About Dr. Gerard Rozing

- **1964-1976:**
  Undergraduate and graduate studies at University of Amsterdam, Netherlands. Majors in Organic Chemistry and Chemical Engineering, Ph.D. Synthetic Organic Chemistry.
- **1977-1979:**
  Post-doctoral research University of Ghent, Belgium and University of Amsterdam.
- **1979-1999:**
  Hewlett-Packard, Waldbronn, Germany. R&D Chemist, group & project Leader, R&D manager, HPLC column, HPLC system, CE capillaries and CE system development.
- **2000:**
  Agilent Technologies University Relations and External Scientific Collaborations Manager, Agilent Research Fellow.
- **Retired September 1, 2012**:
  Since then, working as a freelance consultant.
- **Current:**
  Member of the Strategy Advisory Boards of PharmaFluidics, Ghent, Belgium and Advanced Electrophoresis Solutions, Cambridge, ON, Canada.
  Involved as co-organizer HPLC, MSB and ISC symposium series
- **Contact:**
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Recent Developments in Liquid Phase Separations

Presented by Dr. Gerard P. Rozing

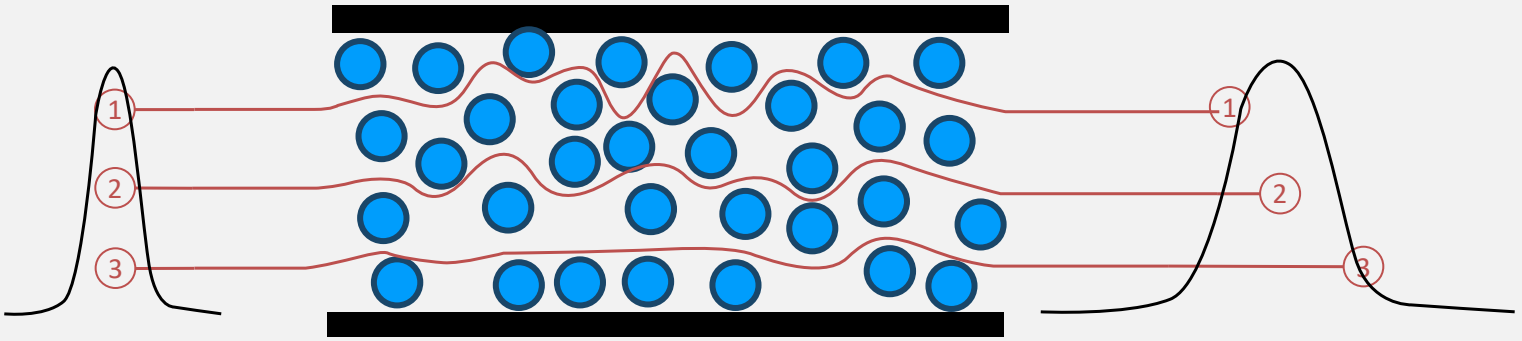
@ KIST Europe, August 27, 2019

µPillar Array Columns (µPAC); a paradigm change in technology for ultra High Resolution micro- and nano-HPLC for bioanalysis

Collaboration with PharmaFluidics, Ghent, Belgium

Performance Limit of Packed HPLC Columns

Zone broadening by unequal pathlengths and velocities of solutes traversing the column bed: "eddy diffusion"

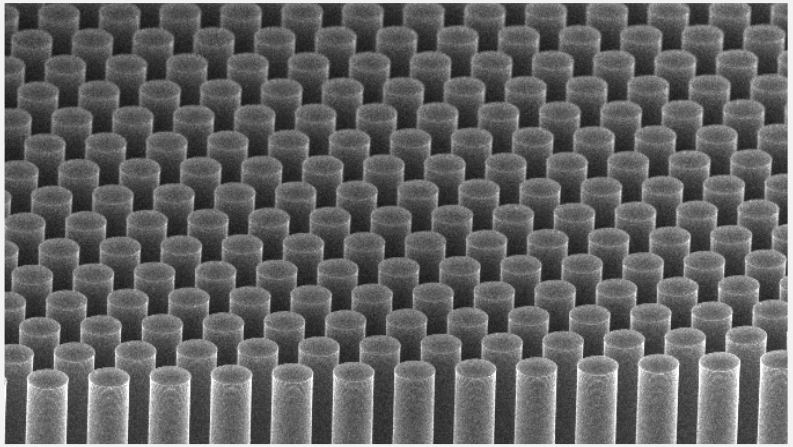


Are caused by inhomogeneous axial and radial density of the packing*

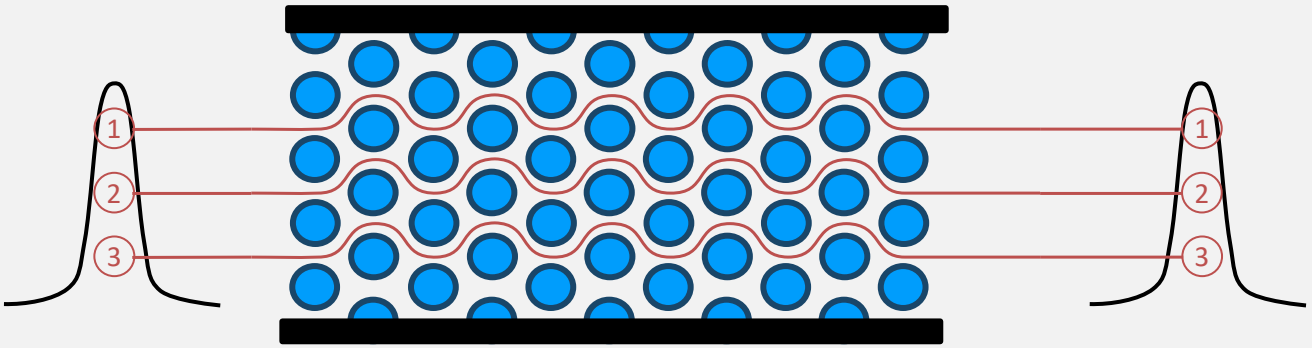
- Disturbance by the wall
- Particle size distribution
- Unequal solvent flow velocity during packing
- Bridge formation during packing ➔ bed instability

*LC-GC Magazine NORTH AMERICA VOLUME 36 NUMBER 2 FEBRUARY 2018 , Page 82.
COLUMN WATCH: Understanding the Science Behind Packing High-Efficiency Columns and Capillaries: Facts, Fundamentals, Challenges, and Future Directions
Fabrice Gritti and M. Farooq Wahab

µPillar Array Columns (µPAC)

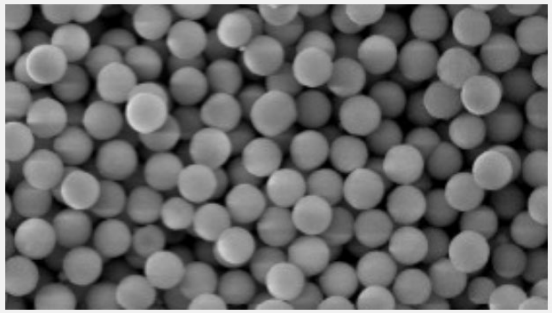


Highly ordered “particles”

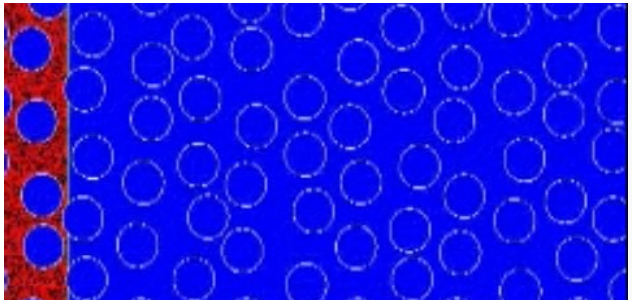


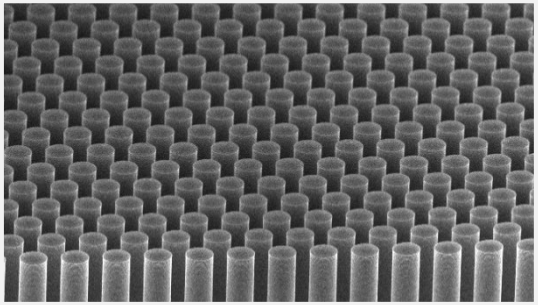
G. Desmet et al.. Anal. Chem., **2007,** 79, 5915-5926 and many publications since then

Unprecedented Separation Performance

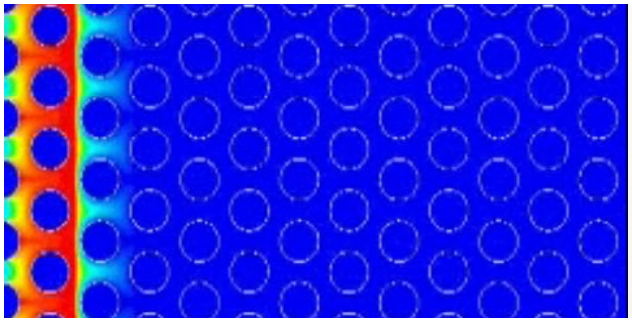


Disorder - Packed Bed



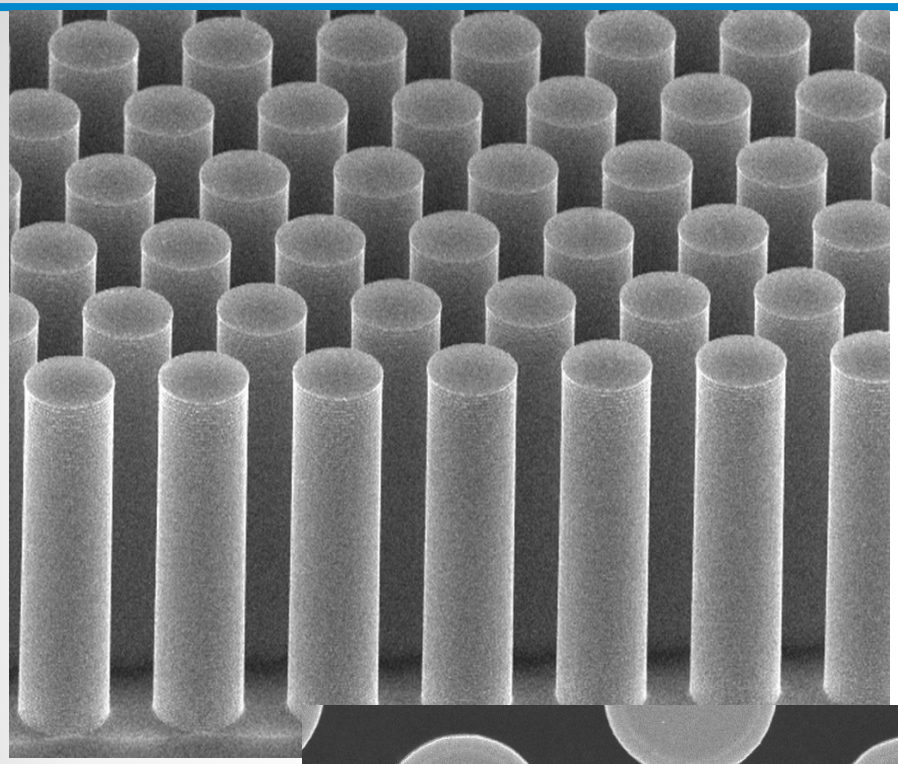


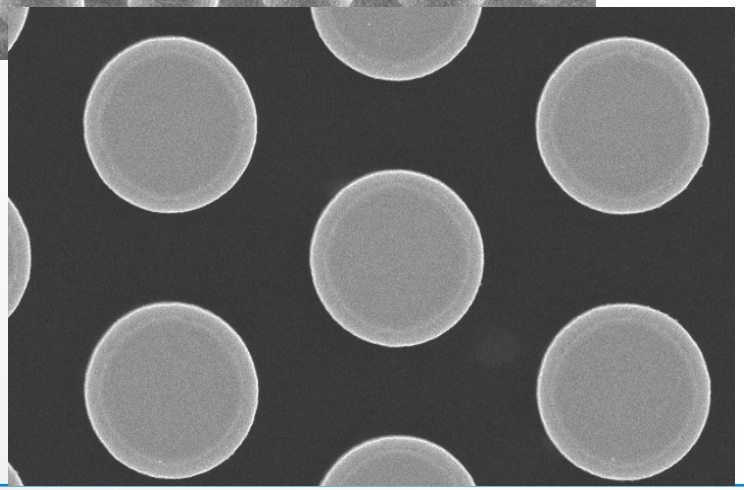
Order – Pillar Array



The benefit of Order versus Disorder

µPillar Array Columns – Some Metrics





Pillars :

- Interpillar distance 2.5 µm
- Diameter ≈ 5 µm
- Height ≈ 20 µm
- Porous layer 0.3 µm deactivated silica
- Surface bonded with C18

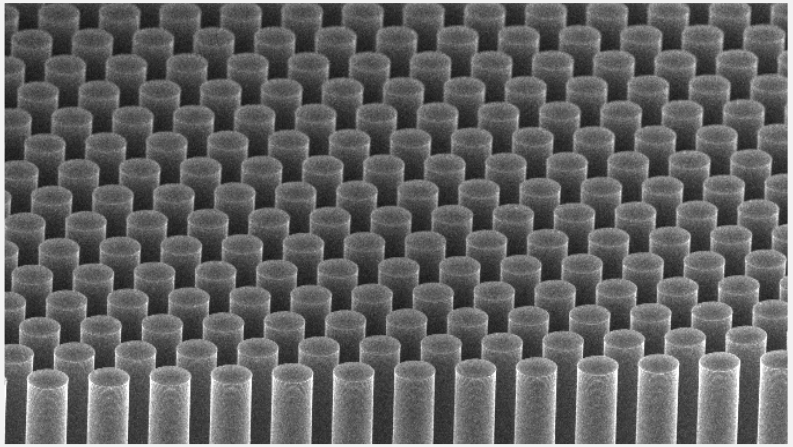
Chips :

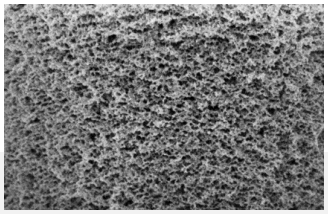
- Channel width 315 µm
- Channel length ≈ 5cm
- Total length 50, 100, 200 cm
- Total volume (2 m) 7 µL
- Inter pillar porosity ≈ 0.6
- Phase ratio ≈ 0.04
- Max pressure 250 bar

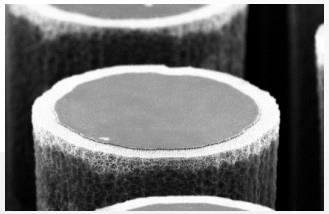
Chromatography :

- Reversed Phase C18
- Permeability ≈ 4×10^{-13} m^2 (50-100x lower common particle packed bed column)
- Reduced Plate Height 1
- Typical flow rate 0.2 – 1 µL/min
- Injection volume up to 1 µL

µPAC Paradigm Changing HPLC Column Technology

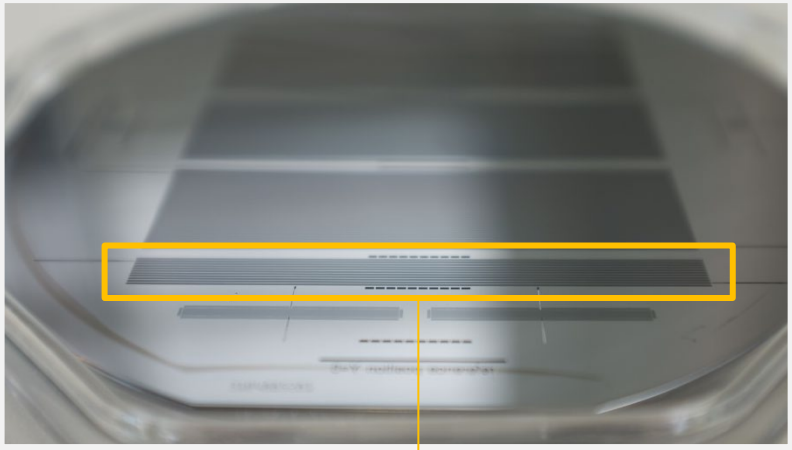


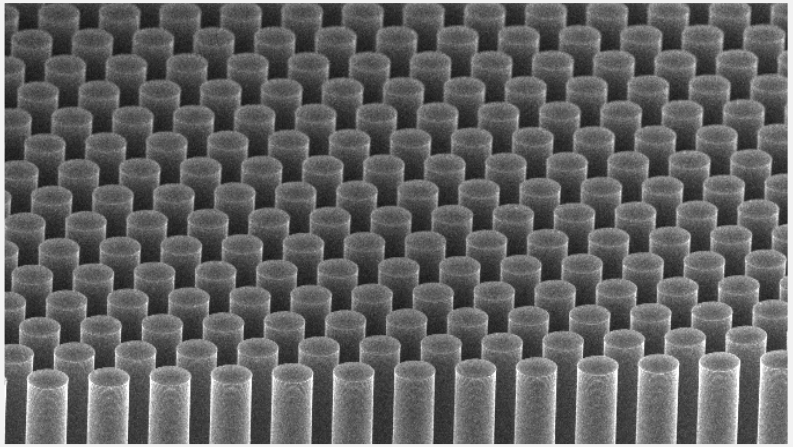




- Silicon wafer
- Photolithographic production process warrants reproducibility
- Etching free-standing pillars
- Surface oxidation makes a silica layer
- Glass bonding

µPAC Paradigm Changing HPLC Column Technology





50 cm µPAC™ column design

- 10 lanes of 5 cm long and 315 µm wide
- Concatenated into a 50 cm long separation bed

µPAC Paradigm Changing HPLC Column Technology

REAL TIME injection

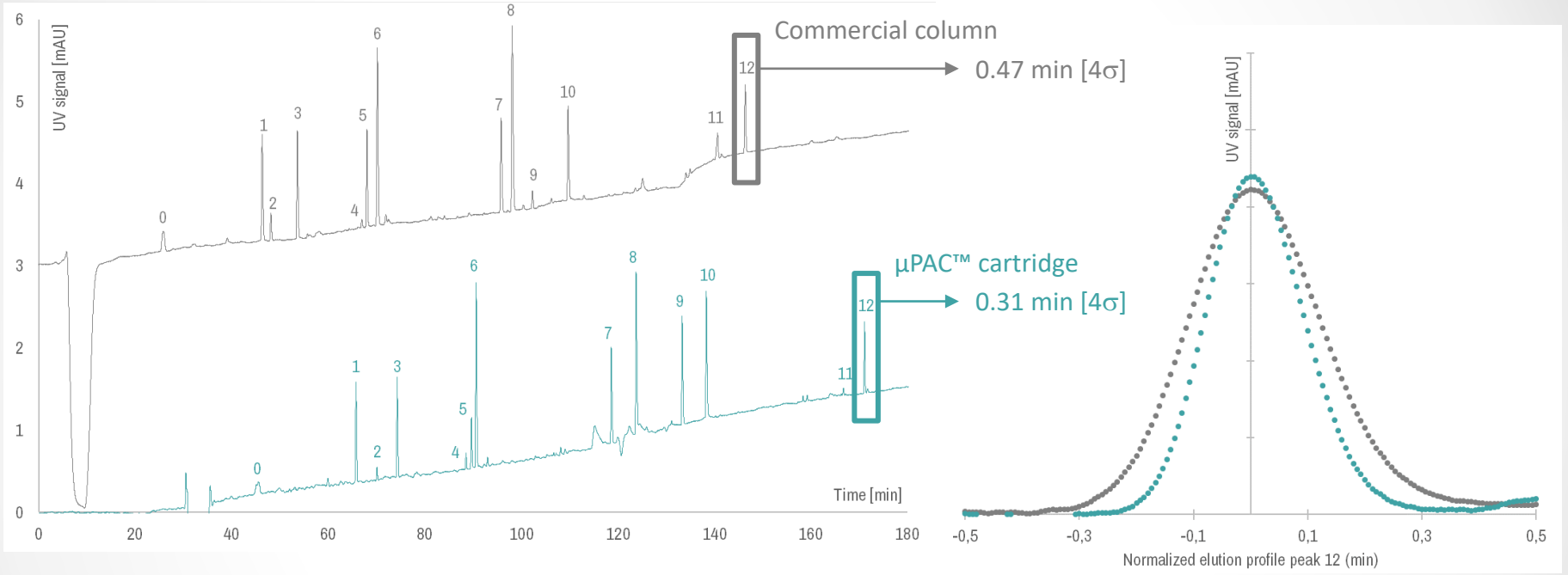
Flow distribution

Unprecedented separation bed length on a small footprint, without additional peak dispersion

TURN structure

µPAC™ - C18 – 200 cm - Separation Performance

µPAC™ cartridge vs packed bed commercial nano-LC column



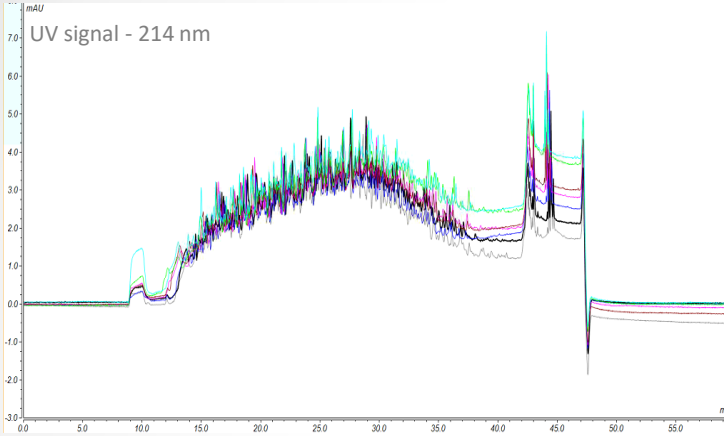
Experimental conditions

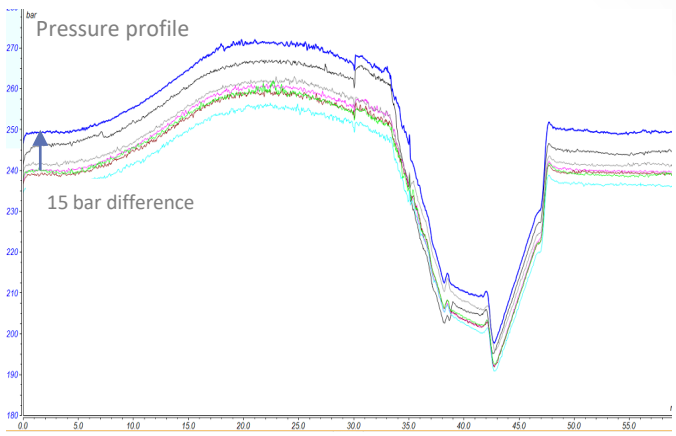
0.5 pmol Cytochrome C digest – 1 µl injection
2 to 40% ACN / 0.1%TFA
180 min gradient / 300 nl/min

LC system: Thermo Scientific Ultimate 3000 nanoRSLC
Detection: UV detection at 214 nm (3 nl flow cell volume)

µPAC™ Stability

Hela digest: Run 1 – 100 – 200 – 300 – 400 – 500 – 600

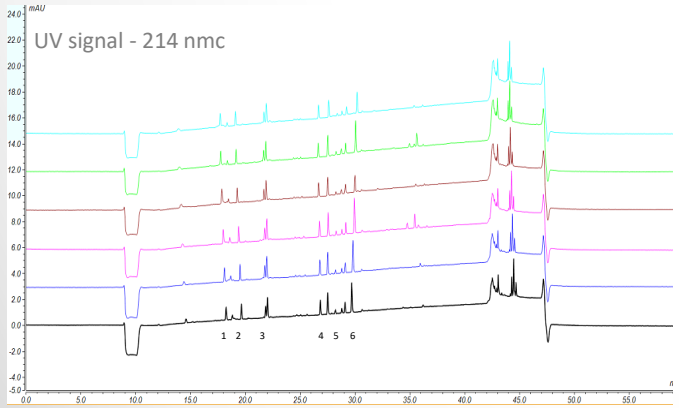


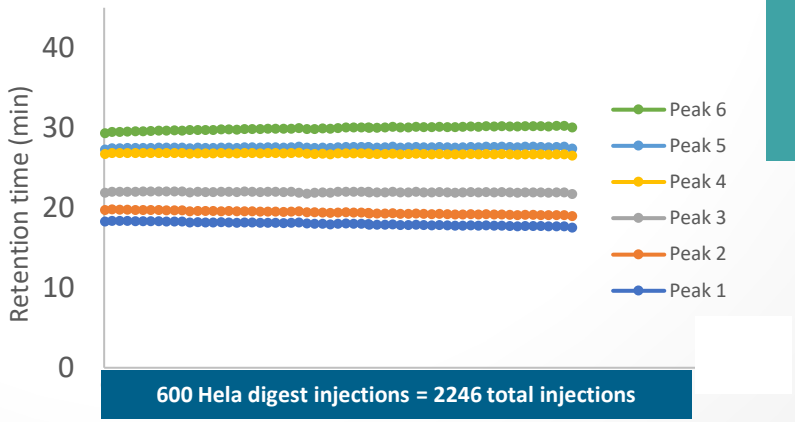


Experimental conditions

100n g Hela digest
1 µl injection
1 to 50% ACN / 0.1%TFA
60 min gradient
1 µl/min

Cytochrome C digest - After Hela digest Run 100 – 200 – 300 – 400 – 500 – 600

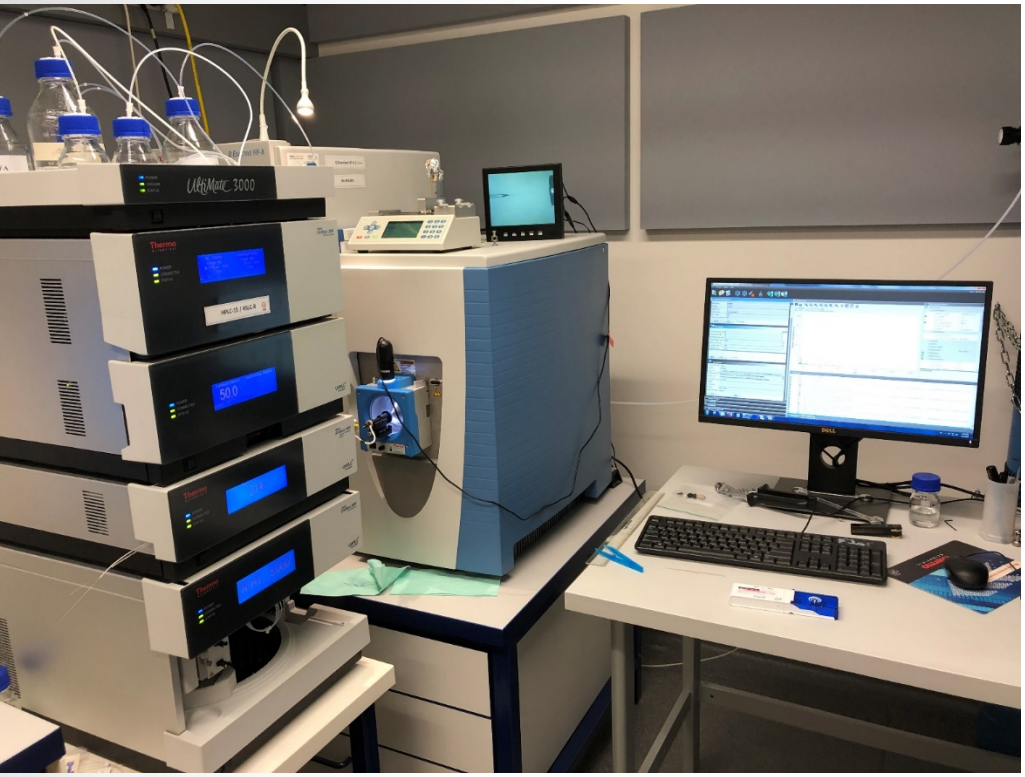


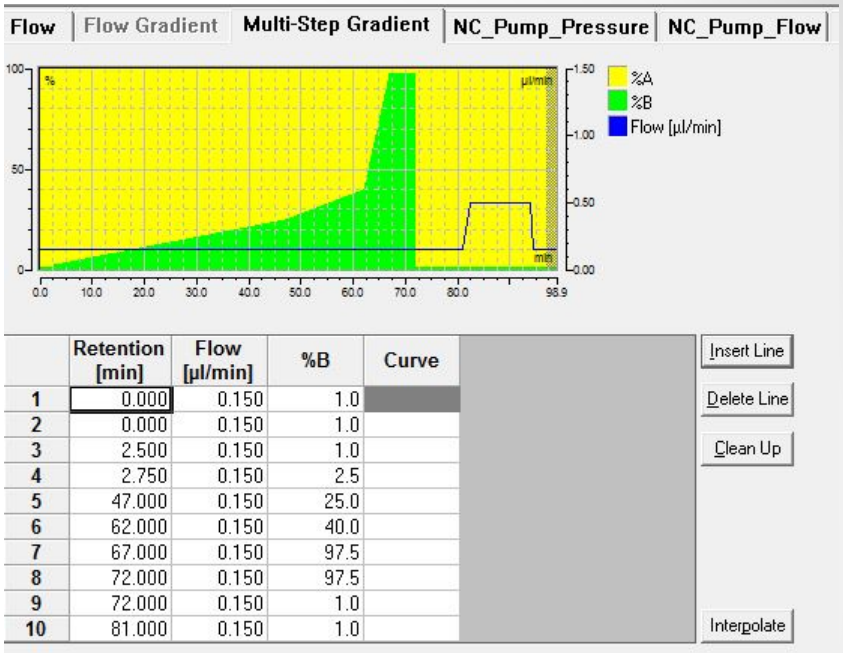


Experimental conditions

0.5 pmol Cyto C digest
1 µl injection
1 to 50% ACN / 0.1%TFA
60 min gradient
1 µl/min

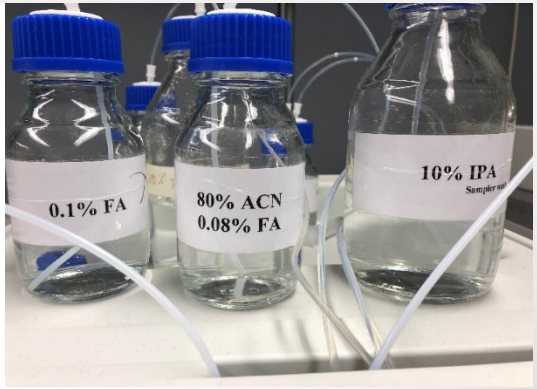
Comparison µPAC and PepMap*





	Retention [min]	Flow [µl/min]	%B	Curve
1	0.000	0.150	1.0	
2	0.000	0.150	1.0	
3	2.500	0.150	1.0	
4	2.750	0.150	2.5	
5	47.000	0.150	25.0	
6	62.000	0.150	40.0	
7	67.000	0.150	97.5	
8	72.000	0.150	97.5	
9	72.000	0.150	1.0	
10	81.000	0.150	1.0	

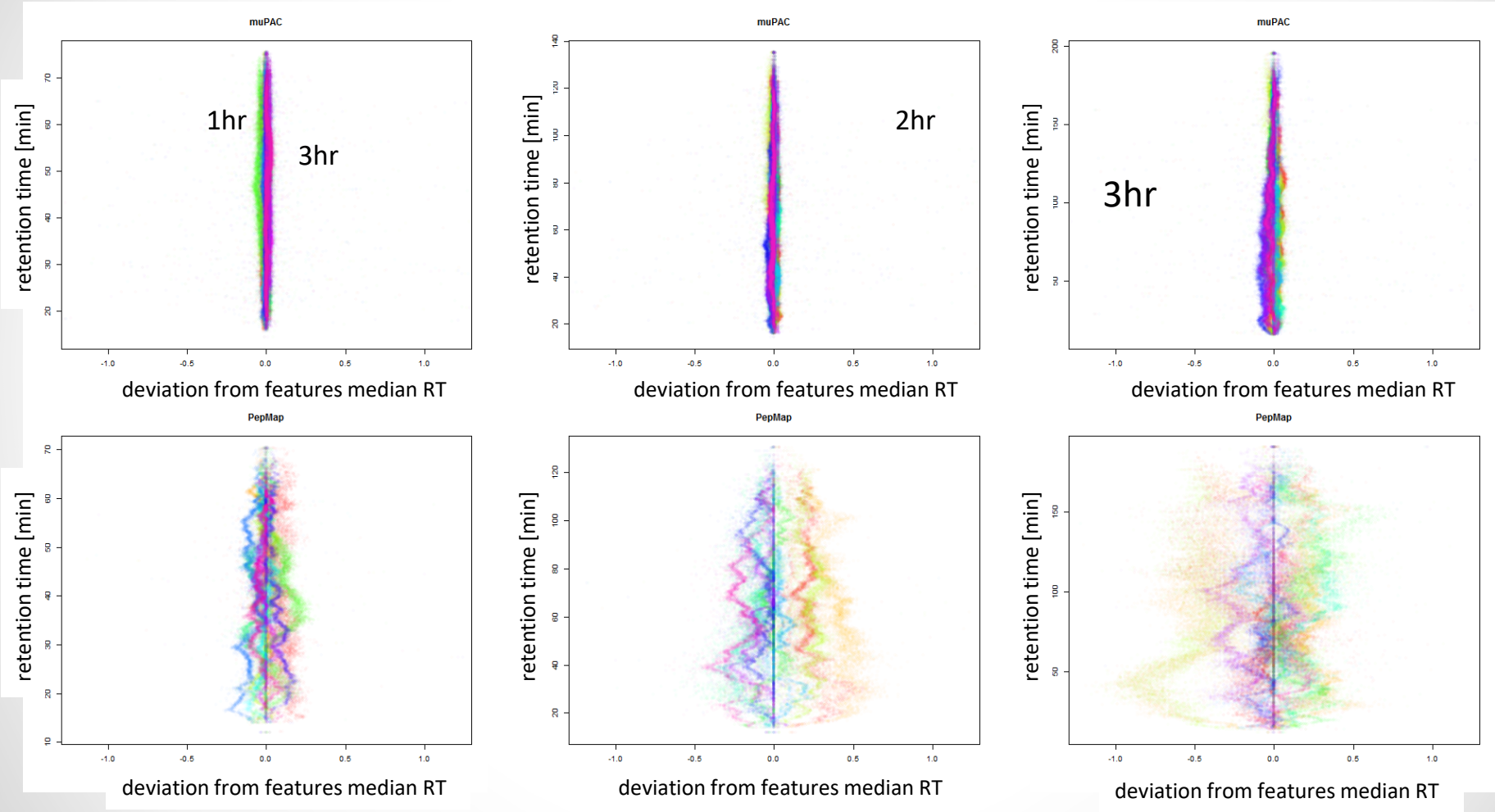
Insert Line
Delete Line
Clean Up
Interpolate



PepMap, 50 cm x 75 µm, 2 µm, C18
µPAC, 50 cm, C18
All conditions the same for both columns

***Courtesy of Dr. Karl Mechtler, Institute of Molecular Biotechnology, Vienna**

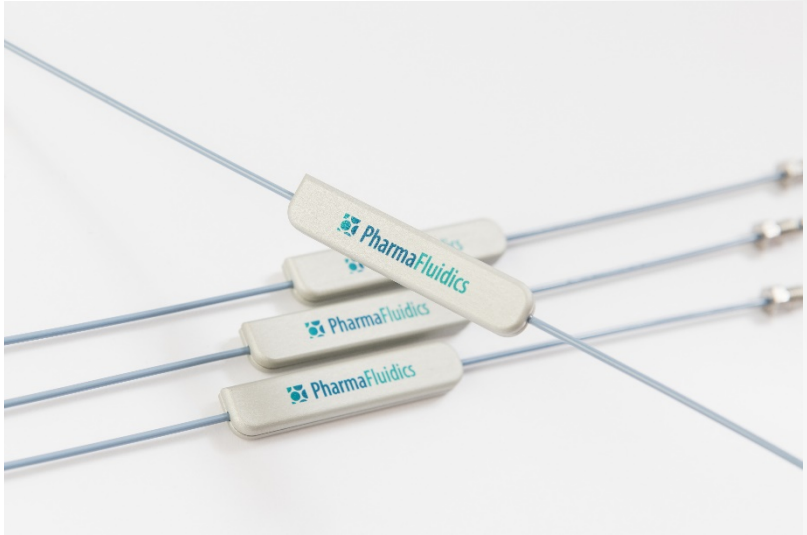
Retention Time Reproducibility



Every color represents a different run; from left to right, gradient time 1, 2, 3, hrs. Top µPAC, bottom PepMap column

New µPAC™ trapping column

- Instant pressurization due to separation bed of perfectly ordered, free-standing pillars
- Perfectly symmetrical and fritless column design, allowing bedirectional use
- Compatible with both switching valve (regular and backflush elution) or vented trapping configuration



µPAC™ Product Portfolio

	200 cm µPAC™ nano	50 cm µPAC™ nano	µPAC™ CapLC
Pillar shape	Cylindrical	Cylindrical	Cylindrical
Pillar diameter [µm]	5	5	5
Interpillar distance [µm]	2,5	2,5	2,5
Channel width [µm]	315	315	**1000**
Channel depth [µm]	18	18	**28**
Column length [cm]	200	50	**50**
Column volume [µl]	9	3	**10**
Surface morphology	Core shell	Core shell	Core shell
Porous layer thickness [µm]	300	300	300
Pore size range [A]	100 - 300	100 - 300	100 - 300
Surface functionalization	C18 + HMDS	C18 + HMDS	C18 + HMDS
Typical flow rate	0.15 – 1 µL/min	0.15 – 1 µL/min	??

Essential Advantages of µPAC Columns

- Ultimate separation performance
- High permeability allows long column length
- Best in class column to column reproducibility
- No frits to terminate particle bed
- Rigid pillars
- Allows bidirectional operation
- Superior longevity and robustness

Summary µPAC

- µPAC is a paradigm in liquid phase separation technology, approaching the ultimate performance of HPLC as predicted by the grounding fathers of HPLC, Knox, Guiochon, and Giddings
- Regard µPAC as Open Tubular Liquid Chromatography in practice
- The first generation µPAC (2.5 µm interpillar distance) has proven feasibility for the separation of a high number of solutes, in the micro- and nanoflow HPLC realm.
- Seamless coupling with all vendor MS systems is key for PharmaFluidics. Adapter kits available.
- Next generation µPACs with shorter interpillar distance will outperform conventional HPLC and eventually UHPLC columns and can be regarded “green” and “smart” separation technology

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