

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

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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

Abstract

This application note demonstrates a high-throughput UPLC-MS/MS research method for the semi-quantitative analysis of various tryptic peptides in human serum samples.

Benefits

- · Targeted, semi-quantitativeUPLC-MS/MS analysis of100 tryptic peptides
- · High throughput analysis meanslarger sample sets can be analyzed
- · Use of a generic LC-MS configurationyields versatility for switching fromone compound class to another

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 partof a Targeted Omics Method Package.

Experimental

Human serum sample preparation

Human serum samples were prepared using the Biognosys Plasma Dive Kit (Biognosys, Schlieren, Switzerland). Briefly, 10 μ L ofsample was denatured, reduced, and alkylated before being diluted and typtically digested using 5 μ L of 0.4 μ g/ μ L typsin. Following acidification, centrifugation, and the addition of a fixed amount of the stable labeled forms of all 100 marker peptides, 6 μ L 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 μ m (2.1 × 30 mm) analytical column. A sample of 6 μ L 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 condition

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 extend able 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 and Discussion

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.

P02763 P19652	Description	Peptide sequence	b/y ions monitored
	Alpha-1-acid glycoprotein 1 (Orosomucoid-1)	SDVVYTDWK	y5, y6, and y7
P01009	Alpha-1-acid glycoprotein 2 (Orosomucoid-2)	EHVAHLLFLR SVLGQLGITK	y6, y7, and y8 y4, y7, and y8
P04217	Alpha-1-antitrypsin Alpha-1B-glycoprotein	LLELTGPK	y4, y7, and y8 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
43652	Afamin (Alpha-albumin)	AESPEVCFNEESPK	y8, y9, and y11
02768	Serum albumin	YLYEIAR	y4, y5, and y6
35858	Insulin-like growth factor-binding protein complex acid labile subunit	LEYLLLSR	y5, y6, and y7
02760 01019	Protein AMBP Angiotensinogen (Serpin A8)	TVAACNLPIVR ALQDQLVLVAAK	y6, y7, and y9 y6, y9, and y10
201008	Angiotensnogen (Serpin Ab) Antithrombin-III (Serpin C1)	EVPLNTIIFMGR	y5, y6, and y8
02647	Apolipoprotein A-I	VSFLSALEEYTK	y7, y8, and y9
02652	Apolipoprotein A-II	EQLTPLIK	y4, y5, and y6
06727	Apolipoprotein A-IV	LAPLAEDVR	y4, y5, and y7
P04114	Apolipoprotein B-100	FSVPAGIVIPSFQALTAR	y9, y10, and y11
02654	Apolipoprotein C-I	EFGNTLEDK	y4, y5, and y7
02655	Apolipoprotein C-II	TAAQNLYEK	y5, y6, and y7
02656	Apolipoprotein C-III Apolipoprotein D	GWVTDGFSSLK	y6, y7, and y9
05090	Apolipoprotein D Apolipoprotein E	NILTSNNIDVK AATVGSLAGQPLQER	y7, y8, and y9 y5, y7, and y11
02049	Apolipoprotein H	VCPFAGILENGAVR	y7, y10, and y12
02743	Apolipoprotein L1	VTEPISAESGEQVER	y7, y8, and y10
95445	Apolipoprotein M	FLLYNR	y3, y4, and y5
43251	Biotinidase	SHLIIAQVAK	y6, y7, and y8
02745	Complement C1q subcomponent subunit A	SLGFCDTTNK	y5, y6, and y8
02746	Complement C1q subcomponent subunit B	GNLCVNLMR	y5, y6, and y7
02747	Complement C1q subcomponent subunit C	FQSVFTVTR	y5, y6, and y7
00736	Complement C1r subcomponent	GLTLHLK	y3, y4, and y5
09871	Complement C1s subcomponent	TNFDNDIALVR	y5, y7, and y8
04003 08185	C₄b-binding protein alpha chain Corticosteroid-binding globulin (Serpin A6)	GYILVGQAK GTWTQPFDLASTR	y4, y5, and y6
43866	CD5 antigen-like (CT-2) (SP-alpha)	IWLDNVR	y4, y5, and y8 y4, y5, and y6
00450	Ceruloplasmin (Ferroxidase)	DIASGLIGPLIICK	y6, y7, and y8
P00751	Complement factor B	YGLVTYATYPK	y6, y7, and y8
08603	Complement factor H (H factor 1)	IDVHLVPDR	y3, y4, and y5
P05156	Complement factor I	IVIEYVDR	y4, y6, and y7
06276	Cholinesterase (EC 3.1.1.8)	IFFPGVSEFGK	y5 and y8(#)
10909	Clusterin (Aging-associated gene 4 protein) (Apolipoprotein J)	ASSIIDELFQDR	y4, y7, and y8
206681	Complement C2	AVISPGFDVFAK	y7, y8, and y9
01024 0C0L4	Complement C3	GYTQQLAFR PVAFSVVPTAAAAVSLK	y3, y5, and y7 b4, y10, and y11
P01031	Complement C4-A Complement C5	TDAPDLPEENQAR	y7, y8, and y10
07357	Complement component C ₈ alpha chain	HTSLGPLEAK	y6, y8, and y9
02748	Complement component C9	LSPIYNLVPVK	y3, y7, and y9
02775	Platelet basic protein (C-X-C motif chemokine 7)	NIQSLEVIGK	y4, y7, and y8
00488	Coagulation factor XIII A chain	STVLTIPEIIIK	y3, y7, and y8
P05160	Coagulation factor XIII B chain	IAQYYYTFK	y4, y6, and y8
00742	Coagulation factor X	ACIPTGPYPCGK	y6, y7, and y9
200740	Coagulation factor IX	SALVLQYLR	y5, y6, and y7
P23142	Fibulin-1	TGYYFDGISR	y4, y6, and y7
902765 9UGM5	Alpha-2-HS-glycoprotein Fetuin-B	FSVVYAK LVVLPFPK	y4, y5, and y6
P02671	Fibrinogen alpha chain	GSESGIFTNTK	y4, y5, and y6 y5, y7, and y8
02679	Fibrinogen gamma chain	DNCCILDER	y4, y5, and y6
P02751	Fibronectin (FN)	SYTITGLQPGTDYK	y6, y9, and y10
06396	Gelsolin (Actin-depolymerizing factor)	AGALNSNDAFVLK	y7, y8, and y9
22352	Glutathione peroxidase 3	FLVGPDGIPIMR	y4, y8, and y9
P68871	Hemoglobin subunit beta	VNVDEVGGEALGR	y7, y8, and y10
02042	Hemoglobin subunit delta (Delta-globin)	LLGNVLVCVLAR	y5, y6, and y7
02790	Hemopexin (Beta-1B-glycoprotein)	NFPSPVDAAFR	y5, y7, and y8
05546	Heparin cofactor 2	TLEAQLTPR	y5, y6, and y7
00738	Haptoglobin	VTSIQDWVQK VGYVSGWGQSDNFK	y5, y6, and y8 y7, y9, and y10
00739	Haptoglobin-related protein		
	Histidine-rich alvconrotein		
04196	Histidine-rich glycoprotein Plasma protease C1 inhibitor	GGEGTGYFVDFSVR	y5, y6, and y7
204196 205155	Plasma protease C1 inhibitor		y5, y6, and y7 y5, y7, and y8
04196 05155 01876		GGEGTGYFVDFSVR LLDSLPSDTR	y5, y6, and y7
204196 205155 201876 201877 201857	Plasma protease C1 inhibitor Ig alpha-1 chain C region Ig alpha-2 chain C region Ig gamma-1 chain C region	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y8 y4 and y7
204196 205155 201876 201877 201857 201859	Plasma protease C1 inhibitor Ig alpha-t Chain C region Ig alpha-2 chain C region Ig gamma-1 chain C region Ig gamma-2 chain C region	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y8 y4 and y7 y4, y5, and y6
204196 205155 201876 201877 201857 201859 201860	Plasma protease C1 inhibitor Ig alpha-1 chain C region Ig alpha-2 chain C region Ig gamma-1 chain C region Ig gamma-2 chain C region Ig gamma-3 chain C region (HDC)	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYVDGVEVHNAK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y8 y4 and y7 y4, y5, and y6 y9, y10, and y11
204196 205155 201876 201877 201857 201859 201860 201860 201871	Plasma protease C1 inhibitor g alpha-1 chain C region g alpha-2 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-3 chain C region g mu chain C region	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYVDGVEVHNAK YAATSQVLLPSK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y8 y4 and y7 y4, y5, and y6 y9, y10, and y11 y3, y4, and y10
204196 205155 201876 201877 201857 201857 201859 201860 201860 201871 205154	Plasma protesse C1 inhibitor Ig alpha-1 chain C region Ig alpha-2 chain C region Ig gamma-1 chain C region Ig gamma-2 chain C region Ig gamma-3 chain C region Ig gamma-3 chain C region Plasma serine protesse inhibitor	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYVDGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y8 y4 and y7 y4, y5, and y6 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9
204196 205155 201876 201877 201857 201859 201860 201871 205154 205154 2019827	Plasma protease C1 inhibitor g alpha-1 chain C region g alpha-2 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-3 chain C region (HDC) g mu chain C region Plasma serine protease inhibitor Inter-alpha-tryps in inhibitor heavy chain H1	GGEGTGYFVDPSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFLAPSSK GLPAPIEK WYVDGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVP	y5, y6, and y7 y5, y7, and y8 y5, y6, and y8 y5, y6, and y7 y5, y6, and y8 y4 and y7 y9, y5, and y6 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y6, y8, and y9
204196 205155 201876 201877 201857 201859 201860 201871 205154 219827 219823	Plasma protease C1 inhibitor g alpha-1 chain C region g alpha-2 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-3 chain C region (HDC) g un chain C region Plasma serine protease inhibitor Inter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H2	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK WYVDGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y7 y4, y5, and y8 y4 and y7 y4, y5, and y6 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y6, y8, and y7
204196 205155 201876 201877 201857 201859 201860 201871 201854 201871 205154 205154 205154 2198227 219823 214624	Plasma protease C1 inhibitor g alpha-1 chain C region g alpha-2 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-3 chain C region (HDC) g mu chain C region Plasma serine protease inhibitor Inter-alpha-tryps in inhibitor heavy chain H1	GGEGTGYFVDPSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFLAPSSK GLPAPIEK WYVDGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVP	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y7 y4, y5, and y6 y9, y10, and y1 y3, y4, and y0 y5, y8, and y9 y4, y6, and y7 y5, y6, and y7
204196 205155 201876 201877 201857 201857 201859 201860 201871 201554 201554 205154 205154 205154 205154 205154 205152 205124 205122 205125 201827 205125 201827 20187 20180 20187 2	Plasma protease C1 inhibitor g alpha-2 chain C region g alpha-2 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-3 chain C region g mu chain C region Plasma serine protease inhibitor Inter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H2 Inter-alpha-trypsin inhibitor heavy chain H4	GGEGTGYFVDPSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVPLAPSSK GLPAPIEK WYDQVEVHNAK YAATSQVLLPSK TLVLADTPFTNFR AAISGENAGLVA FYNQVSTPLLR ILDDLSPR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y7 y4, y5, and y7 y4, y5, and y7 y3, y4, and y10 y5, y8, and y9 y5, y8, and y9 y4, y6, and y7 y5, y6, and y7 y5, y7, and y8
004196 005155 001876 001877 001857 001859 001859 001859 001860 001871 005154 019827 019823 014624 29622 209352 201042	Plasma protease C1 inhibitor g alpha-1 chain C region g alpha-2 chain C region g agmma-2 chain C region g agmma-2 chain C region g agmma-3 chain C region g amma-3 chain C region Plasma serine protease inhibitor nter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H2 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kallistatin (Kallikrein inhibitor)	GGEGTGYFVDFSVR LDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFLAPSSK GLPAPIEK WYVDGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVP FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSGYSLR YFIDFVAR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y4, y6, and y7 y4, y5, and y8 y9, y10, and y1 y3, y4, and y10 y5, y8, and y9 y4, y6, and y1 y5, y8, and y9 y4, y6, and y7 y5, y6, and y7 y5, y7, and y8 y7, y9, and y11 y4, y5, and y8
04196 005155 001876 001857 001857 001859 001860 001860 001851 001859 001860 001852 001852 001852 201823 2019823 2019823 2019823 2019823 2019823 2019823 2019823 2019823 2019823 2019823 2019825	Plasma protease C1 inhibitor g alpha - 1 chain C region g g alpha - 2 chain C region g gamma - 1 chain C region g gamma - 2 chain C region g gamma - 3 chain C region Plasma serine protease inhibitor Inter-alpha - trypsin inhibitor heavy chain H1 Inter-alpha - trypsin inhibitor heavy chain H2 Inter-alpha - trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Klininogen-1 Pigment epithelium-derived factor (PEDF)	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYDQGVEVHNAK YAATSQVLLPSK TLVLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR LGFDLFSK LGFDLFSK LGFDLFSK FVGQSSGYSLR YFIDFVAR ELLDTVTAPQK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y7 y4, y5, and y8 y4, y5, and y8 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y4, y5, and y7 y5, y6, and y7 y5, y6, and y7 y5, y7, and y8
204196 205155 201876 201877 201857 201859 201860 201871 205154 201871 205154 214624 229622 203952 201042 201042 201042 201042 201042	Plasma protease C1 inhibitor g alpha-1 chain C region g alpha-2 chain C region g gamma-2 chain C region g gamma-2 chain C region g umma-2 chain C region g um chain C region Plasma serine protease inhibitor Inter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H2 Inter-alpha-trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kininogen-1 Pigment epithelium-derived factor (PEDF) N-acetyImuramyL-1-alaniae am(dase	GGEGTGYF-VDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFLAPSSK GLPAPIEK WYVDQVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVP FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSGYSLR YFIDFVAR ELLDTVTAPQK AGLLPDVALLGHR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y7 y4, y5, and y8 y9, y10, and y1 y5, y8, and y7 y6, y8, and y0 y5, y8, and y9 y6, y8, and y9 y6, y8, and y9 y5, y6, and y7 y5, y6, and y7 y7, y9, and y11 y4, y5, and y6 y5, y7, and y6 y5, y7, and y6
04196 005155 001876 001877 001877 001859 001860 001860 001871 001854 001871 0015154 019827 019823 019625 001952 001952 001952 001955 00255 96PD5 002776	Plasma protease C1 inhibitor g alpha-1 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-2 chain C region g undera-2 chain C region gu chain C region Plasma serine protease inhibitor nter-alpha-trypsin inhibitor heavy chain H1 nter-alpha-trypsin inhibitor heavy chain H2 nter-alpha-trypsin inhibitor heavy chain H4 Kallistarlin (Kallikrein inhibitor) Plasma kallikrein Kallistarlin (Kallikrein inhibitor) Plasma kallikrein Kallistard Lotalikrein Kallistard Lotalikrein Plasma kallikrein Kallistard Lotard (PCDEDF) N-acetylmuramoyl-L-alanina amidase Plateleit factor 4 (Oncostatin-A)	GGEGTGYF-VDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYDQGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSQYSLR YFIDFVAR ELLDTVTAPQK AGLLRPDYALLGHR ICLDLQAPLYK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y4, y5, y6, and y7 y4, y5, and y6 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y5, y8, and y9 y5, y8, and y7 y5, y6, and y7 y5, y7, and y8 y7, y9, and y1 y4, y5, and y6 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8
204196 205155 201876 201877 201857 201857 201859 201850 201862 201862 201862 201862 201862 201862 2019823 214624 229622 203952 203955 306955 3966D5 202776 202776 202776	Plasma protease C1 inhibitor g alpha-1 Chain C region g alpha-2 chain C region g gamma-2 Chain C region g gamma-3 Chain C region gamma-3 Chain C region Plasma serine protease inhibitor Plasma serine protease inhibitor Inter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H4 Inter-alpha-trypsin inhibitor heavy chain H4 Inter-alpha-trypsin inhibitor heavy chain H4 Inter-alpha-trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kininogen-1 Pigment epithelium-derived factor (PEDF) N-acteyImuramyl-L-alanine amidase Platelet factor 4 (Oncostatin-A) Plasminogen	GGEGTGYFVDPSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVPLAPSSK GLPAPIEK WYVDQVEVHNAK YAATSQVLLPSK TIVLADTPTNFR AAISGENAGLVR FYNQVSTPLLR LGFTDLFSK LGFTDLFSK LGFTDLFSK VFIDFVAR ELLDTVTAPQK AGLLRPDYALLGHR ICLDLQAPLVK HSIFTPETNPR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y5, y6, and y7 y4, y5, and y8 y9, y10, and y1 y3, y4, and y7 y4, y6, and y1 y5, y8, and y9 y4, y6, and y7 y5, y6, and y7 y5, y6, and y7 y5, y7, and y8 y5, y6, and y9
204196 201876 201877 2018877 201857 201860 201871 205154 205154 205154 205154 205154 20552 203622 203952 203952 203952 203952 203952 203952 203952 203955 20766 200747 200747 200747	Plasma protease C1 inhibitor g alpha-1 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-2 chain C region g undera-2 chain C region Plasma serine protease inhibitor Inter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H2 Inter-alpha-trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kininogen-1 Pigment epithelium-derived factor (PEDF) N-acetylmuramoyl-L-alanine amidase Plashiel factor 4 (Oncostatin-A) Plasmingen Serum paraoxonase	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYVDQVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSGYSLR YEIDFVAR ELLDTVTAPQK AGLRPDYALGHR ICLDLQAPLYK HSIFTPETNPR LLIGTVFHK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y8 y4 and y7 y4, y5, and y8 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y5, y8, and y9 y5, y8, and y9 y5, y6, and y7 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y8, and y9 y5, y7, and y8 y5, y8, and y9 y5, y8, and y9
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204196 206155 201876 201877 201857 201857 201860 201871 205154 219827 219823 214624 229622 203952 201042 203955 202776 202776 902773	Plasma protease C1 inhibitor g alpha-1 chain C region g alpha-2 chain C region g gamma-1 chain C region g gamma-2 chain C region g umma-2 chain C region g um chain C region Plasma serine protease inhibitor Inter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H2 Inter-alpha-trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kininogen-1 Pigment epithelium-derived factor (PEDF) N-acetyinuranyl-L-alanian e amidase Platelet factor 4 (Oncostatin-A) Plasminogen Serum paraoxonase Proteoglycan 4 Retinol-binding protein 4	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYVDQEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSGYSLR YFIDFVAR ELLDTVTRPQK AGLLRPVALLGHR ICLDLQAPLYK HSIFTPETNPR LLGTVFHK GLPNVVTSAISLPNIR FSGTWYAMAK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y8 y4, y5, y6, and y7 y4, y5, and y8 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y5, y8, and y9 y5, y6, and y7 y5, y6, and y7 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y8, and y9 y5, y6, and y9 y5, y6, and y10 y5, y8, and y9 y5, y6, and y10 y5, y6, and y10 y5, y6, and y10 y5, y6, and y10 y5, y6, and y10 y6, y7, and y8 y5, y6, and y10 y6, y7, and y8 y5, y6, and y10 y6, y6, and y10 y6, y6, and y10 y6, y6, and y10
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004196 005155 001876 001877 001857 001859 001860 P01810 P01821 119823 114624 229622 003952 001442 936955 002776 002776 902254 902254 49908	Plasma protease C1 inhibitor g alpha - 1 chain C region g gamma - 1 chain C region g gamma - 2 chain C region g gamma - 2 chain C region g gamma - 3 chain C region gamma - 3 chain C region Plasma serine protease inhibitor nter-alpha - trypsin inhibitor heavy chain H1 nter-alpha - trypsin inhibitor heavy chain H2 nter-alpha - trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kininogen-1 Pigment epithelium - derived factor (PEDF) N-acetyimuranyL - Jalanie amidase Platelet factor 4 (Oncostatin-A) Plasminogen Serum paraoxonase Proteoglycan 4 Retinol-binding protein 4 Serum amyloid A - 4 protein Selenoprotein P	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPJEK WYVDGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTGOSSGYSLR YFIDFVAR ELLDTVTAPOK AGLLEPVYALGHR ICDLCAPLYK HSIFTPETNPR LLIGTVFHK GLPNVVTSAISLPNIR GLPNVVTSAISLPNIR FSGTWYAMAK EALQGVGDMGR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y8 y5, y6, and y7 y4, y5, and y8 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y5, y8, and y9 y5, y8, and y9 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y8, and y9 y5, y7, and y8 y5, y8, and y9 y5, y6, and y1 y5, y7, and y8 y5, y6, and y9 y5, y6, and y9 y5, y6, and y9 y5, y6, and y9 y5, y7, and y8
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P04196 205155 201876 201877 201857 201859 201860 201871 201854 201823 214624 229622 201042 229622 201042 203625 201042 203655 202776 902776 902753 235542 249908 204278 205452	Plasma protease C1 inhibitor g alpha - 1 chain C region g alpha - 2 chain C region g gamma - 2 chain C region g gamma - 2 chain C region gamma - 3 chain C region gamma - 3 chain C region Plasma serine protease inhibitor nter-alpha - trypsin inhibitor heavy chain H1 nter-alpha - trypsin inhibitor heavy chain H2 nter-alpha - trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kininogen-1 Pigment epithelium - derived factor (PEDF) N-acetylmuramyl-L - alania emidase Platelet factor 4 (Oncostatin-A) Plasminogen Serum paraxonase Proteoglycan 4 Retinol-binding protein 4 Serum anyloid A - 4 protein Serum anyloid A - 4 protein	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPJEK WYVDGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTGOSSGYSLR YFIDFVAR ELLDTVTAPOK AGLLEPVYALGHR ICDLCAPLYK HSIFTPETNPR LLIGTVFHK GLPNVVTSAISLPNIR GLPNVVTSAISLPNIR FSGTWYAMAK EALQGVGDMGR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y8 y4, and y7 y4, y5, sn ad y8 y4, y5, and y8 y9, y10, and y110 y5, y8, and y6 y5, y8, and y6 y5, y8, and y7 y5, y7, and y8 y7, y9, and y11 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y6, and y7 y5, y7, and y8 y5, y6, and y10 y5, y6, and y10 y5, y6, and y10 y4, y5, and y8 y5, y7, and y8 y6, y7, and y8 y5, y7, and y8
004196 005155 001876 001877 001857 001857 001857 001859 001860 001871 005155 119827 119827 119828 114624 229622 003952 001042 236955 002776 000747 227169 1922544 002753 249908 204278 005452 005543	Plasma protease C1 inhibitor g alpha - 1 chain C region g ghma - 1 chain C region g gamma - 1 chain C region g gamma - 1 chain C region g gamma - 2 chain C region Plasma serine protease inhibitor Inter - alpha - tryps in inhibitor heavy chain H1 Inter - alpha - tryps in inhibitor heavy chain H2 Inter - alpha - tryps in inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Klininogen -1 Pigment epitheium - derived factor (PEDF) N-acetylmuramoyl-L-alanine amidase Plashel tactor 4 (Oncostatin - A) Plasme Kator 4 (Oncostatin - A) Plasme paraxonase Proteoglycan 4 Retinol-binding protein 4 Serum anyloid A - 4 protein Serum parakonase	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYVDGVEVHNAK YAATSQVLPSK TLYLADTLPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSGYSLR YFIDFVAR ELLDTVTAPQK AGLLEPVALGHR ICIDLGAPLYK HSIFTPETNPR LLIGTVFHK GLPNVVTSAISLPNIR FSGTWYAMAK EALQGVGDMGR LDTGELAPPR IALGGLLFPASNLR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y7 y4, y5, y6, and y7 y4, y5, and y8 y9, y10, and y10 y5, y8, and y0 y5, y8, and y10 y5, y8, and y10 y5, y8, and y7 y5, y7, and y8 y7, y9, and y1 y6, y7, and y8 y5, y7, and y8
004196 005155 001876 001877 001857 001857 001859 001860 001811 005155 19827 198282 01042 203952 001042 303955 002776 000747 229654 002753 305542 49908 0056452 0056453 002734 002734	Plasma protease C1 inhibitor g alpha - 1 chain C region g ghma - 1 chain C region g gamma - 1 chain C region g gamma - 1 chain C region g gamma - 2 chain C region g gamma - 2 chain C region P plasma serine protease inhibitor Inter - alpha - tryps in inhibitor heavy chain H1 Inter - alpha - tryps in inhibitor heavy chain H2 Inter - alpha - tryps in inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) P lasma kallikrein Klininogen -1 Pigment epithelium - derived factor (PEDF) N-acetylmuramoyl-L-alanine amidase Plasheit actor 4 (Oncostatin -A) Plasma factor 4 (Oncostatin -A) Plasma protease Proteoglycan 4 Retinol-binding protein 4 Serum anyloid A - 4 protein Selenoprotein P Sek Normone-binding globulin Tetranectin	GGEGTGYF-VDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYDQGVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSGYSLR YFIDFVAR ELLDTUTAPQK AGLLRPDYALLGHR ICIDLQAPLYK HSIFTPETNPR LLGTVFHK GLPNVVTSAISLPNIR EALQGVGDMGR LPTOSELAPR IAIGGLFPASNLR LDTLAQEVALLK	y5, y6, and y7 y5, y7, and y8 y5, y6, and y8 y4, and y7 y4, y5, sn ad y8 y4, y5, and y8 y9, y10, and y110 y5, y8, and y6 y5, y8, and y6 y5, y8, and y7 y5, y7, and y8 y7, y9, and y11 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y6, and y7 y5, y7, and y8 y5, y6, and y10 y5, y6, and y10 y5, y6, and y10 y4, y5, and y8 y5, y7, and y8 y6, y7, and y8 y5, y7, and y8
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004196 005155 001876 001857 001857 001857 001857 001857 001857 001857 001857 001857 001857 001857 001857 001857 001871 005154 002627 000747 002766 002784 002784 002784 002784 002784 002786 002786 002786 002786 002786 002786 002786 002786 002786 002786	Plasma protease C1 inhibitor g alpha - 1 chain C region g g alpha - 2 chain C region g gamma - 1 chain C region g gamma - 1 chain C region g gamma - 3 chain C region g gamma - 3 chain C region Plasma serine protease inhibitor Inter-alpha - tryps in inhibitor heavy chain H1 Inter - alpha - tryps in inhibitor heavy chain H2 Inter - alpha - tryps in inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Klininogen-1 Pigment ejihteinum-derived factor (PEDF) N-acetylmuramoyl-Lalanine amidase Plasleit actor 4 (Oncostatin-A) Plasma factor 4 (Oncostatin-A) Plasma protein A Serum arnyloid A - 4 protein Sek hormone-binding globulin Thryxxine-binding globulin (Serpin A7) Prothombin Sertarning - binding protein A Serum arnyloid J. 4 sprotein Serum arnyloid J. 4 sprotein A Serum arnyloid J. 6 sprin A7) Prothombin Sertarniserrin Thryxxine-binding protein A	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GLPAPIEK WYVDQVEVHNAK YAATSQVLLPSK TLYLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTQGSSGYSLR YEIDFVAR ELLDTVTAPQK AGLLPPOYALGHR GLPNVVTSAISLPNIR ESGTWYAMAK EALQGVQDMGR EALQGVQDMGR LDTDSELAPR IALGGLLFPASNLR LDTVTSELAPR IALGGLLFPASNLR LDTVTSELAPR IALGGLLFPASNLR MALALFVLPK TATSEYQTFFNPR EGYYGYTGAFR AADDTWEPFASGK HLSLLTLSNR	y5, y6, and y7 y5, y7, and y8 y5, y8, and y8 y4 and y7 y4, y5, and y8 y9, y10, and y11 y3, y4, and y10 y5, y8, and y9 y5, y8, and y9 y5, y8, and y9 y5, y8, and y9 y5, y7, and y8 y5, y7, and y8 y6, y7, and y8 y6, y7, and y8 y5, y7, and y8 y5, y7, and y8 y5, y7, and y8
204196 204156 201876 201877 201857 201857 201859 201859 201827 2905154 2905154 290523 290522 290522 290522 290525 290525 290545 290776 202756 202753 202554 202753 205542 205543 204278 205543 205545 205543 205545 205543 205545 205555 205555 205555 2055555 2055555555	Plasma protease C1 inhibitor g alpha-1 chain C region g gamma-1 chain C region g gamma-2 chain C region g gamma-3 chain C region gamma-3 chain C region Plasma serine protease inhibitor Plasma serine protease inhibitor Plasma serine protease inhibitor Inter-alpha-trypsin inhibitor heavy chain H1 Inter-alpha-trypsin inhibitor heavy chain H2 Inter-alpha-trypsin inhibitor heavy chain H4 Kallistatin (Kallikrein inhibitor) Plasma kallikrein Kininogen-1 Pigment epithelium- derived factor (PEDF) N-acteylmuram-oyl-L-alanine amidase Platelet factor 4 (Oncostatin-A) Plasminogen Serum paraxoxnase Proteoglycan 4 Retinol-binding protein 4 Serum anyiold A-4 protein Selenoprotein P Sex hormone-binding globulin Tetranectin Thyroxine-binding globulin (Serpin A7) Prothrombin Serotransferrin	GGEGTGYFVDFSVR LLDSLPSDTR TPLTATLSK DASGATFTWTPSSGK GPSVFPLAPSSK GPSVFPLAPSSK WYVDGVEVHNAK YAATGQVLIPSK TLVLADTFPTNFR AAISGENAGLVR FYNQVSTPLLR ILDDLSPR LGFTDLFSK IAYGTGQSSGYSLR YFIDFVAR ELLDTVTAQK AGLLRPVALLGHR ICDLQAPLYK GLPNVYTSAISLPNIR FSGTWYAMAK EALQGVGMGAR LDTDSELAPR IALGGLLFPASNLR LDTDSELAPR IALGGLLFPASNLR LDTDSELAPR IALGGLLFPASNLR LDTLAGEVALLK NALALFVLPK TATSEYQTFFNPR EGYYGYTGAFR	y5, y6, and y7 y5, y7, and y8 y5, y6, and y8 y4, and y7 y4, y6, y6, and y8 y4, y6, and y8 y9, y10, and y11 y5, y6, and y10 y5, y6, and y10 y5, y6, and y7 y5, y7, and y8 y7, y9, and y11 y4, y5, and y6 y5, y7, and y8 y5, y7, and y8 y6, y7, and y8 y6, y7, and y8 y6, y7, and y8 y5, y7, and y8 y6, y7, and y8 y5, y7, 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. Figures 1 and 2 show example data acquired for six of the 100 proteins. Data for these 100 proteins was acquired details the b and y product ions monitored for the given marker peptide. over two analyses. Each 12-minute analysis was capable of analyzing 50 marker peptides, where three transitions

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 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.

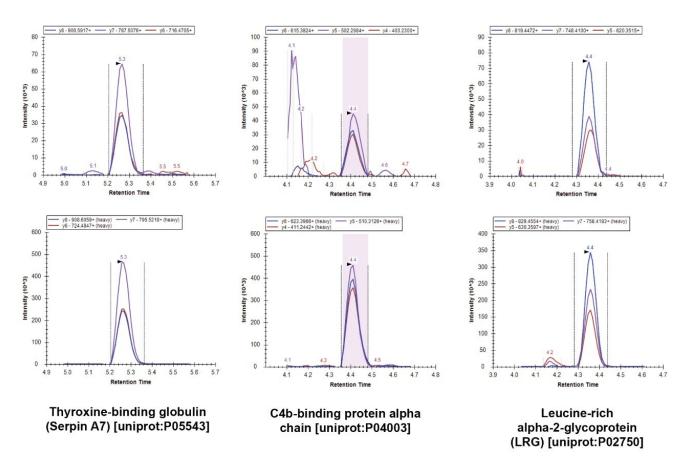


Figure 1. Example chromatograms acquired for three of the 100 proteins analyzed using the Biognosys PlasmaDive kit. (uniprot: P05543, P04003, and P02750). The upper chromatograms show the three transitions for the native peptide, and the lower chromatogram the stable labeled (heavy) reference peptide.

Conclusion

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 inhuman 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 a can canform the basis of a comprehensive suite of targeted multi-omic workflows.

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