Supporting information

Integration of Ion Mobility MS^{E} after Fully Automated, Online, High-Resolution Liquid Extraction Surface Analysis Micro-Liquid Chromatography

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Table S-1. Pituitary gland proteins identified from LESA-µLC-HDMS^E analysis. Data were generated using Progenesis QI for proteomics software (v2.0.5556.29015).

Accession number	Peptide count	Unique peptides	Confidence score	Description
Q7TNJ2	1	1	2.77	ATP-binding cassette sub-family A member 7
P01186	17	17	155.34	Vasopressin-neurophysin 2-copeptin
P01194	25	25	183.60	Pro-opiomelanocortin

Table S-2. Pituitary gland peptides identified from LESA-μLC-HDMS^{E} analysis. Data were generated using Progenesis QI for proteomics software (v2.0.5556.29015).

Accession number	Retention time (min)	Charge	Drift time (ms)	m/z	Measured mass	Mass error (u)	Score	Sequence	Modifications	Description	Present in LESA-MS data? (Y/N; charge state)	Present in LESA-HDMS data? (Y/N; charge state)
P01186	13.53	3	3.81	460.24	1377.69	0.002	5.75	QESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	15.42	2	3.67	481.72	961.42	-0.007	5.64	MEHFRWG		Pro-opiomelanocortin	N	N
P01194	15.42	2	3.67	481.72	961.42	0.017	5.69	NVAENESAE		Pro-opiomelanocortin	N	N
P01186	13.30	3	3.67	482.25	1443.73	0.003	5.77	AGTQESVDSAKPRV		Vasopressin-neurophysin 2-copeptin	N	N
P01194	15.66	2	3.67	487.20	972.39	-0.018	6.04	EGEQPDGLE		Pro-opiomelanocortin	N	N
P01194	15.66	2	3.67	487.20	972.39	-0.018	5.39	ELEGEQPDG		Pro-opiomelanocortin	N	N
P01186	13.49	3	3.81	493.92	1478.74	0.004	6.19	TQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	Y; +3
P01186	13.56	3	3.88	512.93	1535.76	0.003	5.89	GTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	13.84	2	3.88	534.21	1066.41	-0.011	6.11	ESSQCQDLT	[5] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01186	13.61	3	4.09	536.61	1606.80	0.004	6.90	AGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01186	13.84	2	3.81	542.73	1083.45	0.016	5.61	CADELGCFL	[1] Carbamidomethyl C|[7] Carbamidomethyl C	Vasopressin-neurophysin 2-copeptin	Y; +2	N
P01194	15.97	3	3.81	555.61	1663.81	-0.005	5.85	TTESNLLACIRACR	[9] Carbamidomethyl C|[13] Carbamidomethyl C	Pro-opiomelanocortin	Y; +3	N
P01194	15.66	2	4.16	558.32	1114.62	0.001	5.76	EKSQTPLVTL		Pro-opiomelanocortin	N	N
P01186	13.77	3	4.22	562.63	1684.88	0.007	5.77	VQLAGTQESVDSAKPR		Vasopressin-neurophysin 2-copeptin	N	N
P01194	16.32	3	3.88	569.61	1705.82	-0.004	5.38	TTESNLLACIRACR	[1] Acetyl N-TERM|[9] Carbamidomethyl C|[13] Carbamidomethyl C	Pro-opiomelanocortin	Y; +3	Y; +3
P01186	14.08	3	4.36	574.30	1719.88	0.005	7.01	LAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01186	14.46	3	4.50	595.66	1783.95	0.005	6.57	VQLAGTQESVDSAKPRV		Vasopressin-neurophysin 2-copeptin	N	N
P01186	14.27	3	4.57	616.99	1847.94	0.003	6.31	QLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	16.32	7	4.50	627.45	4385.07	-0.042	---	FPLEFKRELEGEQPDGLEQVLEPDTEKADGPYRVEHF	[1] Acetyl N-TERM	Pro-opiomelanocortin	N	N
Q7TNJ2	21.43	2	4.71	639.36	1276.71	0.004	2.77	AVATVAQSFLLSA		ATP-binding cassette sub-family A member 7	N	N
P01194	17.18	4	4.64	640.80	2559.18	0.001	5.65	GPRNSSSAGGSAQRRAEEETAGGDGR		Pro-opiomelanocortin	N	N
P01186	14.70	3	4.71	650.01	1947.02	0.013	7.16	VQLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01186	15.20	3	5.19	687.71	2060.10	0.007	6.30	LVQLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	15.42	3	4.16	706.36	2116.06	-0.002	5.84	RPVKVYPNVAENESAEAFP		Pro-opiomelanocortin	N	N
P01194	16.32	6	4.99	731.85	4385.08	-0.032	5.22	FPLEFKRELEGEQPDGLEQVLEPDTEKADGPYRVEHF	[1] Acetyl N-TERM	Pro-opiomelanocortin	N	N
P01186	14.30	2	5.26	765.40	1528.78	0.004	5.87	VQLAGTQESVDSAKP		Vasopressin-neurophysin 2-copeptin	N	N
P01186	18.02	4	5.19	767.44	3065.71	0.013	6.05	GPARELLLRLVQLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01186	13.56	2	5.47	768.89	1535.76	-0.001	5.89	GTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	18.30	4	4.99	773.63	3090.50	-0.042	5.53	LLLALLLQTSIDVWSWCLESSQCQDL	[17] Carbamidomethyl C|[23] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01186	15.70	3	5.40	777.43	2329.28	0.006	5.97	LRLVQLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	16.04	3	4.64	787.08	2358.20	0.018	6.92	RPVKVYPNVAENESAEAFPLE		Pro-opiomelanocortin	N	N
P01194	16.04	3	4.64	787.08	2358.20	-0.015	5.10	LLACIRACRLDLSAETPVFPG	[4] Carbamidomethyl C|[8] Carbamidomethyl C	Pro-opiomelanocortin	N	N

Table S-2 (continued). Pituitary gland peptides identified from LESA-µLC-HDMS^E analysis. Data were generated using Progenesis QI for proteomics software (v2.0.5556.29015).

Accession number	Retention time (min)	Charge	Drift time (ms)	m/z	Measured mass	Mass error (u)	Score	Sequence	Modifications	Description	Present in LESA-MS data? (Y/N; charge state)	Present in LESA-HDMS data? (Y/N; charge state)
P01194	19.50	4	6.09	800.89	3199.52	-0.038	5.41	PDTEKADGPYRVEHFRWGNPPKDKRY	[1] Acetyl N-TERM	Pro-opiomelanocortin	N	N
P01186	14.96	5	4.29	803.76	4013.77	-0.077	5.77	YFQNCPRGGKRATSDMELRQCLPCGPGGKGRCFGP	[5] Carbamidomethyl C|[21] Carbamidomethyl C|[24] Carbamidomethyl C|[32] Carbamidomethyl C	Vasopressin-neurophysin 2-copeptin	N	N
P01186	14.08	2	5.54	804.41	1606.80	0.004	0.00	AGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	Y; +4
P01186	13.61	2	5.54	804.41	1606.80	0.006	6.90	AGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	Y; +4
P01194	16.01	2	5.82	832.91	1663.80	-0.015	5.85	TTESNLLACIRACR	[9] Carbamidomethyl C|[13] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01194	17.18	3	4.64	836.10	2505.28	0.026	5.44	PLTENPRKYVMGHFRWDRFG		Pro-opiomelanocortin	N	N
P01194	12.53	2	5.68	841.89	1681.77	-0.039	5.44	FRWDRFGPRNSSSA		Pro-opiomelanocortin	N	N
P01186	13.77	2	6.44	843.44	1684.88	0.001	5.77	VQLAGTQESVDSAKPR		Vasopressin-neurophysin 2-copeptin	N	N
P01194	16.32	2	5.96	853.91	1705.81	-0.017	5.38	TTESNLLACIRACR	[1] Acetyl N-TERM|[9] Carbamidomethyl C|[13] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01194	17.18	3	4.78	854.07	2559.18	-0.001	5.65	GPRNSSSAGGSAQRRAEEETAGGDGR		Pro-opiomelanocortin	N	N
P01186	14.08	2	6.02	860.95	1719.88	-0.001	7.01	LAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	16.28	5	5.40	878.02	4385.08	-0.033	5.22	FPLEFKRELEGEQPDGLEQVLEPDTEKADGPYRVEHF	[1] Acetyl N-TERM	Pro-opiomelanocortin	Y; +5	Y; +5
P01194	16.97	2	6.23	929.97	1857.93	0.009	5.68	YGGFMTSEKSQTPLVTL		Pro-opiomelanocortin	N	N
P01194	18.11	6	5.54	945.13	5664.76	-0.144	5.19	VGKKRRPVKVYPNVAENESAEAFPLEFKRELEGEQPDGLEQVLEPDTEKA		Pro-opiomelanocortin	N	N
P01186	14.70	2	7.06	974.51	1947.01	0.007	7.16	VQLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	18.26	6	5.68	988.83	5926.91	0.002	5.46	RFCNSRSGALLLALLLQTSIDVWSWCLESSQCQDLTTESNLLACIRACRL	[1] Acetyl N-TERM|[3] Carbamidomethyl C|[26] Carbamidomethyl C|[32] Carbamidomethyl C|[44] Carbamidomethyl C|[48] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01194	16.97	4	4.78	995.98	3979.88	-0.024	6.40	LEPDTEKADGPYRVEHFRWGNPPKDKRYGGFMTS		Pro-opiomelanocortin	N	N
P01194	17.02	4	5.47	1000.48	3997.90	-0.032	5.71	MPRFCNSRSGALLLALLLQTSIDVWSWCLESSQC	[5] Carbamidomethyl C|[28] Carbamidomethyl C|[34] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01194	17.02	4	5.47	1000.48	3997.90	-0.029	---	TEKADGPYRVEHFRWGNPPKDKRYGGFMTSEKSQ		Pro-opiomelanocortin	N	N
P01194	16.97	4	4.92	1009.96	4035.80	-0.090	5.90	ELEGEQPDGLEQVLEPDTEKADGPYRVEHFRWGNP		Pro-opiomelanocortin	N	N
P01186	17.99	3	6.93	1022.91	3065.69	-0.004	6.05	GPARELLLRLVQLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01186	15.20	2	7.62	1031.06	2060.10	0.013	6.30	LVQLAGTQESVDSAKPRVY		Vasopressin-neurophysin 2-copeptin	N	N
P01194	18.21	6	5.96	1040.17	6235.00	-0.040	5.35	EQPDGLEQVLEPDTEKADGPYRVEHFRWGNPPKDKRYGGFMTSEKSQTPLVTLF	[41] Oxidation M	Pro-opiomelanocortin	N	N
P01186	13.84	1	9.76	1084.45	1083.44	0.002	5.61	CADELGCFL	[1] Carbamidomethyl C|[7] Carbamidomethyl C	Vasopressin-neurophysin 2-copeptin	N	N
P01194	18.11	5	6.16	1093.34	5461.68	-0.095	6.21	YSMEHFRWGKPVGKKRRPVKVYPNVAENESAEAFPLEFKRELEGEQ		Pro-opiomelanocortin	N	N
P01194	16.04	2	7.41	1180.10	2358.19	0.008	6.92	RPVKVYPNVAENESAEAFPLE		Pro-opiomelanocortin	N	N
P01194	16.04	2	7.41	1180.10	2358.19	-0.026	5.10	LLACIRACRLDLSAETPVFPG	[4] Carbamidomethyl C|[8] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01194	18.26	5	6.44	1186.39	5926.90	-0.007	5.46	RFCNSRSGALLLALLLQTSIDVWSWCLESSQCQDLTTESNLLACIRACRL	[1] Acetyl N-TERM|[3] Carbamidomethyl C|[26] Carbamidomethyl C|[32] Carbamidomethyl C|[44] Carbamidomethyl C|[48] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01186	15.47	3	6.02	1200.21	3597.62	-0.078	5.74	MLAMMLNTTLSACFLSLLALTSACYFQNCPR	[13] Carbamidomethyl C|[24] Carbamidomethyl C|[29] Carbamidomethyl C	Vasopressin-neurophysin 2-copeptin	N	N
P01194	15.42	3	5.82	1206.22	3615.63	-0.016	5.46	WDRFGPRNSSSAGGSAQRRAEEETAGGDGRPEPS	[1] Acetyl N-TERM	Pro-opiomelanocortin	N	N
P01194	18.21	5	6.65	1248.01	6235.01	-0.032	5.35	EQPDGLEQVLEPDTEKADGPYRVEHFRWGNPPKDKRYGGFMTSEKSQTPLVTLF	[41] Oxidation M	Pro-opiomelanocortin	N	N
P01194	17.18	2	7.76	1253.64	2505.27	0.017	---	PLTENPRKYVMGHFRWDRFG		Pro-opiomelanocortin	N	N
P01194	16.97	3	6.58	1327.64	3979.89	-0.019	6.40	LEPDTEKADGPYRVEHFRWGNPPKDKRYGGFMTS		Pro-opiomelanocortin	N	N
P01194	17.02	3	6.58	1333.64	3997.90	-0.029	5.71	MPRFCNSRSGALLLALLLQTSIDVWSWCLESSQC	[5] Carbamidomethyl C|[28] Carbamidomethyl C|[34] Carbamidomethyl C	Pro-opiomelanocortin	N	N
P01194	17.02	3	6.58	1333.64	3997.90	-0.026	5.51	TEKADGPYRVEHFRWGNPPKDKRYGGFMTSEKSQ		Pro-opiomelanocortin	N	N

Table S-3. Mouse brain proteins identified from LESA-µLC-HDMS^{E} analysis. Data were generated using Progenesis QI for proteomics software (v2.0.5556.29015).

Accession number	Peptide count	Unique peptides	Confidence score	Description
Q2TA51	1	1	3.90	Keratin-associated protein 5-5 OS=Mus musculus GN=Krtap5-5 PE=2 SV=1
P43277	3	2	8.71	Histone H1.3 OS=Mus musculus GN=Hist1h1d PE=1 SV=2
Q64507	1	1	3.31	Keratin-associated protein 5-1 OS=Mus musculus GN=Krtap5-1 PE=2 SV=1
Q8VI56	1	1	2.08	Low-density lipoprotein receptor-related protein 4 OS=Mus musculus GN=Lrp4 PE=1 SV=3
P43275	1	1	3.34	Histone H1.1 OS=Mus musculus GN=Hist1h1a PE=1 SV=2
Q8C0Q3	1	1	2.76	Tetratricopeptide repeat protein 34 OS=Mus musculus GN=Ttc34 PE=2 SV=2
O55111	1	1	4.30	Desmoglein-2 OS=Mus musculus GN=Dsg2 PE=1 SV=3
P54320	2	2	8.74	Elastin OS=Mus musculus GN=Eln PE=1 SV=2
Q9JHT5	1	1	4.47	AMME syndrome candidate gene 1 protein homolog OS=Mus musculus GN=Ammecr1 PE=2 SV=1
P43276	1	0	4.90	Histone H1.5 OS=Mus musculus GN=Hist1h1b PE=1 SV=2

Table S-4. Mouse brain peptides identified from LESA-µLC-HDMS^E analysis. Data were generated using Progenesis QI for proteomics software (v2.0.5556.29015).

Accession number	Retention time (min)	Charge	Drift time (ms)	m/z	Measured mass	Mass error (u)	Score	Sequence	Modifications	Description	Present in LESA-MS data? (Y/N; charge state)	Present in LESA-HDMS data? (Y/N; charge state)
P54320	24.99	1	5.61	556.31	555.30	-0.004	4.41	LPGVGGG		Elastin OS=Mus musculus GN=Eln PE=1 SV=2	N	N
Q9JHT5	6.12	2	3.81	597.69	1193.36	-0.075	4.47	SGGGGGASSSSHCS	[13] Carbamidomethyl C	AMME syndrome candidate gene 1 protein homolog OS=Mus musculus GN=Ammecr1 PE=2 SV=1	N	N
P54320	21.17	1	4.50	610.37	609.36	0.015	4.32	PGAVPAV		Elastin OS=Mus musculus GN=Eln PE=1 SV=2	N	N
P43277	21.27	1	5.19	646.41	645.40	0.023	4.90	AKKTGAA		Histone H1.3 OS=Mus musculus GN=Hist1h1d PE=1 SV=2	Y;+1	Y;+1
P43276	21.27	1	5.19	646.41	645.40	0.023	4.90	AKKTGAA		Histone H1.5 OS=Mus musculus GN=Hist1h1b PE=1 SV=2	Y;+1	Y;+1
Q8VI56	11.87	1	5.89	668.21	667.21	-0.004	2.08	SGMDGSD		Low-density lipoprotein receptor-related protein 4 OS=Mus musculus GN=Lrp4 PE=1 SV=3	N	N
Q8C0Q3	20.83	1	9.00	718.42	717.41	0.001	2.76	RKVATSG		Tetratricopeptide repeat protein 34 OS=Mus musculus GN=Ttc34 PE=2 SV=2	N	N
Q2TA51	10.41	1	8.38	741.23	740.22	-0.003	3.90	GSCGSCGG	[3] Carbamidomethyl C|[6] Carbamidomethyl C	Keratin-associated protein 5-5 OS=Mus musculus GN=Krtap5-5 PE=2 SV=1	N	N
Q2TA51	11.73	1	6.37	741.23	740.22	0.002	3.46	GSCGSCGG	[3] Carbamidomethyl C|[6] Carbamidomethyl C	Keratin-associated protein 5-5 OS=Mus musculus GN=Krtap5-5 PE=2 SV=1	N	N
Q64507	12.04	1	6.37	741.23	740.22	0.003	3.31	SCGGCGSG	[2] Carbamidomethyl C|[5] Carbamidomethyl C	Keratin-associated protein 5-1 OS=Mus musculus GN=Krtap5-1 PE=2 SV=1	N	N
Q64507	9.80	1	6.44	741.23	740.23	0.005	2.94	SCGGCGSG	[2] Carbamidomethyl C|[5] Carbamidomethyl C	Keratin-associated protein 5-1 OS=Mus musculus GN=Krtap5-1 PE=2 SV=1	N	N
P43277	21.44	1	9.56	788.47	787.47	0.000	3.81	AAGKRKAS		Histone H1.3 OS=Mus musculus GN=Hist1h1d PE=1 SV=2	N	Y;+1
O55111	18.12	1	8.93	806.33	805.32	0.010	4.30	ADEVGSDN		Desmoglein-2 OS=Mus musculus GN=Dsg2 PE=1 SV=3	N	N
P43275	21.33	1	10.11	873.53	872.53	-0.017	3.34	GAAAKKTVK		Histone H1.1 OS=Mus musculus GN=Hist1h1a PE=1 SV=2	N	N

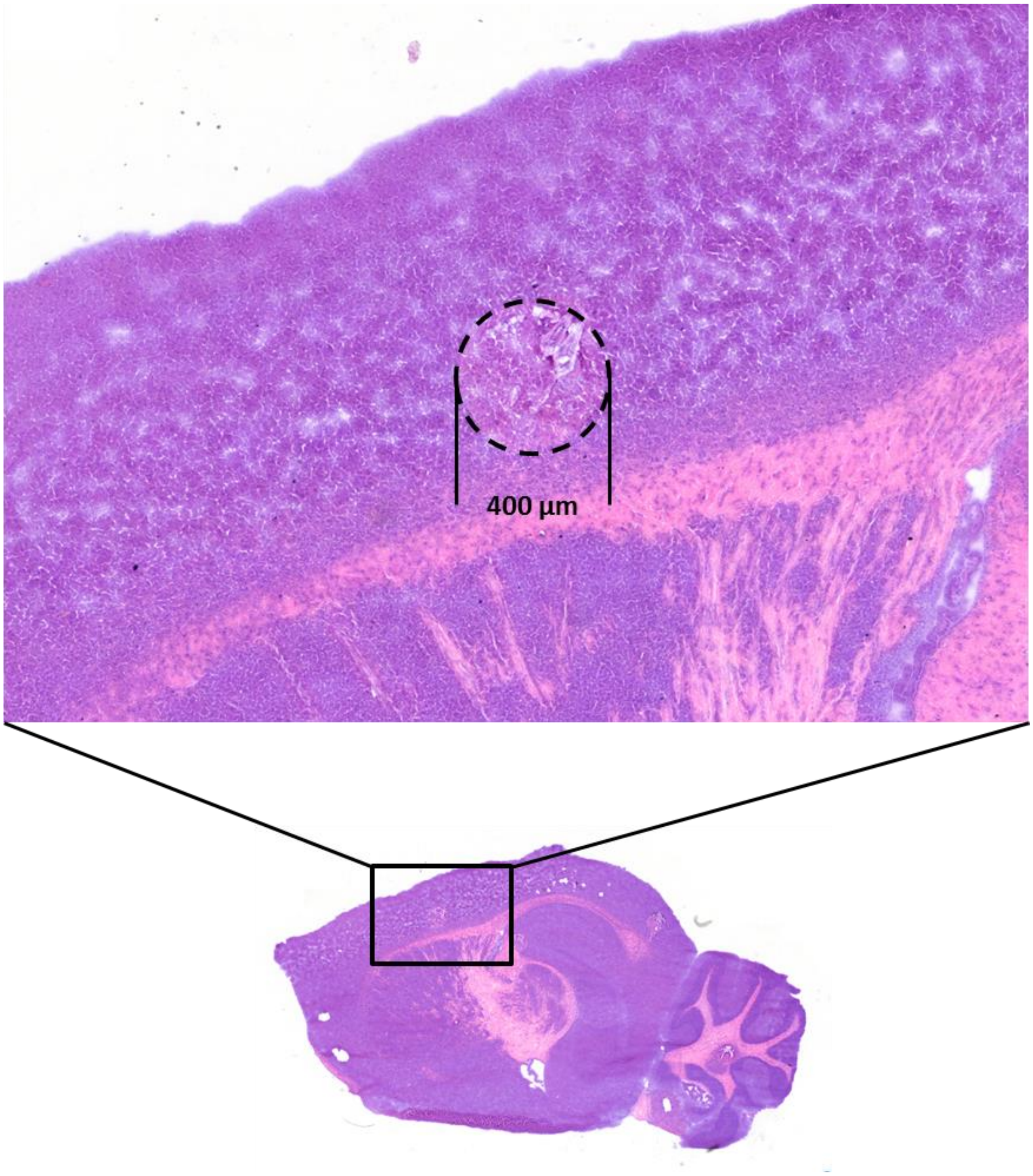

Figure S-1. Evaluation of the HR-LESA extraction spot size in cerebral cortex of wild-type mouse brain. By visual inspection of the H&E image of the tissue section after HR-LESA extraction, the diameter of the area sampled was of 400 µm.

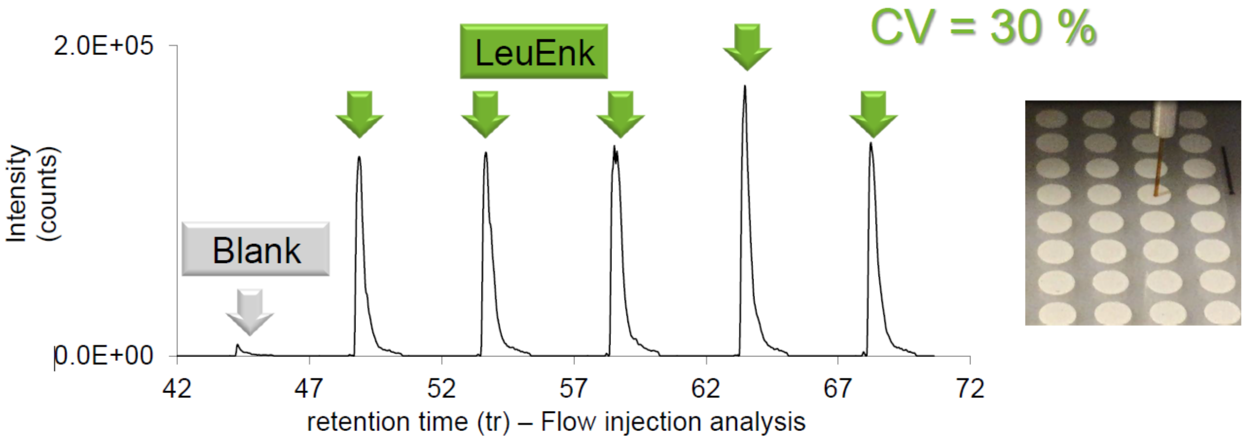

Figure S-2. Evaluation of the reproducibility of the extraction with the silica capillary probe followed by FIA. Results display XIC at m/z 556.28 (protonated Leucine-Enkephalin ion [LeuEnk + H]+) for one blank (glass slide) followed by 5 extractions of LeuEnk using 0.9 µL of 50% ACN + 0.1% HCOOH. Photograph displays the silica capillary probe during the extraction from the plate. CV based on absolute area (n=5)

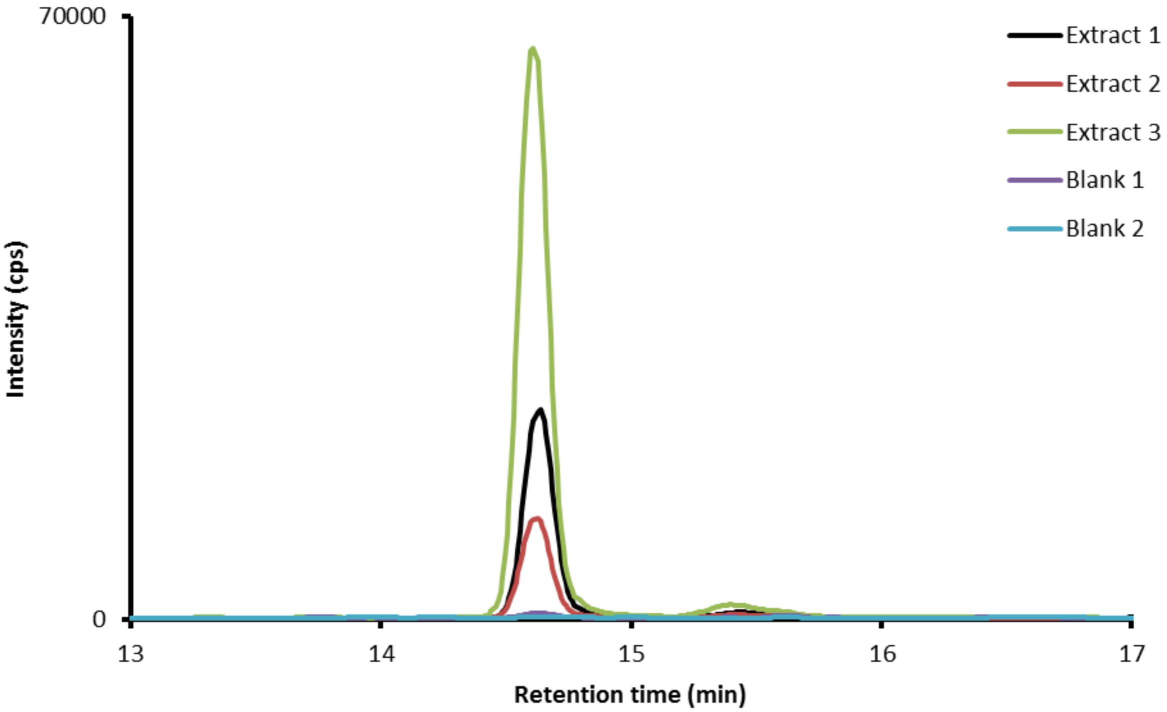

Figure S-3. Reproducibility of LESA-µLC-MS platform using leucine enkephalin standard spotted onto hydrophobic plate. Three extracts were taken, followed by two blank extracts. The relative standard deviation of the retention time of the leucine enkephalin extraction is <0.12% (n=3). The carry-over in the first blank extraction is <1.1% considering the absolute peak area of the last leucine enkephalin extraction versus the absolute peak area of the first blank extraction.

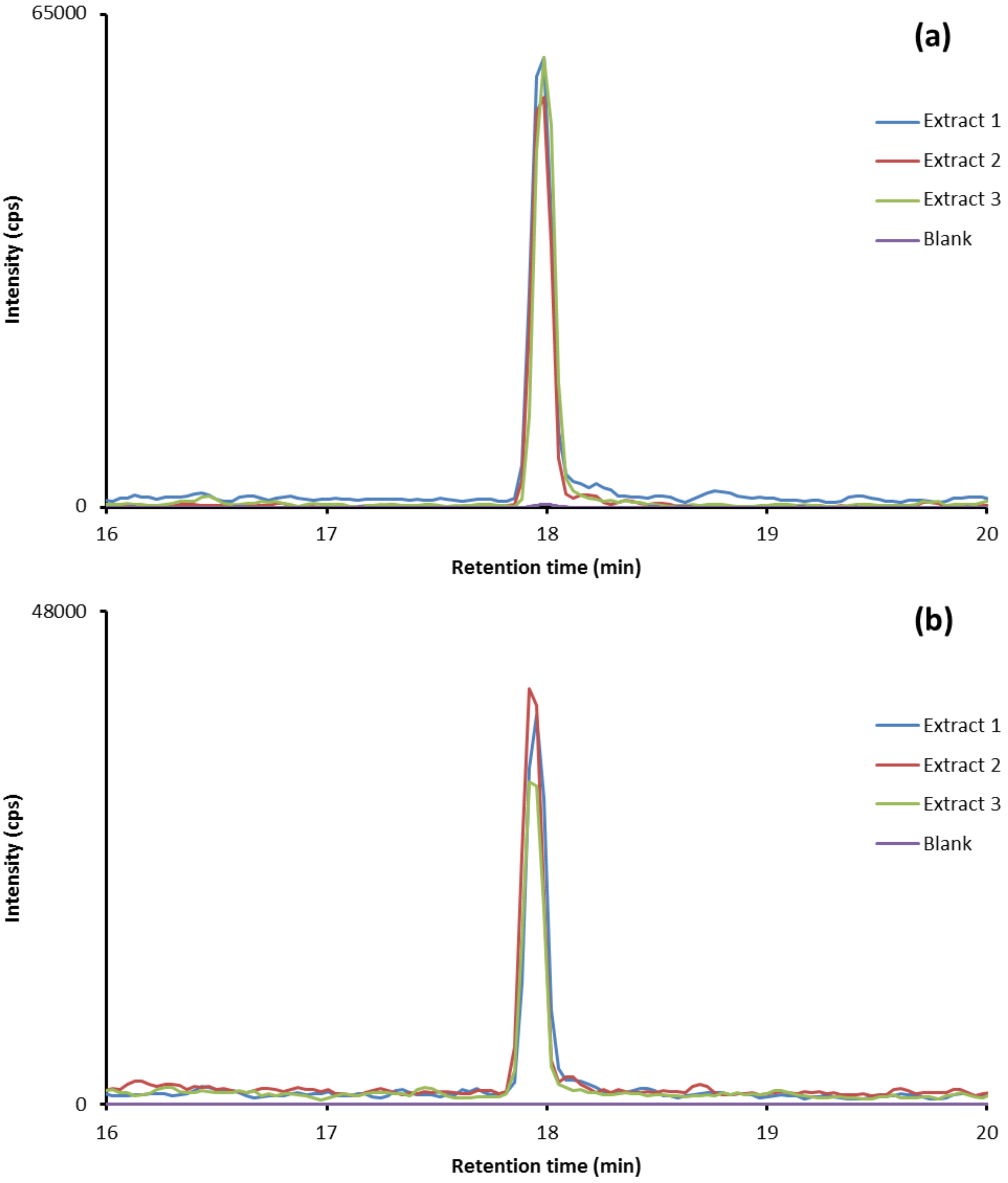

Figure S-4. Reproducibility of HR-LESA-µLC-HDMS^E platform using APP and WT mouse brain tissues. Three extracts from each tissue section were taken from the cerebral cortex region, followed by one blank extract from the glass slide. XIC are shown of *m/z* 634.38 at 6.02 ms drift time from APP brain (a) and WT brain (b). The relative standard deviation for the three extractions (n=3) considering the peak areas are 7.1% (a) and 13.3% (b). The carry-over was assessed by dividing the absolute peak area of the last brain extraction (green) by the absolute peak area of the following blank extraction (purple). Carry-over values were: 0.6% (a) and 0.7% (b).

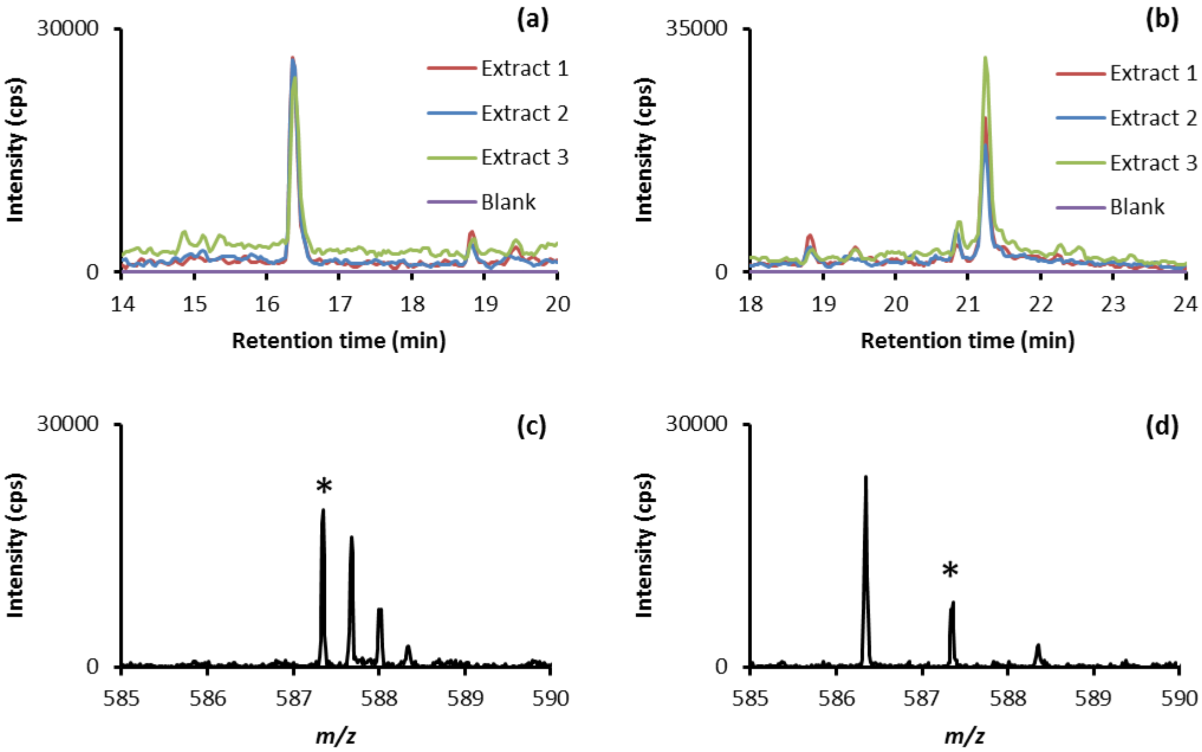

Figure S-5. Isobaric separation of neuropeptides in the cerebral cortex region of APP mouse brain. Three extractions were performed from the tissue section, followed by one blank extract from the glass slide. XIC are shown of *m/z* 587.35 from APP brain with corresponding mass spectrum at retention time 16.36 min with 5.33 ms drift time (a) and 21.23 min with 8.17 ms drift time (b). The relative standard deviation of the three extractions (n=3) at retention time 16.36 min and 21.23 min are 9.3% (a) and 25.4% (b), respectively. The carry-over was assessed by dividing the absolute peak area of the last brain extraction (green) by the absolute peak area of the following blank extraction (purple). Carry-over values were: 0.8% (at 16.36 min; 5.33 ms) and 0.7% (at 21.23 min; 8.17 ms). The asterisk (*) corresponds to *m/z* used to extract the ion chromatogram (XIC).

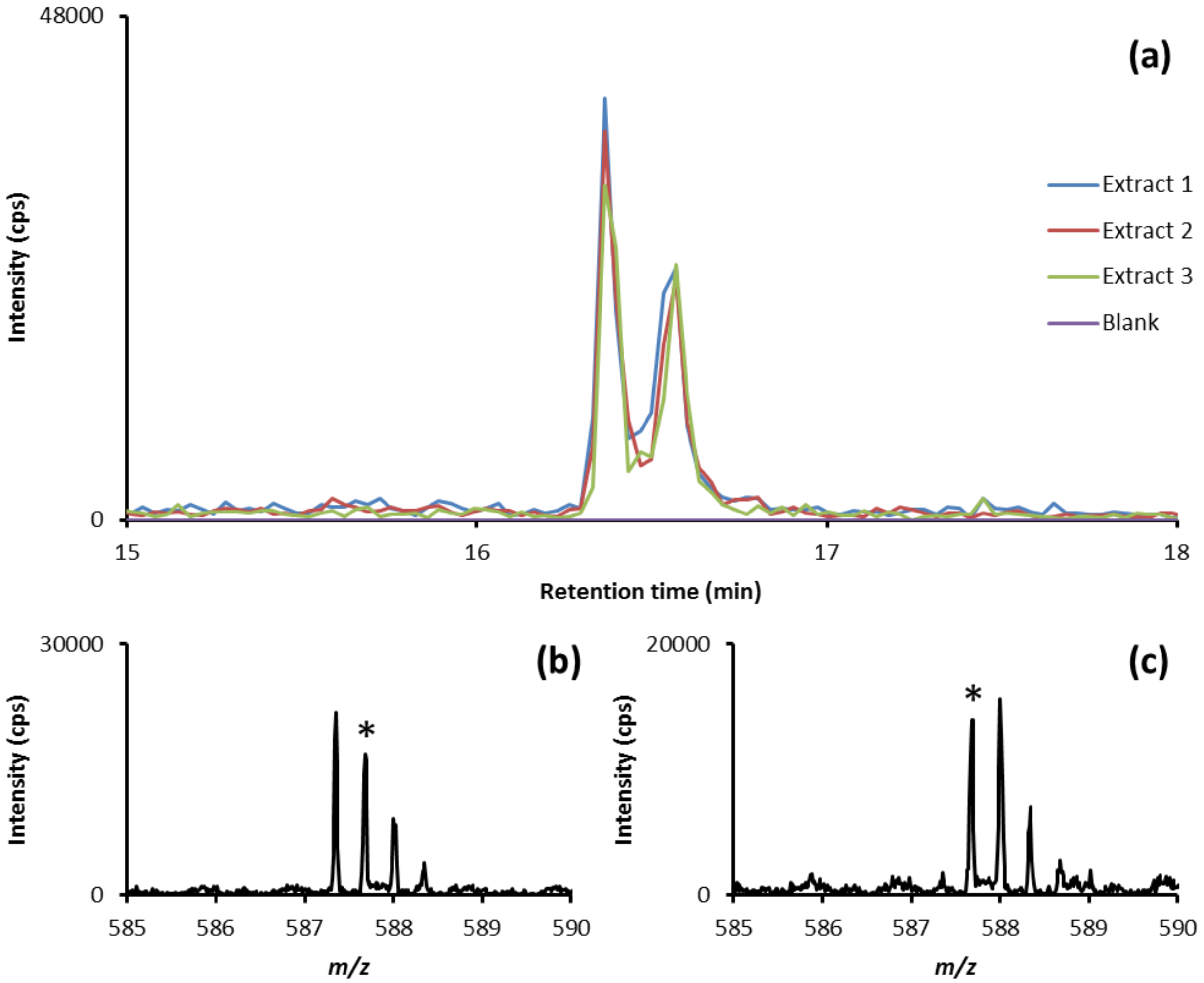

Figure S-6. Isobaric separation of neuropeptides in the cerebral cortex region of APP mouse brain. Three extractions were performed from the tissue section, followed by one blank extract from the glass slide. XIC are shown of *m/z* 587.68 from APP brain (a) with corresponding mass spectrum at retention time 16.36 min (b) and 16.57 min (c) both at 5.26 ms drift time. The relative standard deviation of the three extractions (n=3) at retention time 16.36 min and 16.57 min are 9.0% and 14.4%, respectively. The carry-over was assessed by dividing the absolute peak area of the last brain extraction (green) by the absolute peak area of the following blank extraction (purple). Carry-over values were: 0.5 % (at 16.36 min) and 0.1% (at 16.57 min). The asterisk (*) corresponds to *m/z* used to extract the ion chromatogram (XIC).

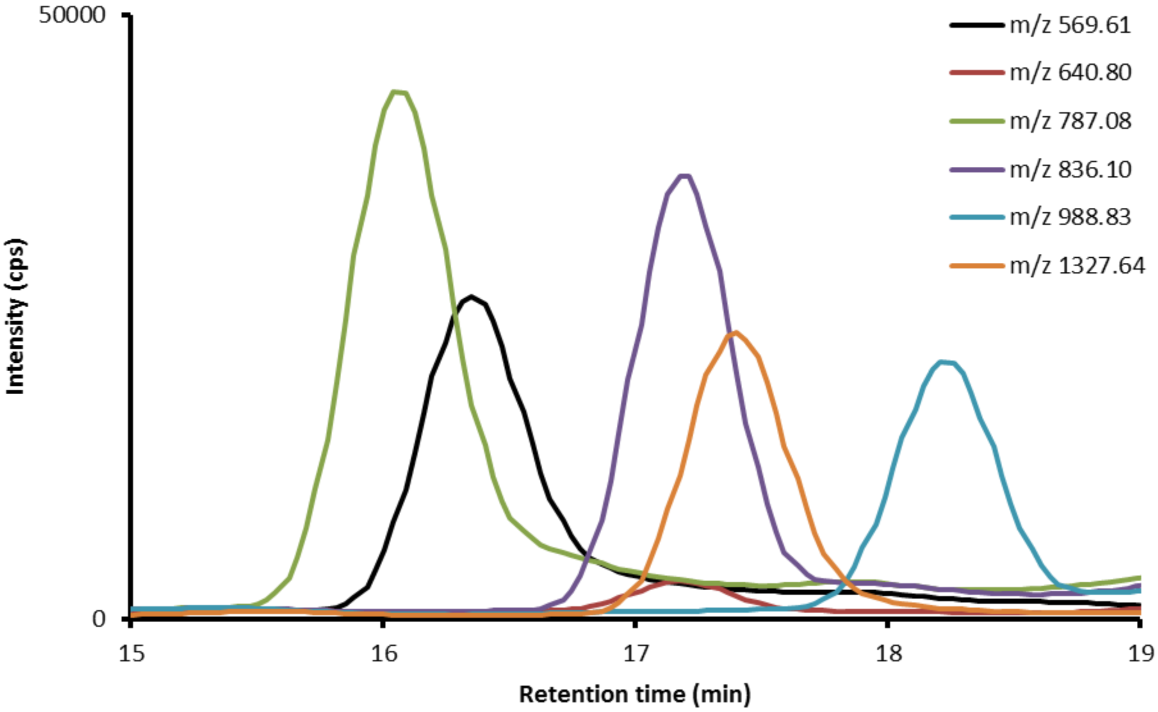

Figure S-7. Extracted ion chromatograms (XIC) of identified neuropeptides from the pituitary gland. The XIC of six neuropeptides are shown at the following retention times: 16.32 min (*m/z* 569.61), 17.18 min (*m/z* 640.80), 16.04 min (*m/z* 787.08), 17.18 min (*m/z* 836.10), 18.26 min (*m/z* 988.83), and 17.40 min (*m/z* 1327.64). These neuropeptides are derived from the pro-opiomelanocortin (POMC) precursor protein as shown in Table S-2.

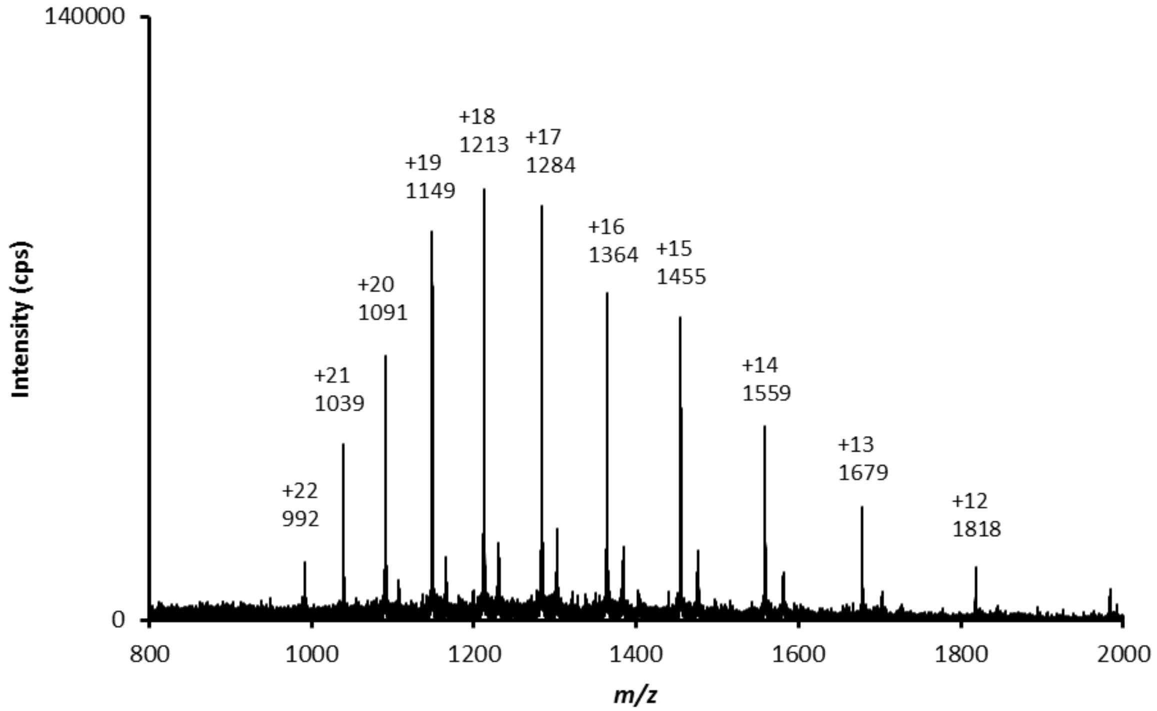

Figure S-8. Intact protein extracted from the adenohypophysis region of the pituitary gland. Deconvolution of the mass spectrum allowed determining the mass of an intact protein at 21832 Da. This protein was identified as POMC.

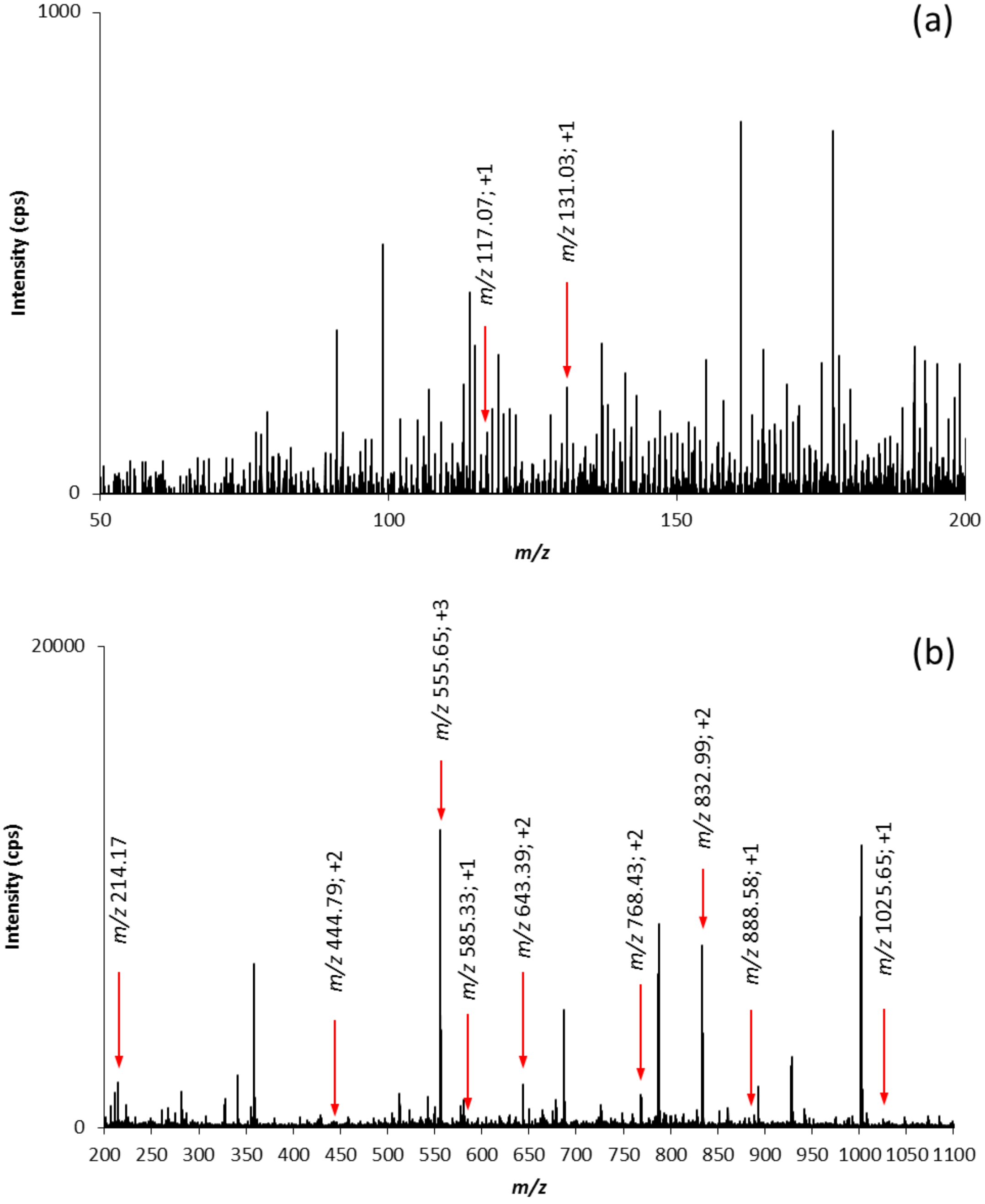

Figure S-9. MS^E data and fragments of alpha-MSH (precursor *m/z* 555.65, +3) extracted from the pituitary gland. Spectra are shown at high (a) and low (b) collision energy after background subtraction. Fragments from the triply charged precursor were identified as follows: *m/z* 117.07 (y_1 ion), *m/z* 131.03 (b_1 ion), *m/z* 214.17 (y_2 ion), *m/z* 444.79 (y_7 ion; +2), *m/z* 555.65 (precursor; +3), *m/z* 585.33 (y_5 ion), *m/z* 643.39 (y_{10} ion; +2), *m/z* 768.43 (y_{12} ion; +2), *m/z* 832.99 (precursor; +2), *m/z* 888.58 (y_7 ion), and *m/z* 1025.65 (y_8 ion). This data correlates with previously published data presented in Hao, G.; Gross, S. S. *Journal of the American Society for Mass Spectrometry* **2006**, 17, 1725-1730.

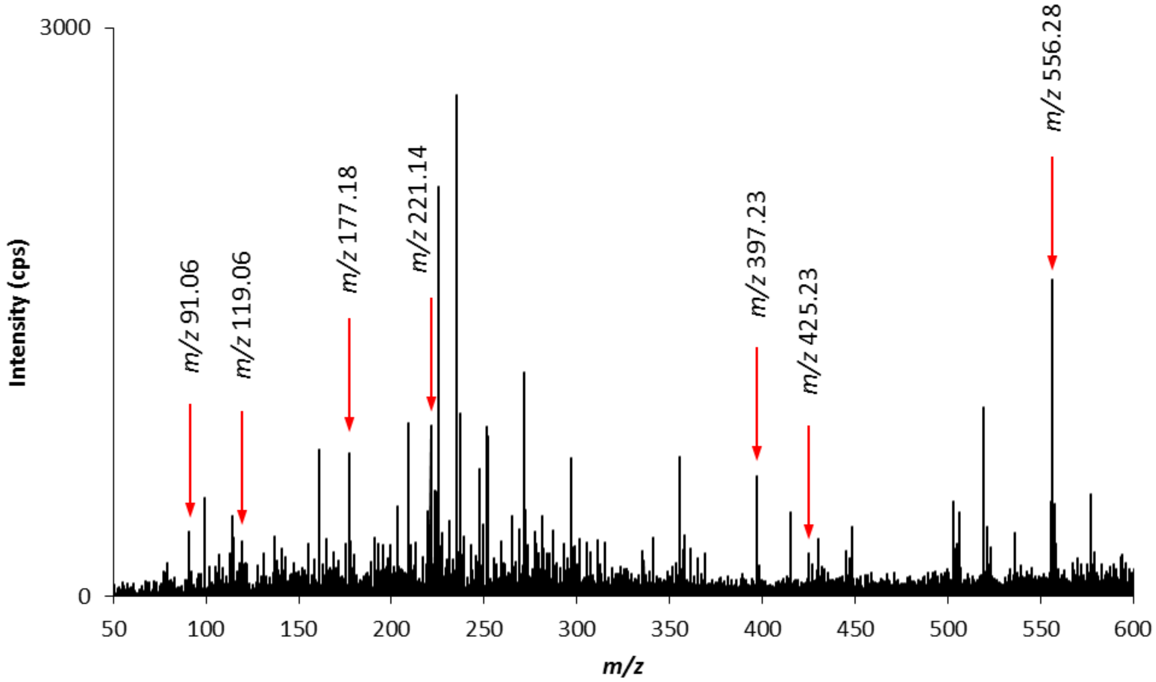

Figure S-10. MS^E data and fragments of leucine enkephalin (precursor *m/z* 556.28, +1) extracted from the pituitary gland. Spectrum is shown at high collision energy at 6.02 ms drift time after background subtraction,3. Fragments from the triply charged precursor were identified as follows: *m/z* 91.06, *m/z* 119.06, *m/z* 177.18, *m/z* 221.14 (b_2 ion), *m/z* 397.23 (a_4 ion) and *m/z* 425.23 (b_4 ion). This data is confirmed with a METLIN database search.