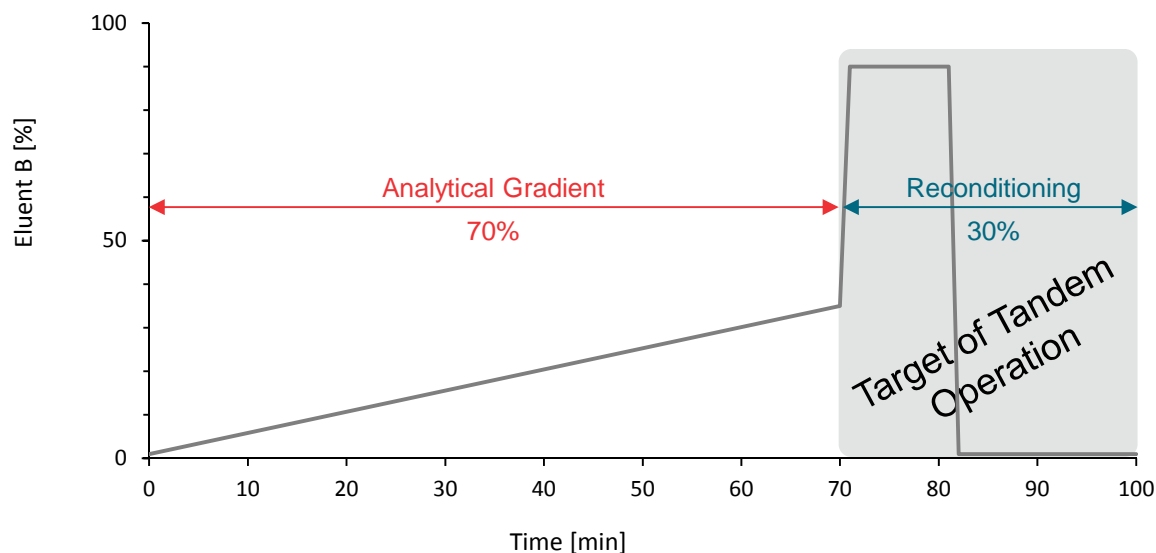# Tandem UHPLC operation for high-throughput LC-MS peptide mapping analyses

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

Thermo Fisher Scientific, Germering, Germany

The world leader in serving science

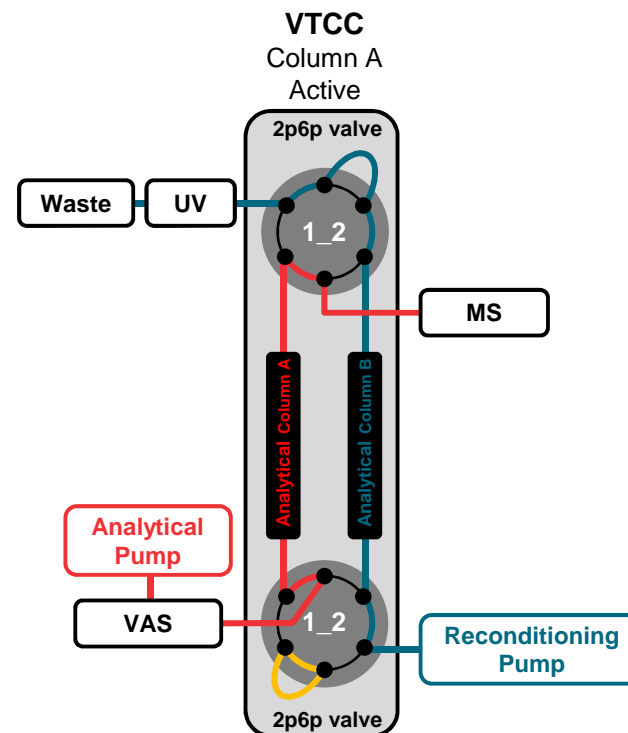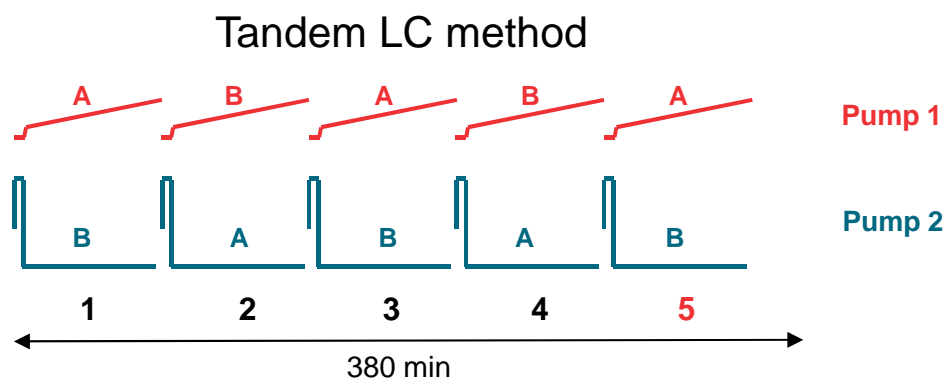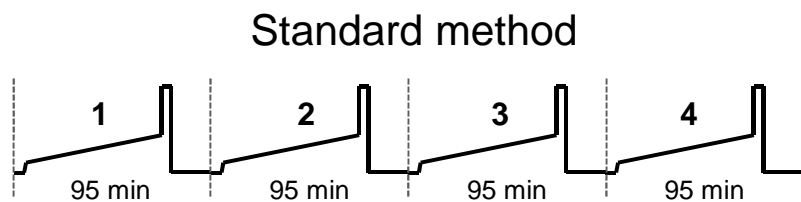# Sections of a common LC/LC-MS method

- **Analytical Gradient Section**
  Chromatographic separation of target analytes

- **Reconditioning Section**
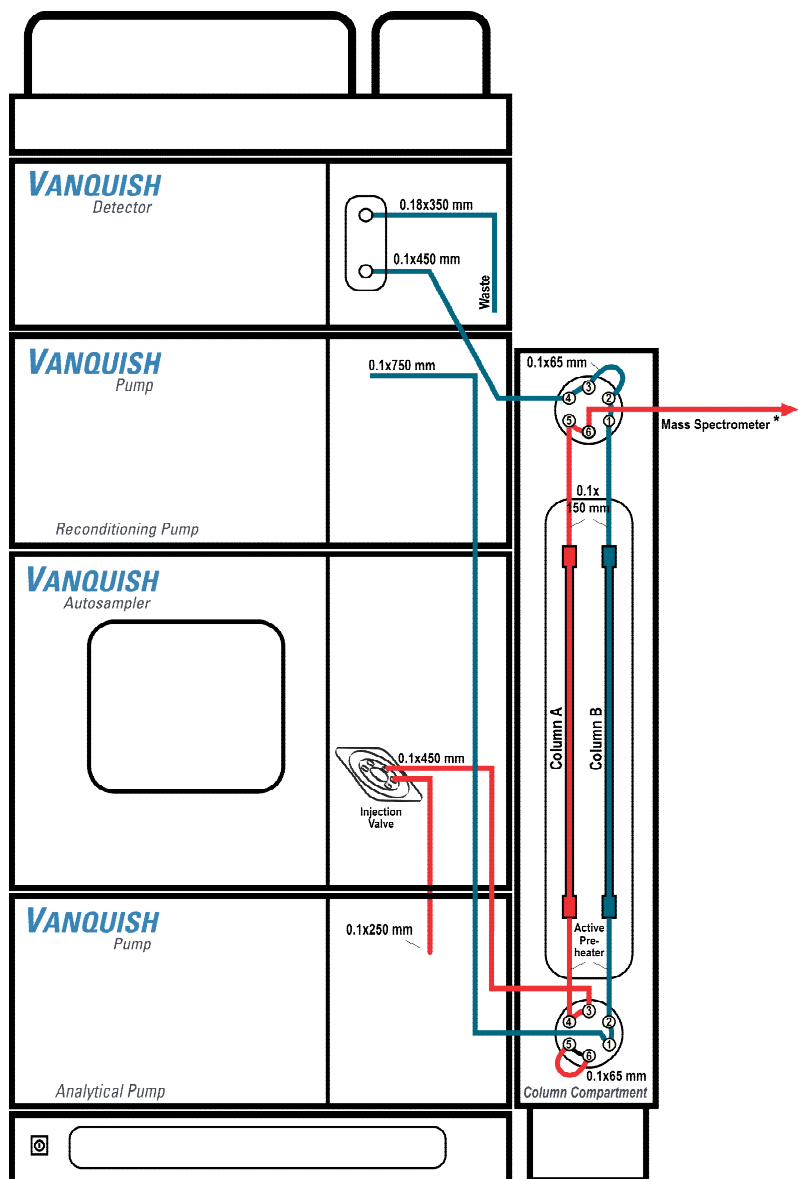 Wash and re-equilibration of the column for the next injection

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Vanquish™ Tandem Operation

Standard method

1   95 min   2   95 min   3   95 min   4   95 min

Tandem LC method

A   B   A   B   A   **Pump 1**

B   A   B   A   B   **Pump 2**

1   2   3   4   5

380 min

VTCC
Column A
Active

2p6p valve

Waste   UV   1_2   MS

Analytical Column A   Analytical Column B

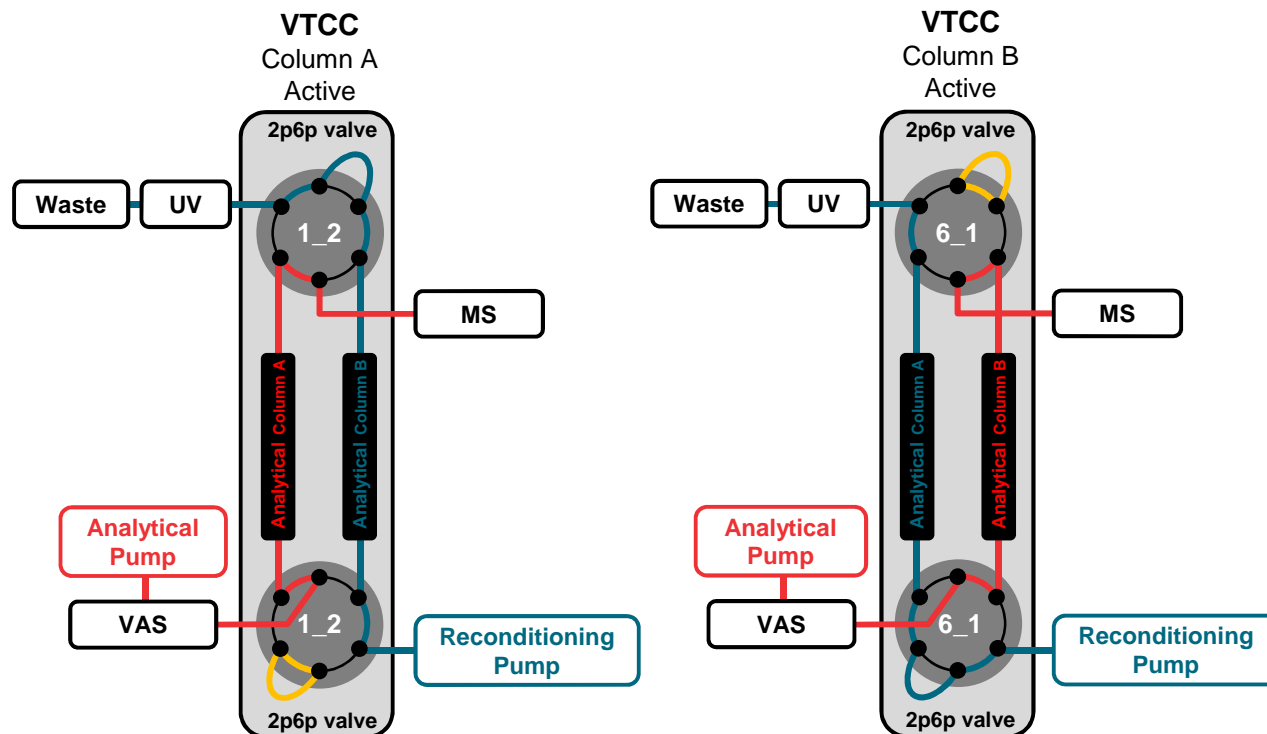Analytical Pump   VAS   1_2   Reconditioning Pump

2p6p valve

- Two flow paths: one for analysis (**red**), one for off-line column wash and re-equilibration (**green**)
- Columns are switched between the two flow paths
- Eliminates the wash and equilibration phase from the run cycle

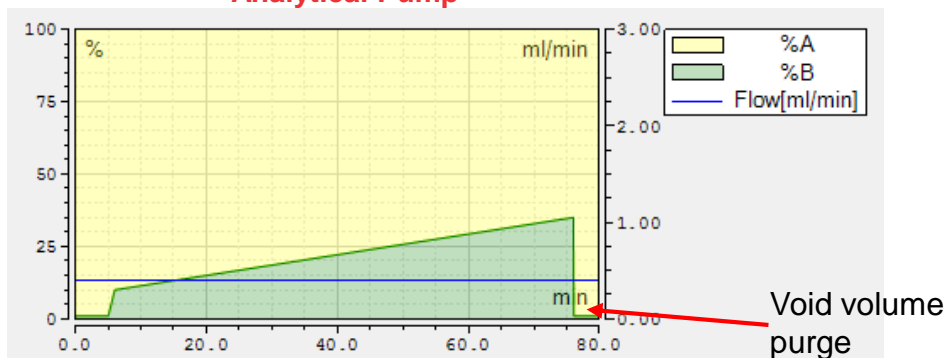## Higher throughput <u>without</u> changing your method!

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Tandem LC Configuration

VTCC
Column A
Active

2p6p valve

Waste — UV

1_2

MS

Analytical Column A | Analytical Column B

Analytical Pump

VAS

1_2

Reconditioning Pump

2p6p valve

VTCC
Column B
Active

2p6p valve

Waste — UV

6_1

MS

Analytical Column A | Analytical Column B

Analytical Pump

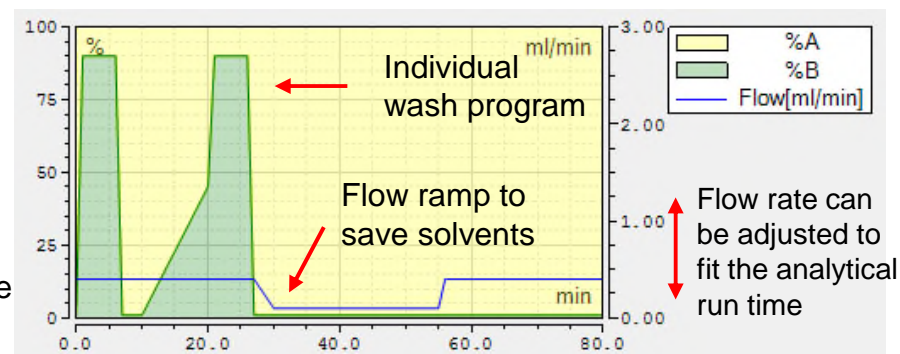VAS

6_1

Reconditioning Pump

2p6p valve

- **Analytical pump** is responsible for the gradient.

- **Reconditioning pump** is always performing the column wash and equilibration.

- The void volume purge is required to purge from the mixing point to the lower valve.
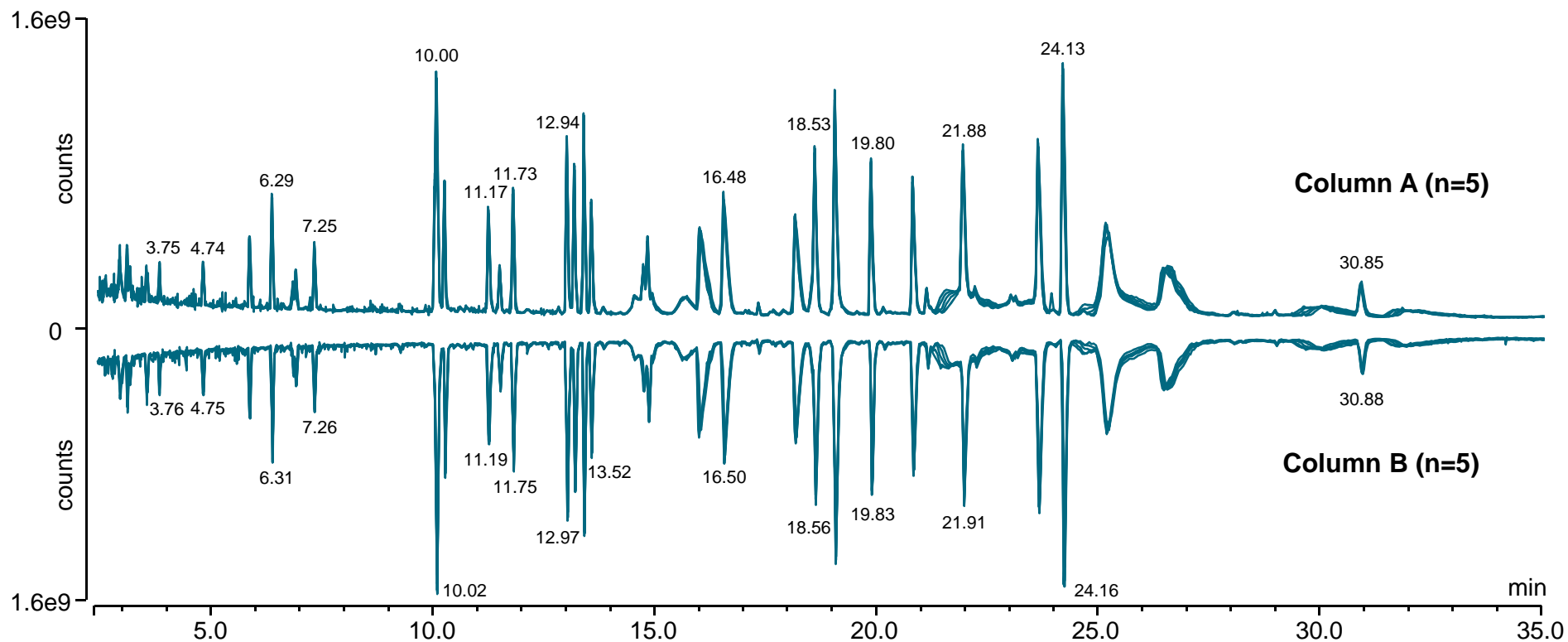
Analytical Pump

%A
%B
Flow[ml/min]

Void volume purge

Reconditioning Pump

%A
%B
Flow[ml/min]

Individual wash program

Flow ramp to save solvents

Flow rate can be adjusted to fit the analytical run time

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SMART Digest™ of Infliximab

Overlay of 5 total ion current chromatograms
Vanquish Tandem Setup: 2x Acclaim™ VANQUISH™ C18 2.1x250mm, 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, R=15k, mass range 140-2000; UV detection@214nm

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SMART Digest of Infliximab - Results

Single Column Setup						Tandem LC Setup			
Column A			Column B					Column A/B	
RT [min] n=5	RT RSD [%] n=5	Area RSD [%] n=5	RT [min] n=5	RT RSD [%] n=5	Area RSD [%] n=5	Abs. RT Shift Column A to B [min]	Rel. RT Shift Column A to B [%]	RT RSD [%] n=10	Area RSD [%] n=10
3.75	0.18	2.72	3.76	0.13	1.64	0.005	0.13	0.16	2.12
4.74	0.054	3.53	4.75	0.11	5.05	0.010	0.21	0.14	4.24
6.29	0.072	2.33	6.31	0.037	1.14	0.020	0.32	0.18	2.19
7.25	0.018	4.94	7.26	0.033	4.72	0.016	0.23	0.12	4.94
10.00	0.032	3.05	10.02	0.037	1.75	0.023	0.23	0.12	2.35
11.17	0.040	3.96	11.19	0.047	2.70	0.022	0.20	0.11	3.22
11.73	0.043	1.64	11.75	0.007	2.59	0.025	0.21	0.12	2.15
12.94	0.014	4.19	12.97	0.012	1.61	0.023	0.18	0.10	3.03
13.49	0.028	1.66	13.52	0.025	3.11	0.024	0.18	0.10	2.36
16.48	0.056	1.02	16.50	0.031	0.78	0.024	0.14	0.087	0.91
18.53	0.019	1.94	18.56	0.020	1.90	0.027	0.15	0.080	2.35
19.80	0.019	0.50	19.83	0.016	0.78	0.029	0.15	0.078	0.62
21.88	0.028	4.35	21.91	0.0075	1.71	0.033	0.15	0.083	3.78
24.13	0.025	1.52	24.16	0.030	0.60	0.031	0.13	0.072	1.09
30.85	0.039	1.56	30.88	0.039	2.00	0.031	0.10	0.064	1.74
Average	**0.045**	**2.59**		**0.039**	**2.14**	**0.023**	**0.18**	**0.11**	**2.47**

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Conclusions

- The Tandem LC setup is feasible with the current Vanquish UHPLC platform
    - Analytical pump: Vanquish Binary H (or Binary F)
    - Reconditioning pump: Vanquish Quaternary F (or Binary H, Binary F)

- Up to 60% higher throughput without changing your method!
- Retention time RSDs below 0.11% for the tandem - and single column setup.
- Supported under Chromeleon™ 7.2 and SII 1.3

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TECHNICAL NOTE 72203

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

Authors
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Key words
Dual column, dual gradient, offline reconditioning, alternating column regeneration, Vanquish, Q Exactive, monoclonal antibody, biotherapeutics, biosimilar
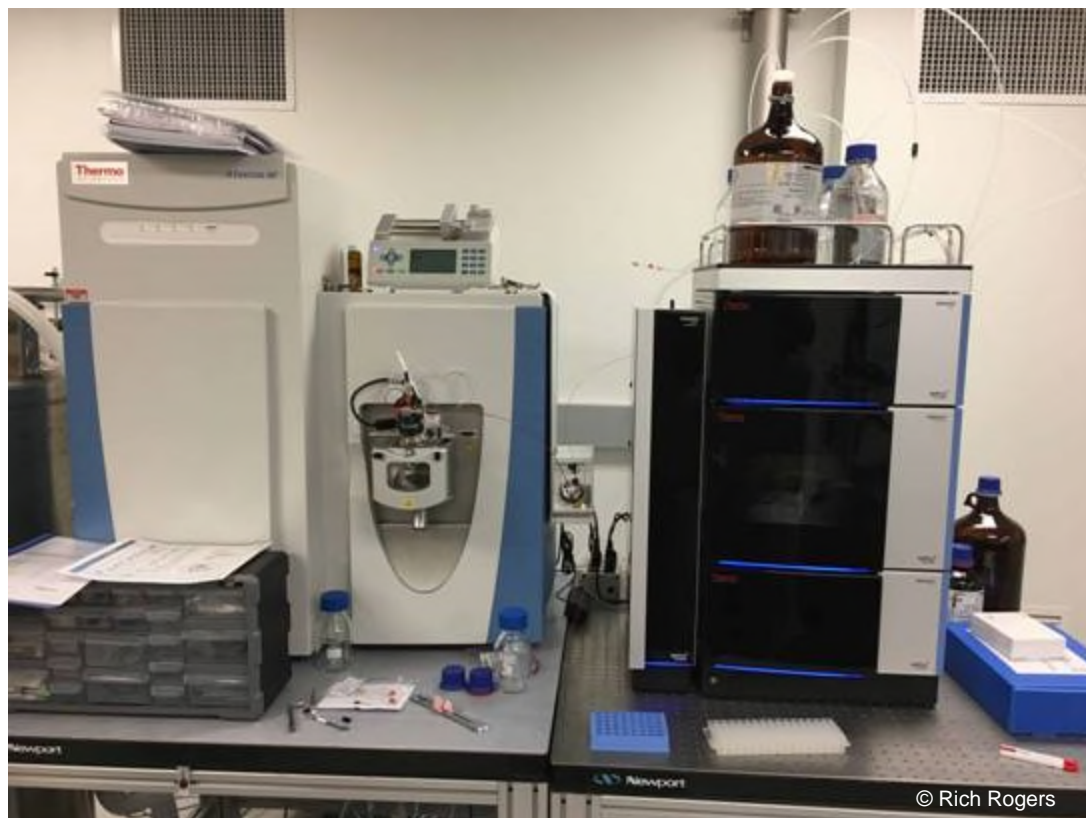
Goal
To demonstrate the use of a multi-pump UHPLC system and enable tandem analysis with two columns in parallel, addressing productivity and throughput improvement of existing LC-MS methods.

Introduction
Common liquid chromatography (LC) methods with gradient elution can be segmented into an analytical gradient section and a reconditioning section. The gradient section is responsible for the actual chromatographic separation, and the reconditioning section is where the column is washed and re-equilibrated for the next injection (Figure 1). The process of column re-equilibration involves replacing the mobile phase between the particles (inter-particle), within the pores of the particles (intra-particle), and in the interfacial region between the mobile phase and stationary phase.[1] Good and accepted practice suggests using at least five column volumes to sufficiently equilibrate the analytical column.[2] If a column is required to be equilibrated with a buffered mobile phase or with a mobile phase containing an ion pair reagent, the required equilibration time is even longer. Depending on the column dimensions, gradient length, and flow rate, typically 10–60% of the total runtime is consumed by these column reconditioning steps within the gradient method.

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ThermoFisher SCIENTIFIC | AppsLab Library   https://appslab.thermofisher.com/App/3695/vanquish-tandemlc

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Vanquish Tandem LC for Biopharma QC

© Rich Rogers

Vanquish Tandem LC (based on Flex Binary) coupled to a Q Exactive HF

Compliant Multiple Attribute Monitoring (MAM) of therapeutic proteins for manufacturing control

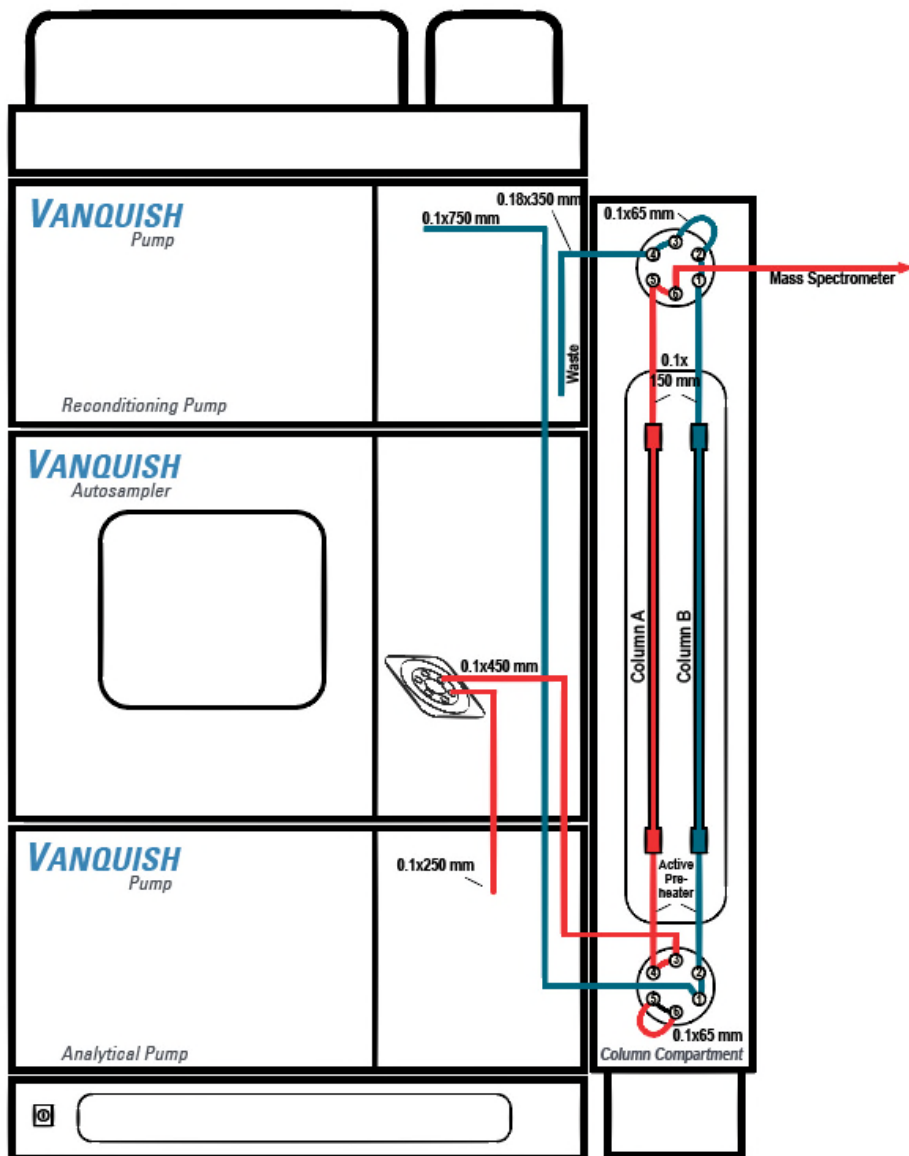JUST Biotherapeutics, Seattle
Rich Rogers lab

Rich Rogers: *"Our new system is installed and pumping out GB of amazing data"*

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Setup Details

- Vanquish Tandem LC w/o UV Detector

- Vanquish Tandem LC w/o UV Detector and TCC on the left side (Conversion Kit)

- Vanquish Tandem LC w/ UV Detector

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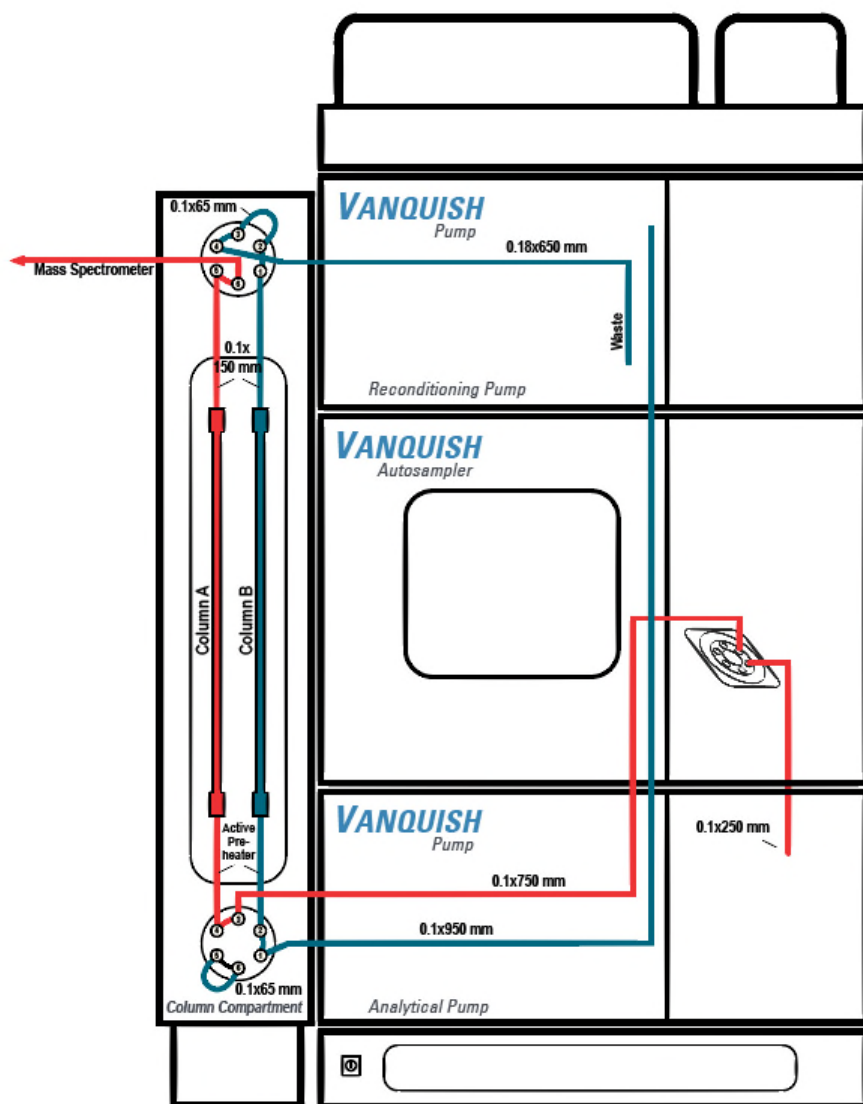
#	Viper™ Capillary	PN
2x	2p6p Valve	6036.1560
2x	0.1x65 mm	6042.2306
2x	0.1x150 mm	6042.2320
1x*	0.1x250 mm	6042.2330
2x	0.1x450 mm	6042.2350
1x	0.1x750 mm	6042.2390
1x	0.18x350 mm	6042.2337
2x*	0.1x380 mm Active PreHeater	6732.0110

* 1 already included in System Base Vanquish Ship Kits

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#	Viper Capillary	PN
2x	2p6p Valve	6036.1560
2x	0.1x65 mm	6042.2306
2x	0.1x150 mm	6042.2320
1x*	0.1x250 mm	6042.2330
1x	0.1x450 mm	6042.2350
1x	0.1x750 mm	6042.2390
1x	0.1x950 mm	6042.2395
1x	0.18x650 mm	6042.2380
2x*	0.1x380 mm Active PreHeater	6732.0110

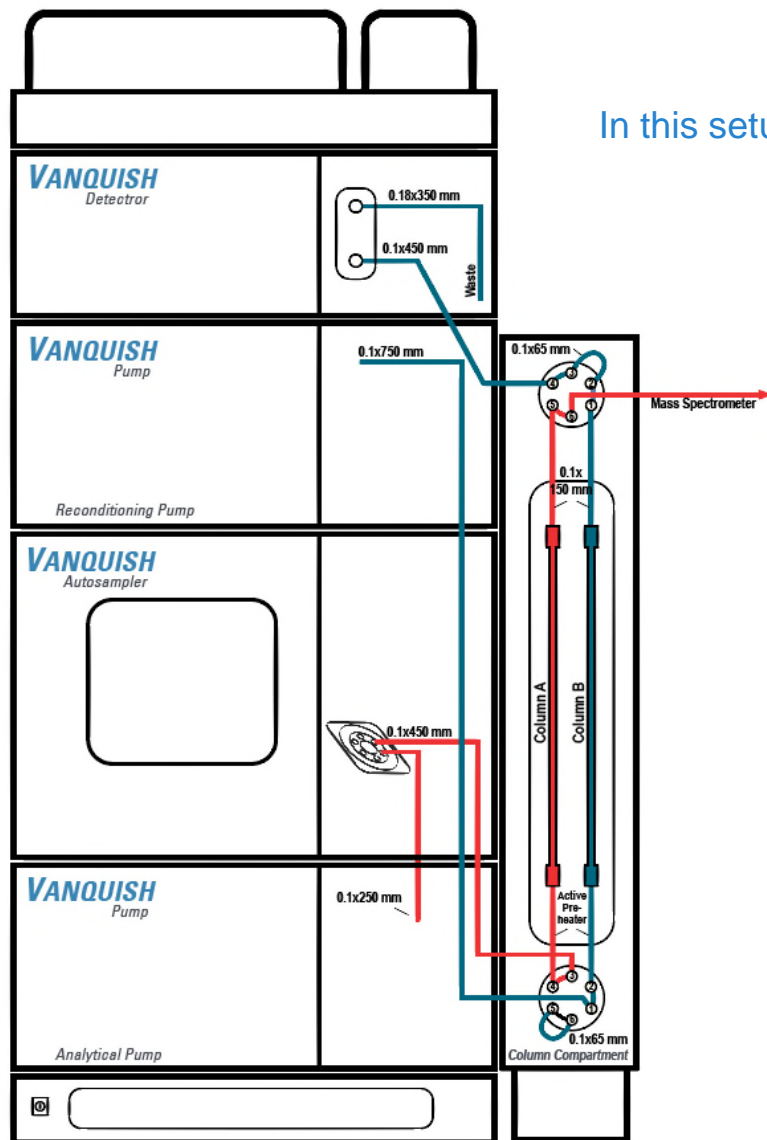* 1 already included in System Base Vanquish Ship Kits

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In this setup the VVWD is used for wash and equilibration monitoring.

#	Viper Capillary	PN
2x	2p6p Valve	6036.1560
2x	0.1x65 mm	6042.2306
2x	0.1x150 mm	6042.2320
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