Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1080 (2005) 148–156
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Confirmation of okadaic acid, dinophysistoxin-1 and dinophysistoxin-2 in shellfish as their anthrylmethyl derivatives using UV radiation

Dorothea F.K. Rawn [a,*], Cathie Ménard [a], Barbara Niedzwiadek [a], David Lewis [a], Benjamin P.-Y. Lau [a], Nathalie Delauney-Bertoncini [b], Marie Claire Hennion [b], James F. Lawrence [a,1]

[a] *Food Research Division, Bureau of Chemical Safety, Health Products and Food Branch, Health Canada, Sir Frederick Banting Research Centre, Address Locator: 2203D, Tunney's Pasture, Ottawa, Ont., Canada K1A 0L2*
[b] *Laboratoire Environnement et Chimie Analytique, Ecole Supérieure de Physique et de Chimie Industrielles, 10 rue Vauquelin, 75231 Paris cedex 05, France*

Received 10 September 2004; received in revised form 9 May 2005; accepted 23 May 2005

Abstract

A rapid and simple method for confirmation of the diarrhetic shellfish poisons (DSP): okadaic acid (OA), dinophysistoxin-1 (DTX-1) and dinophysistoxin-2 (DTX-2) using fluorescence detection following derivatization with 9-chloromethylanthracene, has been established as an alternate to LC/MS. Exposure of the anthrylmethyl derivatives of OA, DTX-1 and DTX-2 to near UV light (300–400 nm) resulted in the loss of these compounds to below detection limits within 30 min, with a concurrent appearance of two additional compounds. Based on the mass spectral evidence, we propose that these newly formed compounds are the decarboxylation products of the derivatized diarrhetic shellfish poisons. UV radiation is, therefore, proposed as a rapid and simple confirmation technique for these DSP in mussel samples.

Keywords: Okadaic acid; DTX-1; DTX-2; Confirmation; Fluorescence; 9-Chloromethylanthracene; UV

1. Introduction

Marine dinoflagellates (*Dinophysis* sp. and *Prorocentrum* sp.) are an important food for filter feeding bivalves (e.g., mussels, clams, etc.) [1]. These phytoplankton are known to produce the diarrhetic shellfish poisons (DSP); okadaic acid (OA) and its analogues, dinophysistoxin-1 (DTX-1) and dinophysistoxin-2 (DTX-2), which are lipophilic polyether compounds (Fig. 1A) associated with severe gastrointestinal disturbances in humans, upon ingestion of contaminated shellfish [2,3]. In addition, OA, DTX-1 and DTX-2 inhibit type 1 and type 2A protein phosphatases [4,5] and are powerful tumour promoting substances [6]. Diarrhetic shellfish poisoning has been occurring with greater frequency worldwide [7] and has been reported in Canada since the early 1990s [8–10].

Measurement of these compounds using classical analytical techniques without the advantage of LC/MS capabilities requires derivatization of samples. Numerous derivatization reagents have been tested and reported in the literature, although, 9-anthryldiazomethane (ADAM) [4,11] has been reported most widely due to its specificity and sensitivity. The ADAM reagent, however, is known to be expensive and unstable at temperatures above −70 °C. Lawrence et al. [12] reported the successful use of 9-chloromethylanthracene (CA) to derivatize OA and its analogues. The resulting product (Fig. 1B) is the same as that obtained when the ADAM reagent is used. The advantage of the CA reagent is that it is commercially available at a reasonable cost and stable if refrigerated [12]. Although known to be successful in the analysis of shellfish, this reagent is reported to be unsuccessful in the derivatization of OA and its analogues

* Corresponding author. Tel.: +1 613 941 8462; Fax: +1 613 941 4775.
E-mail address: thea_rawn@hc-sc.gc.ca (D.F.K. Rawn).
[1] James F. Lawrence has recently retired.

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.05.035

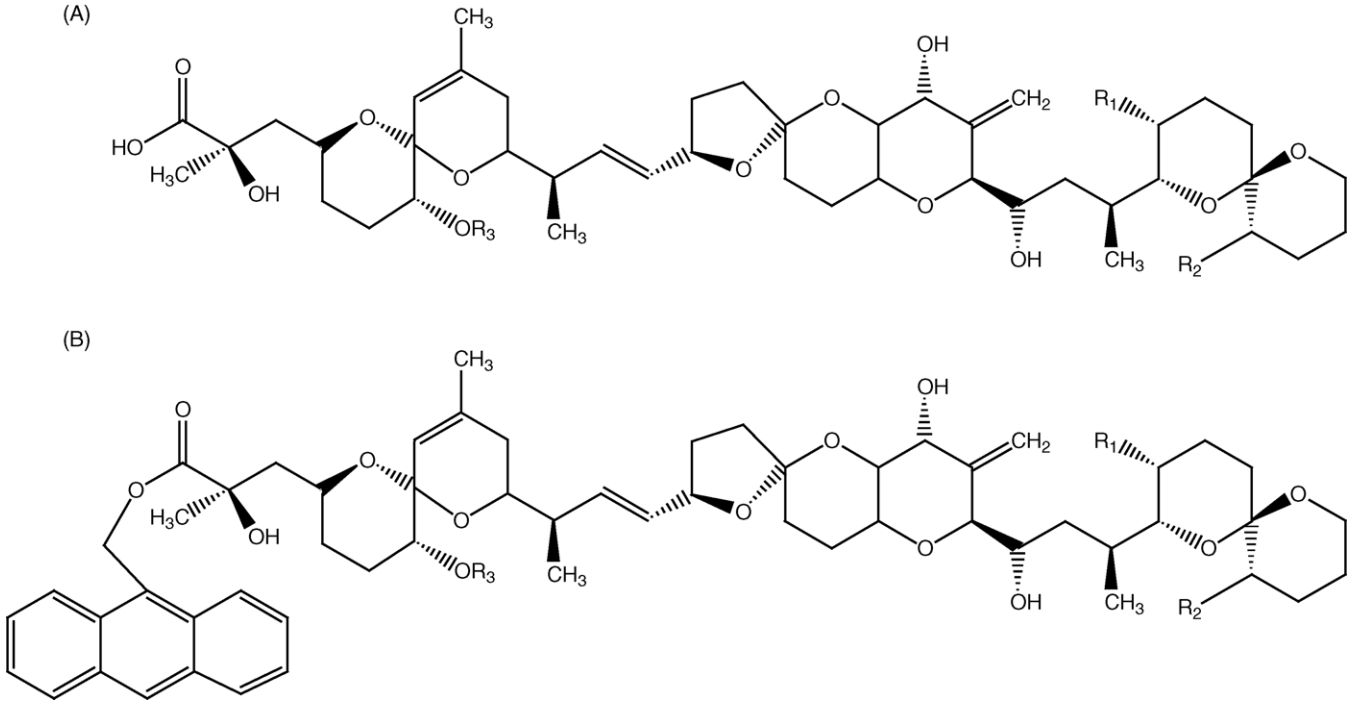

Fig. 1. (A) Structures of okadaic acid (OA) and its analogues. OA: $R_1 = CH_3$, $R_2 = H$, $R_3 = H$, dinophysistoxin-1 (DTX-1): $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = H$, dinophysistoxin-2 (DTX-2): $R_1 = H$, $R_2 = CH_3$, $R_3 = H$. (B) Anthrylmethyl derivatives of OA and its analogues.

in phytoplankton samples, possibly due to interaction between the derivatization reagent and sample matrix [11].

Recently, studies to determine toxin photodegradation (e.g., domoic acid and microcystins) have been performed in water samples with variable levels of humic substances and iron [13–15]. The literature reports of this work indicate that toxins are subject to degradation upon exposure to UV light, however, the use of UV light as a confirmatory tool for toxins in mussels has not, to our knowledge, been previously reported in the literature.

DSP toxin confirmation is routinely performed using LC/MS. This instrumentation, however, is not available in all laboratories and, therefore, alternate confirmatory techniques are required. In the present study, a rapid and simple method for confirmation of OA, DTX-1 and DTX-2 was developed using derivatization with CA and exposure to near UV light. The results obtained using LC with fluorescence detection were confirmed using LC/MS.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a quaternary pump, a vacuum degasser and a multi-wavelength fluorescence detector equipped with an autosampler (Agilent Series 1100, Mississauga, Ontario). The analytical column used was a Symmetry C_{18} column, 5 μm, 150 mm × 3.9 mm (Waters, Milford, MA). An HPLC (Agilent Series 1100) equipped with a binary pump, vacuum degasser, autosampler and a UV detector, coupled to a Quattro II tandem mass spectrometer (Micromass, Manchester, UK) through a Z-spray ESI interface and MassLynx software was used in the confirmation of all compounds. The analytical column used for confirmation was a Jones C_8 column, 3 μm, 150 mm × 2 mm (Hengoed, UK). A Polytron® homogenizer, Mistral 2000 centrifuge, rotary evaporator (Brinkman Büchi Rotavapor-R) and Pierce Reactivial were used in the sample preparation. An ultra-violet lamp (Model UVL.56 black ray long wavelength ultra violet lamp, Ultra-Violet Products Inc., Upland, CA) was used for irradiation of samples.

2.2. Chemicals

Tetramethylammonium hydroxide (TMAH) as an acetonitrile (ACN) solution (25%, w/v) and 9-chloromethylanthracene (CA) were purchased from Sigma–Aldrich (Oakville, ON). CA was refrigerated when not in use. All solvents used in this study, methanol (MeOH), dichloromethane (DCM), hexane and ACN, were either HPLC grade or distilled in glass grade (Omnisolve, EM Science, Gibbstown, NJ). Reagent grade anhydrous sodium sulfate was purchased from Mallinckrodt Baker (Phillipsburg, NJ). Purified water prepared with a Milli-Q water purification system (Millipore, Bedford, MA) was used throughout the study.

2.3. Diarrhetic shellfish toxins and standard materials

The okadaic acid (OA) standard used in the study was purchased from the Institute for Marine BioSciences, National Research Council of Canada, Halifax, Nova Scotia, Canada and received as a 25.3 μg/ml solution in ACN. The solution was diluted with MeOH to 5.06 μg/ml for use in the present study. Dilute solutions of DTX-1 (2 μg/ml) and DTX-2 (5 μg/ml) were received as generous gifts from Dr. M.A. Quilliam (Institute for Marine BioSciences, National Research Council of Canada, Halifax). An anthrylmethyl-OA standard was purchased from Sigma–Aldrich (Oakville, ON) as a solution in ACN, for comparison purposes. The mussel hepatopancreas certified reference material (MUS-2) had been purchased for previous studies from the Institute for Marine Biosciences, Halifax, Canada and prepared for use following the recommendations of the National Research Council. In brief, the mussel was quantitatively transferred to a 50 ml plastic centrifuge tube and diluted to 2 ml using MeOH. Following the original dilution, 4 ml of MeOH:water (80:20) were added to the centrifuge tube and mixed for 3 min using a Polytron®, followed by centrifugation at 4000 rpm for 10 min. The supernatant was collected in a 25 ml volumetric flask and the residue was rinsed with a further 8 ml aqueous MeOH, which had been used to rinse the Polytron® probe and centrifuged. This supernatant was added to the volumetric flask. The Polytron® probe was rinsed with a further 6 ml MeOH:water (80:20) and the solvent transferred to a second centrifuge tube, centrifuged and the supernatant added to the 25 ml volumetric flask. The solution was taken to volume using methanol:water (80:20). All standard solutions were refrigerated when not in use.

2.4. Preparation of shellfish samples

Mussel samples used as the blanks were purchased from the retail market in Ottawa, but were cultured in Prince Edward Island, Canada. Samples were frozen at −20 °C upon receipt until processed. The digestive glands (hepatopancreas) of individual mussels were removed, combined and homogenized using a Polytron®. Extraction was performed following the method described by Lawrence et al. [12]. Subsamples (1 g) were extracted by homogenizing with 6 ml MeOH:water (80:20) for 2 min, followed by centrifugation for 10 min at 2500 rpm. The supernatant was collected and an additional 2 ml MeOH:water (80:20) was added to the residue and centrifuged for a further 10 min at 2500 rpm. The combined supernatant was transferred to a 60 ml separatory funnel and shaken with 3 × 15 ml DCM:hexane (15:85). The organic phase was discarded and 5 ml water was added to the separatory funnel and shaken, followed by further extraction with 3 × 15 ml DCM:hexane (1:1). The DCM layer was removed and dried with anhydrous Na_2SO_4 into a round bottom flask and evaporated to dryness using a rotary evaporator at 40 °C. The remaining residue was taken up in 1 ml MeOH.

2.5. Derivatization

OA, DTX-1 and DTX-2 were derivatized using the method of Lawrence et al. [12]. Standard solutions were taken to dryness in a 4 ml Reactivial at 60 °C using a gentle stream of nitrogen. Once dry, 150 μl 0.4 mM TMAH was added to the remaining residue, followed by heating in a closed container at 60 °C for 2 min and taken to dryness. An 100 μl aliquot of 0.8 mM CA solution was added to the vial and the cap was replaced. This mixture was heated to 90 °C for 1 h under dark conditions, followed by cooling in a refrigerator. Prior to cleanup, 2 ml DCM:hexane (40:60) was added to the vial and mixed.

2.6. Silica gel cleanup

Prior to use, 500 mg silica gel SPE cartridges (Supelco, Oakville, ON) were conditioned with 6 ml DCM, followed by 6 ml DCM:hexane (40:60) and the effluent was discarded. Each solvent was drained to the top of the column bed prior to addition of the next solvent. The derivatized sample was then added to the top of the column and the cartridge was rinsed with 6 ml DCM:hexane (50:50) and 7 ml MeOH:DCM (1:99). Prior to the addition of the DCM:hexane to the silica gel, 4 ml were added to the sample vial as a rinse. The effluents were discarded and 7 ml MeOH:DCM (5:95) were used to elute the OA, DTX-1 and DTX-2 from the cartridge. The eluate was collected in a 50 ml round bottom flask and taken to dryness using a rotary evaporator at 35 °C. The residue was taken up in 1 ml ACN.

2.7. UV radiation of samples

A 100–200 μl aliquot of the clean, derivatized toxin sample was transferred to a clear autosampler vial, prior to exposure to UV light. Samples were exposed to UV light ranging from 320 to 400 nm, with peak emission at 365 nm. The UV light was placed directly above the autosampler vial for periods of 5, 10, 15, 20 and 30 min, prior to capping the vial. Once capped, the vials were shaken and 25 μl were taken for injection on the LC.

2.8. Liquid chromatographic analysis

The solvent flow rate was 1.2 ml/min through each run. The solvent mobile phase was 74% ACN and 26% Milli-Q water for 10 min which was taken to 78% ACN, 22% Milli-Q water using a linear gradient by 12 min. Over the next 2 min, the solvent was taken to 98% ACN, 2% Milli-Q water following a linear gradient, where it remained until 16 min. The solvent system was taken back to the starting solution (74% ACN; 26% Milli-Q water) following a linear gradient by 18 min. The multi-wavelength fluorescence detector was set to an excitation wavelength of 365 nm and an emission wavelength of 412 nm. The gain was set at 10.

2.9. LC/MS confirmation

For the MS1 scans, isocratic chromatography was carried out using 80% ACN, 20% Milli-Q water containing 0.04% formic acid, with a flow rate of 0.150 ml/min. The UV detector was set at 254 nm. The mass spectrometer system was equipped with an electrospray source which was operating in positive ion detection mode. The MS system was tuned using an OA standard solution (2.5 ng/μl) with monitoring of the $[M + H]^+$ ion at 995.4. The capillary voltage was 3.0 kV and the cone voltage was set at 25 V. The source was set to 120 °C and the desolvation temperature was set to 350 °C. Nitrogen was used as the drying gas (350 l/h) and for nebulizing (20 l/h). The collision gas was argon, with a pressure of 8.8×10^{-4} mbar. For daughter scans, the mobile phase consisted of: ACN:0.08% formic acid in water (80:20), while other conditions remained the same. The scanning time was 2.1 s over the mass range of *m*/*z* 600 to *m*/*z* 1200. Resolution was taken at 10% of the valleys between two adjacent masses.

3. Results and discussion

During previous studies, it had been noted that mussel extracts allowed to sit exposed to sunlight for a period of approximately 30 min between extraction and analysis, were found to have reduced OA levels relative to samples processed completely without delay. This led to a full investigation of the effect of UV exposure on OA which is described in the present study.

OA standards were derivatized using the CA reagent to form the anthrylmethyl derivative and cleaned up using SPE cartridges. Samples were then exposed to UV light for periods of 0, 5, 10, 15, 20 and 30 min and taken for HPLC analysis using fluorescence detection. Chromatograms of the samples analyzed without UV exposure were compared to those obtained using samples exposed to the incremental amounts of UV radiation. An inverse relationship between the anthrylmethyl-OA peak area and UV exposure time was observed, with complete disappearance of the anthrylmethyl-OA peak within 30 min UV exposure (Fig. 2).

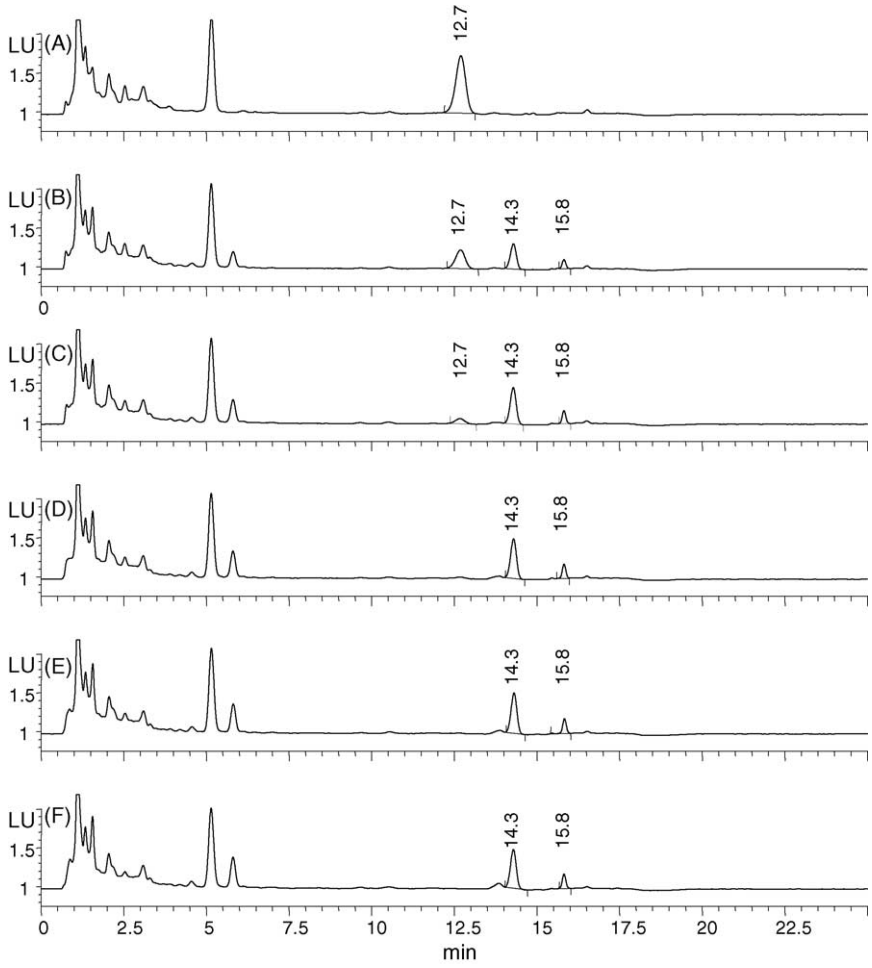

Fig. 2. Chromatograms of the anthrylmethyl derivative of okadaic acid (OA) after incremental exposure to UV radiation. (A) 0 min, (B) 5 min, (C) 10 min, (D) 15 min, (E) 20 min, (F) 30 min obtained using fluorescence detection. Peaks at retention times ~12.7, 14.3 and 15.8 min represent OA, transformation products 1 and 2, respectively.

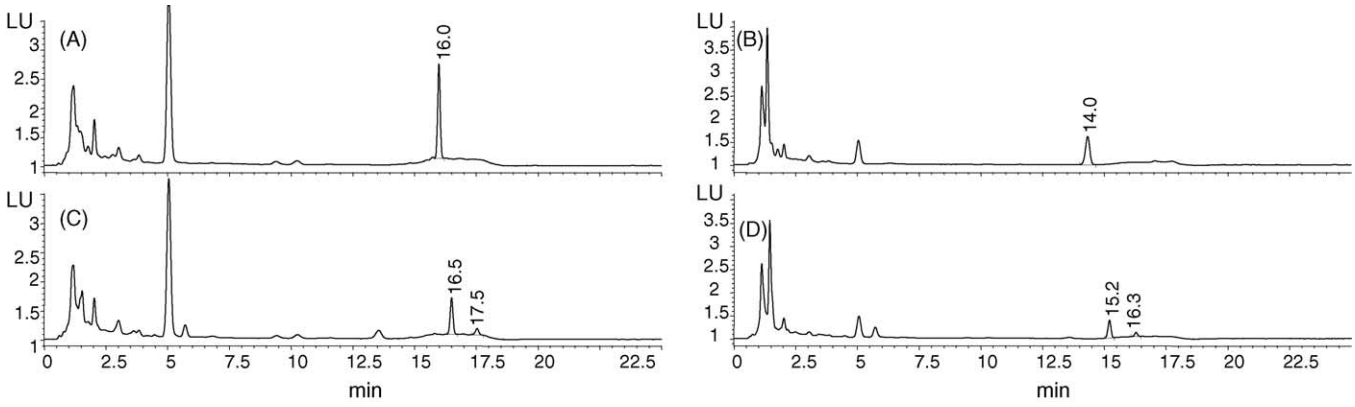

Fig. 3. Chromatograms of OA analogues obtained using fluorescence detection before [(A) DTX-1 and (B) DTX-2] and after 30 min UV exposure [(C) DTX-1 transformation products and (D) DTX-2 transformation products].

Corresponding to the reduction and loss of the anthrylmethyl-OA peak upon UV exposure, was the appearance and increase of two peaks which eluted approximately 1.5 and 3.5 min later than the original peak (Fig. 2). The longer retention times of these products suggests that a transformation from the anthrylmethyl-OA to less polar compounds occurred as a result of UV exposure. Beyond 30 min UV exposure, the response of the two product peaks remained constant, confirming that the transformation from anthrylmethyl-OA to the products was complete.

As anticipated, UV exposure to an anthrylmethyl-OA analytical standard also resulted in the loss of the parent compound, with the concurrent production of two less polar products. By using the analytical standard for comparison, there was no need for the derivatization and clean up steps prior to analysis. Although this was useful in the analysis with fluorescence detection, the sodium adduct overwhelmed the response of the molecular ion during mass spectral confirmations.

Following study of OA the effect of UV exposure to both DTX-1 and DTX-2 was similarly tested. As observed with OA, the anthrylmethyl derivatives of both DTX-1 and DTX-2 decreased with increased UV exposure time and two later eluting peaks appeared approximately 0.5 and 1.5 min, and 1.0 and 2.0 min later than the DTX-1 and DTX-2 derivatives, respectively. Complete loss of the anthrylmethyl analogues of DTX-1 and DTX-2 also was observed upon 30 min UV exposure, similar to OA (Fig. 3).

In addition to the analysis of standards, a certified reference material (CRM) of mussel tissue (MUS-2) known to contain both OA and DTX-1, was extracted, derivatized, cleaned up and exposed to UV light to confirm that similar results would be obtained in mussel samples. Again, a loss in the anthrylmethyl analogues of OA and DTX-1 was observed, with the corresponding appearance of two peaks eluting later in the chromatogram (Table 1). This study was repeated using the CRM extract diluted with blank mussel extract to simulate lower concentrations of OA in tissue, to confirm that the results would be consistent over a variety of concentrations. OA and DTX-1 concentrations in MUS-2, the 1:5 and 1:10 dilutions were calculated using the relative response to analytical standards (Table 1). Concentrations of the two dilutions of the MUS-2 sample confirmed that the response to the anthrylmethyl derivatives of OA and DTX-1 is linear.

The transformation products resulting from UV exposure also were used to determine concentration levels of OA and DTX-1 in the mussel extract and its dilutions. Concentration levels in the UV treated extract were determined using a standard curve established using UV exposed anthrylmethyl standards and found to be very similar to those obtained prior to UV exposure (Table 1). This indicates that UV exposure is an acceptable method for both qualitative and quantitative

Table 1
Concentrations (ppm) of OA and DTX-1 in undiluted and diluted mussel CRM (MUS-2) (certified concentrations 11 and 1 ppm, OA and DTX-1, respectively) before and after UV exposure for 30 min based on fluorescence detection

Compound	UV exposure	
	0 min	30 min
MUS-2 extract		
OA	9.80	–
OA transformation product 1	–	8.86
OA transformation product 2	–	10.20
DTX-1	0.46	–
DTX-1 transformation product 1	–	0.45
DTX-1 transformation product 2	–	0.73
5 × diluted mussel extract		
OA	1.84	–
OA transformation product 1	–	1.79
OA transformation product 2	–	1.95
DTX-1	0.09	–
DTX-1 transformation product 1	–	0.09
DTX-1 transformation product 2	–	0.09
10 × diluted mussel extract		
OA	0.87	–
OA transformation product 1	–	0.98
OA transformation product 2	–	1.01
DTX-1	0.04	–
DTX-1 transformation product 1	–	0.04
DTX-1 transformation product 2	–	0.04

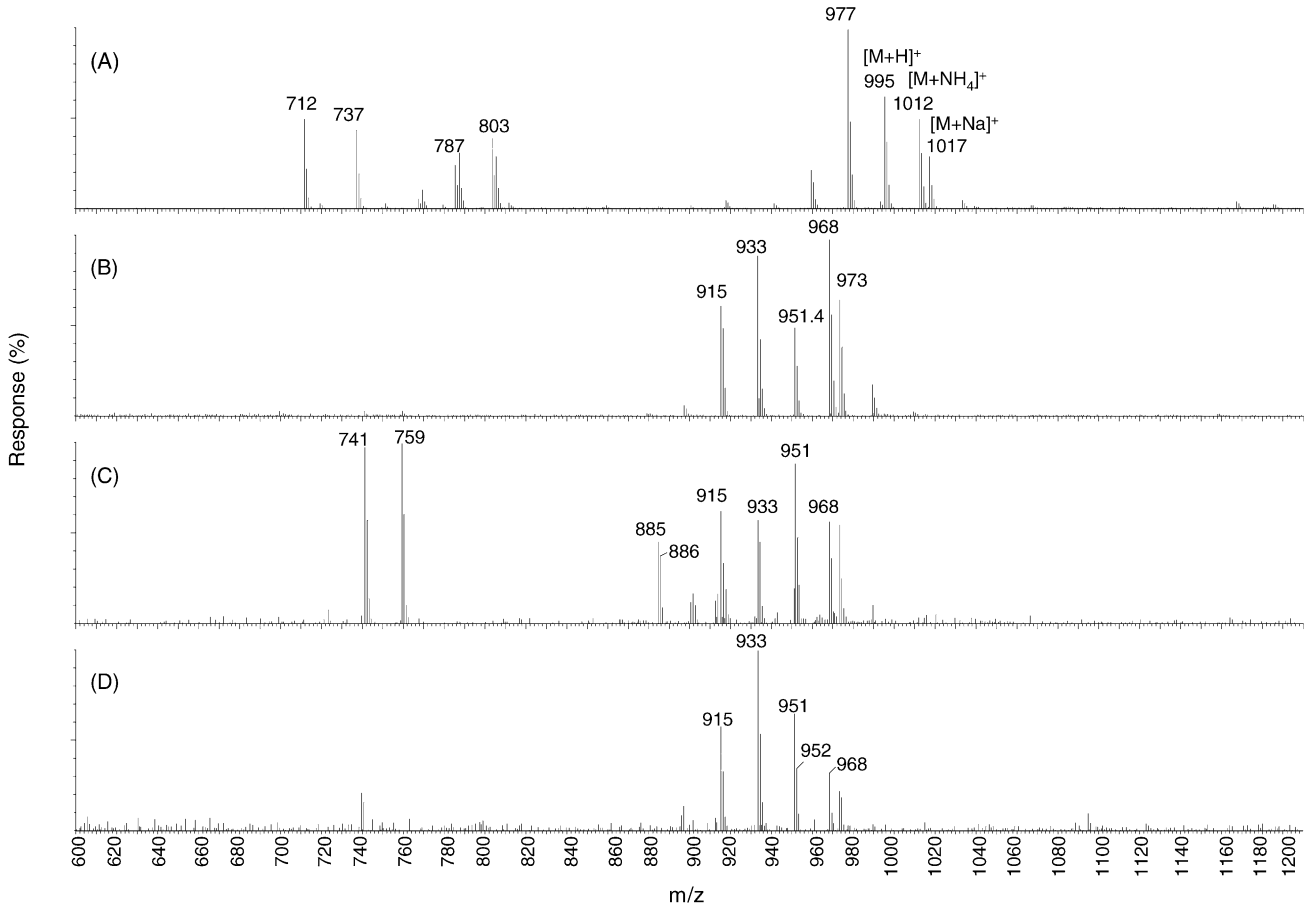

Fig. 4. Mass spectra recorded by LC/ESI-MS of (A) anthrylmethyl-OA (r.t. = ∼10 min) and its transformation products; (B) product 1, r.t. = 10.7 min; (C) product 2, r.t. = 11.7 min and (D) product 3, r.t. = 13.0 min.

confirmation of OA, DTX-1 and DTX-2 in mussel samples and that either of the product peaks can be used to establish concentration levels in a sample.

Hepatopancreas samples from mussels previously determined to contain no OA, DTX-1 or DTX-2 were analyzed following sample extraction, derivatization and cleanup, without exposure to UV radiation and repeated following 30 min exposure to UV light. As anticipated, the anthrylmethyl derivatives of OA, DTX-1 and DTX-2 were not observed in the blank samples prior to UV exposure. The peaks representing the transformation products similarly were missing from the chromatograms of these samples, following UV exposure, confirming that matrix artifacts were not contributing to the observed peaks following UV irradiation of a sample.

Although HPLC analysis with fluorescence detection had shown that UV exposure to the anthrylmethyl derivatives of OA, DTX-1 and DTX-2 resulted in the loss of these compounds with concurrent formation of two additional compounds, the structure of the UV products could not be established. Additional analyses, therefore, were performed using LC/MS to determine the structures of the UV products of anthrylmethyl-OA.

Initially, mass spectral analysis of the parent OA derivative (M.W. 994.54) was performed. Anthrylmethyl-OA produced ions representing ammonium, sodium and potassium adducts (*m*/*z*: 1012, 1017 and 1033, respectively), resulting from exposure to glass during sample preparation and storage. The $[M + H]^+$ ion (995) was observed in chromatograms of anthrylmethyl-OA samples, prior to UV exposure at a retention time of ∼10 min (Fig. 4A). The sequential loss of one, two and three water molecules was observed in mass spectra of the anthrylmethyl-OA sample, prior to UV exposure (*m*/*z* 977, 959 and 941, respectively) (Fig. 4A). Additionally, a fragment was observed at *m*/*z* 803, consistent with the loss of the anthrylmethyl group.

Two large peaks were observed at ∼10.7 and 13.0 min using both UV detection and mass spectral analysis which were established as transformation products in samples exposed to UV light for 30 min. Both of these later eluting, and hence, less polar compounds had similar mass spectra, with the molecular ion fragment observed at *m*/*z* of 951, consistent with the loss of 44 mass units, or loss of a carboxyl group from anthrylmethyl-OA. Following the initial loss of *m*/*z* 44, loss of one and two water molecules (*m*/*z* 933 and 915, respectively) (Fig. 4B and D) were observed. Additionally, the presence of the $[M + NH_4]^+$, $[M + Na]^+$ and $[M + K]^+$ adduct ions were identified at *m*/*z* 968, 973 and 989, respectively, consistent with exposure to reagents.

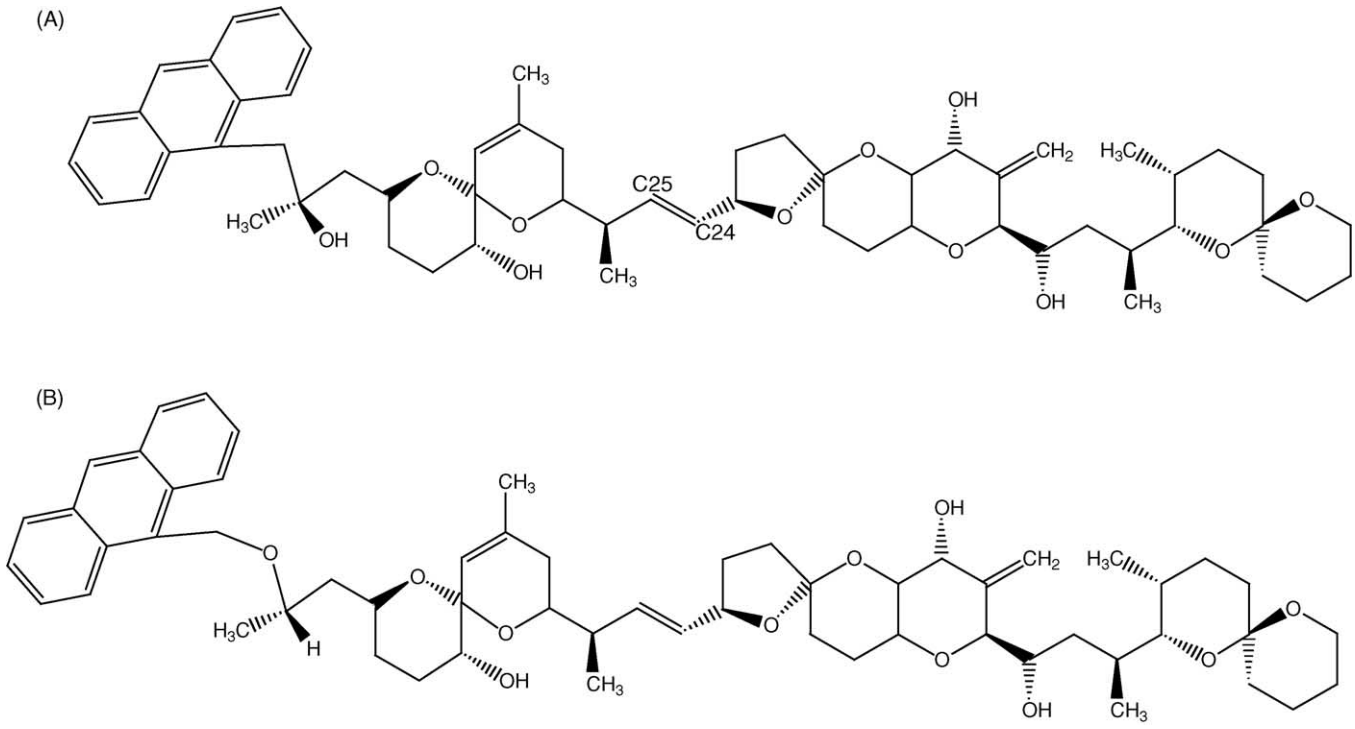

Fig. 5. Proposed structures of transformation products of anthrylmethyl-OA. (A) Photoisomers around alkene bond at C24 and C25 and (B) minor product of UV radiation.

A smaller peak with a retention time (~11.7 min) between the two main peaks also was observed in the total ion chromatogram. The fragmentation pattern established for this peak was similar to those obtained for the other transformation products in the higher mass range. Additional fragments of this smaller peak, however, were observed at *m*/*z* 759.4 and 741.3 (Fig. 4C), consistent with the loss of the anthrylmethyl functional group from the molecular ion and the $[MH - H_2O]^+$ ion, respectively.

The mass spectra were then studied to determine possible structures for these transformation products. We were able to establish two rather than three possible structures, consistent with the loss of a carboxyl group from anthrylmethyl-OA. We proposed the structures identified in Fig. 5 as the UV products of the anthrylmethyl derivatives of OA, which are consistent with two products less polar than the parent molecule.

To further confirm the structures of the three anthrylmethyl-OA UV products, the precursor molecules (*m*/*z* 951.3) of the product compounds were isolated and product ions (MS/MS) scans were performed. Although the product ion scans from the peaks at retention times of 10.7 and 13.0 min were nearly identical, a greater number of daughter fragments were produced by the peak at 11.7 min (Table 2). The additional fragments observed were consistent with the loss of an anthrylmethyl group (*m*/*z* 741) from the parent molecule (Table 2), as observed in the initial scan.

We believe that the UV products appearing at retention times of 10.7 and 13.0 min are *cis*- and *trans*-isomers around the C24 and C25 double bond of the product identified in Fig. 5A. Photoisomerization as a result of UV exposure has been studied both theoretically and experimentally for many years [16,17] and is what we believe to have occurred with okadaic acid and its analogues. Isomerization of microcystins has similarly been observed following UV exposure, which resulted in the production of non-toxic forms [18,19]. The structures we initially proposed also included a product with an ether linkage (Fig. 5B) between the anthrylmethyl group and the OA component of the molecule, which would be susceptible to cleavage. We believe that the peak with a retention time of 11.7 min is the product proposed in Fig. 5B.

After MS analyses were completed, it was necessary to re-examine the chromatograms obtained using fluorescence detection to establish whether a third peak was present in the chromatograms from the initial work. The presence of an additional peak, however, was not observed despite examining results using a reduced scale. The concentrations of the anthrylmethyl-OA solutions and mussel extracts used in the HPLC analyses with fluorescence detection (~100 ng/ml OA) were much lower than required for detection of OA and its transformation products using LC/MS (1–2.5 ng/μl). The samples at the higher concentrations (2.5 ng/μl) used for the MS analyses were reinjected and analyzed using fluorescence detection. At these high concentrations, a very small third peak was observed using fluorescence detection at a retention time of 15.1 min, between the transformation product peaks identified originally (Fig. 6). This indicates that the major UV transformation of the anthrylmethyl derivatives of OA, DTX-1 and DTX-2 is the loss of CO_2, without re-arrangement and

Table 2
Daughter ions produced from the UV products of anthrylmethyl-OA

Parent ions		Peak 1, r.t. = 10.7 min	Peak 2, r.t. = 11.7 min	Peak 3, r.t. = 13.0 min
m/z	Structure			
968	NH_4^+ adduct of products	968	968	968
		951	951	950
		933	933	933
		915	759	915
			741	
			740	
951	Product–CO_2	951	915	951
		933	897	915
		915	741	897
		897	365	
		879		
933	Product–H_2O	933	915	933
		915	896	915
		897	741	897
		879	723	879
		861	705	665
			483	
915	Product–$2H_2O$	915	915	915
		897	897	897
		879	879	879
		861	843	861
			705	843
			569	705
			485	

that conversion to an ether is a minor reaction pathway. Only one isomer of the photo induced re-arrangement product was observed in the present study (Fig. 5B). We believe that if much higher concentrations were studied, both isomers of the OA re-arrangement product, which appear to be minor conversion products, would have been observed.

The lack of stability of DSP was indicated by the systematic loss of CO_2 from the anthrylmethyl derivatives of OA, DTX-1 and DTX-2 that was observed in all standards and tissue extracts upon irradiation with UV light at wavelengths between 320 and 400 nm. Anthrylmethyl derivatives of OA, DTX-1 and DTX-2 consistently form two major products

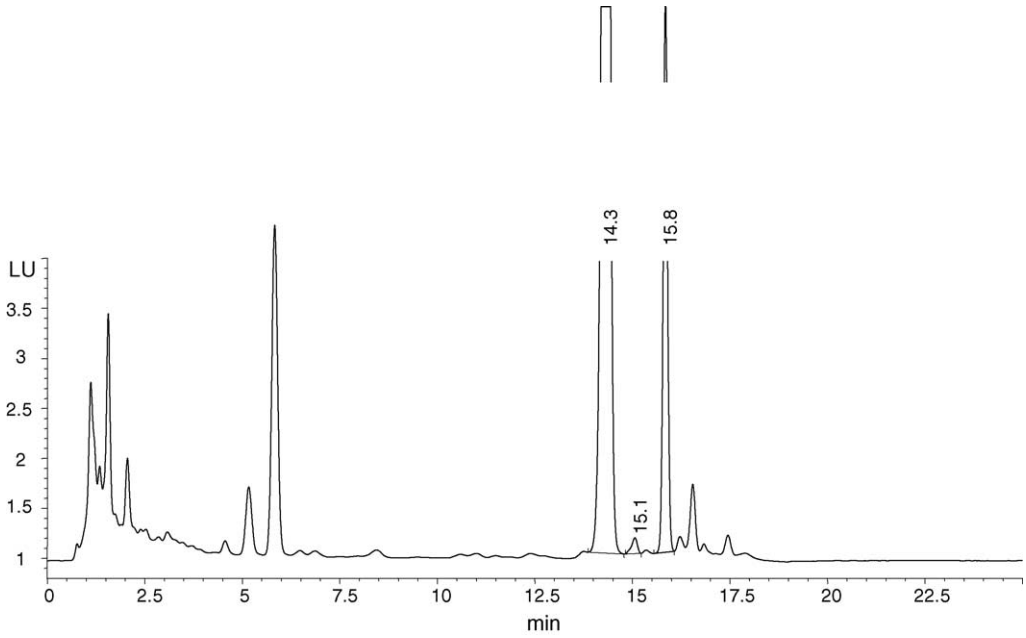

Fig. 6. UV transformation products of 2.5 ng/μl anthrylmethyl-OA, established using fluorescence detection.

following UV exposure, which appear as structural isomers (Fig. 5A) and a minor, third product which has an ether linkage at C37 (Fig. 5B). The limit of detection for OA was found to be 9 ng/ml, based on a three to one signal to noise ratio for the first transformation peak. Based on these results, we propose the use of fluorescence detection of sample extracts exposed to near UV radiation as a rapid, simple and sensitive confirmation tool for OA presence in shellfish samples for laboratories without access to routine use of LC/MS.

References

[1] A.G. Martinez, J.F. Lawrence, in: J.P.F. D'Mello (Ed.), Food Safety Contaminants and Toxicology, CABI Publishing, Cambridge, MA, 2003, p. 47.
[2] C. Garcia, P. Pereira, L. Valle, N. Lagos, Biol. Res. 36 (2003) 171.
[3] H. Terao, E. Ito, T. Yanagi, T. Yasumoto, Toxicon 24 (1986) 1141.
[4] J.C. Marr, L.M. McDowell, M.A. Quilliam, Nat. Toxins 2 (1994) 302.
[5] J.C. González, F. Leira, O.I. Fontal, M.R. Vieytes, F.F. Arévalo, J.M. Vieites, M. Bermúdez-Puente, S. Muniz, C. Salgado, T. Yasumoto, L.M. Botana, Anal. Chim. Acta 466 (2002) 233.
[6] M. Suganuma, H. Fujiki, H. Suguri, S. Yoshizawa, M. Hirota, M. Nakayasu, M. Ojika, K. Wakamatsu, K. Yamada, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 1768.
[7] S.S. Kelly, A.G. Bishop, E.P. Carmody, K.J. James, J. Chromatogr. A 749 (1996) 33.
[8] S. Pleasance, M.A. Quilliam, A.S.W. de Freitas, J.C. Marr, A.D. Cembella, Rapid Commun. Mass Spectrom. 4 (1990) 206.
[9] M.A. Quilliam, M.W. Gilgan, S. Pleasance, A.S.W. De Freitas, D. Douglas, L. Fritz, T. Hu, J.C. Marr, C. Smyth, J.L.C. Wright, in: T.J. Smayda, Y. Shimizu (Eds.), Toxic Phytoplankton Blooms in the Sea, Elsevier Science Publishers, Amsterdam, 1993, p. 547.
[10] M.A. Quilliam, J. AOAC Int. 82 (1999) 773.
[11] M.J. Nogueiras, A. Gago-Martinez, A.I. Paniello, M. Twohig, K.J. James, J.F. Lawrence, Anal. Bioanal. Chem. 377 (2003) 1202.
[12] J.F. Lawrence, S. Roussel, C. Ménard, J. Chromatogr. A 721 (1996) 359.
[13] M. Welker, C. Steinberg, Water Res. 33 (1999) 1159.
[14] A.J. Feitz, T.D. Waite, Environ. Sci. Technol. 37 (2003) 561.
[15] S.S. Bates, C. Léger, M.L. Wells, K. Hardy, in: S.S. Bates (Ed.), Proceedings of the Eighth Canadian Workshop on Harmful Marine Algae, Can. Tech. Rep. Fish. Aquat. Sci., vol. 2498, 2003, p. 30.
[16] A.R. Olson, Trans. Faraday Soc. 27 (1931) 69.
[17] A.R. Olson, W. Maroney, J. Am. Chem. Soc. 56 (1934) 1320.
[18] K. Kaya, T. Sano, Chem. Res. Toxicol. 11 (1998) 159.
[19] K. Tsuji, T. Watanuki, F. Kondo, M.F. Watanabe, S. Suzuki, H. Nakazawa, M. Suzuki, H. Uchida, K.-I. Harada, Toxicon 33 (1995) 1619.