Electronic Supplementary Material (ESI) for Analyst.

Simultaneous quantification of tau and α-synuclein in cerebrospinal fluid by high-resolution mass spectrometry for differentiation of Lewy Body Dementia from Alzheimer's Disease and controls.

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Supplementary Material

Table S1: Targeted α-synuclein and tau peptides, and MS/MS conditions

α-Synuclein	Precursor ion m/z	NCE (%)	Product ion types
14N GVVAAAE	616.33 (z=1)	20	b3 ; b4 ; b5 ; b6 ; y4
15N GVVAAAE	6223.31 (z=1)	20	b3 ; b4 ; b5 ; b6 ; y4
14N GVLYVGSK	412.00 (z=2)	15	b3 ; $y6^{+2}$;$y6^{+2}$ (P+1) ; y5 ;y5 (P+1) ; y6 ; y6 (P+1)
15N GVLYVGSK	416.23 (z=2)	15	b3 ; $y6^{+2}$;$y6^{+2}$ (P+1) ; y5 ;y5 (P+1) ; y6 ; y6 (P+1)
14N GVVHGVATVAE	519.78 (z=2)	20	b9-H2O +2;b9 +2 ;b10+2 ; b7 ; b8-H2O ; b9-H2O ; b9-H20 P+1 ; b9 ;b9 p+11 ; b10 ; b10 p+1
15N GVVHGVATVAE	526.26 (z=2)	20	b9-H2O +2;b9 +2 ;b10+2 ; b7 ; b8-H2O ; b9-H2O ; b9-H20 P+1 ; b9 ;b9 p+11 ; b10 ; b10 p+1
14N QVTNVGGAVVTGVTAVAQK	600.34 (z=3)	17	y3 ; y4 ; y5 ; y6 ; y8 ; y9 ; y10 ; b7 ;b8 ; b9
15N QVTNVGGAVVTGVTAVAQK	607.98 (z=3)	17	y3 ; y4 ; y5 ; y6 ; y8 ; y9 ; y10 ; b7 ;b8 ; b9
14N GAGSIAAATGFVK	575.32 (z=2)	22	b4 ; b5 ; y4 ; y5 ;y6 ;y7 ; y8 ; y9 ; y10 ;y11
15N GAGSIAAATGFVK	582.29 (z=2)	22	b4 ; b5 ; y4 ; y5 ;y6 ;y7 ; y8 ; y9 ; y10 ;y11
14N GAPQEGILE	457.23 (z=2)	17	y2 ; b4 ;y4 ; b5 ; y5 ;b7 ; b8 ; b8 P+1 ; b7
15N GAPGEGILE	462.22 (z=2)	17	y2 ; b4 ;y4 ; b5 ; y5 ;b7 ; b8 ; b8 P+1 ; b7
14N DMPVDPDNE	516.2 (z=2)	17	y4 ; y5 ; y7 ; b8
15N DMPVDPDNE	521.19 (z=2)	17	y4 ; y5 ; y7 ; b8
14N AYEMPSEE	478.19 (z=2)	17	y3 ; y4 ; b4 ; b7
15N AYEMPSEE	482.18 (z=2)	17	y3 ; y4 ; b4 ; b7
14N GYQDYEPEA	536.22 (z=2)	17	y3 ; b5 ; b6 ;b7 ;b8
15N GYQDYEPEA	541.22 (z=2)	17	y3 ; b5 ; b6 ;b7 ;b8
Tau	Precursor ion m/z	NCE (%)	Product ion type
14N TPPAPK	305.68 (z=2)	10	y5 +2 ; y4 ;y5 ;y5 P+1
15N TPPAPK	309.17 (z=2)	10	y5 +2 ; y4 ;y5 ;y5 P+1
14N SGYSSPGSPGTPGSR	697.73 (z=2)	20	b3 ;y4 ; b5 ;y7 ; y7 P+1 ; b8 ; y10 ; y10 P+1 ; y11 P+1 ; y12 ;y12 P+1
15N SGYSSPGSPGTPGSR	706.29 (z=2)	20	b3 ;y4 ; b5 ;y7 ; y7 P+1 ; b8 ; y10 ; y10 P+1 ; y11 P+1 ; y12 ;y12 P+1
14N TPSLPTPPTR	533.80 (z=2)	25	y4 ;y6 ;y6 P+1 ; y7 ;

			y8 ;y8 P+1
15N TPSLPTPPTR	540.28 (z=2)	25	y4 ;y6 ;y6 P+1 ; y7 ; y8 ;y8 P+1
14N SPVVSGDTSPR	551.28 (z=2)	25	y4 ; y6 ; y6 P+1 ;y7 ;y8 ; y8 P+1 ;y9 ;y9 P+1
15N SPVVSGDTSPR	558.26 (z=2)	25	y4 ; y6 ; y6 P+1 ;y7 ;y8 ; y8 P+1 ;y9 ;y9 P+1

Hemoglobin	Precursor ion m/z	NCE (%)	Product ion type
VLSPADK	365.21 (z=2)	20	y6 ;y5 ;y4
VLSPADK[$^{13}C_6$;$^{15}N_2$]	369.22 (z=2)	20	y6 ;y5 ;y4

Table S2: Evaluation of matrix effect in human CSF for synuclein a) and tau b) representative peptides

a)

QC human CSF (n=3)	GVVAAAE			GVLYVGSK			GVVHGVATVAE			GAGSIAAATGFVK			GYQDYEPEA		
	Mean ng/mL	Bias	CV	Mean ng/mL	Bias	CV	Mean ng/mL	Bias	CV	Mean ng/mL	Bias	CV	Mean ng/mL	Bias	CV
Blank (endogenous)	1.79	/	21%	1.58		15%	0.38	/	13%	0.33	/	7.9%	LLOQ	/	/
Spike (+1 ng/mL)	2.86	7.9%	2.9%	2.71	13.0%	9.5%	1.35	3.1%	1.9%	1.46	13.0%	4.9%	LLOQ	/	/
Spike (+5 ng/mL)	6.7	1.6%	3.8%	6.35	4.7%	5.6%	4.94	8.7%	12%	5.0	6.6%	7.5%	5.24	1.7%	4.4%

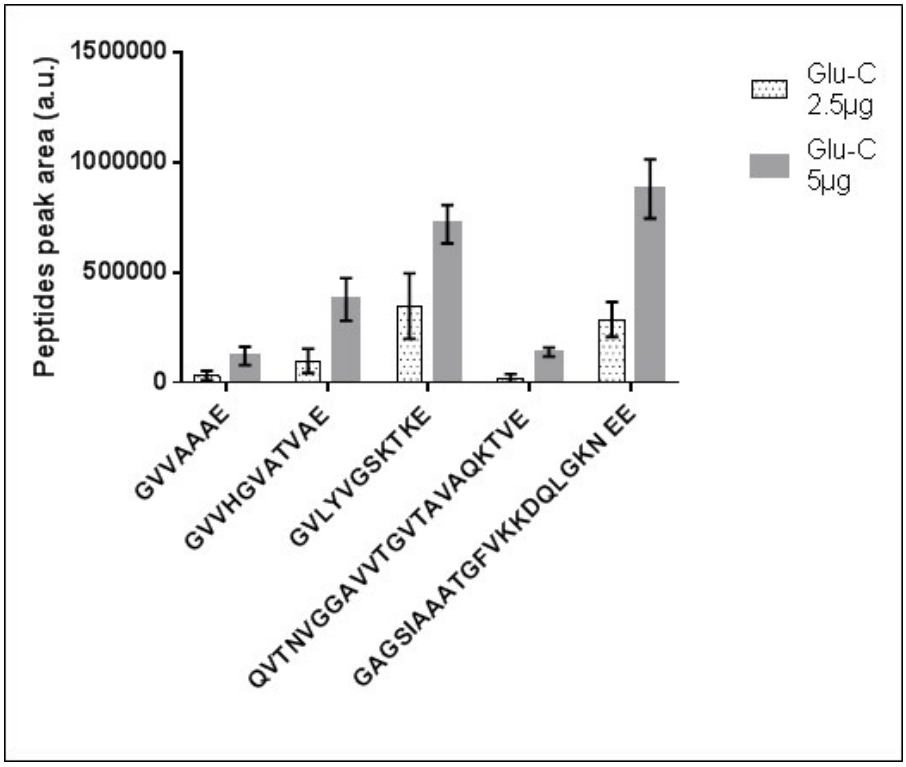
b)

QC human CSF (n=3)	TPPAPK			SGYSSPGTPGSR			TPSLPTPPTR			SPVVSGDTSPR		
	Mean (ng/mL)	Bias	CV	Mean (ng/mL)	Bias	CV	Mean (µg/mL)	Bias	CV	Mean (µg/mL)	Bias	CV
Blank (endogenous)	11.4	/	8%	2.59		20%	4.37	/	13%	LLOQ	/	/
Spike (+2.5 ng/mL)	/	/	/	5.32	9.1%	10.0%	7.13	10.0%	10.0%	1.93	23%	17%
Spike (+12.5ng/mL)	24	-0.3%	1.5%	14.8	2.2%	2.1%	17.3	-3.7%	9.2%	12.0	8.8%	3.3%

Human CSF was spiked with a mix of α-synuclein and tau (low/high concentrations) and back calculated against a calibration curve prepared in artificial CSF. Endogenous α-synuclein and tau concentrations were added to the spiked amount for calculations of bias. Bias and CV% are reported

Figure S1: Optimization of Glu-C digestion: Impact of Glu-C quantity a) and temperature of incubation b) on representative peptide signals (n=2 in each conditions)

a)



b)

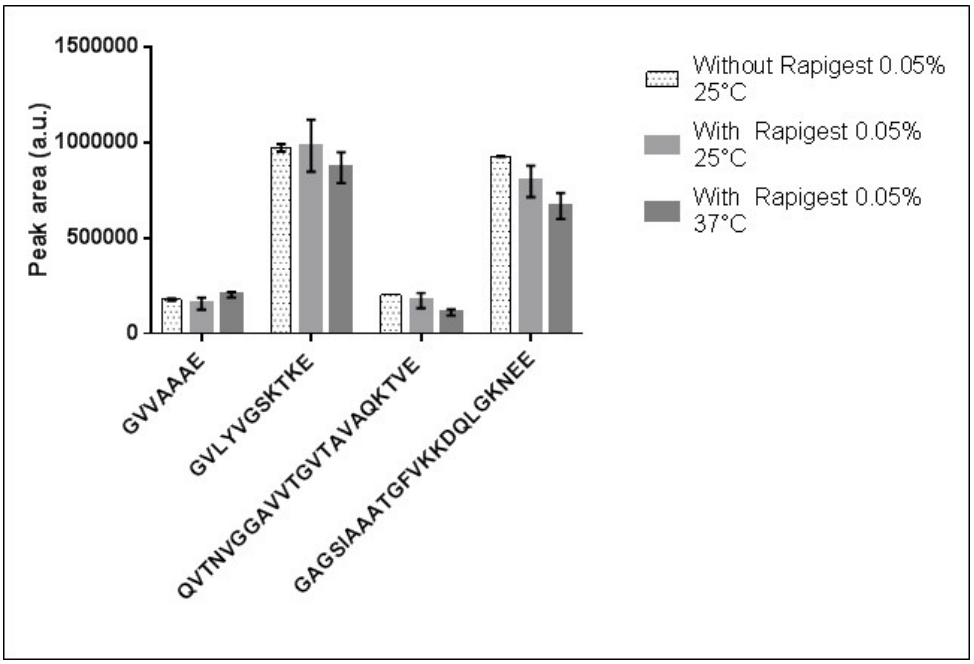


Figure S2: Impact of the sequential Glu-C/trypsin protocol on the release and/or detection of α-synuclein peptides. Mean peak area of α-synuclein peptides (n = 2) detected in both conditions is illustrated.

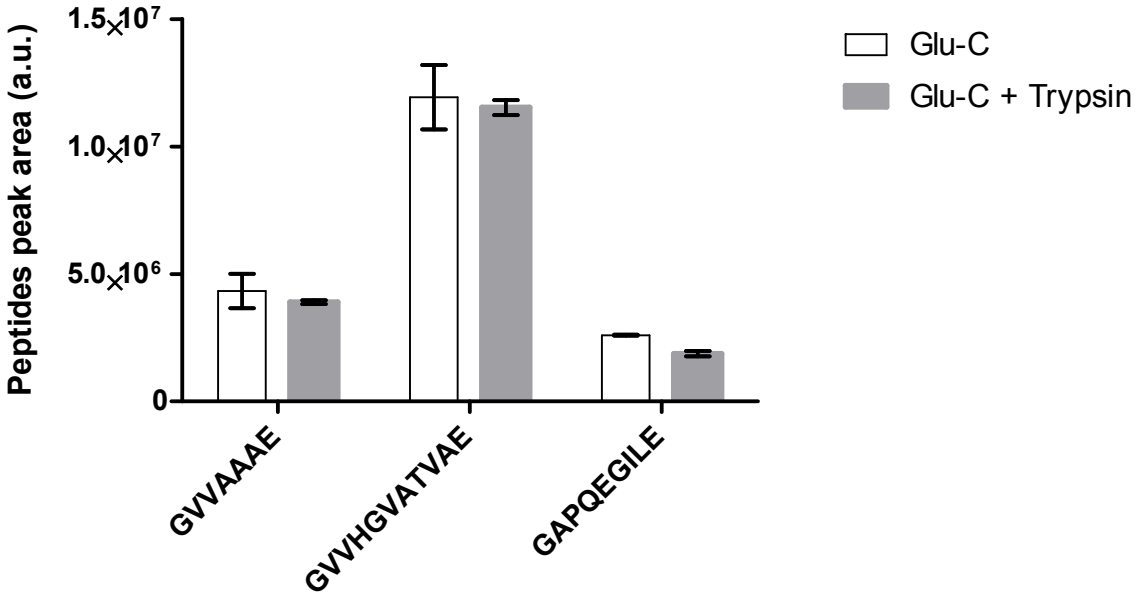


Figure S3: Linearity of tau (representative peptide TPSLPTPPTR) in artificial CSF by overnight digestion and trypsin only digestion (sample preparation as in Barthelemy N, J.Prot Res. 2016). LLOQ was determined at 0.5 ng/mL

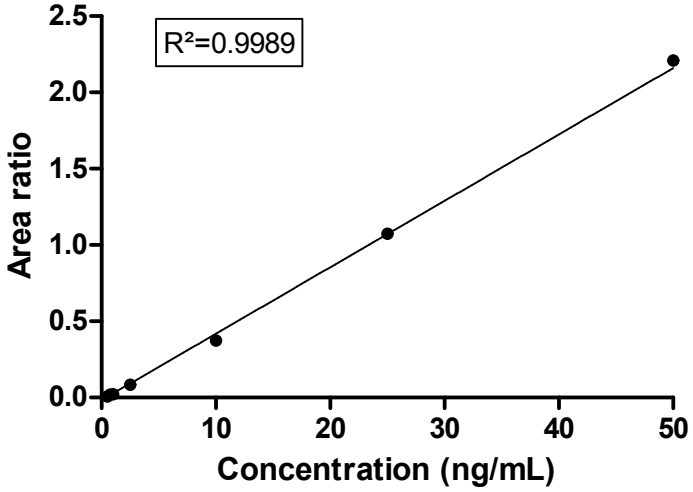


Figure S4: Evaluation of blood contamination based on signal intensity of peptide VLSPADK from hemoglobin.

CSF sample was considered as contaminated when signal of peptide VLSPADK exceeded the highest intensity observed in the calibration curve prepared in artificial CSF. Samples N°9 and 19 were excluded.