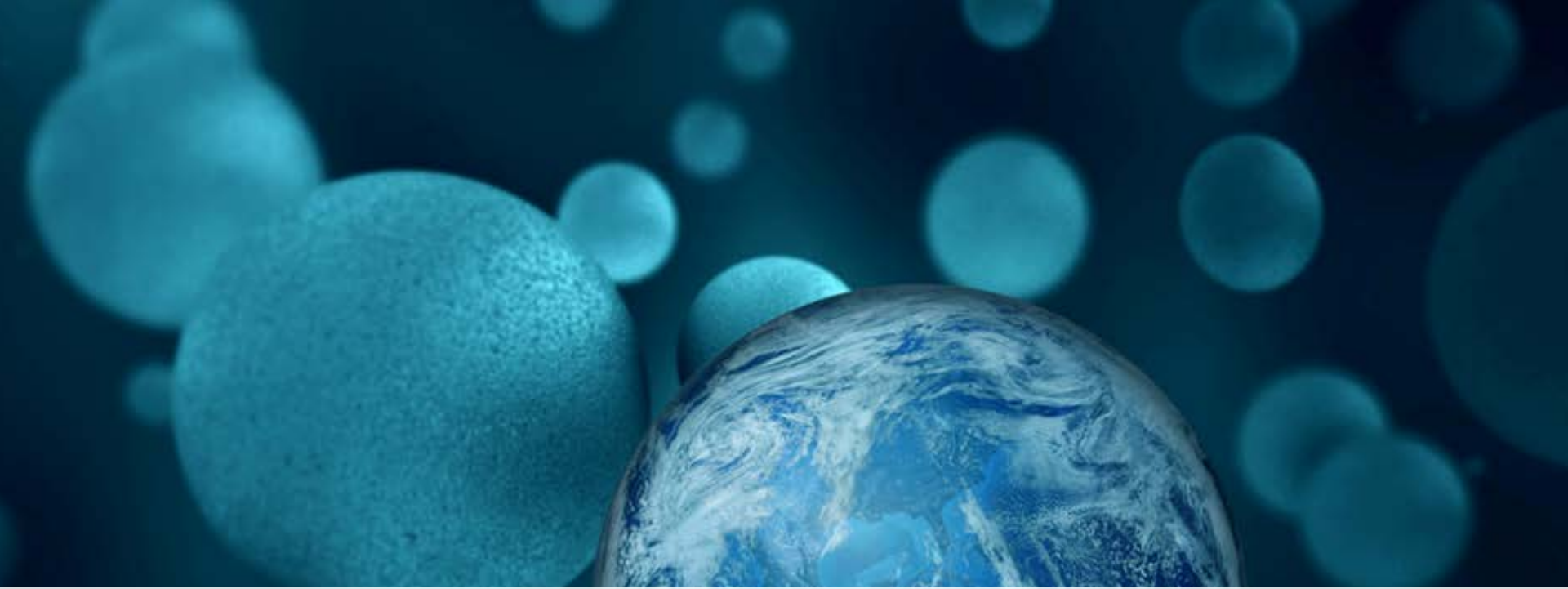


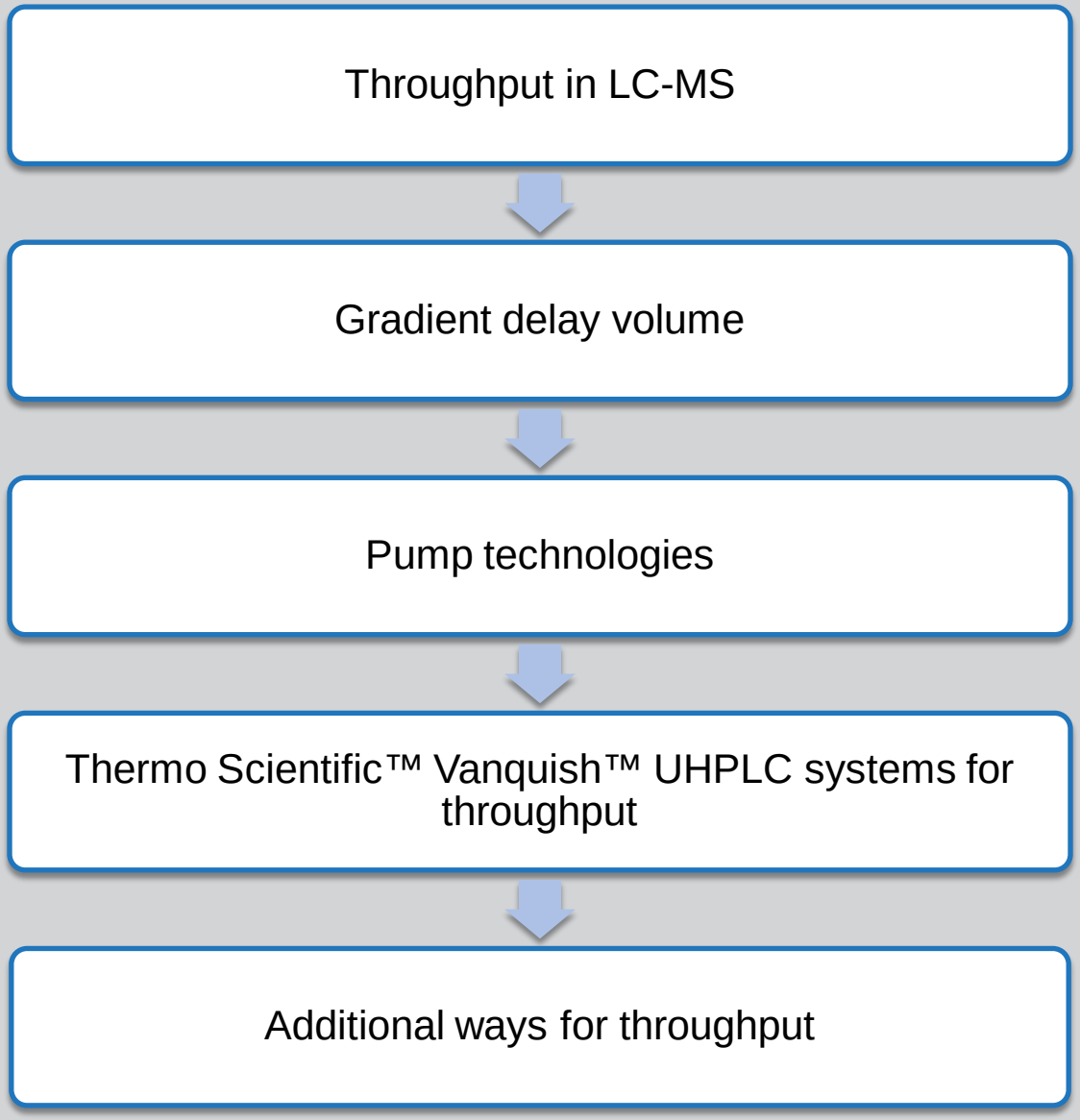
ThermoFisher SCIENTIFIC

Which UHPLC for LC-MS?

Dr. Giorgia Greco
Product Manager HPLC for MS Systems
Thermo Fisher Scientific, Germering, Germany

Proprietary & Confidential

Content of the Webinar



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What is UHPLC?

- ***System back pressure above 600 bar***
- ***Analytical column with sub-2 micron particles***

To achieve:

- ***Faster separation***
- ***Better resolution***
- ***Less waste***

Vanquish Flex systems

Quaternary system
- Quaternary solvent blending
- Pressures up to 1000 bar
- Flow rates of up to 8 mL/min
- Biocompatible

Binary system
- Binary high pressure solvent mixing
- Pressures up to 1000 bar
- Flow rates of up to 8 mL/min
- Biocompatible

Up to 1000 bar

Vanquish Horizon systems

- Highest pressure capability up to 1500 bar
- Flow rates up to 5 mL/min
- Lowest system dispersion and GDV
- Unmatched detection sensitivity and linearity
- Biocompatible

1500 bar

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Why is Throughput in LC-MS Important?

We require sophisticated LC-MS technology.

We must manage regulatory demands.

We need systems with high-resolution, selectivity, sensitivity, robustness, large number of molecules to be discovered, identified and quantified in one single run.

We require high throughput capabilities at the same time.

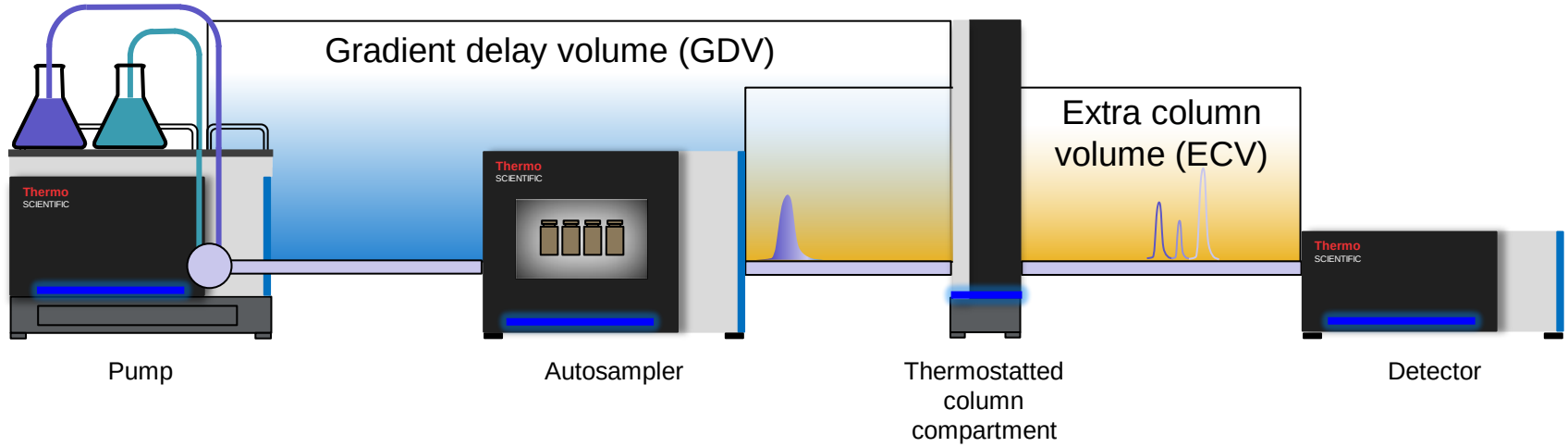
Which UHPLC system is suitable for our high throughput LC-MS analysis?

Vanquish UHPLC system
Thermo Scientific™ Q Exactive™ hybrid quadrupole Orbitrap™ MS

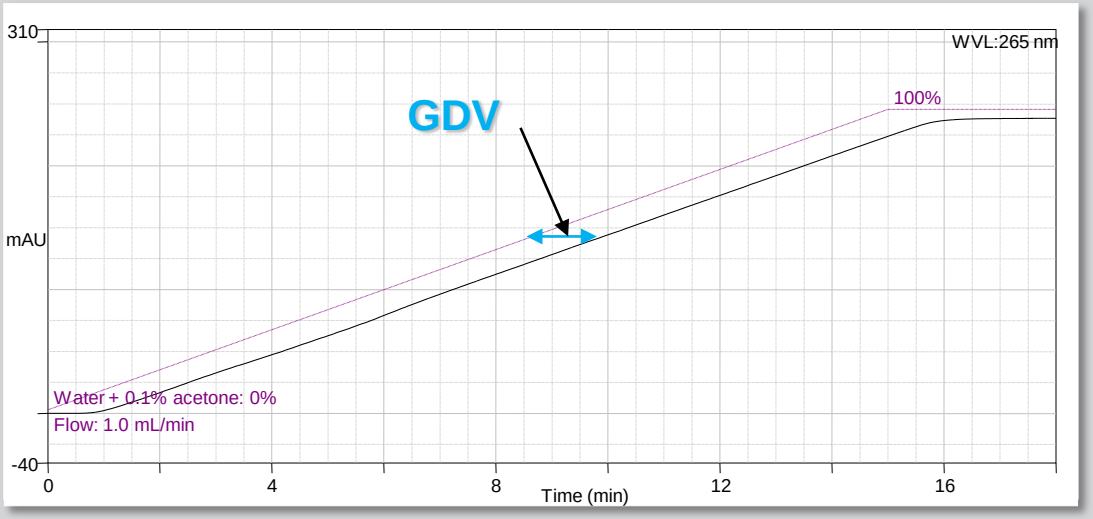
Vanquish UHPLC system
Thermo Scientific™ TSQ Quantiva™ triple quadrupole MS

ThermoFisher SCIENTIFIC

Why is the GDV of your “Front-End” Important for Throughput?



- **Gradient delay volume (GDV):** Volume of fluid between mixing point of the gradient and column head
- **Extra column volume (ECV):** Volume of fluid between sample injection point and midpoint of the detector’s flow cell.



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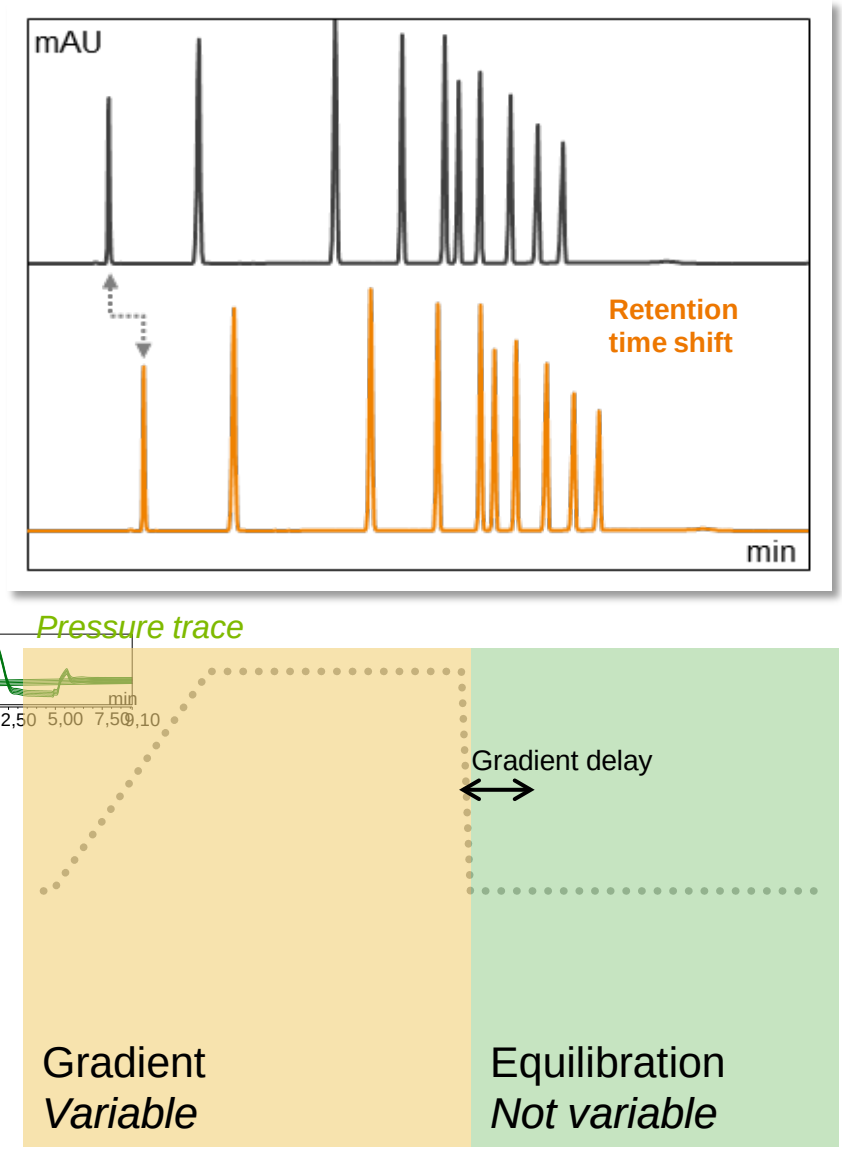
Why is the GDV of your “Front-End” Important for Throughput?



Short runtimes and higher throughput

- Column equilibration time is related to the GDV.
- Small GDV - shorter equilibration times
- Contribution of equilibration time is relevant for short runs.

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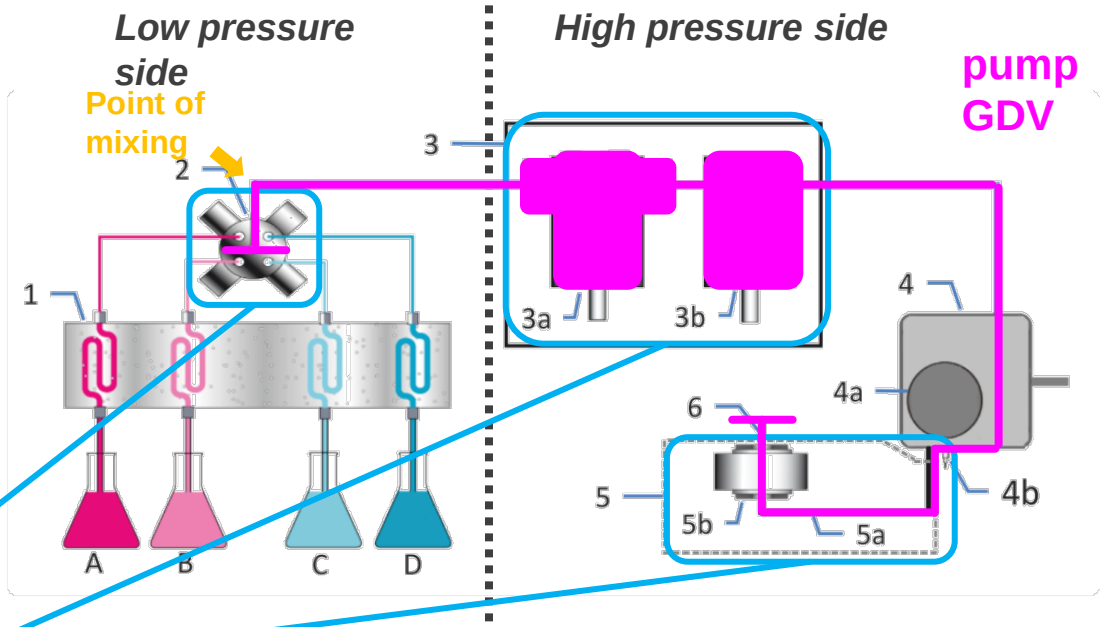
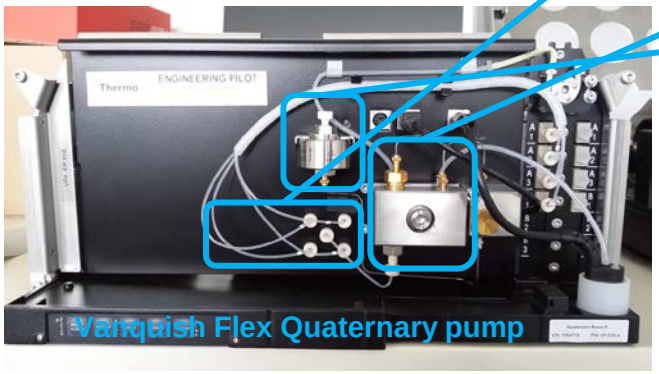
Why do UHPLC Systems have Different System GDVs?

UHPLC systems can use different pump technologies: *Low pressure gradient (**LPG**)*



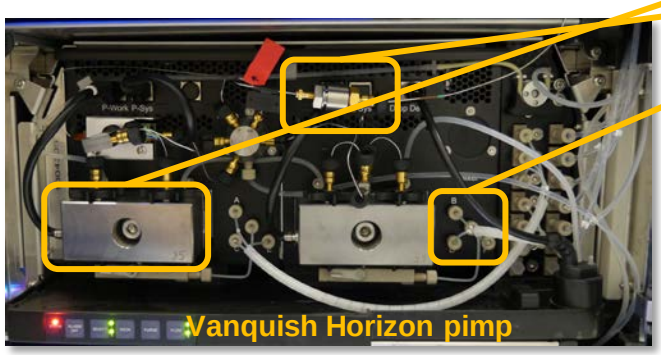


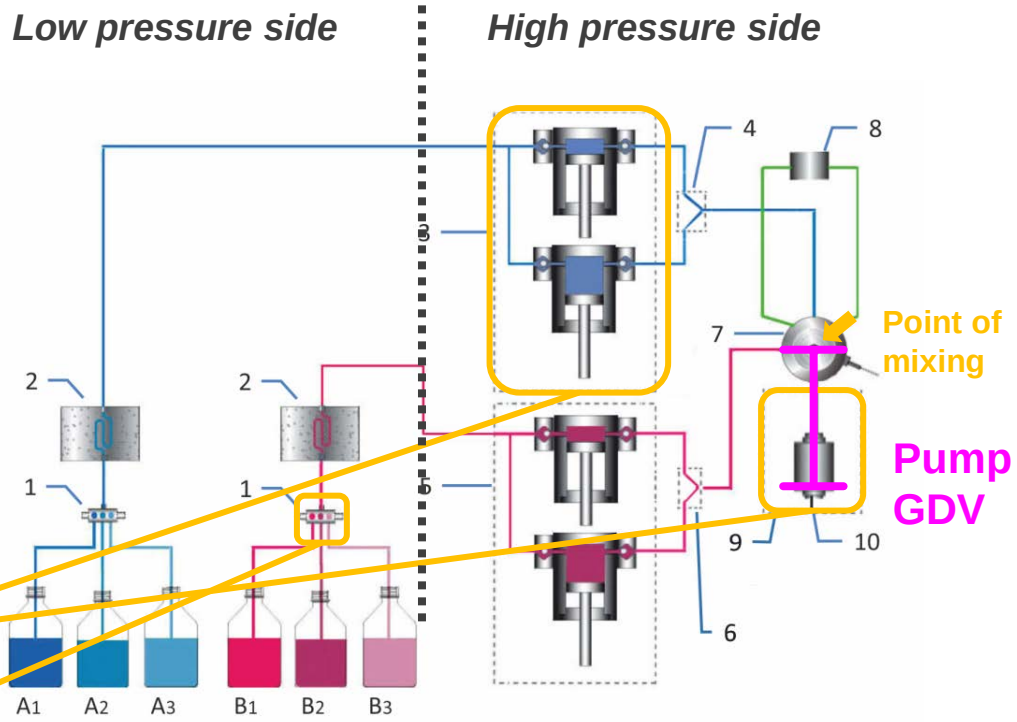
By default, LPG pump systems have large GDVs.

ThermoFisher SCIENTIFIC

Why do UHPLC Systems have Different System GDVs?

UHPLC systems can use different pump technologies: *High pressure gradient (**HPG**)*





By default, HPG pump systems have small GDVs.

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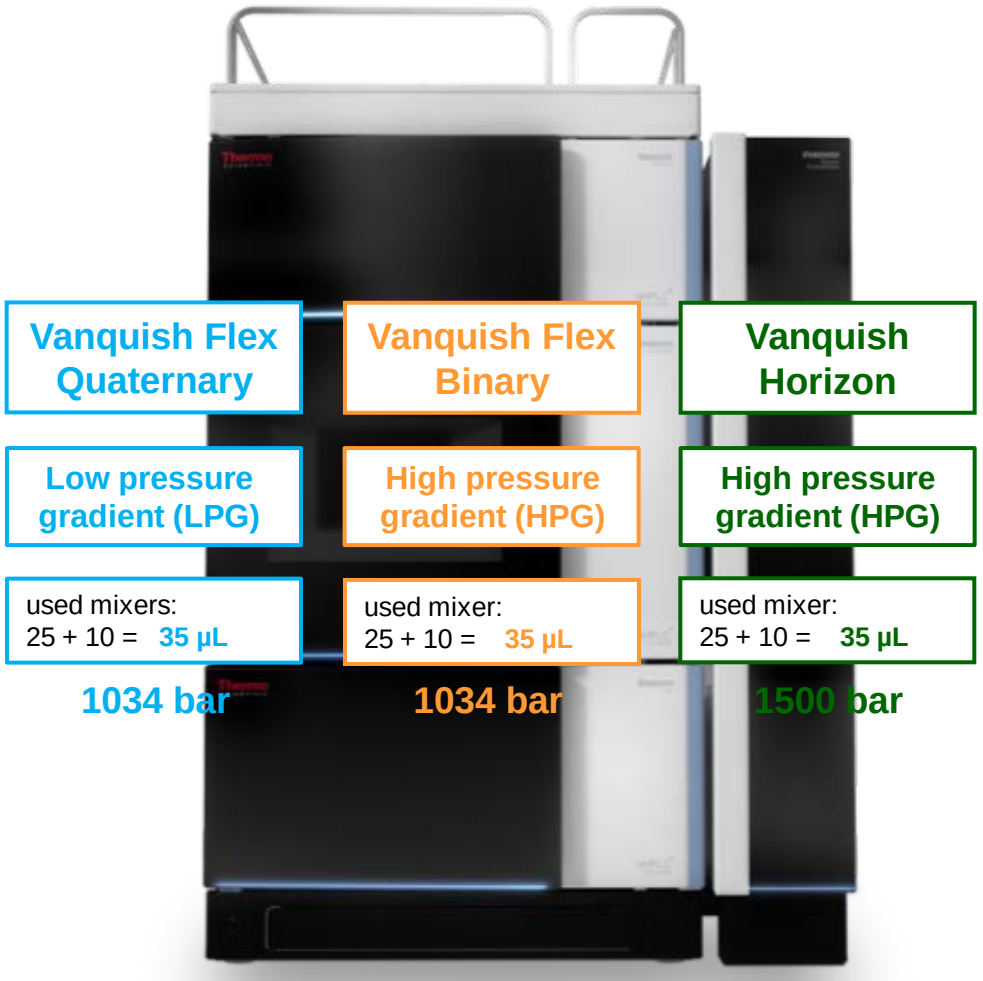
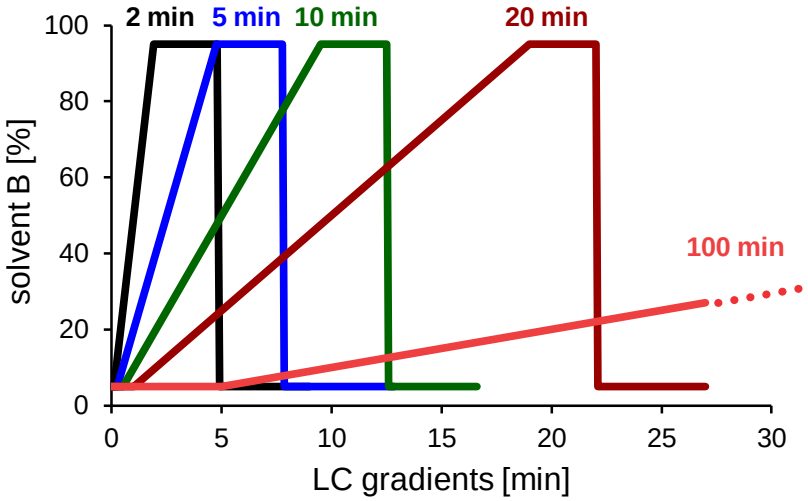
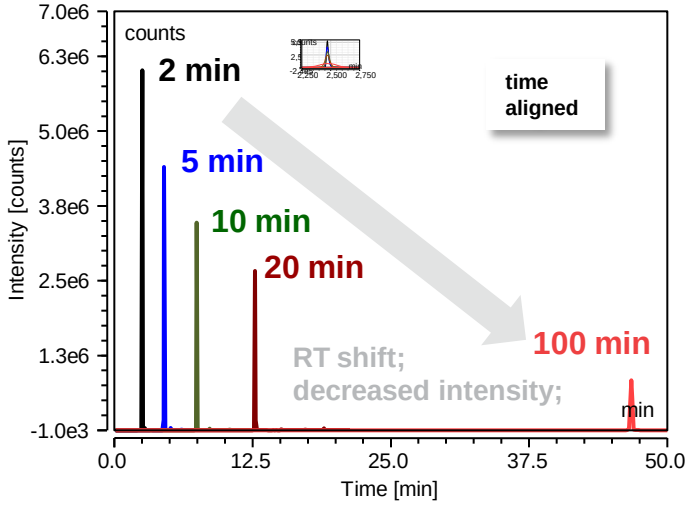
What UHPLC for LC-MS?

Tested Vanquish systems



LC-MS analysis of pesticides





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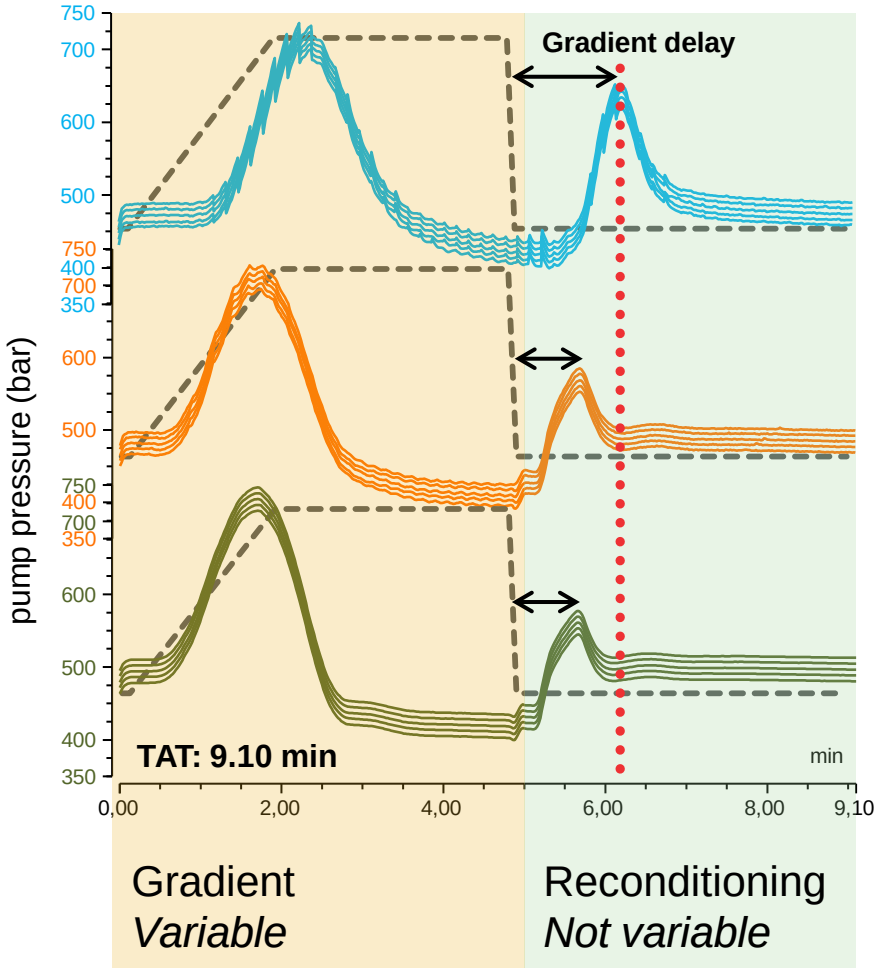
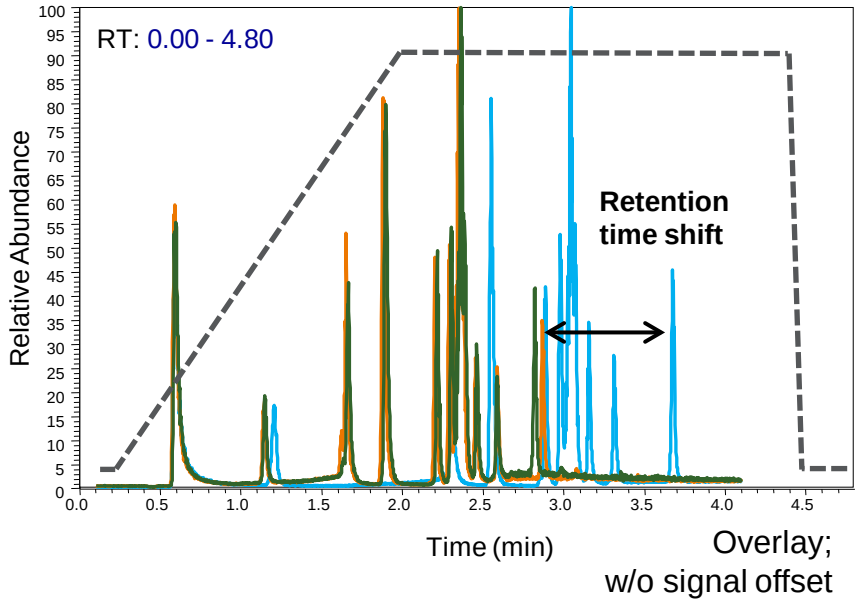
Total Analysis Time (TAT) of Vanquish UHPLC Systems

Pressure traces (2 min gradient)



Overlay (n=5); w/ signal offset

Extracted ion chromatograms



Overlay; w/o signal offset

Vanquish Flex Quaternary

Vanquish Flex Binary

Vanquish Horizon

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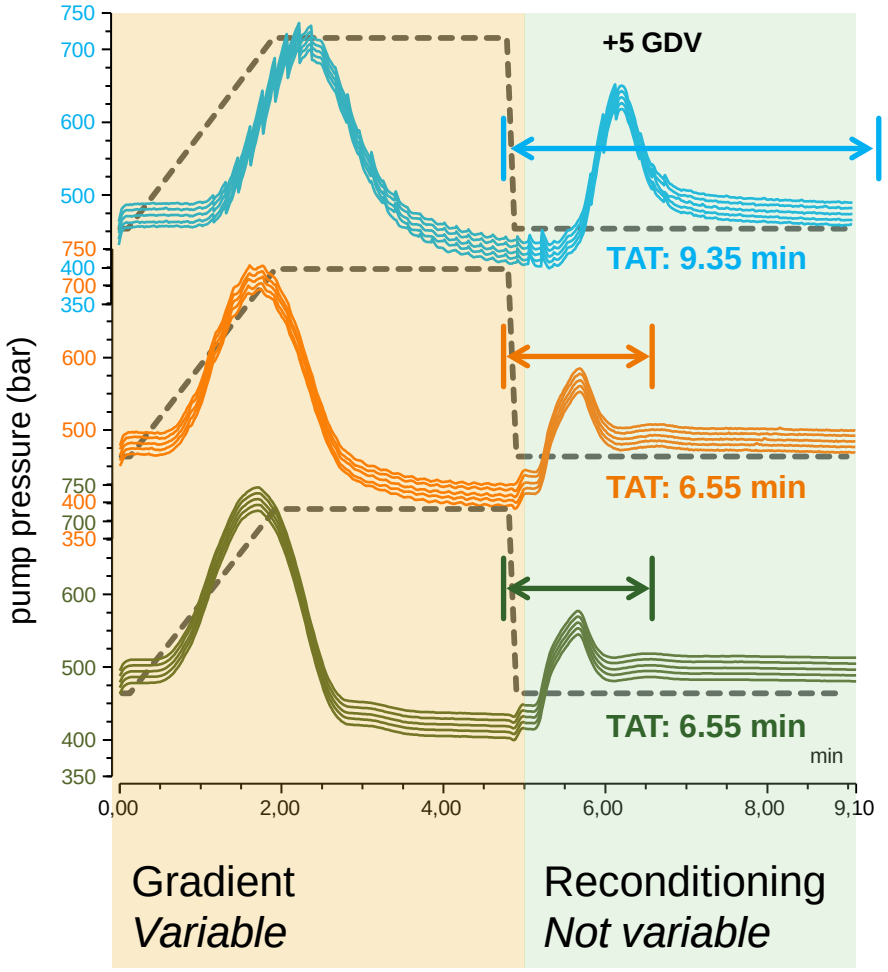
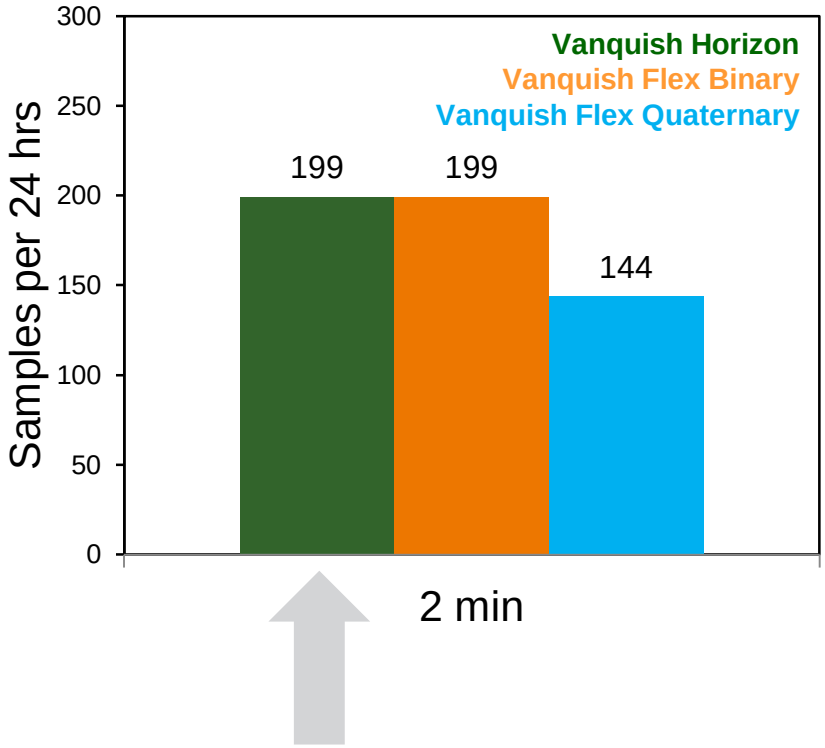
TAT Optimization of Vanquish UHPLC Systems

Pressure traces (2 min gradient)



Overlay (*n*=5); w/ signal offset

Sample throughput



>50 samples more per day with a Vanquish binary system

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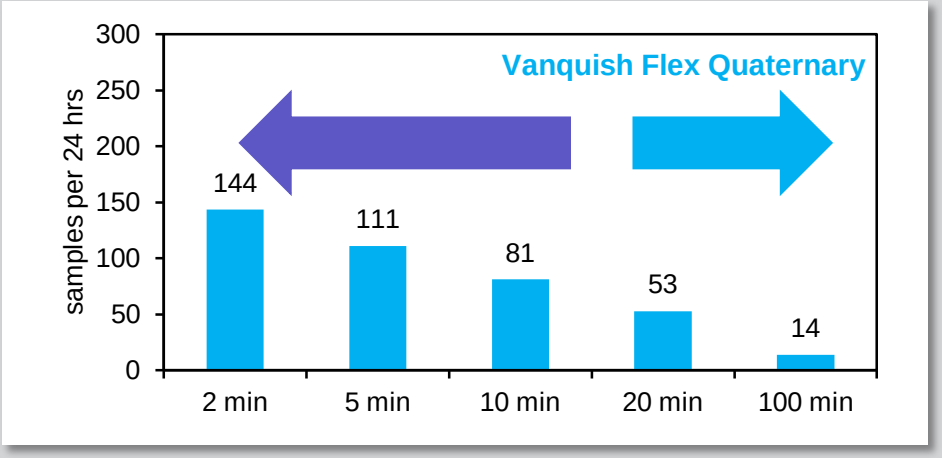
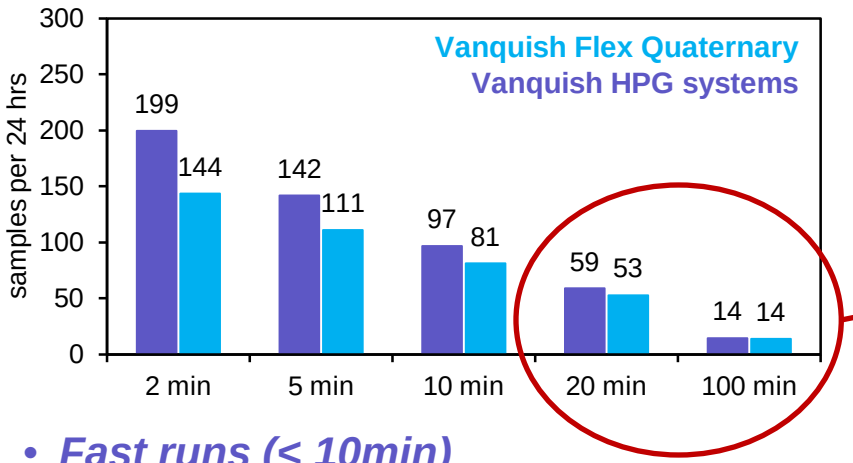
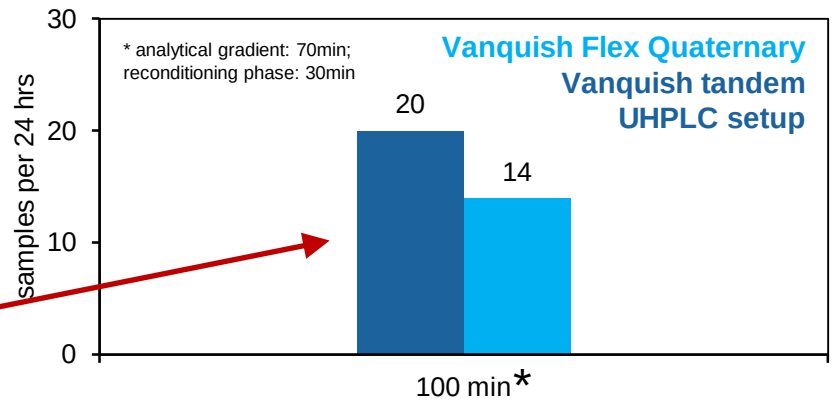
Throughput Considerations





- ***Fast runs (< 10min)***
- ***Throughput increase from 10-30%***

→Vanquish HPG systems for throughput in LC-MS



- ***LC runs (> 20min)***
- ***Long reconditioning phase required***

→ Tandem UHPLC setups as alternative ways to achieve throughput

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Vanquish Flex Binary and Horizon Systems – What‘s the difference?

Vanquish Flex Binary

Vanquish Horizon

Vanquish Horizon system operates with the superior pump technology for critical and challenging applications.

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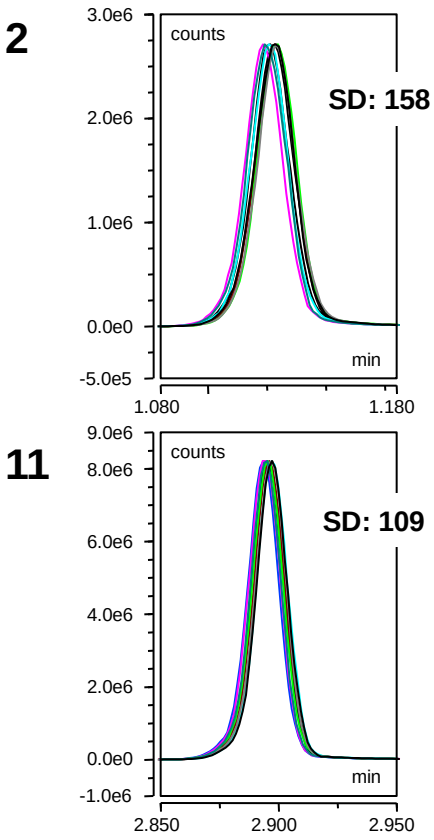
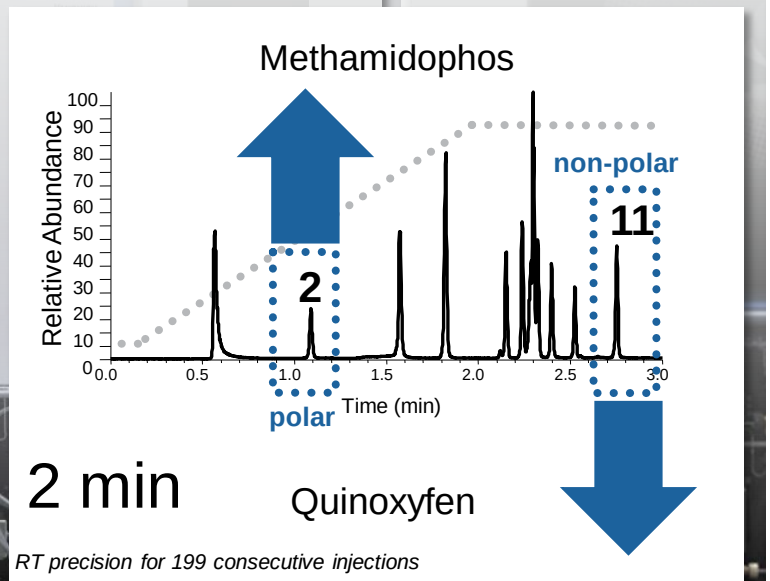
Vanquish Flex Binary and Horizon Systems – What's the difference?

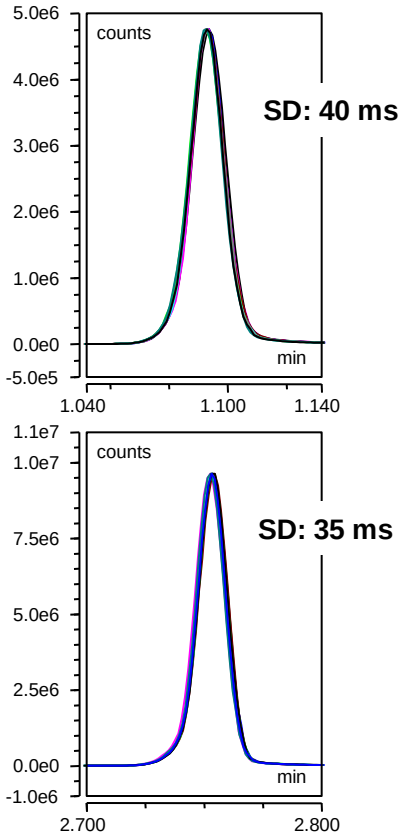
Vanquish Flex Binary



60 ms ≡ 0.001 min



Vanquish Horizon



Higher RT precision of the Vanquish Horizon system can reduce your number of technical replicates, one important aspect to be considered for high-throughput.

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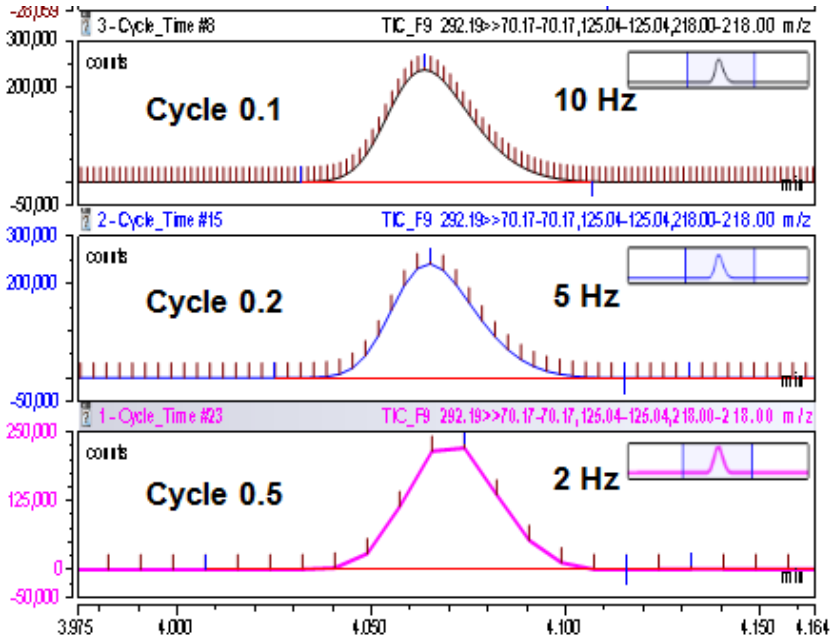
What Level of Throughput is Possible with the Vanquish Horizon?

LC-MS analysis of pesticides

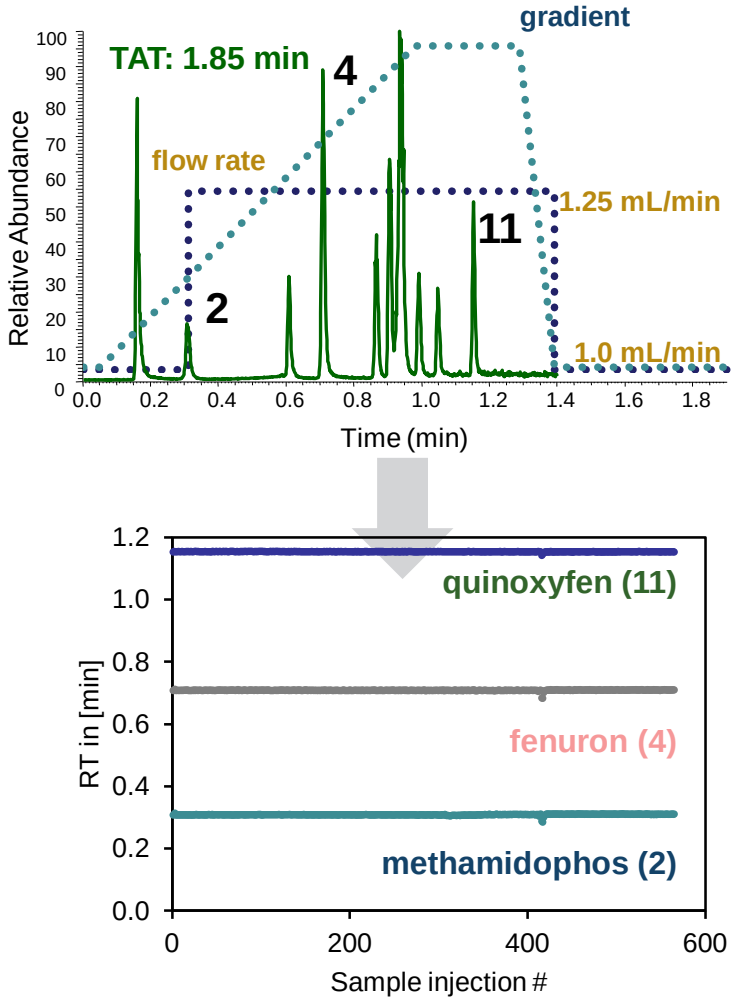
LC conditions	
Column	Thermo Scientific™ Hypersil GOLD™ 50 x 2.1 mm, 1.9 µm

567 samples per day



At least 10 Hz are required to obtain RT SD = 0.001 min for peak width at 50% of 0.025 min (1.5 s).

Throughput maximization



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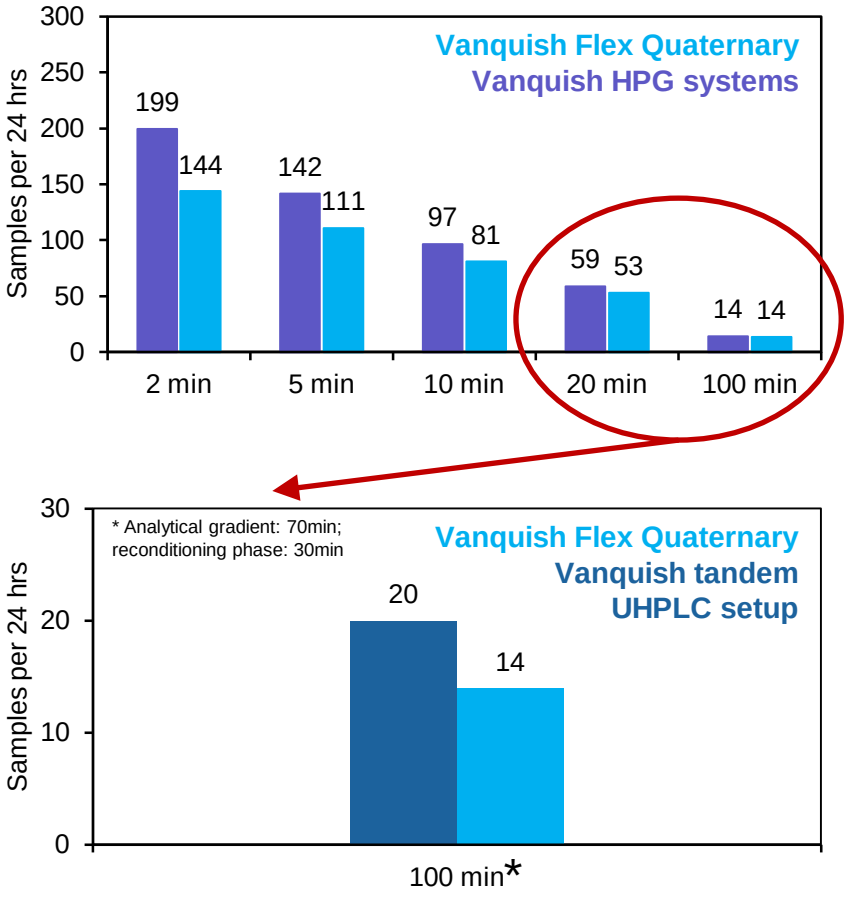
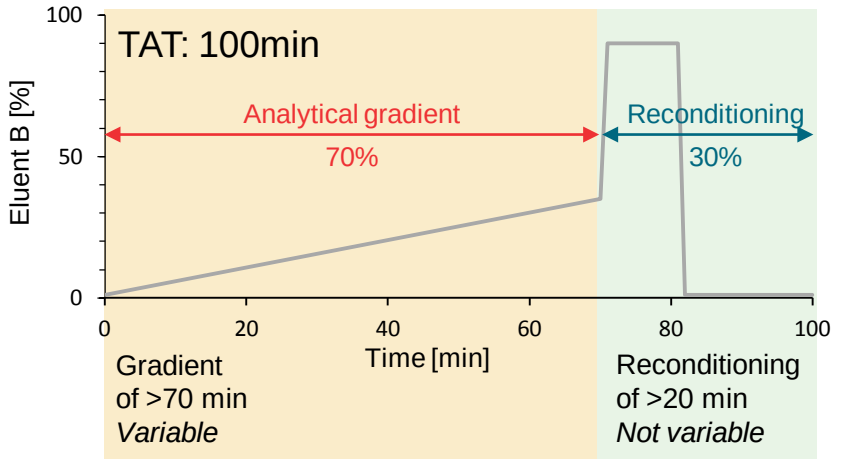
Additional Ways For Throughput

Discussion starter



Shallow LC gradient



Reconditioning time may take more than 20 min until next sample injection.

Target of tandem UHPLC setups!!!

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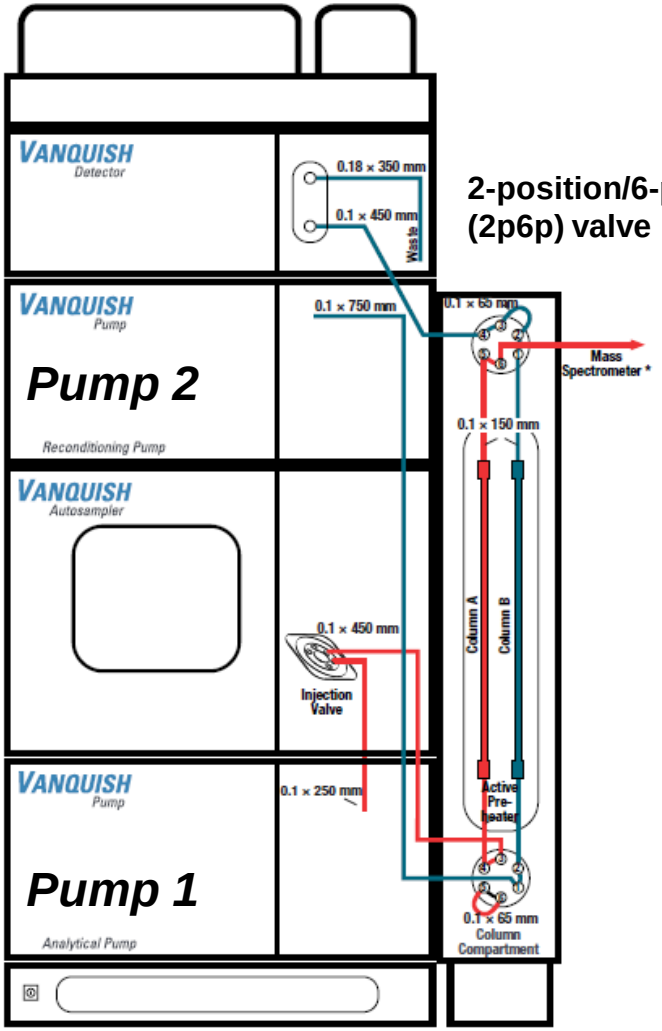
Vanquish UHPLC Tandem LC Setup to Increase Productivity

Vanquish tandem LC and LC-MS workflow



2-position/6-port (2p6p) valve

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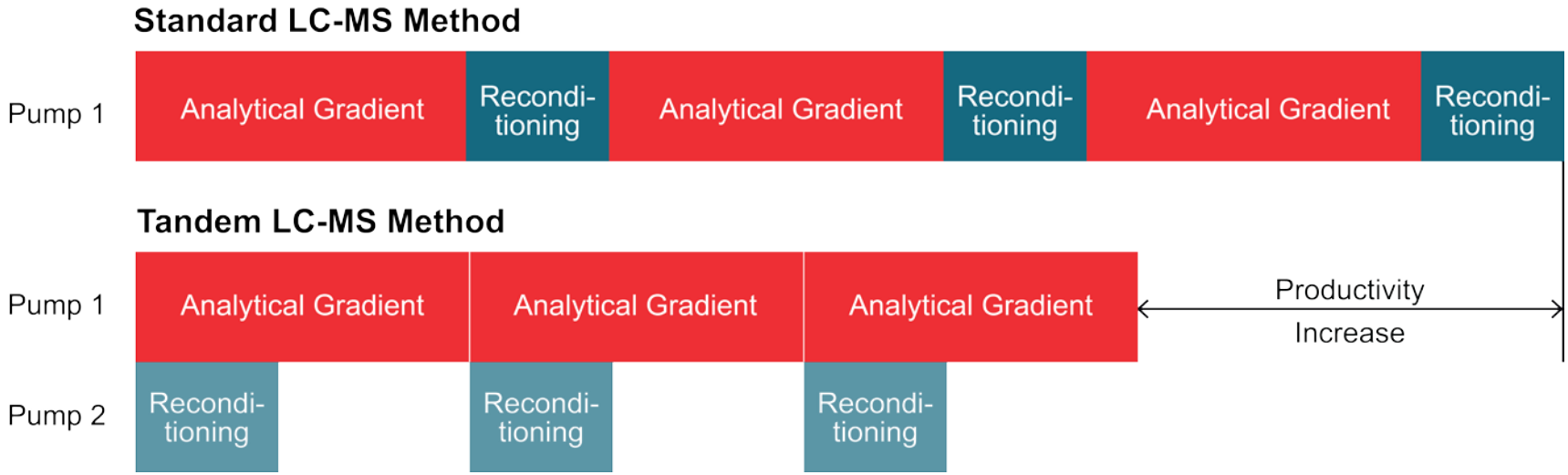
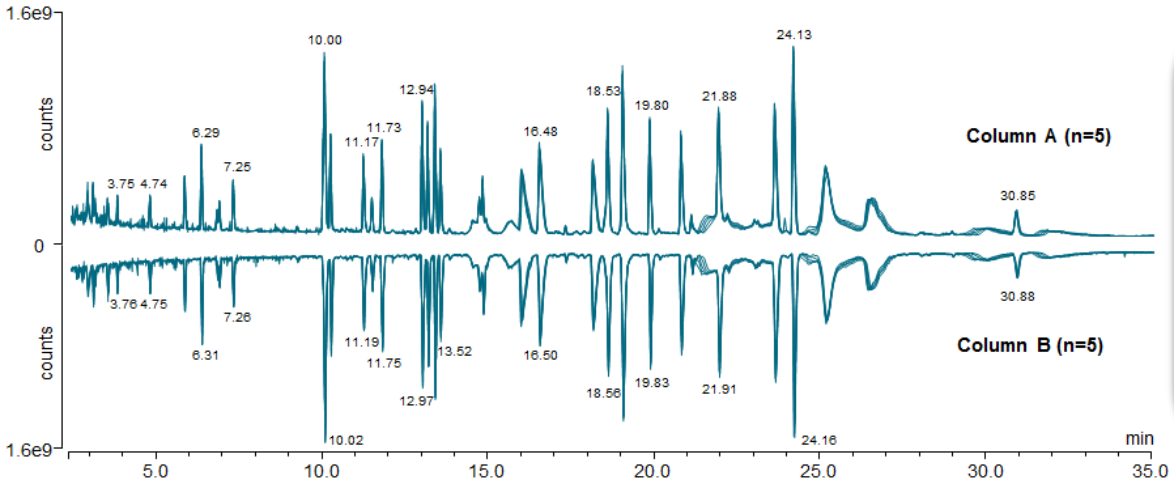
Vanquish UHPLC Tandem LC Setup to Increase Productivity

Vanquish tandem LC-MS workflow





Overlay of 5 total ion current chromatograms Vanquish tandem setup: 2x Thermo Scientific™ Acclaim™ VANQUISH™ column C18 2.1x250 mm, 2.2 µm, Lot No. 1425071; **A:** 0.1/100 FA/Water (v/v); **B:** 0.1/100 FA/Acetonitrile (v/v) **Gradient:** 1-45% B in 40 min, 60 °C; 400 µL/min; Injection volume 5 µL, 1 µg Infliximab SMART digest; **Reconditioning:** Two times 90% B for 5 min, 24 min equilibration at 1% B; **Detection:** Q Exactive HF MS, R=15k, mass range 140-2000; UV detection@214 nm

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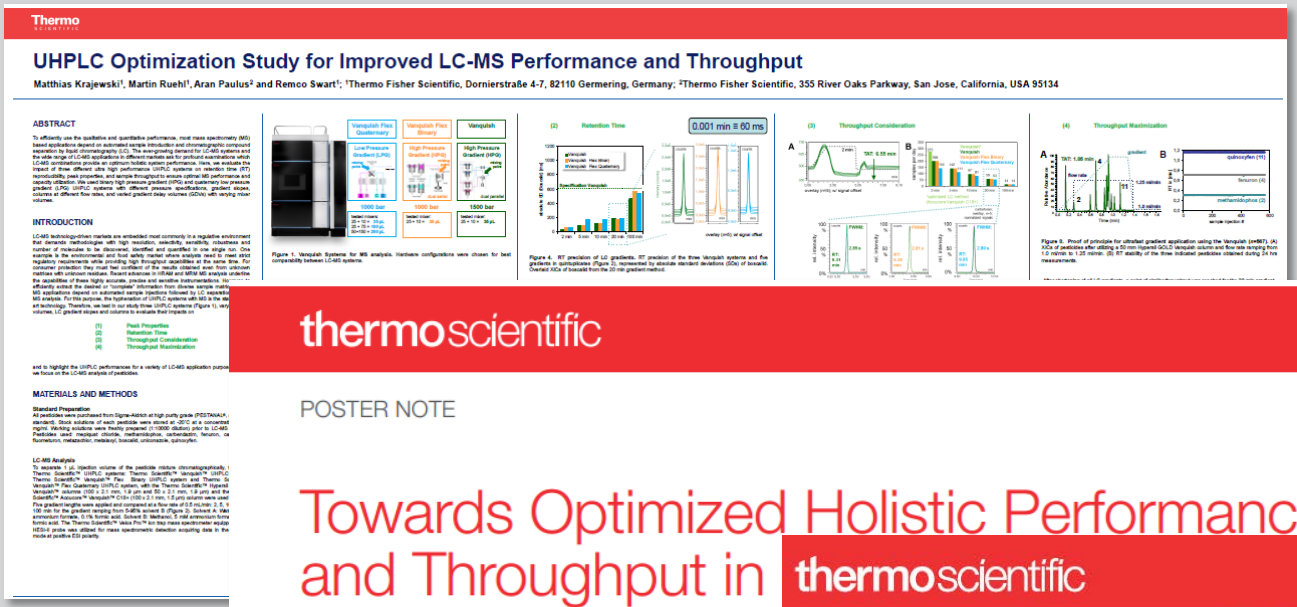
Summary of the Webinar

1. With an UHPLC system you can focus on peak capacity and sample throughput.
2. The system gradient delay volume (GDV) is the crucial parameter for your sample throughput.
3. The operation principle of the pump and the pump mixer configurations contribute significantly to the GDV of your UHPLC system.
4. Low pressure gradient (LPG) or high pressure gradient (HPG) pump technology is chosen in alignment with your application requirements.
5. With HPG pump technology you can achieve maximum sample throughput.
6. With LPG pump technology you can use ternary or quaternary gradients and advanced column chemistries for e.g. method development.
7. For LC-MS applications with very long run times, you can increase your productivity with tandem UHPLC system setups.

Do you want to know more?



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UHPLC Optimization Study for Improved LC-MS Performance and Throughput

Matthias Krajewski[1], Martin Ruehl[1], Aran Paulus[2] and Remco Swart[1]; [1]Thermo Fisher Scientific, Dornierstraße 4-7, 82110 Germering, Germany; [2]Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, California, USA 95134

ABSTRACT

INTRODUCTION

MATERIALS AND METHODS

thermoscientific

POSTER NOTE

Towards Optimized Holistic Performance and Throughput in

Matthias Krajewski, Martin Ruehl, Fran

Thermo Fisher Scientific, Dornierstraß

No. 72

thermoscientific

TECHNICAL NOTE 72203

Tandem UHPLC operation for high-throughput LC-MS peptide mapping analyses

Authors

Martin Samonig, Sabrina Patzelt, Carsten Paul, Martin Rühl, and Remco Swart

Goal

To demonstrate the use of a multi-pump UHPLC system and enable tandem analysis with two columns in parallel, addressing productivity and throughput improvement of existing LC-MS methods.

Contact us:
analyze.eu@thermofisher.com

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Thank you very much for your attention!

Questions?

Do you have additional questions or do you want to talk to an expert from Thermo Fisher Scientific?

Please send an E-Mail to
analyze.eu@thermofisher.com
and we will get back to you.