ThP 455

Screening of Peptide Libraries for the Receptor-Binding Domain (RBD) of SARS-CoV 2 Variants using a Single Quadrupole Mass Spectrometer

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1. Overview

Chromatographic separation and identification of 53 individual peptides representative of the receptor binding domain (RBD) for the spike glycoprotein for different variants of SARS-CoV-2, Delta (B.1617.2) and Omicron (B.1.1.529), was achieved using a Shimadzu LCMS-2050 single quadrupole. This method style can be implemented to detect variations to the RBD of SARS-CoV-2.

2. Introduction

COVID-19 is caused by SARS-CoV-2 or severe acute respiratory syndrome coronavirus 2, a single-stranded RNA virus. This virus infects host cells and replicates itself using a glycosylated spike protein on the viral envelope. Throughout the pandemic, new variants of SARS-CoV-2 continue to emerge. With each new variant comes the need to determine changes to receptor binding domain (RBD) of the spike glycoprotein. Changes in the RBD can increase or decrease the binding affinity and therefore directly affect the transmission of the virus. This study uses a single quadrupole mass spectrometer, LCMS-2050 to screen a peptide library derived from the RBDs for the Omicron and Delta variants.

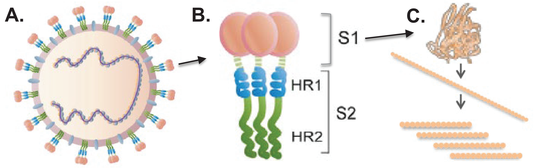

Figure 1 Schematic of SARS-CoV-2 protein and peptide pool[1-2] **A.** Example of SARS-CoV-2 **B**. Structure of S protein **C**. JPT's preparation of peptide pool from Subunit 1 containing RBD

3. Method

Two different peptide pools sourced from JPT Peptide Technologies were chromatographed using a Shimadzu single quadrupole liquid chromatograph mass spectrometer, the LCMS-2050. Each peptide pool consisted of 53 individual peptides and synthetically represent the RBD of the spike glycoprotein for SARS-CoV-2, Delta (B.1617.2) and Omicron (B.1.1.529). The peptide pool samples were diluted to a final concentration of 10 µg/mL in 0.1% formic acid in water. A full scan and targeted screening method was used to determine initial charge states and differentiate between the two samples.

Table 1 Snapshot of LCMS method parameters

LCMS-2050	Parameters	LC-40	Parameters
Ion source	DUIS	**Column**	Restek Raptor ARC-C18 2.1×100 mm, 2.7 µm
Nebulizing gas	2.0 L/min	**Flow rate**	0.4 mL/min
Desolvation temperature	450 °C	**Mobile phase A**	0.1% formic acid in water
DL temperature	200 °C	**Mobile phase B**	0.1% formic acid in methanol
Heating gas	7.0 L/min	**Injection volume**	5 µL
Drying gas	5.0 L/min	**Column Oven temperature**	40 °C
Polarity	Positive and Negative	**Run time**	20 minutes

4. Results

4-1. Targeted Screening

The LCMS-2050 chromatographically separated and identified two different SARS-CoV-2 variants using unique peptide sequences. Each peptide library consisted of multiple peptides with a molecular weight range of 1514.77 to 2017.47 g/mol. Using electrospray ionization in both positive and negative polarity, each peptide was identified. A targeted screening method was then developed using the most abundant charge state and individual retention times. (Figure 2). Initial comparison showed multiple differences in the *m/z* detected resulting from alterations to the amino acid sequence in the RBD of the spike protein.

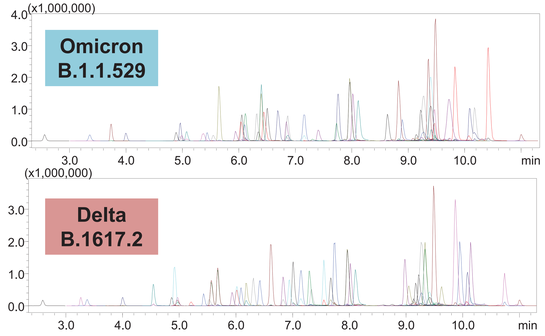

Figure 2 Representative Chromatograms for Targeted Method Top- Omicron (B.1.1.529), Bottom- Delta (B.1617.2)

4-2. Charge State Determination

A full scan method with an *m/z* range of 10-2000 in both positive and negative polarity was used to identify the most abundant charge state for each peptide. The ability to identify multiple charges states allows for additional conformation of peptide sequences. Figure 3 shows an example of a mutation of amino acid 339 in the RBD and the ionization differences in both positive and negative polarity for the intact and +2 charge state.

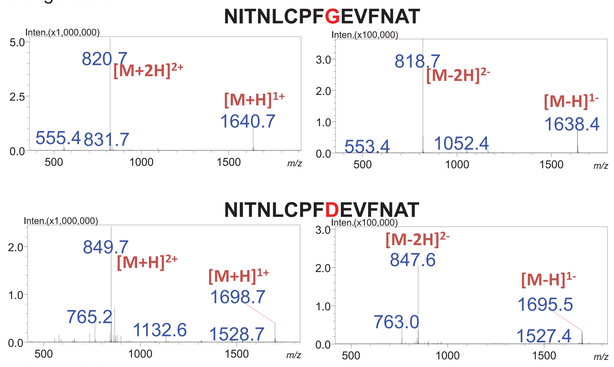

Figure 3 Spectrum of full scan in positive and negative polarity.

4-3. Differentiating between Delta and Omicron

Multiple mutations were identified between Delta (B.1.1.529) and Omicron (B.1.1.529). For example, Omicron has a mutation that changed glycine (G339) to aspartic acid (D339), which could be seen by an *m/z* difference of 58 (Figure 3). A second mutation changed three serines (S371, S373, and S375) to leucine (L371), proline (P373), and phenylalanine (F375), resulting in an *m/z* difference of 96. A third mutation had an *m/z* difference of 14 resulting from the change of lysine (K417) to asparagine (N417).

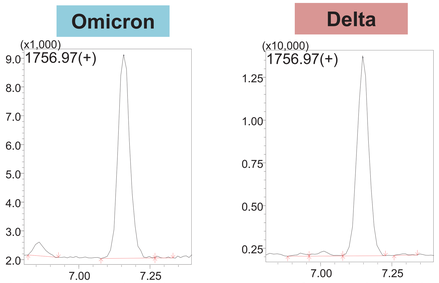

Figure 4 Representative chromatograms of a peptide with no mutation in the RBD of SARS-Cov-2.

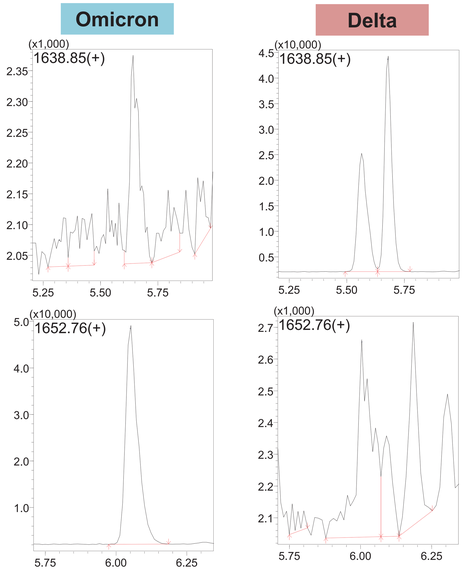

Figure 5 Representative chromatograms of Mutations in the RBD of SARS-CoV-2. Top- Peptide Sequence **APGQTGKIADYNYKL** (*m/z* 1638.85), mutation K0417N. Bottom- Peptide Sequence **DISTEIYQAGSKPCN** (*m/z* 1652.76), mutation S0477N.

5. Conclusions

- A targeted screening method was developed for the receptor binding domain (RBD) for SARS-CoV-2, Delta (B.1617.2) and omicron (B.1.1.529) variants.
- Mutations to the peptide sequences were identified using a single quadrupole mass spectrometer, LCMS-2050, and used to differentiation between two COVID variants .
- The broad scan range and short polarity switching time allowed for simultaneous detection of multiple charge states including the intact charge state in both positive and negative polarity.
- This method could be expanded to include additional mutations of the RBD of SARS-CoV-2 for differentiation of COVID variants.

References

1. Huang Y., Yang C., Xu X.. et al. Acta Pharmacologica Sinica (2020) 41:1141–1149; https://doi.org/10.1038/s41401-020-0485-4
2. Alem A., Drosch M., Reimer U., et. al. JPT Peptide Technologies GmbH (2021) *Peptide Tools to Support the Fight Against COVID-19*