UHPLC to HPLC and back – questions of selectivity

Adrian Clarke[1], Lee Mallard[1], Rachel Wilson[1], John Nightingale[3], Partha Mukherjee[4] and Patrik Petersson[2]

1) Process R&D Avlon/Charnwood, Analytical Chemistry, Loughborough, UK.
Adrian.Clarke@astrazeneca.com
2) Pharmaceutical & Analytical R&D, Lund, Sweden.
3) Process R&D Macclesfield, Analytical Chemistry, Macclesfield, UK.
4) Pharmaceutical & Analytical R&D, Wilmington, USA.

Sept 2009

AstraZeneca
life inspiring ideas

Outline

- AstraZeneca R&D's aims for UHPLC
- UHPLC evaluations and benefits
- Implementation Challenges
  - Strategy & plans
- Method transfer UHPLC ↔ HPLC
  - Theory & requirements
  - Dwell volume differences
  - Frictional heating
  - Stationary phase selectivity
- Column characterisation
  - Modified Tanaka tests
  - Selectivity *vs.* particle size
  - Stability testing
- Conclusions

AZ aims: High Speed/Resolution LC

QUALITY (High resolution = Improved data quality):

- issues with peak co-elution
- Insufficient data points (quantification & identity with MS)

PRODUCTIVITY (speed):

- Maintain (Improve) Resolution/Peak capacity with ↓ analysis time
- Decrease method development times
- Common approach/methods→ speed up/simplify method transfer
- LC instruments: reduce numbers/better utilisation
  - Use existing LC instruments
- Consider all internal and external customers
- Integrate into existing strategies (extension of LC)
  - link with MS & Increase MS data quality
  - method development strategies

Global Process R&D

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Global UPLC evaluations

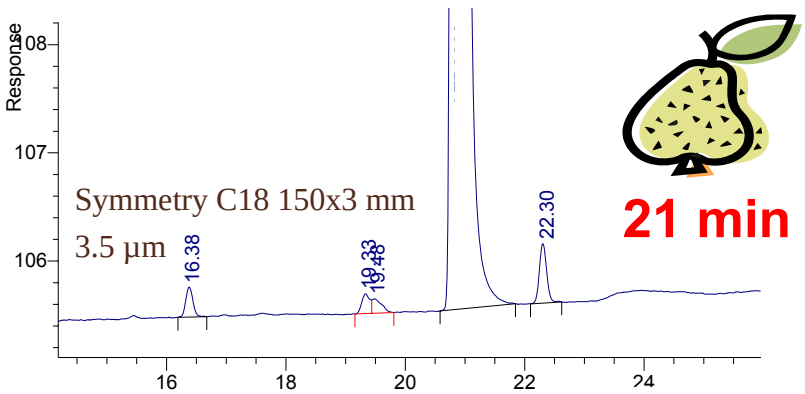
- Stage 1: Instrument Robustness/Performance in hands of “expert” (2007)
- Stage 2: Replace HPLC in ≥1 mid/late stage project per site (2007/2008)
- Stage 3: Implementation/Devolvement & continued monitoring (≥2008)

Stage 1: Instrument performance

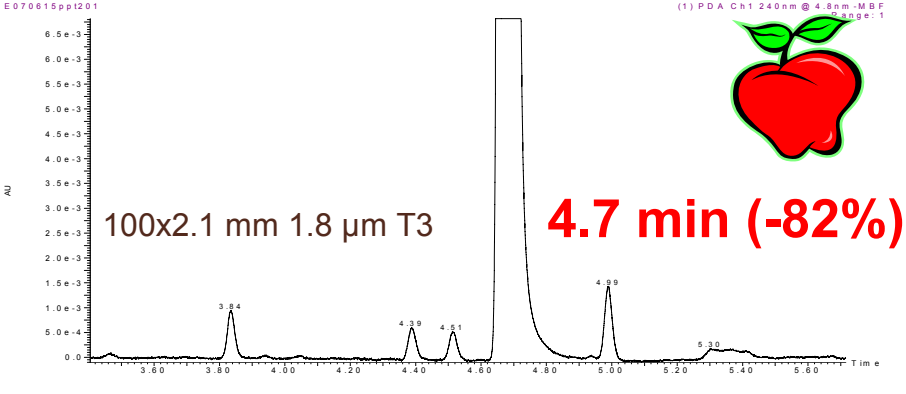
- Head to head comparisons
  - Acquity UPLC had clear advantages over it's competitors (2007)
- Repeated analysis during ~9 months (at ~800 bar)
- UPLC Instrument performance (R_t & area RSDs....) better than/as good as HPLC
- "Downtime" comparable/better then conventional HPLC
- No real special precautions need to be adhered to – just *old fashioned* LC common sense!
- UPLC can be used routinely in Analytical Development

Stage 2: Project evaluations

UPLC used instead of HPLC for ≥1 project/AD site (n=7)



21 min



4.7 min (-82%)

- **Confirms/support the expected advantages**
- UHPLC performance is significantly better than HPLC
  - Increase speed by 3-4 *vs.* HPLC (maintain resolution)
  - Better capability for labile samples
  - Increase resolution/peak capacity
  - Speeds up method dev & provides higher quality data
  - More sensitive -easier processing, better for MS & trace analysis
  - But, require higher speed detectors
- UPLC is as accurate, precise & robust as HPLC

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Readout from Stage 2

- Better utilisation of LCs
  - reduce the number of LCs (2 HPLCs with ≤1 UPLC?)
  - requires adoption of new ways of working
- Reduce the solvent consumption significantly (~80%)
- Training is vital
- Conclusion: UPLC can be used routinely & reliability expected to be acceptable
- A HPLC to U(H)PLC replacement strategy was been agreed within Global Development Analytical Chemistry
  - AZ development adopting & implementing UHPLC (stage 3)

"Implementation of U(H)PLC within a global pharmaceutical company – A new way of working"

Prof. Patrik Petersson (AstraZeneca R&D Lund)

Solvias Science Day

November 2, 2009, Congress Center, Basel

Registration and more info on

http://www.solvias.ch/english/news-events/events/solvias-science-day.html

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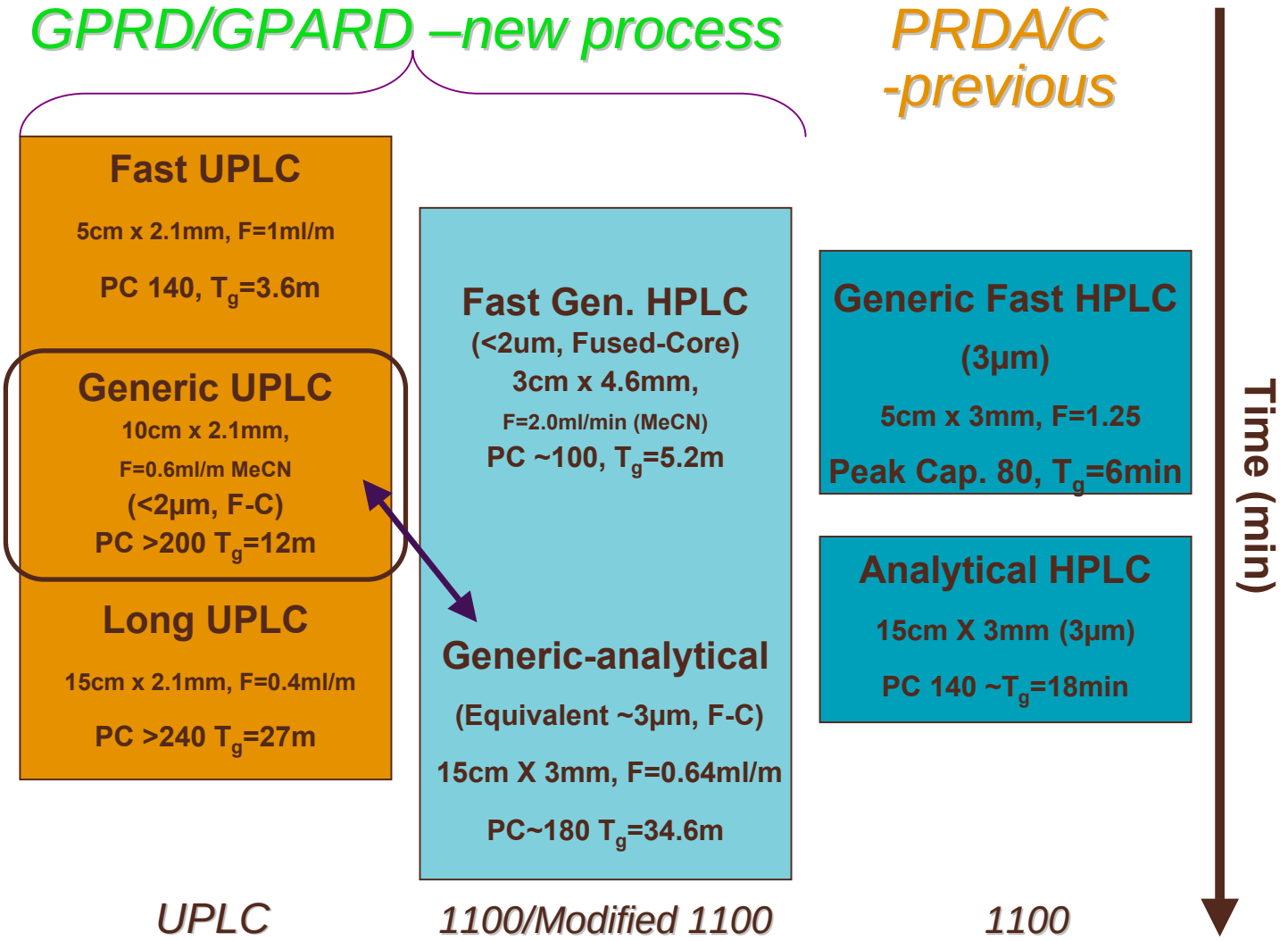
Impact of implementing UHPLC

- Method transfer
- UHPLC availability in AZ
  - Prioritise as have limited UHPLCs in analytical chemistry R&D
  - No UHPLC capability in process chemistry
- AZ operations & Strategic (long term) CROs
  - Will purchase UHPLC, but have less UHPLC experience
  - Ensure CRO/Operations get same level of UHPLC training
  - Tech transfer may take longer
- Tactical (short term) GxP CRO
  - some have/planning to have UHPLC, others are simply keeping an eye on the area
- Non-GMP CROs: most do not have UHPLC
- Need to translate UHPLC methods to HPLC (increased analysis times) for internal (chemistry) & external customers

High Speed/Resolution LC



Aim: Maintain selectivity in HPLC & UPLC (generics)

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Method Transfer

- It is possible to translate to HPLC ...
- In theory translations are simple

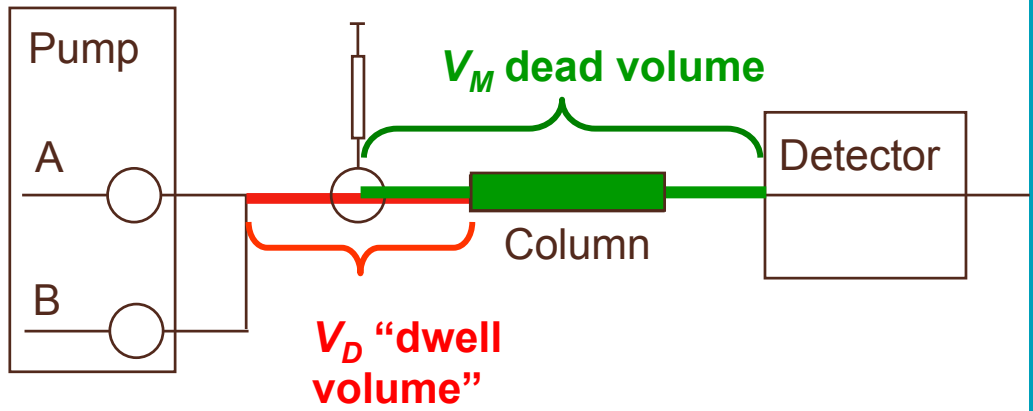
$$\frac{t_1 F_1}{\Delta\Phi_1 V_{M1}} = \frac{t_2 F_2}{\Delta\Phi_2 V_{M2}}$$

- Keep average retention factor (k_g) constant for each segment in the gradient.
  - i.e. Don't change selectivity from mobile phase

$$k_g \propto t_G F/(\Delta\Phi V_M)$$

Method Transfer-Input parameters

- Within AZ use excel translation workbooks
  - Also “HPLC calculator” on University of Geneva website (http://www.unige.ch/sciences/pharm/fanal/lcap/telechargement.htm)
- Column properties
  - Internal diameter
  - Column length
  - Particle size
- Instrument properties
  - Dead volume
  - Dwell volume
- Method related properties
  - Mobile phase gradient
  - Injection volume



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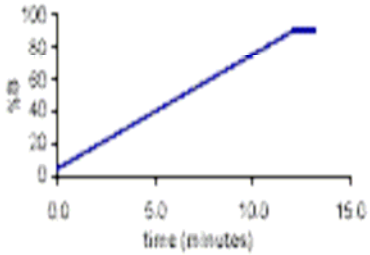
Method Transfer ($t_G F/(\Delta\Phi V_M)$ constant)

Method transfer in gradient mode

Original method (1)

Original column geometry		Original exp. Conditions	
Column length (L_1)	100 mm	Flow-rate (F_1)	600 µL/min
Column diameter (d_{c1})	2.1 mm	Inj. volume (V_{inj1})	2.0 µL
Particle size (d_{p1})	1.7 µm		
Dwell volume (V_{d1})	0.15 mL		



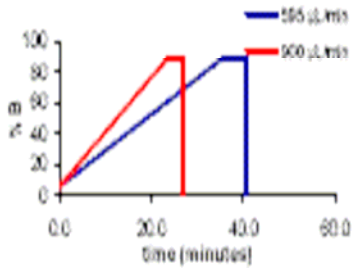
Additional gradient information	
Suggested reconditionning step	4 min

Original gradient profile

Step	time (min)	%A	%B
initial conditions	0.0	95	5
initial hold	12.0	10	90
3	13.2	10	90
4			
5			
6			
7			
8			
9			
10			

Transferred method (2)

Transferred column geometry		Transferred exp. Conditions	
Column length (L_2)	150 mm	Flow-rate (F_2)	595 µL/min
Column diameter (d_{c2})	3.0 mm	Inj. volume (V_{inj2})	6.1 µL
Particle size (d_{p2})	3.5 µm		
Dwell volume (V_{d2})	1.1 mL		



Additional gradient information	
Suggested reconditionning step	12 min

Geometric transfer of the gradient profile

Step	time (min)	%A	%B
initial conditions	0.0	95	5
initial hold	36.0	10	90
3	40.8	10	90
4			
5			
6			
7			
8			
9			
10			

"HPLC calculator" on University of Geneva website
(http://www.unige.ch/sciences/pharm/fanal/lcap/telechargement.htm)

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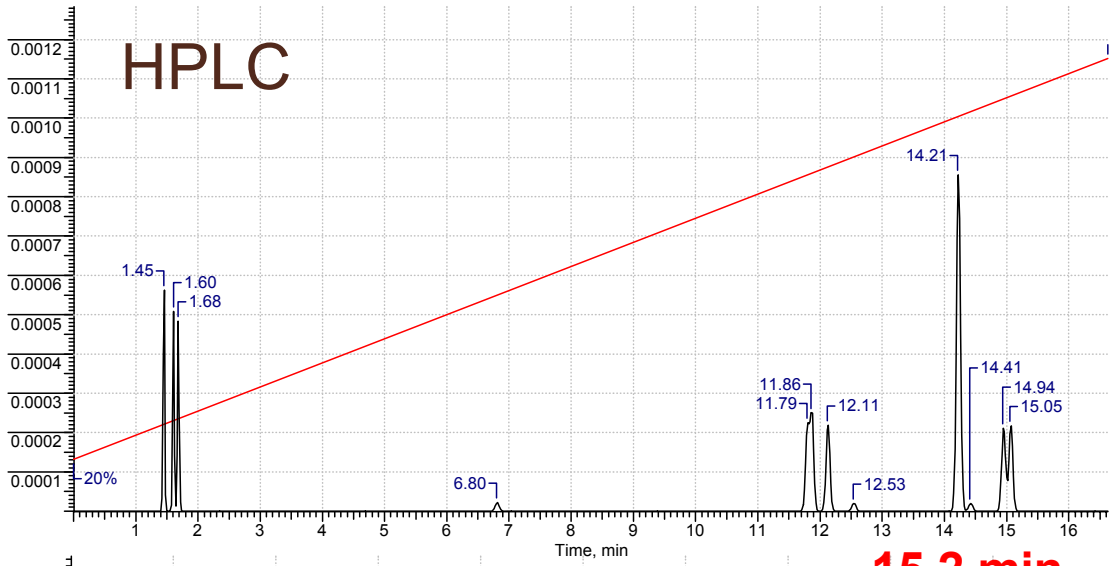
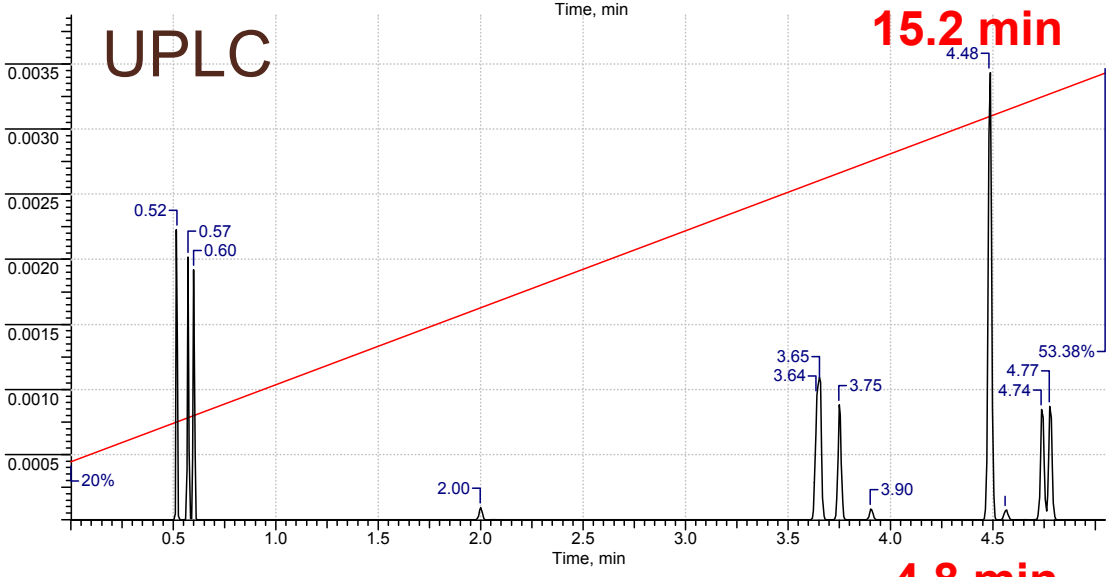
Method Transfer ($t_G F/(\Delta\Phi V_M)$ constant)



15.2 min



4.8 min

HPLC

- 150 x 3 mm, 3.5 µm
- F= 0.6 mL/min
- t_G=21 min
- $\Delta\Phi$=50%
- v_M= 0.68 mL
- V_D= 1.4 mL
- Inj vol=5µl
- $t_G F/(\Delta\Phi * V_M)$= (21x0.6)/(0.5x0.68)=3.7

UPLC

- 100 x 2.1 mm, 1.7 µm
- F= 0.55 mL/min
- t_G=7.5 min
- $\Delta\Phi$=50%
- v_M= 0.22 mL
- V_D= 0.15 mL
- Inj vol=1.7µl
- $t_G F/(\Delta\Phi * V_M)$= (7.5x0.55)/(0.5x0.22)=3.7

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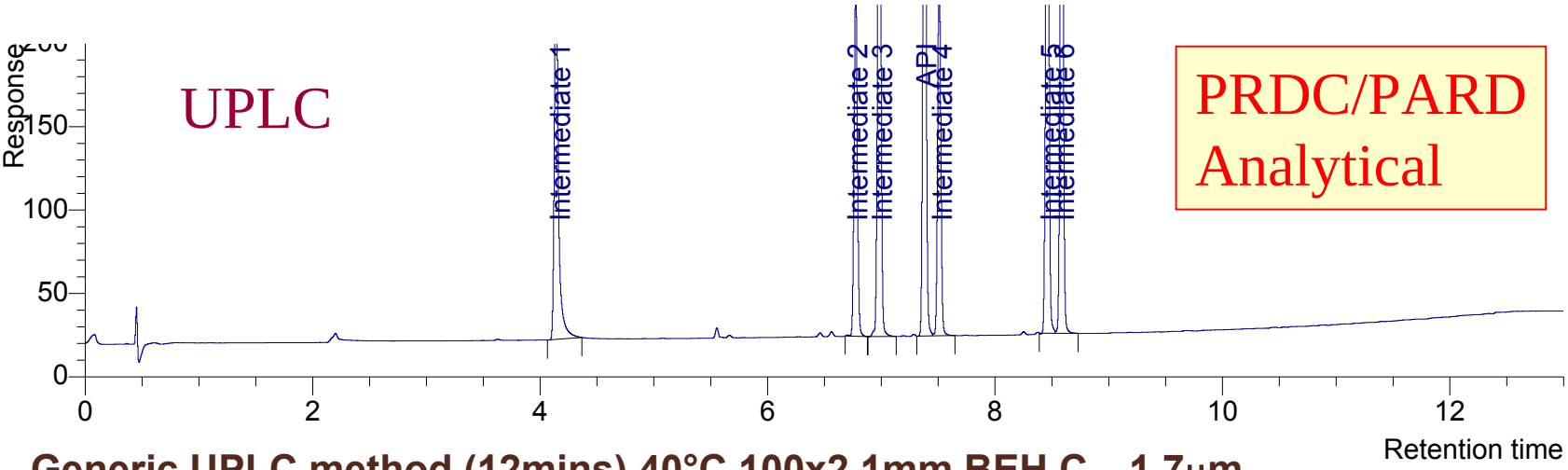
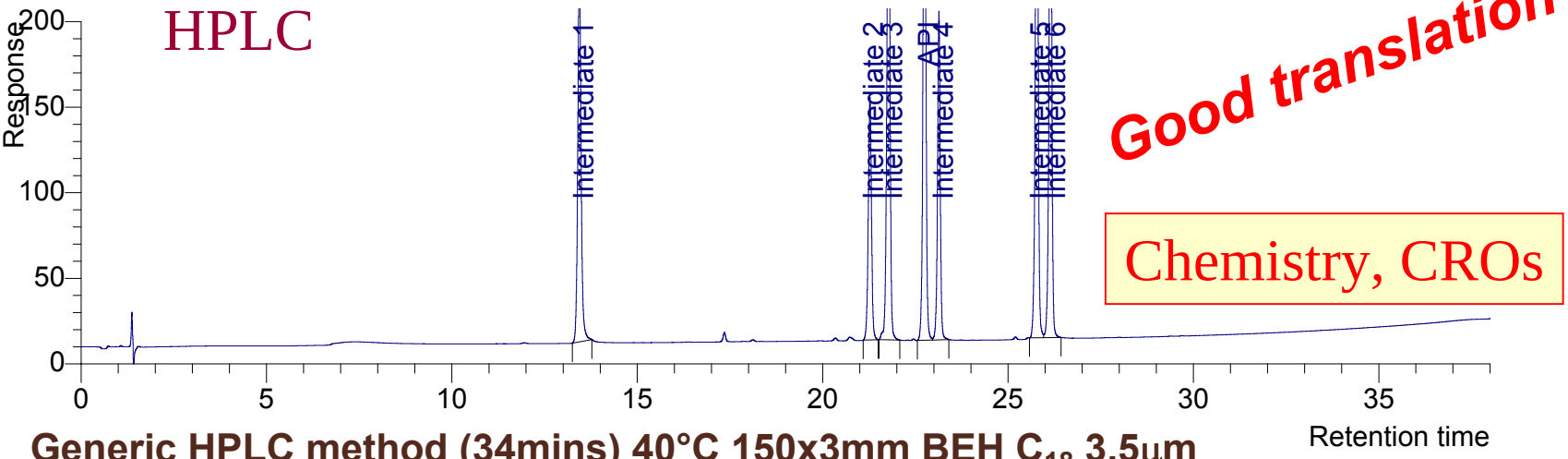
Generic UPLC→HPLC Translation

Aim: Maintain selectivity in HPLC & UPLC (generics)



PRDC/PARD Analytical

Generic UPLC method (12mins) 40°C 100x2.1mm BEH C_{18} 1.7μm



Good translation

Chemistry, CROs

Generic HPLC method (34mins) 40°C 150x3mm BEH C_{18} 3.5μm

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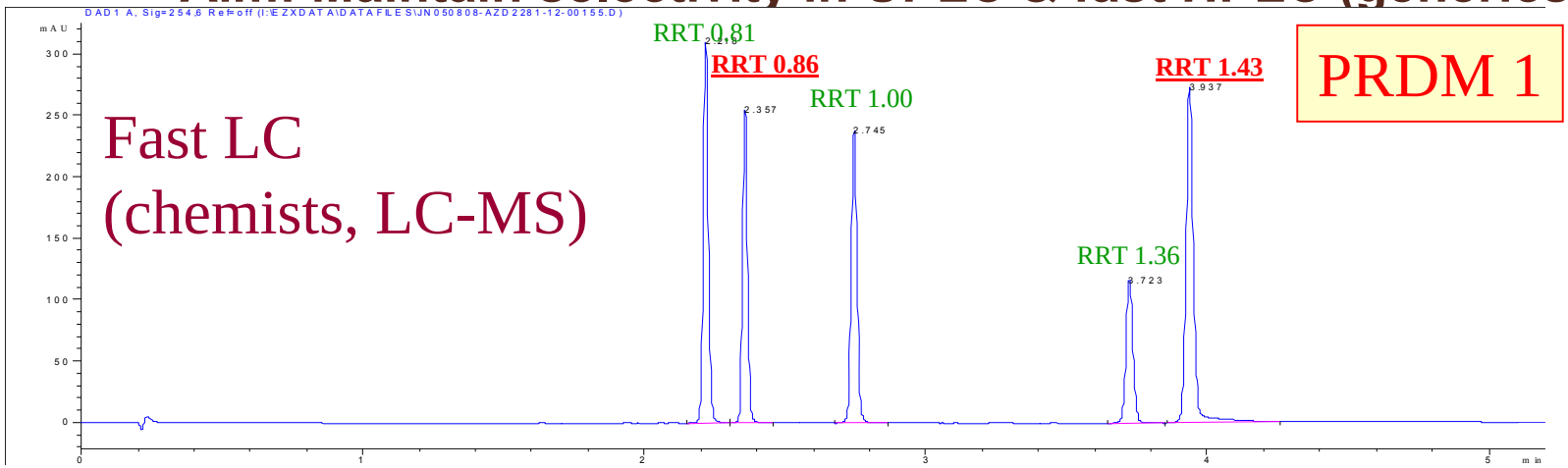
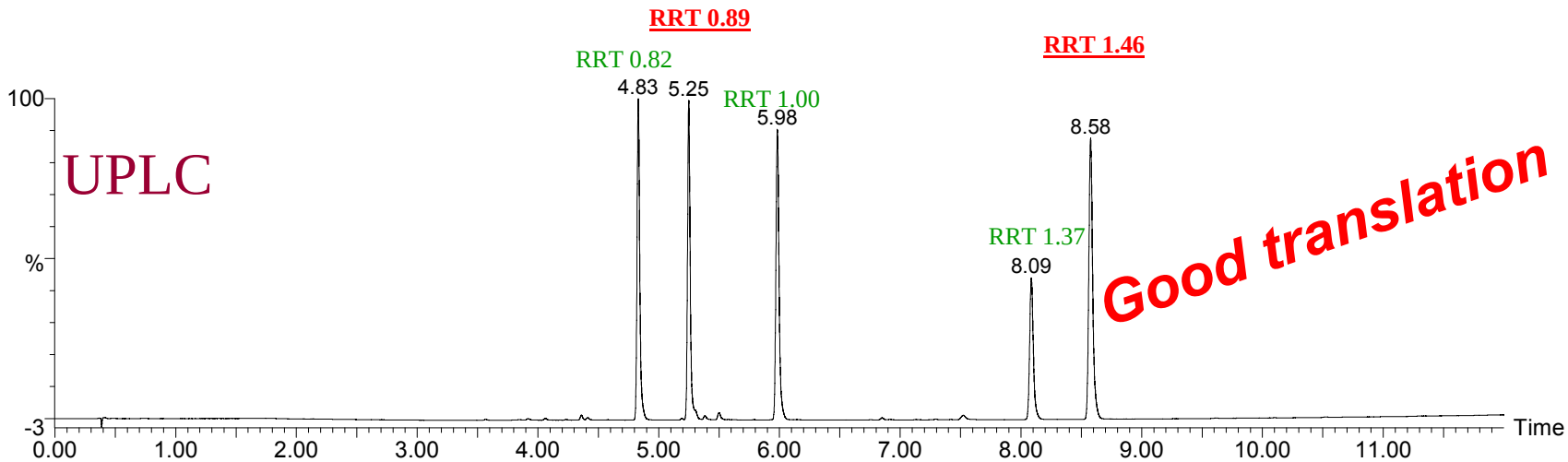
UPLC ↔ Fast HPLC Translation trials

Aim: Maintain selectivity in UPLC & fast HPLC (generics)



Generic Fast HPLC method (5.2mins) 40°C 30x4.6mm Gravity C18 1.7μm



Generic UPLC method (12mins) 40°C 100x2mm Gravity C18 1.7μm

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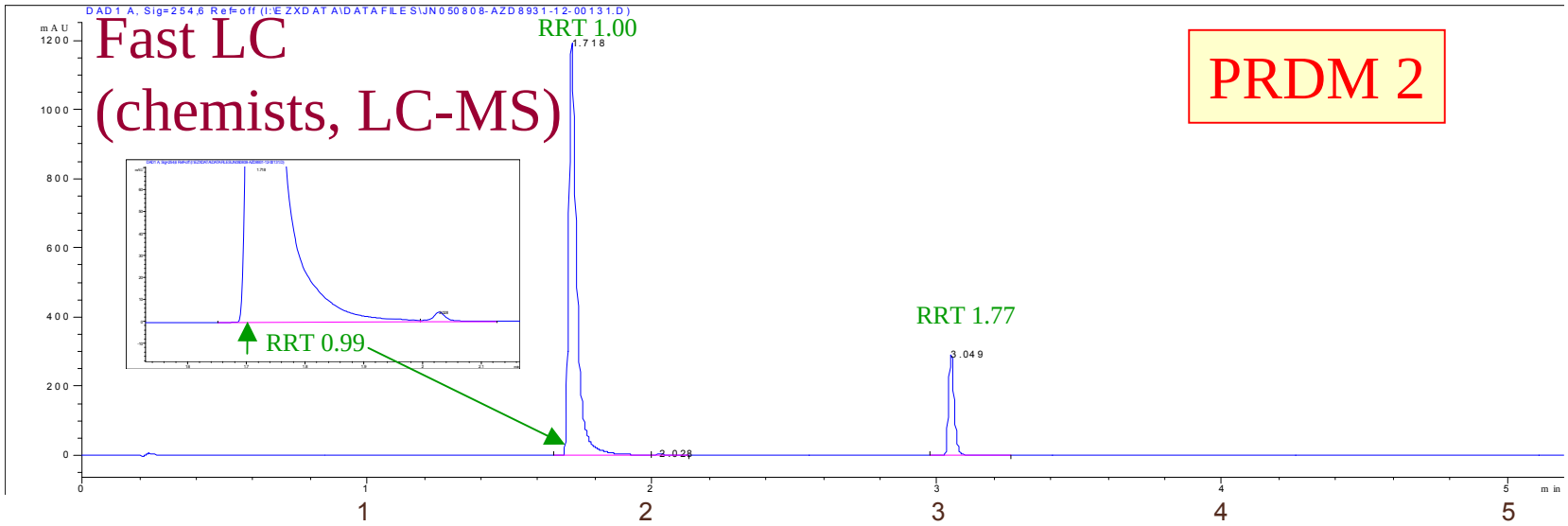
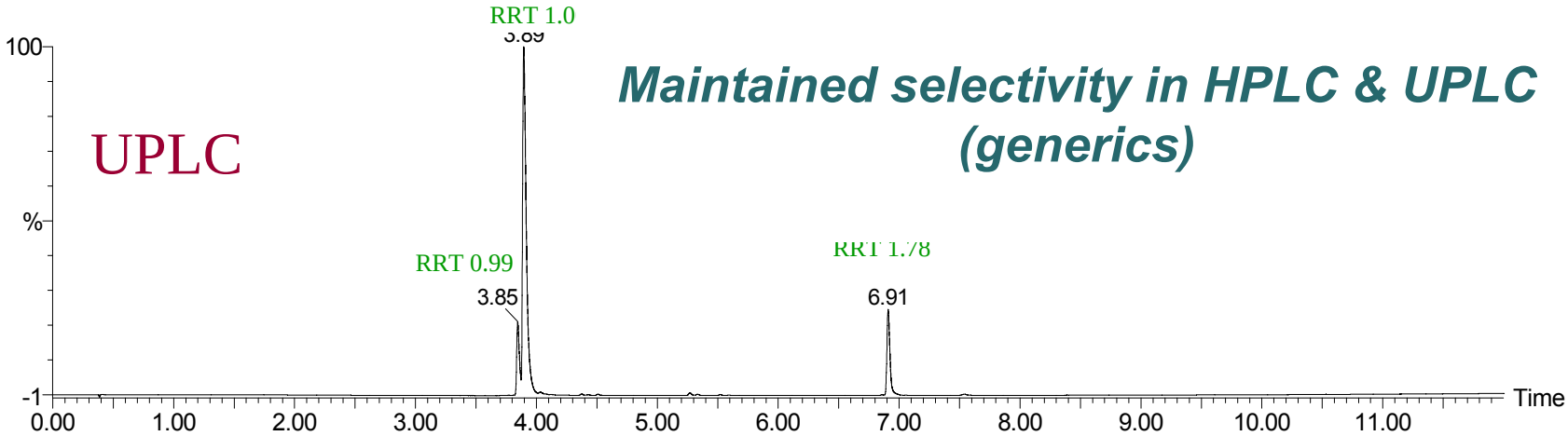
UPLC ↔ Fast HPLC Translation trials



Generic Fast HPLC method (5.2mins) 40°C 30x4.6mm Gravity C18 1.7μm



Maintained selectivity in HPLC & UPLC (generics)

Generic UPLC method (12mins) 40°C 100x2mm Gravity C18 1.7μm

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Method Transfer

- In practice there are complications!!!
  - Differences in instrument dwell volume
  - Heat of friction can also cause problems
  - Differences in selectivity between different particle sizes

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Method Transfer

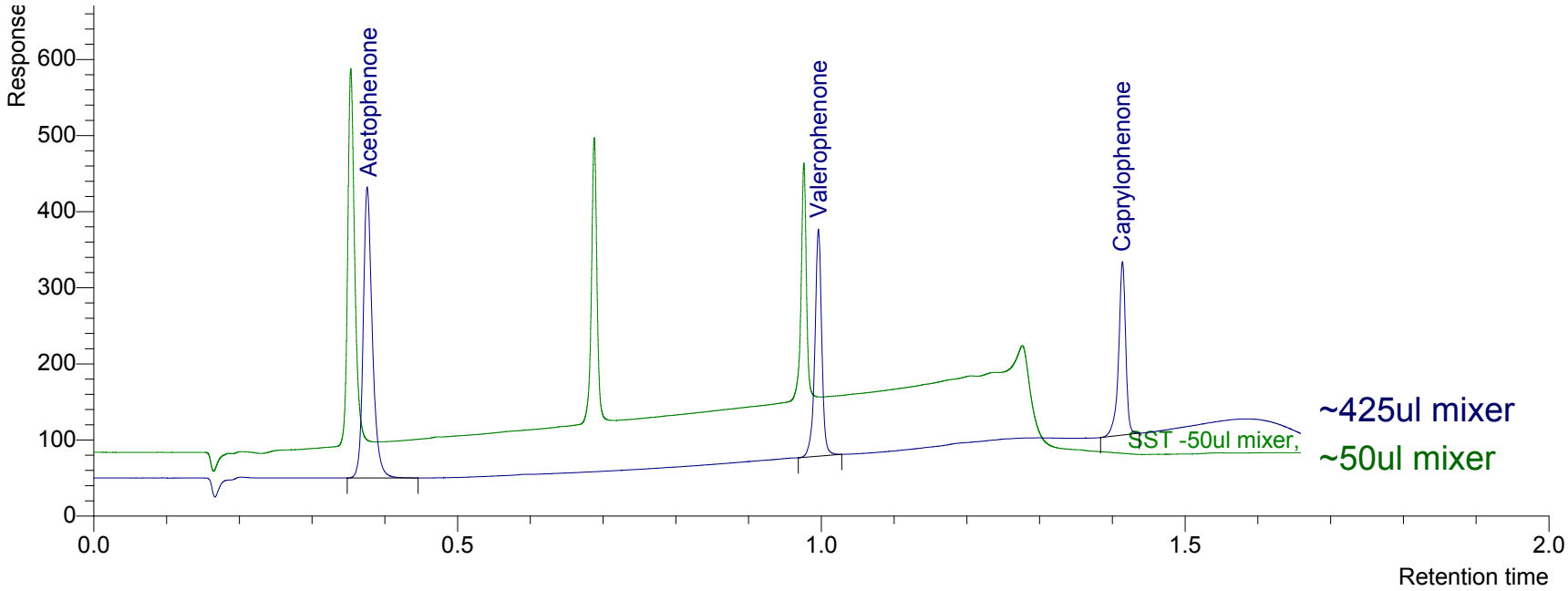
- Differences in dwell volume
  - Possible to compensate a smaller dwell volumes by introducing an isocratic step (HPLC → UHPLC)
  - Not possible to compensate for larger dwell volumes
    - Largest impact for:
      - high dwell vol systems & low vol columns
      - early eluting peaks (aim for k1* >3)
      - analytes that are not closely related
    - UPLC 10cm x 2.1mm to HPLC 15cm X 4.6mm

      ~initial 3% of gradient affected
    - UPLC 10cm x 2.1mm to Fast HPLC 3cm X 4.6mm

      ~initial 15% of gradient affected

Dwell volume effects

UPLC mixers (50µl vs. 425µl peptide mixer)



	~50ul mixer		~425ul mixer	
Peak Name	**Ret. time (min)**	**RRT**	**Ret. time (min)**	**RRT**
Acetophenone	0.353	0.51	0.376	0.38
Valerophenone	0.688	1	0.996	1
Caprylophenone	0.976	1.42	1.414	1.42

Increased dwell volume can change in selectivity for early peaks

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Method Transfer

- Differences in dwell volume (UHPLC → Fast HPLC)
  - Or standardise on initial isocratic step in UHPLC (generic UHPLC methods) to compensate for larger dwell volumes
    - 7 projects studied at PRD Macclesfield using fast HPLC.
    - 4 projects RRT ±0.02
    - 3 projects RRT ±0.04
      - Methods can be translated across formats
  - Or live with potential issues when transferring to Fast HPLC
      - Consider optimisation of HPLCs (25-40% improvement in peak capacities)
  - Best solution is to inject when the gradient hits the column (currently not possible)

Heat of friction

- A temperature gradient is built up over the column
  - The evolved frictional heat is proportional to the flow rate & pressure
  - Measured at constant pressure

L (cm)	F(mL/min)	ΔP(psi)	T_{inlet} (C)	T_{outlet} (C)	ΔT (C)
5.0	1.07	13290	22.3	31.3	9
15	0.36	13250	21.4	25.5	4.1
45	0.12	14150	21	21.7	0.7

Critical comparison of performances of superficially porous particles and sub-2 μm particles under optimized ultra-high pressure conditions, Y. Zhang, X. Wang, P. Mukherjee, P. Petersson, J. Chromatogr. A, 1216 (2009) 4597-4605

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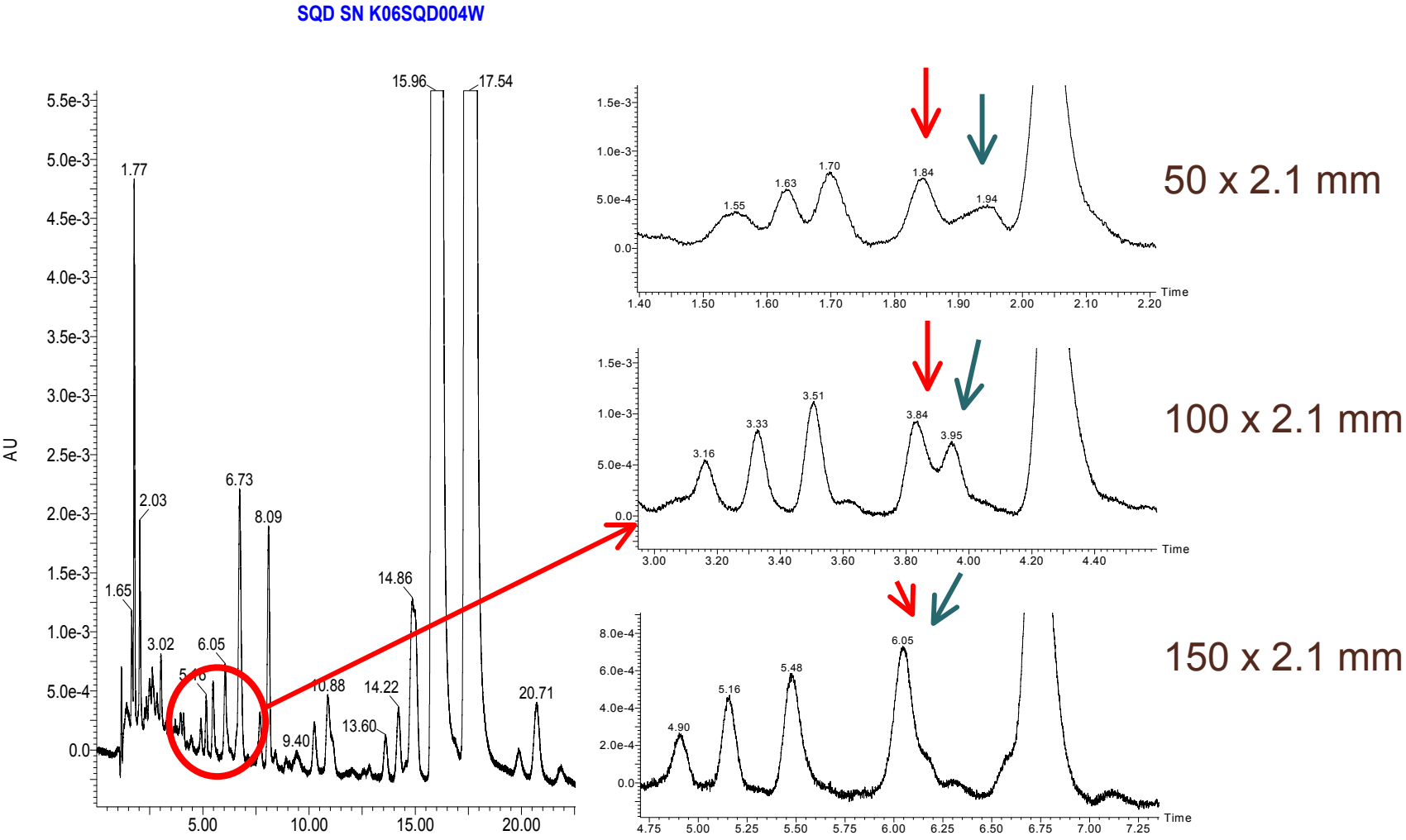
Method transfer-Heat of friction?



Patrik Petersson *et al.* (PARD Lund)

constant flow

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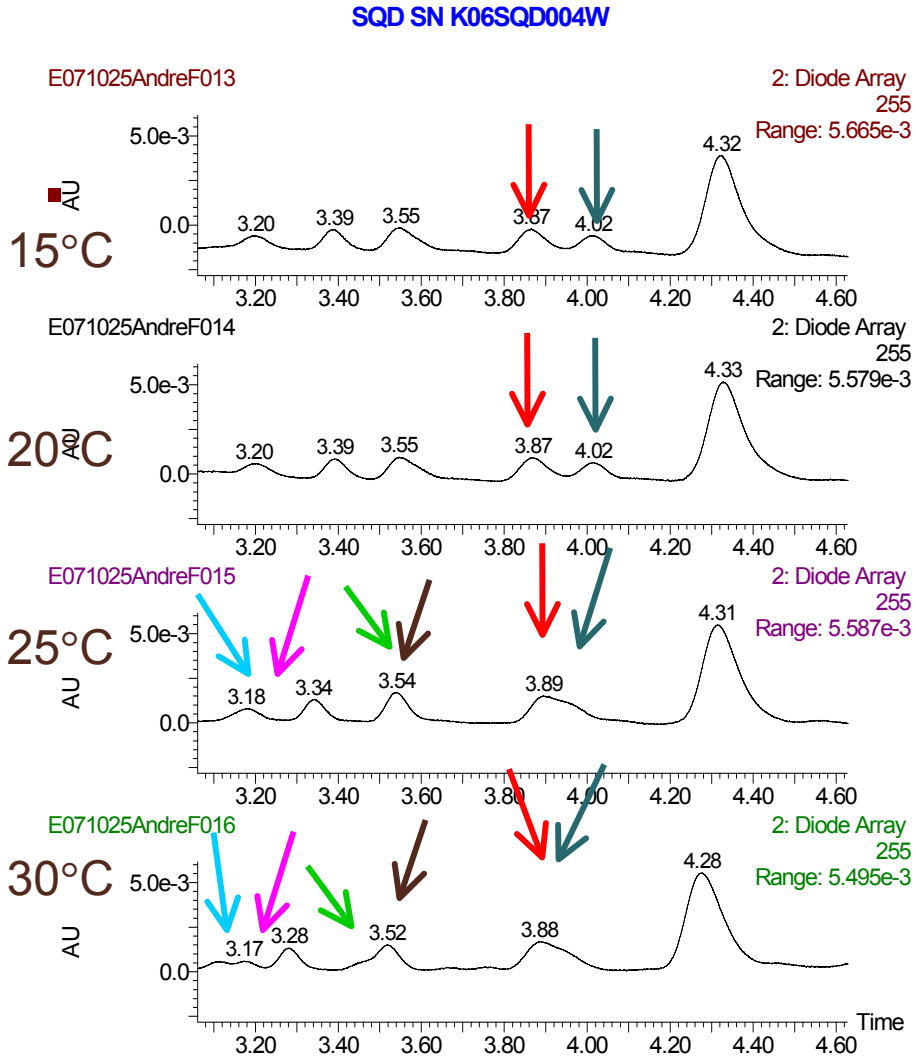
Method transfer-Heat of friction

- Experiments at different temp on **10cm column** supports the assumption of heat of friction
- Charged degradants move more than neutral
- Compensate by decreasing/increasing the temperature!
- In practice rarely a problem
- as analytes are closely related?



Column: 100 x 2.1 mm

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Stationary phase selectivities

- AZ Characterisation: UPLC phases with HPLC equivalent
- Constant selectivity <2 μm *vs.* 3, 5μm
  - Wide pH range, Stable up ≥60°C, Scale to semi-prep
    - Waters BEH/XBridge: C18, C8, Phenyl, Shield RP18
    - MN Nucleodur series + other vendors
- UPLC specific phases
  - prototype equivalent HPLC phases
- Superficially porous columns
  - Ascentis Express/Halo C_{18} (P <600bar)
  - 1 Particle for UHPLC & HPLC –no selectivity differences
  - Limited pH range & stationary phases available (semi prep)
- Cannot assume material with same trade name show same selectivity across all particle sizes-some do not!!!!

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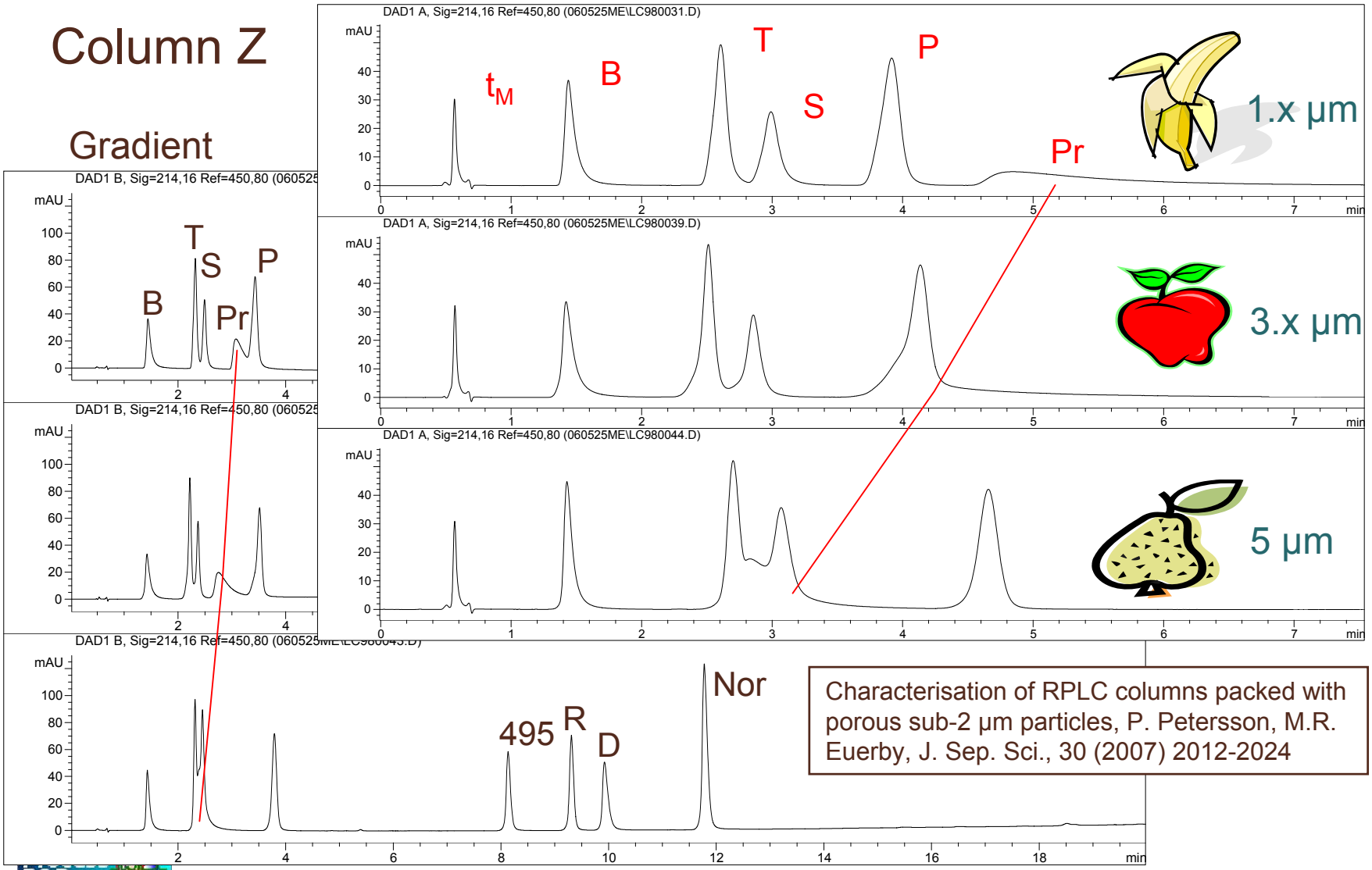
Differences in selectivity between different particle sizes

Isocratic

Column Z

Gradient



Characterisation of RPLC columns packed with porous sub-2 µm particles, P. Petersson, M.R. Euerby, J. Sep. Sci., 30 (2007) 2012-2024

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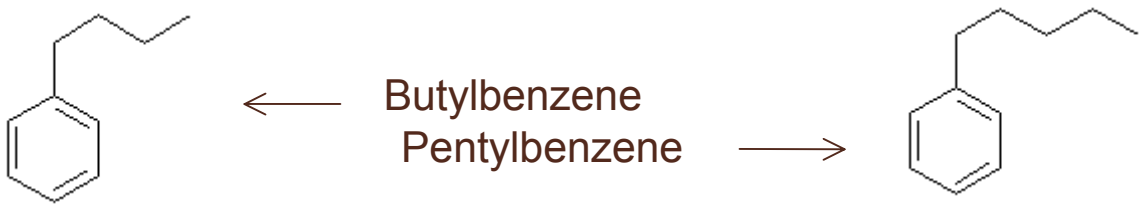
Column Characterisation

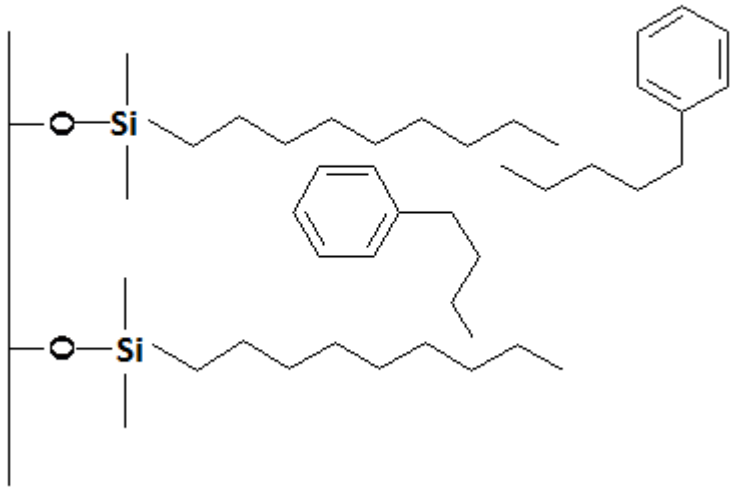
- There is no universal method for characterising columns
- AstraZeneca R&D Charnwood/Lund method based on the Tanaka protocol [Kimata *et al J Chrom Sci* 27 (1989) 721]
- The Tanaka protocol is based on 6 variables which can be used to more accurately define the selectivity of the phase
- All columns undergo testing to give results for these 6 variables
  - Hydrophobicity
  - Hydrophobic Selectivity
  - Steric Selectivity
  - Hydrogen Bonding Capacity
  - Total Ion Exchange Capacity
  - Acidic Ion Exchange Capacity
- Additional tests for aromatic and polar embedded phases

Column Characterisation

Hydrophobicity k_{PB} Mobile Phase: 80/20 MeOH/H_2O
Retention factor of pentylbenzene – indicates ligand density of the stationary phase

Hydrophobic Selectivity α_{CH2} Mobile Phase: 80/20 MeOH/H_2O
Ability to distinguish between molecules that differ by a CH_2 unit.





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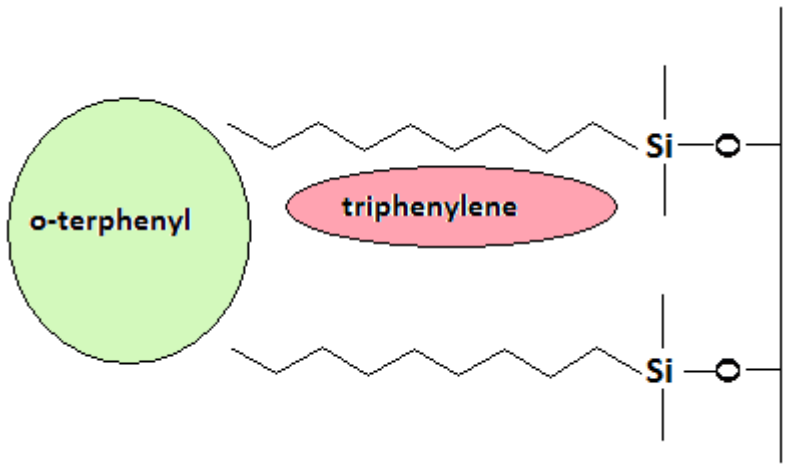
Column Characterisation

Steric Selectivity $\alpha_{T/O}$ Mobile phase: 80/20 $MeOH/H_2O$

measure of ligand density, planar triphenylene can penetrate the space between ligands. However, less dense bonding allows the more bulky o-terphenyl in.
Also a measure of ligand order.



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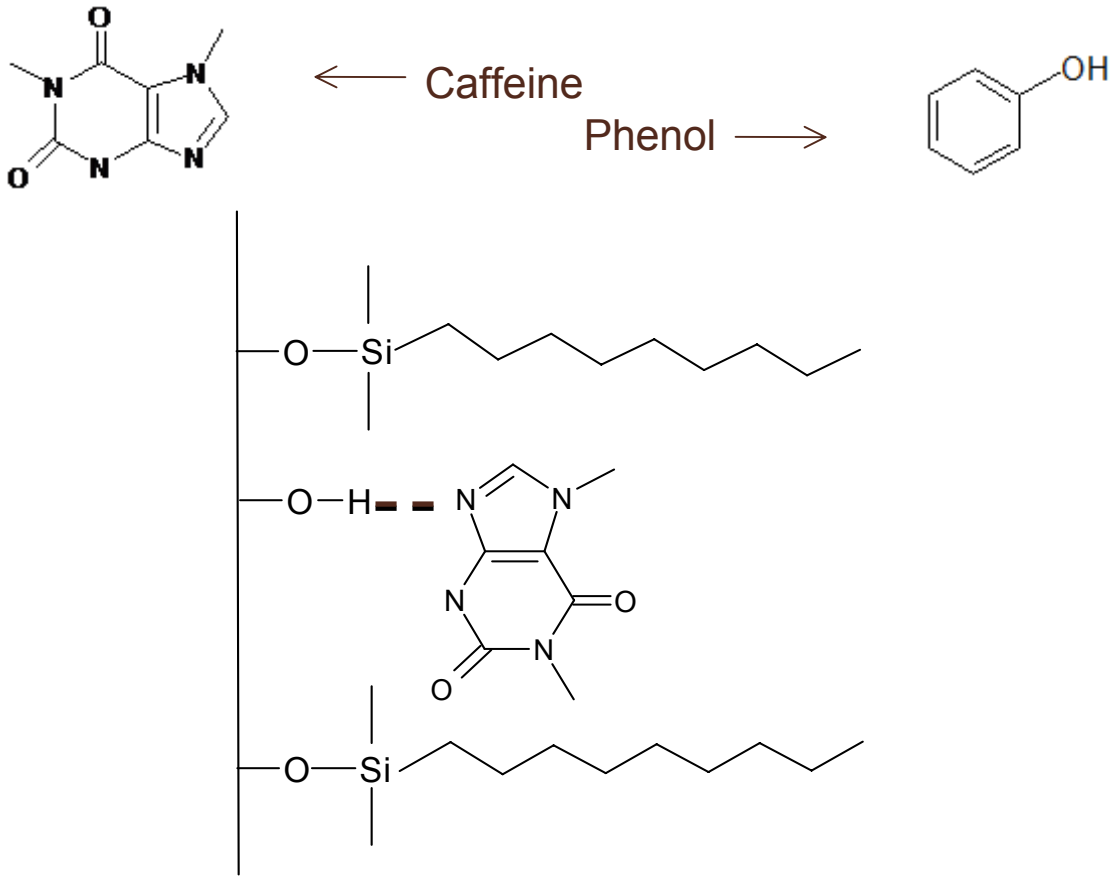
Column Characterisation

Hydrogen bonding $\alpha_{C/P}$ Mobile Phase: 30/70 MeOH/H_2O

A measure of the number of silanol groups in the phase.

Caffeine has extensive hydrogen bonding capability, whereas phenol has less.

More silanol groups would see an increased retention of caffeine over phenol.



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Column Characterisation

Total Ion Exchange Capacity $\alpha_{B/P\ pH\ 7.6}$

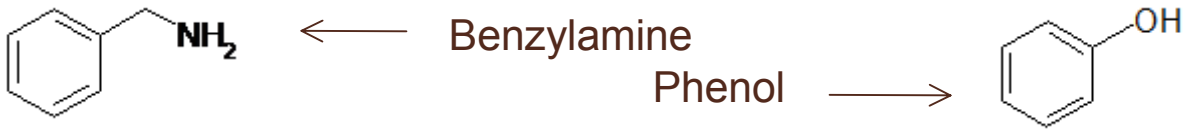
Mobile Phase: 70/30 KH_2PO_4 pH 7.6/MeOH

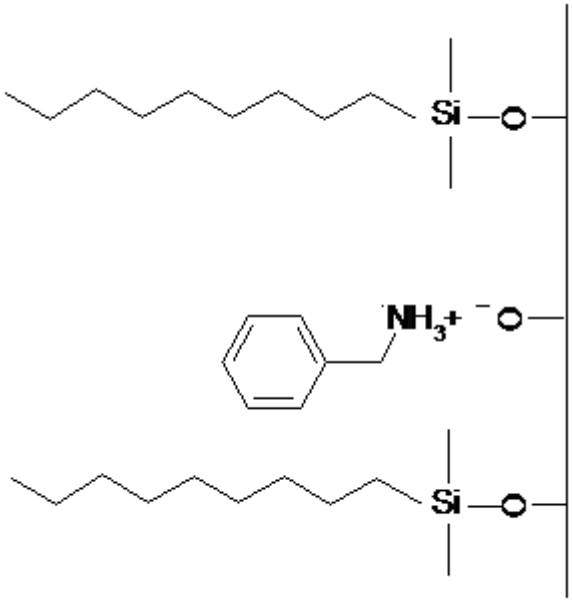
Total ion exchange capacity gives measure of total silanol activity

Acidic Ion Exchange Capacity $\alpha_{B/P\ pH\ 2.7}$

Mobile Phase: 70/30 KH_2PO_4 pH 2.7/MeOH

A measure of the acidic silanol activity





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Additional Characterisation

- Polar Embedded phases are tested to assess the polar embedded group interactions with more polar analytes
  - Testing also used to determine mechanism of polar embedded phase synthesis

- Phenyl phases undergo testing with aromatic analytes to determine if there are π-π interactions aiding retention

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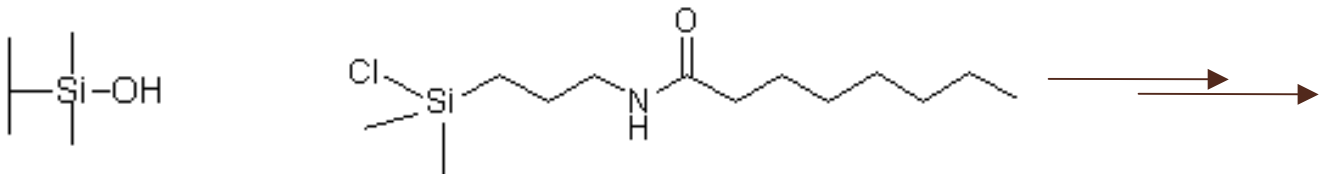
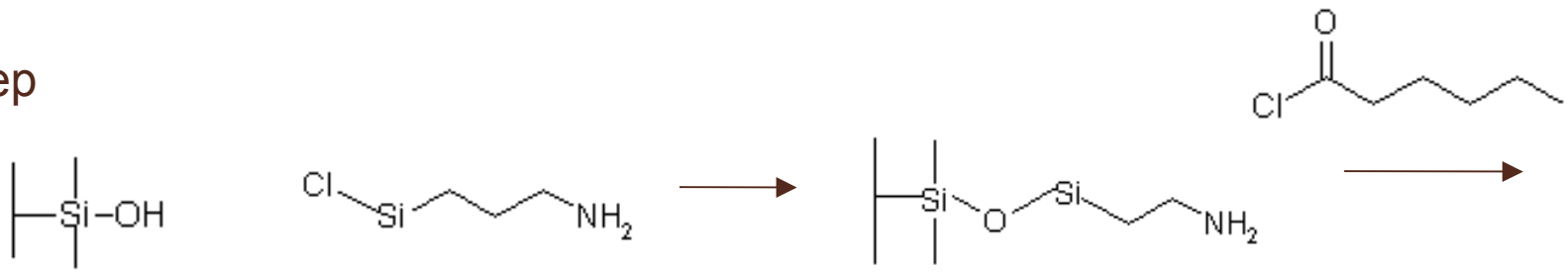
Polar Embedded Phase Synthesis

1 step



2 step



Residual silanol groups from the 2 step synthesis are where benzylamine could interact

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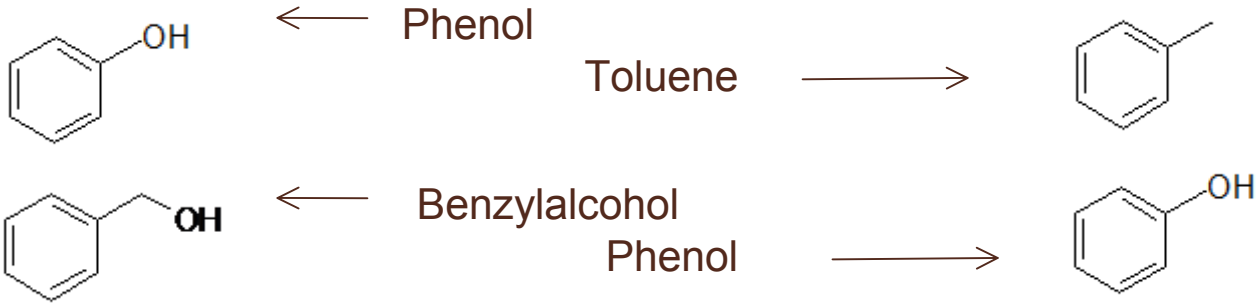
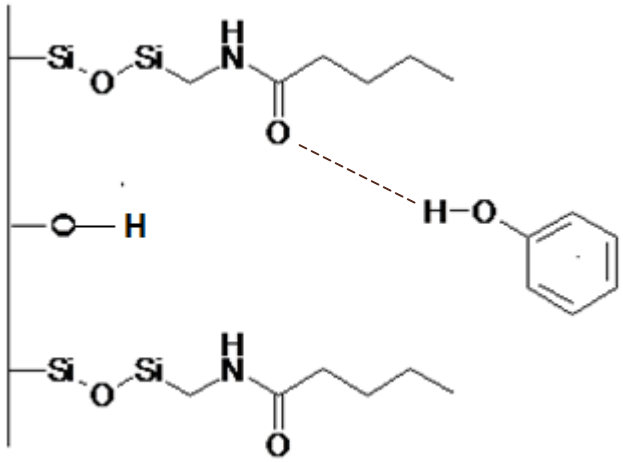
Polar Embedded Phase Extra Characterisation Parameters

Phenolic Selectivity $\alpha_{P/TI}$ $\alpha_{P/BA}$

Mobile Phase: 30/70 kH_2PO_4/MeOH

Measures retention of phenolic analytes compared to non phenolic at pH 2.5, phenol can hydrogen bond to amide links used in polar embedded phases



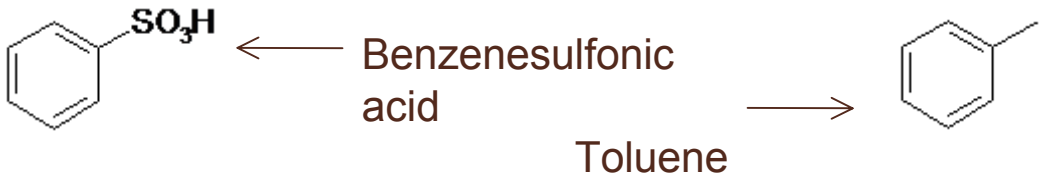


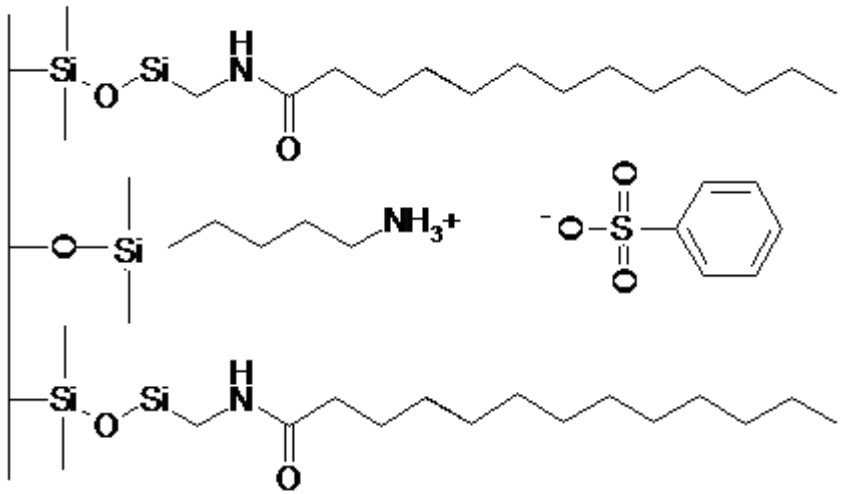
Global Process R&D

Anion-exchange capacity $\alpha_{BSA/TI}$

Mobile Phase: pH 2.5 30/70 KH_2PO_4/MeOH

Retention factor between benzenesulfonic acid, which can be deprotonated to interact with residual amine groups from phase synthesis, & toluene which has no such capability





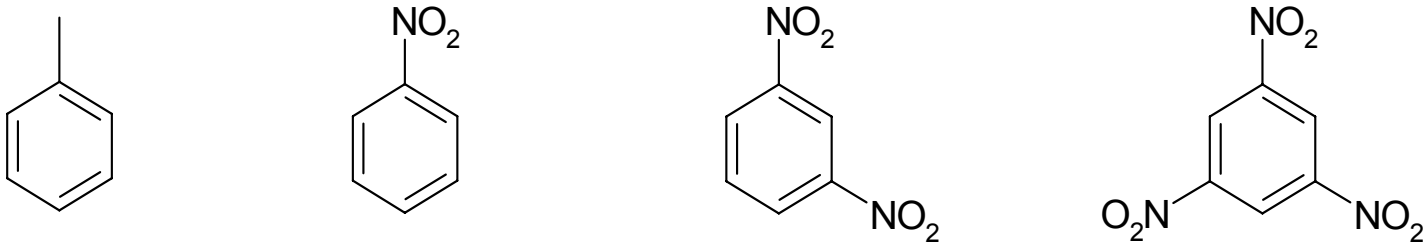
Global Process R&D

Aromatic Phase Probes

Phenolic Selectivity $\alpha_{TNB/NB}$ $\alpha_{DNB/NB}$ $\alpha_{TNB/TI}$

Mobile Phase: 1:1 MeOH/H_2O

Measures retention of aromatic analytes due to π-π interactions. Increased retention of electron deficient analytes (nitrobenzenes) by aromatic ligands of the stationary phase.



Increasing π-π interactions

Global Process R&D

Global Process R&D

Statistical Testing

- Determine if results for a phase with different particle sizes are ‘consistent’

		k PB	α CH2	α T/O	α C/P	α B/P pH 7.6	αB/P pH 2 .6
"New generation" C18 3 µm	Batch A	3.05	1.47	1.58	0.38	0.349	0.113
	Batch B	3.31	1.47	1.58	0.38	0.377	0.114
	Batch C	3.17	1.47	1.61	0.38	0.328	0.113
	Batch D	3.09	1.46	1.57	0.38	0.386	0.114
	% RSD between batch	3.7	0.4	1.0	0.8	7.4	0.5
Traditional "type A" C18 3 µm	Batch A	1.06	1.31	1.77	0.41	10.36	0.29
	Batch B	1.10	1.33	1.69	0.43	9.77	0.36
	Batch C	1.11	1.33	1.71	0.44	10.99	0.29
	Batch D	1.10	1.33	1.81	0.43	10.15	0.27
	% RSD between batch (df = 3)	1.9	0.9	3.2	2.7	5.0	13.0
	Pooled RSD between batch (df = 6)	**2.9**	**0.7**	**2.4**	**2.0**	**6.3**	**9.2**

*Critical value for a one-tailed F-test at 95% confidence level F_{crit} = **5.1 for $F_{2/6}$** for $F_{3/6}$ 4.8 & $F_{1/6}$ 6.0

Statistical data calculated by Patrik Petersson, AstraZeneca Lund.

Global Process R&D

Conventional C18 Phases

- Monomeric bonded C18 Stationary phases

Column Details	Hydrophobicity k_{PB}	Hydrophobic α_{CH2}	Steric $\alpha_{T/O}$	Hydrogen Bonding $\alpha_{C/P}$	Total Ion Exchange $\alpha_{B/P}$ pH 7.6	Acidic Ion Exchange $\alpha_{B/P}$ pH 2.6
Nucleodur Gravity C18 *3.0 µm* 2.1 x 50 mm	7.42	1.52	1.38	0.37	0.27	0.07
Nucleodur Gravity C18 *UPLC 1.8 µm* 2.1 x 50 mm	7.10	1.52	1.39	0.37	0.26	0.07
Vendor D C18 *~3 µm* 2.1 x 50 mm	**7.50**	1.52	1.41	0.45	0.29	0.08
Vendor D C18 *UHPLC ~2 µm* 2.1 x 50 mm	**6.25**	1.51	1.37	0.38	0.42	0.10
Waters Acquity HSS C18* *3.3 µm* 2.1 x 50 mm *prototype	5.72	1.51	1.48	0.36	0.24	0.10
Waters Acquity HSS C18 UPLC *1.8 µm* 2.1 x 50 mm	5.91	1.48	1.47	0.37	0.24	0.11

*prototype. Commercially available in October

Global Process R&D

Global Process R&D

Waters Phases

Column Details	k_{PB}	a_{CH2}	$a_{T/O}$	$a_{C/P}$	$a_{B/P}$ pH 7.6	$a_{B/P}$ pH 2.7
HSS T3 * 3.5 um 2.1 x 50 mm	4.54	1.49	1.18	0.50	0.33	0.11
HSS T3 1.8 um 2.1 x 50 mm	4.95	1.50	1.19	0.50	0.34	0.12
HSS C18 * 3.5 um 2.1 x 50 mm	5.72	1.51	1.48	0.36	0.24	0.10
HSS C18 1.8 um 2.1 x 50 mm	5.91	1.48	1.47	0.37	0.24	0.11
Xbridge C18 3.5 um 2.1 x 50 mm	2.94	1.46	1.38	0.35	0.26	0.14
Acquity BEH C18 1.7 um 2.1 x 50 mm	2.81	1.46	1.36	0.36	0.26	0.14
HSS C18 SB* 3.5 um 3.0 x 150 mm	2.26	1.39	1.89	1.83	5.37	0.11
HSS C18 SB 1.8 um 2.1 x 100 mm	1.76	1.39	1.94	1.86	4.99	0.16
X-Bridge Phenyl 3.5 um 3.0 x 150 mm	1.32	1.30	1.00	0.83	0.39	0.16
Acquity BEH Phenyl 1.7 um 2.1 x 50 mm	0.88	1.30	1.00	0.85	0.41	0.18
X-Bridge Shield RP18 3.5 um 3.0 x 150 mm	2.51	1.38	2.22	0.33	0.26	0.12
Acquity BEH Shield RP18 1.7 um 2.1 x 50 mm	1.26	1.41	2.36	0.30	0.27	0.13

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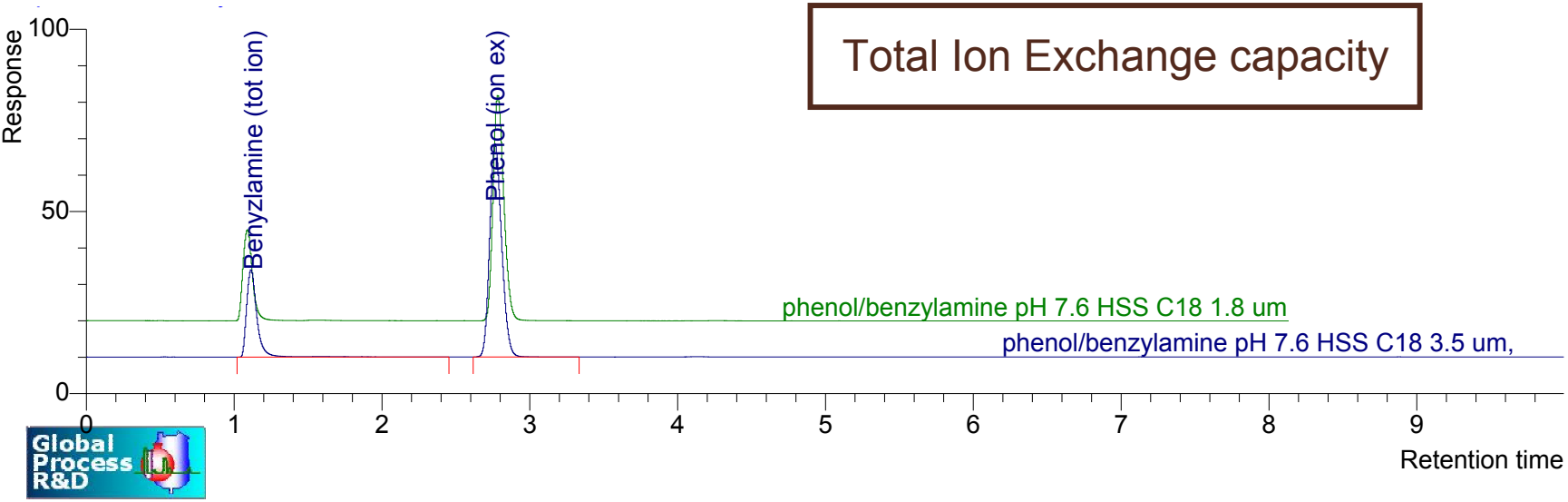
*prototype. Commercially available in October

Global Process R&D

Consistent selectivity

Column Details	Hydrophobicity k_{PB}	Hydrophobic α_{CH2}	Steric $\alpha_{T/O}$	Hydrogen Bonding $\alpha_{C/P}$	Total Ion Exchange $\alpha_{B/P}$ pH 7.6	Acidic Ion Exchange $\alpha_{B/P}$ pH 2.6
Waters Acquity HSS C18* *3.3 µm* 2.1 x 50 mm *available in Oct'09	5.72	1.51	1.48	0.36	0.24	0.10
Waters Acquity HSS C18 UPLC *1.8 µm* 2.1 x 50 mm	5.91	1.48	1.47	0.37	0.24	0.11

- Showing consistent chromatography across particle size.



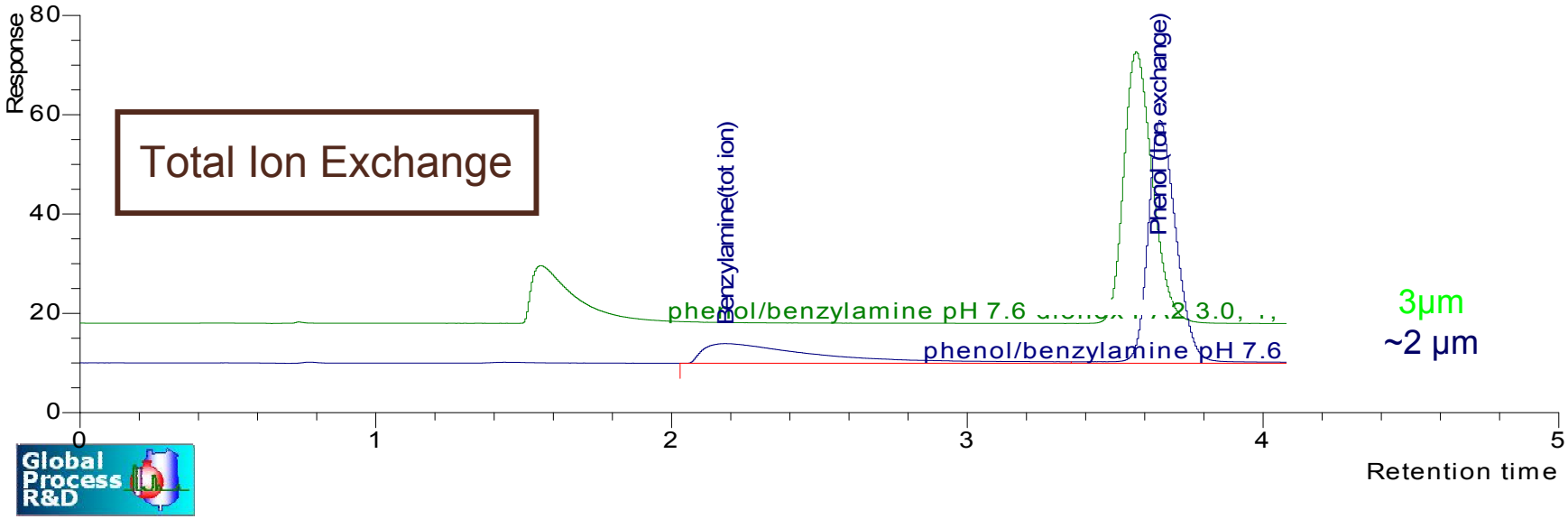
Total Ion Exchange capacity

Global Process R&D

Inconsistent selectivity

Column Details	Hydrophobicity k_{PB}	Hydrophobic α_{CH2}	Steric $\alpha_{T/O}$	Hydrogen Bonding $\alpha_{C/P}$	Total Ion Exchange $\alpha_{B/P}$ pH 7.6	Acidic Ion Exchange $\alpha_{B/P}$ pH 2.6
Vendor D Polar Emb. C18 ~3 µm 4.6 x 50 mm	4.11	1.45	1.87	**0.26**	**0.34**	**0.05**
Vendor D Polar Emb. C18 UHPLC ~2 µm 2.1 x 50 mm	4.03	1.44	1.92	**0.22**	**0.52**	**0.07**

- Modern polar embedded phases use 1 step bonding
- Significant differences seen in half of the characterisation tests.



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Comparing Particle Size

Column Details	Hydrophobicity k_{PB}	Hydrophobic α_{CH2}	Steric $\alpha_{T/O}$	Hydrogen Bonding $\alpha_{C/P}$	Total Ion Exchange $\alpha_{B/P}$ pH 7.6	Acidic Ion Exchange $\alpha_{B/P}$ pH 2.6
Vendor D Polar Emb. C18 *~3 µm* 4.6 x 50 mm	4.11	1.45	1.87	**0.26**	**0.34**	**0.05**
Vendor D Polar Emb. C18 UHPLC *~2 µm* 2.1 x 50 mm	4.03	1.44	1.92	**0.22**	**0.52**	**0.07**
% RSD	1	0	2	**12**	**30**	**24**
$F_{1/6}$*	0.2	0.3	0.6	**33.3**	**22.9**	**6.6**

- Large difference indicates possible different silica surface or bonding density.
  - Later confirmed by the vendor!

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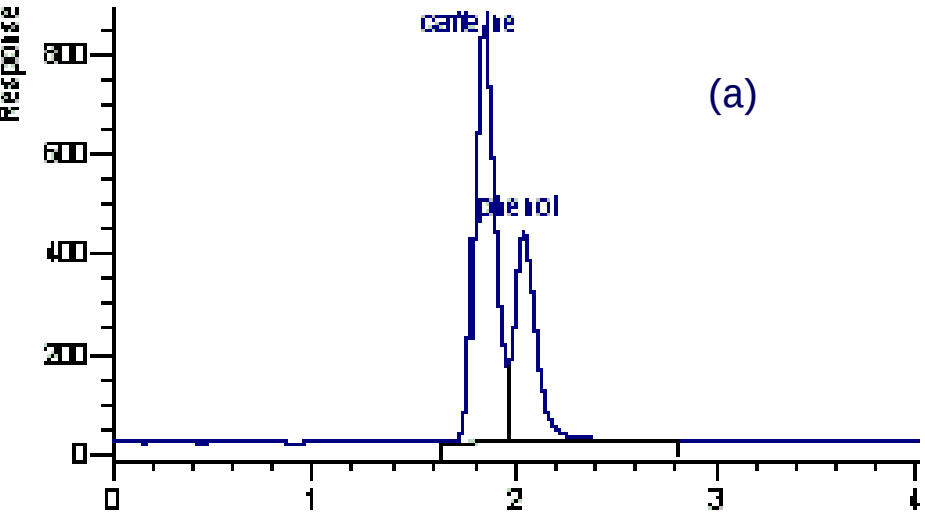
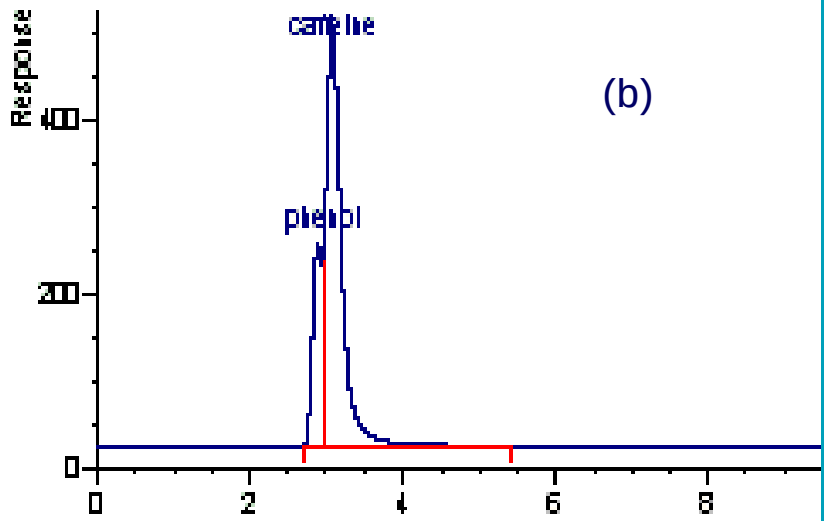
PFP phase

- Significant selectivity differences between two particle sizes
- Hydrogen bonding capacity & total ion exchange capacity between two particle sizes exhibit different elution order of analytes
- Likely transferability problems!

hydrogen bonding results



~3 um PFP phase



<2 um PFP phase

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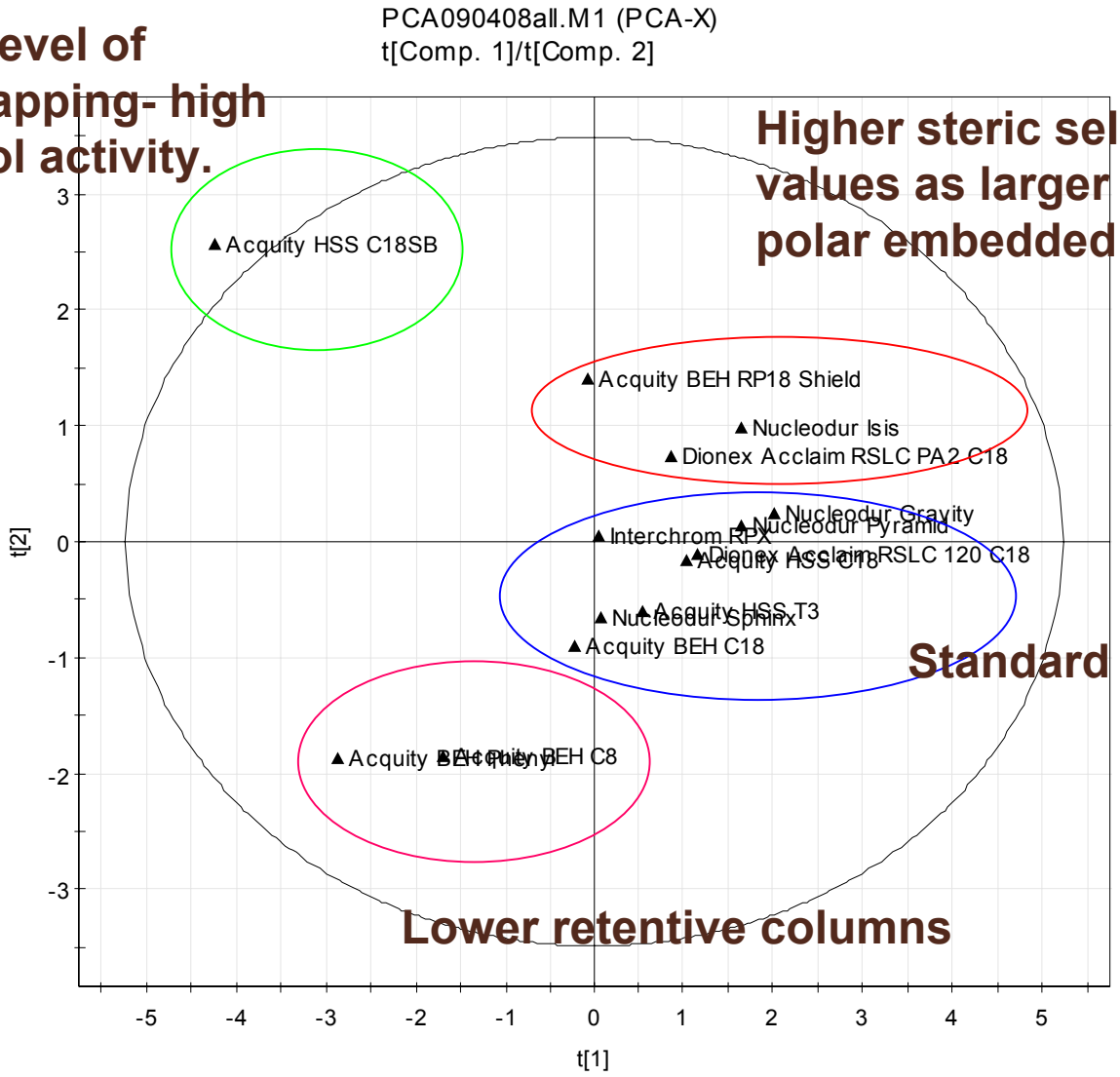
PCA Plots

- Identify phases which differ in selectivity or are similar

Low level of endcapping- high silanol activity.



Higher steric selectivity values as larger slots with polar embedded phases

Standard C18 columns

Lower retentive columns

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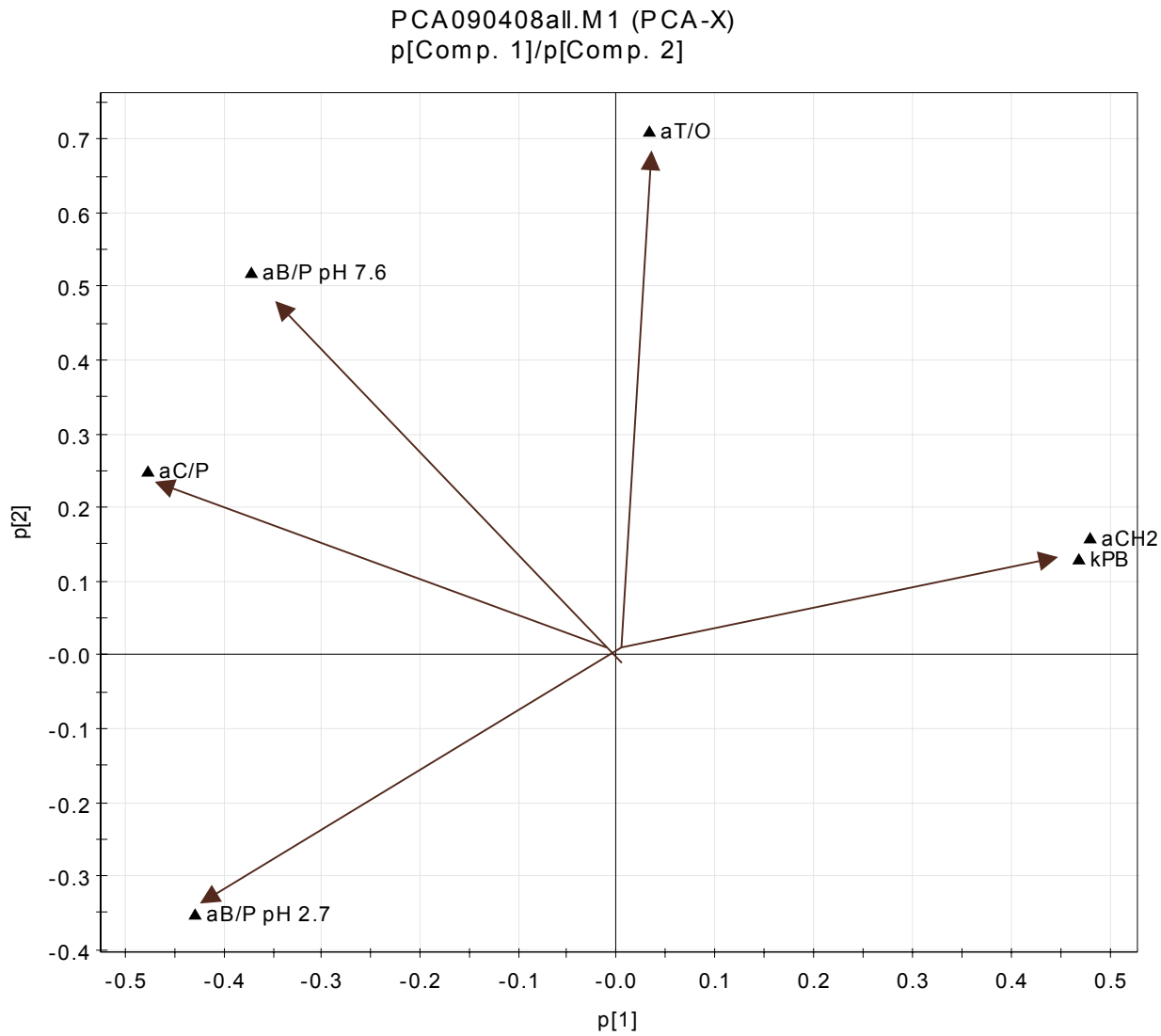
Supporting Loading Plot



R2X[1] = 0.540116 R2X[2] SIMCA-P+ 11 - 2009-03-08 18:48:01

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Column Stability

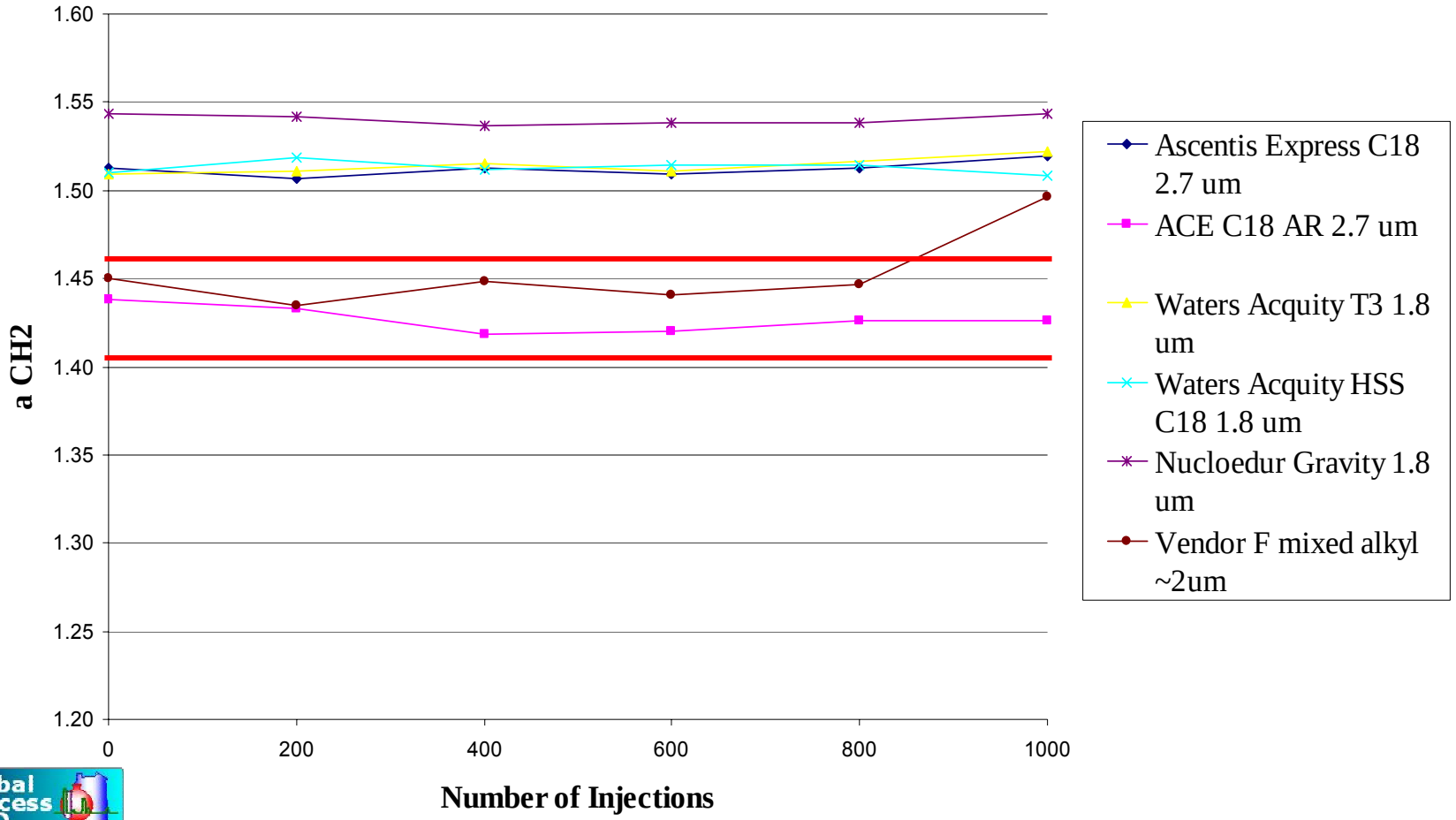
- Accelerated stability testing on 50 x 2.1mm columns
  - 1000 injections at 60°C
  - "rule of thumb" representative of 4000 injections at 40°C
  - Between every 10 injections of blank (methanol) a UPLC SST (system suitability test) sampled.
  - SST is mixture of 7 neutral compounds
  - 0.03%TFA/MeCN gradients
  - P ~800bar, F=1.2ml/min, T_G=3min –cycle time 3.75min

- Initial partial characterisation, repeated every 200 injections
  - Hydrophobic selectivity
  - Shape selectivity
  - Total ion exchange

Global Process R&D

Stability: Hydrophobic α

- Column characterisation test using butyl and pentylbenzene
- Typically no significant change (≤2%) over 1000 injections



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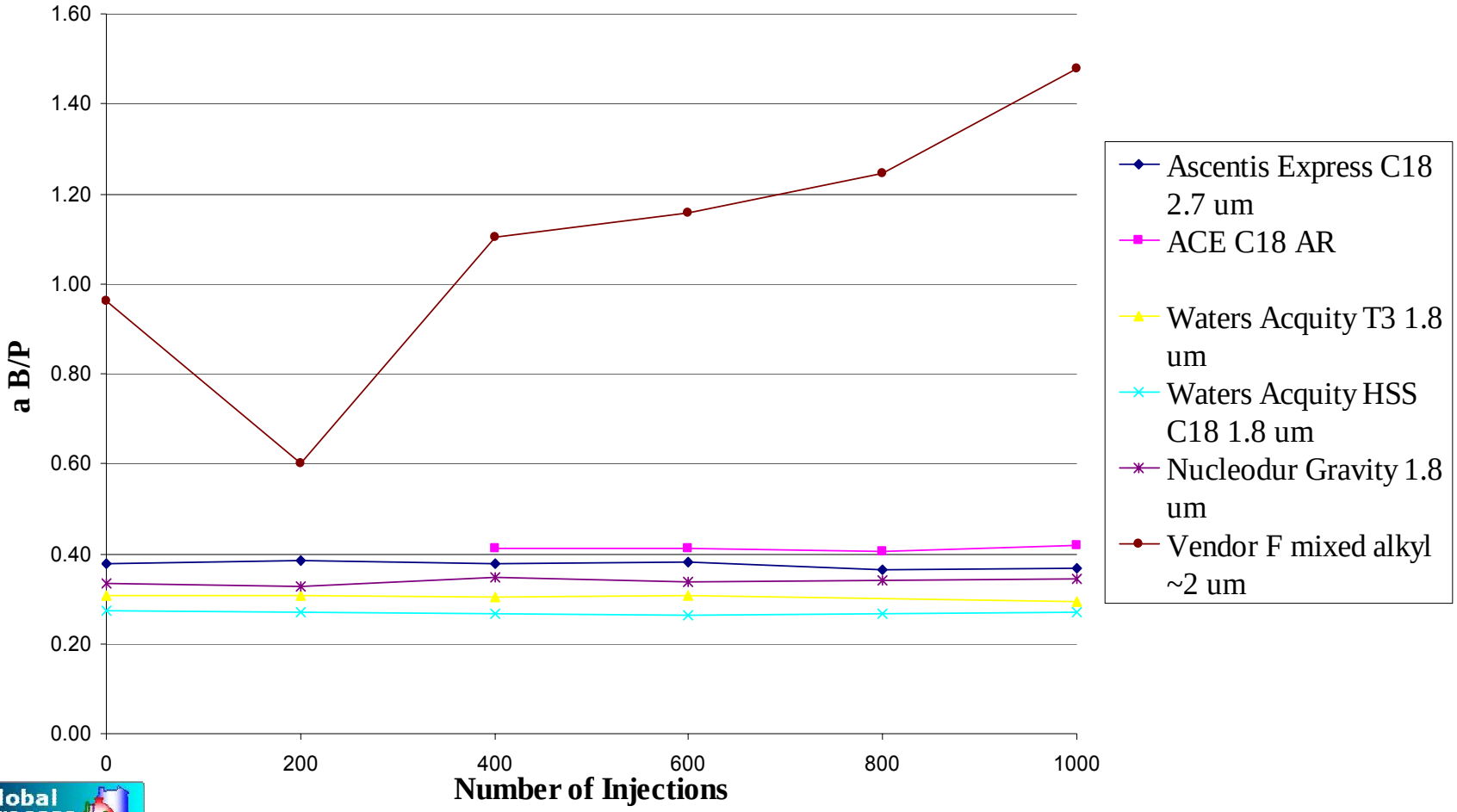
Stability: Total Ion Ex. Capability

- Most variable of all the selectivity terms used in stability testing
- Degradation of the phase affects the number & accessibility of silanol groups



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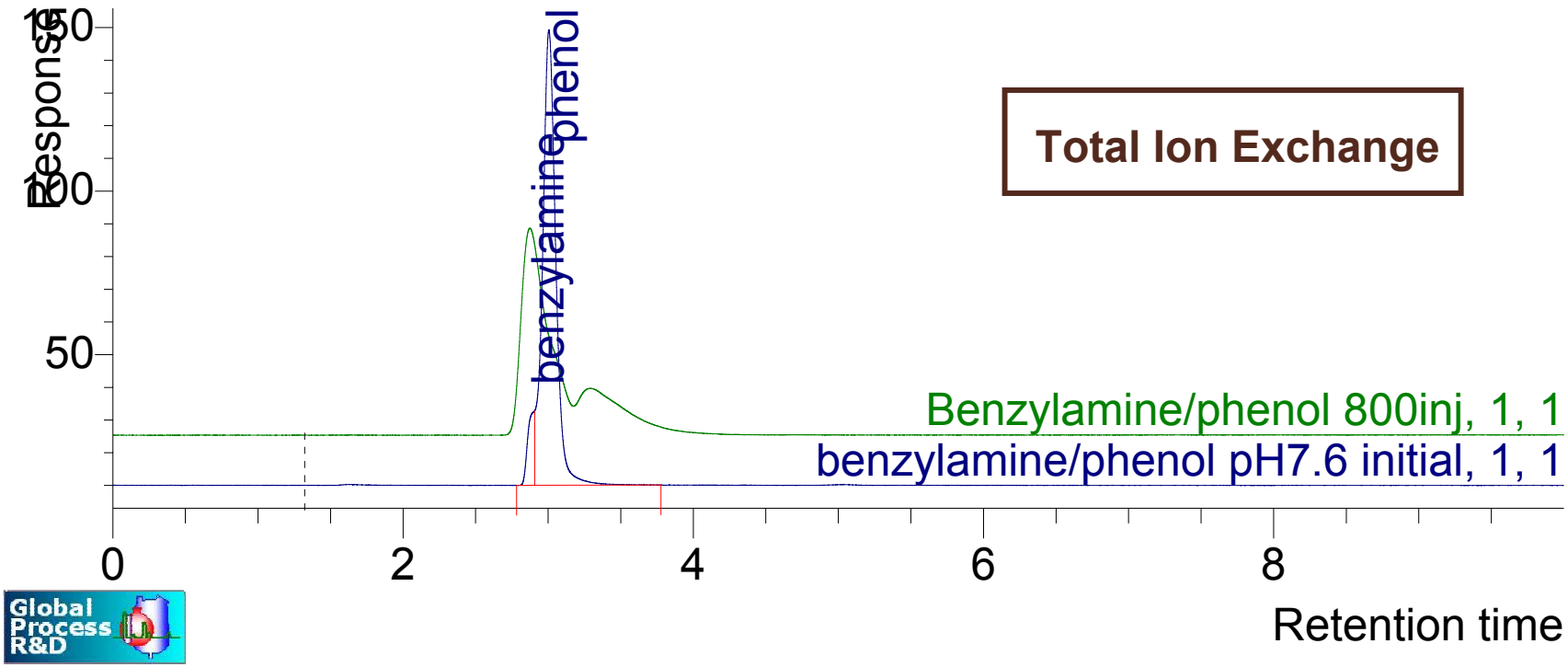
Global Process R&D

Mixed alkyl phase

- Shown to be unstable at low pH
- Large changes in retention of analytes, specifically benzylamine & phenol (reversal of elution)
- Suggests loss of ligand- increase in silanol groups & easier access



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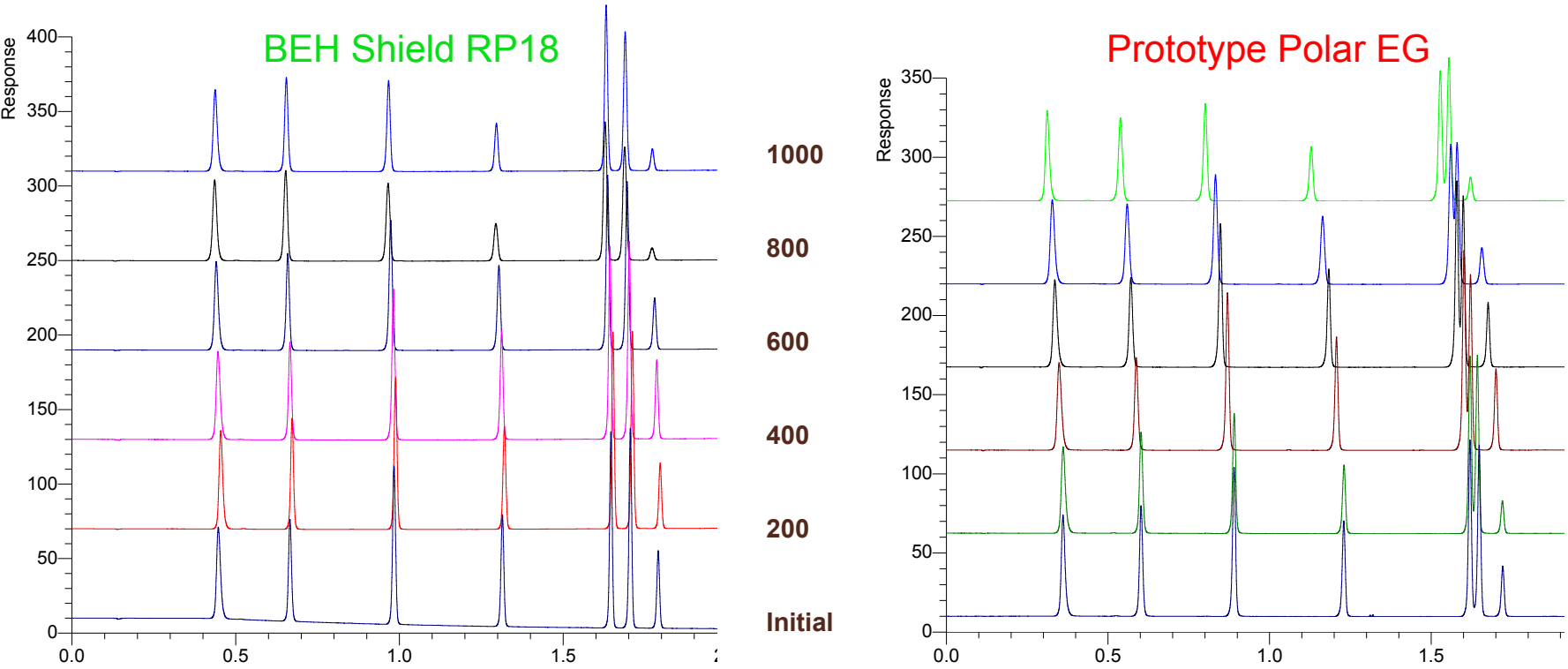
Column Stability

- Aq TFA/MeCN gradients @60°C (1000 injections, P~700bar)



Overlaid gradient separation of Waters neutral SST after multiple series of 200 injections

- See loss in retention –significant?

Global Process R&D

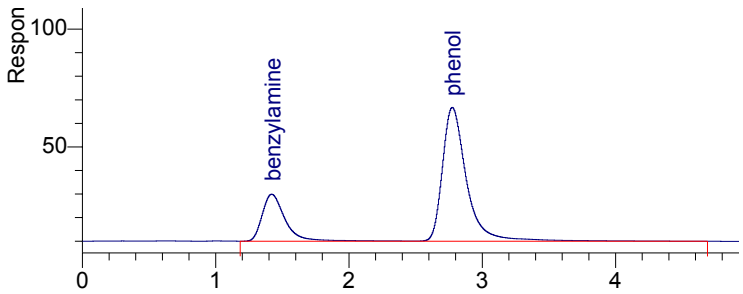
A.M. Clarke, P. Petersson, L. Mallard, M. Meluddin 2008

Column Stability

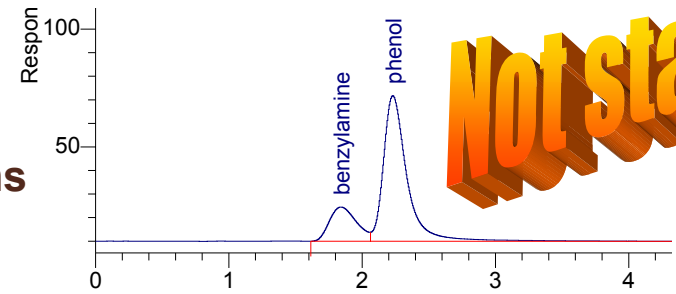
- Selectivity changes following use/testing
- Isocratic separation of benzylamine & phenol at pH 7.6

BEH Shield RP18

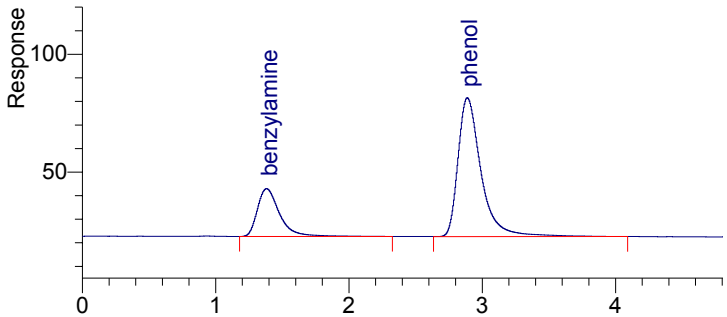
Prototype Polar EG



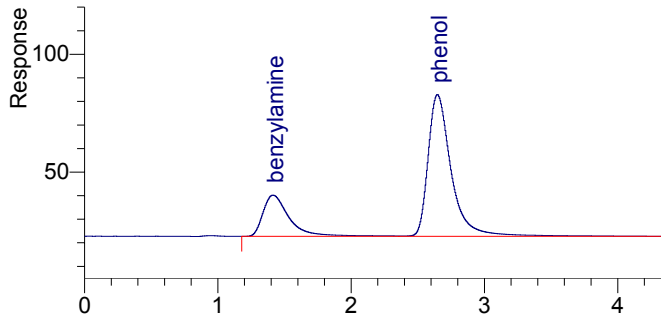
1000 injections



Not stable!



Initial

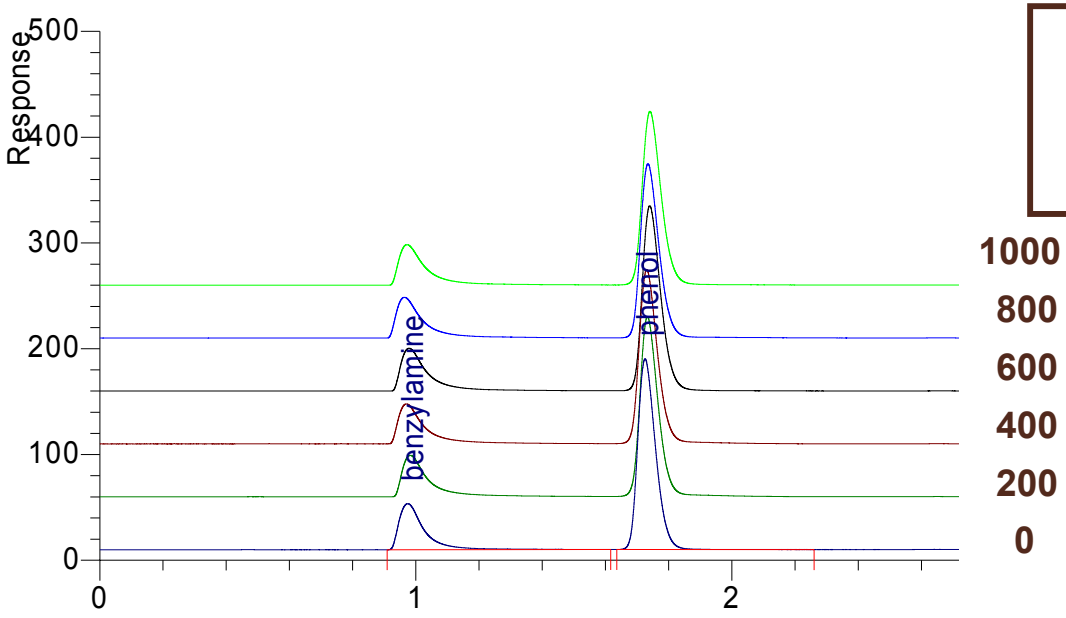


Global Process R&D

Superficially porous phases

- Superficially porous phases have a performance better/comparable to sub-2 µm particles under optimised conditions (at lower pressure)
- Best performance require P >400bar & low dwell volume LC (i.e. UHPLC)

X. Wang et al., J. Chromatogr. A, 1216 (2009) 4597-4605



Stability

Total Ion Exchange

1000
800
600
400
200
0

- Ascentis express C18 2.1 x 50 mm shows similar hydrophobic selectivity & shape selectivity overlays, therefore stable (<600bar, low pH up to 60°C).

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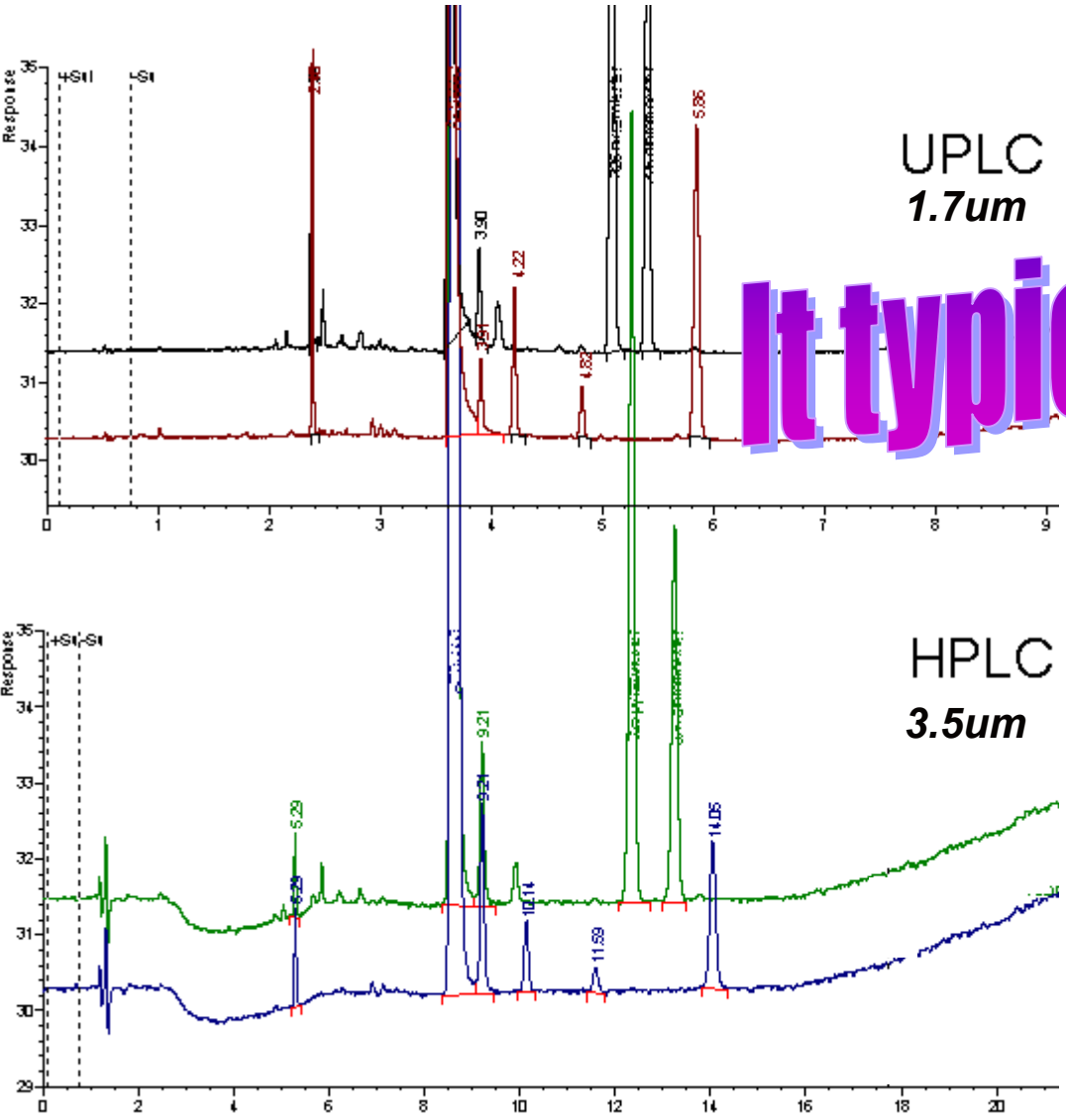
Global Process R&D

Method transfer in practice



It typically works!

Compound	Relative retention time			
	HPLC	UPLC	Δ	Δ [%]
Deg A	0.61	0.66	0.05	5
API	1.00	1.00	0.00	0
Deg B	1.07	1.07	0.00	0
Deg C	1.43	1.40	-0.03	-3
Deg D	1.54	1.49	-0.05	-5
Deg E	1.18	1.16	-0.02	-2
Deg F	1.34	1.32	-0.02	-2
Deg G	1.63	1.60	-0.03	-3

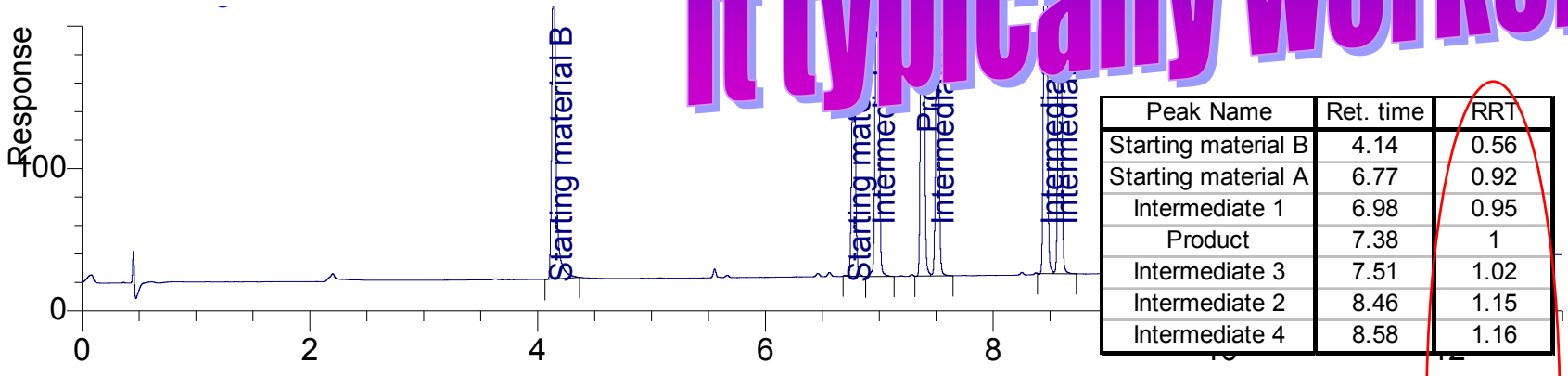
Excellent agreement of RRT's!!!

Geometrical scaled gradient.

Method transfer in practice

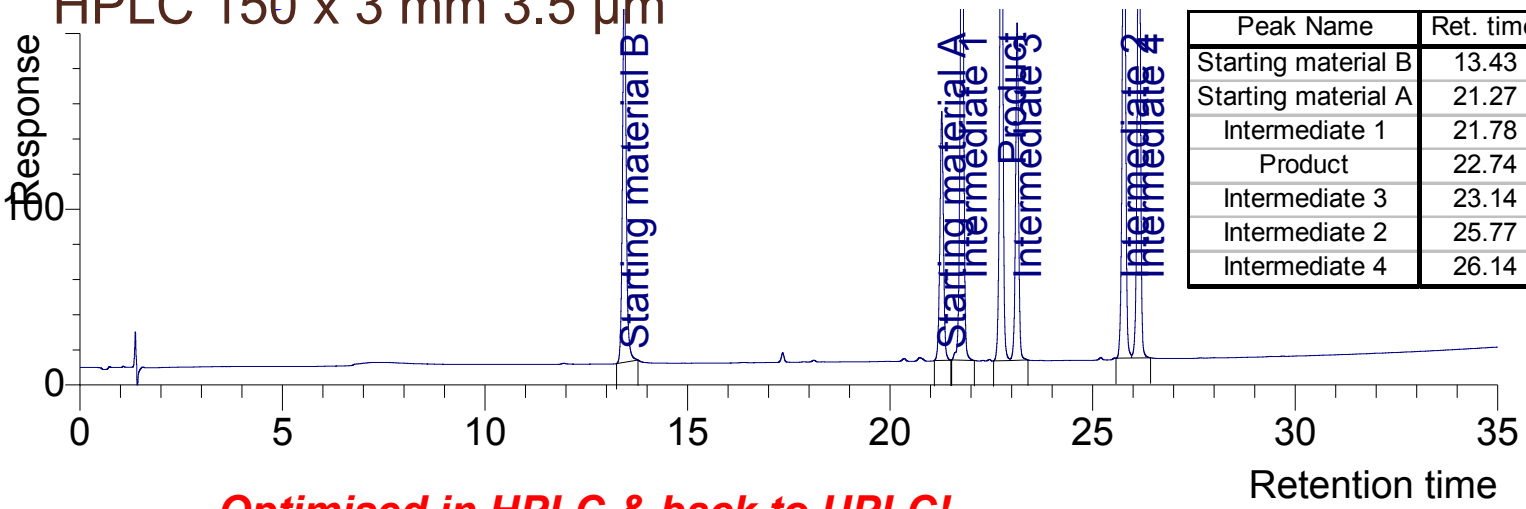
UPLC 100 x 2.1 mm 1.7 µm

It typically works!



Peak Name	Ret. time	RRT
Starting material B	4.14	0.56
Starting material A	6.77	0.92
Intermediate 1	6.98	0.95
Product	7.38	1
Intermediate 3	7.51	1.02
Intermediate 2	8.46	1.15
Intermediate 4	8.58	1.16

HPLC 150 x 3 mm 3.5 µm



Peak Name	Ret. time	RRT
Starting material B	13.43	0.59
Starting material A	21.27	0.94
Intermediate 1	21.78	0.96
Product	22.74	1
Intermediate 3	23.14	1.02
Intermediate 2	25.77	1.13
Intermediate 4	26.14	1.15

Optimised in HPLC & back to UPLC!
Comparable Peak capacities ±5%

Excellent agreement of RRT's!!!

Method transfer UHPLC ↔ HPLC

- How much extra validation is needed?
  - Show selectvity is the same (particle size effect, heat of friction)
    - when validating the original method (samples are available)
  - Show that the linearity is acceptable.
  - Difference in injection principle or materials?
    - e.g. Agilent 1100/1200 has different injection principle (& possibly materials) than an Acquity.
    - In a small no of projects we have seen a difference …
  - SST covers other requirements? (S/N, within range, etc)

- Two methods needed, one for UHPLC and one for HPLC
  - The only differences should be:
    - Different gradient tables
    - Different injection volumes & flow rates
    - Different column dimensions & d_p (same stationary phase type)

- Separation experts debating the current best practice on this ...
- Regulatory views required....

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Conclusions

- **AZ development adopting & implementing UHPLC**
  - Been successful in projects in AZ development
- Transfer from UHPLC to HPLC required to consider all customers
- Identify methods (HPLC ↔ UHPLC transfer by maintaining selectivity)
- Keep mobile phase selectivity constant
  - Maintain average capacity factor constant
  - Optimisation of existing HPLCs can improve translations
- Keep stationary phase selectivity constant
  - Maintain stationary phase selectivity in UHPLC & HPLC
- Successful in practice

Global Process R&D

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Conclusions

- Column characterisation
  - Useful for identifying preferred phases
  - “diverse” phases for generic methods/method development, and
  - Equivalent stationary phases (replacement)
  - Efficiency is not everything –Still need selectivity!
    - More UHPLC phases required
  - Several “good” vendors, but still cannot assume the same trade name = same selectivity for all particle sizes
    - Often have different silica surface properties
    - PFP and polymeric phases difficult to reproduce
    - Modern polar embedded phases use 1 step synthesis
- Cannot assume phases are stable

Global Process R&D

Global Process R&D

Acknowledgements

AstraZeneca Separation Science Global Technical Community

- Adrian Clarke (PRD Avlon/Charnwood, UK)
- Patrick Petersson (PARD Lund, Sweden)
- John Nightingale (PRD Macclesfield, UK)
- Partha Mukherjee (PARD Wilmington, US)
- Lee Mallard (PRD Avlon/Charnwood, UK)
- Rachel Wilson (PRD Avlon/Charnwood, UK)
- Helen Porter (PRD Avlon/Charnwood, UK)
- Mohamed Meluddin (Loughborough University/PRD Avlon/Charnwood, UK)
- Xiaoli Wang (PARD Wilmington, US)
- Simon Linke (PRD Avlon/Charnwood, UK)
- Marita Ransmark (PARD Lund, Sweden)
- Mel Euerby (PARD Charnwood, UK/HiChrom Ltd, UK)

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Questions ?