Determination of the neurotoxin BMAA (β-*N*-methylamino-L-alanine) in cycad seed and cyanobacteria by LC-MS/MS (liquid chromatography tandem mass spectrometry)

Johan Rosén* and Karl-Erik Hellenäs

Received 30th May 2008, Accepted 21st August 2008
First published as an Advance Article on the web 9th October 2008
DOI: 10.1039/b809231a

A highly specific method for the analysis of β-*N*-methylamino-L-alanine (BMAA) by LC-MS/MS (liquid chromatography tandem mass spectrometry) has been developed and applied for cycad seeds and cyanobacteria. BMAA was analysed as a free fraction or as total BMAA after acidic hydrolysis to release any protein-bound BMAA. Deuterium labelled BMAA was synthesised and used as internal standard. The method comprises HILIC (hydrophilic interaction chromatography) and positive electrospray ionisation of the native compound, *i.e.* no derivatisation was used. For safe identification five specific product ions (*m*/*z* 102, 88, 76, 73 and 44), all derived from a precursor ion of *m*/*z* 119 and originating from different parts of the molecule, were detected (typical relative abundance 100%, 16%, 14%, 12% and 22% respectively). Cyanobacteria or muscle tissue was spiked with BMAA (10 to 1000 μg g^{-1}) to validate the method (accuracy 95% to 109%, relative standard deviation 1% to 6%). The detection limit for free and total BMAA in tissue was <1 μg g^{-1} and <4 μg g^{-1} respectively. BMAA was successfully identified and quantified in cycad seeds, whereas previously reported findings of BMAA in samples of cyanobacteria could not be confirmed. Instead, the presence of α-,γ-diamino butyric acid (DAB), an isomer of BMAA, was confirmed in one sample. The possible implications of this finding are discussed.

Introduction

It has been suggested that BMAA (β-*N*-methylamino-L-alanine) is linked to nerve degenerative diseases. The substance was originally isolated from seeds from the cycad tree (*Cycas circinalis*) in 1967.[1] Food derived from the cycad seeds is believed to be involved in the development of ALS-PDC (amyotrophic lateral-sclerosis-parkinsonism dementia complex) in the Western Pacific (*e.g.* the island of Guam), and BMAA is one of the candidates for causing the syndrome.[2] However, other compounds unique for the cycads have also been reported, *e.g.* cycasin,[3] and it is not clear whether other explanations such as hereditary factors play a role. Since 2003 Cox and co-workers have reported findings of BMAA in brain tissue from patients who suffered from ALS-PDC on Guam, and also from Canadian patients who died from Alzheimer's disease and who had not been exposed to any cycad-related material.[4–6] No BMAA was found in brain tissue from a control group who died of causes unrelated to neurodegenerative disease. In that study an HPLC method comprising derivatisation with AQC (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate) and detection by fluorescence was used.[4–7] The group also reported findings of protein-bound BMAA in *e.g.* cycad flour and brain tissue and launched a hypothesis of bioaccumulation.[5,6] Using the same method, Cox's group reported in 2005 findings of free and protein-bound BMAA in common species of cyanobacteria (blue–green algae), and suggested that it could be bioaccumulated in the food chain.[8] On this basis, it was hypothesised that BMAA might be a causative agent for nerve degenerative diseases also outside the Western Pacific islands. If so, the findings could indeed have important medical implications, extending to the control of widespread diseases such as Alzheimer's, and might make BMAA an emerging food safety issue. Consequently, this intriguing hypothesis has triggered considerable research efforts and has attracted strong media interest in some countries. However, the findings of BMAA in brain and cyanobacteria have not yet been verified by others. On the contrary, the occurrence in brain tissue has been questioned by Montine and co-workers.[9]

Confirmation of the presence of BMAA in samples of brain tissue and cyanobacteria would be invaluable, and highly specific methods-using *e.g.* MS/MS-should be developed and applied to these matrices. Moreover, it would be beneficial if these methods were based on direct detection of BMAA, *i.e.* avoiding derivatisation, since there is at least a theoretical risk that a compound similar to BMAA would react to give a BMAA-derivative during the derivatisation procedure. However, the majority of the analytical methods developed for BMAA were based on the derivatisation of BMAA.[4–15] The only exception is a recent paper based on LC/MS (single MS) that could not verify the presence of BMAA in cyanobacteria.[16] Among the MS methods developed[12–16] only one uses tandem MS, still detecting the derivative only.[14] None of the earlier methods have been presented with validation data in terms of both accuracy and precision. In a recent report, analysis, occurrence and toxicity of BMAA are thoroughly reviewed and evaluated.[17] The purpose of the present

National Food Administration, Box 622, SE 751 26 Uppsala, Sweden. E-mail: johan.rosen@slv.se

work was to develop a highly specific method based on LC-MS/MS without derivatisation, validate it, and to apply it to cyanobacteria samples similar to the samples previously reported positive for BMAA.

Experimental

Materials

A synthetic preparation of β-*N*-methylamino-L-alanine (L-BMAA) was supplied as the hydrochloride ((+)-L-β-*N*-methyl-α,β-diaminopropionic acid hydrochloride) by RBI, Research Biochemicals International (Natick, MA, USA). Deuterium labelled methylamine (gas) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA), and α-acetamido acrylic acid was from Alfa Aesar GmbH (Karlsruhe, Germany). HPLC-grade acetonitrile (ACN) was from Lab-Scan (Dublin, Ireland), and water was Milli-Q water from a Millipore purification system. Hydrochloric acid (HCl), formic acid and trichloroactetic acid (TCA) were all from Merck (Whitehouse, NJ, USA). The syringe-driven filter was a Millex-GS, 0.22 μm, and the centrifuge spin filter a Microcon YM-3 regenerated cellulose with cut off 3 kDa, both from Millipore (Bedford, MA, USA). Stock solutions of BMAA 1 mg ml^{-1} were prepared in water and stored at −20 °C. Working standard solutions were obtained by dilutions in water to 10 μg ml^{-1} (for spiking of samples), or by dilutions in 70% (v/v) ACN in water to 0.01, 0.1 and 1 μg ml^{-1} for optimising the mass spectrometers and testing linearity.

Equipment

Mass spectrometry was performed using a triple quadrupole: either Micromass Quattro Ultima (Micromass UK Ltd, Altrincham, Cheshire, UK), or API 4000 QTRAP (Applied Biosystems/MDS SCIEX, Concord, ON, CAN). The HPLC system was a Waters Alliance 2690 (Waters Ltd, Watford, Hertfordshire, UK) or Agilent 1100 (Santa Clara, CA, USA). The HPLC column was a ZIC®-HILIC, 5 μm, 50 × 2.1 mm or 150 × 2.1 mm from SeQuant AB, Chrom Tech AB (Umeå, Sweden).

Samples

Samples of cyanobacteria or extracts thereof were kindly provided by Birgitta Bergman at the Department of Botany, Stockholm University, Sweden. Samples of cyanobacterial blooms from the Baltic Sea were provided by Harri Kankaanpää, Finnish Institute of Marine Research, Helsinki, Finland. *Spirulina platensis*, sold as dietary supplement, was obtained from a local health-food store. Seeds from *Cycas micronesica* were originally collected at the village Yigo on the island Guam in the Pacific Ocean. Homogenate of the kernel of these seeds was kindly provided by Sandra Banack, California State University, Fullerton, CA, USA. The homogenate was dried at 40 °C and milled to a homogenous white powder.

Synthesis of deuterium labelled BMAA (d3-BMAA)

The procedure was essentially as described earlier.[1,12] Water (2.5 ml) was placed in an open 10 ml test tube at room temperature. Deuterium labelled methylamine (CD_3NH_2) was bubbled through the water by means of a Pasteur pipette connected to the gas outlet and aimed at the bottom of the tube. Except from the first bubbles consisting of air from the tubing, the bubbles dissolved readily before they reached the surface, even at >5–10 bubbles per second. When the liquid volume had reached 5.25 ml the gas flow was stopped. The liquid had gained 2.00 g. α-Acetamido acrylic acid (1.12 g) was added, the tube closed, and the solution incubated at 40 °C for 88 h. The water and excess methylamine were then evaporated by a stream of nitrogen, and 1 part of the formed oily product was dissolved in 4 parts of 6 M HCl. The solution was boiled at 110 °C for 3 h and diluted 1 : 50 000 before characterization with LC-MS/MS.

Sample extraction

Sample extraction was performed essentially as described by Cox *et al.*[8] Cycad seeds or cyanobacteria (10 mg dry weight) were spiked with 100 μg g^{-1} of d3-BMAA and extracted in an Eppendorf tube with 0.1 M TCA (2 × 300 μl) or 70% (v/v) acetonitrile in water (2 × 300 μl) or 50% (v/v) ethanol in water. Extraction of cycad was by shaking only. To ensure efficient extraction of unspiked samples of cyanobacteria a plastic homogeniser designed for the Eppendorf tube was used. Also ultrasonication (15 min), and freezing (−70 °C) and thawing of the extract repeatedly were applied. The extract was centrifuged and the supernatant transferred to vials suited to the LC-MS/MS system. For the release of any protein-bound BMAA, acid hydrolysis was accomplished using 6 M HCl (300 μl) as the extraction medium, and incubating for 17 h at 110 °C. Excess HCl was evaporated and the residue dissolved in water (600 μl). After centrifugation the extracts were passed through syringe filters. Ultrafiltration was used when necessary before aliquots were injected into the LC-MS/MS system.

LC-MS/MS analysis

The mobile phase consisted of ACN and 60 mM formic acid in water (65 : 35 or 70 : 30), and the HPLC-flow was 0.4–0.5 ml min^{-1}. The injection volume was 2–10 μl, and the instruments operated in positive electrospray mode. For the Ultima instrument, nitrogen was used as the drying gas (500 l h^{-1}, 400 °C), as the nebulising gas (fully open) and as the cone gas (200 l h^{-1}). Argon was used as the collision gas (2.0×10^{-3} mbar). The source temperature was maintained at 125 °C, the capillary voltage at 2.7 kV, the cone voltage at 10 V and the first hexapole voltage at 10 V. The dwell time for each transition in multiple reaction monitoring mode was 0.10 s, and the interchannel delay was 0.05 s. The instrument was operated at unit resolution, and the multiplier set at 650 V. For the API 4000 instrument, similar settings were used, and nitrogen was used as collision gas.

Results and discussion

The mass of the main isotope of uncharged BMAA ($C_4H_{10}N_2O_2$) is 118 u. Infusing a water solution of BMAA into the LCMS equipment, using positive electrospray with default settings and scanning *m/z* 20–250, gave a main signal at *m/z* 119 corresponding to $[M + H]^+$ (Fig. 1a). Natural occurrence of the isotope *m/z* 120 was also observed, as well as a supposed dimer

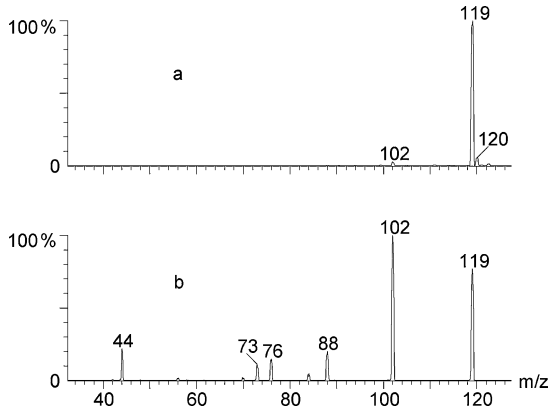

Fig. 1 MS-scan obtained by infusing a standard solution of BMAA into a triple quadrupole LC-MS/MS using positive electrospray. (a) MS-mode. (b) Product ions in MS/MS-mode.

$[2M + H]^+$ at *m/z* 237. An ion at *m/z* 102 was also detected. Since *m/z* 102 also constituted the main fragment at MS/MS (see below), it was concluded that this was a fragment produced in the ion source. The results show that the standard was sufficiently pure to eliminate the risk of selecting an erroneous precursor ion for MS/MS. No significant ion was observed when the mass spectrometer was operated in negative mode.

By tuning MS1 for *m/z* 119 and then scanning MS2 for product ions several fragments were observed (Fig. 1b). The optimal collision energy, relative abundance (using the optimal collision energy), and suggested reactions for the five most intense ions are recorded in Table 1. The fragments correspond to all groups bonded to the α-carbon as well as parts of the side chain. Hence, despite the low molecular weight, enough product ions with sufficiently large intensity were obtained to allow for highly specific identification.

In the synthesis of deuterium labelled BMAA, a CO–N bond was cleaved by acid hydrolysis in the last step. For this purpose 2 M HCl at 100 °C was used in an initial attempt. The liquid remained yellow, and when the product was characterized by LC/MS it was found that *m/z* 164 (uncleaved product) was 30 times more abundant than the expected *m/z* of 122. 6 M HCl at 110 °C produced though a dark solution (with a strong smell of acetic acid) that gave 50 times stronger signal at *m/z* 122, and no detectable amounts of *m/z* 164. No unlabeled BMAA was detected (*m/z* 119 < 0.05% of *m/z* 122). The fragments of *m/z* 122 in MS/MS were all in accordance with what was found for unlabeled BMAA. The yield was estimated to 0.8 g by comparing the signal of *m/z* 122 to a solution of unlabeled BMAA (*m/z* 119).

Several stationary phases were tested for chromatography, including both RP (reversed phase) and HILIC (hydrophilic interaction chromatography, also referred to as aqueous normal phase) systems.[18] Hypercarb (Thermo Hypersil-Keystone), Bidentate C18 (MicroSolv Technologies) as well as standard silica based C18 columns gave either no significant retention or a bad peak shape. However, the ZIC®-HILIC column, which had a sulfobetaine type zwitterionic stationary phase covalently attached to silica particles, showed significant retention with mobile phases of ≥ 60% (v/v) of ACN in water. Concentrations of 65% to 70% were chosen as optimal for analysis of BMAA in the present study. Higher retention can be obtained by increasing the ACN content, if necessary for separation from matrix components that might arise with other sample types or other extraction conditions, but will at the same time broaden the peak. Modifiers (acetic acid, formic acid and ammonium acetate) were tested separately or in combination to minimize tailing. Formic acid improved the peak shape more than acetic acid, while combination with ammonium ions gave no further improvement. The final optimized LC-MS/MS system gave a retention time of 5.7 min (flow rate 0.4 ml min^{-1}, column length 150 mm, 70% (v/v) of ACN in 60 mM formic acid), and a linear response from 0.01 μg ml^{-1} to at least 1 μg ml^{-1}. No difference in retention time between BMAA and d3-BMAA could be observed.

For quantification of BMAA the signal was compared to d3-BMAA. The area ratio of *m/z* 102/105 or *m/z* 44/47 was used, and concentrations were calculated against a standard curve. Validation was performed by adding BMAA in known concentrations (10, 20 and 100 μg g^{-1}) to blank samples of cyanobacteria. BMAA was analysed either as the free fraction, or the samples were subjected to acid hydrolysis. Bias (bias% = $(m - s)/s \times 100$, where m = measured amount and s = spiked amount) varied between −5% and +9% and the relative standard deviation (RSD) between 1% and 6% (Table 2). In order to test the method at higher concentrations, and for other types of samples rich in protein, tissue of bovine muscle was validated in a similar way at 200 and 1000 μg g^{-1}. For free BMAA bias was in average −4% and RSD 1.4%. For spiked muscle tissue subjected to acid hydrolysis bias averaged −3% and RSD 5%.

When injecting an extract of cycad seeds (*Cycas micronesica*), all five characteristic product ions were detected with the same relative ion ratios as for a standard solution of BMAA (Fig. 2). The signal from all detected peaks was approximately 1000 times higher than the noise level. Moreover, for four of the product ions no other peak could be detected in the entire chromatogram. Only for the transition 119 > 73 could one matrix peak be detected. However, this peak was well separated from the BMAA peak. These results demonstrate the high specificity for BMAA

Table 1 Product ions of BMAA and suggested corresponding reactions

m/z	Optimal collision energy/eV	Relative intensity (%)	Corresponding to
119 > 102	10	100	Loss of ammonia
119 > 88	10	16	Loss of methylamine
119 > 76	10	14	Loss of CH_2=N–CH_3
119 > 73	10	12	Loss of formic acid
119 > 44	15	22	$[CH_3–NH=CH_2]^+$

Table 2 Accuracy and precision results from the validation using spiked cyanobacteria samples

Concentration added/μg g^{-1}	*n*	Extraction method	Average concentration found/μg g^{-1}	RSD (%)
10	3	Free	10.4	2
20	3	Free	20.7	6
100	3	Free	95.0	2
10	3	Total	10.9	6
20	3	Total	19.8	3
100	3	Total	99.4	1

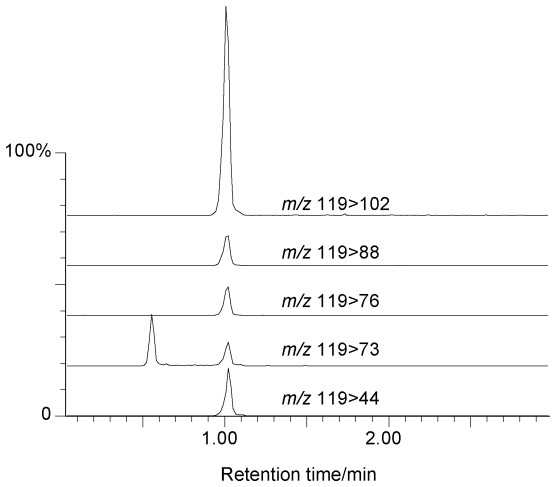

Fig. 2 LC-MS/MS chromatogram obtained by injection of an extract of kernels from cycad seeds. 100% corresponds to 1.9×10^6 cps. (Ultima, column length 50 mm, mobile phase 65% ACN, flow rate 0.5 ml min^{-1}).

of the developed method. The concentration of free BMAA in dried cycad seed was 578 μg g^{-1} ($n = 4$, RSD = 0.6%), and total BMAA was 593 μg g^{-1} ($n = 4$, RSD = 2%). The small difference between total BMAA and free BMAA is within the range for method variation. The results are within the range of earlier reports: 240 μg g^{-1} (*Cycas micronesica*),[7] 21–218 μg g^{-1} (5 *Cycas* species, wet weight),[10] and 290–1560 μg g^{-1} (4 *Cycas* species, dry weight).[19]

The samples of cyanobacteria comprised laboratory raised strains ($n = 30$), blooms from the Baltic Sea ($n = 4$) and commercial *Spirulina* ($n = 2$). Most of the samples were analyzed for both free and protein-bound BMAA. The presence of BMAA could not be confirmed in any of these samples (Fig. 3). The detection limit of the method for these samples was estimated from signal-to-noise ratios (S/N = 3) to be <1 μg g^{-1} for free BMAA and <4 μg g^{-1} for total BMAA. In two samples, traces (<4 μg g^{-1}, total BMAA) were detected at *m/z* 119>102, however, at this low level other transitions could not be used to confirm the identity of BMAA. In order to test the efficiency of the hydrolysis, lysine—a common basic amino acid—was simultaneously detected for reference at *m/z* 147 > 130. Quantification of lysine was aided by the use of an isotopically labelled internal standard (^{15}N-lysine). In general the amount of lysine detected increased by two orders of magnitude after hydrolysis, indicating that the hydrolysis of proteins was successful.

The present results are in contrast with the previous findings of Cox *et al.* who in 2005 reported that 95% of cyanobacteria samples investigated contained BMAA in concentrations of between 3 and 6000 μg g^{-1}.[8] In a more recent paper the same group reported BMAA in all analysed samples of cyanobacterial blooms, scums and mats in British waterbodies.[14] The identity of BMAA was, at least in some samples, confirmed by LC-MS/MS, although only for the derivative.[14] The quantitative data in these studies were obtained using an HPLC method comprising derivatisation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) and detection by fluorescence. The method will detect not only all amino acids, but also any compound that reacts with the fluorescent derivatisation reagent or exhibits

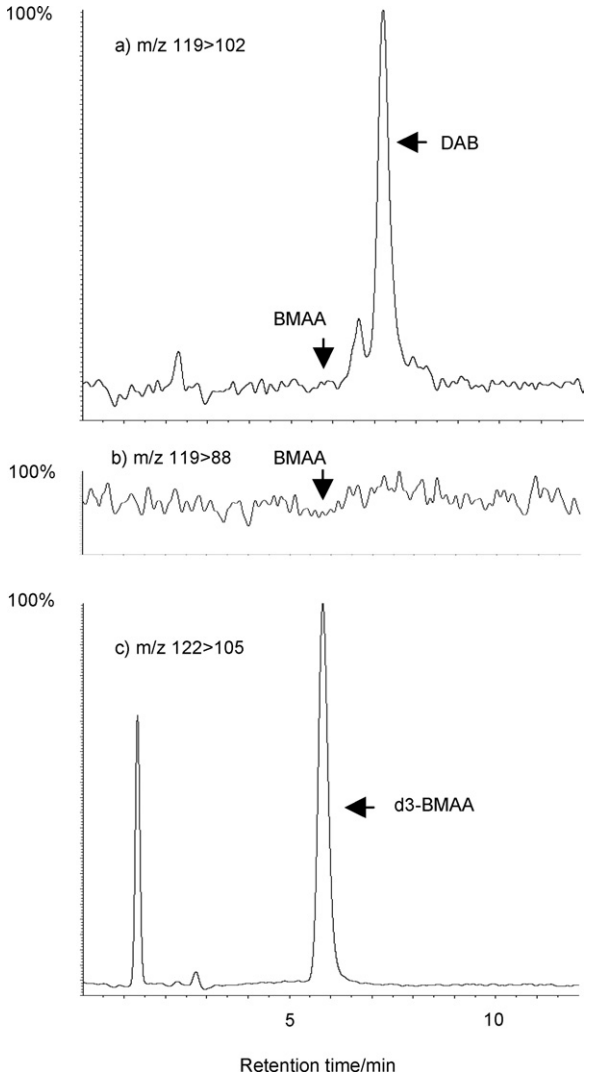

Fig. 3 LC-MS/MS chromatogram representing cyanobacteria (*Calothrix* PCC7103) extracted for free BMAA. The sample was spiked with 100 μg g^{-1} d3-BMAA prior to extraction. (QTRAP, column length 150 mm, mobile phase 70% ACN, flow rate 0.4 ml min^{-1}). (a) BMAA estimated to <1 μg g^{-1}. DAB (quantified as BMAA) 7 μg g^{-1}. 100% corresponds to 800 cps. (b) 100% corresponds to 48 cps. (c) 100% corresponds to 14 000 cps.

fluorescence at the given settings. Retention time is thus the only criterion for identification, and at least 50 compounds in cyanobacteria can be detected by the method, many of them not resolved by baseline separation. Difficulties in obtaining quantitative derivatisation for BMAA by the method have been reported,[20] and validation data, in terms of accuracy and precision obtained from spiked samples, have not been presented. It should be pointed out that fluorescence detection is generally a highly sensitive detection methodology. Thus, there is at least a potential risk that BMAA in the earlier studies was correctly identified but its concentration highly overestimated. Another possible explanation for the diverging results could be that BMAA might only be produced by cyanobacteria under certain circumstances. The samples analysed here were not identical to those used in the earlier study. However, they were as similar as

possible, *i.e.* the same species raised under the same conditions by the same laboratory. Yet another explanation could be that another compound reacts with AQC to give a BMAA-derivative. This might *e.g.* be a carbamate of BMAA[21] (although at least α-*N*-carboxy-BMAA rapidly reverted to BMAA on acidification[22]), or a glucuronide of a carbamate (since glucuronides of similar compounds have been reported to be relatively stable[23]). Another recent study using LC-MS for direct detection of BMAA failed to detect BMAA in the five analysed samples of cyanobacteria.[16]

In the present study a compound with slightly longer retention time and product ions similar to BMAA (*e.g.* *m*/*z* 102 and 44, but not 88) was observed in one of the cyanobacteria samples (*Calothrix* PCC7103) (Fig. 3). It was deduced the compound was a diamino acid not loosing methylamine upon fragmentation in MS/MS. The compound was identified as α-,γ-diamino butyric acid (DAB) by the use of a standard solution of DAB, detection of 6 product ions (*m*/*z* 102, 101, 74, 73, 56, 44), calculation of relative ion ratios (54%, 100%, 19%, 26%, 33%, 14% respectively) and by co-chromatography. Significant amounts, both of the free and protein bound form (approximately 7 and 370 μg g^{-1}, respectively, quantified as BMAA) was detected. DAB is known to be present in free state in many plants.[24] It has also been found in combined form in cell walls of certain species of bacteria,[25] although not yet in cyanobacteria. The actual sample was from a laboratory raised cyanobacteria culture. Therefore, contamination with other bacteria is unlikely. In the present study DAB was also detected after acidic hydrolysis in lower amounts (<50 μg g^{-1}) in many of the cyanobacteria samples. It should though be noted that formation of DAB from glutamine residues has earlier been demonstrated in alkaline treatment of proteins.[26] Thus, the origin of DAB in the present samples is unclear.

Derivatisation of BMAA with AQC, as previously used by Metcalf *et al.*[14] and Banack *et al.*[15] for MS/MS verification, gave the main ions *m*/*z* 459 > 289, 171 and 119. At our laboratory these same main product ions were found both for BMAA and DAB. Therefore comparing relative ion ratios will be crucial if not other fragments are recorded, as pointed out by Banack *et al.*[15] Fragments typical for AQC-derivatised BMAA and DAB were found in the present study to be 258, 88, 76 and 269, 188, 101 respectively. For the underivatised molecules useful product ions from *m*/*z* 119 were found to be 88 for BMAA, and 101 and 74 for DAB.

Interestingly DAB is known as a neurotoxin originally isolated from *Lathyrus latifolius* when searching for the cause of neurolathyrism.[24] Since ingestion of cycad leaves by cattle could produce a similar neurological disorder, Vega and Bell set out to find DAB in tissues of *Cycadaceae.* Instead, their work led to the discovery of BMAA.[1]

In conclusion, LC-MS/MS enabled highly selective identification of BMAA and, when using d3-BMAA, also reliable quantification. The general occurrence of high concentrations of BMAA in cyanobacteria reported earlier could not be confirmed by this study.

References

1 A. Vega and E. A. Bell, *Phytochemistry*, 1967, **6**, 759–762.
2 P. S. Spencer, P. B. Nunn, J. Hugon, A. C. Ludolph, S. M. Ross, D. N. Roy and R. C. Robertson, *Science*, 1987, **237**, 517–522.
3 P. S. Spencer, G. E. Kisby and A. C. Ludolph, *Geriatrics*, 1991, **46**(Suppl. 1), 37–42.
4 P. A. Cox, S. A. Banack and S. J. Murch, *Proc. Natl. Acad. Sci.*, 2003, **100**, 13380–13383.
5 S. J. Murch, P. A. Cox and S. A. Banack, *Proc. Natl. Acad. Sci.*, 2004, **101**, 12228–12231.
6 S. J. Murch, P. A. Cox, S. A. Banack, J. C. Steele and O. W. Sacks, *Acta Neurol. Scand.*, 2004, **110**, 267–269.
7 S. A. Banack and P. A. Cox, *Neurology*, 2003, **61**, 387–389.
8 P. A. Cox, S. A. Banack, S. J. Murch, U. Rasmussen, G. Tien, R. R. Bidigare, J. S. Metcalf, L. F. Morrison, G. A. Codd and B. Bergman, *Proc. Natl. Acad. Sci.*, 2005, **102**, 5074–5078.
9 T. J. Montine, K. Li, D. P. Perl and D. Glasko, *Neurology*, 2005, **65**, 768–769.
10 S. F. Dossaji and E. A. Bell, *Phytochemistry*, 1973, **12**, 143–144.
11 G. E. Kisby, M. Ellison and P. S. Spencer, *Neurology*, 1992, **42**, 1336–1340.
12 M. W. Duncan, J. C. Steele, I. J. Kopin and S. P. Markey, *Neurology*, 1990, **40**, 767–772.
13 M. Pan, T. J. Mabry, P. Cao and M. Moini, *J. Chromatogr.*, 1997, **787**, 288–294.
14 J. S. Metcalf, S. A. Banack, J. Lindsay, L. F. Morrison, P. A. Cox and G. A. Codd, *Environ. Microbiol.*, 2008, **10**, 702–708.
15 S. A. Banack, H. E. Johnson, R. Cheng and P. A. Cox, *Mar. Drugs*, 2007, **5**, 180–196.
16 T. Kubo, N. Kato, K. Hosoya and K. Kaya, *Toxicon*, 2008, DOI: 10.1016/j.toxicon.2008.02.015.
17 U. Beckman Sundh, J. Rosén and C. Andersson, *Analysis, occurrence, and toxicity of β-methylaminoalanine (BMAA)*, Nordic Council of Ministers, TemaNord 2007:561, ISBN 978–92–893–1541–8, Copenhagen, 2007.
18 J. J. Pesek and M. T. Matyska, *LC-GC*, 2006, **24**(3), 1–5.
19 M. W. Duncan, I. J. Kopin, J. S. Crowley, S. M. Jones and S. P. Markey, *J. Anal. Toxicol.*, 1989, **13**, 169–173.
20 J. Eriksson, S. Jonasson, D. Papaefthimiou, U. Rasmussen and B. Bergman, *Amino Acids*, 2008, DOI: 10.1007/s00726-007-0023-4.
21 T. G. Myers and S. D. Nelson, *J. Biol. Chem.*, 1990, **265**, 10193–10195.
22 P. B. Nunn and P. O'Brien, *FEBS Lett.*, 1989, **251**, 31–35.
23 W. H. Schaefer, *Curr. Drug Metab.*, 2006, **7**, 873–881.
24 C. Ressler, P. A. Redstone and R. H. Erenberg, *Science*, 1961, **134**, 188–190.
25 K. H. Schleifer and O. Kandler, *Bacteriol. Rev.*, 1972, **36**, 407–477.
26 R. L. M. Synge, *Biochem. J.*, 1939, 671–678.