Clinical Research

Simultaneous Detection of Respiratory Infectious Diseases using Immunoprecipitation and Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

Purpose: To monitor multiple infectious diseases in a fast and accurate way using immunoprecipitation and selected reaction monitoring.

Methods: Immunoprecipitation (IP) was performed using Thermo Scientific™ Pierce™ MS-Compatible IP Kit (Streptavidin). The IP purified samples were then digested using SMART Digest™ Trypsin Kits and analyzed by Thermo Scientific™ Vanquish™ MD HPLC system hyphenated to Thermo Scientific™ TSQ Altis™ MD mass spectrometer. Data processing was performed using Thermo Scientific™ TraceFinder™ LDT software 1.0**.**

Results: A total of 12 peptides were successfully monitored (2 to 3 peptides per disease type) simultaneously by LC-MS/MS. The entire sample preparation was finalized to less than 1 hour, reduced from the original starting method of 6 hours. LC-MS run time was also optimized to 5 minutes. The protein precipitation and post-sample clean-up were bypassed since IP was selective to enrich the target protein and purify the sample matrix. With criteria of % accuracy from 80 to 120, % RSD < 15, % CV < 15, and R^2 > 0.99, LOQs were determined to be between 0.05 to 1 fmol of the SIL peptides on the LC column.

Introduction

With recent emergences of new infectious diseases and their variants, there is a need to develop a faster and more accurate analytical tool to detect different respiratory infectious disease viral agents such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza viruses. This report describes a targeted approach for the simultaneous detection of different respiratory infectious disease viruses using immunoprecipitation (IP) and selected reaction monitoring (SRM). The types of respiratory infectious disease viruses monitored in this report include SARS-CoV-2, influenza A and B viruses, respiratory syncytial virus (RSV), and human coronavirus (HCoV-229E).

Materials and methods

Sample Preparation

The biotinylated antibody panel was added to the samples containing each nucleoprotein (NP) stored in VTM after removing the nasopharyngeal swabs. Pierce Streptavidin Magnetic Beads were used for immunoprecipitation in VTM followed by trypsin digestion. Stock solutions of different stable-isotope labeled (SIL) peptides concentration points were first prepared using serial dilution to avoid dilution of the sample matrix. Each stock was then added to the sample matrix to make a final concentration of SIL peptides, ranging from 0.005 to 10 fmol/µL.

Test Method(s)

LC separation was performed on a Thermo Scientific™ Vanquish™ MD HPLC system using a Thermo Scientific™ Hypersil™ GOLD C18 column (2.1 x 50 mm, 1.9 µm, Part No. 25002052130). Analysis was performed on a Thermo Scientific™ TSQ Altis™ MD mass spectrometer. LC and MS conditions are described in Table 1. Final SRM transitions of the target peptides from each disease are listed in Technical Note 000749.

Data Analysis

Data processing was performed using Thermo Scientific™ TraceFinder™ LDT software 1.0. Each data point of the calibration curve was analyzed in triplicate and then fitted with % accuracy from 80 to 120, % RSD < 15, % CV < 15, and R^2 > 0.99 to determine the limits of quantitation (LOQ) for each peptide. The limits of detection (LOD) and linear range were also determined for each peptide.

Figure 1. Experimental workflow

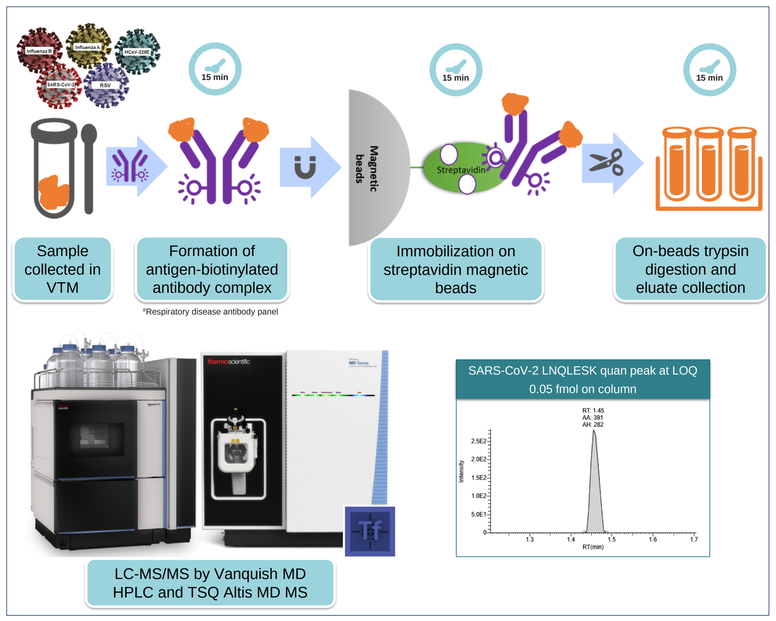


Table 1. LC and MS conditions

LC gradient			
Time (min)	% A	% B	Curve
0.0	98	2	5
0.5	98	2	5
0.7	90	10	5
3.0	40	60	5
3.3	5	95	5
3.8	5	95	5
3.9	98	2	5
5.0	98	2	5
Separation conditions			
Mobile phase A	0.1 % formic acid in water		
Mobile phase B	0.1 % formic acid in 10: 10: 80 water: isopropanol: acetonitrile (v/v/v)		
Flow rate	0.5 mL/min		
Column temperature	40 °C		
Injection volume	10 µL		
MS global parameters			
Source type	Heated electrospray ionization (H-ESI)		
Polarity	Positive		
Spray voltage (V)	3500		
Sheath gas (Arb)	50		
Aux gas (Arb)	10		
Sweep gas (Arb)	2		
Ion Transfer tube temp (°C)	325		
Vaporizer temp (°C)	350		
Divert Valve A	0.0 min: position 1-6 (waste) 0.5 min: position 1-2 (MS) 3.3 min: position 1-6 (waste)		
SRM scan parameters			
Cycle time (sec)	0.35		
Q1 resolution (FWHM)	0.7		
Q3 resolution (FWHM)	0.7		
CID gas (mTorr)	1.5		
Source fragmentation (V)	0		
Chromatographic peak width (sec)	6		
RF Lens (V)	60		

Results

Figure 2. Workflow optimization

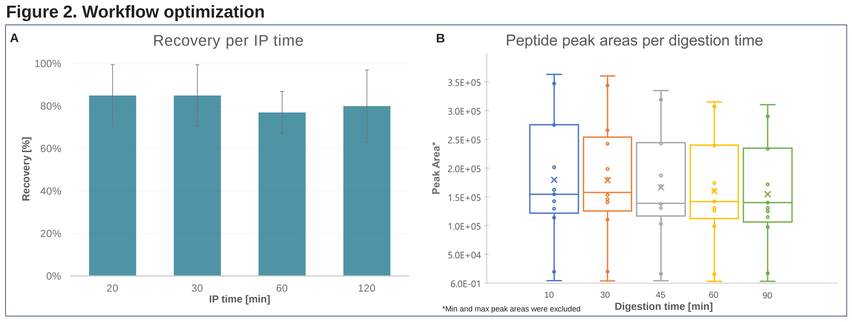


Figure 3. Calibration curves of all peptides over their corresponding linear ranges (A) and the peptides LNQLESK, SALILR, and TIYFSPIR at low calibration points (B)

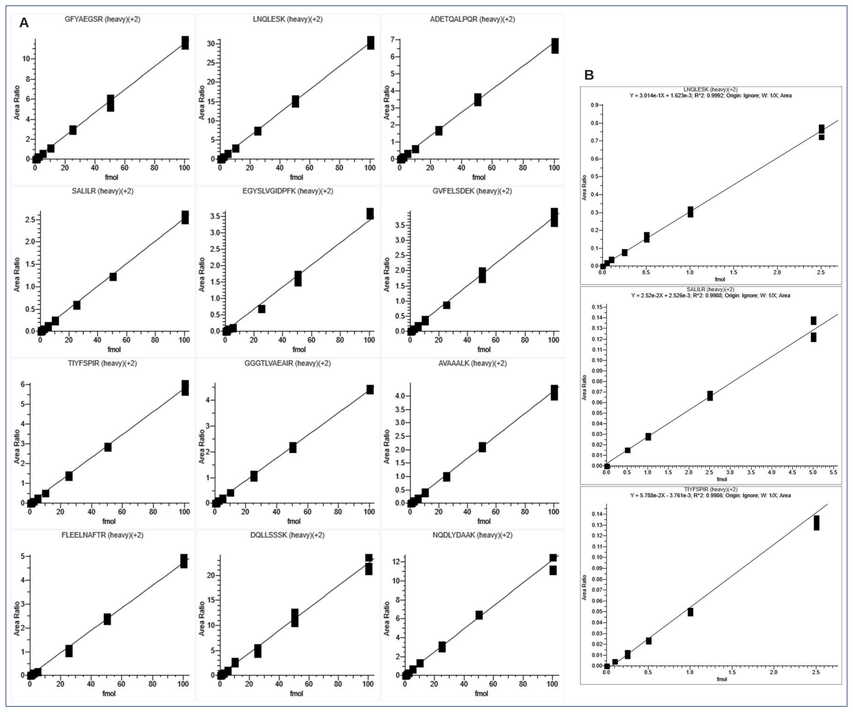
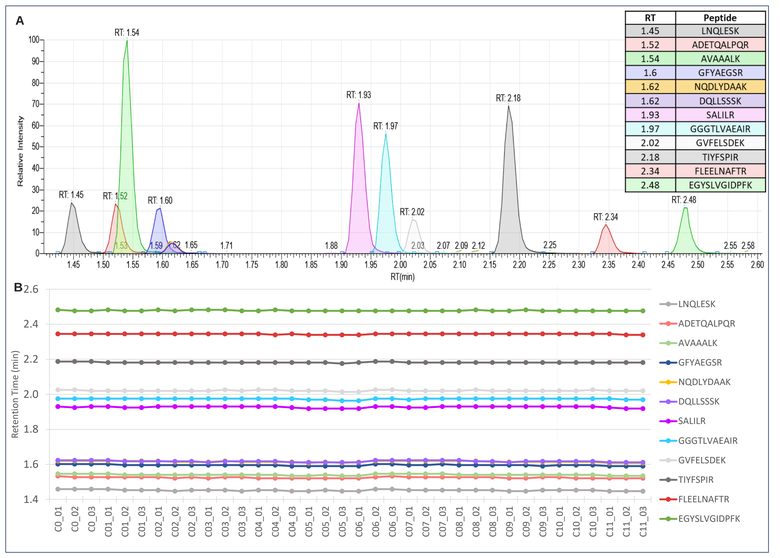


Table 2. Determined analytical properties of the method from the calibration curve including LODs, LOQs, linear range, and R^2 values

	Peptide Sequence	LOD (fmol on column)	LOQ (fmol on column)	Linear Range (fmol on column)	R^2
SARS-CoV-2	GFYAEGSR	0.10	0.25	0.25 - 100	0.9971
	LNQLESK	0.05	0.05	0.05 - 100	0.9992
	ADETQALPQR	0.25	0.25	0.25 - 100	0.9979
Influenza A	SALILR	0.50	0.50	0.50 - 100	0.9988
	EGYSLVGIDPFK	0.10	0.25	0.25 - 100	0.9901
	GVFELSDEK	0.25	0.25	0.25 - 100	0.9970
Influenza B	TIYFSPIR	0.10	0.25	0.25 - 100	0.9985
	GGGTLVAEAIR	0.10	0.10	0.10 - 100	0.9990
HCoV-229E	AVAAALK	1.00	1.00	1.00 - 100	0.9974
	FLEELNAFTR	0.25	0.50	0.50 - 100	0.9951
RSV	DQLLSSSK	0.50	0.50	0.50 - 100	0.9903
	NQDLYDAAK	1.00	1.00	1.00 - 100	0.9939

Figure 4. Representative retention times of all the target peptides (A) and variation of detected retention time (B)



Conclusions

- We have shown successful implementation and optimization of IP and SRM methods to simultaneously monitor four types of infectious diseases by targeting the NP component of enveloped viruses.
- The workflow was optimized to less than 1-hour sample preparation and 5-minute LC-MS analysis.
- The IP method generated a clean and MS-compatible sample matrix, providing reliable quantification of 0.05 to 1 fmol of the peptides on the column.
- This optimized and fast process increases sample throughput and ultimately expedites turn-around time.
- Incorporation of the Thermo Scientific™ KingFisher™ sample purification system can reduce 70% of the manual steps, increase consistency, and facilitate greater sample throughput for high-volume laboratories.
- More details are available online (Technical Note 000749).

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