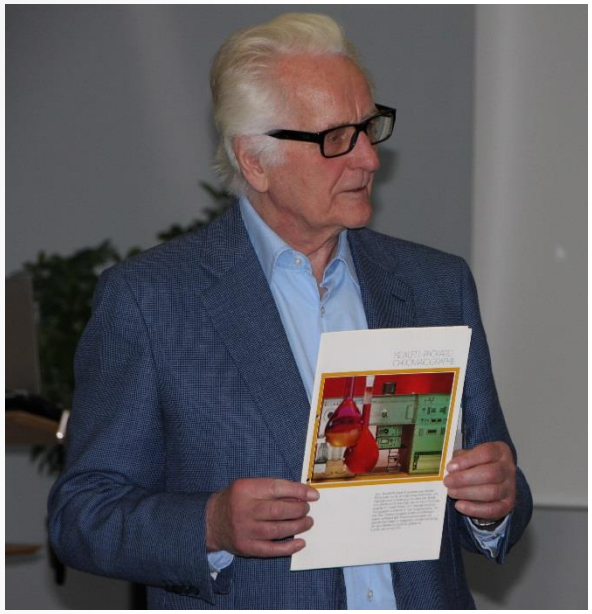
Uwe D. Neue Award in Separation Science

- Uwe D. Neue: A Personal Reflection

- Thank You
  - Permanent Scientific Committee HPLC Conference Series
  - Sponsor of the Uwe D. Neue Award, Waters Inc. in particular Martin Gilar.

- Acknowledgements
  - Colleagues and Senior Management at Agilent Technologies world wide
  - My peers in academia
  - ….

Special Acknowledgements for:



Prof. K-Peter Hupe
Founder HPLC Business HP/Agilent and Scientific Consultant

Dipl.-Ing. FH Fred Strohmeier
Colleague and peer. Now Senior Vice President, Life Sciences and Diagnostics Group

Current and Future Perspectives on UHPLC; Requirements for Improved Abilities and Functionality.

Dwight Stoll[1], Monika Dittmann[2], Konstantin Choikhet[2] , Gerard Rozing[3]

[1]Gustavus Adolphus College, St. Peter, MN, USA
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[3]ROZING.COM Consulting, Karlsruhe, Germany

2004: HPLC evolved to UHPLC

Two key ingredients for this evolution

- Introduction of new ultra-high pressure stable sub-2-µmeter diameter particles followed by the introduction of superficially porous, low diameter particles in 2006

- Next generation HPLC instrumentation capable to deliver solvents at ultra-high pressure and able to conserve the ultra high efficiency separation of columns packed with these new particles

UHPLC – Innovation*

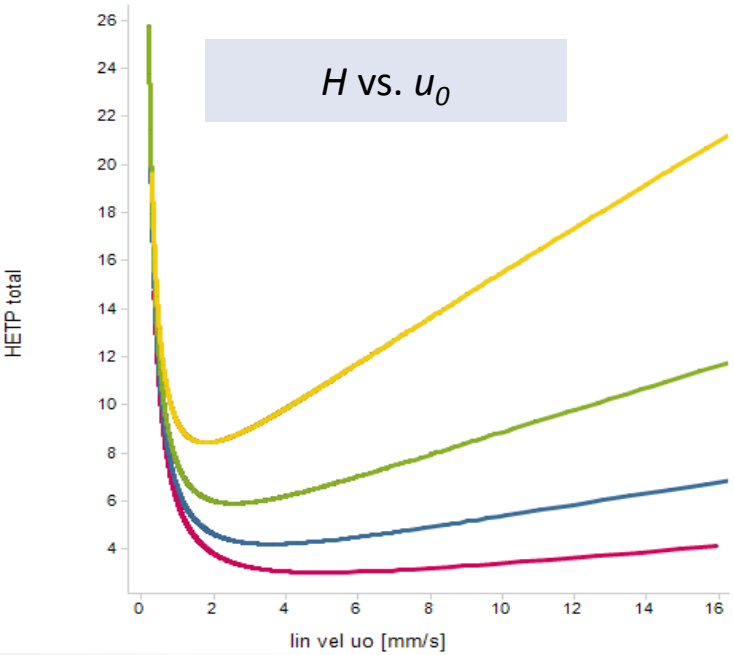
- Pioneered by Waters Inc. as UPLC® system with BEH particle technology (Uwe Neue as contributor) and Advanced Materials Technology with HALO particles (last year's Uwe Neue Award recipient Jack Kirkland).

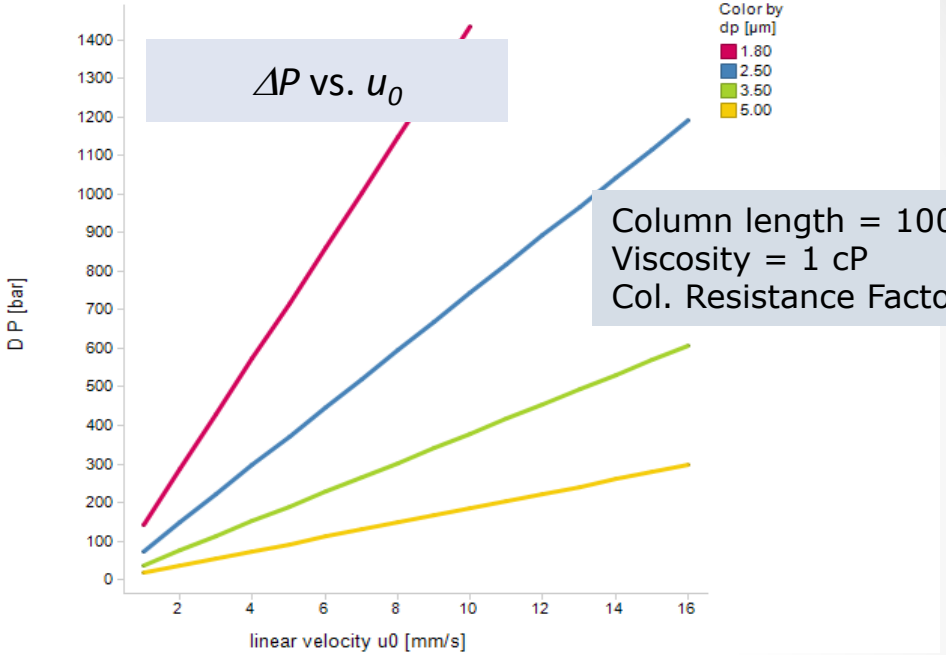
- A plethora of UHPLC systems and column technology has followed since.

*An innovation is something original, new, and important in whatever field that breaks into a market or society. This is accomplished through more effective products, processes, services, technologies, or ideas that are readily available to markets, governments and society.
Source: http://en.wikipedia.org/wiki/Innovation

UHPLC - Essentials

HETP and Pressure Drop vs. Solvent Velocity Dilemma





Column length = 100 mm,
Viscosity = 1 cP
Col. Resistance Factor = 700

Find the best values of L, d_p and u_o to obtain the highest N or (N/t_0) under one constraint: ΔP_{max}

$$t_0 = \frac{L}{u_0}$$

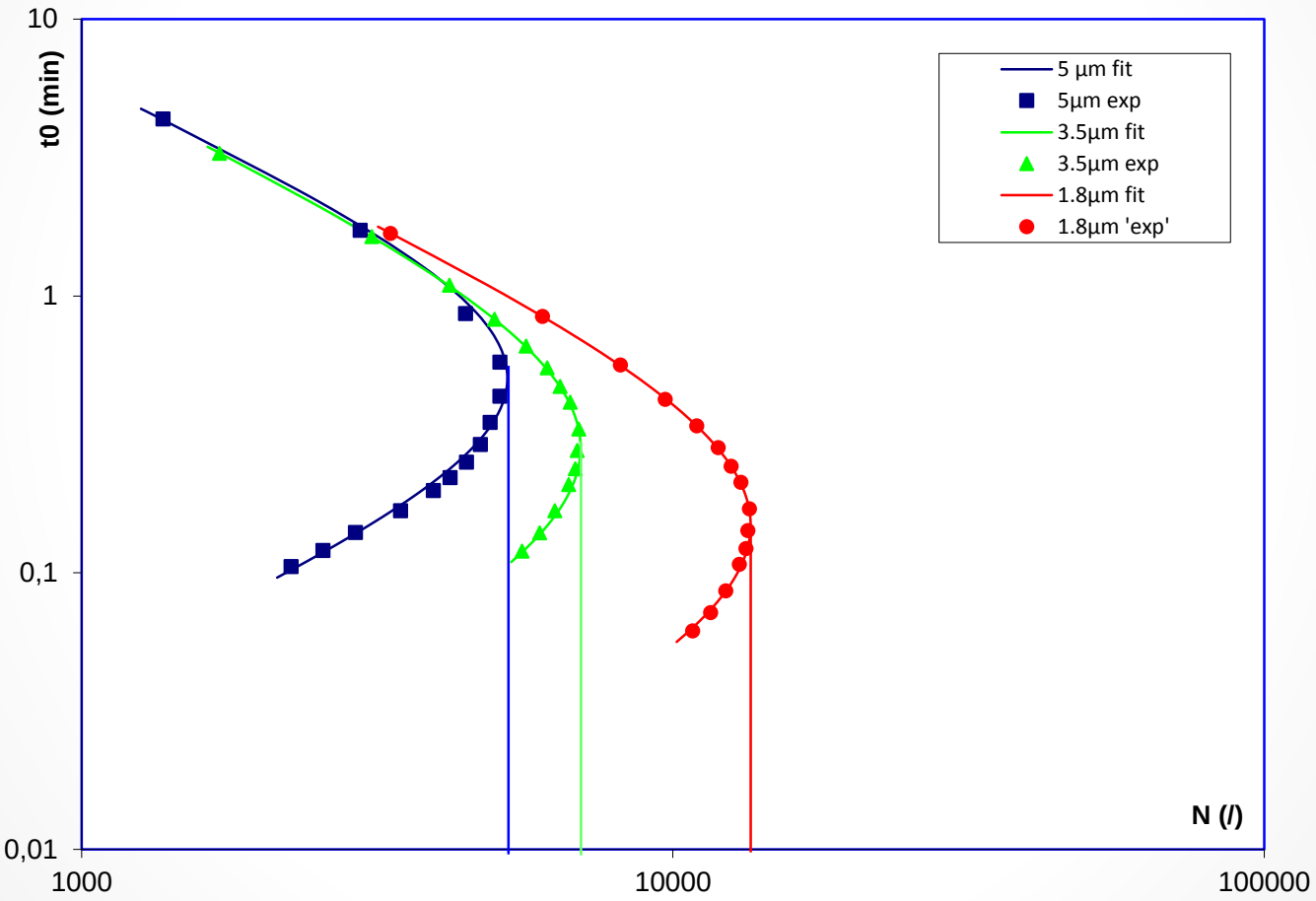
$$N = L / H$$

$$\Delta P_{\max} = u_0 \cdot \eta \cdot L \cdot \frac{\Phi_0}{d_p^2}$$

UHPLC - Essentials

1 - Parameter Kinetic Optimization*

***L* (50 mm) and d_p fixed, select optimal u_o**



*Slide courtesy of Prof. Ken Broeckhoven, Free University of Brussels

UHPLC - Essentials

2- Parameter Kinetic Optimization (e.g. "Poppe Plot" approach)

Poppe's approach: find $u_{0,max}$ and $L(u_{0,max})$ corresponding to a given N_{req} and ΔP_{max} at a fixed particle size

The following relationship exists for "plate time":

$$\frac{t_0}{N} = \frac{H(u_0)}{u_0}$$

$$\frac{H(u_0)}{u_0} = \frac{A \cdot d_p}{u_0} + \frac{B \cdot D_m}{u_0^2} + C \cdot \frac{d_p^2}{D_m}$$

In order to construct the plot of the plate time in dependence of N_{req} after obtaining the coefficients of the van Deemter equation (or e.g. the Knox equation) by non-linear regression, the Poppe plot can be calculated and substitution of the appropriate value for ΔP_{max}.

$$\log(t_0 / N_{req}) = \log C_1 N_{req} \qquad C_1 = \left[h^2 \Phi_0 \eta / \Delta P^{\max}\right] \qquad h = H(u_0)/d_p$$

So, when one wants to decrease the plate time and does not want to sacrifice separation time, the column resistance factor (Φ) should be reduced (e.g. monoliths!), viscosity decreased (η) (e.g. increase temperature!) or increase the max. available pressure

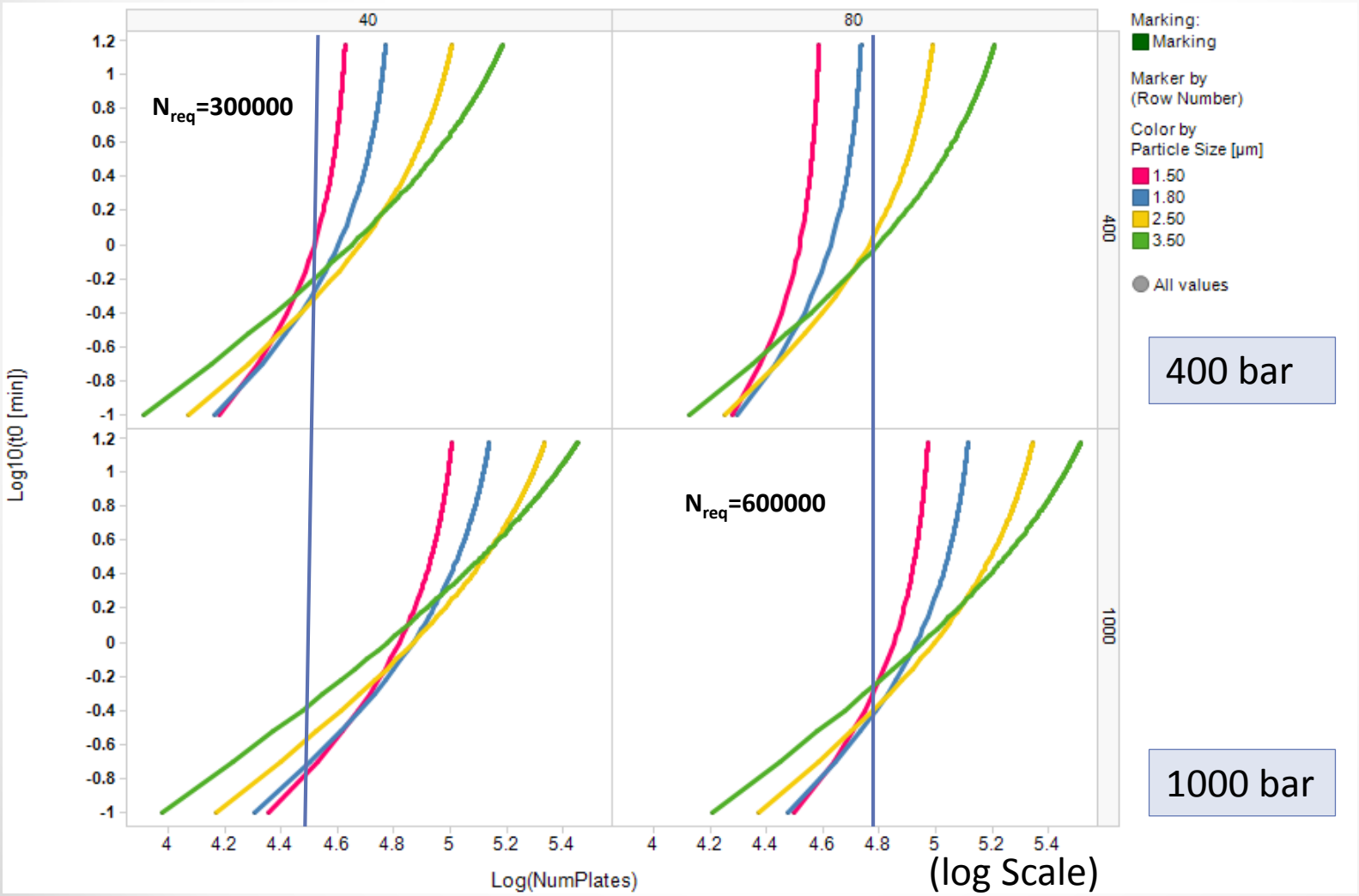
H Poppe, J. Chrom. A, **778**, 3 (1997), G. Desmet et al., Anal. Chem., **77**, 4058 (2005), F. Gritti and G. Guiochon, J. Chrom. A, **1228**, 2, (2012)

UHPLC - Essentials

2-Parameter Kinetic Plots for Different Particle Sizes

40 mm

80 mm



400 bar

1000 bar

(log Scale)

UHPLC – Essentials

3-Parameter Kinetic Optimization (Knox & Saleem)

Find the maximum plate number possible when, u_0, L and d_p are varied to reach any ΔP_{max}

$$u_0^* = \left(\frac{\Delta P_{max} \cdot \nu_{opt}^2}{\Phi \cdot \eta} \right)^{0.25} \cdot t_0^{-0.25} \cdot D_m^{0.5}$$

$$d_p^* = \left(\frac{\Phi \cdot \eta \cdot \nu_{opt}^2}{\Delta P_{max}} \cdot \right)^{0.25} t_0^{0.25} \cdot D_m^{0.5}$$

$$L^* = \left(\frac{\Delta P_{max} \cdot \nu_{opt}^2}{\Phi \cdot \eta} \cdot \right)^{0.25} t_0^{0.75} \cdot D_m^{0.5}$$

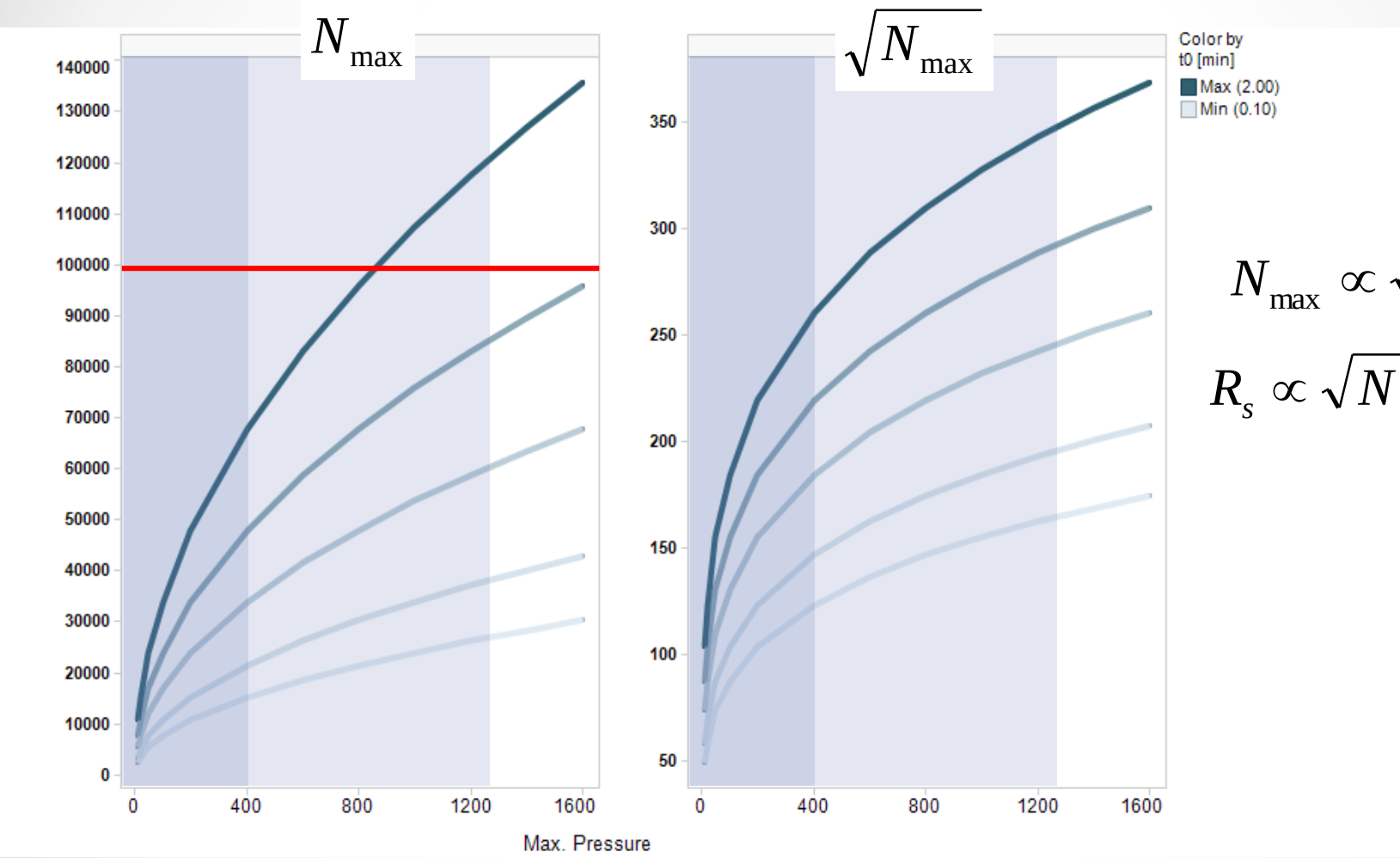
$$N_{\max}^* = \left(\frac{\Delta P_{max} \cdot t_0}{\Phi \cdot \eta} \right)^{0.5} \cdot \frac{1}{h_{\min}}$$

Knox-Saleem limit

J.H. Knox and M. Saleem. J. Chromatogr. Sci., 7 (1969), p. 614
P. W. Carr, X. Wang, *Anal. Chem.* **2009,** *81,* 5342–5353

UHPLC –Essentials

Maximum Achievable Plate Number vs. Pressure*



$$N_{max} \propto \sqrt{\Delta P}$$

$$R_s \propto \sqrt{N} \propto \sqrt{\sqrt{\Delta P}}$$

Preliminary Conclusions and Assumptions

- Kinetic optimization predicts that the max. plate number achievable does not increase proportional with pressure
- More pressure will result in shorter time to obtain N_{req} *

But ……

Such inferences from kinetic plots only apply if**:

- HETP is independent from column length
- physical and chemical properties of solvents and solutes, particle properties and column dimensions are independent of pressure
- frictional heating can be neglected

*K. Broeckhoven and G. Desmet, accepted for publication in Trends in Analytical Chemistry

**F. Gritti and G. Guiochon, J. Chrom., 1228, 2, (2012)

UHPLC – Constraints

Effect of High Pressure on*

- Solvent properties
  - density (ρ), specific volume, viscosity (η), compressibility (χ) and melting point
- Solute properties
  - diffusion coefficient (D_m), molar volume, conformation/3D-structure changes (in particular with high MW substances)
- Retention factors (k) and in particular of ionizable molecules and HMW substances**
- Particle porosity and total porosity of the packed bed (ε_T)
- Column dimensions length and diameter (L and d_c)

Under ultra-high pressure conditions, solute and solvent properties change but not independently!!

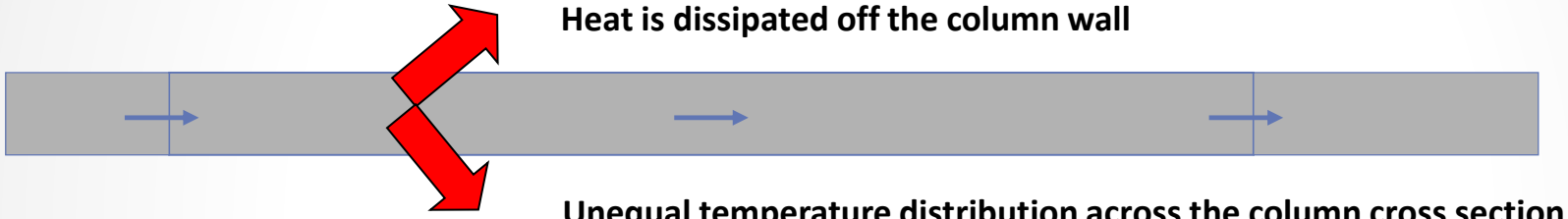
*M. Martin & G. Guiochon, J. Chrom. A, 1090, 16 (2005)
**M.M. Fallas, U.D. Neue, M.R. Hadley, D.V. McCalley, J. Chrom. A, 1209, 195–205 (2008)

UHPLC – Constraints

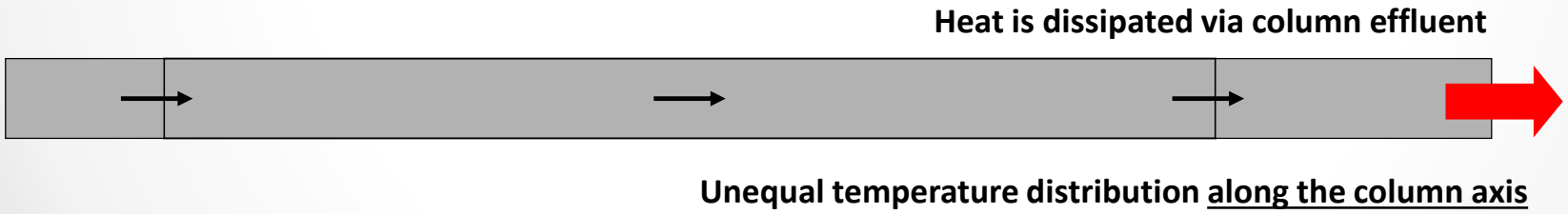
Frictional Heating and Column Environment

$$Power = \Delta P \times F$$

Isothermal: fast heat exchange with constant temperature environment



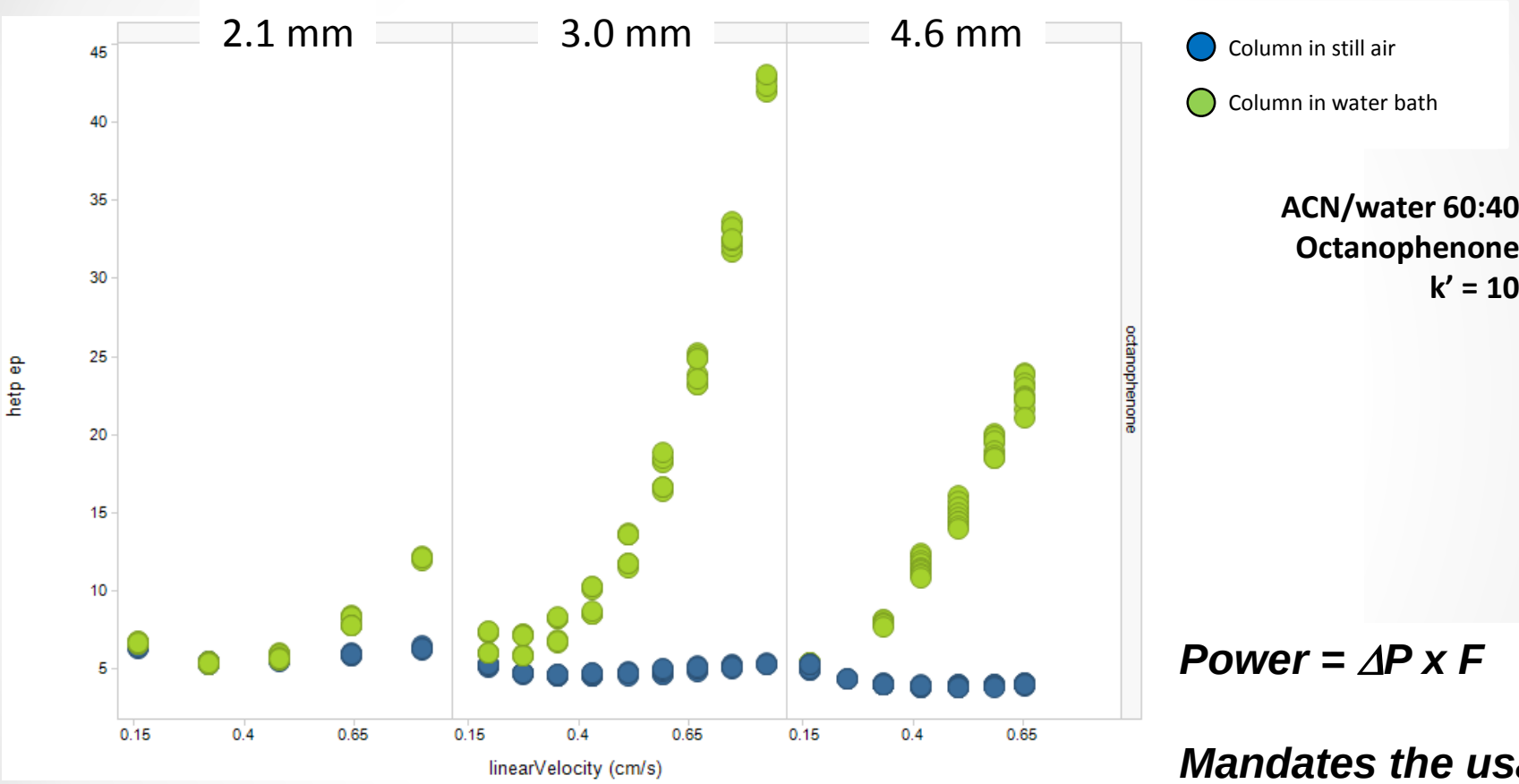
Adiabatic: no heat exchange with environment



Slide courtesy of Prof. G. Desmet et al, Free University of Brussels, CHIS

UHPLC – Constraints

Frictional Heating and Column Environment*



ACN/water 60:40
Octanophenone
k' = 10

Power = ΔP x F

Mandates the usage of lower i.d. columns which raises the bar for following!!

***experimental results courtesy of Monika Dittmann**

UHPLC – Constraints

Extra Column Band-Broadening

$$\sigma^2_{v,tot} = \sigma^2_{v,col} + \sigma^2_{v,cap} + \sigma^2_{v,inj.} + \sigma^2_{v,cell}$$

$$\sigma^2_{v,cap} = \frac{V_{cap} r^2 u}{24 D_m}$$

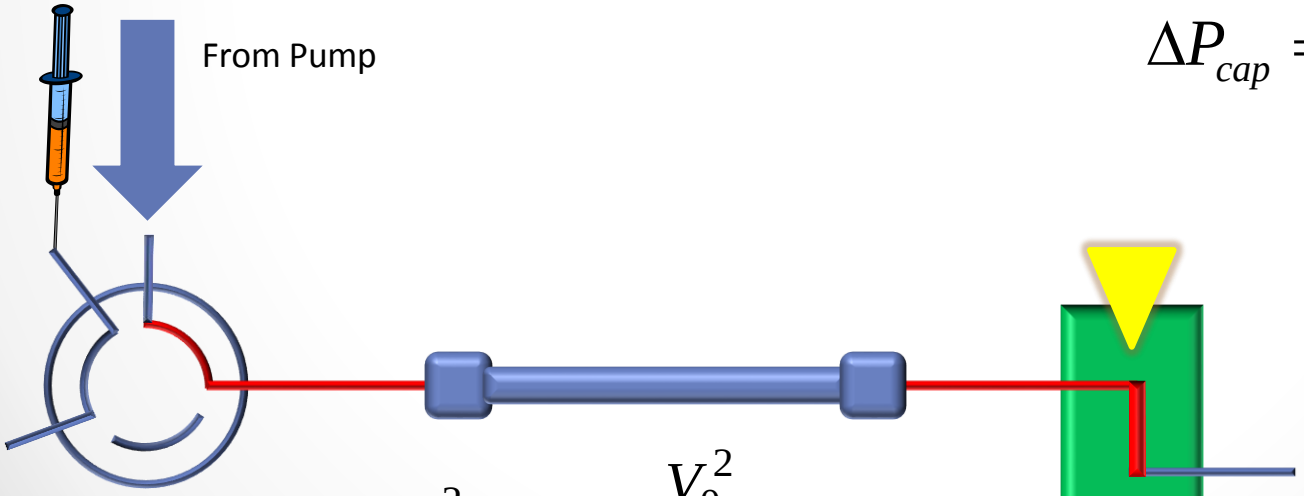
$$\sigma^2_{v,cell} = \frac{V^2_{cell}}{X}$$

X=dispersion factor, 1-12

Typical values for variance

Injector:	**1 µl²**
Capillaries (500x0.12 mm)	**7 µl²**
Capillaries (500x0.08 mm)	**4 µl²**
Column (2.1x50 mm, k' =1)	**3 µl²**
Column (2.1x50 mm, k' =5)	**33 µl²**

$$\Delta P_{cap} = \frac{F \cdot 8\eta \cdot L_{cap}}{\pi r^4_{cap}}$$



$$\sigma^2_{V,column} = \frac{V_0^2}{N} \cdot (1 + k')$$

UHPLC – Constraints at Ultra-high Pressure

Mechanical & Hydraulic Engineering Challenges

- Switching and sealing interfaces, like piston-seal, check and rotary valves will become difficult to realize especially when the material strength limits are reached or materials more readily fatigues
- Pressure cycles will blow and relax the tubing in (sub)micrometer dimensions may damage bed structure and/or particles
- Narrower i.d. columns mandate lower flow rates and lower system volume and ultra-high pressure. Leaks will not noticeable, harder to find and eliminate. Significant influence on flow rate and composition precision and accuracy
- Reliable pumps at ultra high pressure ➔ example

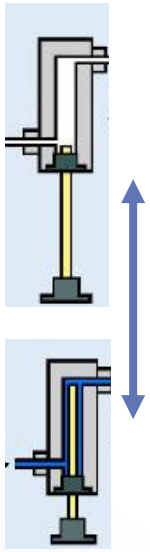
UHPLC – Engineering Requirements

What is the force required to deliver solvents @ 1000 bar?

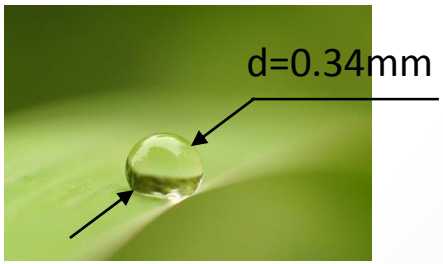
Desired flow rate 0.05 - 5 ml/min
Piston diameter e.g. 3.1 mm, cross-section 7.6 mm^2
Stroke volume up to 100 µl = 100 mm^3
Desired flow rate precision (per channel) ~0.2%

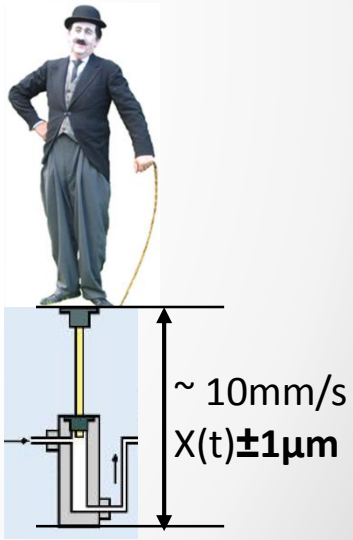
Force
~ 800N

Linear displacement
~13 mm



Required displacement precision:
20 nl vol.
or ~2 µm linear
or better



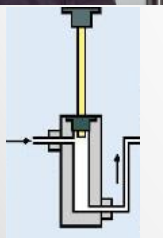


UHPLC – Engineering Requirements

What is the force required to deliver solvents @ 1500 bar?

Desired flow rate 0.05 - 5 ml/min
Piston diameter e.g. 3.1 mm, cross-section 7.6 mm^2
Stroke volume up to 100 µl = 100 mm^3
Desired flow rate precision (per channel) ~0.2%

Force
~ 1200N

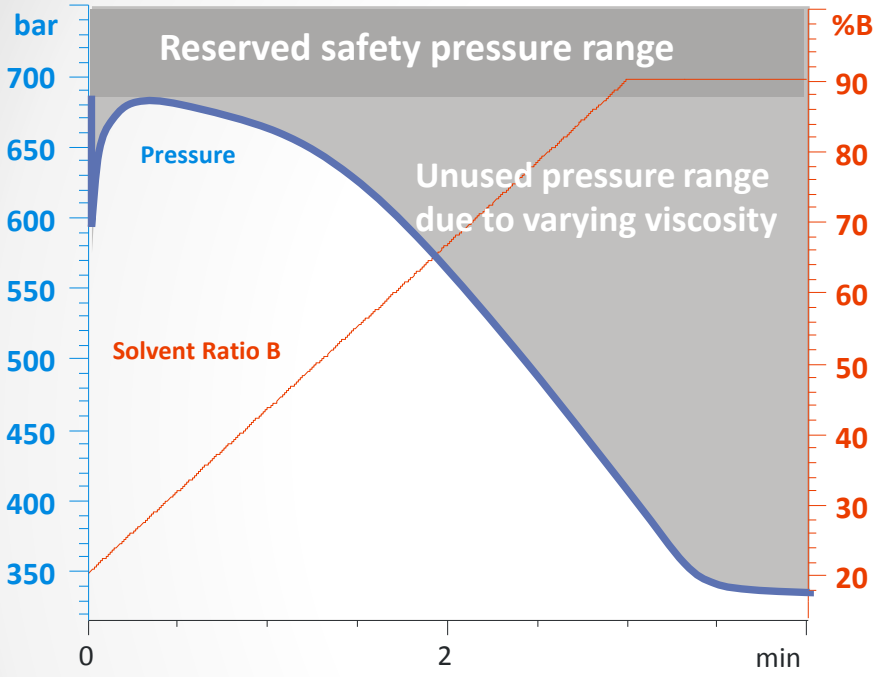


UHPLC – Other pathways

- Constant Pressure Operation
- Multidimensional HPLC

UHPLC

Constant Pressure Operation



BENEFITS

- Super robust operation:
  - no overpressure shutdowns
  - "safety" pressure range necessary
- More efficient use of instrument power range, higher flow at lower viscosity
- Without pressure cycles, less stress on system and column
- 10-30% decrease of run time

UHPLC - Constant Pressure Operation

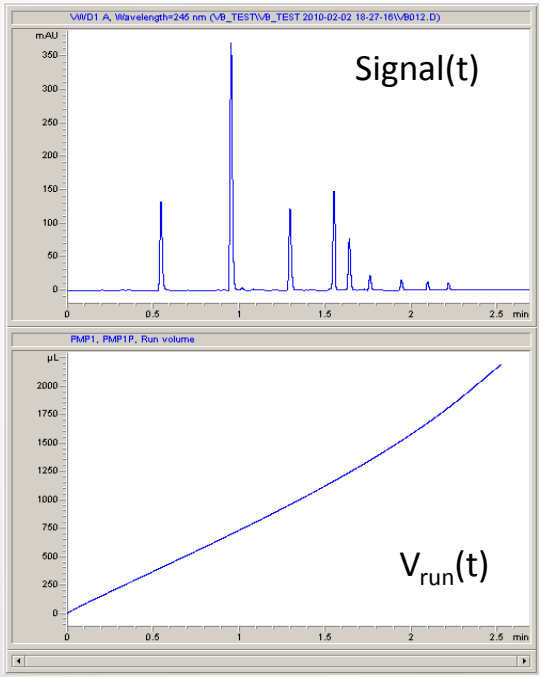
Signal Transformation

Measured run time axis into retention volume axis into equivalent “chromatographic” time axis.

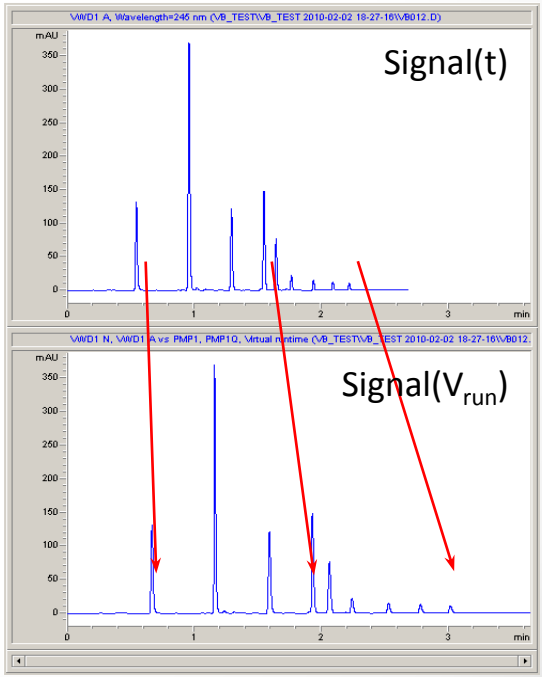
“Chromatographic” time = $\frac{Delivered\ Volume}{Hypothetical\ Constant\ Flow}$

X axis transformation: $V_{run} = f(t) \Rightarrow t = f^{-1}(V_{run})$

$Signal(t) \xrightarrow{t=f^{-1}(V_{run})} Signal(f^{-1}(V_{run})) = Signal(V_{run})$

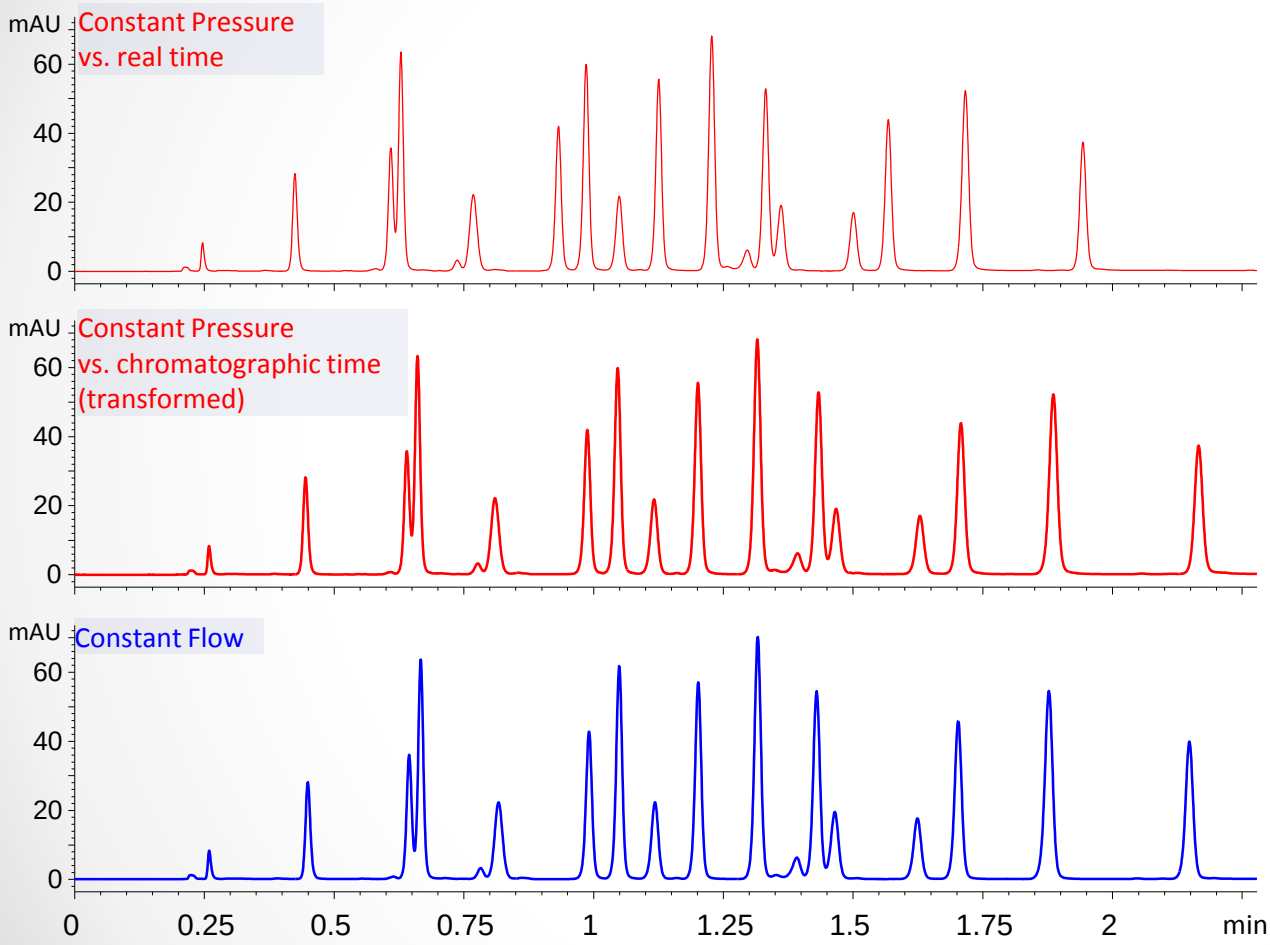


X-axis transformation



UHPLC - Constant Pressure Operation

Application Example



Elution order:

Thiourea, Atrazindesethyl, Hexanzinon, Metoxuron, Cyanazin, Methabenzthiazuron, Chlortoluron, Atrazin, Diuron, Metobromuron, Metazachlor, Nifedipin, Sebuthylazin, Terbuthylazin, Linuron, Prometryn, Nimodipin

Conditions and results:

Column: Eclipse plus C18, 1.8µm, 2.1x100mm

Constant Pressure: 1050bar, 1.05→1.4ml/min

Constant Flow: 1ml/min, 1020→750bar

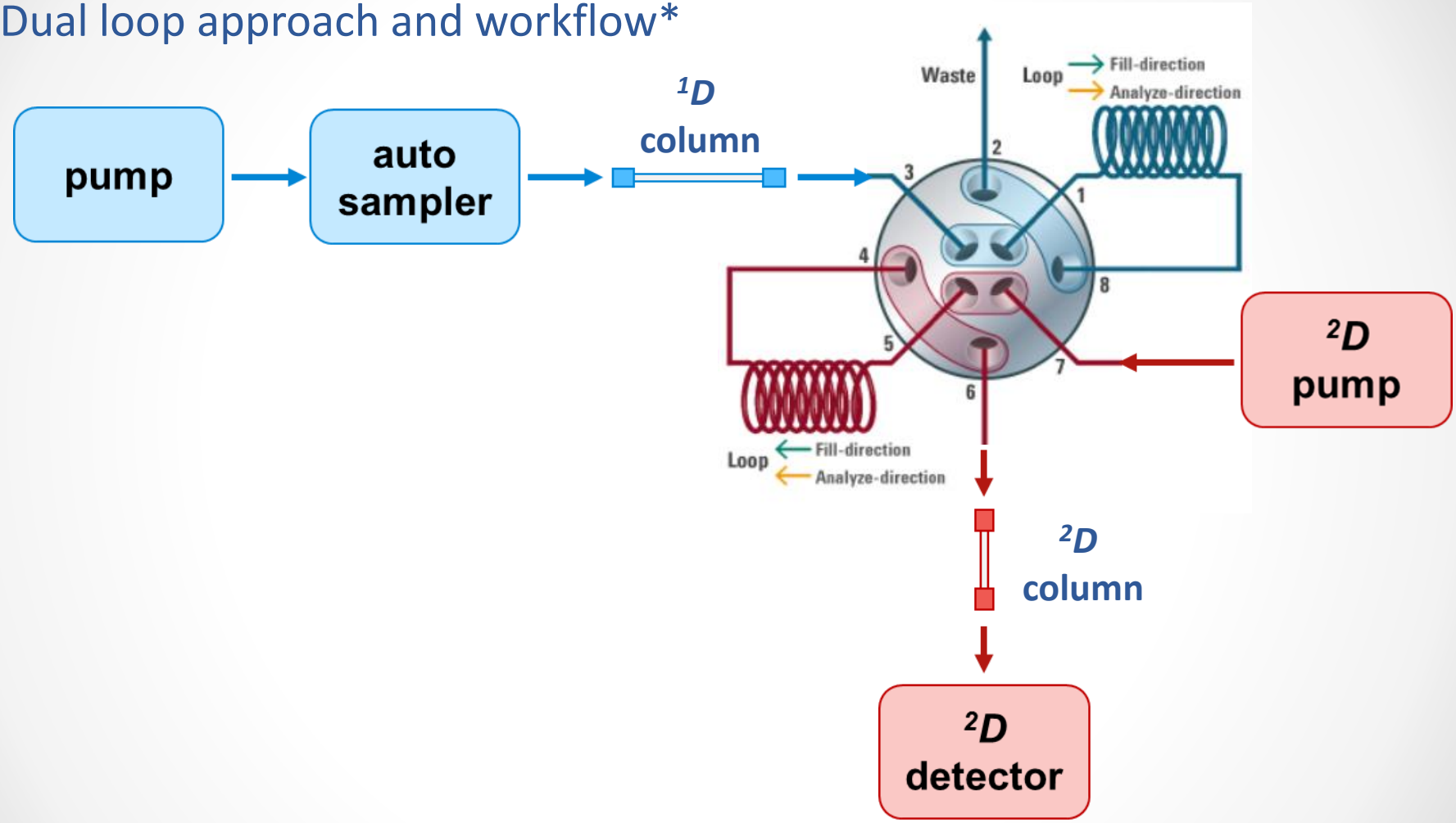
Time gain in Constant Pressure Mode: 10%

Further work, Desmet et al and Gritti & Guiochon

Multidimensional HPLC

Alternative?

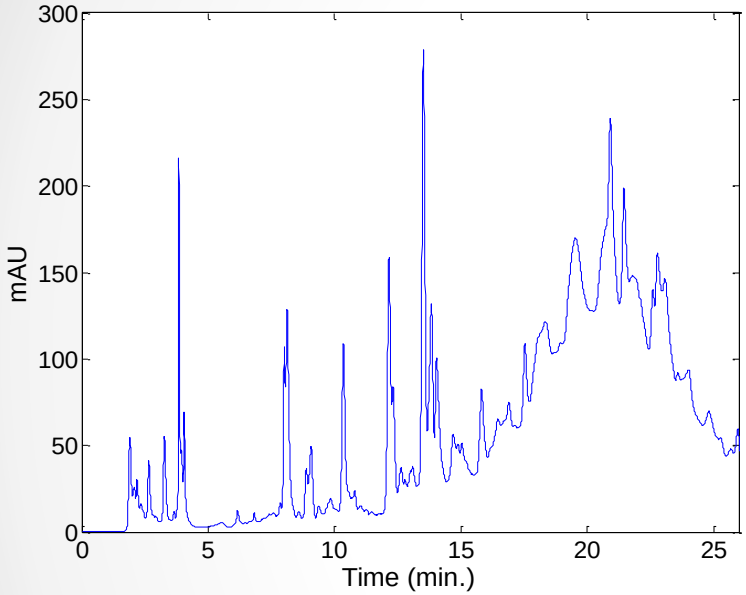
Dual loop approach and workflow*

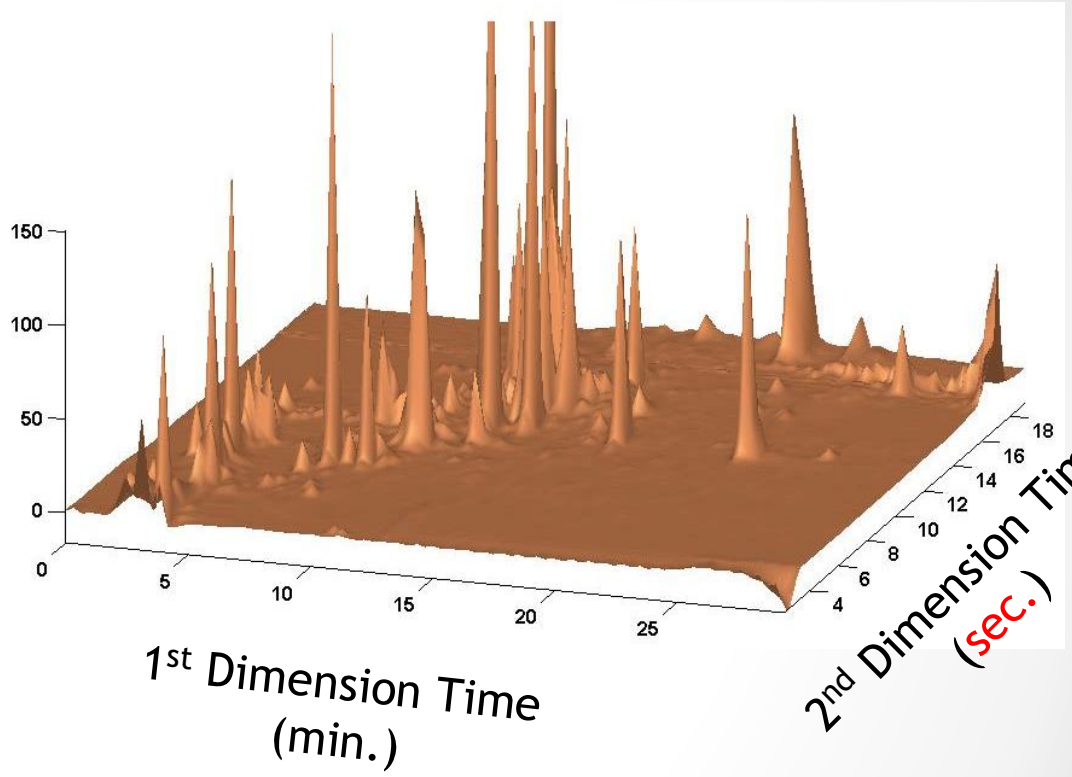


* Slide courtesy of Dr. Stephan Buckenmaier, Agilent Technologies

Multidimensional HPLC

1D LC vs. 2D LC





Peak Capacity$_{1D}$ = 150

Peaks$_{1D}$ = 77

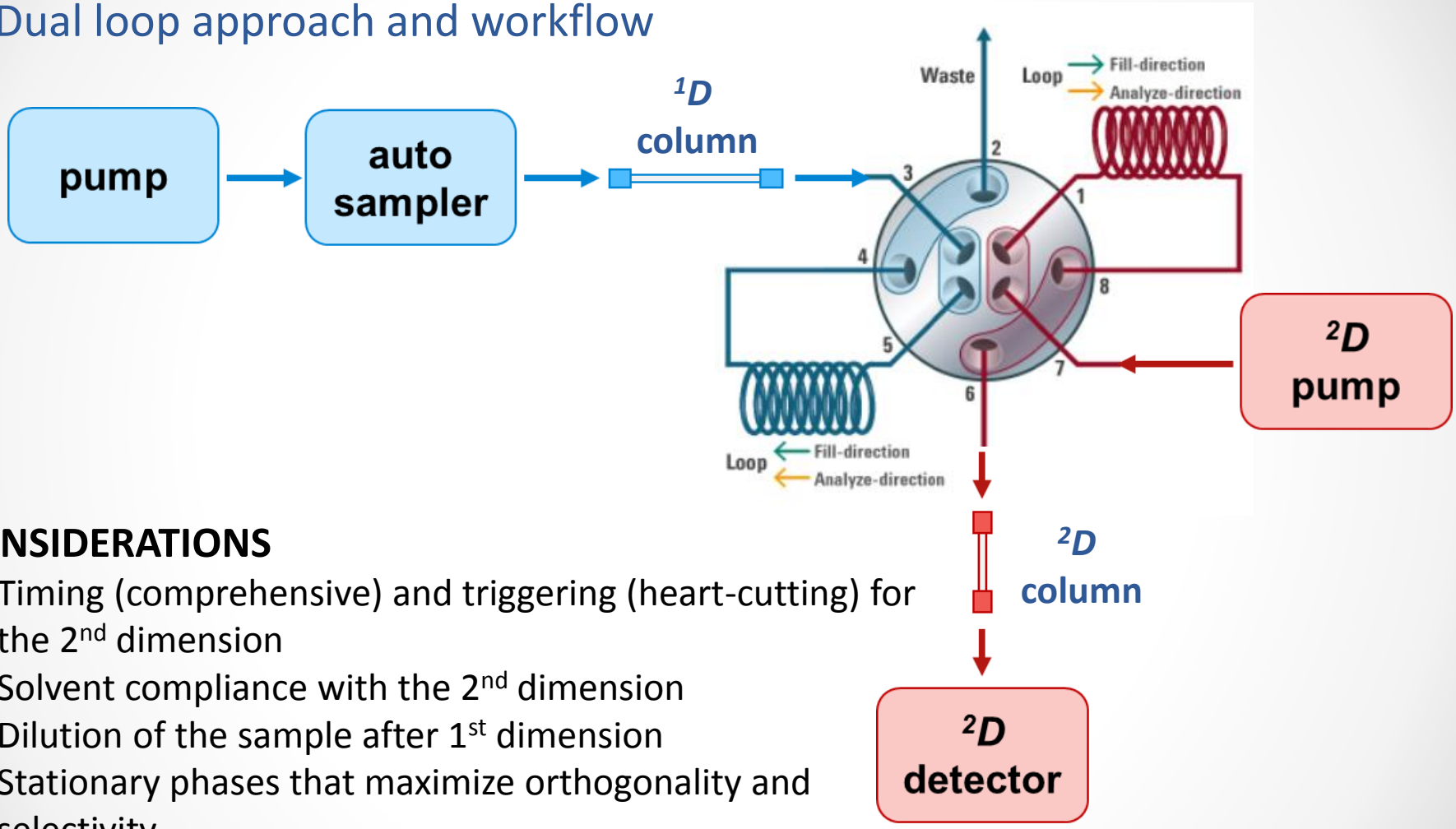
Peak Capacity$_{2D}$ = 900

Peaks$_{2D}$ = 174

Multidimensional HPLC

System Challenges

Dual loop approach and workflow

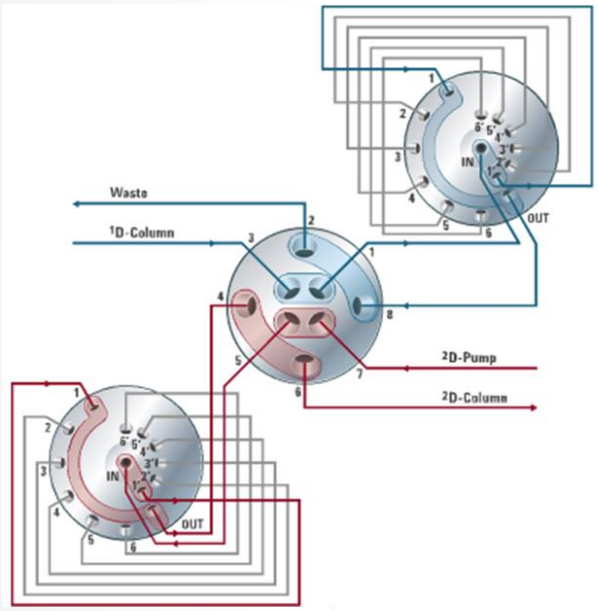


CONSIDERATIONS

- Timing (comprehensive) and triggering (heart-cutting) for the 2nd dimension
- Solvent compliance with the 2nd dimension
- Dilution of the sample after 1st dimension
- Stationary phases that maximize orthogonality and selectivity
- User Interface for system control (including MS detectors)
- Quantitation and data representation

Multidimensional HPLC

Integrated systems and solutions are needed



Tuesday, May 13, 5:15 pm-5:35 pm

L-149: Improvements in Two-Dimensional HPLC Applications Enabled Advances in Instrument Technology.

by Stephan Buckenmaier et al., Agilent Technologies, Waldbronn, Germany

- 2x six loops instead of one gives 12 sampling positions
- High pressure selection valves for 2nd dimension UHPLC

*** Slide courtesy of Dr. Stephan Buckenmaier, Agilent Technologies**

Conclusions

- Raising the pressure bar does not increase *N* proportionally but N_{req} is obtained faster
- At ultra-high pressure, physical, thermal and engineering constraints will make requirements for systems extremely challenging especially since columns have to be of lower i.d.
- Constant pressure operation is a viable option with operational benefits but has yet to be proven in practice
- Multidimensional LC will be a viable pathway for qualitative and quantitative analysis and high peak capacity but:
  - More dedicated and selective stationary phases for versatility and high orthogonality must be available.
  - Intelligent guidance of system set-up, valve control and automation that support the user.
  - Data handling, quantitation and representation will be demanding especially in combination with MS detection

Acknowledgements

My co-authors, Monika Dittmann, Konstantin Choikhet and Dwight Stoll
Ken Broeckhoven, Free University of Brussels
Stephan Buckenmaier, Agilent Technologies, Germany
Waters Inc. Award Sponsor
Organizers of HPLC 2014 for the invitation

A PDF copy of this talk will be available on my website http://www.rozing.com after the talk (registration required)