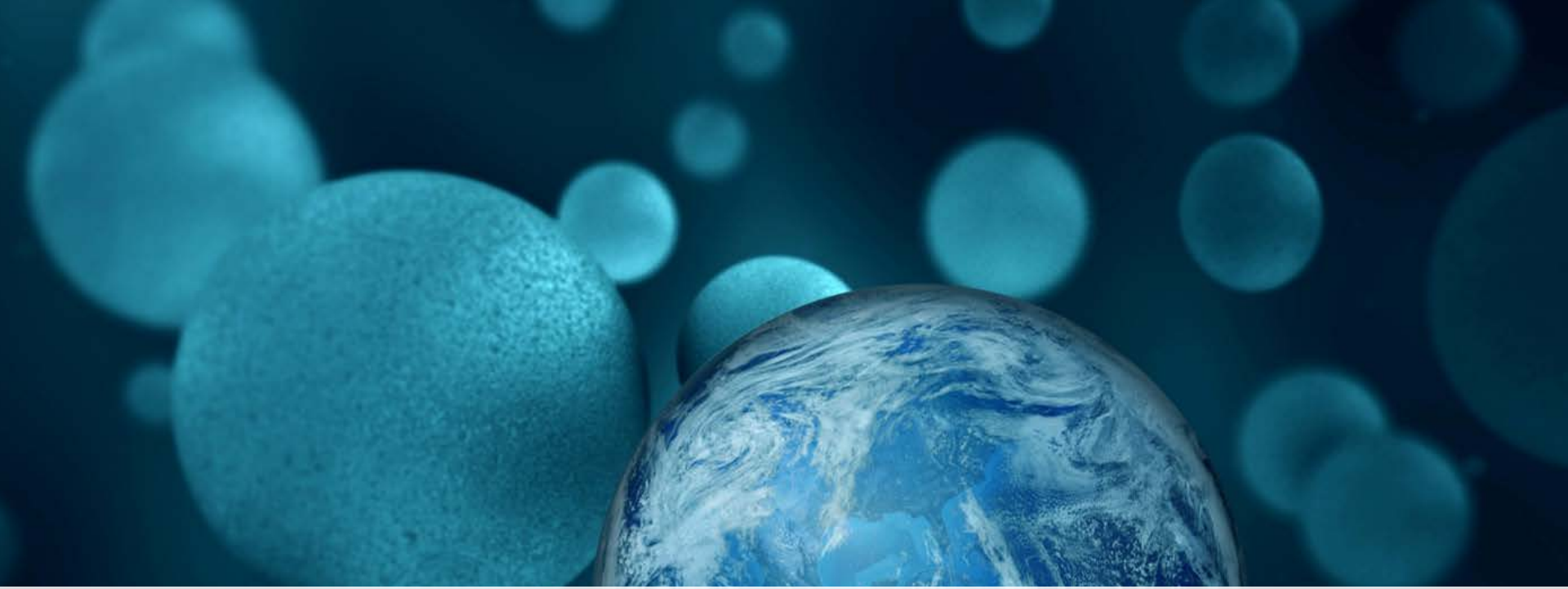

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Raise your nano LC-MS performance with new Thermo Scientific solutions

8 February 2016

The Thermo Scientific™ nano LC portfolio

- Thermo Scientific™ UltiMate™ 3000 RLSCnano with New ProFlow™ technology
  - General system overview
  - ProFlow technology: features and advantages
  - Nano LC-MS application examples

Alex Boychenko

- The new Thermo Scientific™ EASY-nLC™ 1200
  - Established concept
  - New features
  - Application examples

Stephan Meding

- Questions & Answers

Challenges and Goals in nano LC-MS based proteomics

Goals

Protein Identification
- More protein identifications from single run analysis
- Analysis of low sample amounts

Confident Quantitation
- Precise label-free quantification in large cohorts
- Sensitive targeted quantification in complex matrixes

High sample throughput
- Fast samples profiling with high MS sensitivity

Challenges

System Performance
- Chromatographic resolution
- Chromatographic repeatability

System Usability
- Required level of expertise
- Sample throughput

- UltiMate 3000 RSLCnano with ProFlow technology
- EASY-nLC 1200

provide superior nanoLC performance

The Thermo Scientific nano LC portfolio

EASY-nLC 1200

Launched at HUPO,
27-30 September 2015

UltiMate 3000 RSLCnano

ProFlow technology launched at HTC-14, 27-29 January 2016

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The Thermo Scientific nano LC portfolio

EASY-nLC 1200

Dedicated

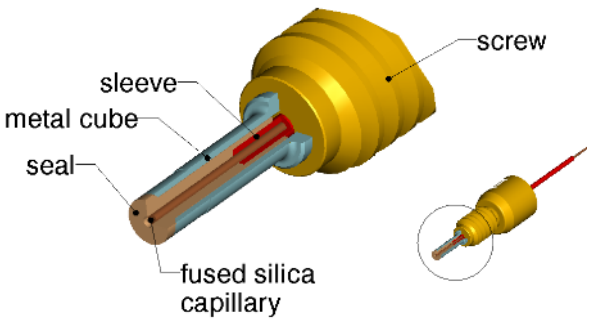
- Optimized, and fully integrated solution for LC/MS based proteomics
- Provides effortless ultra-high performance for non-chromatography experts
- Highest pressure rating available in market – ideal for ultra long column applications

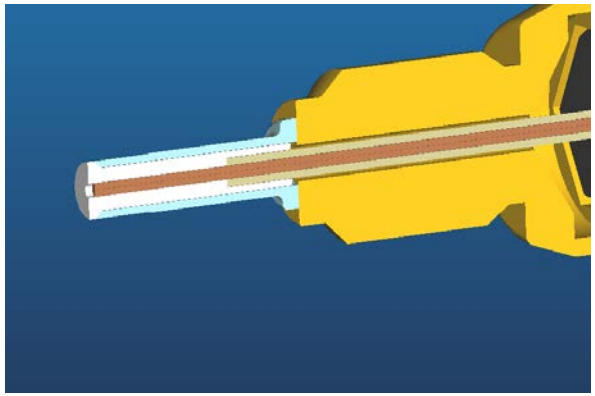
UltiMate 3000 RSLCnano

Versatile

- Optimized ultra-high performance platform for proteomics, metabolomics, and biopharmaceutical analysis
- Routine and advanced workflows
- Wide application range
  - nano-, cap- or micro-, and analytical flows
  - Direct injection, Pre-concentration, online and offline 2D-LC/MS

nanoViper™ – Nano LC Capillary Connections Made Easy





Features

- UHPLC Fingertight connection
- Up to 1200 bar
- Universal fit
- Near zero-dead-volume by design

Values

- Tool-free assembly, easy fluidics
- UHPLC compatible
- Compatible with most LC columns and valves
- High peak capacity and low peak broadening

The standard proteomics workflow

LC-MS
Data Acquisition

Data Analysis
- Identification
- Quantitation

Discovery, Publication, Patent

Sample Preparation
- Extraction
- Digest
- Enrichment / Fractionation

UltiMate 3000 RSLCnano: Flow Range Versatility

Nano Flow

New ProFlow technology

- ***Flow range:***
50-1500 nL/min (recommended)
- ***Max System Pressure:***
860 bar at full flow range

Micro Flow

- ***Flow range:***
5-50 µL/min (recommended)
- ***Max System Pressure:***
800 bar at 25 µL/min

Capillary Flow

- ***Flow range:***
0.5-10 µL/min (recommended)
- ***Max System Pressure:***
800 bar at 5 µL/min

Analytical flow

with LPG pump in NCS-3500RS

- ***Flow range:***
< 2500 µL/min
- ***Max System Pressure:***
620 bar

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UltiMate 3000 RSLCnano: ProFlow technology

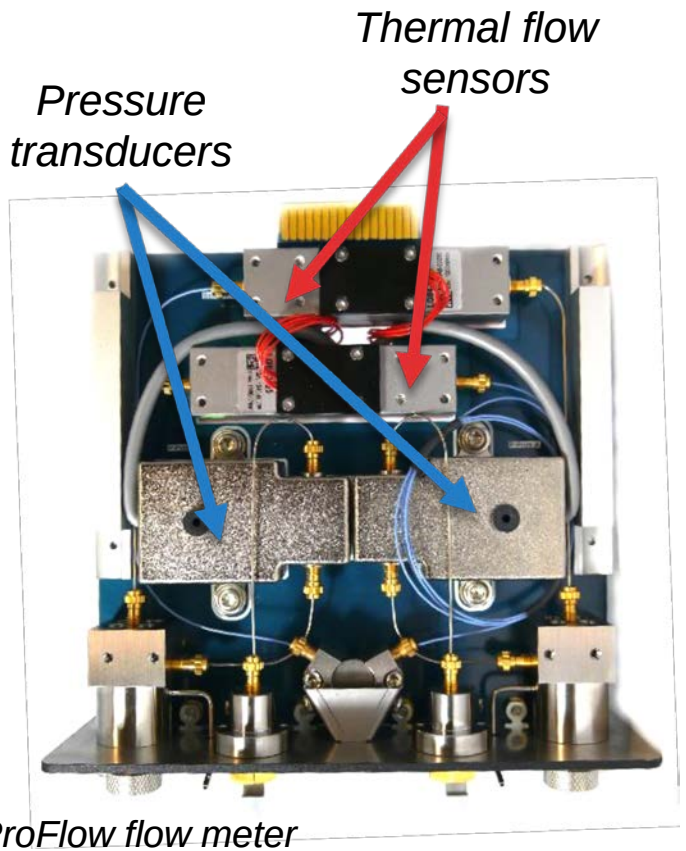
Nano Flow

New ProFlow technology

- ***Flow range:***
50-1500 nL/min (recommended)

- ***Max System Pressure:***
860 bar at full flow range

- Thermal flow sensors dedicated to nano flow rates provide exceptional flow precision
- Robust pressure transducers ensure long term system stability



Bottom view of ProFlow flow meter

Fast and simple system start-up and operation

- **Purging pump heads:**
when changing solvents - 15 min
when refreshing solvents - 5 min
- **Purging flow meter:** *10 min*
- **Equilibrating the System:** 30 min

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UltiMate 3000 RSLCnano: ProFlow Technology

NEW

ProFlow technology for a direct nano flow control

NCS-3500RS

UltiMate 3000 RSLCnano

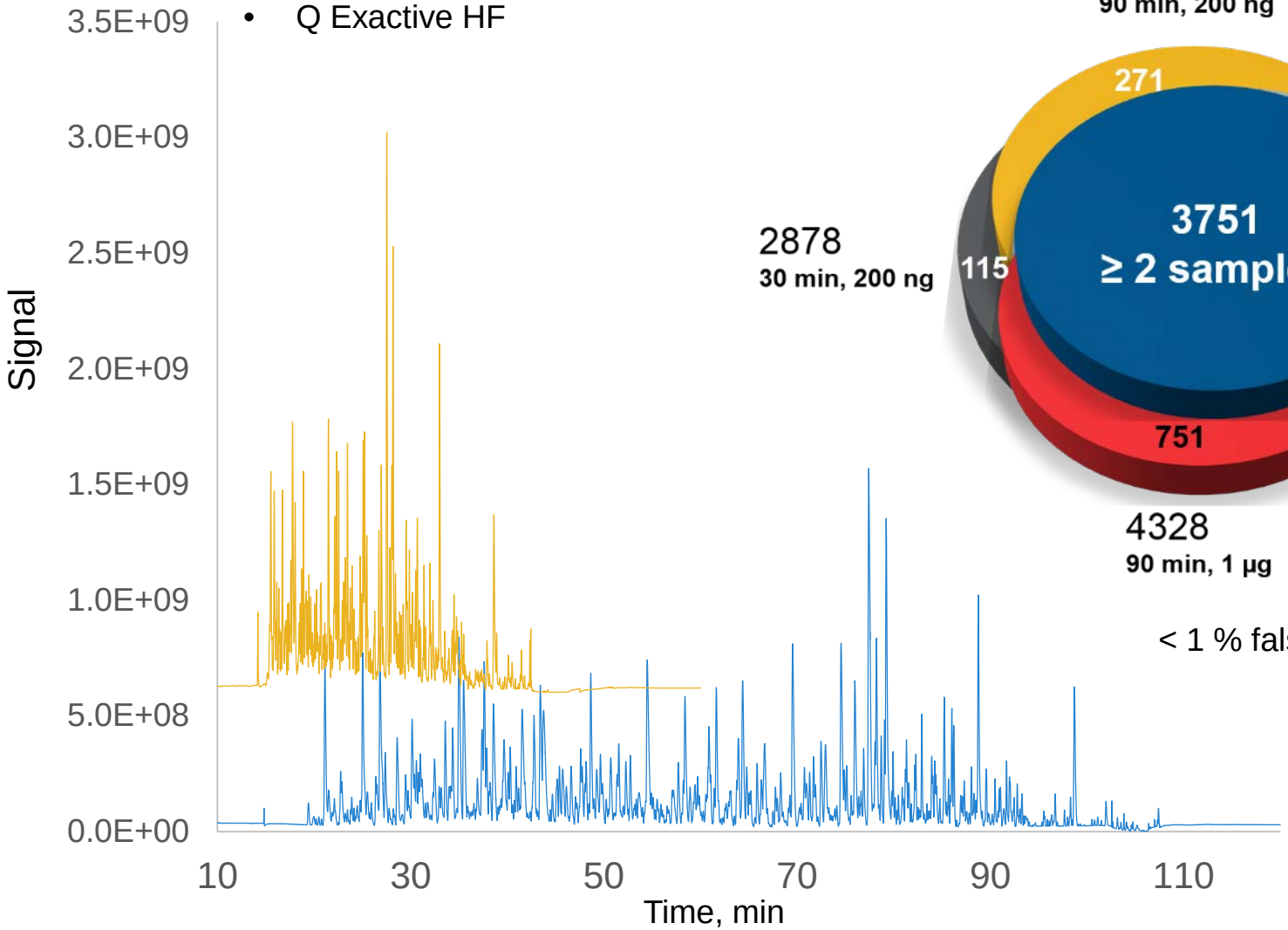
- Better user experience with fast and simple system start-up and operation
  for longer system uptimes and robust nano LC-MS data acquisition
- Enhanced reproducibility through uncompromised retention time precision
  ***for more confident identification and accurate quantification in large* cohorts**
- Wide nano flow – pressure footprint
  for better resolution with longer columns or high throughput
- Standard Instrument Integration (SII) for LC-MS control
  for seamless single software system operation

Applications: Proteomics

- **Nano LC-MS for shotgun proteomics**
- Label-free and targeted quantification
- High throughput analysis

RSLCnano and HR/AM MS for shotgun proteomics

- HeLa cell lysate digest
- EASY-Spray column: 75 µm x 50 cm, 2 µm
- Q Exactive HF



< 1 % false discovery rate (FDR)

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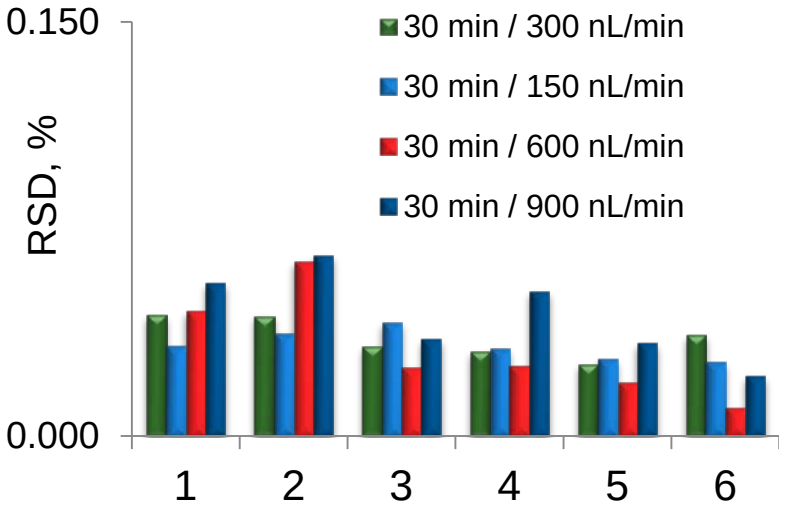
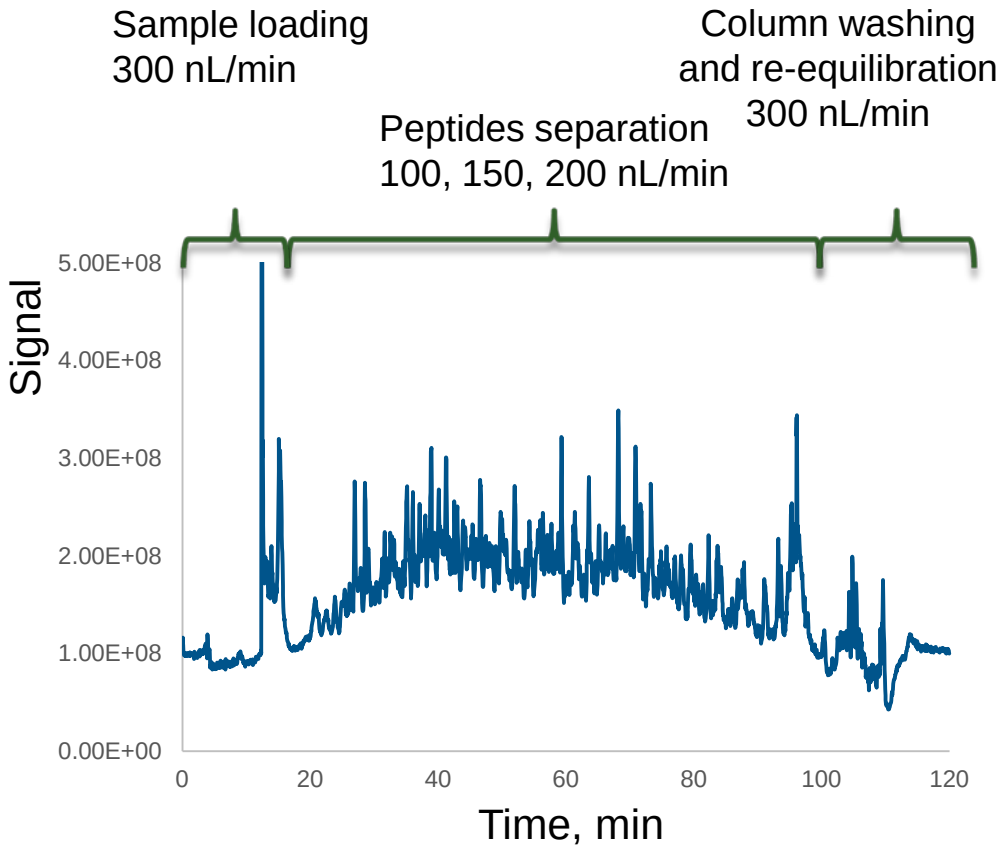
Enhanced sensitivity with low nano-flow rates

Retention time reproducibility



1	TGQAPGFSYTDANK
2	TGPNLHGLFGR
3	EDLIAYLK
4	Heme-CAQCHTVEK
5	GITWGEETLMEYLENPKK
6	GITWGEETLMEYLENPK

Separation conditions

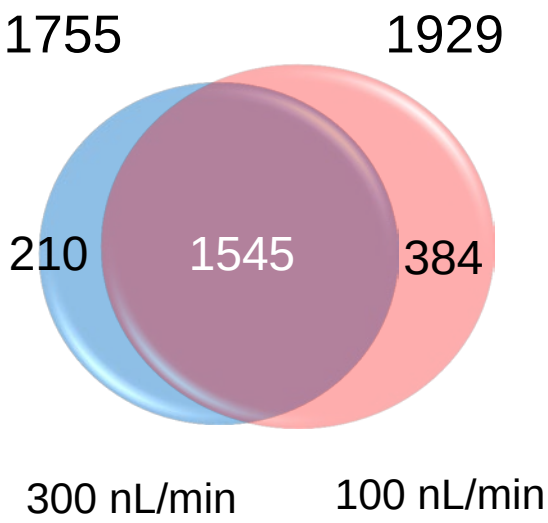


- RSD, % values were averaged for 3 RSLCnano systems with ProFlow flow meter
- Direct injection of Cytochrome C digest
- 12 replicates, 30 min gradient

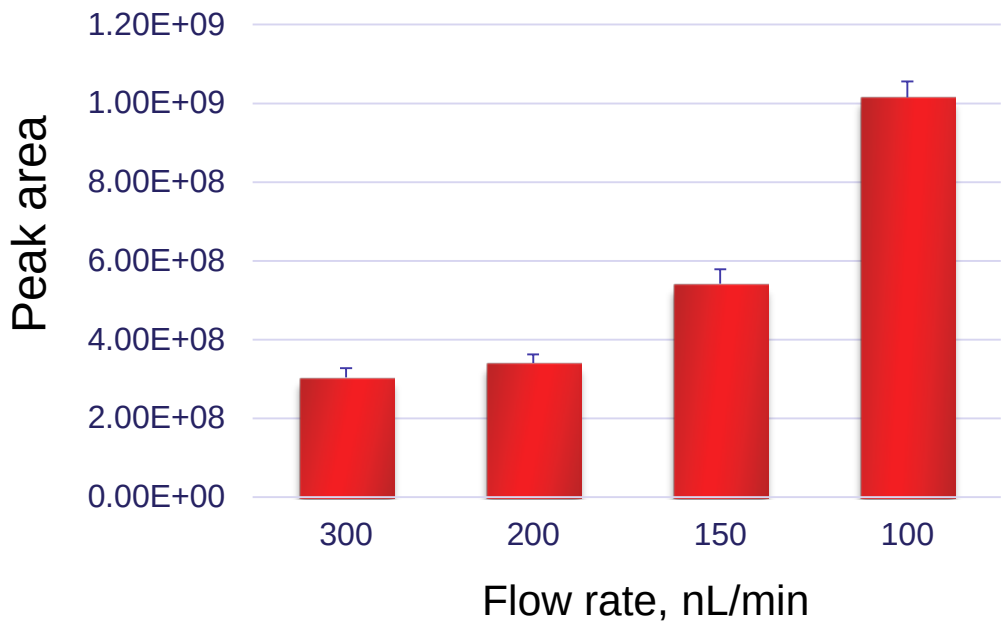
Analysis of small sample amounts

- Flow rates: 100 nL – 300 nL min
- 90 min gradient
- 75 µm x 15 cm, 2 µm Acclaim PepMap
- MS: Q Exactive HF

Protein IDs
20 ng injection



< 1 % false discovery rate (FDR)



Peptide: **R.GC[CAM]HLLVATPGR.L**

Protein: O00571 (DDX3X_HUMAN)

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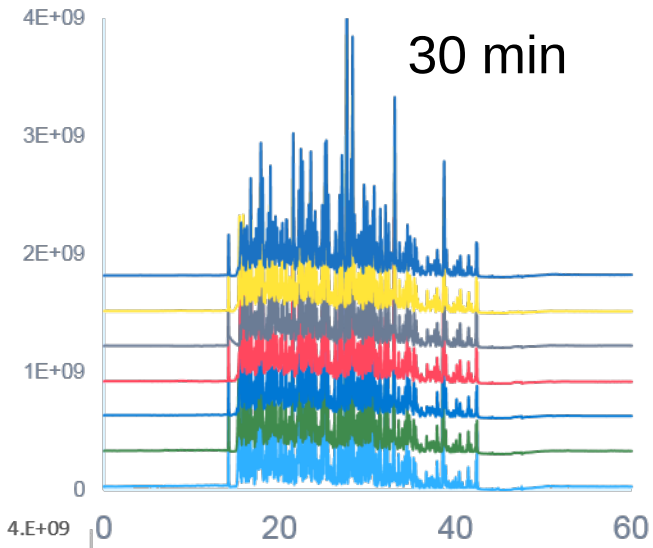
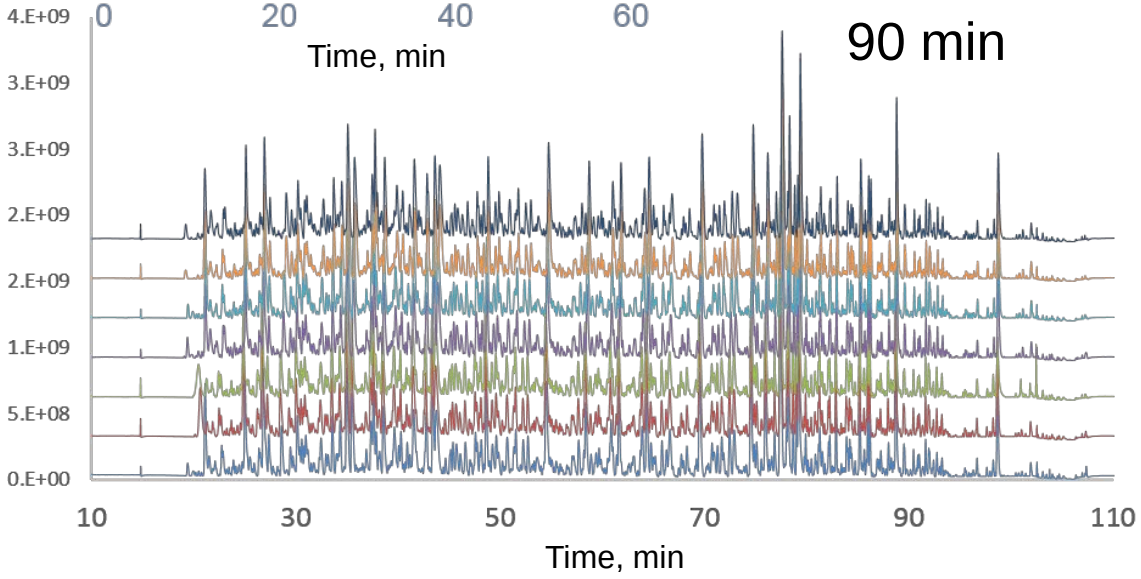
Applications: Proteomics

- Nano LC-MS for shotgun proteomics
- **Label-free and targeted quantification**
- High throughput analysis

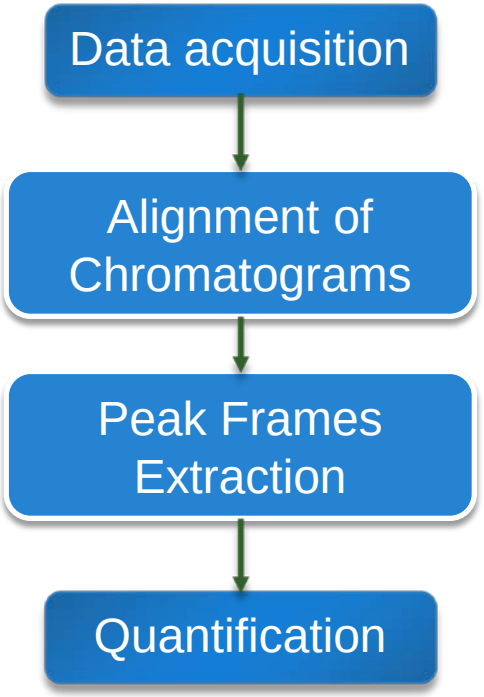
Label-free quantification: analytical variability

High precision of nano LC-MS label-free quantification





- 7 consecutive replicates
- HeLa cell lysate digest
- EASY-Spray 75 µm x 500 mm, 2 µm
- MS detection: Q Exactive HF
- 30 and 90 min gradient
- SIEVE 2.1 software



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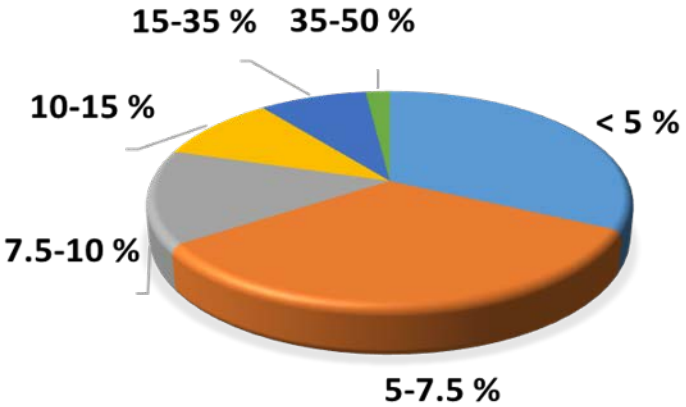
Label-free quantification: analytical variability

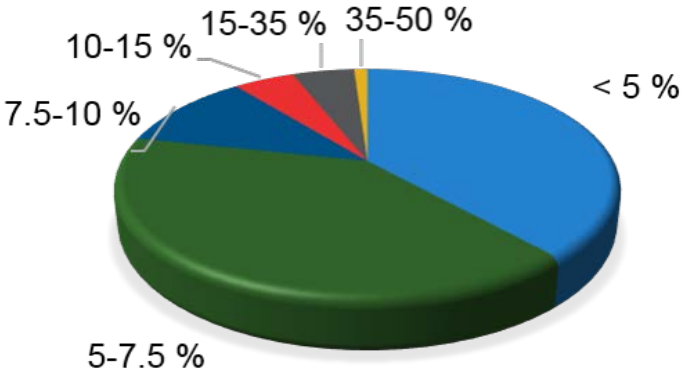
High precision of nano LC-MS label-free quantification

Analytical variability

> 40,000 targets were quantified with less than 15% peak area RSD



30 min gradient



90 min gradient

Number of peak frames that were quantified with corresponding peak area RSD, % values for 7 replicates

50,000 Peak frames in total

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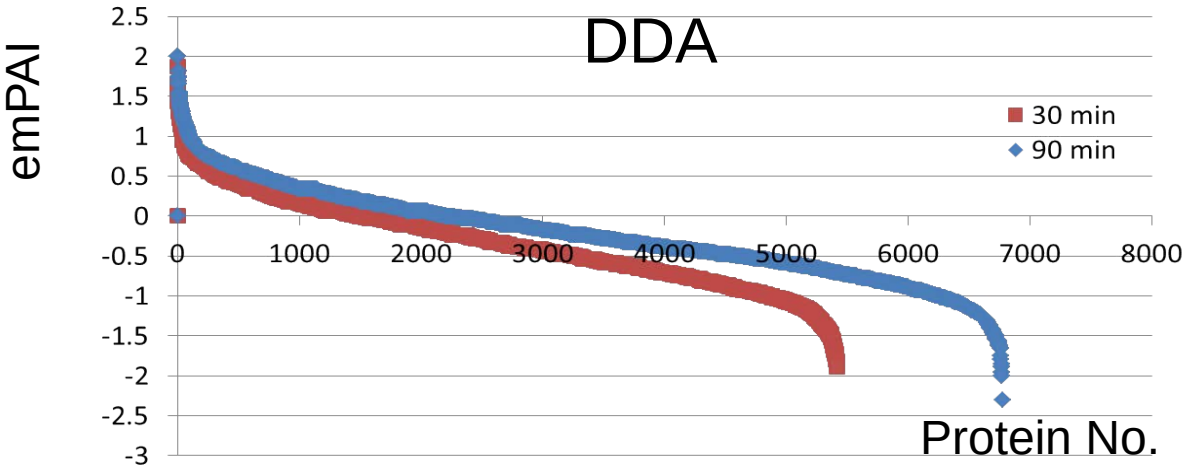
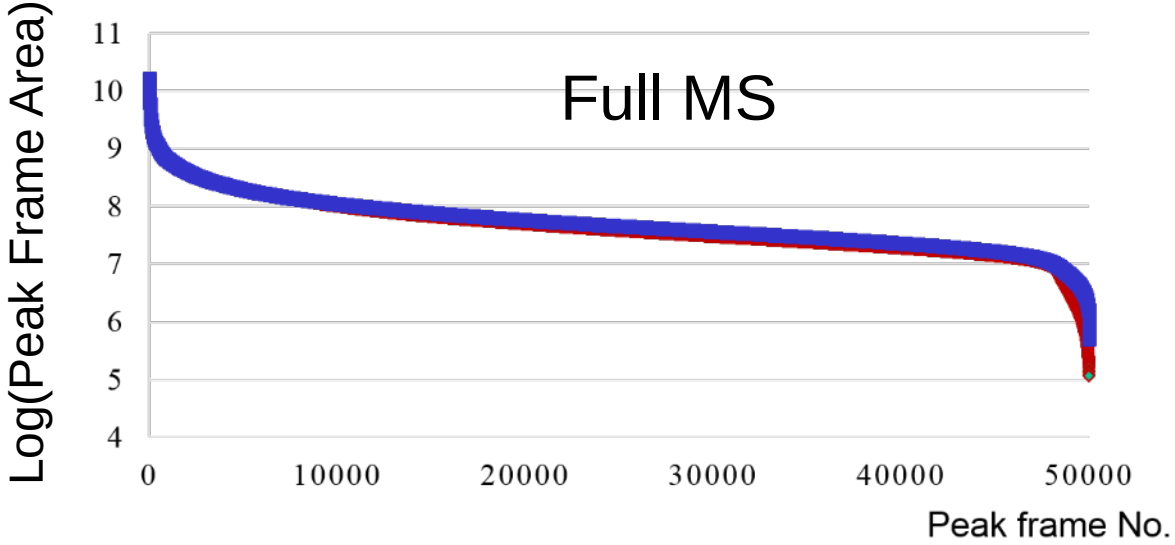
Label-free quantification: deepness of proteome profiling



Log transformed and sorted exponentially modified protein abundance index (emPAI)

4 orders of magnitude

30 min (red) and 90 minute (blue) gradient data.



Log transformed and sorted Peak Frame Areas

> 5 orders of magnitude

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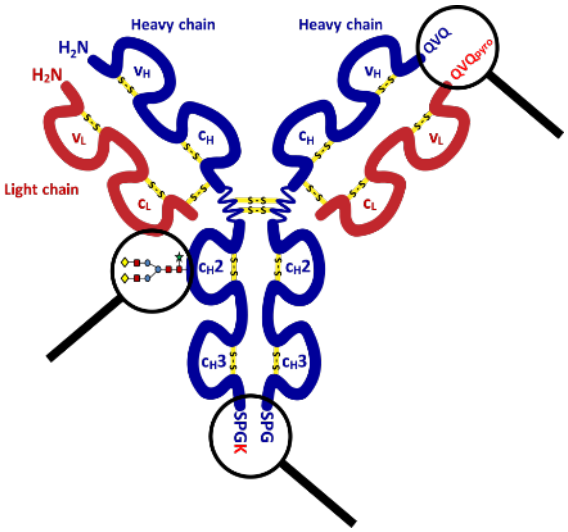
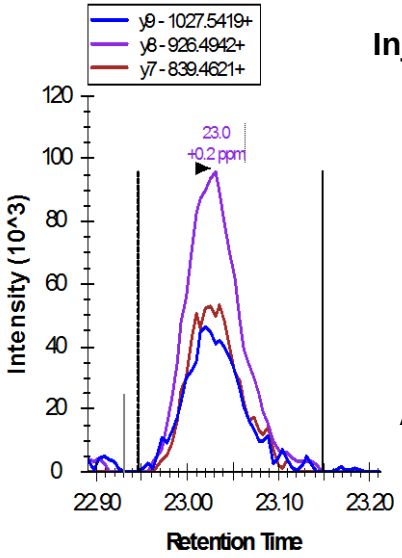
Nano LC HR/AM MS targeted quantification

Multiplexed scheduled PRM analysis of rituximab in human matrix

Heavy chain: **5 unique peptides**
Light chain: **3 unique peptides**



FSGSGSGTSYSLTISR



Inj. Number	Retention time, min
1	23.00
2	23.05
3	23.01
4	23.02
5	23.08
6	23.03
7	23.00
Average	23.03
STD	0.03
RSD, %	0.12

- 7 consecutive replicates
- EASY-Spray 75 µm x 500 mm, 2 µm
- MS detection: Q Exactive HF
- 4 sec peak width
- 30 min gradient

1:10^4 rituximab to HeLa total protein amount

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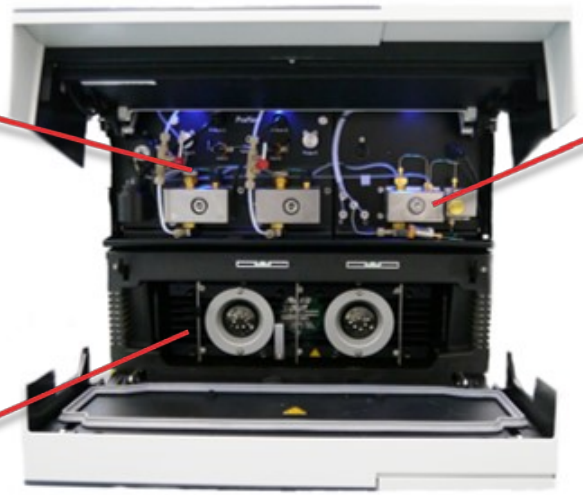
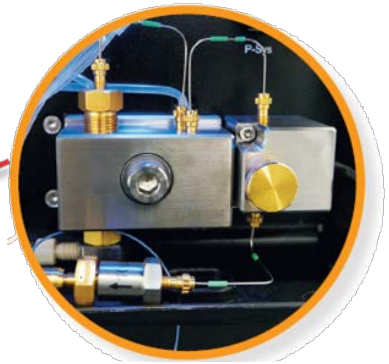
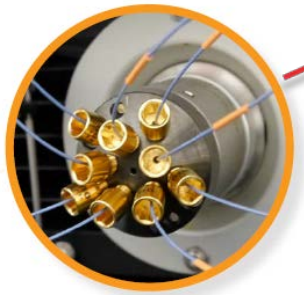
Applications: Proteomics

- Nano LC-MS for shotgun proteomics
- Label-free and targeted quantification
- **High throughput analysis**

Fast separations with ProFlow Technology





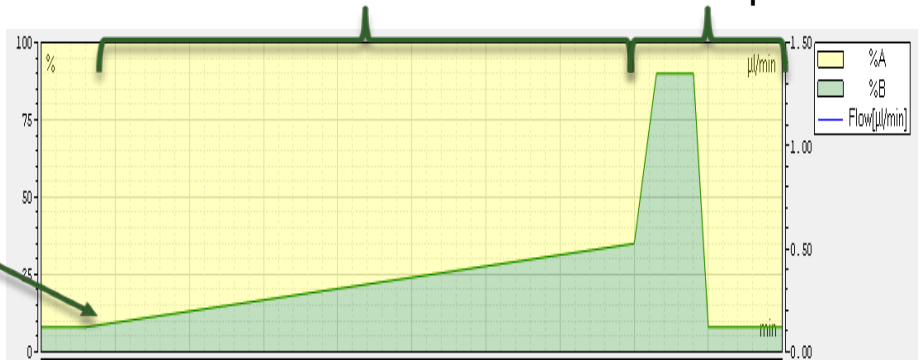


- 5 min total analysis time
- MS detection: Q Exactive HF
- 75 µm x 15 cm Acclaim PepMap
- 20 µL sample loop

Fast sample loading onto trap column with ternary micro pump
80 µL/min

Elution of peptides
Flow rate: 1500 nL/min

Column washing and re-equilibration



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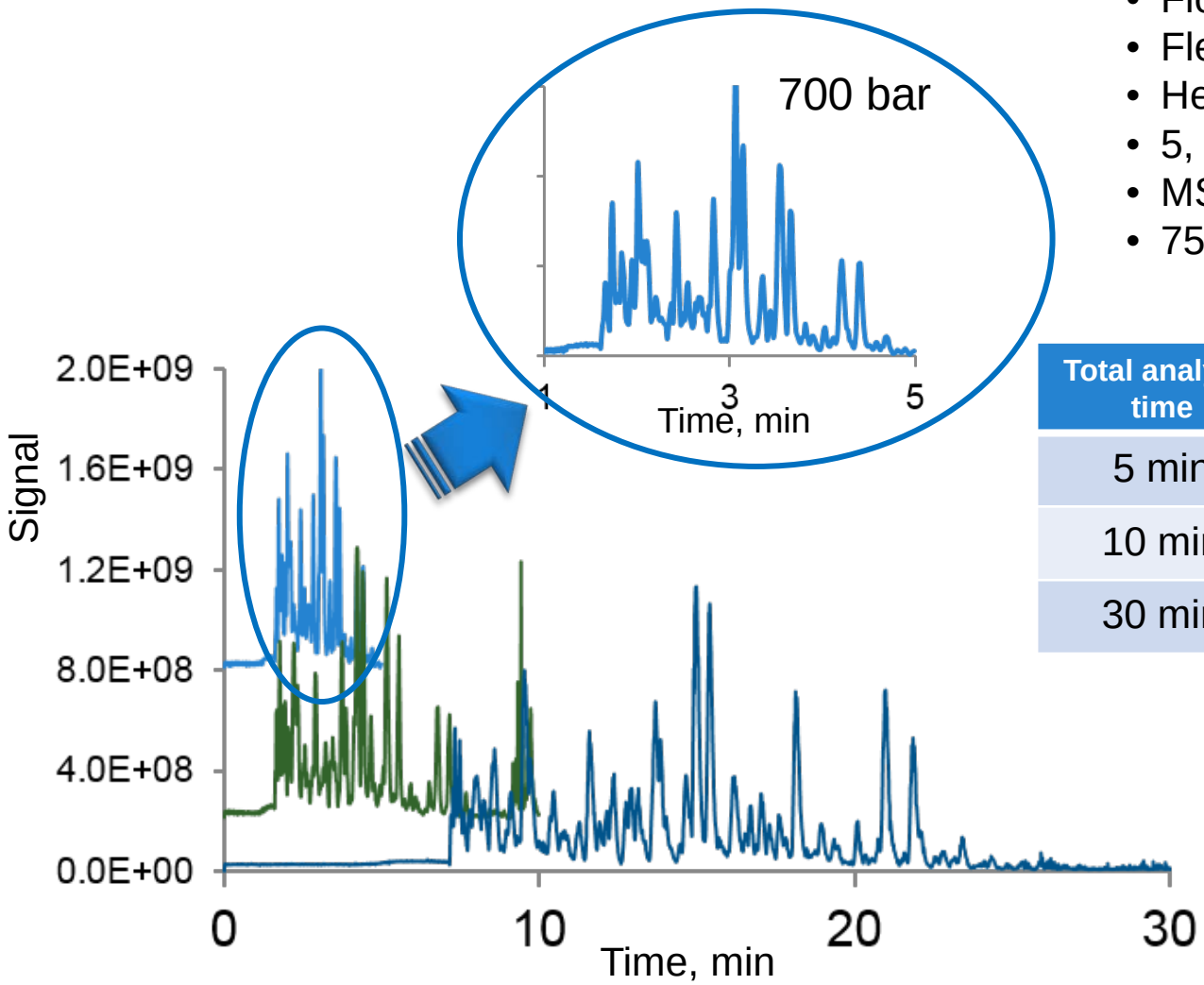
Fast gradients with ProFlow Technology

Wide flow – pressure footprint for a high throughput nano LC-MS analysis



- Flow rate: 1,500 nL/min
- Flex ion source with metal emitter
- HeLa cell lysate digest
- 5, 10 and 30 min total analysis time
- MS detection: Q Exactive HF
- 75 µm x 15 cm Acclaim PepMap

Total analysis time	# of proteins	# of peptides
5 min	635	2406
10 min	982	4092
30 min	1494	6694

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The ultimate solution for all low flow workflows

- Wide UHPLC footprint to tune for highest resolution or throughput
- New ProFlow technology for best-in-class retention time precision in nano LC applications
- Straightforward operation and integration with Thermo Scientific mass spectrometers

UltiMate 3000 RSLCnano with ProFlow technology

The standard proteomics workflow

LC-MS
Data Acquisition

Data Analysis
- Identification
- Quantitation

Discovery, Publication, Patent

Sample Preparation
- Extraction
- Digest
- Enrichment / Fractionation

EASY-nLC 1200 – Design Concept & Established Features

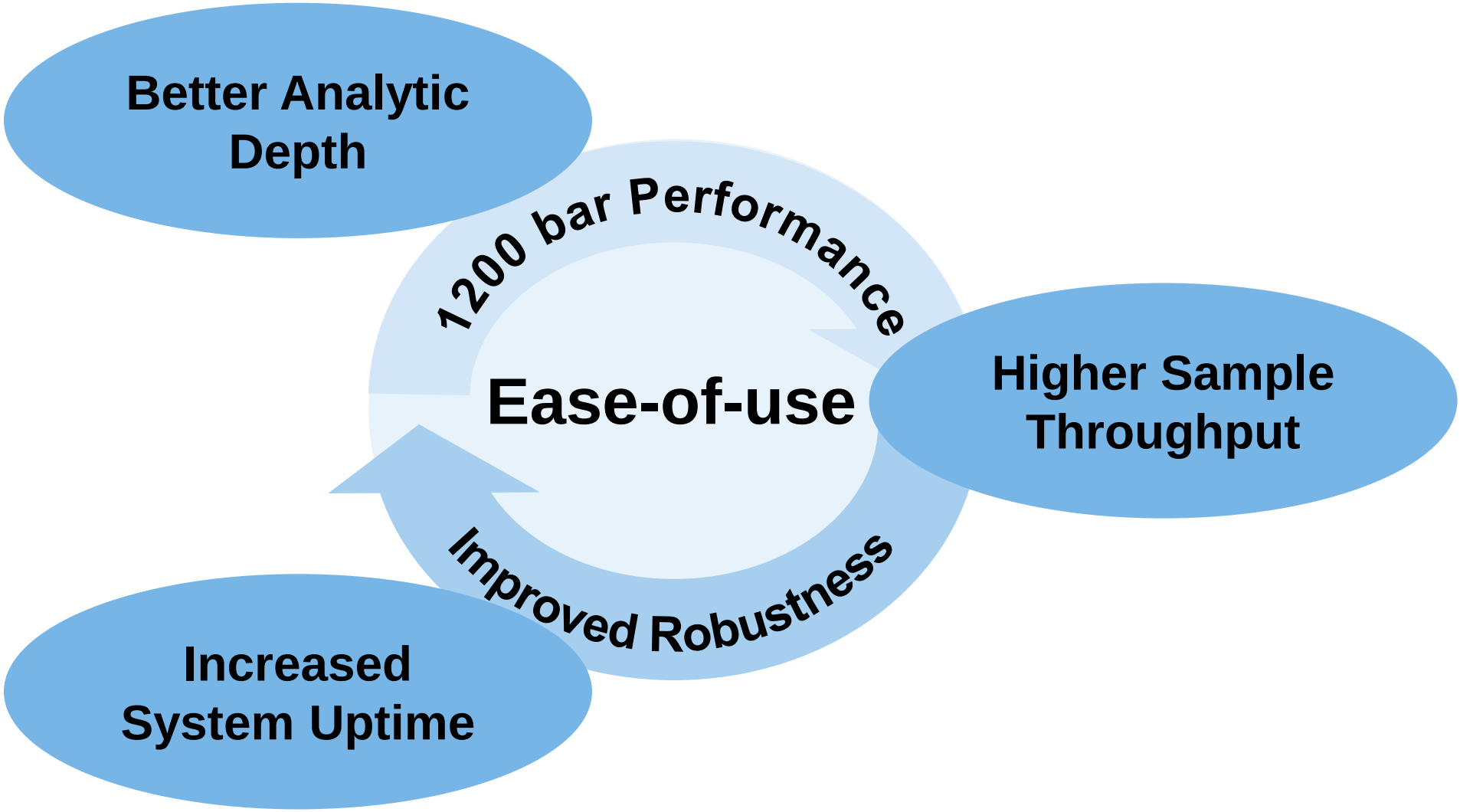
Design Concept

- Integrated System
- Ease-of-use with intuitive system operation
- Wizard style method set-up
- Easy diagnostics and troubleshooting

Established features

- Layout of flow path
- Autosampler with zero sample loss injections
- Integrated Computer & Touchscreen
- Small footprint

EASY-nLC 1200 – Design Rationale for New System



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EASY-nLC 1200 – **New** Features

- 1200 bar system pressure
- Complete nanoViper™ high pressure flow-path
- Maintenance-free valves
- Improved software
  - same concept, new features
- Connection to EASY-Spray™ source for temperature control

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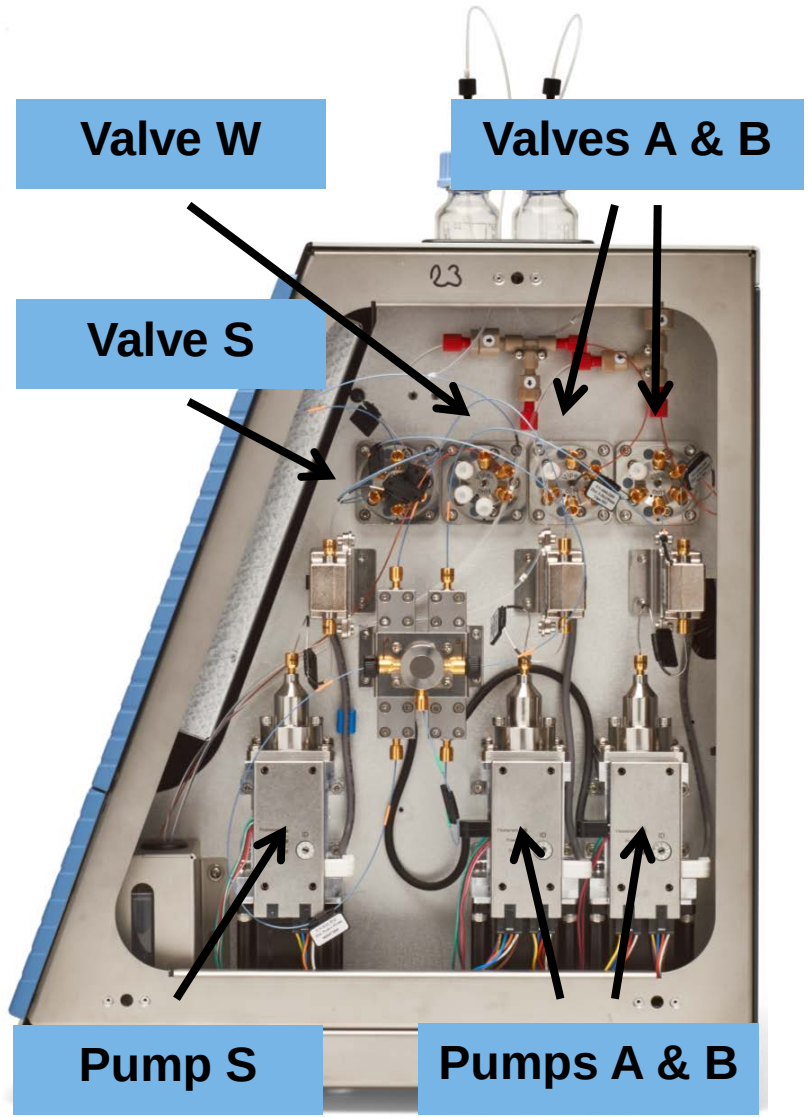
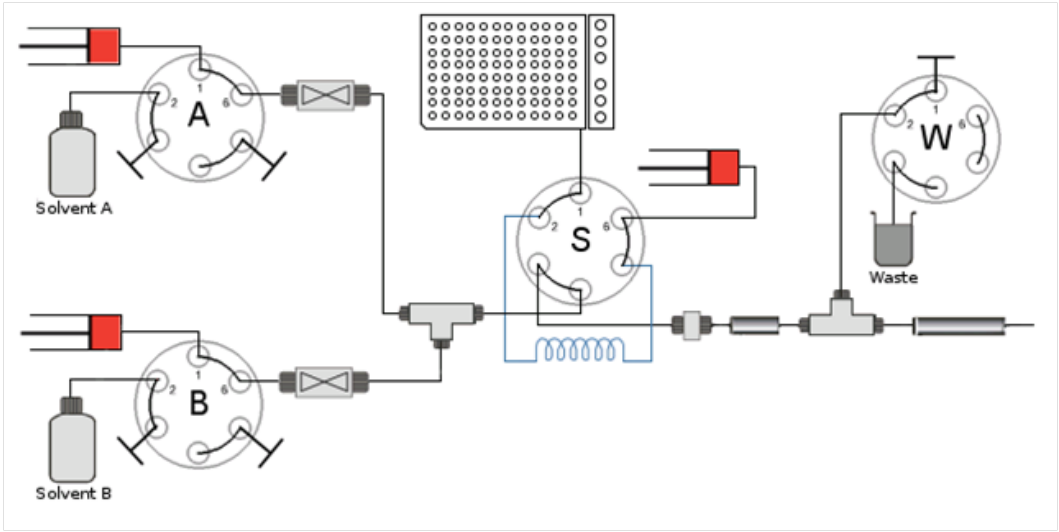
EASY-nLC 1200 – Hardware - Software Integration



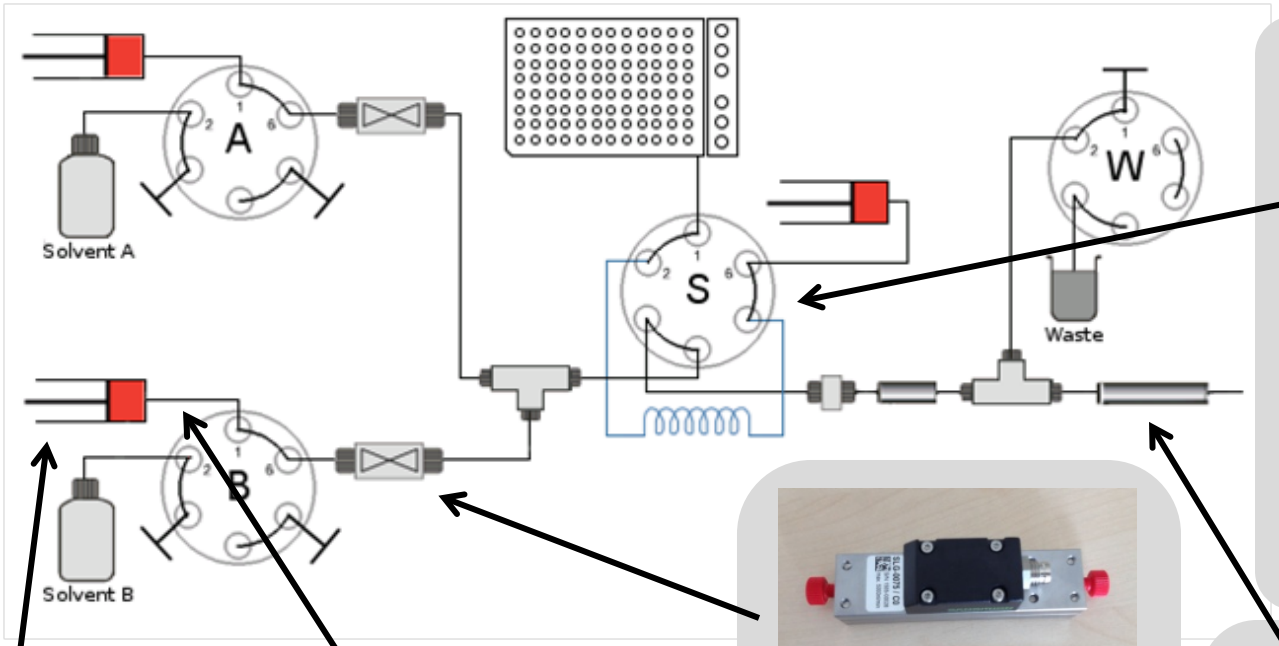
On Screen Display



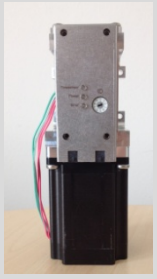
- Actual flow path is displayed on screen
- Components can be interactively controlled by touching them on the screen

EASY-nLC 1200 – **New** Hardware Features

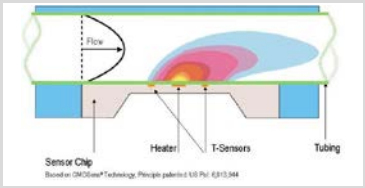
All connections in high pressure flow-path now 1200 bar nanoViper™
(Blue capillary sleeves indicate new pressure rating)



New 1200 bar ceramic valves (all 4)
- Maintenance-free
- nanoViper only
- ≤95% ACN required



New 1200 bar pumps and pressure sensors



New 1200 bar flow sensors

New 1200 bar EASY-Spray™ columns

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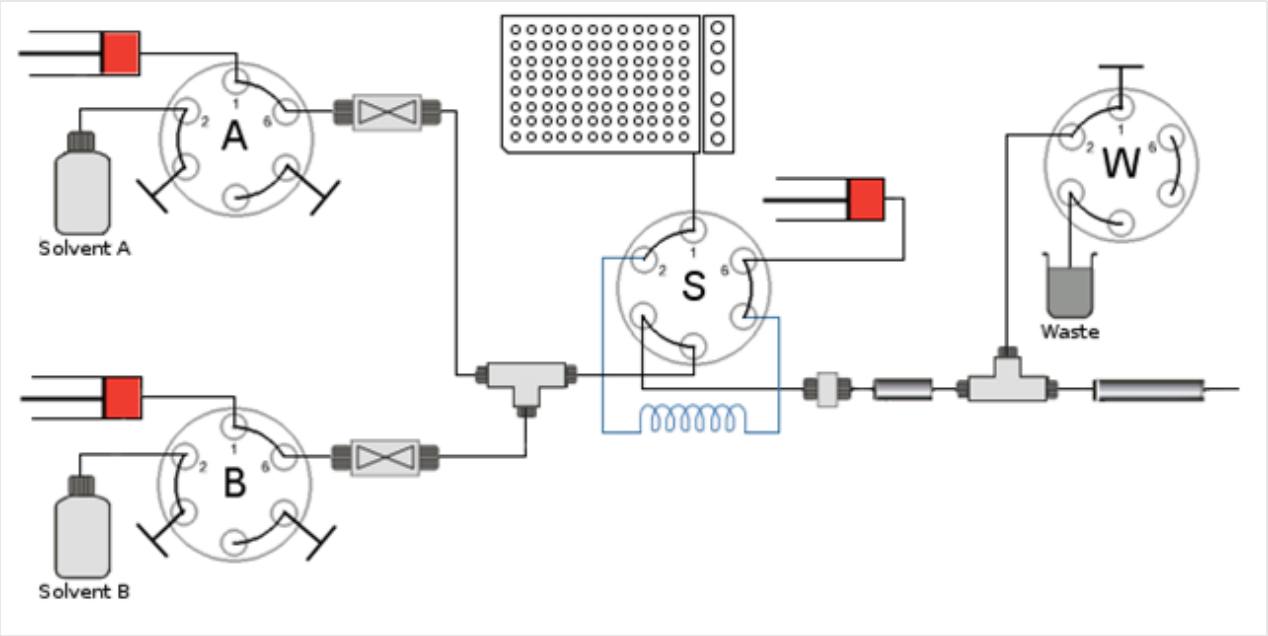
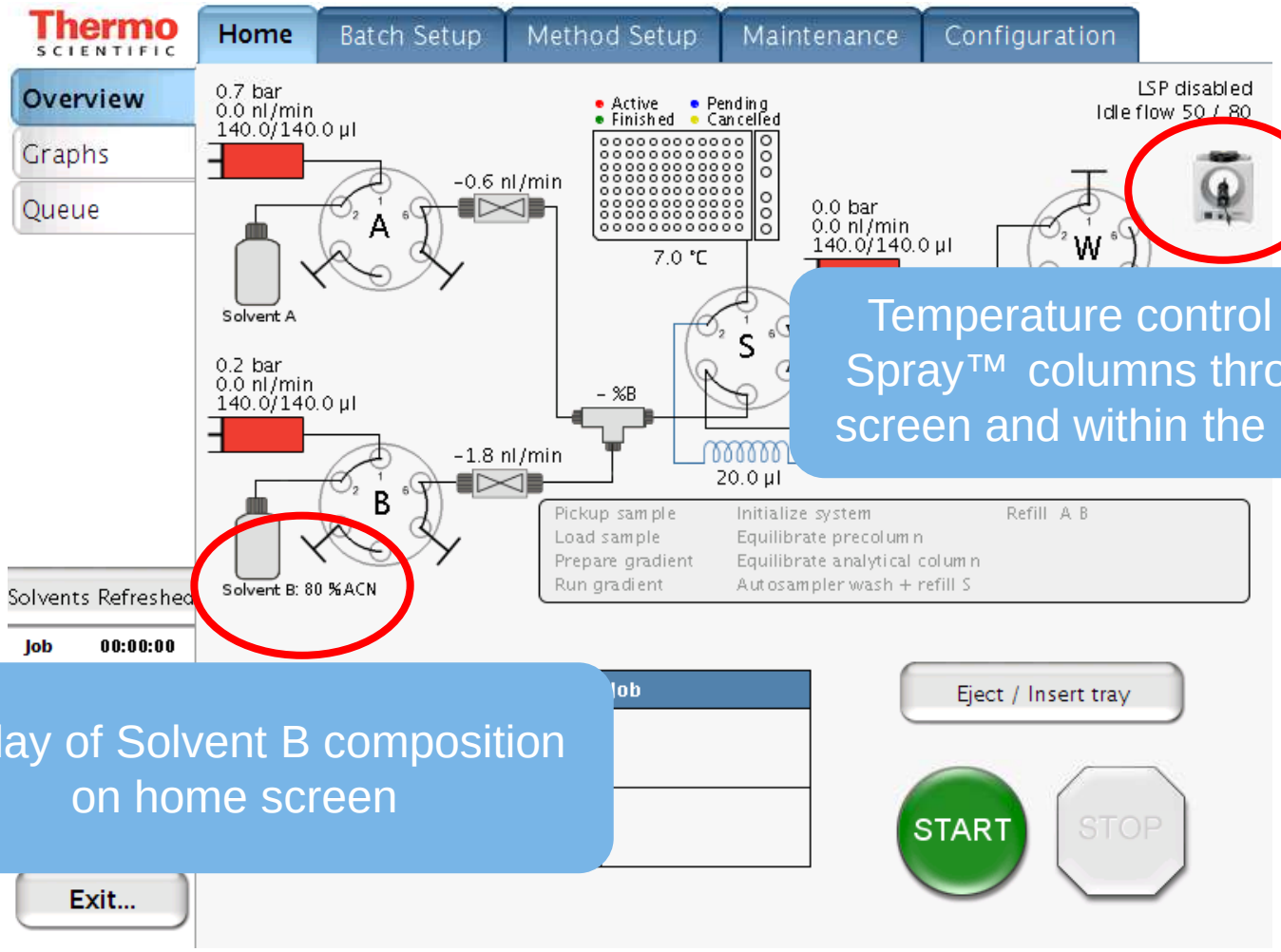
EASY-nLC 1200 – **New** Hardware Features



1200 bar pressure rating	• **Higher sample throughput** • **Better analytic depth**
New components, e.g. valves	• **Improved system robustness**

EASY-nLC 1200 – **New** Software Features



Temperature control of EASY-Spray™ columns through home screen and within the LC method

Display of Solvent B composition on home screen

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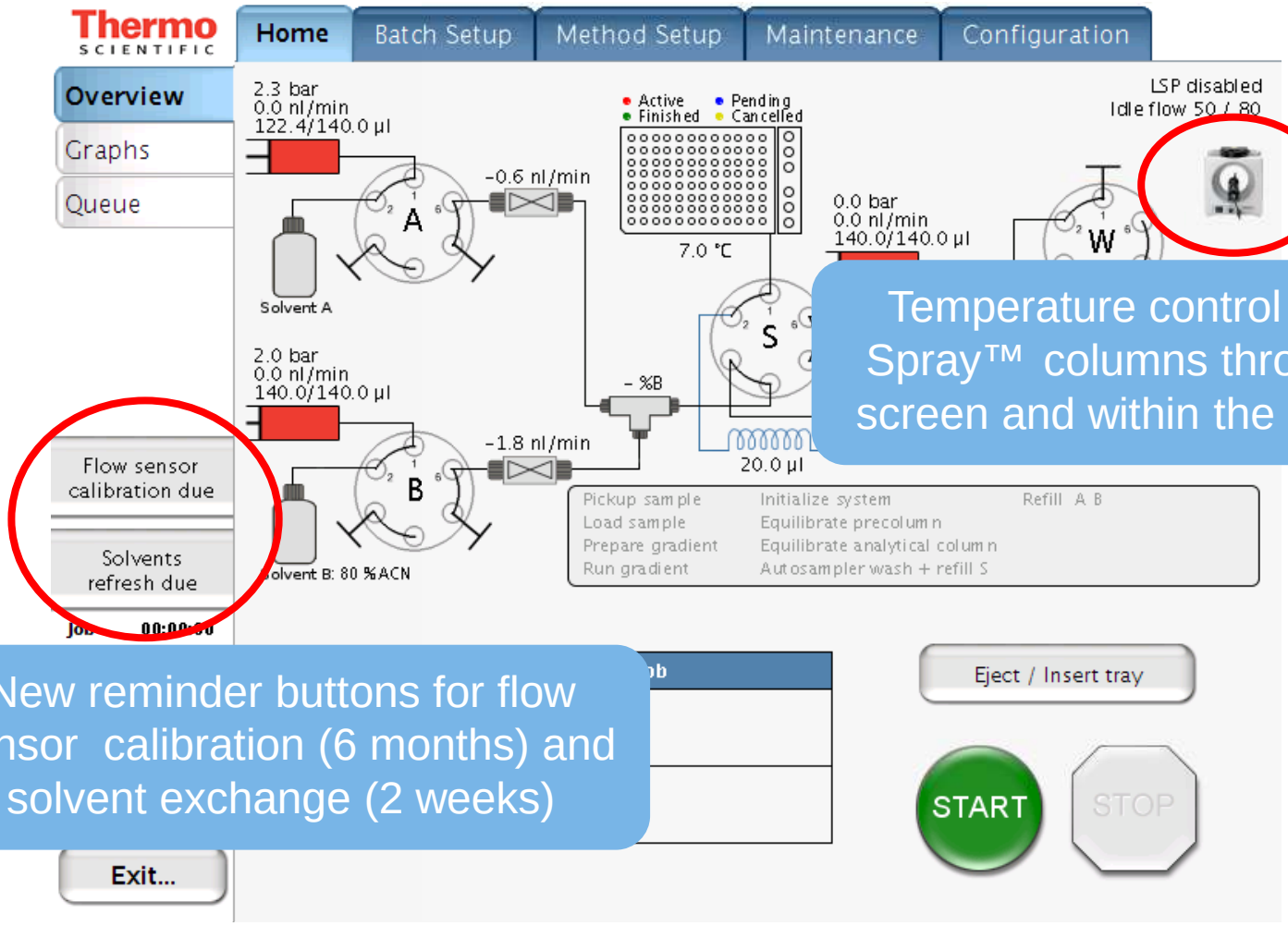
EASY-nLC 1200 – **New** Software Features



Temperature control of EASY-Spray™ columns through home screen and within the LC method

New reminder buttons for flow sensor calibration (6 months) and solvent exchange (2 weeks)

- Easier control of system parameters

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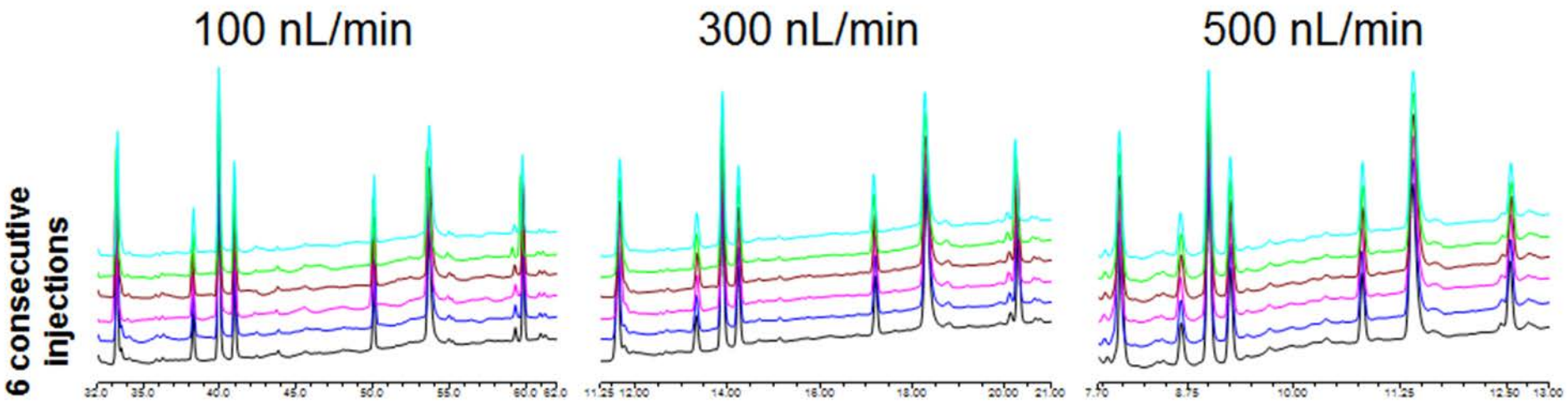
Good Retention Time Precision

Chromatographic separation with standard nano LC flow rates



1 pmol Cytochrome C digest, λ = 214 nm

Peak	# 1	# 2	# 3	# 4	# 5	# 6	# 7
RT (min)	33.3	38.3	40.0	41.0	50.1	53.7	59.8
RSD (%)	**0.12**	**0.07**	**0.10**	**0.08**	**0.07**	**0.05**	**0.09**

Peak	# 1	# 2	# 3	# 4	# 5	# 6	# 7
RT (min)	11.7	13.4	14.0	14.3	17.3	18.4	20.3
RSD (%)	**0.56**	**0.39**	**0.33**	**0.34**	**0.18**	**0.19**	**0.11**

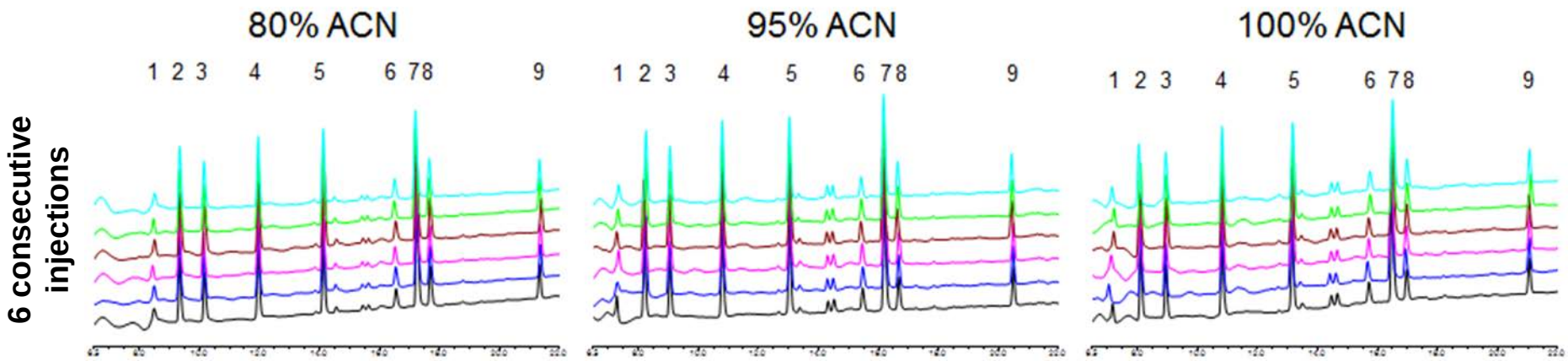
Peak	# 1	# 2	# 3	# 4	# 5	# 6	# 7
RT (min)	8.0	8.7	9.0	9.3	10.8	11.4	12.6
RSD (%)	**0.10**	**0.05**	**0.06**	**0.07**	**0.08**	**0.09**	**0.08**

- Retention time precision <0.4% RSD for flow rates relevant in nano LC-MS applications
- High run-to-run repeatability improves confidence in peptide quantification

80% Acetonitrile as Solvent B

- Reducing acetonitrile concentration is essential for long valve lifetime
  - Specification: ≤95% acetonitrile in water
- Reduced acetonitrile concentration improves chromatographic performance

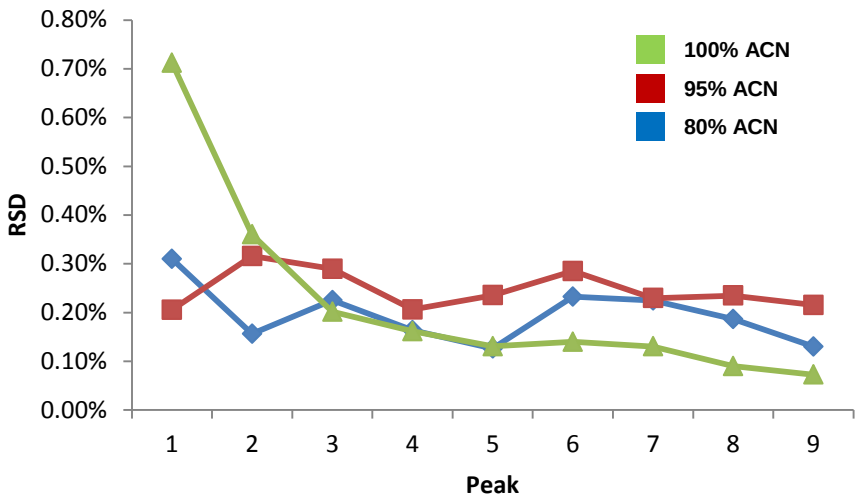
Chromatographic separation with different solvent B compositions



1 pmol Cytochrome C digest
300 nL/min
30 min gradient (adjusted for consistent ACN concentration between experiments)
λ = 214 nm

See Application Note **AN637**

80% Acetonitrile as Solvent B



		Peak								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
80% ACN	RT (min)	8.5	9.3	10.1	11.9	14.1	16.5	17.2	17.7	21.3
	RSD (%)	**0.31**	**0.16**	0.23	0.16	0.13	0.23	0.22	0.19	0.13
95% ACN	RT (min)	7.3	8.2	9.0	10.8	13.0	15.4	16.1	16.6	20.4
	RSD (%)	**0.21**	**0.32**	0.29	0.21	0.24	0.29	0.23	0.23	0.22
100 % ACN	RT (min)	7.2	8.1	8.9	10.8	13.2	15.7	16.5	17.0	21.1
	RSD (%)	**0.71**	**0.36**	0.20	0.16	0.13	0.14	0.13	0.09	0.07

- Better peptide retention with lower acetonitrile concentration at onset of gradient
  - Lower acetonitrile at start of gradient possible (EASY-nLC 1200 aligns gradient with 2% B)
  - Better mixing performance

- **Using 80% acetonitrile will improve data quality**
  - 80% Acetonitrile, 20% water, 0.1% formic acid available through Fisher Scientific (LS122-500, 500 mL bottle)

See Application Note **AN637**

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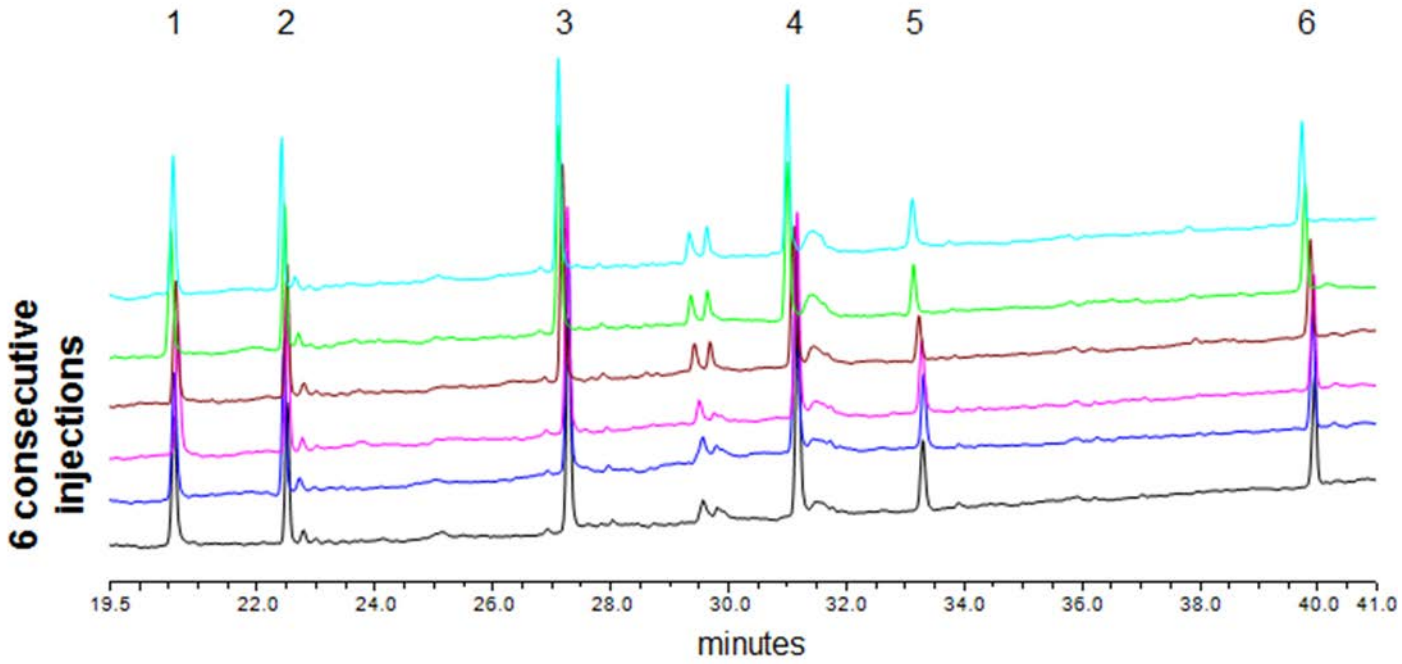
80% Acetonitrile as Solvent B

Retention time stability throughout 135 injections (8 days of measurement)

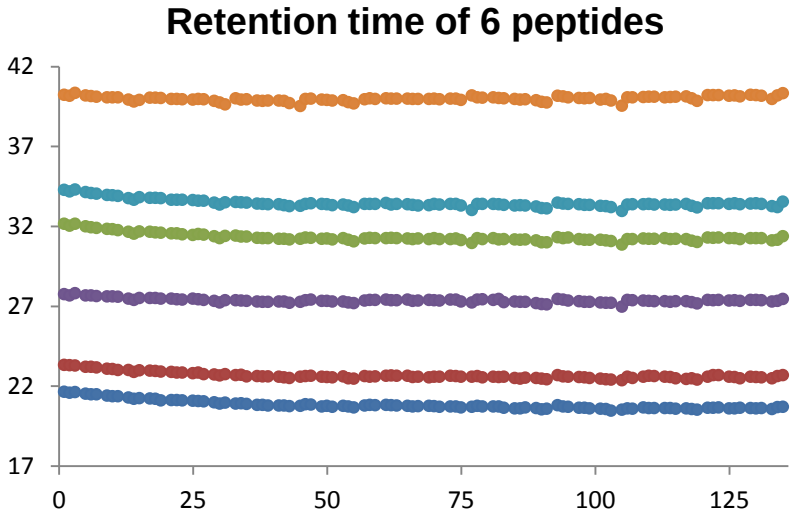


1 pmol Cytochrome C digest
75cm Acclaim™ PepMap™ column
300 nL/min gradient flow
60 min gradient
1180 bar sample loading and column equilibration
900 bar gradient maximum pressure
λ = 214 nm

See Application Note **AN637**

80% Acetonitrile as Solvent B

Retention time stability throughout 135 injections (8 days of measurement)



	Peaks					
	1	2	3	4	5	6
All injections						
RT (min)	20.82	22.66	27.36	31.31	33.45	39.98
RSD	1.32%	0.92%	0.48%	0.79%	0.74%	0.37%
6 consecutive injections during the sequence (injections 64 – 69)						
RT (min)	20.59	22.48	27.21	31.10	33.23	39.86
RSD	0.20%	0.17%	0.29%	0.26%	0.25%	0.22%

- Good retention time stability over the whole sequence of injections
- **Long injections sequences can be reproducibly run on the EASY-nLC 1200**

See Application Note **AN637**

Increase Throughput with IFC™

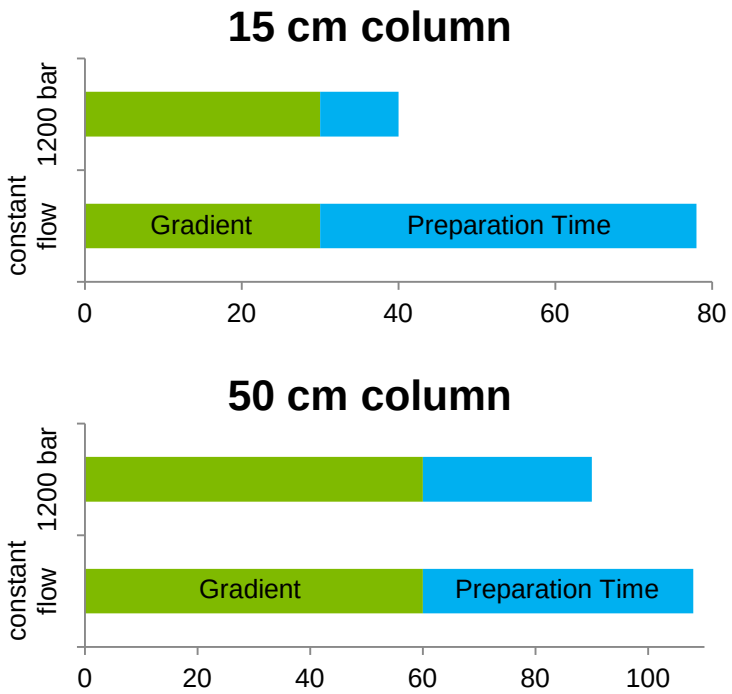
Sample loading and column equillibration are major contributors to cycle time in nano LC-MS analyses

Accelerate sample loading and column equilibration with Intelligent Flow Control (IFC™):

flow rate determined by maximum system pressure

Increase sample throughput

without compromising on data quality



	15 cm column		50 cm column	
Loading & equilibration mode	Constant flow (300 nL/min)	IFC™ (1200 bar)	Constant flow (300 nL/min)	IFC™ (1200 bar)
Gradient length	30 min	30 min	60 min	60 min
Column equilibration, sample pick-up and loading	48 min	10 min	48 min	30 min
Cycle time	78 min	40 min	108 min	90 min
Injections per day	18.5	36.0	13.3	16.0
Additional throughput increase (%)		**49%**		**17%**

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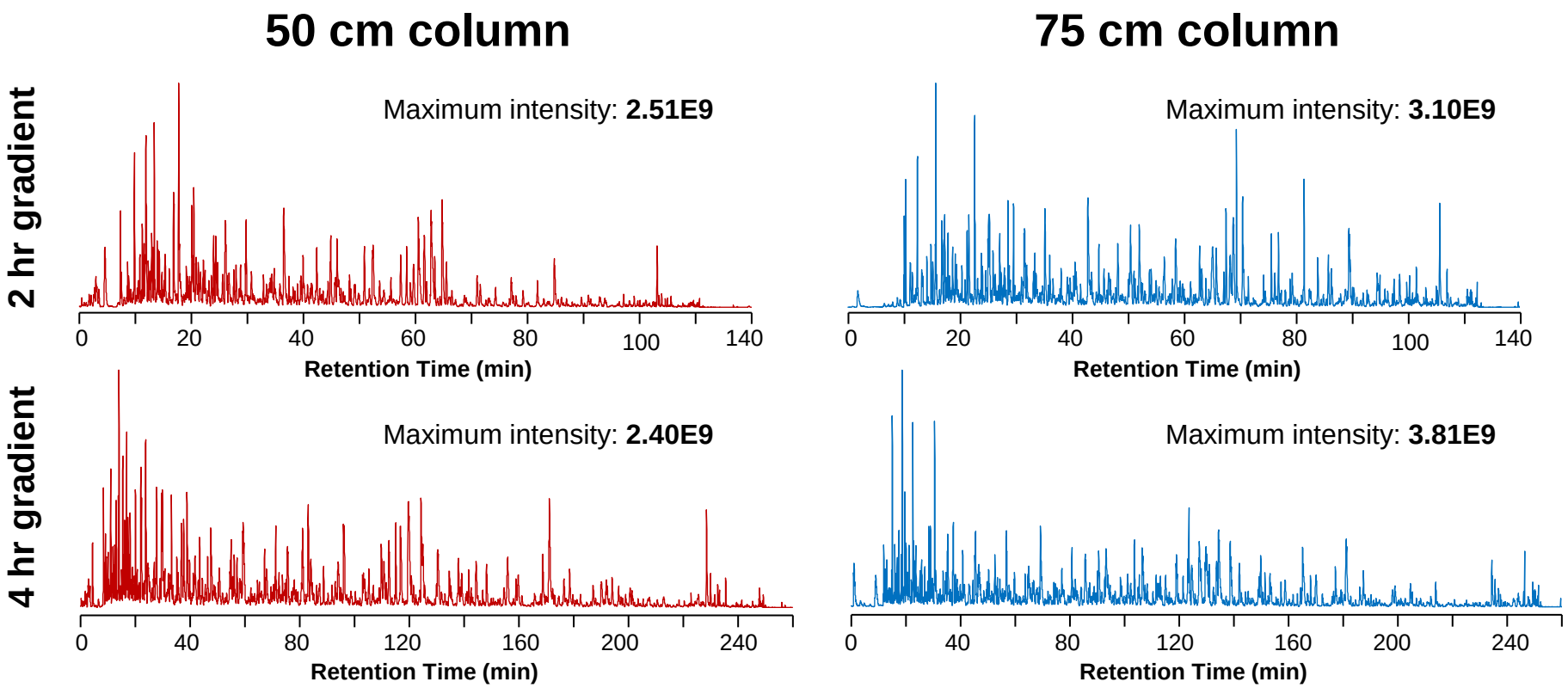
Increased Protein Identification Rates with 75 cm Columns

- Comparison of 75 cm and 50 cm EASY-Spray column
  - Sample: HeLa Digest (1 µg)
  - Gradient: 120 min or 240 min



See Application Note **AN639** and Poster Note **PN64629**

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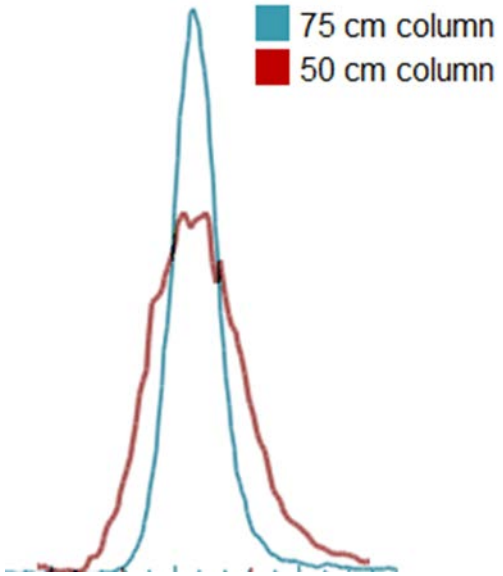
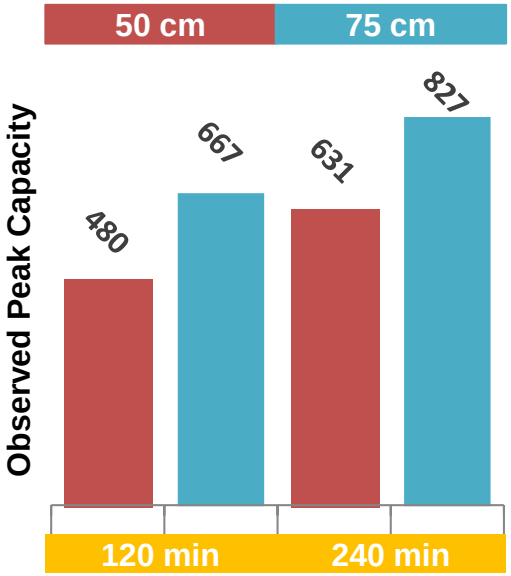
Increased Protein Identification Rates with 75 cm Columns

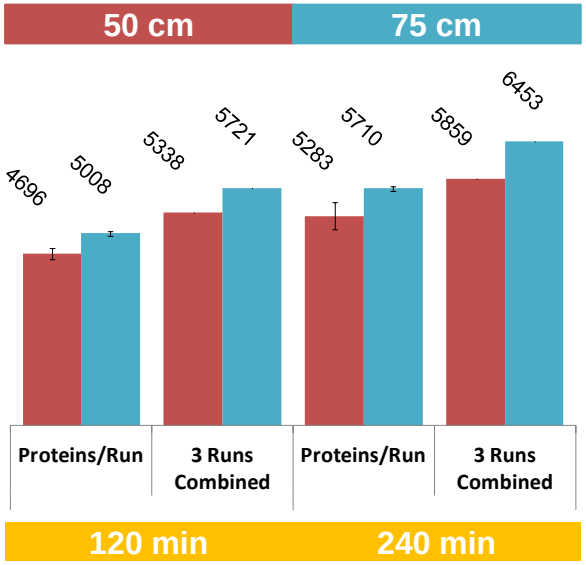
Peak Width



Peak Capacity



Identified Proteins



- Narrower peak width in 75 cm column increases peak capacity
- ~10% higher protein identifications with 75 cm columns possible

See Application Note **AN639** and Poster Note **PN64629**

Effortless ultra high performance

- Optimized for proteomics
- Intuitive system operations
- For every level of expertise

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Conclusion

- Thermo Fisher Scientific offers the right solution for every nano flow application
  - New nano LC technologies for robust and precise nano flow delivery
  - nanoViper connections for fast and reliable fluidics set-up
- New ProFlow technology improves retention time stability
  - more confidence in your analyte identifications
  - better precision for label-free and targeted quantitation
  - increased sensitivity with low nano flow rates
- New EASY-nLC 1200 provides new levels of system performance
  - increased throughput with better system robustness and higher system pressure
  - increased analytic depth with longer analytical columns

Thank you for your attention

- Questions

- Online resources
  - Thermoscientific.com/nanoLCMS
  - Planetorbitrap.com

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