Rapid and reliable quantitation of amino acids and myo-inositol in mouse brain by high performance liquid chromatography and tandem mass spectrometry

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ABSTRACT

Amino acids and myo-inositol have long been proposed as putative biomarkers for neurodegenerative diseases. Accurate measures and stability have precluded their selective use. To this end, a sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS) method based on multiple reaction monitoring was developed to simultaneously quantify glutamine, glutamate, γ-aminobutyric acid (GABA), aspartic acid, N-acetyl aspartic acid, taurine, choline, creatine, phosphocholine and myo-inositol in mouse brain by methanol extractions. Chromatography was performed using a hydrophilic interaction chromatography silica column within in a total run time of 15 min. The validated method is selective, sensitive, accurate, and precise. The method has a limit of quantification ranging from 2.5 to 20 ng/ml for a range of analytes and a dynamic range from 2.5–20 to 500–4000 ng/ml. This LC–MS/MS method was validated for biomarker discovery in models of human neurological disorders.

1. Introduction

Amino acids (AAs) are the building blocks for peptides and proteins serving a broad range of cellular functions including coenzymes, antioxidants, and neurotransmitters [1–3]. AAs such as glutamic acid (Glu), and γ-aminobutyric acid (GABA) have a prominent role in the pathobiology of several neurological disorders [4–6]. Common amongst these diseases is neuronal dysfunction and loss [7–9]. The basis for the dysfunction lies, in part, through alterations in excitatory amino acid (EAA) activities such as Glu, which binds to a variety of EAA ligand-gated ion channels. If, for any reason, receptor activation becomes excessive, target neurons are damaged and eventually die [10]. As Glu and other AAs function as excitatory neurotransmitters within the central nervous system (CNS), their implications for neuronal injury in a range of neurodegenerative disorders that include ischemia, traumatic brain injury, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and amyotrophic lateral sclerosis amongst others are profound [11]. For example, previous studies indicated that AAs such as Asp, GABA, and Glu are significantly reduced in AD brains; whereas other studies showed elevated myo-inositol (MI) levels in the brains and cerebrospinal fluid of AD and Down syndrome patients, respectively [12–14]. As a consequence excitotoxic AAs have been a long term focus of research pursuits in health and into the pathobiology of diseases of the nervous system making their quantitation highly relevant [15]. Therefore, quantification of AAs may play an important role in the diagnosis and understanding the etiology and progression of neurodegenerative diseases [16].

AAs have been previously quantified by gas chromatography–mass spectroscopy (GC–MS) following derivatization with silylation agents such as tert-butyl-dimethylsilyl and t-butylmethylsilyl [17–19]. AAs have also been determined in brain tissues by liquid chromatography (LC) with UV, fluorescence, and coulometric detection [12,14,20]. These methods have several disadvantages such as the tedious extraction procedure, limited selectivity, relatively long run time, and the presence of interfering peaks due to the low selectivity of these detection techniques. Because of its high selectivity and sensitivity, tandem mass spectrometer MS/MS detectors can overcome these problems. Therefore, LC–MS/MS methods were developed for

the quantification of AAs in biological samples [17–19,21,22]. It is difficult to retain highly hydrophilic analytes using reverse-phase chromatography, which may lead to the co-elution of such hydrophilic analytes with other endogenous compounds present in the biological matrix. Ion pairing chromatography is a special form of reverse-phase chromatography for separating complex mixtures of hydrophilic and ionic analytes. Therefore, ion pairing chromatography with ion-pairing reagents such as perfluoro-carboxylic acids (PDFOA) and trifluoroacetic acid was used to improve the chromatographic separation of AAs [18]. However, the use of these ion-pairing reagents results in ion suppression issues as well as irreproducibility of the retention profile over time. The column had to be flushed with organic solvent for 20 min every 6 injections to prevent the accumulation of ion pairing reagents and therefore improve reproducibility of retention time [23].

Another approach to improve the retention of hydrophilic analytes is to use hydrophilic interaction liquid chromatography (HILIC). The mechanism of retention in HILIC columns includes the partitioning of analytes between a water-enriched layer of a stagnant aqueous mobile phase attached to a hydrophilic stationary phase and a relatively more hydrophobic bulk mobile phase [24]. Therefore, hydrophilic molecules can be retained on normal phase columns using solvents typically used in reverse-phase mobile phases such as water and acetonitrile (ACN). Retention of analytes is increased by increasing the ACN content in mobile phase [25]. The high content of organic mobile phases required to retain molecules on a HILIC column improves ionization of analytes in the ESI source, which leads to enhanced MS response compared to reversed-phase chromatography [25]. One LC–MS/MS method was developed for the analysis of AAs in brain micro-dialysates using HILIC [26]. However, only two AAs (GABA and Glu) were quantified using this method. Therefore, we report a valid LC–MS/MS method for the quantification of nine AAs and MI in mouse brain using a HILIC silica column. This method was developed to support multiple studies that aim to study the role of AAs and MI as potential biomarkers for neurological disorders.

2. Experimental

2.1. Chemicals and reagents

Glutamine (Gln), myo-inositol (MI), γ-aminobutyric acid (GABA), glutamic acid (Glu), N-acetyl aspartic acid (NAA), aspartic acid (Asp), taurine (Tau), choline (Cho), creatine (CR), and phospho-choline (PC) were purchased from Sigma–Aldrich (St. Louis, MO). Glu-d5 was purchased from C/D/N ISOTOPES, INC. (Pointe-Claire, Quebec, Canada). HPLC grade methanol, acetonitrile, 1-butanol, acetone, formic acid, and acetic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Instrumentation

A Waters ACQUITY ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) coupled with a 4000 Q TRAP® hybrid quadrupole linear ion trap mass spectrometer with an electrospray ionization (ESI) source (Applied Biosystems, MDS Sciex, Foster City, CA, USA) was used throughout. The UPLC and MS systems were controlled by Empower Pro 6.0 and Analyst 1.4.2 software, respectively. All chromatographic separations were performed using a Waters Atlantis® HILIC silica column (5 μm, 150 mm × 2.1 mm) equipped with a Phenomenex SECURITYGUARD™ C18 (ODS) column (4 mm × 3.0 mm) (Phenomenex, Torrance, CA, USA).

Table 1
MRM transitions and MS parameters for the analytes and IS.

	MRM transition	Declustering potential (V)	Collision energy (eV)	Cell exit potential (V)
Gln	147.0 → 129.9	31	15	22
MI	178.9 → 160.9	−55	−18	−1
GABA	104.0 → 68.9	31	23	12
Glu	148.0 → 84.0	36	25	14
NAA	176.0 → 133.9	36	15	22
Asp	133.9 → 73.9	36	21	12
Tau	125.9 → 107.8	41	17	18
Cho	104.0 → 60.0	56	25	10
CR	131.9 → 87.1	46	23	14
PC	184.0 → 86.0	41	25	14
Glu-d5 (IS)	153.0 → 88.0	41	25	14

2.3. Liquid chromatographic and mass spectrometric conditions

Mobile phase A consisted of 0.1% formic acid in water and mobile phase B comprised of acetonitrile (ACN). The chromatographic separation was achieved using gradient elution. The initial mobile phase composition was 75% B for the first 5.25 min, was gradually decreased to 25% B over 0.25 min, and then held constant at 25% B for 3.5 min. The mobile phase was then reset to the initial conditions at 75% B over 0.25 min and the column was equilibrated under these conditions for 5.75 min. The total run time was 15 min and the injection volume of all samples was 10 μl. The column temperature was set at 23 °C and a total flow rate of 300 μl/min was used.

Mass spectrometer parameters, such as temperature, voltage, and gas pressure were optimized by infusing each analyte and the internal standard (Glu-d5) using a 1 μg/ml solution in H₂O via a Harvard '22' standard infusion syringe pump (Harvard Apparatus, South Natick, MA, USA). Analytes and internal standard (IS) were detected in the positive ionization mode except MI, for which negative ionization mode was used. The following settings were optimized for achieving highest signal intensity, ion spray voltage: ±4500 V, source temperature: 600 °C, curtain gas: 10, gas-1: 40, gas-2: 30, collision gas pressure: high, Q1/Q3 resolution: unit; and interface heater: on. The multiple reaction monitoring (MRM) transitions for each analyte and IS, as well as their respective optimum MS parameters, including declustering potential (DP), collision energy (CE), and cell exit potential (CXP), are shown in Table 1.

2.4. Preparation of standard solutions and calibration curves

Brain homogenates were prepared by homogenizing approximately 100 mg brain tissues, collected from control mice, in methanol (1:3, w/v). The brain homogenate was then diluted 4500-fold with distilled water. 200 μl of the diluted homogenates were spiked with 20 μl of the appropriate working standard solutions containing IS (Glu-d5). Samples were then extracted by protein precipitation using 2 ml methanol. Samples were vortexed and centrifuged at 20,000 × g for 10 min. The supernatants were aspirated and evaporated under vacuum, and the resulting residues were reconstituted in 400 μl of 50% ACN. Therefore, samples were diluted 36,000-fold (4 × 4500 × 2) by the time they are analyzed. The final concentration of IS in the calibration curve was 75 ng/ml. The calibration curves were constructed within the following dynamic ranges for various analytes: 2.5–500 ng/ml for Cho; 5–1000 ng/ml for PC, GABA, Asp, NAA, and Gln; 10–2000 ng/ml for Glu, Tau, and CR; and 20–4000 ng/ml for MI.

2.5. Sample preparation

Brain tissues were collected from mice and stored at −80 °C until analysis by LC–MS/MS. Approximately, 20 mg of brain tissue was homogenized in methanol (1:3, w/v). The brain homogenate was then diluted 1500-fold with distilled water. 200 µl of the diluted homogenate was spiked with 20 µl of IS solution (Glu-d5). Samples were then extracted and reconstituted the same way as described for calibration standards. Therefore, samples were diluted 12,000-fold $(4 \times 1500 \times 2)$ by the time they are analyzed.

2.6. Method validation

The method was validated using 5 QC points for each calibration curve. The concentrations of QC points were 5, 10, 50, 750, and 1000 ng/ml for PC, GABA, Asp, NAA and Gln; 10, 20, 100, 1500, 2000 ng/ml for Glu, Tau, and CR; 20, 40, 200, 3000, 4000 ng/ml for MI; and 2.5, 5, 25, 375, 500 ng/ml for Cho. Five replicates of each QC point were analyzed each day to determine the intra- and inter-day accuracy and precision. This process was repeated 3 times over 3 days using freshly prepared calibration curves. Intra-day accuracy and precision were calculated from the % bias [% (measured−theoretical)/theoretical concentration] and relative standard deviation [%RSD = % standard deviation/mean], respectively, for the 5 replicates of each QC point. Inter-day accuracy and precision were calculated similarly for the 15 replicates of each QC point pooled from the 3 validation runs. The absolute recovery was determined for each of the 5 QC points. Absolute recovery was calculated by comparing the peak area of samples spiked pre-extraction to the peak area of samples in neat solution (50% ACN in water). Stability of stock solutions was determined on the bench, in the −20 °C freezer, and in the −80 °C freezer.

## 3. Results and discussions

3.1. Method development

A sensitive method was developed for the quantification of 9 AAs and MI in mouse brain. Variant columns and mobile phases were evaluated to optimize chromatographic conditions. AAs were not retained longer than 1–1.5 min on reverse phase columns such as C18 and phenyl columns regardless of the composition or the pH of the mobile phase (data not shown). Hydrophilic analytes are retained longer using normal phase, ion pairing, or ion exchange chromatography. However, these techniques are generally not compatible with ESI-MS because of the use of nonvolatile mobile phase solvents, buffers, and or salts. In addition, despite the availability of relatively volatile ion pairing reagents, ion suppression and other issues related to method ruggedness have been reported with LC–MS analysis of AAs as well as other analytes using these volatile ion pairing reagents [18,23,27]. The effect of ion pairing agents in the method development process was investigated. The use of tridecafluoroheptanoic acid (TDFHA) at relatively small concentrations (0.1–1 mM) caused significant ion suppression for most AAs such as Tau (75-fold), MI (70-fold), NAA (12-fold), and GABA and PC (3-fold). TDFHA also resulted in a marked increase in the retention time of Gln and Glu (100%), CR (275%), and GABA and Cho (1000%). However, the retention time of other analytes were not affected by TDFHA. Another approach to analyze hydrophilic analytes is HILIC. One LC–MS/MS method was developed to quantify GABA and Glu in brain microdialysates using HILIC column [26]. However, this method quantified only two AAs (GABA and Glu).

Therefore, we investigated the application of HILIC to support the LC–MS/MS analysis of AAs in mouse brain.

The effect of the mobile phase composition including both the organic and aqueous phases on the signal intensity and chromatography of analytes using HILIC columns were investigated. The use of ACN resulted in a higher MS signal compared to MeOH for most AAs. For examples, the MS signal for Glu and Asp were 10-fold higher when ACN, rather than MeOH, was used in the mobile phase. Although 0.1% acetic acid resulted in a higher signal and a slightly higher retention time than 0.1% formic acid for most of the analytes, it, however, resulted in a poor peak shape for NAA and Asp. The retention time of Gln, MI, GABA, NAA, Asp, and Cho increased with increasing the pH of the mobile phase, but there was a decrease in signal intensity as well. Therefore, 0.1% formic acid was finally selected as the aqueous phase because it provides baseline separation with an acceptable peak shape and high sensitivity for all analytes within a relatively short run time. Fig. 1 shows a representative chromatogram of all the AAs and MI in a mouse brain sample under final chromatographic conditions.

Preparing the calibration curve in the same biological matrices to be analyzed compensates for losses of analytes during sample extraction and ion suppression/enhancement in the ESI source that result from the co-eluting endogenous components of the matrix. Because AAs are endogenous compounds, blank matrices free from AAs are not available. Therefore, the method of standard addition was investigated. Calibration standards were prepared by spiking the biological matrix of interest (brain homogenate) with analyte standards and the background of endogenous levels of AAs in the blank unspiked samples were subtracted. Because of the high endogenous levels of most AAs and the wide variation of the endogenous levels of the various AAs in the brain, it was not possible to produce a calibration curve with the desirable dynamic range for all AAs of interest by directly spiking control tissue homogenates. Therefore, control brain homogenates were diluted three fold more than the actual study samples before they were used to construct the calibration curves, i.e. 1500-fold for brain homogenates of the study samples vs. 4500-fold for the brain homogenate used spiked with calibration standards to construct the calibration curve. We studied the effect of matrix dilution on the extraction recovery to ensure that the matrix of the actual study samples and the "diluted matrix" of the calibration standards produce similar matrix (suppression/enhancement) effects on the AA analytes. Extraction recoveries of all analytes were not affected by sample dilution and were similar in brain homogenates diluted in the range of 10^3–10^5 fold (data not shown).

Several extraction procedures were investigated for the extraction of AAs from brain tissues including protein precipitation and liquid–liquid extraction. Extraction recoveries were higher using methanol for protein precipitation compared with acetonitrile, ethanol, 5% FA in ethanol, or 5% NH$_4$OH in ethanol. Liquid–liquid extraction using methanol/H$_2$O/hexane yielded extraction recoveries similar to protein precipitation with methanol but the latter was selected, as it was simpler to perform. Extraction recoveries of all the analytes are summarized in Table 2. The recoveries of all analytes were higher than 70% at various QC concentrations using methanol–protein precipitation. It has been reported that some AAs undergo rapid degradation during sample preparation due to residual activity of endogenous enzymes [28]. Therefore, the stability of AAs during sample preparation was investigated. Indeed, most of the AAs of interest underwent rapid degradation after homogenizing brain tissues in water. In contrast, these AAs were stable for at least 24 h after brain homogenization in methanol (Fig. 2). This is likely due to the inhibition of the activity of endogenous enzymes responsible for amino acid degradation by the organic solvent. Therefore, the brain tissues were homogenized in methanol in our studies.
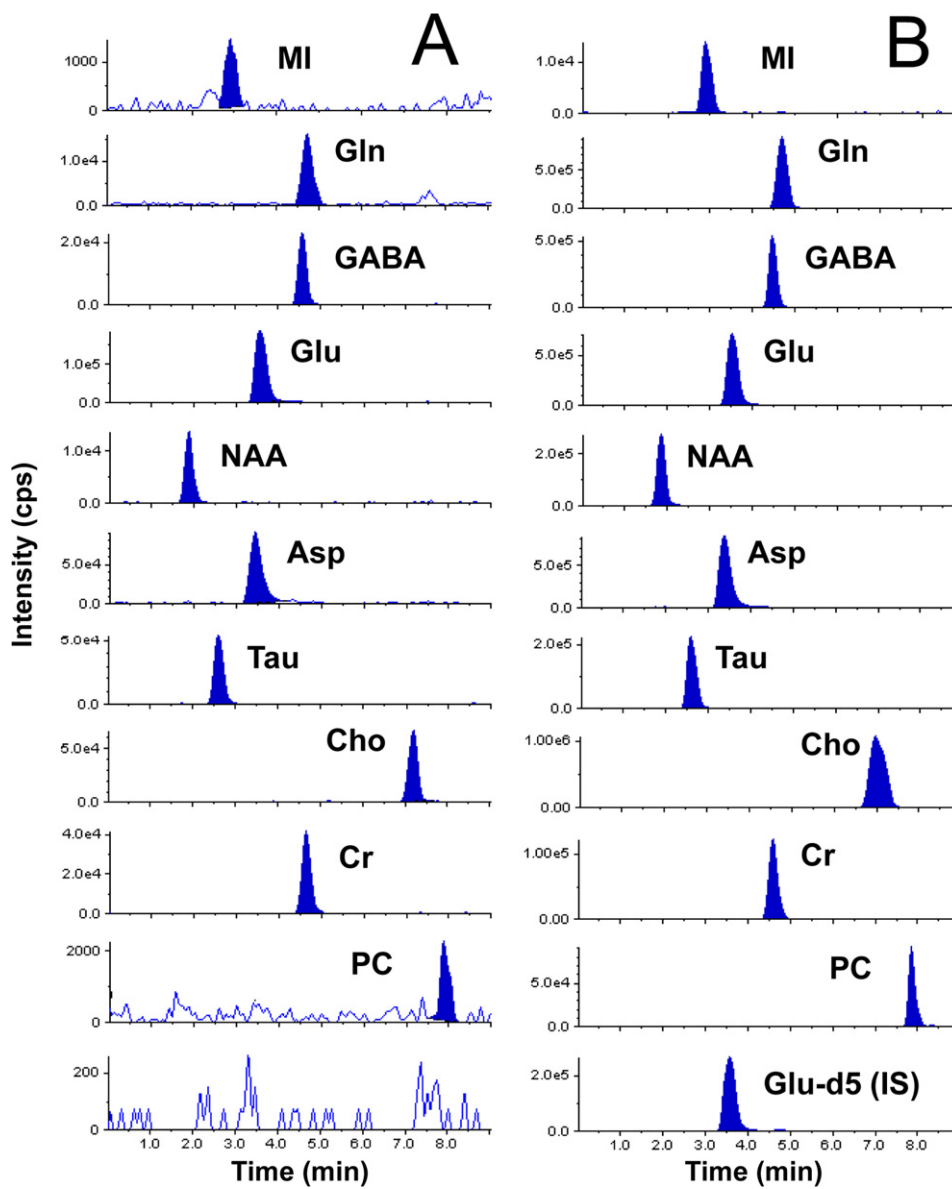
Fig. 1. A representative chromatogram of the endogenous levels of AAs in mouse brain before spiking with analyte standards (A), and after spiking with analyte standard solutions (concentration) and IS (concentration) (B).
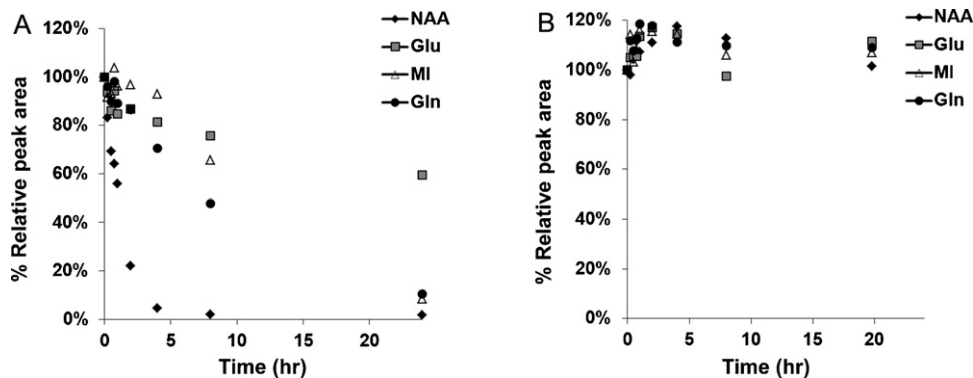
Fig. 2. Degradation profile of some AAs from brains homogenized (A) in (1:3, w/v) water and (B) in (1:3, w/v) methanol. The % relative peak area is calculated by taking the ratio of peak area of analytes at various time points to peak area of analytes at time "0 h".

Table 2
Extraction recoveries of analytes from mouse brain at 5 QC levels.

Nominal conc.	QC1 5 (ng/ml)	QC2 10 (ng/ml)	QC3 50 (ng/ml)	QC4 750 (ng/ml)	QC5 1000 (ng/ml)
PC	98.9 ± 10.3	108.6 ± 3.7	91.7 ± 5.5	101.5 ± 7.2	98.3 ± 4.7
GABA	105.0 ± 15.5	72.6 ± 3.4	80.7 ± 2.7	70.5 ± 2.1	71.4 ± 4.5
Asp	80.2 ± 8.9	101.3 ± 9.2	87.1 ± 6.0	81.0 ± 4.1	75.1 ± 5.0
NAA	84.6 ± 7.7	114.6 ± 11.1	93.5 ± 12.3	87.5 ± 4.2	71.6 ± 11.6
Gln	81.3 ± 12.8	85.7 ± 3.1	78.5 ± 3.3	75.2 ± 2.7	74.8 ± 5.2

Nominal conc.	QC1 10 (ng/ml)	QC2 20 (ng/ml)	QC3 100 (ng/ml)	QC4 1500 (ng/ml)	QC5 2000 (ng/ml)
Glu	80.0 ± 8.3	85.4 ± 9.8	88.4 ± 5.5	81.5 ± 1.2	77.2 ± 2.5
Tau	74.9 ± 10.7	84.8 ± 7.5	77.3 ± 7.4	77.2 ± 5.1	71.5 ± 1.9
CR	72.5 ± 11.9	70.0 ± 5.6	72.1 ± 3.6	75.9 ± 2.1	75.2 ± 1.0

Nominal conc.	QC1 20 (ng/ml)	QC2 40 (ng/ml)	QC3 200 (ng/ml)	QC4 3000 (ng/ml)	QC5 4000 (ng/ml)
MI	72.3 ± 10.0	75.0 ± 12.3	75.8 ± 1.7	80.2 ± 5.6	73.1 ± 11.6

Nominal conc.	QC1 2.5 (ng/ml)	QC2 5 (ng/ml)	QC3 25 (ng/ml)	QC4 375 (ng/ml)	QC5 500 (ng/ml)
Cho	77.3 ± 12.8	86.50 ± 7.8	78.1 ± 5.7	82.3 ± 1.4	78.4 ± 3.5

3.2. Method validation

The reliability and reproducibility of this method was evaluated by determining the inter-day and intra-day (data not shown) accuracy and precision using 5 QC concentrations distributed throughout the dynamic range for each analyte. Table 3 summarizes the results of inter-day accuracy and precision of the method. The precision and accuracy was <20% for all the 10 analytes at LOQ and <15% at the other four QC concentrations. The slopes and intercepts were consistent throughout validation and the regression coefficient (r^2) was >0.95 for all analytes. The LOQ levels ranged from 2.5 ng/ml to 20 ng/ml and the dynamic ranges ranged from 2.5–500 ng/ml to 20–4000 ng/ml for the various analytes. The LOQ and dynamic ranges for the various analytes varied due to the marked differences in their sensitivity and their endogenous levels in brain. The results of stability studies indicate that neat standards of the analytes were stable for at least 1 week, and

2 months, on the bench, and in the −20 °C and −80 °C freezers, respectively.

3.3. AAs in mouse brain

Table 4 summarizes the concentrations of AAs and MI obtained in mouse brain tissue. AA levels were reported by magnetic resonance (MR) spectroscopy, fluorescence detection, GC–MS, and HPLC with UV or electrochemical detection (ECD). Data reported from these studies varied significantly. We report similar concentrations of MI, Asp, CR, Cho, GABA, and PC to those previously reported [29–34]. In contrast, our concentrations of Gln and Tau were about 5-fold lower than previous reports [33,35], whereas, NAA and Glu were 2.5-fold higher than what was reported in previous studies [30,31]. The differences in endogenous levels of AAs reported from the various studies could be due to degradation of AAs during sample extraction due to residual enzymatic activity,

Table 3
Summary of the inter-day accuracy and precision of analytes in mouse brain.

Nominal conc.	QC1 5 (ng/ml)	% RSD	QC2 10 (ng/ml)	% RSD	QC3 50 (ng/ml)	% RSD	QC4 750 (ng/ml)	% RSD	QC5 1000 (ng/ml)	% RSD
PC	4.90	15.79	9.57	11.79	45.49	8.24	803.31	6.97	1057.29	3.85
GABA	5.19	12.93	11.32	10.49	52.80	6.39	712.68	4.55	954.68	2.89
Asp	4.83	14.94	10.55	9.59	51.16	8.68	733.79	5.06	963.45	4.34
NAA	4.90	15.53	10.03	10.15	51.33	9.95	738.14	6.29	997.51	6.16
Gln	4.77	18.61	10.63	12.93	52.97	11.34	696.75	5.94	938.33	5.42

Nominal conc.	QC1 10 (ng/ml)	% RSD	QC2 20 (ng/ml)	% RSD	QC3 100 (ng/ml)	% RSD	QC4 1500 (ng/ml)	% RSD	QC5 2000 (ng/ml)	% RSD
Glu	10.62	16.01	19.24	11.94	108.96	6.38	1437.81	3.41	1878.79	6.59
Tau	9.42	17.40	19.85	12.33	111.89	6.49	1355.18	5.39	1730.06	4.27
CR	10.66	10.19	20.07	12.11	106.96	8.57	1372.24	5.27	1818.87	7.08

Nominal conc.	QC1 20 (ng/ml)	% RSD	QC2 40 (ng/ml)	% RSD	QC3 200 (ng/ml)	% RSD	QC4 3000 (ng/ml)	% RSD	QC5 4000 (ng/ml)	% RSD
MI	19.60	13.46	39.43	12.21	210.70	11.98	3017.25	6.75	3734.61	7.14

Nominal conc.	QC1 2.5 (ng/ml)	% RSD	QC2 5 (ng/ml)	% RSD	QC3 25 (ng/ml)	% RSD	QC4 375 (ng/ml)	% RSD	QC5 500 (ng/ml)	% RSD
Cho	2.72	11.76	4.90	11.26	25.68	7.47	351.61	4.25	441.20	4.36

Table 4
Endogenous levels of AAs and MI in control mice brain obtained from our method ($n = 9$). Values are reported as mean ± SEM.

Analyte	Concentration (mmol/kg tissue)
Gln	5.81 ± 0.27
MI	4.41 ± 0.32
GABA	4.34 ± 0.85
Glu	8.65 ± 0.38
NAA	4.91 ± 0.28
Asp	2.82 ± 0.47
Tau	10.25 ± 0.55
Cho	0.98 ± 0.28
CR	13.30 ± 0.43
PC	0.44 ± 0.07

different analytical procedures used, age, gender, and/or the number of mice used in the study. In addition, one study [31] quantified AAs in specific regions of brain such as the cortex rather than the whole brain.

4. Conclusion

A sensitive, selective, and simple LC–MS/MS method using HILIC was validated for the quantification of nine AAs and MI in mouse brain. The validated method is selective, precise, and accurate. One step methanol–protein precipitation was used for sample extraction. Extraction recovery for all analytes was consistently higher than 70% throughout the dynamic range of the method. Method validation was performed in the range of 2.5–20 to 500–2000 ng/ml to ensure the simultaneous quantification of all analytes of interest in one chromatographic run. This method will be applied to support multiple projects, aim to study the relationship between the levels of AAs in brain and the pathological changes associated with neurological diseases. Furthermore, this LC–MS/MS method will be used along with magnetic resonance spectroscopy to elucidate the neuropathobiology of diseases of the nervous system.

Acknowledgement

References

[1] E. Jellum, E.A. Kvittingen, O. Stokke, Biomed. Environ. Mass Spectrom. 16 (1988) 57.
[2] F. Magni, L. Arnoldi, G. Galati, M. Galli Kienle, Anal. Biochem. 220 (1994) 308.
[3] J.J. Pitt, M. Eggington, S.G. Kahler, Clin. Chem. 48 (2002) 1970.
[4] K. Gajcy, S. Lochynski, T. Librowski, Curr. Med. Chem. 17 (2010) 2338.
[5] M.D. Gottwald, M.J. Aminoff, Ann. Neurol. 69 (2011) 919.
[6] D.T. Proctor, E.J. Coulson, P.R. Dodd, Prog. Neurobiol. 93 (2011) 509.
[7] H. Cheng, K.S. Vetrivel, P. Gong, X. Meckler, A. Parent, G. Thinakaran, Nat. Clin. Pract. Neurol. 3 (2007) 374.
[8] R. Ehehalt, P. Keller, C. Haass, C. Thiele, K. Simons, J. Cell Biol. 160 (2003) 113.
[9] V. Jelic, L.O. Wahlund, Expert Rev. Med. Devices 4 (2007) 475.
[10] Y. Wang, Z.H. Qin, Apoptosis 15 (2010) 1382.
[11] A. Lau, M. Tymianski, Pflugers Arch. 460 (2010) 525.
[12] G. Clarke, S. O'Mahony, G. Malone, T.G. Dinan, J. Neurosci. Methods 160 (2007) 223.
[13] R.A. Rissman, W.C. Mobley, J. Neurochem. 117 (2011) 613.
[14] I. Smolders, S. Sarre, Y. Michotte, G. Ebinger, J. Neurosci. Methods 57 (1995) 47.
[15] P.R. Moult, Biochem. Soc. Trans. 37 (2009) 1317.
[16] J.A. Eckstein, G.M. Ammerman, J.M. Reveles, B.L. Ackermann, J. Neurosci. Methods 171 (2008) 190.
[17] M. Armstrong, K. Jonscher, N.A. Reisdorph, Rapid Commun. Mass Spectrom. 21 (2007) 2717.
[18] J. Qu, W. Chen, G. Luo, Y. Wang, S. Xiao, Z. Ling, G. Chen, Analyst 127 (2002) 66.
[19] W.C. Yang, H. Mirzaei, X. Liu, F.E. Regnier, Anal. Chem. 78 (2006) 4702.
[20] H.L. Rowley, K.F. Martin, C.A. Marsden, J. Neurosci. Methods 57 (1995) 93.
[21] M. Piraud, C. Vianey-Saban, K. Petritis, C. Elfakir, J.P. Steghens, A. Morla, D. Bouchu, Rapid Commun. Mass Spectrom. 17 (2003) 1297.
[22] L. Gu, A.D. Jones, R.L. Last, Anal. Chem. 79 (2007) 8067.
[23] S.A. Gustavsson, J. Samskog, K.E. Markides, B. Langstrom, J. Chromatogr. A 937 (2001) 41.
[24] A.J. Alpert, J. Chromatogr. 499 (1990) 177.
[25] W. Naidong, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 796 (2003) 209.
[26] K. Buck, P. Voehringer, B. Ferger, J. Neurosci. Methods 182 (2009) 78.
[27] M.J. Avery, Rapid Commun. Mass Spectrom. 17 (2003) 197.
[28] J. Battistuta, C. Bjartmar, B.D. Trapp, Neurochem. Res. 26 (2001) 695.
[29] H.U. Shetty, H.W. Holloway, L.D. Acevedo, Z. Galdzicki, Biochem. J. 313 (Pt 1) (1996) 31.
[30] M.A. Jalil, L. Begum, L. Contreras, B. Pardo, M. Iijima, M.X. Li, M. Ramos, P. Marmol, M. Horiuchi, K. Shimotsu, S. Nakagawa, A. Okubo, M. Sameshima, Y. Isashiki, A. Del Arco, K. Kobayashi, J. Satrustegui, T. Saheki, J. Biol. Chem. 280 (2005) 31333.
[31] M.J. Paik, I.S. Cho, I. Mook-Jung, G. Lee, K.R. Kim, BMB Rep. 41 (2008) 23.
[32] S.W. Provencher, Magn. Reson. Med. 30 (1993) 672.
[33] S. Murai, H. Saito, R. Shirato, T. Kawaguchi, J. Pharmacol. Toxicol. Methods 46 (2001) 103.
[34] A. Schwarcz, O. Natt, T. Watanabe, S. Boretius, J. Frahm, T. Michaelis, Magn. Reson. Med. 49 (2003) 822.
[35] J.H. Thurston, R.E. Hauhart, J.A. Dirgo, Life Sci. 26 (1980) 1561.