

Comprehending COVID-19: Application of UniSpray and Electrospray Ionization for the Detection of Proteolytic Digested SARS-CoV-2 Proteins

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

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Abstract

The COVID-19 outbreak has prompted the development of LC-MS based methods to detect viral infection and load as a complement to current tests to determine infection. Targeted mass spectrometry, through the detection of viral proteins in proteolytically digested body fluids, has been suggested as an additional SARS-CoV-2 test method. The work presented here demonstrates the application of UniSpray and Electrospray ionization sources for the detection of selected SARS-CoV-2 tryptic peptides with a Xevo TQ-XS Tandem Quadrupole Mass Spectrometer.

Benefits

- Complementary nature of UniSpray and Electrospray for surrogate peptide-based quantitation of SARS-CoV-2 proteins in nasopharyngeal swabs
- Optimizing quantitation dynamic range through use of a selected peptides that benefit from improved ionization or selectivity characteristics using an UniSpray ionization interface

Introduction

COVID-19 coronavirus disease is a highly pathogenic viral infection caused by SARS-CoV-2 and responsible for the current on-going pandemic. SARS-CoV-2 virus particles are protein rich, with Spike glycoprotein (SPIKE) and Nucleoprotein (NCAP) being the two main constituents. The detection and quantification of SARS-CoV-2 proteins by a targeted LC-MS method is being considered as an alternative technology for COVID-19 viral load determination to complement polymerase chain reaction (PCR) based testing.^{1,2} Therefore, a community-based effort to develop a 'A Universally Adoptable Corona Multiple Reaction Monitoring Assay' was initiated.³ Here, we have compared a novel ionization technique UniSpray with Electrospray ionization to further optimize the LC-MS detection method (720006967 <<https://www.waters.com/nextgen/us/en/library/application-notes/2020/comprehending-covid-19-multiple-reaction-monitoring-transition-selection-and-optimization-strategies-for-lc-ms-based-sars-cov-2-detection.html>> and 720006968 <<https://www.waters.com/nextgen/us/en/library/application-notes/2020/comprehending-covid-19-maximizing-lc-ms-detection-dynamic-range-for-multiple-reaction-monitoring-based-sars-cov-2-analysis.html>>) for selected

SPIKE and NCAP tryptic peptides and investigate the potential increase in dynamic range and selectivity of this LC-MS method.

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.³ 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.

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 ₂ O
Mobile phase B:	0.1% formic acid in acetonitrile

MS Conditions

MS system:	Xevo TQ-XS
Ionization mode:	UniSpray and ESI positive
Acquisition mode:	MRM
Capillary voltage (ESI+):	0.5 kV
Impactor voltage (US+):	0.7 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

Results and Discussion

The developed Multiple Reaction Monitoring (MRM) method described in *Comprehending COVID-19: Maximizing LC-MS Detection Dynamic Range for Multiple Reaction Monitoring Based SARS-CoV-2 Analysis* ([720006968](#)) as part of a community based effort in collaboration with the Cov-MS consortium was applied in this study as well.³ It uses two transitions per peptide in order to maximize duty cycle and signal-to-noise, as well as regular LC methods and conditions in order to maintain robustness and throughput. Both UniSpray and Electrospray ionization interfaces were applied, and the same dilution series samples analyzed as described in the original Cov-MS standard operation procedure (SOP).

The chromatograms shown in Figure 1 are typical in that the application of UniSpray results in higher signal intensities compared to Electrospray. However, in this example, UniSpray also provided improved S/N compared to its Electrospray counterpart. The S/N gain obtained for this peptide at this amount of protein digest injected on-column was approximately two-and-a-half, which is in agreement with previously reported performance metrics for peptides.⁴ The benefit of improved S/N is shown in Figure 2, where the quantitative response of peptide ADETQALPQR from P0DTC9|NCAP_SARS2 obtained with UniSpray and Electrospray interfaces is demonstrated. Peptide ADETQALPQR was previously identified as one of the medium-to-better responding peptides, covering four amounts levels injected on-column. With this peptide and matrix, using UniSpray, an additional amount level could be reached without compromising quantitative performance in terms of residuals and linear regression.

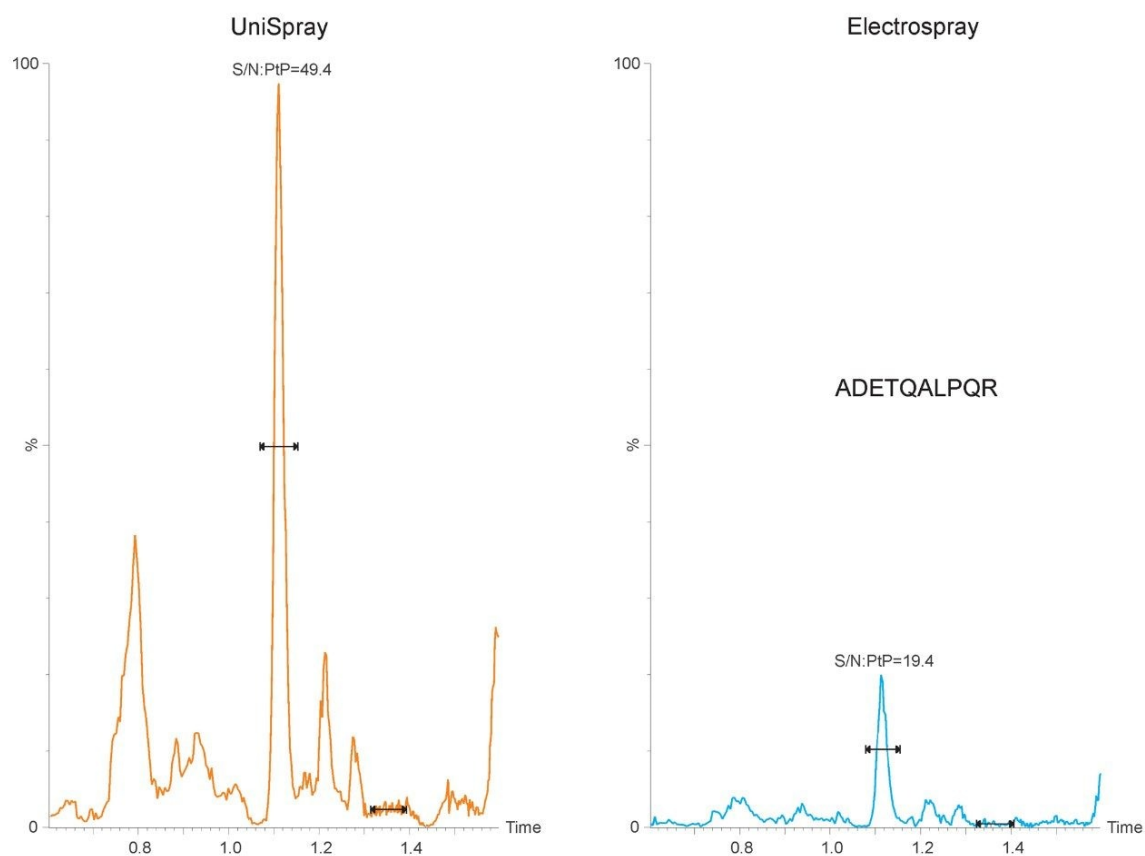


Figure 1. Summed transition (564.8 > 400.2 and 564.8 > 584.4) MRM chromatograms for NCAP peptide ADETQALPQR using UniSpray (left) and Electrospray (right) LC-MS interfaces. Relative response (%) axis are scaled to the same relative level (UniSpray).

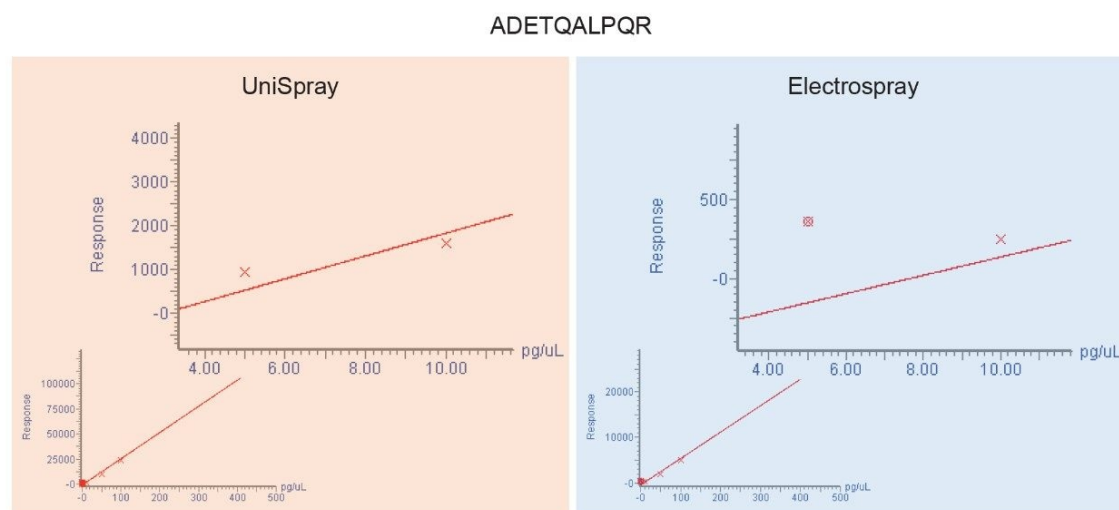


Figure 2. Quantitative analysis of ADETQALPQR from P0DTC9|NCAP_SARS2 using UniSpray (left) and Electrospray (right) interfaces, demonstrating good linear response across at least four amount levels for the NCAP target protein using two transitions per peptide. Shown inset are the two lowest amount levels used with the quantitation curve (lowest level, as indicated by the circle, was not used in the case of Electrospray in order to main adequate analytical quantitative performance).

A comparative results summary is provided in Figure 3, contrasting the peak S/N and area of all MRM chromatograms for the peptides from the NCAP and SPIKE SARS-Cov-2 proteins specified in the Cov-MS SOP, illustrating the complementary nature of the UniSpray and Electrospray interfaces. An average S/N ratio and range was calculated for each peptide by expressing S/N for a given peptide at a particular amount level and contrasting it against the S/N for the same peptide at the same amount level. Next, an average and confidence interval of all S/N ratio values for all amount levels at which the peptide was detected was expressed as illustrated by the quartile distributions shown in the left-hand side set of ratio values of Figure 3. On average, about three peptides showed improved S/N using UniSpray and a similar number of peptides showing improved S/N using Electrospray. However, the ability to have the option to select or use an alternative technology can be critical in being able to detect more confidently particular SARS-Cov-2 peptides in a given matrix, *i.e.* nasopharyngeal swabs, saliva, gargle solution, *etc.* The right-hand side set of distributions, shown in Figure 3, demonstrate the peak area ratio for both ionization techniques, confirming the observation illustrated in Figure 2 and previous studies that the application of the UniSpray interface provided overall larger peak areas, *i.e.* an average 4-fold increase compared to Electrospray, providing better ion statistics, thus potentially improved

reproducibility at lower data signal levels (data not shown).⁴

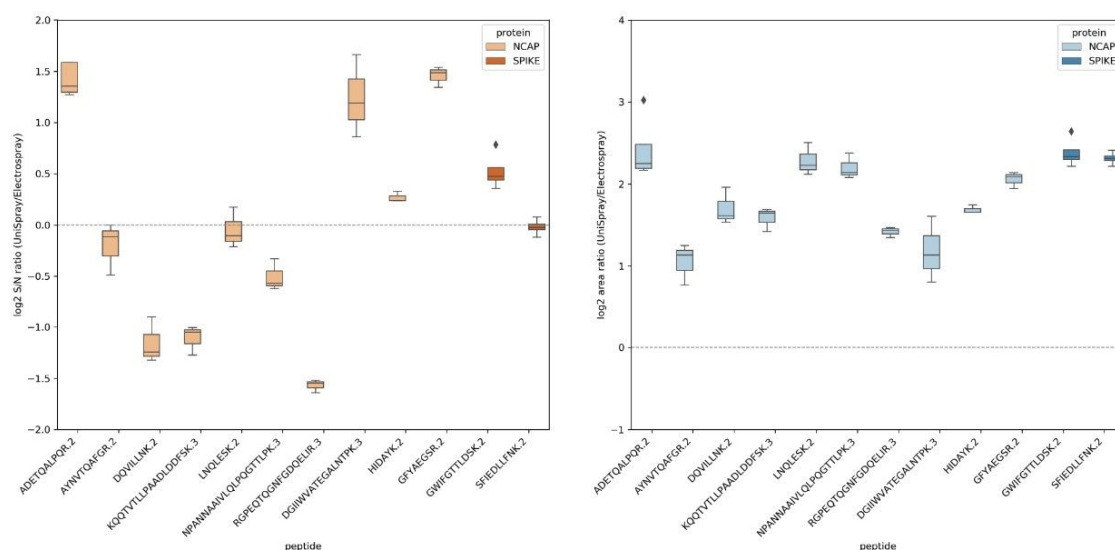


Figure 3. Relative response UniSpray and Electrospray showing the median \log_2 normalized ratio of the average of the peak S/N ratio (left) and the peak area ratio (right) values of UniSpray vs. Electrospray MRM LC-MS analyses of recombinant NCAP and SPIKE protein digests in UTM matrix injected on-column for the peptides specified in the original CovMS SOP.

Conclusion

Since the NCAP and SPIKE proteins are key components of the viral SARS-CoV-2 complement and a direct measure of viral load, LC-MS based technologies are being considered to determine their amounts and concentration in biological matrices. Here, the linear response and LLOD of a number of tryptic NCAP and SPIKE peptides detected in UTM matrix have been investigated to understand the relative response of UniSpray and Electrospray ionization sources and to further characterize the analytical MRM method under development by the Cov-MS consortium. The obtained results indicate that UniSpray and Electrospray are complementary ionization methods for proteolytic peptides and that the developed Xevo TQ-XS Tandem Quadrupole Mass Spectrometer method to detect and quantify SARS-CoV-2 proteins in nasopharyngeal swabs and preserved in

Universal Transport Medium could potentially be further enhanced using a subset of peptides.

References

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