Including peptide enrichment in a mass spectrometry-based workflow for the absolute quantitation of SARS-CoV-2

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ABSTRACT

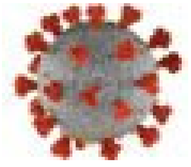
Purpose: Demonstrate that peptide enrichment can enhance a workflow for the absolute quantitation of SARS-CoV-2 by LC-MS.

Methods: Recombinant SARS-CoV-2 nucleocapsid proteins were spiked into nasal fluids and digested to generate peptides. The samples were then enriched to reduce the background and create ultra-pure samples containing target peptides.

Results: LODs and LOQs were demonstrated to be in the low/sub-femtomole on column range. In addition, the increased purity of the samples allowed quicker 2-minute LC-MS run-times.

INTRODUCTION

SARS-CoV-2 is a highly infectious virus that has resulted in over 4 million deaths.1 Containing the virus has only had limited success, partly due to its spread by asymptomatic carriers, emphasizing the need for widespread testing.



P0DTC2 – Spike
P0DTC4 – Envelope
P0DTC6 – Membrane
P0DTC9 – Nucleocapsid

Figure 1. The SARS-CoV-2 viral particle contains numerous copies of each protein

SARS-CoV-2 particles contain numerous proteins that are biomarkers of a COVID-19 infection (Figure 1). One approach capable of detecting such proteins is bottom-up mass spectrometry (MS). Proteolytic digestion of proteins generates peptides, which can be separated by liquid chromatography (LC). A workflow had previously been developed that achieved low on-column detection limits for spiked nasal fluid samples in viral transport media (VTM). Addition of a peptide enrichment step, such as Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA), could increase sample purity. This may reduce background, thereby improving detection limits and reducing LC run-times. Furthermore, SISCAPA could remove any need for sample clean-up and allow the concentration of samples, meaning lower quantities of nucleocapsid protein in nasal fluids could be detected.

MATERIALS AND METHODS

Sample Preparation

A mass spectrometry-based SARS-CoV-2 absolute peptide quantification method was developed using a Thermo Scientific™ Vanquish™ MD HPLC system and a Thermo Scientific™ TSQ Altis™ MD MS. Recombinant SARS-CoV-2 proteins and stable isotope-labeled standards (SISs) were spiked into pooled nasal fluids, before being added to VTM. Samples were then precipitated, centrifuged and enzymatically digested (Thermo Scientific™ SMART Digest™ Trypsin kit). The resulting peptides were enriched with peptide-specific SISCAPA antibodies.

Test Method(s)

Enriched peptides were separated using a 2-minute LC gradient with a Thermo Scientific™ Hypersil GOLD™ C18 column (1.9 mm, 2.1 x 50 mm), coupled with a selected reaction monitoring (SRM) method (Table 1).

Data Analysis

All assays were performed in triplicate and data was analyzed using Thermo Scientific™ TraceFinder™ LDT software.

RESULTS

Absolute quantitation of the targeted SARS-CoV-2 nucleocapsid protein was performed by including corresponding isotope-labeled standards for each peptide to mitigate measurement uncertainty, confirm retention times and correct for any possible matrix effects. Low/sub-femtomole on column detection and quantification was observed for each target peptide, demonstrating the viability of enriching SARS-CoV-2 peptides for detection by LC-MS. The acquisition of calibration curves (Figure 2) demonstrated strong linearity ($R^2 > 0.99$) across analytical measurement ranges between 0.25 and 100 femtomole on column (Table 2). Coefficients of variation and relative standard deviations of less than 15% were achieved for each quantifiable peptide at each concentration within the AMR. Clear chromatographic separation was observed for each of the peptides (Figure 3) and minimal variance in retention times were observed for all peptides in each acquired sample and their corresponding SISs (± 0.01 minutes).

Table 1. SRM conditions and retention times for five peptides targeted by peptide enrichment / LC-MS

Peptide Sequence	Peptide / SIS Mass (Da)	Retention Time (minutes)	Q1 (Da)	Q3 (Da)	CE (V)
KADETQALPQR	1257 1267	1.17	419.6	400.2 673.3 744.4	12
ADETQALPQR	1129 1139	1.19	564.8	400.2 513.3 584.4	20
AYNVTQAFGR	1127 1137	1.25	563.8	679.4 778.4 892.5	20
NPANNAAIVLQL PQGTTLPK	2060 2068	1.30	1030.6	841.5 1082.6 1195.7	33
DGIIWVATEGAL NTPK	1685 1693	1.33	842.9	1001.5 1100.6 1286.7	24

Table 2. Limits of detection (LODs), quantitation (LOQs) and corresponding R^2-values for each targeted peptides. Data was collected in triplicate

Peptide Sequence	LOD	LOQ	R^2
KADETQALPQR	1.0	2.5	0.99
ADETQALPQR	0.50	1.0	0.99
AYNVTQAFGR	0.25	0.25	0.99
NPANNAAIVLQLPQGTTLPK	10	N/A	N/A
DGIIWVATEGALNTPK	10	N/A	N/A

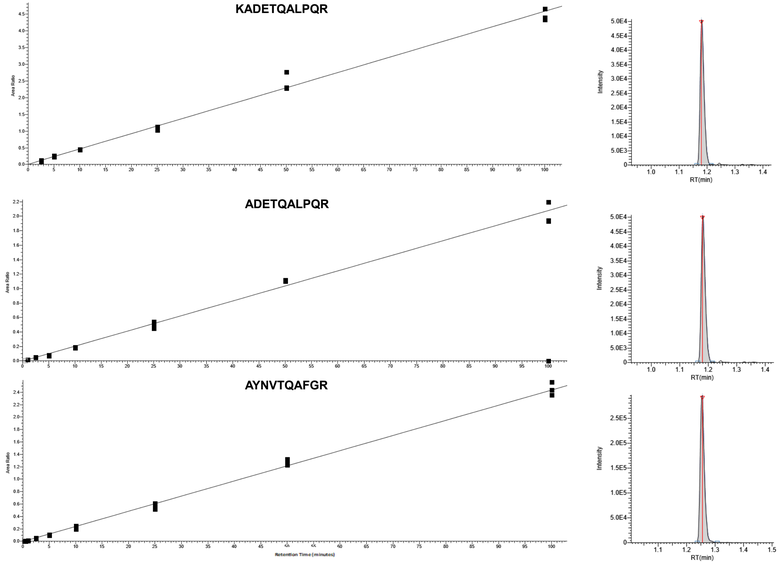


Figure 2. Spectra and calibration curves for targeted peptides from nasal fluid samples

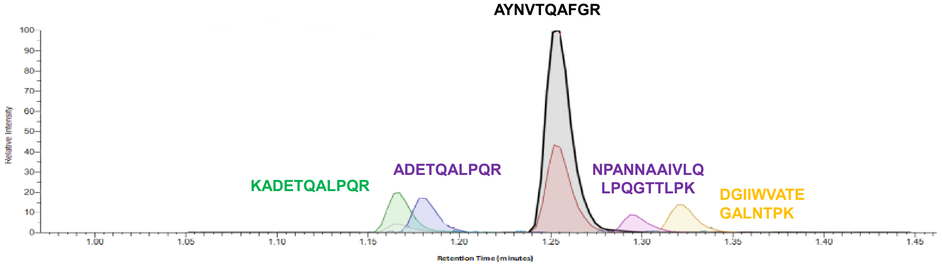


Figure 3. A chromatographic trace demonstrating the separation of the targetted peptides

CONCLUSIONS

Incorporation of peptide enrichment into an existing LC-MS assay has improved the ability to accurately quantify SARS-CoV-2 proteins in nasal fluids by decreasing the required quantity of spiked protein in nasal fluid samples, as well as facilitating 2-minute LC run-times. Moreover, each peptide was detected within a 30-second acquisition window, meaning a multichannel LC system could be capable of further increasing throughput.

Figure 4. Thermo Scientific Vanquish MD HPLC and TSQ Altis MD mass spectrometer

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2. He, J. et al. Diagnostic Performance Between CT and Initial Real-Time RT-PCR for Clinically Suspected 2019 Coronavirus Disease (COVID-19) Patients Outside Wuhan, China. *Respir Med.* **2020**, *168* (105980).

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