An Introduction to the Theory and Practice of LC Method Translations and LC Method Transfers

Gemma Lo

Method Translations: What Are You Trying to Achieve?

- **Faster separations with the same performance?**
  - **eg HPLC → UHPLC methods for increased productivity / sample throughput with similar efficiency, selectivity and resolution?**
- **Converting UHPLC → HPLC methods?**
  - **eg for offshoring or third party labs or manufacturing?**
- **Porous particle → solid core particle method change?**
  - **Take advantage of solid core efficiency, speed or low backpressure?**
- **Faster method development?**
  - **eg reduce overall cycle time?**
- **Higher resolution / peak capacity?**
  - **eg for related substances or complex samples?**
- **Same methods...transferred between instruments?**

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)**

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

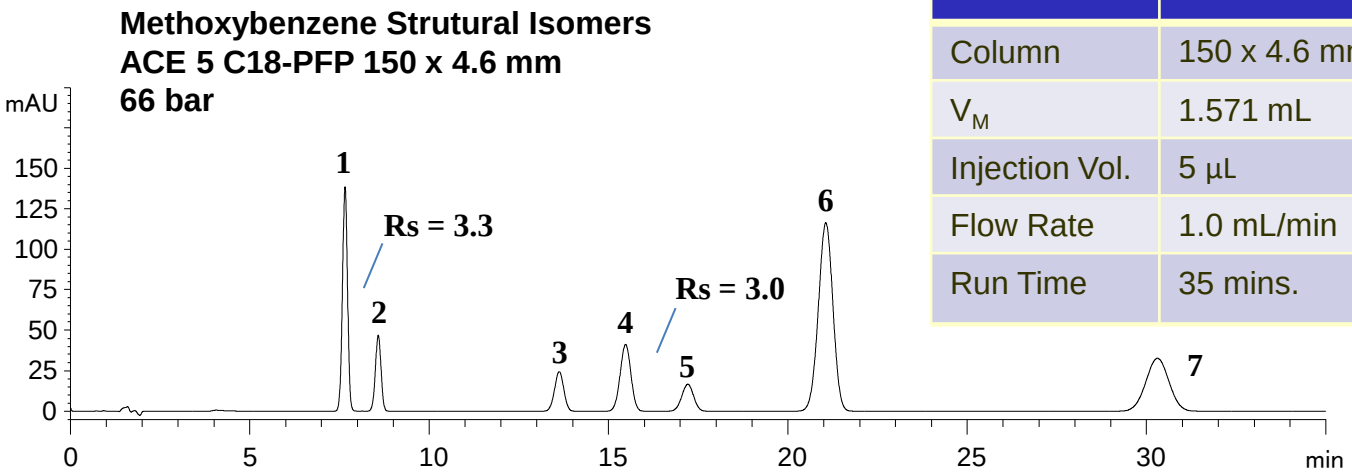
    - **If no change in d_P** $$F_2 = \frac{F_1 \times d_{c2}^2}{d_{c1}^2}$$
    - **If d_P changes can use** $$F_2 = \frac{F_1 \times d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}}$$
  - **Step 3: determine new run time**

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

  - **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



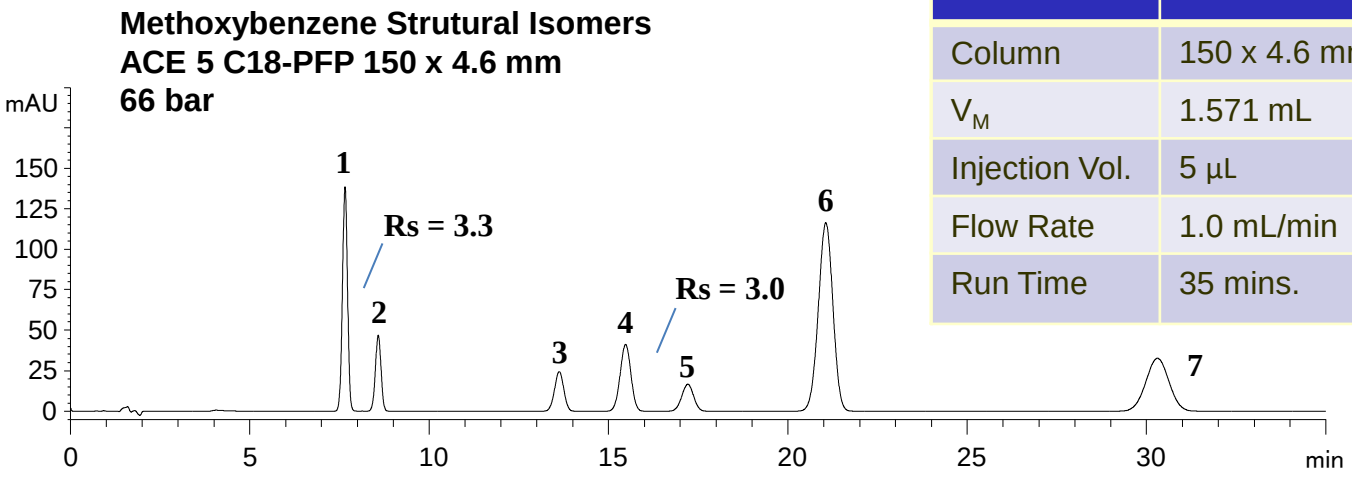
	HPLC Method	UHPLC Method
Column	150 x 4.6 mm, 5µm	**50 x 3.0 mm, 1.7µm**
V_M	1.571 mL	**0.223 mL**
Injection Vol.	5 µL	
Flow Rate	1.0 mL/min	
Run Time	35 mins.	

- **ACE 5 C18-PFP 150 x 4.6 mm** — **V_M = 1.571 mL**
  - **L/d_P = 30,000**
- **ACE 1.7 C18-PFP 50 x 3.0 mm** — **V_M = 0.223 mL**
  - **L/d_P = 29,412**
- **V_M experimentally determined**
  - **Or use**

$$V_M = \pi \left(\frac{d_c}{2}\right)^2 L\varepsilon$$

  - **ε = column porosity = ~0.63 for fully porous, ~0.55 for solid core**

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.	

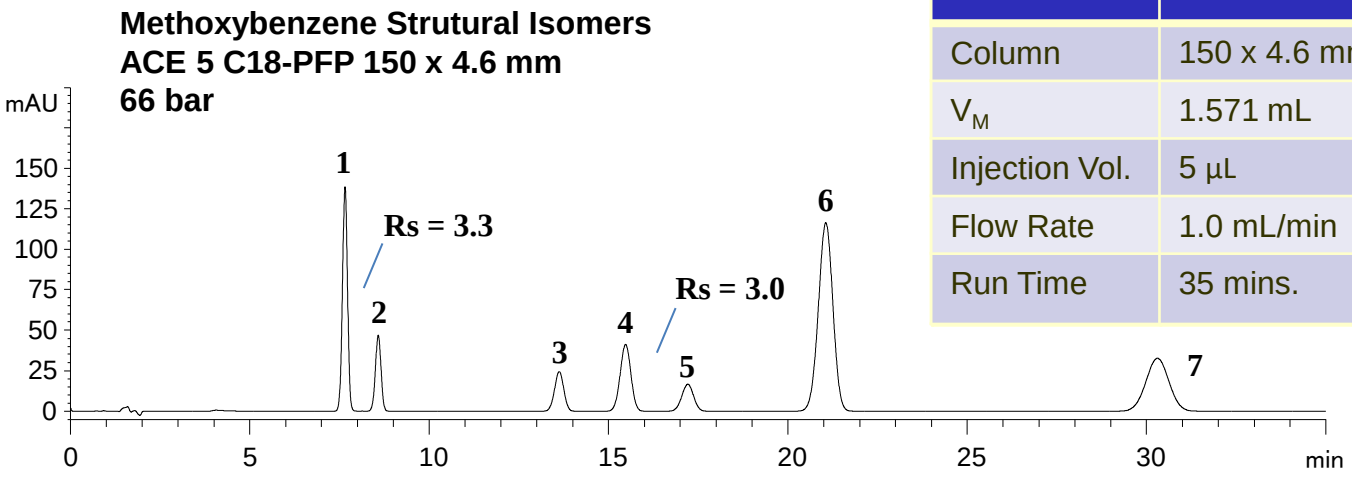
- **Step 1: Scale injection volume**

$$V_{i2} = \frac{V_{i1} \times V_{M2}}{V_{M1}} = \frac{5 \times 0.223}{1.571} = 0.7\mu L$$

- **Step 2: Scale flow rate**

$$F_2 = \frac{F_1 \times d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}} = \frac{1.00 \times 3.0^2 \times 5}{4.6^2 \times 1.7} = 1.25\ mL/min$$

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

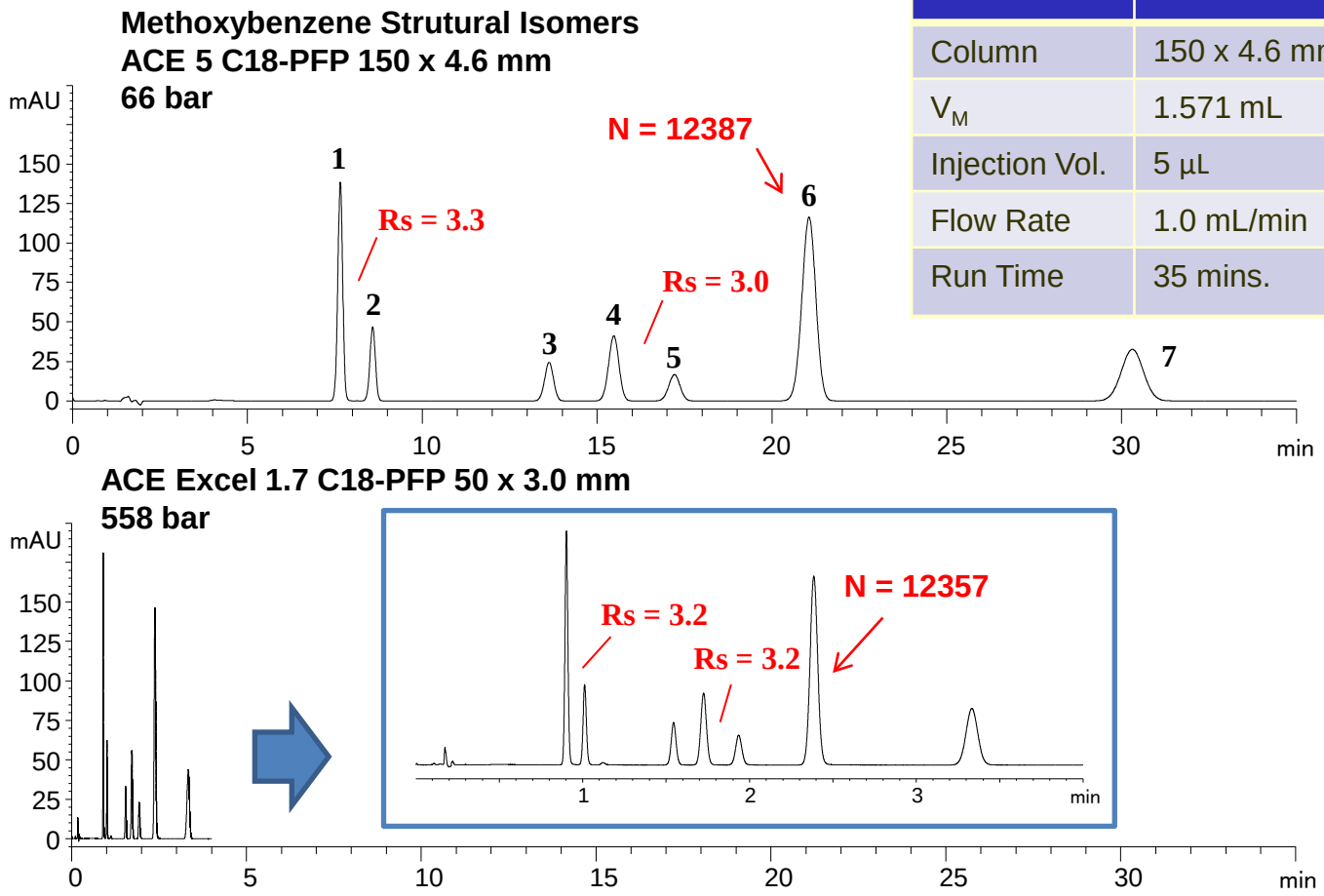
- **Step 3: Calculate UHPLC run time**

$$t_2 = \frac{t_1 F_1 V_{M2}}{F_2 V_{M1}} = \frac{35 \times 1.00 \times 0.223}{1.25 \times 1.571} = 4\ minutes$$

- **We can now estimate the backpressure of the translated method**

$$P_2 = \frac{P_1 \times F_2 \times L_2 \times d_{c1}^2 \times d_{p1}^2}{F_1 \times L_1 \times d_{c2}^2 \times d_{p2}^2} = \frac{66 \times 1.25 \times 50 \times 4.6^2 \times 5^2}{1.00 \times 150 \times 3^2 \times 1.7^2} = 559\ bar$$

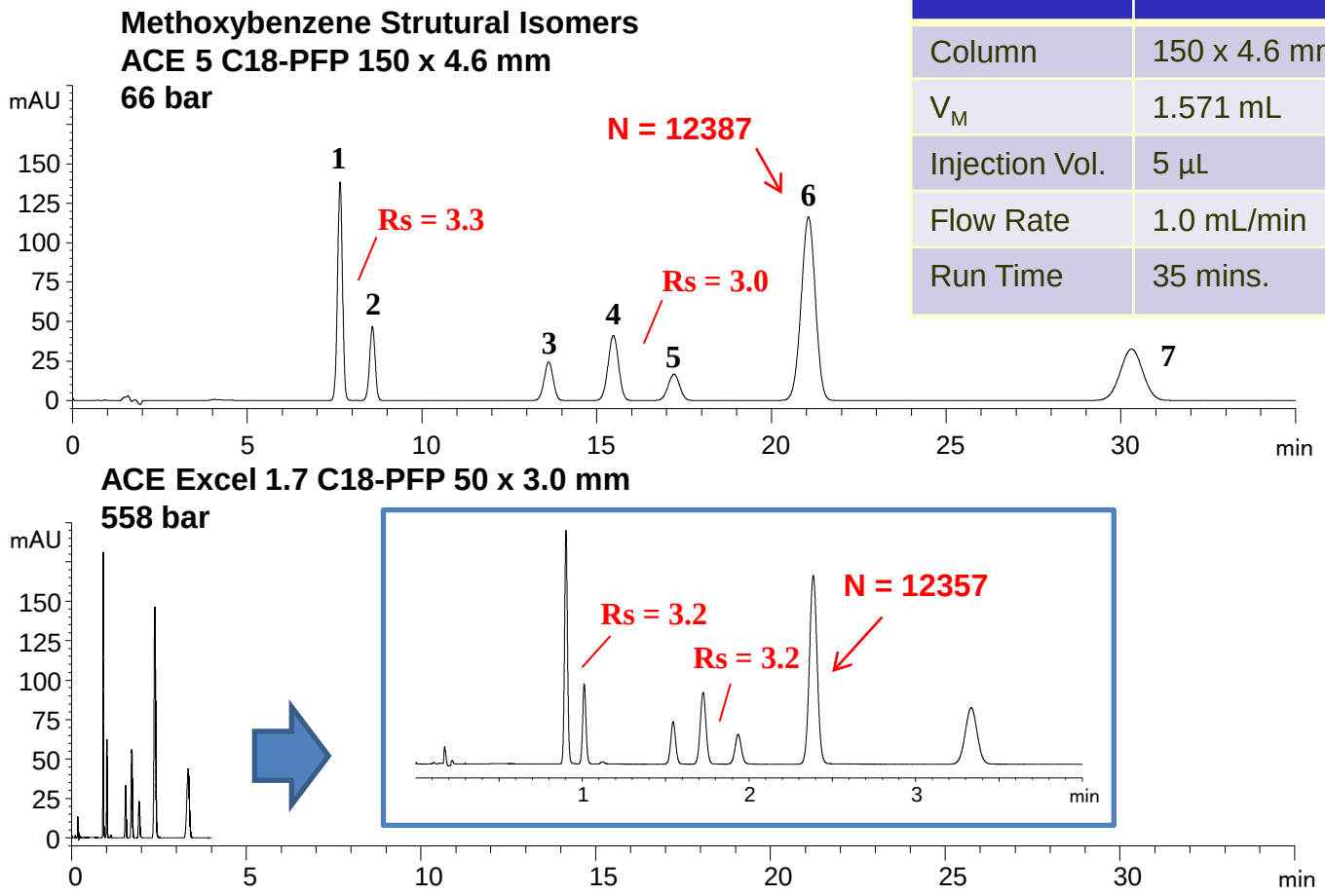
Isocratic Method Translations: HPLC to UHPLC



	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%**

Isocratic Method Translations: HPLC to UHPLC

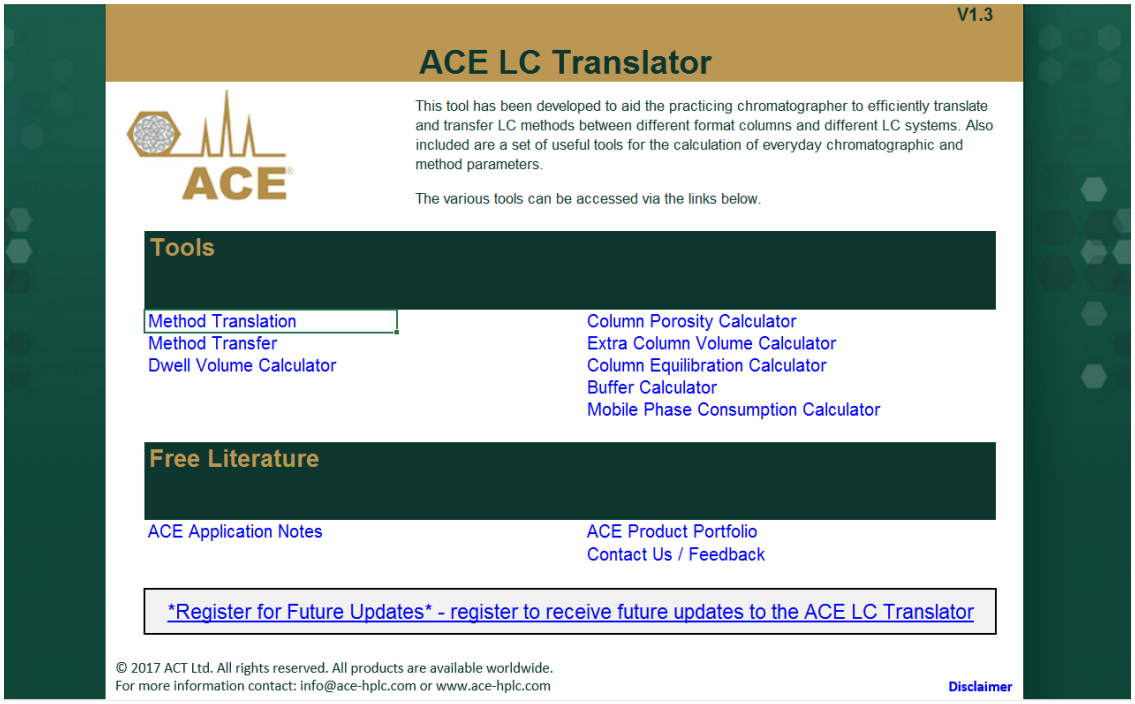


	HPLC Method	UHPLC Method
Column	150 x 4.6 mm, 5µm	50 x 3.0 mm, 1.7µm
V_M	1.571 mL	0.223 mL
Injection Vol.	5 µL	0.7 µL
Flow Rate	1.0 mL/min	1.25 mL/min
Run Time	35 mins.	4 mins

- **Note: the same translation principles are also applicable to UHPLC to HPLC method translations**

ACE LC Translator

- **All of the translation / transfer discussed in this talk have been conveniently combined in the ACE LC Translator**

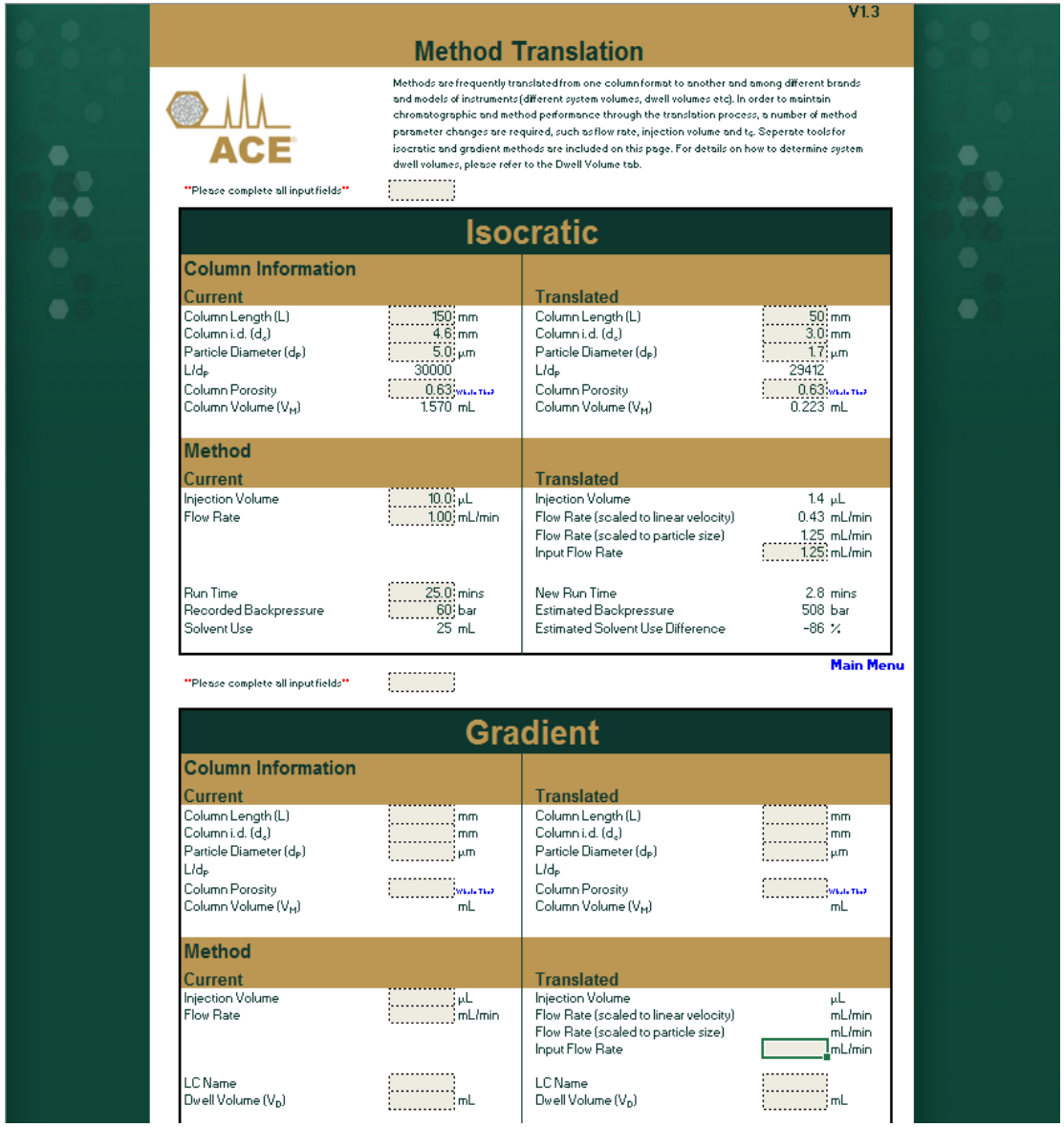


ACE LC Translator

- **All of the translation / transfer discussed in this talk have been conveniently combined in the ACE LC Translator**

- **Download free at:**

  mac-mod.com



V1.3

Method Translation

ACE

Methods are frequently translated from one column format to another and among different brands and models of instruments (different system volumes, dwell volumes etc). In order to maintain chromatographic and method performance through the translation process, a number of method parameter changes are required, such as flow rate, injection volume and t_G. Seperate tools for isocratic and gradient methods are included on this page. For details on how to determine system dwell volumes, please refer to the Dwell Volume tab.

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Isocratic

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	Wide Pore	Column Porosity	0.63	Wide Pore
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
Run Time	25.0	mins	New Run Time	2.8	mins
Recorded Backpressure	60	bar	Estimated Backpressure	508	bar
Solvent Use	25	mL	Estimated Solvent Use Difference	-86	%

Main Menu

"Please complete all input fields"

Gradient

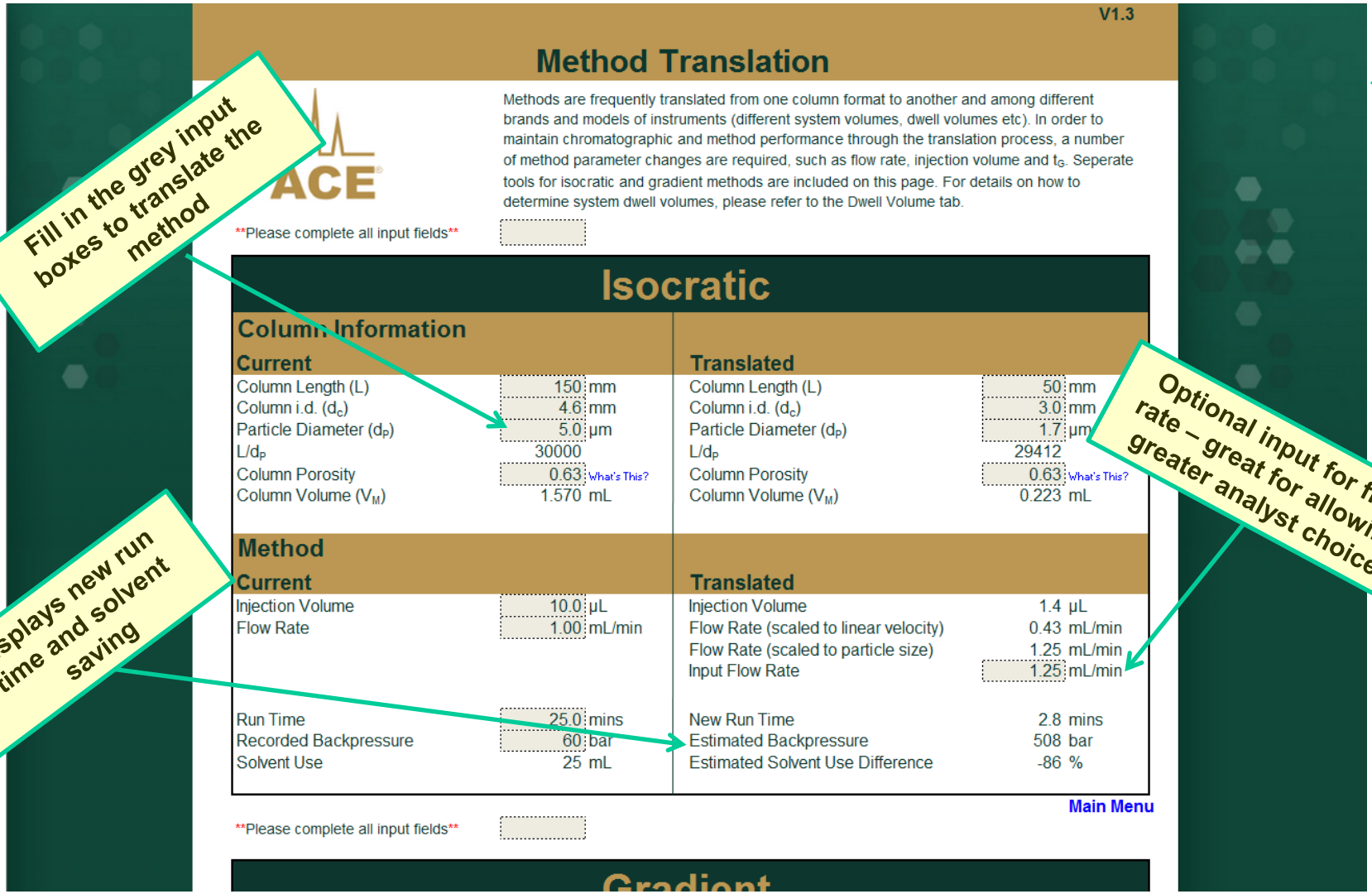
Column Information

Current			Translated		
Column Length (L)		mm	Column Length (L)		mm
Column i.d. (d_c)		mm	Column i.d. (d_c)		mm
Particle Diameter (d_p)		µm	Particle Diameter (d_p)		µm
L/d_p			L/d_p		
Column Porosity		Wide Pore	Column Porosity		Wide Pore
Column Volume (V_M)		mL	Column Volume (V_M)		mL

Method

Current			Translated		
Injection Volume		µL	Injection Volume		µL
Flow Rate		mL/min	Flow Rate (scaled to linear velocity)		mL/min
			Flow Rate (scaled to particle size)		mL/min
			Input Flow Rate		mL/min
LC Name			LC Name		
Dwell Volume (V_D)		mL	Dwell Volume (V_D)		mL

Method Translation Tool Example - Isocratic



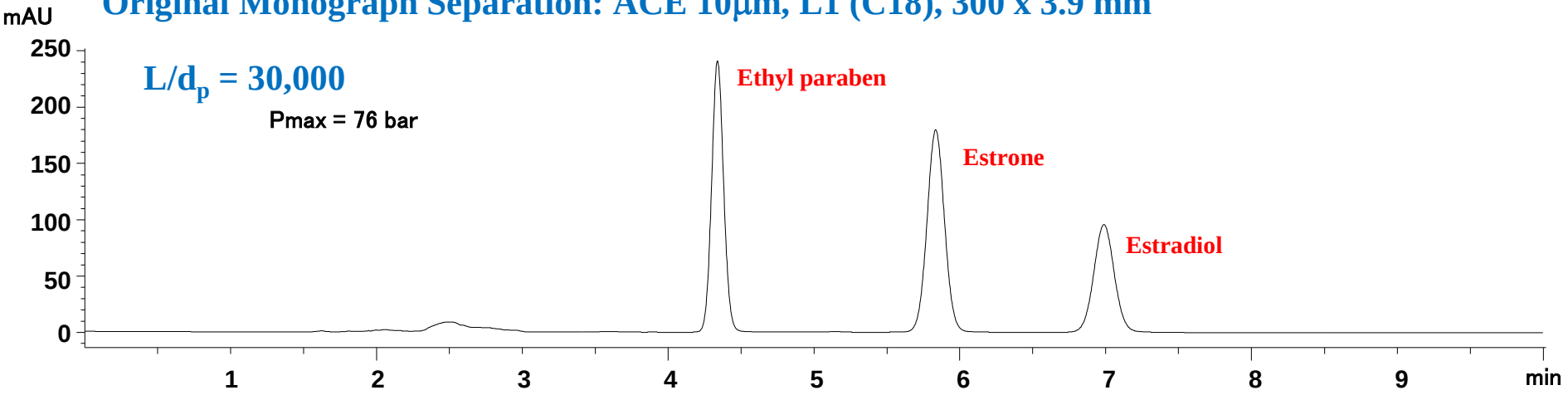
Isocratic Method Translations: Pharmacopeial Methods

- **Revised guidance on allowable parameter changes recently updated for USP <621> chapter***
  - **Significant update to the general chapter – reading recommended**
  - **Isocratic monograph LC methods can be -25% to +50% of the original L/d_p according to the USP guidance**
  - **Also, L/d_p ratios outside this range can be -25% to +50% of the original peak efficiency, N ← large flexibility**
- **Very few changes / flexibility for gradient monograph methods**
- **When translating to smaller columns dimensions (and therefore smaller column volume), system dispersion effects are important**

*** www.usp.org. New guidance in general chapter USP <621> issued August 2014.**

Method Translations: Isocratic Estradiol Porous → Porous (I)

Original Monograph Separation: ACE 10µm, L1 (C18), 300 x 3.9 mm



Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C, 1 mL/min, 25 µL injection

1. **Find suitable 'new' column: 300x3.9mm, 10µm = L/dp of 30,000 = 150x4.6, 5µm**

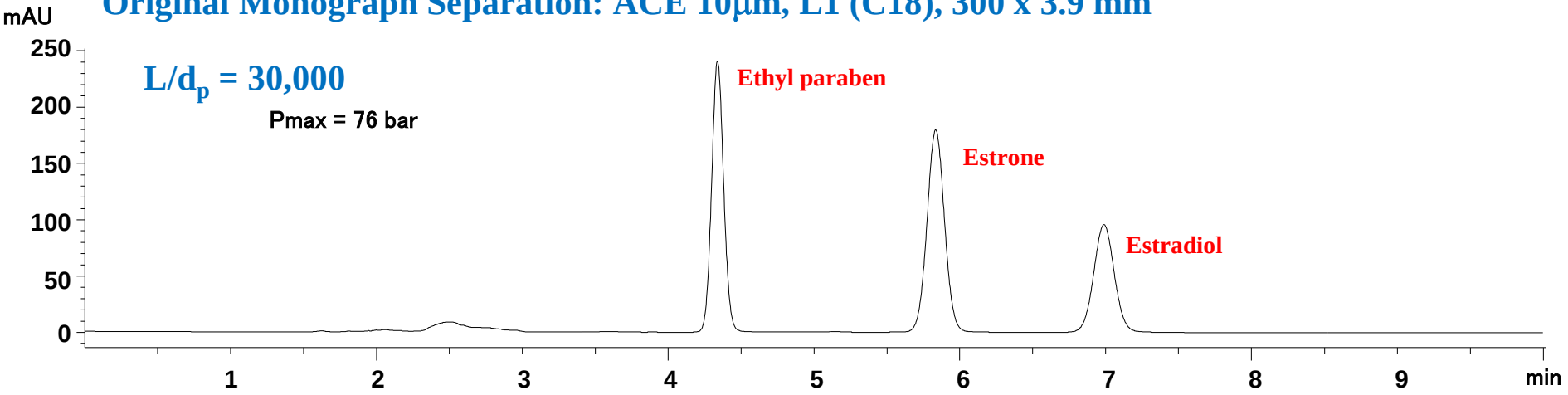
2. **Geometrically scale flow rate:** $F_2 = F_1 \times \frac{{d_{c_2}}^2}{{d_{c_1}}^2}$ **= 1 x 4.6² / 3.9² = 1.39 mL/min**

3. **Volumetrically scale injection:** $Inj_2 = Inj_1 \times \frac{V_{m_2}}{V_{m_1}}$ **= 25 x 1.620 / 2.329 = 17.4 µL**

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

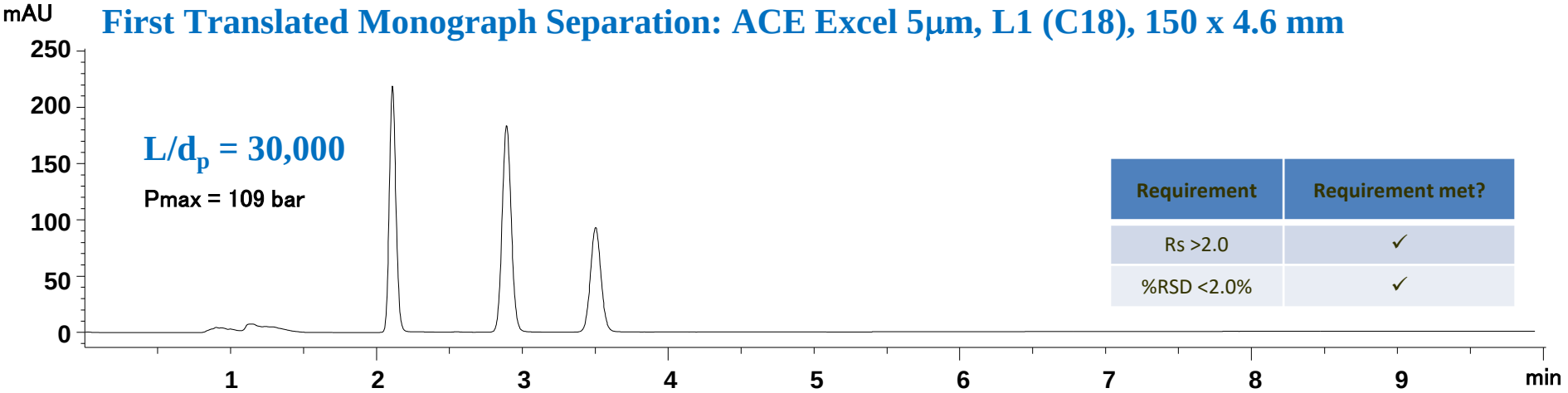
Method Translations: Isocratic Estradiol Porous → Porous (II)

Original Monograph Separation: ACE 10µm, L1 (C18), 300 x 3.9 mm



Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C, 1 mL/min, 25 µL injection

First Translated Monograph Separation: ACE Excel 5µm, L1 (C18), 150 x 4.6 mm



Requirement	Requirement met?
Rs >2.0	✓
%RSD <2.0%	✓

Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C, 1.39 mL/min, 17.4 µL injection

Further examples in A P McKeown, article in Chromatography Today March/April 2015

Method Translations: Isocratic Methods

- **Isocratic monograph LC methods can be -25% to +50% of the original L/d_p according to the USP guidance***

Column Length (mm)	300
Particle Size (um)	10
Lower L/dp (-25%)	22,500
L/dp	30,000
Upper L/dp (+50%)	45,000

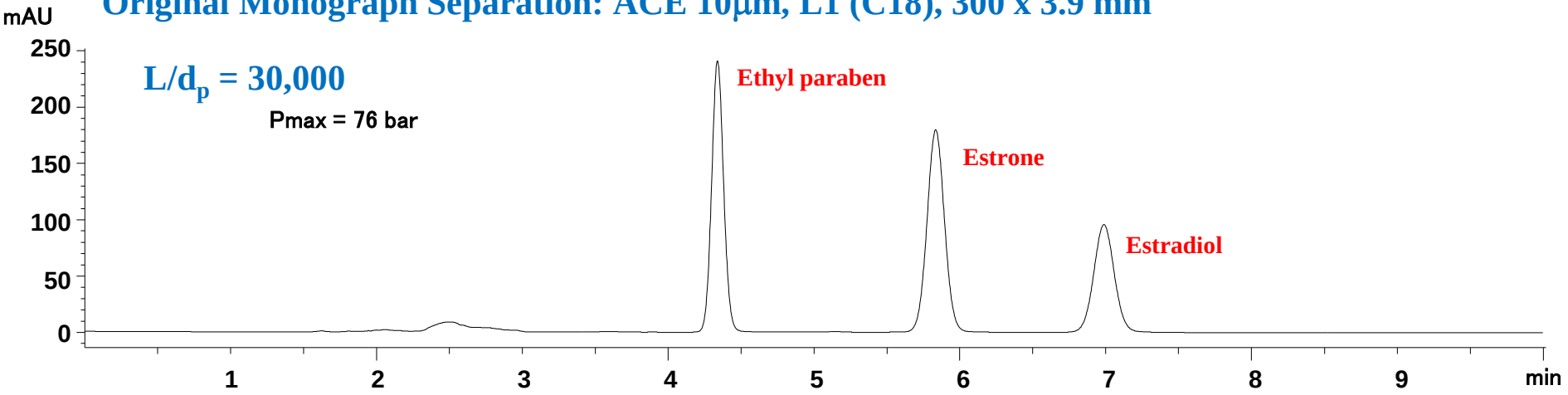
Particle Size (µm)	Column Length (mm) 50	75	100	150	250	300
1.7	29,412	44,118	58,824			
1.8	27,778	41,667	55,556			
1.9	26,316	39,474	52,632			
2	25,000	37,500	50,000	75,000		
2.5	20,000	30,000	40,000	60,000	100,000	
2.6	19,231	28,846	38,462	57,692	96,154	
2.7	18,519	27,778	37,037	55,556	92,593	
3	16,667	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 22,400 to 45,000

* www.usp.org. New guidance in general chapter USP <621> issued August 2014.

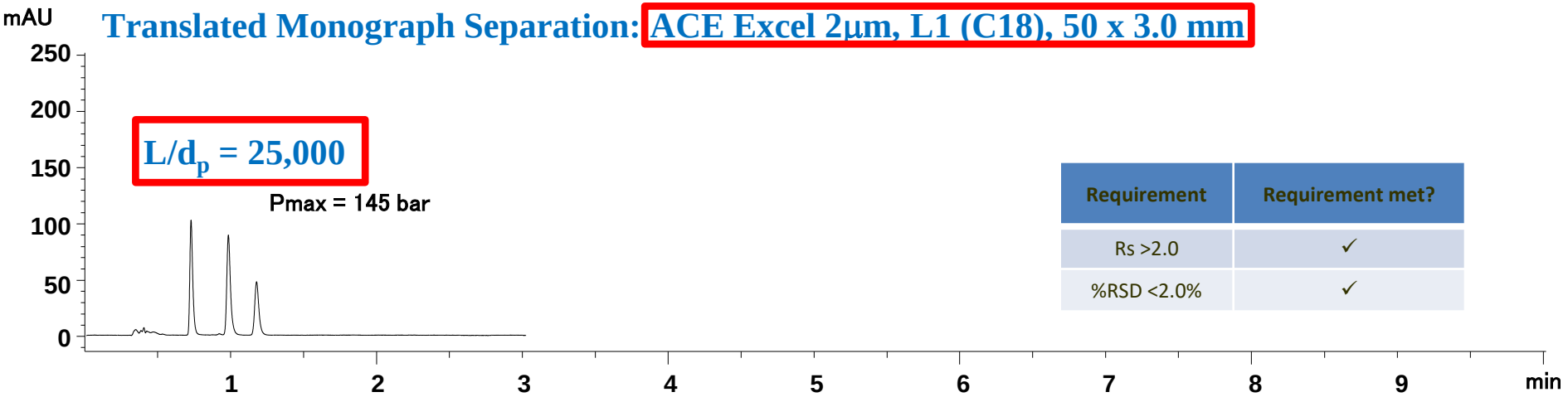
Method Translations: Isocratic Estradiol Porous → Porous (III)

Original Monograph Separation: ACE 10µm, L1 (C18), 300 x 3.9 mm



Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C, 1 mL/min, 25 µL injection

Translated Monograph Separation: ACE Excel 2µm, L1 (C18), 50 x 3.0 mm



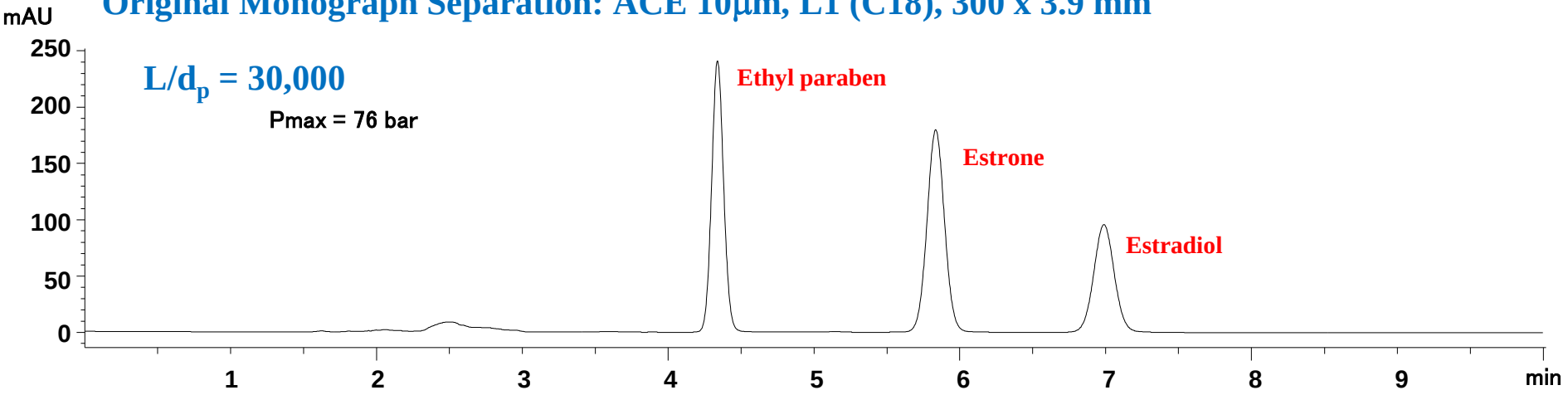
Requirement	Requirement met?
Rs >2.0	✓
%RSD <2.0%	✓

Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C, 0.62 mL/min, 2.4 µL injection

Further examples in A P McKeown, article in Chromatography Today March/April 2015

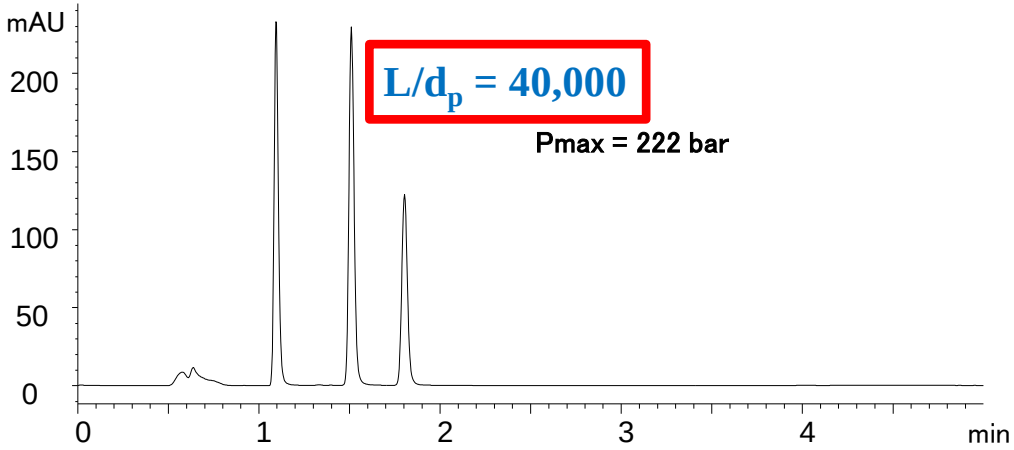
Method Translations: Isocratic Estradiol Porous → Solid Core

Original Monograph Separation: ACE 10µm, L1 (C18), 300 x 3.9 mm



Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C, 1 mL/min, 25 µL injection

Translated Monograph Separation: ACE UltraCore 2.5µm, L1 (SuperC18), 100 x 4.6 mm



Requirement	Requirement met?
Rs >2.0	✓
%RSD <2.0%	✓

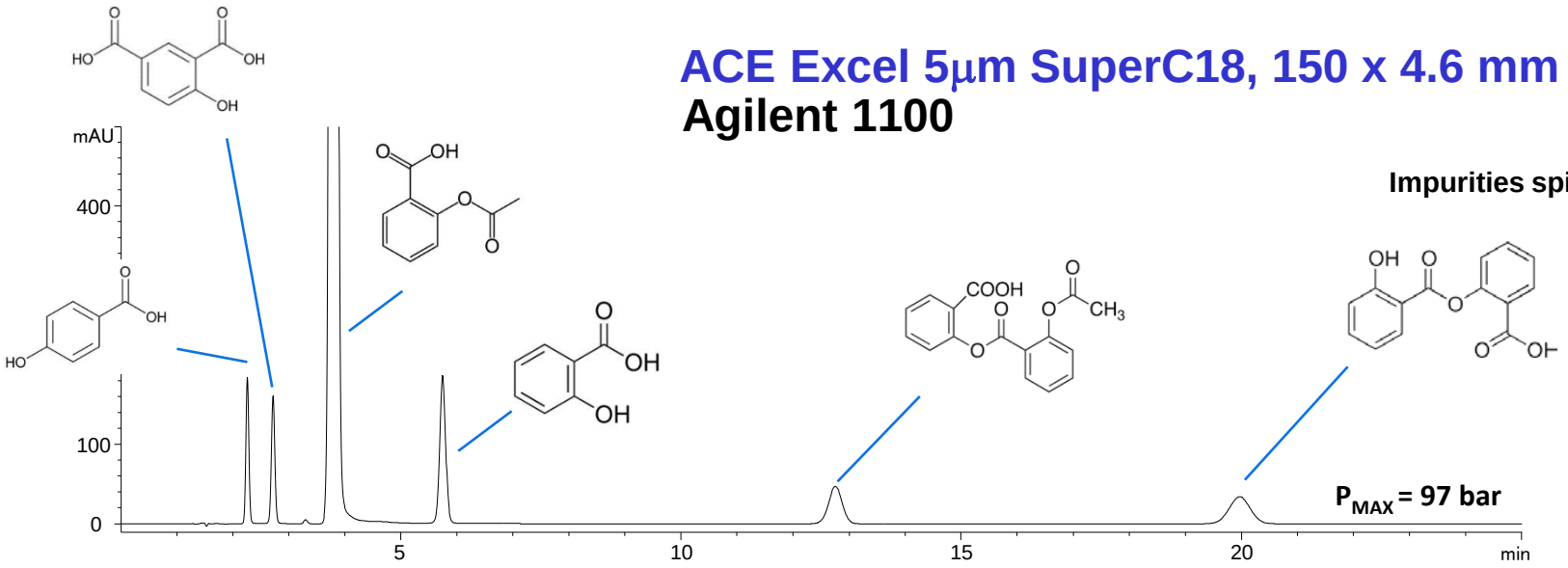
Conditions: 45:55 v/v water:acetonitrile, 205 nm, 22°C, 1.39 mL/min, 10.1 µL injection

- **Look out for negative effects of extra-column band broadening!**

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

Further examples in A P McKeown, article in Chromatography Today March/April 2015

Isocratic Method Translations: Aspirin Porous → Solid Core (I)



ACE Excel 5µm SuperC18, 150 x 4.6 mm
Agilent 1100

Impurities spiked at 0.5%

Translation to ACE UltraCore 5µm SuperC18, 150 x 4.6 mm:

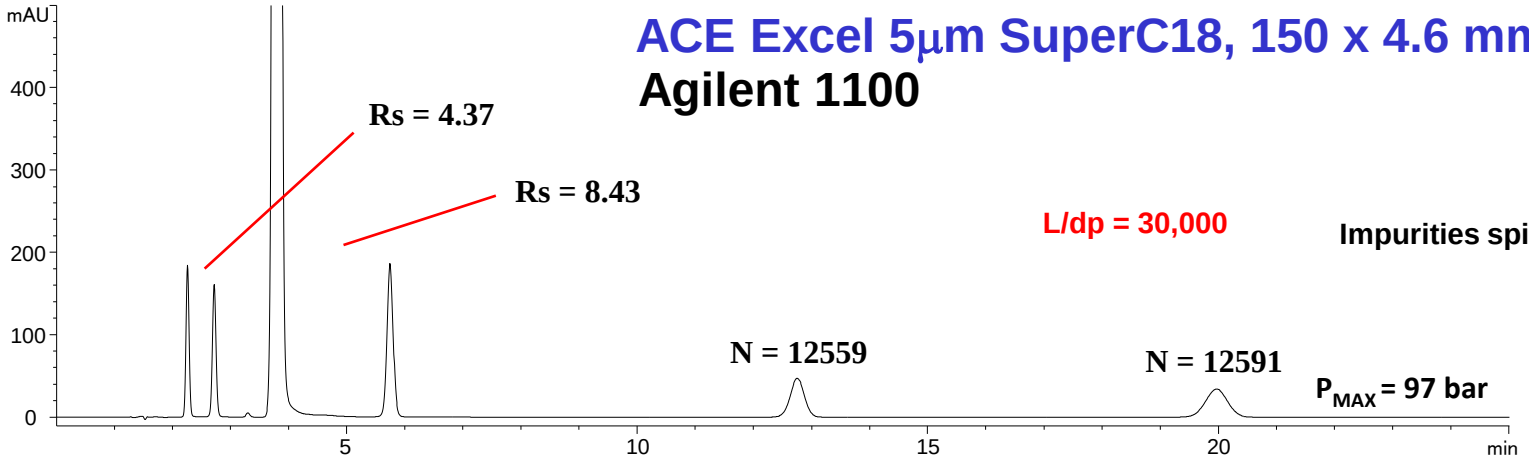
1. **Column dimensions and particle size similar so same flow rate used**
2. **Determine column volumes and scale injection volume**

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

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

Conditions: 60:35:5:0.2 v/v/v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm, 25°C, 1 mL/min, 5 µL injection

Isocratic Method Translations: Aspirin Porous → Solid Core (II)

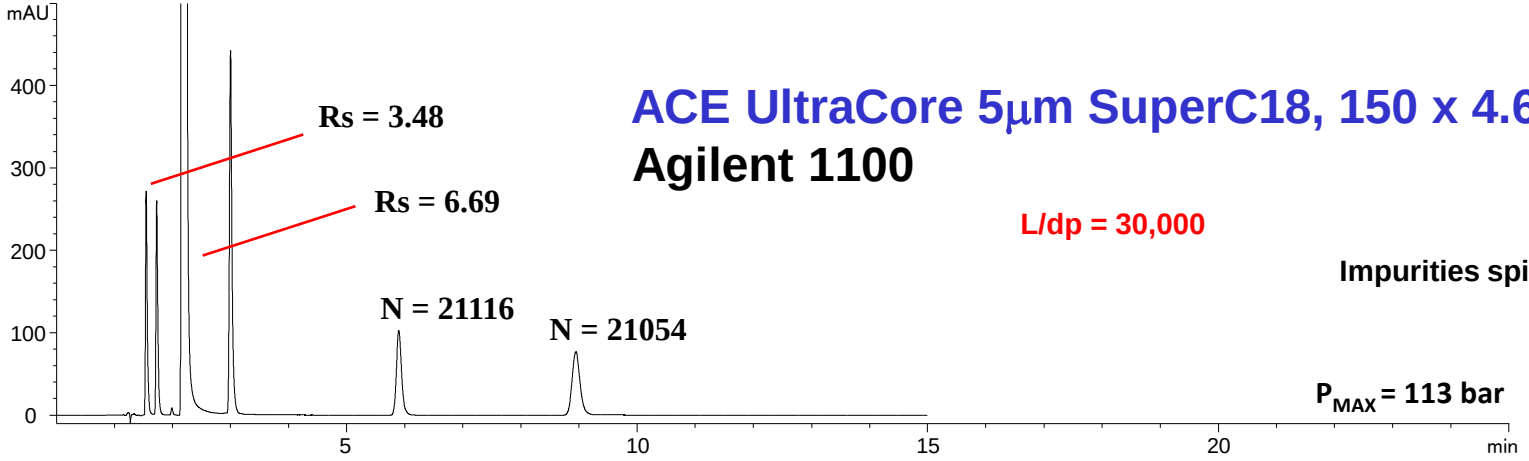


ACE Excel 5µm SuperC18, 150 x 4.6 mm
Agilent 1100

L/dp = 30,000

Impurities spiked at 0.5%

P_{MAX} = 97 bar



ACE UltraCore 5µm SuperC18, 150 x 4.6 mm
Agilent 1100

L/dp = 30,000

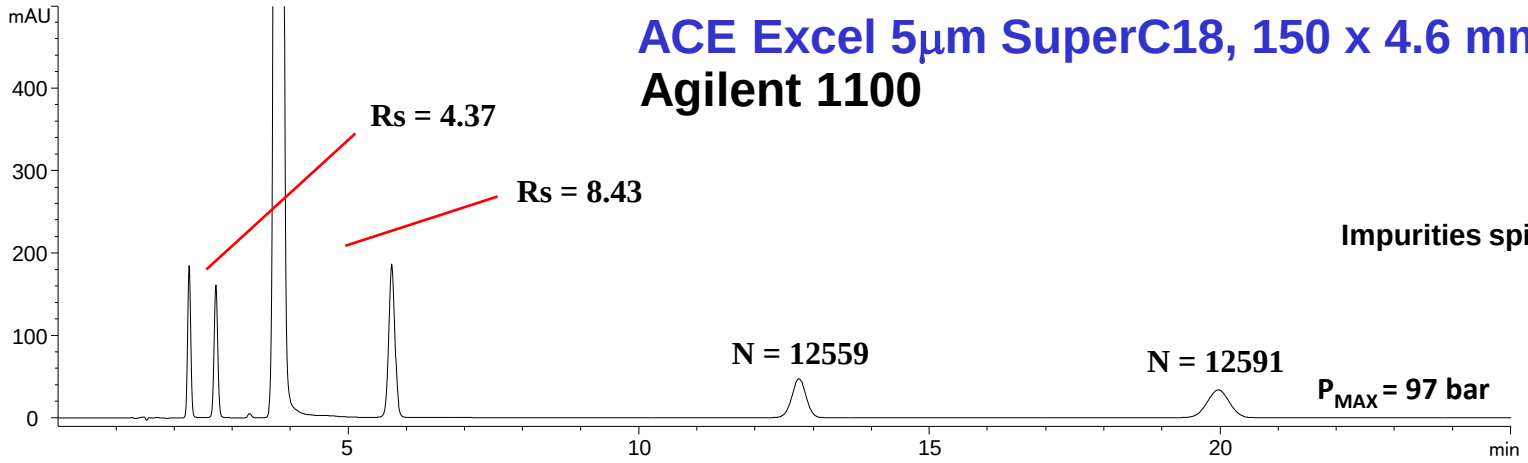
Impurities spiked at 0.5%

P_{MAX} = 113 bar

- **Reduced hydrophobicity of solid core particles leads to 'faster' analysis**
- **Can we go faster...? (within allowable range)**

Conditions: (Top): 60:35:5:0.2 v/v/v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm (2.5 Hz), 25°C, 1 mL/min, 5 µL injection
(Bottom): 60:35:5:0.2 v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm (20 Hz), 25°C, 1 mL/min, 3.9 µL injection

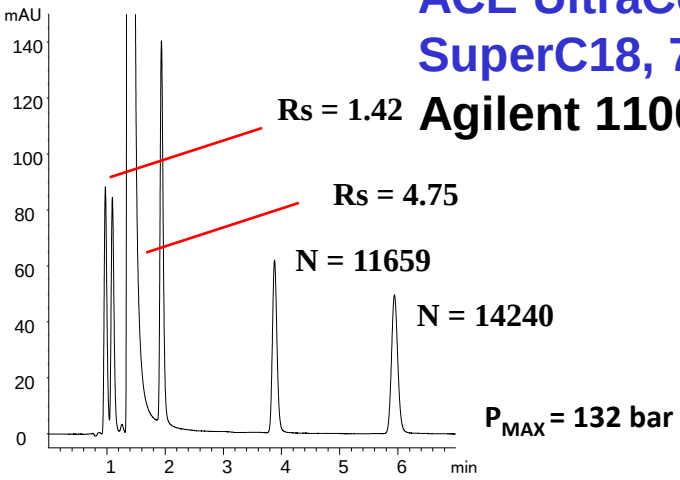
Isocratic Method Translations: Aspirin Porous → Solid Core (III)



ACE Excel 5µm SuperC18, 150 x 4.6 mm L/dp = 30,000
Agilent 1100

Impurities spiked at 0.5%

P_{MAX} = 97 bar



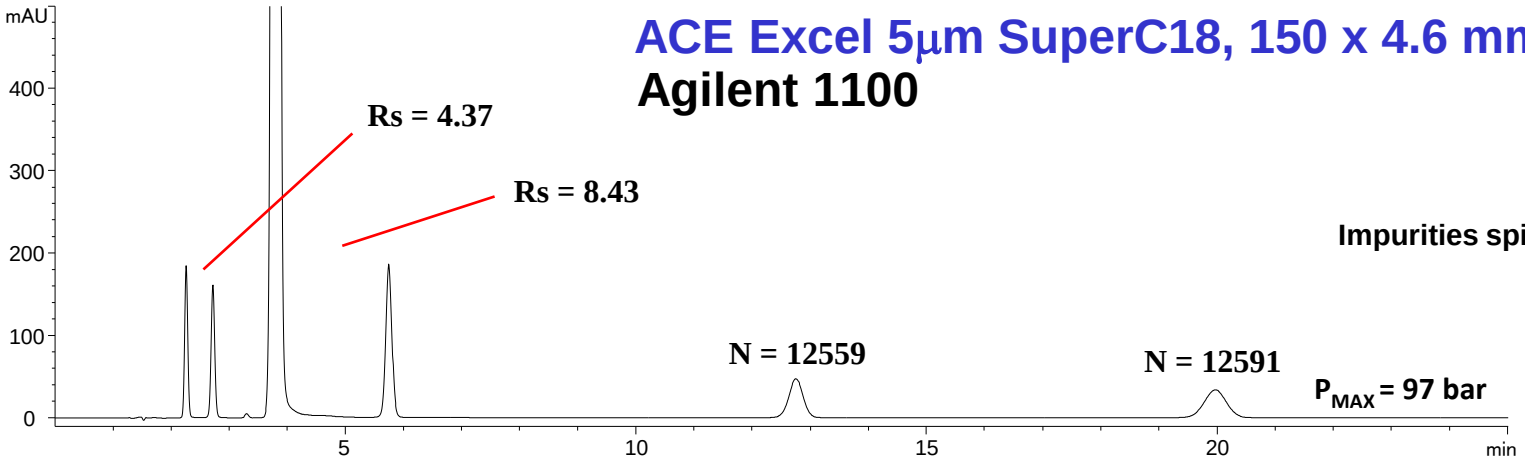
ACE UltraCore 2.5µm SuperC18, 75 x 3.0 mm L/dp = 30,000
Agilent 1100

P_{MAX} = 132 bar

- **N and Rs not maintained. Impact of extra column band broadening of LC system** ☹
- **Increasing i.d. to 4.6 mm (with associated increase in flow and inj. vol.) should help.** ?

Conditions (Top): 60:35:5:0.2 v/v/v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm, 25°C, 1 mL/min, 5 µL injection
(Bottom): 60:35:5:0.2 v/v/v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm (20 Hz), 25°C, 0.43 mL/min, 0.9 µL injection

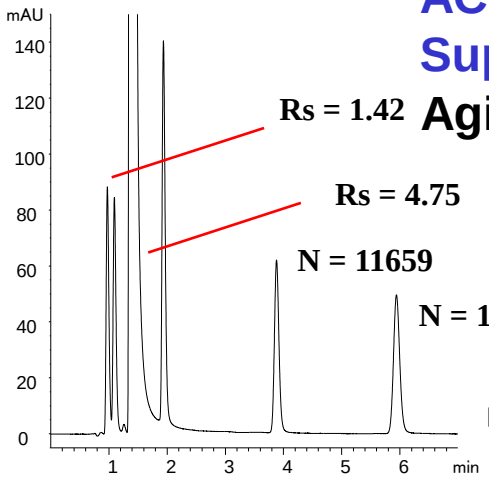
Isocratic Method Translations: Aspirin Porous → Solid Core (IV)



ACE Excel 5µm SuperC18, 150 x 4.6 mm
Agilent 1100

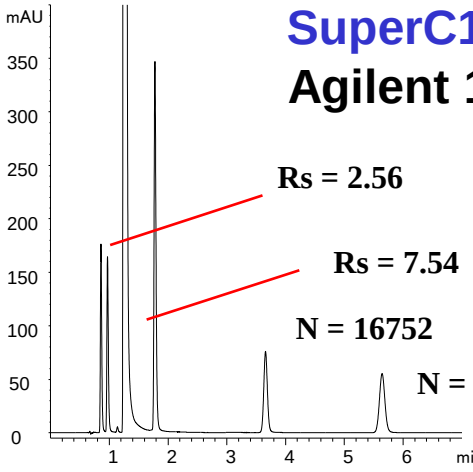
Impurities spiked at 0.5%

P$_{MAX}$ = 97 bar



ACE UltraCore 2.5µm SuperC18, 75 x 3.0 mm
Agilent 1100

P$_{MAX}$ = 132 bar



ACE UltraCore 2.5µm SuperC18, 75 x 4.6 mm
Agilent 1100

P$_{MAX}$ = 146 bar

☺

Conditions **ACE UltraCore 2.5 Super C18 75 x 4.6 mm:**
60:35:5:0.2 v/v/v/v water:acetonitrile:methanol:85% phosphoric acid, 237 nm (20 Hz), 25°C, 1.0 mL/min, 2 µL injection

Gradient Translations

Translations of Gradient Methods – The Basics

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

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

Experimentally determined

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

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

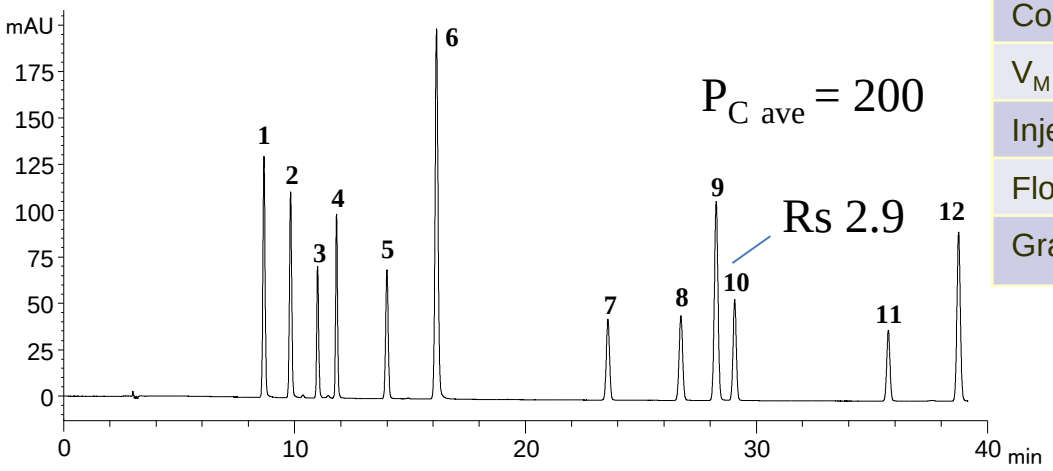
4. **Translate gradient time**
   - To maintain constant k*

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

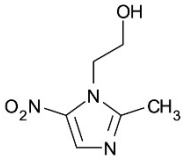
5. **Consider V_D/V_M ratio**

Gradient Method Translations: Porous → Porous

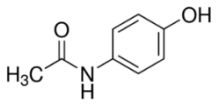
ACE Excel 5 C18-Amide 250 x 4.6 mm, 1 mL/min



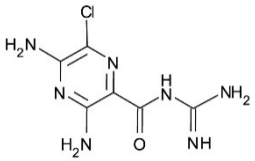
	Original Method
Column	250 x 4.6 mm, 5 µm
V_M	2.668 mL
Injection Vol.	10 µL
Flow Rate	1.0 mL/min
Gradient time	45 min.



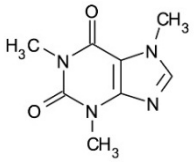
1. Metronidazole



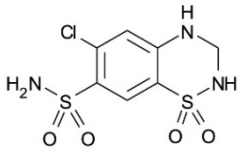
2. 4-acetamidophenol



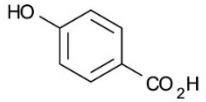
3. amiloride



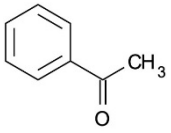
4. caffeine



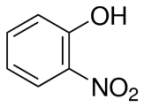
5. Hydrochlorothiazide



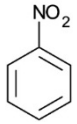
6. 4-Hydroxybenzoic acid



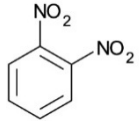
7. Acetophenone



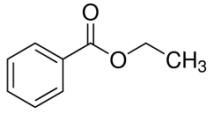
8. 2-nitrophenol



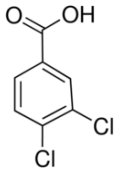
9. Nitrobenzene



10. 1,2-Dinitrobenzene



11. ethylbenzoate

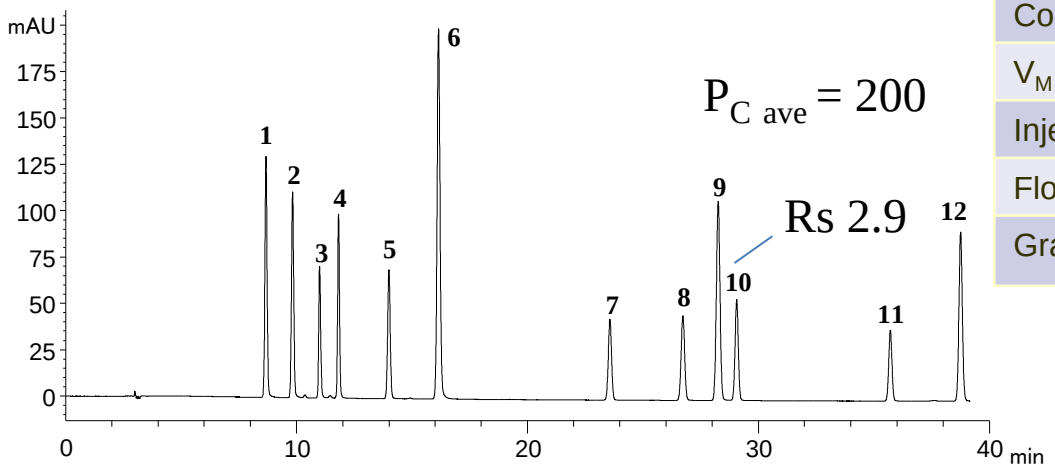


12. 3,4-dichlorobenzoic acid

Gradient analysis, A: 20 mM KH2PO4 pH 2.7 (aq), B: 20 mM KH2PO4 pH 2.7 in MeCN:water 65:35 v/v, 5-100%B in 45.0 mins, hold 100%B for 5.0 mins, 40°C, 1.0 mL/min, 254 nm.
1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2,-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid.

Gradient Method Translations: Porous → Porous

ACE Excel 5 C18-Amide 250 x 4.6 mm, 1 mL/min



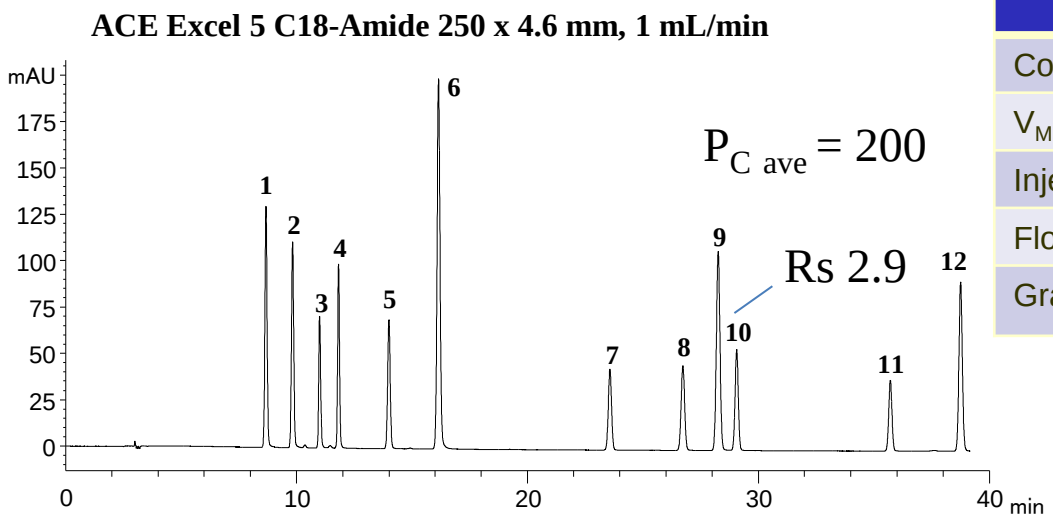
	Original Method
Column	250 x 4.6 mm, 5 µm
V_M	2.668 mL
Injection Vol.	10 µL
Flow Rate	1.0 mL/min
Gradient time	45 min.

- **ACE 5 C18-Amide 250 x 4.6 mm**
  - **L/d_P = 50,000**

V_M = 2.668 mL

Gradient analysis, A: 20 mM KH2PO4 pH 2.7 (aq), B: 20 mM KH2PO4 pH 2.7 in MeCN:water 65:35 v/v, 5-100%B in 45.0 mins, hold 100%B for 5.0 mins, 40°C, 1.0 mL/min, 254 nm.
1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2,-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid.

Gradient Method Translations: Porous → Porous



	Original Method	Translated Method
Column	250 x 4.6 mm, 5 µm	**150 x 4.6 mm, 3 µm**
V_M	2.668 mL	**1.571 mL**
Injection Vol.	10 µL	
Flow Rate	1.0 mL/min	
Gradient time	45 min.	

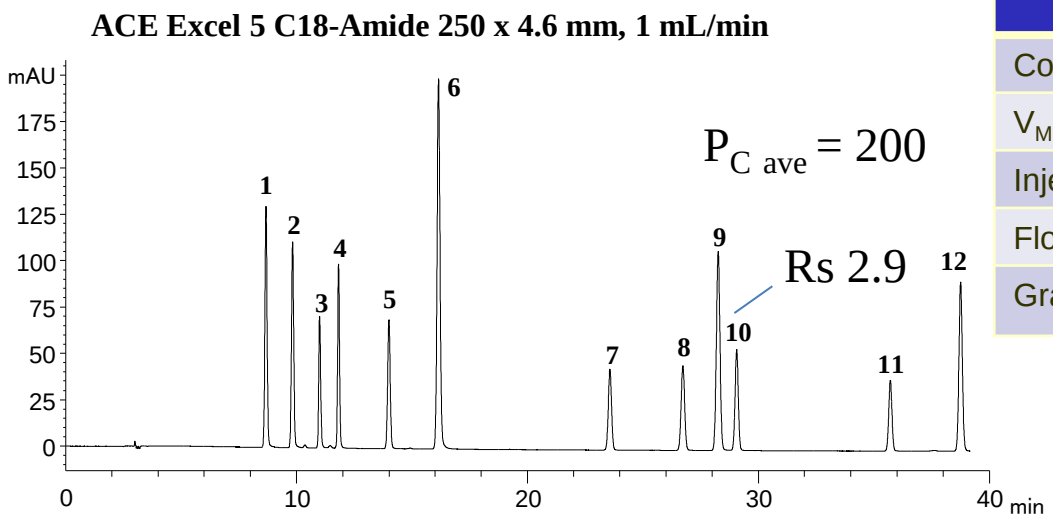
- **ACE 5 C18-Amide 250 x 4.6 mm** — **V_M = 2.668 mL**
  - **L/d_P = 50,000**
- **ACE 3 C18-Amide 150 x 4.6 mm** — **V_M = 1.571 mL**
  - **L/d_P = 50,000**
- **1. V_M experimentally determined**
  - **Or use**

$$V_M = \pi \left(\frac{d_c}{2}\right)^2 L\varepsilon$$

- **ε = column porosity = ~0.63 for fully porous, ~0.55 for solid core**

Gradient analysis, A: 20 mM KH2PO4 pH 2.7 (aq), B: 20 mM KH2PO4 pH 2.7 in MeCN:water 65:35 v/v, 5-100%B in 45.0 mins, hold 100%B for 5.0 mins, 40°C, 1.0 mL/min, 254 nm.
1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2,-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid.

Gradient Method Translations: Porous → Porous



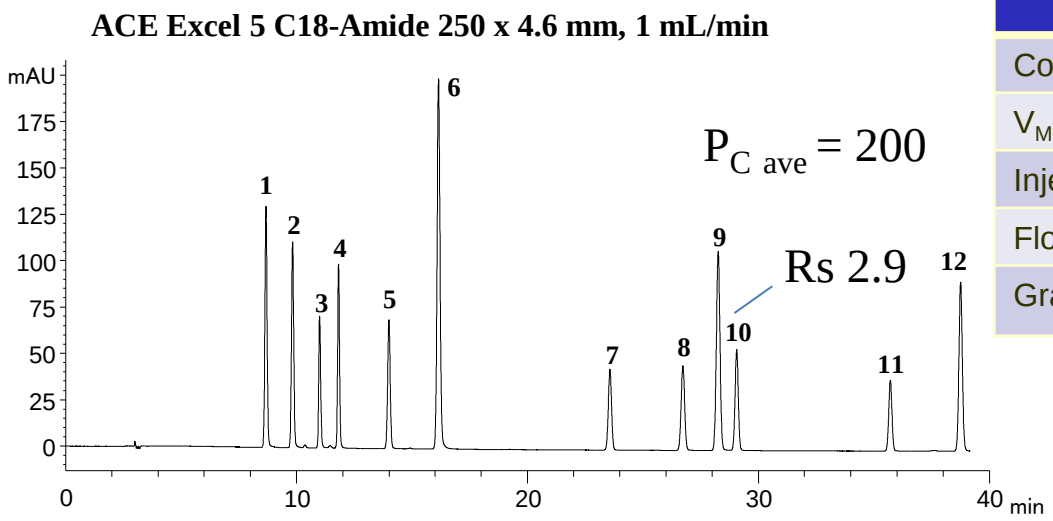
	Original Method	Translated Method
Column	250 x 4.6 mm, 5 µm	150 x 4.6 mm, 3 µm
V_M	2.668 mL	1.571 mL
Injection Vol.	10 µL	**5.9 µL**
Flow Rate	1.0 mL/min	
Gradient time	45 min.	

2. Translate injection volume

$$V_{i2} = \frac{V_{i1} \times V_{M2}}{V_{M1}} = \frac{10 \times 1.571}{2.668} = 5.9\ \mu L$$

Gradient analysis, A: 20 mM KH2PO4 pH 2.7 (aq), B: 20 mM KH2PO4 pH 2.7 in MeCN:water 65:35 v/v,
5-100%B in 45.0 mins, hold 100%B for 5.0 mins, 40°C, 1.0 mL/min, 254 nm.
1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2,-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid.

Gradient Method Translations: Porous → Porous



	Original Method	Translated Method
Column	250 x 4.6 mm, 5 µm	150 x 4.6 mm, 3 µm
V_M	2.668 mL	1.571 mL
Injection Vol.	10 µL	5.9 µL
Flow Rate	1.0 mL/min	**1.67 mL/min**
Gradient time	45 min.	

2. Translate injection volume

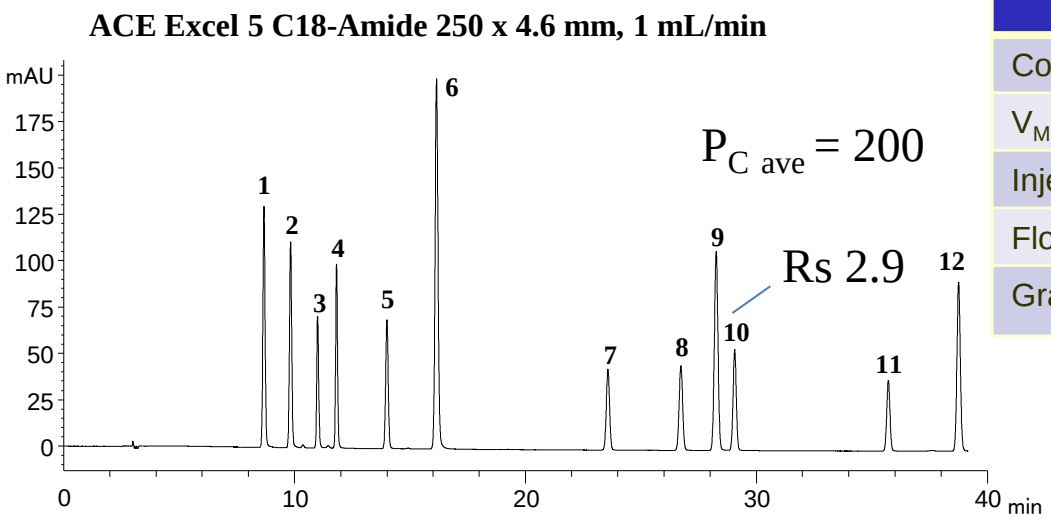
$$V_{i2} = \frac{V_{i1} \times V_{M2}}{V_{M1}} = \frac{10 \times 1.571}{2.668} = 5.9\ \mu L$$

3. Translate flow rate

$$F_2 = \frac{F_1 \times d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}} = \frac{1.00 \times 4.6^2 \times 5}{4.6^2 \times 3} = 1.67\ mL/min$$

Gradient analysis, A: 20 mM KH2PO4 pH 2.7 (aq), B: 20 mM KH2PO4 pH 2.7 in MeCN:water 65:35 v/v, 5-100%B in 45.0 mins, hold 100%B for 5.0 mins, 40°C, 1.0 mL/min, 254 nm.
1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2,-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid.

Gradient Method Translations: Porous → Porous



	Original Method	Translated Method
Column	250 x 4.6 mm, 5 µm	150 x 4.6 mm, 3 µm
V_M	2.668 mL	1.571 mL
Injection Vol.	10 µL	5.9 µL
Flow Rate	1.0 mL/min	1.67 mL/min
Gradient time	45 min.	**15.87 min.**

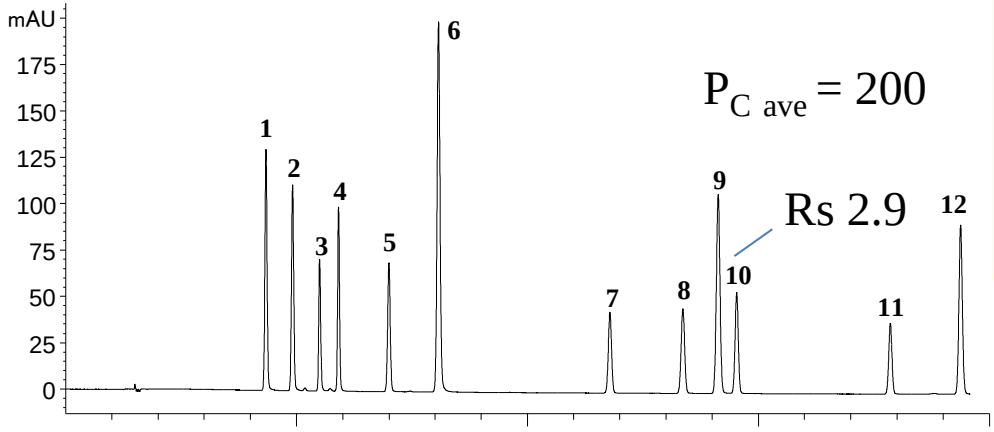
3. Translate gradient time

$$t_{G2} = \frac{t_{G1} V_{M2} F_1}{V_{M1} F_2} = \frac{45 \times 1.571 \times 1.0}{2.668 \times 1.67} = 15.87\ min.$$

Gradient analysis, A: 20 mM KH2PO4 pH 2.7 (aq), B: 20 mM KH2PO4 pH 2.7 in MeCN:water 65:35 v/v, 5-100%B in 45.0 mins, hold 100%B for 5.0 mins, 40°C, 1.0 mL/min, 254 nm.
1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2,-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid.

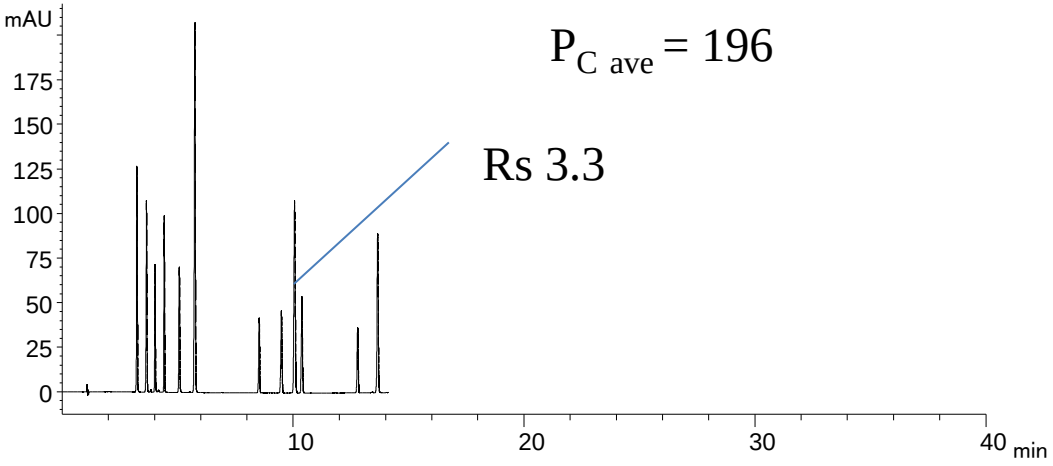
Gradient Method Translations: Porous → Porous

ACE Excel 5 C18-Amide 250 x 4.6 mm, 1 mL/min



	Original Method	Translated Method
Column	250 x 4.6 mm, 5 µm	150 x 4.6 mm, 3 µm
V_M	2.668 mL	1.571 mL
Injection Vol.	10 µL	5.9 µL
Flow Rate	1.0 mL/min	1.67 mL/min
Gradient time	45 min.	15.87 min.

ACE Excel 3 C18-Amide 150 x 4.6 mm, 1.67 mL/min



Total run time: **-64.7%**

Solvent consumption: **-41%**

Gradient analysis, A: 20 mM KH2PO4 pH 2.7 (aq), B: 20 mM KH2PO4 pH 2.7 in MeCN:water 65:35 v/v, 5-100%B in 45.0 mins, hold 100%B for 5.0 mins, 40°C, 1.0 mL/min, 254 nm.
1. metronidazole, 2. 4-acetamidophenol, 3. amiloride, 4. caffeine, 5. hydrochlorothiazide, 6. 4-hydroxybenzoic acid, 7. acetophenone, 8. 2-nitrophenol, 9. nitrobenzene, 10. 1,2,-dinitrobenzene, 11. ethylbenzoate, 12. 3,4-dichlorobenzoic acid.

Step 1. Calculate Column Volumes

- **Due to column volume differences with porous and solid core columns, errors can creep into gradient translation work**

	Porous (0.63) column volumes							
				Column Length				
		20mm	30mm	50mm	75mm	100mm	150mm	250mm
	1mm	0.010	0.015	0.025	0.037	0.049	0.074	0.124
Col. i.d.	2.1mm	0.044	0.065	0.109	0.164	0.218	0.327	0.546
	3.0mm	0.089	0.134	0.223	0.334	0.445	0.668	1.113
	4.6mm	0.209	0.314	0.523	0.785	1.047	1.570	2.617
	Solid core (0.55) column volumes							
				Column Length				
		20mm	30mm	50mm	75mm	100mm	150mm	250mm
	1mm	0.009	0.013	0.022	0.032	0.043	0.065	0.108
Col. i.d.	2.1mm	0.038	0.057	0.095	0.143	0.190	0.286	0.476
	3.0mm	0.078	0.117	0.194	0.292	0.389	0.583	0.972
	4.6mm	0.183	0.274	0.457	0.686	0.914	1.371	2.285

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

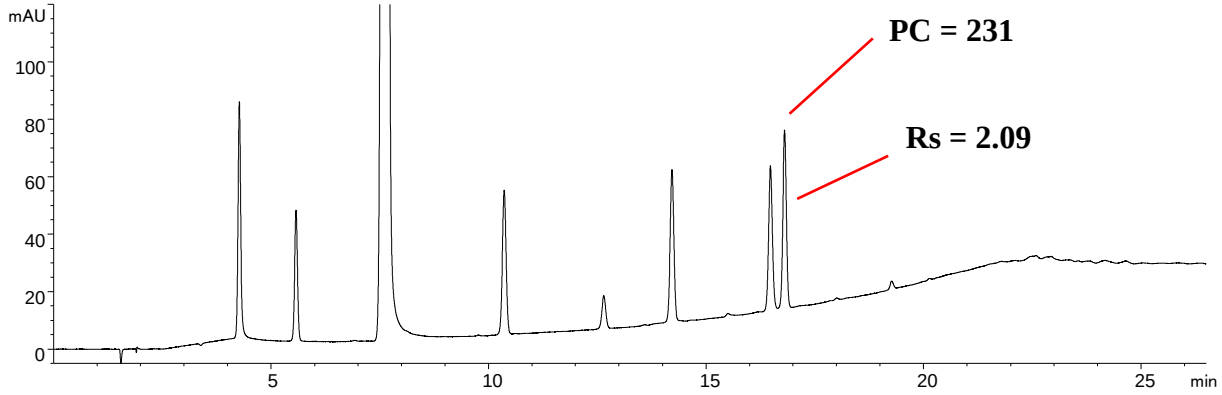
Porosity values:
porous = 0.63
solid core = 0.55

- **Solid core particle columns have ~13% LESS column volume than porous particle columns**

- **Better to experimentally determine porosity for vendor column**

Gradient Method Translations: Acetaminophen Porous → Solid Core

ACE Excel 5µm SuperC18 150 x 4.6 mm



t_G	20 min
Flow:	1 mL/min
Inj. Vol.	5 µL
P_{MAX}	138 bar

$$Peak\ Capacity = 1 + \frac{t_G}{W_{0.5}}$$

Translation to ACE UltraCore 5µm SuperC18 150 x 4.6 mm:

1. Calculate column volumes...solid core porosity differences
2. Translate gradient time $\frac{t_{G1}F_1}{V_{M1}} = \frac{t_{G2}F_2}{V_{M2}}$
3. No flow rate change needed $F_2 = F_1 \times \frac{{d_{c_2}}^2}{{d_{c_1}}^2}$
4. Translate injection volume $Inj_2 = Inj_1 \times \left(\frac{V_{m2}}{V_{m1}}\right)$
5. Calculate whether an injection hold or pre-injection is needed

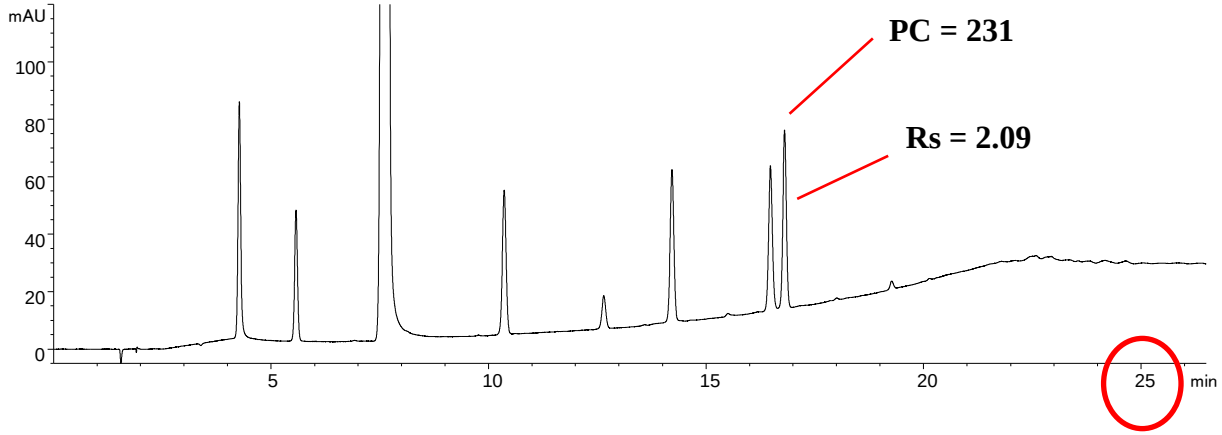
$$V_M \approx \pi \left(\frac{d}{2}\right)^2 L\varepsilon$$

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

150x4.6mm, 5 µm, gradient analysis, A= 20mM ammonium acetate pH 6.0 (aq), B= 20mM ammonium acetate pH 6.0 in MeCN:water 9:1 v/v, 5-95%B in 20.0 mins, hold 95%B for 5.0 mins, 30°C, 1.0 mL/min, 230 nm.
1. 4-aminophenol 2. Hydroquinone 3. acetaminophen 4. 4-acetamidophenol 5. phenol 6. 4-nitrophenol 7. 2-nitrophenol 8. 4-chloroacetanilide

Gradient Method Translations: Acetaminophen Porous → Solid Core

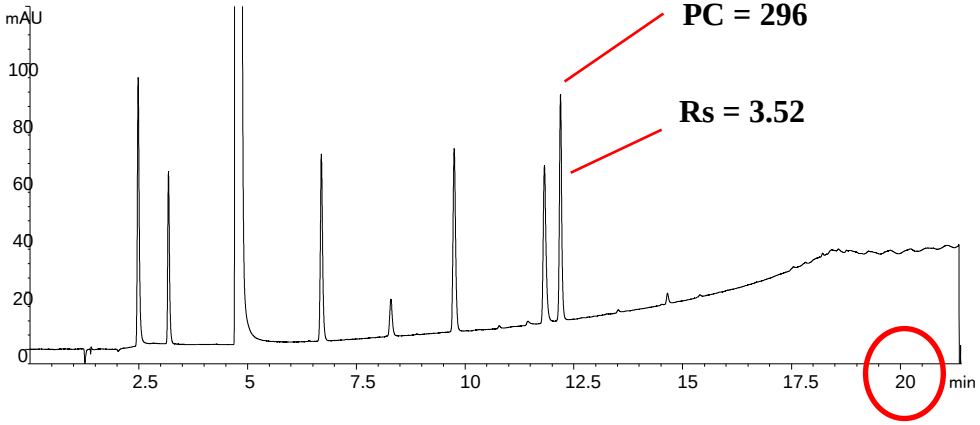
ACE Excel 5µm SuperC18 150 x 4.6 mm



t_G	20 min
Flow:	1 mL/min
Inj. Vol.	5 µL
P_{MAX}	138 bar

$$Peak\ Capacity = 1 + \frac{t_G}{W_{0.5}}$$

ACE UltraCore 5µm SuperC18 150 x 4.6 mm



t_G	16.4 min
Flow:	1 mL/min
Inj. Vol.	3.9 µL
P_{MAX}	159 bar

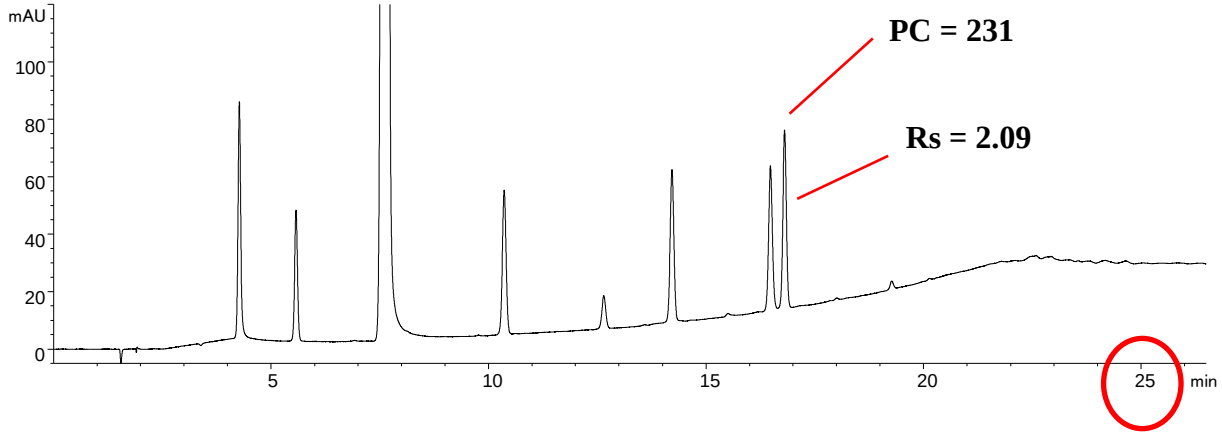
Corrected
- Vm (porosity)
- tG adjusted for constant k*
- Injection volume adjusted

Run time reduced whilst peak capacity and resolution are increased

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

Gradient Method Translations: Acetaminophen Porous → Solid Core

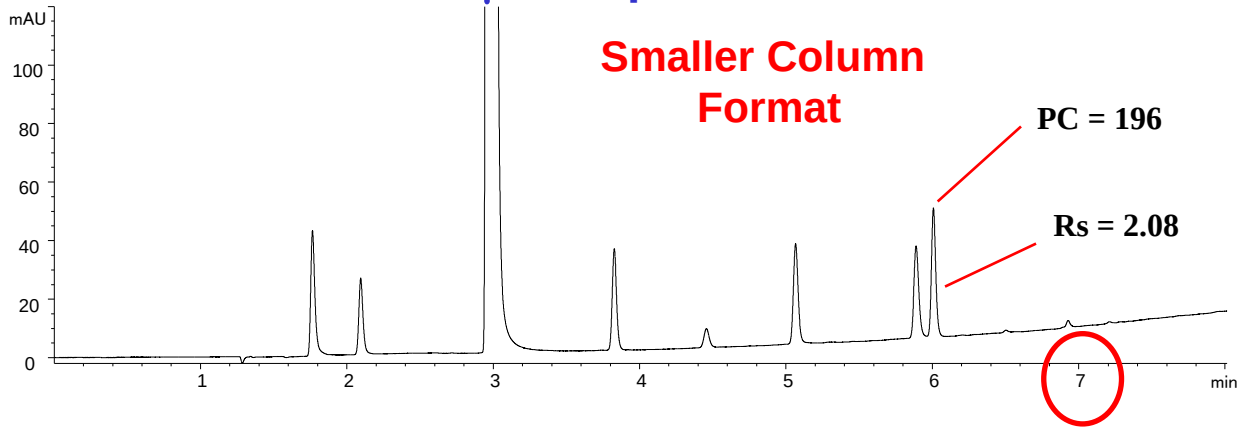
ACE Excel 5µm SuperC18 150 x 4.6 mm



t_G	20 min
Flow:	1 mL/min
Inj. Vol.	5 µL
P_{MAX}	138 bar

$$Peak\ Capacity = 1 + \frac{t_G}{W_{0.5}}$$

ACE UltraCore 2.5µm SuperC18 50 x 3.0 mm



t_G	5.92 min
Flow:	0.43 mL/min
Inj. Vol.	0.63 µL
P_{MAX}	162 bar

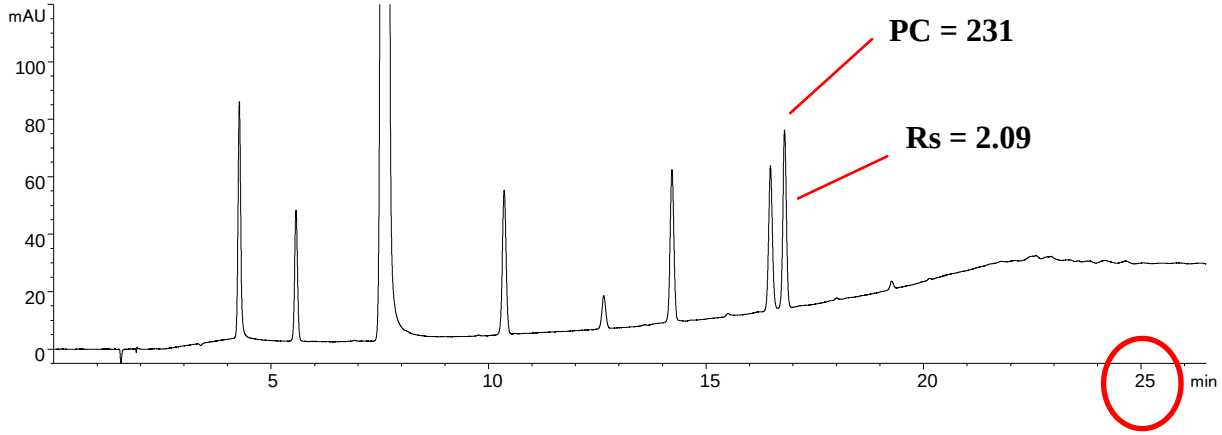
Corrected
- Vm (porosity)
- Constant k* (tG & F)
- Injection volume (Vm)

Significant reduction in run time whilst maintaining resolution

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

Gradient Method Translations: Acetaminophen Porous → Solid Core

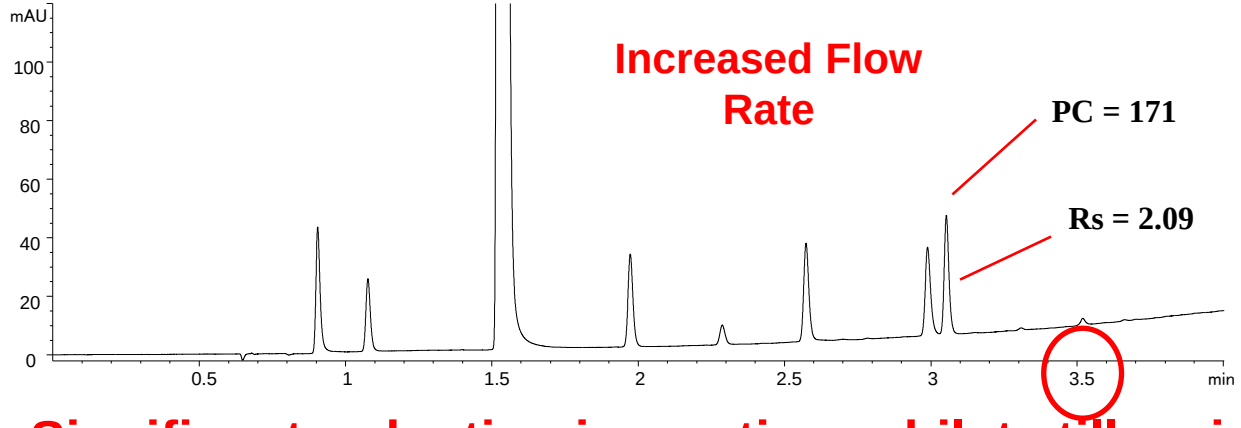
ACE Excel 5µm SuperC18 150 x 4.6 mm



t_G	20 min
Flow:	1 mL/min
Inj. Vol.	5 µL
P_{MAX}	138 bar

$$Peak\ Capacity = 1 + \frac{t_G}{W_{0.5}}$$

ACE UltraCore 2.5µm SuperC18 50 x 3.0 mm



t_G	2.99 min
Flow:	0.85 mL/min
Inj. Vol.	0.63 µL
P_{MAX}	315 bar

Corrected
- Constant k* (tG & F)

Significant reduction in run time whilst still maintaining resolution

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

Gradient Method Translations: Correcting for V_D/V_M

- **It is important when translating to smaller i.d. (therefore small V_M) UHPLC columns to consider the V_D/V_M ratio.**

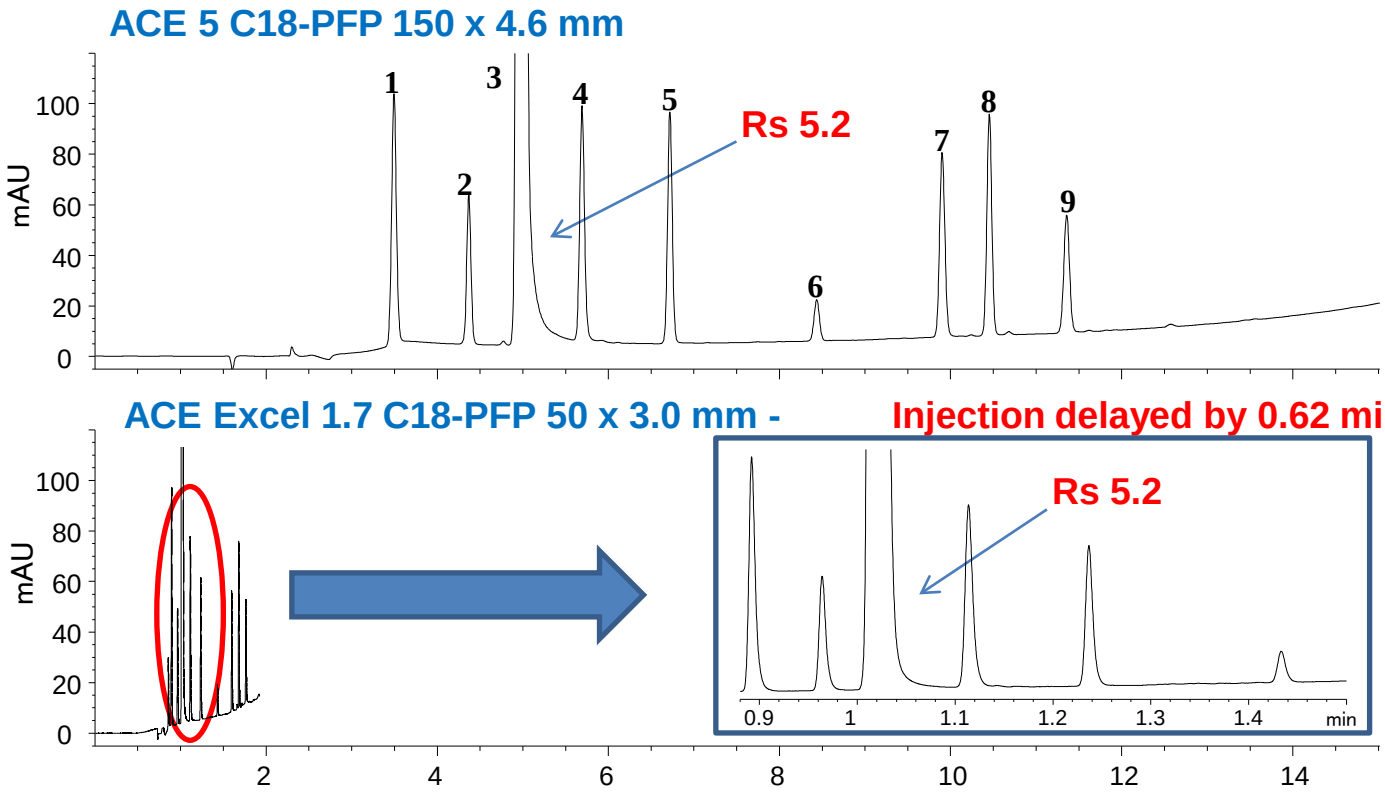
$$\Delta = \left(\frac{V_D}{V_M}\right)_{original} - \left(\frac{V_D}{V_M}\right)_{new} \quad must\ approach\ zero$$

- **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.**
- **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

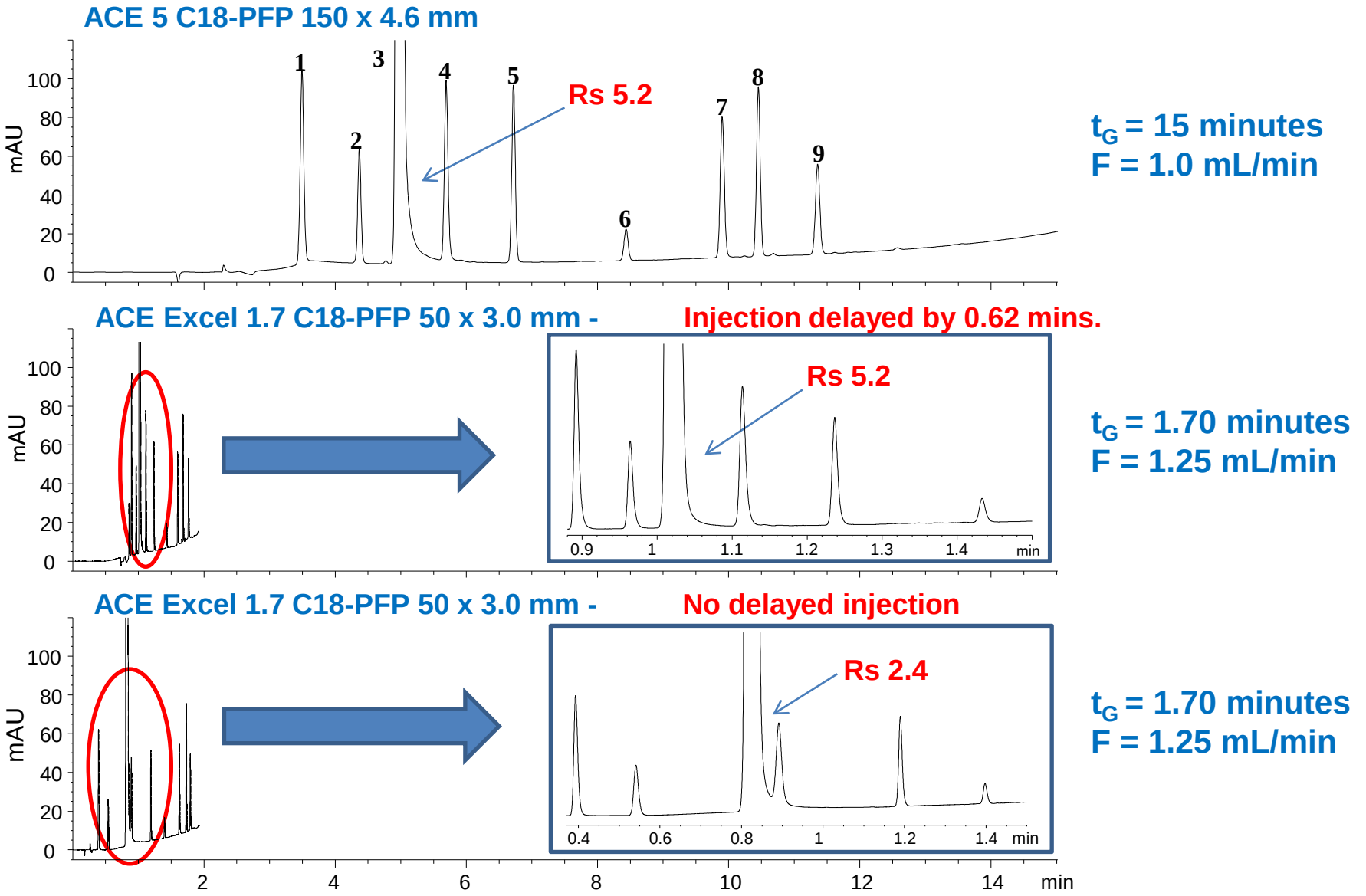
t_G = 1.70 minutes
F = 1.25 mL/min

- **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?



Method Translator Tool Example - Gradient

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

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

Suggested Re-equilibration Time	16.8	mins
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

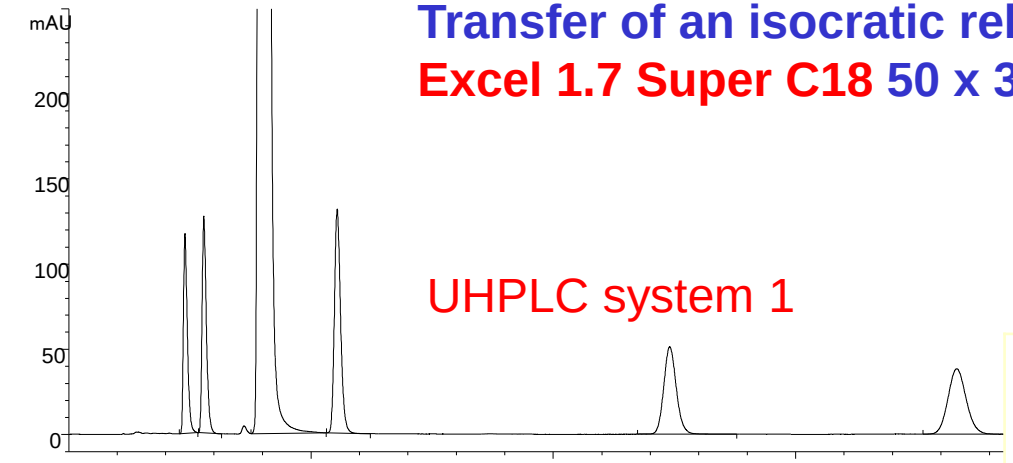
This section tells you if a delayed injection of X mins is required

Transferring Between Instruments

Instrument to Instrument Same Method Transfers

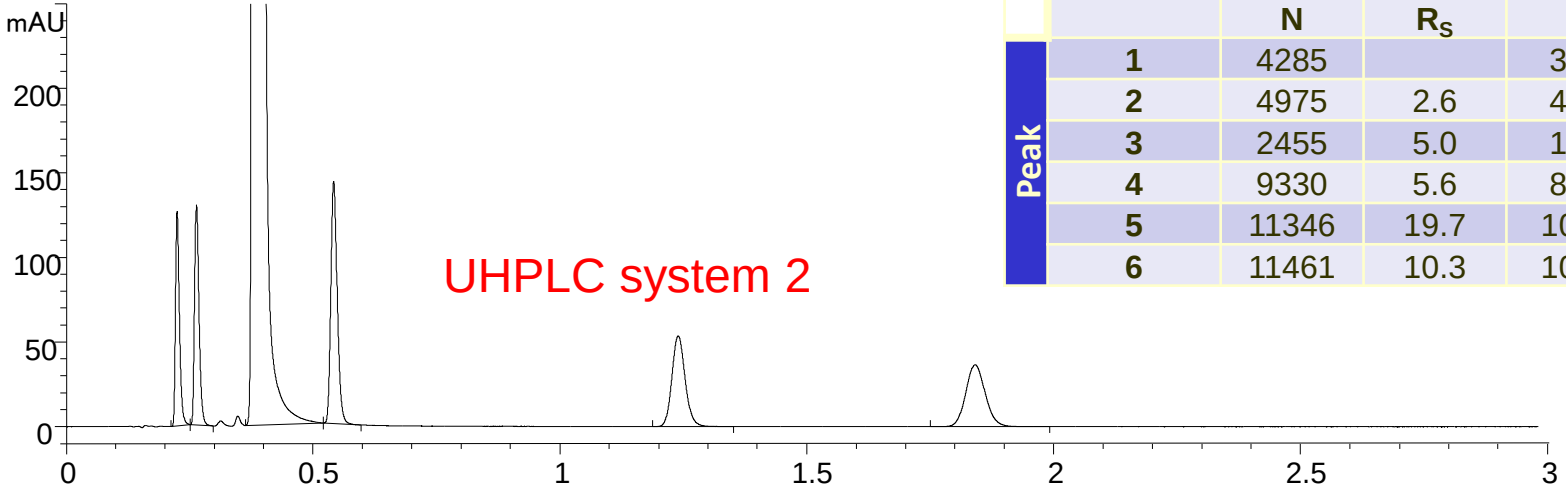
- **Isocratic**
  - **If column format remains identical, no changes necessary.**
  - **(If column format changes, translation required.)**
- **Gradient**
  - **If column format remains identical, need to correct for influence of differing system dwell volumes only. Flow rate, injection volume and gradient 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 Super C18 50 x 3.0 mm between UHPLC systems.

UHPLC system 1

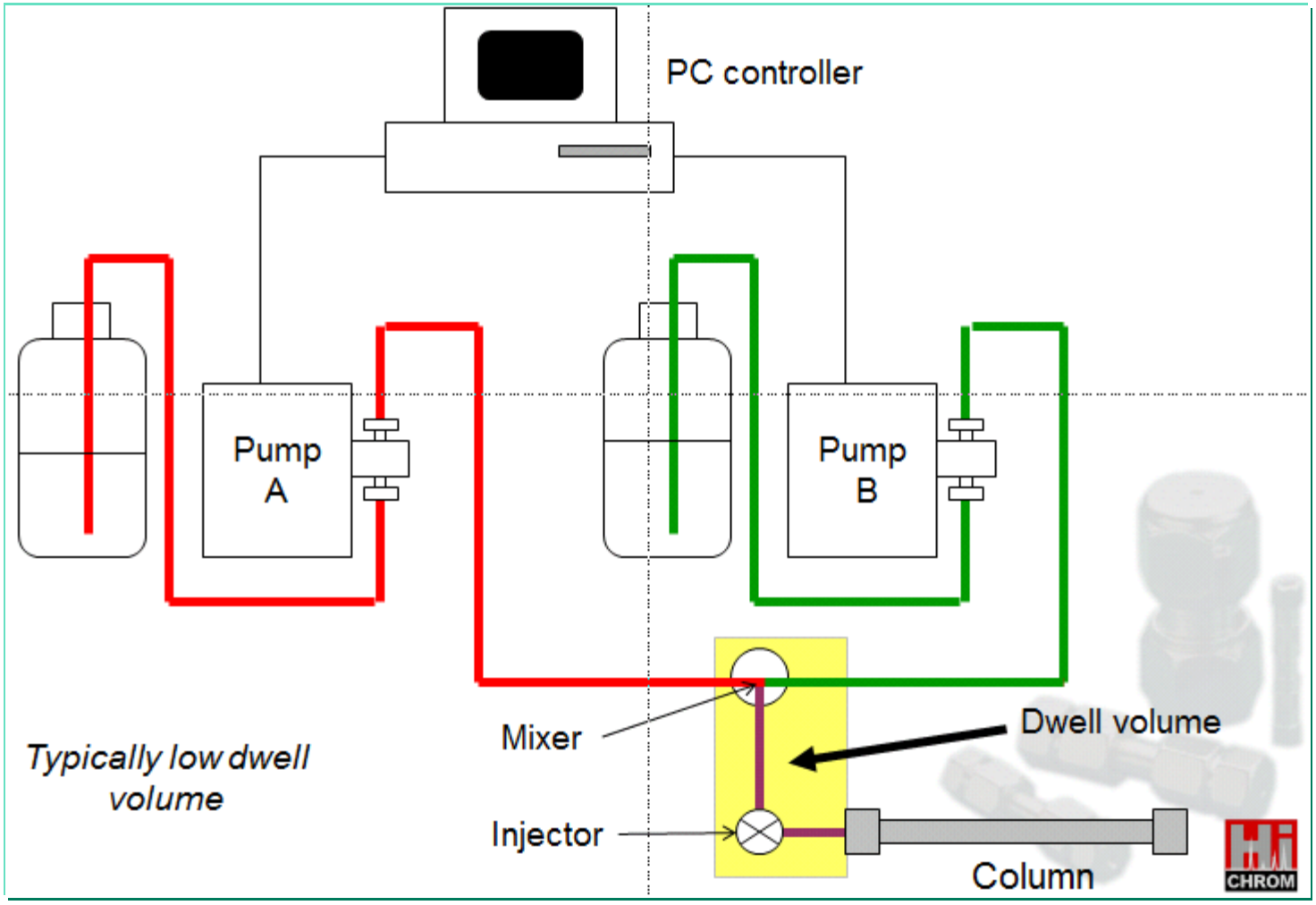


UHPLC system 2

		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

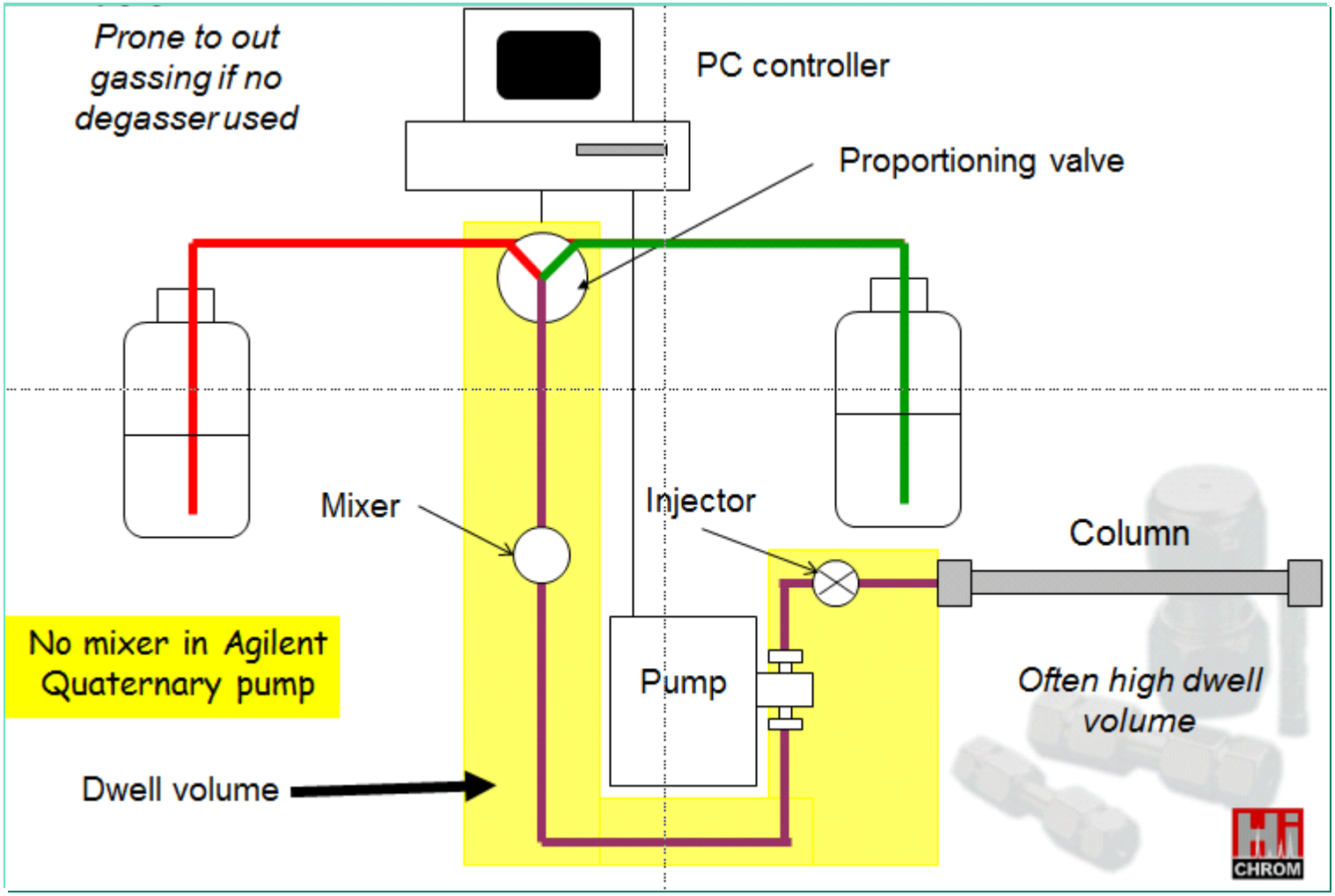
Isocratic analysis 0.2% H_3PO_4 in MeCN:MeOH:water 35:5:60 v/v/v, 40°C, 1.25 mL/min, 0.7 µL , Injection vol., 254 nm. 1. 4-hydroxybenzoic acid, 2. 4-hydroxyisophthalic acid, 3. acetylsalicylic acid (aspirin), 4. salicylic acid, 5. acetylsalicylsalicylic acid, 6. salsalate. Impurities were spiked at 0.5%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

Binary Pump Configuration: High Pressure Mixing



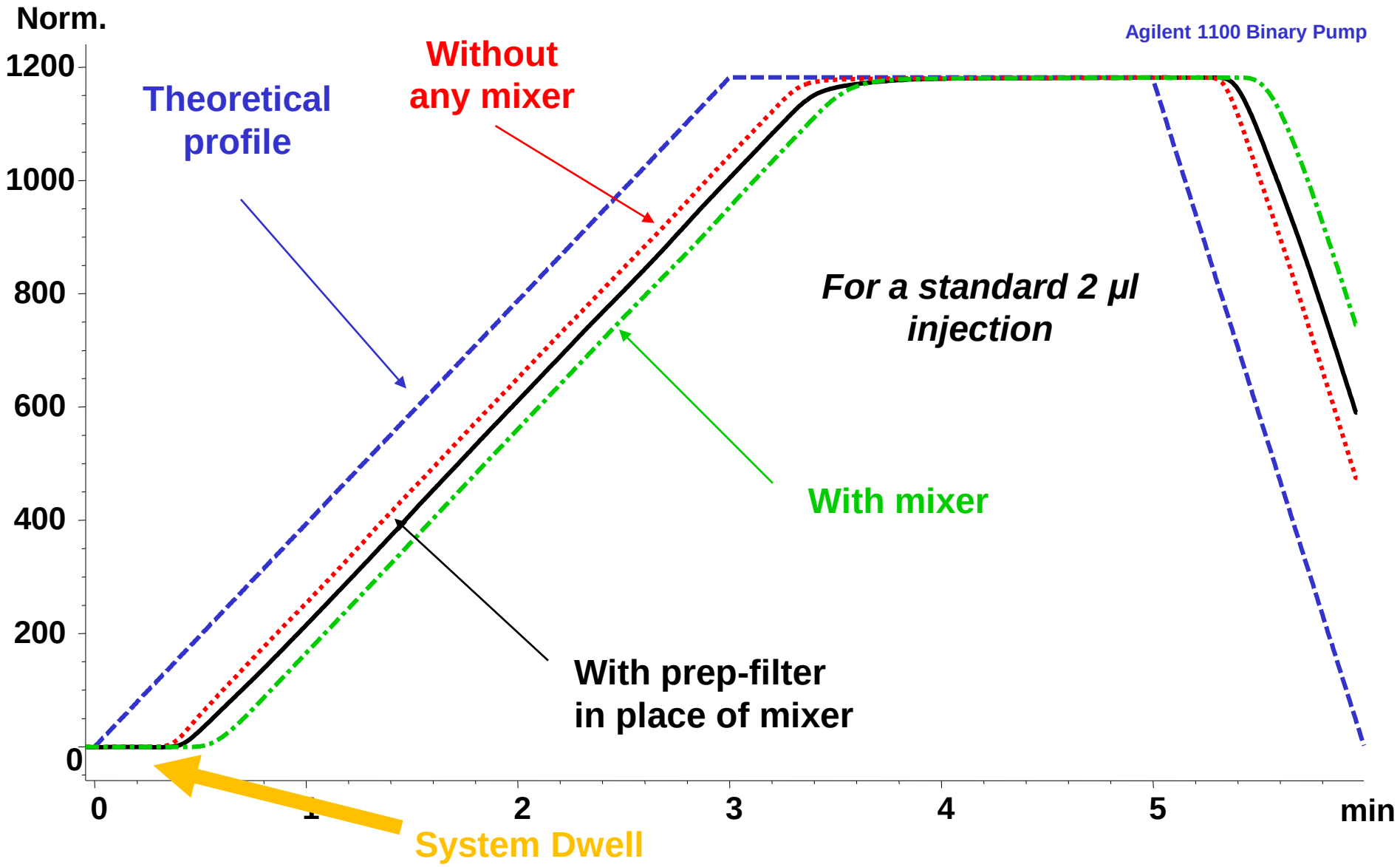
Data courtesy of Euerby & Petersson

Quarternary Pump Configuration: Low Pressure Mixing



Data courtesy of Euerby & Petersson

Gradient Cycles: What Really Happens With Dwell Volume



Instrument Data Can Vary Depending Upon Configuration

Instrument	Typical Dwell Volume (µL)
Agilent HP1100 Binary	180-900
Agilent HP1100 Quaternary	800-1100
Agilent 1200 RRLC	~300
Dionex P680A Quaternary	<400
Thermoquest P4000 Quat	<600
Waters Alliance 2695 Quat	600
Waters Varian 9012 Ternary	1000
Waters Acquity UPLC	~100

	ACQUITY H-Class	1290B (Standard)	1290B (Optimised)	1290Q	Chromaster UltraRs	1200RR	1100 LC125	1200MD	1100 LC94	1100 LC98
ECV (µL)	21.4	13.6	8.3	21.3	18.3	18.7	23.9	29.8	62.0	102.5
Bandwidth	9.96	9.82	7.40	12.78	10.94	11.45	15.48	14.28	25.62	42.87
Dwell volume (µL)	372	198	198	950	262	740	1082	1110	1104	1100

See ACE Knowledge Note #0001 for how to measure system dwell volume

Instrument to Instrument Same Method Transfers

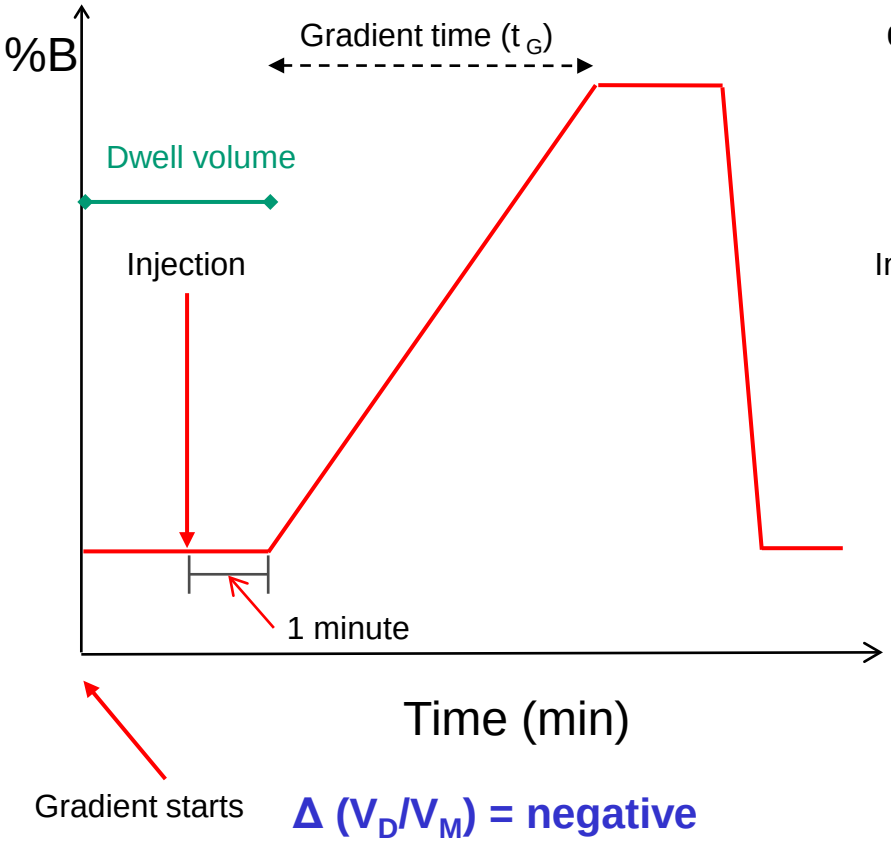
- For gradient method transfer, t_G, F and V_M all remain identical.

- Only need to correct for the system dwell volume
  - 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.
- Positive value: a pre-gradient isocratic hold of x minutes should be added to gradient program.

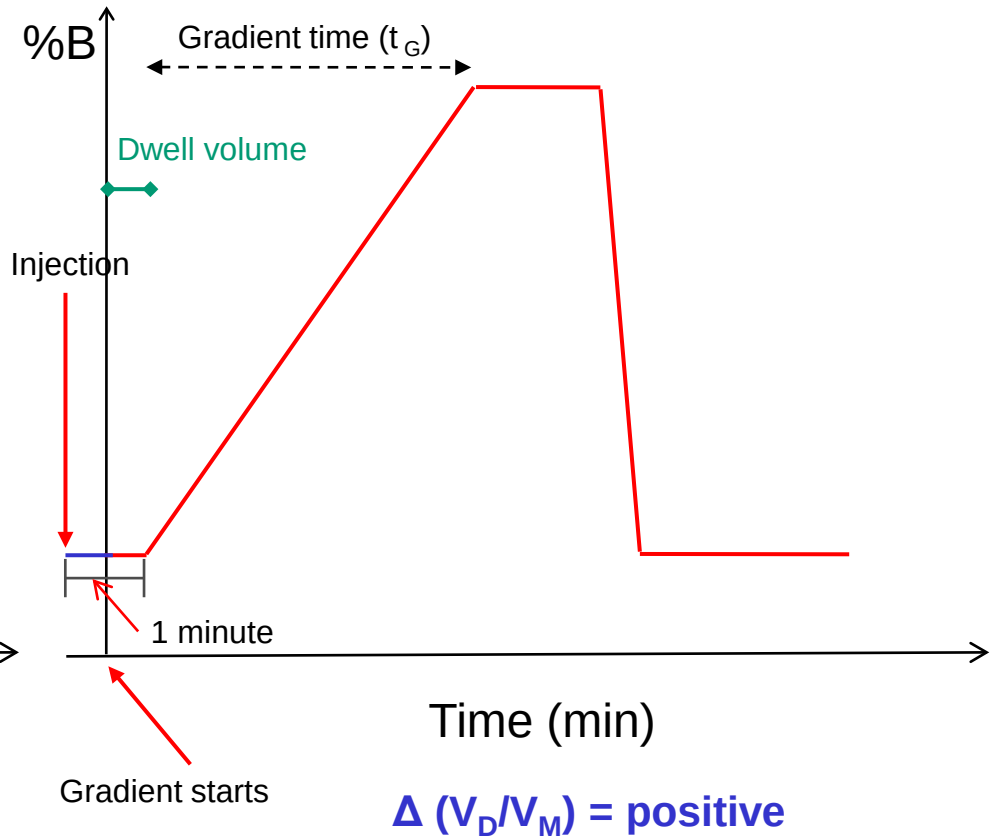
Method Translations: Dwell Volumes / Injection Times



$\Delta (V_D/V_M)$ = negative

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

Injection is delayed until after the start of gradient

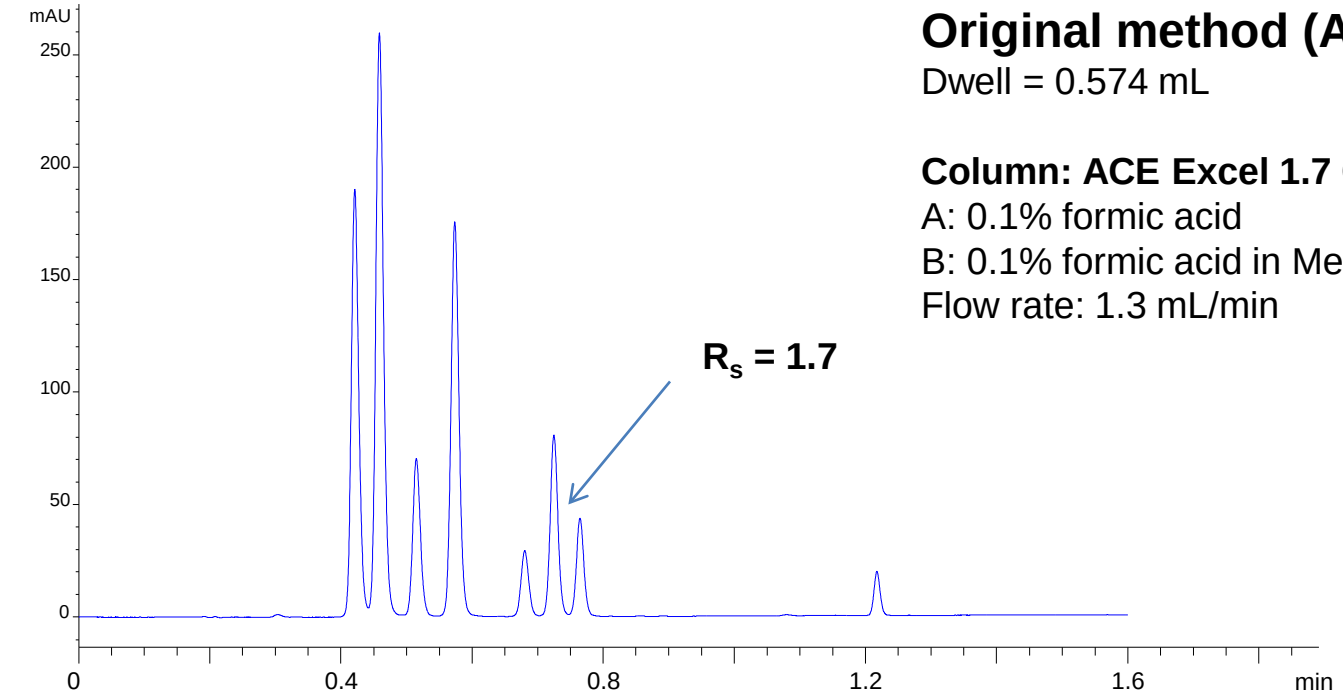


$\Delta (V_D/V_M)$ = positive

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

Pre-gradient hold is added. Effectively extends dwell time

UHPLC Method Transfer - Vanillins



Original method (Agilent 1290 Quaternary)

Dwell = 0.574 mL

Column: ACE Excel 1.7 C18-Amide 50 x 3.0 mm

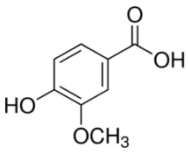
A: 0.1% formic acid

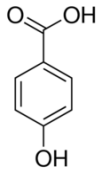
B: 0.1% formic acid in MeCN

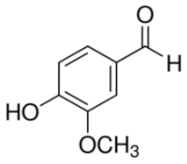
Flow rate: 1.3 mL/min

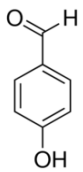
t	%B
0	25
1.32	75
1.49	75
1.6	25

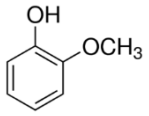
t_G	1.32	
Post time:	2	min
Inj. Vol.	1	µL
P_{MAX}	502	bar

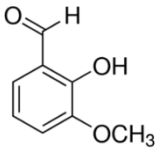

1. vanillic acid

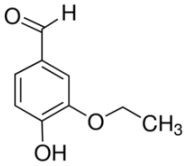

2. 4-hydroxybenzoic acid

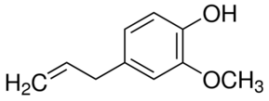

3. vanillin


4. 4-hydroxybenzaldehyde


5. guaiacol


6. *o*-vanillin

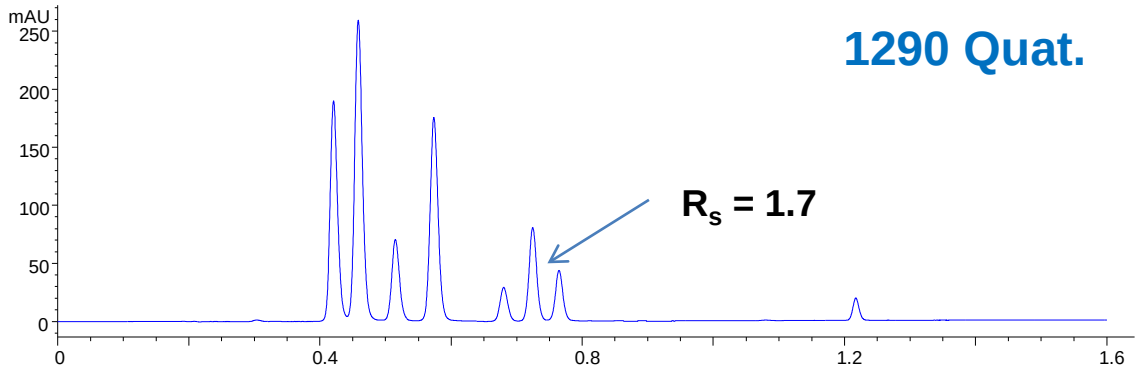

7. ethyl vanillin

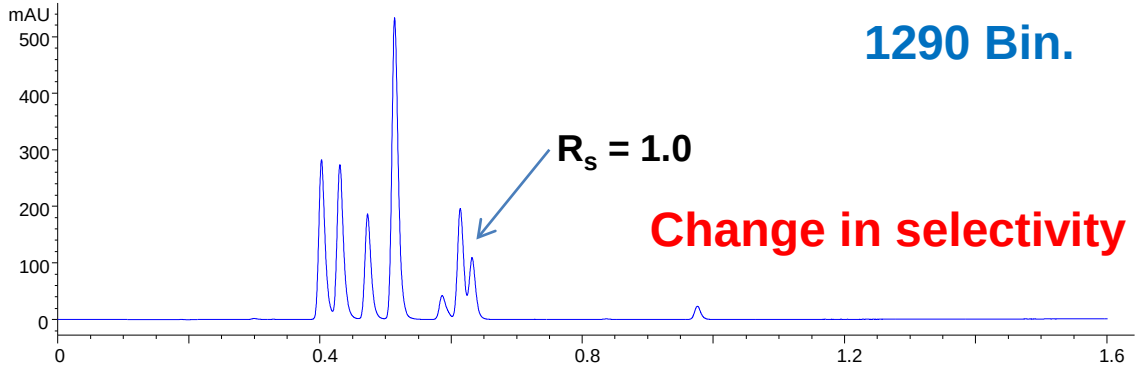

8. eugenol

UHPLC Method Transfer - Vanillins

- **Aim**
  - **To translate the method from Agilent 1290 Quaternary to Agilent 1290 binary**
  - **Need to 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**
  - **A negative value would require a delayed injection**
  - $x = \frac{|\Delta|}{F_2} = 0.29\ min$ **pre-gradient hold**

Instrument to Instrument Method Transfer



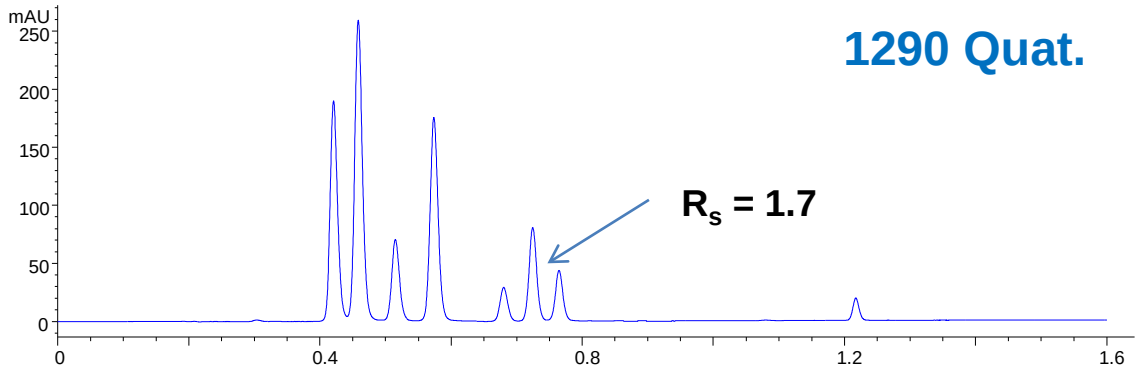


t	%B
0	25
1.32	75
1.49	75
1.6	25

t	%B
0	25
1.32	75
1.49	75
1.6	25

Method transfer – no correction for system dwell

Instrument to Instrument Method Transfer

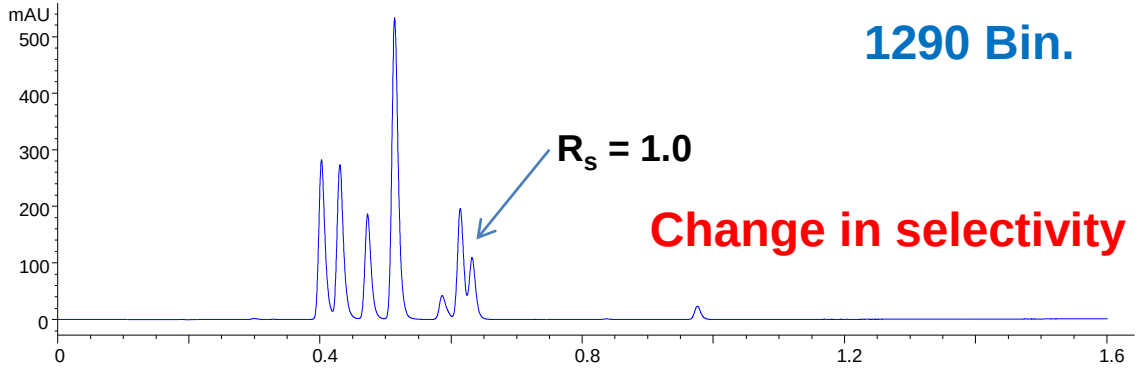


1290 Quat.

R_s = 1.7

t	%B
0	25
1.32	75
1.49	75
1.6	25

Method transfer – no correction for system dwell



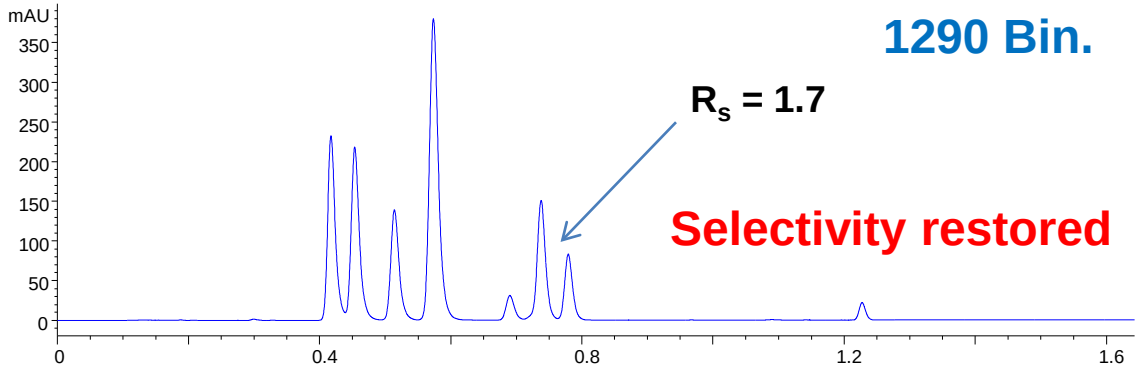
1290 Bin.

R_s = 1.0

Change in selectivity

t	%B
0	25
1.32	75
1.49	75
1.6	25

Method transfer – Corrected for ΔV_D



1290 Bin.

R_s = 1.7

Selectivity restored

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

Method Transfer Tool Example - Gradient

V1.3

Method Transfer

Method transfer involves moving a method from one LC instrument to another, whilst keeping the column format constant. Isocratic analysis is simple with no method changes required. Gradient methods however, should be adjusted to account for any change in system dwell volume in order to ensure accurate method transfer. This tool automatically determines any correction required to ease method transfer. See the Dwell Volume tab for details of how to determine system dwell volume.

Column Information

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

Method

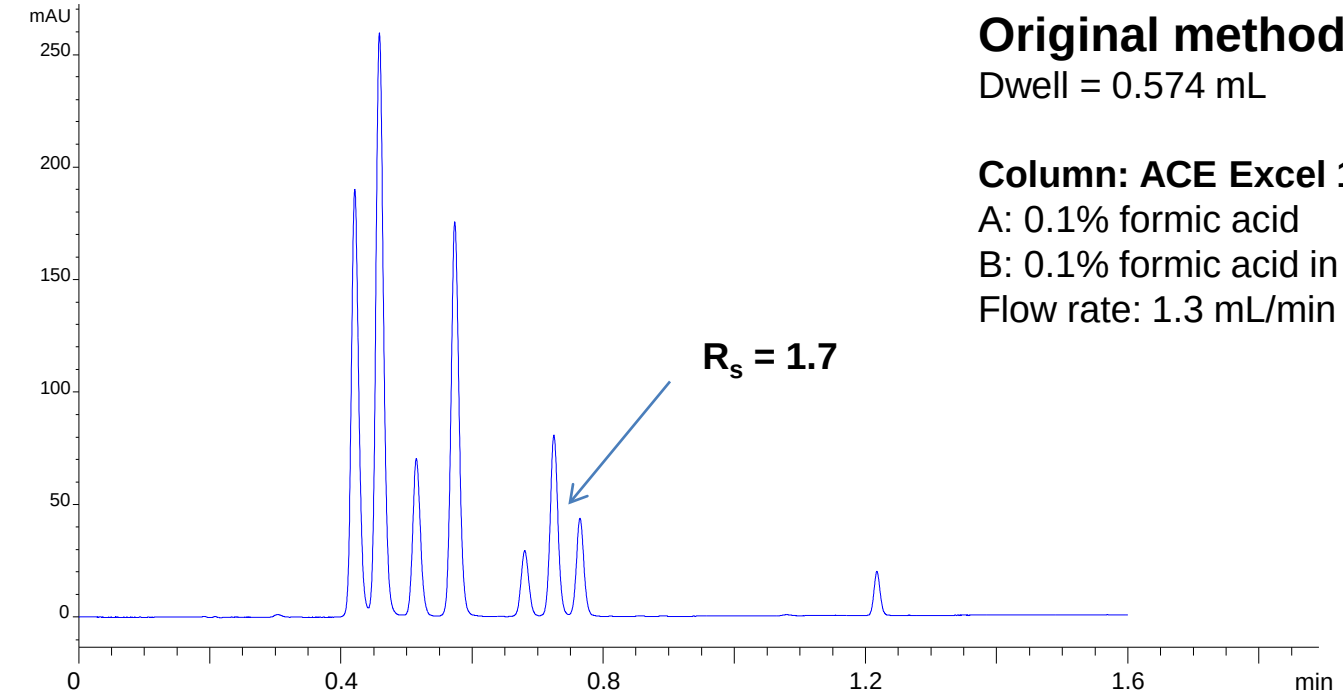
Current			Transferred		
Flow Rate	1.30	mL/min			
LC Name	UHPLC 2		LC Name	UHPLC 3	
Dwell Volume (V_D)	0.574	mL	Dwell Volume (V_D)	0.202	mL

Gradient	Time	%B	Gradient	Time	%B
	0.00	25.0		0.00	25.0
	1.32	75.0		0.29	25.0
	1.49	75.0		1.61	75.0
	1.60	25.0		1.78	75.0
	3.60	25.0		1.89	25.0
				3.89	25.0

New Gradient with isocratic hold

Transferring Between Instruments Part II

UHPLC Method Transfer - Vanillins



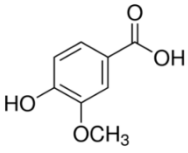
Original method (Agilent 1290 Quaternary

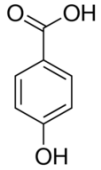
Dwell = 0.574 mL

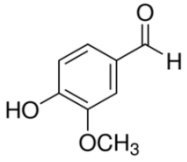
Column: ACE Excel 1.7 C18-Amide 50 x 3.0 mm
A: 0.1% formic acid
B: 0.1% formic acid in MeCN
Flow rate: 1.3 mL/min

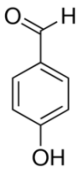
t	%B
0	25
1.32	75
1.49	75
1.6	25

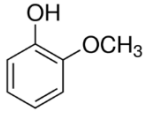
t_G	1.32	
Post time:	2	min
Inj. Vol.	1	µL
P_{MAX}	502	bar

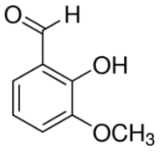

1. vanillic acid

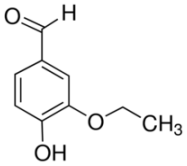

2. 4-hydroxybenzoic acid

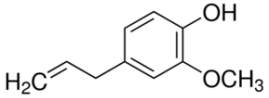

3. vanillin


4. 4-hydroxybenzaldehyde

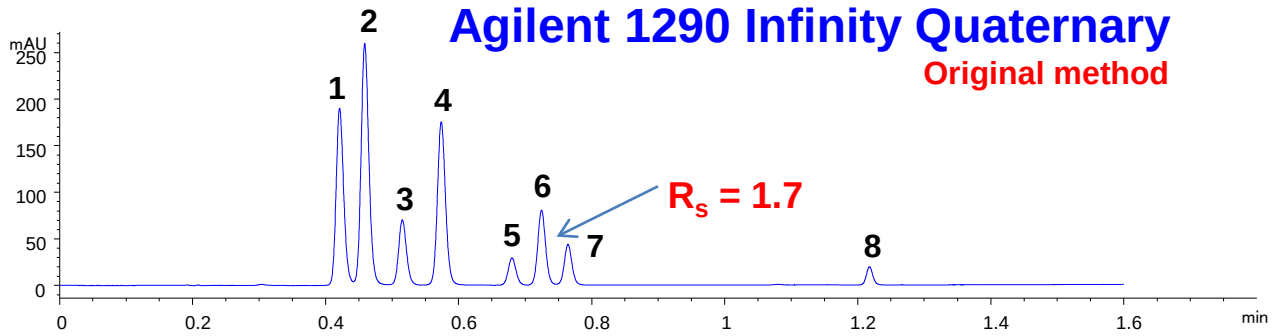

5. guaiacol


6. *o*-vanillin


7. ethyl vanillin

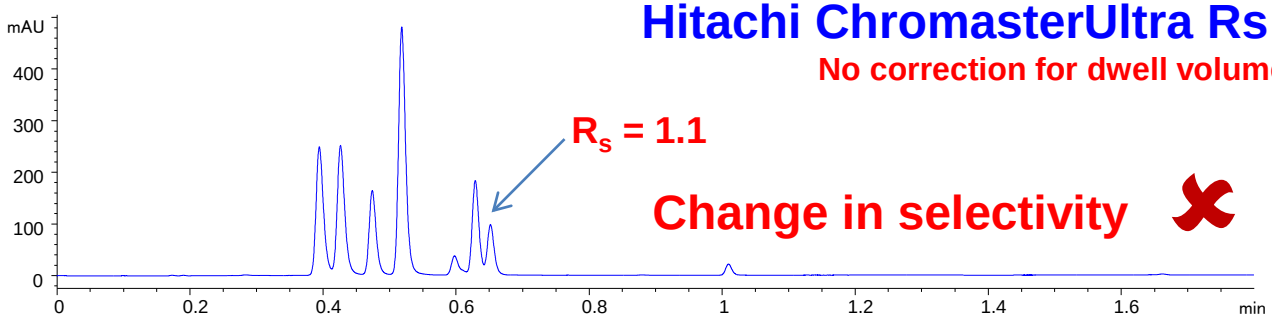

8. eugenol

Instrument to Instrument Method Transfer



t	%B
0	25
1.32	75
1.49	75
1.6	25

no correction for system dwell

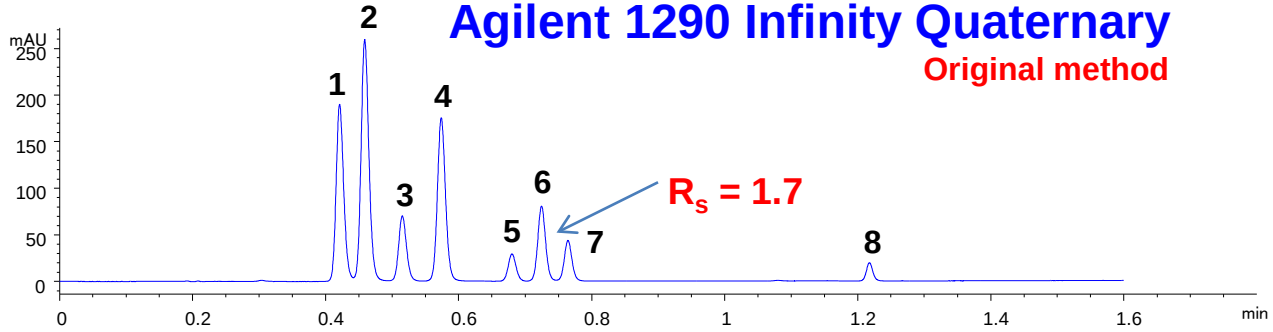


t	%B
0	25
1.32	75
1.49	75
1.6	25

Instrument to Instrument Method Transfer

Agilent 1290 Infinity Quaternary

Original method

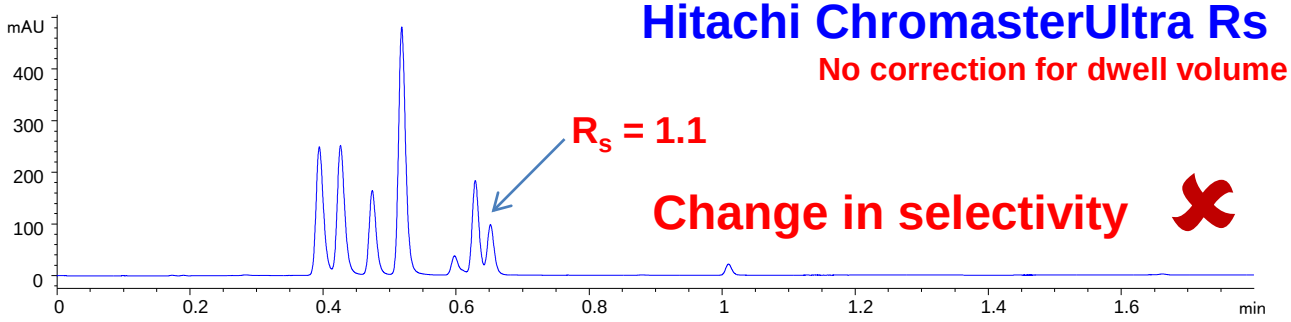


t	%B
0	25
1.32	75
1.49	75
1.6	25

no correction for system dwell

Hitachi ChromasterUltra Rs

No correction for dwell volume



Change in selectivity

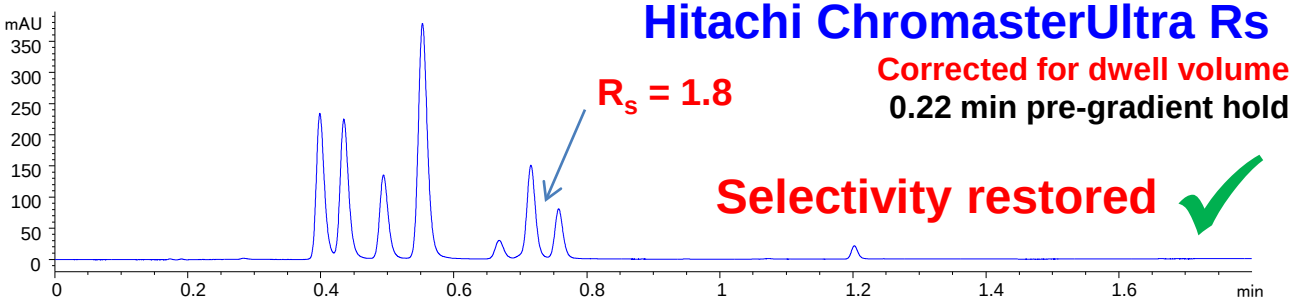
t	%B
0	25
1.32	75
1.49	75
1.6	25

Method corrected for ΔV_D

Hitachi ChromasterUltra Rs

Corrected for dwell volume

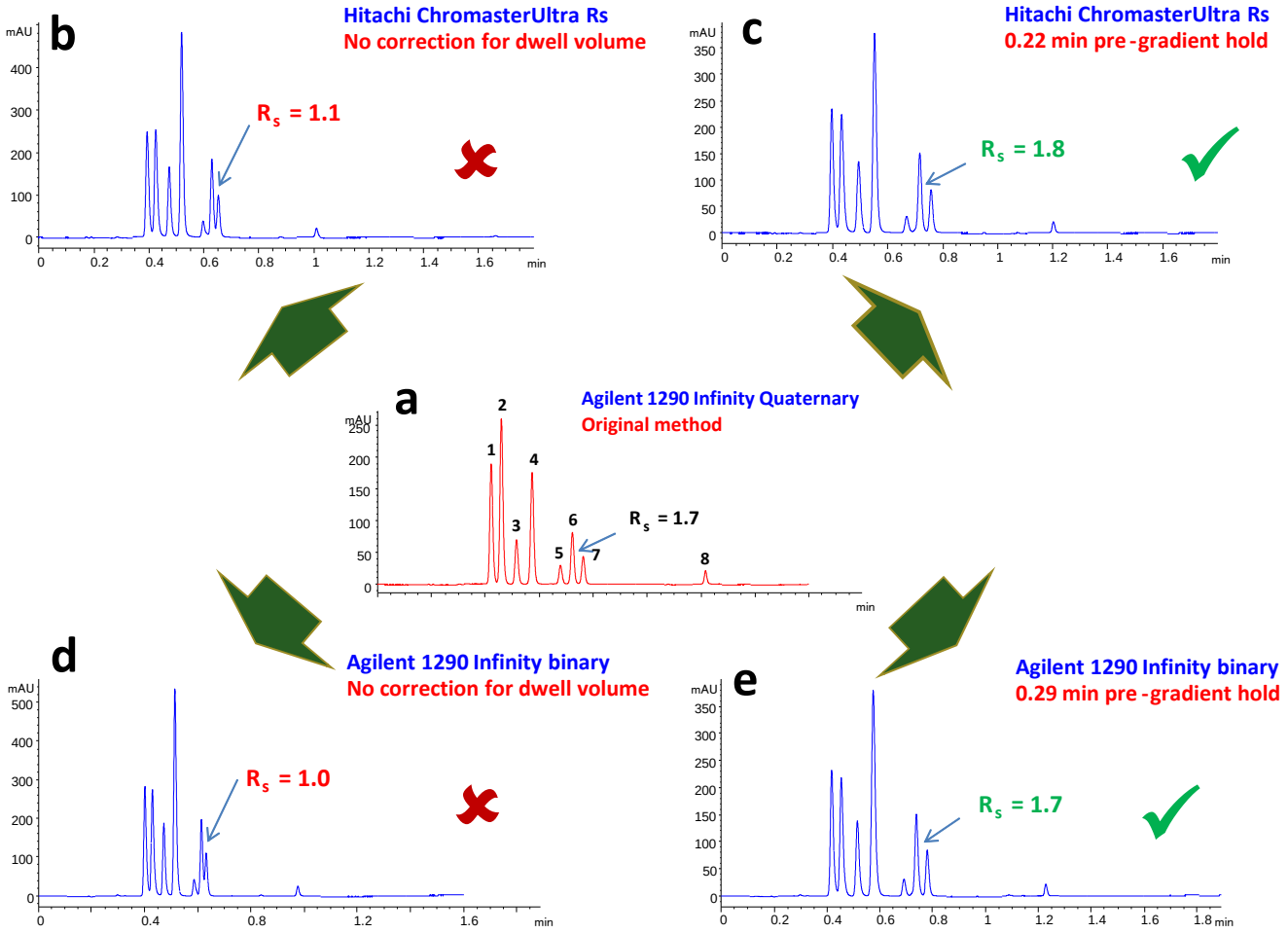
0.22 min pre-gradient hold



Selectivity restored

t	%B
0	25
0.22	25
1.54	75
1.71	75
1.82	25

Transferring Methods - Success or Not?

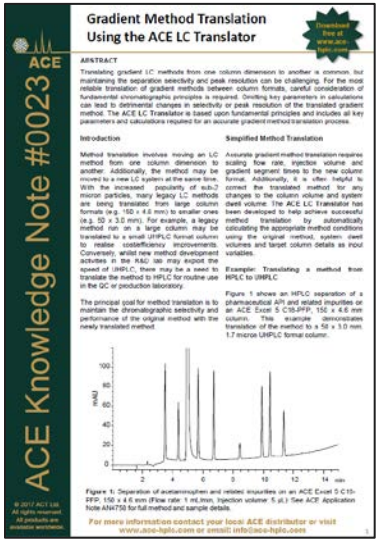


Summary

- **Relatively accurate** method translations are achievable
- **Isocratic** translations
  - **Use L/dp** ratio translations
  - Translate to **columns that have scalable** bonded phases
  - Be aware of **system dispersion effects** on smaller columns
- Gradient translations
  - **More complex** but easy calculations described
  - Consider **V_D/V_m impact** upon translation accuracy ← **few** online calculators currently offer this
- **High peak capacities** are possible for **complex samples** using **column coupling**
- **Instruments transfers** are **simple for isocratic** methods but **gradient methods** must **consider dwell volumes**

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

Download Resources at mac-mod.com

or contact **info@mac-mod.com**

Thank You For Your Attention

info@mac-mod.com

MAC-MOD.com