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# Direct analysis of microcystins by microbore liquid chromatography electrospray ionization ion-trap tandem mass spectrometry

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**Abstract**

Microcystins are a group of structurally similar cyclic heptapeptide hepatotoxins and tumor promoters, produced by cyanobacteria. A microbore liquid chromatography electrospray ionization ion-trap mass spectrometry (LC-ESI-ITMS) method has been developed which is capable of separating and detecting trace amounts of microcystin variants in environmental samples. Extracted water sample was loaded onto a LC trapping column and, using a column switching technique, the compounds of interest were back-flushed onto a 1-mm LC column. Structural elucidation was achieved using ion-trap with tandem mass spectrometry in the data dependent scan mode. Collision-induced dissociation to $MS^3$ allowed tentative identification of these cyclic peptides. Full-scan LC-ESI-MS mass spectrum was obtained when 250 pg of the authentic compound was injected onto the HPLC column, which represents the detection limit for microcystin-LR. This study demonstrated that LC-ESI-ITMS is a reliable and sensitive technique for analysing trace levels of microcystins. 

*Keywords:* Microcystins; Microbore liquid chromatography electrospray ionization ion-trap tandem mass spectrometry; Cyanobacteria

## 1. Introduction

Cyanobacteria (blue-green algae) can occur worldwide in water blooms in eutrophic lakes and drinking water reservoirs [1,2]. The different genera of toxin-producing cyanobacteria, which include *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc*, altogether produce at least 60 toxins, some of which have been characterised [3,4].

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Cyanobacterial microcystins are potent liver toxins and tumor promotors [5,6] and inhibitors of protein phosphatases, thereby causing hyperphosphorylation of cell proteins and disruption of important cellular mechanisms [7–9]. *Microcystis aeruginosa* can produce a wide range of these hepatotoxins which are 7-amino acid cyclic peptides. Their general structure is cyclo (D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha). X and Z are variable L-amino acids. D-MeAsp is D-erythro-methylaspartic acid, Mdha is *N*-methyldehydroalanine, and Adda is (2S,3S,8S,9S)-3-amino-9-methoxy - 2,6,8 - trimethyl - 10 - phenyl - deca - 4,6-dienoic acid. The Adda moiety is essential for the toxicity of microcystins and the Mdha is responsible for covalent binding of the toxin to protein phosphatases [8]. Changes in the stereochemistry about the dienes on Adda result in a loss of toxicity [10], while slight modifications, e.g. demethylation or acetylation at the C-9 position of Adda, retain toxicity [11]. Other modifications include replacement of D-alanine or methyldehydroalanine by D-serine and *N*-methylserine, respectively. Microcystin-LR (MCYST-LR, when leucine and arginine represent the X and Z amino acids, respectively) (Fig. 1, compound I) is the most commonly reported microcystin. The toxicity ($LD_{50}$) of MCYST-LR is $\sim 60$ μg/kg when dosed intraperitoneally in mice [12].

The occurrence of microcystin-containing blooms in freshwaters has been implicated in animal and human poisonings worldwide. Reports have shown increased incidence of primary human liver cancer [13] and animal poisonings [14] in different countries, when people or livestock have consumed water contaminated with microcystins, and a recent tragedy occurred at Caruaru, Brazil [15], where patients at a hemodialysis unit died after treatment with water contaminated with microcystins. The need for rapid, sensitive and reliable analytical methods, which can definitively identify these microcystin variants, can be clearly seen. Immunoassays using monoclonal and polyclonal antibodies have been developed to detect microcystins, but the specificity of these techniques have not been fully investigated and they do not provide any structural information [16].

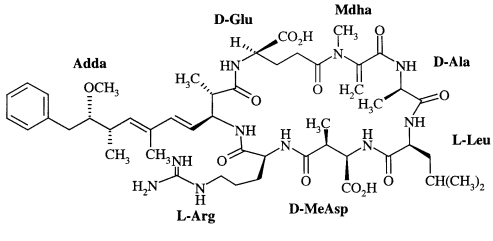



Fig. 1. Structure of microcystin-LR.

The structure of a microcystin was first determined by Botes et al. in 1984 [17]. Since then, extensive structural characterisations of microcystin variants have been carried out [10,18–24]. High resolution fast atom bombardment mass spectrometry (HR/FAB/MS) was used to determine the molecular weights of microcystin analogues. Since collision-induced dissociation (CID) FAB/MS of cyclic peptides produced only a few prominent low mass fragment ions (showing sequential information on 4-amino acids), microcystin variants were purified by high-performance liquid chromatography (HPLC), and converted to the isolated cyclic peptides to linear peptides (ozonolysis followed by sodium borohydride reduction), prior to FAB/CID/MS analysis. A recent application of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) to study microcystin variants directly from cell extracts has been reported [25], although this methodology is less amenable to interface with HPLC separation.

Liquid chromatography electrospray ionization coupled mass spectrometry (LC-ESI-MS) is considered to be a useful and powerful hyphenated technique for sequence analysis of small peptides [26,27]. Using a triple quadrupole mass spectrometer, it has been feasible to characterize microcystins [28–30]. HPLC-ESI-CID mass spectra were obtained for purified MCYST-LR and MCYST-RR authentic compounds [29], whereas with capillary electrophoresis separation ESI-multiple reaction monitoring (MRM) mass spectra of microcystin analogues were acquired from cell extracts [30].

This paper describes the application of microbore LC for the separation of microcystins from aqueous methanol extracts, where ESI selects the protonated molecules and CID produces fragmentation characteristic of the sequence of microcystins. Mass spectrometry was performed with an ion-trap mass spectrometer (IT-MS). IT-MS operates in four stages: (1) ion injection, which involves trapping the injected ions efficiently; (2) ion isolation, where unwanted ions are ejected from the trap while the precursor ions of interest remain trapped in a strong radiofrequency (RF) field; (3) resonance excitation, in which ions undergo CID in the ion trap chamber, and the resonant precursor ion repeatedly collides with helium buffer gas to produce fragment ions; (4) mass analysis. For full scan mass spectrometry, only stages (1) and (4) are involved. All four functions are required for $MS^n$ mode. An advantage of using an ITMS is that the instrument can switch between full-scan MS and a CID product scan in the presence of helium collision gas, with no loss in signal or CID efficiency. Therefore, as demonstrated in this paper, one LC analysis can generate full scan MS and CID MS information.

## 2. Experimental

### 2.1. Toxin sources and extraction

Scum material from a hepatotoxic *Oscillatoria agardhii* bloom was collected from Soulseat Loch, SW Scotland and from a hepatotoxic *Microcystis* sp. bloom, at the Patos Lagoon, southern Brazil. The samples were frozen at −20°C, lyophilised and stored at −20°C until required. Lyophilised cells were removed and extracted in 70% (v/v) aqueous MeOH (50 mg/ml) with ultrasonication. Each extract was clarified by centrifugation at 14 000 rpm (Eppendorf, 5415 centrifuge) for 5 min.

### 2.2. Chemicals

Microcystin-LR was purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile, methanol and ammonium acetate were purchased from J.T. Baker (Phillipsburg, NJ, USA). Formic acid was doubly distilled and purchased from GFS Chemicals (Columbus, OH, USA). Water was obtained in-house from a Barnstead NANOpure ultrafiltration system (Dubuque, Iowa, USA).

### 2.3. Liquid chromatography/mass spectrometry

Microbore LC separation was performed with Ultra Micro Plus LC pumps (manufactured by Micro-Tech Scientific, Sunnyvale, CA). Injection was achieved by column switching, using a PerSeptive Biosystems Integral Workstation (Framingham, MA). MeOH extracts (10 μl) were injected onto a 2 × 1-mm $C_8$ Opti-guard column (Optimize Technologies, Oregon City, OR), washed with water for 5 min and the sample eluted onto a $C_{18}$ Betasil column (100 × 1 mm, Keystone Scientific, Bellefonte, PA), using the LC mobile phase. The mobile phase was 5% acetonitrile: 5% methanol: 0.5% formic acid (A); 49% acetonitrile: 49% methanol: 0.5% formic acid (B). LC gradient elution conditions were initially 50% B to 100% B at 5 min, then 100% B from 5 to 10 min, at a flow rate of 50 μl/min. The entire eluent was directed into the mass spectrometer.

Mass spectrometric analysis was accomplished on a Finnigan LCQ IT-MS (Thermo Separation, San Jose, CA, USA) operated in atmospheric pressure ionization fitted with an electrospray ionization source (ESI). Ions generated from the ESI source were introduced into the mass spectrometer through a heated capillary. The capillary temperature and ionization voltage were maintained at 250°C and 4 kV, respectively. In the single MS scan mode, the maximum number of ions was set to $5 \times 10^7$, for automatic gain control, maximum fill time was 500 ms. Various scan ranges were used, according to the molecular weights of the analytes.

In the CID mode, the maximum number of ions for automatic gain control was set to $2 \times 10^7$ and the trap was filled for up to 500 ms. Different dissociation energies were used and are specified with associated results. Excitation of the ions was accomplished through collisions with helium.

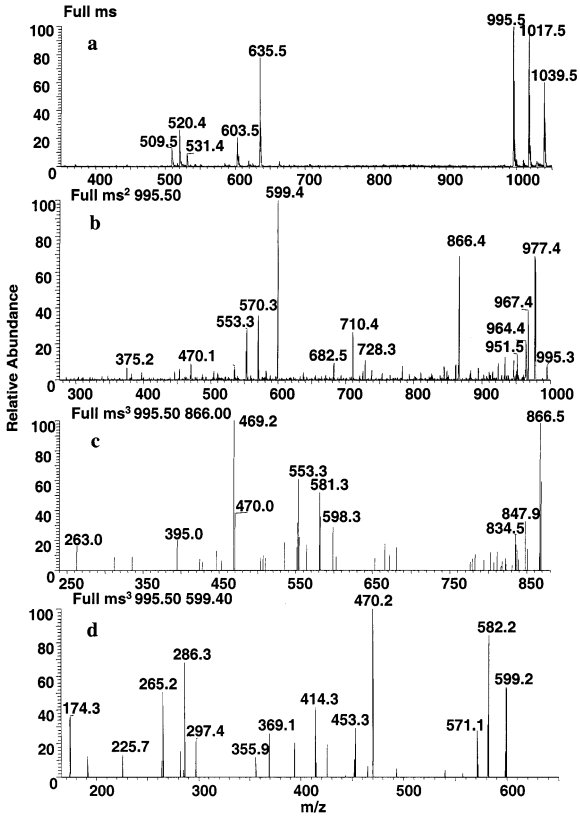



Fig. 2. ESI mass spectra of MCYST-LR: (a) $MS^1$; (b) $MS^2$ of $m/z$ 995.5; isolation window of 1.8 amu and collision energy of 30%; (c) $MS^3$ of $m/z$ 866.3; (d) $MS^3$ of $m/z$ 599.4 product-ion with isolation window of 1.8 amu and collision energy of 15%.

For the Brazilian cyanobacterial extracts, CID data acquisition was automated using the ESI-IT data-dependent scans mode. The ES-ITMS was programmed as follows: (1) acquired a full scan mass spectrum; (2) selected the base peak from the mass spectrum as it met a preset signal-threshold requirement; (3) determined the charge-state; (4) acquired an MS/MS scan with the appropriate mass range for that ion. CID was performed automatically for any components which showed strong intensities on the MS. Collision energy is given as a percentage relative to the maximum voltage which can be applied to the endcaps at the resonance frequency of the selected mass. For all dependent-scan $MS^2$ experiments, a relative collision energy of 40% was used. $MS^3$-dependent scans used 35% relative collision energy. An isolation width for the precursor ion was always set at 3.0 amu. In-source CID mass spectra were obtained using a relative collision energy of 100%, scanning at a mass range of $m/z$ 100–1250.

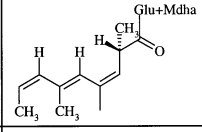
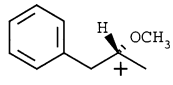
Data acquisition was in the positive ionization centroid mode, using the Finnigan LCQ v. 1.1 software system for data processing. MS tuning and optimization were achieved by infusing MCYST-LR and monitoring the $[M+H]^+$ ion at $m/z$ 995.6.

## 3. Results and discussion

Fragmentation pathways of peptides involve cleavages across the peptide backbone, yielding two main fragment ion series of type b- (containing N-terminal residues) and type y- (C-terminal residues), respectively. The b- type ions may lose the carbonyl moiety, resulting in a satellite series called a- type (immonium ion). The nomenclature used to identify the peptide fragment ions is based on reference [31]. Arginine-containing peptides undergo facile, preferential cleavage adjacent to amino acid residues with acidic side chains [32,33], producing exclusively b- and y- type ions.

ESI/MS analysis of MCYST-LR (compound I, Fig. 1) on a triple quadrupole mass spectrometer produced a singly-charged ion and a doubly-charged ion at $m/z$ 995 and 498, respectively [28]. When high voltage was applied to the orifice at the electrospray source, the Adda side chain $[PhCH_2CH(OCH_3)]$ at $m/z$ 135 was readily generated (data not shown), confirming the presence of microcystin and its analogues. Similarly for the ion-trap mass spectrometer, using helium as the collision gas and in-source CID, the $m/z$ 135 ion was observed in microcystin-LR. When the same in-source CID MS condition was applied to the water bloom extracts, using a scan mass range of $m/z$ 100–1250, it was possible to observe the $m/z$ 135 fragment ions on microcystin-LR and its analogues. However this information alone is insufficient for structural elucidation, and it does not take any structural modification on the Adda moiety into account [34].

Table 1
Proposed fragmentation pattern for MCYST-LR

| *m/z* | $MS^2$ Inference | *m/z* | $MS^3$ Inference | *m/z* | $MS^3$ Inference |
|---|---|---|---|---|---|
| 995 | $[M+H]^+$ | | | | |
| 977 | $[M+H]^+–H_2O$ | | | | |
| 866 | $[M+H]^+$–MeAsp or –Glu | 866 | Precursor ion | | |
| 728 | MeAsp+Arg+Adda+Glu | 553 | H+Mdha+Ala+Leu+MeAsp+Arg | | |
| 710 | $[M+H]^+$–Arg–MeAsp | 470 | H+Ala+Leu+MeAsp+Arg | | |
| 682 | $[M+H]^+$–Adda or H+Arg+Adda+Glu+Mdha | | | | |
| 599 | H+Arg+Adda+Glu or H+MeAsp+Arg+Adda | | | 599 | Precursor Ion |
| 553 | H+Mdha+Ala+Leu+MeAsp+Arg | | | | |
| 470 | H+Arg+Adda or H+Ala+Leu+MeAsp+Arg | | | 470 | H+Arg+Adda or H+Ala+Leu+MeAsp+Arg |
| 375 | $C_{11}H_{14}O$+Glu+Mdha+H   | | | 286 | H+MeAsp+Arg |
| 135 |   | | | 174 | Arg+$NH_3$+H |

Direct infusion of MCYST-LR to an ITMS produced a protonated molecule ($[M + H]^+$) at $m/z$ 995, a sodiated molecule ($[M + Na]^+$) at $m/z$ 1017 and a doubly-charged sodiated molecule ($[M + 2Na]^{2+}$) at $m/z$ 509 (Fig. 2a). Tandem mass spectrometry was performed on MCYST-LR, using the $m/z$ 995 ($MS^2$), 866 ($MS^3$) and 599 ($MS^3$) ions as the precursor ions and their CID mass spectra are displayed in Fig. 2 (b–d), respectively.

Product-ion scan of $m/z$ 995 generated several diagnostic fragment ions, as shown in Table 1. The fragment ion at $m/z$ 866 is 129 mass units less than the parent compound, suggesting the loss of either Glu or MeAsp. The $m/z$ 710 ion (loss of 285 Da) is equivalent to the loss of Arg and MeAsp. The base peak at $m/z$ 599 indicates a loss of 396 mass units; the fragment ion $m/z$ 599 could either be $[H + Arg + Adda + Glu]$ or $[H + MeAsp + Arg + Adda]$. Other minor fragment ions such as the $m/z$ 682 ion could form from either $[H + Arg + Adda + Glu + Mdha]$ or $[M + H - Adda]$. Based on the previous study using $^{15}N$ stable isotope [30], $m/z$ 682 corresponds to $[M + H - Adda]$. The fragment ion at $m/z$ 470 is equivalent to either $[H + Arg + Adda]$ or $[H + Ala + Leu + MeAsp + Arg]$; and $m/z$ 375 consists of $[C_{11}H_{14}O + Glu + Mdha + H]$. In order to clarify the precise amino acid contents of some of

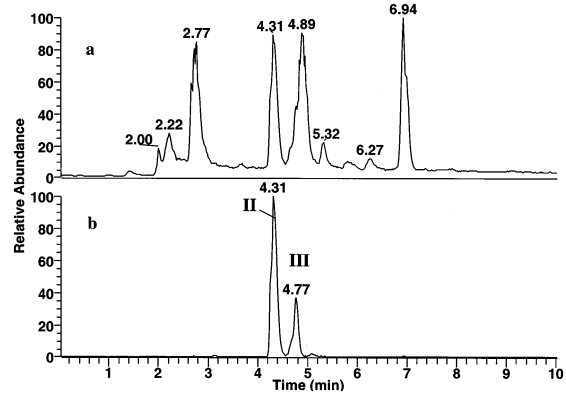



Fig. 3. Total ion chromatograms (a) and reconstructed ion chromatograms (b) obtained from LC-ESI-MS analysis of Soulseat (Scotland) *O. agardhii* bloom extract.

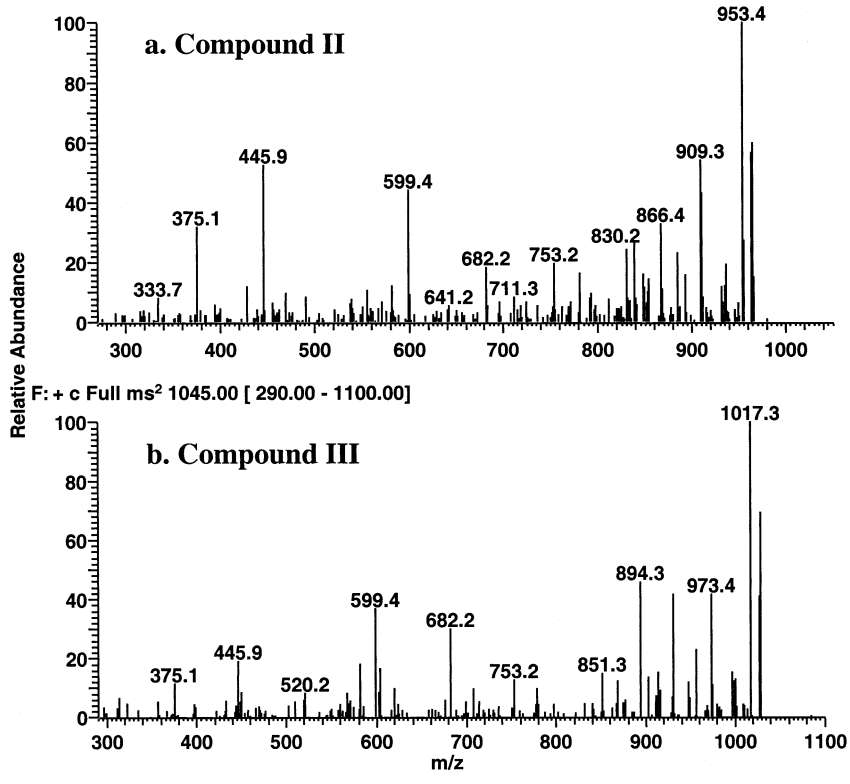



Fig. 4. ESI mass spectra of: (a) desmethyl-MCYST-LR, $MS^2$ of $m/z$ 981; (b) desmethyl-MCYST-HtyR, $MS^2$ of $m/z$ 1045; both with isolation window of 3 amu and collision energy of 40%.

these fragment ions, further investigations were carried out, using $MS^3$.

The $MS^3$ mass spectrum of $m/z$ 866 (Fig. 2c) produced a prominent fragment ion at $m/z$ 553. This base peak corresponds to the sum of [H + Mdha + Ala + Leu + MeAsp + Arg] (Table 1). Since this fragment ion contained MeAsp, this implies that the signal at $m/z$ 866 was due to the loss of Glu from MCYST-LR and not of MeAsp. The $m/z$ 469 ion was a further loss of Mdha from the five amino acid chain.

The $MS^3$ product-ion mass spectrum of $m/z$ 599 (Fig. 2d) produced a fragment ion at $m/z$ 470 (contribution from [H + Arg + Adda] or [H + Ala + Leu + MeAsp + Arg]). Since the $m/z$ 286 ion represents [H + MeAsp + Arg], this confirms the precise structure of $m/z$ 470 and $m/z$ 599 which contained [H + Arg + Adda] and [H + MeAsp + Arg + Adda], respectively (Table 1).

The reconstructed ion chromatograms from the LC-ESI-MS analysis of a cyanobacterial water bloom extract isolated from Soulseat Loch (Scotland) is shown in Fig. 3a. No MCYST-LR was

Table 2
Proposed fragmentation pattern for [Asp³, Dhb⁷] MCYST-LR

| $m/z$ | $MS^2$ inference |
|---|---|
| 981 | $[M+H]^+$ |
| 953 | loss of CO |
| 909 | $[M+H]^+$ — Ala |
| 866 | $[M+H]^+$ — Asp |
| 830 | H + Dhb + Ala + Leu + Asp + Arg + $C_{11}H_{14}O$ + Glu |
| 753 | H + Dhb + Ala + Arg + Adda + Glu |
| 682 | H + Arg + Adda + Glu + Dhb or H + Glu + Dhb + Ala + Leu + MeAsp + Arg |
| 599 | H + Arg + Adda + Glu |
| 470 | H + Ala + Leu + MeAsp + Arg |
| 446 | $C_{11}H_{14}O$ + Glu + Dhb + Ala + H |
| 375 | $C_{11}H_{14}O$ + Glu + Dhb + H |

Table 3
Proposed fragmentation pattern for [Asp$^3$, Dhb$^7$] MCYST-HtyR

| $m/z$ | $MS^2$ inference |
|---|---|
| 1045 | $[M+H]^+$ |
| 1017 | $[M+H]^+$ — CO |
| 973 | $[M+H]^+$ — Ala |
| 930 | H + Arg + Adda + Glu + Dhb + Ala + Hty |
| 894 | $C_{11}H_{14}O$ + Glu + Dhb + Ala + Hty + Asp + Arg + H |
| 753 | H + Arg + Adda + Glu + Dhb + Ala |
| 682 | H + Arg + Adda + Glu + Dhb |
| 603 | (i) H + Arg + MeAsp + Hty + Ala + Dha,<br>(ii) H + Arg + MeAsp + Tyr + Ala + Dhb,<br>(iii) H + Arg + Asp + Hty + Ala + Dhb |
| 599 | H + Arg + Adda + Glu |
| 582 | $m/z$ 599 — 17 Da |
| 446 | $C_{11}H_{14}O$ + Glu + Dhb + Ala + H |
| 375 | $C_{11}H_{14}O$ + Glu + Dhb + H |

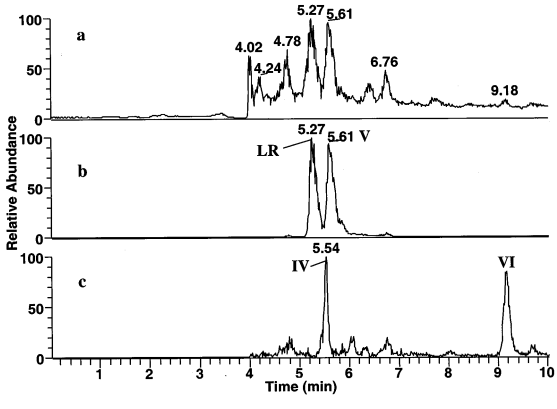



Fig. 5. Total ion chromatograms (a) and reconstructed ion chromatograms (b and c), obtained from LC-ESI-MS analysis of Patos Lagoon (Brazil) microcystis bloom extract.

detected in this sample. Two major signals were observed, at $m/z$ 981 (Fig. 3b, compound II) and 1045 (Fig. 3b, compound III). The component with a protonated molecule at $m/z$ 981 is 14 mass units less than MCYST-LR, which implies a desmethyl MCYST-LR analogue. The loss of a methyl group could occur at Adda, Mdha or MeAsp. Fig. 4a represents the product-ion mass spectrum of this compound II. Similar to MCYST-LR, the following fragment ions were observed at $m/z$ 375, 470, 599, 682 and 866. The presence of the ions at $m/z$ 375 and 599 strongly indicates that the amino acids Adda and Mdha or dehydrobutyrine (Dhb) [22–24] were present (Table 2). The ion at $m/z$ 866 represents a loss of an aspartic acid moiety from the protonated molecule, which further confirms that aspartic acid was present in the cyclic peptide instead of MeAsp. One noticeable feature of this product-ion mass spectrum is the $m/z$ 682 ion which could form from either [H + Arg + Adda + Glu + Mdha (or Dhb)] or [H + Glu + Mdha (or Dhb) + Ala + Leu + Asp + Arg]. The presence of the fragment ion at $m/z$ 753 (71 mass units higher than $m/z$ 682) verifies that $m/z$ 682 consisted of [H + Arg + Adda + Glu + Mdha (or Dhb)]. Sano et al. [35] using 2D-NMR found that dehydrobutyrine is present in the microcystins from Soulseat Loch, replacing methyldehydroalanine, in common with microcystins from other sources [22–24].

The CID mass spectrum ($MS^2$) of the component in Fig. 4b ($[M + H]^+$ at $m/z$ 1045.0) displays a rich array of the commonly observed sequence product-ions. The four known microcystin variants which have a protonated molecule at $m/z$ 1045 include (i) MCYST-desmethyldehydroalanine/homotyrosine/arginine ([Dha]-MCYST-HtyR), (ii) MCYST-tyrosine/arginine (MCYST-YR), (iii) aspartic acid/homotyrosine/arginine ([D-Asp$^3$]-MCYST-HtyR) [28] and (iv) aspartic acid/homotyrosine/arginine/dehydrobutyrine ([D-Asp$^3$, Dhb$^7$]-MCYST-HtyR) [35]. The presence of fragment ions at $m/z$ 682, 446 and 375 confirms the presence of Mdha or Dhb in the molecule (Table 3), and NMR indicated that Dhb was the corresponding amino acid [35]. The difference of 177 mass units between the two fragment ions $m/z$ 930 and 753, was consistent with the loss of homotyrosine. The fragment ion at $m/z$ 930 is 115 mass units less than the protonated molecule, which corresponds to the loss of aspartic acid. This is consistent with the presence of [D-Asp$^3$, Dhb$^7$]-MCYST-HtyR in the Soulseat Loch bloom of cyanobacteria [35].

The LC/ESI/MS total ion chromatogram for the Brazilian bloom extract is presented in Fig. 5. Several microcystin-related components were observed in this sample, their protonated molecules including $m/z$ 995, 1009, 1037 and 1025. Their

CID mass spectra were obtained using the dependent-data scan mode and are shown in Fig. 6 (a–c).

The CID mass spectrum of $m/z$ 1009 was weak, showing very few diagnostic fragment ions (Fig. 6a). An addition of 14 mass units from MCYST-LR suggests that the compound could be: (i) [D-Asp$^3$, ADMAdda] MCYST-LR (ADMAdda represents: *O*-acetyl-*O*-desmethyl-Adda); (ii) MCYST-LHar (Har represents homoarginine); (iii) [GluOCH$_3$] MCYST-LR; (iv) *N*-methyldehydrobutyrine (Mdhb) instead of *N*-methyldehydroalanine MCYST-LR [20]; or (v) MCYST-HilR (Hil represents homoleucine) [21]. The presence of the fragment ion at $m/z$ 599 indicates that the amino acids Adda and Arg were intact; $m/z$ 567 represents the loss of Adda and Glu, eliminating the possibility of methylation on the glutamic acid. A product ion at $m/z$ 710 implies the presence of Adda + Glu + Mdha + Ala + Leu. In-source CID mass spectrum of compound IV contained the $m/z$ 135 fragment ion, which further suggests the modification was not on the Adda side-chain (Fig. 7b). Therefore the product ion mass spectrum suggests an addition of 14 Da on the MeAsp amino acid (Table 4).

The molecular weight of compound V ($m/z$ 1037) is 42 mass units higher than MCYST-LR and had been associated with the analogue [ADMAdda$^5$]MCYST-LHar [36]. For [ADMAdda$^5$]MCYST-LHar, the loss of acetic acid from the amino acid ADMAdda was observed in the FAB/MS/MS spectrum, forming the fragment at $m/z$ 977, whereas the CID mass spectrum of compound V in this current study did not contain a $m/z$ 977 fragment ion (Fig. 6b). Additionally, structural assignments would confirm the presence of Adda, Glu and Mdha because the fragment ions at $m/z$ 135, 375, 470, 599 were detected; $m/z$ 908 and 728 suggested that MeAsp and arginine were present; $m/z$ 965 was derived from the loss of alanine from the molecule (Fig. 6b and Fig. 7c; Table 5). Therefore, the remaining amino acid should possess a residue mass of $m/z$ 155. Compound V is designated MCYST-XR; so far we have not been able to confirm its structure.

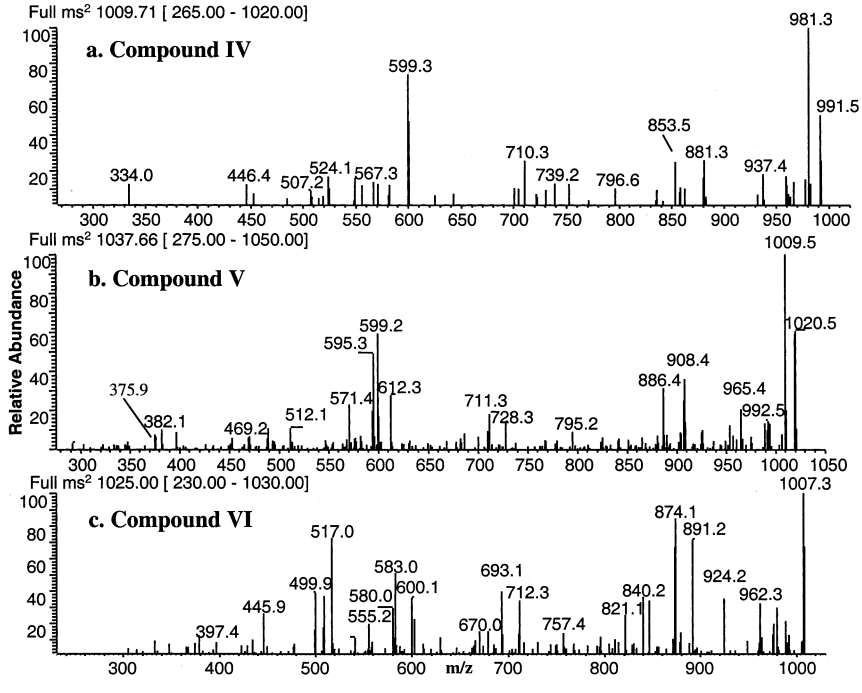



Fig. 6. ESI mass spectra of: (a) methyl-MCYST-LR, MS$^2$ of [M + H]$^+$ ion at $m/z$ 1009.7; (b) MCYST-XR, MS$^2$ of [M + H]$^+$ ion at $m/z$ 1037.6; (c) MCYST-LW, MS$^2$ of $m/z$ 1025.3; all with isolation window of 3 amu and collision energy of 40%.

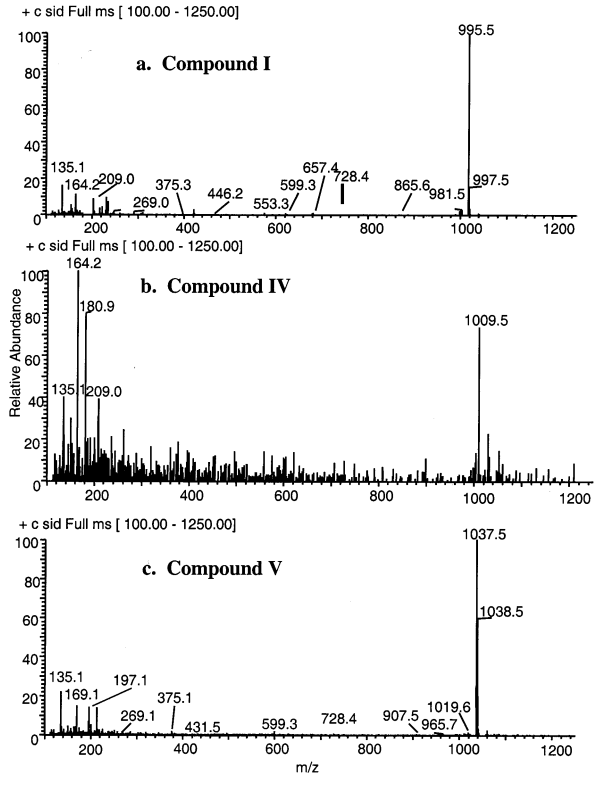



Fig. 7. In-source CID ESI mass spectra of: (a) compound I: MCYST-LR; (b) compound IV; and (c) compound V; obtained from the Patos Lagoon (Brazil) microcystis bloom extract.

Table 4
Proposed fragmentation pattern for methyl-MCYST-LR

| $m/z$ | $MS^2$ inference |
|---|---|
| 1009 | $[M+H]^+$ |
| 991 | $[M+H]^+$ — $H_2O$ |
| 981 | $[M+H]^+$ — CO |
| 937 | $[M+H]^+$ — Ala |
| 881 | $[M+H]^+$ — Glu |
| 853 | $[M+H]^+$ — Arg |
| 710 | H + Adda + Glu + Mdha + Ala + Leu |
| 599 | H + Arg + Adda + Glu |
| 567 | $[M+H]^+$ — Adda — Glu |

Finally, component VI showed a protonated molecule at $m/z$ 1025, which could be attributed to the presence of MCYST-LW (when leucine and tryptophan represent the X and Z amino acids, respectively). This compound has been identified from *Microcystis* PCC 7820 [30] and their CID mass spectra are comparable to each other (Fig. 6c), as described in Table 6.

## 4. Conclusion

This paper describes the application of microbore LC-ESI ITMS to the analysis of microcystins in environmental samples containing cyanobacteria. There are several advantages of using an ion-trap mass spectrometer. For certain

Table 5
Proposed fragmentation pattern for MCYST-XR

| $m/z$ | $MS^2$ inference |
|---|---|
| 1037 | $[M+H]^+$ |
| 1020 | $[M+H]^+$ — 17 Da |
| 1009 | $[M+H]^+$ — CO |
| 965 | $[M+H]^+$ — Ala |
| 908 | $[M+H]^+$ — MeAsp |
| 728 | MeAsp + Arg + Adda + Glu |
| 711 | $m/z$ 728 — 17 Da |
| 612 | Mdha + Ala + (155) + MeAsp + Arg + OH |
| 599 | Arg + Adda + Glu |
| 595 | Mdha + Ala + (155) + MeAsp + Arg |
| 571 | $m/z$ 599 — CO |
| 512 | Ala + (155) + MeAsp + Arg |
| 469 | Arg + Adda |
| 375 | $C_{11}H_{14}O$ + Glu + Mdha |

Table 6
Proposed fragmentation pattern for MCYST-LW

| $m/z$ | $MS^2$ inference |
|---|---|
| 1025 | $[M+H]^+$ |
| 1007 | $[M+H]^+$ — $H_2O$ |
| 891 | $[M+H]^+$ — PhCH=CH(OMe) |
| 874 | H + $C_{11}H_{14}O$ + Glu + Mdha + Ala + Leu + MeAsp + Trp |
| 840 | H + MeAsp + Trp + Adda + Glu + Mdha |
| 712 | H + Glu + Mdha + Ala + Leu + MeAsp + Trp |
| 583 | H + Mdha + Ala + Leu + MeAsp + Trp |
| 517 | H + Mdha + Ala + Leu + MeAsp + Trp + $NH_3$ |
| 500 | H + Ala + Leu + MeAsp + Trp |
| 446 | H + Leu + MeAsp + Trp + $NH_3$ |
| 397 | H + Glu + Mdha + Ala + Leu |

analytes, when comparing the full-scan MS and tandem MS/MS sensitivity of the ion trap mass spectrometer with the triple quadrupole instrument, better detection limits were achieved with the ion-trap instrument, as observed by Dear et al [37]. The improved performance is attributed to: (i) the faster scan rates of the ion-trap mass spectrometer, giving increased ion currents and consequently shorter scan functions, allowing for improved signal averaging; (ii) the fact that the ions are accumulated over a defined period of time. (In contrast, the conventional triple quadrupole mass spectrometer is a flow-through system, which at any time point, selects ions of a specific mass-to-charge ratio while eliminating all the other ions from analysis, the sensitivity of quadrupole mass spectrometers is inherently limited.) When using a triple quadrupole mass spectrometer to analyse samples, it is necessary to set the instrument to acquire the data in the Q1 full-scan mode. Data have to be processed and further experiments have to be set up to obtain the product-ion mass spectra. It is possible to loop two or three experiments simultaneously within one time period. However, if several compounds of interest co-elute within a very short time, in order to obtain the product ion mass spectra of all these compounds the samples have to be analysed more than once. In contrast, using the data-dependent scan mode on the ion-trap, the Q1 full-scan data as well as the product-ion mass spectra can be obtained simultaneously in one analytical run. Finally, the cost of an ion trap mass spectrometer is approximately one third the price of a triple quadrupole mass spectrometer, and the instrument is very easy to use. Hence it is an invaluable research tool for laboratory screening of biological samples.

The drawback of performing CID in an ITMS is the limited scan range. No product-ion information is obtained below the low mass cut-off region. For the analysis of microcystin variants, the low mass scan region was 200 mass units. This could be compensated by using in-source CID or $MS^3$. Good quality $MS^3$ data was available during infusion of MCYST-LR, and precise amino acid sequence information was readily obtained from the CID mass spectra for microcystin variants.

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