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Nota de aplicación

Comprehending COVID-19: Maximizing LC-MS Detection Dynamic Range for Multiple Reaction Monitoring Based SARS-CoV-2 Analysis

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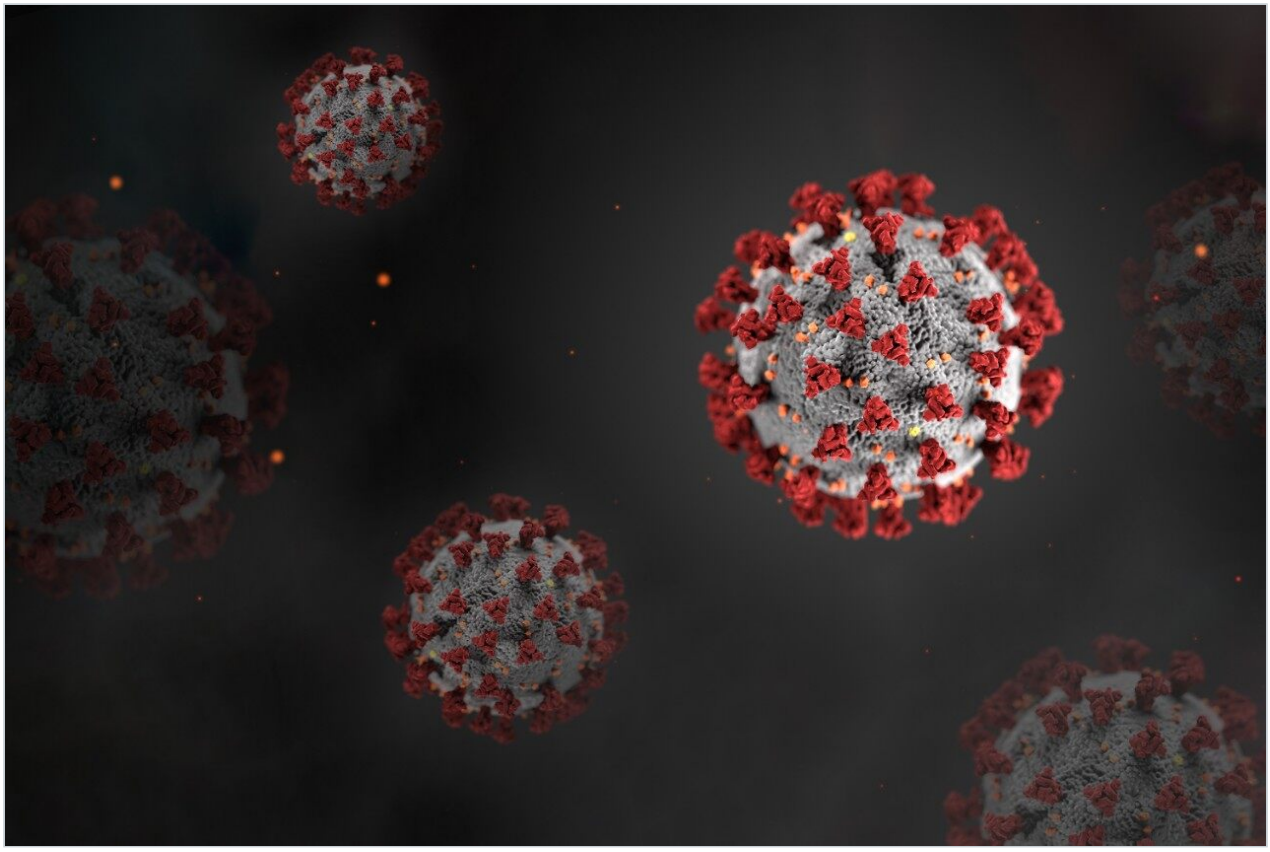

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Targeted MS-based methods show promise to specifically detect SARS-CoV-2 proteins from various types of biological matrices of COVID-19 patients. Here, the combination of selected chemistries and consumables, using the ACQUITY UPLC I-Class PLUS System and Xevo TQ-XS Tandem Quadrupole Mass Spectrometer, are demonstrated for the detection and quantification of SARS-CoV-2 proteins in nasopharyngeal swabs preserved in Universal Transport Medium. A linear response was achieved across five amount levels for recombinant Spike glycoprotein and Nucleoprotein, using at least two transitions per peptide, whilst maintaining good measurement reproducibility across the detection dynamic range.

Benefits

- Maximal quantitation dynamic range through optimized consumable and MRM selection
- Surrogate peptide-based quantitation of SARS-CoV-2 proteins in nasopharyngeal swabs

Introduction

COVID-19 is an ongoing global pandemic caused by the SARS-CoV-2 virus. This outbreak has prompted the development of high-throughput targeted proteomics methods to detect SARS-CoV-2 proteins directly from clinical respiratory tract samples, such as nasopharyngeal swabs and gargle solutions, as an alternative or complement to polymerase chain reaction (PCR) based testing methods. A number of different viral proteins are present in a varying copy number per viral particle.[1,2] Recombinant Spike glycoprotein (SPIKE) and Nucleoprotein (NCAP), the two major protein constituents of the SARS-CoV-2 virion, both responsible, like other corona viruses[3], for an increased % of protein viral particle mass, were digested and spiked into nasopharyngeal swabs from healthy patient samples and preserved in Universal Transport Medium (UTM), quantified via a surrogate peptide-based approach, which was developed as part of a community-based effort to develop 'A Universally Adoptable Corona Multiple Reaction Monitoring Assay'. [4]

Experimental

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 Column 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
5.5	33
5.6	85
7.0	85
7.1	5
8.0	5

Data Management

Software:	MassLynx
	TargetLynx
	Skyline

Results and Discussion

The throughput and chromatographic performance of the developed Multiple Reaction Monitoring (MRM) method described in Comprehending COVID-19: Multiple Reaction Monitoring Transition Selection and Optimization Strategies for LC-MS Based SARS-CoV-2 Detection (720006967EN < https://www.waters.com/nextgen/us/en/library/application-notes/2020/%20comprehending-covid-19-multiple-reaction-monitoring-transition-selection-and-optimization-strategies-for-lc-ms-based-sars-cov-2-detection.html>), using two transitions per peptide in order to maximize duty cycle and signal-to-noise, are exemplified in Figure 1, illustrating the separation and elution of the target peptides of SPIKE_SARS2 and NCAP_SARS2 within a chromatographic window of 5.5 min. Typical baseline peak widths were 4-6 seconds and the total LC-MS MRM method cycle time, including injection, was less than 9 min.

SFIEDLLFNK
NPANNAAIVLQLPQGTTLPK
100
%

MRM chromatograms for the quantitative analysis of P0DTC2|SPIKE_SARS2 and P0DTC9|NCAP_SARS2. The quantitative response of the two-colored labeled MRM chromatograms is detailed in Figures 3 and 4.

The quantitative response was maximized by careful selection of consumables, *i.e.* vials/SPE collection plates and column chemistries, as well as resuspension solvent composition. The cumulative effect of these components is demonstrated in the bottom half of Figure 2, illustrating the average base level MRM response obtained with the original Cov-MS standard operation procedure (SOP), and the additional signal obtained from vial selection (+15%), resuspension solvent composition (+20%), and column chemistry (+10%), providing an overall gain in signal of about ~55%. Shown in the top half of Figure 2 are example % CV transition values for the various spike levels suggesting good method reproducibility and robustness.

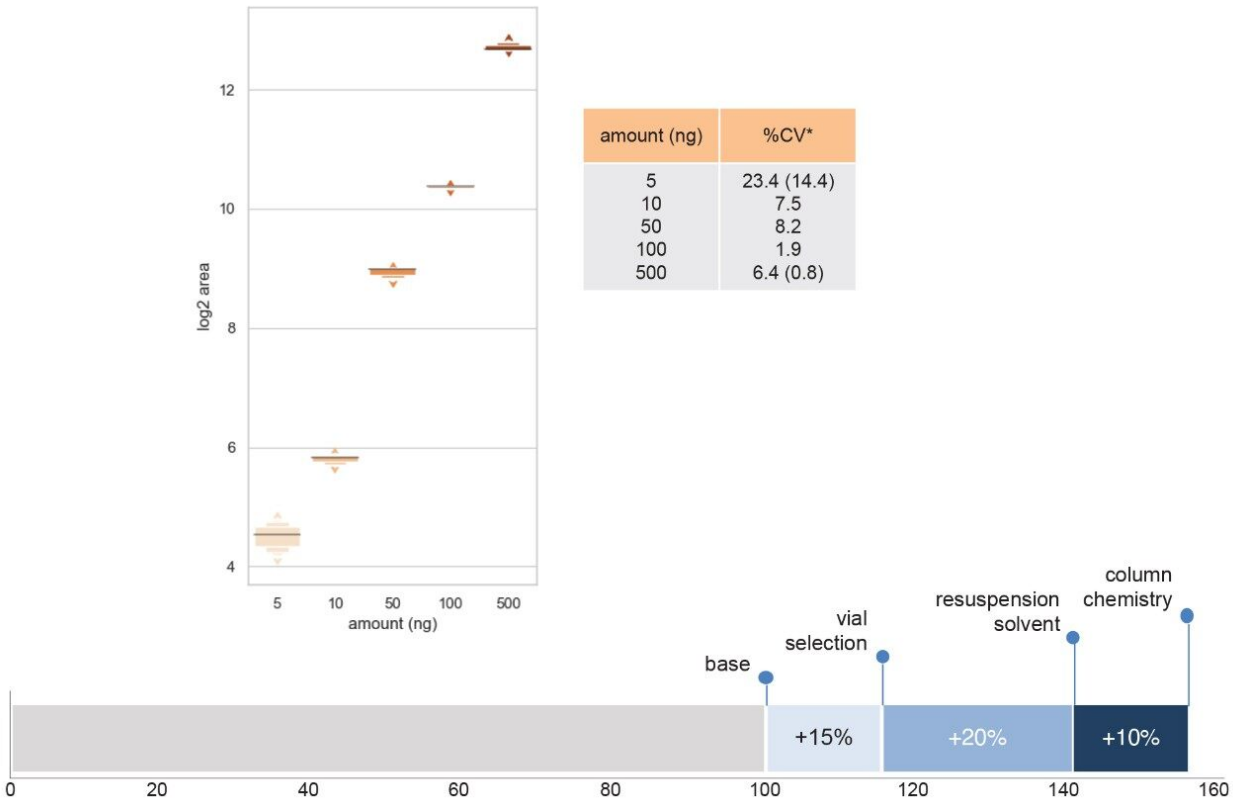

*Figure 2. Incremental MRM signal increase resulting from the application of particular consumables and/or solvents for peptide analysis (bottom) and average % CV MRM reproducibility as a function of spike level amount for SFIEDLLFNK from P0DTC2|SPIKE_SARS2 (top; * = outlier data excluded). Samples were prepared according to the Cov-MS SOP[4], stating that the recombinant SARS2 proteins were originally dissolved in 250 µL UTM matrix and had to be resuspended in 50 µL solvent and 5 µL injected.*

The quantitative figures of merit for two peptides, SFIEDLLFNK from P0DTC2|SPIKE_SARS2 (top) and NPANNAAIVLQLPQGTTLPK from P0DTC9|NCAP_SARS2, both covering five SARS-CoV-2 protein digest spike levels in UTM matrix, are shown in Figure 3, demonstrating good linearity with R^2 values of at least 0.998 and residual values smaller than 15%. Detection of NPANNAAIVLQLPQGTTLPK could be extended by another sample spike level (1 ng, equating to 0.088 fmol NCAP/µL UTM matrix solution, assuming equal amounts of recombinant NCAP and SPIKE present in UTM matrix) using a single MRM transition, but this was not investigated further. Significantly lower limits of detection (LLODs) were observed in the absence of UTM matrix, suggesting that the dynamic range of the method is predominantly limited by the matrix, advocating the use of alternative swab types and/or the development of more efficient clean-up/enrichment techniques to enhance the analytical method further.

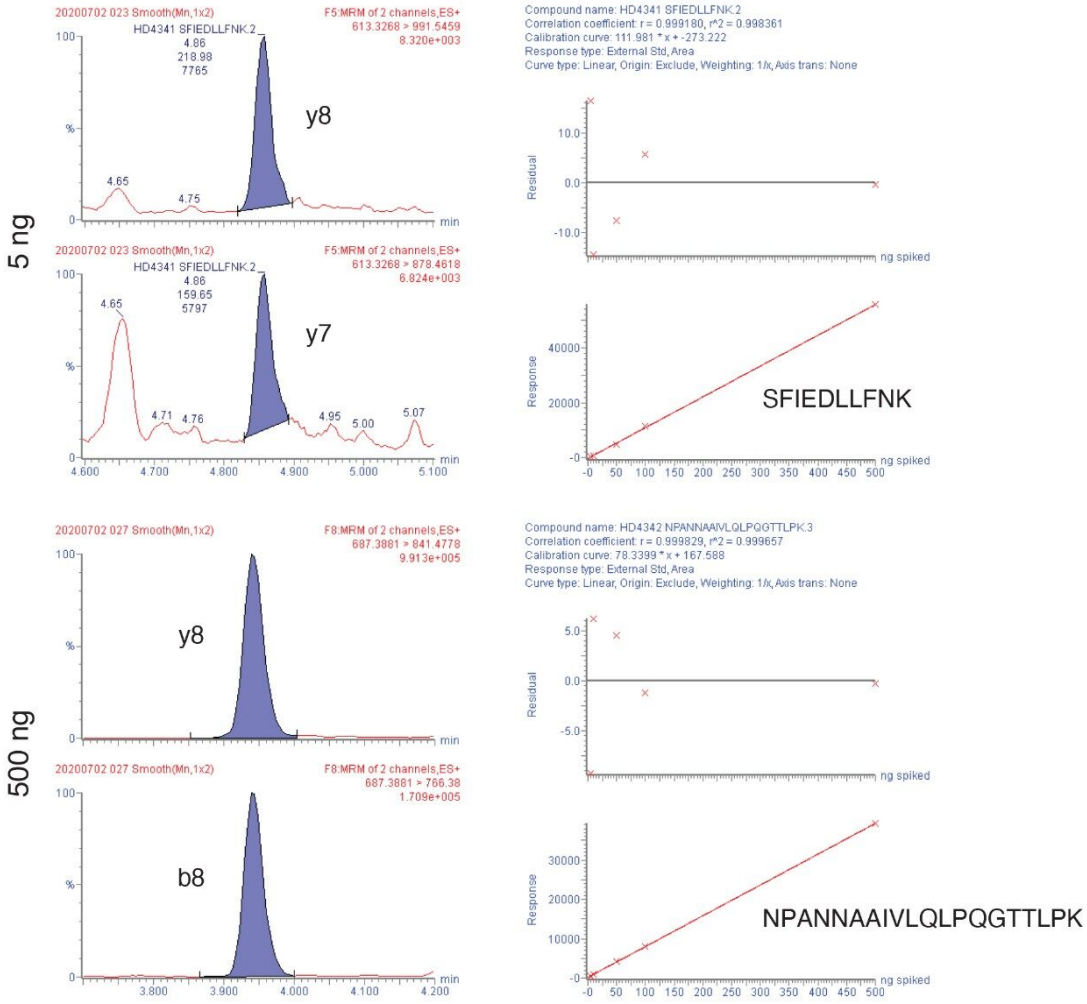

Figure 3. Quantitative analysis of SFIEDLLFNK from P0DTC2|SPIKE_SARS2 (top) and NPANNAAIVLQLPQGTTLPK from P0DTC9|NCAP_SARS2, demonstrating good linear response across 5 amount levels for both target proteins using at least two transitions per peptide. MRM chromatograms are shown for the individual transitions for the lowest spike level for SFIEDLLFNK (5 ng) and the highest spike level (500 ng) for NPANNAAIVLQLPQGTTLPK, respectively.

A semi-quantitative summary of the results is shown in Figure 4, illustrating that all target peptides from the final MRM method could be detected in at least three spike levels. The average number of spike levels detected across all peptides for both proteins was four, with some peptides being identified across five amounts levels. Both proteins, SPIKE_SARS2 and NCAP_SARS2 were identified at the 5 ng sample spike level when present in UTM matrix.

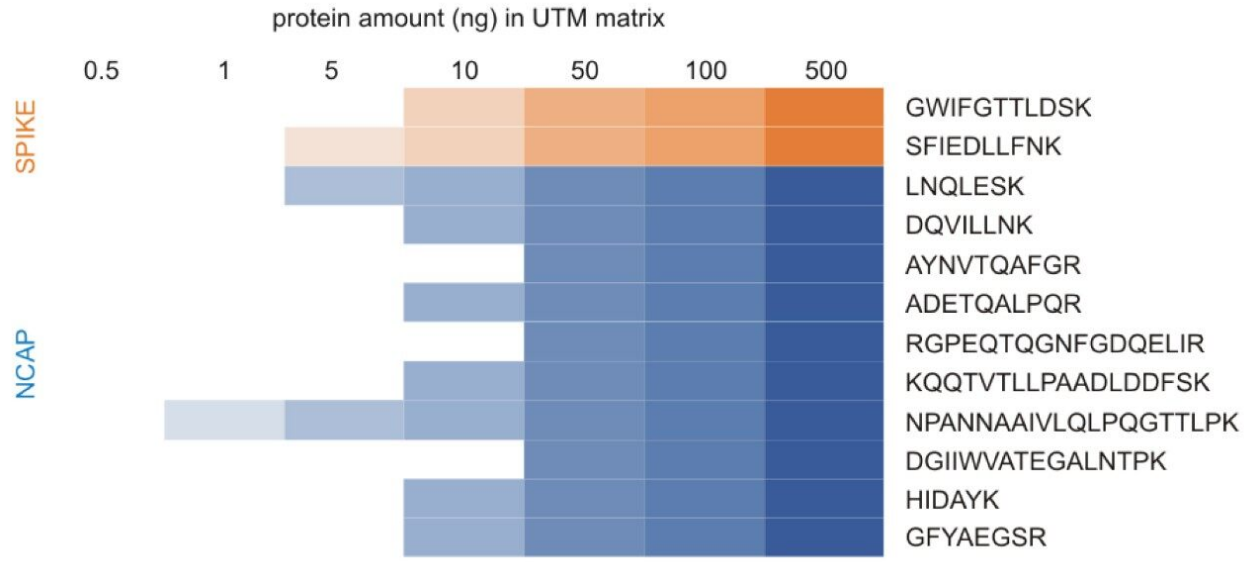

Figure 4. Quantitative results summary of the detection of SPIKE_SARS2 (orange) and NCAP_SARS2 (blue) in UTM matrix, illustrating an average coverage across four spike levels of recombinant SARS-CoV-2 proteins.

Conclusion

The clinical research and development of LC-MS-based COVID-19 MRM-based experiments require thorough analytical characterization of methods. Critical LC-MS specific aspects include specificity, quantitation dynamic range and method robustness/reproducibility. The analytical challenges associated with the novel coronavirus have been met by the careful selection of LC-MS consumables, resulting in an optimized linear dynamic range, reduced non-specific binding, whilst maintaining method throughput, and the application of specific MRM transitions, affording reproducible MRM measurements and improved LLOD. The developed MRM method was successfully applied and evaluated on a Xevo TQ-XS Tandem Quadrupole Mass Spectrometer to detect and quantify SARS-CoV-2 proteins in nasopharyngeal swabs and preserved in Universal Transport Medium.

References

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

2. K Bezstarosti, MM. Lamers, BL Haagmans, JAA Demmers. Targeted Proteomics for the Detection of SARS-CoV-2 Proteins, https://www.biorxiv.org/content/10.1101/2020.04.23.057810v1 < https://www.biorxiv.org/content/10.1101/2020.04.23.057810v1>

3. M Surjit and SK. Lal. "The Nucleocapsid Protein of the SARS Coronavirus: Structure, Function and Therapeutic Potential." Molecular Biology of the SARS-Coronavirus 129–151. 22 Jul. 2009.

4. 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>

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