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A Semi Quantitative Method for the Analysis of Tryptic Peptides in Human Serum: A Rapid, Targeted UPLC-MS/MS Approach Using Biognosys Plasma Dive Kit

Billy Joe Molloy
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Targeted, semi-quantitative UPLC-MS/MS analysis of 100 tryptic peptides
- High throughput analysis means larger sample sets can be analyzed
- Use of a generic LC-MS configuration yields versatility for switching from one compound class to another

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KEYWORDS

Targeted, tryptic peptides, proteins, UPLC, tandem quadrupole, Xevo TQ-S micro, Multiple Reaction Monitoring (MRM), human serum, proteomics

INTRODUCTION

Proteins are important molecules that are involved in almost all biological processes. They are large, high molecular weight molecules, and therefore are analyzed using marker peptides that are produced using proteolytic enzymes like trypsin. Historically these types of analyses have been performed using high-resolution mass spectrometry coupled with micro/nano flow chromatographic systems. These methodologies however are low throughput, and are not suitable for large cohorts of samples. Here we demonstrate a high-throughput UPLC-MS/MS research method for the semi-quantitative analysis of various tryptic peptides in non-depleted, tryptically digested human serum samples. This application note is part of a Targeted Omics Method Package.

EXPERIMENTAL

Human serum sample preparation

Human serum samples were prepared using the Biognosys PlasmaDive Kit (Biognosys, Schlieren, Switzerland). Briefly, 10 µL of sample was denatured, reduced, and alkylated before being diluted and typtically digested using 5 µL of 0.4 µg/µL typsin. Following acidification, centrifugation, and the addition of a fixed amount of the stable labeled forms of all 100 marker peptides, 6 µL of the spiked supernatant was then injected onto the UPLC-MS/MS system.

LC conditions

UPLC separation was performed with an ACQUITY UPLC I-Class System (fixed loop), equipped with a CORTECS T3 2.7 µm (2.1 × 30 mm) analytical column. A sample of 6 µL was injected at a flow rate of 0.15 mL/min. Mobile phase A was 0.01% formic acid $_{(aq)}$ containing 0.2 mM Ammonium Formate and mobile phase B was 50% isopropanol in acetonitrile containing 0.01% formic acid and 0.2 mM Ammonium Formate. After an initial 2.5-minute hold at 1% Mobile phase B, the tryptic peptides were eluted from the column and separated with a gradient of 1–45% Mobile phase B over 2.9 minutes, followed by a 2.5-minute column wash at 85% Mobile phase B. The column was then re-equilibrated to initial conditions. The analytical column temperature was maintained at 60 °C.

MS conditions

Multiple Reaction Monitoring (MRM) analyses were performed using a Xevo TQ-S micro tandem quadrupole Mass Spectrometer. All experiments were performed in positive electrospray ionization (ESI+) mode. The ion source temperature and capillary voltage were kept constant and set to 150 °C and 2.0 kV respectively. The cone gas flow rate was 50 L/hr and desolvation temperature was 650 °C. Cone voltages and collision energies used were those calculated by the Skyline software (MacCoss Lab, University of Washington).

Informatics

Method information was imported onto the LC-MS system using the Quanpedia functionality within MassLynx. This extendable and searchable database produces LC and MS methods as well as processing methods for use in TargetLynx for compound quantification. Skyline was used for the production of MS methods and data visualization.

RESULTS

Table 1 details the 100 marker peptides analyzed, the proteins they represent, and the b and y product ions monitored. Tryptic peptides were detected using a series of MRM transitions. The product ions monitored are detailed in Table 1. These were all singly charged ions, with the exception of the y8 ion for P06276, where both singly and doubly charged ions were monitored. The precursor ions used were the doubly charged ions for all marker peptides, with the exception of P08603 and Q9PD5, where the triply charged precursors were used.

UniProt ID	Description	Peptide sequence	b/y ions monitored
P02763	Alpha-1-acid glycoprotein 1 (Orosomucoid-1)	SDVVYTDWK	y5, y6, and y7
P19652	Alpha-1-acid glycoprotein 2 (Orosomucoid-2)	EHVAHLLFLR	y6, y7, and y8
P01009	Alpha-1-antitrypsin	SVLGQLGITK	y4, y7, and y8
P04217	Alpha-1B-glycoprotein	LLELTGPK	y4, y6, and y7
P08697	Alpha-2-antiplasmin (Serpin F2)	LFGPDLK	Y3, y4, and y5
P02750	Leucine-rich alpha-2-glycoprotein (LRG)	VAAGAFQGLR	y5, y7, and y8
P01023	Alpha-2-macroglobulin (Alpha-2-M)	AIGYLNTGYQR	y6, y7, and y9
P01011	Alpha-1-antichymotrypsin (ACT)	EIGELYLPK	y3, y5, and y7
P43652	Afamin (Alpha-albumin)	AESPEVCFNEESPK	y8, y9, and y11
P02768	Serum albumin	YLYEIAR	y4, y5, and y6
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	LEYLLLSR	y5, y6, and y7
P02760	Protein AMBP	TVAACNLPIVR	y6, y7, and y9
P01019	Angiotensinogen (Serpin A8)	ALQDQLVLVAAK	y6, y9, and y10
P01008	Antithrombin-III (Serpin C1)	EVPLNTIIFMGR	y5, y6, and y8
P02647	Apolipoprotein A-I	VSFLSALEEYTK	y7, y8, and y9
P02652	Apolipoprotein A-II	EQLTPLIK	y4, y5, and y6
P06727	Apolipoprotein A-IV	LAPLAEDVR	y4, y5, and y7
P04114	Apolipoprotein B-100	FSVPAGIVIPSFQALTAR	y9, y10, and y11
P02654	Apolipoprotein C-I	EFGNTLEDK	y4, y5, and y7
P02655	Apolipoprotein C-II	TAAQNLYEK	y5, y6, and y7
P02656	Apolipoprotein C-III	GWVTDGFSSLK	y6, y7, and y9
P05090	Apolipoprotein D	NILTSNNIDVK	y7, y8, and y9
P02649	Apolipoprotein E	AATVGSLAGQPLQER	y5, y7, and y11
P02749	Apolipoprotein H	VCPFAGILENGAVR	y7, y10, and y12
O14791	Apolipoprotein L1	VTEPISAESGEQVER	y7, y8, and y10
O95445	Apolipoprotein M	FLLYNR	y3, y4, and y5
P43251	Biotinidase	SHLIIAQVAK	y6, y7, and y8
P02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	y5, y6, and y8
P02746	Complement C1q subcomponent subunit B	GNLCVNLMR	y5, y6, and y7
P02747	Complement C1q subcomponent subunit C	FQSVFTVTR	y5, y6, and y7
P00736	Complement C1r subcomponent	GLTLHLK	y3, y4, and y5
P09871	Complement C1s subcomponent	TNFDNDIALVR	y5, y7, and y8
P04003	C_4b-binding protein alpha chain	GYILVGQAK	y4, y5, and y6
P08185	Corticosteroid-binding globulin (Serpin A6)	GTWTQPFDLASTR	y4, y5, and y8
O43866	CD5 antigen-like (CT-2) (SP-alpha)	IWLDNVR	y4, y5, and y6
P00450	Ceruloplasmin (Ferroxidase)	DIASGLIGPLIICK	y6, y7, and y8
P00751	Complement factor B	YGLVTYATYPK	y6, y7, and y8
P08603	Complement factor H (H factor 1)	IDVHLVPDR	y3, y4, and y5
P05156	Complement factor I	IVIEYVDR	y4, y6, and y7
P06276	Cholinesterase (EC 3.1.1.8)	IFFPGVSEFGK	y5 and y8(#)
P10909	Clusterin (Aging-associated gene 4 protein) (Apolipoprotein J)	ASSIIDELFQDR	y4, y7, and y8
P06681	Complement C2	AVISPGFDVFAK	y7, y8, and y9
P01024	Complement C3	GYTQQLAFR	y3, y5, and y7
P0C0L4	Complement C_4-A	PVAFSVVPTAAAAVSLK	b4, y10, and y11
P01031	Complement C5	TDAPDLPEENQAR	y7, y8, and y10
P07357	Complement component C_8 alpha chain	HTSLGPLEAK	y6, y8, and y9
P02748	Complement component C9	LSPIYNLVPVK	y3, y7, and y9
P02775	Platelet basic protein (C-X-C motif chemokine 7)	NIQSLEVIGK	y4, y7, and y8
P00488	Coagulation factor XIII A chain	STVLTIPEIIIK	y3, y7, and y8
P05160	Coagulation factor XIII B chain	IAQYYYTFK	y4, y6, and y8
P00742	Coagulation factor X	ACIPTGPYPCGK	y6, y7, and y9
P00740	Coagulation factor IX	SALVLQYLR	y5, y6, and y7
P23142	Fibulin-1	TGYYFDGISR	y4, y6, and y7
P02765	Alpha-2-HS-glycoprotein	FSVVYAK	y4, y5, and y6
Q9UGM5	Fetuin-B	LVVLPFPK	y4, y5, and y6
P02671	Fibrinogen alpha chain	GSESGIFTNTK	y5, y7, and y8
P02679	Fibrinogen gamma chain	DNCCILDER	y4, y5, and y6
P02751	Fibronectin (FN)	SYTITGLQPGTDYK	y6, y9, and y10
P06396	Gelsolin (Actin-depolymerizing factor)	AGALNSNDAFVLK	y7, y8, and y9
P22352	Glutathione peroxidase 3	FLVGPDGIPIMR	y4, y8, and y9
P68871	Hemoglobin subunit beta	VNVDEVGGEALGR	y7, y8, and y10
P02042	Hemoglobin subunit delta (Delta-globin)	LLGNVLVCVLAR	y5, y6, and y7
P02790	Hemopexin (Beta-1B-glycoprotein)	NFPSPVDAAFR	y5, y7, and y8
P05546	Heparin cofactor 2	TLEAQLTPR	y5, y6, and y7
P00738	Haptoglobin	VTSIQDWVQK	y5, y6, and y8
P00739	Haptoglobin-related protein	VGYVSGWGQSDNFK	y7, y9, and y10
P04196	Histidine-rich glycoprotein	GGEGTGYFVDFSVR	y5, y6, and y7
P05155	Plasma protease C1 inhibitor	LLDSLPSDTR	y5, y7, and y8
P01876	Ig alpha-1 chain C region	TPLTATLSK	y5, y6, and y7
P01877	Ig alpha-2 chain C region	DASGATFTWTPSSGK	y5, y6, and y8
P01857	Ig gamma-1 chain C region	GPSVFPLAPSSK	y4 and y7
P01859	Ig gamma-2 chain C region	GLPAPIEK	y4, y5, and y6

UniProt ID	Description	Peptide sequence	b/y ions monitored
P01860	Ig gamma-3 chain C region (HDC)	WYVDGVEVHNAK	y9, y10, and y11
P01871	Ig mu chain C region	YAATSQVLLPSK	y3, y4, and y10
P05154	Plasma serine protease inhibitor	TLYLADTFPTNFR	y5, y8, and y9
P19827	Inter-alpha-trypsin inhibitor heavy chain H1	AAISGENAGLVR	y6, y8, and y9
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	FYNQVSTPLLR	y4, y6, and y7
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	ILDDLSPR	y5, y6, and y7
P29622	Kallistatin (Kallikrein inhibitor)	LGFTDLFSK	y6, y7, and y8
P03952	Plasma kallikrein	IAYGTQGSSGYSLR	y7, y9, and y11
P01042	Kininogen-1	YFIDFVAR	y4, y5, and y6
P36955	Pigment epithelium-derived factor (PEDF)	ELLDTVTAPQK	y5, y7, and y8
Q96PD5	N-acetylmuramoyl-L-alanine amidase	AGLLRPDYALLGHR	y3, y4, and y5
P02776	Platelet factor 4 (Oncostatin-A)	ICLDLQAPLYK	y5, y8, and y9
P00747	Plasminogen	HSIFTPETNPR	y6, y8, and y9
P27169	Serum paraoxonase	LLIGTVFHK	y5, y6, and y7
Q92954	Proteoglycan 4	GLPNVVTSAISLPNIR	y4, y6, and y10
P02753	Retinol-binding protein 4	FSGTWYAMAK	y4, y8, and y9
P35542	Serum amyloid A-4 protein	EALQGVGDMGR	y5, y7, and y8
P49908	Selenoprotein P	LPTDSELAPR	y6, y7, and y8
P04278	Sex hormone-binding globulin	IALGGLLFPASNLR	y6, y7, and y8
P05452	Tetranectin	LDTLAQEVALLK	b3, y7, and y8
P05543	Thyroxine-binding globulin (Serpin A7)	NALALFVLPK	y6, y7, and y8
P00734	Prothrombin	TATSEYQTFFNPR	y5, y7, and y8
P02787	Serotransferrin	EGYYGYTGAFR	y5, y6, and y8
P02766	Transthyretin	AADDTWEPFASGK	y6, y7, and y8
P02774	Vitamin D-binding protein	HLSLLTTLSNR	y6, y7, and y9
P04004	Vitronectin	FEDGVLDPDYPR	y5, y6, and y7
P04275	von Willebrand factor	ILAGPAGDSNVVK	y9, y10, and y11
P25311	Zinc-alpha-2-glycoprotein	AYLEEECPATLR	y5, y6, and y8

 # - Both singly and doubly charged ions monitored for.

Table 1. Names, Uniprot ID's, and marker peptides used for the 100 proteins monitored using the Biognosys Plasma Dive kit. Column 4 details the b and y product ions monitored for the given marker peptide.

Figures 1 and 2 show example data acquired for six of the 100 proteins. Data for these 100 proteins was acquired over two analyses. Each 12-minute analysis was capable of analyzing 50 marker peptides, where three transitions were monitored for both the native and stable labeled forms. If fewer transitions per peptide were monitored, it would be possible to analyze more proteins in a single injection. However, monitoring three transitions per peptide increased the confidence in identification.

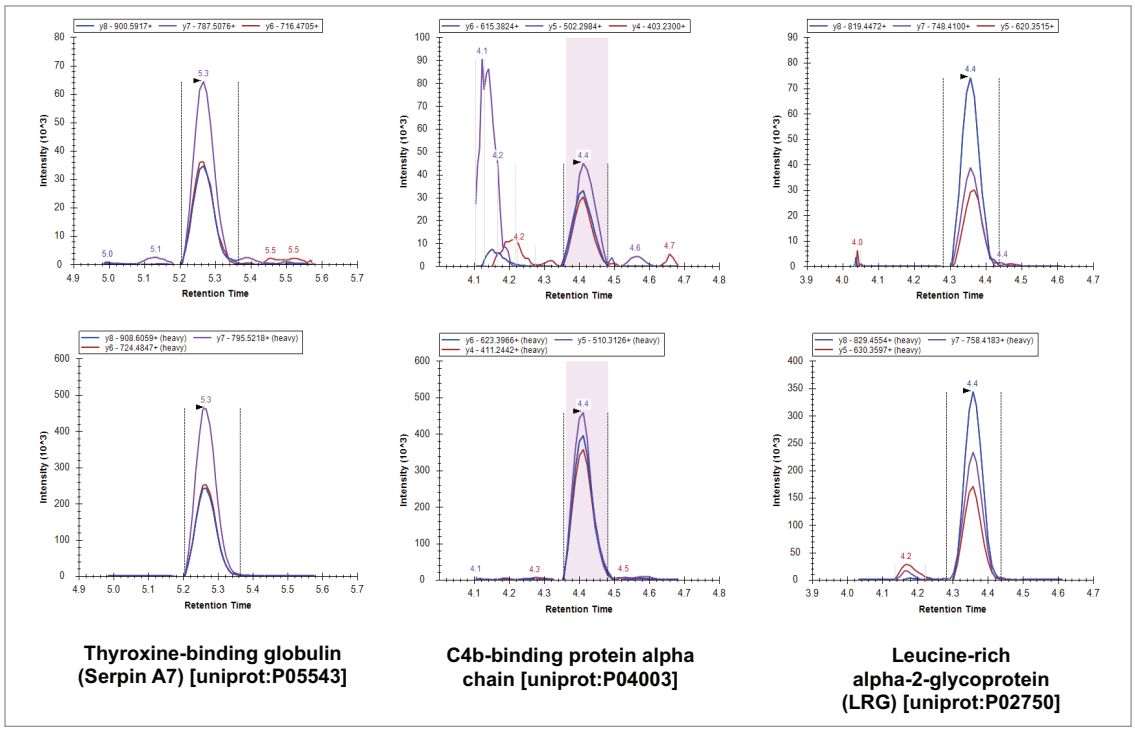
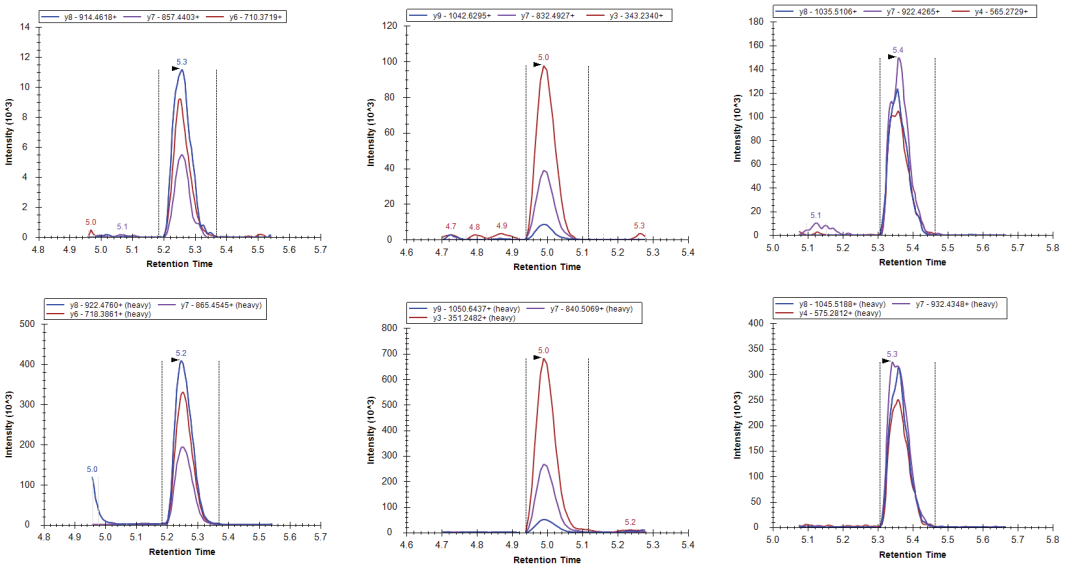


Figure 1. Example chromatograms acquired for three of the 100 proteins analyzed using the Biognosys PlasmaDive kit. (uniprot: P05543, P04003, and P02750). The upper chromatograms show the three transitions for the native peptide, and the lower chromatogram the stable labeled (heavy) reference peptide.



Kallistatin (Kallikrein inhibitor) [uniprot: P29622]

Complement component C9 [uniprot: P02748]

Clusterin (Aging-associated gene 4 protein) (Apolipoprotein J) [uniprot: P10909]

Figure 2. Example chromatograms acquired for three of the 100 proteins analyzed using the Biognosys PlasmaDive kit. (uniprot: P29622, P02748, and P10909). The upper chromatograms shows the three transitions for the native peptide, and the lower chromatogram the stable labeled (heavy) reference peptide.

CONCLUSIONS

A rapid UPLC-MS/MS methodology has been developed for the research analysis of proteins. This method has been demonstrated to be suitable for the analysis of physiologically relevant levels of multiple proteins in human serum. This method utilizes a generic LC-MS platform that can be used for various compound classes (including metabolomics, lipidomics, and proteomics). Deployment of this method in conjunction with other complementary methods available on the Waters MetaboQuan™ website can form the basis of a comprehensive suite of targeted multi-omic workflows.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
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