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Occurrence and risk assessment of domoic acid and its analogs in seafood marketed in South Korea

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ABSTRACT

Amnesic shellfish poisoning (ASP) is a seafood-related neurotoxic disorder caused by domoic acid (DA) and its structural analogs, which can lead to symptoms such as short-term memory loss. Despite its increasing regulatory importance in Korea's seafood safety management, comprehensive data on the occurrence of DA and its analogs remain limited. This study investigated the occurrence of five ASP toxins (DA, epi-DA, and iso-DA A, D, and E) in seafood distributed in Korea and assessed the potential health risks associated with their consumption. A total of 347 seafood samples, representing 19 species of bivalves, crustaceans, fish, and gastropods, were analyzed using two validated methods: high-performance liquid chromatography with UV detection and liquid chromatography-tandem mass spectrometry. ASP toxins were detected in one fish and seven bivalve samples, with iso-DA A being the most frequently detected analog. Contamination was observed from February to November, indicating its presence for most of the year. Risk assessment considered three exposure scenarios: (i) summation of DA and epi-DA, (ii) total ASP with equivalent toxicity, and (iii) total ASP with relative toxicity. Although acute reference dose values remained low overall, they increased sequentially across all scenarios. These findings provide critical baseline data for future risk management and regulatory policies on ASP toxins in Korea's seafood industry.

1. Introduction

Recent climate change has exacerbated various issues, including an increase in harmful algal blooms, significantly enhancing the importance of shellfish toxins that cause foodborne illnesses. According to the Harmful Algal Event Database (http://haedat.iode.org), harmful algal blooms and shellfish toxin occurrences are consistently reported in coastal nations worldwide (Gibble et al., 2021; Hallegraeff et al., 2021; Moriarty et al., 2021).

Domoic acid (DA) is a representative toxin of amnesic shellfish poison (ASP). The risk to human health occurs through the consumption of contaminated marine organisms such as bivalves and crustaceans (Ben-Gigirey et al., 2021; Fehling et al., 2004; Sandoval-Belmar et al., 2023; Zabaglo et al., 2016). The first documented case of human DA poisoning occurred in 1987 in Canada, which subsequently led to extensive research on ASP (Addison & Stewart, 1989). ASP comprises DA as the parent toxin and eight isodomoic acids A, B, C, D, E, F, G, and H (iso-DA A–H) and one diastereomer epi-DA (FAO/WHO, 2016; Kvrgić et al., 2022). DA is naturally produced by diatoms or red algae, including some *Pseudo-nitzschia* or *Chondria* species, respectively (Ben-Gigirey et al., 2021; Dong et al., 2020; Jiang et al., 2014; Lefebvre & Robertson, 2010; Lelong et al., 2012; Maeno et al., 2018). During this biosynthesis, several analogs of DA—such as epi-DA and iso-DA A and C—are often co-produced (Hansen et al., 2011; Holland et al., 2005; Meda et al., 1986; Weber et al., 2021). Additionally, rising temperatures can accelerate the photodegradation of DA, further contributing to the formation

Abbreviations: ASP, amnesic shellfish poison; DA, domoic acid; ELISA, enzyme-linked immunosorbent assay; HPLC-UV, high-performance liquid chromatography-ultraviolet detection; LC-MS/MS, liquid chromatography-tandem mass spectrometry; ARfD, acute reference dose; TDI, tolerable daily intake; EFSA, European Food Safety Authority; EURLMB, European Union Reference Laboratory for Marine Biotoxins; CRM, certified reference material; MeCN, acetonitrile; MeOH, methanol; FA, formic acid; FDA, United States Food and Drug Administration; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; LB, lower bound; UB, upper bound; bw, body weight.

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of these analogs in the marine environment (Ben-Gigirey et al., 2021; Bouillon et al., 2008; Campbell et al., 2004; Zabaglo et al., 2016).

ASP, a neurotoxin, exhibits a similar chemical structure to excitatory amino acids such as glutamic and kainic acids (Takeuchi et al., 1984), enabling it to bind to glutamate receptors, leading to neurotoxic effects such as short-term memory loss and coma, as well as gastrointestinal symptoms, including vomiting and diarrhea (Fehling et al., 2004; Kvrgić et al., 2022; Mos, 2001; Zabaglo et al., 2016). Among ASPs, DA is the most toxic, and epi-DA is equally toxic (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain)Schrenk et al., 2021; FAO/WHO, 2016; Quilliam, 2003). Other analogs, except for epi-DA, have a lower toxicity than DA. The relative toxicities of iso-DAs A, C, and F are 0.55, 0.01, and 0.04, respectively (C. Vale, 2014).

Many countries have enacted regulations and implemented management practices to prevent harm associated with consuming seafood contaminated by ASPs. For example, the European Union sets a regulatory limit of 20 mg/kg for the sum of DA and epi-DA to manage ASPs (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) Schrenk et al., 2021). In Korea, only DA is managed based on a maximum limit of 20 mg/kg (MFDS, 2022). However, from a toxicity perspective, some analogs, such as epi-DA and iso-DA A, are of toxicological relevance and, therefore, should be analyzed (FAO/WHO, 2016).

ASPs can be determined using several methods, including enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography-ultraviolet detection (HPLC-UV), and LC-tandem mass spectrometry (LC-MS/MS) (Chen et al., 2019; Hess et al., 2001; Holland et al., 2003; Kleivdal et al., 2007; Lawrence et al., 1991; McCarron et al., 2014; Quilliam et al., 1995). Utilizing the antigen–antibody reaction, ELISA offers high target specificity, although cross-reactivity may occur between DA and its analogs, making it difficult to distinguish between toxins (Traynor et al., 2006; Zhao et al., 2022). HPLC-UV is the most widely used method for detecting DA, showing a maximum absorption wavelength of 242 nm. Nevertheless, the selective separation of DA and its analogs remains challenging, and the presence of interfering substances such as tryptophan in seafood makes accurate analysis difficult (Nie et al., 2023). LC-MS/MS uses mass-based analysis, which can reduce interference from structurally and chemically similar substances, allowing the confirmation of ASPs (Bouillon et al., 2008; Wang et al., 2007).

Analogs such as epi-DA are often detected alongside DA in bivalves. In Spain, for instance, DA and epi-DA were detected in scallop (*Pecten maximus*) at concentrations of 107.10 and 7.94 mg/kg, respectively (Regueiro et al., 2011). In addition, the calculated levels of DA, iso-DAs A, D, and E, and epi-DA in spiny oyster (*Spondylus squamosus*) from the Philippines were 80.92, 3.11, 6.62, 2.26, and 4.29 mg/kg, respectively (Takata et al., 2009). Analogs including iso-DAs A, D, and E and epi-DA have also been detected simultaneously with DA in oyster (*Crassostrea gigas*, *C. hongkongensis*) and razor clam (*Sinonovacula constricta*) from China (Zheng et al., 2022). Fish such as anchovy (*Engraulidae*) and mackerel (*Scomber scombrus*) have also been reported as vectors for DA (Ben-Gigirey et al., 2021; Kershaw et al., 2021; Sandoval-Belmar et al., 2023).

As the growth of *Pseudo-nitzschia* species that produce several ASPs in seawater can be influenced by numerous environmental, physical, and nutrient parameters, including climate change, salinity, and plankton levels, confirming the production volume of toxins is difficult (Dong et al., 2020; Tanković et al., 2022; Tas et al., 2016). Therefore, the discovery of *P. multiseries* and *P. pungens*, along with several other *Pseudo-nitzschia* species in Korea, suggests a potential risk of ASP occurrence and exposure in seafood, including bivalves and crustaceans (Gang et al., 2003; Kwon & Kang, 2014; Lee et al., 2013, 2021; Yoo, 2003). DA at levels of 2.05 mg/kg has been detected in surf clam (*Mactra veneriformis*) in Korea (Choi et al., 2009).

The acute reference dose (ARfD) and tolerable daily intake (TDI) are commonly used to assess acute and chronic exposure to dietary toxins. Although a TDI for DA has not been established due to insufficient data on its chronic effects, the European Food Safety Authority (EFSA) has set an ARfD of 30 μg/kg body weight (bw)/day for the sum of DA and epi-DA, based on acute toxicity data from humans and experimental animals (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain) Schrenk et al., 2021). In Korea, no cases of ASP toxicity symptoms in humans due to seafood consumption have been reported. Furthermore, the contamination status of DA and its analogs in various seafood samples and the potential health risks remain unclear. Hence, occurrence studies of analogs, including epi-DA, in vectors such as fish and risk assessments based on the resulting data are needed.

In this study, to avoid false positives caused by interfering substances such as tryptophan, which co-elutes with analogs (iso-DAs A, D, and E) and complicates accurate analysis, an analytical method based on HPLC-MS/MS was established and validated to simultaneously determine five ASPs, including these analogs (Quilliam et al., 1995). Although HPLC-UV has limitations in detecting certain analogs, it remains an officially recognized analytical method in countries including South Korea (MFDS, 2022) and members of the European Union (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain)Schrenk et al., 2021). To address the lack of high-cost HPLC-MS/MS equipment, we slightly modified and validated the method of the European Union Reference Laboratory for Marine Biotoxins (EURLMB) for analyzing DA and epi-DA using HPLC-UV as an auxiliary monitoring tool (EURLMB, 2008). Subsequently, we investigated the occurrence of ASP in seafood (bivalve, crustacean, fish, and gastropod) marketed in Korea, after which a risk assessment was conducted using the data obtained.

2. Materials and methods

2.1. Samples

A total of 347 seafood samples, including 12 bivalve, 3 crustacean, 2 fish, and 2 gastropod species distributed in Korea, were purchased from online retailers from January to December 2023 to analyze ASP occurrence. Bivalve and crustacean seafood samples were selected to represent the Korean population's seafood intake and previous DA detection history. Fish and gastropods were selected to investigate their potential as toxin vectors and threats to public health, considering their behavior of feeding on the phytoplankton capable of producing ASP (Ben-Gigirey et al., 2021). The bivalve samples included 22 ark shells (*Scapharca broughtonii*), 14 cockles (*Fulvia mutica*), 24 hard clams (*Mercenaria mercenaria*), 47 mussels (19 *Mytilus coruscus*, 28 *M. galloprovincialis*), 17 oysters (*C. gigas*), 26 pen shells (*Atrina pectinata*), 14 razor clams (*Solen grandis*), 43 scallops (18 *Argopecten irradians*, 25 *Mizuhopecten yessoensis*), 17 short-necked clams (*Ruditapes philippinarum*), and 16 surf clams (*Ma. quadrangularis*). The crustacean samples included 15 morotoge shrimps (*Pandalus japonicu*), 17 red snow crabs (*Chionoecetes japonicus*), and 15 swimming crabs (*Portunus trituberculatus*). Fish samples included 28 anchovies (*Engraulis japonicus*) and 10 mackerels (*Scomber japonicus*), and gastropod samples included 12 abalones (*Haliotis discus hannai*) and 10 conches (*Rapana venosa*). The collected samples were used after identifying each species through morphological characteristics and/or species-specific genetic marker analysis by Professor Kwang-Sik Choi at Jeju National University (Jeju-do, South Korea).

To ensure representativeness, all samples were collected in quantities of at least 200 g, and more than 1 kg of live seafood was purchased. The samples were processed on the day of collection. External contaminants on the samples were removed by washing them under running water, and the samples were carefully opened with a knife to avoid damaging the tissues or internal organs. Thereafter, edible parts were separated according to the Korean Food Code (Ministry of Food and Drug Safety (MFDS), 2023). To remove foreign material from the separated samples, they were washed thrice with 300 mL distilled water and drained in a sieve for 5 min. The drained samples were homogenized using a blender, stored at −20 °C, and thawed before use.

2.2. Chemicals and reagents

Certified reference material (CRM) CRM-DA-h (DA, 96.6 mg/kg; epi-DA, 0.55 mg/kg; iso-DA A, 1.1 mg/kg; iso-DA D, 0.82 mg/kg; iso-DA E, 0.19 mg/kg) and CRM-ASP-Mus-d (DA, 47.6 mg/kg; epi-DA, 1.9 mg/kg) were purchased from the National Research Council Canada (Halifax, NS, Canada). For CRM-DA-h, only DA (96.6 mg/kg) and epi-DA (0.55 mg/kg) are certified, while the concentrations of iso-DA A, D, and E are non-certified reference values. HPLC-grade water, acetonitrile (MeCN), and methanol (MeOH) were purchased from Burdick & Jackson (Muskegon, MI, USA). HPLC-grade trifluoroacetic acid (≥99.0 %) and LC-MS-grade formic acid (FA) (>99.0 %) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Thermo Fisher Scientific (Waltham, MA, USA), respectively. The samples were purified using a Bond Elut SAX Cartridge (500 mg, 6 mL) from Agilent Technologies (Santa Clara, CA, USA).

2.3. Toxin extraction and cleanup

The extraction of toxins for HPLC-UV analysis was performed using a previously described method with slight modification (EURLMB, 2008). Briefly, 4.0 ± 0.1 g of sample was extracted with 16 mL of 50 % MeOH (50:50, v/v) by shaking (300 rpm, 15 min), followed by centrifugation (10,000×*g*, 5 min). Next, the supernatant was filtered through a syringe filter (0.2 μm, polyvinylidene difluoride) and injected into the apparatus.

For HPLC-MS/MS analysis, the initial extraction followed the same procedure, but the supernatant was further cleaned up through a strong anion exchange cartridge. Before use, the cartridge was conditioned using 6 mL MeOH, 3 mL water, and 3 mL 50 % MeOH. After conditioning, 4 mL of the supernatant was loaded. Subsequently, the cartridge was washed with 6 mL 15 % MeCN and dried thoroughly. Next, 2 mL MeCN:water:FA (10:88:2, v/v/v) was applied to the cartridge to elute toxins, and the eluted solvent was filtered through a syringe filter and injected into the apparatus.

2.4. HPLC-MS/MS conditions

The HPLC-MS/MS system consisted of the Agilent 1290 Infinity II UPLC series equipped with the Agilent 6470 triple quadrupole MS detector (Agilent Technologies, Santa Clara, CA, USA). An Acquity HSS C18 Column (2.1 × 100 mm, 1.8 μm) from Waters Corporation (Milford, MA, USA) was used for toxin separation and analysis in a column oven at 45 °C. The mobile phase comprised two solvents: A (0.1 % FA in water) and B (0.1 % FA in MeCN). The gradient elution was started with 5 % Solvent B and increased to 10 % over 15.0 min, after which Solvent B was increased to 50 % over 15.5 min and held for 1.5 min. Thereafter, Solvent B was decreased to 5 % over 17.5 min and held for 2.5 min to re-equilibrate the column. The entire analysis procedure was completed in 20 min. Next, 5 μL of the sample was injected and analyzed at a flow rate of 0.3 mL/min. The five ASPs were detected by positive ion electrospray analysis, and ultrapure nitrogen (>99.999 %) was used as a collision gas. Optimized MS parameters were as follows: gas temperature, 250 °C; gas flow, 11 L/min; nebulizer, 40 psi; sheath gas temperature, 320 °C; sheath gas flow, 11 L/min; and nozzle voltage, −3000 V. The precursor ion ($[M+H]^+$) for the five toxins was 312.3 *m/z*. The product ions ($[M+H]^+$) for quantitative and qualitative analyses were 266 and 248 *m/z*, respectively.

2.5. HPLC-UV conditions

The HPLC-UV system was optimized by modifying an analytical method used to simultaneously determine DA and epi-DA (EURLMB, 2008). An Agilent 1260 Infinity HPLC System (Agilent Technologies) comprising a UV detector (G1314F; Agilent Technologies) was used at a wavelength of 242 nm. A Zorbax Extended C18 column (4.6 × 150 mm, 5 μm; Agilent Technologies) was used for separation in a column oven maintained at 35 °C. The isocratic mobile phase was 10 % MeCN containing 0.1 % trifluoroacetic acid. The injection volume was 20 μL, and the flow rate was 1 mL/min.

2.6. Method performance

Both methods were validated based on United States Food and Drug Administration (FDA) guidelines (2019). The analysis method was validated on three matrices: mussel (*M. galloproinacialis*, bivalve), crab (*Po. trituberculatus*, crustacean), and anchovy (*E. japonicus*, fish). The following parameters were considered for intralaboratory validation: linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. Linearity was evaluated through the coefficient of determination (R^2) of the six-point (0.2, 0.5, 1, 2, 4, and 8 mg/kg, based on the concentration of DA) calibration curves for HPLC-MS/MS and six-point (0.2, 1, 2.5, 5, 10, and 20 mg/kg, based on the concentration of DA) calibration curves for HPLC-UV. When $R^2 > 0.99$, linearity was considered excellent. LOD and LOQ were calculated using the following equation considering the slope of the calibration curve (S) and the standard deviation (σ) of the area from the lowest concentration in the calibration curve: $LOD = 3.3 \times \sigma/S$, $LOQ = 10 \times \sigma/S$. Accuracy and precision were evaluated as the recovery value and relative standard deviation (RSD), respectively, using intraday ($n = 3$) and interday ($n = 3$) replicate assays. Intra- and interday recoveries were measured at three concentrations based on DA concentration: 1, 2, and 4 mg/kg for HPLC-MS/MS and 10, 20, and 40 mg/kg for HPLC-UV. The trueness of the method was evaluated using CRM-ASP-Mus-d with known analyte concentrations. CRM-ASP-Mus-d was measured in triplicate, and the trueness was assessed by comparing the measured values to the certified values of matrix CRM.

To evaluate the performance of the simultaneous analysis method using HPLC-MS/MS, an interlaboratory validation was conducted using three sets of samples in three seafood matrices (mussel, crab, and anchovy) at low, middle, and high concentrations (1, 2, and 4 mg/kg). In addition, the samples consisted of six calibration matrix-matched standards (0.2, 0.5, 1, 2, 4, and 8 mg/kg, based on the concentration of DA). The data were received from three laboratories for the five ASPs, and the reproducibility (%) was determined by the RSD of the recovery rates obtained from three laboratories.

2.7. Risk assessment

Acute dietary exposure to ASPs was determined using the occurrence data acquired from HPLC-MS/MS analysis, seafood consumption, and body weight trends. For each sample, the non-detected results were replaced with a value of zero in the lower bound (LB) and the LOD value in the upper bound (UB) (GEMS/Food-EURO, 1995). Seafood consumption and body weight data were based on the 8th Korea National Health and Nutrition Examination Survey (2019–2021), considering the 95th percentile of extreme intake for both the Korean population and consumer group to reflect the characteristics of various demographic groups. Exposure assessment was conducted and compared using two types of occurrence data: the sum of DA and epi-DA and total ASP (the sum of DA, epi-DA, and iso-DAs A, D, and E). Dietary exposure to total ASP was estimated under two assumptions. First, by applying the same toxicity as DA to all toxins, and second, by applying relative toxicities to each toxin. The relative toxicities used were 0.55 for iso-DA A and 0.004 for iso-DAs D and E (C. Vale, 2014). Risk assessment was conducted by comparing the dietary exposure value with the ARfD values of 30 μg DA/kg bw/day for the sum of DA and epi-DA as set by the EFSA: %ARfD >100 % is recognized as a high-risk level (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain)Schrenk et al., 2021).

3. Results

3.1. Method performance

The performance of both analytical methods used to investigate ASP occurrence in seafood was evaluated to ascertain whether they satisfied the criteria of the FDA guidelines. Representative chromatograms of standard solutions and sample extracts for each matrix are provided in the supplementary material (Fig. S1–S2). The intraday accuracy (expressed as recovery, %), intraday precision (RSD, %), and interday precision (intermediate precision, %) were evaluated for each matrix using HPLC-MS/MS, and the results are summarized in Table 1. The intraday recoveries for DA, epi-DA, and iso-DAs A, D, and E were 83.5–95.5 %, 87.0–100.1 %, 85.4–103.5 %, 92.6–102.7 %, and 82.1–96.1 %, respectively, and the intermediate precisions were 1.5–14.3 %, 0.6–7.2 %, 2.1–15.0 %, 2.3–16.6 %, and 0.8–5.3 %, respectively. Table S1 indicates that the R^2 exceeded 0.99 across the three seafood matrices, demonstrating good linearity in both methods. The LODs for each toxin using the HPLC-MS/MS system ranged from low to high ppb levels, depending on the matrix. In mussel: DA (30 μg/kg), epi-DA (0.98 μg/kg), iso-DA A (2.77 μg/kg), iso-DA D (1.99 μg/kg), iso-DA E (0.16 μg/kg); in crab: DA (48 μg/kg), epi-DA (6.57 μg/kg), iso-DA A (3.88 μg/kg), iso-DA D (4.58 μg/kg), iso-DA E (0.98 μg/kg); in anchovy: DA (30 μg/kg), epi-DA (1.01 μg/kg), iso-DA A (1.80 μg/kg), iso-DA D (1.48 μg/kg), iso-DA E (0.48 μg/kg). For the HPLC-UV system, the combined LODs of DA and epi-DA were higher, ranging from several tens to over one hundred ppb: mussel (72 μg/kg), crab (118 μg/kg), and anchovy (35 μg/kg). The LOD and LOQ were lower when using HPLC-MS/MS. Table S2 presents the intraday recovery and interday precision for the sum of DA and epi-DA using HPLC-UV, showing ranges of 87.2–95.8 % and 0.5–2.2 %, respectively. Additionally, the measured value for matrix CRM via HPLC-MS/MS was 45.3 ± 1.4 mg/kg for the sum of DA and epi-DA, with a trueness of 91.4 ± 2.8 %, as shown in Table 2. As shown in Table S3, a measured value of 44.9 ± 0.3 mg/kg for the sum of DA and epi-DA was obtained from the HPLC-UV analysis method, with a trueness of 90.9 ± 0.7 %, indicating that both methods are suitable for application to the matrices.

The interlaboratory validation results for the simultaneous analysis method using matrix CRM are shown in Fig. 1. The reproducibilities for the five ASPs in three matrices were 2.9–13.6 % for DA, 1.1–9.4 % for epi-DA, 2.6–13.7 % for iso-DA A, 1.6–11.6 % for iso-DA D, and 1.6–11.0 % for iso-DA E, which satisfy the FDA guidelines (Food and Drug Administration (FDA), 2019).

Table 2
Method trueness using mussel matrix certified reference material via HPLC-MS/MS.

	DA	epi-DA	DA + epi-DA
Certified value (mg/kg)	47.6	1.9	49 ± 3
Measured value (mg/kg)	42.0 ± 1.3	1.9 ± 0.1	45.3 ± 1.4
Trueness (%) ± [a]RSD (%)	88.2 ± 2.8	97.7 ± 2.5	91.4 ± 2.8

[a] Relative standard deviation.

3.2. Occurrence of ASP in seafood marketed in Korea

The occurrence of ASP was investigated in 347 seafood samples by species using HPLC-MS/MS and HPLC-UV (Table 3). For the detection of DA, levels of 358.8 and 465.3 μg/kg were detected using HPLC-MS/MS and HPLC-UV, respectively, with 2.6 % of the detection rate in fish. Moreover, iso-DA A was detected in 15 samples (13.7–169.9 μg/kg), iso-DA D in 12 samples (4.6–33.4 μg/kg), and iso-DA E in 10 samples (0.7–3.1 μg/kg). While epi-DA was not detected, other analogs were more frequently detected in bivalves. Specifically, iso-DA A was found at 5.4 %, iso-DA D at 4.6 %, and iso-DA E at 3.8 % in bivalves. In contrast, fish samples showed lower detection rates with iso-DA A at 5.3 %, iso-DA D at 2.6 %, and iso-DA E at 2.6 %.

Fig. 2 shows the mean concentrations of ASP using HPLC-MS/MS in positive samples by species. The highest mean concentrations for each ASP were found in anchovy, with 358.8 μg/kg for DA, 67.2 μg/kg for iso-DA A, 33.4 μg/kg for iso-DA D, and 3.1 μg/kg for iso-DA E. This suggests that ASP, which is managed in bivalves and crustaceans in Korea, can also occur in non-bivalve seafood such as fish.

Fig. 3 shows the monthly ASP detection profiles in individual positive samples and indicates that iso-DA A occurred in all positive samples, with relatively elevated levels detected in April (106.8 μg/kg) and June (169.9 μg/kg). The samples with the highest concentrations of each ASP were as follows: DA and iso-DAs D and E were detected in anchovy (*E. japonicus*), while iso-DA A was detected in scallop (*Mi. yessoensis*). The monthly detection rates of ASP were as follows: February (two samples, detection rate: 9.1 %), March (four samples, detection rate: 8.2 %), April (one sample, detection rate: 2.2 %), May (one sample, detection rate: 1.7 %), June (one sample, detection rate: 2.6 %), July (three samples, detection rate: 10.0 %), August (one sample, detection rate: 2.4 %), September (one sample, detection rate: 4.5 %), and November

Table 1
Intra- and interday precision and accuracy for determining ASP in three seafood matrices via HPLC-MS/MS.

Toxin	Spiked level (mg/kg)	Mussel			Crab			Anchovy		
		Recovery (%)	[a]RSD (%)	Intermediate precision (%)	Recovery (%)	RSD (%)	Intermediate precision (%)	Recovery (%)	RSD (%)	Intermediate precision (%)
		$n = 3$	$n = 3$	$n = 9$	$n = 3$	$n = 3$	$n = 9$	$n = 3$	$n = 3$	$n = 9$
DA	Low	85.8	1.7	1.8	95.5	9.0	14.3	83.7	4.7	4.4
	Middle	83.8	0.8	1.7	93.7	2.7	7.2	83.5	4.5	3.5
	High	86.7	1.5	1.5	94.5	1.5	5.1	86.5	3.7	3.6
epi-DA	Low	97.3	1.3	5.1	92.1	11.9	0.6	100.1	3.4	3.2
	Middle	87.0	1.7	3.4	89.4	10.8	7.2	92.3	2.7	4.3
	High	91.9	3.7	4.0	95.1	0.9	6.1	89.7	2.6	5.0
iso-DA A	Low	103.5	10.1	12.7	93.8	10.0	14.3	96.1	2.8	3.1
	Middle	96.5	5.1	7.2	85.4	1.9	15.0	99.3	7.9	5.4
	High	98.2	3.5	3.8	90.1	2.6	6.3	102.1	0.6	2.1
iso-DA D	Low	97.5	1.0	4.1	95.0	3.0	11.1	102.7	1.2	3.2
	Middle	92.6	5.9	3.0	93.3	1.2	16.6	94.5	2.1	3.7
	High	93.6	1.4	2.3	97.7	4.6	11.4	94.7	1.3	3.2
iso-DA E	Low	82.1	1.8	2.8	96.1	3.2	0.8	94.8	4.5	5.0
	Middle	82.2	2.0	2.8	94.5	5.3	4.4	89.7	1.5	2.2
	High	83.6	1.2	2.0	95.1	2.8	5.3	88.1	0.6	1.5

[a] Relative standard deviation.

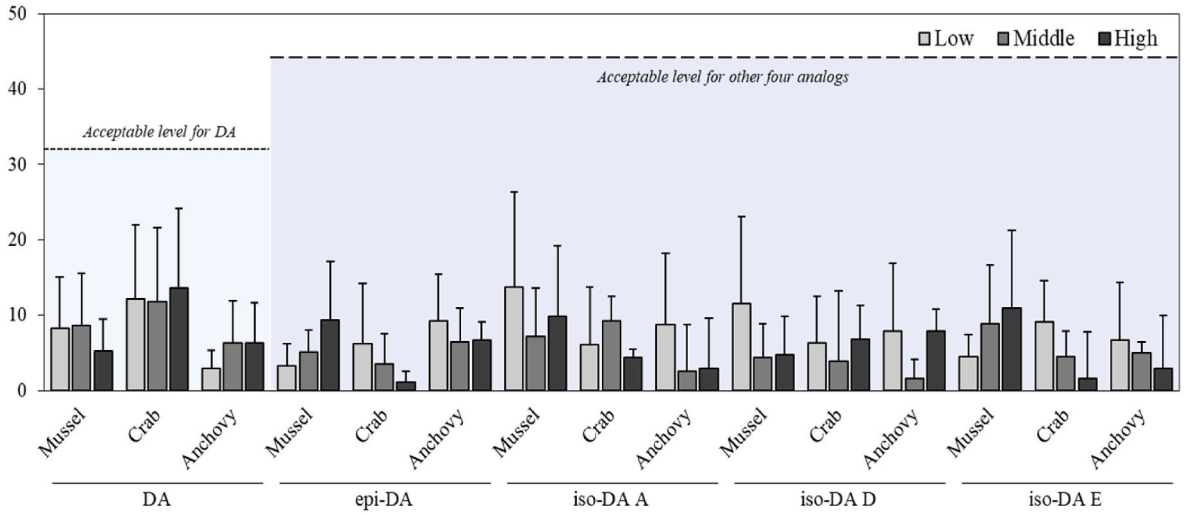

Fig. 1. Results of interlaboratory reproducibility conducted using mussel, crab, and anchovy matrices via HPLC-MS/MS.

Table 3
Occurrence of amnesic shellfish poison in 347 seafood samples collected across Korea determined using two different instruments.

Species	Parameter	HPLC-UV	HPLC-MS/MS				
		DA + epi-DA	DA	epi-DA	iso-DA A	iso-DA D	iso-DA E
Bivalve	No. of samples	240	240	240	240	240	240
	No. of positive samples	–[a]	–	–	13	11	9
	Detection rate (%)	–	–	–	5.4	4.6	3.8
	Range (μg/kg)	–	–	–	13.7–169.9	4.6–18.6	0.7–1.4
	Positive mean (μg/kg)	–	–	–	38.7	9.9	1.0
Crustacean	No. of samples	47	47	47	47	47	47
	No. of positive samples	–	–	–	–	–	–
	Detection rate (%)	–	–	–	–	–	–
	Range (μg/kg)	–	–	–	–	–	–
	Positive mean (μg/kg)	–	–	–	–	–	–
Fish	No. of samples	38	38	38	38	38	38
	No. of positive samples	1	1	–	2	1	1
	Detection rate (%)	2.6	2.6	–	5.3	2.6	2.6
	Range (μg/kg)	465.3	358.8	–	27.6–106.8	33.4	3.1
	Positive mean (μg/kg)	–	–	–	67.2	–	–
Gastropod	No. of samples	22	22	22	22	22	22
	No. of positive samples	–	–	–	–	–	–
	Detection rate (%)	–	–	–	–	–	–
	Range (μg/kg)	–	–	–	–	–	–
	Positive mean (μg/kg)	–	–	–	–	–	–
Total	No. of samples	347	347	347	347	347	347
	No. of positive samples	1	1	–	15	12	10
	Detection rate (%)	0.3	0.3	–	4.3	3.5	2.9
	Range (μg/kg)	465.3	358.8	–	13.7–169.9	4.6–33.4	0.7–3.1
	Positive mean (μg/kg)	–	–	–	42.5	11.9	1.2

[a] Not detected.

(one sample, detection rate: 10.0 %). These findings suggest the potential for the year-round occurrence of ASP in Korea.

3.3. ASP risk assessment

Fig. 4 (A, C, and E) shows the %ARfD based on dietary exposure values using LB and UB occurrence data for the sum of DA and epi-DA and total ASP in the Korean population; Fig. 4 (B, D, and F) shows the consumer group. Both groups exhibited high exposure values from fish consumption. Specifically, Fig. 4 (B, D, and F) indicates that the highest exposure values were observed in the 1–2-year-old consumer group, with LB values corresponding to 0.08, 0.12, and 0.10 % of the ARfD (30 μg/kg bw/day), respectively. UB values corresponded to 0.27, 0.34, and 0.31 % of the ARfD set by the EFSA, respectively. Furthermore, across all age groups, the %ARfD was <0.12 % for LB values and <0.34 % for UB values, indicating no acute health risk. Although the %ARfD values were generally low, they increased sequentially for the %ARfD calculated for the sum of DA and epi-DA, the %ARfD for total ASP with relative toxicity, and the %ARfD for total ASP with equivalent toxicity. This indicates that seafood consumption could result in ASP exposure attributed to analogs.

4. Discussion

We investigated contamination by ASPs comprising DA and its analogs in seafood samples (bivalves, crustaceans, fish, and gastropods) distributed in Korea, using the HPLC-MS/MS method established in this study and a modified HPLC-UV method based on the EURLMB. We then

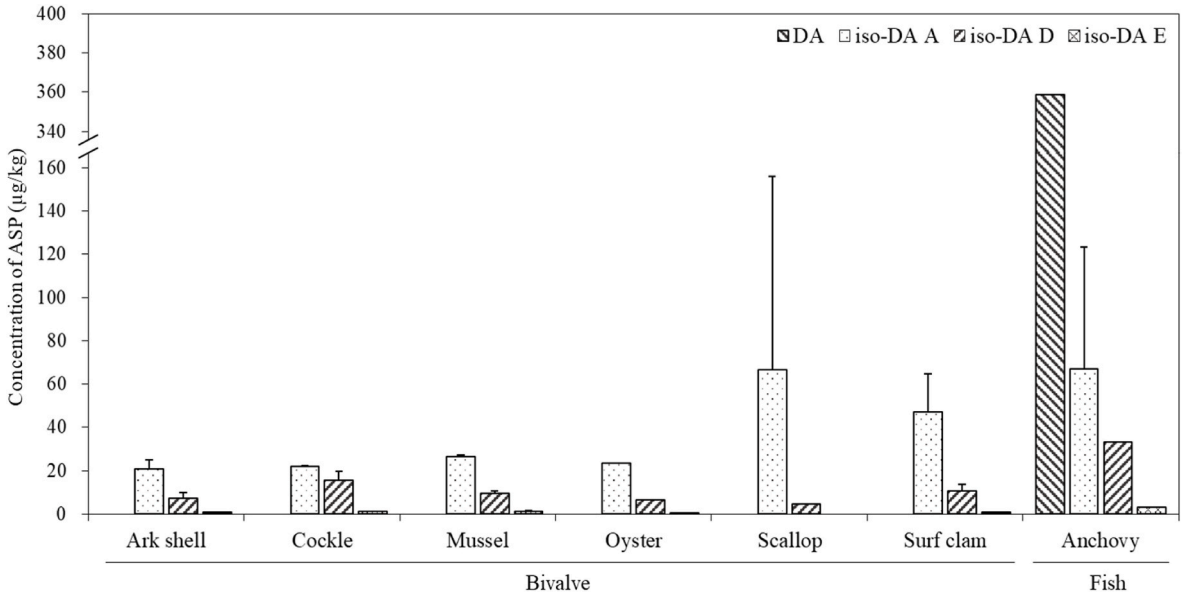

Fig. 2. Positive mean concentration of amnesic shellfish poison in seafood samples collected across Korea.

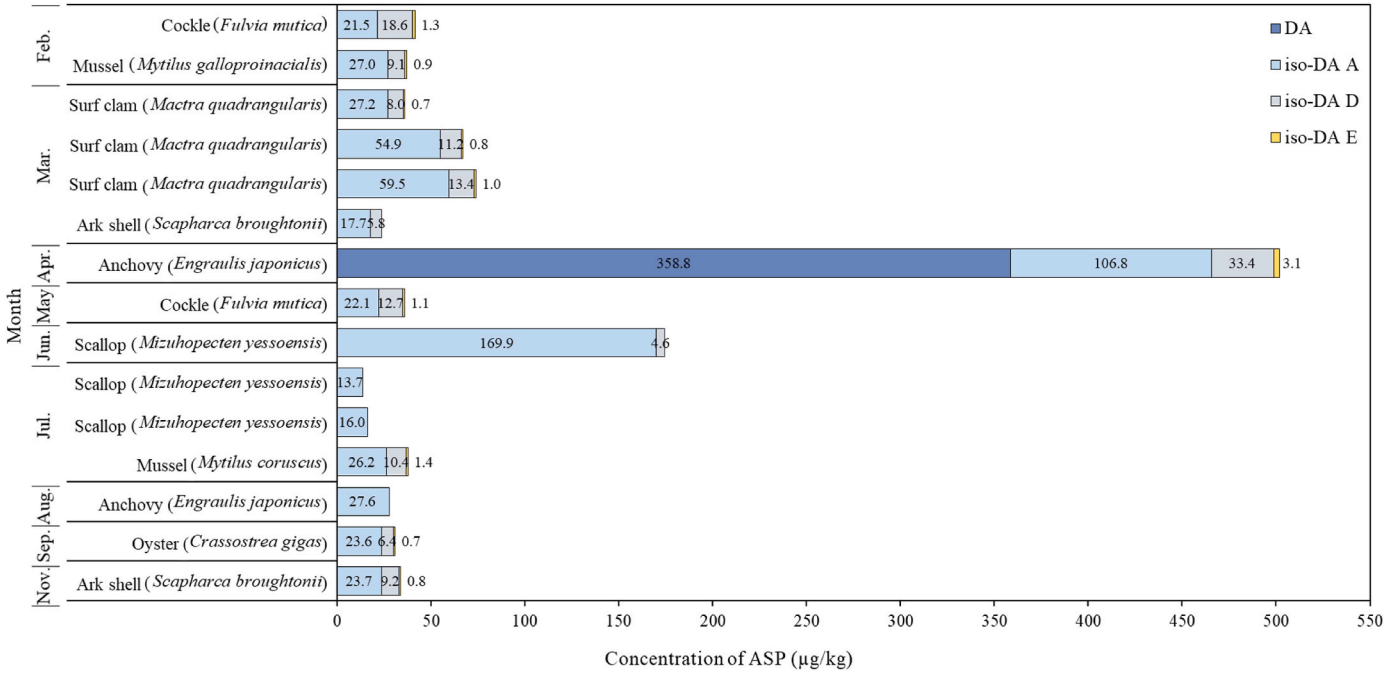

Fig. 3. Monthly amnesic shellfish poison profiles of individual positive samples collected in Korea in 2023.

conducted a risk assessment based on the obtained occurrence data.

Both methods used for determining the ASP contents in seafood satisfied the performance criteria of the FDA guidelines (Food and Drug Administration (FDA), 2019). In one anchovy sample, DA occurred at concentrations of 0.36 mg/kg by HPLC-MS/MS and 0.47 mg/kg by HPLC-UV. These levels are lower than the previously reported 444 and 244 mg/kg of DA detected in the viscera of anchovy (*En. mordax*) and sardine (*Sardinops sagax*), respectively, collected in California (Lefebvre et al., 2002). Additionally, 74.2 mg/kg of DA was detected in the whole body of sardines, sometimes eaten without evisceration in Portugal (P. Vale & Sampayo, 2001). Regarding bivalves, while DA was not detected in the present study, the highest concentration of iso-DA A was found in scallop (*M. yessoensis*) at 0.17 mg/kg. However, previous studies have reported 7.3 and 296.3 mg/kg of DA in the adductor muscle and hepatopancreas of scallop (*Pe. maximus*) collected from the Isle of Man (Bogan et al., 2007). Moreover, 80.9 mg/kg of DA and 4.3 mg/kg of epi-DA were detected in oyster (*S. squamosus*) collected in the Philippines (Takata et al., 2009). Although no ASP was detected in crustaceans and gastropods in our study, previous research reported DA concentrations as high as 90 mg/kg in Dungeness crab (*Cancer magister*) from Washington and maximum DA levels of 674 mg/kg in sea snail (*Nassarius fossatus*), indicating potential ASP contamination in non-bivalve species (Kvitek et al., 2008; Shumway, 1995; Wekell et al., 1994). These findings suggest that planktivorous fish and gastropods may act as vectors for ASP, as noted previously (Costa et al., 2005; Scholin et al., 2000). Although the detected levels were below the regulatory limit of 20 mg/kg, these findings suggest that ASP can also occur in seafood, such as fish, in addition to bivalves and crustaceans, which

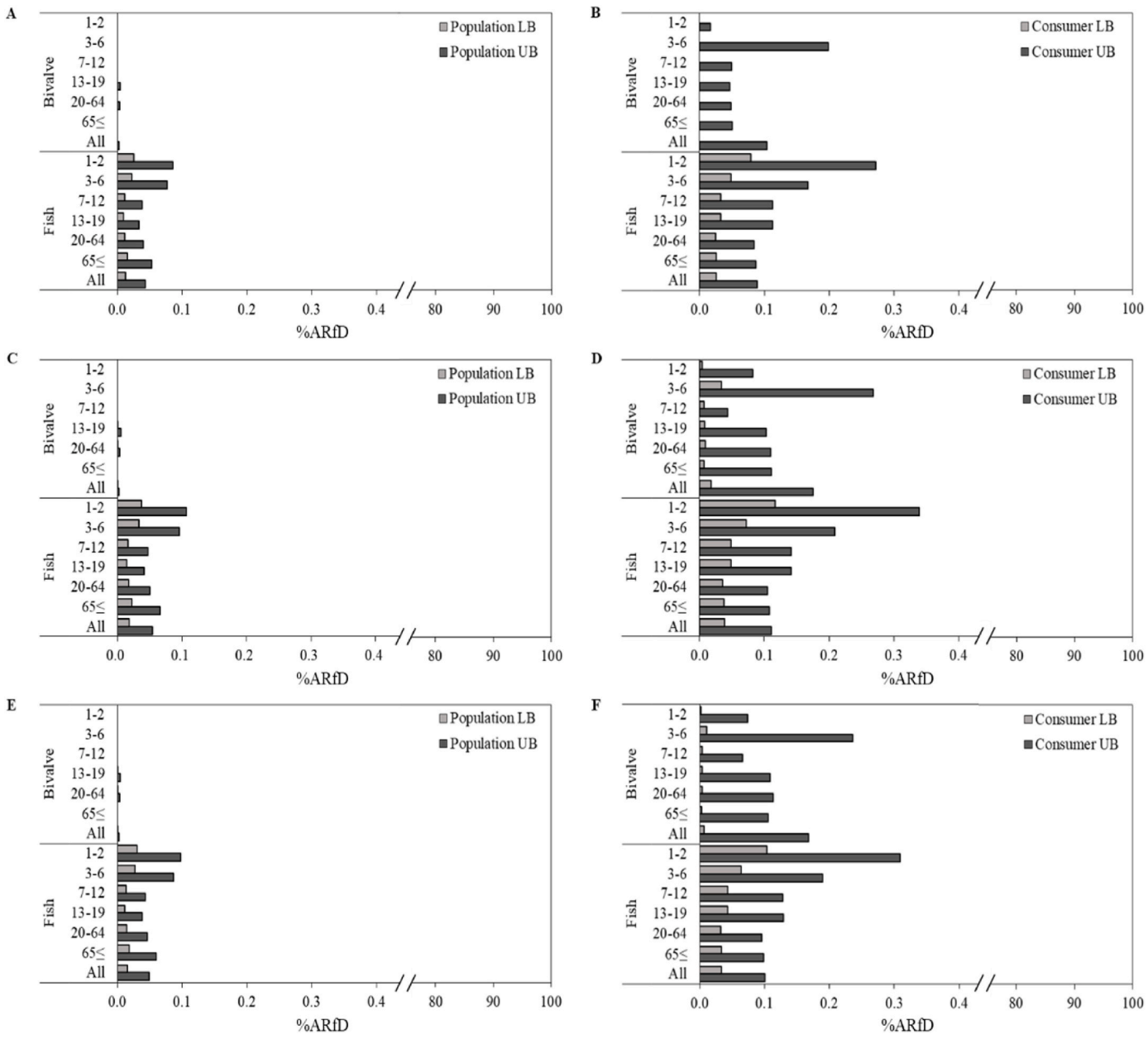

Fig. 4. Percentage acute reference dose (%ARfD) for the general population and consumer group based on lower (LB) and upper bound (UB) occurrence data. (A) %ARfD for the general population using occurrence data of the sum of DA and epi-DA. (B) %ARfD for the consumer group using occurrence data of the sum of DA and epi-DA. (C) %ARfD for the general population using occurrence data of total ASP with equivalent toxicity factor. (D) %ARfD for the consumer group using occurrence data of total ASP with equivalent toxicity factor. (E) %ARfD for the general population using occurrence data of total ASP with relative toxicity factor. (F) %ARfD for the consumer group using occurrence data of total ASP with relative toxicity factor.

are currently managed for ASP in Korea.

The detection rates of 4.3 % for iso-DA A, 3.5 % for iso-DA D, and 2.9 % for iso-DA E were lower than the previously reported levels of 10.9, 11.3, and 8.4 %, respectively (Zheng et al., 2022). It should also be noted that the concentrations of iso-DA A, D, and E in CRM-DA-h are non-certified reference values, which may affect the accuracy of their quantification and thus warrant cautious interpretation. Nonetheless, the maximum concentration of iso-DA A, which has the highest relative toxicity among analogs, was 169.9 μg/kg, i.e., approximately 8-fold higher than the previously reported value of 21.4 μg/kg (Zheng et al., 2022). Although epi-DA was not found in our study, it may occur due to the transformation of DA into epi-DA due to long-term storage and photodegradation (Ben-Gigirey et al., 2021). In Spain, 7.94 mg/kg of epi-DA was detected in scallops (Regueiro et al., 2011). Furthermore, the occurrence data of the sum of DA and epi-DA from 1999 to 2008 showed its detection in the UK, Ireland, Spain, and Portugal at rates 8–43-fold higher than the regulatory limit of 20 mg sum DA/kg (European Food Safety Authority (EFSA), 2009). Therefore, conducting a contamination investigation considering the occurrence of analogs such as epi-DA with equivalent toxicity to DA is necessary.

Despite being found in trace amounts, ASPs occur year-round (Kvrgić et al., 2022; Zheng et al., 2022). However, specifying ASP production and occurrence timing is difficult due to the influence of variables such as temperature, salinity, and acidification (Dong et al., 2020; Tanković et al., 2022). Recent trends in global warming have contributed to climate change, including an increase in temperature. A study investigating the association between DA levels in shellfish from Washington, California, and Oregon and warm ocean conditions during 1991–2015

reported that the warmer the oceans, the more likely it was for DA to exceed the regulatory limit of 20 mg/kg (McKibben et al., 2017). Furthermore, DA toxicity tended to be higher and more widespread, and more DA events tended to occur under these conditions. In Korea, ocean temperatures have risen by ≈ 1.4 °C in the period 1968–2021; accordingly, the occurrence of paralytic shellfish toxins has gradually increased from spring (March–April) to winter (January–February) relative to that more than a decade ago (National Institute of Fisheries Science (NIFS), 2022). In addition to DA being able to convert to analogs such as epi-DA and iso-DAs D, E, and F during photodegradation, this shift suggests the possibility of increased contents of these analogs under the influence of sunlight and temperature (Ben-Gigirey et al., 2021; Bouillon et al., 2008; Clayden et al., 2005). Given that the time for shellfish toxin emergence in Korea is accelerating, ASPs should be continuously monitored year-round.

An ASP risk assessment was performed using the occurrence data obtained from the present study. Under the three assumptions (the sum of DA and epi-DA, total ASP with equivalent toxicity, and total ASP with relative toxicity), the LB values for the 1–2-year-old consumer group who consumed fish were 0.0238, 0.0351, and 0.0310 μg/kg bw/day, respectively. Compared with the sum of DA and epi-DA, these values were 1.5-fold higher for equivalent toxicity and 1.3-fold higher for relative toxicity. This suggests that dietary exposure to several ASPs following seafood consumption is evident even when equivalent and relative toxicities are assigned to other analogs. Furthermore, repeated exposure to low levels of DA may have neurotoxic effects that may impact many people (Grattan, 2022; Grattan et al., 2016, 2018; Stuchal et al., 2020). Considering that the TDI for DA is 0.075 mg/kg based on previous studies, chronic exposure due to repeated toxin intake poses a potential risk to human health despite the lower individual toxicity of analogs (Grattan et al., 2018; Marien, 1996).

In the current study, the relative toxicities of iso-DAs A, D, and E were set at 0.55, 0.004, and 0.004, respectively (C. Vale, 2014). However, the relative toxicities of these analogs have not been definitively established, with some studies suggesting relative toxicities of 0.80, 0.04, and 0.03, respectively (Sawant et al., 2010). This uncertainty complicates accurate exposure assessment. Therefore, to estimate the risk of ASP more accurately, future risk assessments should be conducted based on data with an increased number of monitoring samples and more precise relative toxicities.

5. Conclusion

This study developed and validated a simultaneous analytical method using HPLC-UV and HPLC-MS/MS to detect ASP toxins (DA, epi-DA, and iso-DAs A, D, and E) in seafood distributed in Korea. The methods met FDA performance criteria and were applied to monitor ASP contamination and assess dietary risk. ASPs were detected in bivalves and fish from February to November, with DA found in one sample and iso-DA A in all positive samples. In addition, iso-DA D and iso-DA E were also detected, albeit at low levels, suggesting the year-round presence of various ASP analogs and highlighting the need for continuous monitoring that includes these analogs.

Risk assessment suggested that dietary exposure remains within safe levels. However, the detection of toxic analogs highlights potential health risks, with fish acting as a vector for ASP transmission. This underscores the need to regulate toxic analogs such as epi-DA and expand monitoring beyond bivalves and crustaceans to fish and other seafood. Future risk assessments should incorporate not only conservative approaches using equivalent toxicity but also realistic exposure scenarios considering relative toxicity. This will enable a more accurate evaluation of ASP exposure and inform risk management strategies in Korea.

CRediT authorship contribution statement

Si Eun Kim: Writing – original draft, Investigation, Formal analysis. **Sang Yoo Lee:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Su Been Park:** Visualization, Investigation, Conceptualization. **Songyi Han:** Validation, Formal analysis. **Soon Ho Lee:** Validation, Resources, Investigation. **Hyang Sook Chun:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2025.111413.

Data availability

Data will be made available on request.

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