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Further improvements in the application of high-performance liquid chromatography, capillary electrophoresis and capillary electrochromatography to the analysis of algal toxins in the aquatic environment ☆

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Abstract

The presence of algal toxins in the aquatic environment represents an important socioeconomic concern in many places worldwide, due to the toxicity that these compounds can induce in seafood or freshwater organisms at very low levels. Several analytical alternatives have been proposed over the last years for the control of these contaminants, which acute or chronic toxicity requires low detection levels and demands for the search of sensitive methods for their detection and determination. HPLC has been widely used for this purpose, although several alternatives such as CE or capillary electrochromatography (CEC) are being lately developed with this aim. In this work we report on the application of improved HPLC, as well as CE and CEC, for the analysis of diarrhetic shellfish poisoning toxins, amnesic shellfish poisoning (ASP) toxins and microcystins (MCs) present in different matrices such as water, shellfish or algae. Improvements in sample preparation for increasing sensitivity and selectivity are also shown. While UV and fluorimetric detection are the detection methods generally used, mass spectrometric detection was also applied for ASP toxins and MCs, especially for confirmatory purposes. From the results obtained it can be concluded that both HPLC and CE offer a good potential for a sensitive and selective determination of these algal toxins in such complex matrices. The results obtained for CEC allow also to conclude that this technique can result in a promising technique for such application.
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1. Introduction

The frequence, intensity and geographic distribution of toxic episodes in the aquatic environment, due to the proliferation of harmful algae seems to be increasing in the last decades consequently seriously

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affecting public health and economy in different regions worldwide. The search for accurate and sensitive analytical developments for monitoring these toxins is therefore an important task for scientist working in this field [1,2]. On the other hand, the complexity of the matrices, in which these compounds are usually present, demands improved sample preparation methodologies with the aim of removing interferent compounds avoiding legal implications of false results [3–6]. At this point the use of mass spectrometry (MS) as a detection mode is also very useful, especially for confirmatory purposes [7,8]. Some of the groups of toxins involved in harmful algal blooms, such as diarrhetic shellfish poisoning (DSP) toxins, amnesic shellfish poisoning (ASP) toxins and microcystins (MCs) were selected in this study as examples of the application of the improvements achieved for high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) with UV, fluorescence detection (FL) and MS detection for the analysis of the compounds mentioned

In a previous work we had been developing a CEC method to be applied for the analysis of ASP toxins [9] while immunoaffinity chromatography (IAC) had been also applied for an improved sample preparation for the analysis of MCs [10], and further improvements on both of the newer approaches are also presented in this work.

CEC is a modern technique that ideally combines the main features of CE and HPLC with the aim of providing improvements, specially in terms of sensitivity and efficiency. On the other hand, the use of IAC for improved sample preparation protocols, provides advantages such as higher specificity and selectivity in comparison with conventional solid-phase extraction (SPE) methods used with similar purposes.

2. Experimental

2.1. Toxin and standard samples

Calibration solutions of 25.3 μg/ml okadaic acid (OACS-1) and 100 μg/ml domoic acid (DACS-1B), a certified standard lyophillised mussel material (MUS-2) containing both 11 μg/g okadoic acid (OA) and 1 μg/g dinophysistoxin-1 (DTX-1), and mussel tissue reference material (MUS-1) containing 98.5 μg/g DA, were purchased from the Institute for Marine Biosciences, NRC, Halifax, Canada. Standards of microcystins LR, YR and RR (MC-LR, MC-YR and MC-RR) were purchased from Calbiochem-Novabiochem (Nottingham, UK) (see Fig. 1).

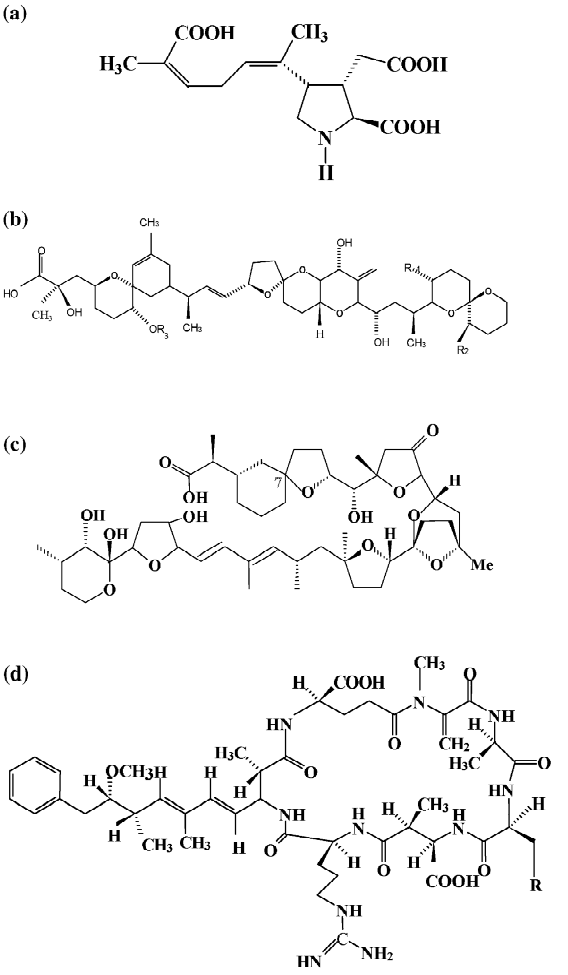

Fig. 1. Chemical structures of algal toxins: (a) Domoic acid. (b) Diarrhetic shellfish toxins. Okadaic acid (OA), R_1=H, R_2=H, R_3=CH_3; dinophysistoxin-1 (DTX-1), R_1=H, R_2=CH_3, R_3=CH_3; dinophysistoxin-2 (DTX-2), R_1=H, R_2=CH_3, R_3=H; dinophysistoxin-3 (DTX-3), R_1=Acyl. (c) Pectenosecoacids. PTX-2SA, C7:R; 7-epi-PTX-2SA, C7:S. (d) Microcystins. MC-LR: R=$CH(CH_3)_2$; MC-RR: R=$CH_2CH_2NHC(NH_2)NH$; MC-YR: R=C_6H_4OH.

Species of *Dinophysis acuta* were collected in August 1996 from the Celtic sea, Ireland, were kindly provided by Dr. Kevin J. James, from the Cork Institute of Technology, Cork, Ireland. Blue green algae samples from a natural bloom in Montargil (Portugal) were kindly provided by Dr. Susana Franca, Instituto Nacional da Saúde Dr. Ricardo Jorge, Lisbon, Portugal. Contaminated water samples were collected from Tamega river (Portugal) after a bloom of toxic cyanobacteria in 1999.

Mussels from Rías Bajas naturally contaminated with DSP toxins were kindly provided by Socomgal and ASP naturally contaminated razor clams from Ría de Vivero (Lugo), were kindly provided by Delegación Provincial de la Consellería de Pesca de Lugo, Xunta de Galicia and contaminated scallops from Kilkerrin Bay (Ireland) were kindly provided by Dr. Kevin James from the Cork Institute Technology. Uncontaminated samples of scallops were provided by Rampesca.

All these samples were kept frozen (−18 °C) until analysis.

2.2. Chemicals

All solvents and chemicals were HPLC or analytical grade. Milli-Q grade water (Millipore) was used for the preparation of aqueous solutions.

Phosphate-buffered saline (PBS) solution for immunoaffinity clean-up of microcystins was prepared with 1.06 g of $NaH_2PO_4 \cdot H_2O$, 3.27 g of $Na_2HPO_4 \cdot 7H_2O$ and 8.18 g of NaCl for 1 l solution and, pH was adjusted to 7.4 with 1 *M* NaOH solution.

Guaranteed grades of reactants such as boric acid, formic acid, sodium dodecyl sulfate (SDS), 50 m*M* sodium borate at pH 9.2 and 50 m*M* phosphate buffer at pH 2.5 (Hewlett-Packard), quinuclidine (Aldrich), *N*-chlorosuccinimide (Sigma) and 9-anthraldehyde hydrazone (synthetized in our laboratory) were used in the different analysis.

C_{18} Empore extraction disks (3M, TX, USA) were used for treatment of water samples containing microcystins.

Solid-phase extraction cleanup for ASP toxins was carried out using LC-SAX cartridges: part No.1210-2044, lot No. 182639, 3 ml capacity, 500 mg, Varian. The immunoaffinity columns used for immunoaffinity cleanup of MCs were Microcystin ImmunoSep (ImmunoKem, Canada).

2.3. Apparatus

HPLC analyses were performed using the following instruments: a HP 1050 liquid chromatograph equipped with both a fluorescence detection system (HP 1046) and a UV diode array detection (DAD) system (HP 1040M) with Chemstation data analysis software (all from Agilent Technologies); and a Jasco PU-980 Liquid Chromatograph with a UV detection system (Perkin-Elmer LC-95 UV–Vis).

Both CE and CEC analyses were performed in a HP ^{3D}CE system equipped with a DAD system and HP ^{3D}Chemstation for instrument control, data acquisition and data analysis (Agilent Technologies).

LC–electrospray ionization (ESI) MS analyses were performed using a 1100 Series LC–MS system equipped with an electrospray source, a single quadrupole mass spectrometer, and HP ^{3D}Chemstation (Agilent Technologies).

2.4. Sample preparation

2.4.1. Amnesic shellfish poisoning toxins

2.4.1.1. Extraction and clean-up

The initial conditions used for the extraction had been proposed by Quilliam et al. [3] and were slightly modified in our laboratory: 16 ml methanol–water (1:1, v/v) was added to 4 g homogenate tissue. The mixture was then homogenized for 3 min and centrifuged for 10 min at 3600 rpm. The supernatant was filtered through a 0.45 μm filter (Millex-HV). The remaining extract was taken for cleaning up purposes or kept in the fridge until further analysis.

Anion-exchange SPE has been used for cleanup purposes under the conditions initially proposed by Zhao et al. [11] with slight modifications [4]: 5.0 ml of sample extract was passed through a strong anion-exchange (SAX) cartridge previously conditioned with 6 ml methanol, 3 ml water and 3 ml methanol–water (1:1). The extract was washed with methanol–water (1:1), then eluted with 5 ml 0.1 *M* formic acid and then analysed by HPLC–UV.

2.4.2. Diarrhetic shellfish poisoning toxins

2.4.2.1. Extraction

Hepatopancreas were carefully removed from whole mussel tissue and then homogenized.

A 2-g amount of homogenized shellfish tissue was extracted with 80% aqueous methanol, after further homogenization at room temperature, washed with 5 ml of hexane (twice) and partitioned with 6 ml of $CHCl_3$ (twice). The combined $CHCl_3$ layers were evaporated to dryness under a stream of nitrogen and the residue was redissolved with 1 ml MeOH.

2.4.2.2. Derivatisation and clean-up

Toxin standards and phytoplankton extracts in methanol were evaporated to dryness under a stream of nitrogen in amber vials (2 ml).

9-Anthryldiazomethane (ADAM) was synthetized according to the conditions described by Quilliam et al. [12]. ADAM is prepared just immediately before the derivatization reaction, to avoid the presence of interferences associated with decomposition products.

Derivatization with ADAM was then accomplished by first adding 100 μl of ''in situ'' ADAM to the evaporated extracts. The solution was then heated at 37 °C for 2 h in the dark. SPE columns were packed with 500 mg of activated silica. The residue from the evaporated ''in situ'' ADAM reaction was redissolved and transferred to the previously conditioned column, using aliquots of 300 μl chloroform–hexane (1:1)×3. The column was washed with 5 ml chloroform–hexane (1:1), followed by 5 ml chloroform and 5 ml methanol–chloroform (1:9) was used as eluent. The eluate was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 500 μl methanol.

2.4.3. Microcystins produced by cyanobacteria

2.4.3.1. Extraction and clean-up

Methanol–water (75:25) was used for the extraction of microcystins from lyophilised blue–green algae. A 20-ml volume of this solution were added to a 0.5 g aliquot of the lyophilised material and mixed for 3 min in an Ultra-Turrax T25 homogenizer, followed by centrifugation at 4500 rpm (3600 *g*) for 10 min at room temperature. The supernatant was collected, and the residue reextracted with 10 ml methanol–water (75:25). After combination, supernatants were concentrated to a final volume of 10 ml under nitrogen stream.

Surface water samples were directly analysed after filtration through a 0.45 μm Millex-HV syringe filters (Millipore, Bedford, MA, USA).

Immunoaffinity cartridges for selective extraction of microcystins were constituted by functionalized Sepharose and silica bed as supports for microcystin-LR polyclonal antibodies. The procedure followed in this clean-up includes conditioning of the cartridges with 3 ml of water and 3 ml of PBS prior to loading sample, must be diluted in PBS to achieve a concentration lower than 15% methanol. The rinse of the cartridge was carried out with 3 ml PBS, 3 ml water and 3 ml methanol–water (25:75), and elution of toxins with 6 ml of methanol–water (80:20) containing 4% (v/v) acetic acid. The effluent was collected in a 50-ml round bottom flask and evaporated to dryness at 35 °C and redissolved in methanol for injection into the HPLC–UV or CE–ESI-MS systems. For the CE–UV analysis, the effluent collected was redissolved in 30 m*M* borate solution, pH 9.5 adjusted with 1 *M* NaOH.

An SPE procedure using C_{18} disks was used for water samples. After conditioning the disks with 5 ml of MeOH–0.1% trifluoroacetic acid (TFA), 10 ml of MeOH and 10 ml of water, followed by loading the water sample (containing 0.5% MeOH), and elution of the toxins, which was carried out by using 20 ml of MeOH–0.1% TFA. After evaporation, samples were redissolved in MeOH or 30 m*M* borate solution for the instrumental analysis.

2.5. HPLC analysis

ASP and DSP analyses were performed by using UV and fluorescence detection, respectively and the analysis of ASP toxins was also performed using MS detection for confirmation purposes. The conditions used for this analysis are as follows.

Isocratic elution using a mobile phase consisting in 12% aqueous acetonitrile with 0.2% formic acid (adjusted to pH 2–3) at a flow-rate of 1.0 ml/min and an injection volume of 20 μl. Separation of ASP toxins was performed in a 250×4.6 mm Prodigy

ODS 0.5 μm column (Phenomenex) and UV detection was carried out at 242 nm.

HPLC–ESI-MS detection was performed under the following conditions: sample introduction was via injector with 20 μl through a 250×2.1 mm I.D. column packed with 5 μm Vydac 201TP52, reversed-phase C_{18}. The mobile phase was 8% aqueous acetonitrile with 0.05% formic acid and the flow-rate was 0.2 ml/min pumped isocratically.

Analysis of DSP toxins was carried out using an isocratic elution with MeCN–MeOH–water (70:15:15) for algae samples and MeCN–water (85:15) for mussel samples, respectively, at a flow-rate of 1.0 ml/min and an injection volume of 20 μl. The separation of DSP toxins was performed in a reversed-phase column, HP-Hypersil ODS (25 cm× 4 mm I.D., 5 μm). The fluorescence detector was operated with 254 nm excitation, 412 nm emission, and xenon lamp pulse frequency of 55 Hz.

2.6. CE analysis

CE–DAD has been applied for the analysis of microcystins and ASP toxins.

The CE conditions used for the analysis of ASP toxins are reported elsewhere [13] and described here briefly: separation was performed in 66 cm×363 μm O.D.×50 μm I.D. bare fused-silica capillaries with an UV window located 15 cm from the end of the capillary at room temperature. UV detection was performed at wavelength of 242 nm. Hydrodynamic injection of the sample was accomplished using a 50 mbar push for 12 s and the applied voltage was 30 kV. The background electrolyte was 25 m*M* borate buffer at pH 9.2.

CE analysis of microcystins was performed using uncoated fused-silica capillaries, 58 cm (49.5 cm effective length)×50 μm I.D.; detection wavelengths were set at 200 and 238 nm. Micellar electrokinetic chromatography (MEKC) was selected as the CE mode of choice using 120 m*M* borate buffer with 20 m*M* SDS as separation electrolyte, with pH adjusted to 9.5 with NaOH. Applied voltage was 30 kV and the capillary temperature was thermostated at 25 °C. Samples were redissolved in 30 m*M* borate at pH 9.5, in order to use field-amplified sample stacking (FASS) for on-line preconcentration of the toxins during the CE analysis; hydrodynamic injection of samples was carried out with 50 mbar for 25 s.

MS detection was also used for the CE analysis of microcystins specially for confirmatory purposes. In this case the separation of the toxins was performed on fused-silica capillaries with 80 cm×50 μm I.D., using 50 m*M* formic acid as separation electrolyte. Hydrodynamic injection of samples was carried out at 50 mbar for 3 s, while separation voltage was 20 kV and temperature in the capillary was 25 °C. The CE–MS interface consisted of a sheath liquid flow system, delivering 7 μl/min of 0.1% formic acid in methanol–water (50:50, v/v). ESI-MS was conducted in the positive ion mode, setting the capillary voltage at 4000 V and the nitrogen gas flow at 3 l/min with a temperature of 150 °C. The system was scanned from m/z 300 to 1100 during detection, and for the selective ion monitoring (SIM) mode, protonated ions were monitored at m/z 1045 and 996, corresponding to $[M+H]^+$ of MC-YR and MC-LR, respectively, and also at m/z 520 which corresponds to $[M+2H]^{2+}$ for MC-RR.

2.7. CEC analysis

CEC of ASP toxins was performed based on the preliminary studies of Leão Martins et al. [9] using a 100 μm I.D.×375 μm O.D. capillary column with a packed bed length of 25 cm (CEC C_{18}, 3 μm particle size) obtained from Agilent Technologies. For all separations, the total column length was the packed bed length plus 8.5 cm of polyimide-coated fused-silica tubing. The column was conditioned with mobile phase by first pressurizing the inlet at 10 bar and ramping the voltage to 12 kV over a 40-min period. Both the inlet and outlet were pressurized at 10 bar and the voltage was maintained at 12 kV during 10 min.

Electrokinetic injection of the sample was done using 10 kV for 10 s and the applied voltage during the run was 12 kV at 25 °C. UV detection was performed at a wavelength of 242 nm. The mobile phase (5 m*M* phosphate buffer, pH 2.5–MeCN, 40:60) was prepared daily and filtered through a 0.2 μm LC poly(vinylidene difluoride) (PVDF) acrodisc filter and degassed for 25 min by ultrasonication before use.

3. Results and discussion

3.1. Liquid chromatographic analysis

The potential of HPLC for the determination of contaminants such as algal toxins has been clearly demonstrated over the last years, nevertheless there are still some drawbacks, usually associated with the lack of selectivity associated with the complexity of the matrices, which need to be overcame [4], with this aim, special attention has been dedicated in this work to improve sample preparation protocols.

3.1.1. For ASP toxins

Different extraction and clean up procedures had been evaluated in a previous study carried out by this group of research [4]. After this evaluation it has been found that water–methanol (1:1, v/v) as proposed by Quilliam et al. [3] offered the best conditions for an efficient extraction of ASP toxins with minimal extracts degradation. The optimal conditions for the clean-up of the extracts of ASP toxins were achieved when using anion-exchange SPE under the conditions reported in the Experimental section. An efficient removal of interferences, specially those coming from tryptophan, which is usually present in this kind of biological matrices, co-eluting with domoic acid (DA) or some of its isomers, as well as high percentages of recovery have been achieved under these conditions.

These improved conditions for sample preparation allowed to achieve a good resolution when using HPLC for the analysis of the naturally contaminated samples under the conditions reported in the Experimental section of this work. The confirmation of the results obtained has been carried out by HPLC–ESI (positive ion mode)-MS-SIM, and under conditions previously optimized [8] which are described in the Experimental section. Good resolution was observed for DA and isomers which were sensitively determined after having removed critical interferences such as tryptophan, present in the matrix using the evaluated and optimized SPE procedure described in the Experimental section. HPLC–MS also allows sensitive determination of DA and isomers present in naturally contaminated samples as can be seen in Fig. 2. A full scan mass spectrum obtained from the extract of naturally contaminated scallop hepatopancreas samples (Bertragh Bay, Ireland) is shown in Fig. 2, and it is characterized by an abundant protonated molecule $[M+H]^+$ at m/z 312 with not significant fragmentation.

3.1.2. For DSP toxins

The results obtained after the application of LC–FL to the analysis of DSP toxins are shown in Fig. 3. The improved conditions for the use of ''in situ'' ADAM for the derivatization of the DSP compounds to convert them into the correspondent fluorescent derivatives, which had been previously evaluated [12] as well as the optimization of the chromatographic conditions reported in the Experimental section allowed one to obtain a complete separation of the DSP analogues (OA, its derivatives and pectenotoxins) present in phytoplankton sample extracts such as *Dinophysis acuta*, being able to sensitively determine the different toxins involved in the contamination of such species. The potency of ''in situ'' ADAM has been clearly demonstrated and the chromatographic conditions used, specially those regarding to the composition of the mobile phase are critical for achieving the required selectivity.

3.2. Capillary electrophoresis analysis

In this work we also report on the results obtained after the application of CE as an alternative for the analysis of ASP toxins and microcystins in algae and water samples.

3.2.1. For ASP toxins

The results obtained (Fig. 4) show that this technique can result in a promising alternative for such analysis, although improvements on its performance are still required in order to increase the sensitivity. In Fig. 4 it is shown an example of the CE to the analysis of ASP toxins present in naturally contaminated scallop hepatopancreas (Bertragh Bay, Ireland). A double anion- and cation-exchange SPE initially proposed by Zhao et al. [11], which had been also previously evaluated [4], was applied for the clean-up of ASP toxins prior to CE analysis, with the aim of making the ASP extracts compatible under CE conditions. The conditions used for this clean-up are also described in the Experimental section.

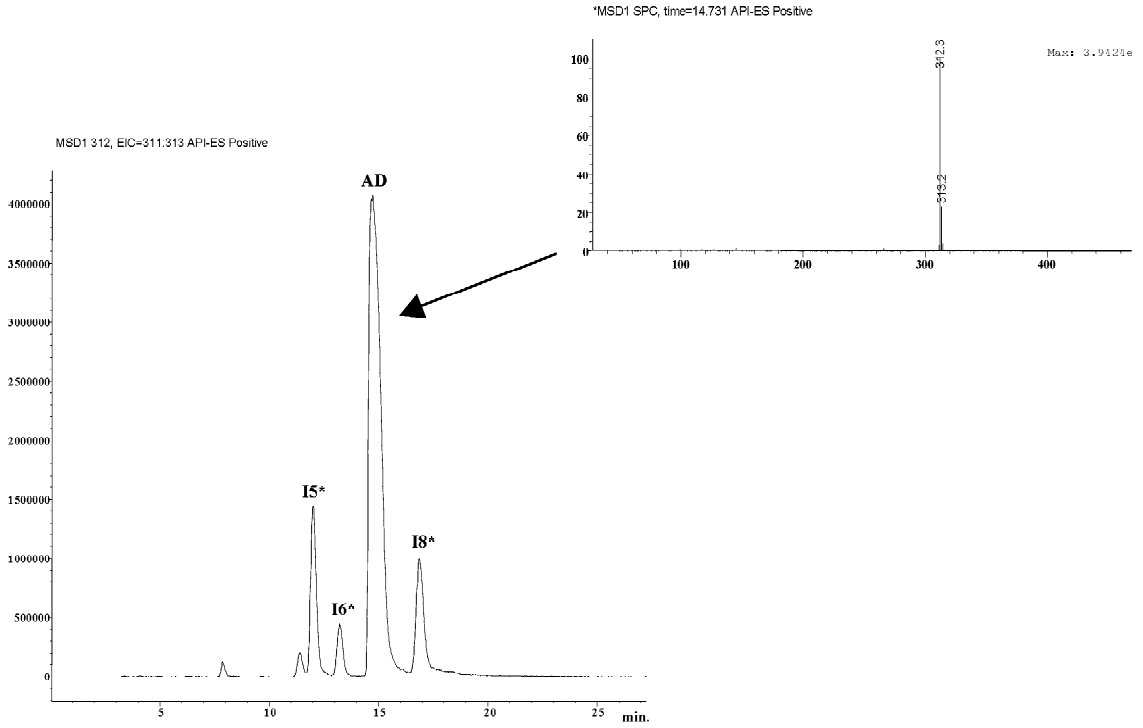

Fig. 2. Analysis of contaminated scallop hepatopancreas samples (Bertragh Bay, Ireland) by HPLC–ESI-MS in the positive ion and full scan modes.

The results obtained after using this conventional capillary zone electrophoresis mode are in accordance with the expected, specially in terms of lack of sensitivity. This low sensitivity did not allow one to identify isomers of DA present in both MUS-1 and naturally contaminated samples, which presence had been confirmed by HPLC–MS. Nevertheless the sensitivity of this technique was enough to determine the amount of DA present in the naturally contaminated samples under the regulatory levels. Improvements on the application of this technique are still in progress, specially with the aim of enhancing the sensitivity using different preconcentration approaches.

3.2.2. For microcystins

CE has been also applied to the analysis of microcystins present in water and algae samples. To overcome the problems associated with the low sensitivity, as well as with the aim of increasing the selectivity, both, sample pretreatment and analysis have been studied. For the sample pretreatment different approaches have been tested, including conventional SPE or IAC for clean-up purposes, which optimization was carried out using HPLC–UV under the conditions described elsewhere [14].

Fig. 5 shows an example of the results obtained after the application of IAC for the sample concentration and clean-up of microcystins. The potential of immunoaffinity for a selective removal of interferences is clearly shown in this figure. The sensitivity of CE has been clearly enhanced using both, IAC for sample pretreatment and FASS for the concentration of the sample, allowing an increase on the sensitivity, which have contributed to a clear determination of the MCs that are present in contaminated samples. The selectivity has also been increased by using the MEKC mode in the CE analysis.

The confirmation of the results previously ob-

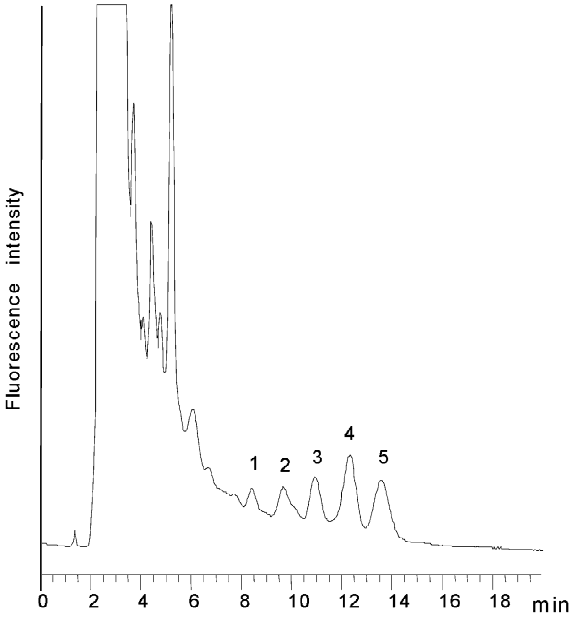

Fig. 3. Chromatogram from the HPLC–FL of an extract from a phytoplankton sample (*D. acuta*) after derivatisation with ''in situ'' ADAM. Peaks: 1=PTX2SA isomer; 2=PTX2SA; 3=OA; 4=7-epi-PTX2SA and 5=DTX-2.

tained by CE was successfully carried out by using CE–ESI-MS. The use of this technique was preceded of a complete optimization of the system [15], including CE conditions, the sheath liquid composition in the ESI interface and different MS parameters. Fig. 5 shows an example of the results obtained for the CE–ESI-MS analysis of the MCs present in extracts of blue–green algae after IAC clean-up. The electropherogram corresponds to the total ion current (TIC) in the positive ion mode, and toxins MC-LR and MC-RR were identified when m/z of 995 and 520 were selected, respectively for these two toxic compounds.

3.3. Capillary electrochromatography

A novel micro(μ)-separation technique recently developed has been also applied in this work to the analysis of ASP toxins. This technique includes some features typical of CE and HPLC. CEC provides high separations efficiency and selectivity, and short analysis times. Preliminary results of the application of CEC for these particular analytes have been reported by Leão Martin et al. [9], and further improvements on the conditions for such application are presented in this work. These improvements allowed an increased sensitivity of CEC, providing the adequate conditions for the analysis of ASP toxins in naturally contaminated samples. These

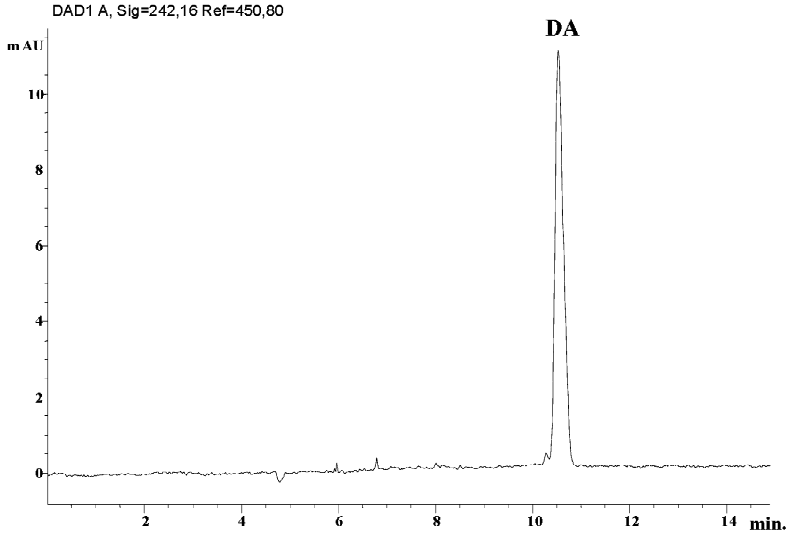

Fig. 4. CE–DAD analysis of naturally contaminated scallop hepatopancreas sample extracts after strong anion-exchange (SAX)/strong cation-exchange (SCX) cleanup.

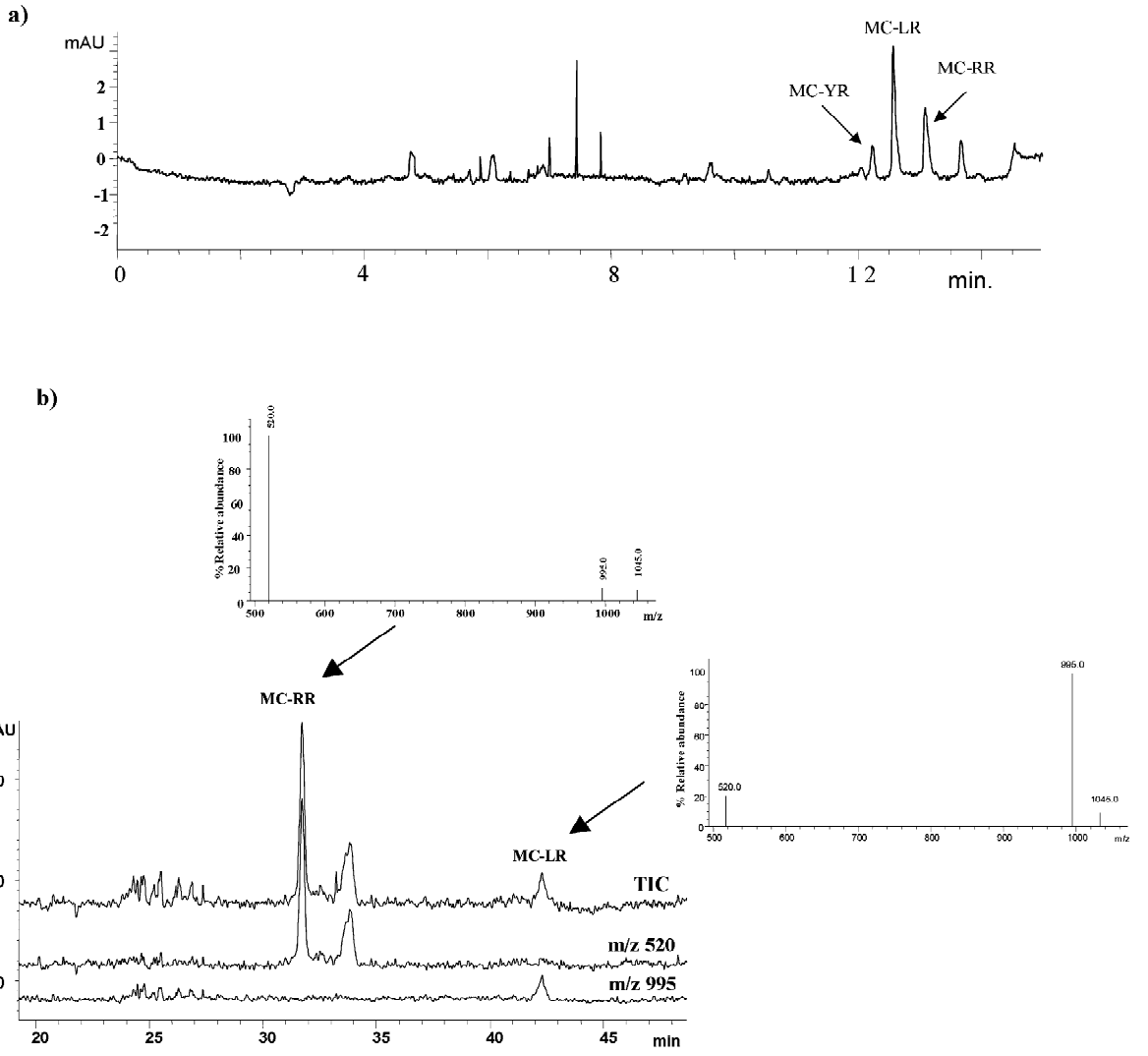

Fig. 5. Analysis of naturally contaminated blue–green algae after IAC cleanup by (a) CE–UV and (b) CE–ESI-MS total ion current chromatogram in the positive ion, full scan mode.

conditions are described in the Experimental section and an example of the results obtained is shown in Fig. 6. The main drawback associated with this technique is the lack of robustness and reproducibility which is mainly due to the bubbles formation into the capillary columns.

Further research is still required for the application of this technique on routine basis. This research should be focus in the search for new stationary phases more adequate to the analysis of these particular compounds.

4. Conclusions

HPLC can be still considered as the most powerful tool for the analysis of algal toxins. Improvements on the performance of this technique to be applied to an efficient monitoring of these toxic compounds at the very low levels at which they can cause damages in human health, has been accomplished by mean of improvements on both sample pretreatment and chromatographic conditions or by selecting an adequate detection mode.

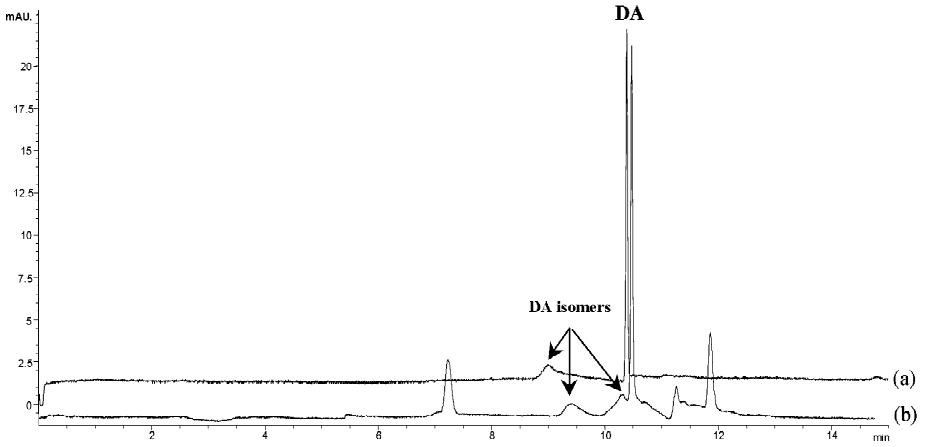

Fig. 6. CEC–DAD analysis of (a) domoic acid standard solution and (b) MUS-1. Mobile phase: 5 m*M* phosphate buffer, pH 2.5–MeCN (40:60). Conditions: 12 kV, 10 bar pressure, 25 °C. Detection: UV at 242 nm. Injection: 10 kV for 10 s.

CE is resulting in a powerful alternative for a fast and simple determination of the named compounds. The lack of sensitivity has been overcame by improving sample pretreatment protocols as well as the electrophoretic conditions, using sample stacking or alternative CE modes such as MEKC.

CEC is emerging as a promising alternative for such applications, allowing the determination of some of this compounds (ASP toxins) taking the main features of HPLC and CE. The performance of CEC can be still improved specially with the aim of enhancing the robustness of this analytical approach.

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References

[1] W.W. Carmichael, Sci. Am. 270 (1994) 78.
[2] M.A. Quilliam, J. AOAC Int. 79 (1996) 209.
[3] M.A. Quilliam, M. Xie, W.R. Hardstasff, J. AOAC Int. 78 (1995) 543.
[4] E. Vaquero, N. Piñeiro, J.M. Leão, A. Gago-Martínez, J.A. Rodríguez Vázquez, manuscript in preparation.
[5] K.P. Bateman, P. Thibault, D.J. Douglas, R.L. White, J. Chromatogr. A 712 (1995) 253.
[6] E.C. Aguete, A. Gago-Martínez, J.A. Rodríguez-Vázquez, S. O'Connell, C. Moroney, K.J. James, Chromatographia 53 (2001) 254.
[7] M.A. Quilliam, in: D. Barceló (Ed.), Applications of LC–MS in Environmental Chemistry, Journal of Chromatography Library, Vol. 59, Elsevier, Amsterdam, 1996, p. 415.
[8] N. Piñeiro, E. Vaquero, J.M. Leão, A. Gago-Martínez, J.A. Rodríguez Vázquez, Chromatographia 53 (2001) 231.
[9] J.M. Leão Martins, A. Gago-Martinez, E. Dabek-Zlotorzynska, R. Aranda-Rodriguez, J.F. Lawrence, J. Sep. Sci. 25 (2002) 342.
[10] J.F. Lawrence, C. Menàrd, J. Chromatogr. A 922 (2001) 111.
[11] J.Y. Zhao, P. Thibault, M.A. Quilliam, Electrophoresis 18 (2) (1997) 268.
[12] M.A. Quilliam, A. Gago-Martínez, J.A. Rodríguez-Váquez, J. Chromatogr. A 807 (1998) 229.
[13] N. Piñeiro, J.M. Leão, A. Gago-Martínez, J.A. Rodríguez Vázquez, J. Chromatogr. A 847 (1999) 223.
[14] E.C. Aguete, A. Gago-Martínez, J.M. Leão, J.A. Rodríguez-Vázquez, C. Menàrd, J.F. Lawrence, Talanta, in press.
[15] E.C. Aguete, A. Gago-Martínez, J.A. Rodríguez-Vázquez, manuscript in preparation.