



ThermoFisher
S C I E N T I F I C

The Thermo Scientific Capillary-Flow LC-MS Solutions

The world leader in serving science

- Thermo Scientific™ LC-MS front-end UHPLC portfolio
- Advantages of CapLC-MS and Fields of Application
- The Thermo Scientific™ CapLC-MS solution
- Application Examples
- Available Additional Material
- Frequently Asked Questions & Answers

Thermo Scientific Low-Flow UHPLC Portfolio



Thermo Scientific™ UltiMate™ 3000 RSLCnano System

*Versatility and unsurpassed
precision*

Nano, capillary and micro-flow
applications



Thermo Scientific™ EASY-nLC™ 1200 System

*Operational simplicity and
excellent performance*

Nano-flow applications

MS Front-End Portfolio from a Flow Rate Perspective

EASY-nLC 1200



UltiMate 3000 RSLCnano



- Thermo Scientific™ Vanquish™ Horizon system
- Thermo Scientific™ Vanquish™ Flex system
- Thermo Scientific™ UltiMate™ 3000 RSLC system
- Thermo Scientific™ UltiMate™ BioRS system
- Thermo Scientific™ UltiMate™ SD system



Nano: <math><1.5 \mu\text{L}/\text{min}</math>

Capillary: 1-15 $\mu\text{L}/\text{min}$

Micro: 10-50 $\mu\text{L}/\text{min}$

Analytical: > 100 $\mu\text{L}/\text{min}$

high throughput & high sensitivity

50 μm 75 μm 150 μm 300 μm

0.5 mm

1.0 mm

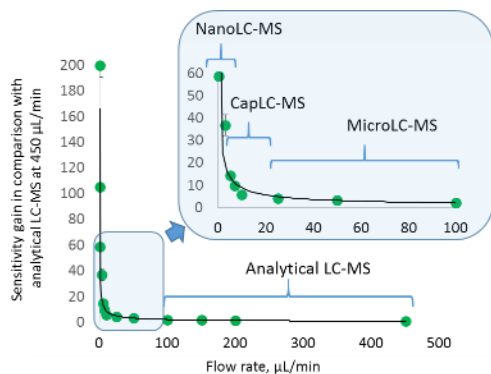
... 2.1- 4.6 mm Column ID

Sensitivity

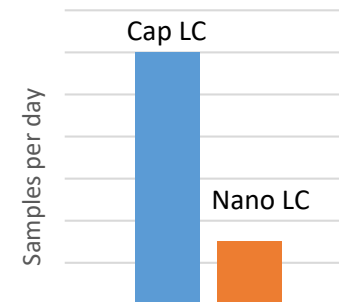
Throughput

Advantages of CapLC-MS and Fields of Application

High sensitivity



High throughput



Reliable, sensitive and quantitative analysis of large sample cohorts

Shotgun Proteomics

Translational Proteomics

Bioanalysis

High repeatability



EASY-handling



The Thermo Scientific Capillary-Flow LC-MS Solution



Large column portfolio and option of 3rd party columns



column

Easy set-up with nanoViper and EASY-Spray technology



emitter



source



State-of-the-art low flow UHPLC system

- Versatile platform for all workflows
- Capable of nano, cap & micro flow



Thermo Scientific™ Chromeleon™ CDS



Thermo Scientific™ Xcalibur™ software



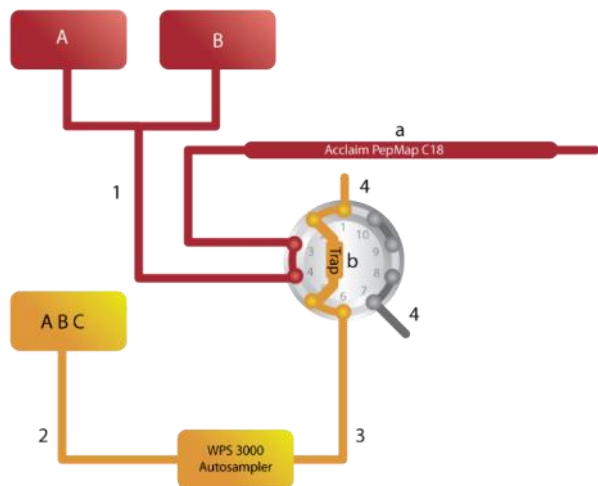
Thermo Scientific™ TraceFinder™ software



Supported LC-MS software

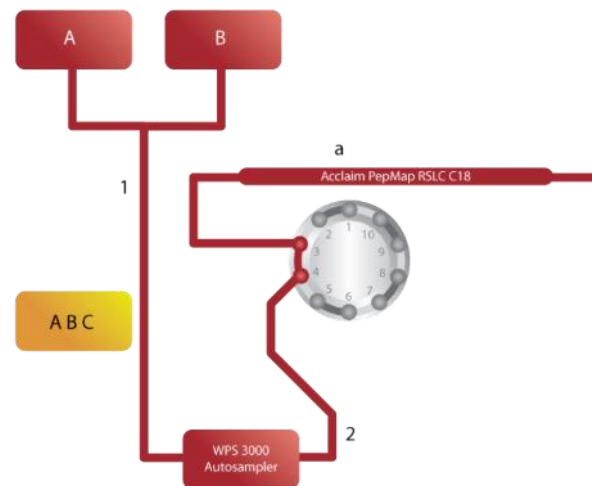
Leading in HRAM MS

Standard Application Set-ups for Capillary-Flow UltiMate 3000 RSLCnano



Pre-concentration (Default option)

- *Use loading pump for sample loading onto trap column*
 - Trap cartridge protects separation column from contaminants in sample
 - Faster loading of sample results in reduced cycle time
 - Near zero-sample loss injections with μL -pick up mode



Direct Injection

- *Sample directly loaded onto analytical column*
 - Better retention of very hydrophilic peptides

Thermo Fisher Scientific Offers Two ESI Source Options



Analytical Column



Trap Column Cartridges

Recommended solution for optimal performance



Thermo Scientific™ EASY-Spray™ source and transfer line (20 µm emitter)

Alternative solution offering good performance



Thermo Scientific™ Ion Max™ source and HESI probe with low flow (50 µm) needle



Column Consumables for the CapLC-MS Solution

We offer:

Stationary Phase	ID	Length	Particle Size
Acclaim PepMap	300 µm	5 cm and 15 cm	2 µm
Acclaim PA2	300 µm	15 cm	3 µm
Hypersil Gold	300 µm	15 cm	3 µm
Hypersil Gold	320 µm	5 and 10 cm	5 µm
Hypersil Gold C8	300 µm	10 cm	3 and 5 µm
Hypersil Gold AQ	300 µm	10 cm	1.9 µm and 3 µm
Hypersil Gold PFP	300 µm	10 cm	3 µm
Hypercarb	320 µm	10 and 15 cm	3 µm
PepSwift	200µm and 500 µm	5 and 25 cm	NA
ProSwift RP-4H	200 µm and 500 µm	10 and 25 cm	NA
ProSwift C4 RP-5H	200 µm and 500 µm	10 and 25 cm	NA

Alternatively 3rd party columns may be used with our solution

Note: For full details see online catalogue:thermo.dirxion.com/chromatography

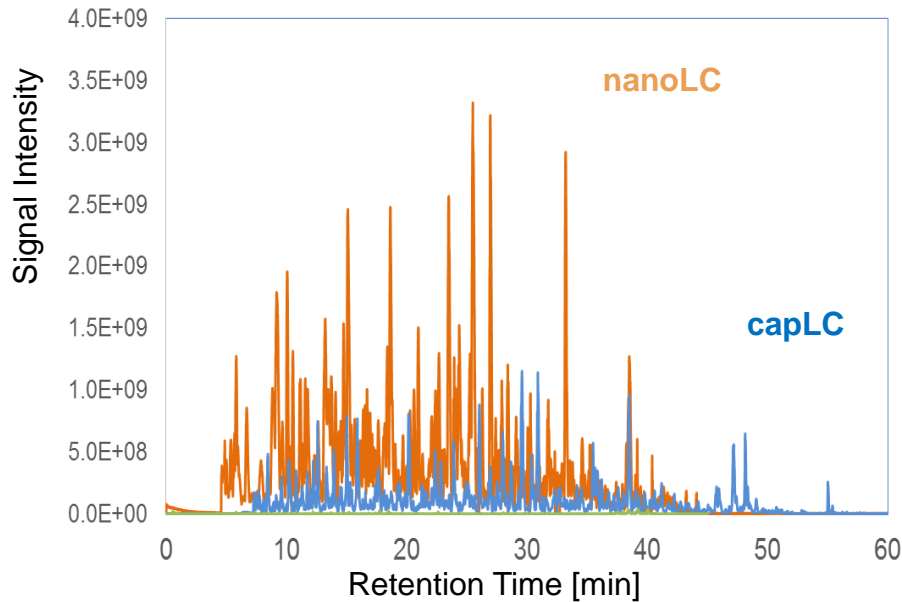
Application Examples



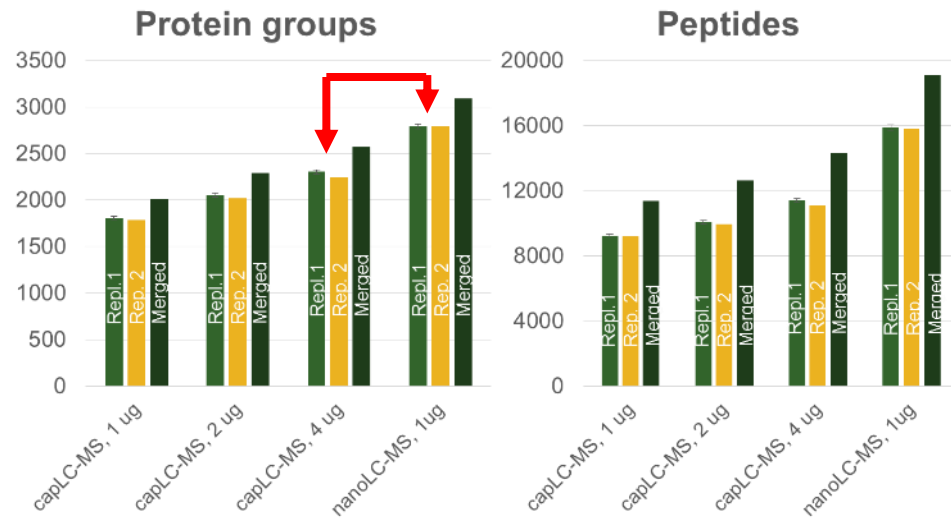
Comparing nanoLC vs. capLC Shotgun Proteomics (DDA)

Cap LC-MS delivers ~2200 protein identifications in a single 60 minute run (~ **80%** of identifications obtained with 60 minute nano LC-MS run)

BPC profiles of HeLa cell lysate digest



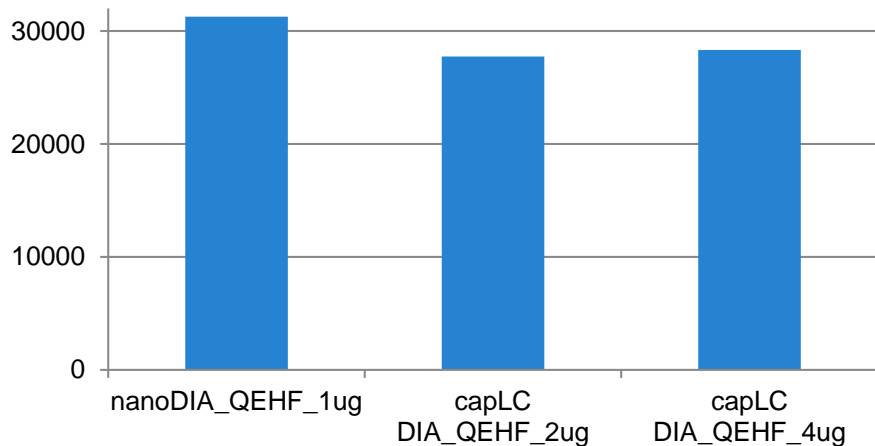
Number of protein groups and peptides identified with nano and cap LC-MS



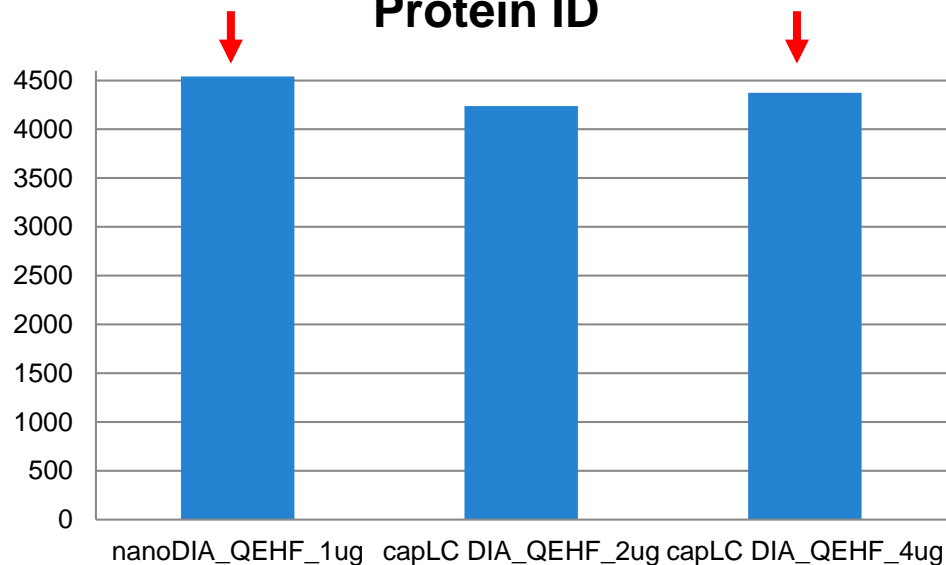
Comparing NanoLC-MS DIA vs. CapLC-MS DIA

- CapLC-MS DIA experiments shows nearly comparable identification rates compared to NanoLC-MS DIA experiments
- In both cases >27,000 peptide precursors and >4000 proteins can be identified with 1% FDR

Peptide Precursors 1% FDR



Protein ID



NanoLC: QE HF, 90 mins run time, 250 nL/min flow rate, average of 4 replicates

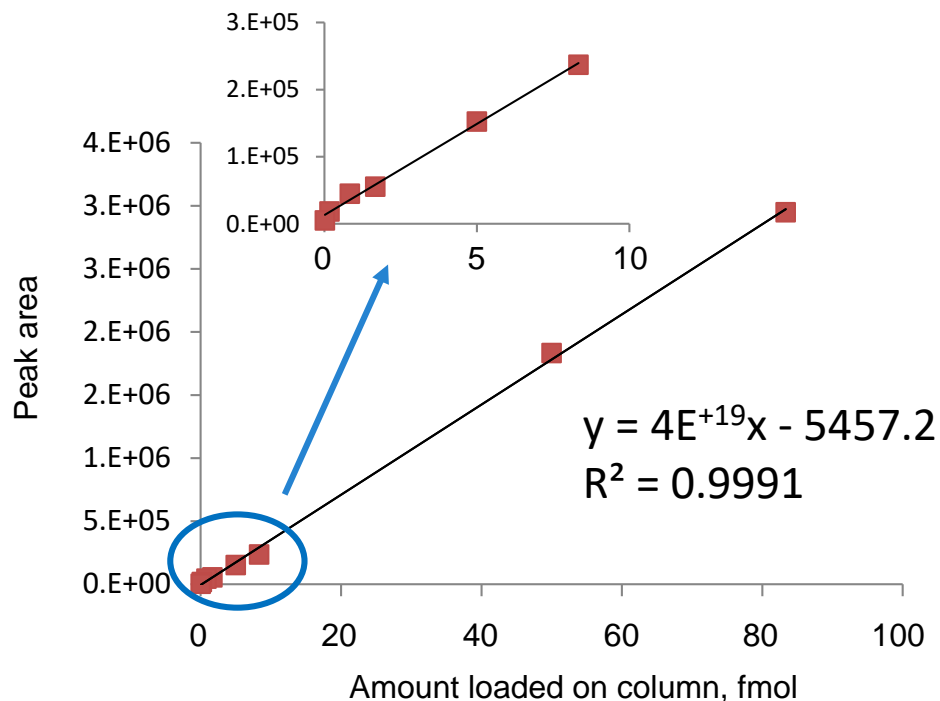
CapLC: QE HF, 60 mins run time, 5 μ L/min flow rate, average of 3 replicates

Sample: HeLa protein digest; Analysis done with Spectronaut v9

Targeted Analysis of Infliximab by PRM

Cap LC-MS allows confident quantification spanning 5 orders of magnitude for neat samples and 3-4 orders of magnitude for analytes in complex matrices

Calibration plot for PRM quantification of Infliximab



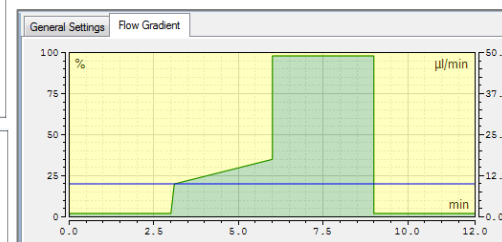
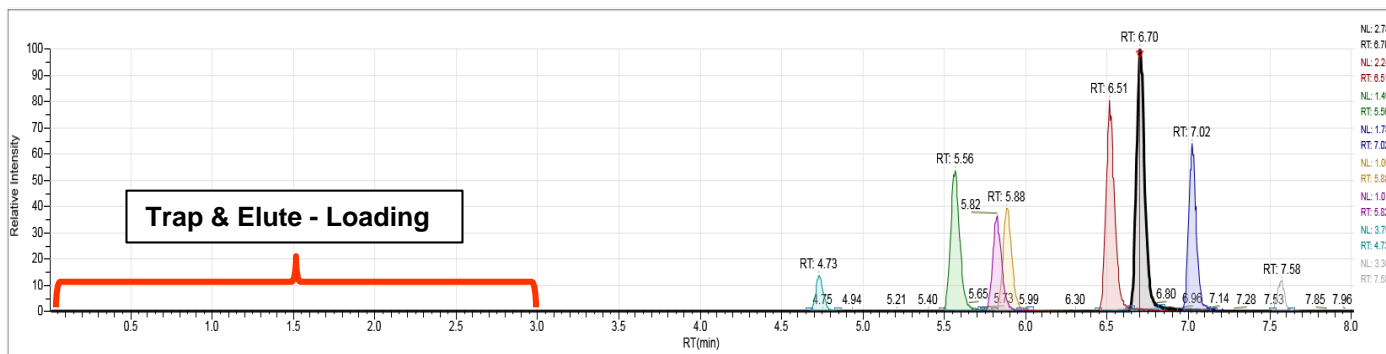
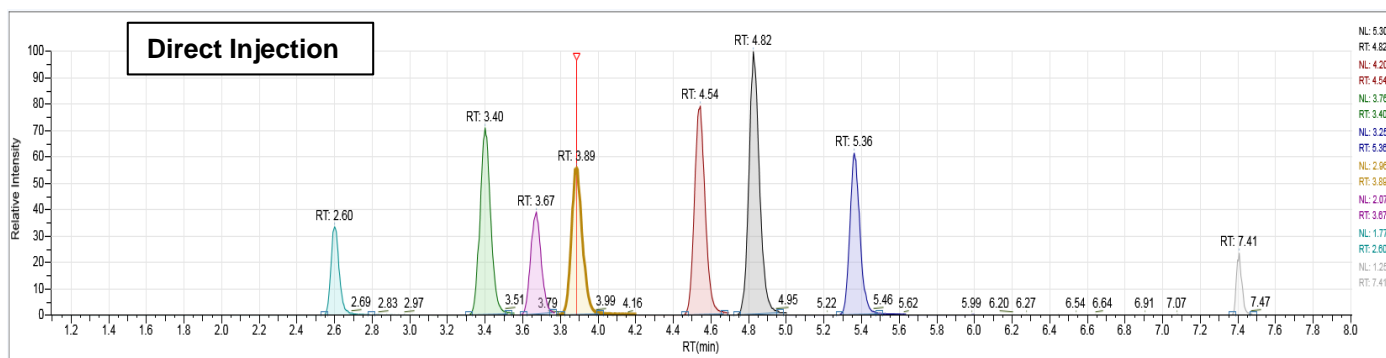
	Neat Infliximab digest	Spiked into 500ng HeLa digest (500 ng)
Lowest calibration point	16 amol	3 fmol
Dynamic range	10^5	10^3 - 10^4

Calibration curve for DILLTQSPAILS SVSPGER peptide (m/z 632.68, charge +3).
Quantification was done on $y7$ ion (m/z 731.38)

Small Molecule Sample Mix – in Rat Plasma

- Good improvement in signal response (3x) with 10 μ L/min flow rate and HESI source (in comparison to analytical flow application)
- Good chromatographic profile both with Direct Injection and Pre-concentration mode

Compound Name
Desomorphine
Fentanyl
Desmethyldoxepin
Flecainide
Midazolam
Clonazepam
Imipramine
Amitriptyline
Fluoxetine
Diazepam

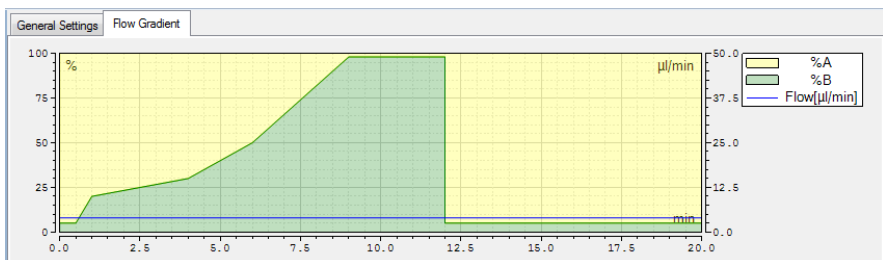


No	Time	Flow [µl/min]	%B	Curve
1	0.000			Run
2	0.000	10.000	2.0	5
3	3.000	10.000	2.0	5
4	3.100	10.000	20.0	5
5	6.000	10.000	35.0	5
6	6.000	10.000	98.0	5
7	9.000	10.000	98.0	5
8	9.000	10.000	2.0	5
9	12.000	10.000	2.0	5
10	New Row			
11	12.000			Stop Run

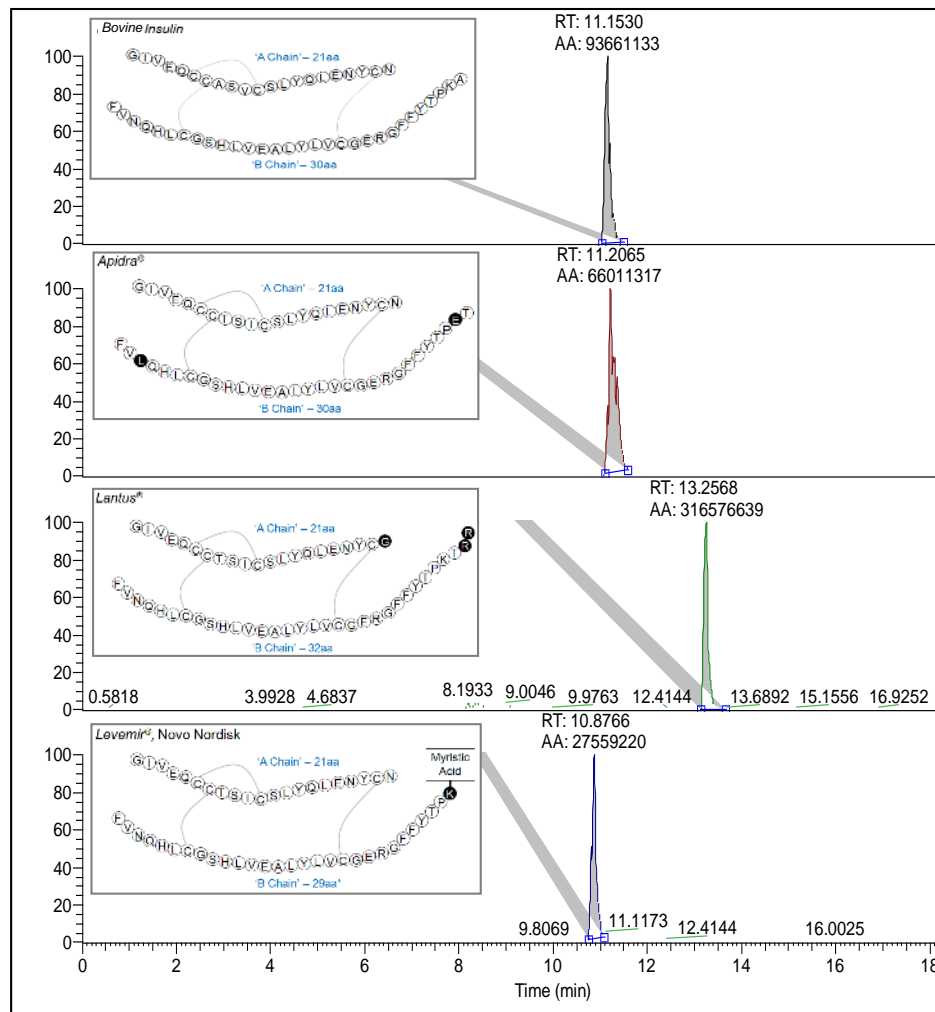
Column: 300 μ m x 15cm Acclaim PepMap
Software: TraceFinder; MS: Q Exactive Focus

Insulin Quantitation – Transfer from Analytical Flow Method

- Good improvement in signal response with 4 $\mu\text{L}/\text{min}$ flow rate and HESI source (in comparison to analytical flow application)
- Successful direct transfer from existing MSIA workflow for analytical flow
- Good chromatographic profile



Column: 200 μm x 25cm ProSwift
 Software: TraceFinder
 MS: Q Exactive Focus



Solution Verification

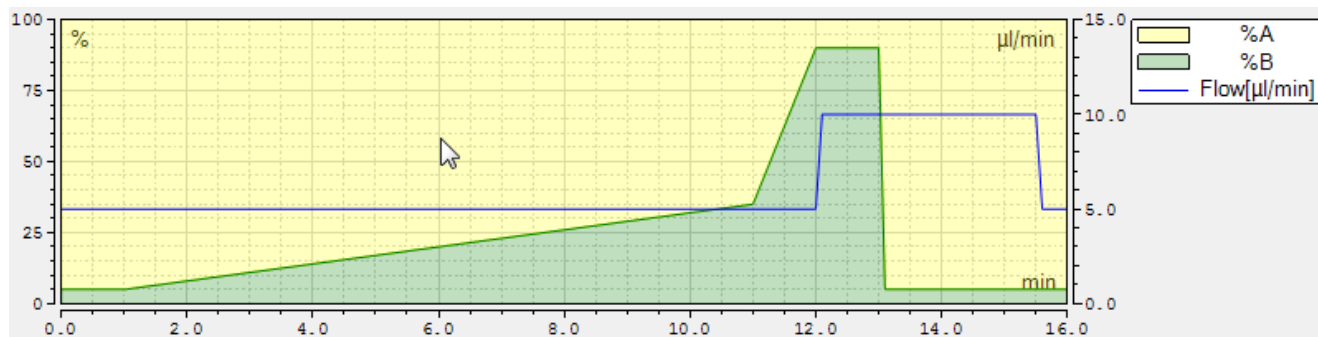
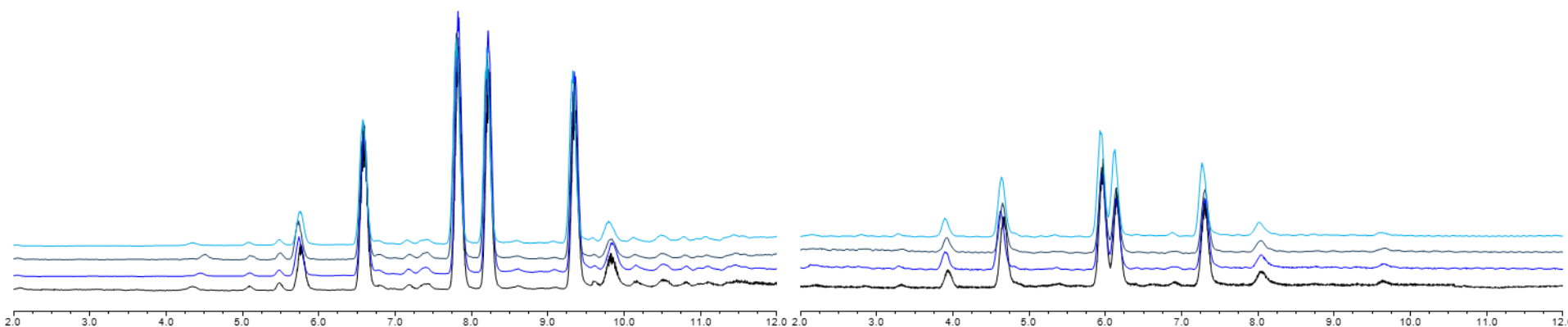


Solution Evaluation – High Throughput Set-Up

10 minute gradients (~16 minutes cycle time) result in good chromatographic separation

15 cm x 300 μ m, 2 μ m Acclaim PepMap

5 cm x 300 μ m, 2 μ m Acclaim PepMap



Trap column set-up

- Loading flow: 100 μ L/min
- Duration: 15 sec (25 μ L)

Analytical flow rate

- Chrom. sep.: 5 μ L/min
- Column equ.: 10 μ L/min

Gradient

- 5-35% ACN, 0.1% FA in 10 min

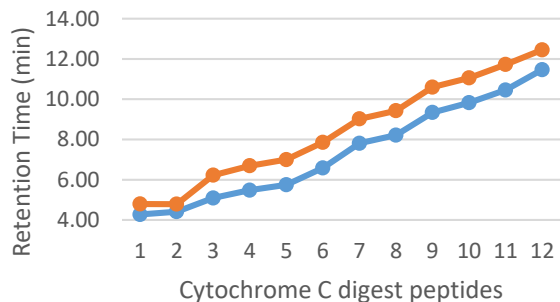
Analyte

- Cytochrome c digest (1 pmol)

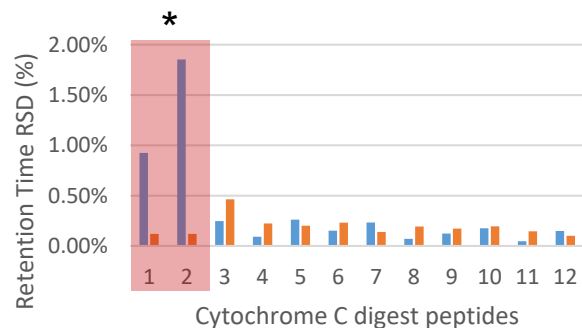
Solution Evaluation – Pre-Concentration vs. Direct Injection Mode

- For “standard” peptides no significant difference in chromatographic performance, e.g. retention time stability and peak widths
- Early eluting, very hydrophilic peptides should be analysed with direct injection mode (*)
- Pre-concentration mode allows fast loading and reduces gradient delay volume compared to direct injection

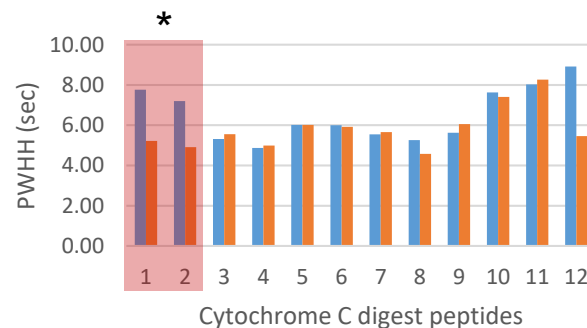
Elution time for Peptide Standard



Retention Time Stability



Peak Width



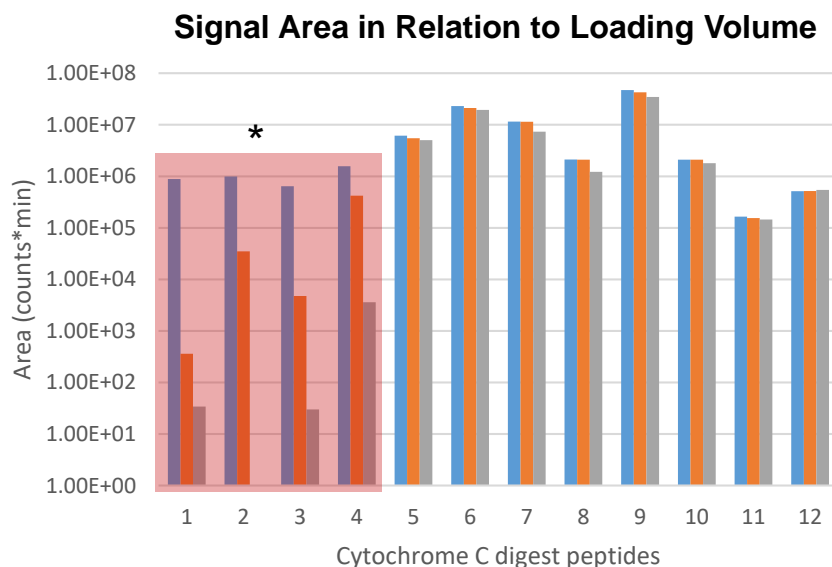
LC-MS Settings:

Sample: 1 pmol Cytochrome c digest
0.3 x 150 mm, 2 µm Acclaim PepMap C18 column
10 min gradient, ~17 min cycle time

■ Pre-concentration mode
■ Direct injection mode

Solution Evaluation – Loading Volume in Pre-Concentration Mode

- Increasing the loading volume can be used for removing salts and other unretained contaminants
- Increasing loading volume has no effect on “standard” peptides detection
- Early eluting, hydrophilic peptides are partially lost by excessive loading (*)
 - Isocratic elution through the trap cartridge



Loading Volume

- 25 µL
- 50 µL
- 75 µL

LC-MS Settings:

Pre-concentration mode

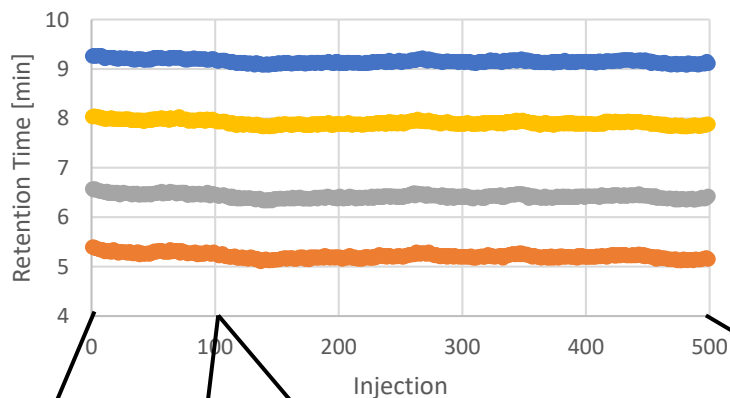
200 µL/min loading flow; 100 % water, 0.1% FA

Sample: 1 pmol Cytochrome c digest

0.3 x 150 mm, 2 µm Acclaim PepMap C18 column

10 min gradient, ~17 min cycle time

Cap LC-MS Delivers High Retention Time and High Signal Stability



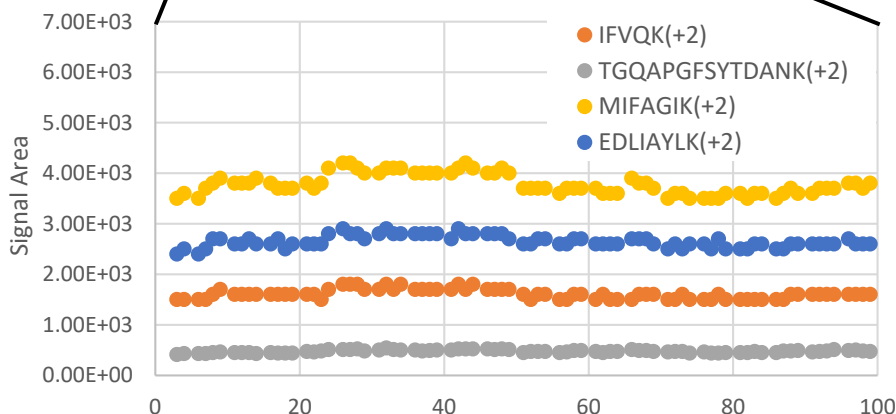
Retention time stability (500 CCD injections)

	IFVQK	TGQAPGFSY TDANK	MIFAGIK	EDLIAYLK
RT RSD	1.02%	0.68%	0.56%	0.43%

Injection sequence



Intermittent analysis of Cytochrome c digest (CCD) in E.coli matrix (100 injections)

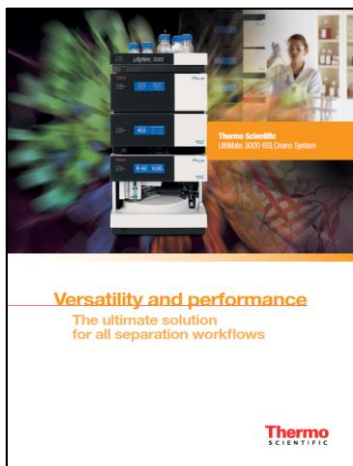


SRM Signal area stability without correction on internal standard (100 CCD / E.coli matrix injections)

	IFVQK	TGQAPGF SYTDANK	MIFAGIK	EDLIAYLK
Average Area of Signal	1.61E+03	4.75E+02	3.78E+03	2.65E+03
RSD	5.8%	5.9%	5.4%	4.3%

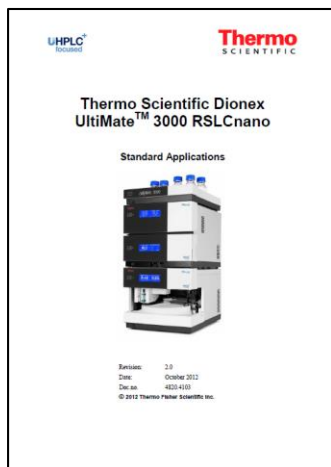
Acquisition on TSQ Quantiva
CCD: 50 fmol, E.coli digest 500 ng

Available Additional Material



UltiMate 3000

RSLCnano Brochure



Standard Applications Manual

CHROMATOGRAPHY TODAY

Capillary Flow LC-MS Unites Sensitivity and Throughput



Webinar by Prof. Jun Qu, SUNY Buffalo

EBF 2016

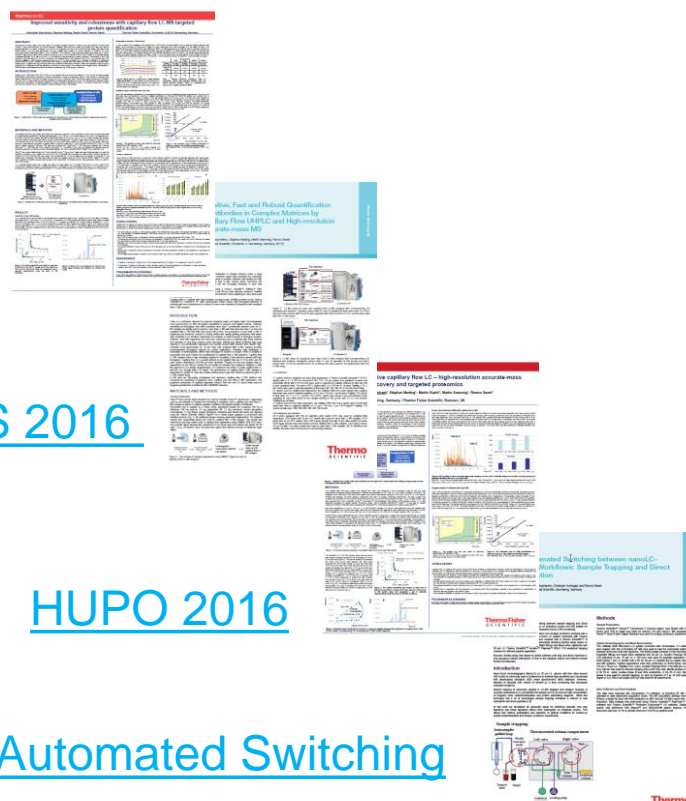
ASMS 2016

HUPO 2016



Low flow Website

Automated Switching



Frequently Asked Questions

Question	Answer
Can I upgrade nano flow UltiMate 3000 RSLCnano systems to capillary flow?	Yes, by exchanging the flowmeter and the respective capillaries.
Which Software can be used?	It is recommended to use SII for Xcalibur. If required DCMSlink can be used as well.
Do I need to buy direct injection and pre-concentration applications kits with a new capillary-flow UltiMate RSLCnano?	New Capillary-flow UltiMate RSLCnano systems come with the all capillaries required to set-up both modes. Dedicated application kits for both modes are available for upgrade.
Do a need an EASY-Spray source?	Thermo Fisher Scientific recommends using an EASY-Spray source for achieving best performance. However, capLC-MS can also be performed with Ion Max HESI sources in low flow set-up.