JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY REVIEW

J. Agric. Food Chem. 2010, 58, 11525–11533 11525
DOI:10.1021/jf1031789

Analytical Approaches for an Important Shellfish Poisoning Agent: Domoic Acid

Yu He,[†,§,⊥,⊗] Agnes Fekete,[§] Guonan Chen,[†,⊥,⊗] Mourad Harir,[§] Lan Zhang,[†,⊥,⊗] Ping Tong,[†,⊥] and Philippe Schmitt-Kopplin*[,§,#]

[†]Key Laboratory of Analysis and Detection for Food Safety, Ministry of Education (Fuzhou University), Fuzhou, Fujian 350002, People's Republic of China, [§]Department of BioGeoChemistry and Analytics, Institute of Ecological Chemistry, Helmholtz Center Munich, German Research Center for Environmental Health, Ingolstädter Landstrasse 1, D-85758, Neuherberg, Germany, [⊥]Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety (Fuzhou University), Fuzhou, Fujian 350002, People's Republic of China, [⊗]Department of Chemistry, Qi Shan Campus, Fuzhou University, 2 Xue Yuan Road, University Town, Fuzhou, Fujian 350108, People's Republic of China, and [#]Center of Life and Food Science, Technische Universität München, Alte Akademie 8, D-85354 Freising, Germany

Domoic acid (DA), a neurotoxic amino acid produced by some strains of phytoplankton, is responsible for the human toxic syndrome amnesic shellfish poisoning. This exocitotoxin results in neuronal degeneration and necrosis in specific regions of the hippocampus. Because DA accumulates mostly in shellfish, causing outbreaks in different countries, screening for DA has been carried out with various assays. Although bioassays and immunoassays have been developed, several liquid chromatographic methods for the determination of DA in different matrices such as shellfish, algae, or seawater have been reported. Additionally, other alternative methods such as capillary electrophoresis and capillary electrochromatography have been described. This paper summaries the toxicology, the chemistry, and the developed determination methods of DA.

KEYWORDS: Domoic acid; shellfish poisoning; determination; sample preparation; HPLC; CE; CEC

INTRODUCTION

"Red tides", a vivid name to indicate the discoloration of seawater, result from the explosive growth (so-called "blooms") of some harmful microscopic planktonic algae (*1*, *2*). There are approximately 5000 marine algal species in the oceans, which are critical foods for shellfish, crustaceans, and finfish. Among these species, around 300 can sometimes contribute to red tides. During blooming, some species produce potent toxins, which are consumed and accumulated by ocean creatures. Besides the environmental impacts, human intoxication accidents associated with the contaminated shellfishes have occurred for some time now. In the past couple of decades, the frequency, intensity, and distribution of this threat have grown. Consequently, increasing attention has been drawn to the issues of "shellfish poisoning", including paralytic shellfish poisoning (PSP), diarrheic shellfish poisoning (DSP), neurologic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), azaspiracid shellfish poisoning (AZP) (*1*, *2*), and ciguatera fish poisoning (CFP) (*3*−*5*).

The main toxins responsible for these poisoning syndromes have some similar characteristics: water-solubility, heat and acid stability, and not inactivation by ordinary cooking methods. Moreover, the symptoms caused by these toxins also present some similarities (listed in **Table 1**).

*Author to whom correspondence should be addressed (fax +49-89-3187-3358; e-mail schmitt-kopplin@helmholtz-muenchen.de).

Domoic acid (DA), the principal causative toxin of ASP, is a natural product of certain species of marine diatom *Pseudo-nitzschia* (*6*). In 1958, DA was originally isolated from the red alga called "doumoi" or "hanayanagi" (*Chondria armata*) in Japan (*7*). Subsequently, DA isomers were identified (*8*, *9*). From the initial outbreak in Canada in 1987, DA has been found in algae or dinoflagellates in Japan, the eastern coasts of North and South America, the western coast of North America, and the Mediterranean region (*10*−*12*).

DA can bioaccumulate in marine organisms (*13*) such as shellfish, anchovies, and sardines that feed on the phytoplankton known to produce this toxin. DA can accumulate at high concentrations in the tissues of these plankton feeders when the toxic phytoplankton is high in concentration in the surrounding waters. Thus, marine animals, seabirds, or even human beings will exhibit acute intoxication after consumption of these contaminated foods.

The first DA outbreak occurred in 1987 in Prince Edward Island, Canada. At that time, ASP caused 3 deaths and 105 cases of acute human intoxication following the consumption of blue mussels (93% of patients suffered from gastrointestinal symptoms and 26%, neurological symptoms). After that, the Canadian authorities imposed an action limit for DA in mussels of 20 mg of DA/g of mussel flesh, which has been adopted elsewhere and is the limit enforced in the European Union, United States, New Zealand, and Australia for DA in a variety of shellfish species.

© 2010 American Chemical Society
Published on Web 10/22/2010

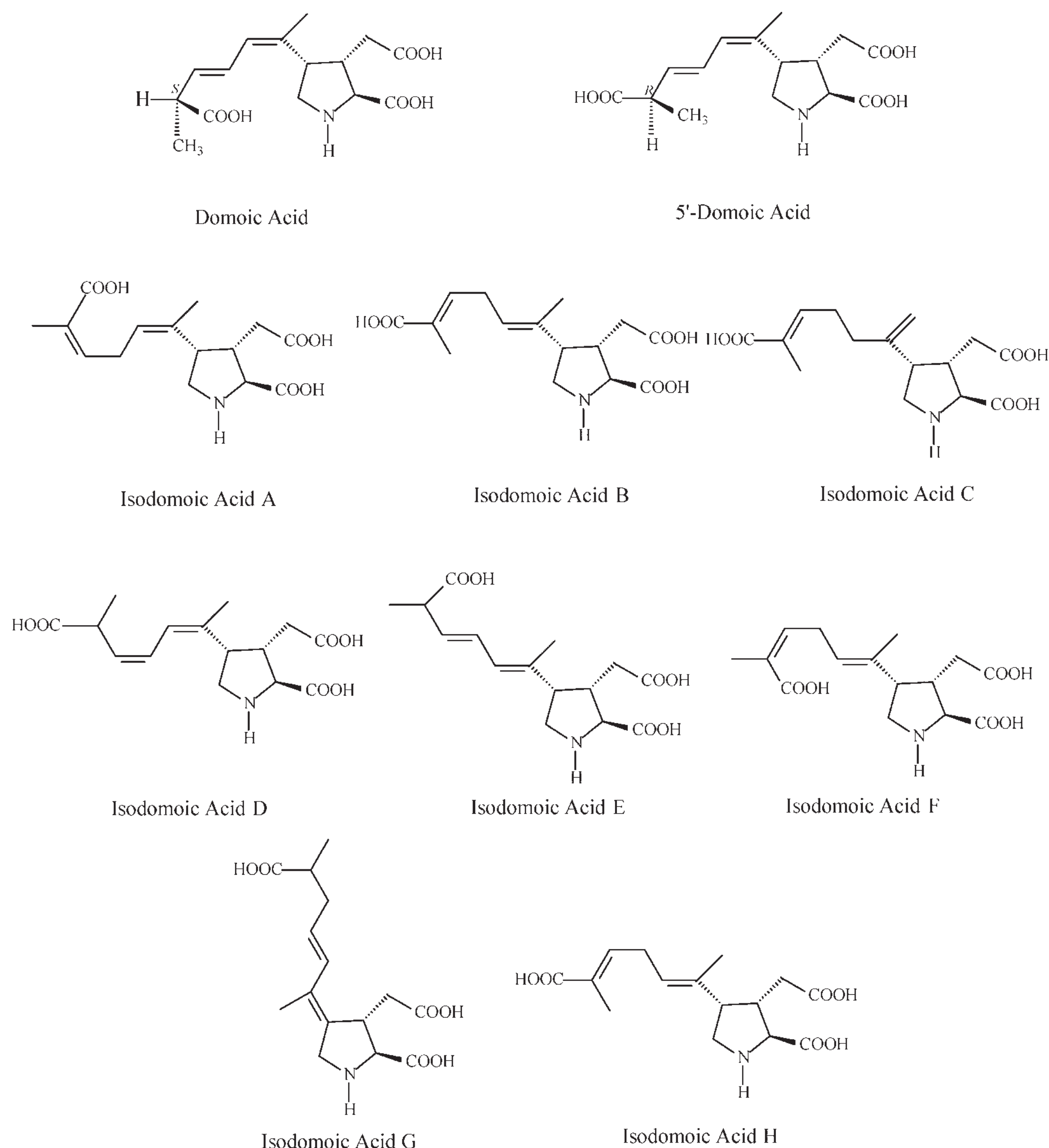

Figure 1. Chemical structures of domoic acid and its isomers.

Table 1. Toxins Responsible for and Symptoms of Six Shellfish Poisonings

shellfish poisoning	acronym	toxin	symptoms
paralytic shellfish poisoning	PSP	saxitoxin (STX)	purely neurological symptoms
diarrheic shellfish poisoning	DSP	okadaic acid (OA)	gastrointestinal symptoms
neurologic shellfish poisoning	NSP	brevetoxin (BTX)	gastrointestinal and neurological symptoms
amnesic shellfish poisoning	ASP	domoic acid (DA)	gastrointestinal and neurological disorders
azaspiracid shellfish poisoning	AZP	azaspiracid (AZA)	gastrointestinal intoxications
ciguatera fish poisoning	CFP	ciguatoxins (CTX)	gastrointestinal, neurological, and cardiovascular symptoms

DA belongs to the kainoid class of compounds, which is a class of excitatory neurotransmitters. It can damage the neurons by activating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors (*14*), causing an influx of calcium. In mammals, including humans, domoic acid acts as a neurotoxin, causing short-term memory loss, brain damage, and, in severe cases, death (*14*−*17*). After the ingestion of bivalve molluscs or possibly fish contaminated with DA, gastrointestinal symptoms appear, including nausea, abdominal cramps, vomiting, diarrhea, and anorexia. The neurological symptoms (headaches, dizziness, ataxia, loss of memory) may occur after a delay of a few hours or up to 3 days according to the outbreak observed in 1987.

DA is a crystalline water-soluble potent neurotoxic amino acid, which has at least nine geometrical isomers (see **Figure 1**) (*6*, *8*, *18*−*20*). Isodomoic acid A, B, and C and domoilactones, found in seaweed, have not been detected in extracts of plankton or shellfish tissue. However, isodomoic acids D, E, and F and the 5′-epi-domoic acid have been isolated from both plankton cells and shellfish tissue (*6*). Formation of these geometrical isomers can be achieved by brief exposure of dilute solutions of DA to UV light. In addition, heat can accelerate the conversion from DA to 5′-epi-domoic acid (*6*). Interestingly, pharmacological studies reported that these DA isomers are not as toxic as DA because they bind less strongly to the kainate receptor proteins than DA itself (*21*). However, 5′-epi-domoic acid and DA have the same or a similar toxin (*6*).

SAMPLE PREPARATION

An AOAC extraction procedure (*22*) can be applied to the extraction of DA from shellfish tissues. In this extraction method, homogenized shellfish tissues are mixed with water followed by ultrasonication, boiling, centrifuging. The supernatant was filtered and used for analysis. An aqueous methanol extraction for DA extract was developed (*23*): mussel material is blended with methanol/water (1:1, v/v) and centrifuged, and the supernatant is filtered for analysis. The latter is demonstrated to be better suited to trace analysis and combines well with a highly selective cleanup based on strong anion exchange (SAX) (*24*).

Solid-phase extraction (SPE) has become the most common method for cleanup of shellfish samples (*24−36*). According to cleanup experiments on DA and confirmation experiments on real-world samples, the cleanup of mussel and scallop tissues with a SAX cartridge can attain valid approaches for routine monitoring of DA in shellfish both for LC-UV and for LC-MS, avoiding false positives, and thus many methods have been based on SAX-SPE (*24*, *26*, *28−30*, *32*, *33*, *35*, *37*, *38*). The SAX-SPE procedure was modified by loading the SAX eluate onto a strong cation exchange (SCX) cartridge to achieve a higher degree of cleanup (*24*).

Nevertheless, Powell et al. (*36*) concluded that an additional step with a SAX-SPE cartridge did not significantly improve the recovery of DA from sand crab samples. Chan et al. (*27*) even used an amorphous titania sorbent, instead of an SPE cartridge, for the preconcentration of DA. This sol−gel titania material is able to adsorb DA from seawater, via the formation of an ester linkage between the carboxylic moieties of DA and the Ti−OH groups on the sorbent surface, at low pH and desorb it at high pH.

Some techniques such as EIA and ELISA sometimes may require little cleanup and have the advantages of being easy and fast (*39*, *40*). However, the cross-reactivity with similar toxins will result in false positives and limit the demonstration of toxicity.

METHODS OF ANALYSIS

Bioassays. The bioassays included mouse bioassay, receptor blinding bioassay, and hippocampal slice preparations. The AOAC mouse bioassay for PSP toxins can also be employed for the detection of the unique toxicity of DA at approximate concentrations of 40 mg/g (*41*), whereas the guideline value in mussels established in Canada, and subsequently adopted by most other countries that have set limits for ASP, is 20 mg of DA/g of mussel tissue. Therefore, the relative insensitivity of this assay precluded its use for regulatory purposes. A receptor binding assay for DA was developed by Van Dolah et al. (*42*). The limit of detection and selectivity of the assay (IC_{50} = 0.89 nM, 0.3 mg) were optimized to be suitable for the analysis of DA in seawater extracts from algae and for the analysis of DA in shellfish in years (*6*, *43*). Hippocampal slice preparation based on a rapid and reversible increase produced by DA in amplitude of the orthodromic population spike and a decrease in field EPSP was also reported as a viable tool for detecting DA (*44*, *45*).

Biochemical Assays. Enzyme-linked immunosorbent assay (ELISA) was effective in the determination of DA as a screening and quantitation method due to its simple format (*39*). It has been employed in the determination of DA in mammalian serum and urine and human body fluids (*40*, *46*). After commercialization in 1998 (*37*), ELISA was improved by Biosense, and the LOQ of the kit was reduced to 10 mg/kg of shellfish.

Chemical Assays. *Amino Acid Analysis*. It was pointed out that amino acid analysis could be applied to shellfish extracts after the necessary cleanup and concentration of the material (*6*). However, crude aqueous extracts of plankton can be analyzed directly. Detection of amino acids could be achieved at 440 nm absorbance measurement, whereas DA could be detected at 550 nm absorbance. Even though the limit of detection for DA of this approach was close to that of LC-UV methods, it was not effective enough, especially when samples contained high concentrations of free amino acids. This could be because the structure and properties of DA are similar to those of some amino acids. In addition, the analysis time was much longer.

Thin Layer Chromatography (*TLC*). Quilliam et al. employed TLC as a method for the determination DA (*25*). TLC was further developed to detect DA after a SAX-SPE cleanup procedure (*25*). In this study, glass TLC plates (10 × 20 cm with a 250 μm thick layer of silica gel) were used to perform separation, and a 3:1:1 butanol/acetic acid/water mixture was selected as the best solvent system. The conclusion was drawn that this method can be used as a routine screening method for shellfish tissues in those laboratories without a LC system. Meanwhile, it was indicated that TLC could be a chemical confirmation method for DA in positive samples tested by assay methods.

Liquid Chromatography (*LC*). At present, LC with UV detection is a usual instrumental method for the determination of DA in shellfish (*6*, *22*, *23*, *26*, *49*), and the methods are summarized in **Table 2**. The detection limit for DA with this method, depending on the sensitivity of the UV detector, is about 0.1−1 mg of DA/g of tissue, which was suitable for regulatory purposes. However, false positives were commonly encountered because of interferences from crude extracts (*26*). Particularly, tryptophan and its derivatives, commonly contained in shellfish and finfish tissues, may be eluted close to DA with some columns and chromatographic conditions used, thus making analysis results inaccurate. Thus, some alternative approaches such as fluorescence detection (FLD) (*27−29*) and chemiluminescence detection (CLD) (*48*) were developed. Analytical methods based on ion exchange chromatography (*49*) and reversed-phase chromatography were also coupled to UV (*47*, *50*, *51*).

Besides these methods, mass spectrometry (MS) has become a dominant detection for LC with its advantage in substance identification (*22*, *30−32*, *47*, *52*, *53*). Compared with the other methods, higher sensitivity and specificity were attained by MS. A method based on hydrophilic interaction liquid chromatography with mass spectrometry (HILIC-MS) was described to determine DA in shellfish (*33*). This method was set up either on an ion spray ion trap MS instrument operating in MS and MS/MS scanning acquisition modes or on a turbo ion spray triple-quadrupole MS system operating in selected ion monitoring (SIM) and multiple reaction monitoring (MRM) acquisition modes. The minimum detection levels for DA in tissue were well below the regulatory limit for DA in tissue. An improved reverse-phase high-performance liquid chromatography (RP-HPLC) method was reported for the determination of DA and analogues (*31*). The complicated SPE cleanup step could be avoided when the pH of the mobile phase was optimized to 2.5. This chromatographic condition was successfully applied to LC-MS^3 to determine DA and epi-DA in scallop tissues. Different ionization sources, namely, ESI, atmospheric pressure ionization (APCI), atmospheric pressure photoionization (APPI), and combined APCI/APPI, were compared to improve the analytical signal (*34*). The optimized method was employed to investigate DA levels in 46 shellfish along the Mediterranean coasts, obtaining results similar to those reported by other authors. An ultraperformance liquid chromatographic (UPLC)-MS/MS method for the determination of dissolved DA in seawater was described (*54*). UPLC was performed on C18 columns (4.6 × 50.0 mm, 1.8 μm particle size) and C18 precolumn (4.6 × 50.0 mm, 1.8 μm particle size) at 70 °C oven temperature

Table 2. HPLC Methods for DA Analysis

analyte	sample	detection	stationary phase	mobile phase	type	performance characteristics	ref
DA	728 shellfish samples from U.K. harvesting site	UV-DAD; MS	UV: C 18 Spherisorb (5 μm, 250 $\times$ 4.6 mm), with Lichrosorb C18 guard cartridge (10 mm), 40 °C MS: Zorbax Rx-C18 (5 μm, 2 $\times$ 150 mm), 20 °C	UV: 0.1% TFA in 10% aqueous ACN MS: (A) water; (B) ACN/water (95:5), both with 50 mM HCOOH and 2 mM NH_4COOH 85% A + 15% B	isocratic elution flow rate, (UV) 1.5 mL/min, (MS) 0.2 mL/min	linear range, 0.5−10.0 μg/mL; LOD, 0.03 μg/mL (UV), 0.07 μg/mL (MS); recovery, 84−93%	*26*
DA, OA, STX, anatoxin-A, nodularin, microcystins, DTX-1	real algae sample	ESI MS	Aqua C18 (5 μm, 25 $\times$ 4.6 mm)	(A) ACN; (B) aqueous 0.01 M TFA containing 0.01% HFBA	gradient elution flow rate, 0.7 mL/min	LOD, 0.5 ng; recovery, 96%	*47*
DA	real shellfish sample and phytoplankton	FLD	Luna C18 (5 μm, 250 $\times$ 4.6 mm), with a precolumn (5 μm, 30 $\times$ 4.6 mm) 35 °C	for NBD-F (DA-NBD), ACN/water (40:60) containing 0.1% TFA	for NBD-F: isocratic elution for the FMOC derivatization: gradient elution flow rate, 1.0 mL/min	linear range, 0.04−2 μg/mL; LOD, $\leq$1 ng/mL; recovery, $\geq$95%	*28*
DA, kainic acid	real mussel tissue	FLD	Nucleosil C18 (5 μm, 100 Å, 250 $\times$ 4.6 mm)	water/ACN (87:13) containing 0.1% TFA	isocratic elution flow rate, 0.7 mL/min	linear range, 50−1500 ppb; LOD, 25 ppb; recovery, 97.6−99.3%	*29*
DA	spiked blue mussels	CLD UV	Chromolich Performance RP-18e (100 $\times$ 4.6 mm)	5 mM phosphate buffer (pH 2.7) /ACN = 9:1 (v/v)	isocratic elution flow rate, 0.5 mL/min	linear range, 1−500 ng/mL; LOD, 8 pg/mL; recovery, 106.2 $\pm$ 2.1% (2 μg/g, n = 6)	*48*
DA, analogues, tryptophan	spiked shellfish sample	UV; MS^3	UV: Luna C18(2) (5 μm, 250 $\times$ 4.6 mm), 40 °C MS: Luna C18(2) (5 μm, 150 $\times$ 2.0 mm), 40 °C	UV: ACN/water/phosphoric acid/triethylamine (120:878:2:0.1 v/v) (pH 2.5) MS: ACN/water (11:89) containing 0.035% TFA (pH 2.5)	isocratic elution flow rate, (UV) 1.2 mL/min, (MS) 0.2 mL/min	linear range, 0.05−5.0 μg/mL (UV), 0.025−10 μg/mL (MS^3); LOD, 25 ng/mL (UV), 0.2 μg/g tissue (MS^3)	*31*

Table 2. Continued

analyte	sample	detection	stationary phase	mobile phase	type	performance characteristics	ref
DA	real scallop tissues	MS^3	Luna C18 (5 μm, 150 × 2.0 mm), 40 °C	ACN/water (5:95 to 40:60) containing 0.05% TFA	gradient elution flow rate, 0.2 mL/min	linear range, 0.05−10 μg/mL (MS), 0.025−10 μg/mL (MS^2), 0.025−10 μg/mL (MS^3); LOD, 0.02 μg/mL (MS), 0.014 μg/mL (MS^2), 0.008 μg DA/mL (MS^3); recovery, 92% (n = 5)	*32*
DA	spiked seawater and phytoplankton	MS/MS (MRM)	Luna C18 (5 μm,150 × 2.0 mm), 40 °C	(A) water; (B) ACN; in a binary system, containing 0.1% formic acid	gradient elution flow rate, 0.2 mL/min	linear range, 0.05−400 ng/mL; LOD, 30 pg/mL; recovery, >90%	*53*
DA	real shellfish samples	MS/MS; APCI; APPI; APCI/APPI	Luna C18 (5 μm, 150 × 2.1 mm), 40 °C	ACN/water (1:9, v/v) containing 0.035% TFA (pH 2.5)	isocratic elution flow rate, 0.2 mL/min	linear range, 0.05−5 μg/mL; LOD, 0.2 μg/mL; recovery, 81−95%	*34*
dissolved DA, DA isomers	spiked seawater	MS/MS	Zorbax Eclipse XDB C18 (1.8 μm, 4.6 × 50.0 mm), with a Zorbax Eclipse Plus C18 (1.8 μm, 4.6 × 50.0 mm), 70 °C	(A) 100% water + 0.1% HCOOH; (B) 100% ACN + 0.1% HCOOH	gradient elution flow rate, 0.75 mL/min	linear range, 0.1−10 ng/mL; LOD, 0.02 ng/mL; recovery, 92.1−110.6%	*54*
DA, kainic acid	real shellfish sample	UV	Lichrospher C18 (5 μm, 250 × 4.0 mm), with Lichrospher C18 guard column (5 μm, 40 × 4.0 mm)	(A) ACN (5−35%); (B) 0.05% TFA/water	gradient elution flow rate, 1.0 mL/min	linear range, 25−500 ng/g; LOD, <25 ng/g; recovery, 73.8−92.8%	*49*
DA, AZA1, GYM, OA, PTX2, YTX	real mussel sample	MS/MS, MRM	Luna C18 (2) (5 μm, 150 × 2 mm), 30 °C	(A) ACN/water (1:9, v/v); (B) ACN/water (9:1, v/v); (C) 33 mM ammonium hydroxide and 500 mM formic acid in water	gradient elution flow rate, 0.2 mL/min	LOD, 0.015 mg/kg; recovery, 79−125%	*51*
DA	real scallops and *Pseudonitzschia*	UV-DAD MS/MS	VYDAC 201TP54 (5 μm, 250 × 4.6 mm), with a VYDAC guard column 201 GK54T (5 μm, 10 × 4 mm), 40 °C	0.1% TFA in MeOH/water (1−95%)	gradient elution flow rate, 0.2 mL/min		*52*
DA, DSP	real mussel sample	UV ESI-MS	UV: Prodigy ODS (0.5 μm, 250 × 4.6 mm) EIS-MS: 250 × 2.1 mm column packed with 5 μm Vydac reversed phase C18	UV: 12% aqueous ACN with 0.2% formic acid (adjusted to pH 2−3) MS: 8% aqueous ACN with 0.05% formic acid	isocratic elution flow rate, (UV) 1.0 mL/min, (MS) 0.2 mL/min	linear range, 1.5−8 μg/mL; LOD, 0.47 μg/mL	*30*

Table 2. Continued

analyte	sample	detection	stationary phase	mobile phase	type	performance characteristics	ref
DA	real shellfish samples	MS	Toso Haas column (TSK-GEL Amide-80 material, 5 μm, 250 × 2 mm), room temperature	(A) water, (B) 95% ACN/water solution, both A and B containing 2 mM NH_4COOH and 3.6 mM HCOOH. for DA, 75% B; for DA and PSP, 75−45% B	isocratic elution (DA) gradient elution (DA and PSP) flow rate, 0.2 mL/min	linear range, 0.123−10 μg/mL; LOD, 63 ng/g (positive), 190 ng/g (negative)	*33*
DA	spiked seawater	FLD	Beckman C18 column (5 μm, 250 × 4.6 mm), with a C18 Beckman guard column (5 μm, 45 × 4.6 mm), 35 °C	ACN/water (38:62) containing 0.05% TFA	isocratic elution flow rate, 1.0 mL/min	linear range, 0−50 ng/mL; LOD, 120 pg/mL; recovery, 89%	*36*

and 750 μL/min flow rate. The complete resolution between DA and its isomers was achieved in <3 min. The LOD and LOQ obtained with the whole method have been 0.020 and 0.060 ng/mL, respectively, which allows the determination of DA dissolved in seawater at very low concentration levels.

Capillary Electrophoresis (*CE*). CE was initially demonstrated to be suitable for determining DA (**Table 3**) and its isomers by Quilliam et al. (*55*) and Nguyen et al. (*56*) as a rapid, high-resolution analysis method. Zhao et al. (*24*) have applied CE-UV for the analysis of DA and isomers with a method detection limit of 150 ng/g in tissues. The optimal analysis was performed in phosphate or borate buffers at a pH of approximately 9.0. With the addition of β-cyclodextrin to the borate buffer, the separation of DA and several of its isomers (isodomoic acids) was superior to that achieved with LC. This method was modified by combining CE with UV/diode array detection and applied to the identification of DA in some contaminated razor clams as well as in mussel tissue reference material (NRC CRM-ASP-MUS-b) (*57*). Another modified CE-UV method was also presented for the analysis of different toxins including DA produced by algae (*35*). Different methods (HPLC, CE, and CEC) were compared in the determination of these algal toxins in complex matrices, and the results indicated that CE offered as good a potential for the sensitive and selective determination of DA as LC (*30*). Moreover, a CE method coupled with online capillary isotachophoresis was developed to analyze shellfish samples and food supplements containing algae extract (*58*). The optimized cITP-CZE electrolyte system was 10 mM HCl + 20 mM β-alanine (BALA) + 0.05% hydroxyethylcellulose (leading electrolyte), 5 mM caproic acid (terminating electrolyte), and 20 mM caproic acid + 20 mM BALA+ 0.1% HPMC (background electrolyte). The cITP-CZE method contributed speed of analysis, ease of operation, high sensitivity, and low running cost for DA determination.

Capillary Electrochromatography (*CEC*). CEC, similar to CE, also can provide faster analysis with higher selectivity, making it an attractive and promising alternative to HPLC for DA monitoring (*30*). The determination of DA was achieved with the combination of CEC and photodiode array detection (PAD) as shown in **Table 4**. Analysis was performed with a reverse phase column (C18) and a mobile phase consisting of 5 mM phosphate buffer (pH 2.5)/acetonitrile (40:60) with an applied voltage of 7 kV (*59*). Wu et al. used pressurized capillary electrochromatography (pCEC) to quantify DA (*60*) from spiked shellfish matrix in 6 min.

SUMMARY

DA, as the main constituent of ASP, is widely distributed in the world's oceans. It has been a concern for public health as well as having serious economical consequences to recreational and commercial fisheries, since the major toxic incident in Canada occurred in 1987. To a great extent, the occurrence of DA is related with human activities. Sewage from industry and urban living increases continuously, increasing water pollution and water eutrophication, which cause red tides to breed rapidly or abundantly. Besides, the development of marine farming also results in serious environmental contamination. Raising the environmental awareness of the public is the best way to resolve the problem of ocean contamination. Nevertheless, in the present serious pollution situation, it is an urgent issue to establish a rapid, accurate monitoring technology for the determination and unequivocal confirmation of DA or its isomers in shellfish. This is of importance to reduce or eliminate ocean toxins in the shellfish culture environment, to inhibit toxin accumulation in shellfish,

Table 3. CE Methods for DA Analysis

analyte	sample	detection	BGE	capillary length	capillary dimension	injection	voltage	performance characteristics	ref
DA sample constituents	real shellfish sample and food supplement containing algae extract	UV	10 mM HCl + 20 mM alanine (BALA) + 0.05% hydroxyethylcellulose (leading electrolyte), 5 mM caproic acid (terminating electrolyte) and 20 mM caproic acid + 20 mM BALA + 0.1% HPMC (background electrolyte)	PTFE preseparation capillary, 90 mm; PTFE analytical capillary, 250 mm; effective length, 150 mm	PTFE preseparation capillary, 0.8 mm i.d.; PTFE analytical capillary, 0.3 mm i.d.	injected by a valve with fixed internal sample loop (30 μL)		linear range, 0−200 μg/L; LOD, 1.5 μg/L; recovery, 101 ± 3%	*58*
DA	real mussel sample	UV MS	UV: 25 mM borate buffer (pH 9.2) MS: 50 mM formic acid	UV: 66 cm MS: 80 cm	UV: 50 μm i.d., 363 μm o.d. MS: 50 μm I.D.	UV: 50 mbar × 12 s MS: 50 mbar ×3 s	UV: 30 kV MS: 20 kV		*30*
DA isomers	real shellfish sample	UV	22.5 mM sodium tetraborate (pH 9.2) with 20 mM β-cyclodextrin	60 cm (total length = 75 cm)	50 μm i.d., 363 μm o.d.		30 kV	mass LOD, 3 pg/mL; method LOD, 150 ng/mL; recovery, 91 ± 2% (n = 4)	*24*
PSP ASP YTX, microcystins, aquatic toxins	real shellfish sample	UV	25 mM borate buffer	51 cm (total length = 66 cm)	50 μm i.d., 363 μm o.d.	50 mbar × 12 s	30 kV	linear range, 1.5−8 μg/mL	*35*

Table 4. CEC Method for DA Analysis

analyte	sample	detection	mobile phase	capillary dimension	length	voltage	performance characteristics	ref
DA	real mussel sample	UV	5 mM phosphate buffer (pH 2.5)/MeCN, 40:60	100 μm i.d., 75 μm o.d. for ASP, C18 (3 μm)	for ASP: bed length of 25 cm for all toxins: bed length plus 8.5 cm of polyimide-coated fused silica tubing	12 kV		*30*
DA, other compound	spiked shellfish sample	PAD	5 mM phosphate buffer (pH 2.5) /ACN (40:60)	chromatographic columns: RP-C18 bonded silica, 3 μm capillary columns: 20 cm × 75 μm (i.d.)	20 cm (total length = 27 cm)	7 kV		*59*
DA	spiked shellfish sample	UV	5 mM Tris buffer (pH 8.0)/CAN (40:60) isocratic elution flow rate, 0.050 mL/min	100 μm i.d., 375 μm o.d. packed with 3 μm octadecyl silica particles	20 cm (total length = 55 cm)	−13 kV	linear range, 1.0−100.0 μg/mL; LOD, 0.5 μg/mL; recovery, 83−86%	*60*

and to minimize adverse impacts on human health. Thus, the developed methods are summarized here. Additionally, tables comparing the different methods are presented.

LITERATURE CITED

(1) Hallegraeff, G. M. Harmful algal blooms: a global overview. In *Manual on Harmful Marine Microalgae*; Hallegraeff, G. M., Anderson, D. M., Cembella, A. D., Eds.; UNESCO: Paris, France, 1995; pp 1−22.

(2) Lindahl, O. Occurrence and monitoring of harmful algae in the marine environment. In *Mycotoxins and Phycotoxins − Developments in Chemistry, Toxicology and Food Safety*; Miraglia, M., Van Egmond, H., Brera, C., Gilbert, J., Eds.; Proceedings of the IX International IUPAC Symposium on Mycotoxins and Phycotoxins: Fort Collins, CO, 1998; pp 409−423.

(3) Legrand, A. M. Ciguatera toxins: origin, transfer through the food chain and toxicity to humans. In *Harmful Algae, Proceedings of the VIII International Conference on Harmful Algae*; Reguera, B., Blanco, J., Fernandez, M., Wyatt, T., Eds.; UNESCO: Paris, France, 1997; pp 39−43.

(4) Lehane, L. Ciguatera update. *Med. J. Aust*. **2000**, *172* (4), 176−179.

(5) Lehane, L.; Lewis, R. J. (Review) Ciguatera: recent advances but the risk remains. *Int. J. Food Microbiol*. **2000**, *61*, 91−125.

(6) Wright, J. L. C.; Quilliam, M. A. Methods for domoic acid, the amnesic shellfish poisoning (ASP) toxin. In *Manual on Harmful Marine Microalgae*; Hallegraeff, G. M., Anderson, D. M., Cembella, A. D., Eds.; UNESCO: Paris, France, 1995; pp 115−135.

(7) Takemoto, T.; Daigo, K. Constitutents of *Chondria armata*. *Chem. Pharm. Bull*. **1958**, *6*, 578−580.

(8) Wright, J. L. C.; Falkl, M.; McInnes, A. G.; Walter, J. A. Identification of isodomoic acid D and two new geometrical isomers of domoic acid in toxic mussels. *Can. J. Chem*. **1990**, *68*, 22−25.

(9) Zaman, L.; Arakawa, O.; Shimosu, A.; Onoue, Y.; Nishio, S.; Shida, Y.; Noguch, T. Two new isomers of domoic acid from a red alga, *Chondria armata*. *Toxicon* **1997**, *35* (2), 205−212.

(10) Fritz, L.; Quilliam, M. A.; Wright, J. L. C.; Beale, A.; Work, T. M. An outbreak of domoic acid poisoning attributed to the pennate diatom *Pseudonitzschia australis*. *J. Phycol*. **1992**, *28*, 439−442.

(11) Trainer, V. L.; Hickey, B. M.; Horner, R. A. Biological and physical dynamics of domoic acid production off the Washington coast. *Limnol. Oceanogr*. **2002**, *47* (5), 1438−1446.

(12) Miguez, A.; Fernandez, M. L.; Fraga, S. First detection of domoic acid in Galicia (NW Spain). In *Harmful and Toxic Algal Blooms*; Yasumoto, T., Oshima, Y., Fukuro, Y., Eds.; UNESCO: Paris, France, 1996; pp 143−145.

(13) Amzil, Z.; Fresnel, J.; Le Gal, D.; Billard, C. Domoic acid accumulation in French shellfish in relation to toxic species of *Pseudo-nitzschia* multiseries and *P. pseudodelicatissima*. *Toxicon* **2001**, *39*, 1245−1251.

(14) Hampson, D. R.; Manolo, J. L. The activation of glutamatereceptors by kainic acid and domoic acid. *Nat. Toxins* **1998**, *6*, 153−158 (from ref 13).

(15) Hampson, D. R.; Huang, X. P.; Wells, J. W.; Walter, J. A.; Wright, J. L. C. Interaction of domoic acid and several derivatives with kainic acid and AMPA binding sites in rat brain. *Eur. J. Pharmacol*. **1992**, *218*, 1−8.

(16) Brown, J. A.; Nijjar, M. S. The release of glutamate and aspartate from rat brain synaptosomes in response to domoic acid (amnesic shellfish toxin) and kainic acid. *Mol. Cell. Biochem*. **1995**, *151*, 49−54.

(17) Xi, D.; Peng, Y. G.; Ramsdell, J. Domoic acid is a potentneurotoxin to neonatal rats. *Nat. Toxins* **1997**, *5*, 74−79.

(18) Quilliam, M. A. Chemical methods for domoic acid, the amnesic shellfish poisioning (ASP) toxin. In *Manual on Harmful Marine Microalgae, Monographs in Oceanographic Methodology*; Hallegraeff, G. M., Anderson, D. M., Cembella, A. D., Eds.; UNESCO: Paris, France, 2003; pp 247−266.

(19) Takemoto, T.; Daigo, K. Constituents of *Chondria armata*. *Chem. Pharm. Bull*. **1958**, *6*, 578−580.

(20) Walter, J. A.; Falk, M.; Wright, J. L. C. NMR study of the protonation state of domoic acid. *Can. J. Chem*. **1992**, *70*, 1156−1161.

(21) Munday, R.; Holland, P. T.; McNabb, P.; Selwood, A. I.; Rhodes, L. L. Comparative toxicity to mice of domoic acid and isodomoic acids A, B and C. *Toxicon* **2008**, *52*, 954−956.

(22) Quilliam, M. A.; Xie, M.; Hardstaff, W. R. A rapid extraction and cleanup procedure for the liquid chromatographic determination of domoic acid in unsalted seafood. *J. AOAC Int*. **1995**, *78*, 543−554.

(23) Quilliam, M. A.; Sim, P. G.; McCulloch, A. W.; McInnes, A. G. High performance liquid chromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton. *Int. J. Environ. Anal. Chem*. **1989**, *36*, 139−154.

(24) Zhao, J. Y.; Thibault, P.; Quilliarn, M. A. Analysis of domoic acid and isomers in seafood by capillary electrophoresis. *Electrophoresis* **1997**, *18*, 268−216.

(25) Quilliam, M. A.; Thomas, K.; Wright, J. L. C. Analysis of domoic acid in shellfish by thin-layer chromatography. *Nat. Toxins* **1998**, *6* (3/4), 147−152.

(26) Hess, P.; Gallacher, S.; Bates, L. A.; Brown, N.; Quilliam, M. A. Determination and confirmation of the amnesic shellfish poisoning toxin, domoic acid, in shellfish from Scotland by liquid chromatography and mass spectrometry. *J. AOAC Int*. **2001**, *84* (5), 1657−1667.

(27) Chan, I. O. M.; Tsang, V. W. H.; Chu, K. K.; Leung, S. K.; Lam, M. H. W.; Lau, T. C.; Lam, P. K. S.; Wu, R. S. S. Solid-phase extraction-fluorimetric high performance liquid chromatographic determination of domoic acid in natural seawater mediated by an amorphous titania sorbent. *Anal. Chim. Acta* **2007**, *583*, 111−117.

(28) James, K. J.; Gillmana, M.; Lehanea, M.; Gago-Martinez, A. New fluorimetric method of liquid chromatography for the determination

of the neurotoxin domoic acid in seafood and marine phytoplankton. *J. Chromatogr., A* **2000**, *871*, 1−6.
(29) Maroulis, M.; Monemvasios, I.; Vardaka, E.; Rigas, P. Determination of domoic acid in mussels by HPLC with post-column derivatization using 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) and fluorescence detection. *J. Chromatogr., B* **2008**, *876*, 245−251.
(30) Gago-Martínez, A.; Piñeiro, N.; Aguete, E. C.; Vaquero, E.; Nogueiras, M.; Leao, J. M.; Rodríguez-Vázquez, J. A.; Dabek-Zlotorzynska, E. 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. *J. Chromatogr., A* **2003**, *992*, 159−168.
(31) López-Rivera, A.; Suárez-Isla, B. A.; Eilers, P. P.; Beaudry, C. G.; Hall, S.; Fernández Amandi, M.; Furey, A.; James, K. J. Improved high-performance liquid chromatographic method for the determination of domoic acid and analogues in shellfish: effect of pH. *Anal. Bioanal. Chem.* **2005**, *381*, 1540−1545.
(32) Furey, A.; Lehane, M.; Gillman, M.; Fernandez-Puente, P.; James, K. J. Determination of domoic acid in shellfish by liquid chromatography with electrospray ionization and multiple tandem mass spectrometry. *J. Chromatogr., A* **2001**, *938*, 167−174.
(33) Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, G. S.; Tartaglione, L.; Quilliam, M. A.; Tubaro, A.; Poletti, R. Hydrophilic interaction liquid chromatography/mass spectrometry for determination of domoic acid in Adriatic shellfish. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2030−2038.
(34) Pardo, O.; Yusa, V.; Leon, N.; Pastor, A. Development of a pressurised liquid extraction and liquid chromatography with electrospray ionization-tandem mass spectrometry method for the determination of domoic acid in shellfish. *J. Chromatogr., A* **2007**, *1154*, 287−294.
(35) Gago-Martinez, A.; Piñeiro, N.; Aguete, E. C.; Vaquero, E.; Nogueiras, M.; Leão, J. M.; Rodriguez-Vázquez, J. A.; Dabek-Zlotorzynska, E. An application of capillary electrophoresis for the analysis of algal toxins from the aquatic environment. *Int. J. Environ. Anal. Chem.* **2003**, *83*, 443−456.
(36) Powell, C. L.; Ferdin, M. E.; Busman, M.; Kvitek, R. G.; Doucette, G. J. Development of a protocol for determination of domoic acid in the sand crab (*Emerita analoga*): a possible new indicator species. *Toxicon* **2002**, *40*, 485−492.
(37) Rhodes, L.; Scholin, C.; Garthwaite, I.; Haywood, A.; Thomas, A. Domoic acid producing *Pseudo-nitzschia* species educed by whole cell DNA probe-based and immunochemical assays. In *Harmful Algae*; Reguera, B., Blanco, J., Fernandez, M. L., Wyatt, T., Eds.; Xunta de Galicia and IOC of UNESCO: Vigo, Spain, 1998; pp 274−277.
(38) McMahon, T.; Silke, J.; Hess, P. Biotoxins in bivalve molluscs in Ireland: monitoring and management. In *Proceedings of the Second International Conference on Harmful Algae Management and Mitigation*; Zhu, M., Zou, Y., Cheong, L., Hall, S., Eds.; UNESCO: Qingdao, China, 2001; p 76.
(39) Yu, F. Y.; Liu, B. H.; Wu, T. S.; Chi, T. F.; Su, M. C. Development of a sensitive enzyme-linked immunosorbent assay for the determination of domoic acid in shellfish. *J. Agric. Food Chem.* **2004**, *52* (17), 5334−5339.
(40) Smith, D. S.; Kidds, D. D. A competitive enzyme-linked immunoassay for domoic acid determination in human body fluids. *Food Chem. Toxicol.* **1994**, *32* (12), 1147−1154.
(41) AOAC. Official Method 959.08, Paralytic shellfish poison, biological method. In *Methods of Analysis of the Association of Official Analytical Chemists*, 17th ed.; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, 2000; pp 59−61.
(42) Van Dolah, F. M.; Finley, E. L.; Haynes, B. L.; Doucette, G. J.; Moeller, P. D.; Ramsdell, J. S. Development of rapid and sensitive high throughput pharmacologic assays for marine phycotoxins. *Nat. Toxins* **1994**, *2* (4), 189−196.
(43) Van Dolah, F. M.; Leighfield, T. A.; Haynes, B. L.; Hampson, D. R.; Ramsdell, J. S. A microplate receptor assay for the amnesic shellfish poisoning toxin, domoic acid, utilizing a cloned glutamate receptor. *Anal. Biochem.* **1997**, *245*, 102−105.
(44) Kerr, D. S.; Briggs, D. M.; Saba, H. I. A neurophysiological method of rapid detection and analysis of marine algal toxins. *Toxicon* **1999**, *37*, 1803−1825.
(45) Saba, H. I.; Briggs, D. M.; Kerr, D. S. A neurophysiological method of marine biotoxin detection. *Soc. Neurosci. Abstr.* **1997**, *23* (1−2), 1473.
(46) Smith, D. S.; Kitts, D. D. Enzyme immunoassay for the determination of domoic acid in mussel extracts. *J. Agric. Food. Chem.* **1995**, *43*, 367−371.
(47) Dahlmann, J.; Budakowski, W. R.; Luckas, B. Liquid chromatography−electrospray ionisation−mass spectrometry based method for the simultaneous determination of algal and cyanobacterial toxins in phytoplankton from marine waters and lakes followed by tentative structural elucidation of microcystins. *J. Chromatogr., A* **2003**, *994*, 45−57.
(48) Kodamatani, H.; Saito, K.; Niina, N.; Yamazaki, S.; Muromatsu, A.; Sakurada, I. Sensitive determination of domoic acid using high-performance liquid chromatography with electrogenerated tris(2,2′-bipyridine) ruthenium(III) chemiluminescence detection. *Anal. Sci.* **2004**, *20*, 1065−1068.
(49) Jaime, E.; Hummert, C.; Hess, P.; Luckas, B. Determination of paralytic shellfish poisoning toxins by high-performance ion-exchange chromatography. *J. Chromatogr., A* **2001**, *929* (1−2), 43−49.
(50) Lawton, L. A.; Edwards, C.; Codd, G. A. Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. *Analyst* **1994**, *119* (7), 1525−1530.
(51) McNabb, P.; Selwood, A. I.; Holland, P. T. Multiresidue method for determination of algal toxins in shellfish: single-laboratory validation and interlaboratory study. *J. AOAC Int.* **2005**, *88*, 761−772.
(52) Campbell, D. A.; Kelly, M. S.; Busman, M.; Bolch, C. J.; Wiggins, E.; Moeller, P. D. R.; Morton, S. L.; Hess, P.; Shumway, S. E. Amnesic shellfish poisoning in the king scallop, *Pecten maximus*, from the west coast of Scotland. *J. Shellfish Res.* **2001**, *20* (1), 75−84.
(53) Wang, Z.; King, K. L.; Ramsdell, J. S.; DoucetteWang, G. J. Determination of domoic acid in seawater and phytoplankton by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2007**, *1163*, 169−176.
(54) De La Iglesia, P.; Giménez, G.; Diogène, J. Determination of dissolved domoic acid in seawater with reversed-phase extraction disks and rapid resolution liquid chromatography tandem mass spectrometry with head-column trapping. *J. Chromatogr., A* **2008**, *1215*, 116−124.
(55) Quilliam, M. A.; Ayer, S. W.; Pleasance, S.; Sim, P. G.; Thibault, P.; Marr, J. C. Recent developments in instrumental analytical methods for marine toxins. In *Seafood Science and Technology*; Bligh, E. G., Ed.; Fishing Book News: Oxford, U.K., 1992; Chapter 41, pp 376−386.
(56) Nguyen, A. L.; Luong, J. H. T.; Masson, C. Capillary electrophoresis for detection and quantitation of domoic acid in mussels. *Anal. Lett.* **1990**, *23*, 1621−1634.
(57) Piñeiro, N.; Leão, J. M.; Gago-Martínez, A.; Rodríguez Vázquez, J. A. Capillary electrophoresis with diode array detection as an alternative analytical method for paralytic and amnesic shellfish toxins. *J. Chromatogr., A* **1999**, *847*, 223−232.
(58) Kvasnička, F.; Ševčík, R.; Voldřich, M. Determination of domoic acid by on-line coupled capillary isotachophoresis with capillary zone electrophoresis. *J. Chromatogr., A* **2006**, *1113*, 255−258.
(59) Martins, J. M. L.; Gago-Martinez, A.; Dabek-Zlotorzynska, E.; Aranda-Rodriguez, R.; Lawrence, J. F. Preliminary results on the application of capillary electrochromatography to the analysis of domoic acid. *J. Sep. Sci.* **2002**, *25*, 342−344.
(60) Wu, W.; Wu, X.; Lin, X.; Xie, Z.; Giesy, J. P. Quantification of domoic acid in shellfish tissues by pressurized capillary electrochromatography. *J. Sep. Sci.* **2009**, *32*, 2117−2122.

Received for review June 16, 2010. Accepted October 5, 2010. We are grateful to the National Nature Sciences Foundation of China (20735002, 20877019, 40940026), the foundation from the Sino-German Center for Research Promotion, the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (708056), the Nature Sciences Funding of Fujian Province (2008J0228, 2007F5068), and the China Scholarship Council for financial support.