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Monolithic column-based reversed-phase liquid chromatography separation for amino acid assay in microdialysates and cerebral spinal fluid

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Abstract

The development of a HPLC method using a monolithic C_{18} column is described using fluorescence detection for the assay of 21 amino acids and related substances with derivatisation using *ortho*-phthaldialdehyde (OPA) in the presence of 3-mercaptopropionic acid (3-MPA). The method employs a tertiary gradient and has a run time of 24 min. Linearity (r^2) for each amino acid was found to be greater than 0.99 up to a 10 μM concentration; reproducibility across all analyses (relative standard deviation (R.S.D.)) was between 0.97 and 6.7% and limit of detection (LOD) between 30 and 300 fmol on column. This method has been applied to the analysis of amino acids in both spinal microdialysis and cerebral spinal fluid samples.
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1. Introduction

The study of amino acids has long been of interest to the biological sciences and there is continual endeavour to accurately measure their concentrations under physiological conditions [1,2]. Within the central nervous system (CNS) the amino acids including aspartate, glutamate, glycine and γ-amino butyric acid (GABA) have been shown to act as neurotransmitters and are principal components in both excitatory and inhibitory processes [3,4]. They are also of interest because of their potential involvement in neurological disorders such as schizophrenia and epilepsy [5,6]. Other amino acids are of interest as a consequence of their downstream formation following synaptic activity, for example, citrulline, which is formed from arginine in the production of nitric oxide [7,8]. Aside from the CNS, amino acids are of importance in peripheral tissue where levels can provide insights into physiological and pathophysiological processes involved in, for example, donor organ transplantation [9]. One method that can be employed for the *in vivo* monitoring of amino acids is microdialysis, which allows simple diffusion across a semi-permeable hollow fibre membrane placed into either the CNS or peripheral tissues. In turn, this enables the biochemical content of the extracellular space to be quantified [10].

In order to measure these substances at physiological concentrations an accurate separation and sensitive detection system is required. HPLC has traditionally been employed using columns with a stationary phase consisting of octadecylsilane (ODS) C_{18} beaded particles. However, this material possesses certain limitations when used as a stationary phase, with slow mass transfer and a large void volume leading to high back pressure and ultimately a low sample throughput [11]. As a typical example, the time taken for a standard separation in our own laboratory was in the order of 50 min for separation of 24 amino acids when employing gradient elution. This is an important factor when employing a technique such as microdialysis which may have a short sampling interval and thus generate a large number of samples for analysis. Therefore, there is demand for a high throughout amino acid assay that is not possible with particulate columns.

Monolithic columns are composed of a continuous porous rod, which eliminates the problems encountered when using the particulate C_{18} material. Since their inception in the early 1990s [12], monoliths have enabled much faster separation of

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compounds as a consequence of their increased porosity and reduced void volume, which enables a faster flow rate to be used without back pressure affecting column performance [13]. Here we describe a method to separate and detect 21 amino acids and related substances commonly found in biological fluids. This was employed in conjunction with the well established derivatisation procedure using *ortho*-phthaldialdehyde/3-mercaptopropionic acid (OPA/3-MPA), allowing a simple and rapid assay methodology [14]. We also report the application of this separation for the quantification of amino acid levels in samples taken from two sources; rodent spinal microdialysate and cerebrospinal fluid (CSF), thereby illustrating the potential utility of this methodology.

2. Experimental

2.1. Chemicals and reagents

Twenty four amino acids and related substances (aspartate, glutamate, serine, glutamine, glycine, histidine, threonine, citrulline, β-alanine, arginine, alanine, taurine, γ-amino butyric acid, tyrosine, ethanolamine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, ornithine, lysine and putrescine; all Sigma, Dorset, UK) were used in the preparation of stock standard solutions. HPLC grade methanol and acetonitrile (ACN) (Fisher Scientific, Leicestershire, UK), water (typically 18.2 MΩ resistivity, Elga Water Systems, UK) and sodium acetate tri-hydrate (HPLC grade, VWR International, Dorset, UK) were used in mobile phase preparation. The derivatisation solution was prepared from 'incomplete' OPA solution (1 mg/ml in potassium borate buffer, pH 10.4; Sigma, Dorset, UK) and 3-MPA (Sigma, Dorset, UK).

2.2. HPLC Instrumentation

The mobile phase was delivered using a PM-80 twin-reciprocating pump with LC-26A vacuum degasser (BAS Technicol, Cheshire, UK) to a RP-18e Chromolith® Performance column (0.46 cm i.d. × 10 cm; VWR International, Dorset, UK) coupled to a monolithic phase guard column (0.46 cm i.d. × 5 mm; VWR International, Dorset, UK). The temperature of the column was maintained at 30 °C using a column block heater (Jones Chromatography Ltd., Mid Glamorgan, UK). Sample derivatisation and injection was carried out using a CMA/200 refrigerated autosampler (8 °C) and fluorescence of derivatised amino acids was measured by a CMA/280 fluorescence detector (maximum excitation, 340–360 nm; maximum emission, 495 nm; logarithmic amplification scale from 1 to 1000) (both CMA/Microdialysis, Sweden). Integration of the detector signal was achieved using a chromatographic data handling package (EZChrom; Aston Scientific, UK).

2.3. Amino acid standards

Standard solutions (2.5 mM) containing 24 amino acids and related substances were prepared by dissolving in 0.1 M hydrochloric acid and stored at −80 °C before dilution as required in deionised water (18.2 MΩ) to give 0.25, 1.0, 2.5 and 10 μM solutions. Individual amino acids were prepared and stored in the same manner and used for the purpose of peak identification.

2.4. Derivatisation reagents and protocol

A 10% (v/v) 3-MPA solution was prepared weekly by diluting with methanol and stored at 4 °C. The derivatisation reagent was prepared daily by the addition of 25 μl of this solution to 975 μl of incomplete OPA solution to give the derivatisation reagent. Sample or standard (10 μl) was mixed with 3 μl of derivatising reagent and allowed to react for 1 min; 10 μl of this solution was then injected onto the column.

2.5. Mobile phase preparation

The gradient profile employed in this separation consisted of three components (A) deionised water (18.2 MΩ); (B) a mixture of methanol, ACN and deionised water; and (C) 100 mM sodium acetate buffer containing 5% (v/v) methanol. During the development of this method, both the relative ratios of the components of (B) and the pH of the sodium acetate buffer were varied in order to optimise peak separation. In order to further optimise separation, the gradient profile of the eluent in relation to time and the flow rate were also altered.

2.6. Method application

Potential applications of this method were demonstrated analysing aliquots of both dialysate samples collected from the intrathecal space and CSF collected from the cisterna magna of the rat. CSF was deproteinised by passing through an Ultrafree-MC centrifugal filter (5000 MW cut-off, Cat. No.—UFC3LCC25; Fisher Scientific, Leicestershire, UK) prior to their injection. Samples were obtained in accordance with the UK Animals (Scientific Procedures) Act 1986.

3. Results and discussion

3.1. Method optimisation

Optimisation of peak separation was achieved by sequentially altering (a) the relative ratios of methanol, ACN and water in mobile phase component B; (b) sodium acetate buffer pH; (c) gradient proportionality; and (d) flow rate. Decreasing the ratio of ACN to methanol in component B was able to alter the separation selectively by partially separating the two diastereomers of L-isoleucine. Increasing the pH of the sodium acetate solution (C) in a range from pH 6.1 to 6.9 was found to specifically enhance the separation of histidine, glycine and threonine, but have a detrimental effect on the resolution of aspartate and glutamate, and arginine, taurine and alanine. Gradient proportionality was optimised to achieve resolution between β-alanine, citrulline, arginine, alanine and taurine. Since these amino acids proved to be the most difficult to elute, their separation was viewed as an end-point for an optimised method.

One major feature of the monolithic column is that it requires a very short equilibration time between each sample injection when using a gradient separation system. Therefore, unlike the traditional beaded ODS C_{18} packing material, the next sample can be injected as soon as the previous run has completed its solvent wash. Thus, the length of the separation was determined as 24 min as dictated by the previously established parameters. One advantage of derivatisation with OPA/3-MPA is it only involves a one-step process lasting 3 min, therefore sequential samples were injected every 27 min following the derivatisation process. However, higher throughput is attainable by overlapping the subsequent sample derivatisation with the solvent wash at the end of the previous run.

3.2. *Optimised final method*

The optimum conditions for this method were found to be a flow rate of 2 ml min^{-1} and column temperature set at 30 °C. The final parameters established for the tertiary gradient system in this method were water (component A), methanol:ACN:water in a ratio of 40:50:10 (component B) and 100 mM sodium acetate buffer containing 5% (v/v) methanol adjusted to pH 6.3 using phosphoric acid (VWR International, Dorset, UK) (component C). The gradient profile employed is detailed in Table 1 along with the back pressure range through the gradient cycle. Of the 24 amino acid standard used in the development of this method the final separation resolved 21 of these amino acids (putrescine eluted in the solvent wash period 21–22 min), with histidine and threonine co-eluting (Fig. 1c).

3.3. *Method verification*

Linearity was calculated following injection of the 21 amino acids and related compounds standard over a range of 0–10 μM, repeated in triplicate, to obtain interday and intraday variation. Linear correlation coefficients (r^2) were found to be greater than or equal to 0.992 for each amino acid or related compound (Tables 2 and 3) quantified using GraphPad Prism version 4.00 (GraphPad Software, Inc., San Diego, USA)

Table 1
Method gradient profile

Time (min)	% Mobile phase in eluent			Back pressure (bar ± σ)
	A	B	C	
0	46	4	50	488 ± 13
8	42	8	50	488 ± 6
10	31	19	50	503 ± 6
20	10	40	50	548 ± 3
21	0	100	0	532 ± 6
22	0	100	0	348 ± 8
23	46	4	50	277 ± 8
24	46	4	50	495 ± 13

The percentage drawn from each component of the tertiary gradient system over the 24 min run time are presented. A, water; B, methanol:ACN:water in a ratio of 40:50:10 (v/v/v); and C, 100 mM sodium acetate buffer containing 5% (v/v) methanol adjusted to pH 6.3; σ, standard deviation.

Table 2
Assessment of intraday variation in linearity

Amino acid	$r^2 \pm \sigma$	Slope ± σ
Aspartate	0.999 ± 0.001	14818 ± 169
Glutamate	0.999 ± 0.001	52800 ± 1095
Serine	0.995 ± 0.003	52376 ± 721
Glutamine	0.999 ± 0.001	25499 ± 214
Glycine	0.992 ± 0.011	54291 ± 1416
Citrulline	0.999 ± 0.000	52158 ± 411
β-Alanine	0.999 ± 0.000	63413 ± 571
Arginine	0.999 ± 0.001	53763 ± 631
Alanine	0.999 ± 0.001	31497 ± 1836
Taurine	1.000 ± 0.000	48973 ± 5474
GABA	0.999 ± 0.000	7110 ± 76
Tyrosine	0.999 ± 0.000	52278 ± 314
Ethanolamine	0.999 ± 0.000	57073 ± 305
Valine	0.998 ± 0.002	51765 ± 650
Methionine	0.997 ± 0.004	59074 ± 656
Tryptophan	0.999 ± 0.000	50661 ± 207
Phenylalanine	0.999 ± 0.000	51618 ± 135
Isoleucine	1.000 ± 0.000	57322 ± 150
Leucine	0.999 ± 0.000	57899 ± 557
Ornithine	0.997 ± 0.001	8866 ± 41
Lysine	0.999 ± 0.000	26931 ± 228

Values obtained following injection (10 μl) of a standard mixture of 24 amino acids in triplicate at 0.25, 1.0, 2.5 and 10 μM. Correlation coefficients (r^2) calculated by GraphPad Prism version 4.00; σ, standard deviation; slope is defined as (peak area/concentration) with the exception of aspartate, GABA and ornithine where slope is defined as (peak height/concentration).

Table 3
Assessment of interday variation in linearity

Amino acid	$r^2 \pm \sigma$	Slope ± σ
Aspartate	0.999 ± 0.001	15008 ± 192
Glutamate	0.999 ± 0.001	52905 ± 3370
Serine	0.997 ± 0.001	52584 ± 686
Glutamine	0.999 ± 0.001	25367 ± 79
Glycine	0.993 ± 0.011	54198 ± 1474
Citrulline	1.000 ± 0.000	51314 ± 750
β-Alanine	0.998 ± 0.000	63177 ± 2482
Arginine	0.999 ± 0.001	56867 ± 5241
Alanine	1.000 ± 0.000	34547 ± 9276
Taurine	1.000 ± 0.001	46460 ± 12372
GABA	0.999 ± 0.001	7426 ± 267
Tyrosine	1.000 ± 0.000	51554 ± 827
Ethanolamine	0.999 ± 0.000	55907 ± 1108
Valine	0.999 ± 0.000	49612 ± 4032
Methionine	0.999 ± 0.000	55003 ± 6021
Tryptophan	0.999 ± 0.001	50557 ± 87
Phenylalanine	0.999 ± 0.001	51622 ± 109
Isoleucine	0.999 ± 0.000	57409 ± 139
Leucine	0.999 ± 0.000	57595 ± 289
Ornithine	0.992 ± 0.008	8356 ± 799
Lysine	0.999 ± 0.000	27007 ± 369

Values obtained following injection (10 μl) of a standard mixture of 24 amino acids in triplicate on separate days at 0.25, 1.0, 2.5 and 10 μM. Correlation coefficients (r^2) calculated by GraphPad Prism version 4.00; σ, standard deviation; slope is defined as (peak area/concentration) with the exception of aspartate, GABA and ornithine where slope is defined as (peak height/concentration).

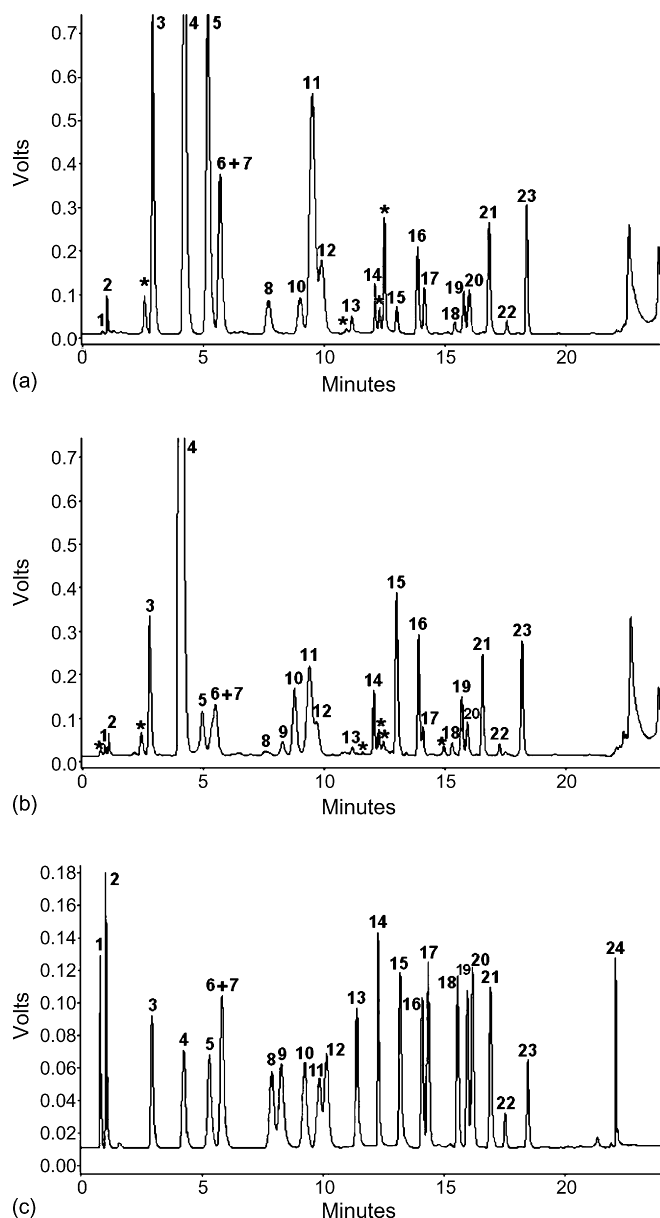

Fig. 1. Representative chromatograms to illustrate method application. (a) Chromatogram of a 10 μl microdialysate sample from the intrathecal space in the rat; (b) chromatogram of a rat cerebrospinal fluid (CSF) sample; and (c) chromatogram of a standard mixture of 24 amino acids, all at a concentration of 10 μM. The amino acids are labelled accordingly: 1, aspartate; 2, glutamate; 3, serine; 4, glutamine; 5, glycine; 6, histidine; 7, threonine; 8, citrulline; 9, β-alanine; 10, arginine; 11, alanine; 12, taurine; 13, γ-amino butyric acid (GABA); 14, tyrosine; 15, ethanolamine; 16, valine; 17, methionine; 18, tryptophan; 19, phenylalanine; 20, isoleucine; 21, leucine; 22, ornithine; 23, lysine; 24, putrescine; (*) represents unidentified peaks.

with the linear regression curve forced through the origin. Intraday reproducibility was calculated from 10 consecutive injections of aliquots (10 μl) of the 2.5 μM amino acid standard, giving relative standard deviations (R.S.D.s) between 1.0 and 5.0%; interday reproducibility was obtained by comparing the mean of these replicates over three consecutive days, giving R.S.D.s between 1.6 and 6.3% (Table 4). Retention times were compared in the same manner; elution of arginine showed the greatest variation of 0.45% (Table 5). The limit of detection (LOD) was assessed for each amino acid and related compound by determining the lowest concentration that retained a S/N ratio of 3:1 in relation to baseline noise. This was determined to be between 3 and 30 nM

Table 4
Assessment of peak area intraday and interday reproducibility

Amino acid	Relative standard deviation	
	Interday	Intraday
Aspartate	4.3	3.5
Glutamate	3.2	3.8
Serine	1.0	5.0
Glutamine	1.2	1.8
Glycine	1.0	4.2
Citrulline	1.1	5.6
β-Alanine	1.2	1.6
Arginine	1.8	4.7
Alanine	5.3	2.2
Taurine	5.0	1.6
GABA	4.1	6.3
Tyrosine	5.0	5.3
Ethanolamine	3.3	3.7
Valine	2.1	5.2
Methionine	1.2	4.3
Tryptophan	1.3	4.6
Phenylalanine	1.7	3.8
Isoleucine	1.8	3.0
Leucine	1.5	2.3
Ornithine	2.9	2.7
Lysine	4.0	2.5

Intraday values were obtained by comparing 10 replicate injections of a 10 μl aliquot of a 2.5 μM amino acid standard. Interday values were obtained by the mean of 10 replicate injections over three consecutive days.

Table 5
Assessment of variation in interday retention times

Amino acid	Mean retention times (min ± σ)
Aspartate	0.83 ± 0.01
Glutamate	0.99 ± 0.01
Serine	2.87 ± 0.03
Glutamine	4.25 ± 0.04
Glycine	5.23 ± 0.06
Citrulline	7.74 ± 0.06
β-Alanine	8.22 ± 0.12
Arginine	8.90 ± 0.08
Alanine	9.15 ± 0.04
Taurine	9.46 ± 0.05
GABA	11.30 ± 0.08
Tyrosine	12.30 ± 0.07
Ethanolamine	13.13 ± 0.01
Valine	14.28 ± 0.01
Methionine	14.51 ± 0.03
Tryptophan	15.90 ± 0.02
Phenylalanine	16.30 ± 0.02
Isoleucine	16.57 ± 0.03
Leucine	17.42 ± 0.04
Ornithine	18.52 ± 0.13
Lysine	19.65 ± 0.06

Values obtained by comparing the mean of three replicate injections of a 10 μl aliquot of a 2.5 μM amino acid standard over three consecutive days. Values given are in min ± standard deviation (σ).

Table 6
Estimated limits of detection and quantification

Amino acid	Limit of detection (fmol/10 μl)	Limit of quantification (fmol/10 μl)
Aspartate	30	100
Glutamate	30	100
Serine	100	100
Glutamine	100	300
Glycine	100	100
Citrulline	100	300
β-Alanine	200	500
Arginine	50	200
Alanine	50	200
Taurine	300	600
GABA	100	300
Tyrosine	100	300
Ethanolamine	100	300
Valine	50	200
Methionine	100	300
Tryptophan	100	300
Phenylalanine	100	300
Isoleucine	100	300
Leucine	50	300
Ornithine	300	600
Lysine	200	500

This was assessed for each quantifiable amino acid by sequentially diluting a standard containing all 24 amino acids; limit of detection was determined as the lowest concentration on column that retained a S/N ratio of 3:1. The limit of quantification was determined as the lowest concentration on column that retained a S/N ratio of 10:1.

(30–300 fmol on column). Also, the limit of quantification (LOQ) was determined as the lowest concentration that retained a S/N ratio of 10:1 in relation to baseline noise and was found to be between 10 and 60 nM (100–600 fmol on column) (Table 6).

In order to assess the accuracy and precision of this method, 10 μl microdialysate samples were assayed in triplicate in order to determine the basal levels of each amino acid. A 5 μl aliquot of this sample was then spiked with 5 μl of a standard containing a total concentration of 10 pmol of each analyte; these were carried out in triplicate. Example chromatograms of this sample spiking are displayed in Fig. 2. This figure shows the correlation between peaks in the basal and spiked samples, thus confirming peak identity in the microdialysis sample. The data relating to precision is presented as a percentage of the expected concentration in each sample as calculated from the basal levels and following the artificial addition of a known amount of each analyte (Table 7). These data illustrate the high level of accuracy achieved using this method, with the largest error observed for serine and ornithine, which gave levels only 86.8 and 89.9%, respectively, of the expected amount present following sample spiking.

Previously published work using monolithic technology [15] has used naphthalene-2,3-dicarboxaldehyde in the presence of cyanide (NDA/CN^-) as the fluorogenic reagent for amino acid detection, which has been shown to form stable derivatives and offers improved sensitivity in comparison to OPA/2-mercaptoethanol (2-ME) derivatisation [16]. Our assay uses OPA/3-MPA derivatisation, which forms more stable isoindole

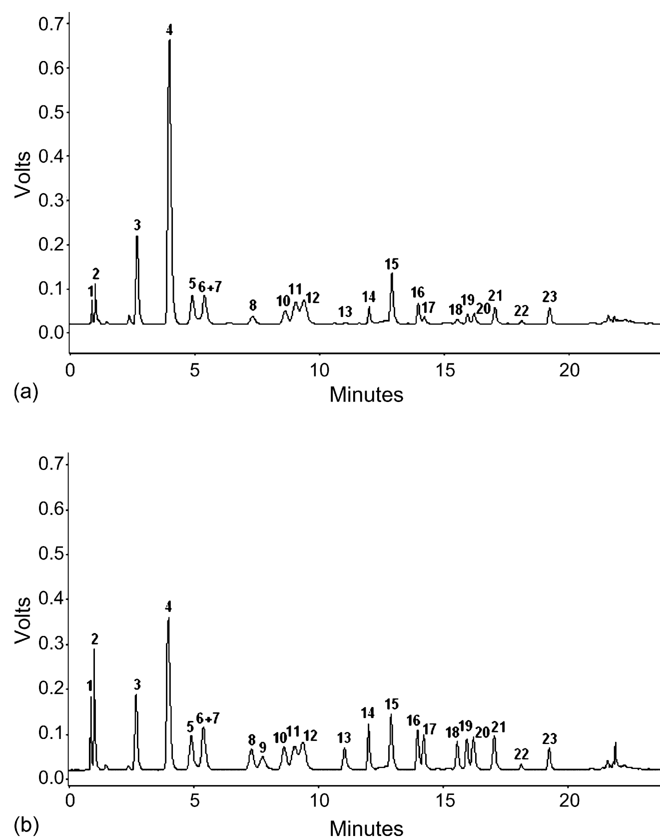

Fig. 2. Representative chromatograms to illustrate assessment of method accuracy and precision. (a) Chromatogram of a 10 μl microdialysate sample from the intrathecal space in the rat; (b) chromatogram of a 5 μl microdialysate sample from the intrathecal space of the rat spiked with 5 μl of a standard solution containing 10 pmol of each analyte. The amino acids are labelled accordingly: 1, aspartate; 2, glutamate; 3, serine; 4, glutamine; 5, glycine; 6, histidine; 7, threonine; 8, citrulline; 9, β-alanine; 10, arginine; 11, alanine; 12, taurine; 13, γ-amino butyric acid (GABA); 14, tyrosine; 15, ethanolamine; 16, valine; 17, methionine; 18, tryptophan; 19, phenylalanine; 20, isoleucine; 21, leucine; 22, ornithine; 23, lysine.

derivatives that those produced by OPA/2-ME [17]. In addition, the faster separation time of this method to some extent negates the issue of degradation. As stated previously, derivatisation with OPA/3-MPA involves a one-step process lasting 3 min, unlike NDA/CN^- derivatisation, which requires the components to be prepared as separate solutions and requires 15 min incubation [15]. Therefore, higher throughput is possible with OPA/3-MPA derivatisation.

3.4. Method application

Representative chromatograms in Fig. 1 show the application of this optimised method to the analysis and assay of biological samples with separation of (a) microdialysate sample from the intrathecal space of the rat; (b) a rat CSF sample in comparison to a standard containing 24 amino acids at 10 μM (c). The differences in concentration for the various amino acids that exist within an *in vivo* sample are large, therefore it is imperative that peak resolution is maintained. As shown in these chromatograms, the separation of peaks closely resem-

Table 7
Assessment of method accuracy and precision

Amino acid	% of expected value following sample spiking (mean ± σ)
Aspartate	101.7 ± 2.1
Glutamate	118.4 ± 2.3
Serine	86.8 ± 3.1
Glutamine	98.9 ± 2.2
Glycine	100.1 ± 3.1
Citrulline	98.9 ± 4.8
β-Alanine	92.4 ± 7.0
Arginine	95.1 ± 4.1
Alanine	98.9 ± 2.3
Taurine	102.3 ± 3.7
GABA	94.3 ± 2.5
Tyrosine	99.5 ± 4.6
Ethanolamine	107.2 ± 4.5
Valine	103.0 ± 3.5
Methionine	97.1 ± 7.1
Tryptophan	96.0 ± 6.2
Phenylalanine	99.7 ± 5.4
Isoleucine	99.0 ± 5.3
Leucine	96.7 ± 4.4
Ornithine	89.9 ± 3.9
Lysine	95.7 ± 2.3

Ten microliters microdialysate samples were assayed in triplicate in order to determine the basal levels of each amino acid. Five microliters of this sample was then spiked with 5 μl of a standard containing a total concentration of 10 pmol; this was carried out in triplicate. The data is presented as a percentage of the expected concentration for each analyte as calculated from the basal levels and spiked addition; σ, standard deviation.

bles that shown in the 24 amino acid standard (Fig. 1c) and are within the limits of detection, allowing accurate measurement of amino acid concentrations within these biological samples. Putrescine is not quantifiable in either of these samples because it is eluted in the solvent wash at 21–22 min. There are also unknown peaks that have been resolved but are not present in the 24 amino acid standard (labelled * in Fig. 1a and b). On examining the sample chromatograms it can be seen that certain amino acids can be present in very high levels, such as glutamine that can often exceed the range of quantification (Fig. 1a and b), whereas others such as GABA are present at very low concentrations. In these cases, samples can be diluted or assayed using higher sensitivity parameters, respectively. The inability to quantify histidine, threonine and putrescine using this method is not a significant issue when assaying CSF and microdialysis samples for amino acids and related compounds that are involved in neuronal activity. It is of greater importance to ensure that amino acids with known participation in neurotransmission such as aspartate, glutamate and GABA are accurately quantifiable. However, in terms of usefulness of the method for a wide range of applications, the ability to measure as many amino acids and related compound as possible is important.

4. Conclusion

In summary, we have developed a high throughput HPLC assay for 21 known amino acids and related substances employing a new monolithic phase column and using OPA/3-MPA derivatisation with suitable parameters validated for analysis and assay of biological samples. This gave a faster separation time than traditional particulate columns due to the increased porosity and reduced void volume of the monoliths, allowing much increased eluent flow rates to be employed whilst retaining peak resolution for the amino acids of interest. The method was shown to be reproducible and linear over a wide concentration range up to 10 μM, with a limit of detection between 30 and 300 fmol on column, and limit of quantification between 100 and 600 fmol on column. The accuracy and specificity of this assay was also assured with the spiking of real samples with a standard of known concentration. In order to demonstrate its use in a range of applications, the method was applied to the analysis of both rat CSF and dialysate samples. This yielded not only peaks corresponding to those present in the standard solution, but also unknown compounds whose future identification would enhance the usefulness of this method.

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References

[1] J. Kehr, J. Chromatogr. B Biomed. Sci. Appl. 708 (1998) 27.
[2] T.P. Piepponen, A. Skujins, J. Chromatogr. B Biomed. Sci. Appl. 757 (2001) 277.
[3] S. Ozawa, H. Kamiya, K. Tsuzuki, Prog. Neurobiol. 54 (1998) 581.
[4] N.G. Bowery, T.G. Smart, Br. J. Pharmacol. 147 (S1) (2006) S109.
[5] D.C. Javitt, Mol. Psychiatry 9 (2004) 984.
[6] G. Sperk, S. Furtinger, C. Schwarzer, S. Pirker, Adv. Exp. Med. Biol. 548 (2004) 92.
[7] I. Cavus, W.S. Kasoff, M.P. Cassaday, R. Jacob, R. Gueorguieva, R.S. Sherwin, J.H. Krystal, D.D. Spencer, W.M. Abi-Saab, Ann. Neurol. 57 (2) (2005) 226.
[8] J. Watts, L. Fowler, P.S. Whitton, B. Pearce, Brain Res. Bull. 65 (2005) 521.
[9] M.A. Silva, D.A. Richards, S.R. Bramhall, D.H. Adams, D.F. Mirza, N. Murphy, Transplantation 79 (2005) 828.
[10] N. Plock, C. Kloft, Eur. J. Pharm. Sci. 25 (2005) 1.
[11] H. Poppe, J. Chromatogr. A 778 (1997) 3.
[12] Q.C. Wang, K. Hosoya, F. Svec, J.M. Frechet, Anal. Chem. 64 (1992) 1232.
[13] H. Zou, X. Huang, M. Ye, Q. Luo, J. Chromatogr. A 954 (2002) 5.
[14] P. Lindroth, K. Mopper, Anal. Chem. 51 (1979) 1667.
[15] L.A. Dawson, A.J. Organ, P. Winter, L.P. Lacroix, C.S. Shilliam, C. Heidbreder, A.J. Shah, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 807 (2004) 235.
[16] P. De Montigny, J.F. Stobaugh, R.S. Givens, R.G. Carlson, K. Srinivasachar, L.A. Sternson, T. Higuchi, Anal. Chem. 59 (1987) 1096.
[17] R. Hanczko, I. Molnar-Perl, Chromatographia 57 (2003) S103.