Fundamentals, Optimization and Practical Aspects of UHPLC Part 3

The Role of Temperature, Column Technology, Totally Porous and Superficially Porous Particles, Monoliths, Method Translation

Role of Temperature in (U)HPLC

Temperature increase

- Decrease in the mobile phase viscosity*
  - Viscosity is reduced 1 to 2 % per ºC increase
  - Lower back pressure
- Increase of solute diffusivity
  - Lower HETP-value

$$D_{m,T} = D_{m,298} \frac{\eta_{298}}{\eta_T} \frac{T}{298}$$

- Decrease in the mobile phase polarity
  - Increasing temperature 4 to 5 ºC is comparable to increasing the methanol or acetonitrile concentration by 1% in a reversed phase system
  - Less organic solvent in the eluent (in RP separations)

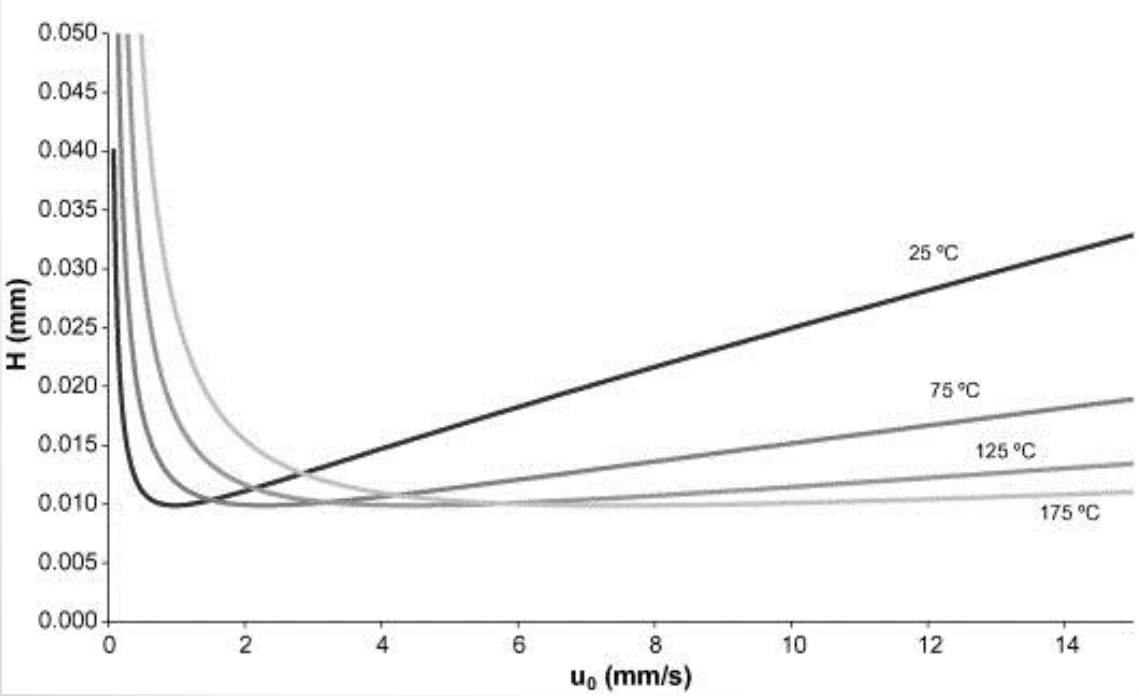
***J. Billen et al., J. Chrom. A, 1210, 30 (2008)**

Role of Temperature in (U)HPLC

Theoretical based on the Knox equation

Theoretical plate-height curves for 5 µm particles calculated with equation below assuming values of: A = 0.66, B = 3.00 and C = 0.05. Diffusion coefficients for phenol in 40/60 acetonitrile/water were calculated at each temperature according to Wilke-Chang

$$H = d_p \left[A \left(\frac{u_0 d_p}{D_m} \right)^{1/3} + B \left(\frac{D_m}{u_0 d_p} \right) + C \left(\frac{u_0 d_p}{D_m} \right) \right]$$

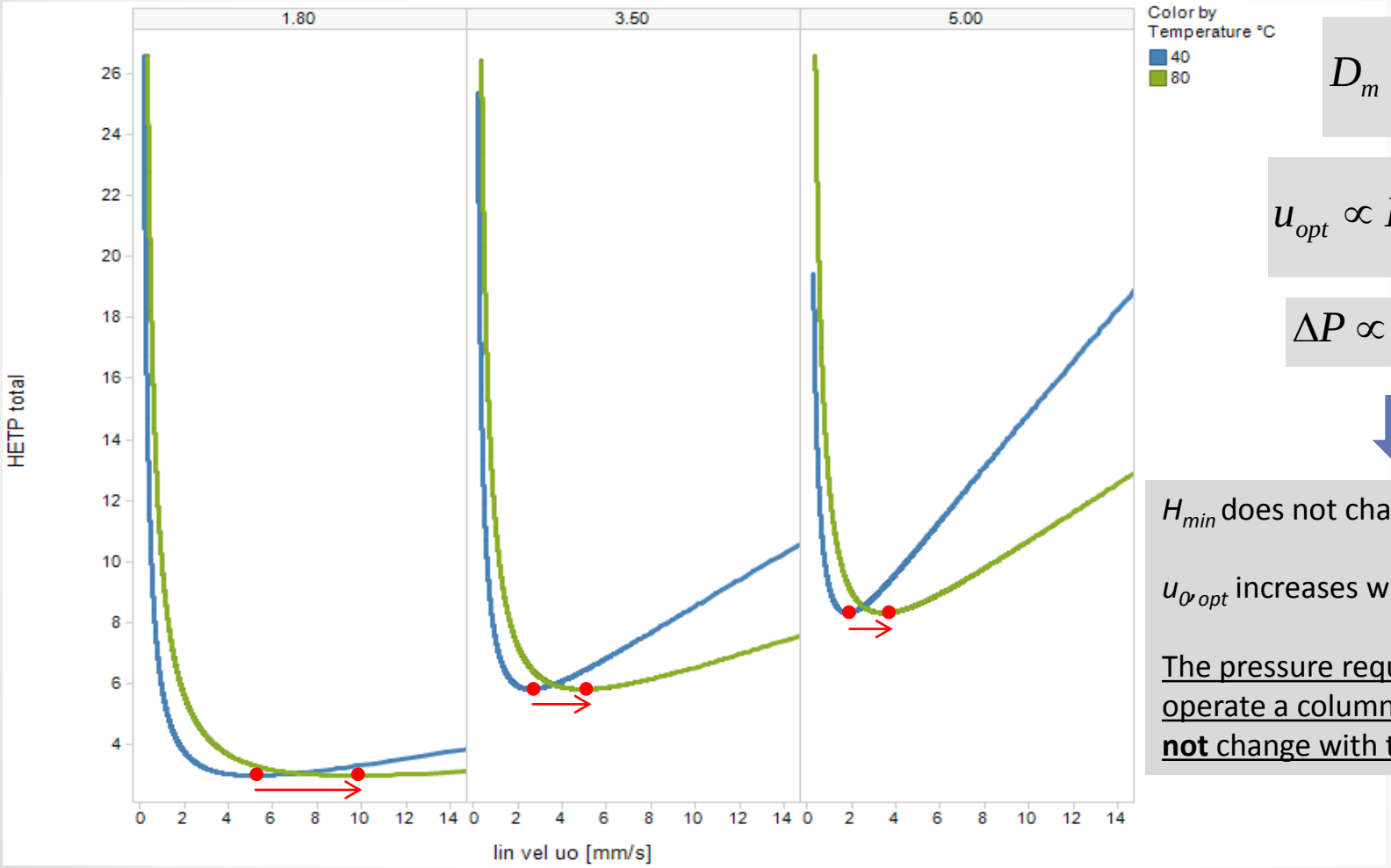


a. Minimum in the HETP plot is not affected by temperature increase
b. The C-term region flattens.

F. Lestremau et al., J. Chrom. A, 1138, 2007, 120 - 131

Role of Temperature in (U)HPLC

Theoretical based on the Knox equation



$$D_m \propto \frac{1}{\eta}$$

$$u_{opt} \propto D_m \propto \frac{1}{\eta}$$

$$\Delta P \propto u_{opt} \cdot \eta$$

H_{min} does not change with T

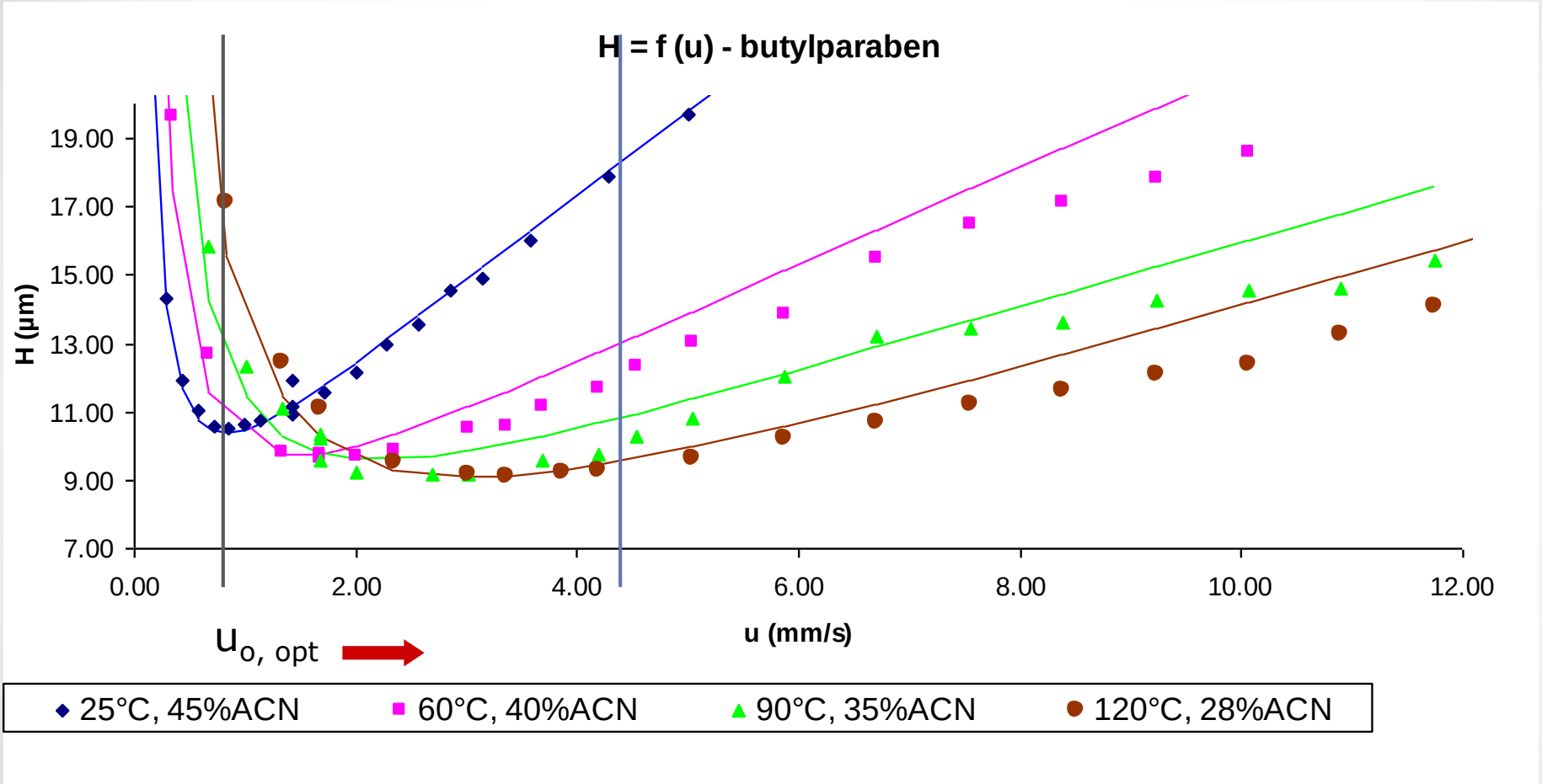
$u_{0,opt}$ increases with T

The pressure required to operate a column at $u_{0,opt}$ does **not** change with temperature

Slide courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

Role of Temperature in (U)HPLC

Experimental Result*



*Results courtesy of Dr. Davy Guillarme, Univ. Geneva

Role of Temperature in (U)HPLC

Experimental Result*

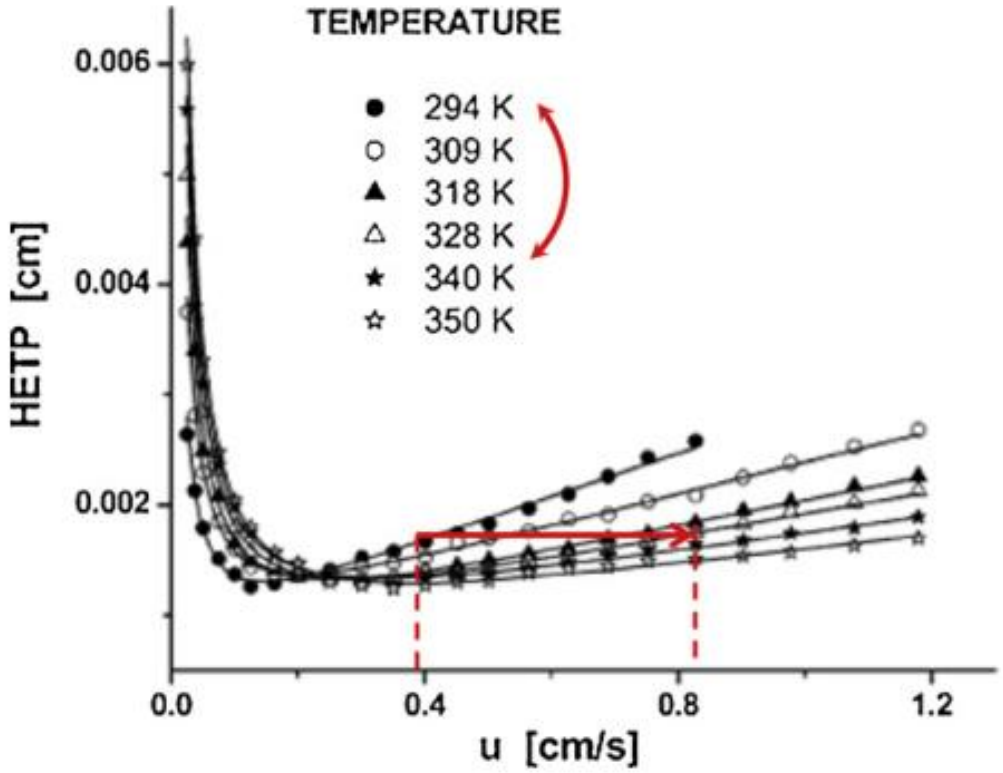
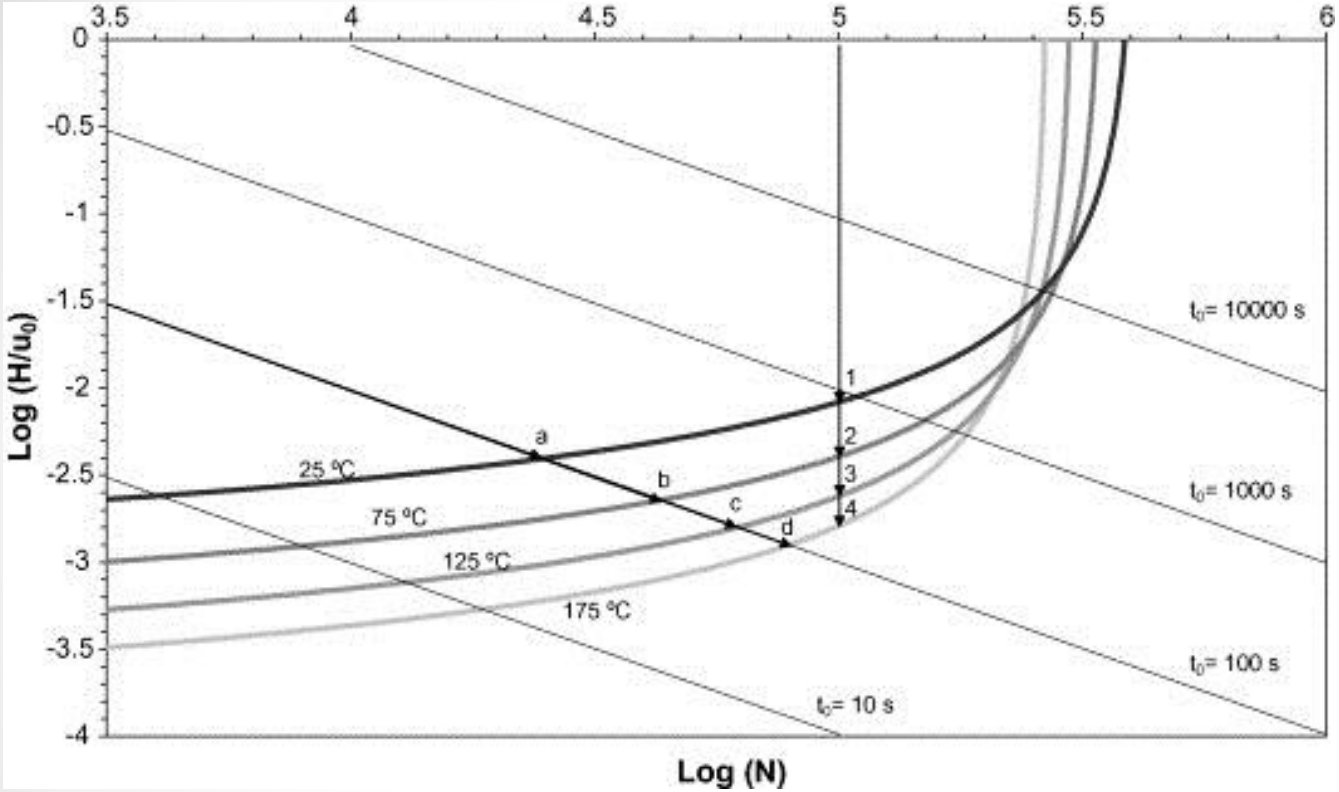


Fig. 2. Effect of the temperature on the HETP of a 5 μm Sunfire-C_{18} column for phenol eluted with a mixture of acetonitrile and water (15/85, v/v). The eluent viscosity decreases from 1.09 to 0.37 cP when the temperature increases from the ambient temperature to 77 °C.

*Guiochon & Gritti, J. Chrom. A, **1228** 2 (2012)

Role of Temperature in UHPLC

Poppe Plot*

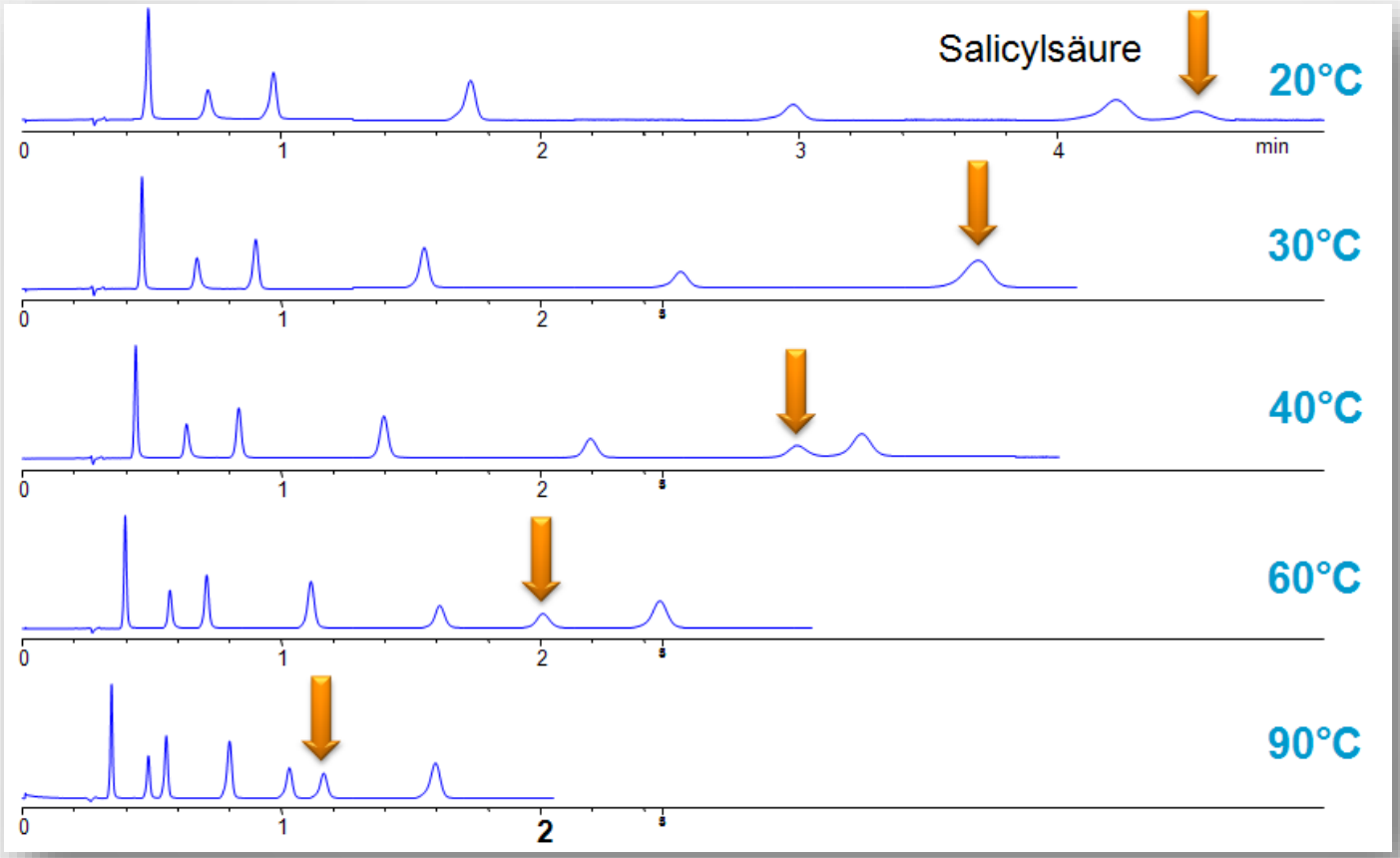


Kinetic plots for 5 µm particles at different temperatures (black: 25◦C, dark grey: 75◦C, grey: 125◦C, light grey: 175◦C). Assumed parameters: maximum pressure, P = 400 bar; mobile phase 40/60 acetonitrile/water; viscosity, η = 8.28×10^{-4} Pa s (25◦C), 4.11×10^{-4} Pa s (75◦C), 2.44×10^{-4} Pa s (125◦C), 1.62×10^{-4} Pa s (175◦C) calculated with flow resistance factor, $\phi_0 = 1000$; diffusion coefficients, Dm = 1×10^{-9} m2/s (25◦C), 2.35×10^{-9} m^2/s (75◦C), $4.54 \times 10^{-9} m^2/s$ (125◦C), 7.68×10^{-9} m^2/s (175◦C); Knox parameters: A = 0.66, B = 3.00 and C = 0.05.

F. Lestremau et al., J. Chrom. A, 1138, 2007, 120 - 131

Role of Temperature in UHPLC

Selectivity changes



Column: ZORBAX SB-C18 4.6 x 50 mm, 1.8 µm
Solvent: A: Water + 0.1% formic acid B: Acetonitrile + 0.1% formic acid (85:15), Flowrate: 1 mL/min

Data and slide courtesy of Dr. Udo Huber, Agilent Technologies, Germany

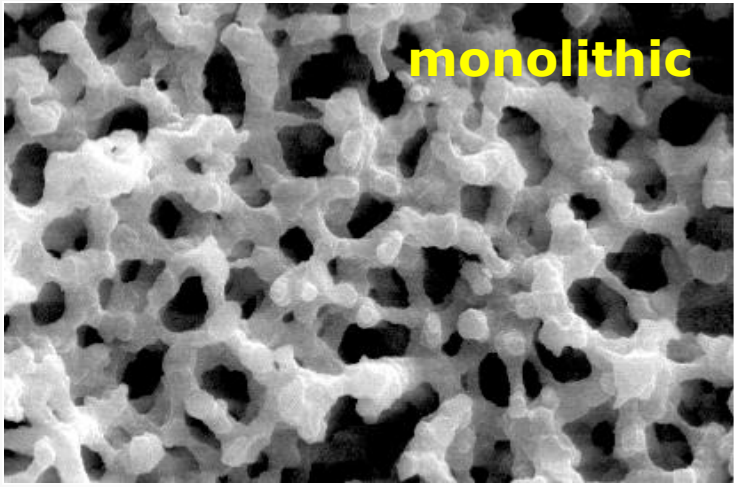
Lessons learned about the role of temperature in UHPLC

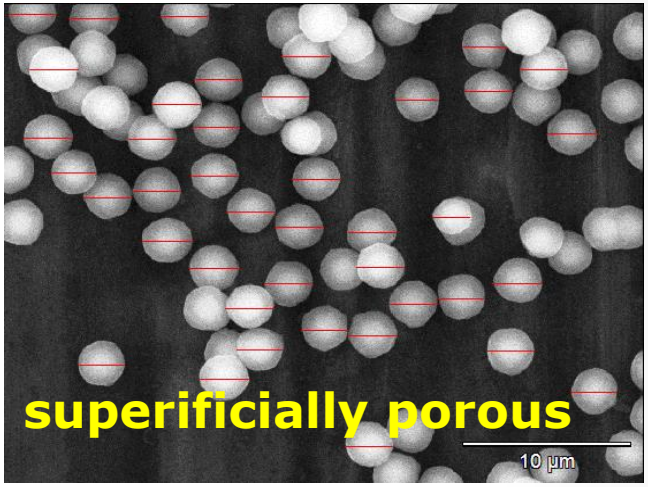
- $u_{o,opt}$ increases with higher temperature which cancels the pressure reduction by the lower mobile phase viscosity.
- In case one operates at velocities higher than $u_{o,opt}$ temperature increase will significantly reduce the HETP. Therefore, high temperature UHPLC allows to work at higher flow rate with gain in efficiency
- At higher temperature, the same retention factor is obtained with less organic solvent ➔ "green" chromatography!
- Be aware of selectivity changes with temperature!
- Stability of the stationary phase will be an issue!
- Standard column thermostats are limited to 100 ◦C. Special heater required

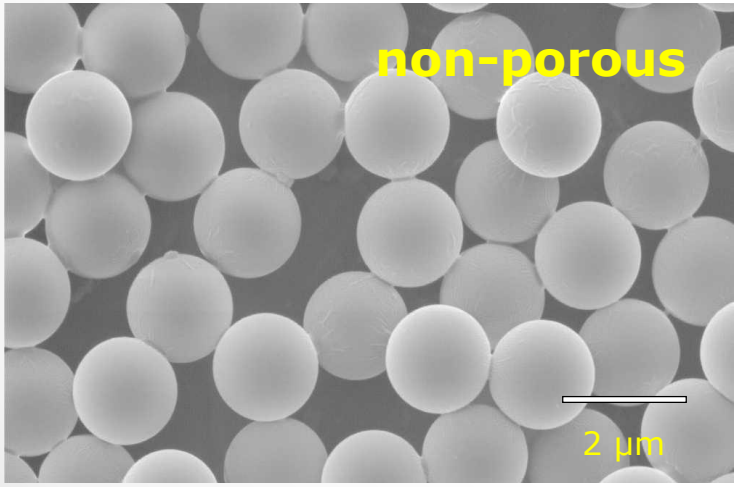
Silica-based Column Technology for UHPLC

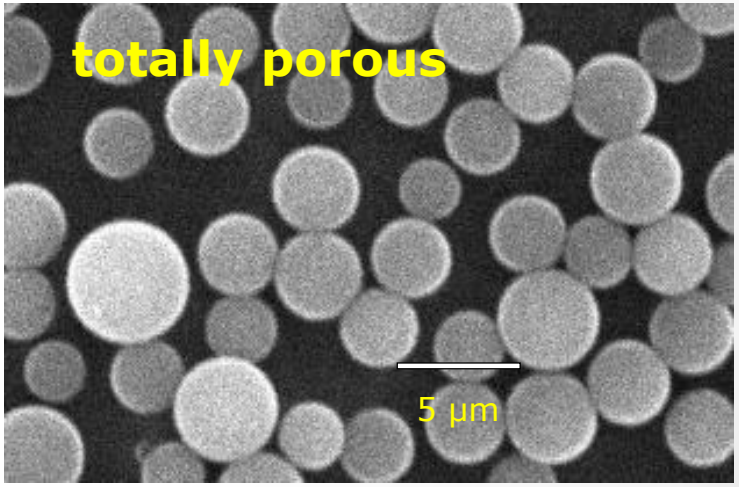
Particle Morphology	Characteristic Size	Requirements, Applications
Totally Porous (TP) Particles	2.5 – 10 µm 8 – 30 nm pores	Standard materials since many years; standard equipment, routine HPLC

Column Technologies for UHPLC









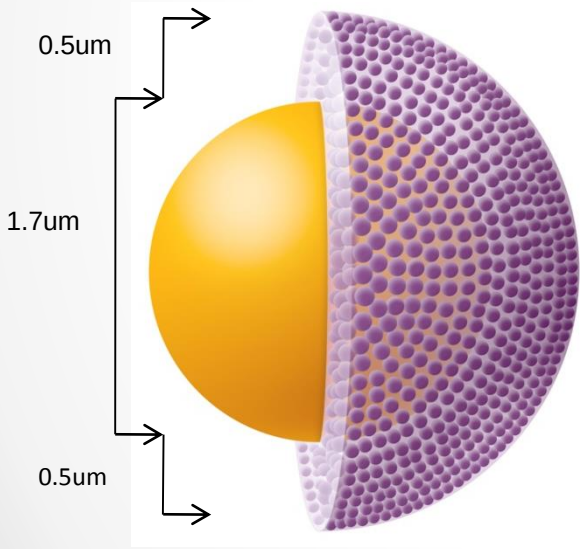
Photos courtesy of Dr. Bill Barber, Agilent Technologies

Column Technology for UHPLC

Superficially Porous Particles

Superficially Porous Particles were introduced already in the 60ties by Cs. Horvath and modernized by J.J. Kirkland et al in the late 90ties (Poroshell 300) and in 2005 (Halo series)

The particle has 2.7 µm outer diameter with a solid core (1.7 µm) and porous outer layer with a 0.5 µm diffusion path. The average pore diameter is 120 Å. The core has 25% of the particle volume. 75% of the particle volume is porous. (Poroshell 120)



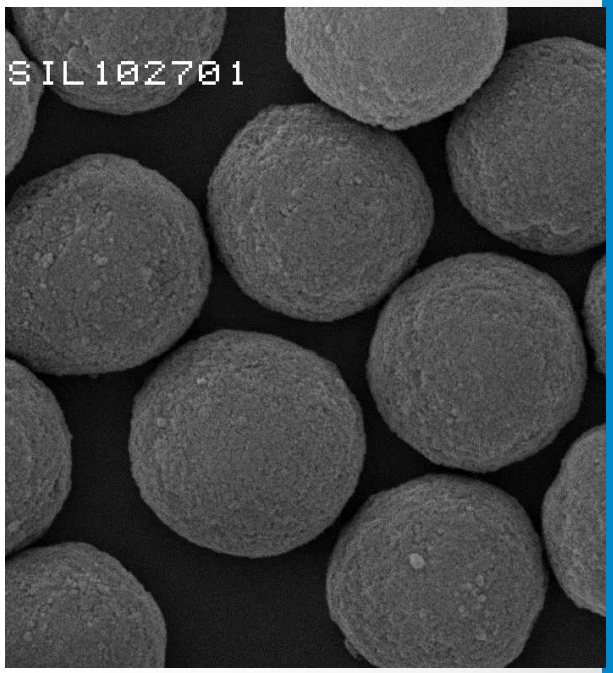
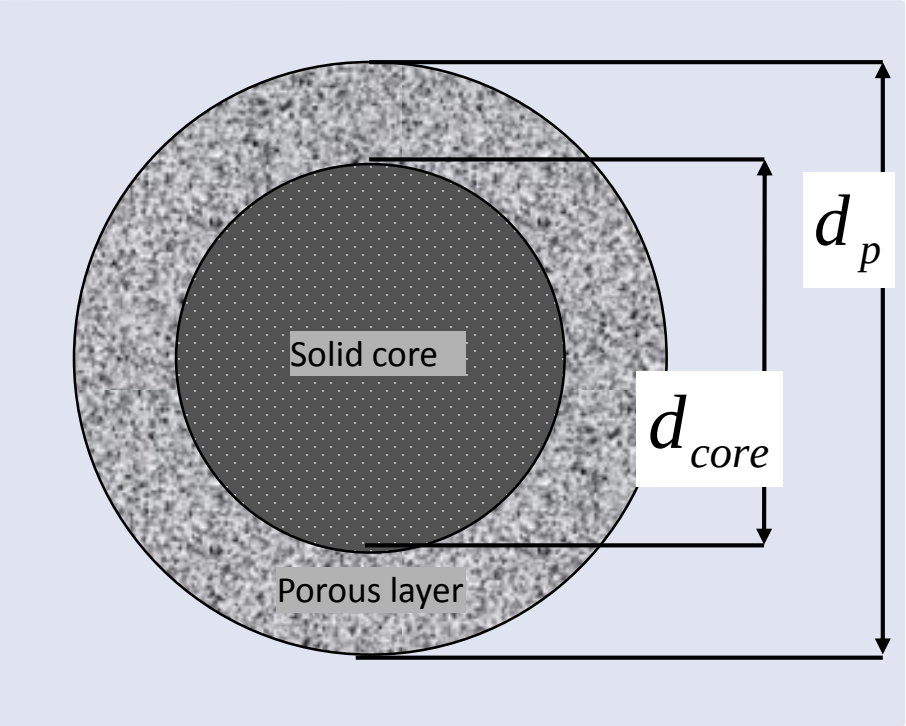


Figure and photo courtesy of Dr. Bill Barber, Agilent Technologies

Morphology of Superficially Porous Particles



The porous volume fraction φ of a superficially porous particle is given by

$$\varphi_{PV} = 1 - \left(\frac{d_{core}}{d_p} \right)^3 \cong 0.75$$

The internal porosity ε_i of a superficially porous particle is assumed to be

$$\varepsilon_i = \varepsilon_{i,\,fully\ porous} \cdot \varphi_{Vporous}$$

Slide courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

Table II: Superficially porous particle columns for reversed-phase chromatography and HILIC

Product	Company	Particle Size (µm)	Pore Diameter (Å)	Phases	Comments
Accucore	Thermo Fisher Scientific	2.6	80	C18, RP-MS, aQ, PFP, phenyl-hexyl, HILIC	Average particle size distribution (D90/D10): 1.12; column dimensions: 30–150 mm × 2.1, 3.0, and 4.6 mm; guard columns (10-mm length) available with separate holder, validation and method development kits available; custom columns available.
Aeris Peptide	Phenomenex	1.7, 3.6	100	C18 (for peptides)	For peptides; shell thickness (3.6-µm particle): 0.5 µm; core diameter: 2.6-µm; shell thickness (1.6-µm particle): 0.2 µm; core: 1.25 µm; column dimensions: 50–250 mm × 2.1 and 4.6 mm.
Aeris Wide Pore	Phenomenex	3.6	200	C4, C8, C18 (for polypeptides and proteins)	For polypeptides and proteins; shell: 0.2 µm; core: 3.2 µm; column dimensions: 50–250 mm × 2.1 and 4.6 mm.
Ascentis Express	Supelco/ Sigma Aldrich	2.7	90	C8, C18, phenyl-hexyl, RP-Amide, PFP, HILIC, ES-Cyano, OH5 (HILIC)	Shell thickness: 0.5 µm; pore size: 90 Å; pressure limit: 600 bar; dimensions: 30–150 mm × 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available
Ascentis Express	Supelco/ Sigma Aldrich	2.7	160	Peptide ES-C18 (for peptides)	For peptides and polypeptides; shell thickness: 0.5 µm; 600 bar; dimensions: 30–150 mm × 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available
Brownlee SPP	PerkinElmer	2.7	90	C8, C18, phenyl-hexyl, RP-Amide, PFP, HILIC (Silica), ES-CN	Shell thickness: 0.5 µm; pressure limit: 600 bar; dimensions: 20–15 mm × 2.1, 3.0, and 4.6 mm; endcapped and nonendcapped phases; guard column holder and columns (5-mm lengths) available; sold in packs of three; ultralow extracolumn volume optimization kit available for instrument modification
Brownlee SPP	PerkinElmer	2.7	160	Peptide ES-C18 (for peptides)	For peptides or polypeptides; shell thickness: 0.5 µm; pressure limit: 600 bar; dimensions: 30–150 mm × 1.0, 2.1, 3.0, and 4.6 mm.
Elroshell	Irish Separation Science Cluster	1.7	100	C18 (not a commercial product)	Shell thickness: 0.35, 0.25, and 0.15 µm; these are research products and are not commercially available
Halo	Advanced Material Technology	2.7	90	C8, C18, phenyl-hexyl, RP-Amide, PFP, HILIC, ES-CN, penta HILIC (Pentanol functionality)	Shell thickness: 0.5 µm; pore size: 90 Å; pressure limit: 600 bar; dimensions: 30–150 mm × 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available
Halo	Advanced Material Technology	2.7	160	Peptide ES-C18 (for peptides)	For peptides or polypeptides: shell thickness: 0.5 µm; pressure limit: 600 bar; dimensions: 30–150 mm × 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available
Kinetex	Phenomenex	1.7, 2.6	100	C8, C18, XB-C18 PFP	PFP = pentafluorophenyl; shell thickness: 0.35 µm (2.6-µm particle) and 0.23 µm (1.7-µm particle); pressure limit: 800 bar (2.6 µm), 1000 bar (1.7 µm); dimensions: 30–150 mm × 1.0, 2.1, 3.0, and 4.6 mm; capillary columns available
Nucleoshell	Macherey-Nagel	2.7	90	C18, PFP, HILIC	Shell thickness: 0.5 µm; core: 1.7 µm; column dimensions: 50–150 mm × 2–4.6 mm; maximum pressure: 600 bar; pH range: 1–11; multiendcapped for improved peak shape with basic compounds; HILIC is zwitterionic ammonium-sulfonic acid phase
Poroshell 120	Agilent Technologies	2.7	120	SB-C18, EC-C18, EC C8, HILIC (silica)	Shell thickness: 0.50 µm; core diameter: 1.7 µm; pressure limit: 600 bar; column dimensions: 30–150 mm × 2.1, 3.0, and 4.6 mm; double endcapped and onendcapped phases.
Poroshell 300	Agilent Technologies	5.0	300	SB-C3, SB-C8, SB-C18, Extend (for proteins)	Extend = Bidentate C18, upper pH limit = 11; shell thickness: 0.25 µm; core diameter: 4.5 µm; pressure limit: 400 bar; column dimensions: 17 mm (guard for 1.0-mm), 12.5 mm (guard for 2.1-mm), and 75 mm × 0.5, 1.0, and 2.1 mm; capillary columns available
Sunshell	ChromaNik Technologies	2.6	80	C18, C8, PFP	Shell thickness: 0.5 µm; core: 1.6 µm; pH limits: 1.5–10; endcapped; phase coverage C18: 7% carbon; average particle size distribution (D90/D10): 1.15; column dimensions: 20–150 mm × 2.1, 3.0, and 4.6 mm; marketed in US by Advantec USA

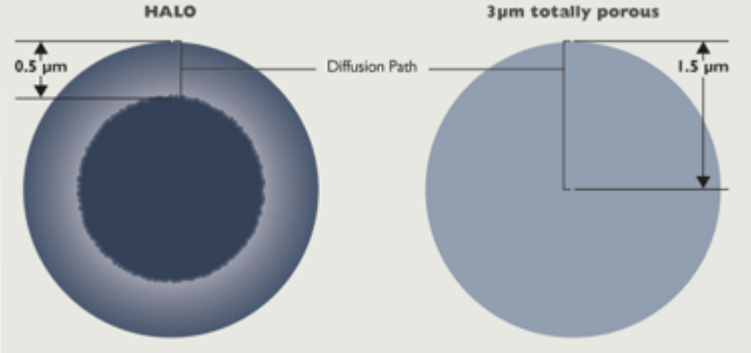
Bonshell, 2.7 µm, 0.5 µm porous shell
Bonna-Agela Technologies, Wilmington, USA

Phenomenex, 5 µm Kinetex

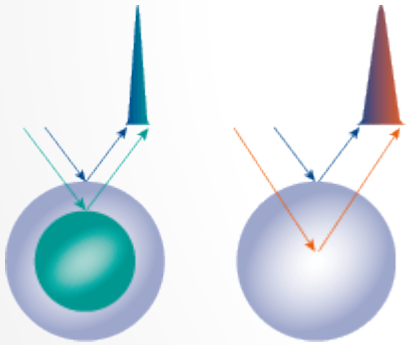
Brownlee (now Perkin Elmer) SPP columns

Table taken from : R. Majors, LCGC North America, Special Supplement on HPLC Column Technology, April, 2012

Columns packed with superficially porous particles will deliver significantly higher efficiencies than columns packed with totally porous particles of the same diameter*



The shorter diffusion path of HALO® particles reduces axial dispersion of solutes and minimizes peak broadening. A Halo particle has only a 0.5 µm diffusion path compared to the approximately 1.5 µm diffusion path of a 3 µm totally porous particle. **.

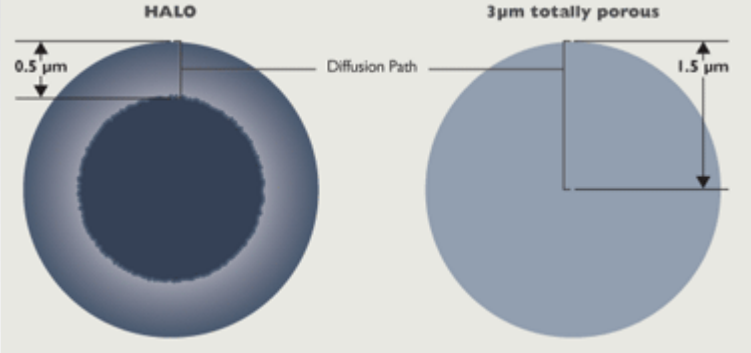


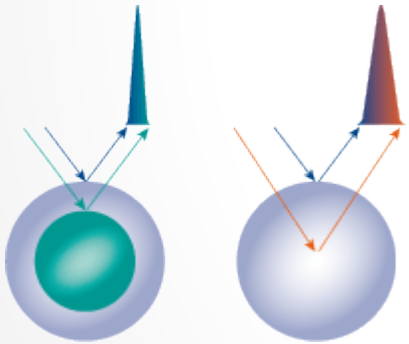
Benefits of core-shell technology particles vs. totally porous silica gel***

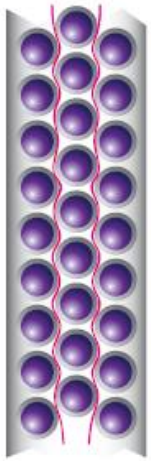
- Short diffusion paths resulting in fast mass transfer (C-term of van Deemter equation)
- Narrow particle size distribution (d90/d10 ~ 1.1)

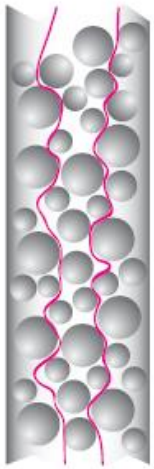
* Quoted from http://www.phenomenex.com/Kinetex/CoreShellTechnology (not actual)
**Quoted from http://www.advanced-materials-tech.com/halo.html
*** Quoted from http://www.mn-net.com/tabid/11635/default.aspx

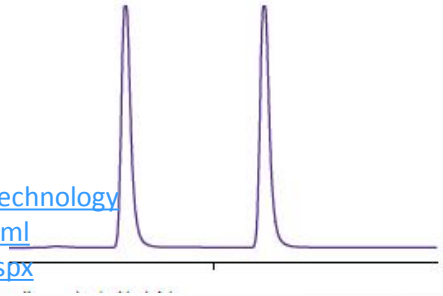
Columns packed with superficially porous particles will deliver significantly higher efficiencies because of the narrower particle size distribution*

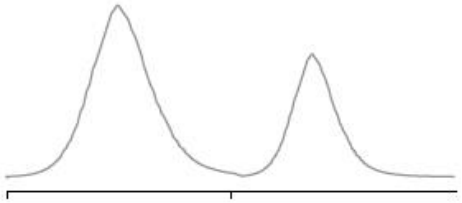












* Quoted from http://www.phenomenex.com/Kinetex/CoreShellTechnology
**Quoted from http://www.advanced-materials-tech.com/halo.html
*** Quoted from http://www.mn-net.com/tabid/11635/default.aspx

HETP vs. *u* according to Knox (and others)

Knox Equation!

$$H = A \cdot d_p \cdot u^{0.33} + \frac{B \cdot D_m}{u} + C \cdot \frac{d_p^2}{D_m} \cdot u$$

A-term B-term C-term

Knox Equation (reduced form)

$$h = A \cdot \nu^{0.33} + \frac{B}{\nu} + C \cdot \nu$$

$$h = H / d_p$$

$$\nu = \frac{u \cdot D_m}{d_p}$$

A-term:
describes the solvent velocity inequalities through the bed and depends on the particle size

B-term:
describes the influence of solute diffusivity and decreases with solvent velocity

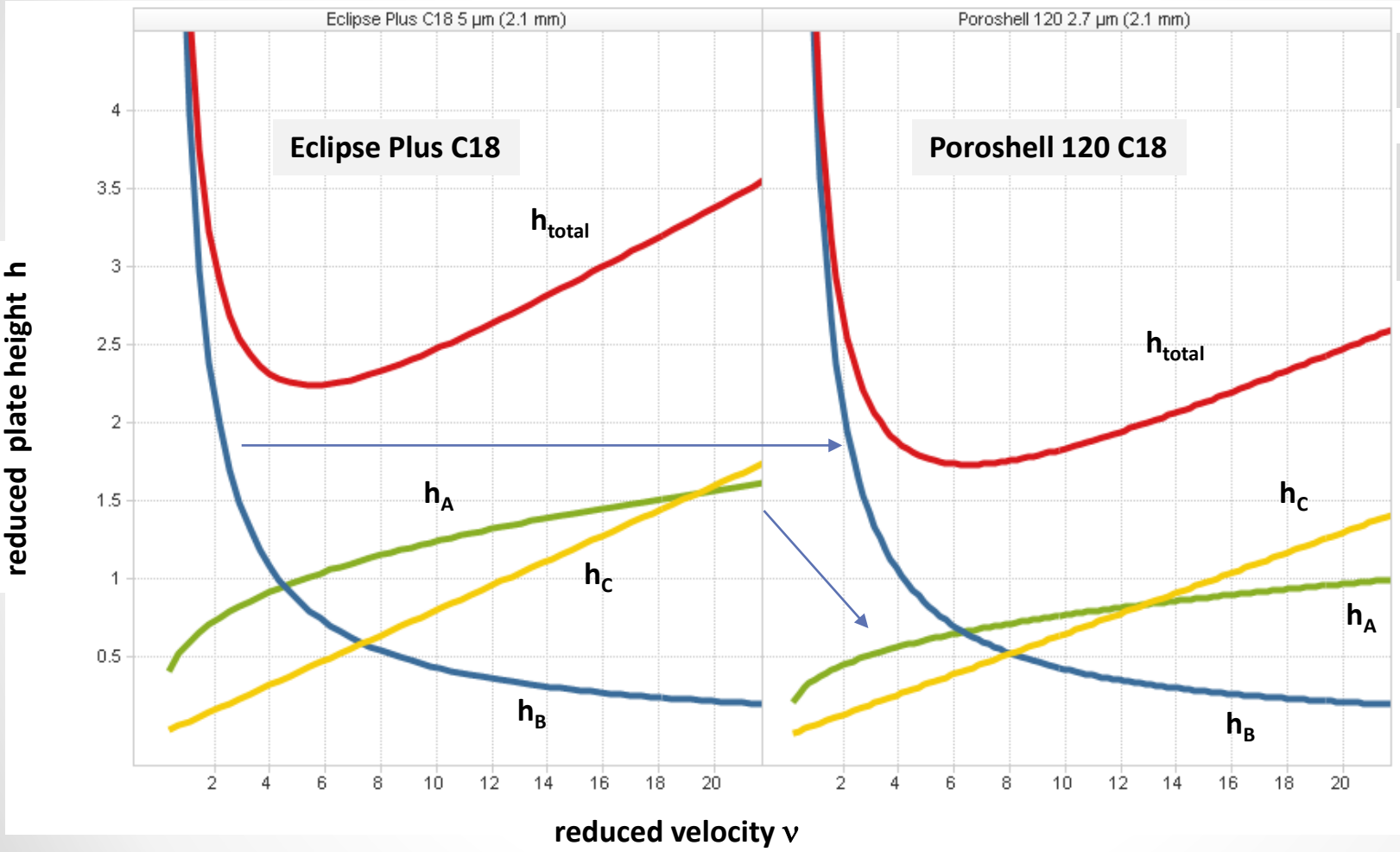
C-term:
describes the mass transport in and out of the particles, depends on particle size and solute diffusivity and increases with solvent velocity

$$H = A \cdot d_p + \frac{B \cdot D_m}{u} + C \cdot \frac{d_p^2}{D_m} u$$

A-term B-term C-term

Van Deemter Equation

Knox Plot TP and SP Particle Columns



$$h = H / d_p$$

$$\nu = \frac{u \cdot D_m}{d_p}$$

Data and slide courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

Experimental Investigation*

H – u curves were measured on 4 TP particle and 1 SP particle columns

Column A	totally porous	1.8 µm
Column B	totally porous	2.5 µm
Column C	totally porous	2.8 µm
Column D	totally porous	3.5 µm
Column E	superficially porous	2.7 µm (core 1.7 µm), φ = 0.75

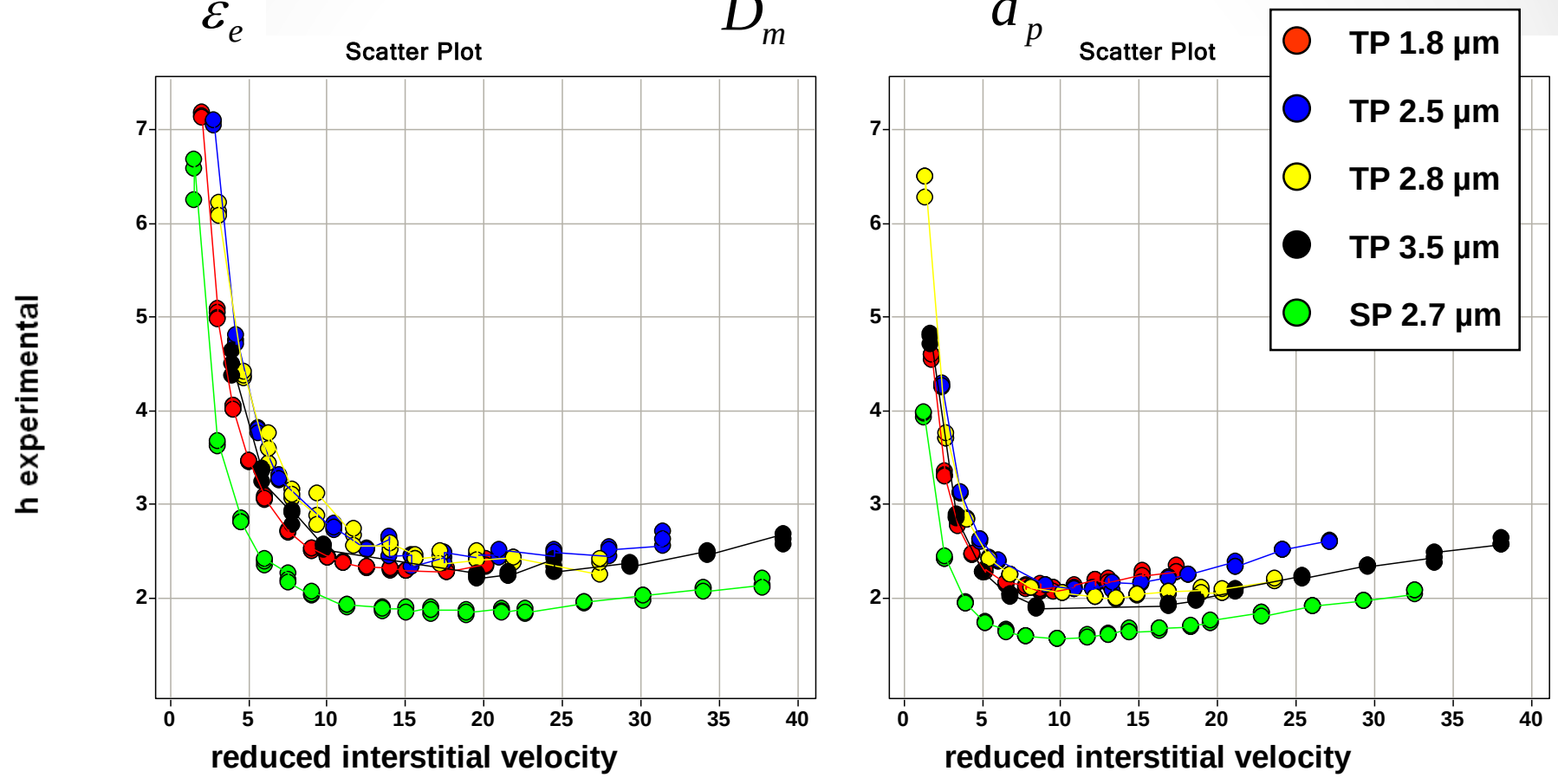
Conditions:

Column dim.:	50x4.6 mm
Solvent:	Acetonitrile/water 60:40
Temperature :	25 °C
Sample:	series of homologous alkyl phenones

***Results on the next 4 pages contributed by Dr. Monika Dittmann, Agilent Technologies, Germany; presented at HPLC2008)**

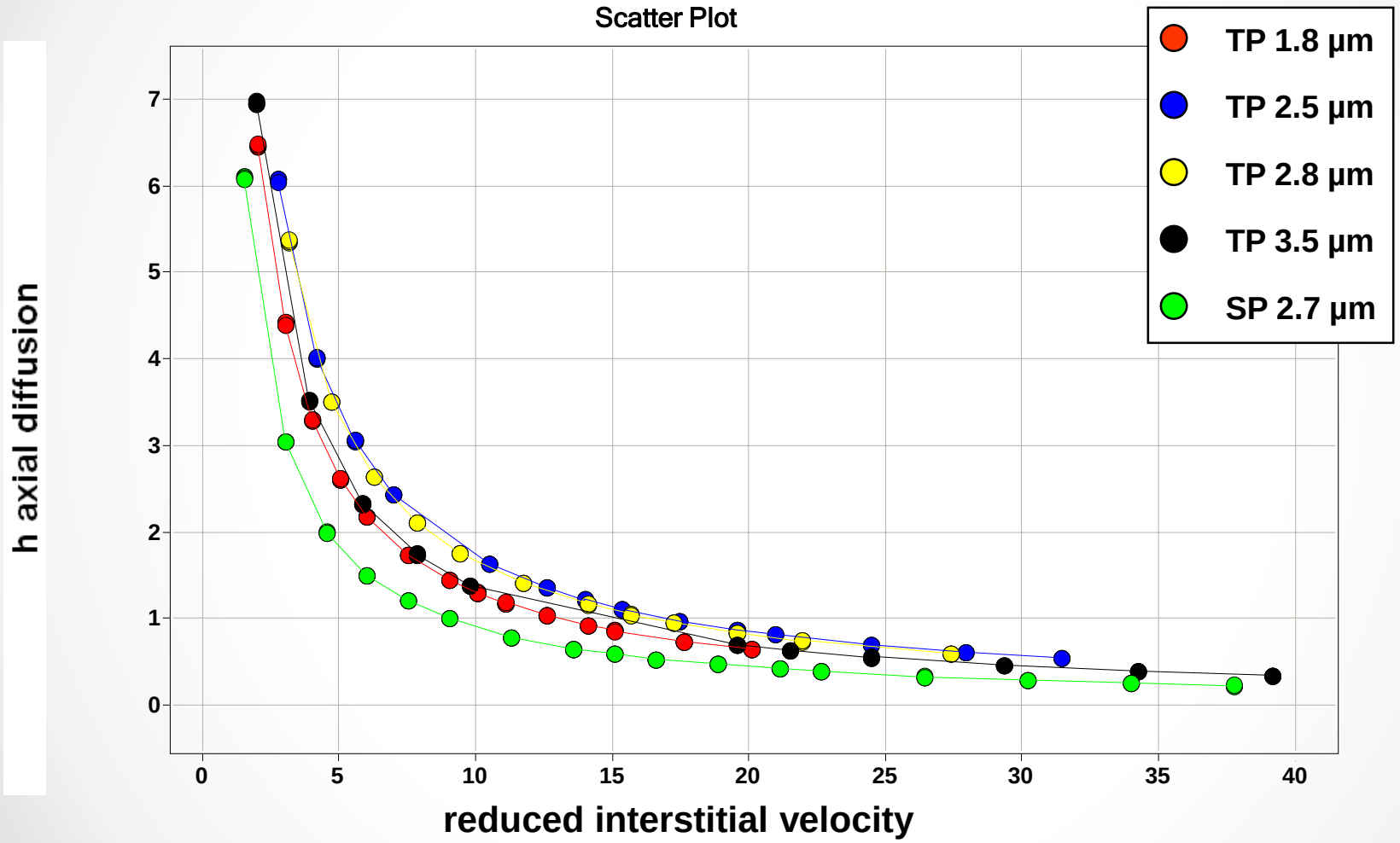
Reduced van Deemter Plots for Octanophenone and Valerophenone

$$u_e = u_0 \cdot \frac{\varepsilon_T}{\varepsilon_e} \qquad \nu_e = u_e \cdot \frac{d_p}{D_m} \qquad h = \frac{H}{d_p}$$



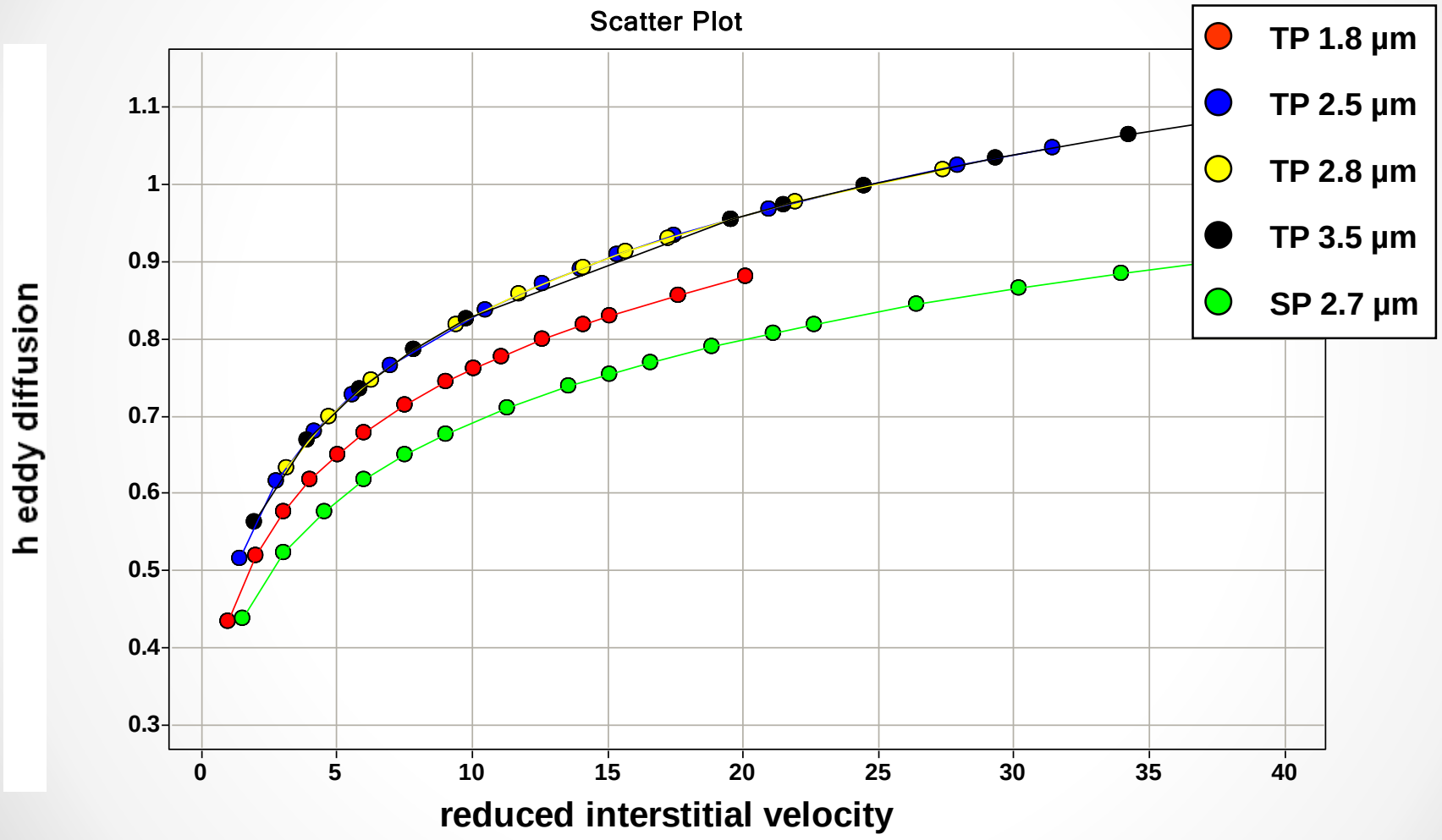
Data and slide courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

Longitudinal Diffusion (h_{ax}) Contribution



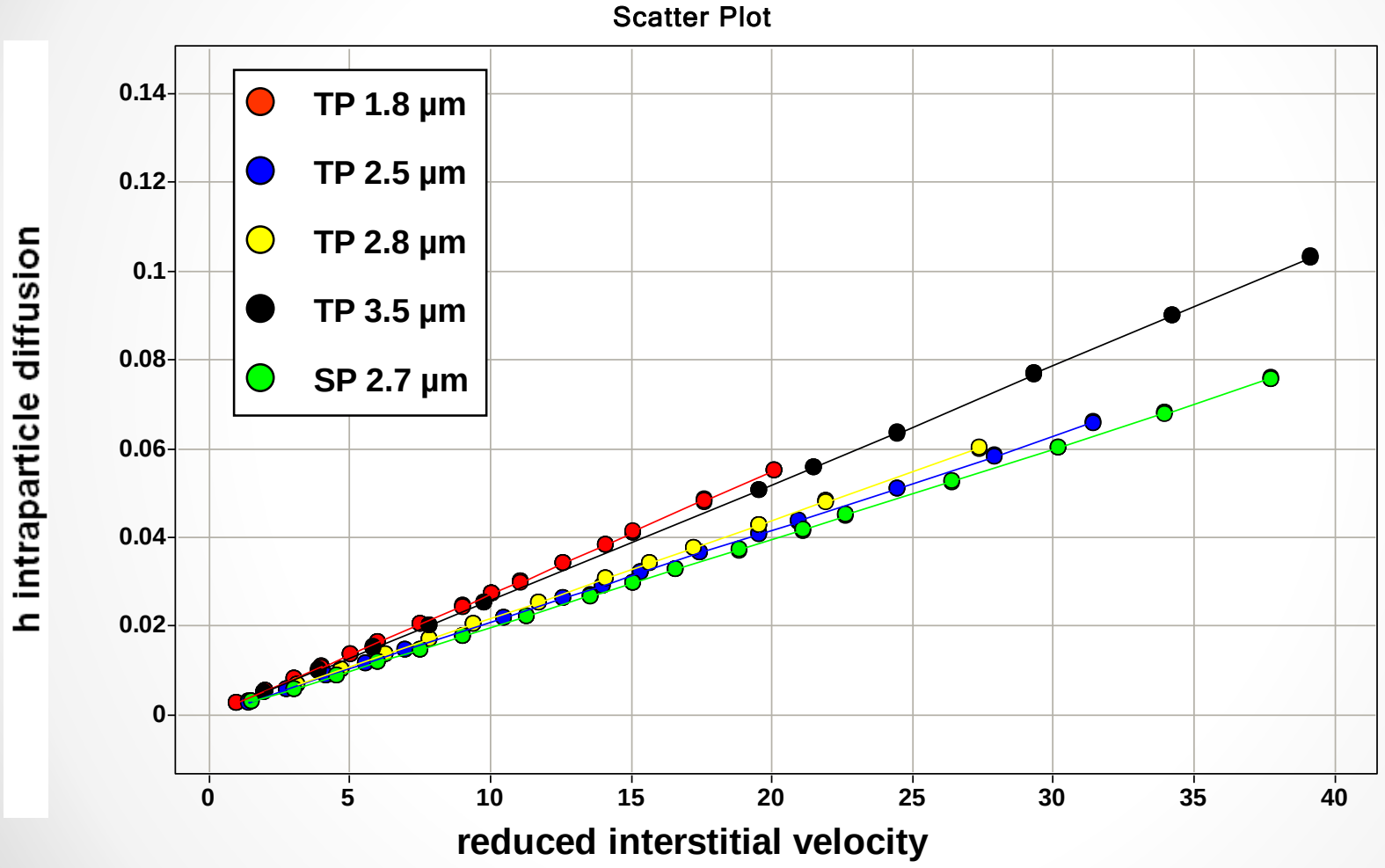
Data and slide courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

Eddy Diffusion (h_{eddy}) Contribution



Data and slide courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

Intraparticle Diffusion (h_{Cs}) Contribution for Different Columns



Data and slide courtesy of Dr. Monika Dittmann, Agilent Technologies, Germany

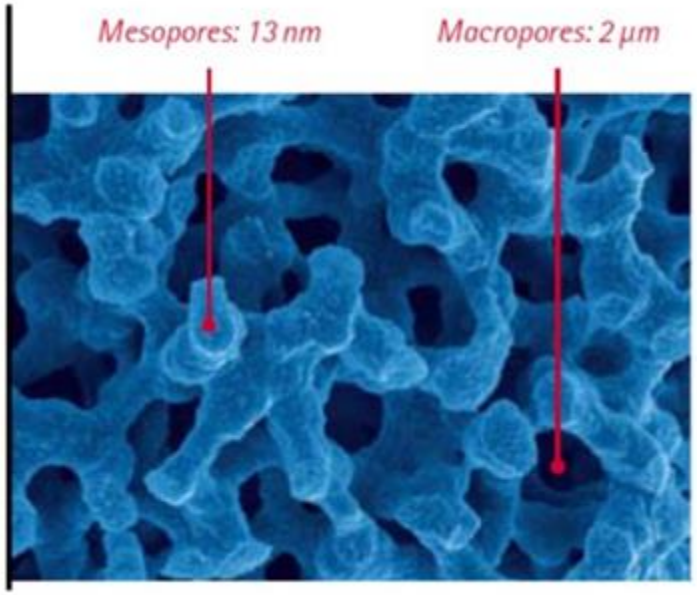
Facts and Legends on Columns packed with sub-3 µm Porous Core-Shell Particles*

- The assumption that the shorter average diffusion path in SP particles leads to better performance (supplier brochures) is wrong
- In many explanations, the contribution of the B-term to the optimum HETP value (25%) is systematically neglected
- Also it is incorrectly assumed that the eddy dispersion term is independent of solvent velocity
- And it is incorrectly assumed that narrow particle size distribution leads to lower Eddy dispersion.

***G. Guiochon & F. Gritti, LCGC North America, Vol. 30(7), 586 (2012)**

Monolithic Columns

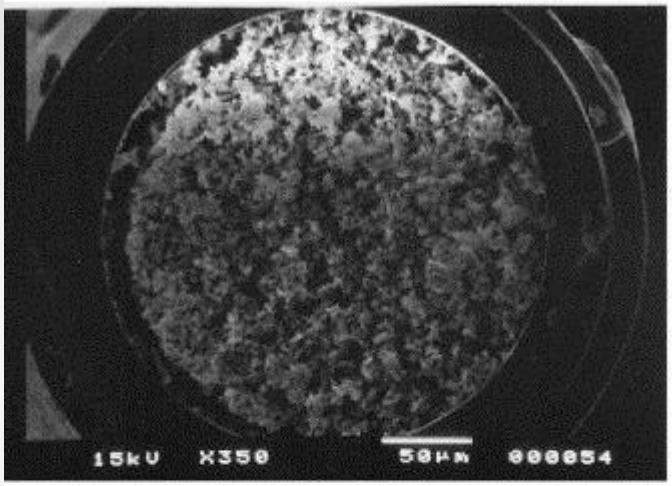
Pro's:
High permeability → low backpressure
Compatible with HPLC equipment
Good efficiency esp. 2nd generation monoliths
No column frit → high tolerance for " dirty" samples
Good mechanical stability and longevity



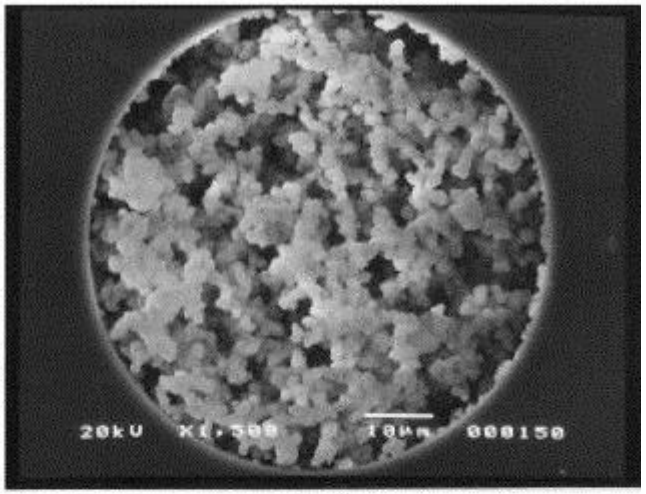
Con's:
Max. pressure 200 bar
Few vendors
Column to column reproducibility
Polymeric monoliths lower performance than silica monoliths

Picture taken from Merck Chromolith brochure

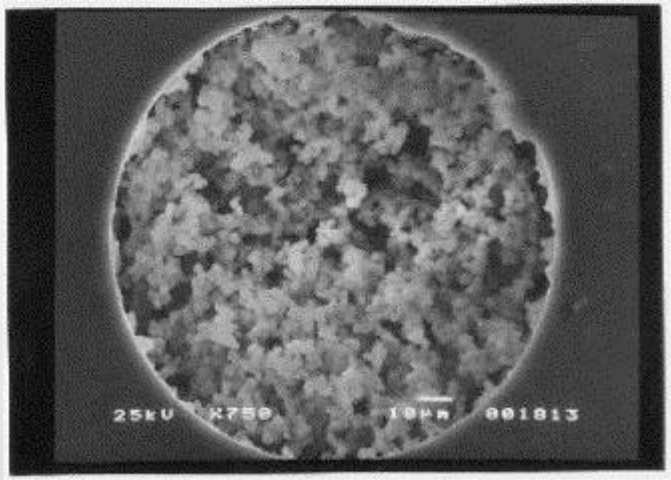
Monolithic Columns



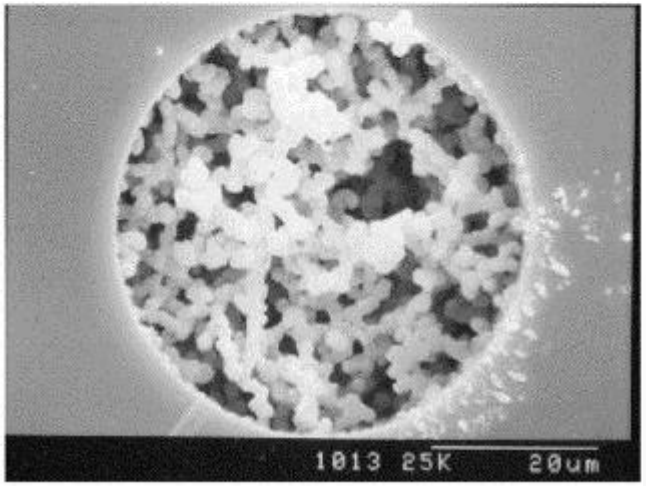
(a)



(c)



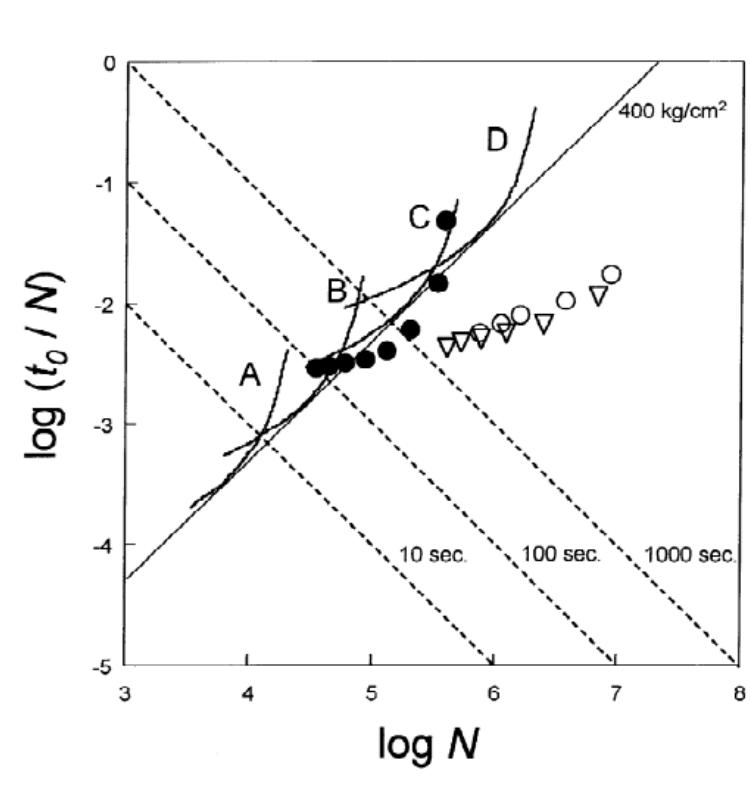
(b)



(d)

Monolithic Columns

Poppe Plot comparison with particle column*



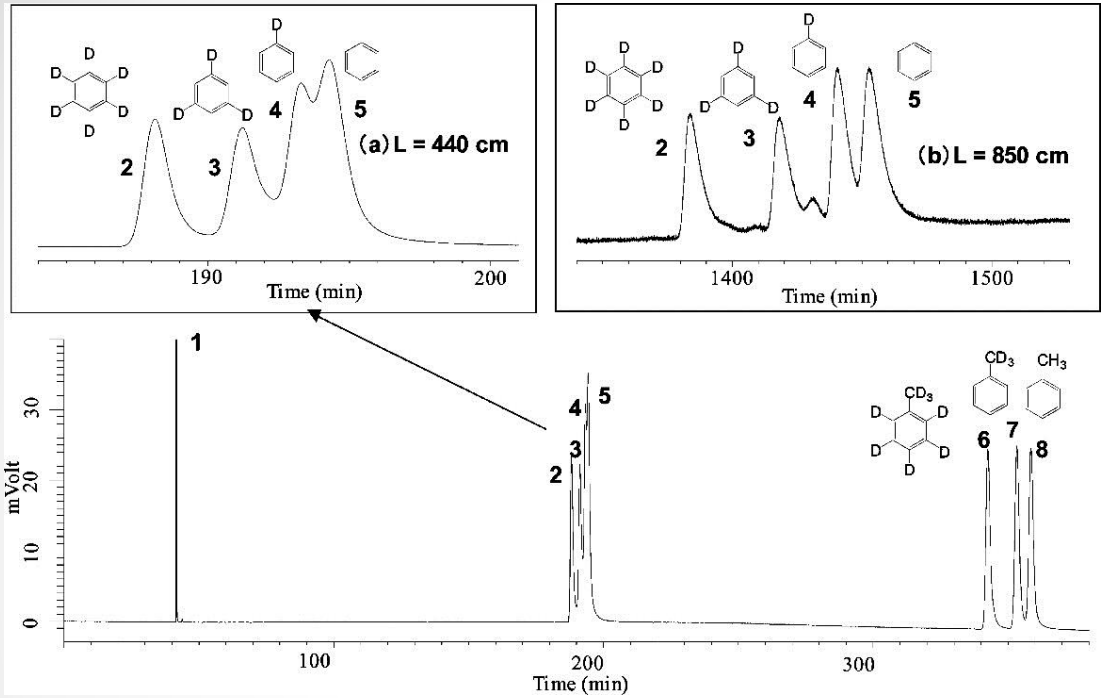
Plots of plate time, (t0 / N), vs. plate number with particle-packed column and monolithic columns

The curves for a column packed with particles were obtained by assuming the parameters, maximum pressure: 400 kg/cm2, η = 0.00047 Pa / s, ϕ = 1000, D = 2.131029 m2/s, and Knox equation
Particle diameter: (A) 1 μm, (B) 2 μm, (C) 5 μm, (D) 10 mm. The points are based on the experimental results in 80% acetonitrile on a monolithic column, MS(50)-A (s), MS(50)-C (,) and a particle-packed column, Mightysil RP18 (d), extrapolated to the limiting conditions of 400 kg / cm2. The dashed lines indicate the required t0 values in seconds.

***N. Tanaka et al., J. Chromatogr. A 965 (2002) 35–49**

Monolithic Columns

1.000.000 Plates!



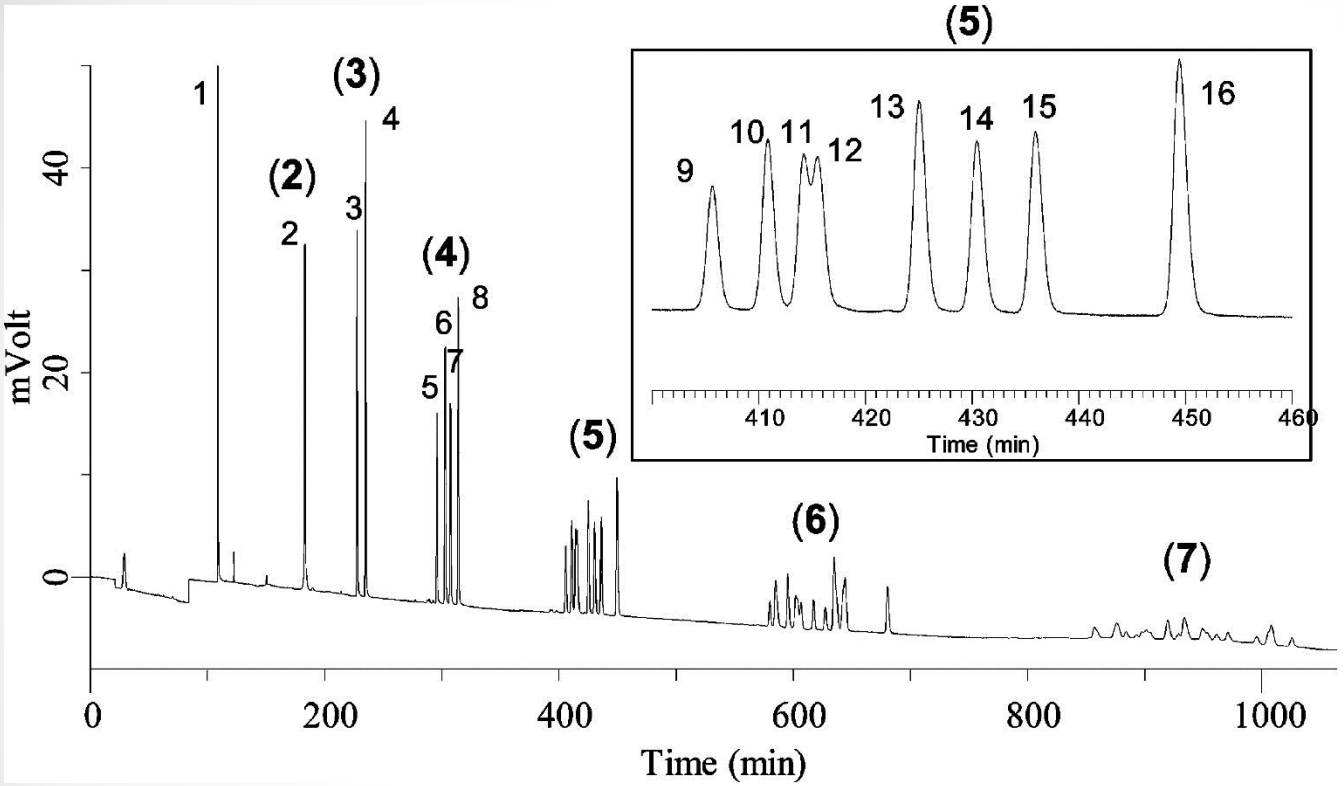
$N_r > 1{,}000{,}000$

Separation of benzene and toluene isotopologues. Mobile phase: acetonitrile–water (30/70). **Column: monolithic silica, 440 cm**. Detection: 210 nm. Temperature: 30 °C. u = 1.76 mm/s. ΔP = 39.6 MPa. Sample: 1, thiourea; 2, benzene-*d*6; 3, benzene-1,3,5-*d*3; 4, benzene-*d*; 5, benzene; 6, toluene-*d*8; 7, toluene-α,α,α-*d*3; and 8, toluene. The inset (a) is a magnification of part of the chromatogram at 185–200 min. The inset (b) is a chromatogram for the mixture of benzene isotopologues obtained with two monolithic silica capillary columns connected in series, length 850 cm (500 cm + 350 cm) in a ternary mobile phase, acetonitrile–methanol–water (10/5/85). u = 1.02 mm/s. ΔP = **34 MPa**.

*Nobuo Tanaka et al., *Anal. Chem.* **2008,** 80, 8741-8750.

Monolithic Columns

1.000.000 Plates!



N_r > 1,000,000

*Separation of styrene oligomers (molecular weight standard for MW = 580). The numbers in parentheses indicate the number of styrene units in the oligomer. Column: **monolithic silica, effective length: 1130 cm**. Mobile phase: acetonitrile–water (95/5). **Δ*P* = 39.5 MPa**. *u* = 1.73 mm/s. Detection: 210 nm. Temperature: 30 °C. The inset is a magnification of the chromatogram for the pentamers.

***Nobuo Tanaka et al., *Anal. Chem.* 2008, 80, 8741-8750.**

Lessons learned

Superficially Porous Particles

- Superficially porous particles are an excellent compromise between efficiency of totally porous particles < 2 µm and permeability of larger particles (3 µm).
- The stationary phase volume of a SP particle is about 25% less than a totally porous particle
- The main reasons for the better efficiency of SP particle columns compared with TP particle columns are:
  - Lower contribution to HETP by Eddy dispersion (A-term)
  - B-term contribution is less since there is less volume in the particle
- Silica based monoliths have too large thru pores and therefore are more suited for very long columns for high plate number

Method Translation

HPLC ➔ UHPLC Isocratic Separations

- Plate number scales linearly with length and inversely with particle size

$$N \sim \frac{L}{d_p}$$

Same time, better resolution
Shorter time, same resolution
Shorter time, lower resolution

Column length (mm)	N, 5 µm	N, 3.5 µm	N, 1.8 µm
150	13050	18650	36250
100	8700	12400	24150
50	4350	6200	12100

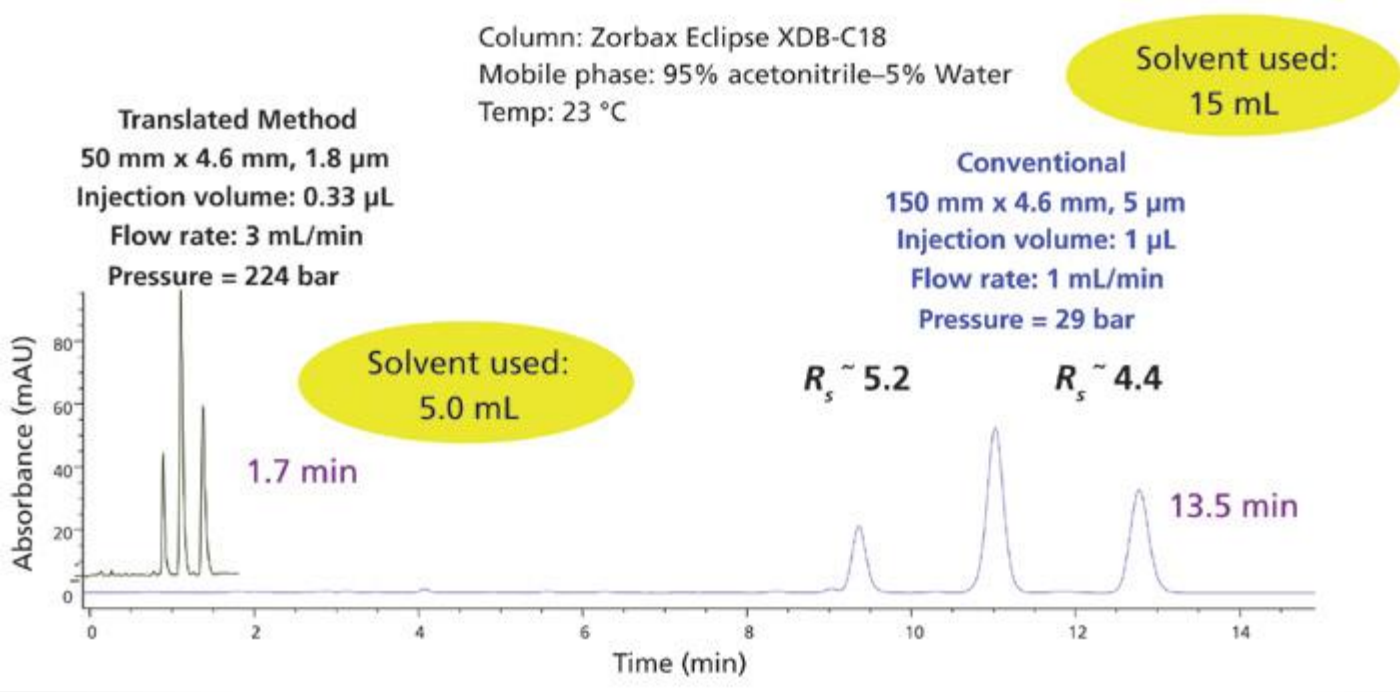
- Flow rate scales quadratical with the column diameter

$$F_2 = \frac{{d_{c2}}^2}{{d_{c1}}^2} \cdot F_1 = \frac{2.1^2}{4.6^2} \cdot 1\text{mL/min} = 0.21\text{mL/min}$$

Data and slide courtesy of Dr. Udo Huber, Agilent Technologies, Germany

Method Translation

HPLC ➔ UHPLC Isocratic Separations

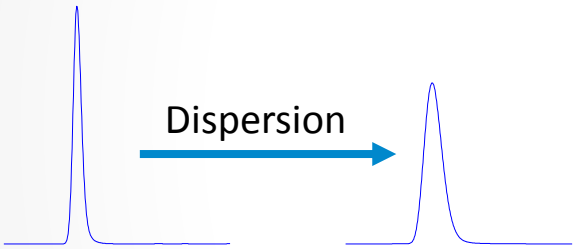


Ronald E. Majors, LCGC North America, Volume 29, Issue 6, pp. 476-485

Method Translation

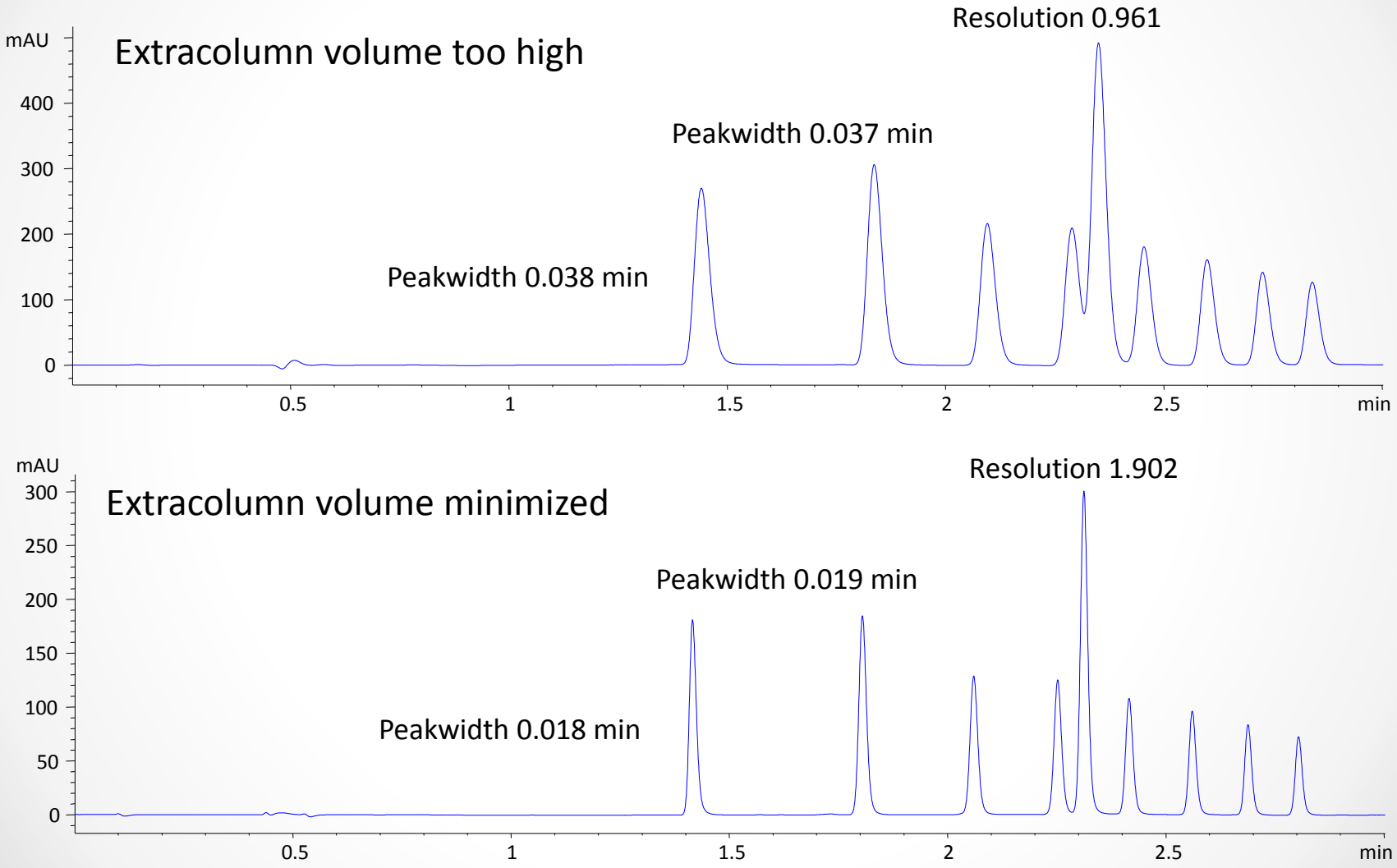
HPLC ➔ UHPLC Isocratic Separations

- Extra column dispersion
  - Defined as the sample zone spreading or dilution which occurs in connecting tubing, sample valves, flow cells and in column end-fittings in the system
  - Particular concern in case the column volume is reduced



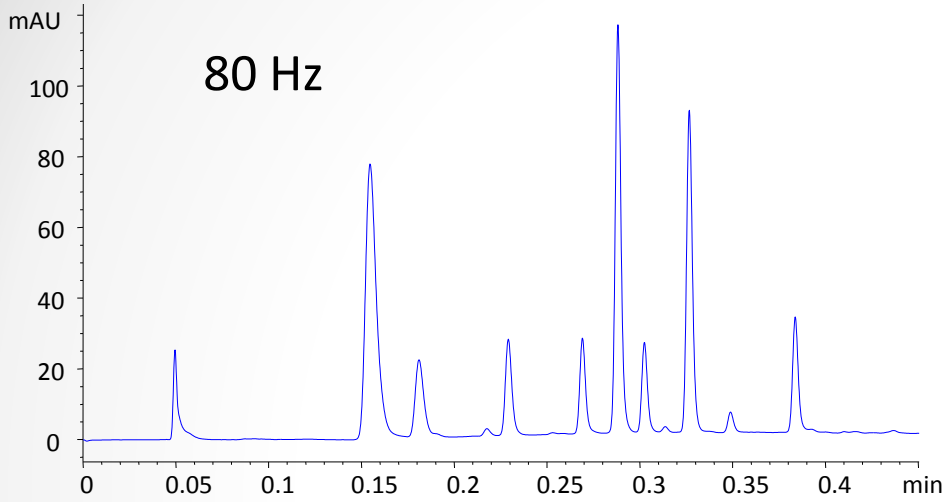
Method Translation

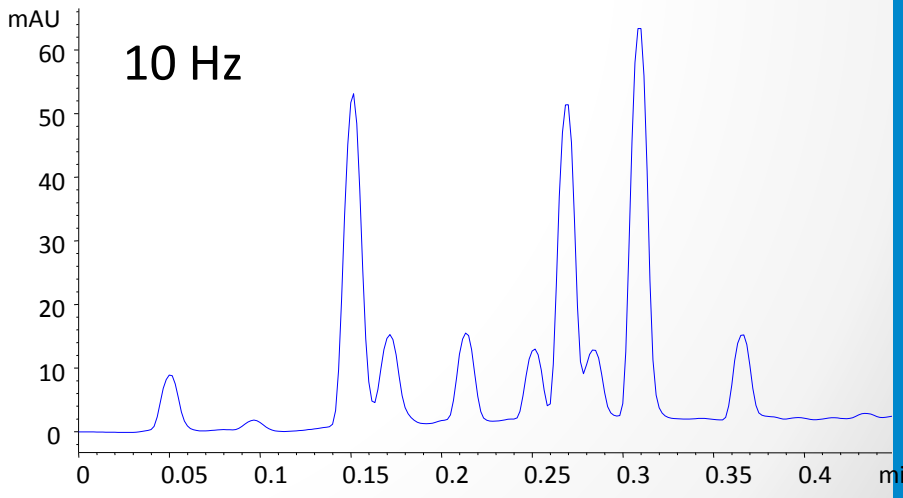
HPLC ➔ UHPLC Influence of extra column volume



Method Translation

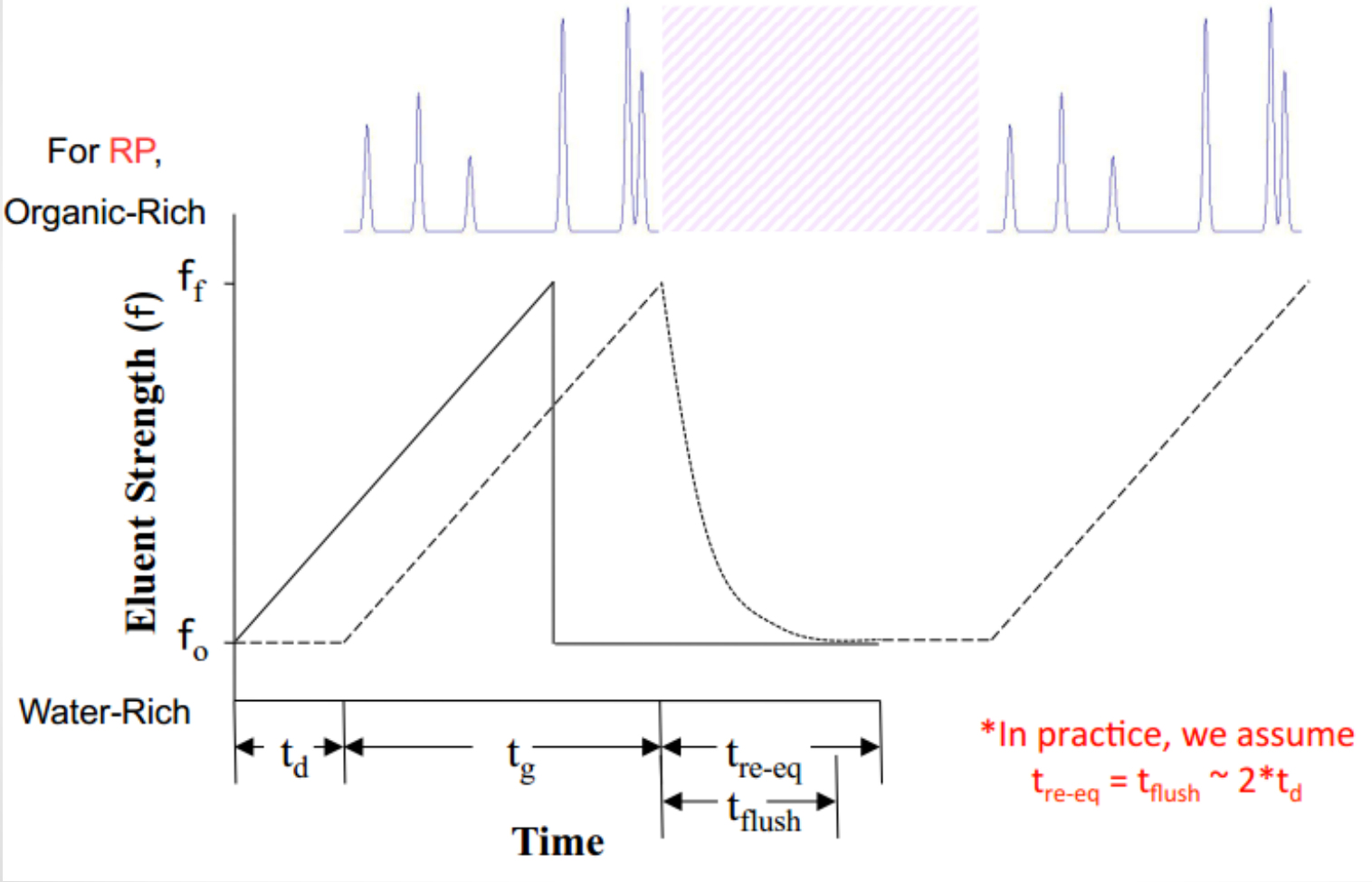
HPLC ➔ UHPLC Influence of detector signal registration frequency





Method Translation

HPLC ➔ UHPLC Gradient Separations

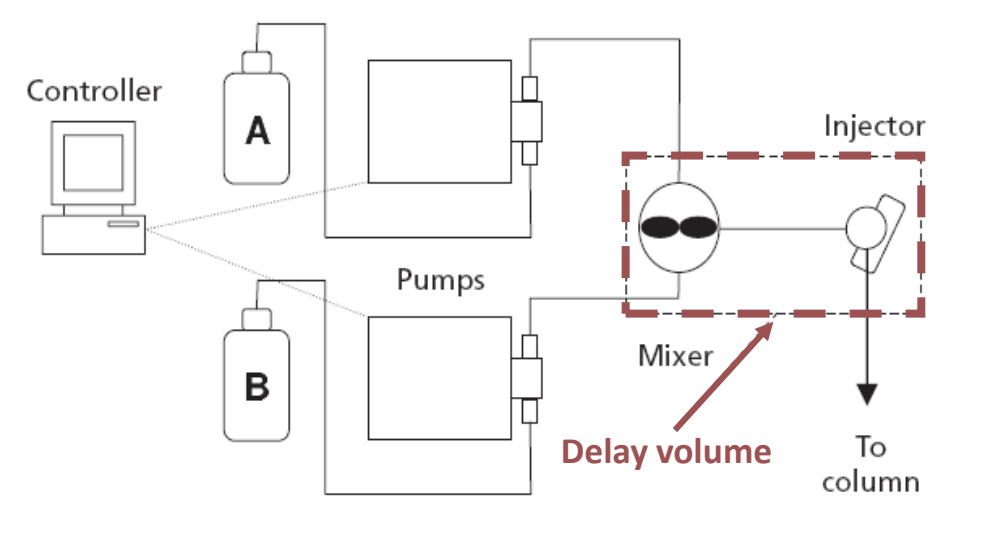


Slide courtesy of Prof. P. Carr & Dr. D. Stoll

Method Translation

HPLC ➔ UHPLC Gradient Separations; definition of delay volume

Delay volume or dwell volume defined as the “volume from the point of mobile phase mixing to the column head”



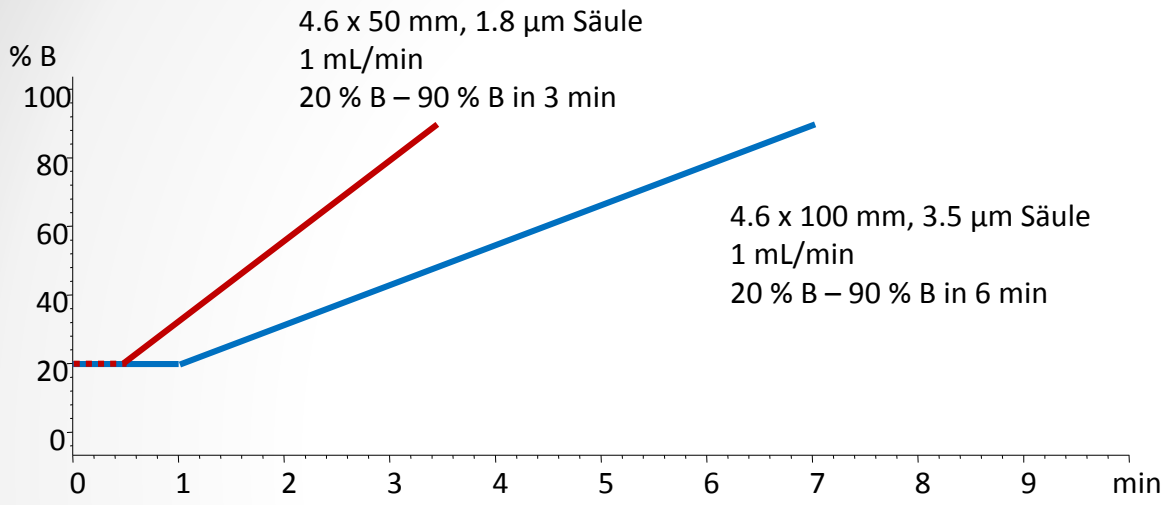
- Delays the arrival of the gradient at the head of the column
- Extends the time by which the solutes on the column are under isocratic separation conditions.

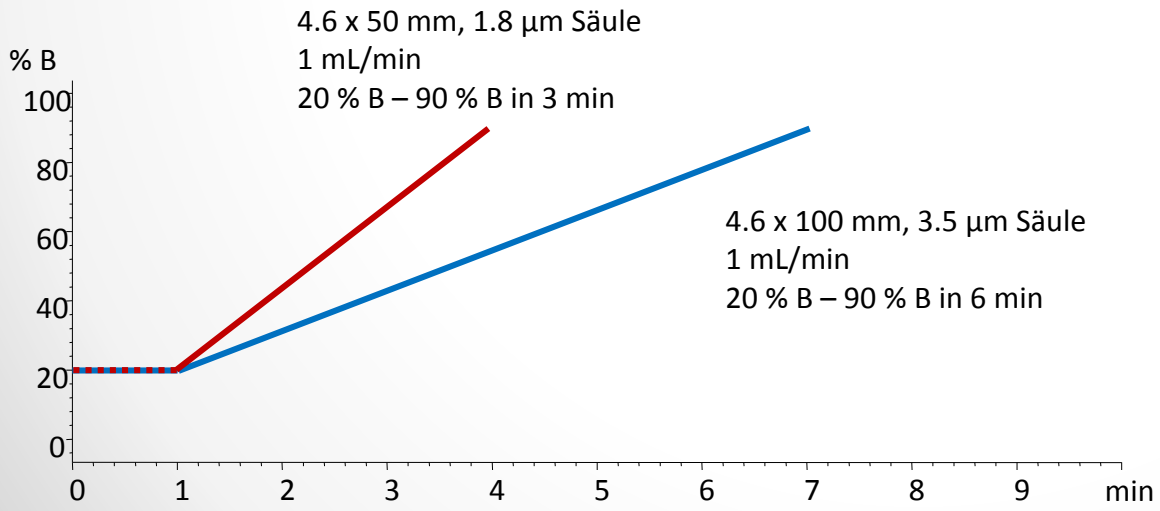
J.W. Dolan LCGC 2006 Vol 24, No 5, 458-466)

Method Translation

HPLC ➔ UHPLC Gradient Separations; Influence of delay volume

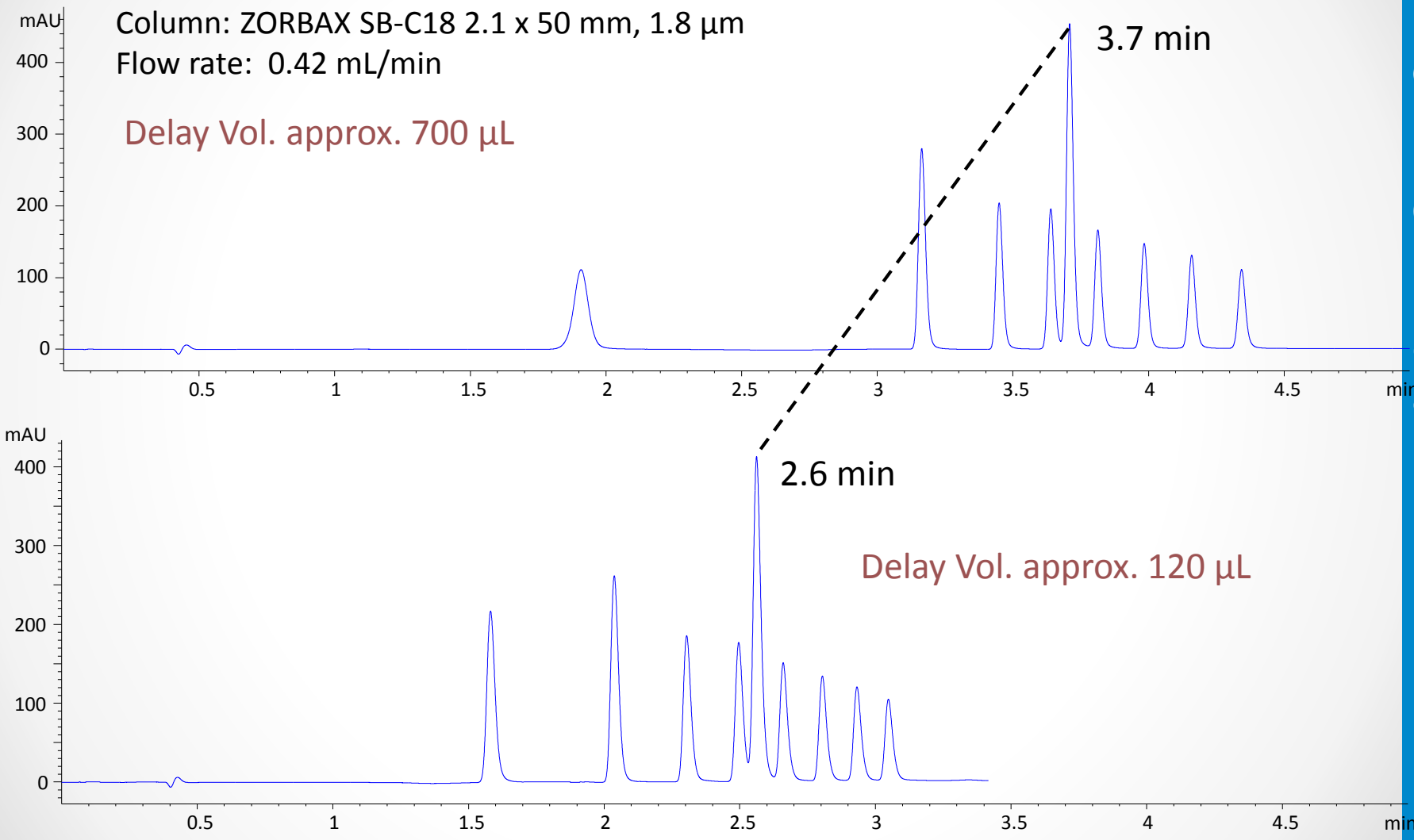
Delay volume 1 mL





Method Translation

HPLC ➔ UHPLC Gradient Separations; Influence of delay volume



Method Translation Literature and Software

- http://www.chem.agilent.com/Library/technicaloverviews/Public/5990-9213EN.pdf
  - Agilent Technologies LC Calculator App for iPhone (isocratic only)
- http://www.americanpharmaceuticalreview.com/Featured-Articles/36760-Direct-Method-Scaling-from-UHPLC-to-HPLC-Is-this-feasible-for-Pharmaceutical-Methods/

- Intelligent System Emulation Technology (ISET) Agilent Technologies for Method Transfer
  - Emulates other HPLC systems (both Agilent and competitors) on Agilent 1290 system
  - Run existing HPLC methods on Agilent 1290 Infinity without modifying your method or system

Acknowledgements

- Dr. Monika Dittmann, Dr. Bill Barber, Dr. Udo Huber, Agilent Technologies
- Prof. Georges Guiochon, Knoxville, USA
- Dr. Davy Guillarme, University of Geneva

Thank You for Your Attention

谢谢

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