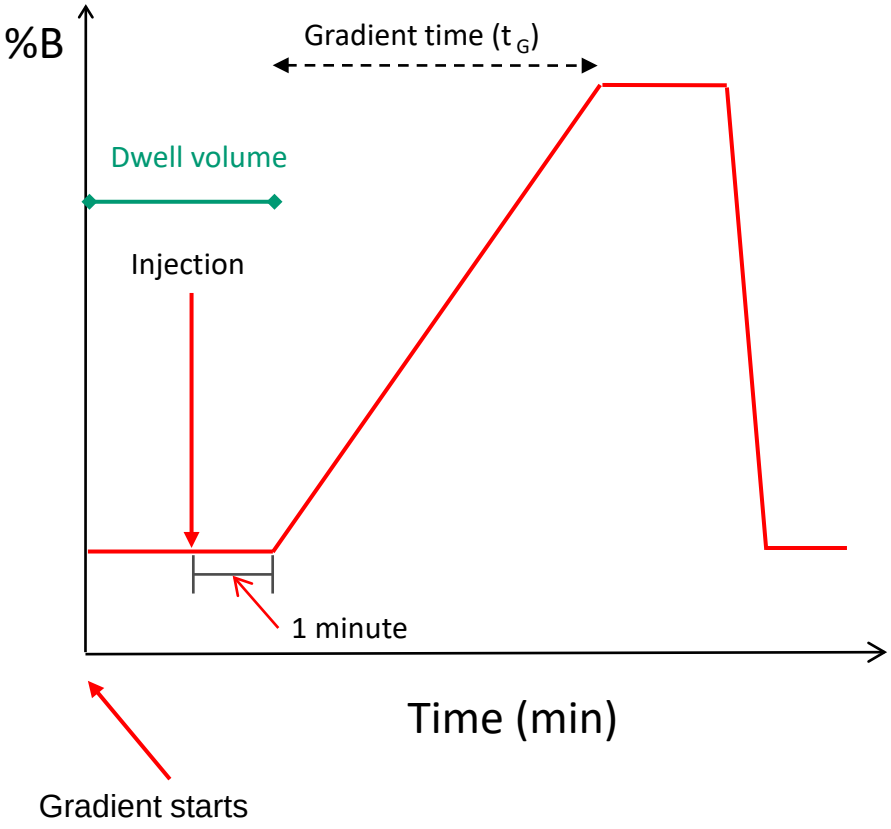
- Only need to correct for the system dwell volume differences
  - Given that V_M does not change for method transfer, the V_D/V_M equation simplifies to:

$$x = \left(\frac{V_{D1} - V_{D2}}{F}\right)$$

  - Negative value: injection must be delayed x minutes after gradient starts so injection is made onto the gradient.
  - Positive value: a pre-gradient isocratic hold of x minutes should be added to gradient program.

Method Transfers: Dwell Volumes / Injection Times

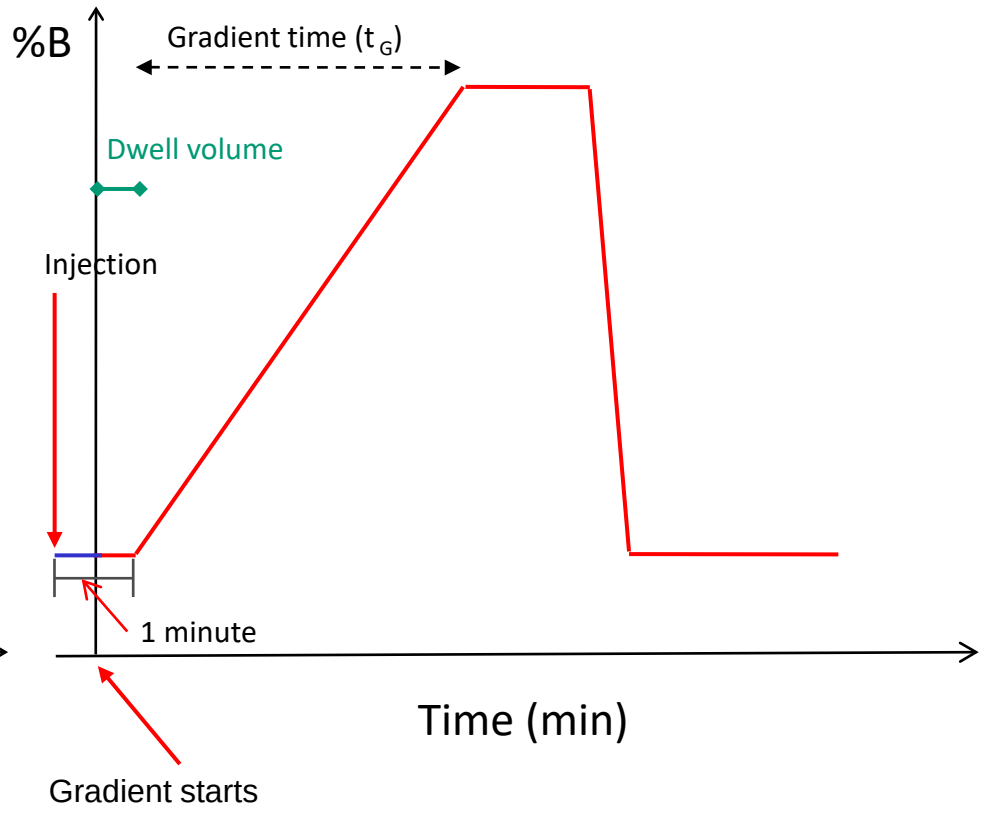
e.g. small column on a high dwell volume system. The dwell time is artificially long.



$\Delta (V_D/V_M)$ = negative

Injection is delayed until after the start of gradient

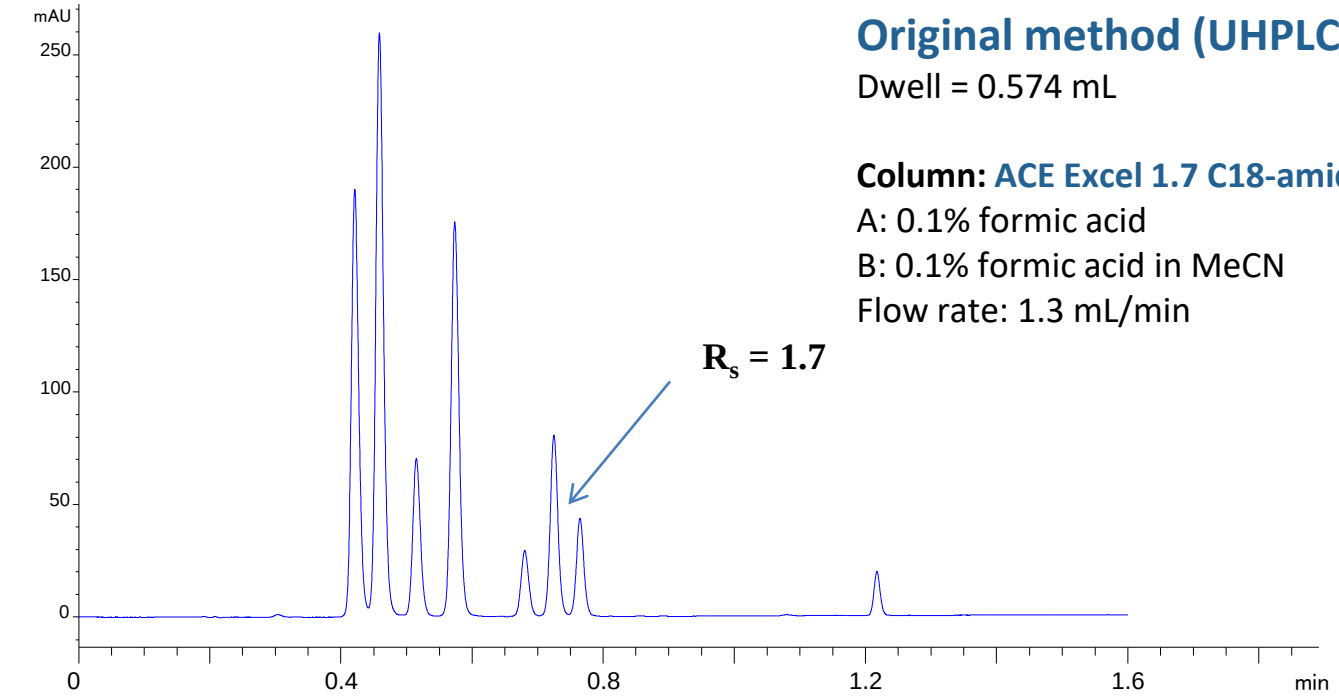
e.g. large column on a low dwell volume system. The dwell time is artificially short.



$\Delta (V_D/V_M)$ = positive

Pre-gradient hold is added. Effectively extends dwell time

UHPLC method transfer - Vanillins



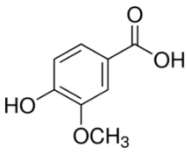
Original method (UHPLC System 1)

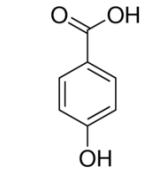
Dwell = 0.574 mL

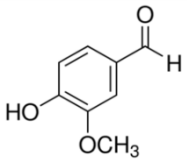
Column: ACE Excel 1.7 C18-amide 50 x 3.0 mm
A: 0.1% formic acid
B: 0.1% formic acid in MeCN
Flow rate: 1.3 mL/min

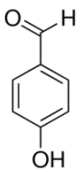
t	%B
0	25
1.32	75
1.49	75
1.6	25

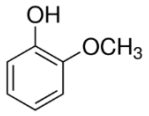
t_G	1.32	
Post time:	2	min
Inj. Vol.	1	µL
P_{MAX}	502	bar

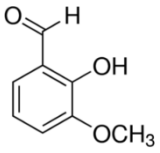

1. vanillic acid

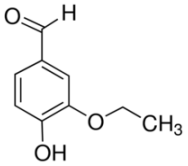

2. 4-hydroxybenzoic acid

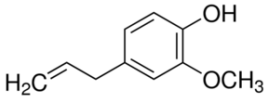

3. vanillin


4. 4-hydroxybenzaldehyde


5. guaiacol


6. *o*-vanillin


7. ethyl vanillin

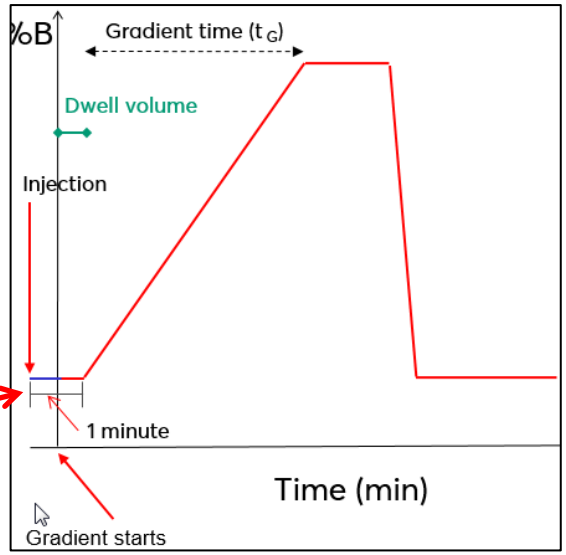

8. eugenol

UHPLC method transfer - Vanillins

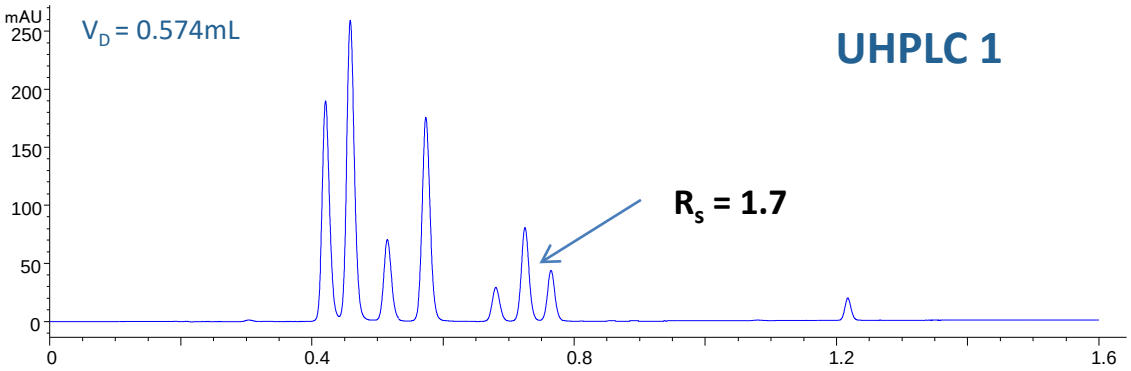
- **Aim**
  - **To translate the method from UHPLC System 1 (Quaternary) to UHPLC System 2 (Binary)**
  - **Correct for system dwell volume only**
  - $V_{D1} - V_{D2} = \Delta = 0.574 - 0.202 = 0.372$ mL
  - **Positive value, therefore a pre-gradient hold (*x*) is required**

$$x = \frac{|\Delta|}{F_2} = 0.29\ min$$ **pre-gradient hold**

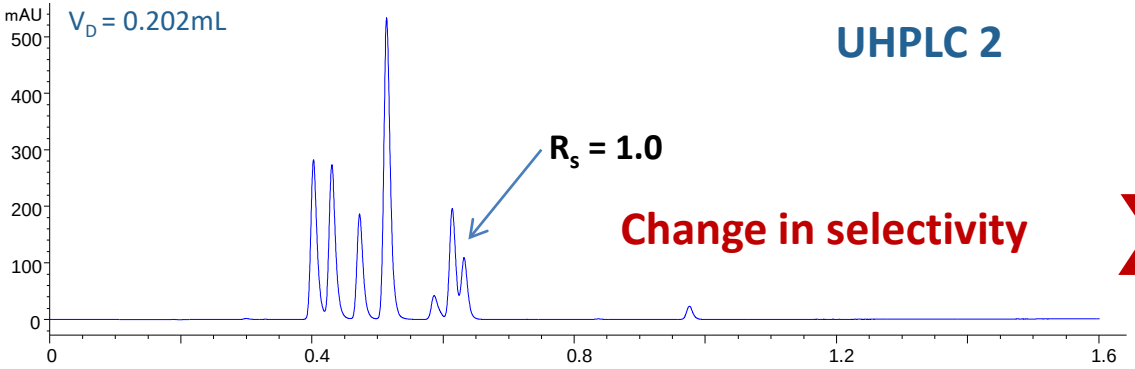
Essentially introducing an isocratic hold prior to gradient start



Instrument to instrument method transfer



t	%B
0	25
1.32	75
1.49	75
1.6	25

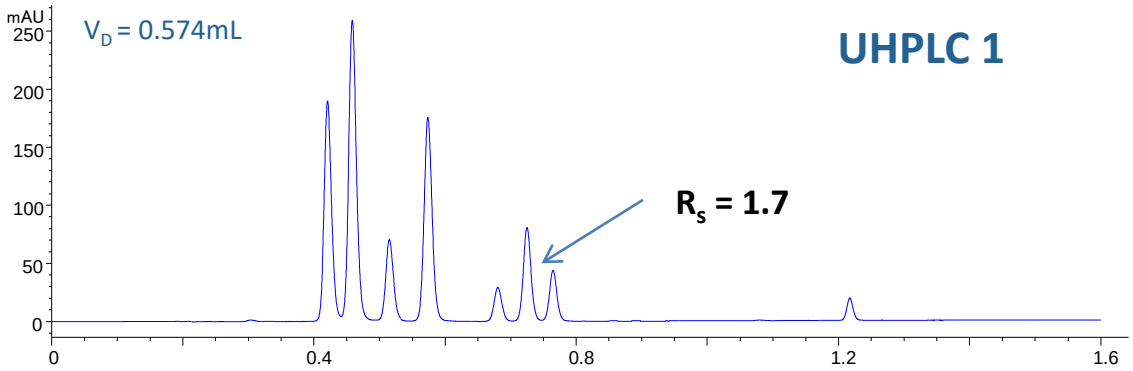


X

t	%B
0	25
1.32	75
1.49	75
1.6	25

Without isocratic hold

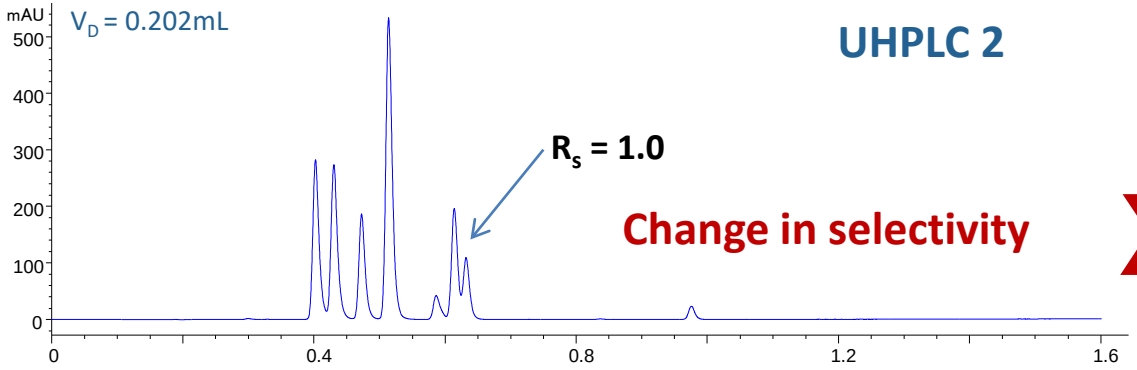
Instrument to instrument method transfer



UHPLC 1

R_s = 1.7

t	%B
0	25
1.32	75
1.49	75
1.6	25



UHPLC 2

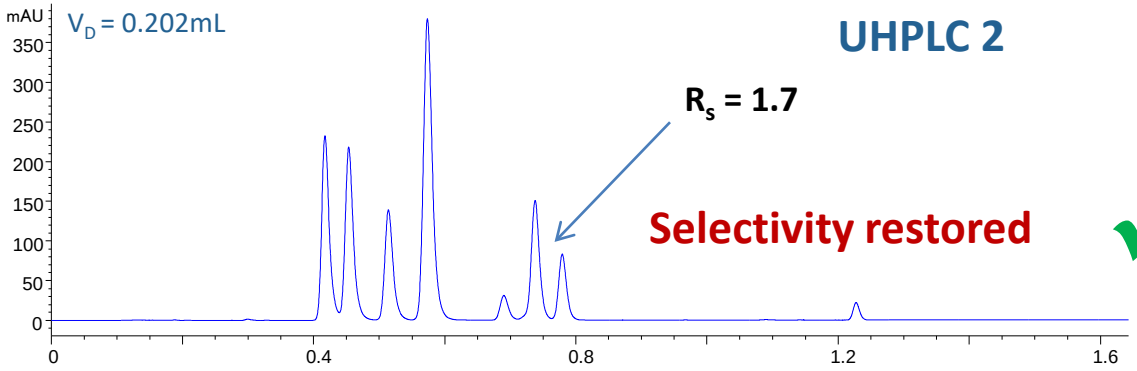
R_s = 1.0

Change in selectivity

X

t	%B
0	25
1.32	75
1.49	75
1.6	25

Without isocratic hold



UHPLC 2

R_s = 1.7

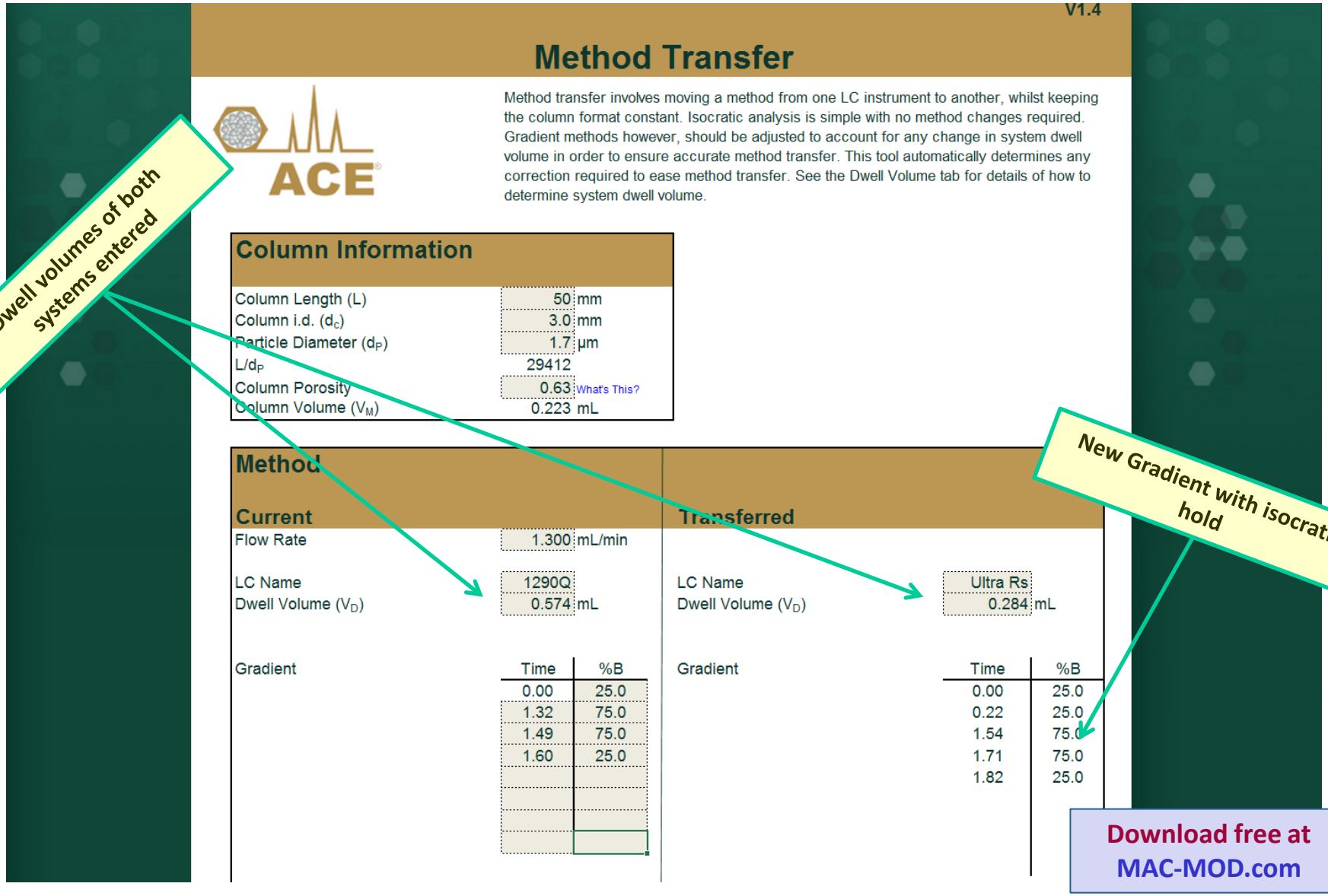
Selectivity restored

✓

t	%B
0	25
0.29	25
1.61	75
1.78	75
1.89	25

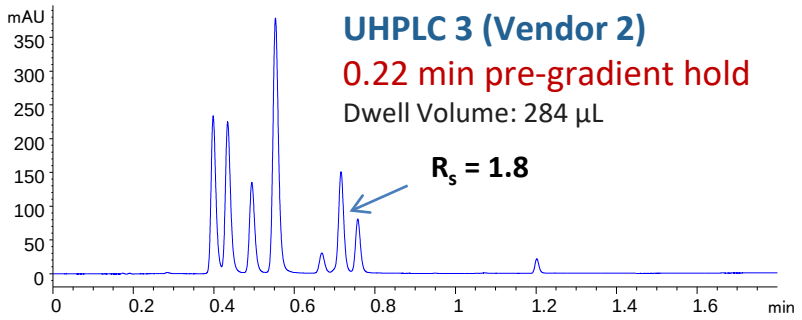
With correction for ΔV_D

Method Transfer Tool Example - Gradient

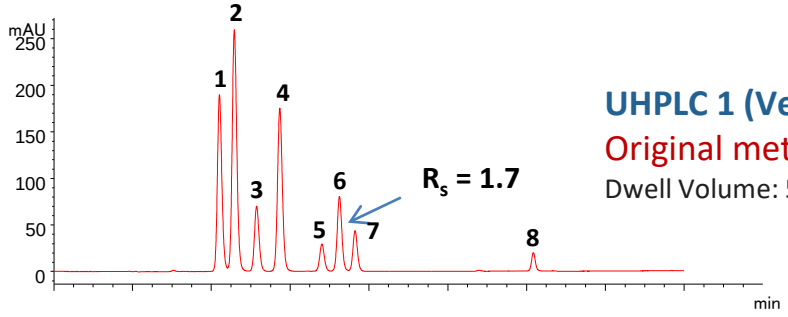


Instrument to instrument method transfer

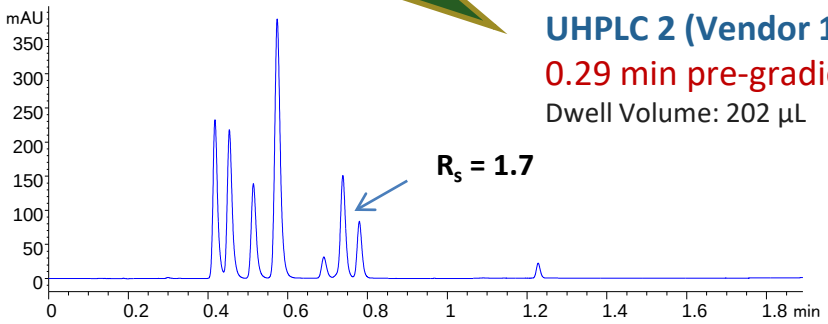
- Using the ACE Method Transfer Tool, gradient method transfer between different vendor LC systems is easily achieved.



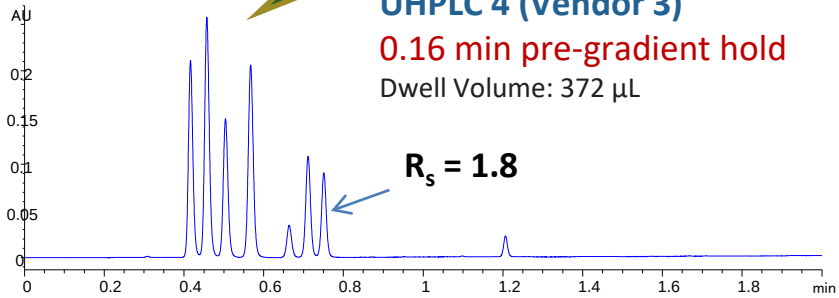
UHPLC 3 (Vendor 2)
0.22 min pre-gradient hold
Dwell Volume: 284 µL



UHPLC 1 (Vendor 1)
Original method
Dwell Volume: 574 µL



UHPLC 2 (Vendor 1)
0.29 min pre-gradient hold
Dwell Volume: 202 µL



UHPLC 4 (Vendor 3)
0.16 min pre-gradient hold
Dwell Volume: 372 µL

Summary #1

- UHPLC can provide many benefits:
  - Increased separation speed
  - Increased resolution
  - 'Ultra-resolution' with column coupling for complex samples
- Stationary phase selectivity is a powerful tool for UHPLC (and HPLC!) method development
  - Different stationary phases provide different mechanisms of analyte interaction.
  - Five **ACE novel phases** (C18-AR, C18-PFP, C18-Amide, CN-ES and SuperC18) plus ACE C18 provide total solution for column screening.

Summary #2

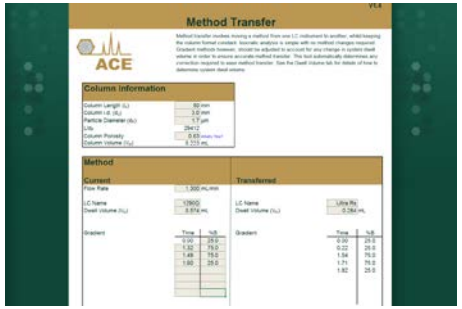
- Translation of HPLC methods to UHPLC can be accomplished by using a few key equations:
  - Isocratic: fairly simple
  - Gradient: more complex (translate t_G and correct for dwell volume)
- Method transfers between instruments also straightforward

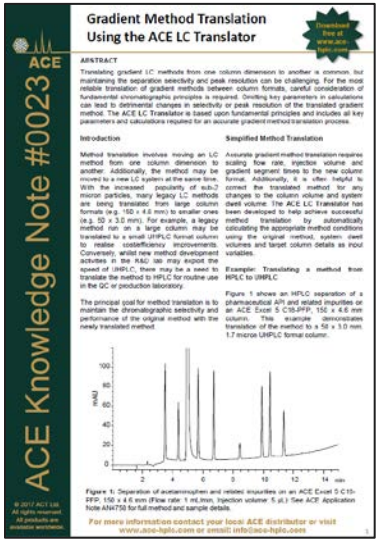
The ACE LC Translator simplifies method translation and instrument transfer

Download free at MAC-MOD.com

Useful Resources

- ACE Translation Tool:
  - (+help file)
  - (+ AKN#0023)





- ACE Knowledge Notes (AKNs):
  - **AKN0001** - How to Determine System Dwell Volume
  - **AKN0006** – UHPLC Column Connections
  - **AKN0011** – Practical UHPLC
  - **AKN0012** - Understanding the Relationship between Particle Size, Performance and Pressure
  - **AKN0017** - How to Determine Extra Column Dispersion and Extra Column Volume
  - **AKN0023** - Gradient Method Translation Using the ACE LC Translator

- ACE Method Development Kit Brochure and Webinar

- Download Resources at mac-mod.com
  or info@mac-mod.com

Thank you for your attention

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