



ThermoFisher
SCIENTIFIC

Limited Resources? Unlimited Opportunities! New Dual HPLC Overcomes Limitations

Patrick Bohman

The world leader in serving science

A Mission We are Proud of



We enable our customers to make the world healthier, cleaner and safer.

Software and Services

- Gold-standard Chromeleon CDS
- Comprehensive services
- Multi-vendor support



Unity Lab Services



Consumables & Chemicals





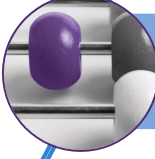

- Comprehensive offering
- IC, LC, GC columns
 - Vials, septas, fittings
 - Solvents and standards
 - Global chemicals



Instruments

- IC, LC, GC, MS
- Sample prep
- LC multiplexing



-  Goals of the modern laboratory - **Productivity**
-  Doubling throughput with **dual LC**
-  **Single quadrupole** mass detection
-  Maximizing detector utilization with **tandem LC**
-  Improving CAD quantification with **inverse gradient**
-  Summary











Reliable Productivity

- More information from every sample
- More done in the same amount of time with no additional effort
- System automation and control
- Faster data processing



Thermo Scientific LC Portfolio – A System for Virtually Every User’s Need

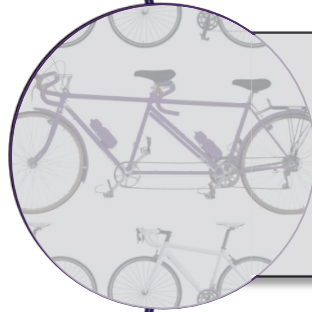
HPLC Systems		UHPLC Systems		
Routine analysis		Low flow	Flexibility, more performance and method development	Research and high throughput
<p>Thermo Scientific™ UltiMate™ 3000 Basic Automated HPLC Systems</p> <ul style="list-style-type: none"> Highly economic and reliable 		<p>Thermo Scientific™ UltiMate™ 3000 SD HPLC Systems</p> <ul style="list-style-type: none"> Workhorse for standard HPLC applications 		
<p>Thermo Scientific™ UltiMate™ 3000 RSLCnano UHPLC System</p>		<p>Thermo Scientific™ EASY-nLC™ 1200 System</p> <ul style="list-style-type: none"> UHPLC systems for Nano/Cap/Micro range Ideal front end for proteomics applications 		
<p>Thermo Scientific™ UltiMate™ 3000 RS/BioRS UHPLC Systems</p> <ul style="list-style-type: none"> Specialty workflow support Binary and quaternary UHPLC systems 		<p>Thermo Scientific™ Vanquish™ Duo System Workflows</p> <p>Thermo Scientific™ Vanquish™ Flex UHPLC Systems</p> <ul style="list-style-type: none"> High-pressure binary and low-pressure quaternary solvent mixing options Two thermostating modes Biocompatible Integration of multiple detection technologies    		
620 bar		800–1200 bar		
		Up to 1000 bar		
		1500 bar		

Three Workflows. Two Flow Paths. One Integrated UHPLC Solution.



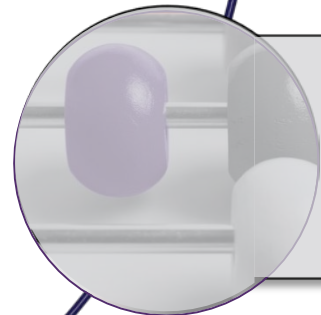
Dual LC

- A two-channel LC system with standard footprint



Tandem LC or LC-MS

- System for efficient detector utilization



Inverse Gradient

- System for advanced quantification capabilities

Thermo Scientific™ Vanquish™ Flex Dual Pump



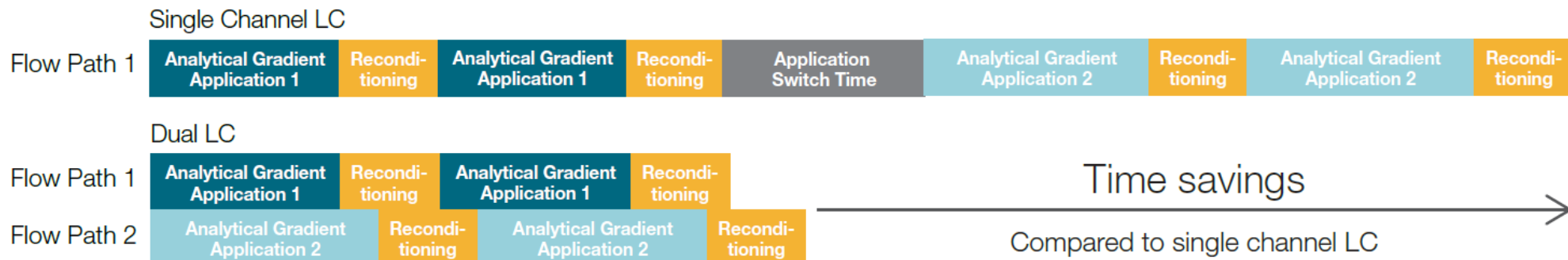
- 2 ternary pumps in one housing
- 1000 bar (15,000 psi) version
- Delivers 2 completely independent gradients

Thermo Scientific™ Vanquish™ Dual Split Samplers



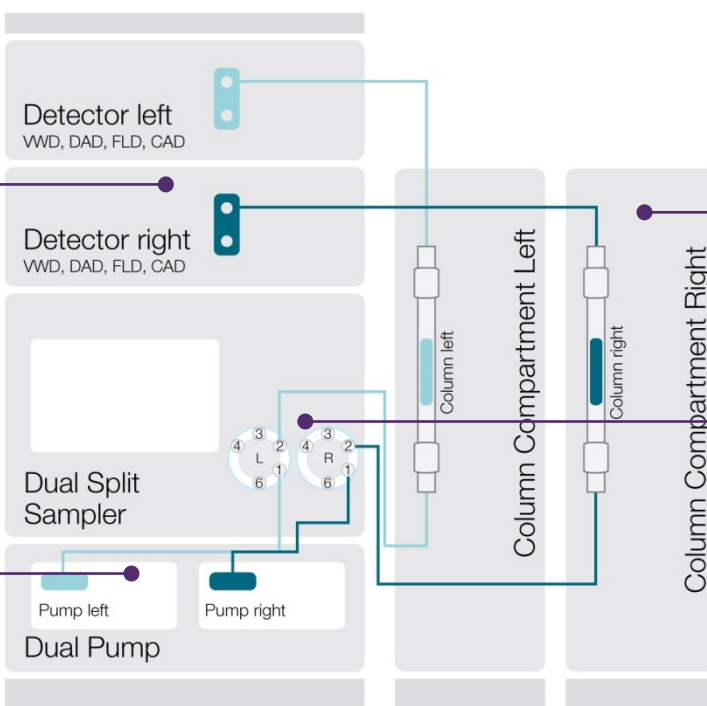
- 2 injection units in one autosampler
- 1000 and 1500 bar (15,000 or 22,000 psi) version
- Independent flow paths

Thermo Scientific Vanquish Duo System for Dual LC



Detectors

Any combination of two detectors ensuring sensitive and universal detections



Column Compartment

Two columns in one or two column compartments for full application flexibility

Autosampler

Two injection valves allowing the simultaneous analysis of completely different applications

Pump

Two independent pumps in one housing

What Can Be Accomplished with Thermo Scientific Vanquish Duo System for Dual LC?

Dual LC



Run two identical columns

Dual LC



Run your complementary

“The instrument offers a fantastic increase in throughput when running two different separation chemistries, enabling us to generate large amounts of characterization data”

Dr. Jonathan Bones, NIBRT

Be finished in half the time

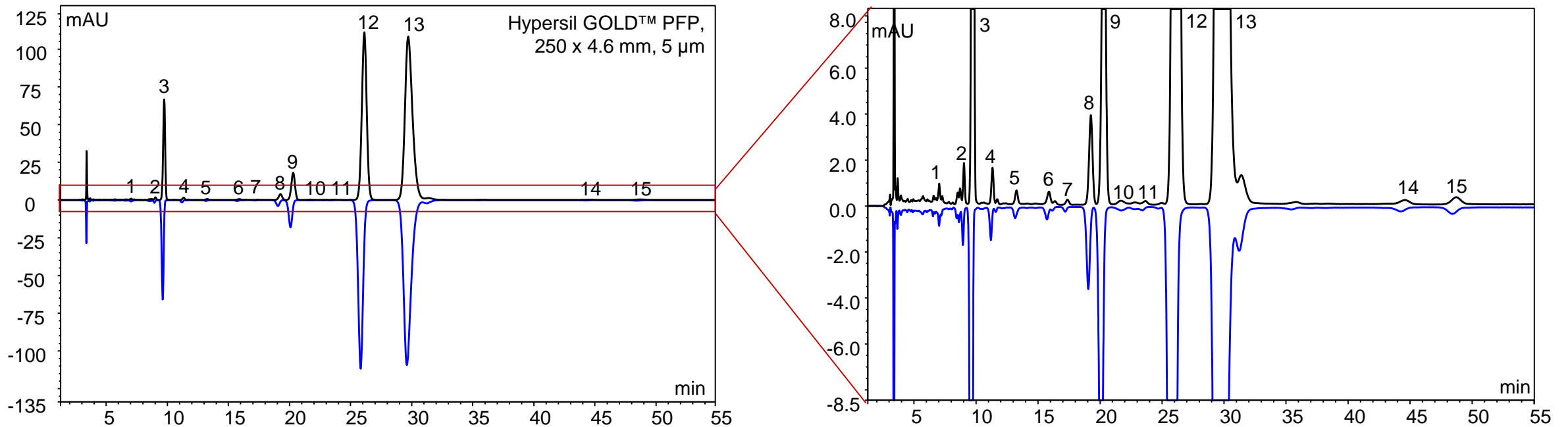
Finished in half the time

Get more information out of every sample

Efficient characterization

Thermo Scientific Vanquish Duo System for Dual LC – Impurity Analysis

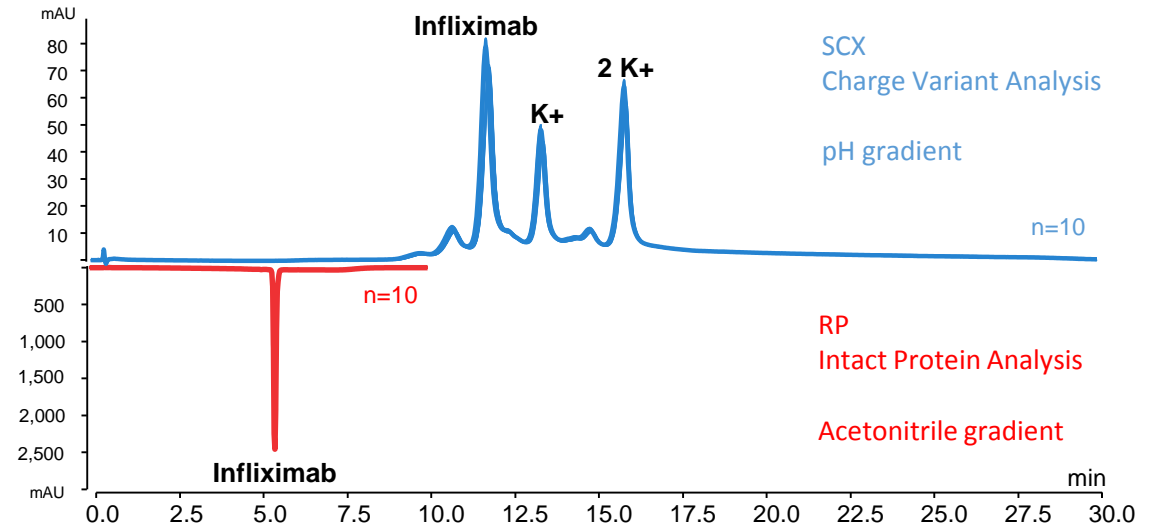
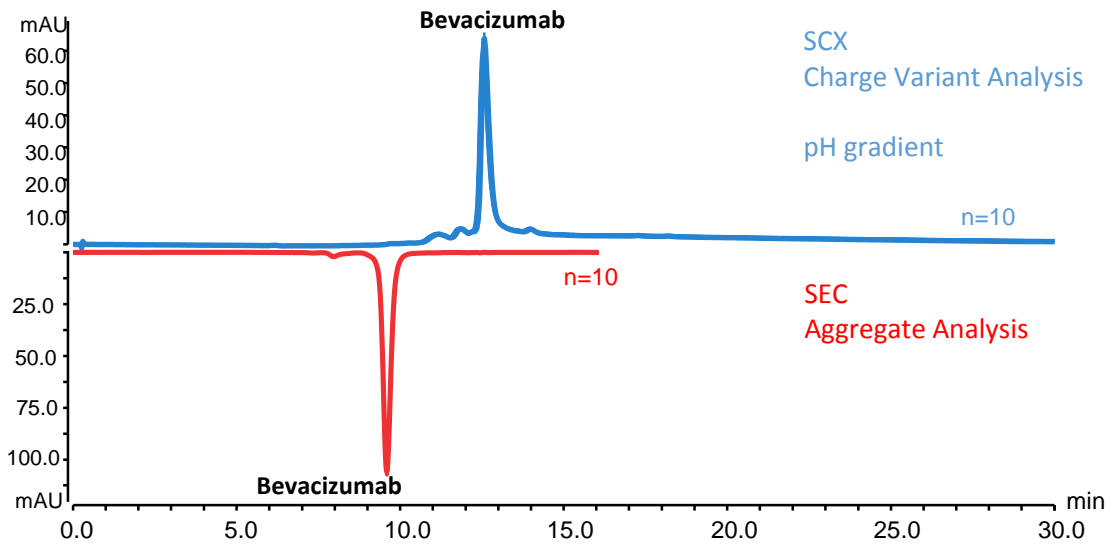
- Combinatorial therapy used for treatment of hyperlipidemia
- Analysis of simvastatin and ezetimibe and their impurities
- Long 55 min isocratic step followed by 10 min column wash and re-equilibration



Doubled throughput of validated isocratic methods without changing method parameters

Thermo Scientific Vanquish Duo System for Dual LC – Antibody Characterization

- Multiple methods used for characterization of biopharmaceuticals
- Two fluidic paths enable simultaneous characterization with complementary methods



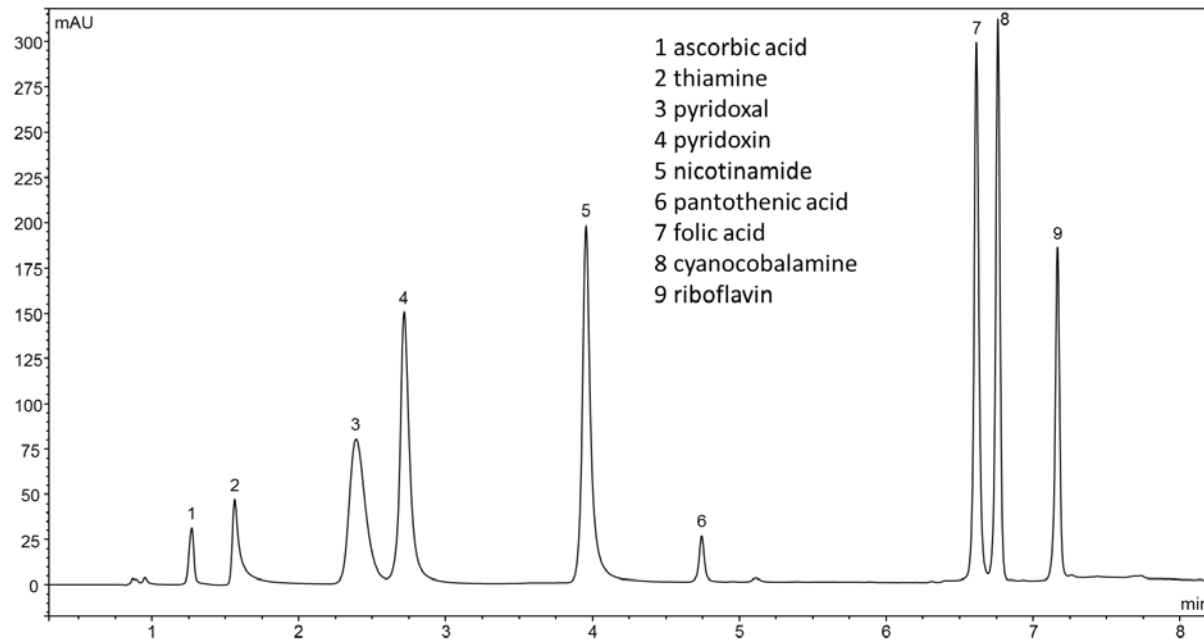
Efficient characterization of antibodies by performing 2 different assays simultaneously

Thermo Scientific Vanquish Duo System for Dual LC – Vitamin Analysis

- Vitamins are divided into waters soluble (WSV) and fat soluble vitamins (FSV)
- So far no good way to routinely analyze both groups simultaneously

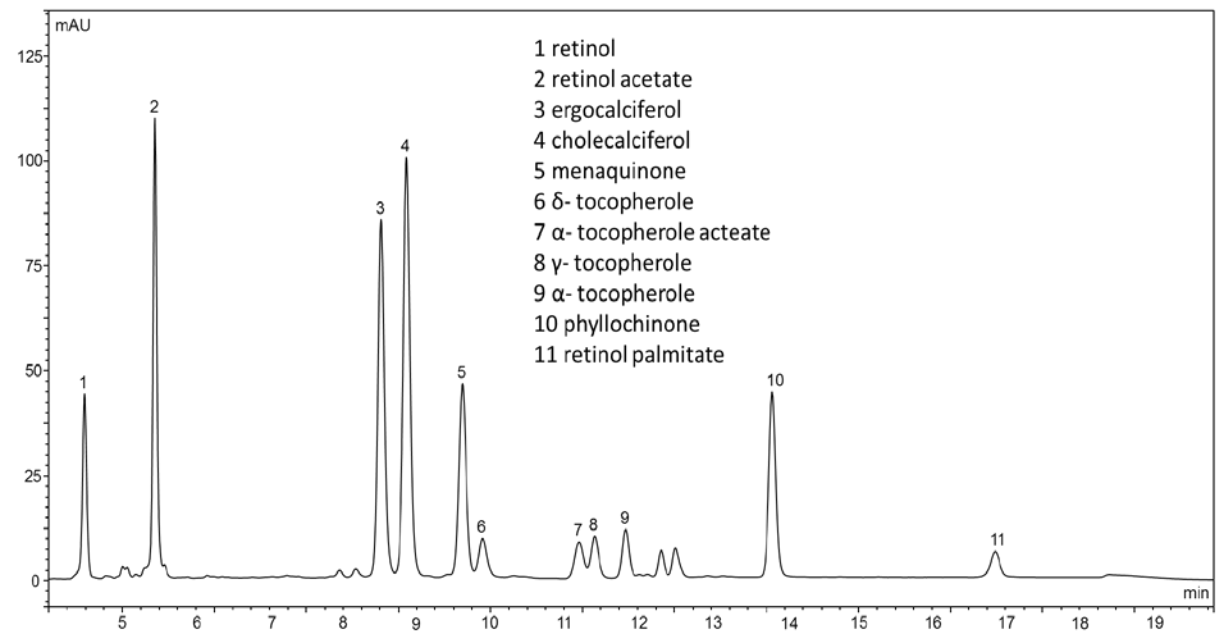
Water soluble vitamins

Thermo Scientific™ Acclaim™ Polar Advantage 150x2.1 mm



Fat soluble vitamins

Thermo Scientific™ Acclaim™ Polar Advantage 250x2.1 mm



Reliable and fast quantification of both vitamin groups

Key Benefits – Thermo Scientific Vanquish Duo System for Dual LC

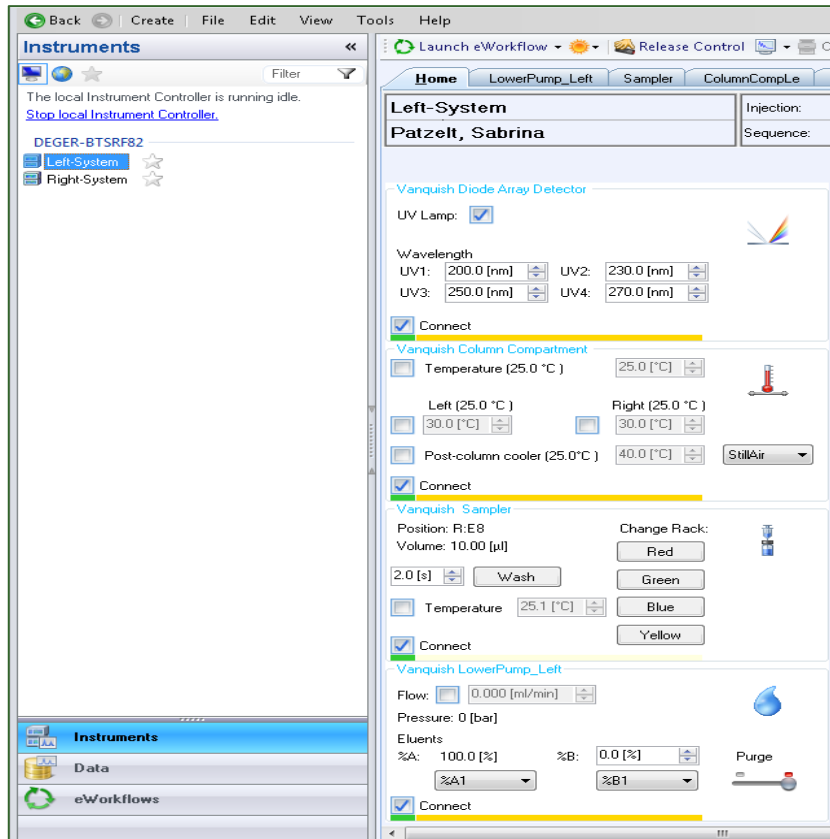
Easy-to-use as any other Thermo Scientific™ Vanquish™ system

1.

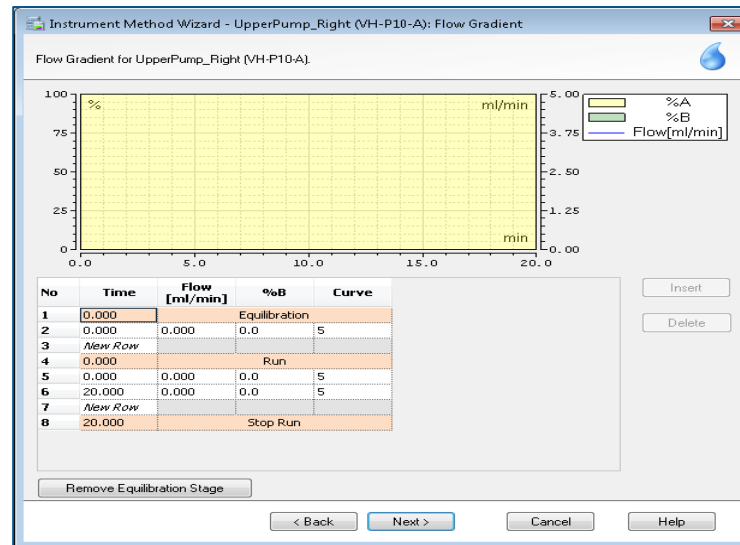
2.

3.

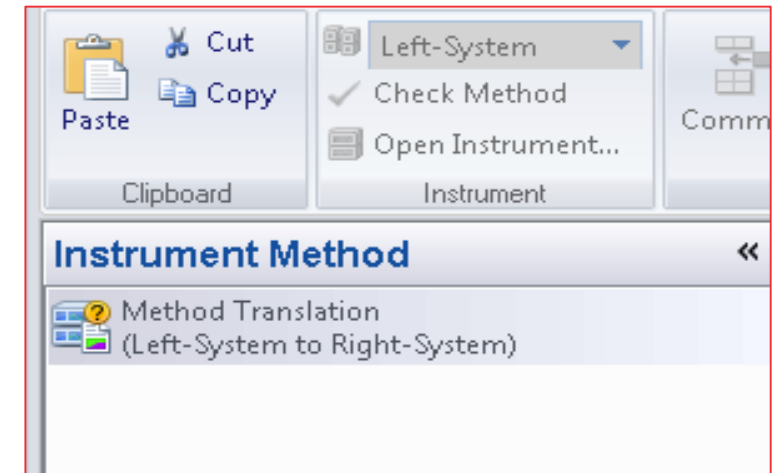
Simple instrument selection



Easy method setup with method wizard



Automatic method translation



Key Benefits – Thermo Scientific Vanquish Duo System for Dual LC

No additional bench space needed

Vanquish Duo System for Dual LC



Vanquish UHPLC Systems



Thermo Scientific Vanquish Detector Portfolio

Integrated detector options

Advantages

Thermo Scientific™ Vanquish™ LightPipe Diode Array Detector (DAD H)	Outstanding signal to noise performance and linearity
Thermo Scientific™ Vanquish™ Flex Diode Array Detector (DAD FG)	Leading Linearity, robustness and smooth MS integration
Thermo Scientific™ Vanquish™ Variable Wavelength Detector (VWD)	Supports a wide operating range
Thermo Scientific™ Vanquish™ Fluorescence Detector (FLD)	High precision and sensitivity
Thermo Scientific™ Vanquish™ Charged Aerosol Detector (CAD)	Offers sensitive, universal detection with a near uniform response
Thermo Scientific™ ISQ™ EC Mass Spectrometer	Single quadrupole MS for robust and easy mass detection

Thermo Scientific ISQ EC/EM Single Quadrupole Mass Spectrometers

- For ion chromatography (IC-MS) and liquid chromatography (LC-MS)
- Built for **routine** analysis and long-term signal stability
- Controlled by Thermo Scientific™ **Chromeleon™** Chromatography Data System (CDS) Software



Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer

- Mass range 10-1250 m/z
- HESI only
- Perfect for IC-MS or LC-MS



Thermo Scientific™ ISQ™ EM Single Quadrupole Mass Spectrometer

- Mass range 10-2000 m/z
- HESI and APCI options
- Perfect for LC-MS

Thermo Scientific ISQ EM Single Quadrupole Mass Spectrometer – Key Features

- Enhanced performance from 10-2000 m/z
- High-performing HESI II and APCI source
- Durable, high-quality design
- Ultra-fast electronics
- Rugged and robust operation
- Innovative, smart software



An ideal single quadrupole MS for novices and experts



- ✓ Robust source **maintains ionization stability** over a wide range of flow rates
- ✓ Convenient built-in **spray needle calibration** adjustment tool further aids robustness
- ✓ **Enhanced spray stability** with redesigned needle tip
- ✓ Orthogonal spray geometry and automated gas flow streams for **solid performance** with dirty samples
- ✓ **Worry-free operation** with intelligent, experiment-based method editor and built-in leak sensor

Designed for continuous operation and maximum uptime

The New Single Quadrupole MS – Key Product Features

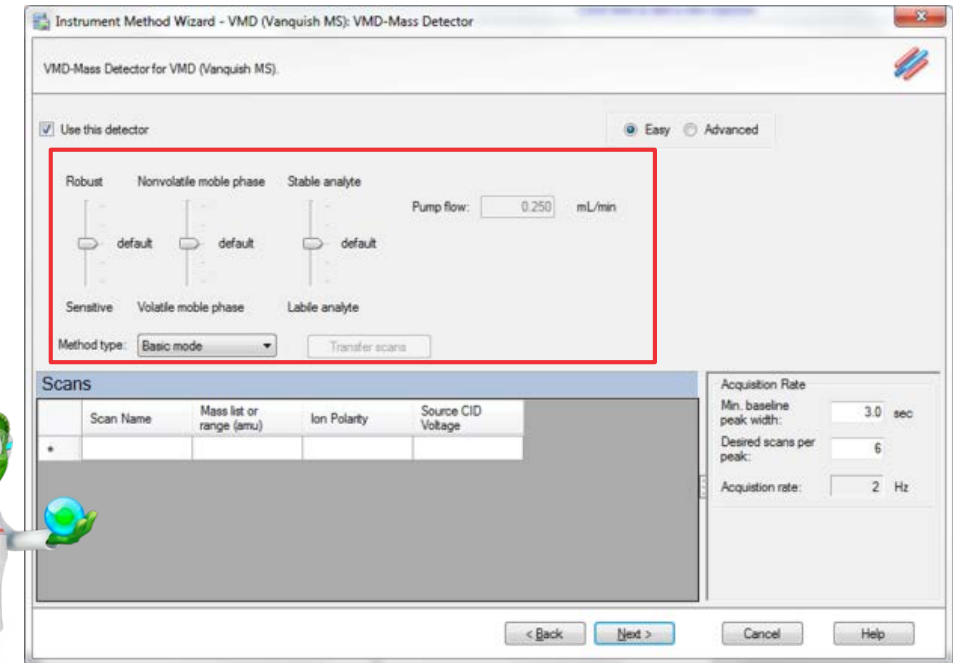
- Durable, high-quality design
- Enhanced low MW performance
- High performing heated electrospray ionization (HESI) II source
- Ultra-fast electronics
- Rugged and robust operation
- 100% Chromeleon CDS integrated
- Innovative, smart software



- ✓ Robust source **maintains ionization stability** over a wide range of flow rates
- ✓ Convenient built-in **spray needle calibration** adjustment tool further aids robustness
- ✓ Orthogonal spray geometry and automated gas flow streams for **solid performance** with dirty samples

The New Single Quadrupole MS – Key Product Features

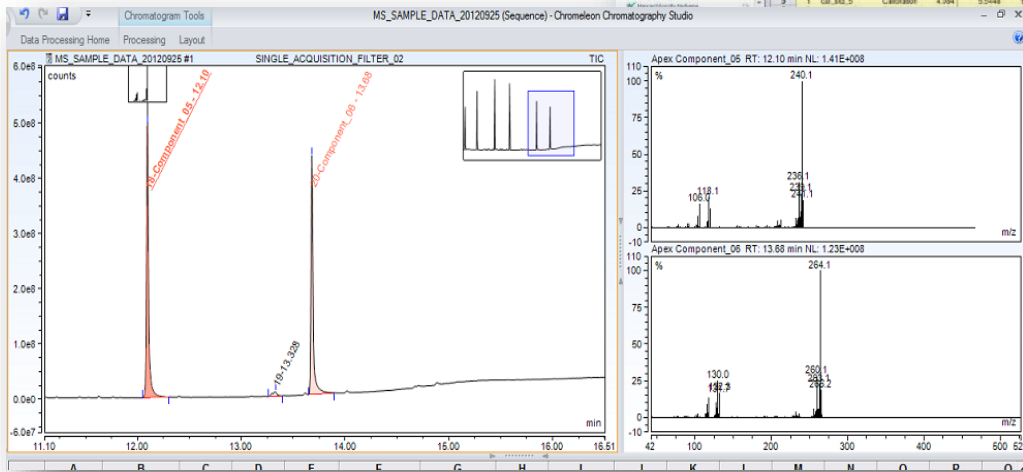
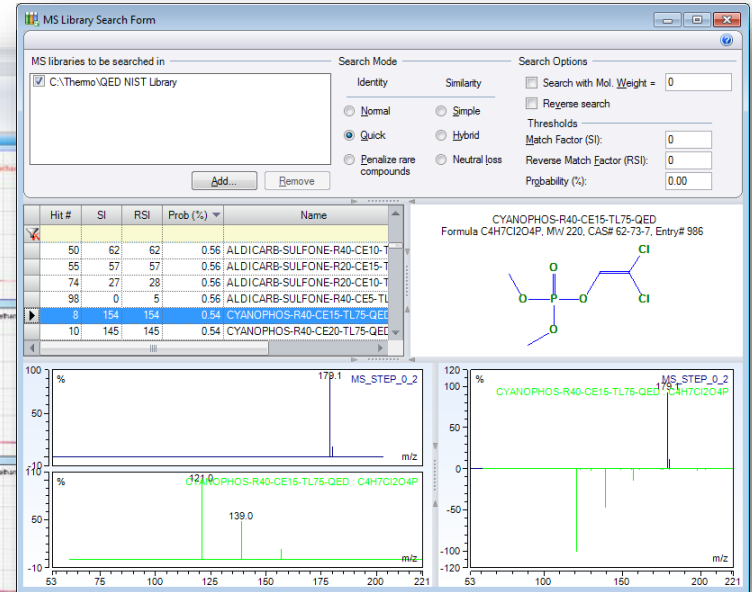
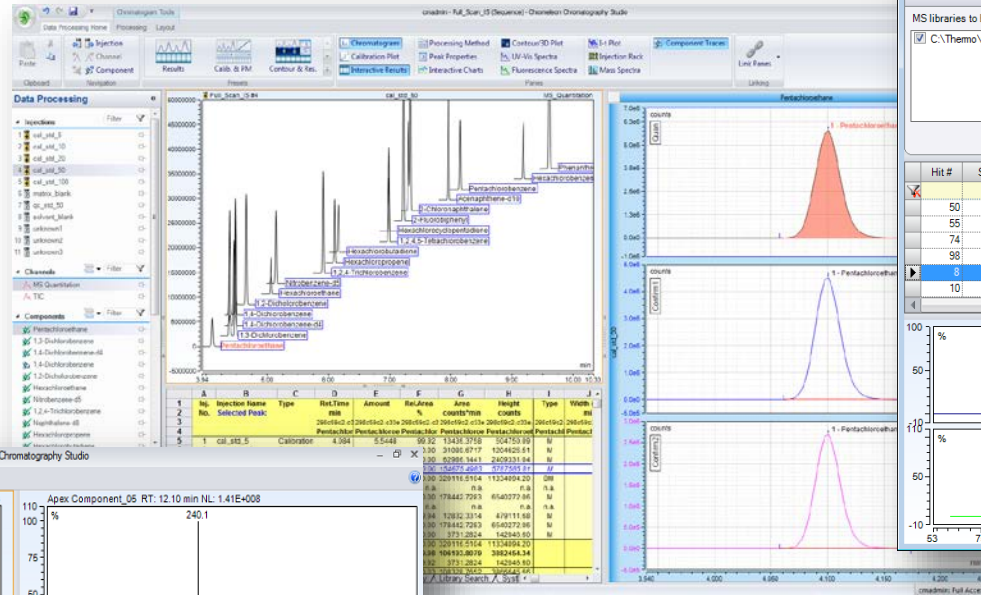
- Enhanced low MW performance
- Durable, high-quality design
- High performing heated electrospray ionization (HESI) II source
- Ultra-fast electronics
- Rugged and robust operation
- 100% Chromeleon CDS integrated
- Innovative, smart software



- ✓ Unique **Autospray** software technology **translates physical properties** of the analyte, mobile-phase and system-wide instrument settings into **optimal method parameters**
- ✓ Easy Mode for the novice user, advanced tools for the MS expert

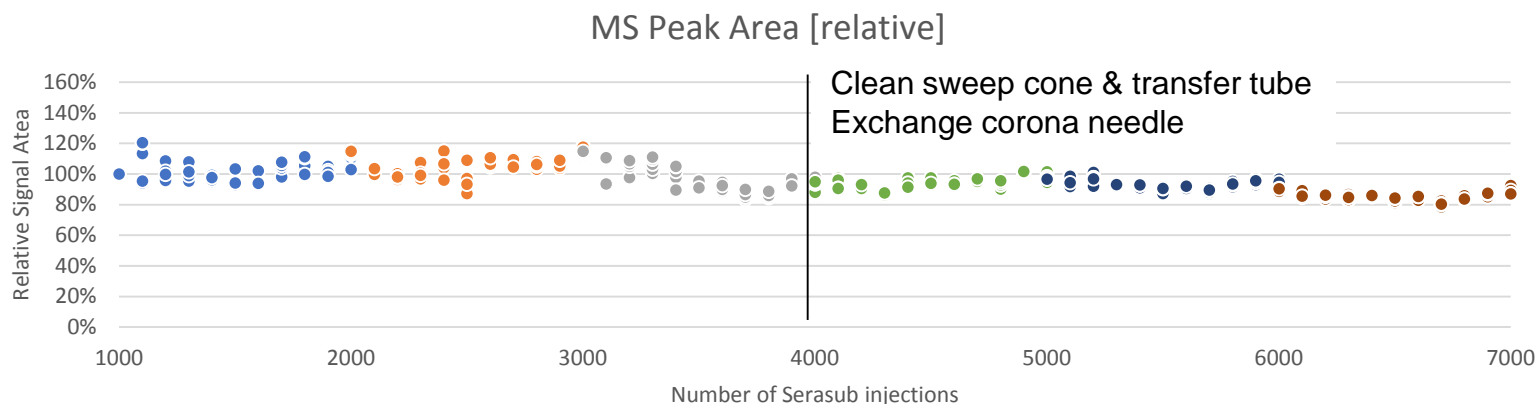
Overall Value Thermo Scientific ISQ EC/EM Mass Spectrometer for Routine Labs

First CDS to support MS with all main front-end separation techniques (GC, LC, IC)
No compromises on data integrity or compliance



Thermo Scientific ISQ EM Robustness (Flow injections of serum analog)

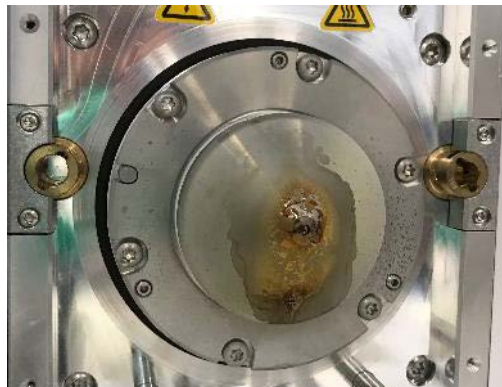
Flow injection set-up without column. Continuously inject high concentration of serum analog (Serasub®, 5% in water, 10 µL injections). Measure 5 replicates of hydrocortisone (10 ng/µL, 1 µL injections) every 100 Serasub injections. 1 color = 1 hydrocortisone & sersub vial.



Sweep cone after 4000 injections



... after 7000 injections



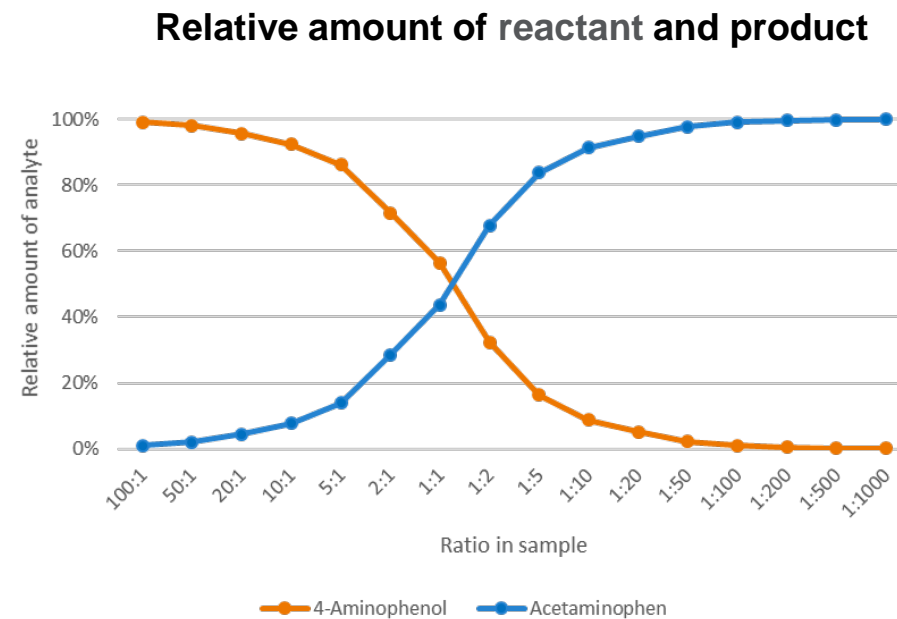
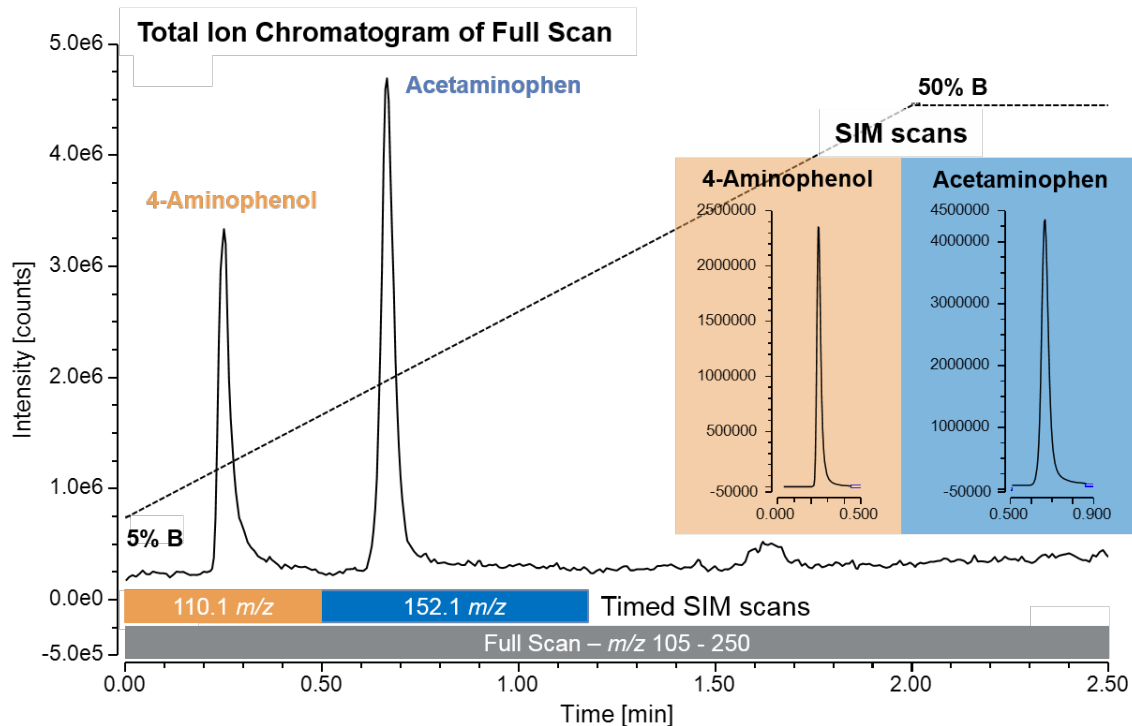
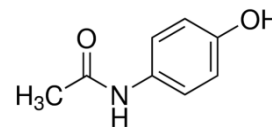
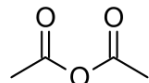
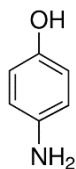
Less than 20% signal loss over 7,000 injections.

7,000 flow injections are the equivalent of 45 days of measurement with 10 min runs.

Conditions are harsher than in usual application settings:

- Very high concentration of serum analog
- No sample clean-up before injection
- No (guard) column

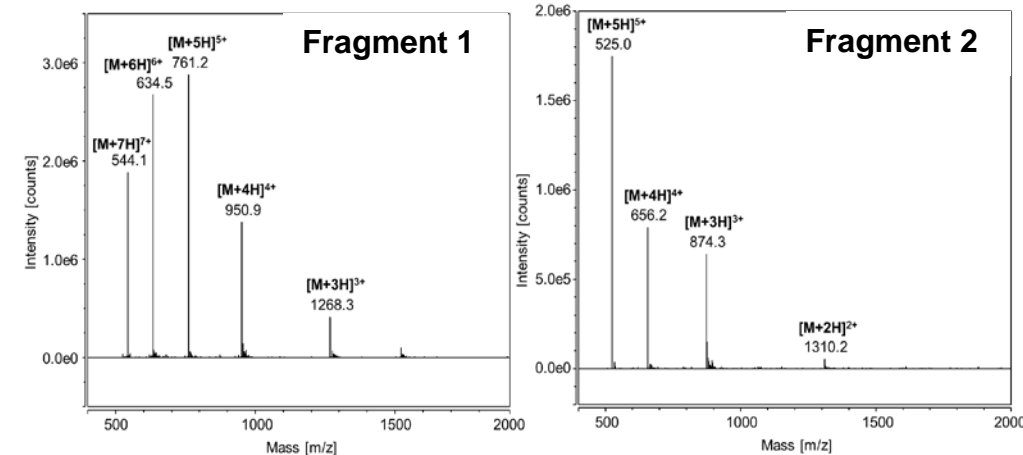
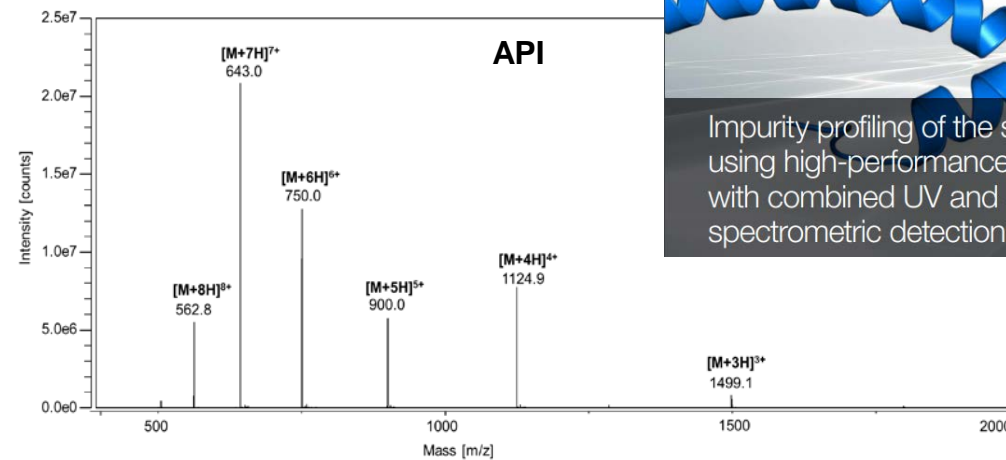
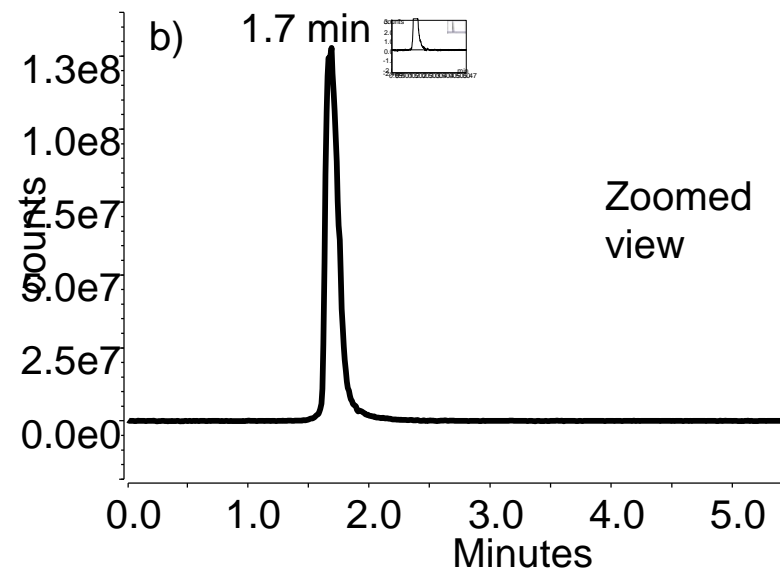
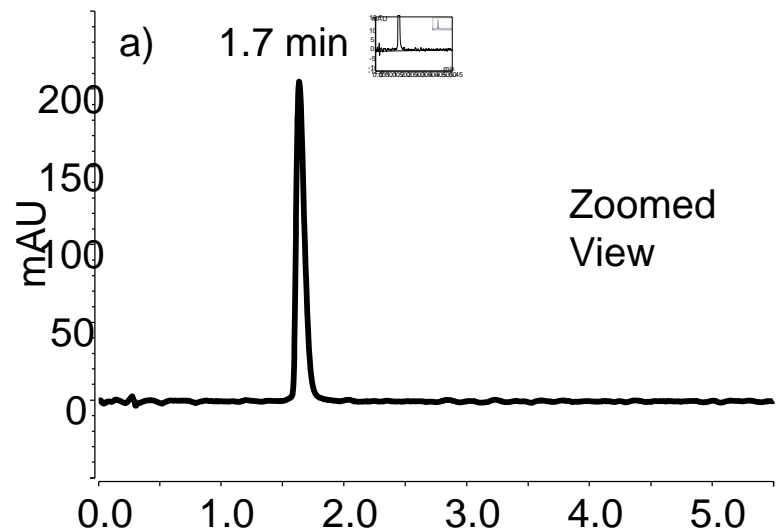
System performs robustly over extended period in very harsh conditions



Thermo Scientific Application Note 72385

Accurate and precise high-throughput reaction monitoring

Peptide Impurity Profiling



Thermo Scientific Application Note 72818

Mass-based compound confirmation and peak purity assessment

Three Workflows. Two Flow Paths. One Integrated UHPLC Solution.



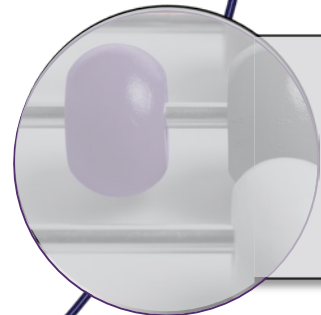
Dual LC

- A two channel LC system with standard footprint



Tandem LC or LC-MS

- System for efficient detector utilization

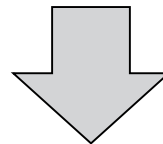
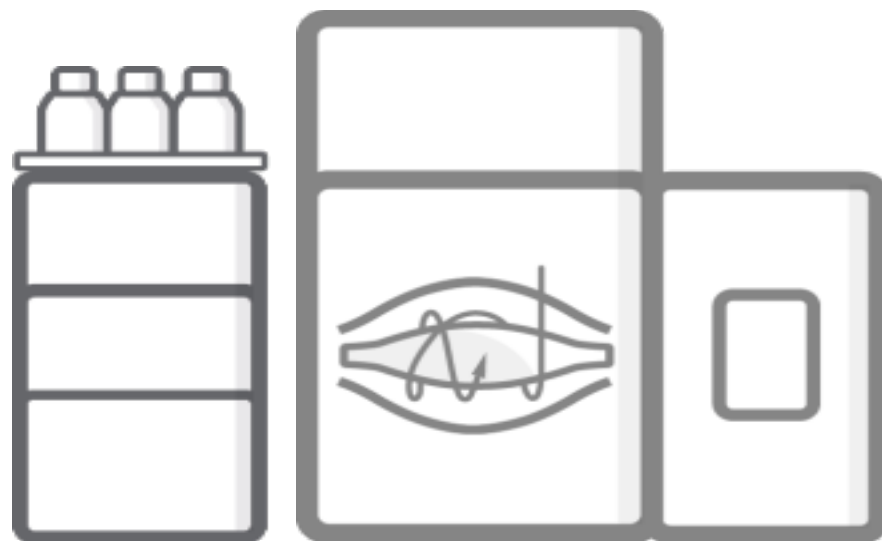


Inverse Gradient

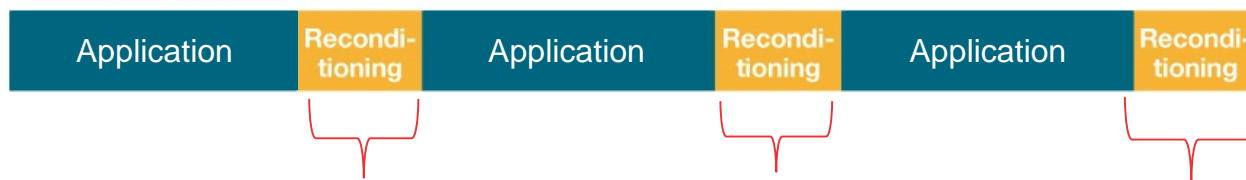
- System for advanced quantification capabilities

Analytical Challenge – Tandem LC or LC-MS

High investments in modern instrumentation



Standard LC / LC-MS



Time without acquisition of meaningful data

Not fully utilizing of instrumentation

Asked to maximize the instrument outcome

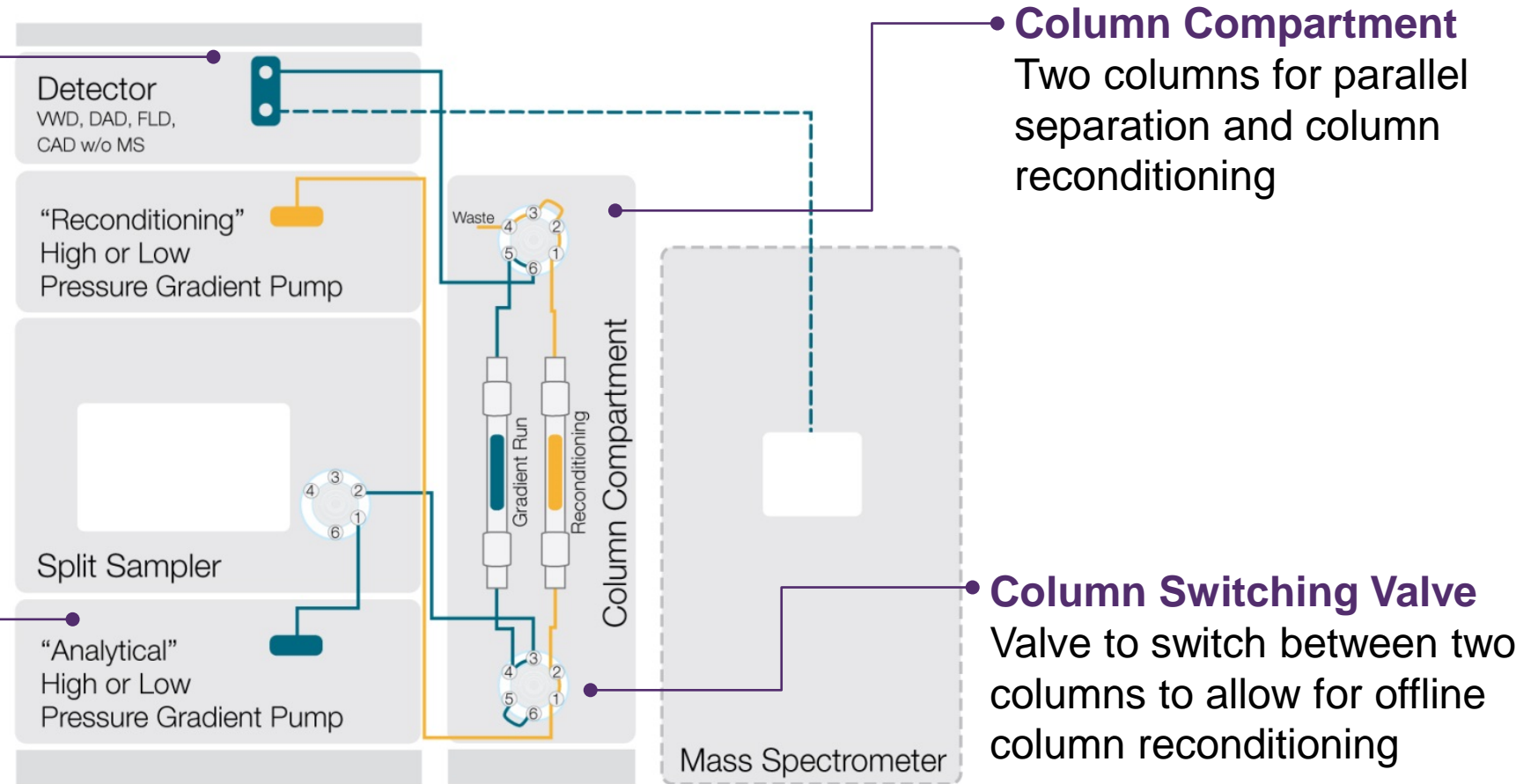
Thermo Scientific Vanquish Duo UHPLC System for Tandem LC or LC-MS

Detectors

Optional UV detector with pressure-resistant, low-dispersive flow cells

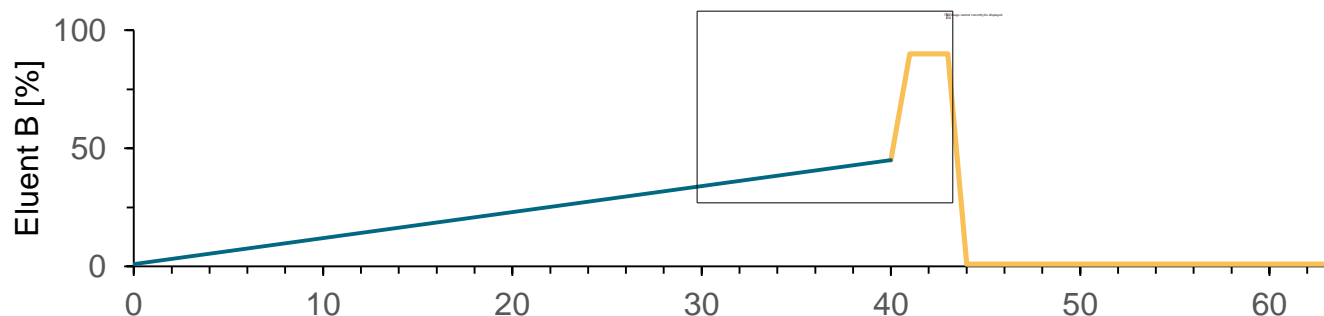
Pump

Flexible pump selection. Use high-pressure gradient pump here for analytical gradient.

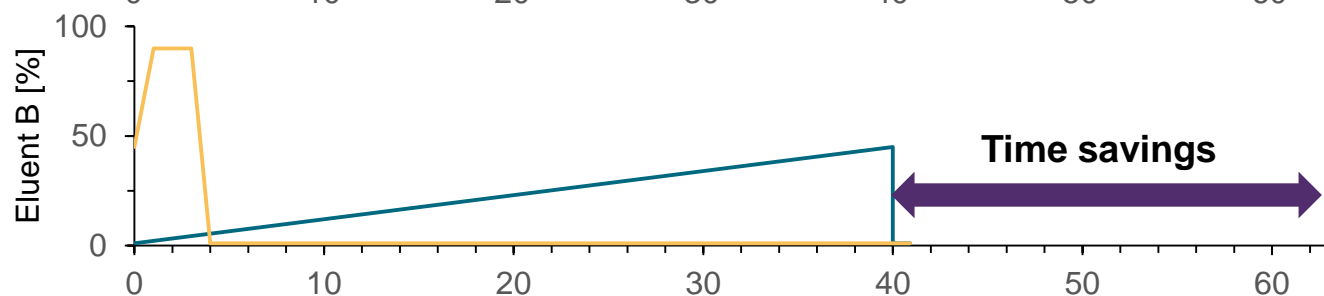


Key Benefits – Thermo Scientific Vanquish Duo UHPLC for Tandem LC or LC-MS

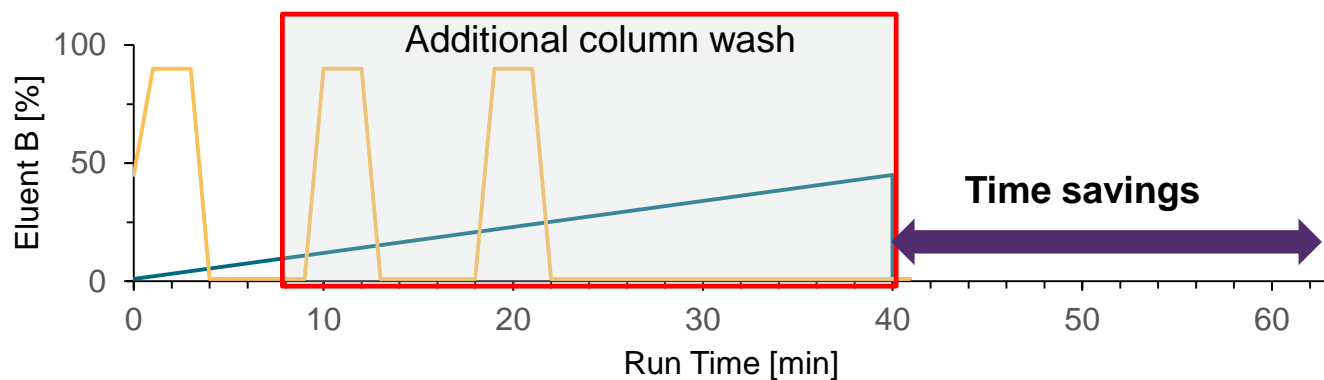
Advanced column wash without sacrificing throughput



Standard gradient profile



Tandem mode allows for time savings

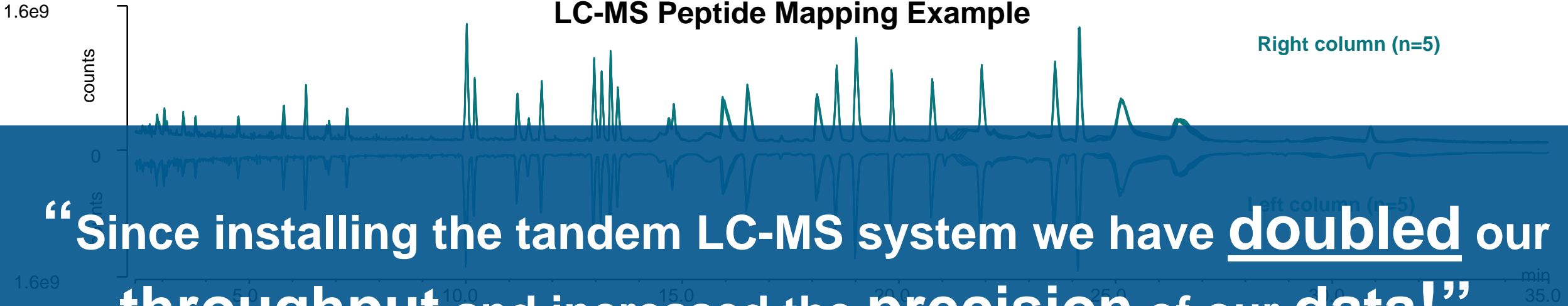


Extra column washing steps without sacrificing throughput

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

Increased throughput

LC-MS Peptide Mapping Example

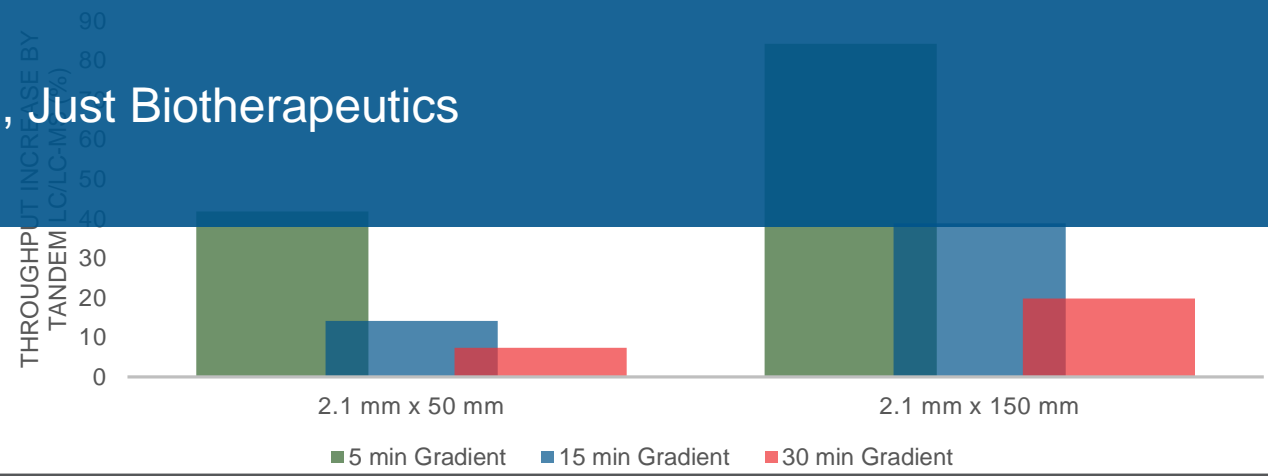


“Since installing the tandem LC-MS system we have doubled our throughput and increased the precision of our data!”

Throughput increase by Tandem LC or LC-MS

Vanquish Duo for Tandem			
Abs. RT Shift Column left to right [min]	Rel. RT Shift Column left to right [%]	RT RSD [%] n=10	Area RSD [%] n=10
0.023	0.18	0.11	2.47

Dr. Richard Rogers, Just Biotherapeutics









Thermo Scientific Technical Note 72203

Thermo Scientific Vanquish Detector Portfolio

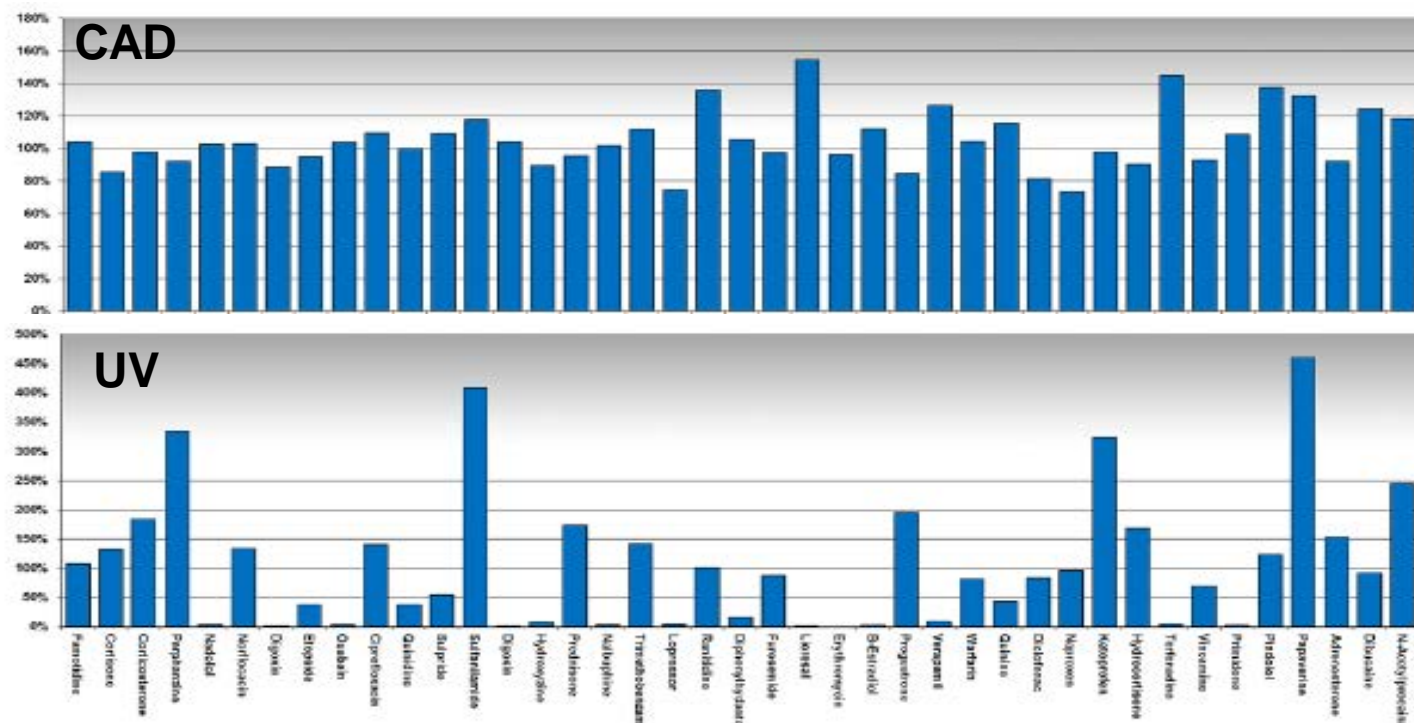
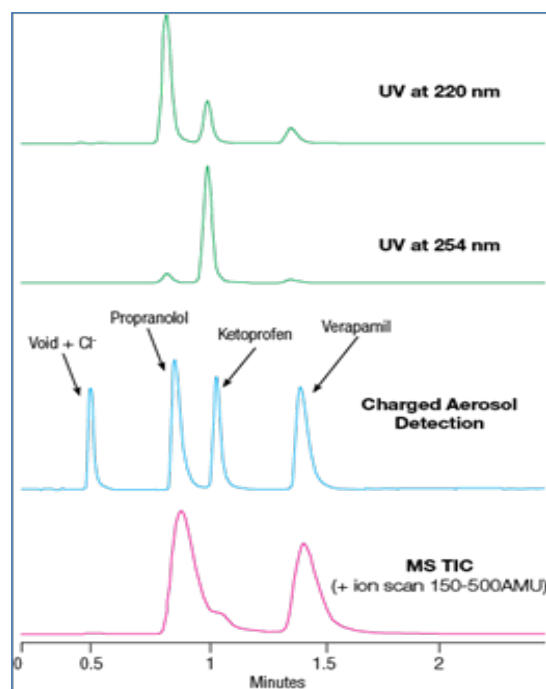
Integrated detector options

Advantages

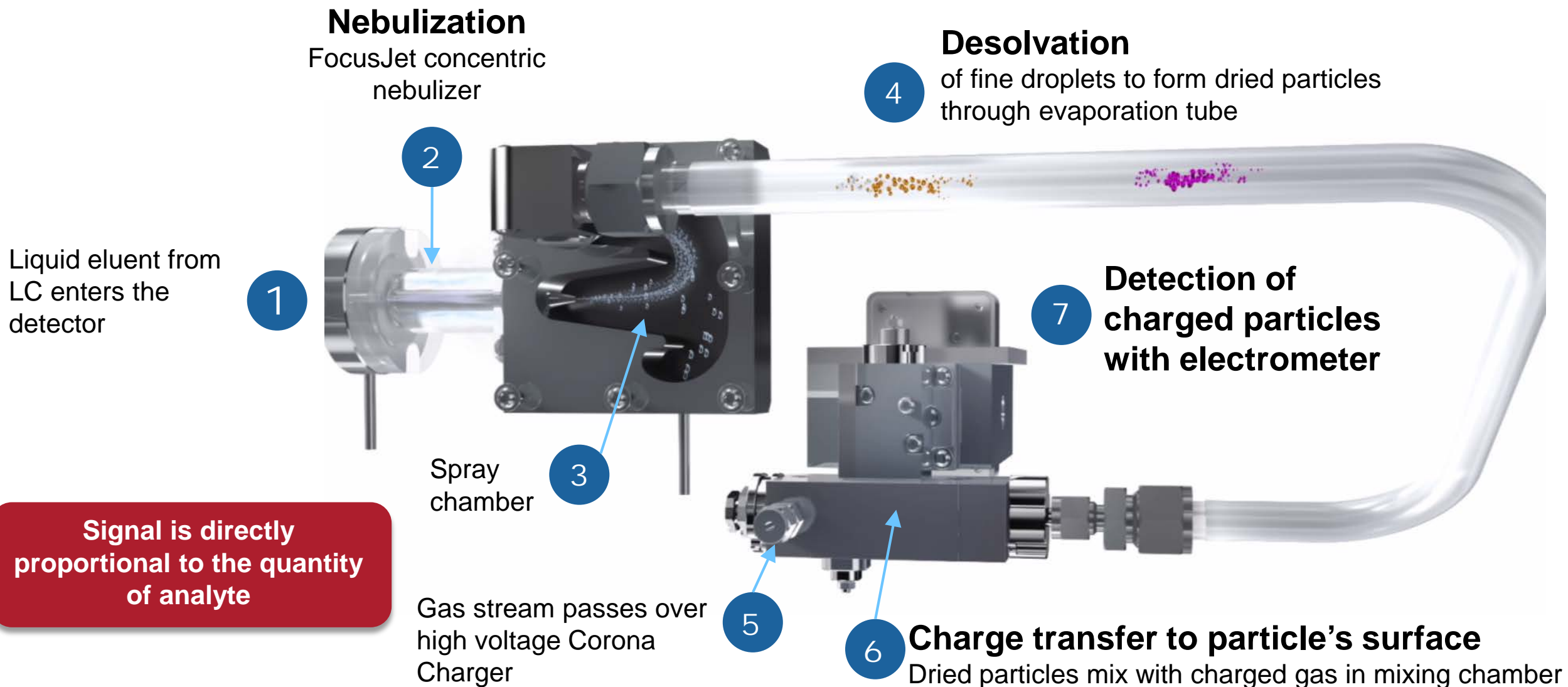
 Vanquish LightPipe Diode Array Detector (DAD H)	Outstanding signal to noise performance and linearity
 Vanquish Flex Diode Array Detector (DAD FG)	Leading Linearity, robustness and smooth MS integration
 Vanquish Variable Wavelength Detector (VWD)	Supports a wide operating range
 Vanquish Fluorescence Detector (FLD)	High precision and sensitivity
 Vanquish Charged Aerosol Detector (CAD)	Offers sensitive, universal detection with a near uniform response
 ISQ EC Mass Spectrometer	Single quadrupole MS for robust and easy mass detection

Unique Detectors: Charged Aerosol Detection (CAD)

- Detection of substances without a chromophore
- Consistent analyte response independent of chemical structure
- Dynamic range up to four orders of magnitude and highest sensitivity
- Relative quantification using virtually any standard



How it Works: Charged Aerosol Detection

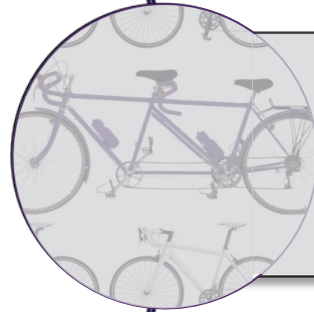


Three Workflows. Two Flow Paths. One Integrated UHPLC Solution.



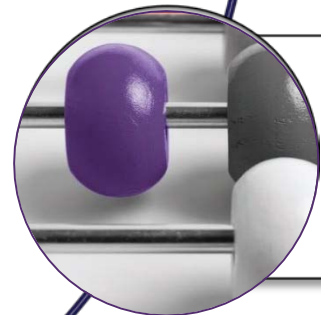
Dual LC

- A two channel LC system with standard footprint



Tandem LC or LC-MS

- System for efficient detector utilization

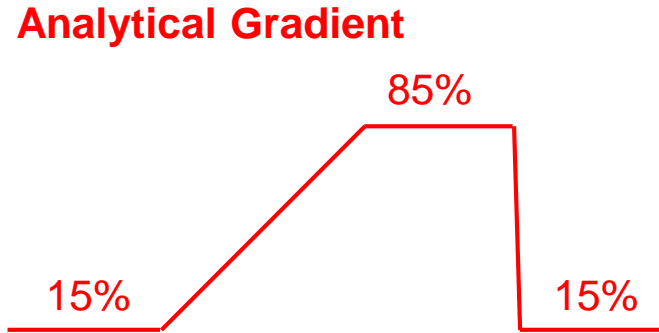


Inverse Gradient

- System for advanced quantification capabilities

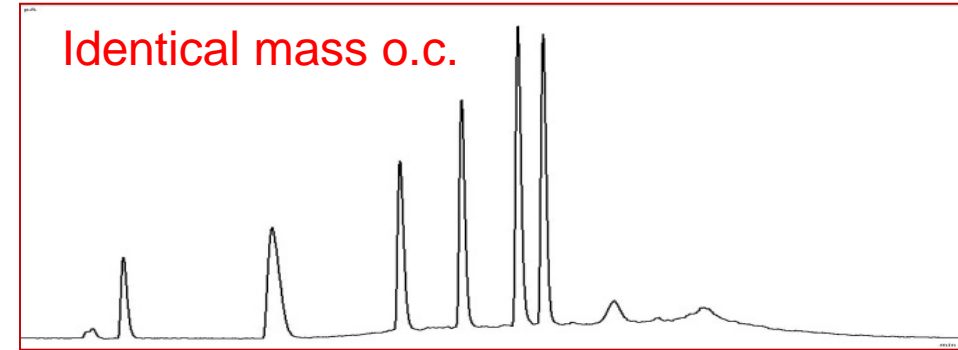
The problem

- Eluent-dependent analyte response during gradient elution



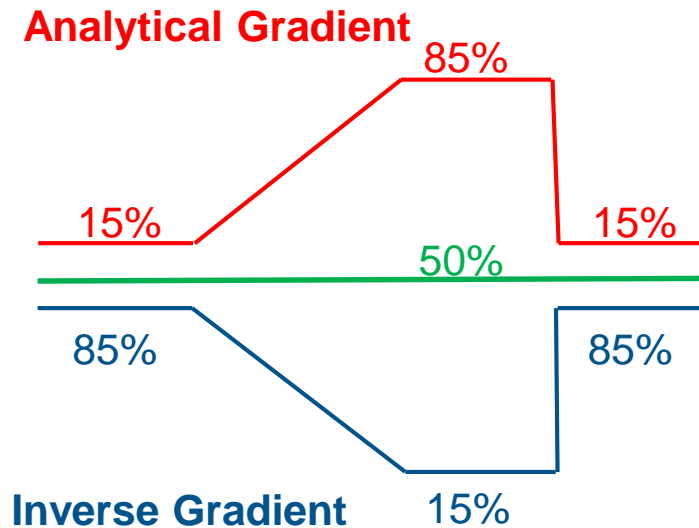
Eluent at detector

Conventional Gradient Elution



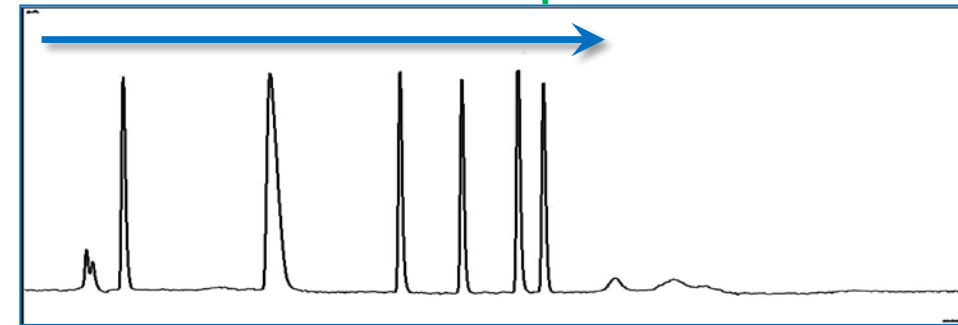
The solution

- Compensate with inverse gradient, giving constant composition in detector



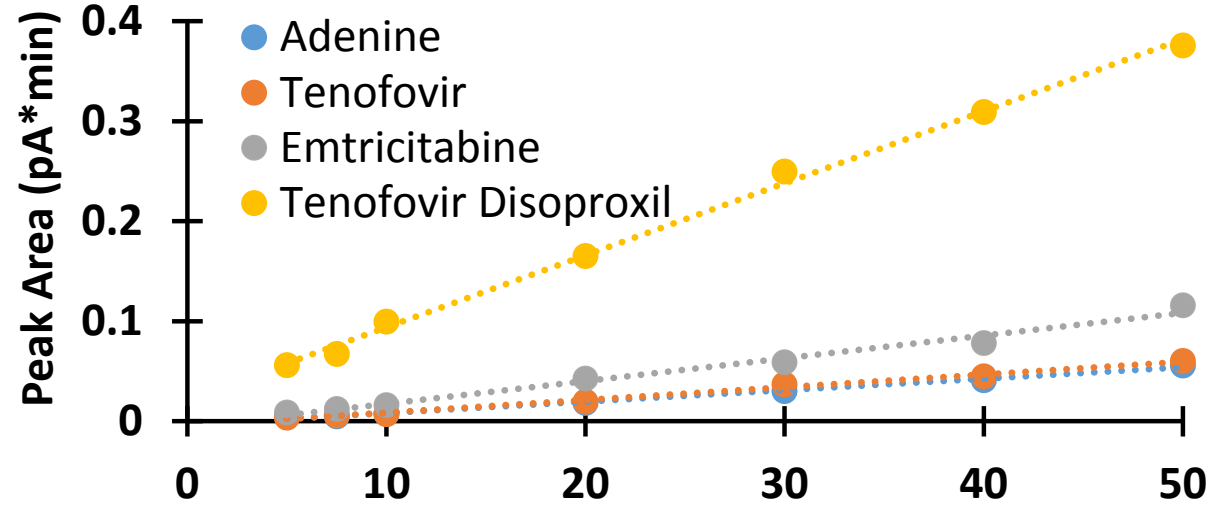
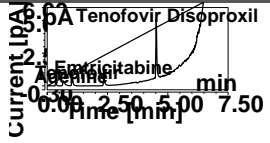
Eluent at detector

Inverse Gradient Compensation

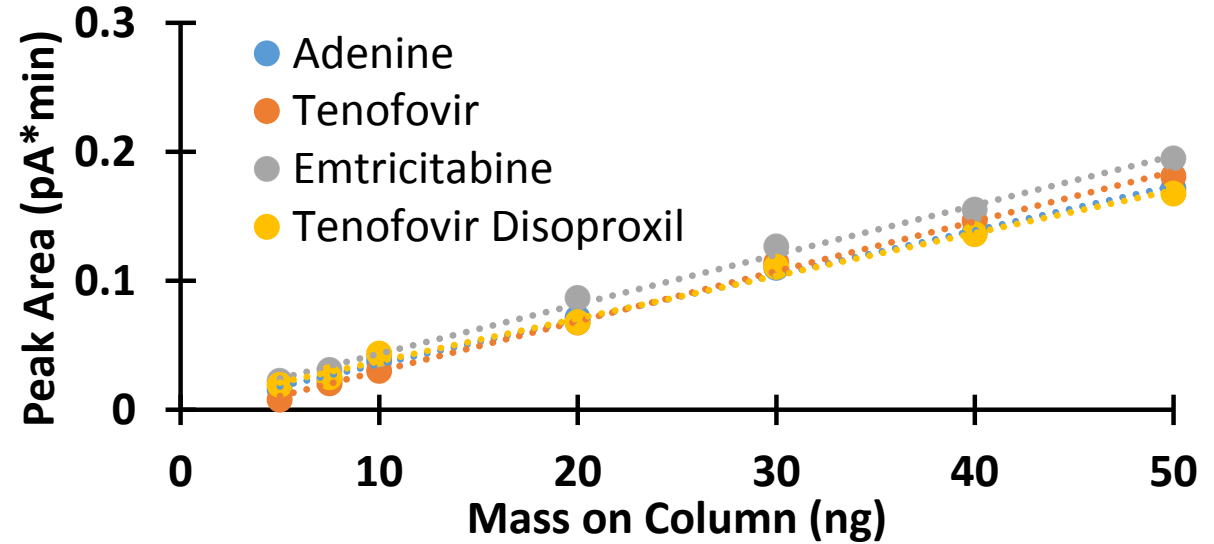
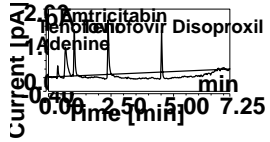


Inverse Gradient for Tenofovir

Conventional
Gradient Elution



Inverse
Gradient Compensation



Inverse gradient compensation results in improved quantification

Key Benefits – Thermo Scientific Vanquish Duo UHPLC System for Inverse Gradient

Easy to implement

1.

Automatic instrument recognition

Instrument Method Wizard - Instrument Method Workflow Selection

Chromeleon found one or more workflows suitable for your instrument configuration. Please select a workflow.

Inverse Gradient
Compensate the analytical gradient with an inverse gradient to get isocratic conditions at the detector. Use this option to optimize your method for charged aerosol detectors (CAD).

Regular Instrument Method
Create a regular instrument method without any optimization.

< Back Next > Cancel Help

2.

Automatic method setup for compensation gradient

Instrument Method Wizard - Inverse Gradient: Flow Gradients

Specify the gradient of the analytical pump. The gradient of the inverse gradient pump will be updated automatically.

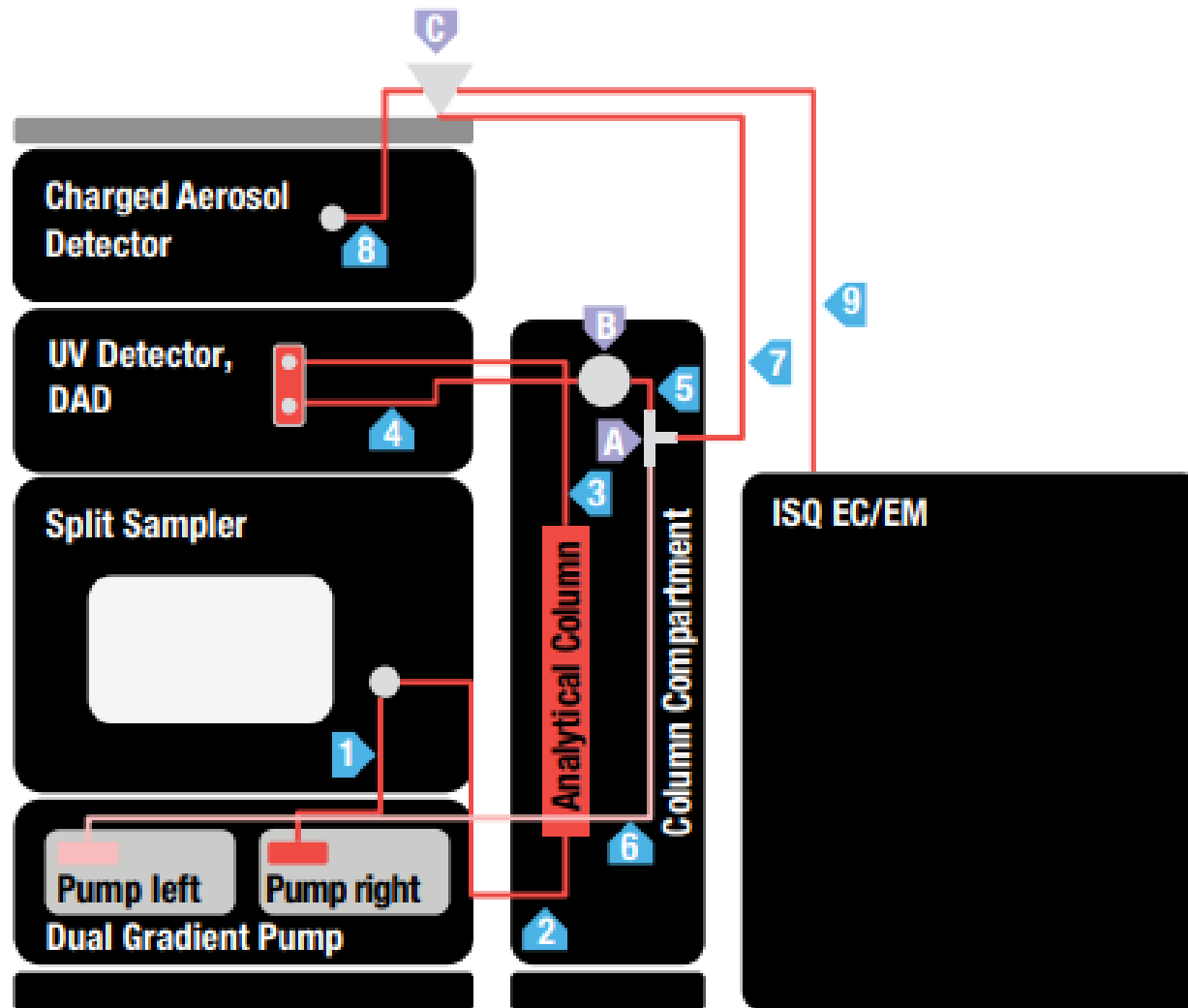
Analytical Gradient (Gradient)

Inverse Gradient (Inverse)

No	Time	Flow [ml/min]	%B	%C	Curve
1	0.000				Equilibration
2	New Row				
3	0.000				Run
4	0.000	1.000	5.0	0.0	5
5	1.000	1.000	5.0	0.0	5
6	5.000	1.000	80.0	0.0	5
7	6.000	1.000	80.0	0.0	5
8	6.000	1.000	5.0	0.0	5
9	New Row				
10	10.000				Stop Run

Insert Row
Delete Row
Remove Equilibration Stage

Multi-detector Set-up: Combining UV, CAD and MS



CAD
Uniform response
quantification



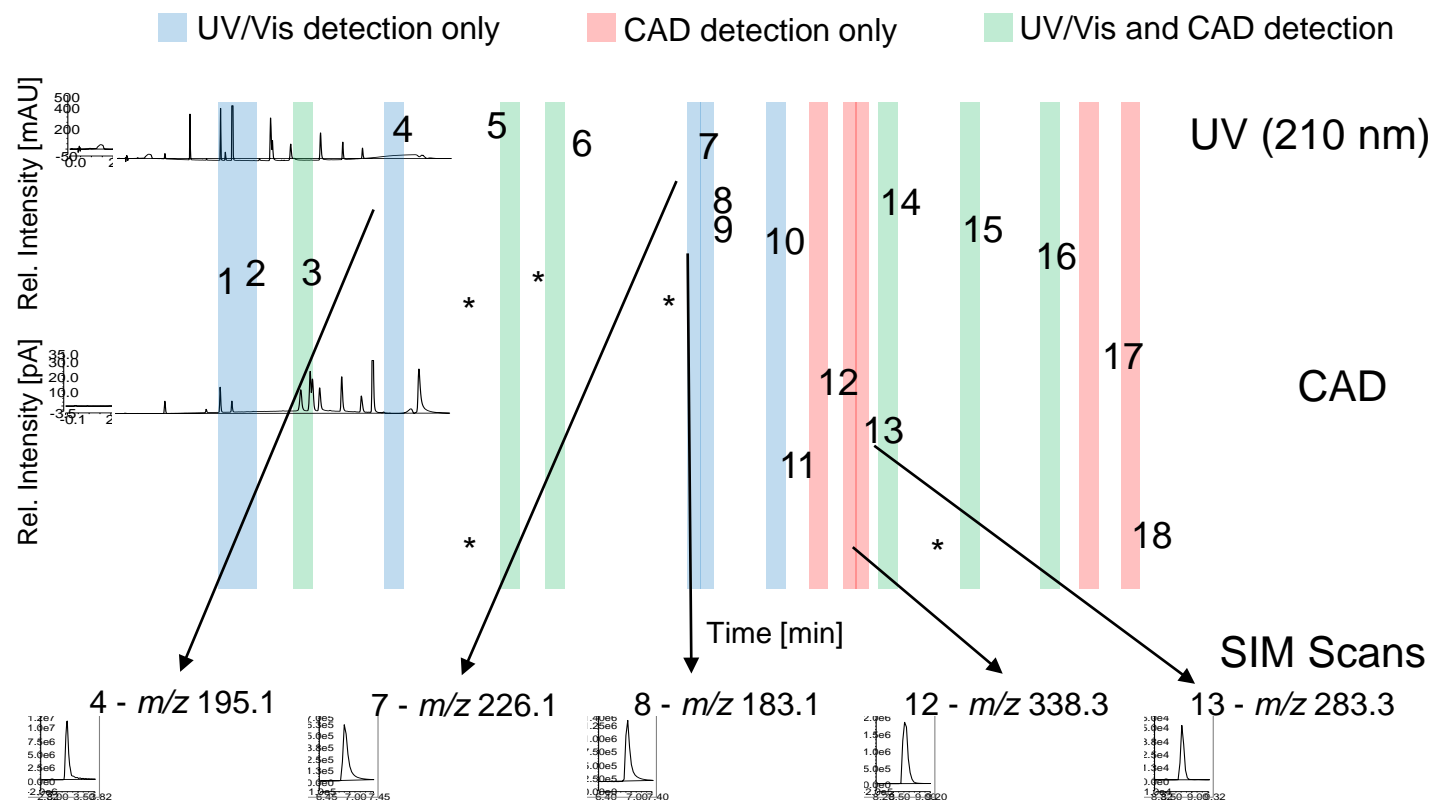
UV
Additional information, LC
control, transfer to routine



MS
Analyte confirmation

Complementary Detectors

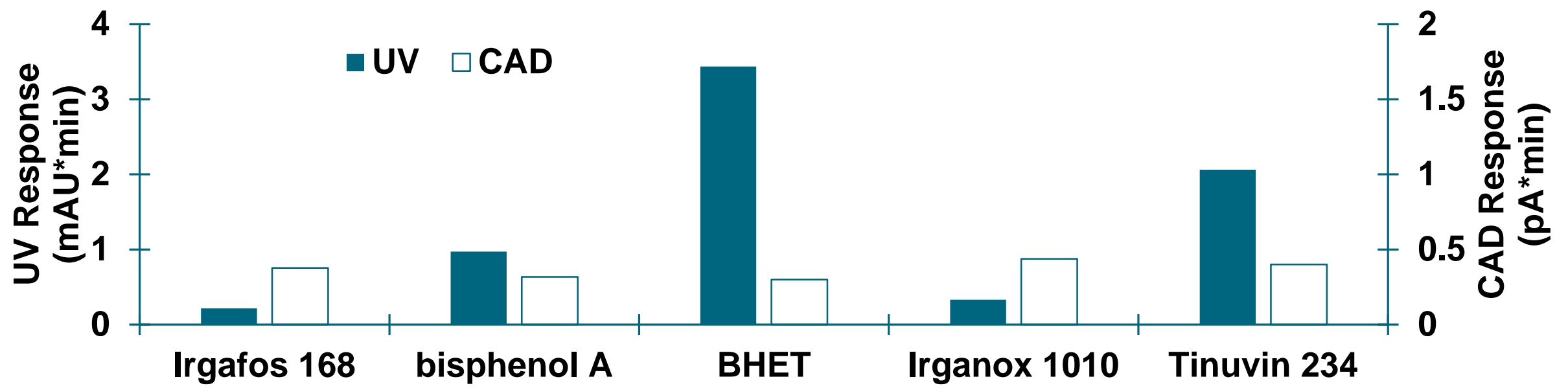
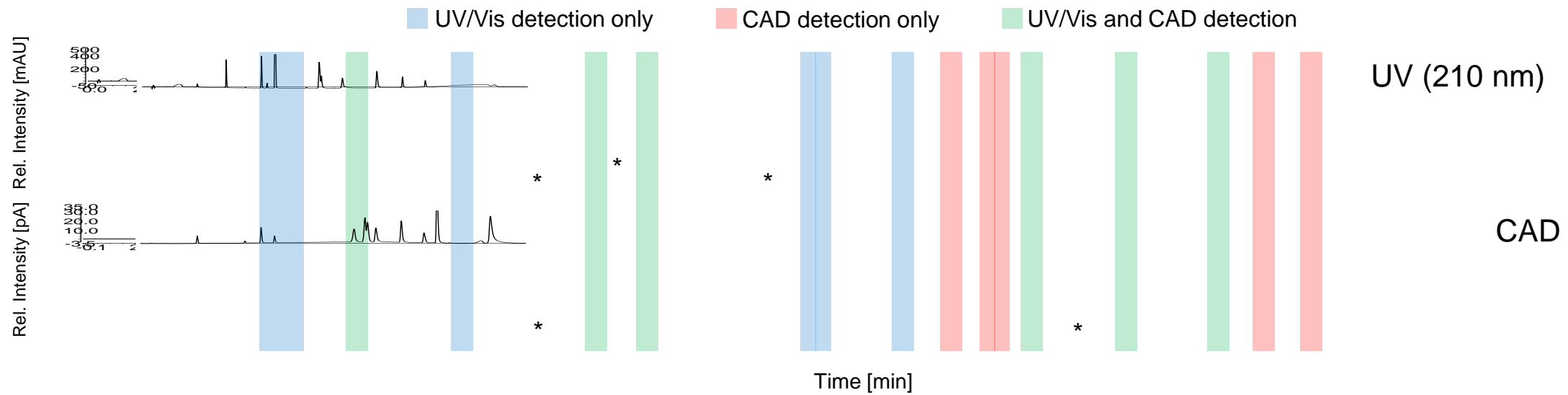
18 extractables analyzed with standard multi-detector setup



#	Analyte	UV	CAD	MS	Mass Found	LOQ (µg/mL)
1	Phthalide	✓		✓	135.1	5 (UV)
2	Phthaldialdehyde	✓		✓	135.1	5 (UV)
3	BHET	✓	✓	✓	255.1	1
4	Dimethyl phthalate	✓		✓	195.1	1 (UV)
5	Bisphenol A	✓	✓	✓*	227.2	1
6	Butylparaben	✓	✓	✓	195.1	50
7	Tinuvin P	✓		✓	226.1	1 (UV)
8	Azobenzene	✓		✓	183.1	1 (UV)
9	2,4-di-t-Butylphenol	✓				1 (UV)
10	BHT	✓		✓	219.2	1 (UV)
11	Palmitic acid		✓	✓	255.2	1
12	Erucamide		✓	✓	338.3	1
13	Stearic acid		✓	✓	283.3	1
14	Tinuvin 234	✓	✓	✓	448.2	1
15	Irganox 1010	✓	✓	✓	1193.8	1
16	Irgafos 168	✓	✓	✓	645.4	1
17	Eicosane		✓			10
18	Tetracosane		✓			10

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Advantage of CAD Over UV Quantification





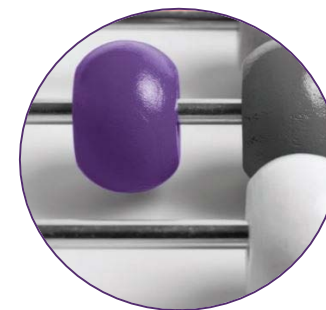
Vanquish Duo UHPLC System for Dual LC

- 2 flow paths in one instrument
- High throughput
- Parallel analysis
- Better productivity



Vanquish Duo UHPLC System for Tandem LC

- Excluding detector idle time
- Increased throughput
- Better productivity
- Easy instrument wizards



Vanquish Duo UHPLC System

- Constant eluent composition at CAD
- Uniform response
- No reference standards required
- Easy instrument wizards

Thermo Fisher Scientific Total Solution



Quality



Productivity



Costs



Saving time

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