A Highly Robust SPE-LC-MS/MS workflow for quantitation of endogenous amyloid β in cerebrospinal fluid

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ABSTRACT

Amyl β-amyloid (Aβ) are peptides resulting from amyloid precursor proteins (APP) during the deposition of amyloid plaques in the brain. Critical events in Alzheimer's disease (AD) involve an imbalance in the production and clearance of Aβ in the brain. Therefore the Aβ concentrations in CSF are considered as critical biomarkers for AD stage diagnostics and drug study for AD treatment.
We developed a robust SPE-LC-MS/MS based work flow to quantify levels of 3 key Aβ peptides in human CSF. A µelution SPE plate was used to decrease the required sample volume and a 13 min LC gradient was applied to enhance the workflow efficiency. The MRM transitions selected for quantitation are 1033.5→1000.5 for 1-38, 867→843.4 for 1-40 and 1129.5→1079.0 for 1-42, with all MS parameter optimized. We were able to quantify the Aβ levels successfully in human CSF with high sensitivity, accuracy and reproducibility.

INTRODUCTION

Alzheimer's disease (AD) is the most common underlying cause of dementia and the leading cause of years lost to disability globally. Critical events of AD involve a balance disturbance between production and clearance of Aβ peptides from amyloid precursor protein in human cerebrospinal fluid (CSF) (Figure 1). The CSF concentration of these peptides can therefore provide a valuable biomarker for potentially predicting the state of disease and monitoring the efficacy of a drug aiming to inhibit the formation of amyloid plaques[1-4].
The three most important Aβ peptides are peptides with amino acids 1-38, 1-40 and 1-42 (Table 1). The traditional Aβ quantitation methods heavily relied on immuno-based techniques, which is time-consuming, labor-dependent and expensive[5,6]. Herein, we report an efficient, sensitive, highly accurate and reproducible SPE-LC-MS/MS based method for quantitation of 3 key Aβ peptides in human CSF (Figure 2).

MATERIALS AND METHODS

Sample Preparation:
Aβ peptide standards 1-38, 1-40 and 1-42 were mixed with 95% artificial CSF and 5% rat plasma for calibration curve, while N15 labeled Aβ peptides were used as internal standards. Standard Aβ and real human CSF samples were denatured by mixing and vortexing with guanidine hydrochloride to avoid peptide aggregation followed by SPE extraction. The SPE eluents were diluted with water and directly subjected into LC-MS/MS analysis.

HPLC Conditions:
An Eksigent ekspert ultraLC 100-XL UHPLC system was utilized. A Waters Acquity UPLC BEH300 C18 column (2.1 x 150 mm, 1.7 µm) with a 5.5 min linear gradient of mobile phase A 0.3% NH_4OH in water and mobile phase B 10/90 0.3% NH_4OH in water/ACN from 10% to 45% B was used at a flow rate of 220 µL/min. A 2-step autosampler needle wash method was set as: strong wash with 600 µL 60/36/4 IPA/ACN/NH_4OH, followed by weak wash/equilibration with 400 µL 90/10 0.3% NH_4OH in water/ACN. The injection volume was set to 10 µL.

MS/MS Conditions:
An AB Sciex QTRAP® 6500 LC/MS/MS system with IonDrive™ Turbo V ion source and Electrospray Ionization (ESI) probe was used.
All compound parameters and source/gas parameters were optimized based on direct injection or split T injection of peptide standards. The following MRM transitions provided the best S/N and selected for quantitation: 1033.5→1000.5 for 1-38, 867→843.4 for 1-40 and 1129.5→1079.0 for 1-42. Data processing was done using MultiQuant™ software.

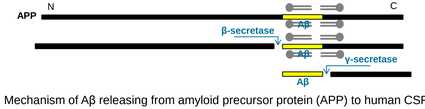


Figure 1. Mechanism of Aβ releasing from amyloid precursor protein (APP) to human CSF.

Peptide	Sequence	Molecular Weight (g/mol)
1-38	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG	4132
1-40	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGV	4330
1-42	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA	4516

Table 1. Peptide sequences and molecular weights of three target Aβ peptide.

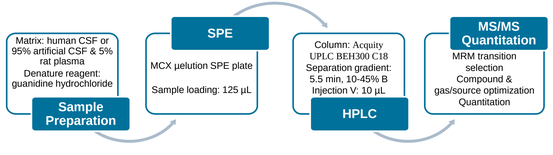


Figure 2. The SPE-LC-MS/MS workflow for Aβ quantitation.

RESULTS

LC-MS/MS based workflow for large amyloid peptides quantitation was reported to be very difficult to develop. With adsorption, aggregation and non-specific adherence being serious issues, samples preparation and analysis need to be complete within several hours to obtain the optimal results. A 5.5 min linear gradient at a flow rate of 220 µL/min (Figure 3) has been approved to obtain appropriate system pressure, LC peak width and apex separation of the 3 Aβ peptides (Figure 4).

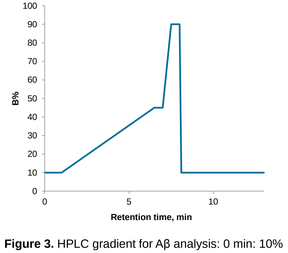


Figure 3. HPLC gradient for Aβ analysis: 0 min: 10% B; 1 min: 10% B; 6.5 min: 45% B; 7 min: 45% B; 7.5 min: 90% B, 8.0 min: 90% B; 8.1 min: 10% B.

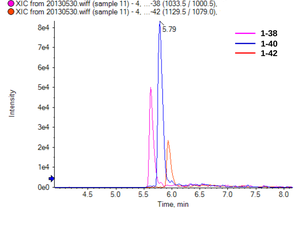


Figure 4. Extract ion chromatograms (XIC) of the target Aβ peptides

With very limited sample amount (125 µL) and small injection volume (10 µL), Aβ levels in real human CSF sample were quantified accurately as 0.81±0.06, 1.43±0.18 and 0.46±0.05 ng/mL (Figure 5). The LLOQs obtained were: 25 pg/mL for 1-38 & 1-40 fragments and 50 ng/mL for 1-42 fragment, along with a wide dynamic range (25-20000 pg/mL and 50-20000 pg/mL) (Figure 6). With higher concentration, the aggregation issue with these compounds was the limiting factor for the ULOQ level.

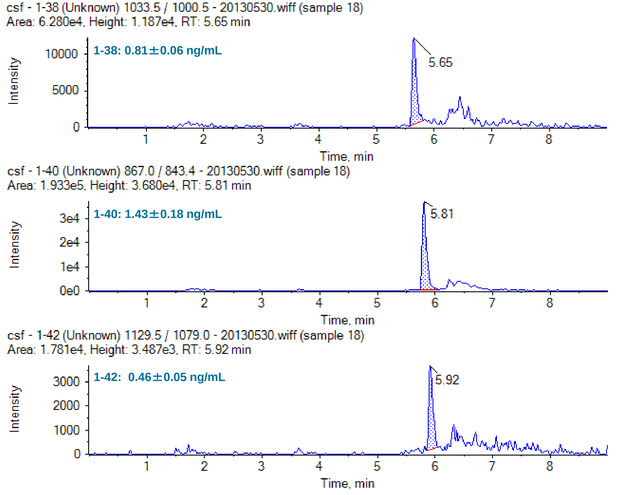
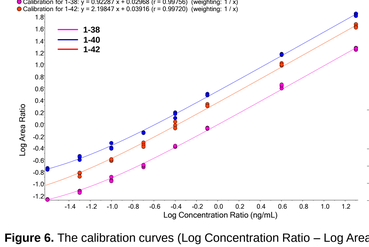


Figure 5. XICs of MRM transitions 1003.5/1000.5 (1-38), 867.0/843.4 (1-40), 1129.5/1079 (1-42) in human CSF sample.



Peptide	LLOQ (pg/mL)	Linear* Range (pg/mL)	R
1-38	25	25-20000	0.998
1-40	25	25-20000	0.999
1-42	50	50-20000	0.997

Figure 6. The calibration curves (Log Concentration Ratio – Log Area Ratio) of the target Aβ peptides.
* The ULOQ levels are determined by the peptide aggregation nature instead of MS detector limit.

More importantly, due to the nature of these peptides, getting good accuracy and reproducibility for quantitation at the low levels have been very challenging. Here, we were able to get good quantitation accuracy (between> 85% and <120%) and reproducibility (< 15% CVs at LLOQ level with triplicate injections) (Figure 7). In addition, the peptide extraction efficiency was >70%.

a)

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	1-38	0.0250	3 of 3	2.558e-2	7.888e-4	3.08	102.31
2	1-38	0.0500	3 of 3	4.611e-2	2.654e-3	5.76	92.22
3	1-38	0.1000	3 of 3	9.386e-2	1.105e-2	11.77	93.86
4	1-38	0.2000	3 of 3	1.779e-1	8.991e-3	5.05	88.96
5	1-38	0.4000	3 of 3	4.147e-1	6.231e-3	1.50	103.66
6	1-38	0.8000	3 of 3	8.733e-1	1.517e-2	1.74	109.17
7	1-38	4.0000	3 of 3	4.505e0	3.209e-1	7.12	112.63
8	1-38	20.0000	3 of 3	1.944e1	6.230e-1	3.20	97.19

b)

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	1-40	0.0250	3 of 3	2.503e-2	1.631e-3	6.52	100.10
2	1-40	0.0500	3 of 3	5.325e-2	5.983e-3	11.24	106.49
3	1-40	0.1000	3 of 3	9.563e-2	1.405e-2	14.70	95.63
4	1-40	0.2000	3 of 3	1.759e-1	2.102e-3	1.17	89.94
5	1-40	0.4000	3 of 3	3.859e-1	4.572e-2	11.85	96.47
6	1-40	0.8000	3 of 3	8.614e-1	2.838e-2	3.30	107.68
7	1-40	4.0000	3 of 3	4.191e0	9.849e-2	2.35	104.77
8	1-40	20.0000	3 of 3	1.978e1	9.026e-1	4.56	98.92

c)

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	1-42	0.0250	0 of 3	N/A	N/A	N/A	N/A
2	1-42	0.0500	3 of 3	4.668e-2	4.946e-3	10.60	93.35
3	1-42	0.1000	3 of 3	9.212e-2	2.858e-3	3.10	92.12
4	1-42	0.2000	3 of 3	1.827e-1	1.746e-2	9.56	91.33
5	1-42	0.4000	3 of 3	4.114e-1	5.709e-2	13.88	102.86
6	1-42	0.8000	3 of 3	8.993e-1	3.281e-2	3.65	112.41
7	1-42	4.0000	3 of 3	4.417e0	1.812e-1	4.10	110.42
8	1-42	20.0000	3 of 3	1.950e1	1.257e0	6.45	97.50

Figure 7. Statistic summary for Aβ peptides (a: 1-38; b: 1-40, c: 1-42). Peptide name, theoretical and averaged calculated concentrations (ng/mL), Standard deviation, CV and accuracy are listed.

CONCLUSIONS

In this study we show a total solution combining sample preparation and mass spectrometric analysis for quantitation of endogenous β-amyloid peptides in human CSF with high accuracy and reproducibility.

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TRADEMARKS/LICENSING