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응용 자료

Advancing Research with the SARS-CoV-2 LC-MS Kit (RUO)

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

Abstract

Detection and quantification of SARS-CoV-2 is important in clinical research to understand the impact of SARS-CoV-2[1] and the significance of prolonged viral shedding.[2]

The SARS-CoV-2 LC-MS Kit (RUO) enables the direct detection and quantification of SARS-CoV-2 Nucleocapsid (NCAP) peptides. The Andrew+ Pipetting Robot was used to streamline and automate the sample preparation process, minimizing hands-on preparation time and reducing operator error. Utilizing the ACQUITY UPLC I-Class with an ACQUITY Premier Column allows for the rapid separation of the target peptides (2.5 minutes cycle time), with the Xevo TQ-XS Mass Spectrometer providing analytically sensitive, selective, and precise detection of viral peptides at 3 amol/µL (inter-day precision ≤17.4% CV, and intra-day precision ≤17.1% CV). The method was shown to be linear over the range 3–50,000 amol/µL.

Scientists can harness the flexibility and reproducibility of UPLC-MS to advance critical SARS-CoV-2 research using the SARS-CoV-2 LC-MS Kit (RUO) to directly detect and quantify SARS-CoV-2 signature NCAP peptides, without the need to amplify the target analytes as required for Polymerase Chain Reaction (PCR).

The Waters' SARS-CoV-2 LC-MS Kit is for research use only (RUO) and is not intended for use in diagnostic procedures.

Benefits

- A clinical research kit that contains all components that enable the direct quantification of SARS-CoV-2 NCAP peptides
- Andrew+ Pipetting Robot automates the sample preparation process to maximize throughput and minimize error
- ACQUITY UPLC I-Class with Xevo TQ-XS Mass Spectrometer provides rapid run times with analytically sensitive and selective analysis

Introduction

Detection and quantification of SARS-CoV-2 is important in clinical research to understand the impact of SARS-CoV-2[1] and the significance of prolonged viral shedding.[2]

The application of LC-MS to detect tryptic digest peptides of SARS-CoV-2 proteins has been successfully demonstrated.[3] However, these studies also highlight that the technique suffers from matrix effects due to interferences arising from the constituent components of the sample matrix, such as viral transport medium (VTM), limiting the analytical sensitivity of the methods. Therefore, to improve detection levels, there is a need for sample clean-up and enrichment.[4,5]

Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) is an enrichment technique that provides three advantages in analyzing SARS-CoV-2 in complex matrices:

- Greater analytical sensitivity through sample concentration and removal of interferences that suppress MS ionization of target peptides i.e. tryptic peptides
- Faster LC-MS sample throughput through selective removal of background interference that would otherwise contribute to longer chromatographic run times
- Improved LC-MS method robustness with extended column lifetimes and reduced instrument downtimes as a

result of cleaner samples for injection

The SARS-CoV-2 LC-MS Starter Kit (RUO), shown in Figure 1, provides all the necessary reagents and consumables to quantify SARS-CoV-2 Nucleocapsid (NCAP) peptides at low concentrations. The SARS-CoV-2 LC-MS Kit (RUO) contains pre-weighed lot-to-lot reproducible reagents that aid in the harmonization of SARS-CoV-2 clinical research methods, providing confidence in the results obtained over the course of longitudinal research studies.

Figure 1. The SARS-CoV-2 LC-MS Starter Kit (RUO) for the analysis of SARS-CoV-2 NCAP peptides using LC-MS/MS.

The Andrew+ Pipetting Robot can be used to streamline and automate the sample preparation process, minimizing hands-on preparation time and reducing operator error. OneLab is used to control the Andrew+ Pipetting Robot, which is a cloud-based software that is intuitive to use and facilitates the rapid deployment of automated methods across laboratories.

The SARS-CoV-2 LC-MS Kit (RUO) allows for the quantification of viral proteins to be used alongside infection biomarkers to identify potential prognostic biomarkers or investigate the impacts of the virus in research studies.

The Waters' SARS-CoV-2 LC-MS Kit is for research use only (RUO) and is not intended for use in diagnostic procedures.

Experimental

The SARS-CoV-2 LC-MS RUO Kit was used to perform the studies detailed in this application note, following the Instructions for Use (IFU).

The Kit workflow describes the denaturation, digestion, enrichment, and UPLC-MS/MS analysis of three (3) peptides; AYNVTQAFGR (AYN), NPANNAAIVLQLPQGTTLPK (NPA), and ADETQALPQR (ADE) derived from the SARS-CoV-2 NCAP protein. The sample is denatured and digested to generate tryptic peptides. The three SARS-CoV-2 NCAP peptides are selectively captured along with their associated stable labeled internal standard using anti-peptide antibodies immobilized onto magnetic beads to facilitate the enrichment process. The peptides are washed to remove unbound matrix/sample interferences and eluted as a clean and concentrated sample, ready for UPLC-MS/MS.

Sample Description

Using the SARS-CoV-2 Peptide Calibrator, peptide calibrator samples were prepared over the range 3–50000 amol/µL in Viral Transport Medium (VTM, Liofilchem, Italy), with QCs prepared at 3, 10, 400, and 25000 amol/µL in VTM.

Sample preparation was performed using the SARS-CoV-2 LC-MS Kit (RUO) on the Andrew+ Pipetting Robot.

Denaturation:

180 µL Calibrator and QC sample is added to a 96-well plate. 20 µL of the denaturant (RapiGest for SARS-CoV-2) is added to each sample and the plate shaken. The 96-well plate is then sealed and heated at 56 °C for 15 minutes.

Digestion:

20 µL of Trypsin solution is added to each well, the plate is shaken and placed in an incubator or thermomixer for 30 minutes at 37 °C. To inhibit further digestion, 20 µL of TLCK solution is added to each well and mixed for 5 minutes at room temperature. 20µL of Stable Isotope Labeled (SIL) NCAP peptide mixture is added and mixed for 30 seconds.

Enrichment:

Using the SARS-CoV-2 LC-MS Enrichment Set (RUO) 30 µL of antibody magnetic bead conjugate was added to each sample, containing equal volumes of bead conjugate that target the AYN, ADE, and NPA peptides. The 96-well plate is thoroughly mixed to resuspend the beads, followed by mixing for 1 hour, ensuring mixing is sufficient

to maintain suspension of the beads throughout. The 96-well plate is then transferred to a magnetic plate array and left for one minute before removing the aqueous solution. 150 µL of Wash Buffer is added to each well, mixed for 30 seconds, transferred to a magnetic plate array, left for one minute and the wash solution removed. This wash step is repeated once more. 50 µL of elution buffer is added to each well and mixed for 5 minutes. The plate is then transferred to a magnetic plate array, left for one minute and the elution solution is transferred to a QuanRecovery 96-well plate. The plate is placed on an autosampler magnetic plate, prior to injection on the UPLC-MS/MS system.

Method Conditions

Example parameters are shown below and are available in the SARS-CoV-2 LC-MS Kit (RUO) Instructions For Use (IFU).

LC Conditions

LC system:	ACQUITY UPLC I-Class FTN
Sample needle:	30 µL
Column:	ACQUITY Premier Peptide BEH C_{18}, 300 Å, 2.1 mm x 30 mm, 1.7 µm
Column temp.:	40 °C
Sample temp.:	8 °C
Injection volume:	20 µL
Flow rate:	0.8 mL/min
Mobile phase A:	0.1% formic acid (v/v) in water
Mobile phase B:	0.1% formic acid (v/v) in acetonitrile

Run time:	1.8 minutes

Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.8	95	5	Initial
0.15	0.8	95	5	6
0.35	0.8	85	15	6
0.60	0.8	85	15	6
1.00	0.8	75	25	6
1.20	0.8	10	90	11
1.50	0.8	95	5	11

MS Conditions

MS system:	Xevo TQ-XS Mass Spectrometer
Ionization mode:	Positive ESI
Capillary voltage:	0.5 kV

MRM Parameters

Peptide	MRM		Cone (V)	Collision (V)	Scan window (min)
ADE	564.8>400.2	Quantifier	35	19	0.41–0.75
	564.8>584.4	Qualifier	35	20	
	564.8>712.4	Qualifier	35	24	
	569.8>410.2	SIL	35	19	
AYN	563.8>679.4	Quantifier	35	19	0.76–1.1
	563.8>578.3	Qualifier	35	18	
	563.8>892.5	Qualifier	35	19	
	568.8>689.4	SIL	35	19	
NPA	687.4>841.5	Quantifier	35	18	1.11–1.45
	687.4>766.4	Qualifier	35	23	
	687.4>865.5	Qualifier	35	23	
	690.4>849.5	SIL	35	18	

Method Events

Time (min)	Event	Action
0.41	Flow state	LC
1.44	Flow state	Waste

Data Management

MS software: MassLynx v4.2 with TargetLynx XS

Results and Discussion

The calibration range was shown to be linear from 3–50000 amol/µL across the three peptides. Using a $1/x^2$ weighting function, the correlation coefficient (r^2) of the calibration lines were >0.99 over five separate occasions.

The analytical sensitivity of the method was evaluated over three occasions at 2, 3, 6, 10, and 20 amol/µL synthetic peptides spiked into VTM. Five replicates were analyzed over three occasions. The lower limit of quantification (LLoQ) was determined to be at 3 amol/µL for AYN, ADE, and NPA, which provide a concentration with precision <20%, bias within ±20% and S/N >10:1 (PtP) (Figure 2A–B).

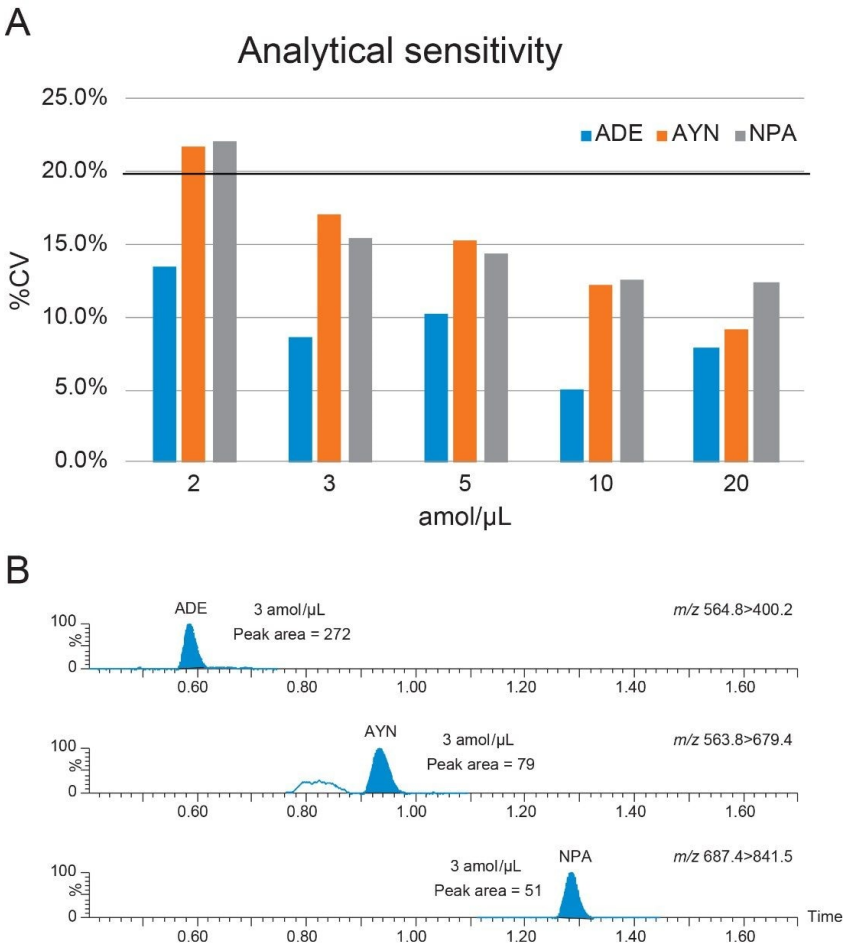

Figure 2A. Analytical sensitivity of the ADE, AYN, and NPA peptides in VTM at 2, 3, 5, 10, and 20 amol/µL following antibody enrichment. 2B. Chromatogram of the ADE, AYN, and NPA peptides at 3 amol/µL in VTM after antibody enrichment.

The precision of the method was evaluated at 3, 10, 400, and 25000 amol/µL for both NCAP derived peptides and synthetic peptides spiked into VTM. Samples were analyzed in replicates of five over five separate occasions.

The inter- and intra-day precision of the method was shown to be ≤17.4% (Figure 3).

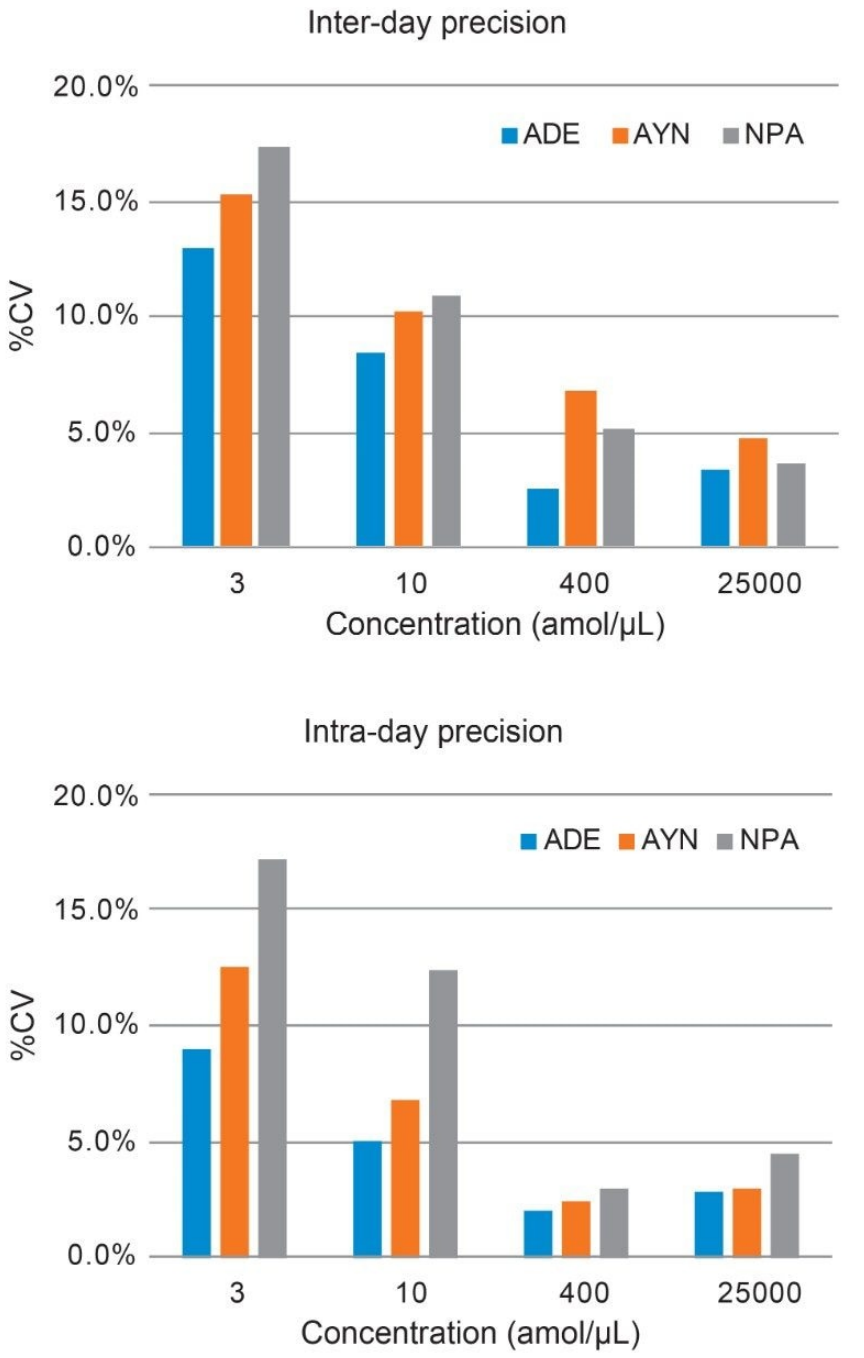

Figure 3. Intra-day and inter-day precision for ADE, AYN, and NPA peptides at 3, 10, 400, and 25000 amol/µL in VTM after enrichment.

QC samples were shown to be stable on the autosampler at 10 °C over 48 hours following re-analysis and comparison to a previously analyzed calibration line.

Conclusion

Using VTM as an example sample matrix, the SARS-Cov-2 LC-MS Kit (RUO) was demonstrated to be able to quantify NCAP peptides over the range 3–50000 amol/µL, with a precision performance of ≤17.4% CV and a lower limit of quantification of 3 amol/µL.

The SARS-Cov-2 LC-MS Kit (RUO) provides direct detection of SARS-CoV-2 NCAP peptides with quantitative measurement that can be used to compare and harmonize results across LC-MS systems and even across research laboratories. By using the key benefit of LC-MS analysis, the ability to detect and measure more than one analyte at a time, the system and Kit could be used to directly detect and quantify SARS-CoV-2 peptides and monitor biomarkers for research studies in a single analysis.

References

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SARS-CoV-2 LC-MS Kit (RUO) <https://www.waters.com/waters/nav.htm?cid=135083368>

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