Rome Wasn’t Built in a Day – Give Me 12 Hours. Productivity Enhancements for UHPLC

Eddie Lim, HPLC APAC Marketing Manager, Thermo Fisher Scientific
July 2018

Thermo Scientific Vanquish Duo - Our Promise

If a company could help with

productivity,

in terms of instrumentation, service, uptime and support, then we would go with that company.

-Patheon

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Thermo Scientific LC Portfolio Overview

HPLC Systems

Routine Analysis

UltiMate 3000 Basic Automated

- Highly economic & reliable

UltiMate 3000 SD

- Workhorse for standard HPLC applications

620 bar

UHPLC Systems

Low-Flow

UltiMate 3000 RSLCnano

EASY-nLC 1200

- UHPLC systems for Nano/Cap/Micro range
- Ideal front-end for proteomics applications

800-1200 bar

Flexibility, More Performance and Method Development

UltiMate 3000 RS/BioRS

- Specialty workflow support
- Binary and Quaternary UHPLC systems

Up to 1000 bar

Research & HT

Vanquish Duo Workflows

Vanquish Flex

- High pressure binary and low pressure quaternary solvent mixing options
- Two thermostatting modes
- Biocompatible
- Integration of multiple detection technologies

Vanquish Horizon

- High pressure binary solvent mixing
- Industry-leading precision and accuracy
- Two thermostatting modes
- Unmatched detection sensitivity
- Biocompatible
- Integration of multiple detection technologies

1500 bar

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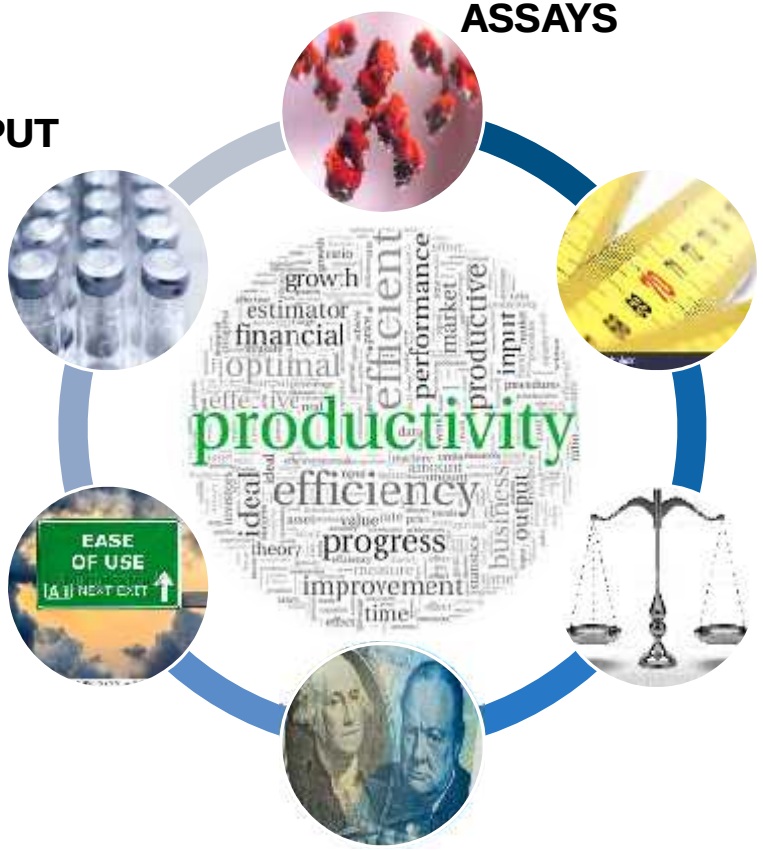
Improve productivity



COMPLEMENTARY ASSAYS

BENCH SPACE

MEANINGFUL RESULTS

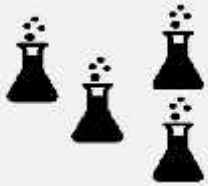
RETURN ON INVESTMENT

LEARNING SOFTWARE

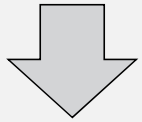
HIGH THROUGHPUT

Analytical Challenges

Accelerate time to results



Increased number of samples

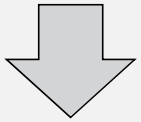


Process more samples with decreasing number of resources

Asked to <u>do</u> more with less

Need for efficient characterization

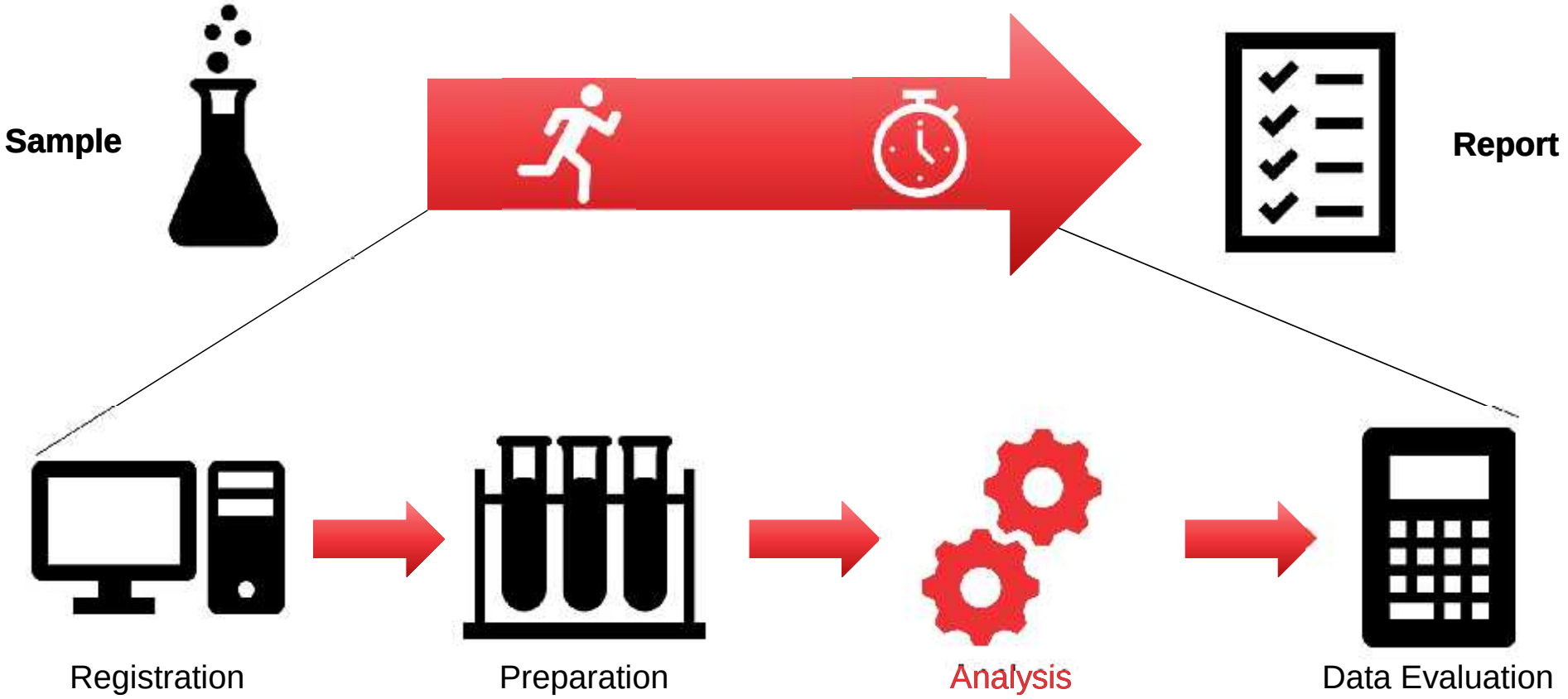
Increased complexity of samples



Need for complementary characterization methods

Asked to <u>know</u> more with less

Rome Wasn‘t Built in a Day – Give me 12 Hours



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How to be productive?

Total Analysis Time

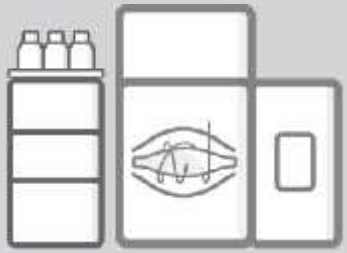
Total Samples

Applications per Sample

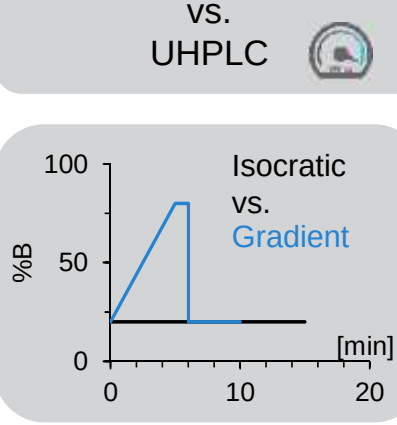
Separation Conditions

Run Time

Single LC-MS method vs. multiple LC-UV methods



HPLC vs. UHPLC



?

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Application Example Peptide Mapping

Applications per Sample = 1

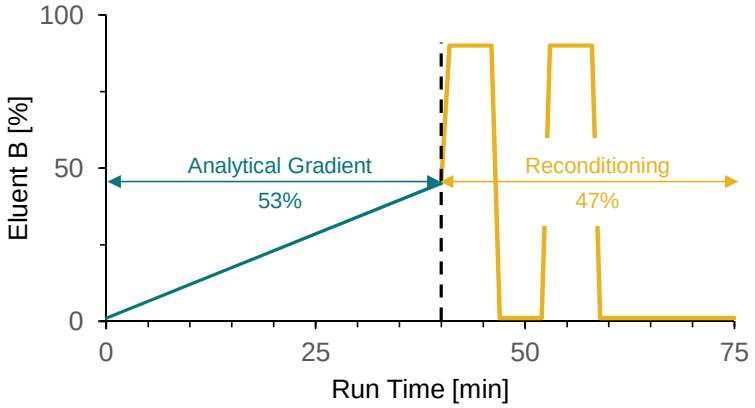
Peptide Mapping with digested proteins

Sample: 1 µg Rituximab SMART digest; Injection volume 5 µL

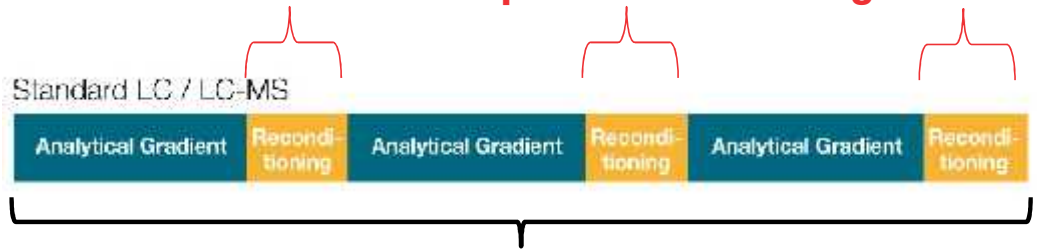
Separation Conditions = Gradient

Run Time = 60 min

A: 0.1/100 FA/Water (v/v); B: 0.1/100 FA/Acetonitrile (v/v); Gradient: 1-45% B in 40 min; 400 µL/min; Reconditioning: two times 90% B for 5 min, 16 min equilibration at 1% B, Column: Thermo Scientific™ Acclaim™ VANQUISH™ C18 2.1x250mm, 2.2 µm; 60 °C



Time without acquisition of meaningful data



Total Samples per Day = 19

Time spent on reconditioning per day = 11h

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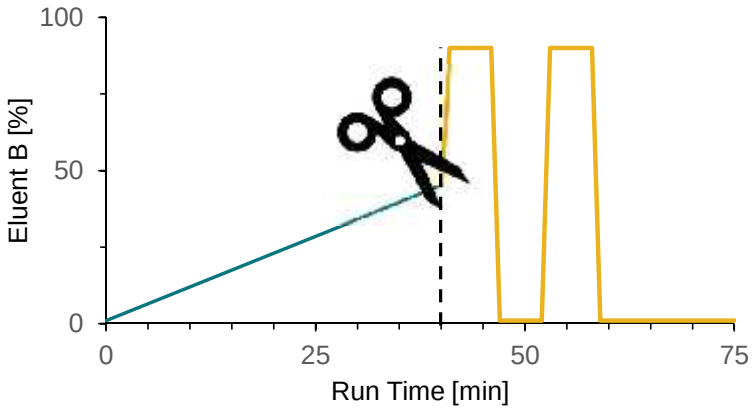
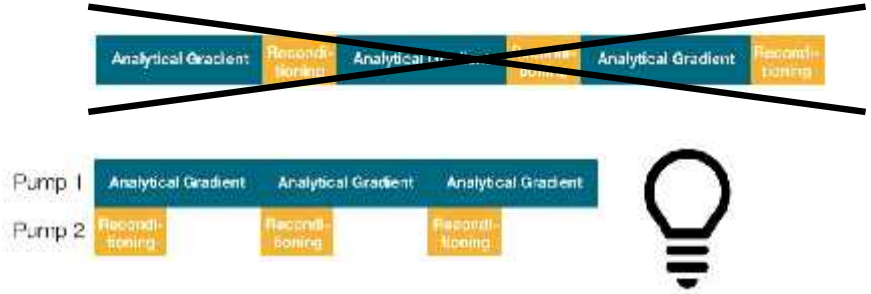
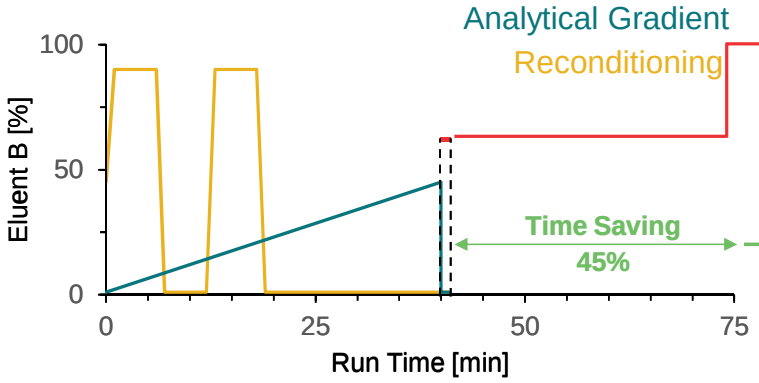
Overlapping Gradients







Flush time of system delay volume

Reduced Detector Idle Time by Tandem LC

Total Samples per Day = 30

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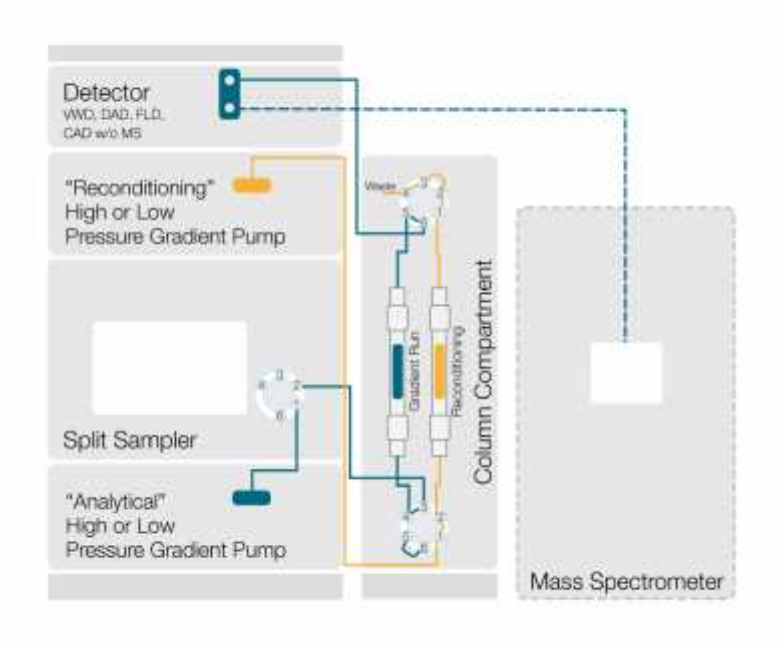
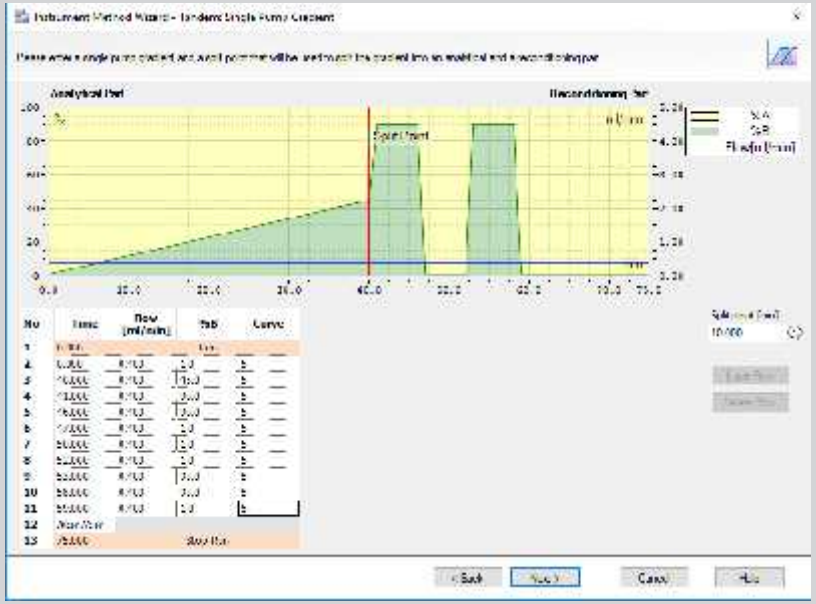
Tandem LC/LC-MS Technical Realization



Thermo Scientific™ Vanquish™ Duo UHPLC System for Tandem LC/LC-MS



Thermo Scientific™ Chromeleon™ 7.2.8 Chromatography Data System or Thermo Scientific™ SII for Xcalibur 1.4

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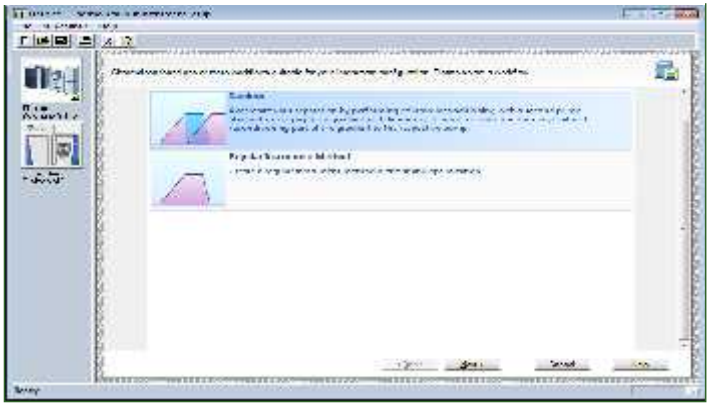
Key Benefits – Thermo Scientific Vanquish Duo System for Tandem LC or LC-MS

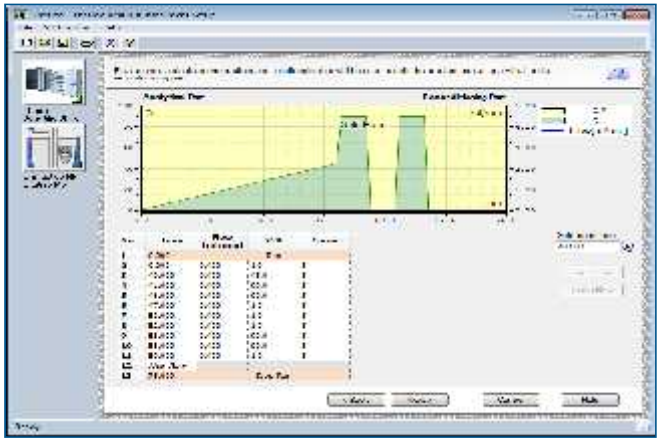
Easy to implement

1. 2. 3.

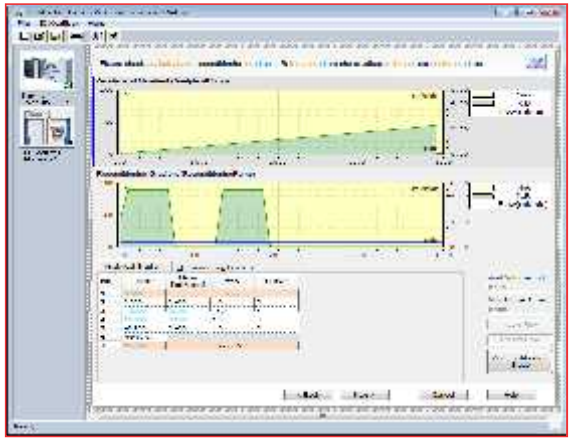
Automatic instrument recognition and fluidic knowledge



Standard instrument method setup

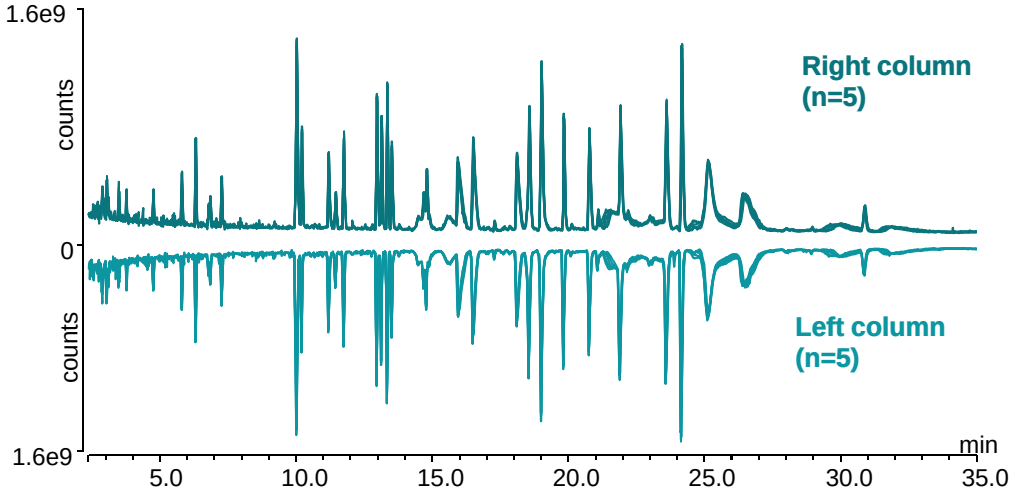


Automatic method setup for analytical and reconditioning pump



Vanquish Duo for Tandem LC/LC-MS Performance

100% sequence coverage for light and heavy chain with both columns



Overlay of 5 total ion current chromatograms
1 µg Rituximab SMART digest ; Injection volume 5 µL; Detection: Thermo Scientific™ Q Exactive HF, R=15k, mass range 140-2000; UV detection: 214nm

Single Column Setup

	Column Left		Column Right	
	RT RSD [%] n=5	Area RSD [%] n=5	RT RSD [%] n=5	Area RSD [%] n=5
Average	0.045	2.59	0.039	2.14

Vanquish Duo for Tandem

	Column Left/Right			
	Abs. RT Shift Column left to right [min]	Rel. RT Shift Column left to right [%]	RT RSD [%] n=10	Area RSD [%] n=10
Average	0.023	0.18	0.11	2.47

Vanquish Duo for Tandem LC/LC-MS:

+ Reduces detector idle time
+ Increases productivity by 45% with RT RSD < 0.11%
+ Is most effective for short analytical gradients

- Is limited to one application at a time

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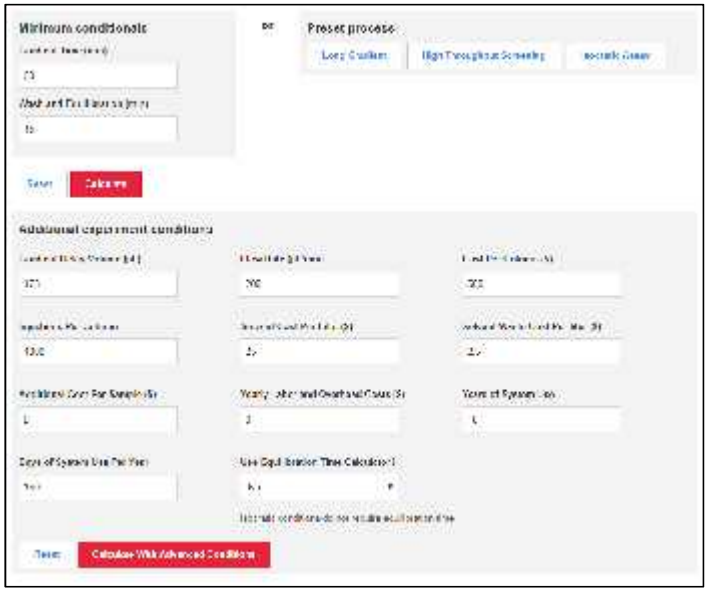
Key Benefits – Thermo Scientific Vanquish Duo System for Tandem LC or LC-MS

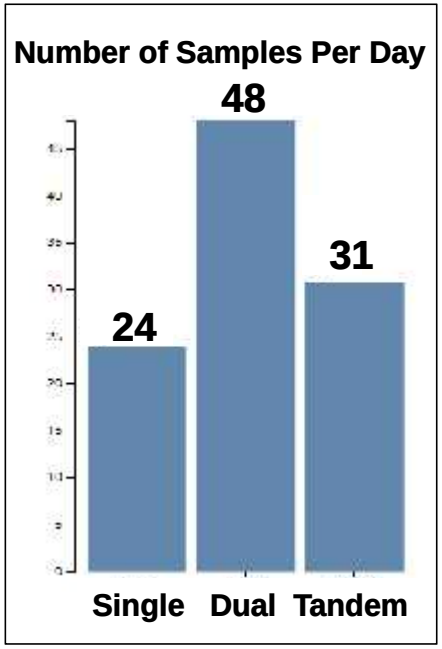
Lower costs per sample

Cost of ownership calculator:

Throughput increase
Cost per sample



Long Gradient experimental conditions



Throughput and costs comparison between standard single and Vanquish Duo workflows

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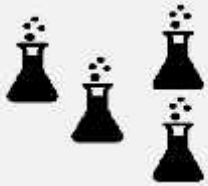
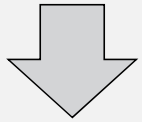
Analytical Challenges

Accelerate time to results



Increased number of samples

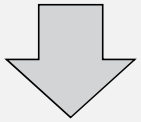


Process more samples with decreasing number of resources

Asked to <u>do</u> more with less

Need for efficient characterization

Increased complexity of samples



Need for complementary characterization methods

Asked to <u>know</u> more with less

Do you run two applications out of a sample?

Applications per Sample = 2

Characterization of monoclonal antibodies

Sample: Infliximab 10 mg/mL in water

Reversed Phase

A: 0.1/100 TFA/Water (v/v);
B: 0.1/9.9/100 TFA/Water/Methanol (v/v/v);
Gradient: 20-50% B in 5.5 min; 600 µL/min;
Reconditioning: 50% B for 0.5 min, Equilibration: 20% B for 3.5 min
Column: Thermo Scientific™ MAbPac™ RP, 4 µm, 2.1 x 100 mm; 80 °C
Injection volume: 2 µL

Gradient | 80°C | 10 min

Strong Cation Exchange

A: 1/10 CX-1 buffer A (pH 5.6)/Water (v/v);
B: 1/10 CX-1 buffer B (pH 10.2)/Water (v/v); Gradient: 15-40% B in 25 min; 200 µL/min; Reconditioning: 85% B for 5 min;
Column: Thermo Scientific™ SCX-10 RS, 5 µm, 2.1 x 50 mm; 30 °C
Injection Volume: 1 µL

Gradient | 30°C | 30 min

Standard Method

Sequential analysis with intensive flushing for incompatible mobile phases

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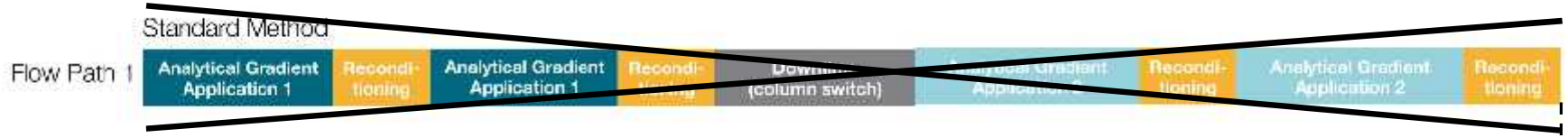
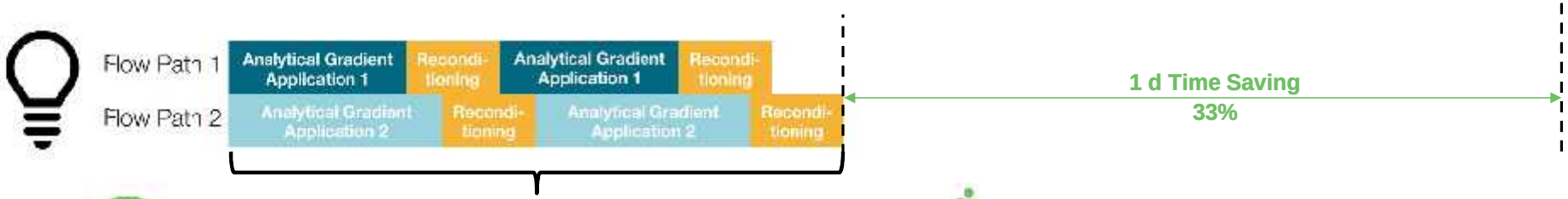
Overlapping Applications



Application	Reversed Phase	Downtime	Strong Cation Exchange
Samples	96		96
Run Time	10 min		30 min
Duration	16 h	8 h	48 h
Total Time	3 d		



48 h Total Analysis Time by Dual LC
No additional Downtime

Doubled Sample Information
Out of a Single Aliquot

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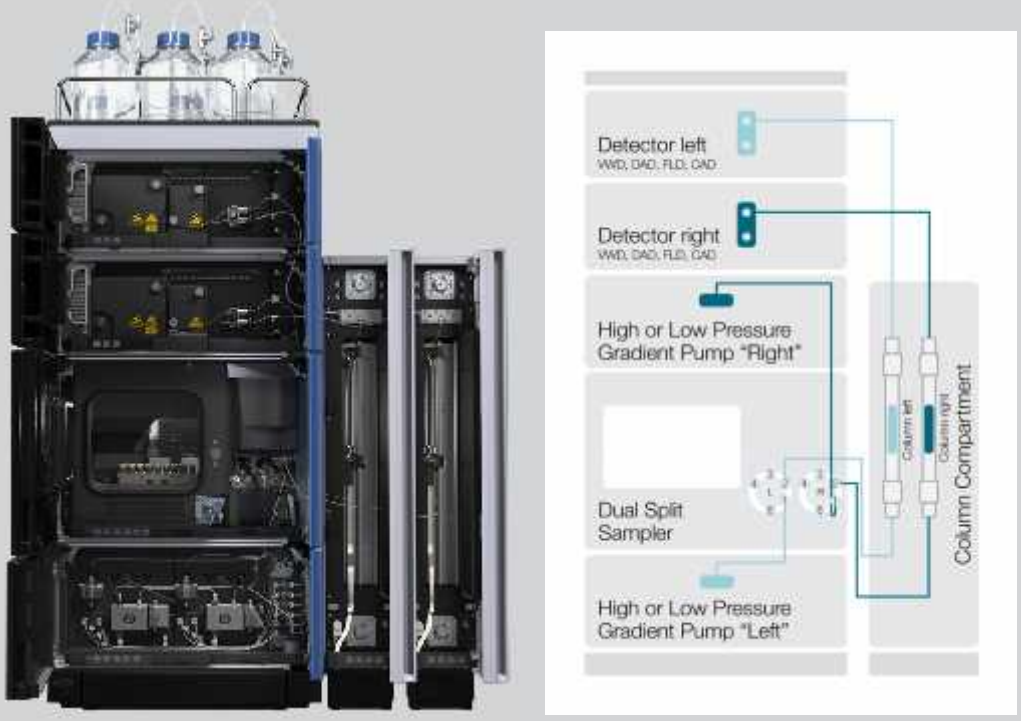
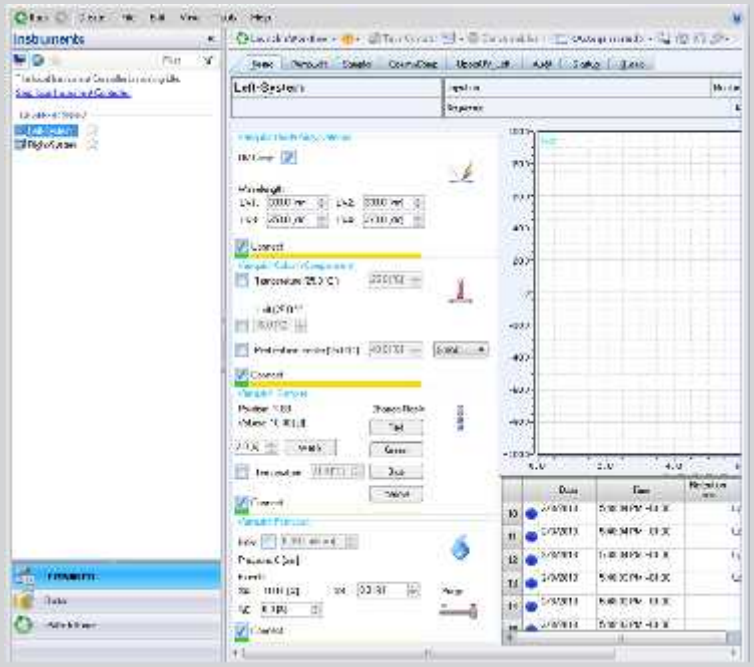
Dual LC Technical Realization



Thermo Scientific™ Vanquish™ Duo UHPLC System for Dual LC



Thermo Scientific™ Chromeleon™ 7.2.8 Chromatography Data System

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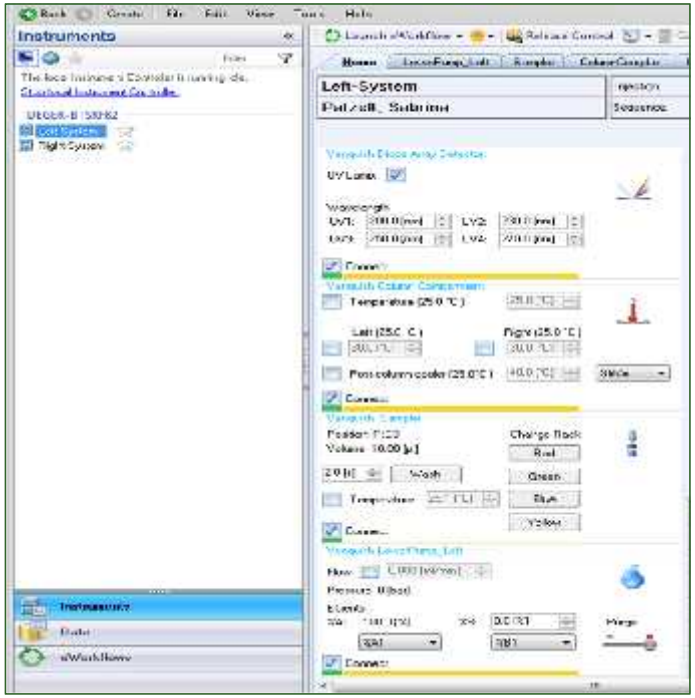
Key Benefits – Thermo Scientific Vanquish Duo System for Dual LC

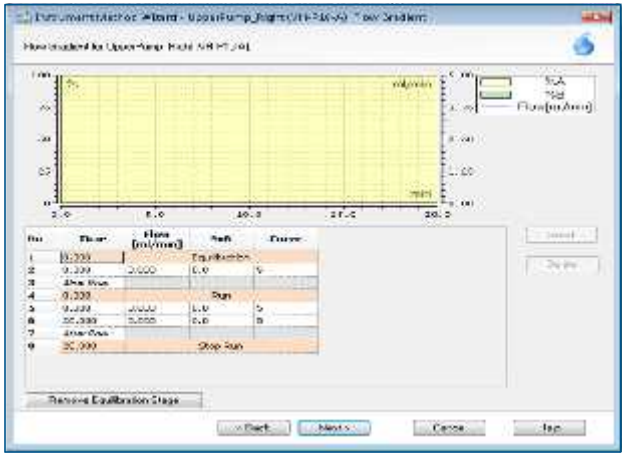
Easy-to-use as any other Thermo Scientific™ Vanquish™ system

1. 2. 3.

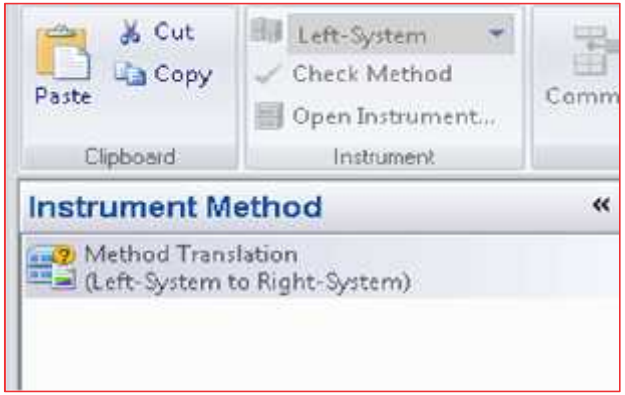
Simple instrument selection



Easy method setup with method wizard



Automatic method translation



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Vanquish Modules

Thermo Scientific™ Vanquish™ Flex Dual Pump

- 2 ternary pumps in one housing
- 1000 bar (15,000 psi) version
- Delivers 2 completely independent gradients

Thermo Scientific™ Vanquish™ Dual Split Samplers

- 2 injection units in one autosampler
- 1000 and 1500 bar (15,000 or 22,000 psi) version
- Independent flow paths

Key Benefits – Thermo Scientific Vanquish Duo System for Dual LC

No additional bench space needed

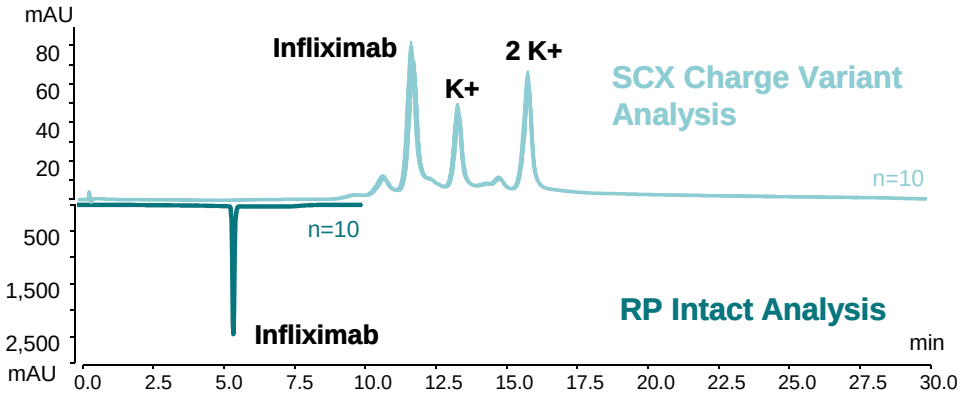
Vanquish Duo System for Dual LC

55 cm

Vanquish system

55 cm

Vanquish Duo for Dual LC Performance



Overlay of 10 LC-UV chromatograms for the left (blue) and right (orange) LC channel
Infliximab 10 mg/mL in water, UV detection: 214nm, 280 nm

	RT (min)	Relative Peak Area (%)
Average (n=10) SCX	11.86	54.08
% RSD (n=10) SCX	0.15	0.57
Average (n=10) RP	5.48	100.00
% RSD (n=10) RP	0.07	0.00

Vanquish Duo for Dual LC:

+ Doubles sample information out of a single aliquot
+ Avoids additional downtime by separate flow paths without increasing bench space
+ Increases productivity up to 100%

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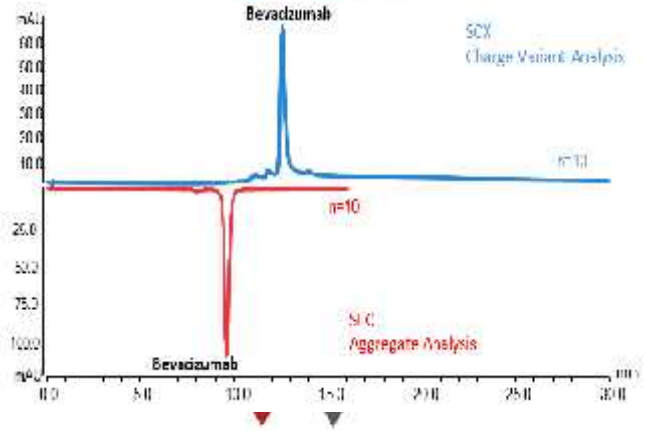
What else can you do with a Vanquish Duo for Dual LC

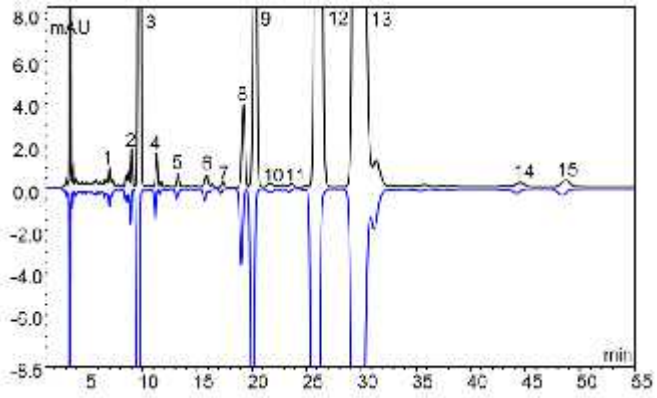
Run your complementary columns in parallel



Get more information out of every sample

Efficient characterization

Run two similar columns in parallel



Be finished in half the time

Finished in half the time

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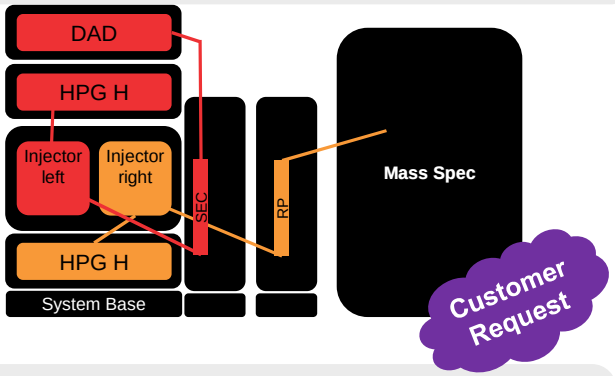
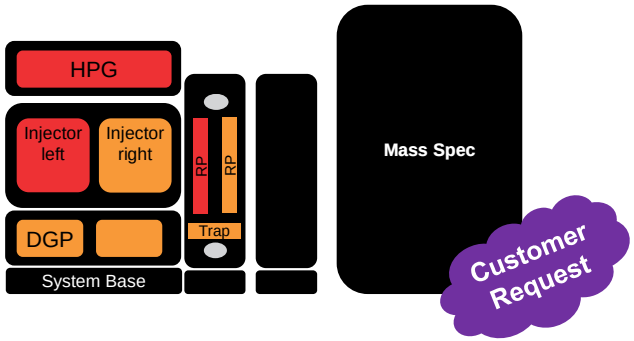
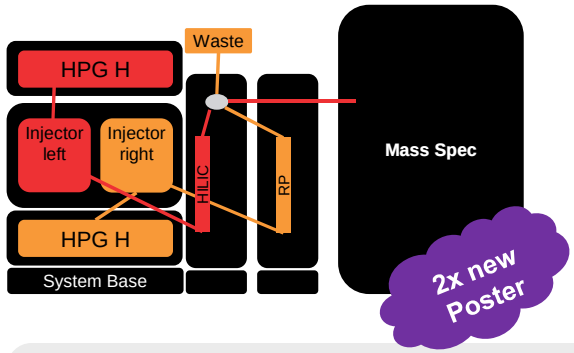
Potential Configurations with Dual Sampler for LC-MS

	Biopharma Characterization System	Trap&Elute Automated Switching	Dual LC-MS for Metabolomics
Setup			
Description	**Channel 1:** Size Exclusion Chromatography for mAbs with UV detection – Aggregate Analysis **Channel 2:** Reversed Phase Chromatography for LC-MS Peptide Mapping of mAbs **Simultaneous use of LC-UV and LC-MS channel**	**Channel 1:** Direct injection of high concentrated sample **Channel 2:** Trap&Elute configuration for dirty and low concentrated samples **Use of the direct injection or the Trap&Elute setup w/o the need of any hardware changes**	**Channel 1:** HILIC **Channel 2:** Reversed Phase **Sequential analysis of metabolomics samples using RP and HILIC w/o the need of any hardware changes**
Software	Chromeleon only **Simultaneous use of channels**	Chromeleon and SII support **Sequential use of channels**	Chromeleon and SII support **Alternate use of channels**

Vanquish Duo for Dual LC

Simultaneous Determination of water and fat-soluble vitamins in Tablets and energy drinks

Column:	Acclaim RSLC Polar Advantage II (PN 074812)
Mobile phase A:	25 mM potassium dihydrogen phosphate, pH 3.8 adjusted with phosphoric acid
Mobile phase B:	70:30 acetonitrile/25 mM potassium dihydrogen phosphate, pH 3.8
Gradient:	Time (min) B (%) 0 0 3 30 7 35 10 100 11 0 20 0
Flow rate:	0.4 mL/min
Temperature:	25 °C with passive pre-heater

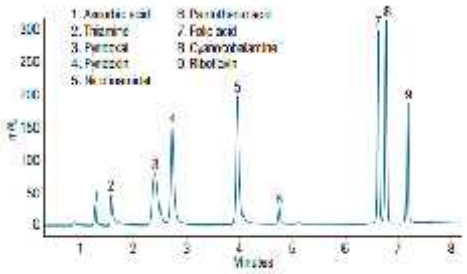


Figure 2. Separation of water-soluble vitamins standard on an Acclaim PA2 column (150 × 2.1 mm, 2.2 µm) recorded at 210 nm.

Peak	Common Name	Chemical Name	UV [nm]
1	Vitamin C	Ascorbic acid	245
2	Vitamin B1	Thiamine	245
3	Vitamin B6-derivate	Pyridoxal	210
4	Vitamin B6	Pyridoxin	210
5	Vitamin B3	Nicotinamide	210
6	Vitamin B5	Pantothenic acid	210
7	Vitamin B9	Folic Acid	280
8	Vitamin B12	Cyanocobalamin	210
9	Vitamin B2	Riboflavin	270

Column:	Acclaim RSLC Polar Advantage II (PN 074814)
Mobile phase A:	Water
Mobile phase B:	Methanol
Gradient:	Time (min) B (%) 0 80 7 95 7.5 100 11 100 11.1 80 20 80
Flow rate:	0.4 mL/min
Temperature:	35 °C with passive pre-heater

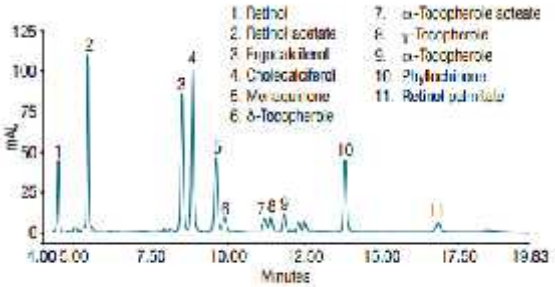
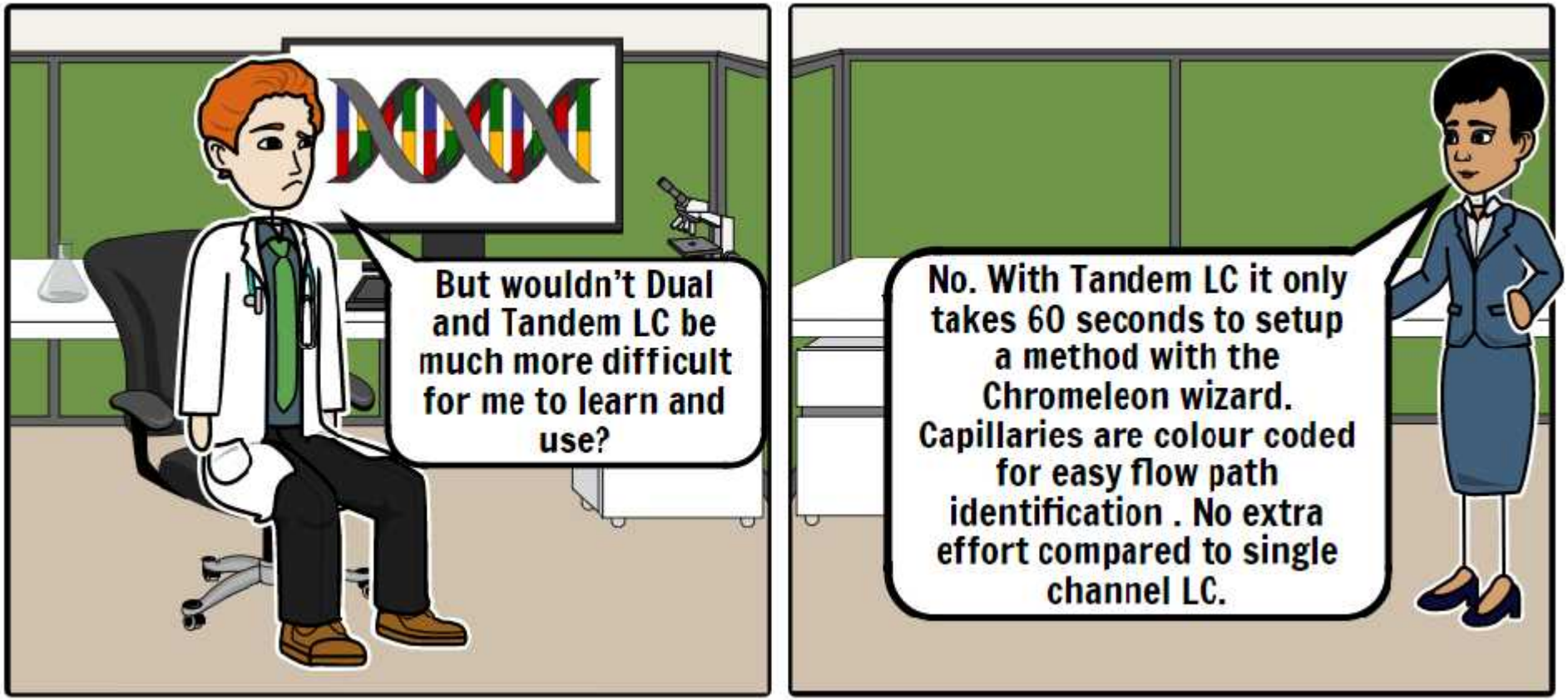


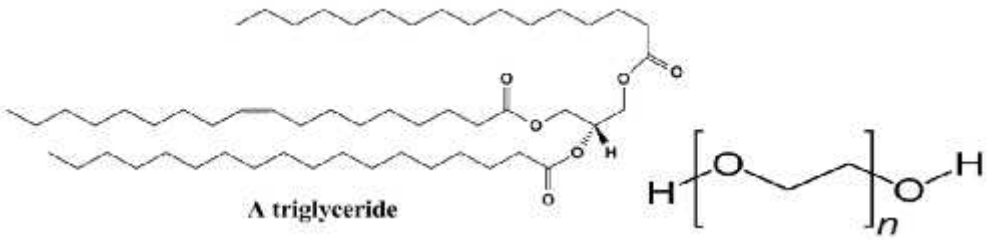
Figure 3. Separation of fat-soluble vitamins standard on an Acclaim PA2 column (250 × 2.1 mm, 2.2 µm) recorded at 280 nm.

Peak	Common Name	Chemical Name	UV [nm]
1	Vitamin A	Retinol	325
2	Vitamin A-derivate	Retinol acetate	325
3	Vitamin D2	Ergocalciferol	265
4	Vitamin D	Cholecalciferol	265
5	Vitamin K2	Menaquinone	265
6	Vitamin E	δ-Tocopherole	280
7	Vitamin E-derivate	α-Tocopherole acetate	280
8	Vitamin E	γ-Tocopherole	280
9	Vitamin E	α-Tocopherole	280
10	Vitamin K1	Phylloquinone	265
11	Vitamin A-derivate	Retinol palmitate	325



Analytical Challenge – Thermo Scientific Vanquish Duo System for Inverse Gradient

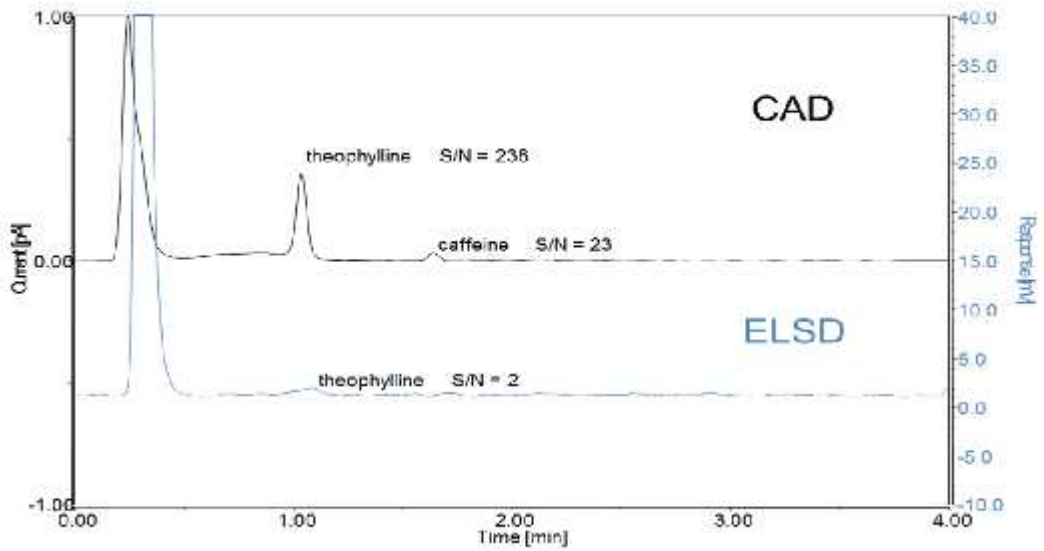
Need to detect any compound in a sample, even without chromophore



Individual standards for each compound required for quantitative measurements

Asked to improve detection and quantification capabilities

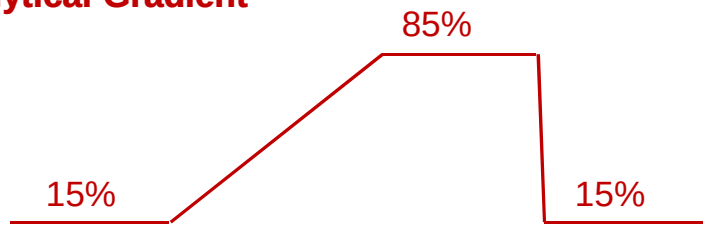
High detector sensitivity



	Evaporative Light Scattering Detector		Charged Aerosol Detector	
	Theophylline	Caffeine	Theophylline	Caffeine
Actual LOD S/N ≥3	8 ng o. c.	16 ng o. c.	**0.5** ng o. c.	**4** ng o. c.

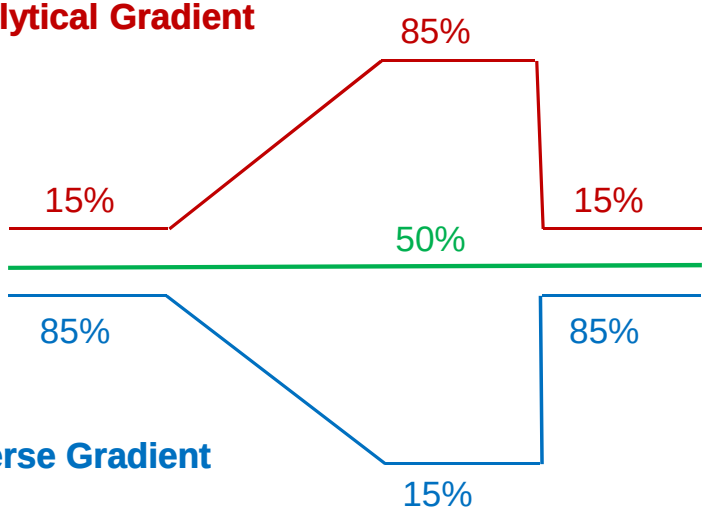
Inverse Gradient Compensation for Uniform Response

Analytical Gradient



Eluent at detector

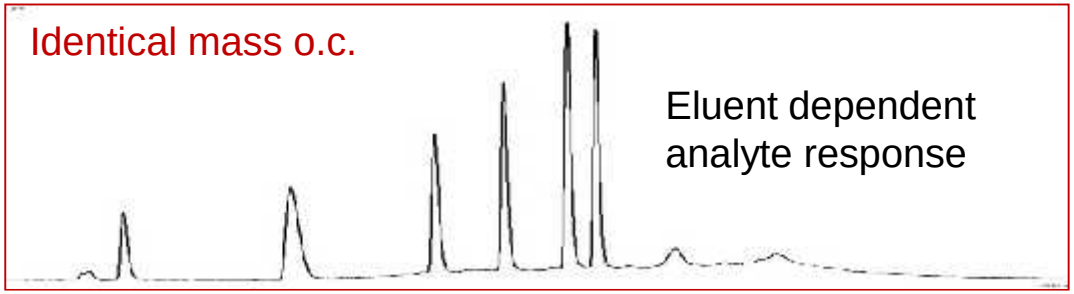
Analytical Gradient



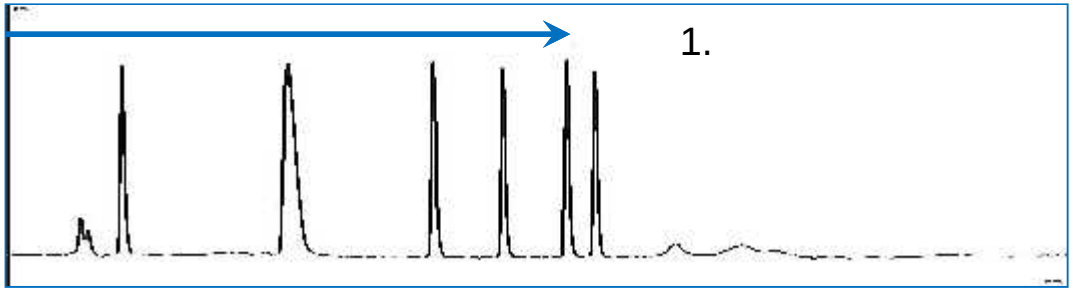
Inverse Gradient

Eluent at detector

Conventional Gradient Elution



Inverse Gradient Compensation



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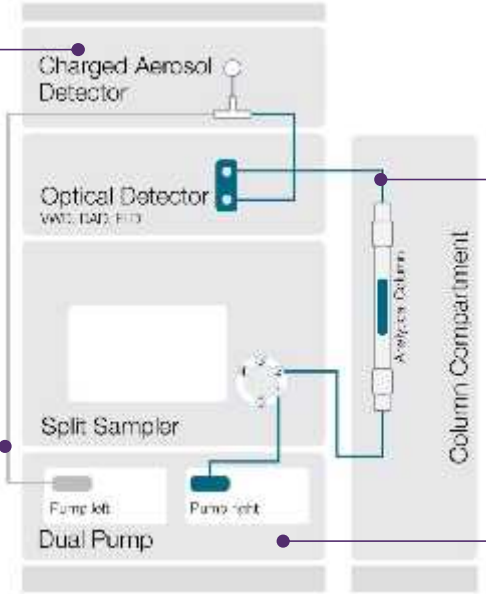
Inverse Gradient Technical Realization

Detector
Universal Charged Aerosol Detector to detect any non-volatile analyte

Compensation Flow
Keeps eluent at detector constant



Analytical Flow Path
With separation column

Vanquish Dual Pump
Delivers analytical and compensation gradient

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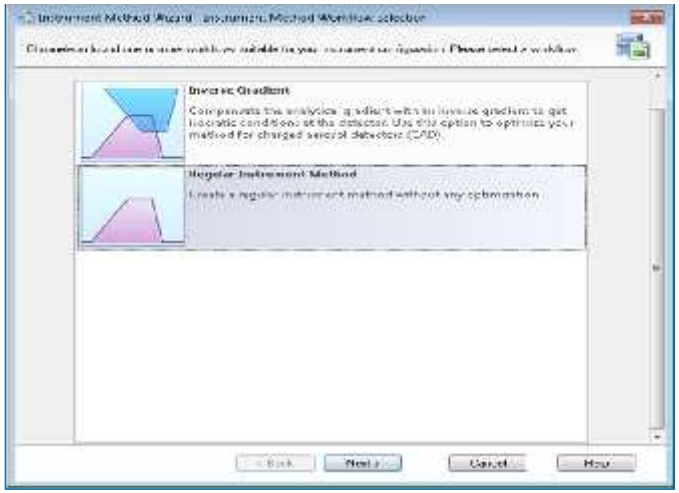
Key Benefits – Thermo Scientific Vanquish Duo System for Inverse Gradient

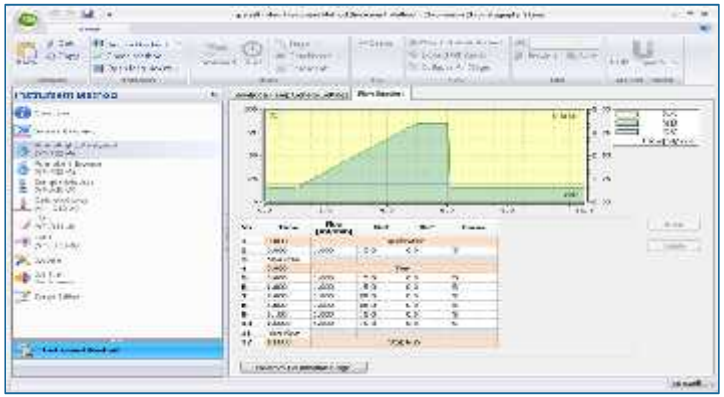
Easy to implement

1. 2. 3.

Automatic instrument recognition and fluidic knowledge



Standard instrument method setup



Automatic method setup for compensation gradient

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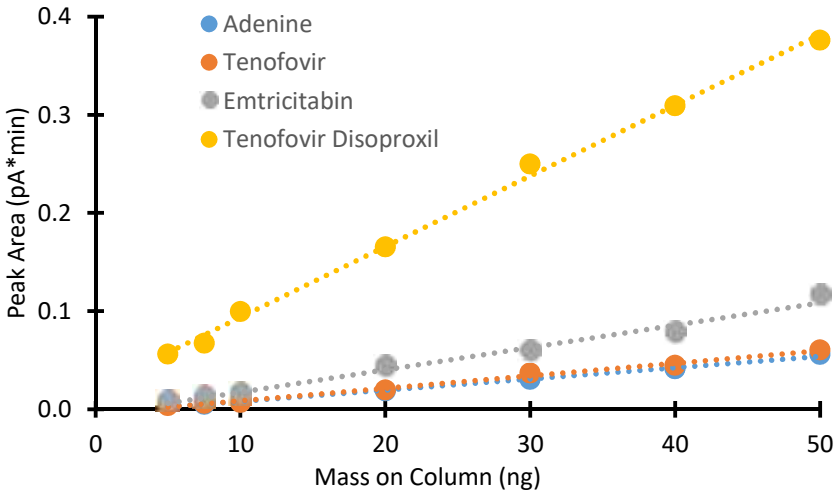
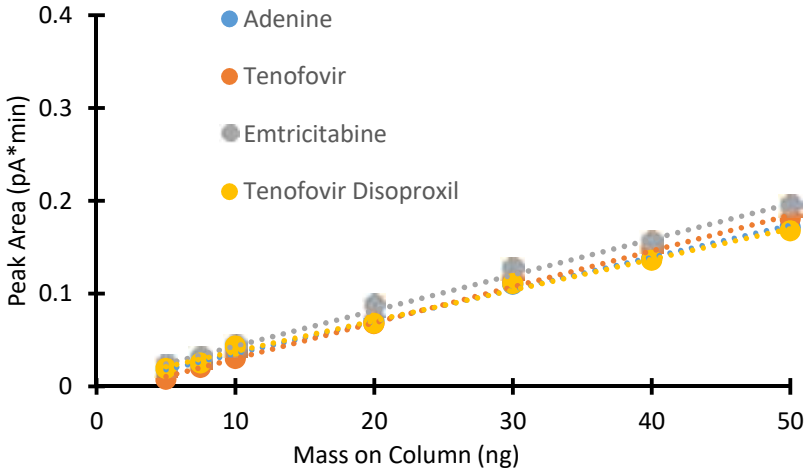
Key Benefits – Thermo Scientific Vanquish Duo System for Inverse Gradient

Standard free quantification

Without gradient Compensation



With Inverse Gradient Compensation



Similar response curves for all analytes after gradient compensation

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Summary

Higher Productivity by

Thermo Scientific™ Vanquish™ Duo UHPLC System for Tandem LC/LC-MS

Shorter Analysis Time by Reduced Detector Idle Time

Increased Samples per Day

Limited to one Application at a Time

Thermo Scientific™ Vanquish™ Duo UHPLC System for Dual LC

Shorter Analysis Time by Overlapping Applications

Doubled sample information out of a single aliquot

Two Applications at a Time Through Separate Flow Paths in a Single System Bench Space

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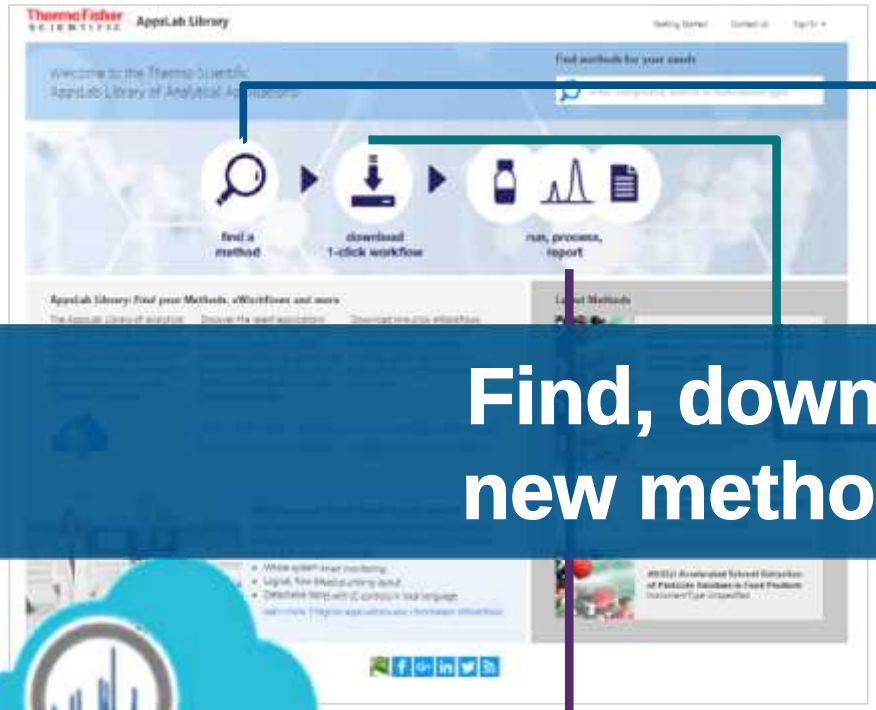
Remco Swart
Frank Steiner
Mauro De Pra

References

Title	27-Feb	28-Feb	01-Mar
High throughput, flexible chromatographic analysis of monoclonal antibodies (910 – 4)	10 – 12 am		
How to squeeze 48 hours into one day: Productivity enhancement by Dual LC technology (1550 – 4)	10 – 12 am		
A Dedicated System for Quantitation of APIs and Impurities by Inverse Gradient Reversed Phase Chromatography With Charged Aerosol Detection (960 – 4)	10 – 12 am		
Use of A Novel UHPLC System for the Simultaneous UHPLC Analysis of Water-Soluble and Fat-Soluble Vitamins (960 – 5)	10 – 12 am		
No Chromophore? No Problem! Universal HPLC Detection.	12 – 1 pm		
Single Quadrupole Mass Spectrometry: A Must-Have in the Chromatography Lab	2 – 3 pm		
Tandem UHPLC operation for high-throughput LC-MS peptide mapping analyses (1320 – 3)		10 – 12 am	
Capillary-flow LC-MS platform for robust and sensitive high-throughput proteomics (1320 – 9)		10 – 12 am	
Mass spectrometry for every chromatographer – A new single quadrupole mass spectrometer to overcome common detection challenges (1940 – 3)			10 – 12 am

Thermo Scientific AppsLab Library of Analytical Applications



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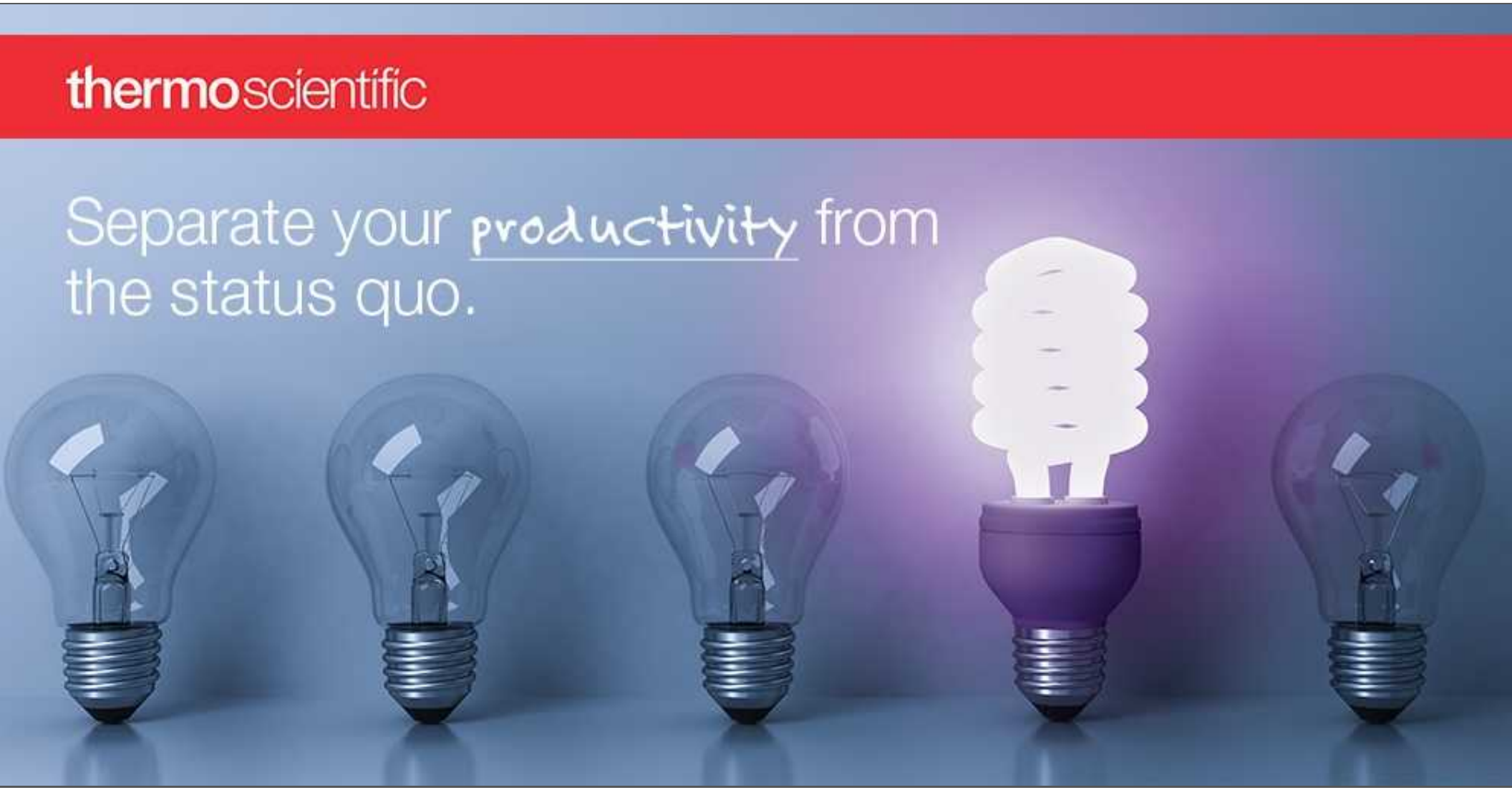
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