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Data Article

A peptide dataset for target analysis of human complement system proteins

Thais Cristina Tirado[a,b,*], Kelly Cavalcanti Machado[c], Patrícia Shigunov[d], Michel Batista[c,1,*], Fabiano Borges Figueiredo[a,b,1]

[a] *Laboratório de Biologia Celular, Instituto Carlos Chagas, Fundação Oswaldo Cruz (FIOCruz), Curitiba, Paraná, Brazil*
[b] *Laboratório de Referência em Leishmanioses, Instituto Carlos Chagas, Fundação Oswaldo Cruz (FIOCruz), Curitiba, Paraná, Brazil*
[c] *Plataforma de Espectrometria de Massas RPT02H, Instituto Carlos Chagas, Fundação Oswaldo Cruz (FIOCruz), Curitiba, Paraná, Brazil*
[d] *Laboratório de Biologia Básica de Células-Tronco, Instituto Carlos Chagas, Fundação Oswaldo Cruz (FIOCruz), Curitiba, Paraná, Brazil*

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ABSTRACT

The targeted LC-MS/MS method has been widely applied for peptide quantification, offering sensibility, specificity, and reproducibility to the analysis. However, it requires the prior selection of targets, including the construction of a spectral library. Here, we present a dataset comprising peptide mass spectra for targeted LC-MS/MS method setup, applied to a set of human complement system proteins. Additionally, we selected a group of peptides and demonstrated their stability and reproducibility in quantification. This dataset is invaluable for studies aiming at the quantification of the complement system proteins by targeted LC-MS/MS, as it provides data for spectral library construction and a list of selected peptides.

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* Corresponding authors.
E-mail addresses: thais.tirado@gmail.com (T.C. Tirado), michel.batista@fiocruz.br (M. Batista).

[1] These authors have contributed equally.

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Specifications Table

Subject	Omics: Proteomics
Specific subject area	Standardization of the targeted LC-MS/MS method for evaluation of complement system proteins and other biomarkers in biological fluids. Protein-targeted LC-MS/MS method setup.
Data format	.xlsx .pdf .docx .jpg .raw
Type of data	Table, Figure, Supplementary material, Raw data
Data collection	Data were acquired using the mass spectrometers Orbitrap Fusion Lumos and/or Orbitrap Exploris 120.
Data source location	Data were collected at the Carlos Chagas Institute (Curitiba, Paraná, Brazil) and stored at ProteomeXchange with the identifier PXD045089.
Data accessibility	Repository name: PRIDE Data identification number: PXD045089 Direct URL to data: http://www.ebi.ac.uk/pride

1. Value of the Data

- This study leverages the established MS acquisitions DDA and PRM, along with the user-friendly bioinformatics portal Expasy, to generate a dataset that enables the quantification of human complement system proteins. This is achieved by combining the precision, resolution, and accuracy of targeted LC-MS/MS analysis.
- The data presented here can support researchers aiming at the quantification of complement system proteins, as it contains mass spectra essential for building libraries for PRM acquisition and evaluating peptide stability.
- The method introduced in this study can facilitate the increased use of targeted proteomics in serum and other biological fluids, as it does not require expertise in DIA or specific algorithms. This workflow is adaptable for experiments aiming at the quantification of one to several proteins, thereby optimizing research time and improving the quality of results through quantitative proteomics.

2. Background

Biological fluids are crucial samples for biomarker discovery. Blood serum is a protein-rich fluid, encompassing components of the complement system. These components play important roles in infectious processes, mainly in opsonization, phagocytosis, and lysis of pathogens. The levels of these proteins are directly associated with susceptibility or resistance to various human infirmities; thus, enhancing research in this field is crucial. The levels of these proteins are directly associated with susceptibility or resistance to various human diseases; thus, enhancing research in this field is essential. The PRM method is a proteomic approach that performs multi-target analysis in a single scan, increasing the likelihood of identifying relevant targets. Therefore, this study aims to propose a standardized PRM method to evaluate multiple components in biological fluids and to contribute to expanding knowledge in this field.

3. Data Description

In this study, we explored various strategies to construct a spectral library and compile a list of peptides suitable for targeted LC-MS/MS analysis applied to 12 proteins within the complement system (Supplementary Material 1). The data were generated by integrating DDA and

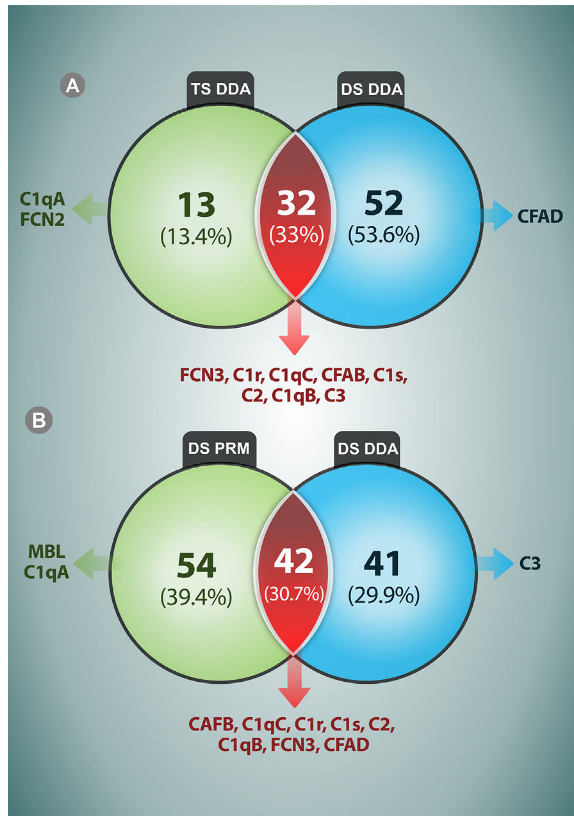


Fig. 1. Representation of peptides in various approaches to spectral library construction. A: Peptides identified by DDA from Total Serum (TS) and Depleted Serum (DS), showing exclusive and shared peptides between both groups. B: Peptides identified by PRM and DDA from Depleted Serum (DS), illustrating exclusive and shared peptides between both groups.

PRM MS acquisitions on both total and high-abundant protein-depleted sera. Low-level proteins in human serum are primary biomarker targets, and serum depletion is essential for their identification among high-level molecules [1], such as albumin. Our findings indicate that out of 418 potential precursors 166 peptide MS precursors were detected, with 53.6% of the targets exclusively identified following serum depletion. This enabled the detection of CFAD peptides (Fig. 1A), one of the less abundant complement system components, present at 1-2 µg/mL in healthy individuals [2]. The DDA method detected fewer targets compared with PRM, with exclusive identifications accounting for 29.9% of the targets. Notably, only PRM managed to detect MBL targets (Fig. 1B), found in concentrations ranging approximately from 1 to 150 µg/mL in human serum [3,4].

Combining optimal features such as intensity, library match (dot product – dotp), and MS peak area linearity across different injections, we selected 12 peptides (13 precursors) representing proteins listed in Table 1. It is worth noting that the linearity test might not accurately represent peptide behavior at varying concentrations, as serum matrix concentration was not maintained consistently across different sample masse injections. Nevertheless, this approach facilitates the selection of peptides detectable even in low sample mass injections.

The stability of the selected peptides was assessed, showing low variability in peptide MS peak areas both between analyses performed on different days and across various sample prepa-

Table 1
Selected peptides by PRM method. Peptides sequence represented with unlabeled and labeled m/z, charge, and their respective retention time.

Protein	Peptide sequence	m/z (unlabeled)	m/z (labeled)	Charge	Retention Time
C3	TIYTPGSTVLYR	685.8694	690.8735	2+	54.8
CFAB	YGLVTYATYPK	638.3346	642.3417	2+	55.4
CFAD	RPDSLQHVLLPVLDR	586.6722	593.3444	3+	65.9
C1qA	SLGFCDTTNK	571.761	575.7681	2+	34.3
C1qB	FDHVITNMNNNYEPR	932.4258	937.4299	2+	45.1
		621.9529	625.289	3+	
C1qC	FQSVFTVTR	542.7929	547.7971	2+	55.0
C1R	VLNYVDWIK	575.3188	579.3259	2+	72.7
C1S	LLEVPEGR	456.7611	461.7652	2+	38.4
C2	AVISPGFDVFAK	625.8426	629.8497	2+	70.7
MBL	FQASVATPR	488.7642	493.7683	2+	30.4
FCN2	LGEFWLGNDNIHALTAQGTSELR	848.0943	851.4304	3+	74.4
FCN3	YGIDWASGR	512.746	517.7501	2+	51.9

rations, with CV mean ranging from 0.05 for C2 to 0.50 for C1qA (Supplementary Material 2 and Table 2). Moreover, this dataset shows the impact on reproducibility by normalizing peptide MS peak areas through heavy peptides. Results indicated a decrease in CV among replicates for most peptides when heavy peptides were used for normalization, thus enhancing the reliability of replicates and the reproducibility of experiments (Supplementary Material 3). Additionally, employing synthetic labeled peptides (heavy peptides) enabled the quantification of eight complement system proteins in serum. The concentrations determined via the PRM method closely match those reported in the literature for most proteins, as detailed in Supplementary Material 4. Although the data were not obtained from a cohort analysis, the determined protein concentrations reflect a strong alignment with existing literature values. These data reinforce and complement the importance of investigating these targets in view of their association with infectious [5] and degenerative [6] diseases, among others [7], since both previously investigated peptides and new targets are mentioned in this study.

4. Experimental Design, Materials and Methods

Twelve proteins of the human complement system were targeted for this study. *In-silico* digestion was performed using the online ExPASY Translate tool to obtain the m/z ratio. The experimental approach was conducted on both depleted and non-depleted human sera, with samples analyzed by mass spectrometry, combining the PRM and DDA methods to construct a peptide library. To select the peptides for PRM, they were accessed for their uniqueness, intensity, stability, and reproducibility. Additionally, an evaluation with synthetic labelled peptides was performed to improve their quantification.

4.1. *Protein* in-silico *digestion*

We targeted 12 proteins of the human complement system for this study (Table 3).

We used the protein IDs retrieved from Uniprot to calculate the mass/charge (m/z) ratios of peptides generated by each protein, using the Expasy online tool [8] (https://web.expasy.org/peptide_mass/). The *in-silico* digestion settings included treating cysteines with Iodoacetamide (IAA), selecting peptides with a charge of 2+ and those containing histidine residue with a charge of 3+, and designating trypsin as the protease (Supplementary Material 5). We then selected peptides with m/z values ranging from 300 to 1000 to improve the MS signal response.

Table 2
Replicates' fragment peak areas variation between injections on different days.

Protein	Day	Replicate 1	Replicate 2	Replicate 3	CV	CV Mean
C3	0	9.09E+08	9.76E+08	9.09E+08	0.04	0.09
	1	8.31E+08	9.64E+08	8.79E+08	0.08	
	2	8.77E+08	7.49E+08	1.00E+09	0.15	
	3	9.58E+08	1.15E+09	1.11E+09	0.10	
CFAB	0	1.32E+08	1.69E+08	1.45E+08	0.12	0.11
	1	1.30E+08	1.58E+08	1.50E+08	0.10	
	2	1.51E+08	1.26E+08	1.46E+08	0.09	
	3	1.45E+08	1.92E+08	1.72E+08	0.14	
CFAD	0	4.95E+06	4.98E+06	4.84E+06	0.01	0.04
	1	4.74E+06	4.87E+06	4.54E+06	0.04	
	2	4.54E+06	4.86E+06	4.84E+06	0.04	
	3	5.53E+06	6.47E+06	6.07E+06	0.08	
C1qA	0	2.16E+07	7.63E+06	2.51E+07	0.51	0.50
	1	2.11E+07	7.80E+06	1.98E+07	0.45	
	2	1.77E+07	6.34E+06	2.25E+07	0.54	
	3	2.01E+07	7.34E+06	2.21E+07	0.48	
C1qB	0	1.59E+07	1.53E+07	1.71E+07	0.06	0.09
	1	1.66E+07	1.52E+07	1.47E+07	0.06	
	2	1.61E+07	1.53E+07	1.78E+07	0.08	
	3	1.99E+07	2.53E+07	1.88E+07	0.16	
C1qC	0	1.84E+08	1.83E+08	1.80E+08	0.01	0.08
	1	1.79E+08	1.74E+08	1.77E+08	0.01	
	2	1.81E+08	1.49E+08	1.85E+08	0.11	
	3	1.70E+08	2.32E+08	1.87E+08	0.16	
C1r	0	1.60E+07	1.75E+07	1.85E+07	0.07	0.07
	1	1.71E+07	1.70E+07	1.57E+07	0.05	
	2	1.62E+07	1.57E+07	1.63E+07	0.02	
	3	1.67E+07	1.94E+07	1.51E+07	0.13	
C1s	0	7.13E+07	4.75E+07	7.04E+07	0.21	0.22
	1	7.15E+07	4.55E+07	6.61E+07	0.23	
	2	6.52E+07	4.04E+07	6.98E+07	0.27	
	3	7.23E+07	5.01E+07	6.86E+07	0.19	
C2	0	1.96E+07	2.21E+07	2.16E+07	0.06	0.06
	1	2.07E+07	2.35E+07	1.92E+07	0.10	
	2	2.00E+07	2.16E+07	1.98E+07	0.05	
	3	2.12E+07	2.30E+07	2.24E+07	0.04	
MBL	0	3.03E+06	1.82E+06	3.41E+06	0.30	0.31
	1	3.06E+06	1.74E+06	3.39E+06	0.32	
	2	2.91E+06	1.45E+06	3.36E+06	0.39	
	3	3.10E+06	1.98E+06	3.18E+06	0.24	
FCN 2	0	5.01E+06	5.11E+06	4.13E+06	0.11	0.15
	1	5.04E+06	4.18E+06	3.40E+06	0.20	
	2	3.89E+06	3.76E+06	3.54E+06	0.05	
	3	3.57E+06	5.75E+06	4.83E+06	0.23	
FCN3	0	5.77E+07	5.53E+07	5.73E+07	0.02	0.16
	1	5.50E+07	5.06E+07	4.80E+07	0.07	
	2	4.64E+07	3.82E+07	5.76E+07	0.21	
	3	4.82E+07	3.21E+07	6.51E+07	0.34	

4.2. Sample preparation

Human samples of non-depleted serum (total) serum and depleted serum, stored at -80°C, were utilized. Serum depletion was performed using the Agilent Human 14 Multiple Affinity Removal System 5188-6560 kit (Agilent Technologies®), following the manufacturer's instructions. The depleted serum was processed on a Microcon® 5 kDa centrifugal filter and washed with Tris-HCl (pH 7.5). The volume retained was quantified by tryptophan fluorescence, and a volume corresponding to 40 µg of protein was dried in a Speedvac. Disulfide bonds were reduced with

Table 3
Protein targets assessed in the present study.

Protein	Complement pathway*	Uniprot ID	Theoretical precursors (*in silico*)	Precursors with MS2	Precursors after selection
C3	All	P01024	107	43	1
CFAB	AP	P00751	40	25	1
CFAD	AP	P00746	13	1	1
C1qA	CP	P02745	10	2	1
C1qB	CP	P02746	14	8	2
C1qC	CP	P02747	13	8	1
C1r	CP	P00736	38	20	1
C1s	CP	P09871	28	19	1
C2	CP	P06681	52	23	1
MBL	LP	P11226	13	7	1
FCN 2	LP	Q1548	18	2	1
FCN 3	LP	O7563	20	8	1

*AP: Alternative pathway; CP: Classical pathway; LP: Lectin pathway.

5 mM dithiothreitol (DTT) and 8 M urea, followed by incubation with 15 mM iodoacetamide (IAA). The mixture was then diluted with 50 mM ammonium bicarbonate to reduce urea concentration to 1 M, and trypsin was added at a 1:50 protease-to-protein mass ratio for incubation at 37°C for 16-18 h. Digestion was halted with 0.5% trifluoroacetic acid (TFA), and the samples were desalted using C18 Stage Tips.

4.3. LC-MS/MS analysis

Samples were analyzed at the mass spectrometry facility RPT02H / Carlos Chagas Institute, Fiocruz state of Paraná, Brazil. Reversed-phase liquid chromatography (LC) was performed using nano chromatography at a flow rate of 250 nL/min. Sample volumes ranging from 62.5 to 500 ng were injected onto a C18 in-house packed emitter (75 µm I.D., 150 mm length, and 3 µm particles, Dr. Maisch®). Phase A consisted of 0.1% formic acid, and phase B of 95% acetonitrile, 0.1% formic acid. Separation was achieved on an Ultimate 3000 RSLC (Thermo Scientific) using a linear gradient from 5 to 40% of phase B over 120 min. For the nano electrospray ionization, 2.3 kV was applied. Two MS acquisition methods were utilized: DDA or PRM, on an Orbitrap Fusion Lumos (for spectral library runs) or an Orbitrap Exploris 120 spectrometer (both from Thermo Scientific).

In DDA, MS1 and MS2 scans were performed in the Orbitrap analyzer at resolutions of 120,000 and 15,000, respectively, with a MS1 scan range of 300 – 1500 m/z. AGC settings were standard for MS1 and 3 × 104 for MS2, with maximum injection times of 50 ms for MS1 and 22 ms for MS2. The most intense ions underwent MS2 analysis (max. of 2 s per cycle) using HCD fragmentation with a normalized collision energy of 30%; a dynamic exclusion list of 60 s was used; internal calibration was enabled for MS1 analysis.

For PRM, MS1 and MS2 scans were acquired in the Orbitrap spectrometer at resolutions of 120,000 and 30,000, respectively, with a scan range of 350 – 1050 m/z. AGC settings were standard for MS1 and targeted MS2, with maximum injection times of 50 ms for MS1 and 54 ms for MS2. Targeted MS2 scans were set for the list of selected peptides, with peptide isolation occurring in the quadrupole using a 1.6 Da window and HCD fragmentation at a normalized collision energy of 30%. Each scan cycle included the full target list (loop control), and internal calibration was enabled for the MS1 scans.

The acquired spectra were analyzed using MaxQuant 2.2.0.0 [9,10] and/or Skyline 21.1.0.146 [11] software. In MaxQuant, specific trypsin search was configured, with methionine oxidation and protein N-terminal acetylation as variable modifications, and cysteine carbamidomethylation as a fixed modification. The PSM search utilized the UniProt human database, containing 81,791

entries (downloaded on April 24, 2023). Skyline setup details are provided in Supplementary Material 6. Mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium via the PRIDE partner repository [12] with the dataset identifier PXD045089.

4.4. Building the peptide spectral library in Skyline

The peptide library was established from five different injections, matching different sample preparation and MS acquisition methods: Total Serum (TS) using DDA and PRM (150 targets and 53 targets), and Depleted Serum (DS) using DDA and PRM (311 targets). The raw data, MaxQuant msms.txt, and mqpar.xml files related to these five runs were used to create independent libraries in Skyline. These libraries were used independently to verify the precursors identified in each run or all together for peptide selection.

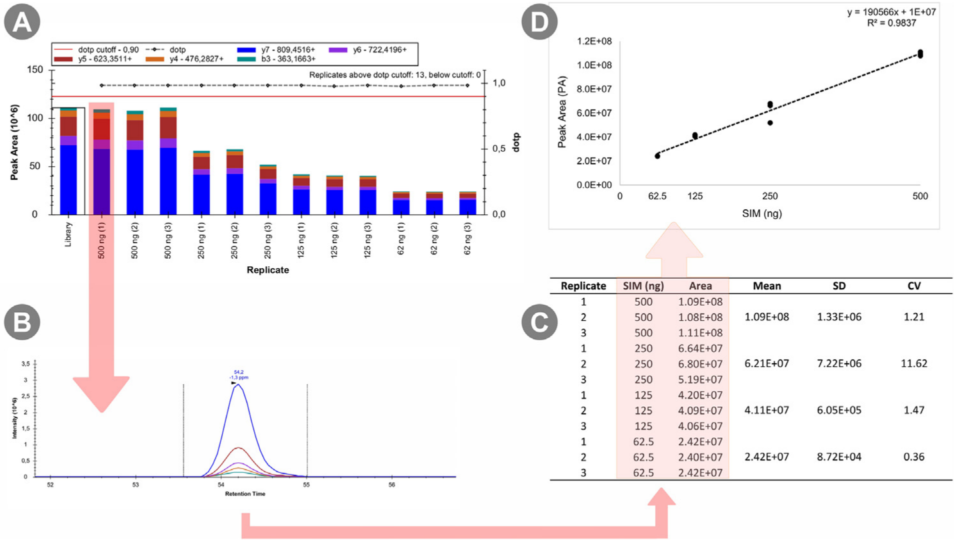
4.5. Peptide selection

To select the best peptides for PRM analysis, three steps were followed:

- Step 1: The MaxQuant peptide tables obtained from the search utilizing the five runs mentioned were used to verify the uniqueness of the *in-silico* peptide target list – peptides that are not common to more than one protein in the human database, ensuring the representativeness of the protein in the sample. Then the peptide-targeted unique sequences were imported into the Skyline software for spectrum visualization.
- Step 2: The five runs used in the spectrum library were also imported into Skyline as "results". This allows for the selection of only those peptides with a high MS peak area (sum of fragment areas), at least five detected transitions, and an absence of cysteine and methionine.
- Step 3: Total serum samples were submitted to PRM analysis (targets selected in Step 2) in four different sample injection masses (SIM) in triplicate of injection: 500, 250, 125, and 62.5 ng, assessing the peptides' intensity variations (Fig. 2A). The peptide fragmentation patterns were compared to the spectral library by analyzing the dotp value. In Skyline, the chromatograms were inspected to confirm that each peptide eluted at a similar retention time across the injections (Fig. 2B). The dotp value, which corresponds to the similarity of the experimental spectra with the spectral library, ranging from 0 to 1, was also considered. For selection, peptides with both higher dotp and MS peak area values were prioritized. For each peptide, the Peak Area (PA) was extracted and transferred to Microsoft Excel® to calculate the mean (M), standard deviation (SD), and coefficient of variation (CV) for three replicates of each injected mass (Fig. 2C). From SIM and peptide PA values, a trend line was obtained, following the line equation and R^2 estimation (Fig. 2D). Aiming for one peptide per protein, peptides detected across all SIMs and presenting the highest linearity were selected.

4.6. Peptide stability and sample preparation reproducibility

Peptide stability and sample preparation reproducibility were assessed by pooling three human serum samples and preparing them in triplicate according to Section 2.2. The samples were injected in duplicate on four different days (day 0 to day 3), following the procedure described in Section 2.3. On day 0, the samples were placed into the autosampler at 7°C and remained there until the end of the test on day 3. The raw data were imported into Skyline, and the PAs were obtained and exported to Excel. The mean (M), standard deviation (SD), and coefficient of variation (CV) were calculated, enabling the assessment of the MS peak area variation of each peptide across the different days.



Replicate	SIM (ng)	Area	Mean	SD	CV
1	500	1.09E+08			
2	500	1.08E+08	1.09E+08	1.33E+06	1.21
3	500	1.11E+08			
1	250	6.64E+07			
2	250	6.80E+07	6.21E+07	7.22E+06	11.62
3	250	5.19E+07			
1	125	4.20E+07			
2	125	4.09E+07	4.11E+07	6.05E+05	1.47
3	125	4.06E+07			
1	62.5	2.42E+07			
2	62.5	2.40E+07	2.42E+07	8.72E+04	0.36
3	62.5	2.42E+07			

Fig. 2. Peptide intensity linearity. A: Peptide peak areas from Skyline. Injection at four different Sample Injected Masses (SIM): 500, 250, 125, and 62.5 ng, in triplicate, compared to the library. B: Chromatogram from Skyline displaying all the peptide fragment XICs in different colors. C: Data exported from Skyline, including Sample Injected Mass (SIM), MS Peak Area (Area), mean of triplicates, Standard Deviation (SD), and Coefficient of Variation (CV). D: Scatter plot of Peak Area (PA) *vs*. Sample Injected Mass (SIM) for the triplicates of the four different Sample Injected Masses (SIM), showing the trend line equation and R^2 value. Example: FQSVFTVTR peptide (C1qC protein).

4.7. Synthetic labelled peptides

Synthetic labelled peptides [13C,15N in lysine (K) and arginine (R) residues] were customized (Synbio Technologies) to improve the quantification of targets and decrease the CV along the replicates. This methodology was applied to peptides such as C1qC, C1s, C2, C3, CFAB, FCN2, FCN3, and MBL. Lyophilized peptides were reconstituted in ultrapure water according to the manufacturer's instructions to a concentration of 1 µg/µL (stock solution). This solution was diluted 100 times, and the peptides were pooled at 0.5 pmol/uL each and analyzed by PRM, either mixed or not with the same three serum samples used for the stability test. The results were evaluated in Skyline using the settings described in Section 2.5. The light-to-heavy peak area ratio for each peptide was obtained, and the CV between samples was calculated and compared with those obtained on day 0 of the stability test (without mixing with the synthetic peptides).

This workflow is outlined in Supplementary Material 7.

Limitations

Not applicable.

Data Availability

PXD045089 (Original data) (Pride).

Ethics Statement

The study was approved by the Research Ethics Committee of FIOCruz under protocol CEP FIOCruz/IOC 5.018.434 and was performed following the Code of Ethics of the World Medical Association (Declaration of Helsinki).

CRediT Author Statement

TCT, KCM, PS, MB and FBF: Study conception and design. TCT, KCM and MB: Data collection. TCT, KCM, and MB: Data analysis. TCT, KCM and MB: Data interpretation. TCT and MB: Drafting of the manuscript. KCM, PS and FBF: Manuscript revison. MB and FBF Study supervision. All authors contributed to the article and approved the submitted version.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2024.110217.

References

[1] A. Zougman, J.P. Wilson, RE. Banks, A simple serum depletion method for proteomics analysis, Biotechniques 69 (2) (2020) 149–152.
[2] M.L. Henriksen, C. Nielsen, D. Pedersen, G.R. Andersen, S. Thiel, Y. Palarasah, et al., Quantification of the pro-form of human complement component factor D (adipsin), J. Immunol. Methods (2022) 507.
[3] M. Káplár, S. Sweni, J. Kulcsár, B. Cogoi, R. Esze, S. Somodi, et al., Mannose-binding lectin levels and carotid intima–media thickness in Type 2 diabetic patients, J. Diabetes Res. 2016 (2016).
[4] A.K. Abbas, A.H. Lichtman, S. Pillai, Imunologia Celular e molecular, Mecanismos Efetores da Imunidade Humoral 8 (2015).
[5] E. Willems, W. Alkema, J. Keizer-Garritsen, A. Suppers, M. van der Flier, R. Philipsen, et al., Biosynthetic homeostasis and resilience of the complement system in health and infectious disease, EBioMedicine 45 (2019) 303–313.
[6] I.E. Acar, E. Willems, E. Kersten, J. Keizer-Garritsen, E. Kragt, B. Bakker, et al., Semi-quantitative multiplex profiling of the complement system identifies associations of complement proteins with genetic variants and metabolites in age-related macular degeneration, J. Pers. Med. 11 (12) (2021).
[7] E. Willems, L. Lorés-Motta, A. Zanichelli, C. Suffritti, M. van der Flier, R.G. van der Molen, et al., Quantitative multiplex profiling of the complement system to diagnose complement-mediated diseases, Clin. Transl. Immunol. 9 (12) (2020).
[8] E. Gasteiger, A. Gattiker, C. Hoogland, I. Ivanyi, R.D. Appel, BA. ExPASy, The proteomics server for in-depth protein knowledge and analysis, Nucleic Acids Res. 31 (13) (2003) 3784–3788.

[9] J. Cox, M. Mann, MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification, Nat. Biotechnol. 26 (12) (2008) 1367–1372.
[10] J. Cox, N. Neuhauser, A. Michalski, R.A. Scheltema, JV. Olsen, M. Mann, Andromeda: a peptide search engine integrated into the MaxQuant environment, J. Proteome Res. 10 (4) (2011) 1794–1805.
[11] B. MacLean, D.M. Tomazela, N. Shulman, M. Chambers, G.L. Finney, B. Frewen, et al., Skyline: an open source document editor for creating and analyzing targeted proteomics experiments, Bioinformatics 26 (7) (2010) 966–968.
[12] Y. Perez-Riverol, J. Bai, C. Bandla, D. García-Seisdedos, S. Hewapathirana, S. Kamatchinathan, et al., The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences, Nucleic Acids Res. 50 (D1) (2022) D543–D552.