SPECIAL GUEST EDITOR SECTION

Determination and Confirmation of the Amnesic Shellfish Poisoning Toxin, Domoic Acid, in Shellfish from Scotland by Liquid Chromatography and Mass Spectrometry

PHILIPP HESS[1], SUSAN GALLACHER, LESLEY A. BATES, and NIGEL BROWN
Fisheries Research Services-Marine Laboratory Aberdeen, Agency of the Scottish Executive Rural Affairs Department (SERAD), Victoria Rd, PO Box 101, Aberdeen, AB11 9DB Scotland, UK
MICHAEL A. QUILLIAM
Institute for Marine Biosciences, National Research Council Canada, Halifax, 1411 Oxford St, NS, B3H 3Z1 Canada

During 1998 and early 1999, shellfish samples from sites in Scotland were found to contain the amnesic shellfish poisoning toxin, domoic acid (DA). Two different techniques, liquid chromatography (LC) with UV diode-array detection and LC with mass spectrometric (MS) detection, were used to detect and confirm DA in shellfish extracts. The LC/UV method was validated for routine monitoring by recovery experiments on spiked mussel and scallop tissues with a certified mussel tissue used as reference material. Crude extracts of selected samples as well as extracts cleaned with strong anion exchange (SAX) were analyzed by both LC/UV and LC/MS. Good correlation (linear regression r^2 = 0.996, slope = 0.93) between the 2 methods was found for cleaned extracts. Analyses of crude extracts by LC/UV produced false-positive results in 2 crab samples, whereas LC/MS analyses gave accurate results. It was concluded that LC/UV is a valid approach for routine monitoring of DA in shellfish when cleanup is performed with a SAX cartridge to prevent false positives. A variety of shellfish species were surveyed for DA content, including *Pecten maximus* (king scallops), *Chlamys opercularis* (queen scallop), *Mytilus edulis* (blue mussels), *Cancer pugaris* (crab), and *Ensis ensis* (razor fish). The highest concentration of DA was 105 μg/g in *Pecten maximus*.

Amnesic shellfish poisoning (ASP) toxins are a group of water-soluble neurotoxins, containing domoic acid (DA) as the principal compound (1). The production of DA has been related to the pennate diatom *Pseudo-nitzschia* spp., which may accumulate in filter-feeding bivalves and pose a risk to human health. Todd (2) described the toxic effects of ASP in humans after an incident in 1987 on the Canadian coast, where 107 people became ill with gastrointestinal and neurological symptoms. Three patients died and 14 displayed severe neurological poisoning.

The occurrence of *Pseudo-nitzschia* spp. in the waters of several countries raised concerns that these toxins may occur in shellfish harvested in Europe. This led to an amendment of the Shellfish Hygiene Directive of the European Community, which states that the maximum value of DA in the whole animal or any edible part separately should not exceed 20 μg/g. Plans were made to include this toxin species in the Directive, and a pilot study was conducted in 1998 to determine if ASP toxins occur in UK shellfish.

Several methods have been proposed for the determination of DA in marine environmental matrixes. Although the mouse bioassay can detect DA at high levels, its sensitivity is insufficient for detection of DA at the regulatory level (1). The most commonly used technique is liquid chromatography (LC) with UV absorbance detection, first developed in 1989 (3, 4). DA was extracted from tissues with boiling water or aqueous methanol. Lawrence et al. (4, 5) developed a method incorporating the same acid extraction procedure used for analysis of paralytic shellfish poisoning (PSP) toxins. This method has the advantage of providing a single extract for both ASP and PSP assays but suffers from the fact that DA is not stable in an acidic extract. Pocklington et al. (6) presented a sensitive LC–fluorimetric method for determination of DA in plankton and in seawater, but this method has not yet been extended to shellfish tissues. The present study focuses on the method proposed by Quilliam et al. (7), which is based on rapid aqueous methanol extraction followed by strong anion exchange (SAX) cleanup and LC with UV diode-array detection (DAD). This method for various shellfish matrixes was validated by using spike recovery experiments, a certified reference material, and parallel analyses by another method based upon LC with mass spectrometric detection (LC/MS; 8). Results from validation of

Guest edited as a special report on "Phycotoxins in Seafood and Drinking Water" by Michael A. Quilliam.
Presented at the 113th AOAC INTERNATIONAL Annual Meeting and Exposition, September 26–30, 1999, Houston, TX.
[1]Corresponding author present address: Marine Institute, Snugboro Rd, Abbotstown, Dublin 15, Ireland; e-mail: philipp.hess@marine.ie.

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

Table 1. Recovery of DA from mussel tissue (*Mytilus edulis*) as determined by LC/UV after cleanup with SAX cartridges

Tube No.	DA added, μg/g	DA determined, μg/g	Recovery, %
1	14.5	14.1	97
2	16.5	16.8	102
3	16.0	16.0	100
4	8.3	9.1	109
5	6.7	6.6	99
6	6.7	6.2	93
7	3.7	3.6	98
8	3.9	3.7	93
9	3.2	3.2	97
10	1.7	2.0	114
11	1.8	1.8	109
12	1.7	1.8	104
13	1.0	1.1	102
14	0.8	0.8	96
15	1.1	1.1	105
Mean			101
STD			± 6

this procedure and data confirming the presence of DA in shellfish from Scottish waters are described.

Experimental

Reagents

(**a**) *Water*.—Deionized using a Millipore (Watford, UK) membrane filtration system.

(**b**) *Methanol and acetonitrile*.—LC grade, Rathburn Chemicals Ltd. (Walkerburn, Scotland, UK).

(**c**) *Trifluoroacetic acid*.—Analytical grade, Sigma Chemical Co. (St. Louis, MO).

(**d**) *Citric acid monohydrate and triammonium citrate (analytical grade)*.—Merck, Darmstadt, Germany.

(**e**) *Citric acid buffer (0.5M, pH 3.2)*.—Prepared by dissolving citric acid monohydrate (20.2 g) and triammonium citrate (7.0 g) in deionized water (200 mL). The buffer was prepared fresh each week. Acetonitrile (25 mL) was added to the buffer and the volume was adjusted to 250 mL in a volumetric flask.

(**f**) *DA calibration standards*.—Dilutions of certified DA solutions (DACS-1C, NRC Certified Reference Materials Program, Halifax, Nova Scotia, Canada).

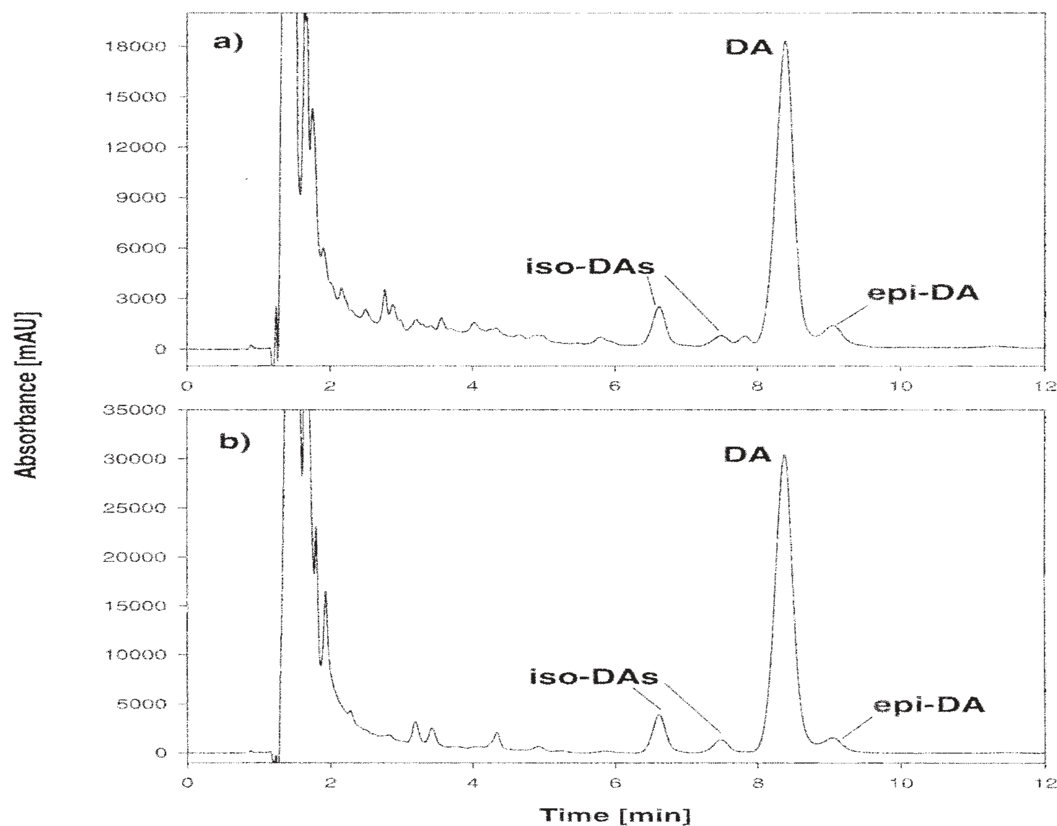

Figure 1. Chromatograms (wavelength 242 nm) obtained from LC/UV analyses of: (a) certified mussel tissue reference material, MUS-1B; and (b) scallop sample harvested from the west coast of Scotland.

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

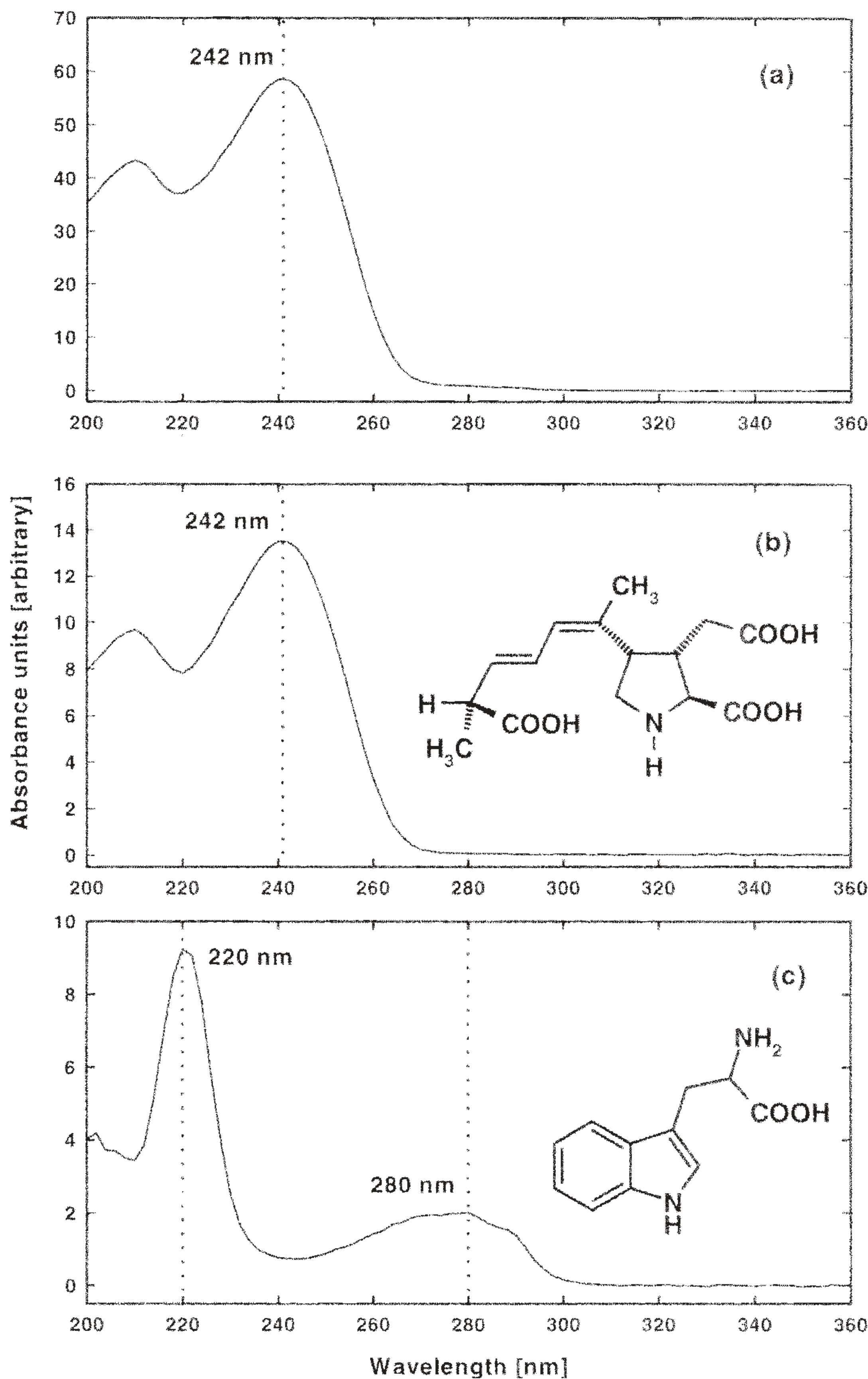

Figure 2. UV/diode-array spectra of: (a) the component eluting at 9.0 min in the LC/UV analysis of a Scottish scallop extract; (b) certified DA standard; and (c) tryptophan standard.

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

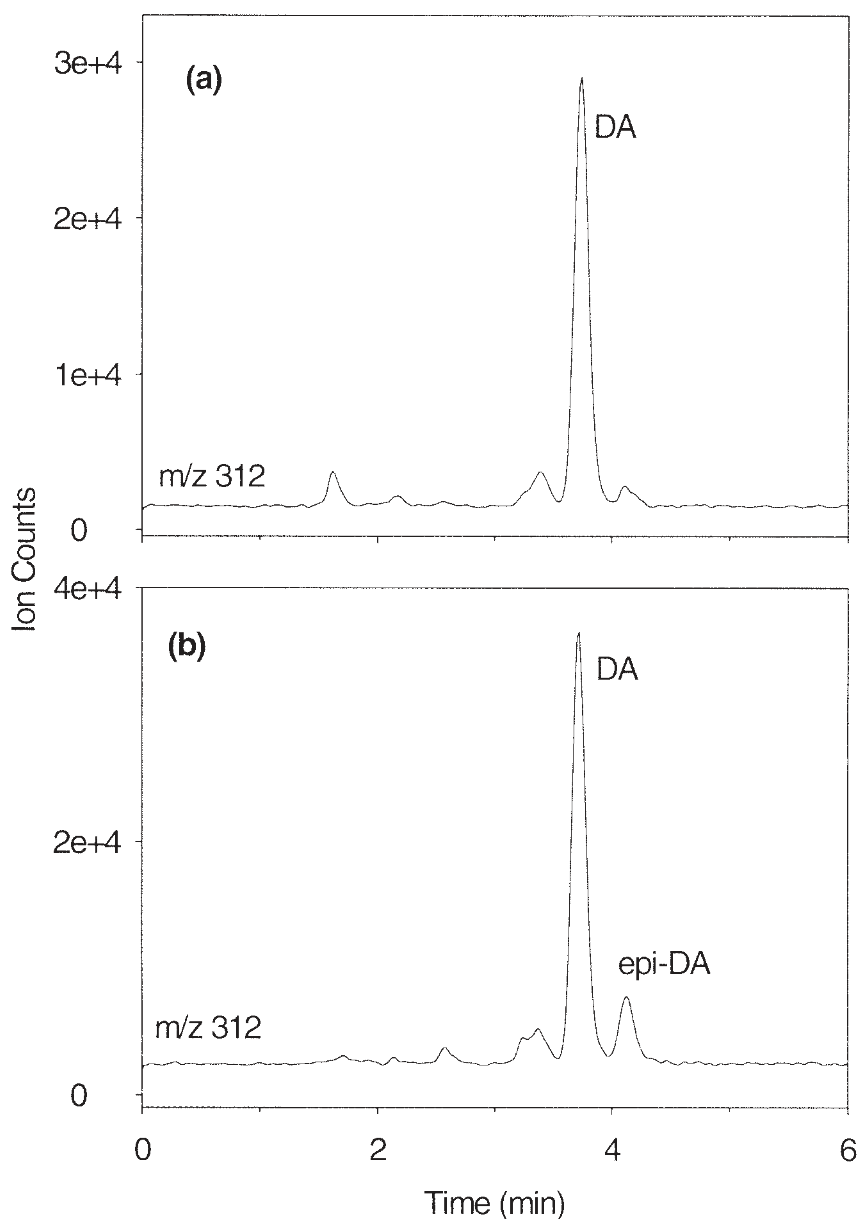

Figure 3. Comparison of $[M+H]^+$ (*m/z* 312) chromatograms obtained from selected ion monitoring LC/MS analyses of: (a) scallop sample harvested from Scottish waters; and (b) mussel tissue reference material (MUS-1, diluted 10-fold).

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

(**g**) *Calibration solutions*.—Diluted and ampuled under nitrogen in amber glass ampules to prevent degradation through oxidation.

(**h**) *Spiking standards*.—Made from solid DA (>95%, Calbiochem, LaJolla, CA) and checked for actual concentration against the calibration standards.

(**i**) *Certified mussel tissue reference material (MUS-1B)*.—Purchased from the NRC Certified Reference Materials Program.

(**j**) *Another mussel tissue reference material MUS-1*.—Also used for some experiments comparing isomer distributions.

(**k**) *Tryptophan*.—Bought as reagent grade D and L isomers from Sigma.

Spiking, Tissue Extraction, and Cartridge Loading Experiments

Samples were extracted according to Quilliam et al. (7) with some modifications. Briefly, shellfish tissues (50–500 g) were removed from the shell, pooled, and homogenized in a food blender. Subsequently 2–6 g homogenate was extracted with 16 mL water–methanol (1 + 1, v/v) in a high-speed blender (Ultraturrax®, Norlab Instruments, Aberdeen, UK) for 1 min at high speed. The extract was centrifuged for 10 min at

3500 rpm in a Centaur 300® Norlab Instruments centrifuge. The crude, centrifuged extract was decanted into a graduated 25 mL polypropylene screw cap universal vial, and diluted to 20 mL with water–methanol (1 + 1, v/v). For recovery experiments, a mussel sample free of DA was homogenized. Subsamples of the homogenized tissue (ca 4 g) were weighed into 50 mL centrifuge tubes. DA standards of various concentrations were added to the tissue; resultant concentrations are reported in Table 1.

Cartridge loading experiments were performed with naturally contaminated extracts. Homogenates (100 g) were prepared for whole scallops (ca 50 μg DA/g tissue) and scallop gonads (ca 12 μg/g). Several portions (4 g each) of the homogenates were extracted, pooled together, and diluted to 100 mL for each of the 2 extracts. The whole animal extract contained 0.176 g tissue equivalent/mL extract, whereas the gonad extract contained 0.28 g tissue equivalent/mL extract. Increasing amounts (0.25, 0.5, 1, 2, 5, and 10 mL) of naturally contaminated scallop extracts were loaded onto SAX cartridges to evaluate the loading capacity of the cartridges. The lipid contents of the tissue homogenates were determined separately by the method described by Bligh and Dyer (9).

For screening, this crude extract was filtered through 0.45 μm Whatman® (Maidstone, UK) filters before LC analysis. To remove interfering compounds, the crude extract (1–5 mL) was subjected to SAX (Waters, Hertsford, UK, QMA 500 mg) cleanup as described by Quilliam et al. (7). Elution was performed with 2 mL citric acid buffer. The filtered crude extract and the SAX-cleaned extract were analyzed by LC–UV–DAD or LC/MS.

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

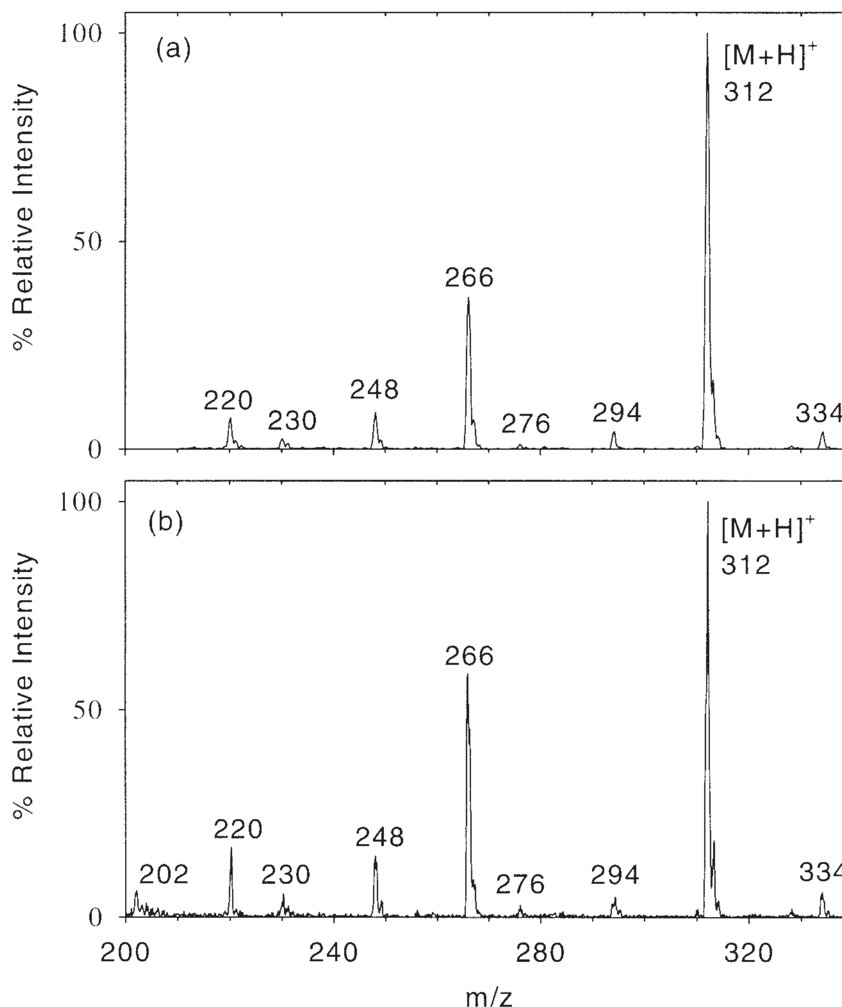

Figure 4. Comparison of full scan ionspray mass spectra of: (a) DA standard; and (b) component observed in a scallop sample (Scottish north coast) acquired after SAX cleanup and 10-fold preconcentration. Ion assignments: *m/z* 334 = $[M+Na]^+$; *m/z* 312 = $[M+H]^+$; *m/z* 294 = $[M+H-H_2O]^+$; *m/z* 276 = $[M+H-2H_2O]^+$; *m/z* 266 = $[M+H-HCOOH]^+$; *m/z* 248 = $[M+H-HCOOH-H_2O]^+$; *m/z* 230 = $[M+H-HCOOH-2H_2O]^+$; *m/z* 220 = $[M+H-2HCOOH]^+$; *m/z* 202 = $[M+H-2HCOOH-H_2O]^+$. *Note*: The spectrum in (a) was scanned only down to 210, hence the absence of the *m/z* 202 ion.

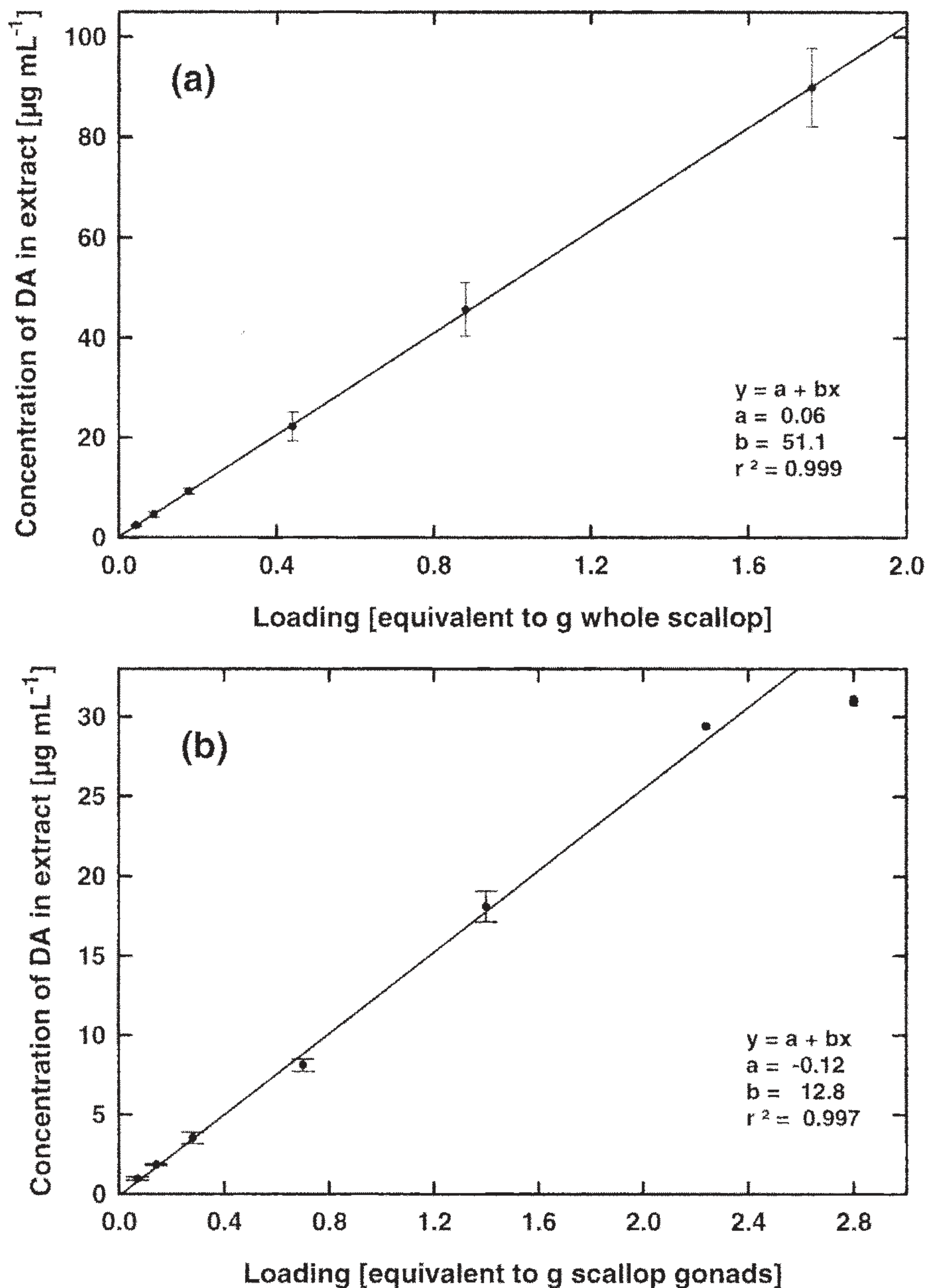

Figure 5. Results from experiments designed to test recovery of DA from homogenized whole scallops (a) and homogenized scallop gonads (b), using SAX cleanup of different extracts loadings. The lines are curves from linear regressions, and error bars represent standard deviation of triplicates. In (b), the result from the highest loading was excluded from the regression curve.

LC–UV/DAD

The LC system consisted of a Thermoseparation Products (Manchester, UK) degasser, a P4000 pump, an AS3000 autosampler, and a UV6000 UV/DAD detector. The column used was a 250 × 4.6 mm Spherisorb C_{18} column (5 µm particle size), with 10 mm Lichrosorb C_{18} guard cartridge, both heated to 40°C. The flow rate was 1.5 mL/min 0.1% trifluoroacetic acid in 10% aqueous acetonitrile throughout the run. The injection volume was 10 µL unless otherwise stated. The software was TSP1000, an OS/2-based software by Thermoquest (Manchester, UK).

Calibration was performed externally, and a full set of 5 calibration standards of DA (0.5, 1.0, 2.0, 5.0, and

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

Table 2. Recovery of DA from certified mussel tissue reference material (MUS-1B), as determined by LC/UV after cleanup with SAX cartridges

Analysis	Recovery, %
1	91
2	91
3	92
4	84
5	88
6	89
7	93
Mean	90
STD	± 3

10.0 µg/mL) was run every 10–15 samples; calibration curves were always linear, with correlation coefficients >0.99. The UV 6000 detector can acquire data in a simple UV mode or in a diode-array mode, allowing collection of spectral information. Spectra can be acquired over the range of 190–800 nm at bandwidths from 1 to 13 nm, and rates of collection from 1 to 10 Hz. The conditions of the diode-array were optimized for acquisition as follows: wavelength range was limited to 200–360 nm, acquiring a data point every 2 nm; collection frequency was 2 Hz. Under these conditions, the signal-to-noise ratio for the 0.5 µg/mL calibration standard of DA was 50. The detection limit for the LC/UV system is 0.03 µg/mL for 10 µL injected (S/N of 3), which corresponds to a method detection limit of ca 0.2 µg/g shellfish.

LC/MS

LC/MS experiments were performed on a Sciex API-165 mass spectrometer (Toronto, Canada) using ionspray ionization, with orifice and ring voltages at 50 and 240 V, respectively. Selected ion monitoring (SIM) was performed using 4 ions: *m/z* 312, 266, 248, and 220, with dwell times of 200 ms/ion. The LC column was a Zorbax (Ontario, Canada) Rx-C18, 2 × 150 mm (5 µm particle size) maintained at 20°C. The mobile phase was 85% A and 15% B, where A = water, B = acetonitrile–water (95 + 5), both with 50mM formic acid and 2mM ammonium formate. The flow rate was 0.2 mL/min and injection volume was 5 µL unless stated otherwise. The detection limit of the LC/MS system was 0.07 µg/mL for a 5 µL injection, which corresponds to ca 0.4 µg/g shellfish.

The bias of LC/MS versus LC/UV was calculated as follows:

$$\text{Bias} = 100 \times (1 - b_1)/1$$

where b_1 is the slope of the regression curve between the quantitative results.

Results and Discussion

Detection and Confirmation of DA in Scottish Shellfish

A pilot study that began in 1998 led to the confirmation that DA was indeed present in shellfish harvested from Scottish waters. Figure 1a shows the chromatogram resulting from the LC/UV analysis of a SAX-cleaned extract of a scallop sample harvested from Scottish waters. The peak eluting at 9.0 min exactly matched the retention time of DA in both the calibration standard and an extract of the mussel tissue reference material, MUS-1B (Figure 1b). Other peaks eluting close to DA were also observed in the scallop extract, and their retention times matched well with peaks also observed in the MUS-1B chromatogram that correspond to isodomoic acids, the *cis-trans* isomers of DA, and epi-DA, the C5′-diastereomer of DA. UV spectra acquired at the apex of LC/UV peaks of sample components gave >99% similarity with the spectra acquired from DA and its isomers in MUS-1B eluting at corresponding retention times. Figure 2a shows the UV spectrum of the suspected DA peak from the scallop extract, and Figure 2b shows the spectrum acquired for a DA standard.

Further evidence for the identification of DA was provided by LC/MS analysis. Figure 3 compares results of the SIM LC/MS analyses of extracts of another mussel tissue reference material, MUS-1, and another scallop sample obtained from Scottish waters. Retention times of the peaks in the *m/z* 312 ($[M+H]^+$) ion chromatogram of the sample matched those of DA and its isomers in the reference material. Furthermore, 3 fragment ions ($[M+H–HCOOH]^+$, *m/z* 266; $[M+H–HCOOH–H_2O]^+$, *m/z* 248; and $[M+H–2HCOOH]^+$, *m/z* 220) were monitored in the same analysis and their retention times and peak area ratios matched well between scallop sample and reference material. Finally, acquisition of full scan mass spectra during an LC/MS analysis of a SAX-cleaned and 10-fold concentrated scallop extract provided additional confirmation of DA. Figure 4 shows a good match between the mass spectra of a DA standard and the presumed DA present in a scallop sample. This preponderance of evidence (retention times, UV spectra, and mass spectra) confirmed that DA was present in Scottish shellfish samples. To our knowledge, this is the first published report of DA in shellfish harvested in UK waters. Identification of the causative organism is currently being investigated by FRS-SERAD, and will be reported separately.

Method Validation

Although the SAX cleanup and LC/UV analysis method had been validated previously for determination of DA in mussels (7), a revalidation was needed for implementation of a UK monitoring program and particularly to test the suitability of the method for local shellfish.

The first concern was the possibility of false positives caused by interfering substances. Therefore, various negative control tissues were tested with and without the SAX cleanup. None of the SAX-cleaned samples tested showed peaks that

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

Table 3. Comparison of concentrations of DA in shellfish tissue as determined by LC/UV and LC/MS before and after SAX cartridge cleanup[a]

Sample identifier	Species	LC/MS crude, µg/g	LC/UV crude, µg/g	LC/MS cartridge, µg/g	LC/UV cartridge, µg/g
1	*Mytilus edulis*	9.5	8.8	7.9	7.0
2	*Chlamys opercularis*	3.0	3.6	3.0	2.5
3	*Pecten maximus*	19	22	18	17
4	*Pecten maximus*	67	65	57	54
5	*Pecten maximus*	23	24	20	18
6	*Pecten maximus*	26	26	22	20
7	*Chlamys opercularis*	7.2	8.4	6.0	5.7
8	*Chlamys opercularis*	11	13	9.8	9.4
9	*Ensis ensis*	2.4	3.2	2.3	2.2
10	*Ensis ensis*	0.6	4.6[b]	0.5	0.3
11	*Ensis ensis*	1.8	3.1[b]	1.6	1.5
12	*Mytilus edulis*	ND[a]	0.6[b]	ND	ND
13	*Mytilus edulis*	ND	4.5[b]	ND	ND
14	*Pecten maximus*	22	22	21	18
15	*Pecten maximus*	31	31	18	15
16	*Chlamys opercularis*	6.5	7.1	5.7	4.6
17	*Pecten maximus*	23	25	21	17
18	*Pecten maximus*	22	21	17	14
19	*Ensis ensis*	0.6	4.1[b]	0.7	ND
20	*Pecten maximus*	10	12	8.6	6.7
21	*Pecten maximus*	13	16	12	10
22	*Pecten maximus*	8.9	8.3	7.9	6.9
23	*Pecten maximus*	22	23	20	16
24	*Pecten maximus*	4.0	4.2	4.2	3.4
25	*Pecten maximus*	2.9	2.7	3.0	2.3
26	*Cancer pugaris*	ND	46[b]	0.4	0.7
27	*Cancer pugaris*	ND	53[b]	ND	1.0

[a] ND = not detected; detection limits were 0.4 µg/g for the LC/MS method and 0.2 µg/g for LC/UV method.
[b] These high figures represent false positive LC/UV results as confirmed by SAX cleanup and LC/MS.

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

interfered with the detection of DA or its isomers. Crude extracts, on the other hand, often showed a peak that eluted close to that of DA. This peak gave a retention time difference with the DA peak of 0.2–0.4 min (0.96–0.98 relative retention time) with a peak width at half height of 0.3 min. The interfering compound had the same retention time as that of tryptophan and produced a UV spectrum that matched to 99.5% the spectrum of commercially available tryptophan (Figure 2c), which is distinctly different from the spectrum of DA (Figure 2b). Initial studies by Quilliam et al. (3) showed a relative retention time of tryptophan of 0.86 compared with that of DA. The difference in separation between the initial study and the present study is due in part to the different stationary phases: Spherisorb-C_{18} in the present and Vydac-201TP-C_{18} in the former. Other compounds similar to tryptophan have also interfered with detection of DA even with the Vydac-201TP-C_{18} column (3); therefore, it appears prudent to use SAX cleanup to avoid false negatives.

An important concern with any method based upon solid-phase extraction is the loading capacity of the cartridge. Too much of some sample matrixes loaded on the SAX cartridge can cause a breakthrough of DA during sample loading and result in lower recovery (7). In addition, because of differences in manufacturing, the loading capacity of a cartridge

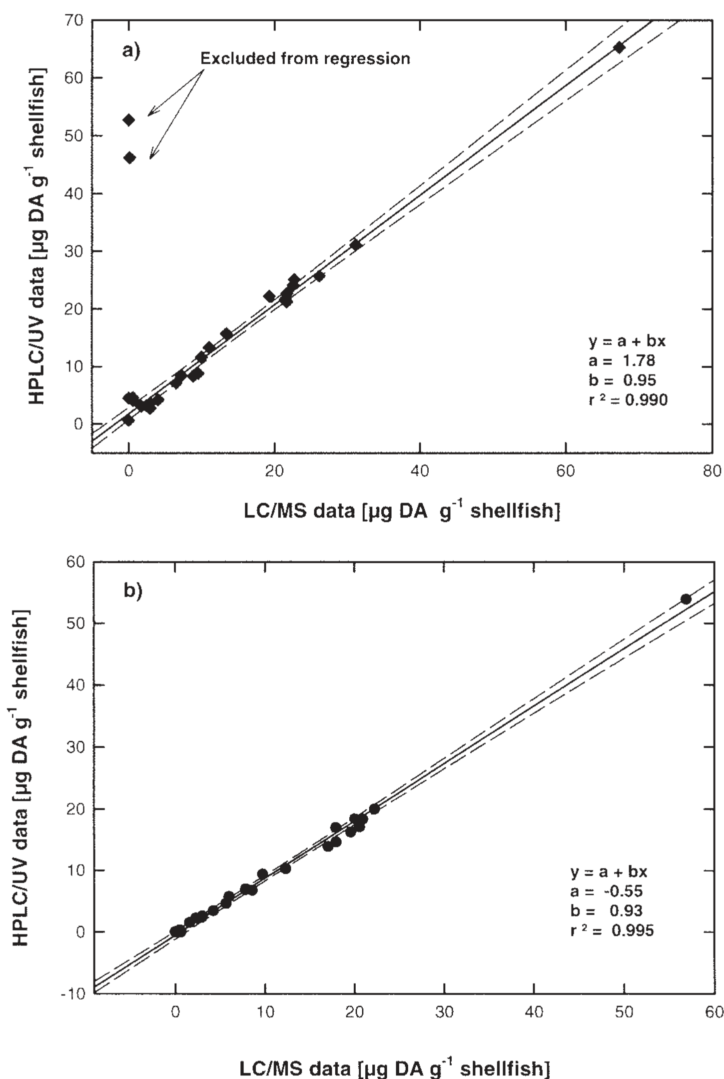

Figure 6. Comparison of results from LC/UV and LC/MS analyses of 27 samples of shellfish harvested in Scotland, 1998: (a) analyses of crude extracts (2 data points were excluded from the regression because interference was shown by diode-array analysis); and (b) analyses of extracts cleaned up by using SAX cartridges. Straight lines are curves for linear regression; dotted lines are 99% confidence intervals.

from a specific manufacturer should always be tested in a validation study. Therefore, the loading capacity of the SAX cartridges was tested by loading different amounts of contaminated scallop extracts (*Pecten maximus*) onto the cartridge and by determining the concentration of DA recovered in the eluent. *P. maximus* was chosen for this experiment because its high lipid content could be expected to cause the greatest interference. The cartridges were tested with extracts from 2 different tissues, namely, the gonads and the whole animal (adductor muscle, mantle, digestive organs, and gonads). Before the experiment the lipid content of the samples was determined to be 0.67 ± 0.06% (n = 8) for the whole animal and 2.1 ± 0.1% (n = 6) for gonads alone. The recovery of DA was independent of loading for the extract that excluded the gonads up to the maximum weight tested (1.76 g equivalent; Figure 5a). For the more fatty gonad extracts, 2.8 g tissue, which contained 57 mg lipids, caused a slight decrease in recovery (Figure 5b). These experiments indicated that the amount of lipid loaded onto the cartridge should be limited. For this study we concluded that the amount of tissue equivalent loaded onto a 0.5 g SAX cartridge should not exceed 1.5 g to allow for slight variations in the lipid content of individual tissues.

Experiments were undertaken to assess recovery of DA from both Scottish mussel (*Mytilus edulis*) tissues and a certified mussel tissue reference material. The recoveries of DA from mussels spiked with a range of DA concentrations varied between 93 and 114%, with an average of 101 ± 6% (n = 15; Table 1). Analysis of 7 different batches of certified mussel tissue reference material, MUS-1B, containing DA at 36 µg/g, resulted in an average recovery of 90 ± 3% (n = 7) compared with the certified value (Table 2). The lower than expected recovery from MUS-1B suggested that some of the DA in the

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

Table 4. Shellfish samples analyzed for DA during the 1998 monitoring program, grouped by species, and levels of DA obtained by LC/UV analysis during the 1998 monitoring program

Species	No. of samples	No. of positives	% Positives	No. of samples 0–2.5 µg/g	No. of samples 2.5–20 µg/g	No. of samples >20 µg/g
Mytilus edulis	523	137	26	120	17	0
Pecten maximus	137	100	73	52	33	15
Chlamys opercularis	29	18	62	6	12	0
Crassostrea spp.	77	25	33	24	1	0
Ensis ensis	8	4	50	4	0	0
Cancer pugaris	3	2	67	2	0	0
Cerastoderma edulis	5	3	60	1	2	0
Total	782	289		209	65	15

reference material was not extracted because of its stronger binding to the tissue, as compared with a simple DA spike. Such an interpretation was recently confirmed by the CRM producer (10). The MUS-1B certification was performed by an exhaustive, volumetric procedure, whereas the above trial was conducted with a one-step dispersive extraction which is typical for routine monitoring, where speed of sample preparation is required for high sample throughput. The coefficients of variance for analysis of reference material and freshly spiked tissues were 3 and 6%, respectively. Both were better than target values of 12% for the determination of most organic and inorganic trace contaminants in proficiency testing schemes such as "Food Analysis Performance Assessment Scheme" (FAPAS) or "Quality Assurance in Marine Environmental Matrixes in Europe" (QUASIMEME).

To further validate the LC/UV method, a set of 27 different shellfish species, containing a range of DA concentrations, was chosen for comparison of the LC/UV and LC/MS methods. Table 3 lists the results obtained for crude and SAX-cleaned extracts by each method. Some of the LC/UV results for crude extracts were positive for DA, whereas LC/MS results were negative. For 2 of these samples (Nos. 26 and 27), examination of UV spectra confirmed that the signals were due to tryptophan and not DA. In other cases (Nos. 10, 11, and 21) the concentration determined by LC/UV was significantly higher than that by LC/MS. For these samples, concentration was insufficient to acquire UV spectra, but it was possible in some cases to determine that the retention time of the peak first identified as DA was not exactly the same as that of DA. When extracts were taken through the SAX cleanup, quantitative results obtained by LC/UV matched very well with those obtained by LC/MS. The correlation coefficients were 0.99 for both crude and SAX-cleaned samples (Figure 6). The bias of the UV method compared with the MS method was 5% for crude extracts and 7% for SAX-cleaned extracts. This bias seems acceptable because the analyses were made in different laboratories with different detection methods. It is possible that this estimation would have been reduced if a greater number of samples had been analyzed. The range of concentrations (0–57 µg DA/g tissue) confirmed through the method comparison was particularly relevant to the monitoring of DA which has a regulatory limit of 20 µg/g.

Shellfish Survey

A total of 782 samples of shellfish from UK harvesting sites were analyzed for DA from April 1998 to March 1999. DA was found in 289 samples (37%) and the toxin concentrations varied greatly between species (Table 4). DA concentrations in shellfish exceeded the regulatory limit on several occasions during 1998, and harvesting of shellfish in these areas was prohibited during the time of occurrence. Although the bulk of the 7 shellfish species tested consisted of *Mytilus edulis*, the largest percentage of toxic samples was from *Pecten maximus*. This species also contained the highest concentrations of DA, up to 105 µg/g in whole tissue. In some instances the combined tissues of gonads and adductor muscle also contained concentrations >20 µg/g. Further studies are necessary to determine if scallops remain the dominant toxic species and if this is due to differences in uptake and metabolism or simply different levels of exposure.

Acknowledgments

This project was funded by SERAD and MAFF. (Ministry of Agriculture, Fisheries and Food). We thank Godfrey Howard, David Basford, John Turriff, Shona Kinnear, Margaret McCann, and Bill Hardstaff for their cooperation in obtain-

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022

ing and processing samples. We also thank Sharleen Alexander for her contribution to establishing the program, and Christian Hummert for initial analysis of samples by LC/MS.

References

(1) Quilliam, M.A., & Wright, J.L.C. (1989) *Anal. Chem.* **61**, 1053A–1060A

(2) Todd, E.C.D. (1993) *J. Food Prot.* **56**, 69–83

(3) Quilliam, M.A., Sim, P.G., McCulloch, A.W., & McInnes, A.G. (1989) *Int. J. Environ. Anal. Chem.* **36**, 139–154

(4) Lawrence, J.F., Charbonneau, C.F., Menard, C., Quilliam, M.A., & Sim, P.G. (1989) *J. Chromatogr.* **462**, 349–356

(5) Lawrence, J.F., Charbonneau, C.F., & Menard, C. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 68–72

(6) Pocklington, R., Milley, J.E., Bates, S.S., Bird, C.J., de Freitas, A.S.W., & Quilliam, M.A. (1990) *Int. J. Environ. Anal. Chem.* **38**, 351–368

(7) Quilliam, M.A., Xie, M., & Hardstaff, W.R. (1995) *J. AOAC Int.* **78**, 543–554

(8) Quilliam, M.A., Thomson, B.A., Scott, G.J., & Siu, K.W.M. (1989) *Rapid Commun. Mass Spectrom.* **3**, 145–150

(9) Bligh, E.G., & Dyer, W.J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917

(10) NRC Certified Reference Material Program (1999) *MUS-1B Mussel Tissue Reference Material Certificate (Revision 99-2-18)*, National Research Council of Canada, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada

Downloaded from https://academic.oup.com/jaoac/article/84/5/1657/5656906 by U.S. Department of Justice user on 17 August 2022