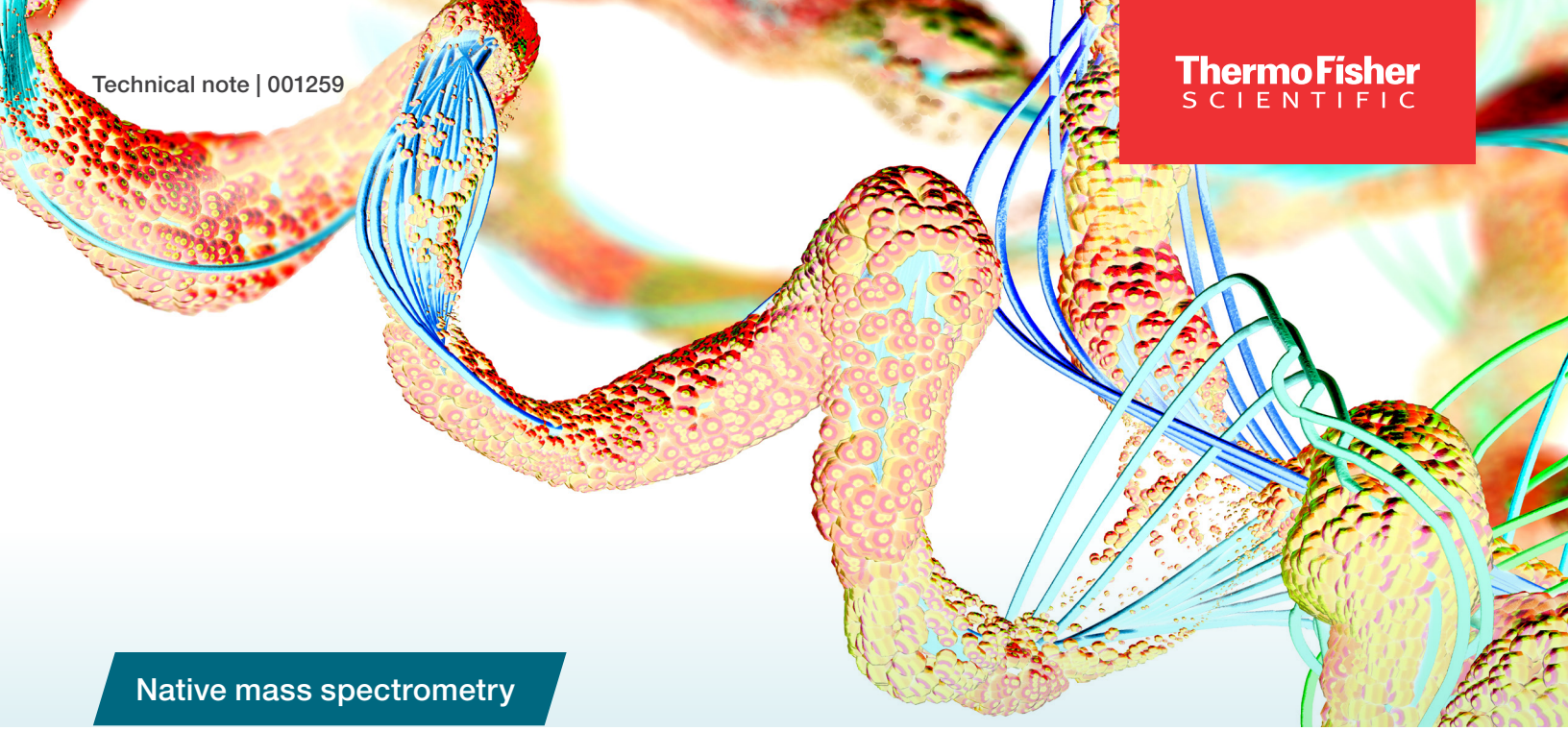


Technical note | 001259

Native mass spectrometry

Rapid online buffer exchange

Solutions for high-throughput analysis of large biomolecules by native mass spectrometry

Authors

Weijing Liu[1], Samy Memmi[2], Terry Zhang[1], Shane Bechler[3], Andrew Norris[4], Rosa Viner[1], Stella Lai[5], Florian Busch[5], Zac VanAernum[5] Vicki Wysocki[5];

[1]Thermo Fisher Scientific, San Jose, CA, USA,
[2]Thermo Fisher Scientific, Merelbeke, Belgium,
[3]Thermo Fisher Scientific, Sunnyvale, CA, USA,
[4]Thermo Fisher Scientific, Reinach, Switzerland,
[5]The Ohio State University, Columbus, OH, USA

Keywords

Online buffer exchange, Native MS, high-throughput, NativePac OBE-1 column, Q Exactive UHMR

Why do we need rapid online buffer exchange?

- Native mass spectrometry (nMS) has become an important tool in large biomolecule analysis due to its ability to retain non-covalent interactions during measurements, making it possible to obtain structural information with high sensitivity and at high speed.
- Interferences from the presence of non-volatiles are typically alleviated by offline buffer exchange, which is time-consuming and difficult to automate.
- Online buffer exchange (OBE) nMS[1] allows for direct screening of structural features of large biomolecules, such as pre-purified proteins, protein complexes, or clarified cell lysates even if such biomolecules are not very stable in MS friendly buffers.
- Information obtained by OBE nMS can be used for fast (<5 min) quality control and can further guide downstream processes (e.g., protein expression protocols and purification optimization).

How does OBE work?

- The separation of proteins and non-volatile small molecules is accomplished by a short size-exclusion column which removes salts and contaminants less than 8kDa.[1]
- OBE nMS must accommodate aqueous mobile phases containing enough ammonium acetate to provide sufficient ionic strength to maintain native protein structure and prevent interactions between analytes and the stationary phase.
- Thermo Scientific™ Vanquish™ Flex UHPLC System or Thermo Scientific™ UltiMate™ 3000 RSLCnano System can be used in different configurations to perform OBE nMS:
  1. OBE using a dual pump setup
  2. OBE using a single pump and the syringe drive
  3. OBE using a single pump, the syringe drive and divert valve
  4. OBE using a single pump
  5. OBE using UltiMate 3000 RSLCnano system and divert valve

Suitable columns for OBE nMS

- The main purpose of the stationary phase in OBE is to separate proteins from small non-volatiles within a short amount of time at a given flow rate (Figure 1 and 2), thereby limiting:
  - Sample dilution
  - The extent to which biomolecular interactions with high k_{off} rates dissociate
- For optimal OBE performance, a column should be chosen that has an exclusion limit below the mass of the proteins to be buffer-exchanged.
- This allows the buffer-exchanged protein to elute prior to the total column void volume where the non-volatile components are eluted shortly afterward.
- Column for OBE nMS:
  - Thermo Scientific™ NativePac OBE-1 SEC column, 2.1 mm x 50 mm, 80 Å (P/N 43803-052130)[2]

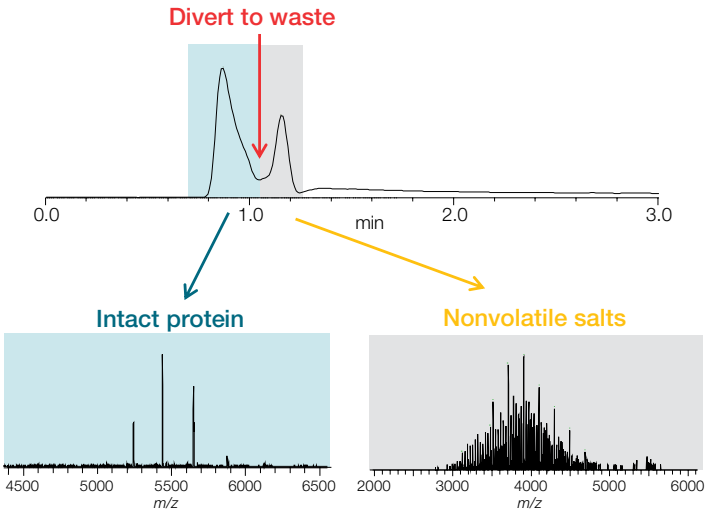
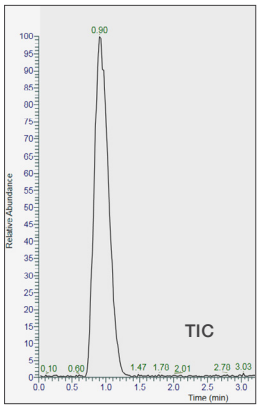
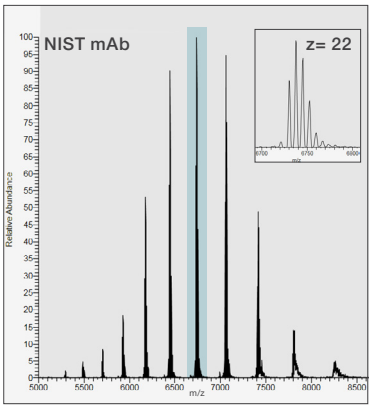


Figure 1. A typical chromatogram from the OBE method

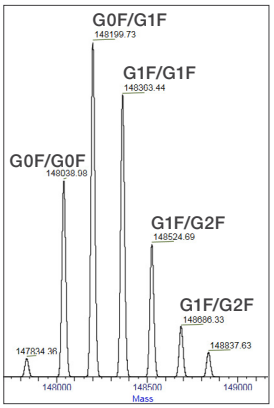
Sample	1 µg NIST mAb in PBS
LC setup	Vanquish Flex UHPLC–dual pump
LC flow rate	0.1 mL/min
LC buffer	200 mM ammonium Acetate
OBE column	NativePac OBE-1, 2.1 × 50 mm 3 µm, 80 Å
MS instrument	Thermo Scientific™ Q Exactive™ UHMR MS
Scan mode	HCD–Extended trapping 120 V
Resolution	12,500
Scan range	*m/z* 1,000–14,000



Short elution time



NISTmAb exchanged from PBS into ammonium acetate

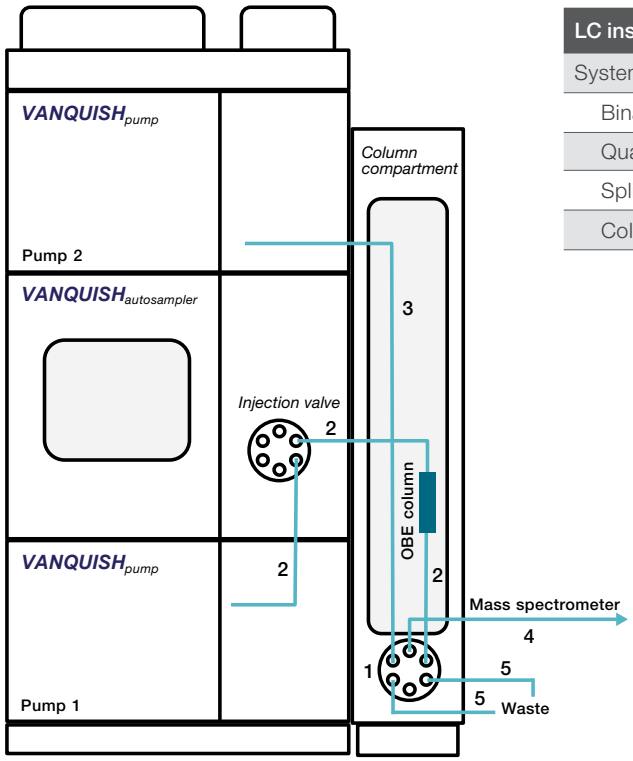


Multiple glycoforms detected without the presence of salt adducts

Figure 2. Efficient removal of PBS buffer by OBE

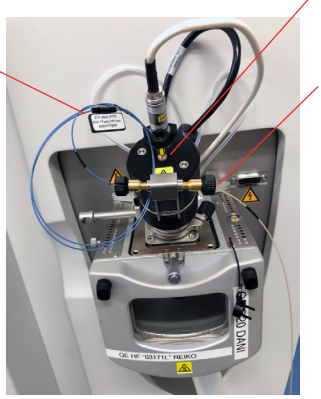
1. OBE using Vanquish Flex UHPLC system with a dual pump setup

LC instrumentation and recommended capillaries



LC instrumentation	P/N
System Base Vanquish Flex	VF-S01-A
Binary Pump F	VF-P20-A **(Pump 1)**
Quaternary Pump F	VF-P20-A **(Pump 2)**
Split Sampler FT	VF-A10-A-02
Column Compartment H	VH-C10-A-03

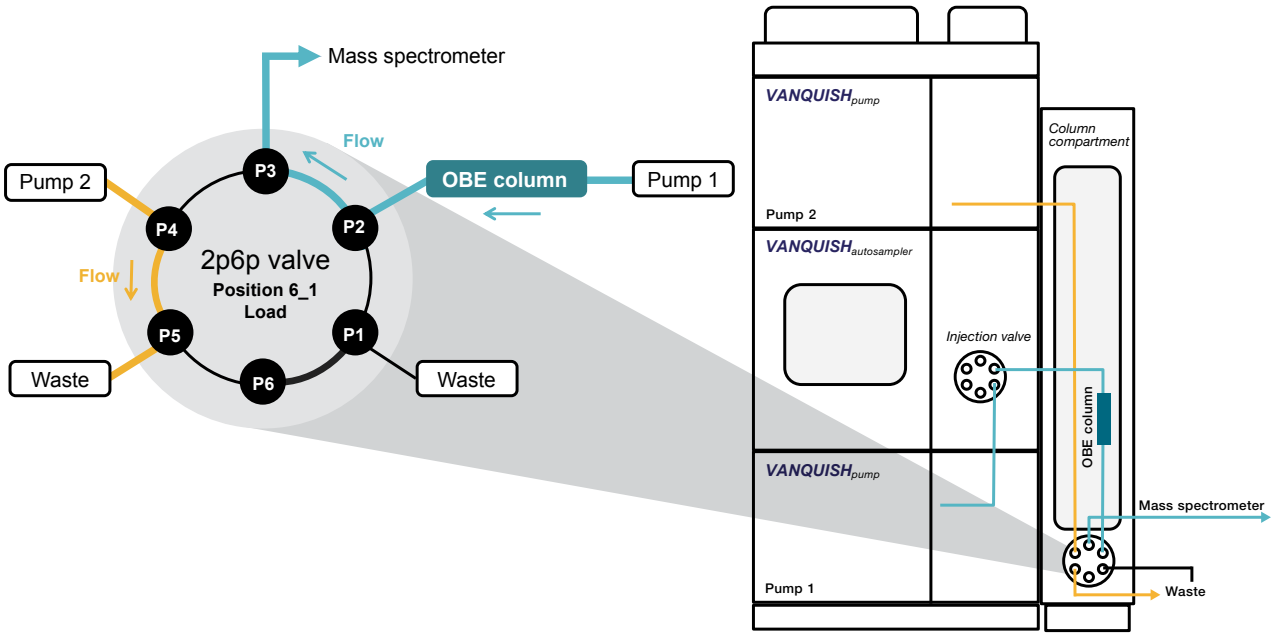
No.	Amount	Viper capillary	P/N	2p6p valve port assignment
1	1×	Biocompatible 2-position/6-port (2p6p) column switching valve	6036.1560	
2	3×	Thermo Scientific™ Viper™ Capillary, MP35N, biocompatible, 0.1 × 350 mm	6042.2340	Port 2 – OBE column Autosampler – OBE column Pump 1 – Injection valve
3	1×	Viper Capillary, MP35N, biocompatible, 0.1 × 750 mm	6042.2390	Port 4 – Second pump
4	1×	Thermo Scientific™ nanoViper™ Fingertight Fittings, 0.1 × 550 mm	6041.5815	Port 3 – Resistor tubing MS
5	2×	nanoViper Fingertight Fittings, 0.1 × 650 mm	6041.5824	Port 5 – Waste Port 1 – Waste



A 75 µm × 650 mm (P/N 6041.5775) “resistor” tubing is fitted between the ESI probe and ground to reduce the spray current and make it possible to utilize mobile phases with high ionic strength

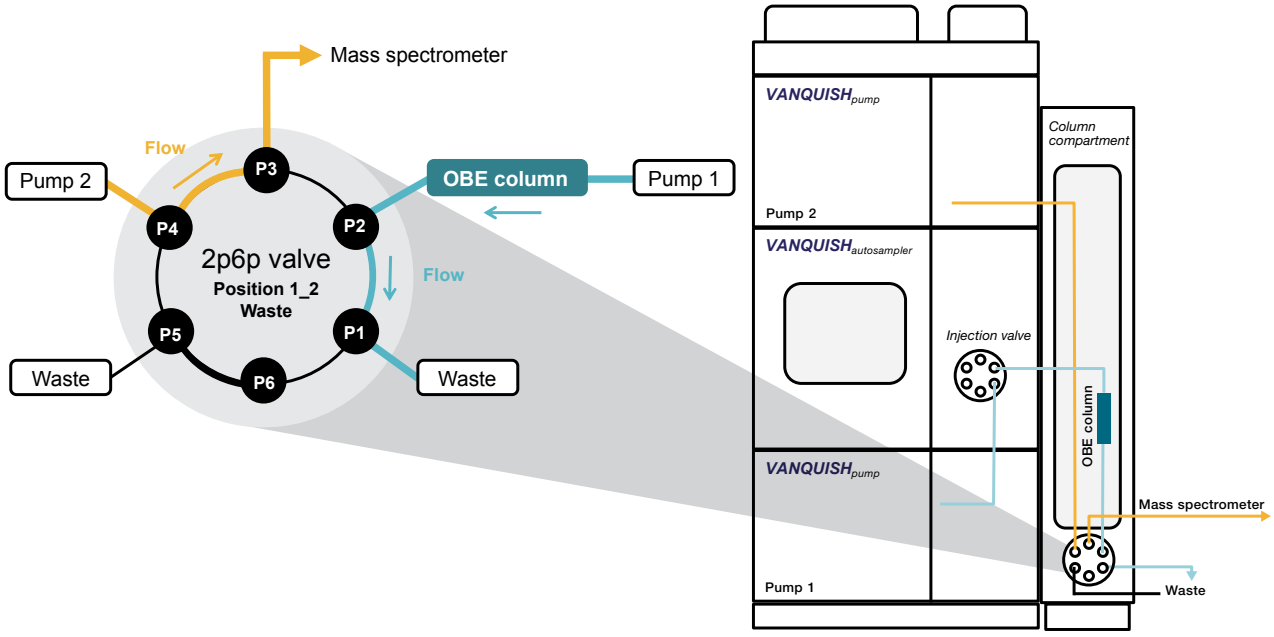
Column switching valve configuration: Position 6_1 LOAD

- This is the configuration at the start of the run when the sample is injected.
- Analytes are eluted with aqueous ammonium acetate solution.
- Analytes will start to elute rather quickly as they are not retained on the column.



Column switching valve configuration: Position 1_2 WASTE

- Pump 2 continues pushing the large analytes to the MS, whereas subsequently eluting non-volatile small molecules are diverted to waste (column regeneration).



Method recommendations

- Use filtered and degassed 50–200 mM ammonium acetate as the mobile phase for both pumps.
- Isocratic flow rates are typically between 50–100 µL/min.
- Scheduling of the 2p6p valve position is done in the LC method window under the column compartment tab.

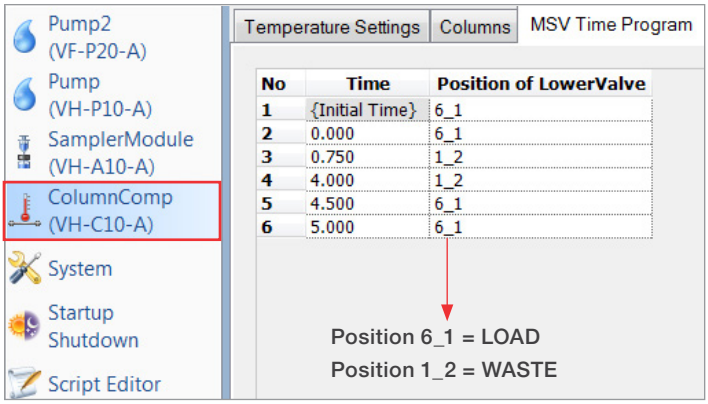


Figure 3. Time scheduling of the 2p6p valve switching from the instrument method editor

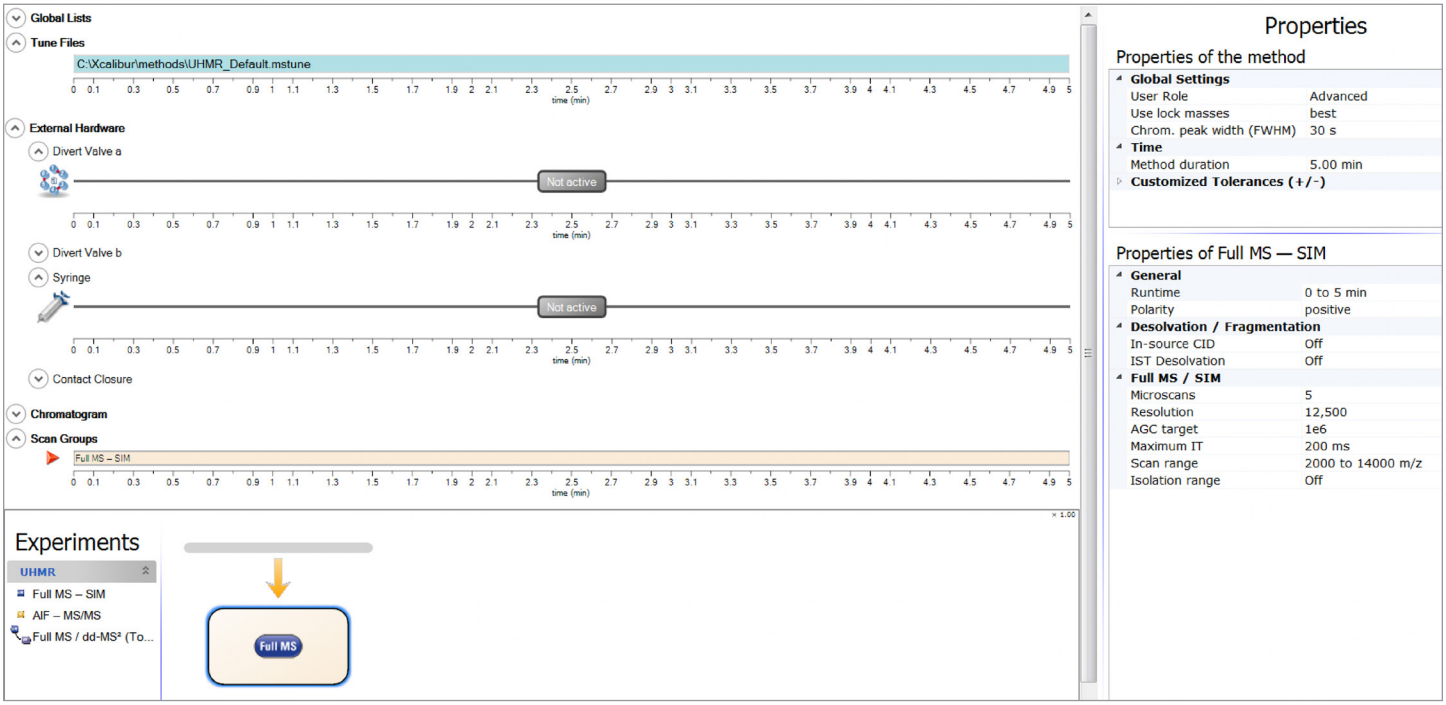
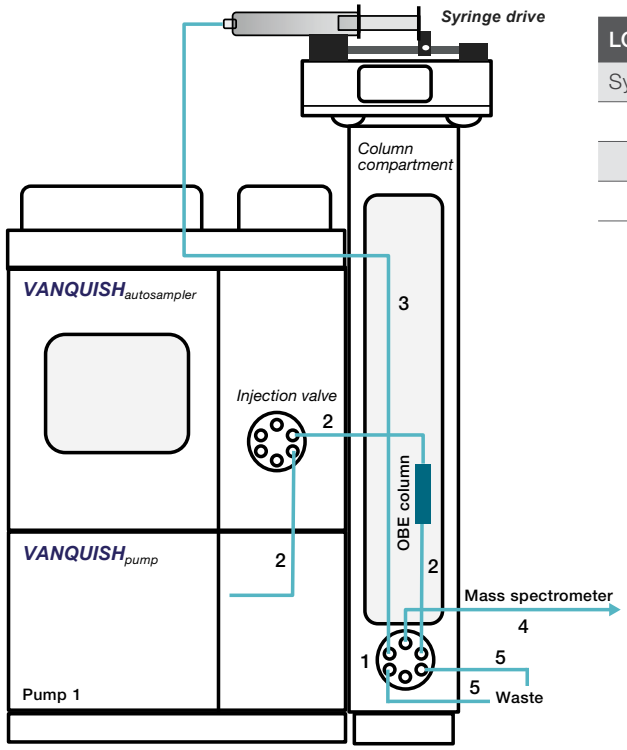


Figure 4. Q Exactive UHMR mass spectrometer standard method example for OBE Native MS

2. OBE using a Vanquish Flex UHPLC with single pump and syringe drive

LC instrumentation and recommended capillaries

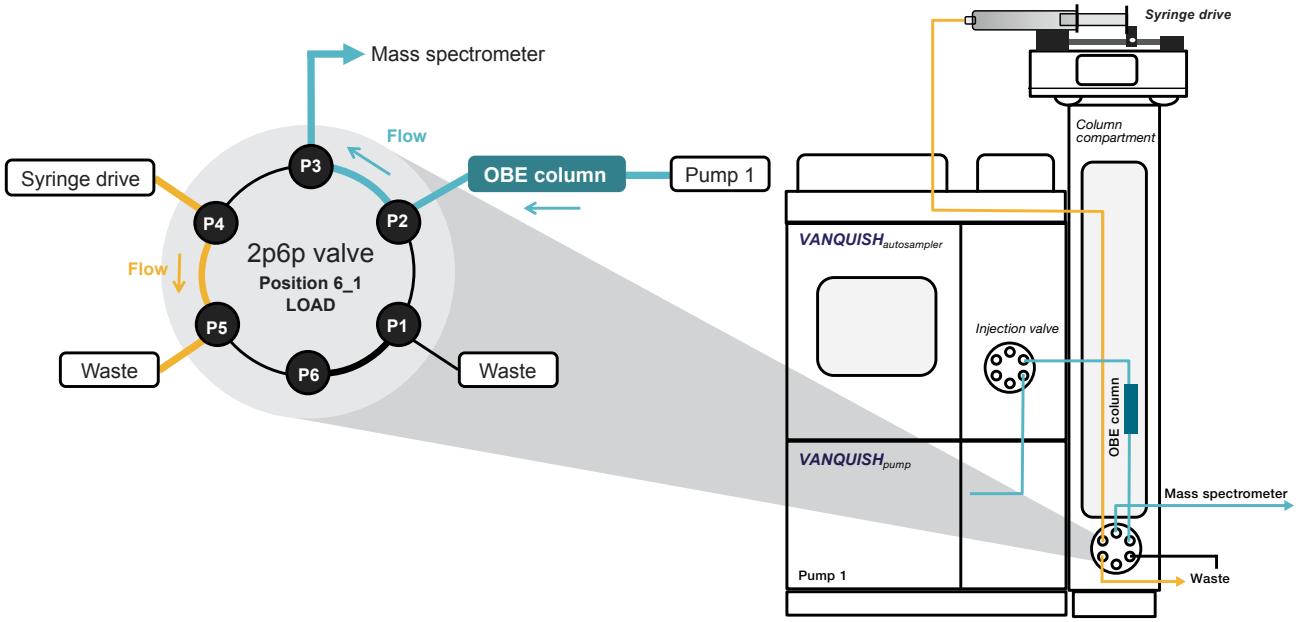


LC instrumentation	P/N
System Base Vanquish Flex	VF-S01-A
Binary Pump F	P/N VF-P10-A-01 **(Pump 1)**
Split Sampler FT	VF-A10-A-02
Column Compartment H	VH-C10-A-03

No.	Amount	Viper capillary	P/N	2p6p valve port assignment
1	1×	Biocompatible 2-position/6-port (2p6p) column switching valve	6036.1560	
2	3×	Viper Capillary, MP35N, biocompatible, 0.1 × 350 mm	6042.2340	Port 2 – OBE column Autosampler – OBE column Pump 1 – Injection valve
3	1×	Viper Capillary, MP35N, biocompatible, 0.1 × 750 mm	6042.2390	Port 4 – Syringe drive
4	1×	nanoViper Fingertight Fittings, 0.1 × 550 mm	6041.5815	Port 3 – Resistor tubing MS
5	2×	nanoViper Fingertight Fittings, 0.1 × 650 mm	6041.5824	Port 5 – Waste Port 1 – Waste

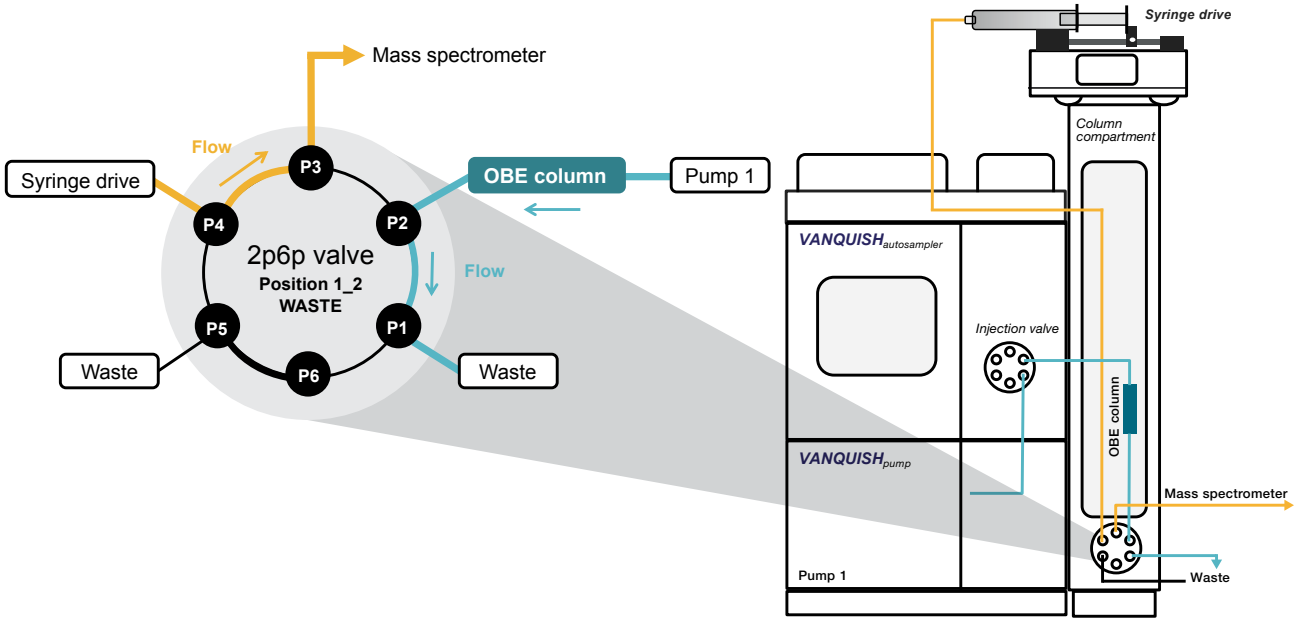
Column switching valve configuration: Position 6_1 LOAD

- This is the configuration at the start of the run when the sample is injected.
- Analytes are eluted with aqueous ammonium acetate solution.
- Analytes will start to elute rather quickly as they are not retained on the column.



Column switching valve configuration: Position 1_2 WASTE

- The **syringe drive** continues pushing the large analytes to the MS, whereas subsequently eluting non-volatile small molecules are diverted to waste (column regeneration).



Method recommendations

- Use filtered and degassed 50–200 mM ammonium acetate as the mobile phase for both the pump and the syringe.
- Isocratic flow rates are typically between 50–100 µL/min.
- Scheduling of the 2p6p valve position is done in the LC method window under the column compartment tab.
- The setup for the syringe drive is done in the MS section of the instrument method.

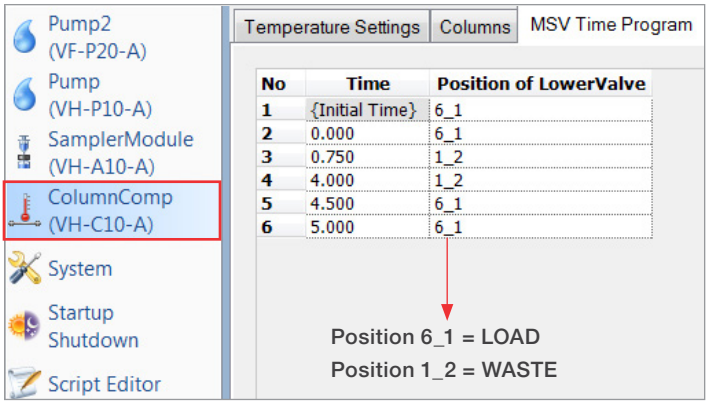


Figure 5. Time scheduling of the 2p6p valve switching from the instrument method editor

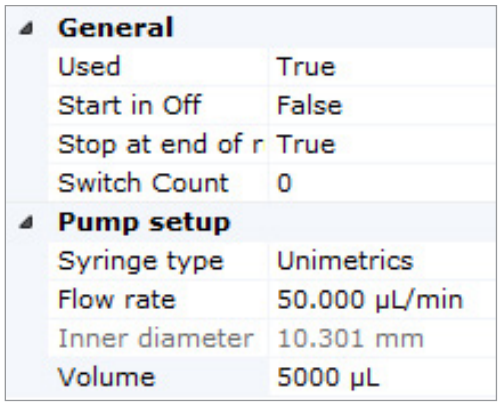


Figure 6. Properties of the syringe drive setup in the instrument method editor

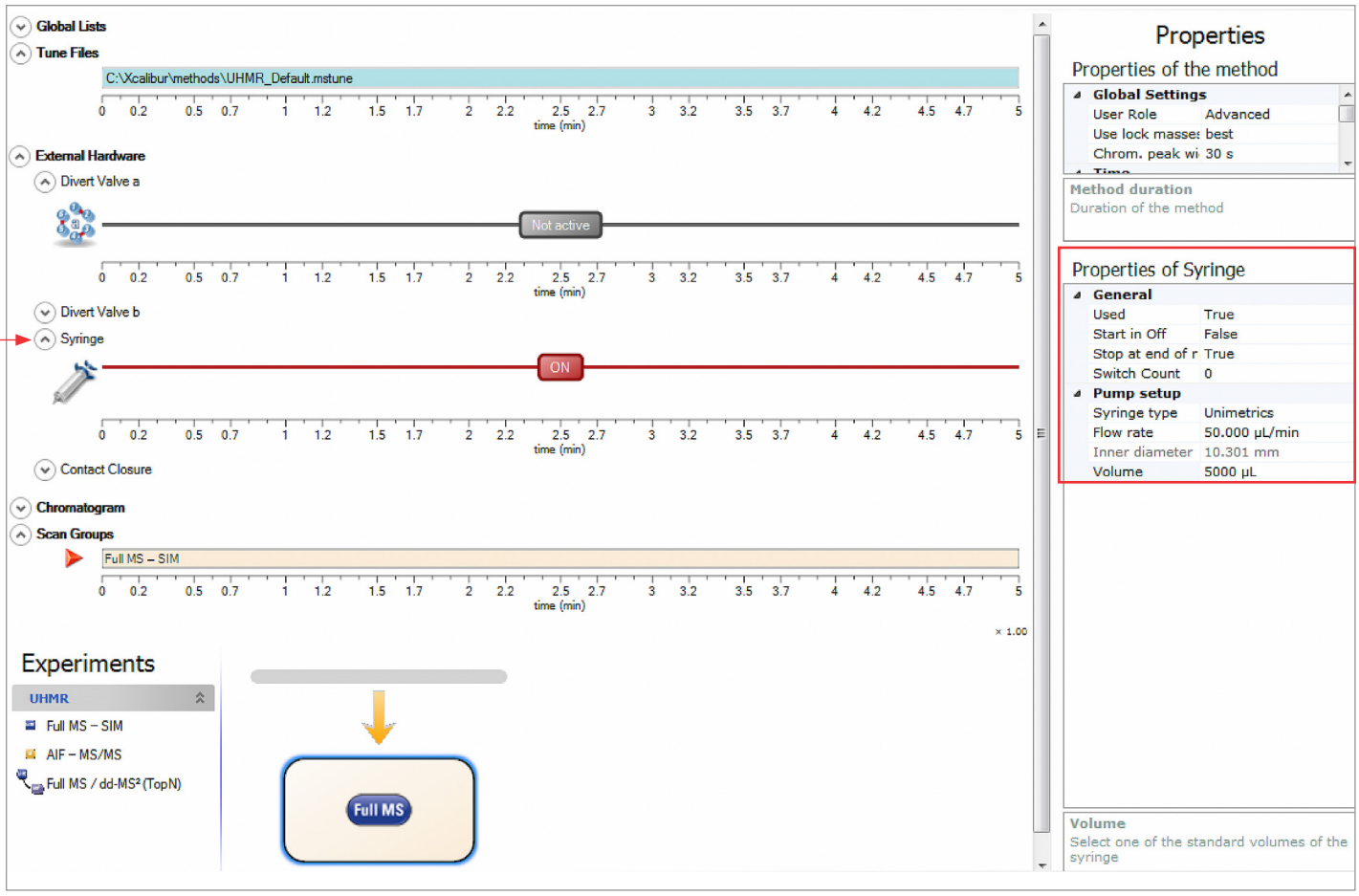
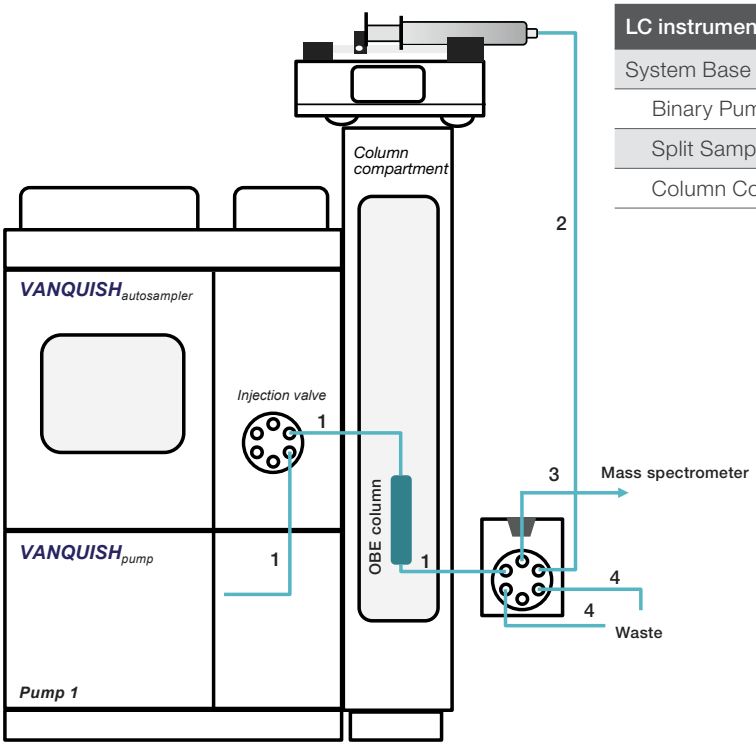


Figure 7. Q Exactive UHMR method with syringe drive control turned on

3. OBE using a Vanquish Flex UHPLC with single pump, syringe drive and divert valve

LC instrumentation and recommended capillaries

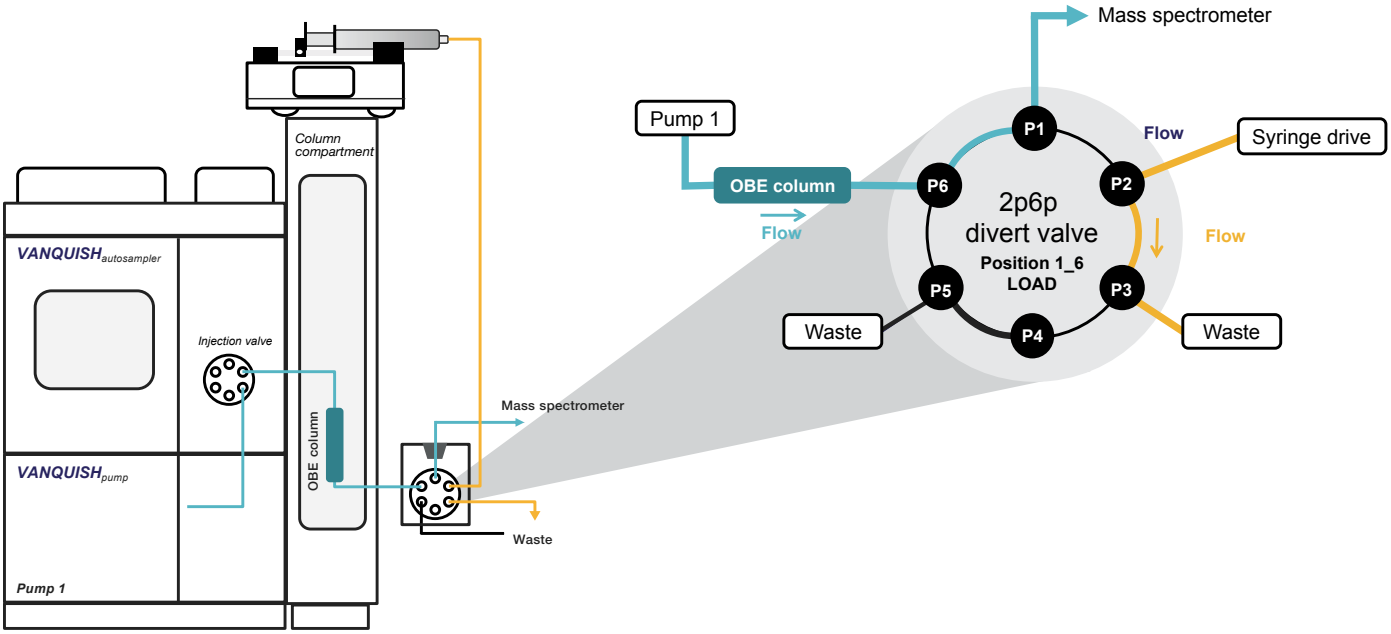


LC instrumentation	P/N
System Base Vanquish Flex	VF-S01-A
Binary Pump F	VF-P20-A **(Pump 1)**
Split Sampler FT	VF-A10-A-02
Column Compartment H	VH-C10-A-03

No.	Amount	Viper capillary	P/N	2p6p valve port assignment
1	3x	Viper Capillary, MP35N, biocompatible, 0.1 × 350 mm	6042.2340	Port 6 – OBE column Autosampler – OBE column Pump 1 – Injection valve
2	1x	nanoViper Fingertight Fittings, 0.1 × 750 mm	6041.5816	Port 2 – Syringe drive
3	1x	nanoViper Fingertight Fittings, 0.1 × 450 mm	6041.5814	Port 1 – Resistor tubing MS
4	2x	nanoViper Fingertight Fittings, 0.1 × 650 mm	6041.5824	Port 5 – Waste Port 3 – Waste

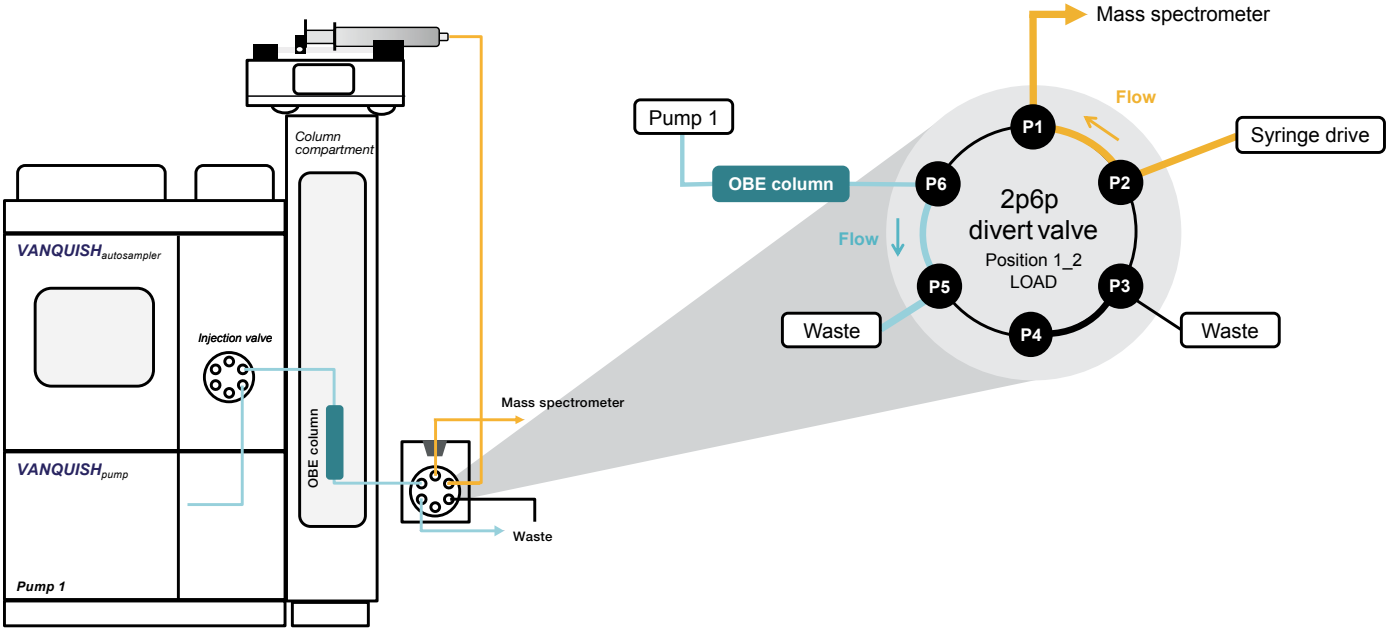
Divert valve configuration: Position 1_6 LOAD

- This is the configuration at the start of the run when the sample is injected.
- Analytes are eluted with aqueous ammonium acetate solution.
- Analytes will start to elute rather quickly as they are not retained on the column.



Divert valve configuration: Position 1_2 WASTE

- The **syringe drive** continues pushing the large analytes to the MS, whereas subsequently eluting non-volatile small molecules are diverted to waste (column regeneration).



Method recommendations

- Use filtered and degassed 50–200 mM ammonium acetate as the mobile phase for both the pump and the syringe.
- Isocratic flow rates are typically between 50–100 µL/min.
- Scheduling of the 2p6p divert valve as well as the setup for the syringe drive is done in the MS section of the instrument method.

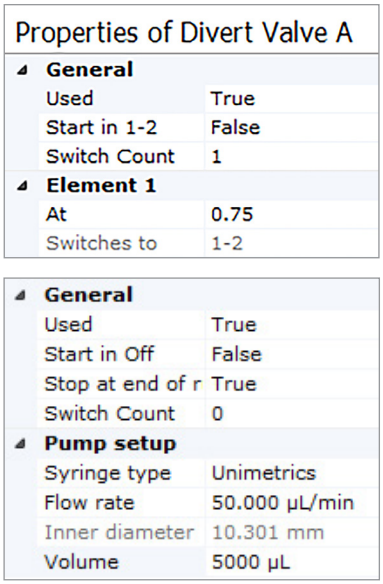


Figure 8. Properties of the divert valve and syringe drive setup in the instrument method editor

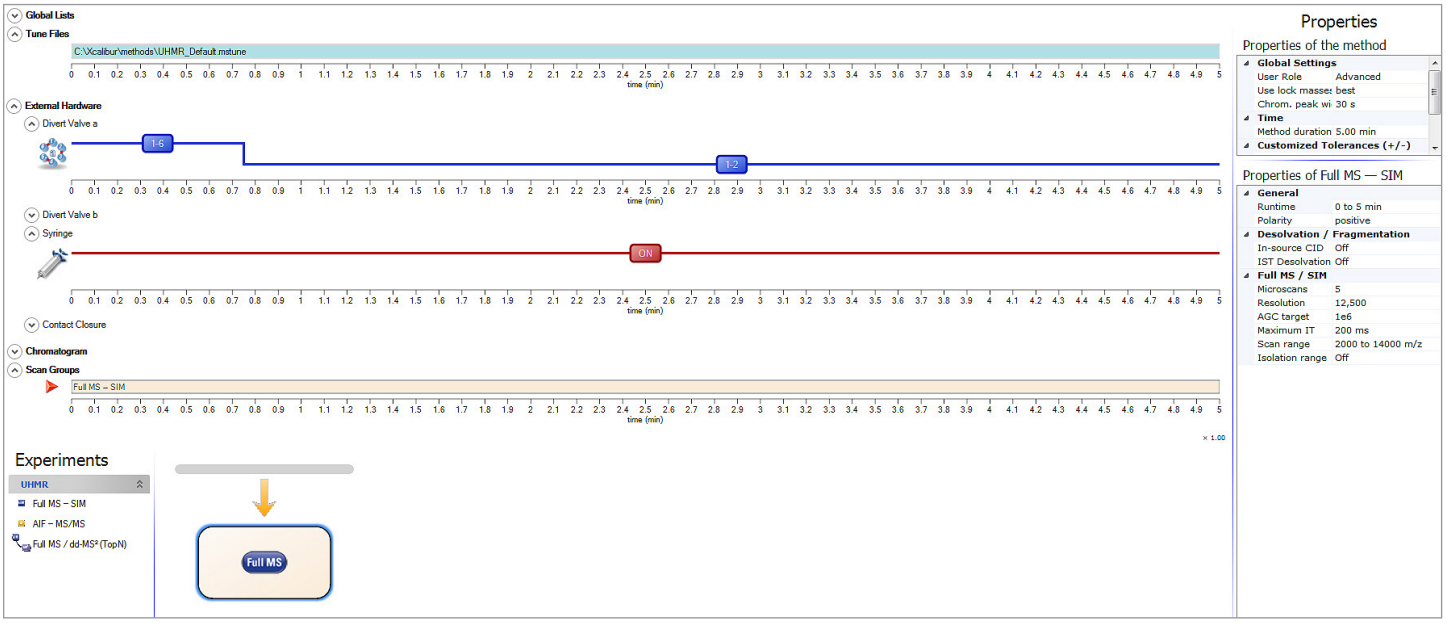
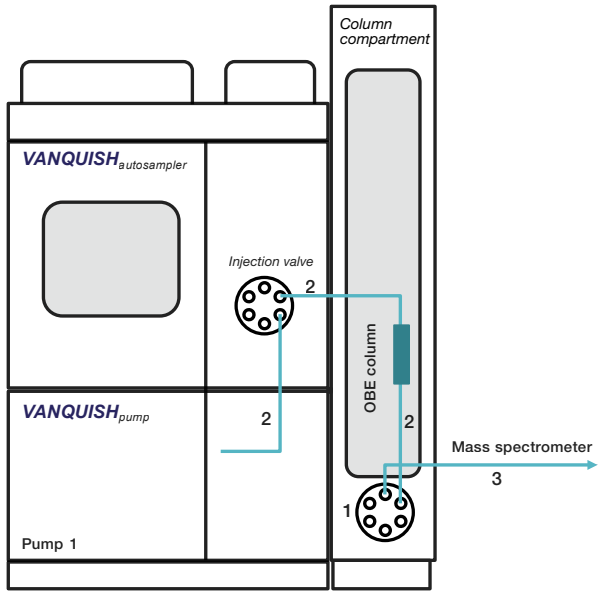


Figure 9. Q Exactive UHMR standard method with syringe control turned on

4. OBE using a Vanquish Flex UHPLC with single pump

LC instrumentation and recommended capillaries



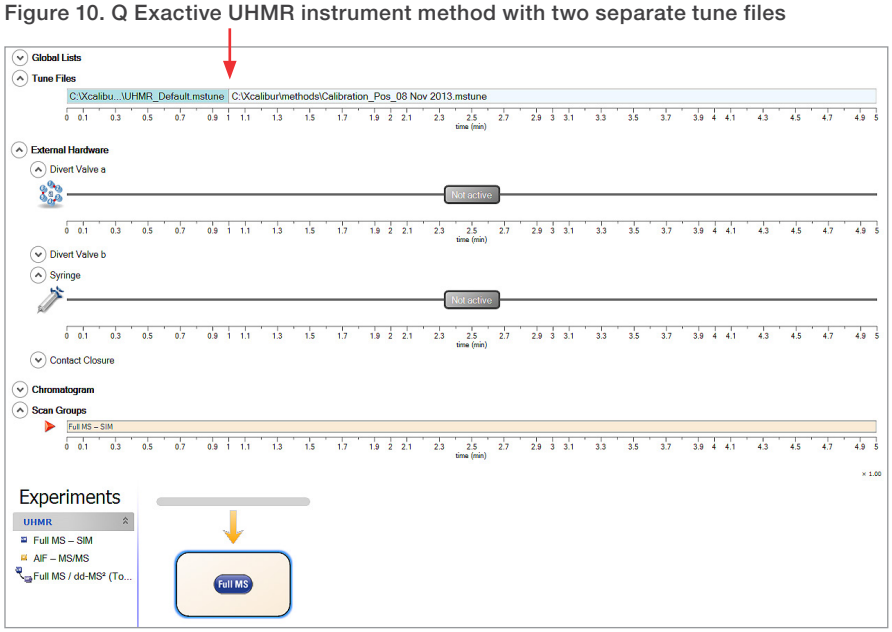
LC instrumentation	P/N
System Base Vanquish Flex	VF-S01-A
Binary Pump F	VF-P10-A-01 **(Pump 1)**
Split Sampler FT	VF-A10-A-02
Column Compartment H	VH-C10-A-03

No.	Amount	Viper capillary	P/N	2p6p valve port assignment
1	1x	Biocompatible 2-position/6-port (2p6p) column switching valve	6036.1560	
2	3x	Viper Capillary, MP35N, biocompatible, 0.1 × 350 mm	6042.2340	Port 2 – OBE column Autosampler – OBE column Pump 1 – Injection valve
3	1x	Viper Capillary, MP35N, biocompatible, 0.1 × 750 mm	6041.5815	Port 2 – Resistor tubing MS

Method recommendations

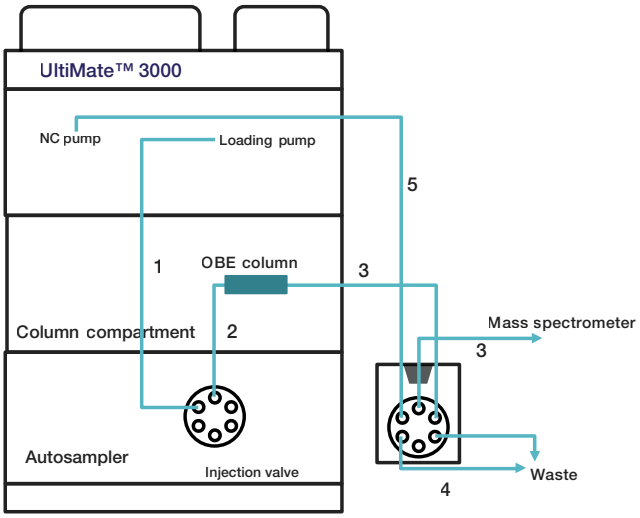
- Use filtered and degassed 50–200 mM ammonium acetate as the mobile phase.
- Isocratic flow rates are typically between 50–100 µL/min.
- With a single pump setup, we use two different tune files, where the second tune file has the spray voltage set to 0 kV to prevent the nonvolatile salts from entering into the MS during the column regeneration.

Figure 10. Q Exactive UHMR instrument method with two separate tune files



5. OBE using UltiMate 3000 RSLCnano with micro flow selector (6041.7903A flowmeter, Biocomp., Micro NCS-3X00a) and divert valve

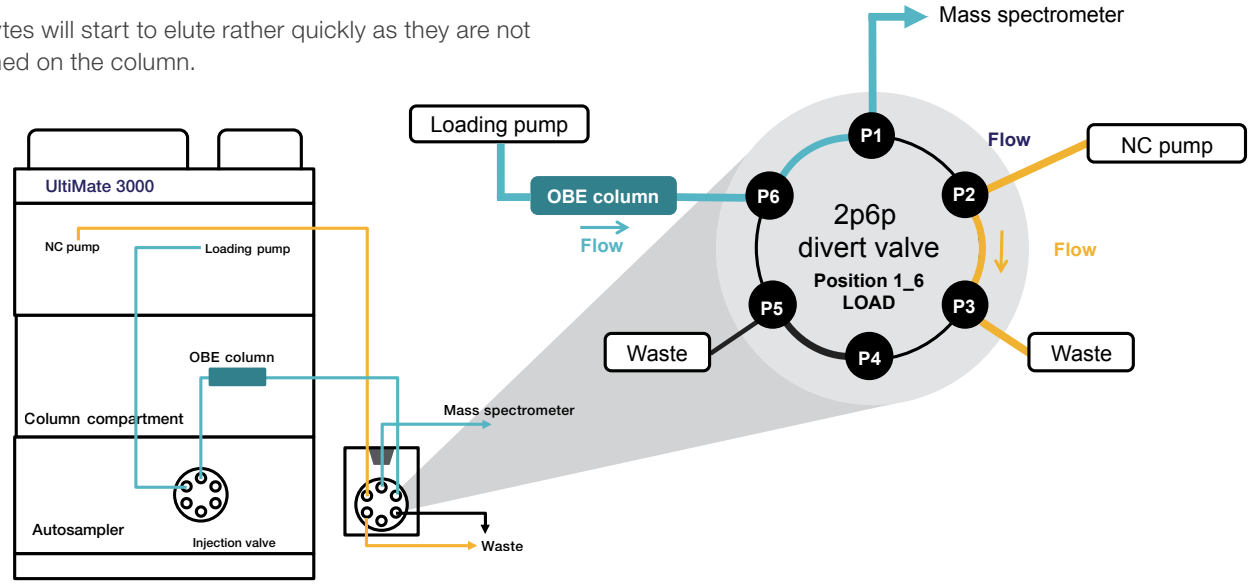
LC instrumentation and recommended capillaries



No.	Amount	Viper capillary	P/N	2p6p valve port assignment
1	1x	nanoViper Fingertight Fittings, 0.1 × 550 mm	6041.5815	Loading pump – Injection valve
2	1x	nanoViper Fingertight Fittings, 0.1 × 350 mm	6041.5813	Autosampler – OBE column
3	2x	nanoViper Fingertight Fittings, 0.1 × 450 mm	6041.5814	Port 1 – Resistor tubing MS Port 6 – OBE column
4	2x	nanoViper Fingertight Fittings, 0.1 × 650 mm	6041.5824	Port 5 – Waste Port 3 – Waste
5	1x	nanoViper Fingertight Fittings, 0.1 × 750 mm	6041.5816	Port 2 – NC pump

Divert valve configuration: Position 1_6 LOAD

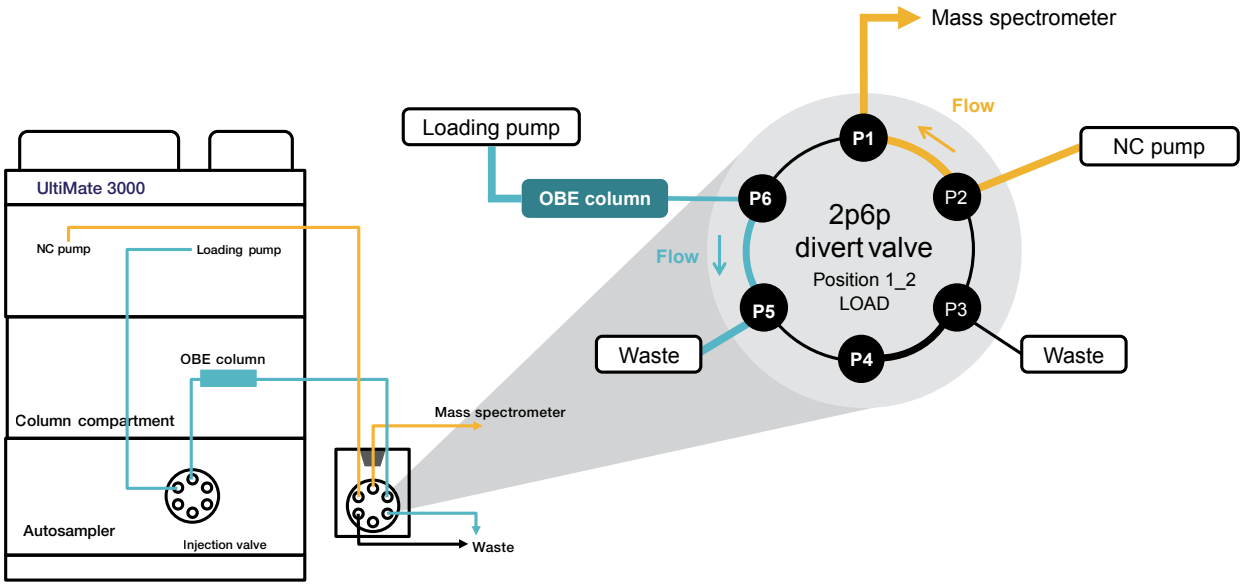
- This is the configuration at the start of the run when the sample is injected.
- Analytes are eluted with aqueous ammonium acetate solution.
- Analytes will start to elute rather quickly as they are not retained on the column.



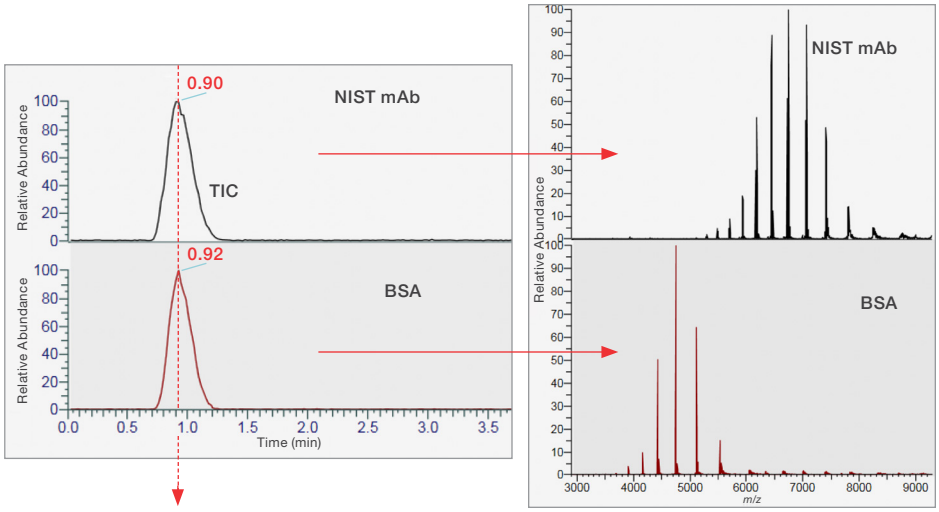
Divert valve configuration: Position 1_2 WASTE

The **NC pump** continues pushing the large analytes to the MS, whereas subsequently eluting non-volatile small molecules are diverted to waste (column regeneration).

*Please note the flow rate of NC pump is up to 50 µL/min with micro flow selector.



Sample	1 µg NIST mAb in PBS 1 µg BSA in PBS
LC setup	Vanquish Flex UHPLC–dual pump
LC flow rate	0.1 mL/min
LC buffer	200 mM ammonium Acetate
OBE column	NativePac OBE-1, 2.1 × 50 mm 3 µm, 80 Å
MS instrument	Q Exactive UHMR MS
Scan mode	HCD–Extended trapping 120 V
Resolution	12,500
Scan range	*m/z* 1,000–14,000



As the proteins used here are above the exclusion limit of the resin (approximately 6 kDa), all proteins elute from the column in the void volume which allows for the development of a single LC-MS method regardless of the size of the biomolecule. Elution time for an individual column remains constant.

Figure 11. OBE workflow example: Efficient removal of PBS buffer

Removal of non-volatiles

- A variety of buffers are used during protein expression and purification, based on the pH range, ionic strength, and chemical properties to stabilize the native structure of the biomolecule of interest.
  - Common buffers mimicking physiological conditions are phosphate-buffered saline (PBS), tris-buffered saline (TBS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer.[1]
- In addition, solution additives such as preservatives, metal chelators and cryoprotectants are often included to further stabilize and protect the biomolecule of interest.
  - Commonly used additives are glycerol, imidazole, and DMSO.

Note: For additional information, see this webinar:
Automating Native Mass Spectrometry through Online Buffer Exchange (OBE)

References

1. VanAernum, Z.L., Busch, F., Jones, B.J., Jia, M., Chen, Z., Boyken, S.E., Sahasrabuddhe, A., Baker, D. and Wysocki, V.H. Rapid online buffer exchange for screening of proteins, protein complexes and cell lysates by native mass spectrometry Nature Protocols 2020, volume 15, pages 1132-1157.
2. Liu, W., Zhang, T., Bechler, S. and Viner, R. Rapid Online Buffer Exchange for Protein Screening https://assets.thermofisher.com/TFS-Assets/MSD/Flyers/rapid-online-buffer-exchange-fl0170.pdf.

Learn more at **thermofisher.com/nativeMS-proteomics**