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Optimization of solid-phase extraction and liquid chromatography–tandem mass spectrometry for the determination of domoic acid in seawater, phytoplankton, and mammalian fluids and tissues☆

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ABSTRACT

We previously reported a solid-phase extraction (SPE) method for determination of the neurotoxin domoic acid (DA) in both seawater and phytoplankton by liquid chromatography–tandem mass spectrometry (LC–MS/MS) with the purpose of sample desalting without DA pre-concentration. In the present study, we optimized the SPE procedure with seawater and phytoplankton samples directly acidified with aqueous formic acid without addition of organic solvents, which allowed sample desalting and also 20-fold pre-concentration of DA in seawater and phytoplankton samples. In order to reduce MS contamination, a diverter valve was installed between LC and MS to send the LC eluant to waste, except for the 6-min elution window bracketing the DA retention time, which was sent to the MS. Reduction of the MS turbo gas temperature also helped to maintain the long-term stability of MS signal. Recoveries exceeded 90% for the DA-negative seawater and the DA-positive cultured phytoplankton samples spiked with DA. The SPE method for DA extraction and sample clean-up in seawater was extended to mammalian fluids and tissues with modification in order to accommodate the fluid samples with limited available volumes and the tissue extracts in aqueous methanol. Recoveries of DA from DA-exposed laboratory mammalian samples (amniotic fluid, cerebrospinal fluid, plasma, placenta, and brain) were above 85%. Recoveries of DA from samples (urine, feces, intestinal contents, and gastric contents) collected from field stranded marine mammals showed large variations and were affected by the sample status. The optimized SPE–LC–MS method allows determination of DA at trace levels (low pg mL^{-1}) in seawater with/without the presence of phytoplankton. The application of SPE clean-up to mammalian fluids and tissue extracts greatly reduced the LC column degradation and MS contamination, which allowed routine screening of marine mammalian samples for confirmation of DA exposure and determination of fluid and tissue DA concentrations in experimental laboratory animals.

1. Introduction

Domoic acid (DA) is a neurotoxin produced by phytoplankton, primarily the diatom *Pseudo-nitzschia*, and can cause intoxication and even mortality of humans and marine mammals [1–4]. *Pseudo-nitzschia* species are distributed worldwide in coastal waters. The concentration of DA in seawater and natural phytoplankton samples is generally low, with a reported high level of DA only about 12 ng mL^{-1} [5,6]. Analytical methods for quantitation of trace levels of DA require either sensitive detectors or sample pre-concentration. The initial published analytical methods for quantitation of DA in seawater employed DA derivatization followed by LC separation and fluorescence detection (FLD) [6–8]. LC–FLD methods are generally labor-intensive and sometimes show poor selectivity [9]. Later, LC–MS based methods emerged and provided unequivocal confirmation and/or quantitation of DA in seawater and phytoplankton samples [5,9–12]. The analysis of DA in shellfish by LC–MS has been well established for over 15 years; however, unlike shellfish samples, seawater with or without phytoplankton contains high levels of salts, which can cause severe MS contamination if salts are not removed before LC injection or diverted to waste before LC eluant enters MS [5]. Reversed-phase solid-phase extraction (SPE) cartridges [5] and disks [11], and

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resin-based SPE [12] have been employed for sample desalting and clean-up for LC–MS analysis of DA. Reports on pre-concentration of DA in samples are very limited. Chan et al. used an amorphous titania sorbent to extract and concentrate DA from seawater [13], but the high ionic strength buffer used to release DA from the sorbent limits its direct injection for LC–MS analysis. de la Iglesia et al. used reversed-phase extraction disks to pre-concentrate DA in seawater about 20-fold with a 75 mL sample volume [11]. However, 14.5% of DA was lost during loading of 50 mL seawater to SPE disks and the extraction efficiency of DA was around 85% with a loading volume of 75 mL seawater. They claimed an internal calibration instead of an external calibration corrected the DA loss at the SPE step, which brought the DA measurement trueness to above 90%; however, no internal calibration method is described in their report [11]. Pre-concentration of DA at the LC column head using an LC gradient starting with a weak mobile phase [11], in combination with a trifluoroacetic acid (TFA) addition to reduce the total charge of DA in solution and also as an ion pairing agent to increase DA retention on LC, allows large-volume injections of sample extracts [9]; An injection volume of 100 μL simplifies sample pre-treatment and enables UV to quantify trace levels of DA. However, DA showed degradation with time after the addition of TFA to samples and LC–MS showed variable results for quantitation especially at low DA concentrations [9]. Since the samples were not desalted, the turbo gas temperature (Tem) for the MS was set at 275 °C [9], which was out of the general working range for that MS system with LC running at a flow rate of 0.2 mL min^{-1}, and thus reduced the sensitivity of DA detection. We previously reported an SPE method that enabled DA to be adsorbed by a C18 reversed-phase sorbent for desalting seawater and phytoplankton samples for LC–MS/MS quantitation, but the DA concentration was diluted 2-fold [5]. In this report, we improved the SPE method to allow both sample desalting and a 20-fold pre-concentration of DA in seawater and phytoplankton samples.

DA-producing phytoplankton are prominent among the groups of harmful algae associated with severe wildlife mortality events. Over the last two decades, the number of marine mammal deaths in the U.S. attributed to DA intoxication was estimated in the thousands [14]. In a DA-associated, large scale mortality of California sea lions (over 400 sea lions died during May and June 1998), the reported DA concentrations using RBA (detection limit 0.05 μg mL^{-1}), LC-UV (detection limit 0.5 μg mL^{-1}), and LC–MS/MS (qualitative confirmation), ranged from undetectable to 182 μg mL^{-1} in feces [3,15], from undetectable to less than 15 μg mL^{-1} by LC-UV and RBA in urine, generally undetectable for all the methods except for 2 samples with DA detected by RBA in serum, undetectable in kidney, stomach washing, cerebrospinal fluid, and brain samples [15]. The negative DA values for gastric contents during the 1998 sea lion mortality event were assumed to be related to empty stomachs caused by vomiting and the time elapse between digestion and post-mortem examination [15]. High detection limits of RBA and LC-UV, and a lack of effective sample clean-up for the LC–MS/MS method may have contributed to the undetectable DA levels in these samples. Tissues and fluids from stranded marine mammals in the U.S. are routinely screened for DA by LC–MS/MS in the authors' laboratory. Common samples available from stranded marine mammals for DA analysis are urine, gastric contents, feces, and intestinal contents. Samples from marine mammals were initially screened for DA by RBA [16]; however, the RBA can exhibit matrix interference with certain tissues from such samples (unpubl. observ., NOAA). Antibody based assays in various formats (e.g., enzyme-linked immunosorbent assay (ELISA) [17] and surface plasmon resonance (SPR) [18]) have been used for DA analysis, but LC–MS/MS is generally required for the confirmation of DA in marine mammalian samples. Tissues and fluids from marine mammals generally contain high concentrations of salts and/or biological macromolecules, which interfere with LC–MS analysis through increased LC column backpressure, degradation of LC columns, and loss of MS sensitivity. Severe contamination by charging the MS ion optics in the high vacuum region (e.g., the first scanning quadrupole of a triple quadrupole MS) can be caused by DA samples with a high salt concentration since DA elutes at a high percentage of water. It is also very common that aqueous methanol extracts of mammalian samples show precipitates after storage in refrigerators and these precipitates remain insoluble even after vortex mixing at room temperature. Marine mammals found stranded hours to days after death generally have more complex matrix problems on LC–MS analysis than live or healthy animals (e.g., blood-like material observed in some feces and urine samples from stranded mammals and gas generation from some of the acidified urine samples). Acidification of "ink" samples (a type of intestinal contents unique to marine mammals of the genus *Kogia*) caused precipitation, which could create LC column problems when using an acidic mobile phase for direct injection without SPE clean-up. We found the above reversed-phase SPE method developed for seawater and phytoplankton samples was also suitable for clean-up of mammalian samples after modification. The modified SPE method accommodates mammalian fluid samples with limited available sample volume and tissue extracts in aqueous methanol, and thus reduces the frequency of instrumental maintenance required for consistent performance.

Lab animals exposed to DA are commonly used as models for human and marine animals and analysis of DA is required for toxicokinetic evaluation. DA concentrations in fluids and tissues of lab animals dosed with DA are generally low (ng mL^{-1}) [19] and samples from these DA-exposed animals have biological matrix characters similar to those from marine mammals. These samples were subjected successfully to the same or similar SPE clean-up procedures used for marine mammals for determination of DA by LC–MS.

2. Experimental

2.1. Chemicals

Domoic acid certified calibration solutions were purchased from Certified Reference Materials Program, National Research Council, Halifax, NS, Canada. Acetonitrile, methanol, and water were of HPLC grade. For sample preparation, Milli-Q water was used. Formic acid (Guaranteed Reagent, EMD brand, minimum 98%) was purchased from VWR International (USA). Ammonium hydroxide (28–30%) and ammonium acetate (Sigmaultra) were purchased from Sigma (USA).

2.2. SPE method development

The SPE breakthrough volume and elution volume for DA were evaluated on Agilent Bond Elute C18 SPE cartridges in different formats of sorbent mass and cartridge volume (Bond Elut C18, 40 μm; Agilent, CA, USA; previously as Varian, CA, USA). DA (0.5 or 1 μg) dissolved in 1 mL SPE washing solvent was loaded on an SPE cartridge pre-conditioned with methanol first and then water. The washing solvent for the cartridge was tested with different percentages of formic acid in 5% (5:95, v/v) methanol/water or in water. DA was eluted in 50% (50:50, v/v) methanol/water as before [5] or 20 mM ammonium acetate in 50% methanol at pH 8. The DA elution buffer was prepared by dissolving ammonium acetate (40 mM) in Milli-Q water, adjusting pH to 8.00 using 1% ammonia with a pH meter (Denver Instrument, Arvada, CO, USA) and then adding an equal volume of methanol. The elution volume of DA from a specific cartridge was examined with 50% methanol and 50% methanol containing ammonium acetate.

2.3. Sample extraction and clean-up

2.3.1. Seawater samples without phytoplankton for dissolved DA (extracellular DA) determination

Natural seawater (salinity ca. 30) was filtered using a Durapore membrane (0.22 μm pore size; Millipore, Billerica, MA, USA). A 30.0 mL aliquot of filtered seawater sample was placed in a 50 mL plastic conical centrifuge tube and acidified with 0.303 mL 20% aqueous formic acid (formic acid: water, 20:80, v/v) to yield 0.2% formic acid in the sample. After vortex mixing, the sample was desalted and extracted for DA by solid-phase extraction (SPE) using a Bond Elut C18 LRC 10 mL column (200 mg, 40 μm; Agilent). The SPE column was conditioned with 1 column volume of methanol followed by 1 column volume of water. The acidified seawater sample was passed slowly through the SPE column drop by drop (flow rate ca. 1 mL min^{-1}) using a vacuum manifold, followed by 10 mL of 0.2% aqueous formic acid (formic acid:water, 0.2:99.8, v/v) as a rinse of the sample tube and the SPE column. DA adsorbed to the cartridge was eluted with 1500 μL 50% methanol/water or 20 mM ammonium acetate in 50% methanol at pH 8 into a 2 mL microcentrifuge tube. The microcentrifuge tube was vortexed to homogenize the solution, which was then transferred to an LC vial for LC–MS analysis. Determination of DA recovery from seawater was performed by adding DA followed by 30.0 mL of filtered and autoclaved DA-negative seawater to a 50 mL plastic conical centrifuge tube and then vortexed. DA was extracted according to the procedure described above.

2.3.2. Seawater samples containing phytoplankton for dissolved (extracellular), particulate (intracellular), and total (extracellular and intracellular) DA determination

A clone of DA-producing *Pseudo-nitzschia multiseries* CLNN17, provided by Dr. S. Bates (Dept. of Fisheries & Oceans Canada, Moncton, NB, Canada), was used for this study. Batch cultures of 250 mL were maintained at 20 °C in two 500 mL glass Erlenmeyer flasks. Cultures were grown in natural seawater (salinity: 30) amended with *f*/2 nutrients (+104 μM Si) and trace metals with 0.01 μM selenium added [20] on a 14 h:10 h light:dark cycle with a photon flux density of ~80 μmol m^{-2} s^{-1} (model QSL; Biospherical Instruments, San Diego, CA, USA). The culture contained 40,100 cells mL^{-1} at the time of harvesting.

The two 250 mL *Pseudo-nitzchia* cultures from two flasks were combined into a 1000 mL solvent reservoir bottle. Immediately before each aliquot was removed for processing, the culture was mixed gently to homogenize. Whatman GF/F glass fiber filters (25 mm diameter; Whatman, USA) were used to separate and collect the cells from 30.0 mL of medium. The combined filtrates were homogenized by vortexing. For determination of dissolved DA in the culture medium, 30.0 mL of filtrate were pipetted into 50 mL plastic conical centrifuge tubes for extraction of DA as described above for extraction of DA from seawater without phytoplankton. Each filter was placed in a small tube and stored at −20 °C at least overnight (field samples with phytoplankton collected on filters are frozen at least overnight before processing). The method of Bates et al. was used to directly measure particulate DA in seawater and cultures [21]. The filter and accompanying cell pellet were placed in a dual smooth glass grinding tube with a fitted teflon pestle, and 2.5 mL of 10% methanol/water (methanol:water, 10:90, v/v) were added to the tube. The filter was ground with the teflon pestle at 250 rpm (model RZR-2000; Caframo, Wiarton, ON, Canada) at room temperature until no filter paper particles were observed. The filter paper solution was vortexed and then transferred to a 15 mL plastic conical centrifuge tube. The cells were disrupted by sonication using 500 W sonic disrupter at 30% amplitude of power setting for 2 min (1/8 in. micro tip probe, Sonifier 450; Branson, Danbury, CT, USA) on an ice slurry to release intracellular DA into solution. The sample was centrifuged and then filtered with a 0.2 μm GHP syringe filter into an LC vial for LC–MS analysis. For direct determination of total DA (including dissolved and particulate DA), a 30.0 mL whole culture sample was sonicated with the micro tip probe in a 50 mL conical centrifuge tube on an ice slurry to disrupt the cells and release intracellular DA into the seawater. A volume of 0.3 mL of 20% aqueous formic acid was added to the sample tube to yield about 0.2% formic acid in the sample. After vortexing, the sample tube was centrifuged (IEC centra CL3R; Thermo Scientific Waltham, MA, USA) at 2750 × *g* for 5 min and the supernatant was loaded on a 200 mg C18 SPE cartridge (10 mL volume format) preconditioned with 1 column volume of methanol followed by 1 column volume of water. Ten milliliters of 0.2% aqueous formic acid were used to rinse the sample tube. After vortexing and centrifuging the sample tube, the supernatant was transferred to the SPE cartridge. DA was eluted in the same way as described for dissolved DA in seawater samples.

2.3.3. Mammalian fluid samples

Mammalian fluids, if frozen, were first thawed at room temperature and then vortexed. Urine was centrifuged before sampling. A certain volume of the fluid (1000 μL for urine supernatant, 500 μL for plasma, and < 500 μL for amniotic fluid and cerebrospinal fluid; sample volumes ≤ 500 μL were limited by the material available.) was placed in a 2 mL microcentrifuge tube. For urine samples from field stranded mammals, 5 mL microcentrifuge tubes were used as gas generation after acidification could occur and force open caps of the smaller tubes resulting in sample loss. For urine samples, 26 μL of 20% aqueous formic acid were added to yield 0.5% formic acid in the sample and the solution was vortexed (for urine samples from field stranded marine mammals, pH should be checked by applying ~0.3 μL of the sample solution to pH paper, as any gas generation consumes acid; more 20% aqueous formic acid should be added until pH < 3). For plasma, amniotic fluid, and cerebrospinal fluid, aqueous formic acid was added to yield a final concentration of 0.5% formic acid in a 1 mL total volume. The sample was then vortexed and centrifuged for about 5 min at ~2750 × *g* (IEC centra CL3R or Minispin plus; Brinkmann Instruments, Westbury, NY, USA). The supernatant was loaded on a Bond Elut C18 SPE (50 mg, 1 mL) preconditioned with 1.5 column volumes of methanol followed by 1 column volume of water. The sample tube was washed with 1.5 mL of 0.2% aqueous formic acid, centrifuged (1 mL wash volume was used for sample volumes ≤0.5 mL), and the supernatant transferred to the SPE cartridge. The cartridge was further washed with 1 mL 0.2% aqueous formic acid (this step was omitted for sample volumes ≤0.5 mL). In general, DA was eluted with 1000 μL 20 mM ammonium acetate in 50% methanol at pH 8. However, an elution volume of 800 μL was used for cerebrospinal fluid to increase the toxin concentration in SPE eluates, as the available sample volumes were <200 μL and more than 95% of DA was eluted with the first 750 μL of solvent (for non-urine samples, 1000 μL 50% methanol were also used as a DA elution solvent).

Mammalian plasma (heparinized), amniotic fluid, and cerebrospinal fluid samples were available only from lab animals (i.e., pregnant Sprague-Dawley female rats dosed with DA at 1.0 mg kg^{-1} by I.V.). Insufficient or no material for these sample types were available from field stranded marine mammals. Urine from stranded marine mammals generally contained precipitates. For evaluation of possible adsorption of DA on urine precipitates, urine sampling (1000 μL) prior to centrifugation was also tested: a urine sample was thawed at room temperature, vortexed, and then settled for about 10 min so that part of the precipitate sank to the bottom. A sample volume of 1000 μL was placed in a microcentrifuge tube and acidified for extraction of DA.

2.3.4. Mammalian tissue samples

For marine mammalian tissue samples, DA was extracted by adding four volumes of 50% methanol/water to a homogenized sample. After 2 min of probe sonication (1/8 in. micro tip probe, Sonifier 450) on an ice slurry, the sample was centrifuged (IEC centra CL2; Thermo Scientific, Waltham, MA, USA) at 3400 × *g* for 10 min. A volume of 500 μL of the supernatant was diluted to a total volume of 5 mL with water and 250 μL of 20% aqueous formic acid to yield 1% formic acid in the final volume (for gastric samples from stranded mammals, pH should be checked by applying ~0.3 μL of the sample solution to pH paper; sufficient 20% aqueous formic acid should be added until pH < 3). The sample was vortexed and then centrifuged at ~2750 × *g* for about 5 min. The supernatant was loaded on a 200 mg, 10 mL Bond Elut C18 SPE cartridge pre-conditioned with 1column volume each of methanol and then water. The sample tube was further washed with 3 mL 0.2% aqueous formic acid. After mixing and centrifuging, the supernatant was transferred to the SPE cartridge. DA was eluted with 1250 μL 20 mM ammonium acetate in 50% methanol at pH 8 into a 2 mL microcentrifuge tube.

For tissues of laboratory mammals exposed to DA, toxin was extracted using a previously reported method [19]. Briefly, tissues (brain and placenta; same animals used in the method development for lab animal fluid samples) were homogenized with an equal volume of 10 mM PBS with 10% methanol and 0.05% Tween 20 and then 3 times the homogenate volume (or aliquot thereof) of 50% methanol were added for extraction. After centrifugation, a volume of supernatant was pipetted into a 15 mL centrifuge tube. Water and formic acid were added to yield 5% or less of methanol and 1% formic acid in the final 5 mL solution. The acidified tissue extracts were processed with the SPE procedure described above for the marine mammalian tissues with 0.5% instead of 0.2% aqueous formic acid as the washing solvent and 50% methanol instead of 50% methanol in buffer as the elution solvent.

2.4. Liquid chromatography–mass spectrometry (LC–MS)

The LC–MS method used to determine DA is similar to that outlined by Wang et al. [5] with some modifications. The method utilized an HP1100 binary LC system (Agilent Technologies, Palo Alto, CA, USA) coupled to an AB Sciex API 4000 triple quadrupole mass spectrometer equipped with a Turbo V^{TM} source (AB Sciex, Foster city, CA, USA). A diverter valve was installed before the MS to divert the LC eluant to waste or to the MS. LC separations were performed on a Luna C18(2) column (150 mm × 2 mm, 5 μm; Phenomenex, Torrance, CA, USA) with a flow rate of 0.2 mL min^{-1}. Mobile phase consisted of water (A) and acetonitrile (B) in a binary system, with 0.1% formic acid as an additive. For evaluation of the relative amount of DA at each elution step of the SPE method development, the LC was operated under isocratic conditions: 25% or 30% B for 4 min with an injection volume of 5 μL. For quantitative determination of DA in all the samples except urine, the LC was operated using a gradient elution: 3 min of 5% B, linear gradient to 35% B at 16 min, 90% B at 18 min, hold for 5 min, then return to initial conditions at 24 min and hold for 5 min before the next injection; the injection volume was 10 μL for seawater samples, algal samples, lab mammalian fluids and tissues samples, and 5 μL for marine mammalian samples. For quantitative determination of DA in urine samples, the LC gradient conditions were: 5% B for 2 min, linear gradient to 12% B at 2.5 min and hold for 2.5 min, linear gradient to 24% B at 12 min then 85% B at 14 min and hold for 5 min, return to 5% B at 20 min, and hold for 5 min before the next injection; the injection volume was 5 μL. The detection of DA by mass spectrometer was achieved by multiple reaction monitoring (MRM) in positive ion mode. Four MRM transitions from the protonated DA ion were monitored: *m*/*z* 312 → 266, *m*/*z* 312 → 248, *m*/*z* 312 → 193, and *m*/*z* 312 → 161. The ion spray voltage (IS) was 5 kV and the declustering potential (DP) was 69 V. The turbo gas temperature (Tem) was 425 °C for SPE eluates in 50% methanol and 400 °C for SPE eluates in 50% methanol containing ammonium acetate. The collision energy (CE) was 23 eV for the precursor/product pairs *m*/*z* 312 → 266 and *m*/*z* 312 → 248, 26 eV for *m*/*z* 312 → 193, and 33 eV for *m*/*z* 312 → 161. For all the quantitative analysis of DA, the LC eluant was sent to waste except for the 6 min window bracketing the retention time of DA that was sent to the MS. For routine sample analysis, DA standards were prepared in 10% acetonitrile/water (10:90, v/v) as DA is more stable in acetonitrile/water than methanol/water (Section 3.1.2). DA LC–MS chromatograms showed no discernible differences (retention time, peak shape, and peak area) with DA standards prepared in 10% acetonitrile/water, 20 mM ammonium acetate in 50% methanol at pH 8, or 50% methanol when the LC elution gradient started with a high percentage of water (e.g., 95%).

3. Results and discussion

3.1. SPE method development

3.1.1. Development of solid-phase extraction procedures

DA is hydrophilic due to its four chargeable groups (three carboxyl groups with pK_a 1.85, 4.47, and 4.75 and one amino group with pK_a 10.6 [22]); acidic conditions partially protonate the carboxyl groups and thus enhance the hydrophobicity of the DA molecule, which allows DA to be reproducibly retained on the Bond Elut C18 SPE sorbent with a low percentage of formic acid in the SPE loading and washing solvents [5]. We previously reported the breakthrough volume of DA on a 500 mg (10 mL) SPE cartridge with low percentages of methanol in the SPE loading and washing solvents: DA started eluting from the cartridge at the 3rd 5 mL application of 5% methanol/water with formic acid (1% or 2% formic acid as the loading and washing solvents); DA started eluting at the 2nd 5 mL with the methanol concentration increased to 12%, and the 1st 5 mL application with the methanol level increased to 15% in the loading and washing solvents. We did not reach the breakthrough volume in the absence of organic solvents in the loading and washing solutions [5]. As the recovery of DA was not affected by the percentage of formic acid in the range of 0.1–2% in the SPE loading and washing solvents [5], the breakthrough volume of DA with water containing a low percentage of formic acid as the loading and washing solvents was tested for the Bond Elut C18 SPE cartridges (500 mg, 10 mL) (Table 1). DA was not detected in the 98 mL loading and washing solvents with the formic acid additive at both 0.2% and 0.5% levels, whereas DA started eluting (less than 0.5% of total DA) in the following 12 mL of solvent wash. The above results clearly indicated that the absence of organic solvents (e.g., methanol) in the washing solvent greatly increased the breakthrough volume of DA on the Bond Elut C18 sorbent (i.e., about 10-fold increase compared to the addition of 5% of methanol in the loading and washing solvents on a 500 mg sorbent, 10 mL cartridge). Since DA detection was not consistent with the drying of DA SPE eluates [5,11], the SPE eluates were directly injected into LC for analysis after vortex mixing. With the reduction of sorbent mass, the elution volume of DA should be reduced too, which would allow use of a smaller sample volume for extraction of DA without greatly reducing the pre-concentration factor. Therefore, the breakthrough volume and the elution volume of DA on different formats of Bond Elut C18 cartridges with sorbent mass equal to or less than 500 mg were investigated with a low percentage of formic acid aqueous solution as the washing solvent and 50% methanol as the elution solvent. The results of breakthrough volume tests are summarized in Table 1. Washing solvent with 0.1% formic acid aqueous solution was not

Table 1
Breakthrough volume of DA on different formats of Bond Elut C18 SPE cartridges.

SPE cartridge format (mg, mL)	Loading and washing solvents	Volume (mL) with no DA detected	Additional volume (mL) with DA detected (percentage)
500, 10	0.2% or 0.5% HCOOH in H_2O	98	12 (<0.5%)
200, 10	0.2% HCOOH in H_2O	40	5 (~0.3%)
100, 10	0.2% HCOOH in H_2O	14	5.5 (~0.2%)
100, 3	0.2% HCOOH in H_2O	9	4 (<0.1%)
50, 1	0.2% or 0.5% HCOOH in H_2O	4	4 (<0.3%)
50, 3	0.5% HCOOH in H_2O	0	4 (~0.4%)
500, 10	2% HCOOH 5% CH_3OH in H_2O	10	5 (<0.3%)
500, 10	1% HCOOH 5% CH_3OH in H_2O	10	5 (<0.1%)
200, 10	1% HCOOH 5% CH_3OH in H_2O	8	4 (~3%)
200, 10	0.5% HCOOH 5% CH_3OH in H_2O	8	4 (~0.7%)
200, 10	0.2% HCOOH 5% CH_3OH in H_2O	4	4 (<0.1%)

considered in the SPE method development as the recovery of DA from algal cultures using the method described in Section 2 was several percent less using 0.1% formic acid (vs. slightly higher concentrations) as the added acid concentration in the seawater and the washing solvent. The results in Table 1 indicate that sorbent packing affects the breakthrough volume of DA. For the 100 mg sorbent mass, the diameter of the sorbent bed in the 10 mL cartridge is much smaller than in the 3 mL cartridge whereas the length of sorbent bed is longer. Similar discrepancies exist between the 50 mg sorbent mass in 1 mL versus 3 mL cartridge formats with a 1 mL cartridge having smaller diameter and longer sorbent bed. Breakthrough volume of DA was higher for the small diameter (cross section) sorbent bed cartridges. The SPE works analogously to HPLC [23]. The calculated retention volume V_R of the loaded DA can be estimated from the equation: $V_R = V_m(1 + k)$. As the sorbent retention volume V_m is the same for the same sorbent mass in different formats (e.g., 100 mg sorbent mass in a 10 mL or 3 mL cartridge) and the retention factor k is the same regardless of the cartridge format as the stationary phase and the mobile phase are the same for DA application. The calculated retention volume V_R for DA is the same for different diameters of the sorbent bed with the same sorbent mass. The calculated peak volume V_P of DA can be estimated from the equation: $V_P = 4V_m(1 + k)/N^{1/2}$. When the flow rate of the cartridge is optimized to give an equivalent linear velocity that is proportional to the square of the diameter of the sorbent bed, the column plate height is not changed and therefore the column plate number N decreases with the short sorbent bed length; the peak volume V_P of DA is higher for the bigger diameter of the sorbent bed with the same sorbent mass and thus the breakthrough volume of DA is reduced corresponding to the increase of diameter of the sorbent bed. It can be deduced that the DA breakthrough volume will be greatly reduced when the sorbent bed is packed even thinner to a disk format to increase the SPE flow rate for speeding up the processing of large sample volumes (e.g., seawater samples), which may result in the elution of a small amount of DA during the sample loading step. Around 90% or higher of DA was eluted in 50% methanol/water for different cartridge formats with their elution volume ratio equal to their sorbent mass ratio; a slightly larger elution volumes were adopted to allow maximum recovery of DA from the SPE cartridges. For DA sample application in this report, the choice of the sorbent packing format for an SPE cartridge was based on the DA concentration in the matrices, pre-concentration factor after SPE extraction and clean-up, available sample volume, and reasonable sample processing time on SPE.

3.1.2. Stability of DA in SPE eluates

DA was reported unstable in acidic conditions [9,24]. We did not observe DA degradation in SPE eluates with 50% methanol as elution solvent after repeating the initial LC–MS analysis of seawater samples after six weeks of storage at 10 °C; however, when repeating the LC–MS analysis 6 months after the initial tests of the same solutions we did observe that DA degraded about 20% for SPE extracted seawater samples. For lab mammalian fluid and tissue samples, DA loss was above 50% after 14 months of storage of the SPE processed samples at 10 °C. Relative amounts of residual formic acid inside the SPE cartridge system after the DA SPE loading and washing steps were evaluated by replacing the elution solvent with Milli-Q water for accurate pH meter measurement; in general, the pH was about or below 3 for all the SPE methods described above. DA stability was tested by splitting the SPE eluates (6 mL 0.5 M NaCl in Milli-Q water spiked with DA to replace a 30 mL seawater sample and 50% methanol/water or 50% acetonitrile/water as the SPE elution solvent) into several fractions: fraction 1, direct injection to LC–MS; fraction 2, re-dissolved in the same volume of water after drying in a turboevaporator at 34 °C; fraction 3, re-dissolved in water after drying the eluate with the addition of a small volume of formic acid to yield 0.1% formic acid in the solution; fraction 4, re-dissolved in water after drying the eluate with the addition of 10 mM CH_3COONH_4 aqueous solution at pH 9.5 in a 22% volume ratio. Addition of CH_3COONH_4 aqueous solution to the SPE eluates (50% CH_3OH or CH_3CN) or formic acid to reach 0.1% in 50% CH_3CN eluates did not show DA loss after drying in comparison with non-dried eluates. Addition of formic acid to reach 0.1% formic acid in 50% CH_3OH eluates could result in more DA loss (about 50% DA from total) after drying. DA generally showed losses after drying in 50% CH_3OH eluates, but losses were not always apparent after drying in 50% CH_3CN eluates. CH_3OH instead of CH_3CN was used in the elution solvent as DA showed peak splitting with increasing CH_3CN in the injection solution and the SPE eluates are injected directly for analysis [5]. In order to overcome DA instability in the acidic eluates, 0.2% aqueous formic acid was used as the washing solvent in the last SPE cartridge wash step and 20 mM ammonium acetate in 50% methanol at pH 8 was used as an alternate or replacement for the SPE elution solvent. The pH of the eluates was increased to about 5 or above 5 for all the SPE methods described above. A value of pH 8 for the SPE elution solvent was used as the Bond Elut C18 sorbent is a silica-based sorbent. The effect of elution buffer concentration on DA detection by LC–MS is discussed in the LC–MS conditions (Section 3.2). On the other hand, the problem of DA instability in the acidic SPE eluates can be solved by increasing its pH through the addition of the buffer solution (e.g., ammonium acetate buffer at pH 9–9.5; 100 μL of the buffer can be added to 1400 μL of 50% methanol eluate of a DA seawater sample with 1400 μL as the SPE elution volume or to 900 μL of 50% methanol eluate of a mammalian fluid sample with 900 μL as the SPE elution volume; for mammalian tissue samples, the buffer can be added to 1250 μL eluates.). For seawater samples and laboratory DA-exposed mammalian fluid and tissue samples, no difference in DA analysis by LC–MS was observed for the SPE elution solvent with or without ammonium acetate buffer; however, for field marine mammalian fluid and tissues samples, the SPE elution solvent with ammonium acetate buffer in general yielded less matrix interference for DA LC–MS analysis than the elution solvent

without the buffer even though the matrix problem varied with the individual sample (Section 3.4).

3.2. LC–MS conditions

Four fragments (*m*/*z* 161, 193, 248, and 266) of the protonated DA ion at *m*/*z* 312 were selected for analysis by the MRM method. The major fragment was the one at *m*/*z* 266; therefore, the MRM channel *m*/*z* 312 → 266 was generally used for quantitation. The relative intensity of the other three fragments decreased in the sequence of *m*/*z* 161, 193, and 248 under the experimental conditions described above. The MRM channel of *m*/*z* 312 → 161 was generally used for confirmation with the S/N at least 3 to 1. Occasionally, a non-primary fragment was used for quantitation when other DA fragments with higher relative intensity were also present as matrix ions. For marine mammalian samples, it is not uncommon to observe the matrix ions with two MRM channels overlapped with those of DA at the same LC retention time.

As we routinely analyze many DA samples in different types of matrices by LC–MS, DA sample extracts without SPE clean-up were also tested for direct injection into LC–MS with the adjustment of the LC–MS method in order to reduce the sample preparation time. The static mixer in the LC binary pump was replaced with a tube to reduce the system dead volume and the LC column equilibration time. The LC gradient elution for DA quantitation started with 6 min instead of 3 min of 5% acetonitrile to wash out the salts from the injected samples into waste through the diverter valve. The MS parameter that has a significant effect on the MS contamination on the DA application is the turbo gas temperature (Tem). DA is a hydrophilic compound and can elute at a high percentage of aqueous mobile phase. Increasing the turbo gas temperature (Tem) usually increases the sensitivity of DA detection, but also the amount of the salts present in the samples and the mobile phase into the MS thereby increasing the MS contamination. The use of a diverter valve combined with an LC gradient elution and the reduced turbo gas temperature (425 °C) greatly reduced the MS contamination but still could not avoid the occasional contamination of Q0 and Q1 in the high vacuum region of the MS when DA samples were not cleaned by SPE.

Ammonium acetate used in the SPE elution solvent is a volatile salt, which reduces its deposition on the MS interface; however, like nonvolatile salts, it can cause MS signal instability when present in the samples and LC system above a certain level and MS signal suppression at high concentrations. When the MS turbo gas temperature (Tem) was set at 425 °C, the drift of the slope of DA calibration curves (10 μL injection; at least 6 DA standards with DA concentration from 0.2 to 50 ng mL^{-1} bracketing 12 seawater samples with 20 mM ammonium acetate in 50% methanol as the SPE elution solvent) for MRM channel *m*/*z* 312 → 266 was less than 0.5%; the drift of the slope was about 4.5% for the 12 marine mammalian tissue samples. After reducing the Tem to 400 °C and injection volume to 5 μL, the drift of the slope decreased to 1.2% for the same set of standards bracketing the same set of marine mammalian samples. Therefore, the concentration of the ammonium acetate in the SPE elution solvent was set at 20 mM and the Tem was set at 400 °C when the SPE elution solvent contained ammonium acetate and 425 °C when the SPE elution solvent contained no buffer.

For daily analysis of the SPE processed DA samples, the instrumental performance of the LC–MS methods was maintained at a limit of quantitation (LOQ) of about 2 pg DA or less on the LC column with the primary MRM channel *m*/*z* 312 → 266 having a signal to noise ratio of 10 or slightly higher and a limit of detection (LOD) of about 1 pg DA or less on the LC column with the MRM confirmation channel *m*/*z* 312 → 161 having a signal to noise ratio of 3 or slightly higher. For samples expected to contain DA (e.g., lab cultures known to produced DA, field seawater with the presence of DA-producing phytoplankton, and samples from lab animals dosed with DA for toxicokinetic studies), the ion ratio of the confirmation channel to the quantitation channel (e.g., *m*/*z* 312 → 161:*m*/*z* 312 → 266) should be within ±20% of the standards run in the same batch for the samples to be reported as DA positive samples and the specific quantitation channel used for DA concentration calculation. If the ion ratio is <−20% of the standard, the quantitation channel (e.g., *m*/*z* 312 → 266) may have matrix problems. In this case, channel *m*/*z* 312 → 161 and channel *m*/*z* 312 → 193 are evaluated as the quantitation channel and confirmation channel, respectively, and the signal to noise ratios of the corresponding peaks are examined to confirm the requirement of ≥3. If the ion ratio is >20% of the standard, the confirmation channel (e.g., *m*/*z* 312 → 161) may have a matrix issue. For this case, channel *m*/*z* 312 → 193 is evaluated as the confirmation channel. As a worst case scenario, when the two least intense MRM channels were used for quantitation (*m*/*z* 312 → 193) and confirmation (*m*/*z* 312 → 248), the LOQ was about 4 pg DA on the LC column with the *m*/*z* 312 → 193 channel having a signal to noise ratio of ∼10 and the LOD was about 1.2 pg DA with the *m*/*z* 312 → 248 channel having a signal to noise ratio of ∼3. For samples requiring both DA identification and quantitation, all the four MRM channels should have signal to noise ratios of ≥3 and both the ion ratio and the peak shapes of at least two MRM channels match the standards run in the same batch while ion ratios from other MRM channels ≥ those of the standards; otherwise, further sample clean-up is required to reduce the matrix interference. For samples to establish DA identity (e.g., new phytoplankton species and marine mammal species with DA never being analyzed), complete product ion spectra should be examined against the DA standard.

3.3. Analysis of DA in seawater and phytoplankton

Using the Bond Elut C18 (200 mg, 10 mL) cartridges with a sample volume of 30.0 mL and an SPE elution volume of 1500 μL, DA was pre-concentrated 20-fold. The accuracy of the method for the seawater application was evaluated by spiking DA-free autoclaved natural seawater. The mean recovery for all three concentration levels was ≥94% and the precision determined as relative standard deviation (RSD) was ≤5% using 50% methanol as the SPE elution solvent (Table 2). The results of DA extraction using 20 mM ammonium acetate in 50% methanol at pH 8 as the SPE elution solvent was similar to those obtained using 50% methanol without the buffer as the SPE elution solvent (e.g., for seawater spiked with DA at a concentration of 0.25 ng mL^{-1}, the measured DA concentration was 0.246 ± 0.002 ng mL^{-1} with the recovery of 100% and RSD of 1% for $n = 3$). The LOQ was 10 pg mL^{-1} of DA in seawater with a 10 μL injection at a signal: noise ratio of 10 or slightly higher for the primary MRM channel *m*/*z* 312 → 266 and LOD was about 5 pg mL^{-1} at a signal: noise ratio of 3 for the MRM confirmation channel *m*/*z* 312 → 161. The recovery value for DA from control non-autoclaved seawater was about 5% less than autoclaved seawater.

A clone of *Pseudo-nitzschia multiseries* was used to examine the DA SPE extraction method for seawater containing DA-producing phytoplankton. Particulate DA (intracellular DA) was extracted

Table 2
Recovery of DA spiked in seawater with 50% methanol as the SPE elution solvent.

Spiked concentration (ng mL^{-1})	Measured concentration (ng mL^{-1})	RSD (%) ($n = 3$)	Recovery (%)
0.025	0.025 ± 0.001	2	99
0.25	0.25 ± 0.01	5	99
7.5	7.0 ± 0.1	2	94

Table 3
Analysis of DA in an algal culture with 50% methanol as the SPE elution solvent.

Measured concentration (ng mL^{-1}) mean ± SD, n = 3 (n = 5 for cell)				
Filtrate	Filtrate with DA 0.075 ng mL^{-1} added	Cell	Whole culture	Whole culture with DA 0.5 ng mL^{-1} added
0.074 ± 0.003	0.142 ± 0.002	0.28 ± 0.01	0.40 ± 0.01	0.87 ± 0.02

using the traditional field DA sampling method as outlined in Section 2 [19]. Dissolved DA (extracellular DA) in culture filtrates and total DA (intracellular and extracellular DA) in whole cultures were extracted using the SPE method described in Section 2.3. The accuracy of the SPE extraction of DA in phytoplankton was evaluated with the recovery tests. The quantity of DA standard added was about or slightly above 100% of the original concentration determined through the pre-analysis of one filtrate and one whole culture sample by LC–MS without addition of DA standard. Subsequently, all samples including filtrates without and with DA spikes (n = 3 for each set; DA was spiked to each filtrate sample tube) and whole culture samples without and with DA spikes (n = 3 for each set; DA was spiked to each whole culture sample tube) were analyzed on the same day. The results are summarized in Table 3, using 50% methanol without ammonium acetate as the SPE elution solvent. The recovery of DA added to the filtrate was 91% and the recovery of DA added to the whole culture was 94%. The concentration of particulate DA obtained through the difference between the whole culture and the filtrate was 0.326 ng mL^{-1}, which is 18% higher than that obtained through DA extraction from the cells collected on the glass fiber filters using the traditional method. Using 50% methanol with ammonium acetate at pH 8 as the SPE elution solvent, the measured DA concentration in the whole culture (0.395 ± 0.002 ng mL^{-1}) was comparable to that (0.40 ± 0.01 ng mL^{-1}) obtained with 50% methanol without ammonium acetate as the SPE elution solvent. The DA cell quota for the culture was about 9.9 fg cell^{-1}.

3.4. Analysis of DA in mammalian fluid samples

As the available sample volume was limited, urine from the same and different marine mammal species (whales, dolphins, and sea lions) were used to evaluate the SPE extraction method and using a single spiked DA concentration level of 20 ng mL^{-1} throughout. Table 4 (DA-negative samples) and Table 5 (DA-positive samples) show DA recovery tests for the field collected stranded marine mammal urine samples using 20 mM ammonium acetate in 50% methanol at pH 8 as the SPE elution solvent. For screening DA in field samples, the urine supernatant was sampled after centrifugation for the SPE clean-up as sampling after centrifugation yielded a slightly better DA recovery (e.g., Urine 1 without blood components showed DA recoveries 98% vs. 90% with and without centrifugation, respectively), or no difference in the DA results (e.g., Urine 6 and the recovery test for Urine 4 with both samples containing precipitates and blood) using 50% methanol as SPE elution solvent (at an early method development stage). Using 20 mM ammonium acetate in 50% methanol at pH 8 instead of 50% methanol as the SPE elution solvent could reduce the matrix interference for DA detection (e.g., the DA recovery of Urine 6 was 80% vs. 47% analyzed with the LC gradient for seawater samples). The LC elution gradient was modified to be less steep than for seawater and plankton samples in order to increase resolution, which frequently yielded much higher DA recoveries (e.g., Urine 3, 81% vs. 72%; Urine 4, 96% vs. 68%; Urine 6, 104% vs. 80%). Stranded animals may show a significant difference in urine from healthy individuals, which complicates the analysis of DA (e.g., Urine 4 and Urine 6 contained a blood-like material with dark red color; Urine 2 and Urine 5 produced gas after addition of formic acid, which required ~2.6% instead of 0.5% acid in the final volume to generate an acidic condition for SPE processing).

The amniotic and cerebrospinal fluids were sampled based on the available volume of individual rats (amniotic fluid < 500 μL, cerebrospinal fluid < 200 μL; each fluid was split into two equal volumes with one receiving a DA spike of ~10 ng mL^{-1} and the other one containing only DA from the dosing). For the amniotic fluid, recoveries were 95% and 96% for two different samples with 0.5% formic acid as the SPE washing solvent and 50% methanol as the elution solvent. For cerebrospinal fluids, recoveries were both 87% for two different samples with 0.2% formic acid as the SPE washing solvent and 50% methanol containing 20 mM ammonium acetate at pH 8 as the elution solvent. For maternal plasma spiked with DA at ~10 ng mL^{-1}, recoveries were 89% and 93% for two different samples with 0.5% formic acid as the SPE washing solvent and 50% methanol as the SPE elution solvent. For maternal plasma using 0.2% formic acid as the SPE washing solvent and 50% methanol containing 20 mM ammonium acetate at pH 8 as the elution solvent, the recovery was 93% for one sample receiving a DA spike of 12 ng mL^{-1} (RSD = 3%, average DA = 1.03 ng mL^{-1} for the original sample set in triplicate; RSD = 1%, average DA = 12.2 ng mL^{-1} for the spiked sample set in triplicate) and 102% for a second sample spiked with 300 ng DA mL^{-1} (RSD = 2%, average DA = 6.4 ng mL^{-1} for the original sample set in triplicate; RSD = 0.6%, average DA = 311 ng mL^{-1} for the spiked sample set in triplicate).

3.5. Analysis of DA in mammalian tissues

Due to the diversity of marine mammals and their food sources, reversed-phase SPE was considered the first step for sample clean-up. The 50% aqueous methanol extracts of gastric contents, intestinal contents, and feces were diluted and acidified to 1% formic acid in the extracts before loading on the SPE cartridges. For most of the samples, addition of formic acid in extracts to 0.2% produced an acidic solution; however, some extracts (e.g., certain gastric contents) required 1% formic acid in the solution to produce

Table 4
Recovery of DA spiked in DA-negative urine from stranded marine mammals.

Sample	Species	Recovery (%)	RSD (%), n = 3
Urine 1[a]	*Tursiops truncatus*	101 (98)	– (2)
Urine 2	*Eumetopias jubatus*	98	2
Urine 3	*Zalophus californianus*	81	1
Urine 4	*Phocoena phocoena*	96	3

[a] For urine 1, due to the limited total sample volume, only one trial was done with ammonium acetate in 50% methanol as the SPE elution solvent; three trials were done with DA eluted from the SPE using 50% methanol without ammonium acetate and the results were listed inside the parentheses.

Table 5
Recovery of DA spiked in DA-positive urine from stranded marine mammals.

Sample	Species	Measured DA concentration (ng mL^{-1}) mean ± SD, n = 3		Recovery (%)
		Original urine	Urine with DA 20 ng mL^{-1} added	
Urine 5	*Tursiops truncatus*	2.4 ± 0.1	18.2 ± 0.7	79
Urine 6	*Kogia sima*	1.5 ± 0.1	22.4 ± 0.2	104

Table 6
Recovery of DA spiked in aqueous methanolic extracts of DA-negative feces and gastric contents from marine mammals ($n = 3$).

Sample	Species	Recovery (%)[a]	Recovery (%)[b]
Feces 1	*Eubalaena australis*	92.5 ± 0.3	101.2 ± 0.9
Feces 2	*Zalophus californaianus*	97.6 ± 0.9	100 ± 2
Gastric contents 1	*Tursiops truncaus*	97.2 ± 0.4	99 ± 1
Gastric contents 2[c]	*Tursiops truncaus*	90.3 ± 0.9	–

[a] In the SPE method, the cartridges were washed with 0.5% aqueous formic acid and eluted with 50% methanol.
[b] In the SPE method, the cartridges were washed with 0.2% aqueous formic acid and eluted with 20 mM ammonium acetate in 50% methanol at pH 8.
[c] Recovery using ammonium acetate in 50% methanol as the SPE elution solvent was not done.

an acidic condition prior to loading on the SPE. For examination of the SPE clean-up procedures for field marine mammalian samples, DA was spiked into sample extracts at 50 ng DA mL^{-1} which was equivalent to about 250 ng DA g^{-1} tissue. Recoveries were above 90% for DA spiked into DA-negative 50% aqueous methanolic extracts of fecal and gastric content samples tested (Table 6). For ink samples, no negative-control samples were available. Two ink samples from *Kogia breviceps* (pygmy sperm whale) were tested and the recovery of DA from the SPE method was obtained through the measurement of the samples before and after addition of DA standard ($n = 3$ for each set) to the sample extracts. The relative standard deviation (RSD) for all the DA concentration measurements was less than 3% and the recovery was 97% or 103% for 'Ink 1' and 89% or 102% for 'Ink 2' using 50% methanol or 20 mM ammonium acetate in 50% methanol at pH 8 as the SPE elution solvent respectively.

The SPE clean-up showed recoveries above 90% for the limited number of fecal, gastric content, and intestinal content (ink) samples with DA spiked into the aqueous methanolic extracts. However, recoveries varied considerably from ~20% to >90%, even within the same species with DA spiked directly into the original tissue samples (Table 7; DA spike level 125 ng DA g^{-1} tissue). Application of the less steep LC elution gradient for urine samples to Feces 3 did not improve its DA recovery, likely due to the loss of toxin via reactions of the sample matrix. The extraction of DA from marine mammalian tissues was based on the single-step dispersive extraction with 50% methanol used with shellfish for regulatory applications [25]. Currently for samples collected from field stranded marine mammals, DA results are only qualitative and are used for routine screening of samples for confirmation of DA exposure. Recovery tests of DA fortified unknown samples should be performed along with direct measurement of original samples without DA spikes to provide quantitative results. Conducting recovery tests for individual samples greatly increases the work load and is not an efficient solution for routine quantitation of numerous field marine mammalian samples. Addition of internal standards to the samples at the extraction step may aid in tracking DA loss during sample processing and evaluating the effect of sample matrix on MS responses.

Table 7
Recovery of DA spiked into the tissues of feces and gastric contents from marine mammals ($n = 3$).

Sample	Species	Recovery (%)
Feces 3	*Balaenoptera acutorostrata*	21.7 ± 0.3
Feces 4	*Balaenoptera acutorostrata*	78 ± 2
Feces 5	*Balaenoptera acutorostrata*	92 ± 2
Feces 6	*Grampus griseus*	88 ± 3
Gastric contents 3	*Kogia breviceps*	93 ± 6
Gastric contents 4	*Tursiops truncaus*	83 ± 3
Gastric contents 5	*Tursiops truncaus*	80.8 ± 0.8

The accuracy of the SPE–LC–MS method on the extraction of DA from rat unborn fetal brain and placenta tissues was evaluated by recovery tests of spiking less than 20 ng DA g^{-1} tissue to each fetal brain and placenta homogenate aliquots from pregnant rats dosed with DA. The recovery of DA was 91%, 105%, and 90% for different placenta samples and 86% and 87% for two different fetal brain samples. Brain sample extracts seemed to contain significant particulate matter, as on one occasion a sample clogged the SPE cartridge even though it had been centrifuged at above 10,000 × g for more than 6 min.

4. Conclusion

SPE–LC–MS methods have been optimized for the determination of the algal neurotoxin domoic acid in seawater with/without plankton and in mammalian tissues and fluids. The optimized SPE method enabled pre-concentration of DA 20-fold in seawater with recovery above 90% by LC–MS determination. The SPE method for DA extraction from seawater was modified to accommodate mammalian tissue extracts in aqueous methanol and fluids with limited available sample volume. The recovery of DA in the laboratory-based mammalian samples (amniotic fluid, cerebrospinal fluid, plasma, placenta, and brain) after SPE clean-up was above 85%. The recovery of DA for samples (urine, feces, intestinal contents, and gastric contents) collected from field stranded marine mammals showed large variations and appeared to be affected by the animals' conditions at the time of sampling. DA showed instability under acidic conditions and ammonium acetate buffer was used as either an alternate or replacement of SPE elution solvent to increase pH of the SPE eluates for long-term storage of the SPE-processed samples. The optimized SPE–LC–MS methods greatly reduces the LC column degradation and MS contamination introduced by these sample types and allows routine monitoring of DA in seawater and biological samples while maintaining consistent instrumental performance for long periods.

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