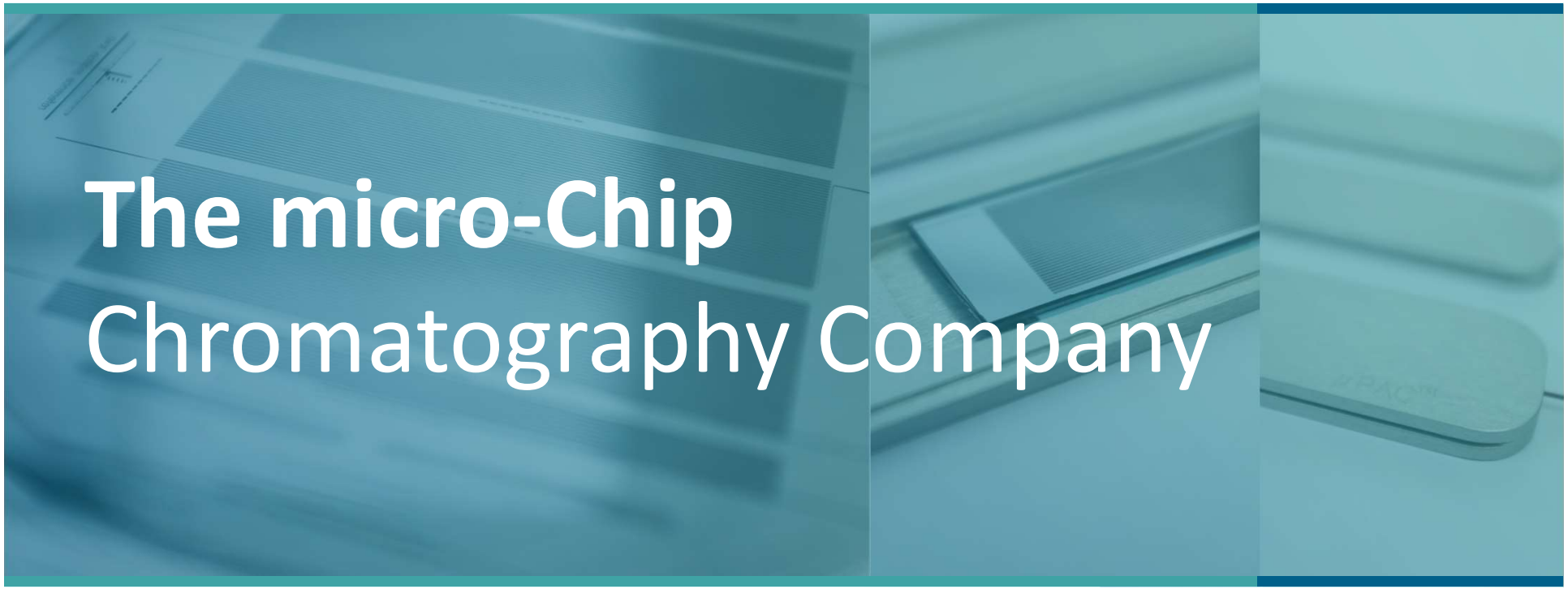
PharmaFluidics



The micro-Chip Chromatography Company

PharmaFluidics Team
Please visit www.pharmafluidics.com

MD Scientific

MD Scientific is an authorized distributor in Denmark
www.md-scientific.dk - +45 7027 8565

PharmaFluidics - Company Profile

Spin-off of Vrije Universiteit Brussel

Located near Ghent, Belgium in “Flanders’ Life Sciences valley”

Industry:

- ALDA : Global Analytical Lab, Life Sciences & Diagnostics Instrumentation Industry
- **HPLC/MS : High Performance Liquid Chromatography / Mass Spectroscopy Segment**

Key expertise

- Design, lithographic production, surface treatment of micro-Chip based HPLC columns

Product: µPAC™ - an new generation of HPLC columns

USP:

Detect more molecules, with higher sensitivity, in tiny, complex biological samples

Market:

Premium segment “ultra-high resolution” LC/MS market

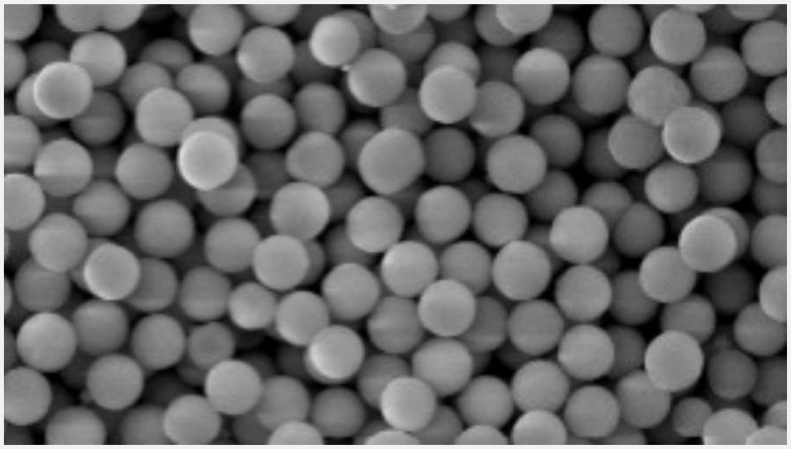
Sales:

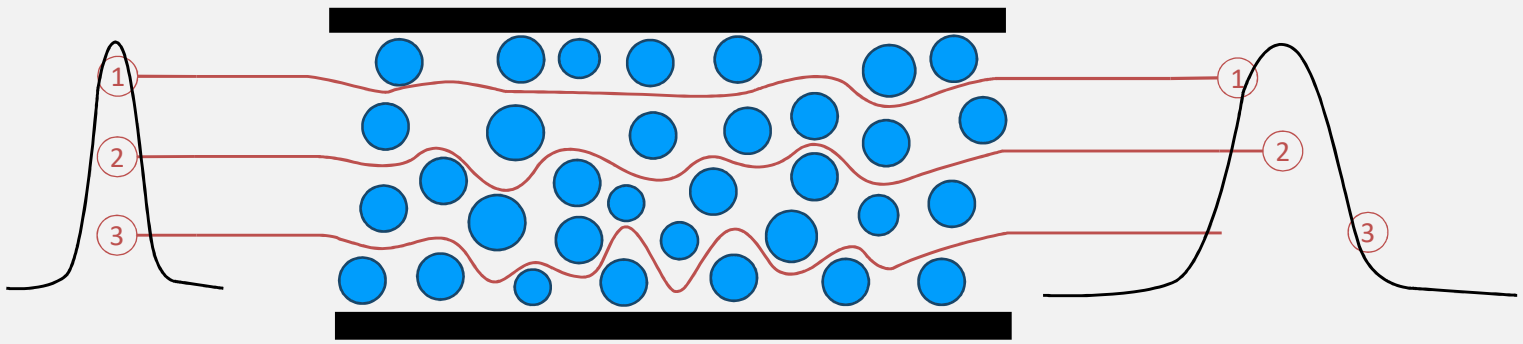
- Presented to market at specialist trade shows in the USA and Europe since spring 2017
- Priced in June 2017
- Sold since September 2017 for Proteomics, Metabolomics and Lipidomics profiling, to Bio-Pharma customers with bio-marker, diagnostics and drug research & development applications

The Micro Pillar Array Column (µPAC™)

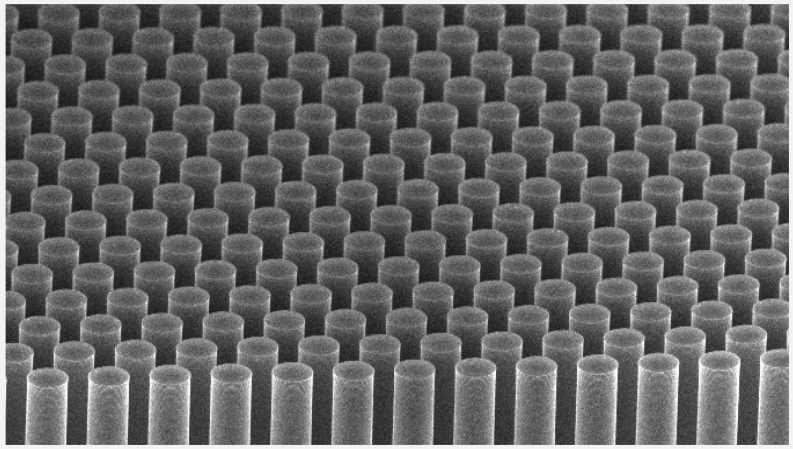
PharmaFluidics the micro-Chip Chromatography Company

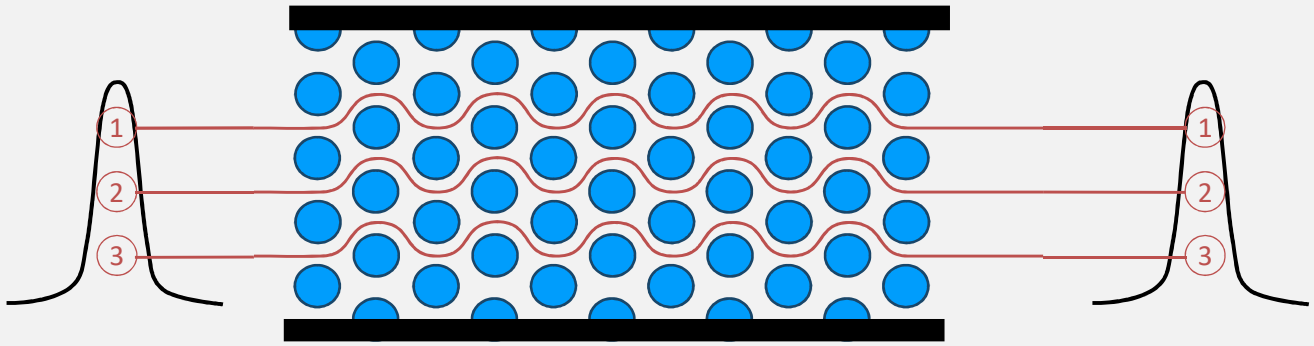
Traditional HPLC columns





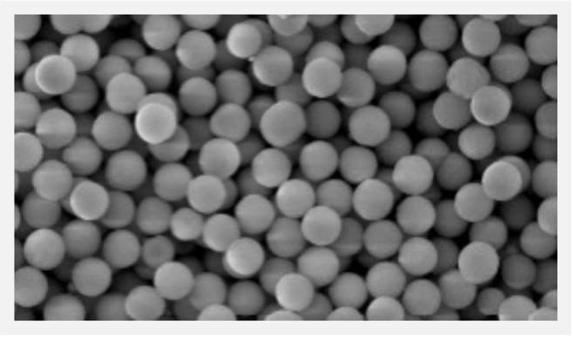
PharmaFluidics' game-changing technology



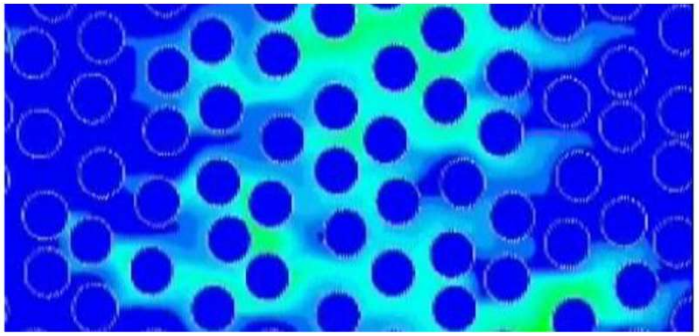


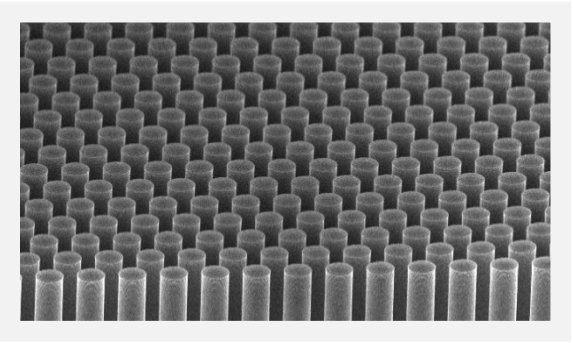
Unprecedented separation performance

The benefit of Order versus Disorder

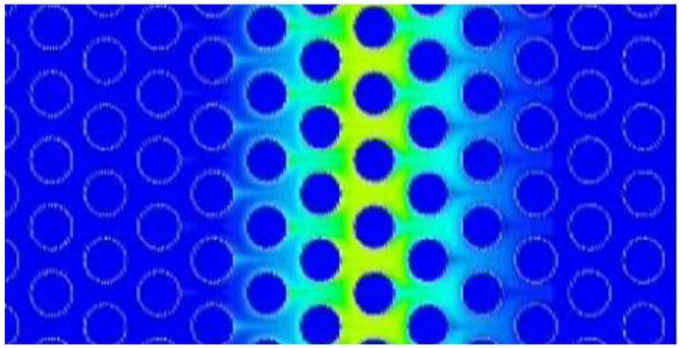


Disorder - Packed Bed



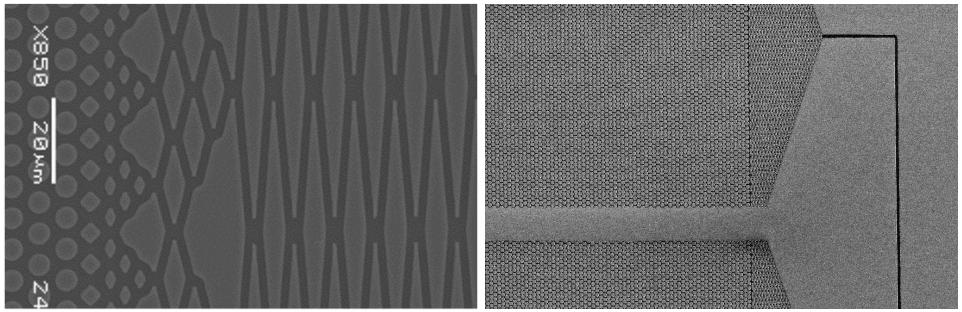


Order – Pillar Array

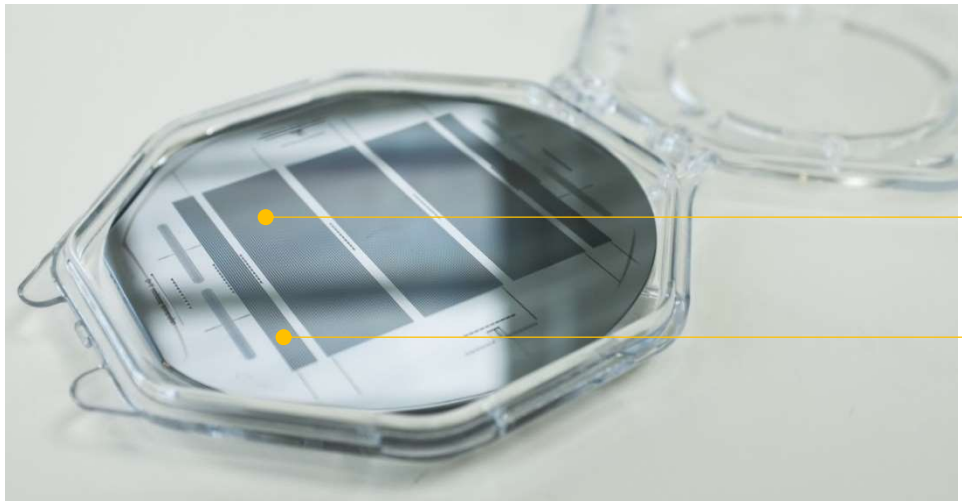


Unprecedented separation performance

µPAC™ - Pillar Array Columns

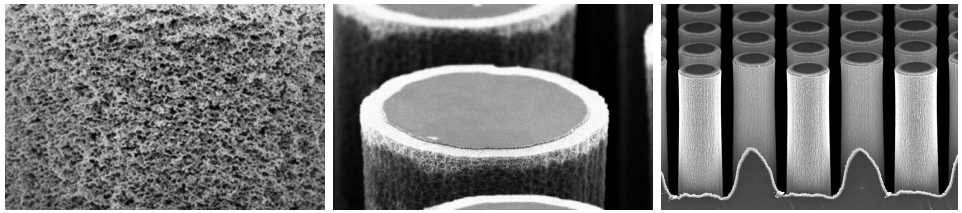


Flow distribution for perfect plug conservation in inlets and turns



200 cm column

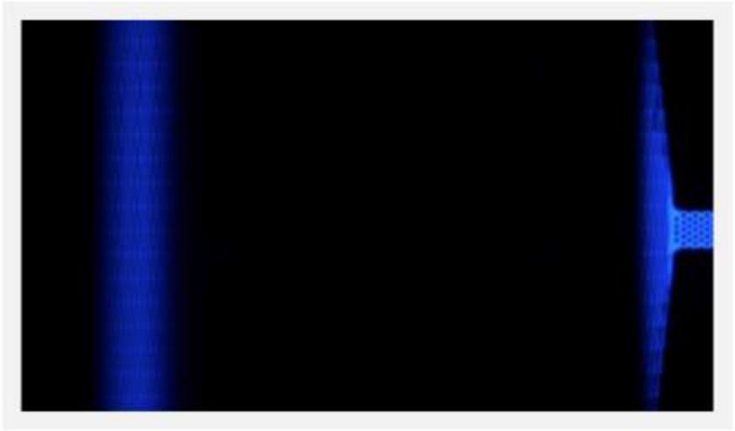
50 cm column



Stationary Phase controlled by design

Unprecedented separation performance

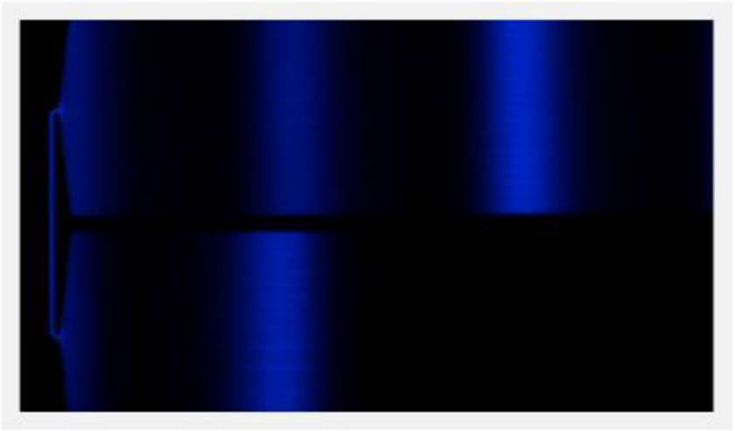
µPAC™ - Pillar Array Columns



From narrow in- and outlet to wider separation channels

Flow distribution

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



Connecting parallel Separation channels

µPAC™ - C18 – 200 cm/50 cm

micro-Chip
Liquid Chromatography Cartridges
for use with
Any 3rd party HPLC/MS equipment

Highly reliable and robust nano-LC solution with:

- The highest resolution
- The best data productivity
- State-of-the-art repeatability

Allowing unique modes of operation

- Pressure at optimal flow rate of 300 nl/min ~ 100 bar (200cm µPAC™) or ~ 40 bar (50cm µPAC™)
- Compliant up to 350 bar (5,000 psi) allowing a maximum flow rate of ~ 1 µl/min (200cm) or ~ 2.5 µl/min (50cm)

- ✓ Length of the column: 200cm
- ✓ Coating: C18-End capped
- ✓ Flow: max. 1µl/min (at 50°C)
- ✓ Max. pressure allowed: 320 bar/4641psi
  - ✓ At 300 nl/min (30°C): 90 – 120 bar/1305,34 – 1740,45psi
  - ✓ At 1µl/min (50°C): 80 – 320 bars/ 1160,30 – 4641,21psi

- ✓ Pillar distance: 2,5µm
- ✓ Pillar diameter: 5µm
- ✓ Pore size: 10 – 30nm
- ✓ Inlet union: 1/32” – 1/32”
- ✓ Outlet union: 1/32” – 360 OD

- ✓ Void volume: 9µl
- ✓ Outer dimensions:
  - L: 11cm/4.33inch
  - W: 3cm/1.18inch
  - H: 0,6cm/0,23inch
- ✓ Sample volume: >4nl

Unprecedented separation performance

See more from less sample

Performance	µPAC™ Chip Cartridge	State-of-the-art HPLC	Standard HPLC
Plate number	400.000	250.000/m	150.000/m
Peak capacity	Towards 1.200	700	300

Product USP

More PLATES

More PEAKS

More IDENTIFICATIONS

Nano SAMPLE VOLUME

CONVENIENCE in Use

Simplified WORKFLOW

Reproducible & Robust

Target markets and applications

Biomarker discovery

Launch segment: metabolomics / lipidomics

Challenges

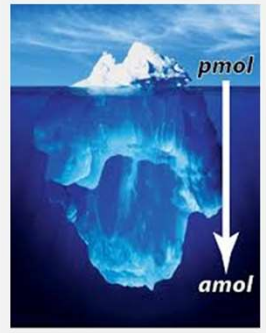
- Rare molecules in complex mixtures
- Tiny biopsies

Bio-pharmaceuticals & Bio-similars development

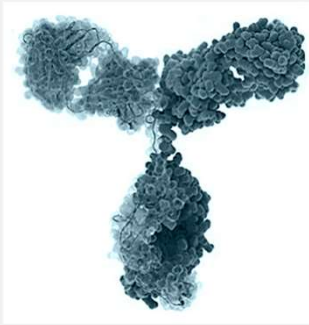
Challenges

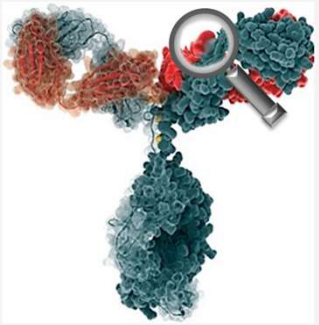
- Tiny differences in deamidation & glycosylation patterns of proteins or mono-clonal-antibodies
- Analytics supported FDA/EMA approvals

Trace amounts in complex samples



Subtle modifications





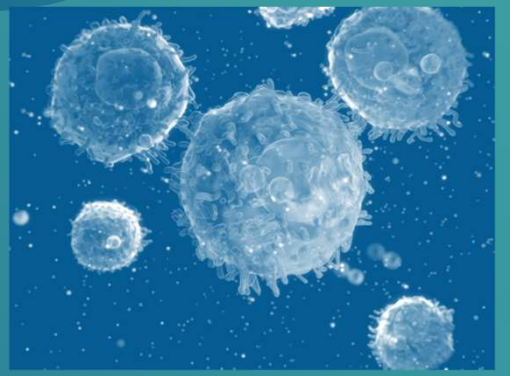
Data and results

- µPAC™-MS Proteomics Platform
- µPAC™-MS Lipidomics Platform
- µPAC™-MS Metabolomics Platform
- µPAC™-MS Biopharmaceuticals

PharmaFluidics the micro-Chip Chromatography Company

Data and results

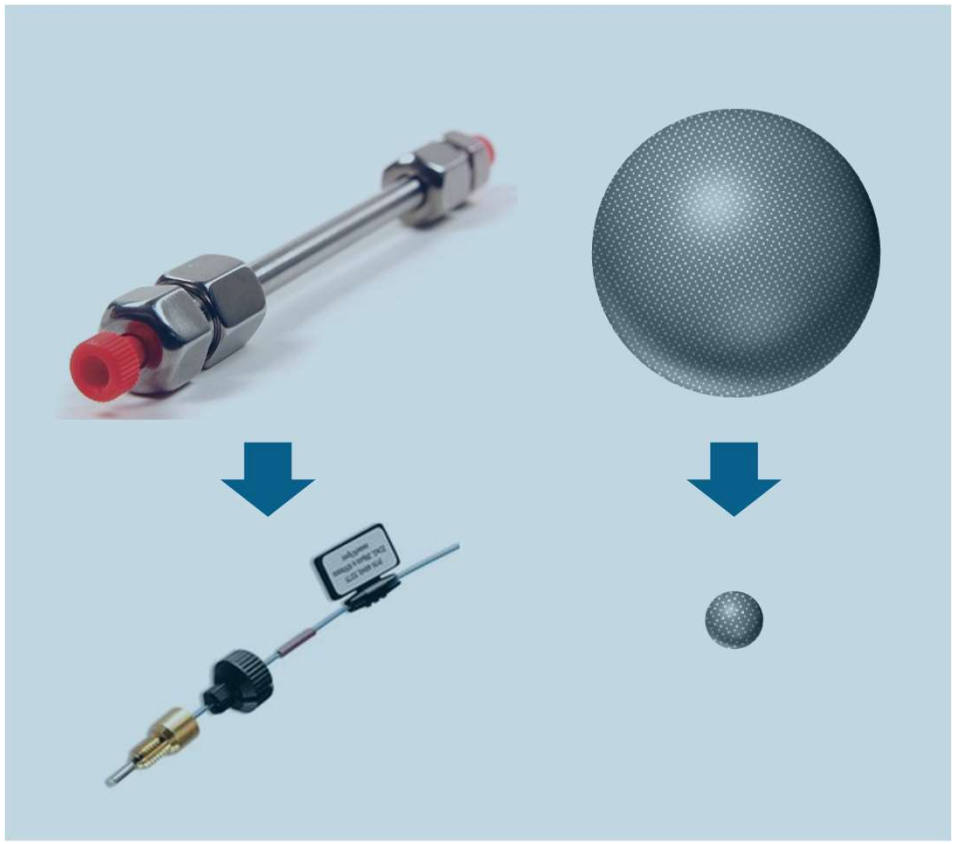
- **µPAC™-MS Proteomics Platform**
- µPAC™-MS Lipidomics Platform
- µPAC™-MS Metabolomics Platform
- µPAC™-MS Biopharmaceuticals



PharmaFluidics the micro-Chip Chromatography Company

State of the art in bottom-up proteomics

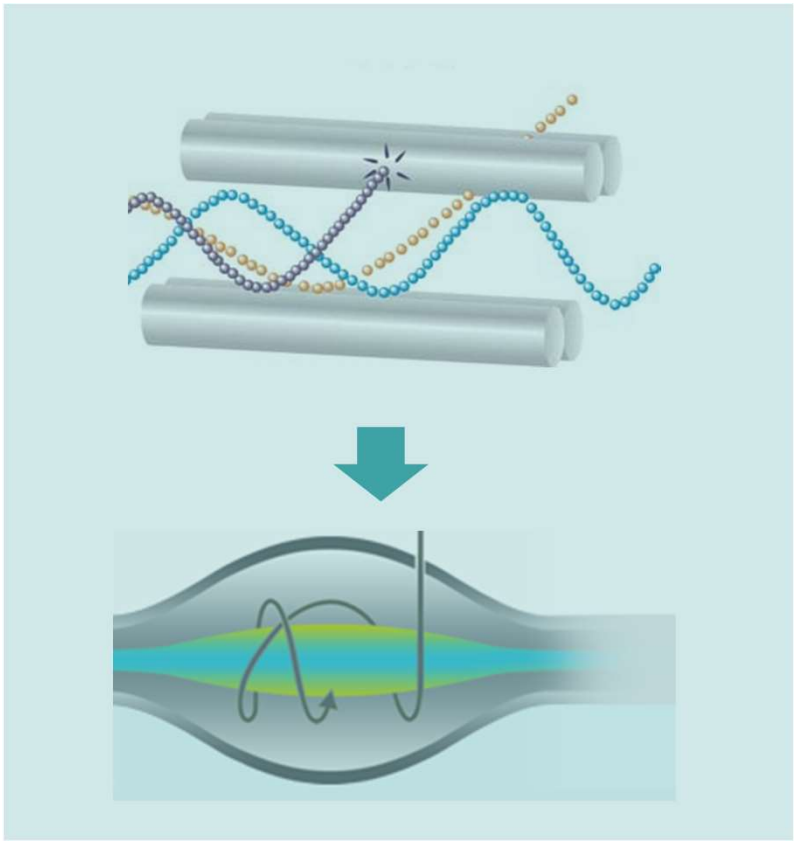
LC



Sensitivity ↑

Efficiency ↑

MS



Resolution & Sensitivity ↑

State of the art in bottom-up proteomics

LC systems

Flow rate: 50-1,500 nl/min
Pressure: ≤ 1,200 bar
(17,000 psi)

LC columns

ID: 75 µm
Packing: ≤ 2 µm C18 silica
Length: ≥ 50 cm

Mass analyzers

Orbitrap-quadrupole hybrid
…

Identification of over 4,000 proteins in a single 90-140 min gradient run

Traditional HPLC column limitations

- High backpressure observed for long (≥ 50 cm) LC columns
  → loss of data, upper pressure limit exceeded
  → wear & tear of LC pump system and fluidics

- Column packing is a delicate process
  → poor column-to-column reproducibility

How to increase column performance even further?

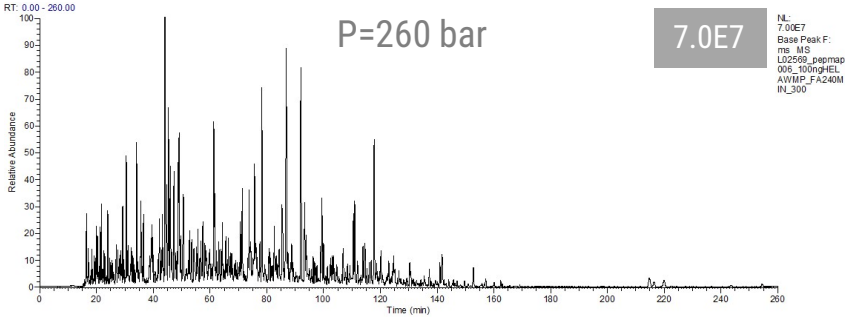
- Reduction of particle diameter → Increase in backpressure

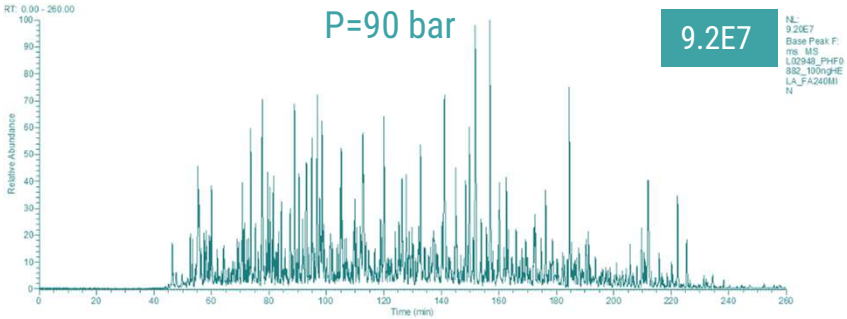
- Increase in column length → Increase in backpressure

µPAC™-MS Proteomics Platform

0.75 mm x 15 cm packed bed C18 (2 µm)	
# unique PEPTIDE identifications	# PROTEIN identifications
6196	**1219**

200 cm µPAC™ C18	
# unique PEPTIDE identifications	# PROTEIN identifications
10560 (GROUND)	**1736** (GROUND)





Conclusion

µPAC™ vs packed bed = **70%** increase in peptide ID's, **42%** increase in protein ID's

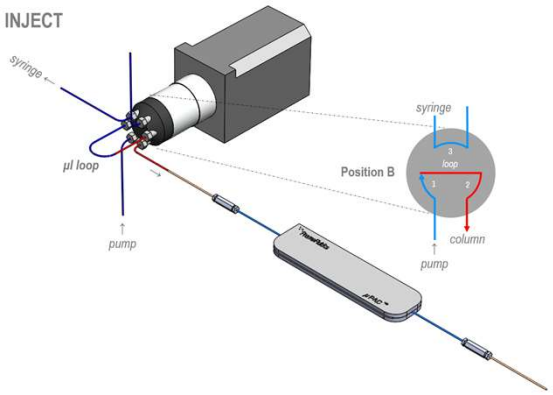
Obtained with LTQ Orbitrap

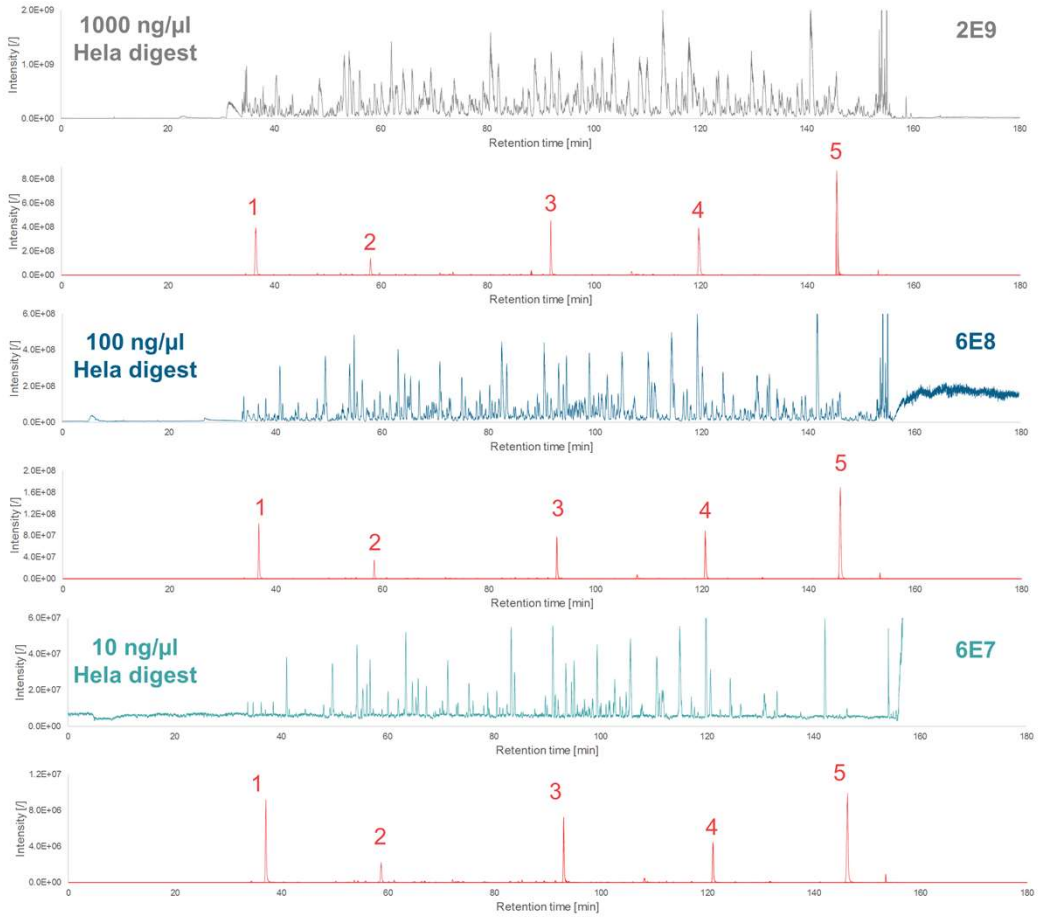
Experimental conditions

Column(s):	200 cm µPAC™ C18 15 cm Pepmap C18
Flow:	0.3 µL/min
Mobile Phase A:	0.1% FA
Mobile Phase B:	0.1% FA in 80% ACN
Gradient:	1-50% B in 240 min
Temp:	30°C
Inj vol:	1 µL
Sample:	500 ng/µl HeLa cell digest
Detection:	Thermo LTQ Orbitrap XL MS

µPAC™-MS Proteomics Platform

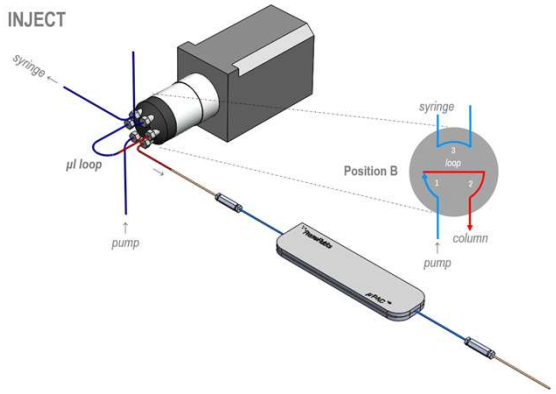
Hela cell digest – 200 cm µPAC™ connected to Orbitrap™ Fusion™ Lumos™ Tribrid™

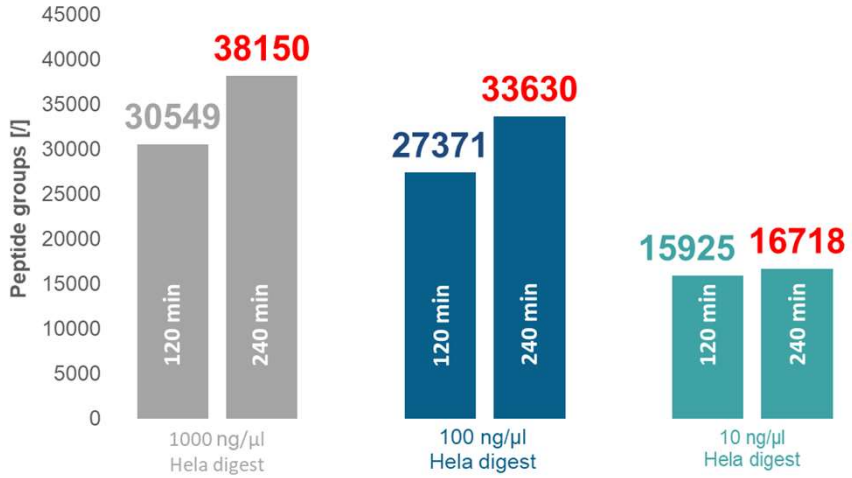


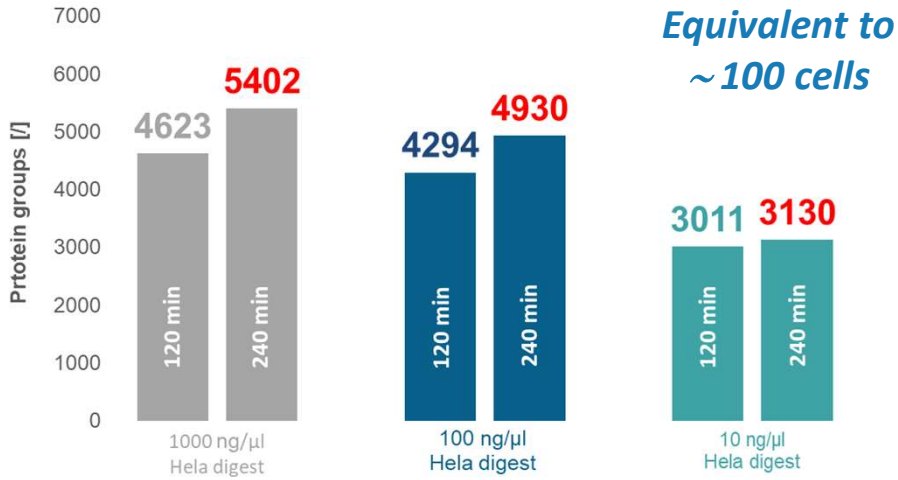


µPAC™-MS Proteomics Platform

Hela cell digest – 200 cm µPAC™ connected to Orbitrap™ Fusion™ Lumos™ Tribrid™





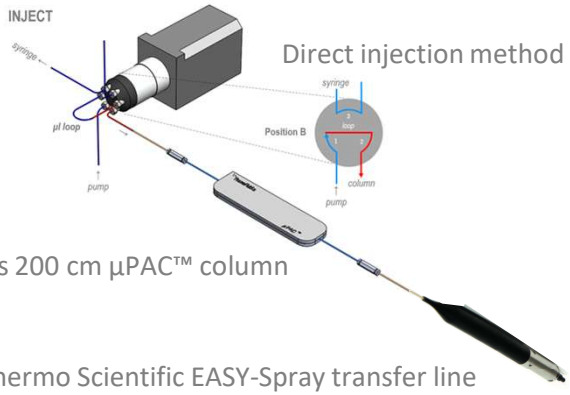


Equivalent to ~100 cells

µPAC™ Flexibility

Experimental conditions

Column:	200 cm µPAC™ C18
Flow:	**1 µL/min**
A:	0.1% FA
B:	0.1% FA in 80% ACN
Gradient:	1-50% B in 30-60-90 min
Temp:	50°C
Inj vol:	2-0.1 µL
Sample:	1000-500-250-100-50-10-2 ng/µl HeLa cell digest
Detection:	Thermo Orbitrap Q Exactive HFx



Direct injection method

PharmaFluidics 200 cm µPAC™ column

Thermo Scientific EASY-Spray transfer line

- **EASY-spray transfer line** as **ESI emitter**
- A dilution series of **HeLa cell tryptic digest** (1000 - 2 ng) separated using **30, 60 and 90 min** solvent gradient times.
- All samples spiked with an **internal peptide standard** (100 fmol/µl Pierce™ retention time calibration mixture) to evaluate the **chromatographic metrics** throughout the entire experiment.

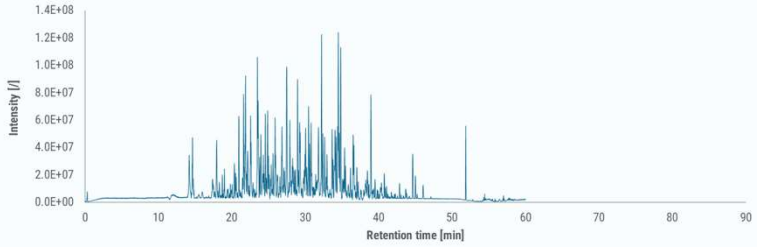
µPAC™ Flexibility

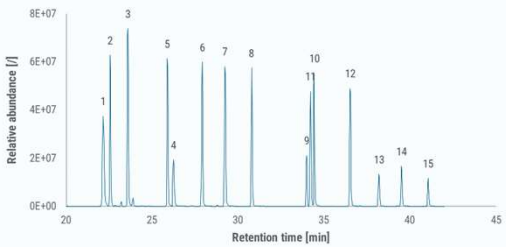
BPC 50 ng HeLa digest

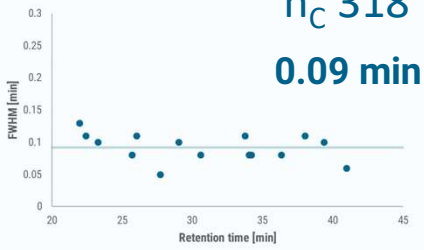
EIC 50 fmol PRTC

FWHM 50 fmol PRTC

30 min



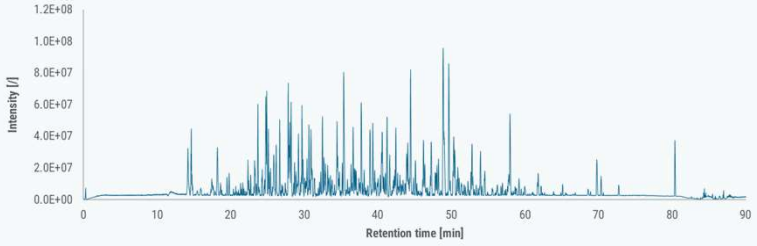


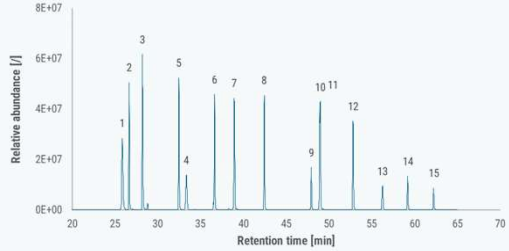


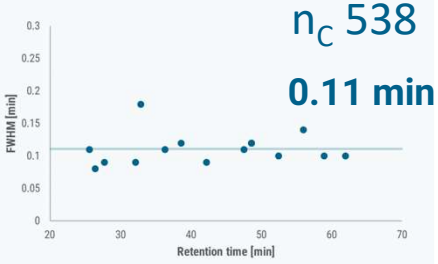
n_C 318

0.09 min

60 min



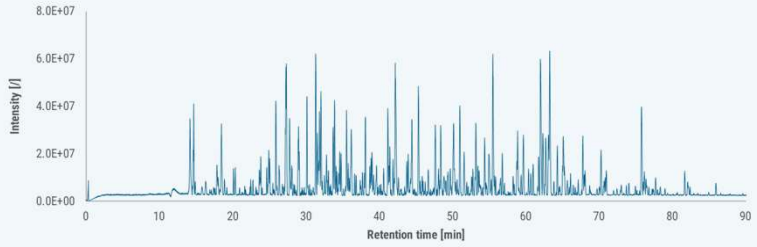


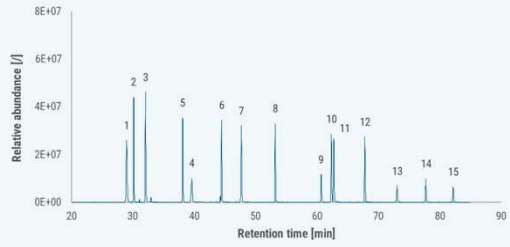


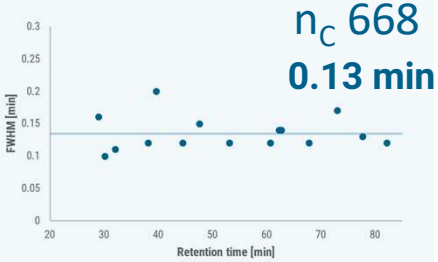
n_C 538

0.11 min

90 min







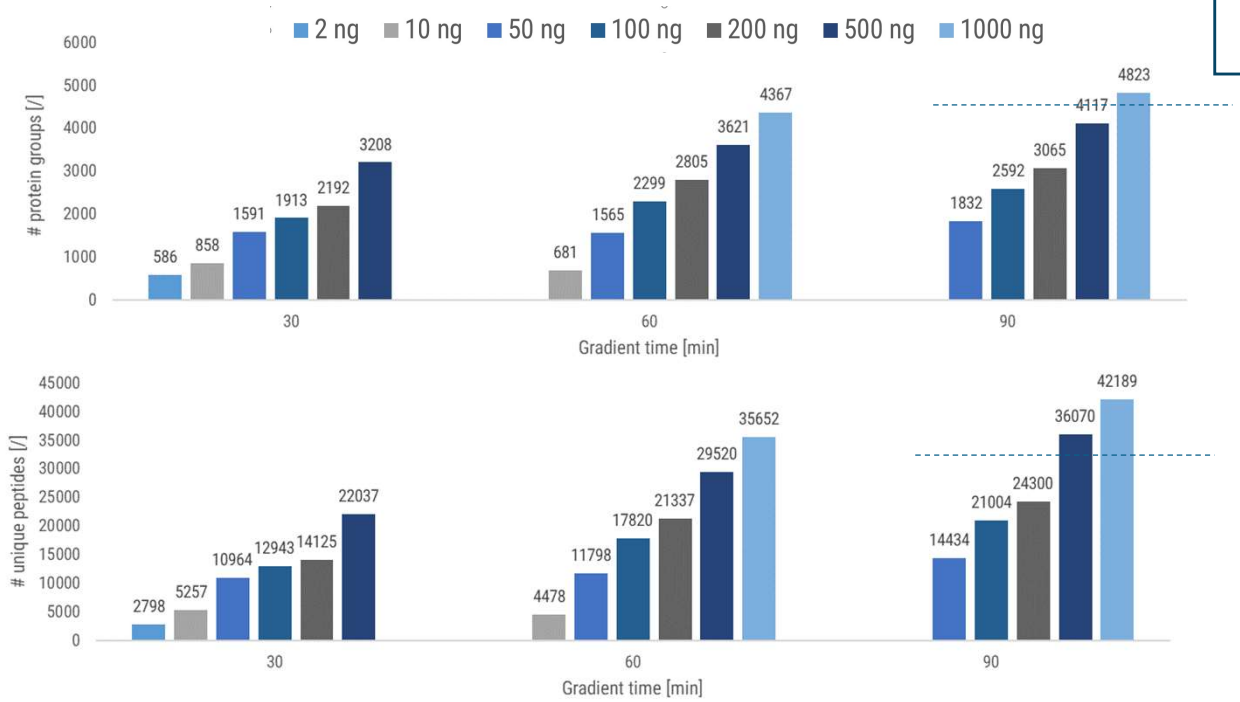
n_C 668

0.13 min

µPAC™ Flexibility

Identifications HeLa cell digest separation

200 cm µPAC™ column



Conclusions

- **Retention time variation of 0.5% CV observed** for 15 QC peptides (50-200 fmol/µl)
- **Number of identifications** with **a 90 min gradient 'capillary' flow** similar to a **120 min 'nano' flow** run for routine sample amounts (1 µg)

Performance test 50cm µPAC

PharmaFluidics the micro-Chip Chromatography Company

µPAC™ 50cm - Benchmarking

Experimental conditions

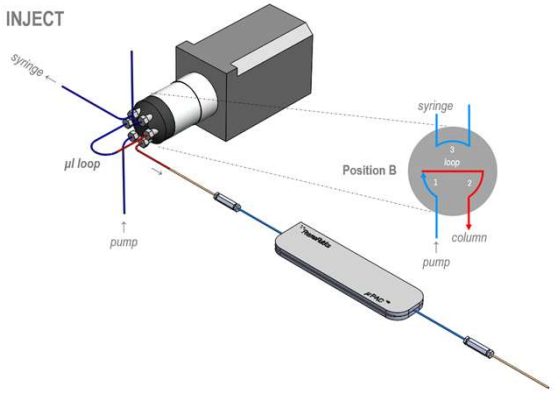
Chromatography

Instrument:	Thermo Scientific™ Ultimate 3000 RSLC nano
Injection:	1µL, user defined injection
Column temp:	50°C
Solvent A:	0.1% FA
Solvent B:	0.1% FA in 80% ACN
Gradient:	1-45% B in 30-60-90 min

Mass Spectrometry

Instrument:	Thermo Scientific™ LTQ Orbitrap Elite (DDA)
Resolution:	60,000 (MS1); 15,000 (MS2)
Mass range:	300-2000 m/z
Max IT:	80 ms

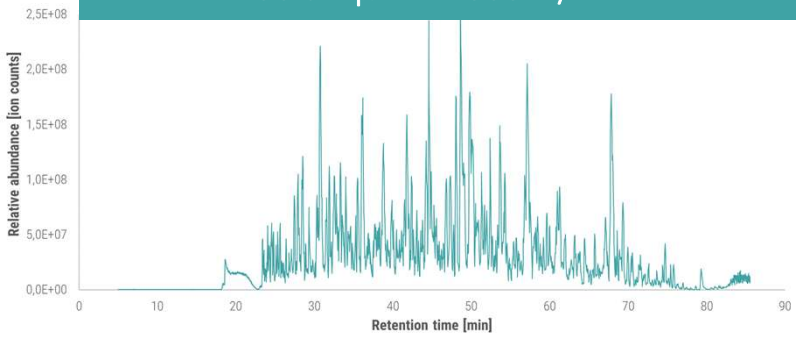
Direct injection method



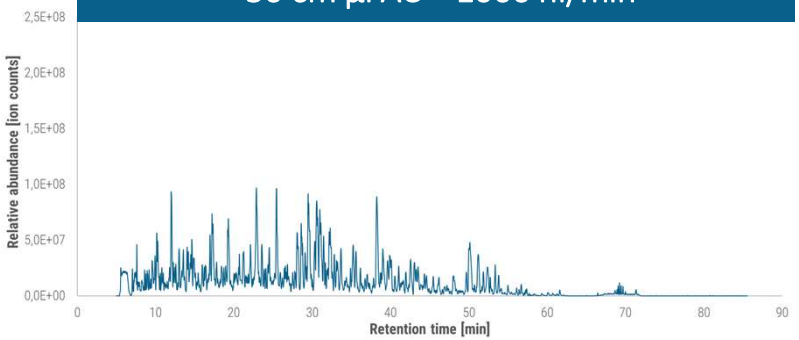
- Benchmarking µPAC™ 50cm column against conventional packed bed column (2µm particles; 15cm x 75µm)
- 500 ng **HeLa cell tryptic digest** was separated using **30, 60 and 90 min** solvent gradient times.
- All measurements were performed in triplicate

50 cm µPAC™ - Benchmarking in Bottom-up Proteomics

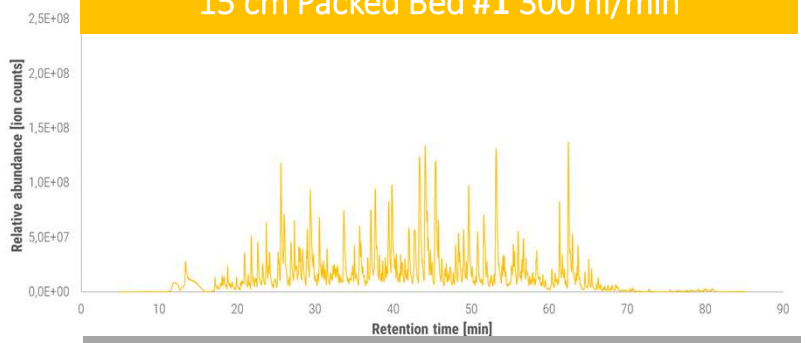
50 cm µPAC™ 300 nl/min



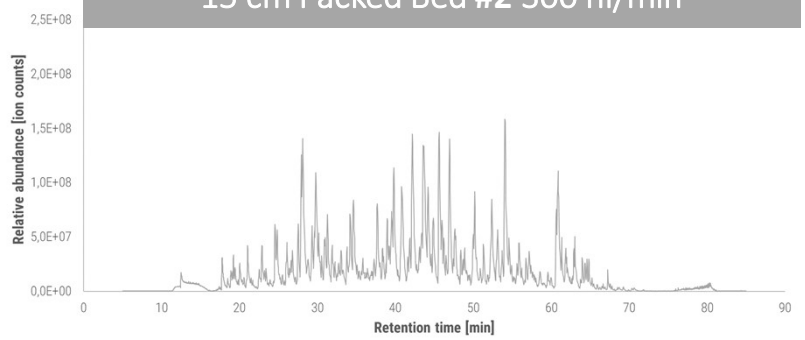
50 cm µPAC™ 1000 nl/min



15 cm Packed Bed **#1** 300 nl/min



15 cm Packed Bed **#2** 300 nl/min



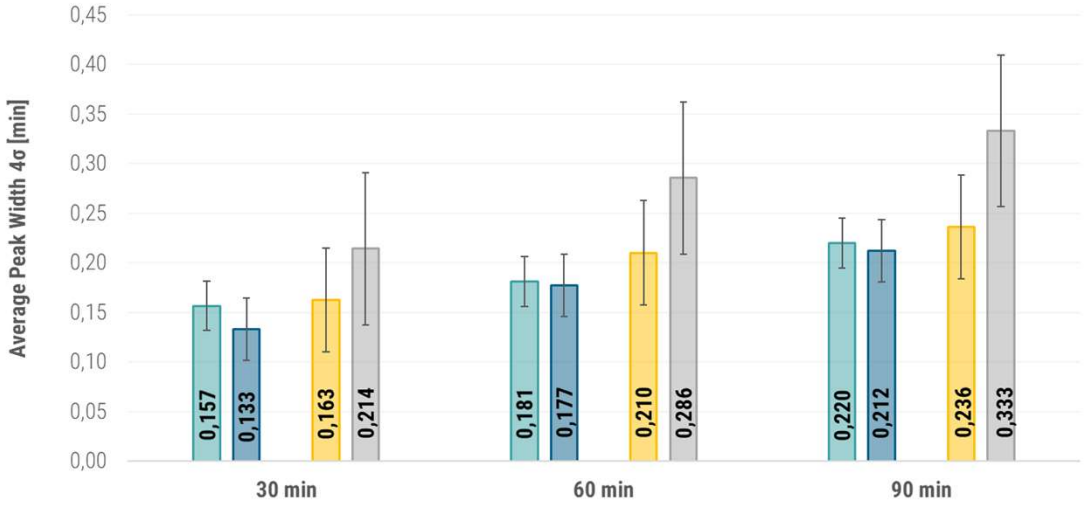
Instrumental Set-up

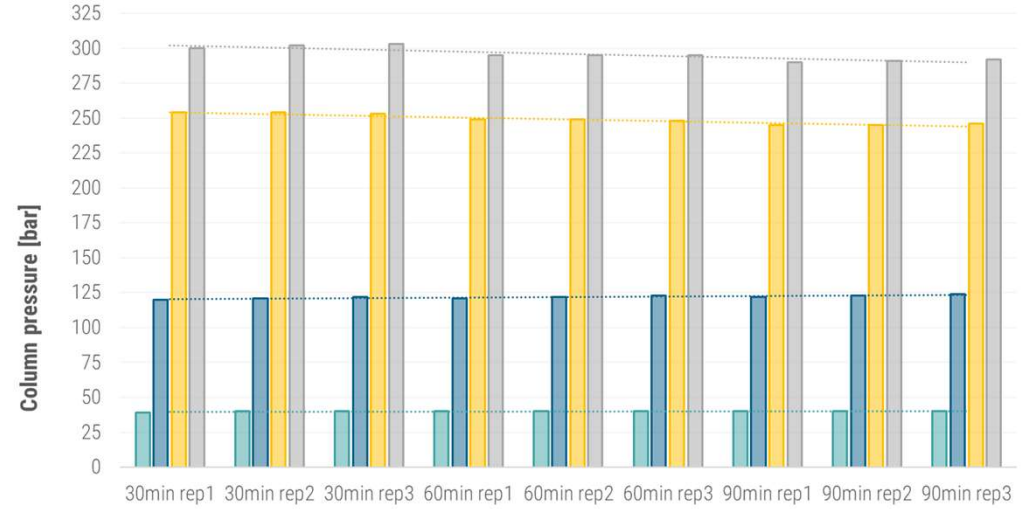
Thermo Scientific™ Ultimate 3000 RSLC nano
Thermo Scientific™ LTQ Orbitrap Elite™ (DDA)

1 µL direct injection
500 ng commercial HeLa digest + 50 fmol PRTC mix
Gradient: 1-45% B
A: H2O + 0.1% FA;
B: 80% ACN + 0.1% FA

50cm µPAC™ column
15 cm Packed Bed #1: (150 x 0.075 mm, 2.0 µm particles)
15 cm Packed Bed #2: (150 x 0.075 mm, 1.7 µm particles)

50 cm µPAC™ - Benchmarking in Bottom-up Proteomics





Peptide Peak Width

30 min gradient: 0.13 - 0.16 min
60 min gradient: 0.17 - 0.18 min
90 min gradient: 0.21 - 0.22 min

→ 4 - 60% reduction

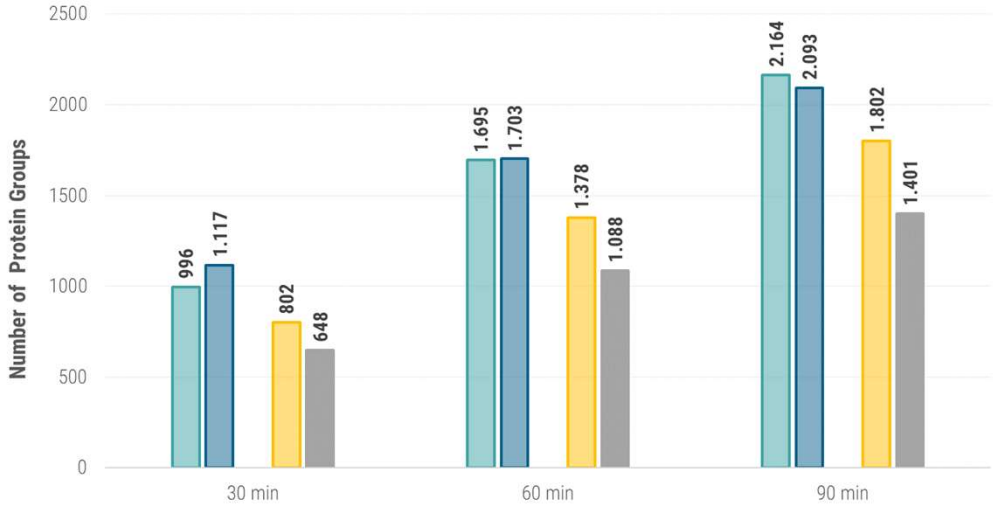
50 cm µPAC™	300 nl/min
50 cm µPAC™	1000 nl/min
15 cm Packed Bed **#1**	300 nl/min
15 cm Packed Bed **#2**	300 nl/min

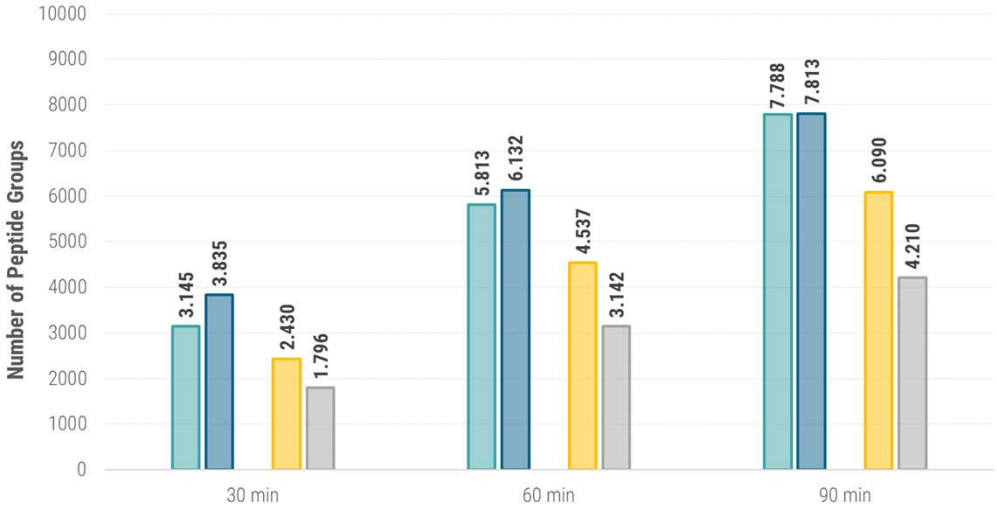
Column Backpressure

300 nl/min: 40 bar
1000 nl/min: 120 bar

→ 6-8 times lower

50 cm µPAC™ - Benchmarking in Bottom-up Proteomics

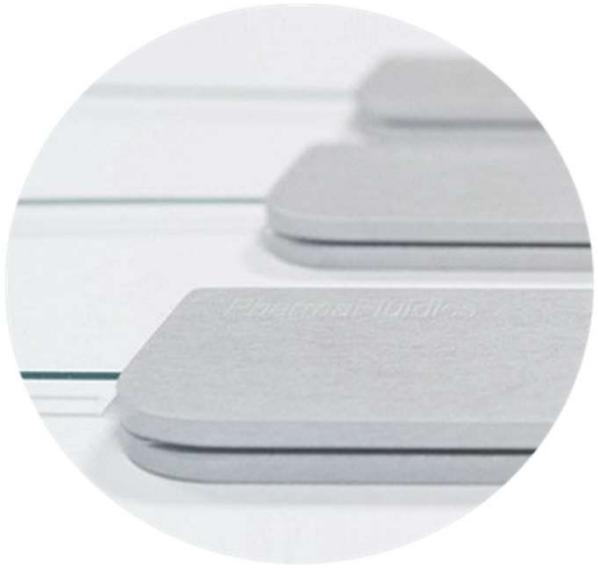


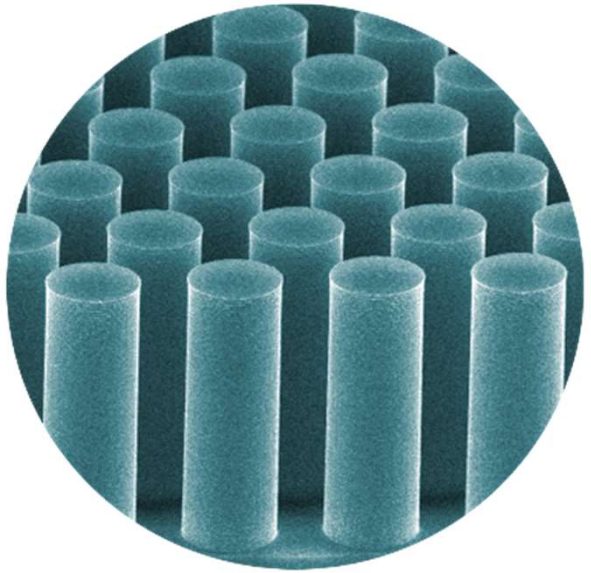


Peptides
300 nl/min → 28 - 75% increase
1000 nl/ min → 28 - 114% increase

50 cm µPAC™	300 nl/min
50 cm µPAC™	1000 nl/min
15 cm Packed Bed **#1**	300 nl/min
15 cm Packed Bed **#2**	300 nl/min

Potein Groups
300 nl/min → 20 - 56% increase
1000 nl/ min → 16 - 72% increase

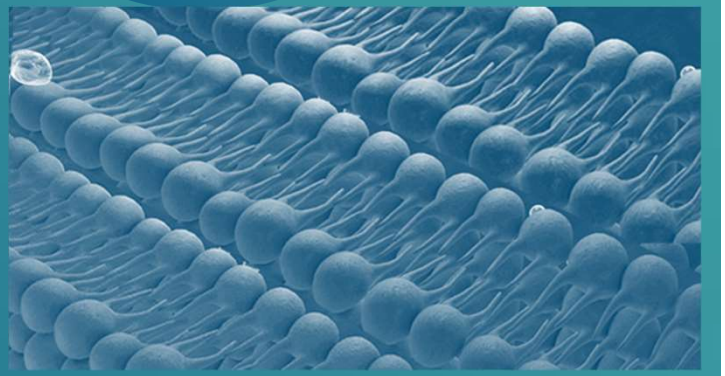




50 cm µPAC™	200 cm µPAC™
➢ 3 µl column volume ➢ 0.1 – 2 µl/min ➢ 30 min – 2h analysis time	➢ 9 µl column volume ➢ 0.1 – 1 µl/min ➢ 2h – 8h analysis time
Increased Throughput	Unique Proteome Coverage

Data and results

- µPAC™-MS Proteomics Platform
- **µPAC™-MS Lipidomics Platform**
- µPAC™-MS Metabolomics Platform
- µPAC™-MS Biopharmaceuticals



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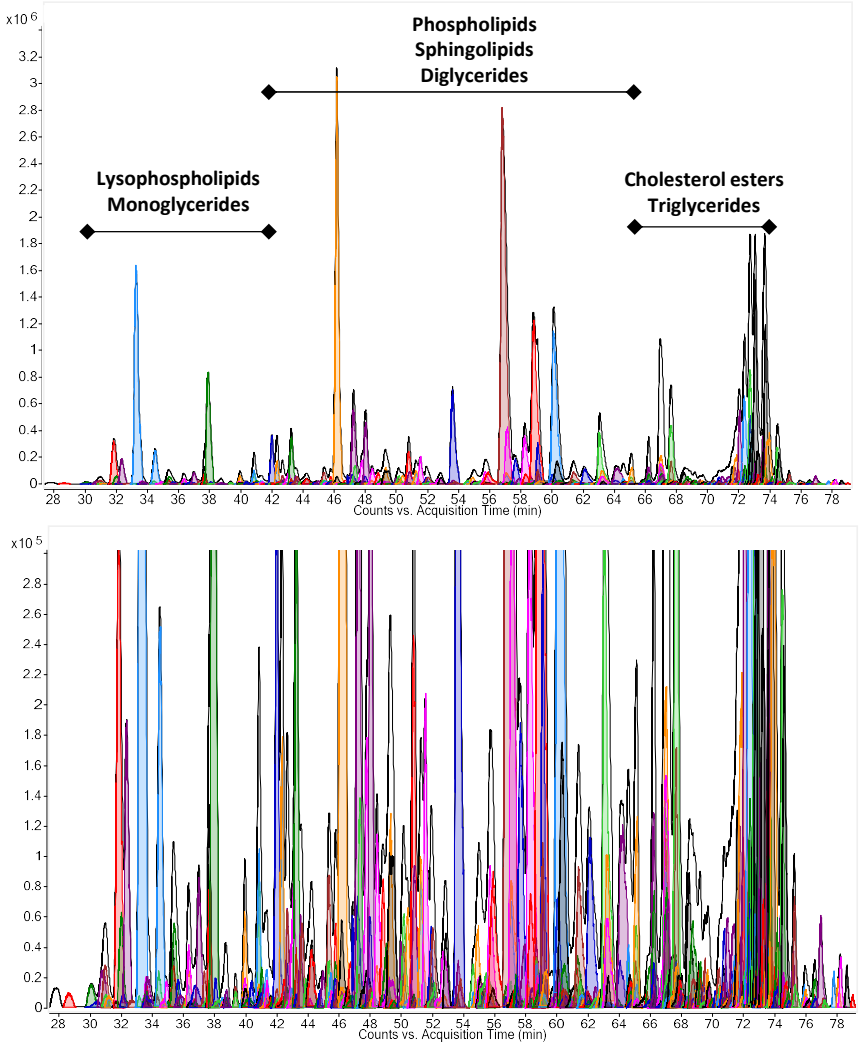
µPAC™-MS Lipidomics Platform

Separation of human blood plasma lipid extract



Enormous sample complexity is revealed, illustrative for µPAC-MS resolving power!

INTER-class lipid separation

- All major lipid classes are detected
- Location indicated on the chromatogram

INTRA-class lipid separation

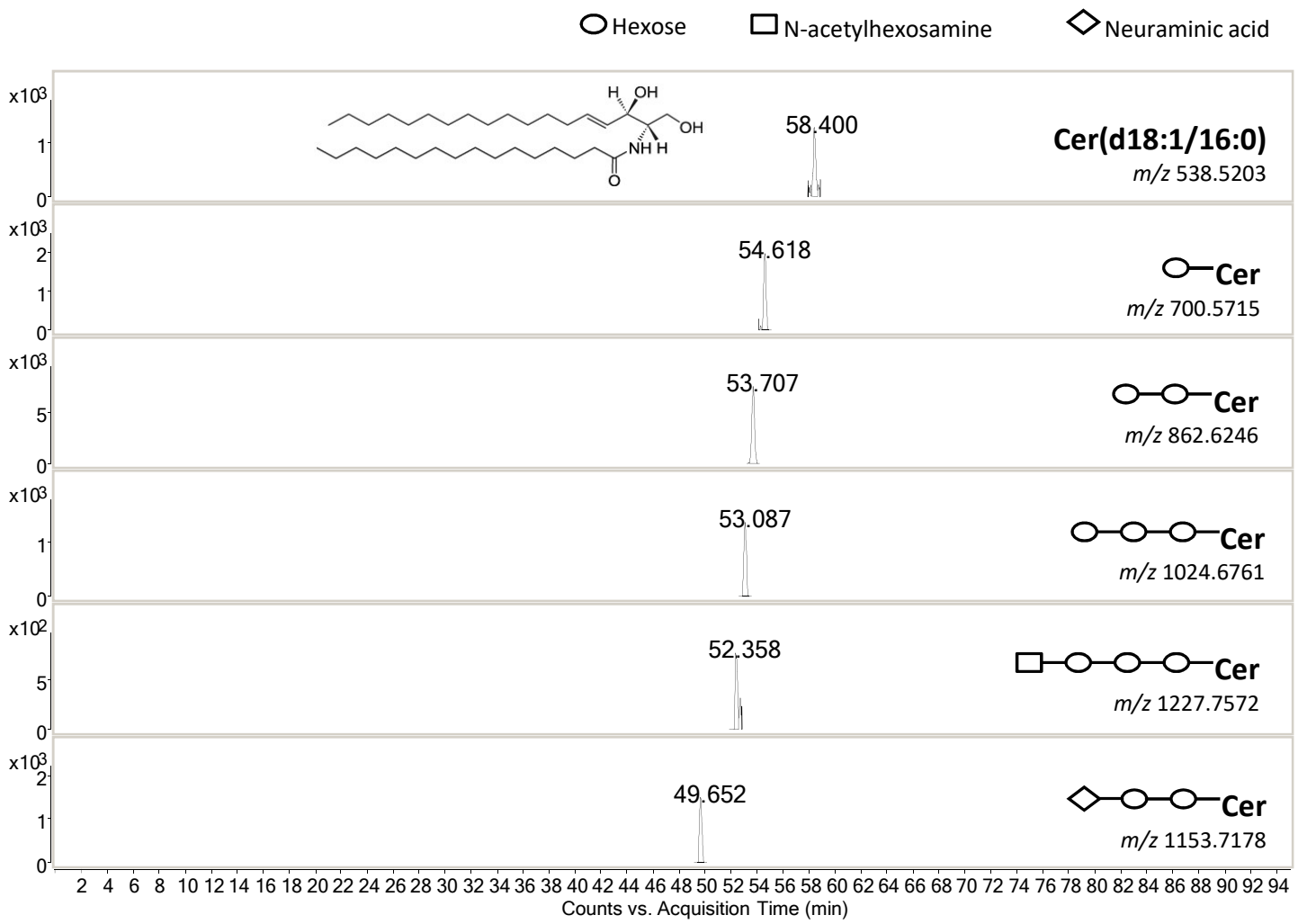
- Number of carbons
- Degree of saturation in fatty acid side chains
- Fatty acid side chain position & composition

Experimental conditions

Lipid extract from 100 µl EDTA blood plasma – 50 nl injection
30 to 98% B in 60 min – 750 nl/min
A: 20mM NH4HCO2 (PH 5)
B: C3H7OH/CH3OH (90/10) (v/v)

µPAC™-MS Lipidomics Platform

Inter-class separation of ceramide glycosphingolipids



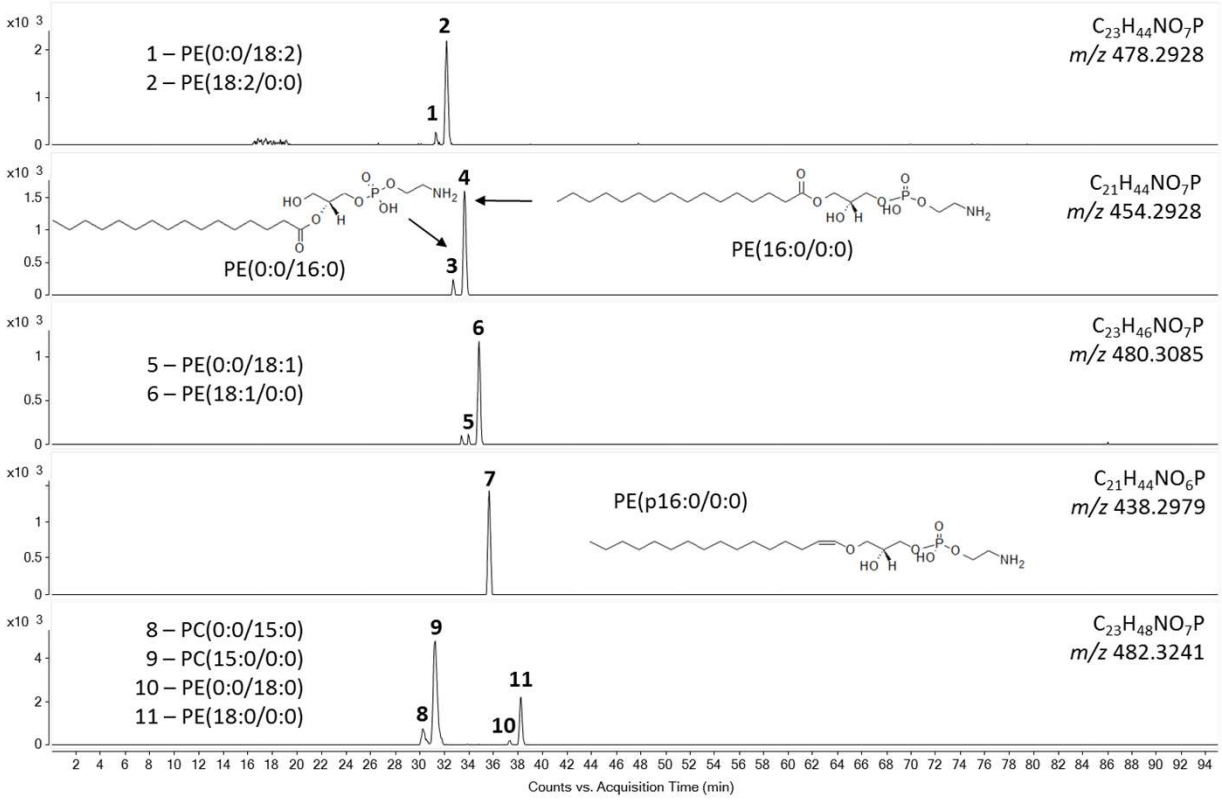
Separation by:

→ Glycosylation pattern

- Polarity increases with number of sugar chains
- More polar molecules elute earlier in RPLC

µPAC™-MS Lipidomics Platform

Intra-class separation of Lysophosphatidylethanolamines



Separation by:

→ Number of carbon atoms

- Lyso-PE (16:0) → **peaks 3 & 4**
- Lyso-PE (18:0) → **peaks 10 & 11**

→ Degree of saturation

- Lyso-PE (18:2) (2 C=C bonds) → **peaks 1 & 2**
- Lyso-PE (18:1) (1 C=C bond) → **peaks 5 & 6**
- Lyso-PE (18:0) (no C=C bonds)→ **peaks 10 & 11**

→ Fatty acid chain position

- Lyso-PE (0:0/16:0) (sn-2 position) → **peak 3**
- Lyso-PE (16:0/0:0) (sn-1 position) → **peak 4**

→ Fatty acid composition

- Lyso-PC (15:0) → **peaks 8 & 9**
- Lyso-PE (18:0) → **peaks 10 & 11**

µPAC™-MS Lipidomics Platform

Conclusions

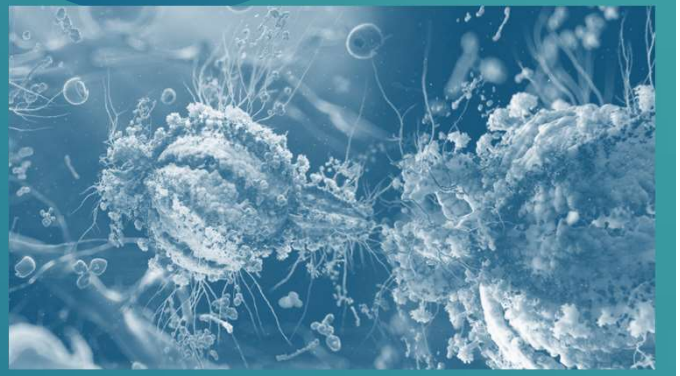
- The use of the µPAC™ column in lipidomics has been illustrated

- In combination with high resolution mass spectrometry:
  - high lipidome coverage can be obtained
  - inter- and intra-class separation can readily be achieved
  - isomeric lipids can be resolved

- The µPAC™ technology holds great potential in lipidomics studies.

Data and results

- µPAC™-MS Proteomics Platform
- µPAC™-MS Lipidomics Platform
- **µPAC™-MS Metabolomics Platform**
- µPAC™-MS Biopharmaceuticals



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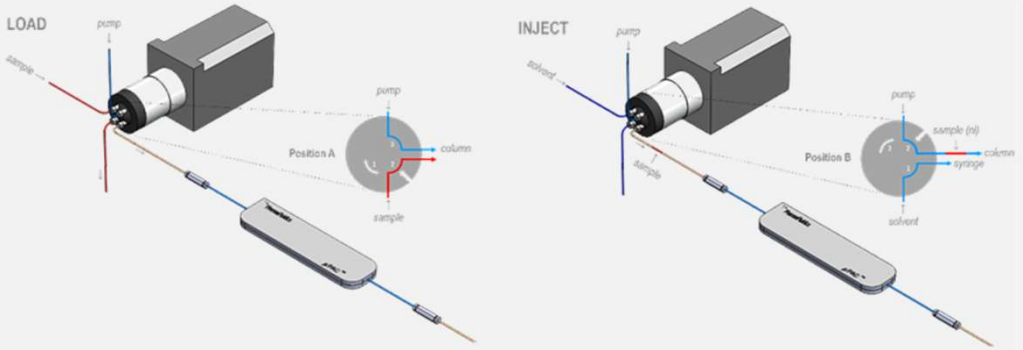
µPAC™-MS Metabolomics Platform

Plant Systems Biology
A VIB-UGENT DEPARTMENT

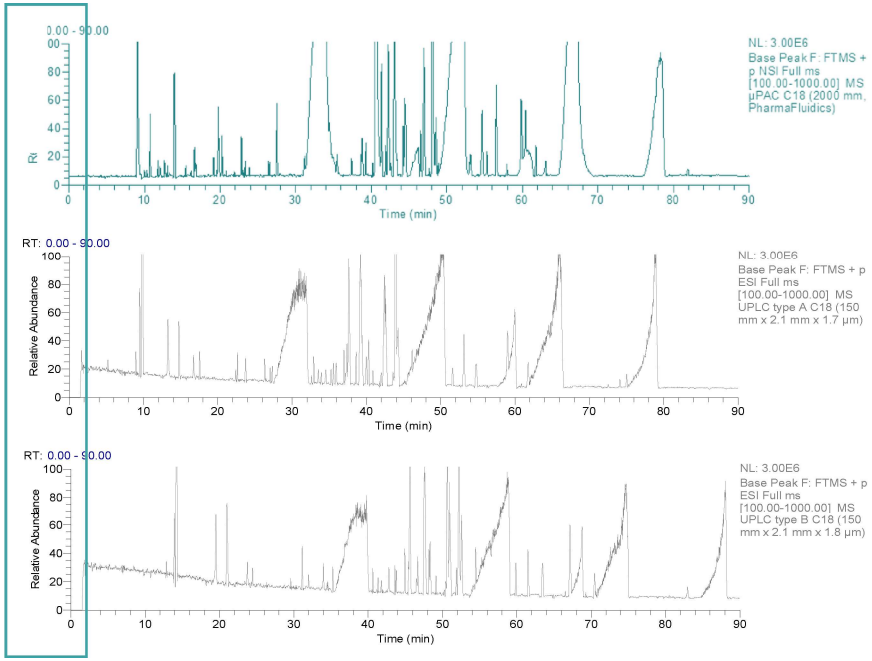
Plant Metabolomics



Increased intensity for a factor 1,250 less sample load

1260 Infinity Nanoflow LC
Nano ESI (Agilent)
LTQ-FT Ultra

Accela (Thermo)
ESI
LTQ-FT Ultra



µPAC™ 200 cm; C18
Poplar bark metabolite extract – 4 nl injection
1 to 50% B in 120 min – **1 µl/min**

Ref A: C18 (15 cm x 2.1 mm; 1.7 µm particles)
Poplar bark metabolite extract – 5 µl injection
1 to 50% B in 120 min – **300 µl/min**

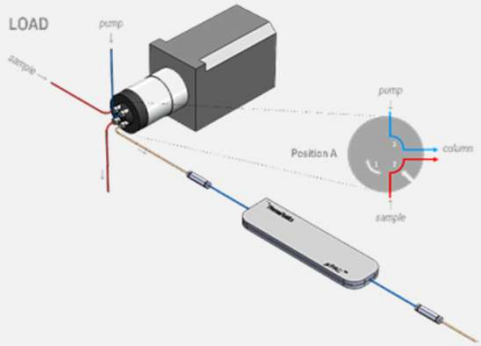
Ref B: C18 (15 cm x 2.1 mm; 1.8 µm particles)
Poplar bark metabolite extract – 5 µl injection
1 to 50% B in 120 min – **300 µl/min**

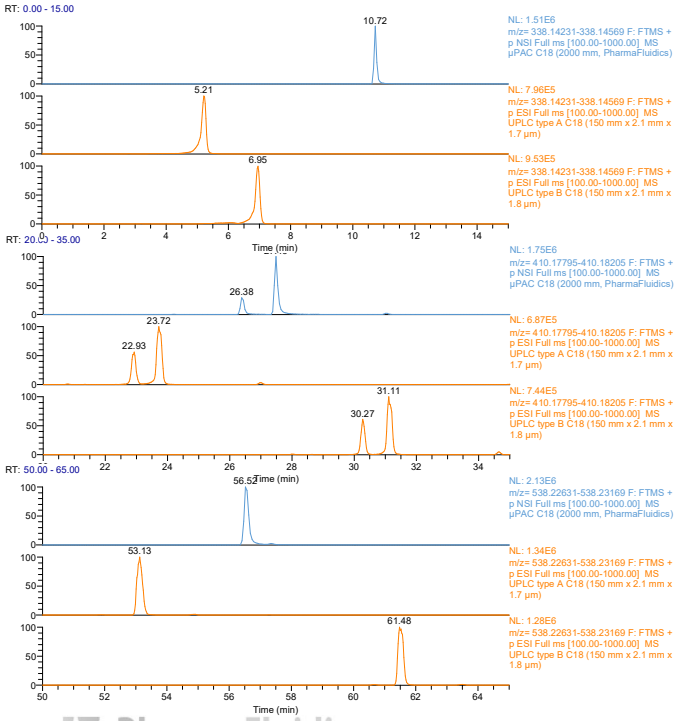
A: H2O (100%) with 0,1% FA; B: C2H3N/H2O (80/20) with 0,1% FA

µPAC™-MS Metabolomics Platform

Plant Systems Biology
A VIB-UGENT DEPARTMENT

Plant Metabolomics





	RT [min]	FWHM [min]	Intensity [/]
PharmaFluidics µPAC™ nano LC C18 (200 cm)	10.72	0.11	1.51E+06
UPLC Type A C18 (15 cm x 2.1 mm x 1.7 µm)	5.21	0.19	7.71E+05
UPLC Type B C18 (15 cm x 2.1 mm x 1.8 µm)	6.95	0.18	9.19E+05

	RT [min]	FWHM [min]	Intensity [/]
PharmaFluidics µPAC™ nano LC C18 (200 cm)	27.49	0.11	1.74E+06
UPLC Type A C18 (15 cm x 2.1 mm x 1.7 µm)	23.72	0.19	6.61E+05
UPLC Type B C18 (15 cm x 2.1 mm x 1.8 µm)	31.11	0.18	7.25E+05

	RT [min]	FWHM [min]	Intensity [/]
PharmaFluidics µPAC™ nano LC C18 (200 cm)	56.52	0.15	2.12E+06
UPLC Type A C18 (15 cm x 2.1 mm x 1.7 µm)	53.13	0.21	1.33E+06
UPLC Type B C18 (15 cm x 2.1 mm x 1.8 µm)	61.48	0.20	1.27E+06

Data and results

- µPAC™-MS Proteomics Platform
- µPAC™-MS Lipidomics Platform
- µPAC™-MS Metabolomics Platform
- **µPAC™-MS Biopharmaceuticals**

PharmaFluidics the micro-Chip Chromatography Company

Next-generation mAbs

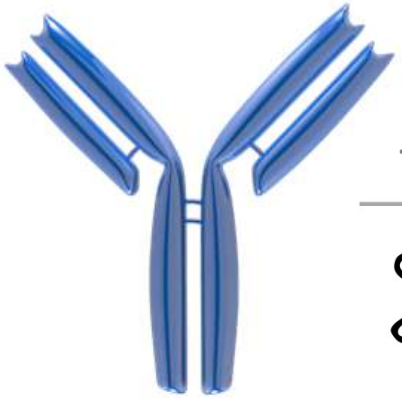
- The success of mAbs as therapeutics has triggered the development of various next-generation formats such as:
  - **Antibody-drug conjugates (ADC)**
  - Bispecific mAbs
  - Antibody fragments (Fab, Fc, Nanobodies)
  - Fc fusion proteins
  - Glyco-engineered mAbs

- ADCs consist of an antibody that targets a specific tumor cell and a highly potent cytotoxin

- Promise of ADCs: selective delivery of toxic compounds to tumor cells (side effect much lower compared to classical chemotherapy)

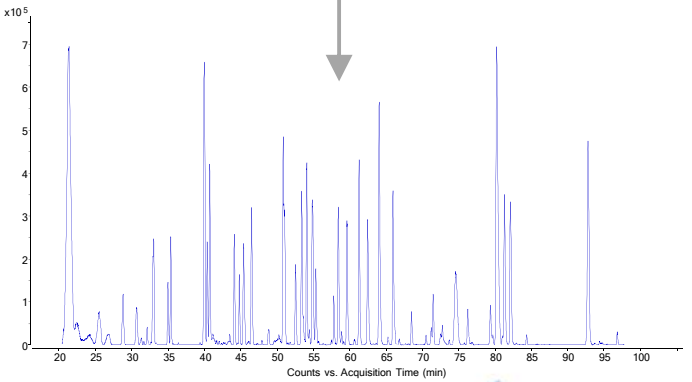
µPAC™-MS Biopharmaceuticals

Workflow



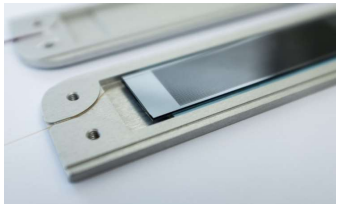
Trypsin

DIQMTQSPSSLSASVGDR
VTITCR
ASQDVNTAVAWYQQKPGK
APK
LLIYSASFLYSGVPSR
…

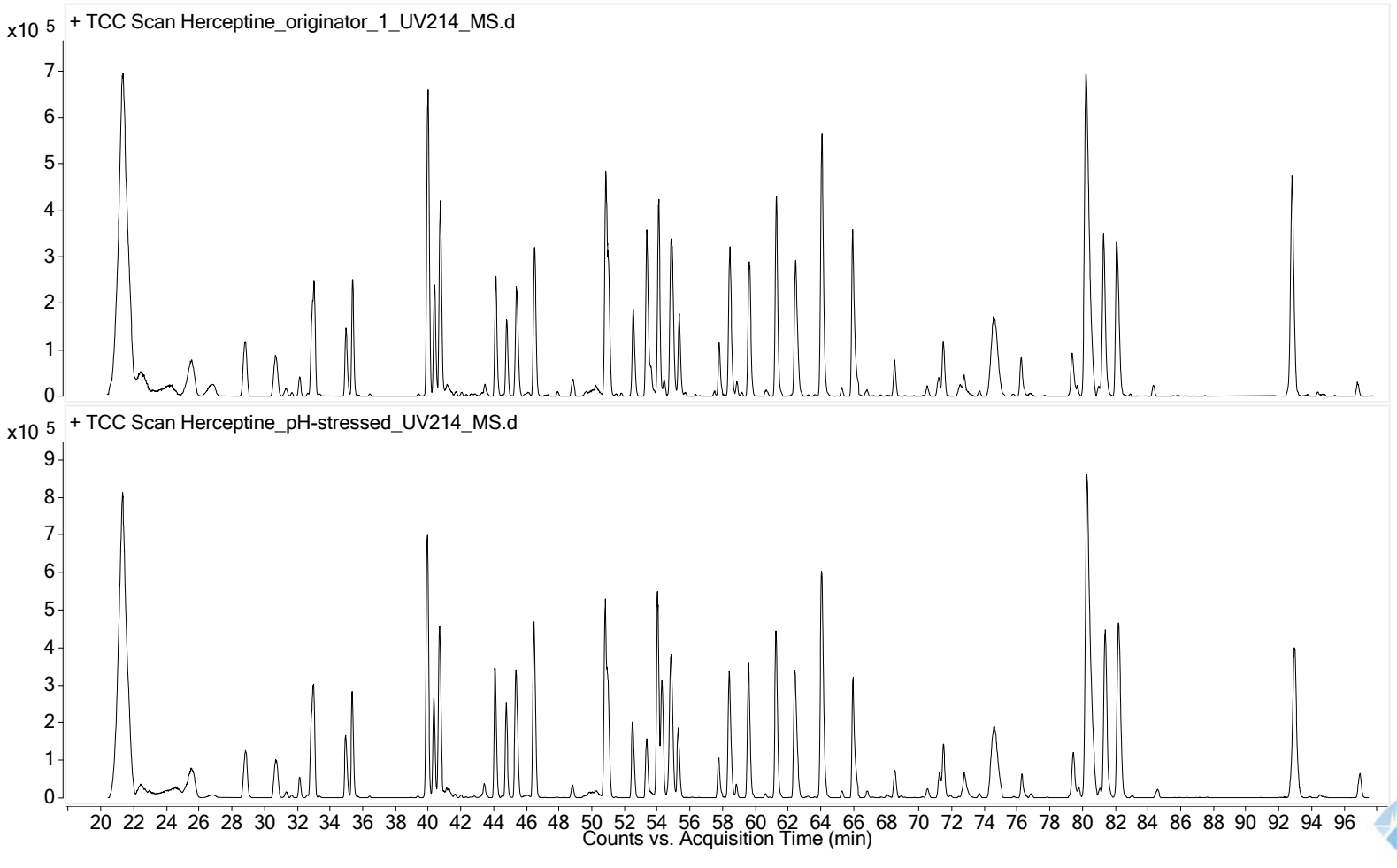


RIC

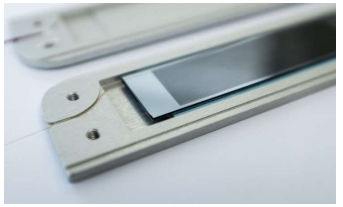
µPAC™-MS - Biopharmaceuticals



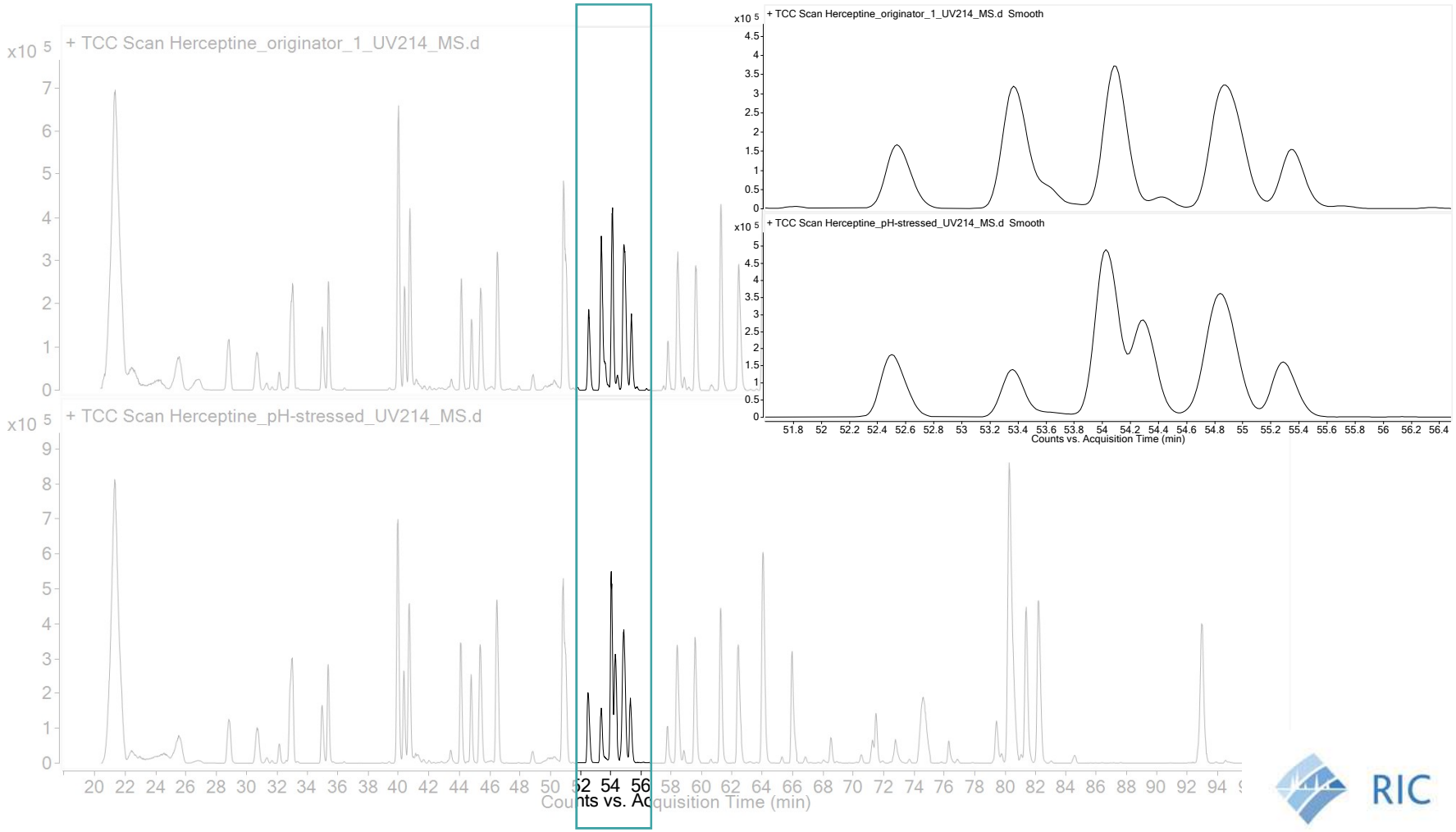
Herceptin – Originator versus high pH-stressed



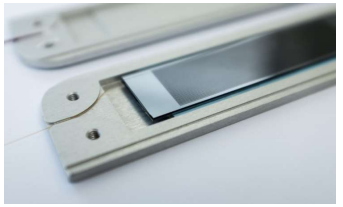
µPAC™-MS - Biopharmaceuticals



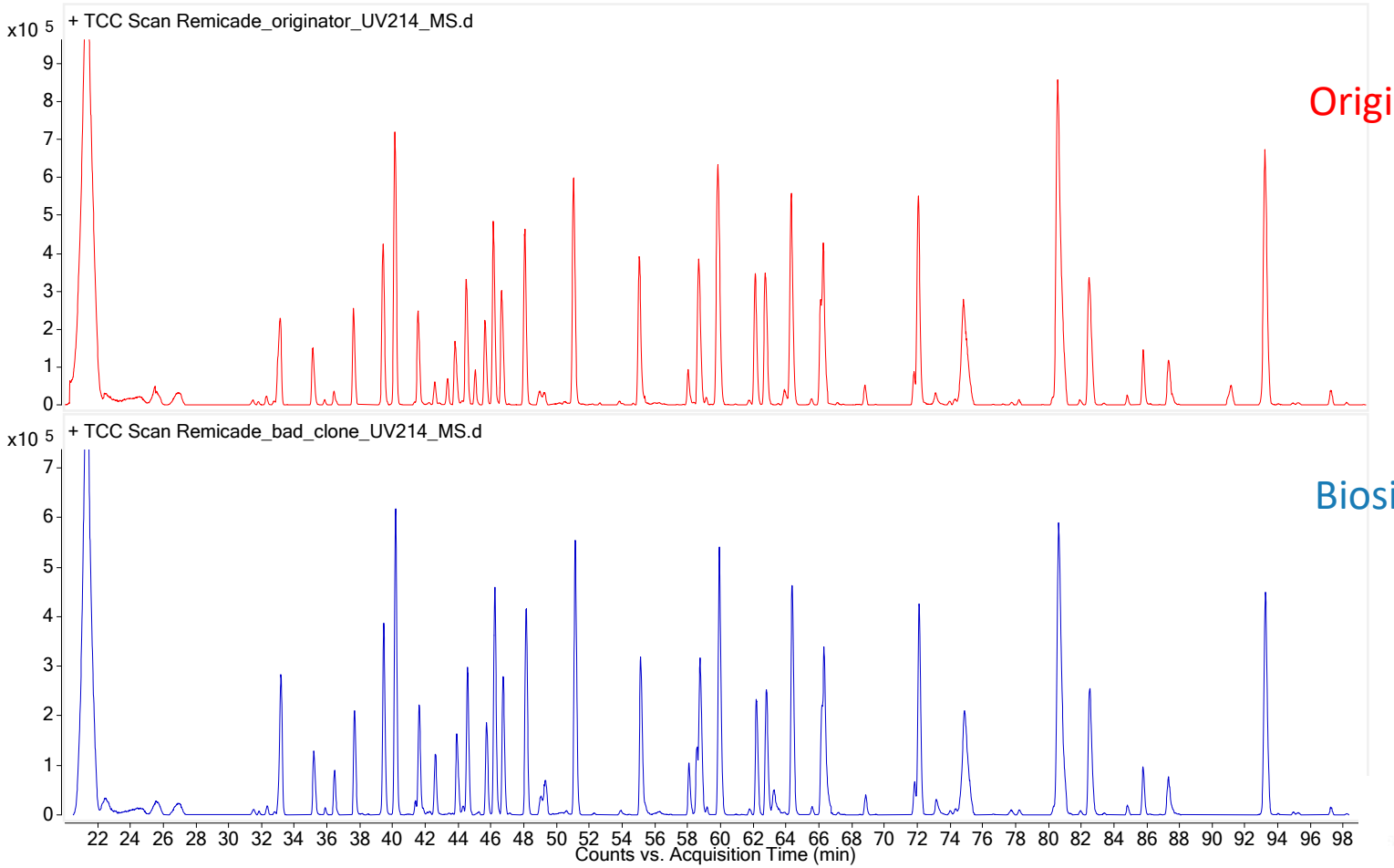
Herceptin – Originator versus high pH-stressed



µPAC™-MS - Biopharmaceuticals

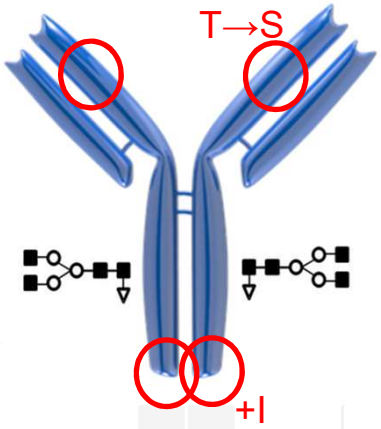


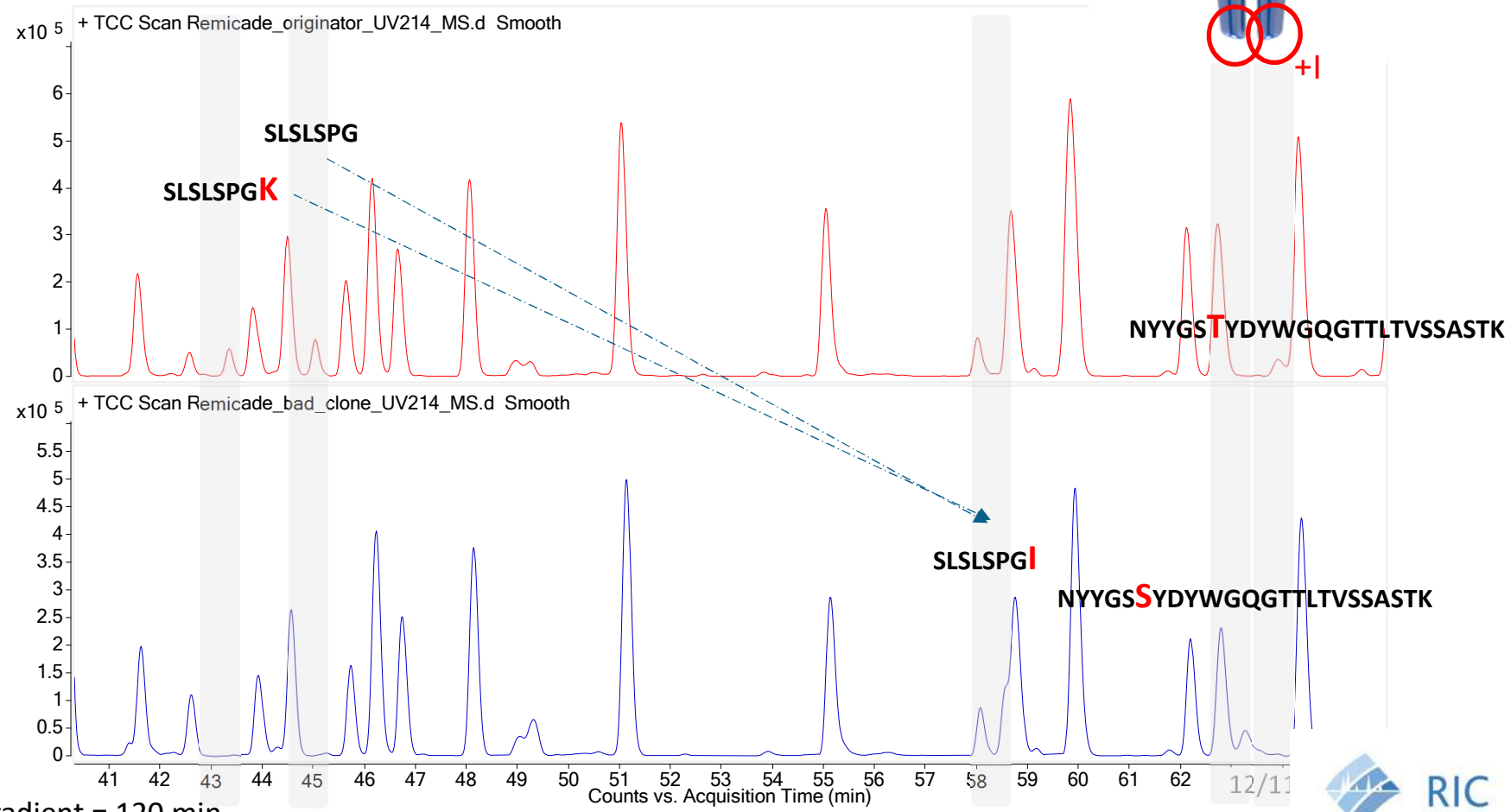
Remicade - originator vs bad clone



µPAC™-MS - Biopharmaceuticals

Remicade - originator vs bad clone





Gradient = 120 min

RIC

µPAC™-MS - Biopharmaceuticals

Conclusions

- The use of the µPAC™ column in the characterization and comparison of mAbs and ADCs has been illustrated.

- In combination with high resolution mass spectrometry, the µPAC™ allows to:
  - confirm the primary structure (sequence)
  - highlight post-translational modications and amino acid substitutions
  - identify drug conjugations sites

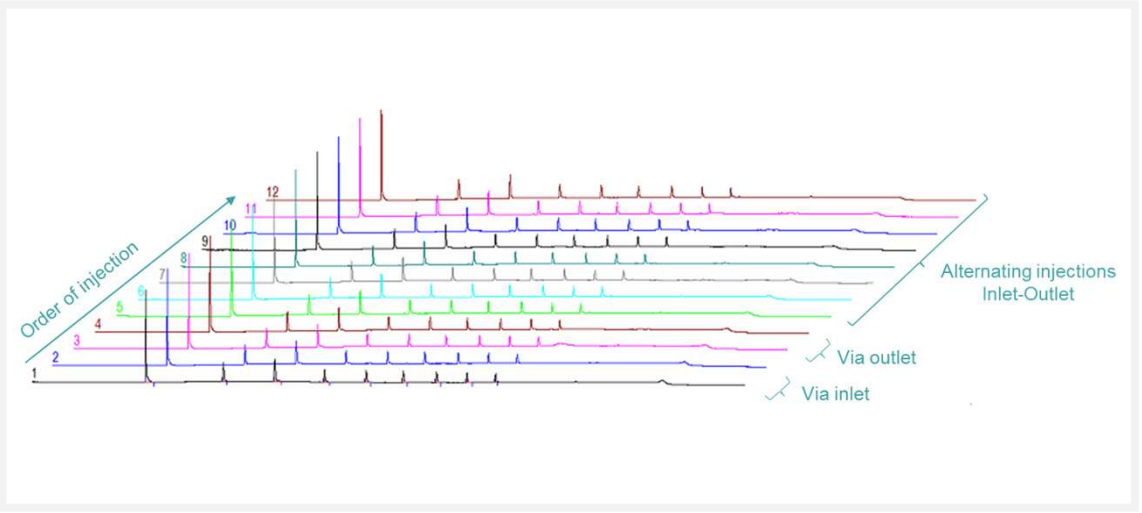
- The µPAC™ column holds great potential in biopharmaceutical applications.

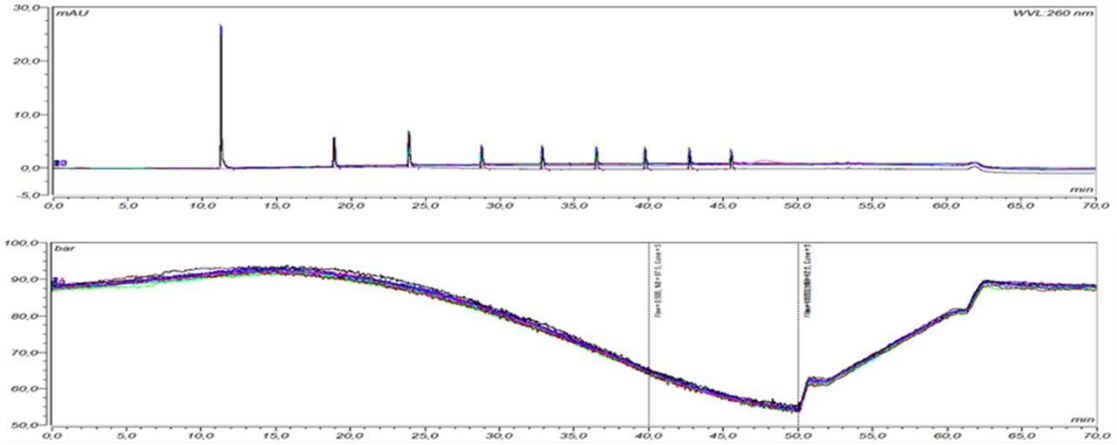
Micro Pillar Array Column (µPAC™)

Robustness

PharmaFluidics the micro-Chip Chromatography Company

Robustness - Bidirectionality





Experimental conditions

9 phenones – 4nl injection
1 to 40% ACN / 0,05% FA
40 min gradients / 500nl/min

Conclusions

Bidirectional use
µPAC™ can be used in both straight and reverse directions without loss in performance

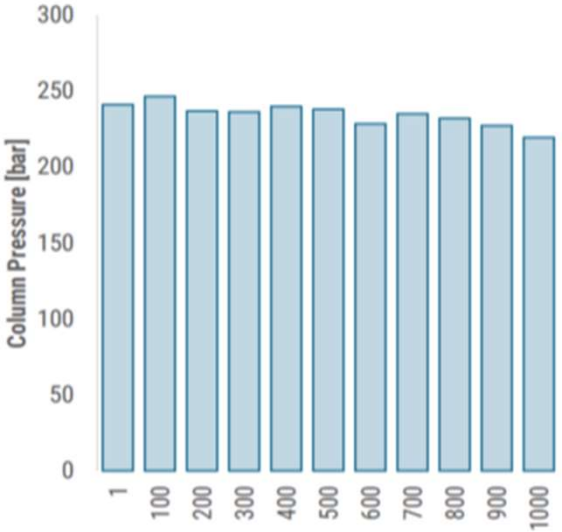
Longevity - Robustness – Acidic pH resistance

➢ Number of HeLa cell digest injections	1000
➢ Total number of injections	3.526
➢ Total volume through column (ml)	195,4
➢ Total amount of column volumes	21.707

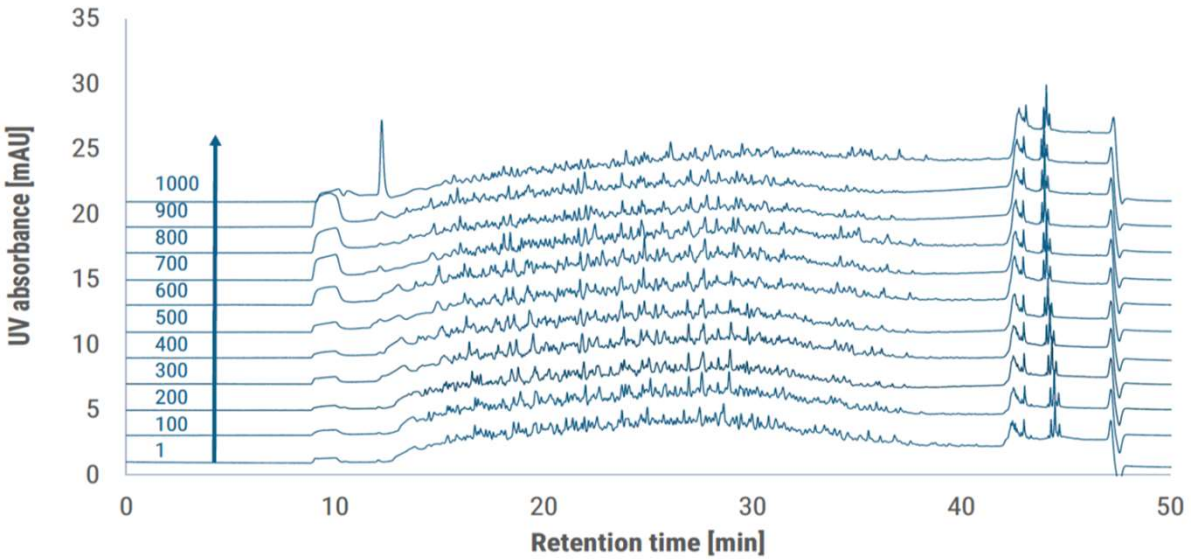
1 µl injection
30 min gradient 1% B -> 50% B
0.1% TFA (pH 1,98)
UV detection: 214nm

100 ng Hela digest
Blank
500 fM Cyto C digest

Pressure profile – HeLa digest



UV profile – HeLa digest



Longevity - Robustness – Acidic pH resistance

➢ Number of HeLa cell digest injections	1000
➢ Total number of injections	3.526
➢ Total volume through column (ml)	195,4
➢ Total amount of column volumes	21.707

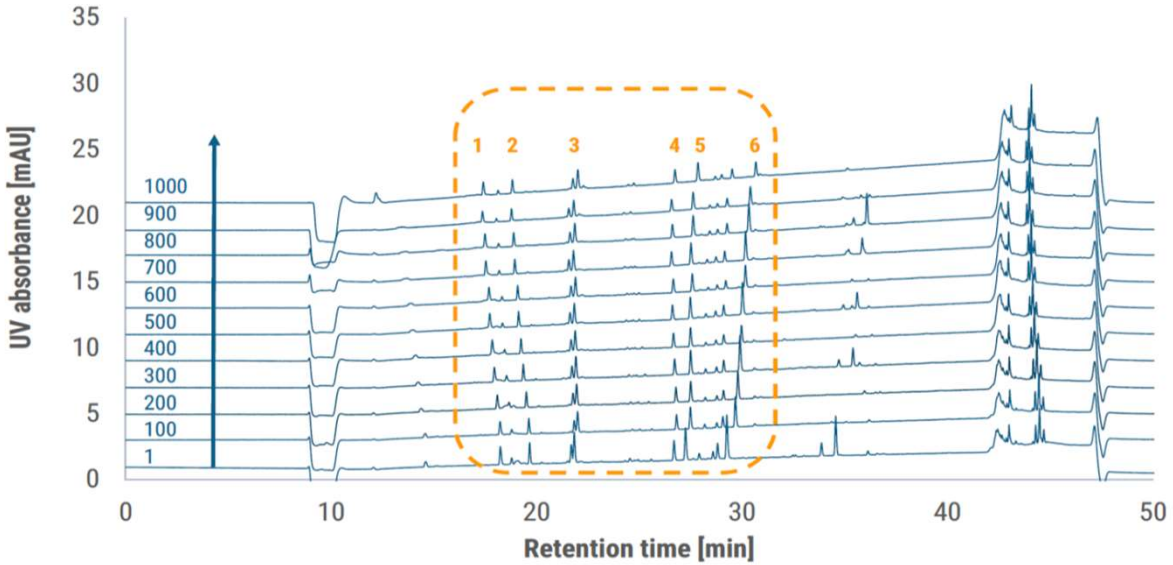
1 µl injection
30 min gradient 1% B -> 50% B
0.1% TFA (pH 1,98)
UV detection: 214nm

100 ng Hela digest
Blank
500 fM Cyto C digest

Chromatographic metrics – Cyto C

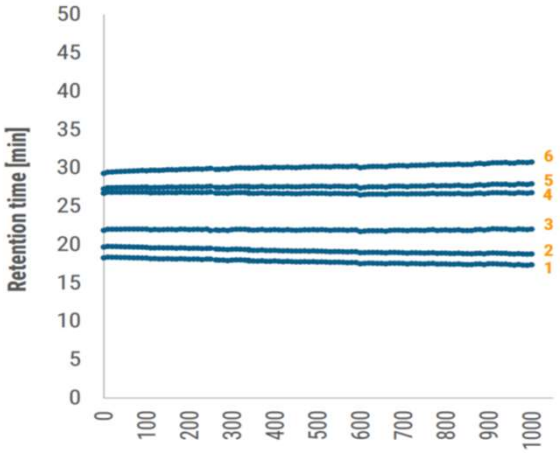
Peptide	Value (min)	Std DEV	CV (%)
1	17.76	0.30	1.72
2	19.17	0.31	1.59
3	21.91	0.07	0.32
4	26.70	0.08	0.31
5	27.59	0.13	0.48
6	30.12	0.35	1.15
Average PW	0.14		

UV profile – Cyto C digest

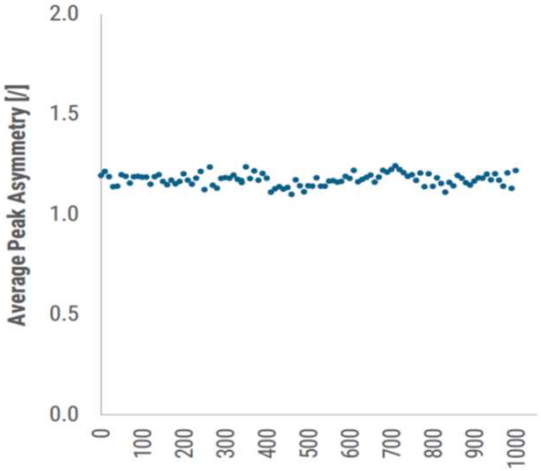


Longevity - Robustness – Acidic pH resistance

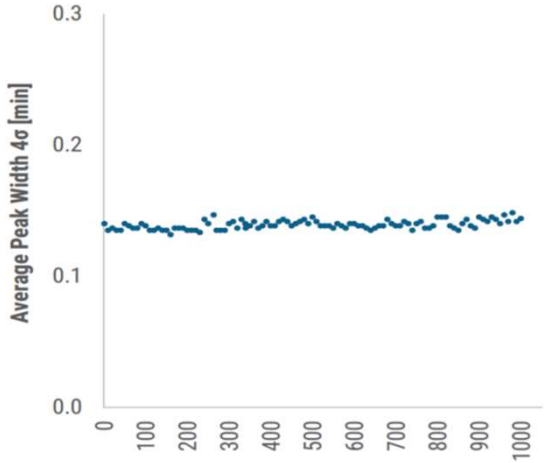
Retention



Peak asymmetry



Peak width



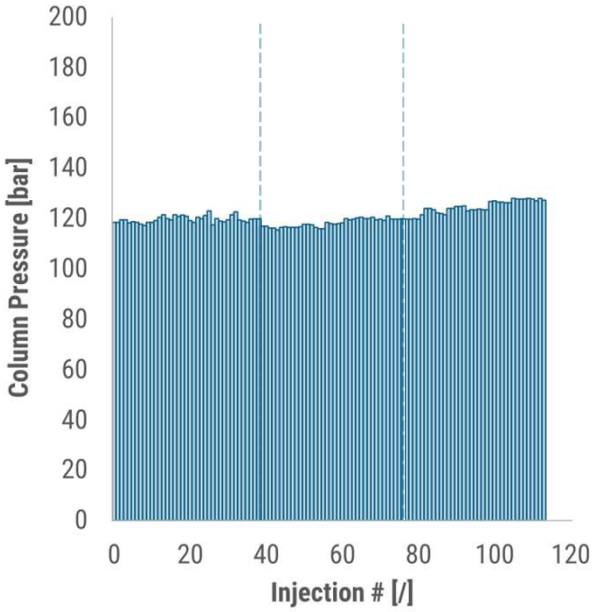
Conclusion

The µPAC™ can be used up to 1000 injections of commercial HeLa cell digest (> 3500 total injections) in a low pH range without any loss of performance or pressure increase.

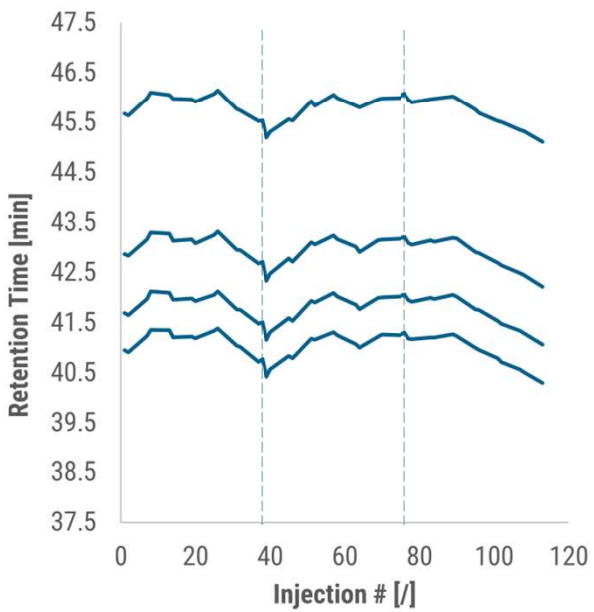
Robustness - Longevity – Challenging samples

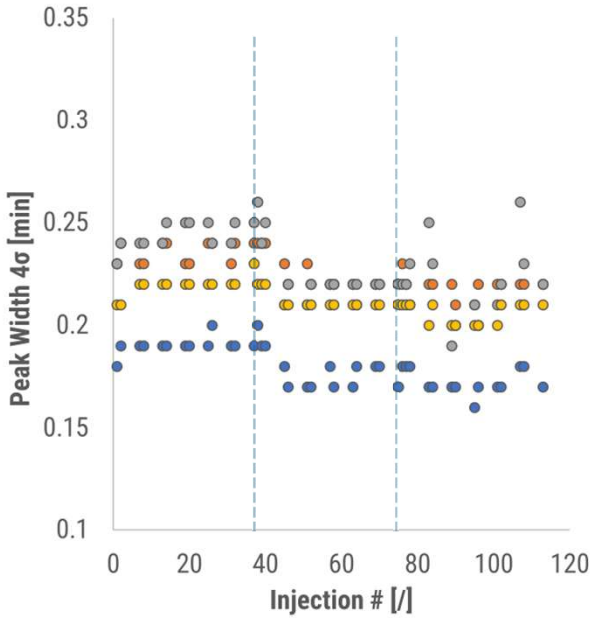
3 different sets of "challenging" samples (n=6):
Set 1: Human cell line treated with detergent (Triton X-114)
Set 2: Plant material lysed with detergent (NP-40)
Set 3: TnT quick coupled transcription/translation system with black precipitation

All injections



Cytochrome C digest reference peptides





Conclusion
No significant increase in column backpressure and excellent RT stability (CV < 1%) was observed.

Micro Pillar Array Column (µPAC™)
RT Stability

PharmaFluidics the micro-Chip Chromatography Company

RT stability – PRTC mixture

Experimental conditions:

Pierce RT calibration mixture (P/N 88320) – 50 fmoles
spiked in 100 ng HeLa digest
1 to 40% ACN / 0,1% FA
30 min gradient / 750 nl/min

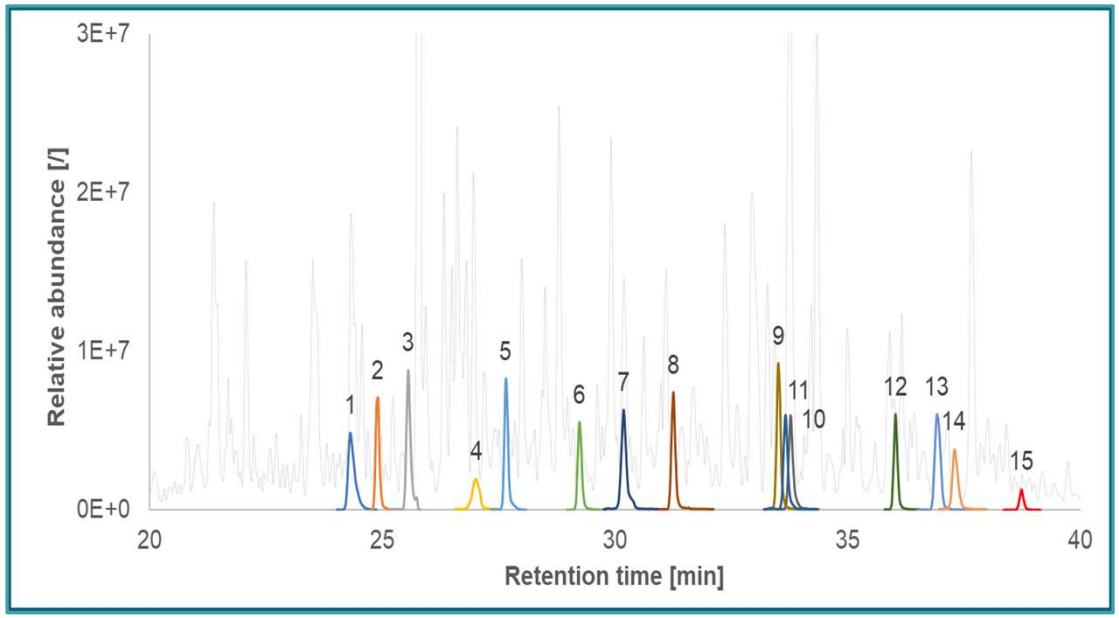
Instrumental setup:

Thermo Scientific™ Ultimate 3000 RSLC
1 µL direct injection
200 cm µPAC column @ 30°C
Thermo Scientific™ LTQ Orbitrap XL™

Chromatographic metrics (n=3)

Peptide	Sequence	m/z	RT [min]	
			Value	% CV
1	SSAAPPPPPR	493.768	23.98	1.27
2	GISNEGQNASIK	613.317	24.58	1.28
3	HVLTSIGEK	496.287	25.23	1.20
4	DIPVPKPK	451.283	26.69	1.17
5	IGDYAGIK	422.736	27.34	1.08
6	TASEFSAIAQDK	695.832	28.93	1.02
7	SAAGAFGPELSR	586.8	29.87	1.04
8	ELGQSGVDTYLQTK	773.896	30.92	1.06
9	GLILVGGYGTR	558.326	33.18	0.91
10	GILFVGSGVSGGEEGAR	801.412	33.45	0.89
11	SFANQPLEVVYSK	745.392	33.33	0.91
12	LTILEELR	498.802	35.73	0.83
13	NGFILDGFPR	573.303	36.64	0.74
14	ELASGLSFPVGFK	680.374	37.01	0.72
15	LSSEAPALFQFDLK	787.421	38.42	0.79
			Mean CV:	***0.99***

Elution profiles – base peak chromatogram



RT stability – PRTC mixture

Experimental conditions:

Pierce RT calibration mixture (P/N 88320) – 50 fmoles
spiked in 100 ng HeLa digest
1 to 40% ACN / 0,1% FA
240 min gradient / 300 nl/min

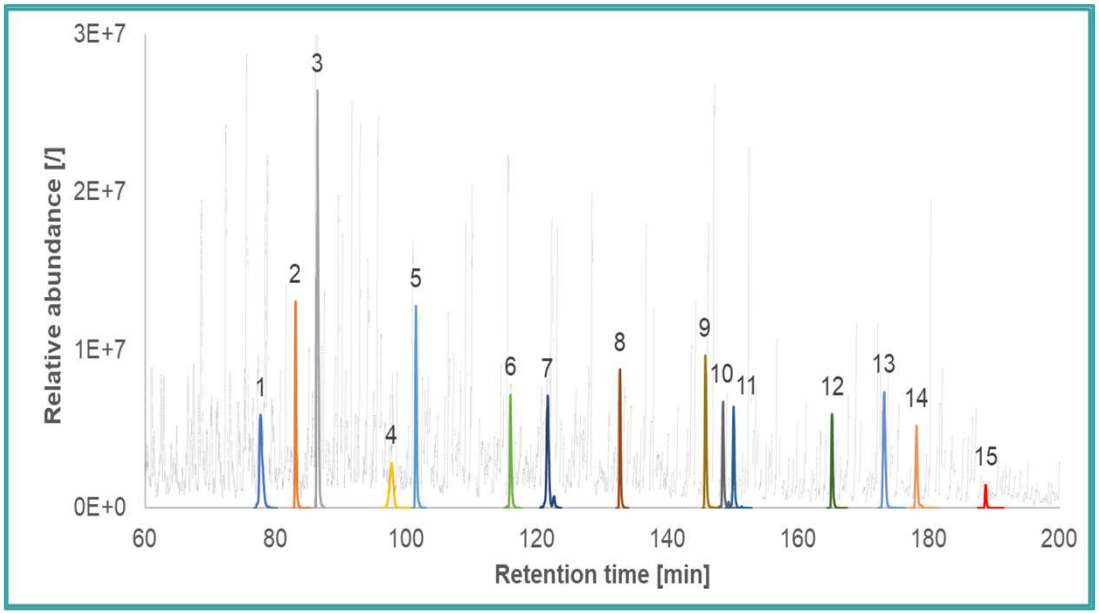
Instrumental setup:

Thermo Scientific™ Ultimate 3000 RSLC
1 µL direct injection
200 cm µPAC column @ 30°C
Thermo Scientific™ LTQ Orbitrap XL™

Chromatographic metrics (n=3)

Peptide	Sequence	m/z	RT [min]	
			Value	% CV
1	SSAAPPPPPR	493.768	77.87	0.69
2	GISNEGQNASIK	613.317	83.17	0.61
3	HVLTSIGEK	496.287	86.52	0.64
4	DIPVPKPK	451.283	97.48	0.82
5	IGDYAGIK	422.736	101.16	0.86
6	TASEFSAIAQDK	695.832	115.38	0.87
7	SAAGAFGPELSR	586.8	121.06	0.84
8	ELGQSGVDTYLQTK	773.896	132.07	0.78
9	GLILVGGYGTR	558.326	145.01	0.77
10	GILFVGSGVSGGEEGAR	801.412	147.73	0.78
11	SFANQPLEVVYSK	745.392	149.25	0.75
12	LTILEELR	498.802	164.26	0.80
13	NGFILDGFPR	573.303	172.08	0.80
14	ELASGLSFPVGFK	680.374	177.03	0.77
15	LSSEAPALFQFDLK	787.421	187.60	0.75
		Mean CV:		***0.77***

Elution profiles – base peak chromatogram



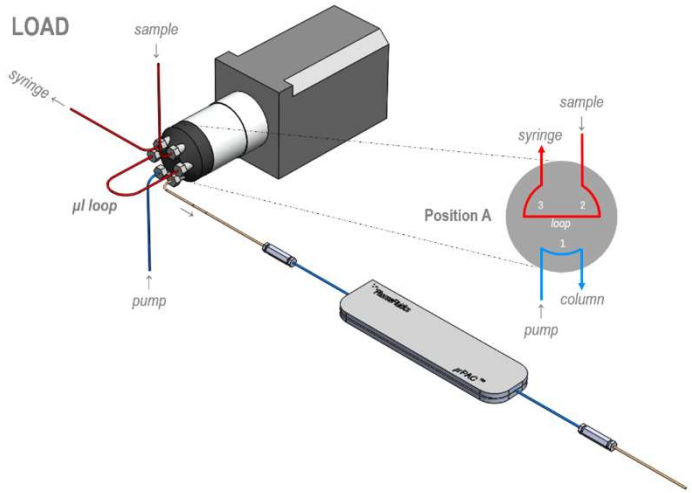
Conclusion
Excellent RT stability was observed with average CV values <1.0% for 750 nL/min and <0.8% for 300 nL/min.

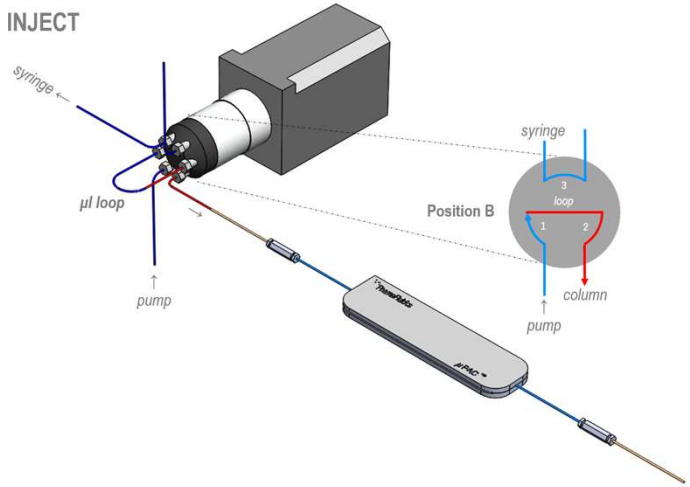
Connectivity

PharmaFluidics the micro-Chip Chromatography Company

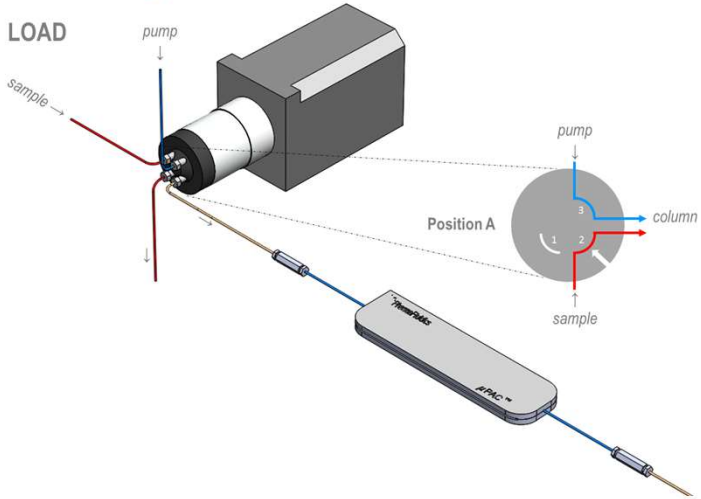
µPAC™ Connectivity - Injection

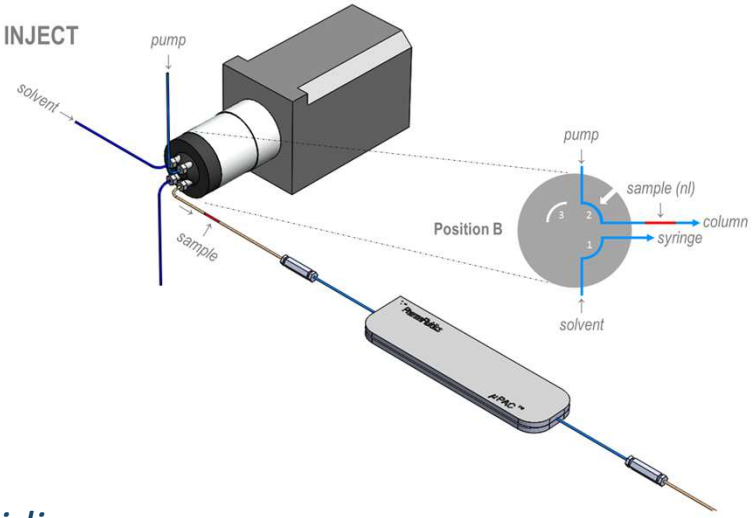
Direct injection method – External loop





Direct injection method – Internal loop

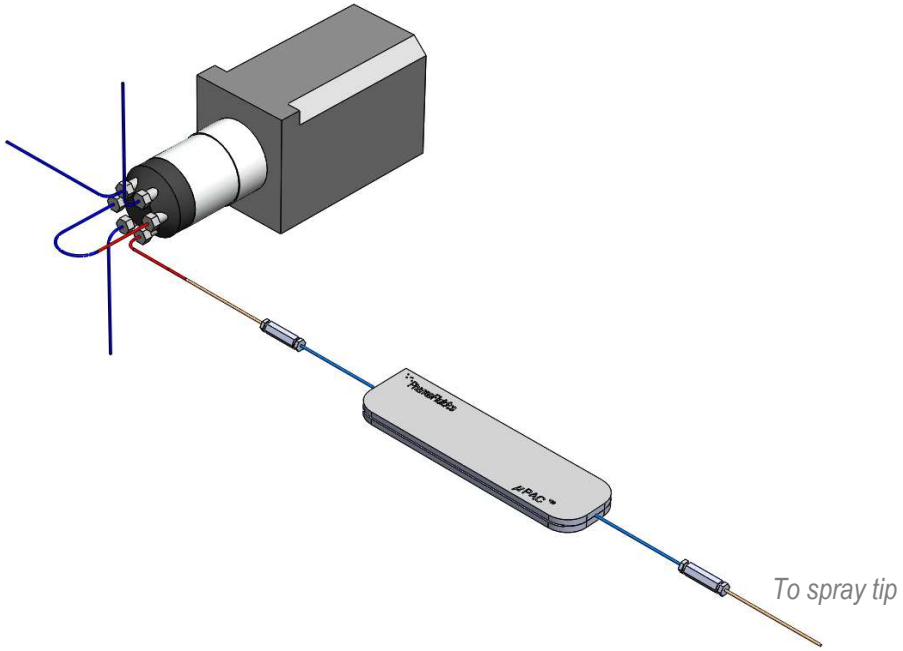




support@pharmafluidics.com

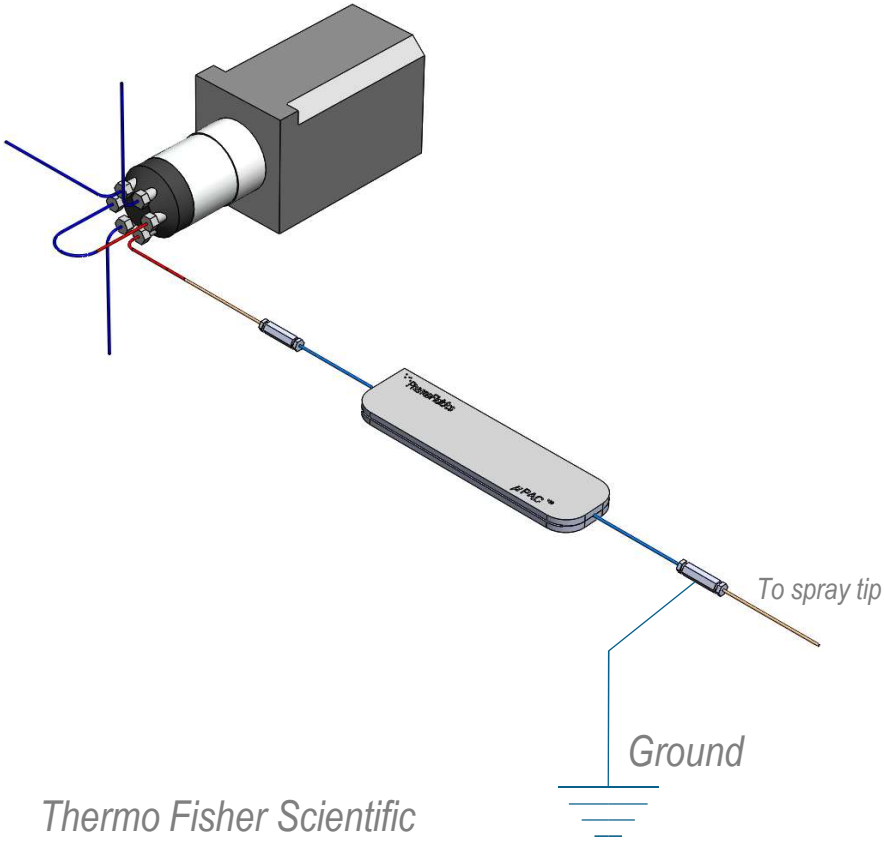
µPAC™ Connectivity – MS Interfacing

Grounded spray tip systems



Agilent
Bruker
Sciex

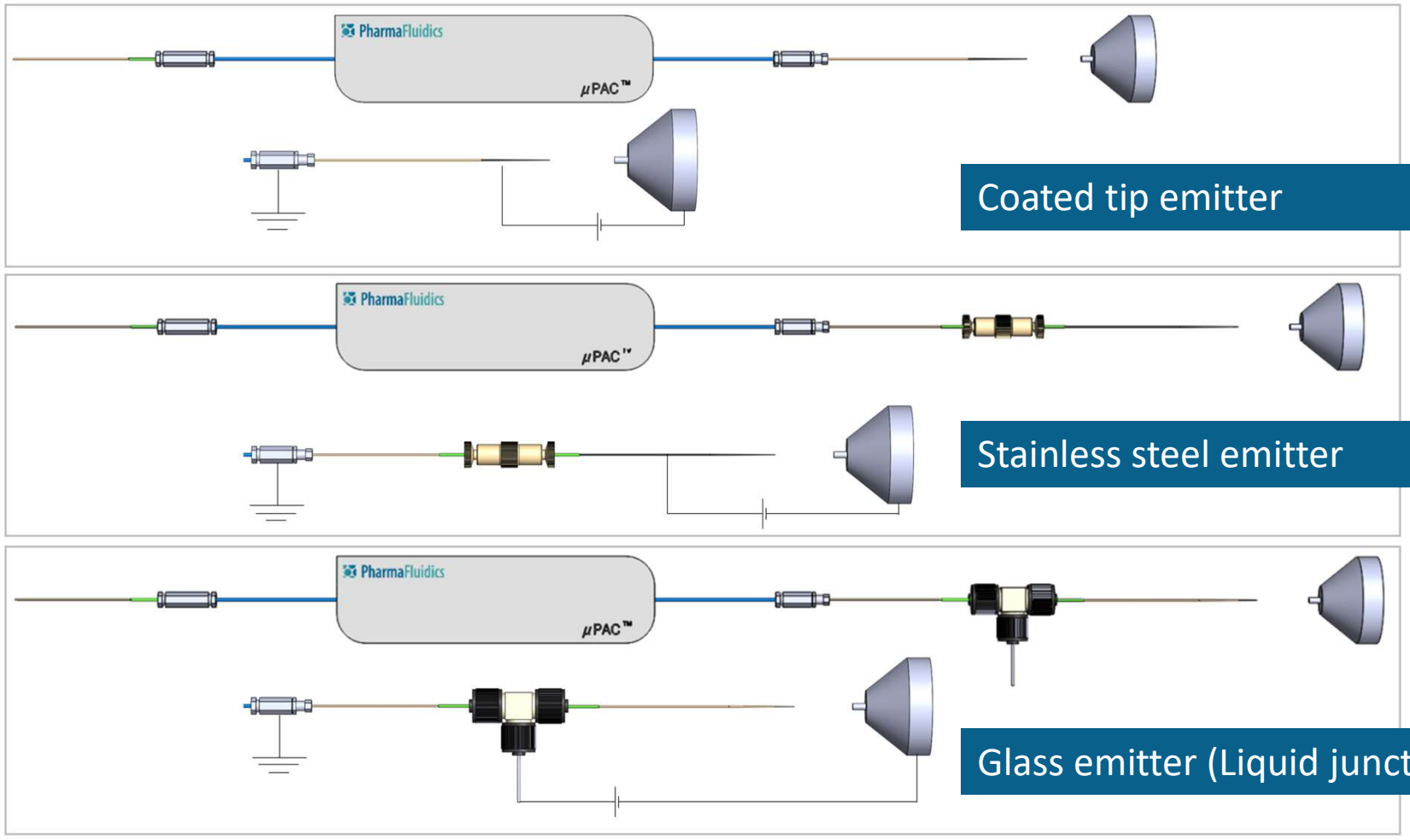
HV spray tip systems



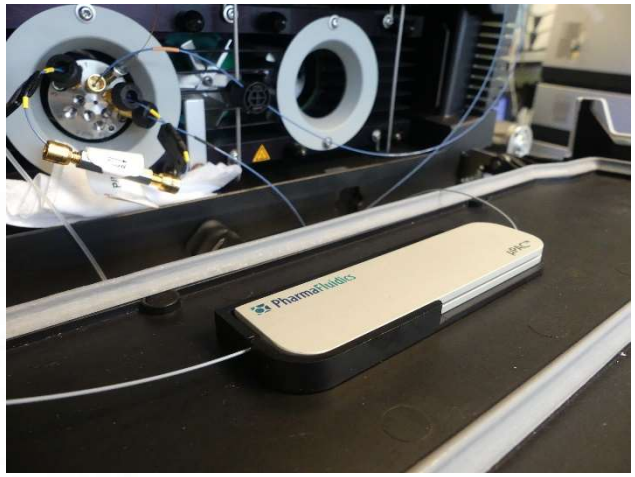
Thermo Fisher Scientific
Waters

support@pharmafluidics.com

Connectivity Esi-MS interfacing



µPATCH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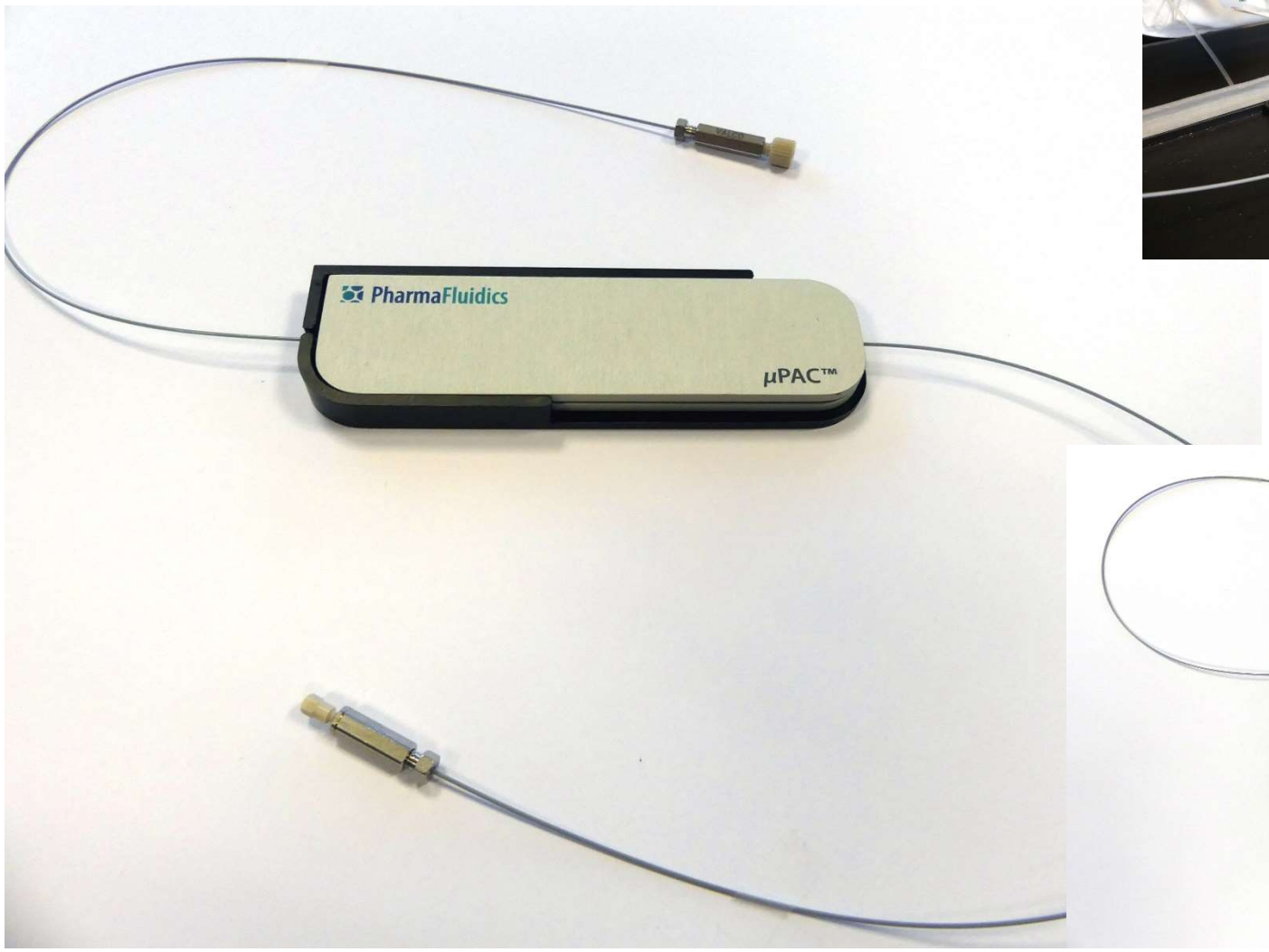
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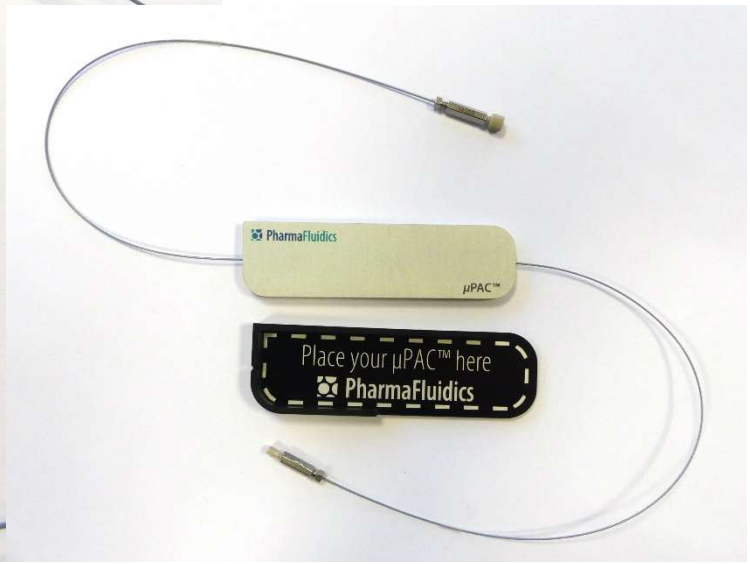
µPAC™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µPAC™
Place your µPAC™ here
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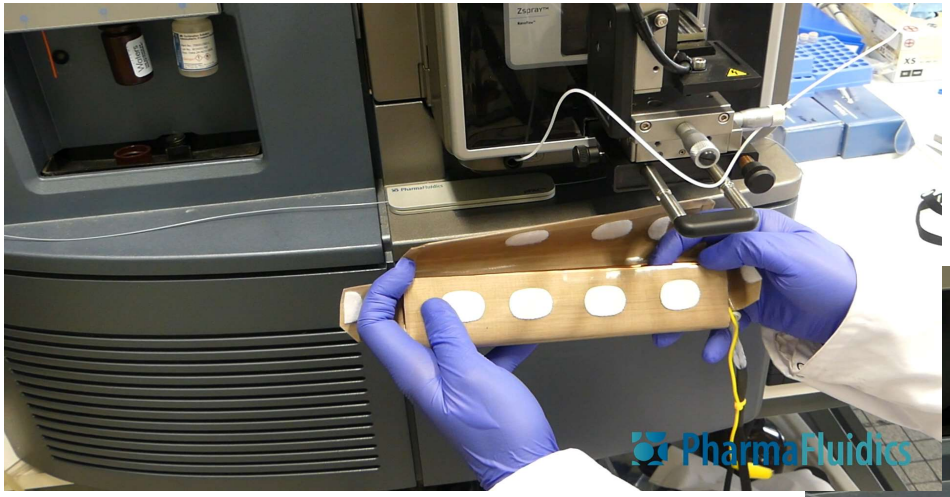
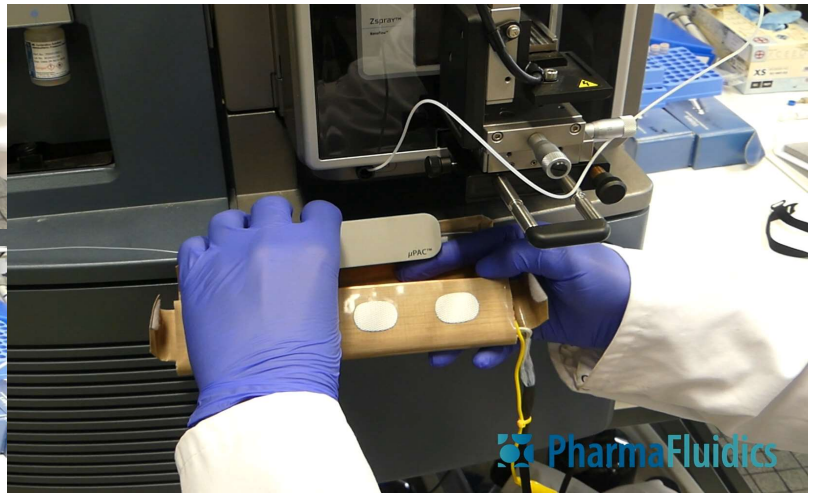
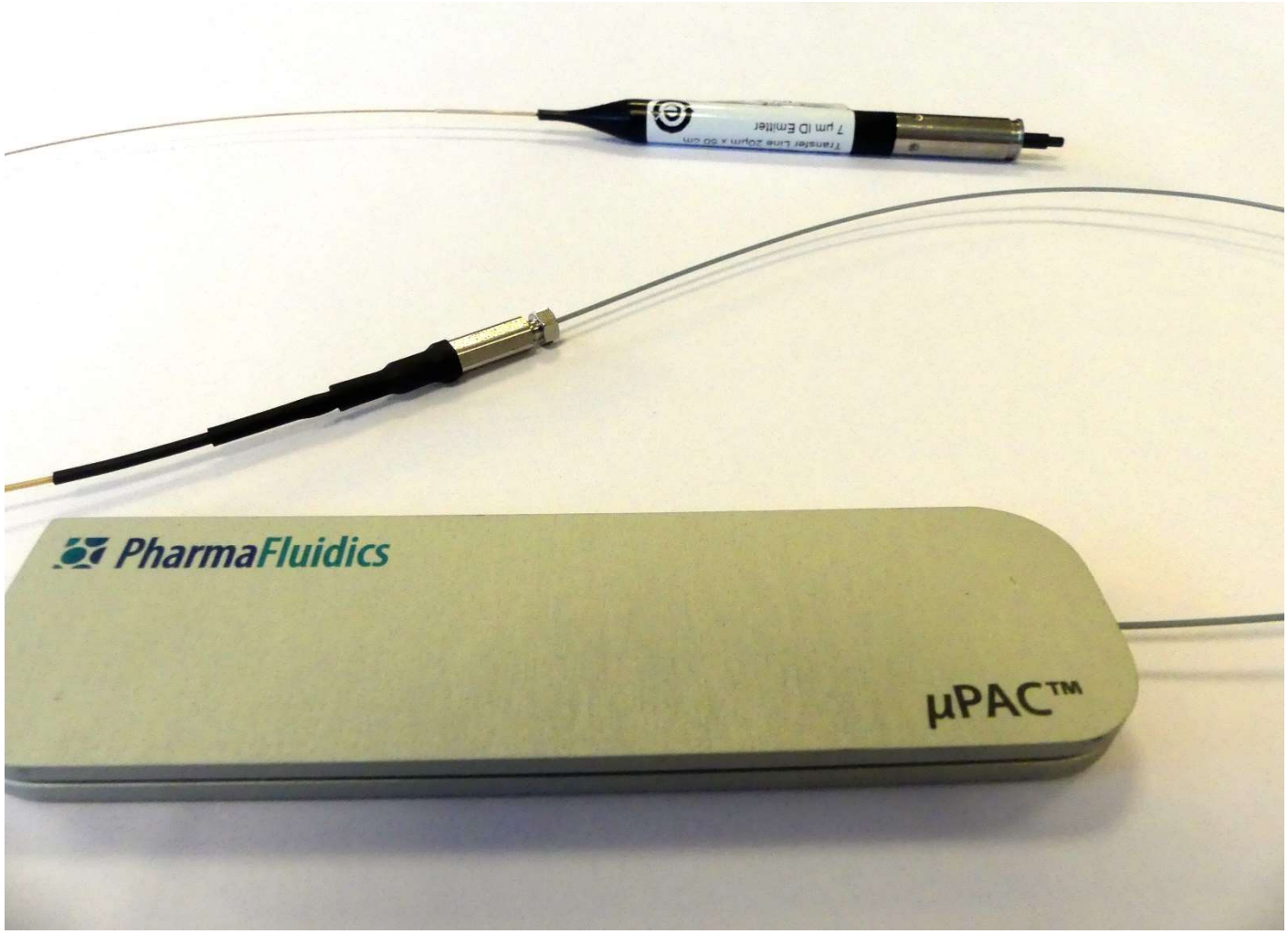
Butterfly heater





µPAC™ Compatible EASY-Spray™ Emitter



Do you work with short gradients?

Do you work with a 15cm packed bed?

Are you working in a routine lab?

Then our 50cm µPAC™ is perfect for you!

- 0.1 – 2 µl/min
- 30 min – 2h gradient time
- 30% increase in prot ID

Do you work with long gradients?

Do you work with a 50cm packed bed?

Are you working with comprehensive proteomics?

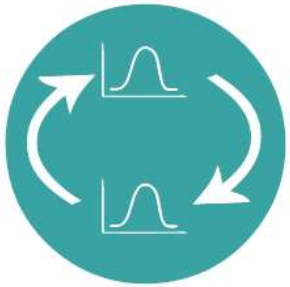
Then the 200cm µPAC™ is your best choice!

- 0.1 – 1 µl/min
- 4h– 8h gradient time
- Unique proteome coverage

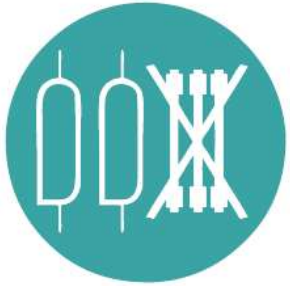
Why should you use µPAC™?

Increased sensitivity
Due to high degree of order

Unrivalled robustness and number of injections
Due to the solid silicon backbone



Outperforming reproducibility
Due to the mask-bases etching process



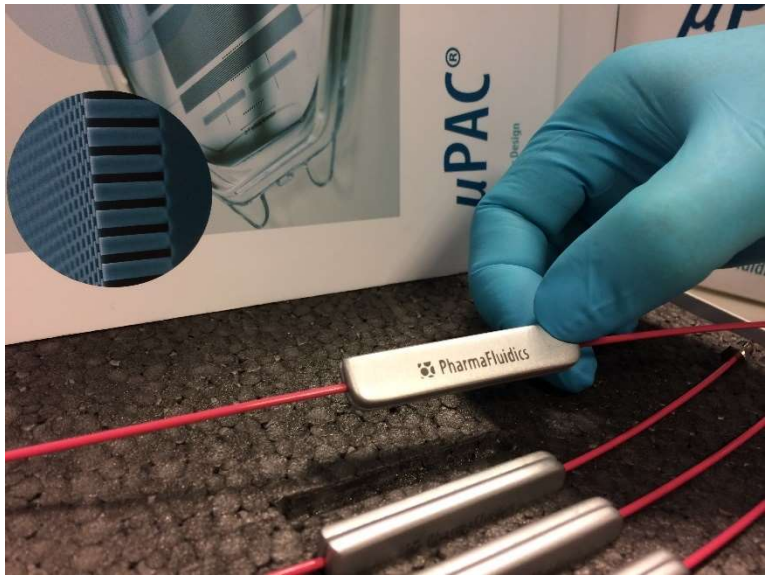
Less column replacements
Due to the high number of injections

Less prone to clogging
Due to high column permeability

Sharper peaks and excellent separation power
Due to exceptional column length

New µPAC™ trapping column

- Reducing sample loading times to a minimum with significant higher flow rates
- 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



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Stationary phase chemistry is currently focusing on reversed phase HPLC separations.

Product	Stationary phase	Product number
50 cm µPAC™ column	C18 endcapped	5525031518200
200 cm µPAC™ column	C18 endcapped	552503151850
µPAC™ Compatible EASY-Spray™ Emitter	NA	PhF80000530

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