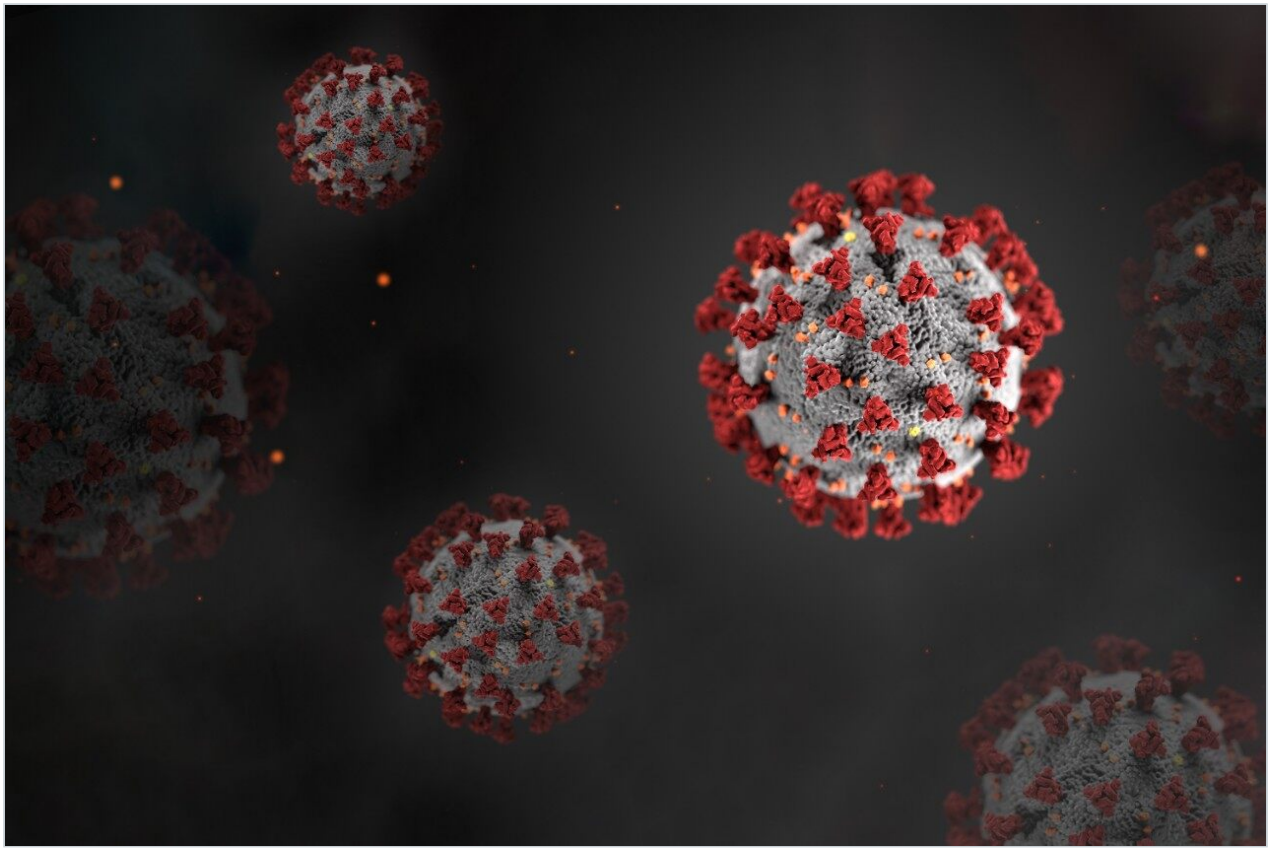
Waters™

Application Note

Comprehending COVID-19: Multiple Reaction Monitoring Transition Selection and Optimization Strategies for LC-MS Based SARS-CoV-2 Detection

Laurence Van Oudenhove, Nikunj Tanna, Jan Claereboudt, Hans Vissers, Bart Van Puyvelde, Simon Daled, Dieter Deforce, Katleen Van Uytfanghe, Maarten Dhaenens

GHENT UNIVERSITY



For research use only. Not for use in diagnostic procedures.

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The COVID-19 pandemic has resulted in the development of mass spectrometry based methods to characterize, identify, and quantify proteins. These methods are aimed at understanding the structural biology and interaction mechanisms of SARS-CoV-2, or as a complementary method to detect relevant markers. Targeted mass spectrometry, through the detection of viral peptides in proteolytically digested body fluids, has been suggested as a SARS-CoV-2 detection method.[1] The work presented here demonstrates application of the MassLynx Skyline Interface for automated peptide Multiple Reaction Monitoring selection and optimization with a Xevo TQ-XS Tandem Quadrupole Mass Spectrometer.

Benefits

- Automated generation and optimization of MRM transitions

- Surrogate peptide detection for SARS-CoV-2 protein detection and quantitation

Introduction

COVID-19 is an ongoing global pandemic caused by the SARS-CoV-2 virus. Efforts to overcome limitations in the current standard polymerase chain reaction (PCR) diagnostic testing capacity and associated reagent shortages have driven the quest for new diagnostics.[2,3] The SARS-CoV-2 virion is unusually protein rich, with Spike glycoprotein (SPIKE) and Nucleoprotein (NCAP) accounting for the majority of the protein complement. SPIKE recognizes human angiotensin-converting enzyme 2 in the initial stage of infection. NCAP is a structural component of the viral particle involved in replication and transcription of the genome.[4] The detection and quantification of SARS-CoV-2 proteins by a targeted LC-MS method is therefore being considered as an alternative method for COVID-19 viral load determination. As a result, LC-MS methods are currently under development as part of a community-based effort to develop a 'A Universally Adoptable Corona Multiple Reaction Monitoring Assay'.[5] Here, we have applied several complementary approaches for selecting and identifying surrogate peptides, including Multiple Reaction Monitoring (MRM) transitions, to detect and quantify SARS-CoV-2 proteins.

Experimental

Tryptic-Lys C peptides from a combined digestion procedure of recombinant SARS-CoV-2 SPIKE and NCAP proteins, as individual standards and spiked in Universal Transport Medium (UTM) matrix, respectively, were obtained in freeze-dried form from Cov-MS.[5] The resulting peptides were analyzed in MRM mode of analysis using an ACQUITY UPLC I-Class PLUS System interfaced to a Xevo TQ-XS Tandem Quadrupole Mass Spectrometer. Quantitative data analysis was conducted with TargetLynx and Skyline.[6]

LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS
Vials:	QuanRecovery Vials with MaxPeak HPS

Column(s):	ACQUITY PREMIER Peptide BEH C_{18} 300 Å, 2.1 mm x 50 mm, 1.7 µm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	5 µL
Flow rate:	0.6 mL/min
Mobile phase A:	0.1% formic acid in H_2O
Mobile phase B:	0.1% formic acid in acetonitrile

MS Conditions

MS system:	Xevo TQ-XS
Ionization mode:	ESI positive
Acquisition mode:	MRM
Capillary voltage:	0.5 kV
Collision energy:	peptide/transition optimized
Cone voltage:	35 V

Gradient

Time (min)	%B solvent
0.0	5

Time (min)	%B solvent
5.5	33
5.6	85
7.0	85
7.1	5
8.0	5

Data Management

Software	MassLynx
	TargetLynx
	MassLynx Skyline Interface
	Skyline

Results and Discussion

The NCAP amino acid sequence and coverage, as defined in the original Cov-MS standard operation procedure (SOP), is shown in Figure 1. Together with the primary amino acid sequence of the SPIKE protein, these form the basis for the MRM selection and optimization process. The MassLynx Skyline Interface (MSI) process was followed for automated optimization and fine tuning of a tandem quadrupole MRM method,[7] as well as the detection of any additional candidate signature peptides. In short, MSI conducts an automated four-step process whereby first the retention time of the peptides is determined. Next, the most sensitive precursor/product ion pairs (MRM transitions) for every peptide are determined using a default calculated collision induced dissociation (CID) fragmentation energy. This is followed by optimization of the individual transition collision energies. Finally, an MRM method with appropriate acquisition windows is created. As

shown on the left hand side of Figure 2, the appropriate peptide and transition settings are specified within a Skyline document. Next, as shown on the right hand side of Figure 2, the document is specified in MSI alongside with other methods files (tune page, acquisition method, LC gradient), sample position and injection volume.

Figure 1. Protein structure (https://swissmodel.expasy.org/interactive/UfqxZJ/models/03) and stepping quadrupole DIA based sequence coverage (blue highlight = DIA identified) map P0DTC9|NCAP_SARS2.

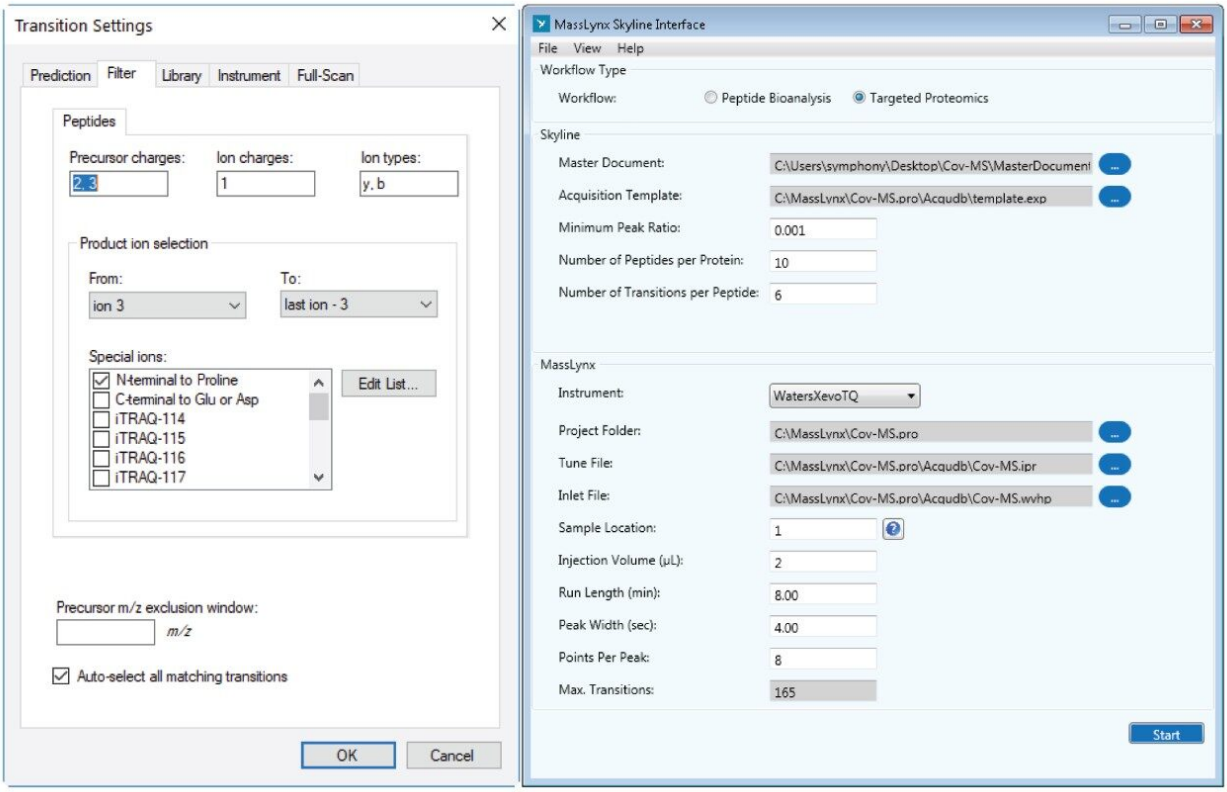


Figure 2. Master Skyline document Transition Settings (left) and Mass Skyline Interface (right).

The NCAP and SPIKE proteins were *in-silico* digested allowing for one sequence motif specific missed cleavage to optimize the MRM transition settings for the discovery identified peptides and potentially identify any additional relevant peptides. At this stage of the optimization process, the number of transitions per peptide was set at six to retain flexibility in transition selection during downstream acquisition and quantification experiments. The detected peptides are overviewed in Table 1. The result summarised in Table 1 overview (1) the peptides specified in the original Cov-MS SOP, identified using a discovery-based data-independent analysis (DIA) method and validated by means of MRM, (2) the SARS-CoV-2 peptides identified by the MSI process, and (3) the peptides that were retained in the final MRM method based on LC-MS response and suitability/specificity.

Peptide	Charge	Discovery/Cov-MS SOP*	MSI	Final MRM assay
SPIKE_SARS2				
GWIFGTTLDSK	2	✓	✓	✓
SFIEDLLFNK	2	✓	✓	✓
RSFIEDLLFNK	2	✓	✓	
LNDLCFTNVYADSFVIR	3		✓	
VYSTGSNVFQTR	2		✓	
HTPINLVR	2		✓	
IADYNYK	2		✓	
GVYYPDK	2		✓	
NCAP_SARS2				
GQQQQGQTVTK	2	✓		
LNQLESK	2	✓	✓	✓
ADETQALPQR	2	✓	✓	✓
EDLKFPR	2	✓	✓	
RGPEQTQGNFGDQELIR	2	✓	✓	✓
AYNVTQAFGR	2	✓	✓	✓
DQVILLNK	2	✓	✓	✓
GQGVPINTNSSPDDQIGYYR	2	✓	✓	
GQGVPINTNSSPDDQIGYYR	3	✓	✓	
KQQTVTLLPAADLDDFSK	3	✓	✓	✓
NPANNAAIVLQLPQGTTLPK	2	✓	✓	
NPANNAAIVLQLPQGTTLPK	3	✓	✓	✓
DGIIWVATEGALNTPK	2	✓	✓	
DGIIWVATEGALNTPK	3	✓	✓	✓
IGMEVTPSGTWLTYTGAIK	2	✓	✓	✓
HWPQIAQFAPSASAFFGMSR	2	✓	✓	
MAGNGGDAALALLLLDR	2	✓	✓	
MAGNGGDAALALLLLDR	3	✓	✓	
ITFGGPSDSTGSNQNGER	3		✓	
GFYAEGSR	2		✓	✓
QQTVTLLPAADLDDFSK	3		✓	
KADETQALPQR	3		✓	
LDDKDPNFK	3		✓	
MKDLSPR	3		✓	
HIDAYK	2		✓	✓

*underlined = peptide MRM method detected in UTM matrix according to Cov-MS SOP

Table 1. Candidate target peptides Cov-MS MRM method identified by a discovery method and MSI, as well

To illustrate the importance of using the correct number of transitions/fragment ions for quantitation, MRM

2. WHO Laboratory Testing for 2019 Novel Coronavirus (2019-nCoV) in Suspected Human Cases. Interim guidance. 2020 Jan 17. https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 <https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117>

3. N. Subbaraman. Coronavirus Tests: Researchers Chase New Diagnostics to Fight the Pandemic, *Nature*, 2020 Mar 23 . doi: 10.1038/d41586-020-00827-6.

   , 2020 Mar 23 . doi: 10.1038/d41586-020-00827-6.

4. JM Parks, JC Smith. How to Discover Antiviral Drugs Quickly. *N Engl J Med*. 2020 Jun 4;382(23):2261-2264. doi: 10.1056/NEJMcibr2007042.

5. M Dhaenens *et al*. http://genesis.ugent.be/uvpublicdata/Cov-MS_launch.mp4 < http://genesis.ugent.be/uvpublicdata/Cov-MS_launch.mp4> and https://www.youtube.com/watch?v=-yV8WJAr1Lc&t=2724s <https://www.youtube.com/watch?v=-yV8WJAr1Lc&t=2724s>

6. B MacLean, DM Tomazela, N Shulman, M Chambers, GL Finney, B Frewen, R Kern, DL Tabb, DC Liebler, MJ MacCoss. Bioinformatics, Skyline: An Open Source Document Editor for Creating and Analyzing Targeted Proteomics Experiments. 2010 Apr 1;26(7):966-8.

7. N Tanna, C Dunning, B Molloy. MassLynx-Skyline Interface (MSI): A New Automated Tool to Streamline MRM Method Development and Optimization for Large Molecule Quantification (720006813EN < https://www.waters.com/waters/library.htm?cid=511436&lid=135053941&locale=en_MY>).

Acknowledgements

The Cov-MS consortium is kindly acknowledged for making evaluation kits available as part of a community-based effort to design a SARS-Cov-2 MRM method.

Laurence Van Oudenhove, Nikunj Tanna, Jan Claereboudt and Hans Vissers (Waters Corporation); Bart Van Puyvelde, Simon Daled, Dieter Deforce and Maarten Dhaenens (Pharmaceutical Biotechnology, University of Ghent); Katleen Van Uytfanghe (Department of Bioanalysis, University of Ghent).

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Xevo TQ-XS Triple Quadrupole Mass Spectrometry <https://www.waters.com/134889751>

MassLynx MS Software <https://www.waters.com/513662>

MassLynx Skyline Interface <https://www.waters.com/135058435>

720006967, August 2020