



A rapid and reliable liquid chromatography/mass spectrometry method for SARS-CoV-2 analysis from gargle solutions and saliva

Marc Kipping^{1,2} · Dirk Tänzler^{1,2} · Andrea Sinz^{1,2}

Received: 28 May 2021 / Revised: 15 July 2021 / Accepted: 9 August 2021 / Published online: 24 August 2021
© The Author(s) 2021

Abstract

We describe a rapid liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the direct detection and quantitation of SARS-CoV-2 nucleoprotein in gargle solutions and saliva. The method is based on a multiple-reaction monitoring (MRM) mass spectrometry approach with a total cycle time of 5 min per analysis and allows the detection and accurate quantitation of SARS-CoV-2 nucleoprotein as low as 500 amol/μL. We improved the sample preparation protocol of our recent piloting SARS-CoV-2 LC-MS study regarding sensitivity, reproducibility, and compatibility with a complementary reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) analysis of the same sample. The aim of this work is to promote diagnostic tools that allow identifying and monitoring SARS-CoV-2 infections by LC-MS/MS methods in a routine clinical environment.

Keywords Liquid chromatography · Mass spectrometry · Multiple-reaction monitoring · Triple quadrupole · SARS-CoV-2

Introduction

The COVID-19 pandemic is one of the biggest challenges of our times. During the past 1.5 years, we have seen a rapid spreading of the disease with increasing numbers of infections and fatalities. The numbers are so dynamic that it is difficult to pencil them in exact counts as are they are obsolete within hours. To date, there have been more than 140 million of SARS-CoV-2 infections and close to 3 million of fatalities worldwide [1], with ~120 million of infected individuals having recovered and ~ 750 million of vaccine doses administered.¹ An infection with SARS-CoV-2 does not necessarily lead to serious health problems; often, the patients do not experience any symptoms. This is one of the greatest challenges when dealing with SARS-CoV-2, making it one of

the most contagious and successfully spreading viruses. Therefore, reliable diagnostic tools allowing a rapid detection of SARS-CoV-2 are of enormous importance. Currently, the gold standard for SARS-CoV-2 diagnostics is reverse transcription quantitative polymerase chain reaction (RT-qPCR). Despite its strengths, RT-qPCR also suffers a number of drawbacks [2]. At the beginning of the COVID-19 pandemic in early 2020, the biggest challenge was the availability of high-throughput tools to diagnose a SARS-CoV-2 infection. This has been successfully addressed by the development of cost-efficient, ready-to-use antigen detection tests, which have become broadly available [3]. Another important issue that needs to be dealt with is the occurrence of SARS-CoV-2 mutations that have to be detected in routine diagnostics [4].

Mass spectrometry (MS) has already been spotted in April 2020 as a valuable complementary method for SARS-CoV-2 diagnostics from gargle solutions of COVID-19 patients [5]. In the meantime, the potential of MS has become evident [6] and numerous reports on SARS-CoV-2 identification by MS methods have been published since the beginning of the pandemic [7–11]. In the present work, we describe our latest efforts in developing a rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method for SARS-CoV-2 diagnostics from gargle solutions and saliva. We report an MS-compatible sample preparation strategy and a

✉ Marc Kipping
marc.kipping@pharmazie.uni-halle.de

✉ Andrea Sinz
andrea.sinz@pharmazie.uni-halle.de

¹ Department of Pharmaceutical Chemistry & Bioanalytics, Institute of Pharmacy, Kurt-Mothes-Str. 3, 06120 Halle (Saale), Germany

² Center for Structural Mass Spectrometry, Kurt-Mothes-Str. 3, 06120 Halle (Saale), Germany

quantification protocol that is based on selected peptides from SARS-CoV-2 nucleoprotein by an LC-multiple-reaction monitoring (MRM)-MS/MS approach.

Experimental section

Sample collection and preparation

For LC-MS/MS method development, two sample types—gargle solutions and saliva—were employed. Gargle solutions were collected from six healthy individuals by gargling with 20 mL of isotonic (0.9% (w/v)) NaCl solution for 30 s. Saliva samples were directly collected from three healthy individuals by spitting ~1 mL of saliva into a 50-mL tube. In initial experiments, our original sample preparation procedure [5] was employed, adding 1 mL of acetone (−20 °C) to 750 µL of gargle solution. After storing the samples at −20 °C overnight, samples were centrifuged (14,000g, 10 min), and the protein pellets were digested using the SMART Digest (Thermo Fisher Scientific) protocol with immobilized trypsin beads. In further experiments, we optimized sample preparation in respect to shorter preparation times, higher sensitivity, and improved compatibility with RT-qPCR by using TRIZOL reagent (100 mL of solution contain 9.5 g guanidinium thiocyanate, 3.1 g ammonium thiocyanate, 3.5 mL 3 M sodium acetate, 5 g glycerol, 48 mL Roti Aqua-Phenol (Roth)) for protein denaturation. A total of 500 µL of TRIZOL reagent was added to 100 µL of saliva and the solutions were loaded on centrifugation filter units (Amicon, 30 kDa molecular weight cutoff, Millipore). Afterwards, the filter units were treated according to the following filter-aided sample preparation (FASP) protocol: filter units were washed twice with 500 µL of 50 mmol/L ammonium bicarbonate, followed by centrifugation (14,000g, 10 min) and incubated at 55 °C for 2 h with trypsin (Promega) (1 µg in 50 µL of 50 mmol/L ammonium bicarbonate). Tryptic peptides were collected by adding 25 µL of 0.5 M NaCl, followed by a centrifugation step (14,000g, 10 min). After adding 2.5 µL of 10% (v/v) trifluoroacetic acid (TFA), sample volumes were adjusted to 70 µL, before the samples were subjected to LC-MS/MS analysis.

Liquid chromatography/tandem mass spectrometry (LC-MS/MS)

Four selected synthetic peptides derived from SARS-CoV-2 nucleoprotein (Arg41-Lsy61, Arg41-Lys65, Met210-Arg226, and Met210-Lys233) and their heavy isotope (¹³C and ¹⁵N)-labeled variants (Arg41-Lsy*61, Arg41-Lys*65, Met210-Arg*226, and Met210-Lys*233, SpikeTide-TQL peptides, where * denotes [¹³C₆¹⁵N₄]-Arg or [¹³C₆¹⁵N₂]-Lys) were purchased from JPT Peptide

Technologies and used for method development and as quantitation standards. LC separation of peptides was performed on a UPLC I-Class FTN system (Waters) equipped with a BEH C18 column (2.1 mm × 50 mm, 1.7 µm, Waters). The UPLC system was directly coupled to a Xevo TQ-XS mass spectrometer (Waters) equipped with electrospray ionization (ESI) source. MS acquisition was performed using an MRM method of two selected transitions per peptide with optimized collision energies.

Experimental setup to estimate LOD/LOQ

Isotope-labeled peptides (see above) were diluted in 50 mmol/L ammonium bicarbonate to concentrations between 5 amol/µL and 500 fmol/µL and digested with trypsin. Isotope-labeled peptides were spiked at different concentrations (0.5 to 50 fmol/µL) to gargle solution samples after tryptic digestion (SMART digestion protocol). Alternatively, isotope-labeled peptides were spiked at the same concentrations (0.5 to 50 fmol/µL) to saliva samples after tryptic digestion (TRIZOL/FASP protocol). The obtained results served to determine the limit of detection (LOD) and the limit of quantitation (LOQ) of our LC-MS/MS method.

Results and discussion

Optimization of LC-MS/MS method

The aim of our LC-MS/MS method development was to obtain highly sensitive detection and accurate quantitation of the tryptic peptides Arg41-Lsy61, Arg41-Lys65, Met210-Arg226, and Met210-Lys233 from SARS-CoV-2 nucleoprotein. It has to be noted that these peptides exhibit lower ionization efficiencies compared to other tryptic peptides in the digestion mixture of SARS-CoV-2 nucleoprotein. Additionally, they are challenging for MS analysis due to their size and charge. We still selected these specific SARS-CoV-2 peptides as they were recently shown to be generated from authentic material of infected patients [5]. The two peptides starting with Arg41 are specific for a group of coronaviruses, while the two peptides starting with Met210 are unique for SARS-CoV-2. Methionine-containing peptides are not ideally suited for LC-MS/MS quantitation as they might undergo oxidation processes. However, we were able to monitor oxidation products with our synthetic peptide standards and observed only <3% of Met-oxidized variant (data not shown). We therefore felt confident to include the respective Met-containing peptides in our experiments. We sought to achieve the highest possible sensitivity, together with short run times in a robust setup that might be used in a clinical routine environment. First, we had to switch from our initial nano-HPLC setup

(300 nL/min) with an LC time of more than 3 h [5] to a normal-flow UPLC setup. The most important constraint was to achieve a baseline LC separation of the four peptides from SARS-CoV-2 nucleoprotein that had been identified previously in gargle samples [5]. For this, we applied the LC gradient as displayed in Fig. 1 with a total cycle time of 5 min. It should be noted that short LC-MS/MS cycle times are crucial for conducting medium- to high-throughput analyses in MS-based SARS-CoV-2 routine diagnostics. While sample preparation can be parallelized enabling hundreds of samples to be processed at the same time, LC-MS/MS sample throughput relies on short cycle times and the number of available mass spectrometers. Consequently, LC-MS/MS cycle times of 5 min allow 12 samples per hour (288 samples per day) to be analyzed on each instrument. Another important aspect to be considered for LC-MS/MS analysis in a clinical setting is a low sample carryover from injection to injection. Carryover between samples was reduced in our setup to non-detectable amounts by using 0.2% (v/v) TFA as purge solution and 0.2% (v/v) formic acid plus 0.2% (v/v) TFA in water/acetonitrile (50:50 (v/v)) as washing solution of the UPLC system (data not shown).

Next, we had to move from the high-resolution mass spectrometer (Orbitrap Fusion Tribrid) that had been employed in our previous study [5] to a quadrupole instrument of the highest sensitivity. Sensitivity is one of the key points for developing MS-based, reliable SARS-CoV-2 diagnostic methods as viral proteins are directly detected. This stands in contrast to RT-qPCR that relies on the amplification of the virus' genetic material. Therefore, the detection limits for SARS-CoV-2 are directly related to the sensitivity of the LC-MS/MS method. The most sensitive method using triple-quadrupole mass spectrometers is MRM scanning. MRM is a targeted method that has to be specifically optimized for each analyte by evaluating the optimum conditions for collisional activation and thereby identifying the most abundant and characteristic fragment ions.

As outlined above, optimization of our LC-MS/MS method was based on synthetic variants of four peptides derived from SARS-CoV-2 nucleoprotein that had been identified in our previous study [5]. For these four peptides, we identified the most suitable MRM transitions and collision energies with two transitions per peptide (Table 1). We also added MRM transitions of selected tryptic peptides

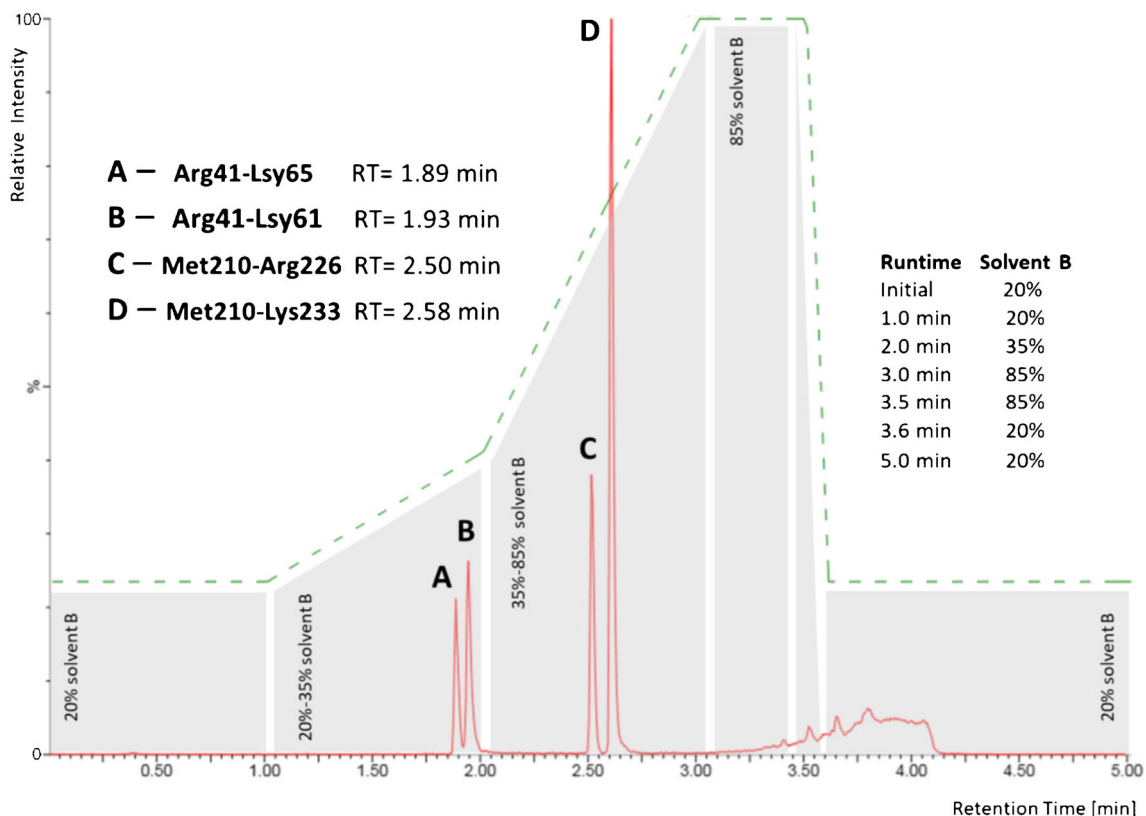


Fig. 1 LC/MRM chromatogram of the four peptides (A) Arg41-Lsy61, (B) Arg41-Lys65, (C) Met210-Arg226, and (D) Met210-Lys233 derived from SARS-CoV-2 nucleoprotein. Peptides A–D were separated at a flow

rate of 400 μ L/min with the LC gradient as indicated; solvent A: 0.2% (v/v) formic acid in water, solvent B: 0.2% (v/v) formic acid in acetonitrile. RT retention time

Table 1 MRM transitions (specific combinations of precursor ion m/z and fragment ion m/z) and collision energies of SARS-CoV-2 peptides, isotope-labeled peptides (*), and selected keratin peptides. Two transitions were selected per peptide

Peptide	Precursor charge	Precursor m/z	Fragment m/z	Collision energy
NLDLDSIIAEVR (keratin)	2+	679.77	903.02	30
NLDLDSIIAEVR (keratin)	2+	679.77	1131.26	30
Arg41-Lys65	4+	703.53	552.65	25
Arg41-Lys65	4+	703.53	881.48	25
VDSLNDINFLK (keratin)	2+	704.28	302.30	30
VDSLNDINFLK (keratin)	2+	704.28	993.10	30
Arg41-Lys*65	4+	705.52	552.65	25
Arg41-Lys*65	4+	705.52	885.46	25
Arg41-Lys61	3+	775.86	341.39	40
Arg41-Lys61	3+	775.86	552.65	35
Arg41-Lys*61	3+	778.51	349.33	40
Arg41-Lys*61	3+	778.51	552.65	35
Met210-Lys233	3+	828.96	906.08	30
Met210-Lys233	3+	828.96	1141.80	25
Met210-Lys*233	3+	831.61	910.06	30
Met210-Lys*233	3+	831.61	1145.77	25
Met210-Arg226	2+	836.48	1069.32	30
Met210-Arg226	2+	836.48	1241.46	30
Met210-Arg*226	2+	841.44	1079.25	30
Met210-Arg*226	2+	841.44	1251.39	30

from other abundant proteins present in our samples and monitored them during the optimization process for sample preparation (see below).

All four synthetic SARS-CoV-2 peptides were employed as heavy isotope (^{13}C and ^{15}N)-labeled versions (SpikeTide-TQL peptides) that were added to the sample solutions to allow for an accurate detection and quantification of SARS-CoV-2 peptides. Heavy isotope-labeled peptides only differ in the masses of precursor and fragment ions, but yield identical fragmentation patterns as the non-labeled SARS-CoV-2 peptides upon collisional activation in the MRM approach. Also, the chromatographic behavior of non-labeled and labeled peptides is identical. Therefore, MRM chromatograms of the synthetic heavy isotope-labeled peptides cannot only be used for the absolute quantification of SARS-CoV-2 nucleoprotein in patient samples, but will also exclude the assignment of false positives in highly complex matrices.

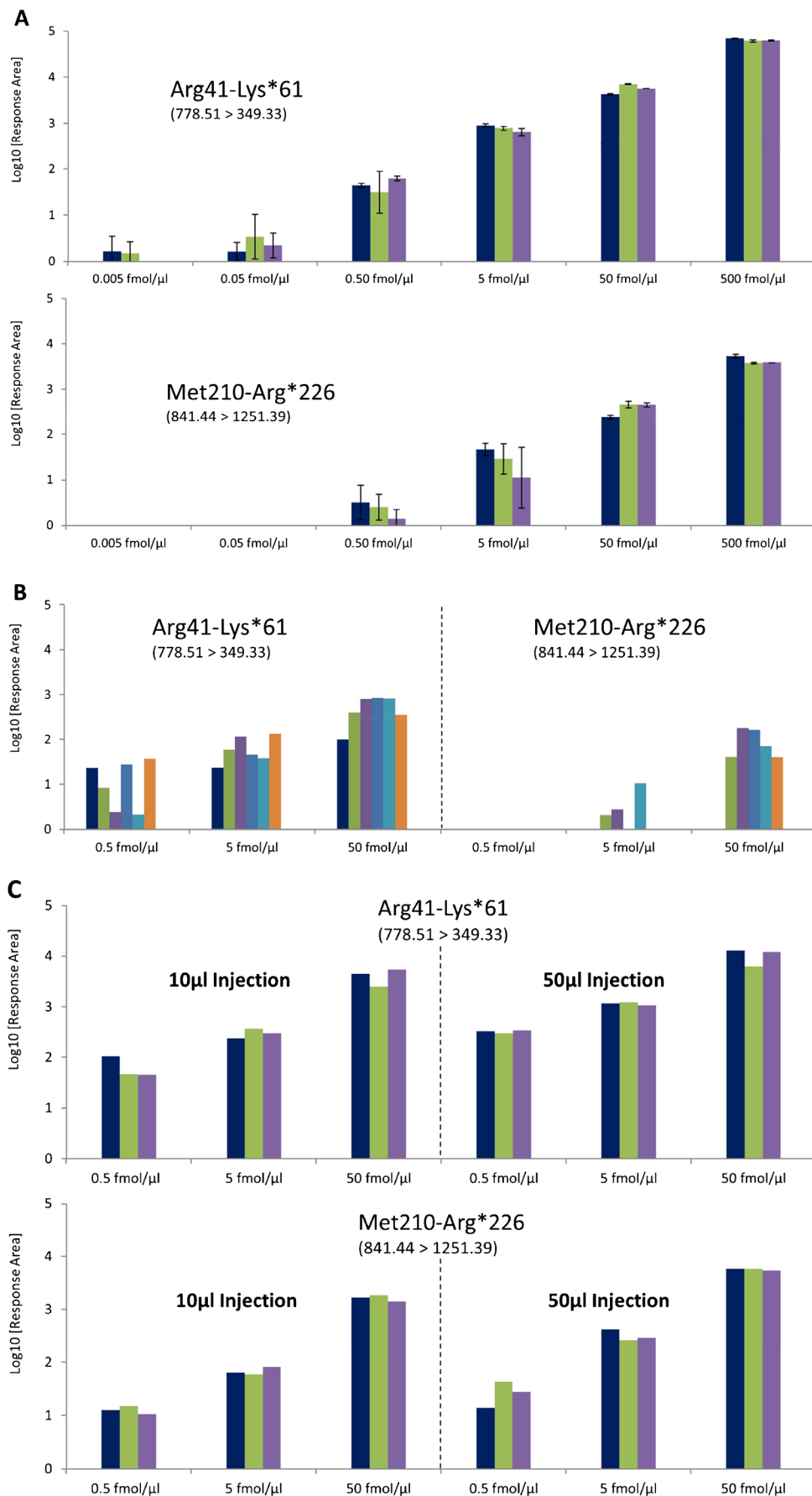
Sensitivity of LC-MS/MS method and estimation of LOD/LOQ

In further experiments, we then used our established LC-MS/MS method for optimizing sample preparation. The peptides from SARS-CoV-2 nucleoprotein that had been identified in our previous study [5] comprise amino acid sequences Arg41-Lys65 and Met210-Lys233. These

peptides contain one missed cleavage site each as our initial digestion procedure was apparently incomplete. The respective SARS-CoV-2 nucleoprotein peptides without missed cleavage sites are composed of amino acid sequences Arg41-Lys61 and Met210-Arg226. The optimized sample preparation (see below) now allows a complete enzymatic digestion, yielding peptides Arg41-Lys61 and Met210-Arg226 from SARS-CoV-2 nucleoprotein.

For that reason, we only employed isotope-labeled SpikeTide-TQL peptides Arg41-Lys*61 and Met210-Arg*226 in all following experiments. SpikeTide-TQL peptides are extended by a C-terminal tag for enhanced solubility and photometric quantitation that is removed during tryptic digestion. It is not possible to directly add these peptides to gargle solutions or saliva as the peptides do not precipitate and

Fig. 2 MRM detection of isotope-labeled SARS-CoV-2 peptides Arg41-Lys*61 and Met210-Arg*226 under different conditions. For each peptide, the first transition was used for quantitation (quantifier), while the second transition served as confirmation (qualifier). The qualifier signal (data not shown) was always present when the quantifier transition had been observed as indicated. (A) Dilution series of peptides in ammonium bicarbonate buffer without adding protein matrix. All experiments were performed in three replicates each (sample preparation and LC-MS/MS measurements). (B) Peptides were added at three concentrations to gargle solutions, SMART digestion protocol was applied. (C) Peptides were added at three concentrations to saliva samples, TRIZOL/FASP protocol was applied



will not be retained using centrifugation filter devices with a molecular weight cutoff value of 30 kDa. Therefore, isotope-labeled peptides were added after tryptic digestion in all experiments.

Figure 2A displays the LOD and LOQ of Arg41-Lsy*61 and Met210-Arg*226 isotope-labeled peptides (see “Experimental section”) that had been digested with trypsin in 50 mmol/L ammonium bicarbonate without adding protein matrix. Both LOD and LOQ were determined to be lower than 500 amol/ μ L. In further experiments, isotope-labeled peptides were spiked to protein pellets of gargle solutions before the SMART digestion protocol [5] was applied. Figure 2B displays a dramatic decrease in sensitivity and reproducibility for the SARS-CoV-2 peptides upon adding a protein matrix, underlining the need to optimize sample preparation.

Optimization of sample preparation

Improving our sample preparation protocol included sample collection, sample homogeneity, sample concentration, and sample preparation times. In principle, nasopharyngeal swabs, gargle solutions, and saliva are suited to detect SARS-CoV-2 [5, 11–13]. A number of challenges regarding non-MS-compatible contaminations have been reported for swab samples [11], where the sample volume is usually less than 10 μ L. Additionally, the quantitative reproducibility of swab sample collection is a matter of debate [13], making gargle solutions a true alternative for sample collection instead of the commonly used nasopharyngeal swab samples. Saliva of COVID-19 patients should contain even higher concentrations of SARS-CoV-2 particles than gargle solution and as saliva is easy to collect, sample quality should be highly reproducible.

Conclusively, we considered saliva to be the optimum sample for a reproducible and quantitative analysis of SARS-CoV-2 and focused our sample preparation optimization on saliva samples. Saliva is a complex matrix making sample preparation challenging. Also, handling and inactivating presumably infectious saliva samples is an issue that requires special attention during method development. First, 100 μ L of saliva was treated with TRIZOL reagent for a complete dissolution of all sample components. TRIZOL reagent is typically used for RNA preparation [14, 15] and presents one of the most potent protein denaturants. It allows a rapid and complete inactivation of RNases, therefore enabling the isolation of intact RNA from cell lysates. Treating our samples with TRIZOL reagent leads to homogenous solutions and is therefore compatible with our FASP protocol for efficient washing and tryptic digestion of proteins. The pellet originating from acetone precipitation, as used in our previous study [5], was sometimes found to be incompletely dissolved in SMART digest buffer. Further advantages of the TRIZOL treatment are the direct availability of the sample as no

overnight protein precipitation step is needed, and the possibility to split samples for parallel analyses by LC-MS/MS and RT-qPCR. The TRIZOL/FASP protocol is compatible with adding the isotope-labeled peptides after tryptic digestion has been performed. Strikingly, the observed LOD/LOQ values (Fig. 2C) were comparable to values obtained without protein matrix (Fig. 2A).

One initial finding when optimizing the TRIZOL/FASP protocol was the detection of high amounts of cytoskeletal keratins in a number of saliva samples. Therefore, we added four specific MRM transitions for highly abundant keratin peptides (Table 1) and monitored their detection. Apparently, keratins contained in the saliva samples were efficiently removed by a simple centrifugation step before applying the FASP protocol and tryptic digestion. We speculate that without centrifugation, the TRIZOL reagent completely dissolves the keratin-containing cells in the saliva making them accessible for a subsequent tryptic digestion. This additional simple centrifugation step of saliva samples will not interfere with the overall sample preparation procedure. Nevertheless, there is a slight chance that virus particles might be attached to cells contained in saliva, which will then be removed by centrifugation. For that reason, we tested the influence of keratins on the detection of SARS-CoV-2 nucleoprotein peptides. Interestingly, neither LOD nor LOQ values were negatively affected by the presence of high amounts of keratin peptides in the samples (Fig. 3). On the other hand, loading very low protein amounts (below 1 μ g) on the centrifugation filter devices resulted in a loss of spiked SARS-CoV-2 peptides, which is probably caused by protein and peptide binding to the membrane of the filter units. A minimum protein load of 1 μ g per filter unit was obtained from treating 100 μ L of saliva with TRIZOL. Additionally, sample loading might be increased to further improve sensitivity, albeit at the cost of longer sample preparation times. It is important to note that neither sample reduction nor alkylation steps were performed in our digestion protocol as the respective SARS-CoV-2 peptides do not contain cysteine residues and proteolytic accessibility of these peptides is not restricted by disulfide bonds. In conclusion, the overall time for sample preparation can be reduced to less than 3 h and can be performed in a highly parallelized fashion. Therefore, the direct use of saliva in combination with our optimized TRIZOL/FASP protocol should improve both sample collection and sensitivity of SARS-CoV-2 detection.

Comparison with RT-qPCR

It has been demonstrated that 10 genomes, i.e., DNA plasmids [11], can be detected in a volume of 10 μ L with cycle times (ct) of 38 in RT-qPCR experiments. A RT-qPCR detection with a ct value of 18 would require the presence of

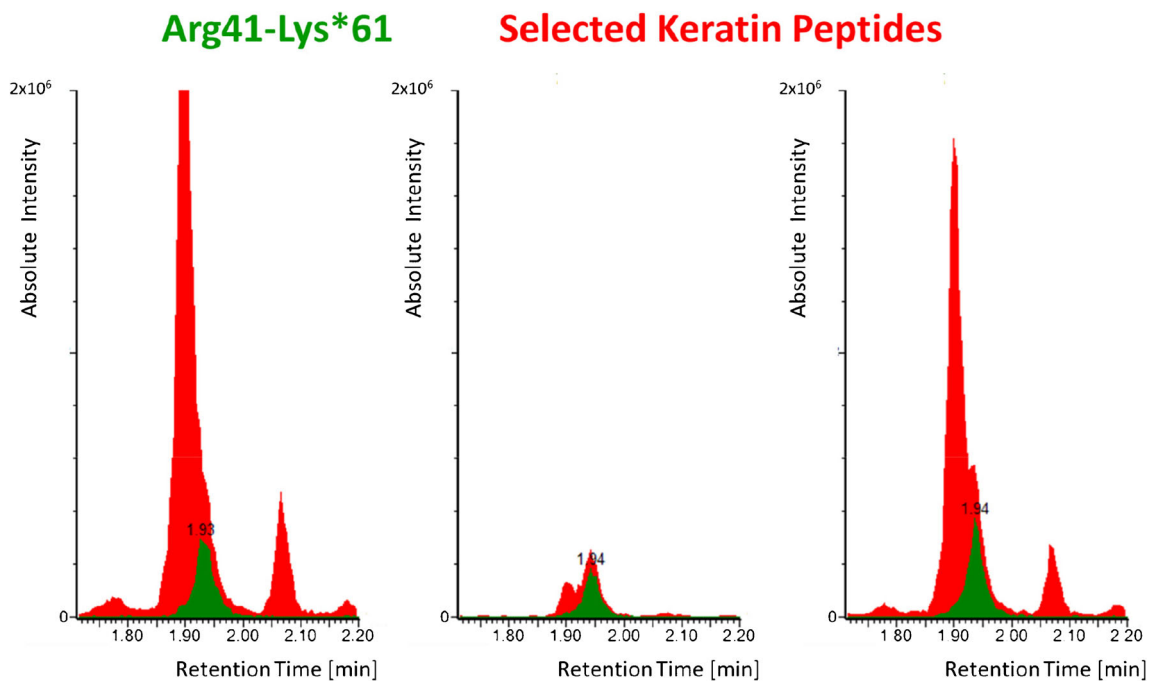


Fig. 3 LC-MS/MS MRM detection of isotope-labeled SARS-CoV-2 peptide Arg41-Lys*61 at 50 fmol/ μ L (green) in the presence of keratin peptides (red). Selected transitions are presented: 778.51 \rightarrow 349.33 (Arg41-Lys*61), 704.28 \rightarrow 302.30 (keratin peptide

VDSLNDIEINFLK), and 679.77 \rightarrow 903.02 (keratin peptide NLDLDSIIAEVR). The signal intensity of the SARS-CoV-2 peptide is not suppressed by the keratin background

10,485,760 genome equivalents in 10 μ L. Calculations with 300 molecules of nucleoprotein per SARS-CoV-2 virus particle [11] result in a nucleoprotein concentration of \sim 520 amol/ μ L, corresponding to a ct value of 18 [11]. In our experiments, we used 100 μ L of saliva and generated 70 μ L of sample solution for LC-MS/MS analysis. Under these conditions, the concentration of SARS-CoV-2 nucleoprotein peptides would be 743 amol/ μ L, which is above our estimated LOD/LOQ of \sim 500 amol/ μ L (see above). It should be noted that this reflects the most conservative analytical calculation. It is likely that a “real-world” RT-qPCR experiment is less sensitive than the one performed under the ideal conditions described in [11] as RNA recovery is rarely complete. Therefore, RT-qPCR experiments with specific ct values would be based on higher virus numbers, resulting in de facto higher nucleoprotein concentrations. Patient samples from our earlier study [5] exhibited ct values of 22 and we were able to detect peptides from SARS-CoV-2 nucleoprotein with a nano-HPLC- nano-ESI-Orbitrap-MS/MS approach. In direct comparison, our previous LC-MS/MS method was found to be less sensitive than the LC-MS/MS approach we describe herein (data not shown). Therefore, we are confident to assume that ct values of higher than 22 will easily be obtained with our new LC/MRM-MS method. The sensitivity of our LC/MRM-MS approach is likely to be further improved by using higher sample amounts, i.e., 200 or 400 μ L of saliva, and by enriching the tryptic SARS-CoV-2 peptides during sample preparation.

Conclusions

We successfully developed an LC-MS/MS method based on an MRM approach that can be used for the direct detection of SARS-CoV-2 from saliva samples. Our approach is complementary to RT-qPCR methods and existing antigen tests, and can be used in a routine clinical environment where quadrupole mass spectrometers are available. Sample preparation time is less than 3 h and sample handling can be parallelized for hundreds of samples (both similar to RT-qPCR). LC-MS/MS analysis time of our method is only 5 min, allowing selected SARS-CoV-2 peptides to be detected and quantified at concentrations as low as 500 amol/ μ L. This concentration of SARS-CoV-2 peptides is obtained by digesting 100 μ L of saliva from infected patients with RT-qPCR ct values of 22. Even higher ct values will be achieved if SARS-CoV-2 peptides enrichment are enriched by antibodies. In general, LC-MS/MS methods exhibit an improved quantitation accuracy compared to RT-qPCR as no amplification (10^6 to 10^9 times) of the analytes is involved. It is still a matter of debate if/how different sample collection protocols influence SARS-CoV-2 detection. MRM-MS-based methods could fill this gap and allow delivering more precise and accurate quantitative data for a direct comparison between different methods.

The outstanding advantage of LC/MRM methods is their potential to detect SARS-CoV2 mutations. The vast majority of amino acid exchanges will result in mass shifts that are easily detected by MS, but there will probably be no drastic change in the physicochemical properties of the respective SARS-CoV-2

peptides, such as ionization efficiency and fragmentation pattern. Virus mutations can be easily monitored by adapting selected MRM transitions using simple mathematical calculations and by synthesizing novel SARS-CoV-2 standard peptides.

Conclusively, we consider the development of novel methods to be of outstanding importance to tackle some of the most urgent issues of the COVID-19 pandemic. It seems out of question that fighting this pandemic urgently requires interdisciplinary collaborations between virologists, clinicians, and analytical chemists.

Abbreviations ACN, Acetonitrile; ct, Cycle time; ESI, Electrospray ionization; FA, Formic acid; FASP, Filter-aided sample preparation; HPLC, High-performance liquid chromatography; LC, Liquid chromatography; LOD, Limit of detection; LOQ, Limit of quantitation; MRM, Multiple-reaction monitoring; MS, Mass spectrometry; MS/MS, Tandem mass spectrometry; RT-qPCR, Reverse transcription quantitative polymerase chain reaction; TFA, Trifluoroacetic acid; UPLC, Ultra-performance liquid chromatography

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00216-021-03614-y>.

Acknowledgements Sinz acknowledges financial support by the DFG (RTG 2467, project number 391498659 “Intrinsically Disordered Proteins – Molecular Principles, Cellular Functions, and Diseases”), the Federal Ministry for Economic Affairs and Energy (BMW, ZIM project KK5096401SK0), the region of Saxony-Anhalt, and the Martin Luther University Halle-Wittenberg (Center for Structural Mass Spectrometry). The Waters Corporation is gratefully acknowledged for providing the Xevo TQ-XS instrument and Dr. Mike van Oosterhout, Dr. Hans Vissers, and Gunnar Weibchen for continuous support. The authors would like to thank Xiaohan Wang for excellent technical support and Dr. Christian Ihling for fruitful discussions and additional LC-MS measurements. Prof. Dr. Mike Schutkowski is acknowledged for giving access to the I-Class UPLC system to conduct initial LC-MS experiments.

Author contribution All the authors have given approval to the final version of the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

Ethics approval The study has been approved by the ethics committee of the Martin Luther University Halle-Wittenberg and has been conducted in accordance with ethical standards.

Informed consent The authors have got the informed consent of all volunteers from whom saliva samples and gargle solutions were obtained.

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included

in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- <https://covid19.who.int>, Accession date: Apr 19, 2021.
- Bezier C, Anthoine G, Charki A. Reliability of real-time Rt-Pcr tests to detect Sars-Cov-2: a literature review. *Int J Metrol Qual Eng.* 2020;11:13.
- Toptan T, Eckermann L, Pfeiffer AE, Hoehl S, Ciesek S, Drosten C, Corman VM. Evaluation of a SARS-CoV-2 rapid antigen test: potential to help reduce community spread? *J Clin Virol.* 2021;13:104713.
- Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, Kalinich CC, Petrone ME, Casanovas-Massana A, Muenker MC, Moore AJ, Klein J, Lu P, Lu-Culligan A, Jiang X, Kim DJ, Kudo E, Mao T, Moriyama M, Oh JE, et al. Analytical sensitivity and efficiency comparisons of Sars-Cov-2 Rt-Qpcr primer-probe sets. *Nat Microbiol.* 2020;5:1299–305.
- Ihling C, Tänzler D, Hagemann S, Kehlen A, Hüttelmaier S, Arlt C, Sinz A. Mass spectrometric identification of SARS-CoV-2 proteins from gargle solution samples of Covid-19 patients. *J Proteome Res.* 2020;19:4389–92.
- Struwe W, Emmott E, Bailey M, Sharon M, Sinz A, Corrales FJ, Thalassinou K, Braybrook J, Mills C, Baran PE. The COVID-19 MS coalition-accelerating diagnostics, prognostics, and treatment. *Lancet.* 2020;395:1761–2.
- Gouveia D, Grenga L, Gaillard JC, Gallais F, Bellanger L, Pible O, Armengaud J. Shortlisting SARS-CoV-2 peptides for targeted studies from experimental data-dependent acquisition tandem mass spectrometry data. *Proteomics.* 2020;20:2000107.
- Nikolaev EN, Indeykina MI, Brzhozovskiy AG, Bugrova AE, Kononikhin AS, Starodubtseva NL, Petrotchenko EV, Kovalev GI, Borchers CH, Sukhikh GT. Mass-spectrometric detection of SARS-CoV-2 virus in scrapings of the epithelium of the nasopharynx of infected patients via nucleocapsid N protein. *J Proteome Res.* 2020;19:4393–7.
- Nachtigall FM, Pereira A, Trofymchuk OS, Santos LS. Detection of SARS-CoV-2 in nasal swabs using MALDI-MS. *Nat Biotechnol.* 2020;38:1168–73.
- Cazares LH, Chaerkady R, Weng SH, Boo CC, Cimburo R, Hsu HE, Rajan S, Dall'Acqua W, Clarke L, Ren K, McTamney P, Kallewaard-LeLay N, Ghaedi M, Ikeda Y, Hess S. Development of a parallel reaction monitoring mass spectrometry assay for the detection of SARS-CoV-2 spike glycoprotein and nucleoprotein. *Anal Chem.* 2020;92:13813–21.
- Van Puyvelde B, Van Uytendange K, Tytgat O, Van Oudenhove L, Gabriels R, Bouwmeester R, Daled S, Van Den Bossche T, Ramasamy P, Verhelst S, De Clerck L, Corveleyn L, Debonne N, Wynendaele E, De Spiegeleer B, Judak P, Roels K, De Wilde L, Van Eenoo P, et al. Cov-MS: a community-based template assay for clinical MS-based protein detection in SARS-CoV-2 patients. *JACS Au.* 2021;1(6):750–65.
- Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Warren JL, Geng B, Muenker MC, Moore AJ, CBF V, Petrone ME, Ott IM, Lu P, Venkataraman A, Lu-Culligan A, Klein J, Earnest R, Simonov M, et al. *New Engl J Med.* 2020;383:1283–6.
- Sakanashi D, Asai N, Nakamura A, Miyazaki N, Kawamoto Y, Ohno T, Yamada A, Koita I, Suematsu H, Hagihara M, Shiota A, Kurumiya A, Sakata M, Kato S, Muramatsu Y, Koizumi Y,

- Kishino T, Ohashi W, Yamagishi Y, Mikamo H. Comparative evaluation of nasopharyngeal swab and saliva specimens for the molecular detection of SARS-CoV-2 RNA in Japanese patients with COVID-19. *J Infect Chemother.* 2021;27:126–9.
14. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162:156–9.
 15. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nat Protoc.* 2006;1:581–5.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.