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Abstract

Amnesic shellfish poisoning is a potentially lethal human toxic syndrome which is caused by domoic acid (DA), a neurotoxin produced by marine phytoplankton, principally from *Pseudonitzschia genus*. In this report, a method to identify and quantify the DA toxin, with simultaneous identification of its photodegradation products has been developed. It uses an Ultra High Performance Liquid Chromatography coupled to a Quadrupole-Time-Of-Flight tandem mass spectrometer (UHPLC-QTOF) after solid-phase extraction An unambiguous identification of DA was carried out by considering both the retention time of DA in UHPLC and the exact mass of protonated DA molecule ($[M+H]^+$= 312.1447 m/z) and of the most intense fragment ion (m/z 266.1391), The quantification was conducted using protonated DA molecule with protonated Glafenin as internal standard, obtaining a LOD of 0.75 µg L^{-1} . Large screening with UHPLC-QTOF could also give structural informations about degradation products of DA present in samples after UV-irradiation. This method was applied for the determination of DA in complex liquid samples after solid-phase extraction, and is applicable for environmental monitoring of this toxic substance in the aquatic environment.

Keywords: Domoic Acid, Toxin, Seawater, Liquid chromatography, Mass spectrometry, Accurate mass

1. Introduction

Domoic acid (DA) was identified as a marine neurotoxin at the end of the 1980s following human poisoning incident in Canada, after consumption of cultured blue mussels *Mytilus edulis* [1]. Red algae and diatoms were found to be primary producers of DA [2], but it is the accumulation of DA in filter-feeding marine organisms which poses the biggest threat to human health. Symptoms produced by this algal toxin include, among other clinical signs, in many of the seriously intoxicated individuals, persistent short term memory loss. The syndrome was thus called amnesic shellfish poisoning (ASP) [3]. DA intoxication in wild animals, such as anchovies, sea lions, whales, sea birds and fishes, has been reported [2, 4-7]. DA is a water soluble, polar, non-protein amino acid, whose chemical structure was determined by NMR [2] and then confirmed following total synthesis [8]. It consists of a proline ring, three carboxyl groups and an imino group, which leads to four chargeable groups that can exist in up to five charged states from
-3 to 1 depending on the pH (Figure 1). At room temperature, DA is relatively stable and does not degrade [9]. At neutral pH, DA has an absorption maximum of 242 nm due to its conjugated diene moiety [5]. DA elimination in the marine environment is essentially by photodegradation *via* sunlight mediated reactions [10]. DA has at least nine geometrical isomers. Among them isodomoic acids D, E and F and the 5'-epi-domoic acid have been isolated from plankton cells and shellfish tissue and have been found to be less toxic than DA [11].

To protect human health and seafood safety, the European Union has established that total DA content must not exceed 20µg DA/g in the edible parts of molluscs [12]. This limit is employed worldwide for harvesting and consumption of shellfish resources to protect human health [13]. Numerous liquid chromatographic methods with ultraviolet diode array detection (HPLC-UVD) can be used following extraction of DA from homogenised tissue by solvent and SPE (solid phase extraction) clean-up [14]. The diene chromophore of DA permits its detection by HPLC–UVD at concentrations as low as 4–80 µg L^{-1} depending on the sensitivity of the detector [15]. To further decrease the LOD, liquid chromatography with fluorimetric detection methodologies (HPLC-FLD)

after derivatisation has been developed in research laboratories for monitoring DA in seafood and marine phytoplankton [13]. Indeed a laboratory culture of diatom genus *Pseudonitschia* produces DA at levels ranging from 1 to 20 pg/cell, with less than1 µg L^{-1} found in the culture medium [16]. In both HPLC-UVD and HPLC-FLD methods, DA is identified based on the coincidence of LC retention time of the suspected chromatographic peaks, with those of DA standard peaks; however, the suspected toxin peaks may represent compounds other than DA. An unambiguous method such as LC–mass spectrometry (LC–MS) must be used to confirm the presence of DA, especially for newly suspected source organisms or for confirming the appearance of DA in a new geographical region. So, even if HPLC-UV methods is often the only analytical tool available in many research institutes and regulatory agencies responsible for monitoring the occurrence of DA, many mass spectrometry methods were developed in different research laboratories [14,17, 18, 19].

Moreover for researchers, the development of very sensitive methods to determine DA in seawater is still a challenge. Indeed the role of dissolved DA in seawater, its distribution patterns across the trophic webs and its production by minimally toxic phytoplankton species are not fully understood.

This study describes a method for unequivocal confirmation of DA and its quantitative analysis in seawater and in complex liquid media by using ultra high performance liquid chromatography coupled to quadrupole-orthogonal time-of-flight tandem mass spectrometer (UHPLC- QTOF), Xevo G2 QTof MS (Waters, Milford, USA), with an electrospray ionization (ESI). Assalts adversely affect ESI performance by making ion formation less reproducible, a SPE method was developed to simultaneously extract DA and remove salts from samples. Conditions affecting the stability of DA were also investigated. The MS^E data collection technique was used, which allows to obtain fragmentation information for all compounds in a single run. Indeed two separate acquisition functions are sequentially measured in full scan mode: one for MS of precursors acquired at low collision cell energy and one for collecting fragmentation data at elevated collision cell energies. The correlation of product to precursor ions is achieved, after deconvolution, by using reconstructed retention time apices and chromatographic peak shapes. In the present case, as

fragmentation information is obtained in advance for all compounds in a single run, it was possible to simultaneously quantify DA and identify non-target degradation products of DA after UV-irradiation in a single run. This UV treatment was performed in order to simulate *in vitro* natural sun degradation of DA. This constitutes a real novelty, offered by the possibility of performing retrospective full data examination, without re-injecting sample.

2. Experimental

2.1. Chemicals and Reagents

Domoic acid (DA) (powder form stored at -20°C) and formic acid (FA) were purchased from VWR International LLC (Radnor, PA, USA). Leibovitz's L-15 medium and fetal bovine serum (FBS, S1520-500) were from Sigma (Steinheim, Germany).

2.2. Extraction of DA from liquid samples by SPE

Oasis® HLB, Hydrophilic-Lipophilic-Balanced, 1 cc Vac Cartridge, 30 mg Sorbent per Cartridge (Waters, Milford, USA) was used for extraction. The choice of this cartridge was also based on pH stability from 0 to 14, absence of silanol interactions, and large use for acid, base and neutral compounds extraction. Leibovitz's L-15 culture medium, which contains many amino acids, vitamins and salts, supplemented with 10% (v/v) foetal bovine serum was used as complex liquid medium, to optimize SPE step. Samples were first spiked with 200 µg L^{-1} of DA, then acidified with 2% FA, vortex-mixed,centrifuged 10 min at 10,000 g and submitted to SPE. No vacuum was applied during sample loading to ensure optimal binding of DA on sorbent. During following steps of extraction, the vacuum was kept approximately at -17kPa. An optimized HLB cartridge protocol was applied as follows: the cartridge was first conditioned with 1mL of methanol (MeOH) and 1mL of water containing 2% FA. Afterwards, 1mL of sample previously acidified with 2% of FA was loaded and washed with 1 mL of H_2O. Finally, DA was eluted with 1mL of MeOH:$H_2$0 (40:60, v/v) with 2% FA. Then 85 µL of the eluate were transferred to ultra high performance liquid

chromatography (UHPLC) vials containing 15 µL of Glafenin (GLF) (5 mg/L) as the internal standard (IS). To investigate the efficiency of the SPE method for DA, RE (Recovery of the Extraction) was determined by comparing the mean peak areas of replicate analyses (n=5) of DA quantification (ratio of DA to IS) obtained before and after SPE extraction (DA spiked at 200 µg L-1), as described by Matuszewski [20]. Assessment of ME (Matrix Effect) was realized by comparing the mean peak area of replicate analyses (n=5) of DA quantification (ratio of DA to IS) obtained in culture medium spiked with DA and IS after SPE extraction and in neat solution standards, as described by Matuszewski [20].

2.3. Salinity measurement

Artificial seawater (33 g/L) was prepared with ready-to-use sea salt containing all 70 trace elements found in natural seawater (Tropic Marin®, Wartenberg, Germany). One part of this artificial seawater solution was spiked with DA (final concentration 340µg L^{-1}) to constitute the sample, and the other part constituted the control. SPE was performed such as previously described in paragraph 2.2 with salvage of each liquid fraction getting through the SPE cartridge.

Salinity and temperature were measured by a conductivity meter Cond 3110 with standard conductivity measuring cell TetraCon 325 (WTW, Germany). Conductivity measuring cell was immersed in a tube with 7 ml of replicate and after 1 minute for stabilization.

2.4. Photodegradation of DA

Photodegradation of DA was obtained by irradiating a solution of 340 µg L^{-1} DA in artificial sea water, in glass container without lid with UV radiation at 254 nm, 6 W, 710 µW/cm^2. The UV lamp was from Vilber Lourmat (Torcy, France). The irradiation experiments were conducted for 3 h with the control sample kept in the dark.

2.5. UHPLC-MS/MS Method

Analyses were performed using an Acquity UPLC H-Class (Waters, Milford, USA) coupled to a

Xevo G2 S Q-TOF mass spectrometer equipped with an electrospray ionization (ESI) source. The chromatographic system consisted of a quaternary pump (Quaternary Solvent Manager) and an autosampler (Sample Manager-FTN) equipped with a 10 µL sample loop. 5 µL of the sample was injected into a Waters Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm). The system was operated under the following gradient elution program: solution A (0.01% FA in H_2O) in solution B (0.01% FA in MeOH) at a flow rate of 300 µL/min as follows: 0-0.2 min, 3% B; 0.2-0.25 min, 3-20% B; 0.25-1 min, 20-55% B; 1-1.5 min, 55-100% B; 1.5-3.5 min, 100% B; 3.5-3.6 min, 100-3% B; 3.6-4.5 min, 3%B. The column and the autosampler were maintained respectively at +25°C and +7°C.

ESI was shown as the optimum ion source interface for DA analysis [21]. Optimization of mass spectrometry parameters was performed in two steps: first, by direct infusion of DA at constant flow of 20 µL min^{-1} and second, by infusion combined with liquid chromatography flow equal to 50µL min^{-1}. Final ESI conditions were: source temperature 120°C, desolvation temperature 500°C, cone gas flow 50 L h^{-1}, desolvation gas flow 1000 L h^{-1}, capillary voltage 2.5 kV, sampling cone 35 and source offset 80. The instrument was set to acquire over the m/z range 50-1200 with a scan time equal to 0.15 s. These conditions gave a resolution equal to 30000 for protonated DA molecule ($[M+H]^+$= 312.1447 m/z. Data were collected in the positive (ESI+) electrospray ionization modes. The MS and the MS/MS experiments were performed using the MS^E function in centroid mode. A MS^E approach consists in MS and MS/MS data acquisitions in a single same run, with no collision energy in function 1 (MS experiment) and a collision energy ramp of 15-45 V in function 2 (MS/MS experiment). Leucine Enkephalin ($[M+H]^+$ = 556.2771 m/z) (1 ng μL^{-1}) was used as lock mass for mass shift correction. The mass spectrometer was calibrated before analyses using 0.5mM sodium formate solution.

DA quantitation was obtained by calibration curve of DA standard reference at the following concentrations: 2.5, 5, 10, 25, 50, 100, 250, 500, 1000µg L^{-1} , prepared by cascade dilution in MeOH:H_20 (40:60, v/v) with 2% FA before each run. After vortex-mixing, 85 µL of each standard

was transferred to UHPLC vials containing 15 µL of GLF (5 mg L^{-1}) as internal standard.

2.6. Analytical validation

Intraassay precision was studied by preparing and analysing five independent replicates of DA quality controls prepared as described above at different concentrations (20, 40, 80, 200, 400, and 800µg L^{-1}) on a given day. Interassay precision and linearity were evaluated from the analysis of a calibration set each day during 5 days.

To evaluate the stability of DA in MeOH:H_20 (40:60, v/v) with 2% FA, that correspond to the injection conditions of DA, extraction of DA was performed as described in 2.2. One aliquot of elution was analysed immediately. Four aliquots of the same sample supernatant were kept at +7°C in the autosampler for 6 h and 22 h, at +4°C in a refrigerator for 4 days and 15 days and at -20°C in a deep-freeze for 24 h prior to analysis. A sixth aliquot was used to study the stability of DA over three freeze (-20°C)-thaw (room temperature) cycles. Three replicates of each aliquot were analysed and compared with independently and extemporaneously prepared calibration curves with DA in powder form stored at -20°C. The mean concentration of DA immediately analysed in triplicate was used as control for comparison with other samples.

2.7. Data analysis

Post-acquisition analyses were performed using the MassLynx™ V4.1 program (Waters, Milford, USA). Using ChromaLynx™ application, compounds were first identified based on their retention time, mass accuracy and fragment confirmation. Then, positively identified compounds in each sample were transferred to quantification using the software TargetLynx™. The MetaboLynx™ application automates the process of peak detection, comparison of data between DA control sample and DA sample after photodegradation and also for filtering the matrix-related peaks. Peaks only present in DA sample after photodegradation are considered as molecules produced by transformation of parent. MetaboLynx™ software used elemental composition to suggest formula

of degradation products. Elemental composition parameters were: 5 ppm mass tolerance, with 0 to 50 for the number of carbon atoms, from 0 to 100 for the number of hydrogen atoms, from 0 to 20 for the number of nitrogen atoms, from 0 to 20 for the number of oxygen atoms and from 0 to 1 for the number of sodium atoms.

3. Results and discussion

3.1. Fragmentation of DA standard

Ionization of DA was better in positive mode than in negative mode and MS conditions were optimized (see detailed values in 2.3 “Material and methods”). DA identification was confirmed by MS and MS/MS fragmentation patterns (Fig. 2). Full mass spectra from DA standard shows the major molecular ion for the toxin at m/z 312.1447 $[M+H]^+$, and a peak at m/z 334.1263 is attributed to the $[M+Na]^+$ sodium adduct ion. The fragmentation profile produced in high collision energy function consists mainly in water (H_2O), formic acid (CH_2O_2) and CO losses (Fig. 2). Table 1 displays elemental composition, corresponding fragmentation or adduct ion, theoretical mass, measured mass and mass errors in ppm of reference DA and its major fragment ions. The maximum mass errors between theoretical and observed values were less than 5 ppm, which means high resolution and good accuracy of measures by theselected method.

Exact mass of the fragmentation of the $[M+H]^+$ adduct ion of DA is detailed in Table 1 and Fig. 2B. The most intense fragment ion (m/z 266.1391) of DA is due to the loss of a H_2O molecule (18 Da). Based on this information, the different fragmentation pathways of DA are proposed in Fig. 3.

Results obtained by exact mass measurements coincide with those reported by other authors using single quadrupole [22], ion-trap single quadrupole [17,23,24], or triple quadrupole with [13,15,25] or without trap technology [21] mass spectrometer.

3.2. UHPLC method optimisation of DA

Efficient separation of DA and GLF from impurities was performed in 4.5 minutes. Peaks of DA and GLF were in the middle of the chromatogram, with retention times respectively equal to 2.22 and 2.39 min (Fig.4)Column temperature was tested, from +25°C to +80°C, with a step of +5°C. No significant difference in DA quantification was observed, except at +80°C where the analyte started to be degraded (data not shown).

In several previous studies the column temperature chosen for liquid chromatographic separation was between +40°C and +70°C [13,15,21,25] but with our system, heating the column above 25°C did not increase the detection nor the quantification limits of DA. The temperature of +25°C was thus adopted.

Mass spectrometer parameters were first optimized by injecting standard reference solution of DA in infusion mode and finalized in combined mode, namely by using a combination of infusion mode with an UHPLC flux (0.05 mL/min.). Generally, for small molecules, the best tension capillary is 0.5 kV in combined mode, but for DA detection, better response was obtained with 2.5 kV, close to value used in infusion mode, 3 kV. DA identification was possible in both positive and negative mode, but positive mode was more sensitive.

DA was identified from MS^E acquisition data by together its retention time (2.22 min.), its mass accuracy given by the elemental composition ($C_{15}H_{21}NO_6^+$) and one chosen fragment (m/z 266.1392) corresponding to elemental composition $C_{14}H_{20}NO_4^+$ to confirm the presence of the molecule. For GLF identification, both retention time (2.39 min.) and mass accuracy ($C_{19}H_{18}N_2O_4Cl^+$): $[M+H]^+$= 373.0955 *m/z* were used. Integration parameters were optimized and mean function was chosen as smoothing method. Itconsists in taking the arithmetical mean of the intensities of the data points in each window along the chromatogram. Identified analytes were quantified using GLF as internal standard.

3.3. Extraction of DA from liquid samples by SPE

According to the HLB generic method, complex liquid samples spiked with DA were acidified

before SPE. This allowed a complete retention of DA on the SPE cartridge, while eluting unretained matrix, with a wash step using 100% water. To finalize the elution step of DA, an optimization approach was used, using 20 different mixtures as elutant solutions, containing 2% FA or 2% ammonium hydroxide (AH) in MeOH:H_2O mixtures with increasing MeOH amounts. MeOH:$H_2$0 (40:60, v/v) with 2% FA solution was selected to be the best elutant solution. It was just strong enough to elute DA while retaining the most hydrophobic interferences on the sorbent. In these conditions, RE of DA was equal to 96 ± 2%, which corresponds to an excellent recovery of DA. ME was equal to 95 ± 2%. This value indicates very slight ionization suppression in the extract compared to the neat solution.

3.4. Desalination of sample by solid-phase extraction

Removing salt in sample before analysis without loss of the molecules of interest is essential for subsequent mass spectrometry. The measure of salinity was performed at each step of the solid phase extraction. The salt concentration in both starting samples (control: artificial sea water and sample: artificial sea water + 0.34 ng/µL DA) was equal to 28.7 g/L. The salts were recovered almost entirely in the "load fraction", respectively at 27.8 and 27.7 g/L, showing that the SPE cartridge did not retained salts on the column. The salinity of the "wash" and "elution" fractions were measured with the conductivity meter Cond 3110. They contained respectively salt concentrations equal to 0.7 and 0.5 g/L, showing that the SPE allowed an almost complete desalinisation of the samples.

3.5. Analytical validation

Calibration curve obtained using linear regression with a weighting factor of $1/x^2$ gave regression correlation coefficients R^2=0.994. The quantification method showed good intraassay precision, with mean relative error (MRE) less than 17.4% and relative standard deviation (RSD) always less than 7.4%. Interessay precision was also good over the concentration range, with MRE inferior to

19.3% and RSD inferior to 13.7%.

DA compound was found to be stable in its injection solvent (MeOH:H_20 (40:60, v/v) with 2% FA) for at least 15 days at +4°C and at least 24 h at -20°C and to tolerate three freeze/thaw cycles, with maximal deviation from initial time equal to 16.6%, 7.7% and 10.2% respectively . The stability of DA in the autosampler at +7°C was demonstrated over 22 h, with 2.9% maximal deviation compared to initial time.

The mean signal/noise ratio (S/N) was obtained thanks to software TargetLynx^{TM}, by using 10 different blank injections. Limits of detection (LOD) was then estimated on the basis of signal/noise ratio (S/N) of three, by injecting solutions with lower and lower DA concentrations. LOD was found to be equal to 0.75 µg L^{-1}. This detection limit value, with Q-TOF method was lower than UV detection (4-80 µg L^{-1}, depending on the sensitivity of the detector [15]), because of the sensitivity and the specificity of the mass detector, and without false positives commonly encountered with UV method. It was also better than MS single quad or orbitrap detection and was equivalent with the LOD obtained with triple-quadrupole MS) [26, 27, 28]. Furthermore,the disadvantage of these SRM or MRM scanning acquisitions is the impossibility to visualize other ions than those isolated as precursor ion prior to the analysis. In the present case however, with MS^E data acquisitions used with Q-TOF, all analytes in sample are detected and saved, including all precursors and their fragments. This allowed to perform the following additional investigation about DA photodegradation products, by reprocessing the data, without performing a new sample injection.

3.6. Identification of DA photodegradation products

UV-irradiation of DA induced the appearance of several peaks after extraction at m/z 312.14 ± 0.02 Da (Fig. 5), that potentially corresponded to degradation products of DA. MetaboLynx^{TM} analysis allowed to identify geometrical isomers of DA based on their measured mass (Table 2, expected products). Fragmentation pattern of these molecules was then manually confirmed from MS^E data, which was similar to the parent DA (Fig.2). Regarding unexpected products coming from photodegradation of DA, two peaks appeared significant in irradiated sample compared to control.

For each of these peaks, many possibilities of elemental composition were proposed by the software (Table 2, unexpected products). With i-FIT values linked to a proposed elemental composition for a given measured mass, from mass error (ppm), retention time (2.54 min.) and assumingimpossible incorporation of more than one nitrogen, the list of proposed elemental composition diminished to finally go to decarboxylated molecules of DA: $C_{14}H_{22}NO_4^+$ (m/z 268.1550) and $C_{14}H_{21}NO_4Na^+$ (m/z 290.1368).

Precursor ion informations with fragment analysis MS^E (MetaboLynx^{TM}) gave complete visualisation of affiliation parent-daughter and daughter-parent ions. Fragment ions combined with elemental composition searched on spectra allowed to determine the photodegradation products (Table 3). The software could also give the probability of the position of the transformation by photodegradation of DA, which allow the structure shown on Figure 6 to be proposed.. Indeed, each major fragment ions of photodegradation product of DA corresponded to the major fragment ions of DA with a loss of CO_2 (m/z 43.9898). For confirmation of the decarboxylated of DA, a MS/MS analysis was realized on m/z 268.15 (Figure 7). All major fragment ions of the decarboxylated molecule were found. Thus, the algorithm used allowed the detection and identification of unknown degradation product, after extraction of ion chromatograms for expected transformation products, based on predicted or unpredicted molecular changes relative to the parent compound DA.

This result is in accordance with previous studies, in which exposure of DA to sunlight modified its chemical structure and produced a suite of isomers (isodomoic acids D, E, or F) and products tentatively identified as decarboxylated derivatives [28]. More recently, the presence of a DA photodegradation product corresponding to a decarboxylation product of DA ($[M+H]^+$ =268) was observed in seawater matrices, after exposure to a solar simulator [29]. In the same study, it was shown that high halides concentrations in sea water increased DA photodegradation and altered its transformation pathway, with the production of a predominant, but unidentified, product ($[M+H]^+$ = 344). This product was not recovered in the present case.

4. Conclusion

The proposed UHPLC–ESI- Quadrupole-Time-Of-Flight tandem mass spectrometry MS^E method after SPE is a useful tool for the rapid and sensitive detection and structural characterization of DA from complex samples. UHPLC gives higher separation efficiency and resolution with much lower solvent consumption than classic HPLC. Q-TOF mass spectrometer allows an unambiguous identification of researched analytes with exact mass determination and simultaneous quantification of DA with a LOD equal to 0.75µg L^{-1}. Moreover, supplementary post-acquisition treatment can be performed to find possible DA transformation products, thanks to specific MS^E acquisition mode of Q-TOF mass spectrometer. This therefore could be an important tool for routine analysis of DA in complex matrices and for the environmental monitoring of this toxic substance in the aquatic environment.

Acknowledgements.

This study was funded by the CPER “Plateforme Littoral” sub-action “Valorisation Biotechnologique des ressources marines littorales” and the Programme Opérationnel FEDER 2007-2013, « Compétitivité Régionale et Emploi » Poitou-Charentes.

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425 Table 1. Accurate mass measurements of domoic acid and its major fragment ions.

Elemental composition	Fragmentation/Adduct ion	Theorical mass m/z	Measured mass m/z	Error (ppm)
$C_{15}H_{22}NO_6Na^+$	$M+H+Na]^+$	334.1266	334.1263	-0.9
$C_{15}H_{22}NO_6^+$	$[M+H]^+$	312.1447	312.1447	0.0
$C_{15}H_{20}NO_5^+$	$[M+H-H_2O]^+$	294.1342	294.1339	-1.0
$C_{14}H_{20}NO_4^+$	$[M+H-CH_2O_2]^+$	266.1392	266.1391	-0.4
$C_{14}H_{18}NO_3^+$	$[M+H-CH_4O_3]^+$	248.1287	248.1283	-1.6
$C_{13}H_{18}NO_2^+$	$[M+H-C_2H_4O_4]^+$	220.1338	220.1335	-1.4
$C_{13}H_{16}NO^+$	$[M+H-C_2H_6O_5]^+$	202.1232	202.1230	-1.0
$C_{12}H_{17}O_2^+$	$[M+H-C_3H_5NO_4]^+$	193.1222	193.1225	+1.5
$C_{12}H_{15}O^+$	$[M+H-C_3H_7NO_4]^+$	175.1123	175.1115	-4.7
$C_{11}H_{13}O^+$	$[M+H-C_4H_9NO_4]^+$	161.0966	161.0965	-0.6

426

427 Table 2. MetaboLynx analysis of artificial seawater spiked with 0.34 ng/µL versus irradiated

428 artificial seawater spiked with 0.34 ng/µL.

Measured mass m/z	Retention time (min)	Area (%)	Elemental composition	Theorical mass m/z	Error (ppm)	Product Name	i-FIT
Expected products of control artificial seawater							
312.1449	2.21	100	$C_{15}H_{22}NO_6^+$	312.1447	+0.7	Domoic acid	-
Expected products of irradiated artificial seawater							
312.1449	1.90	6.51	$C_{15}H_{22}NO_6^+$	312.1447	+0.7	Isodomoic acid	-
312.1449	1.98	13.08	$C_{15}H_{22}NO_6^+$	312.1447	+0.7	Isodomoic acid	-
312.1447	2.09	10.81	$C_{15}H_{22}NO_6^+$	312.1447	+0.0	Domoic acid	-
312.1449	2.18	50.54	$C_{15}H_{22}NO_6^+$	312.1447	+0.7	Isodomoic acid	-
312.1448	2.26	19.06	$C_{15}H_{22}NO_6^+$	312.1447	+0.4	Isodomoic acid	-
Unexpected products of irradiated artificial seawater							
268.1550	2.54	87.01	$C_{15}H_{18}N_5^+$	268.1562	-4.5	-	21.6
			$C_{15}H_{21}N_2ONa^+$	268.1552	-0.7	-	23.3
			$C_{14}H_{22}NO_4^+$	268.1549	+0.4	Decarboxylated domoic acid	24.1
			$C_{13}H_{19}N_5Na^+$	268.1538	+4.5	-	25.0
			$H_{17}N_{14}O_2Na^+$	268.1557	-2.6	-	34.7
290.1368	2.54	12.99	$C_{14}H_{21}NO_4Na^+$	290.1368	0.0	Decarboxylated domoic acid	9.6
			$C_{14}H_{18}N_4O_3^+$	290.1379	-3.8	-	11.0
			$C_{12}H_{16}N_7O_2^+$	290.1365	+1.0	-	16.2
			$C_{15}H_{17}N_5Na^+$	290.1382	-4.8	-	16.3
			$C_{13}H_{22}O_7^+$	290.1366	+0.7	-	16.6
			$C_{12}H_{19}N_4O_3Na^+$	290.1355	+4.5	-	17.4

429 i-FIT: isotopic fit value. The lower the value, the better the fit.

Table 3. Accurate mass measurement of decarboxylated domoic acid and its major fragment ions.

Elemental composition	Theorical mass m/z	Measured mass m/z	Error (ppm)
$C_{14}H_{21}NO_4Na^+$	290.1368	290.1368	+0.0
$C_{14}H_{22}NO_4^+$	268.1549	268.1550	+0.4
$C_{14}H_{20}NO_3^+$	250.1443	250.1445	+0.8
$C_{13}H_{20}NO_2^+$	222.1494	222.1493	-0.5
$C_{13}H1_8NO^+$	204.1388	204.1388	0.0
$C_{12}H_{18}N^+$	176.1439	176.1440	+0.6
$C_{11}H_{17}^+$	149.1330	149.1325*	-3.4

* Molecular ion found in noise, by eliminating CO_2 molecule from $C_{12}H_{17}O_2^+$, the lowest fragment ion of domoic acid including O_2 (m/z 193.1229).

Figure Legends

Figure 1

Structure of domoic acid.

Figure 2

Representative profiles mass spectrum with low energy collision in MS^E mode (parent ion of domoic acid)(A) and mass spectrum with hight energy collision in MS^E mode (fragment ions of domoic acid)(B).

Figure 3

Possible fragmentation pathway for domoic acid.

Figure 4

Separation of DA and GLF by optimized UHPLC method. DA and GLF Extraction Chromatogram and Total Ion Chromatogram. Compound a corresponds to DA at 2.22 min. Compound b corresponds to GLF at 2.39 min and compounds c, d, e,f , g correspond to plastics pollutants.

Figure 5

LC-MS/MS chromatogram after extraction at m/z 312.14 ± 0.02 Da, domoic acid in seawater, before (A) and after (B) UV-irradiation as described in Materials and methods.

Figure 6

Probability of the position of the transformation by photodegradation of domoic acid. Weighted % of the spectral data supporting photodegradation transformation at the position shown.

460 Figure7

461 Representative profiles of MS (A) and MS/MS (B) analysis of the decarboxylated domoic acid.

462

463 Figure 1

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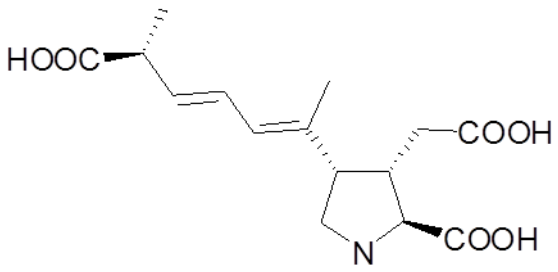

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468 Figure 2

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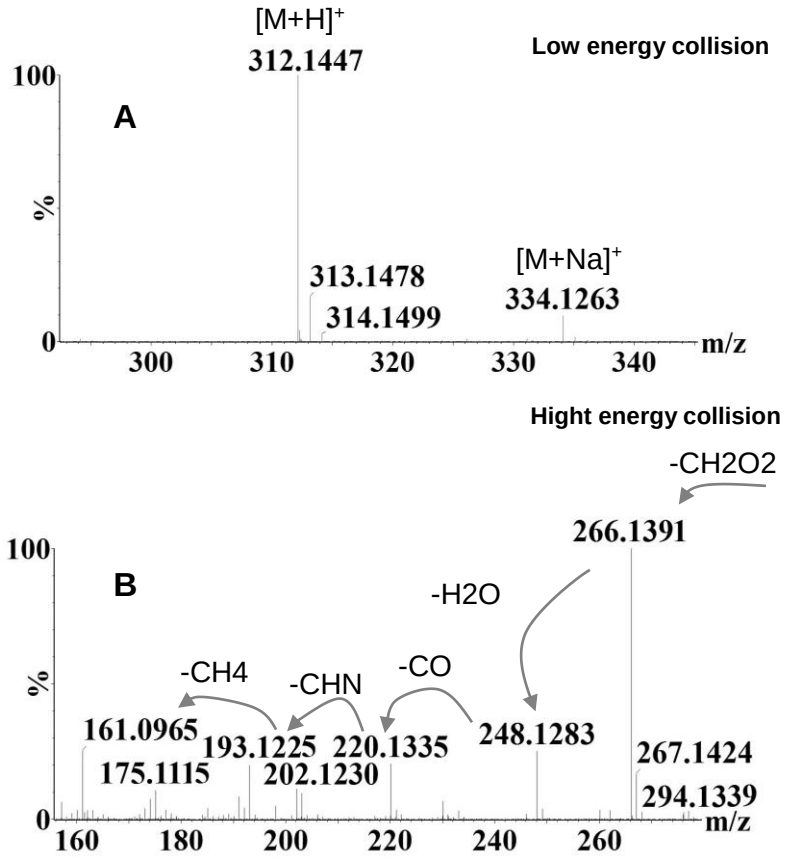

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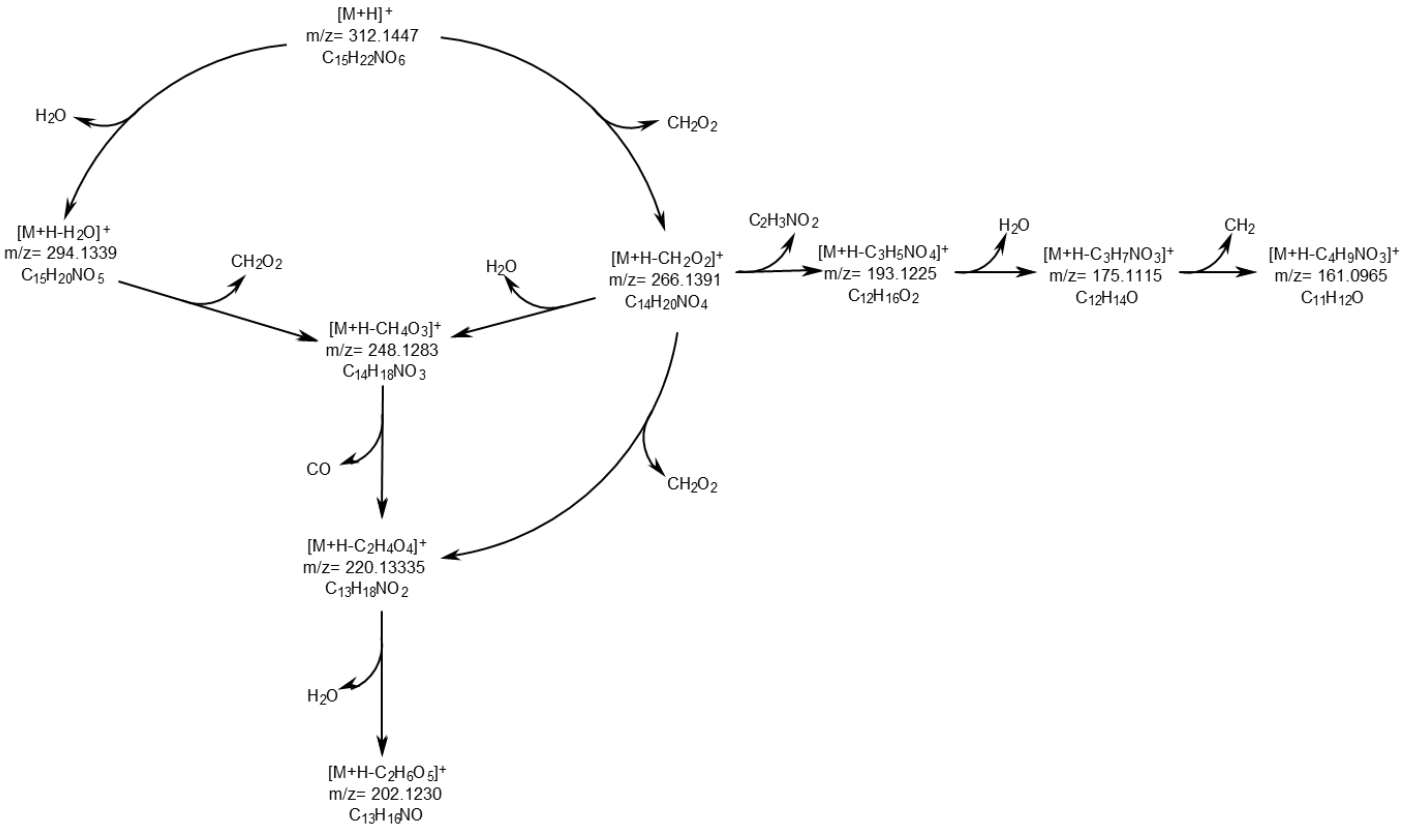
472 Figure 3



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474 Figure 4

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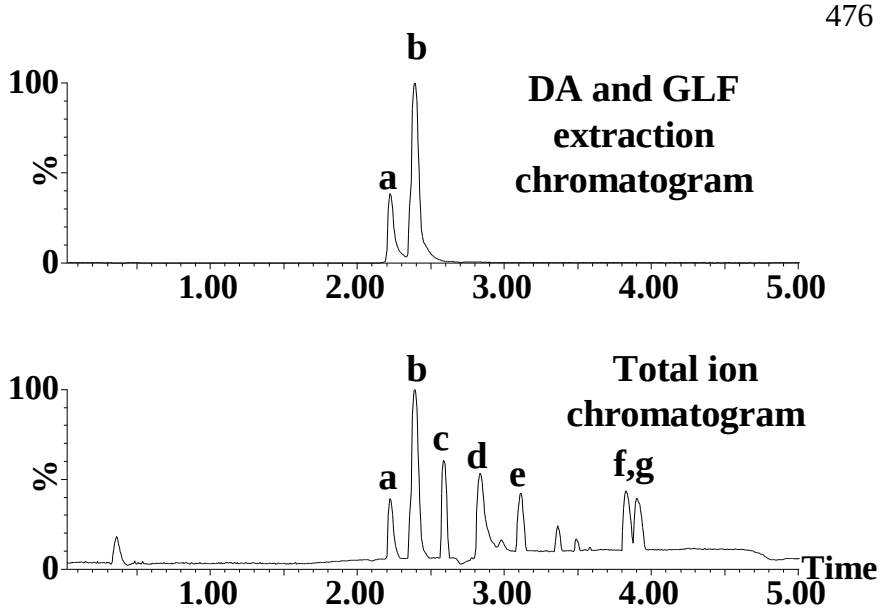

477 Figure 5

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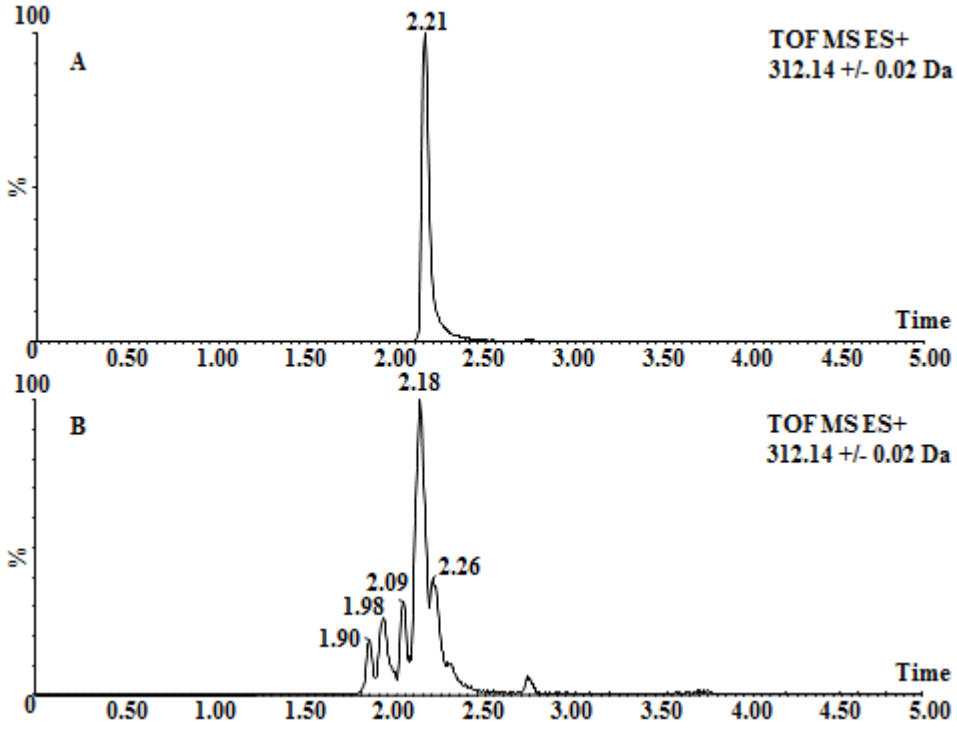

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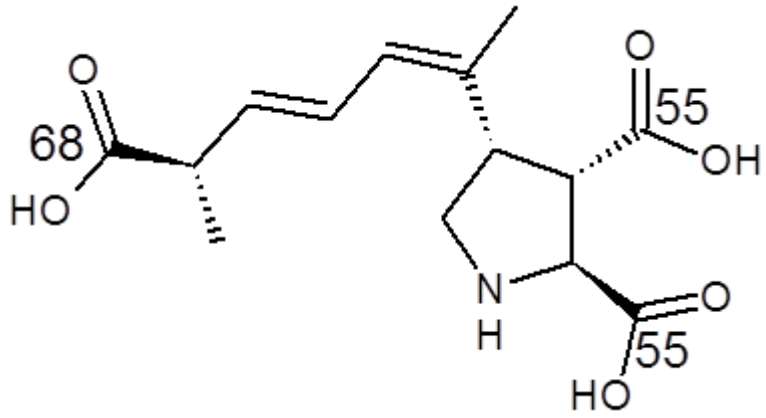
482 Figure 6



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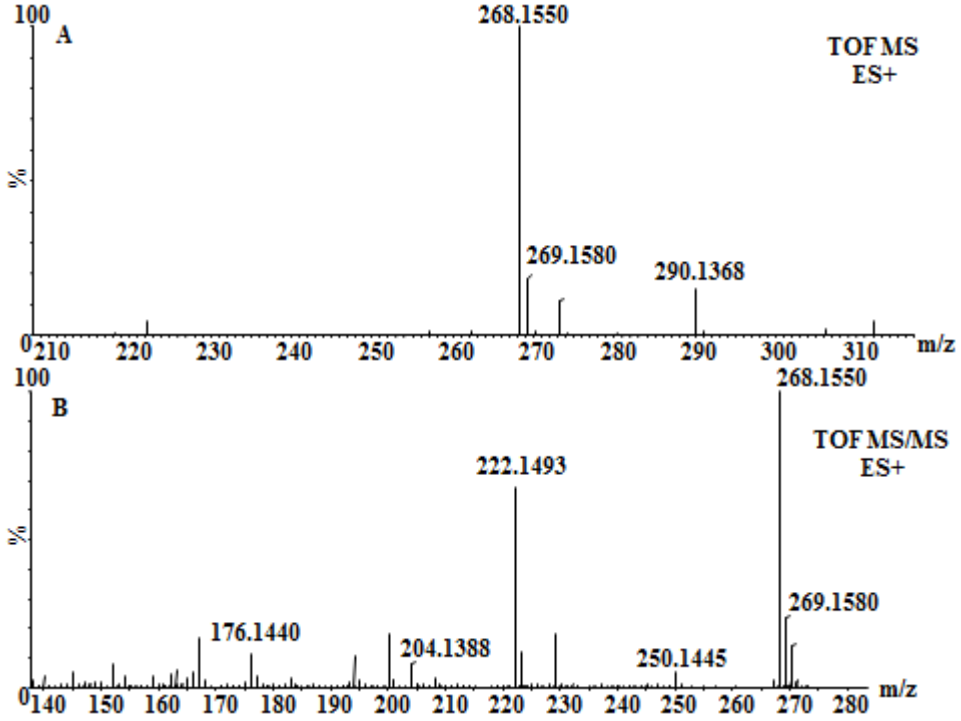
486 Figure 7



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