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Review

Tetrodotoxin and Its Analogs: A Review of Analysis Methods and Levels in Pufferfish

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ABSTRACT - Pufferfish is a major source of tetrodotoxin (TTX), a potent neurotoxin that can lead to death in humans if consumed. The content of TTX and its analogs in different pufferfish species and the distribution in various fish organs are poorly understood. In this study, we compared TTX analysis methods, including mouse bioassay, enzyme-linked immunosorbent assay, high-pressure liquid chromatography (HPLC) coupled with a fluorescence detector, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry (LC-MS) methods. Different HPLC columns (e.g., amide, hydrophilic interaction liquid chromatography, and C_{18} columns) and mobile phases were compared for TTX analysis using LC-MS. Additionally, published extraction methods were compared for LC-MS analysis. The content of TTX and its analogs in different pufferfish species and their distribution in various organs (e.g., liver, muscle, intestine, testis, ovary, and skin) were summarized. This information can help understand the available TTX analysis methods, variation in TTX contents in pufferfish species from different regions, and TTX distribution in various organs.

Key words: Tetrodotoxin, Marine toxins, Neurotoxin, LC-MS/MS, Monitoring

Pufferfish belong to the order Tetraodontiformes, and they mostly reside in the oceans of tropical and subtropical areas. They are also found in freshwater environments and large river estuaries. Many pufferfish species contain tetrodotoxin (TTX), an extremely potent and dangerous neurotoxin that acts on sodium channels in the muscle membranes and nervous tissue[1)]. Pufferfish has been consumed since historical times in worldwide. In Korea, pufferfish were possibly consumed in the Neolithic period approximately 5000 years ago. This tradition has also been preserved in ancient Chinese and Egyptian murals and records[2)]. Most pufferfish contain the toxic compound TTX, and the high consumption of pufferfish in Asia has led to TTX poisoning incidents. Poisoning incidents have typically been noted in Asian countries, such as China, Taiwan, Bangladesh, Malaysia, and the United States and Mexico[3-6)]. Japan, in particular, has had a long history of pufferfish poisoning. Potent TTX toxicity has also been reported in invasive pufferfish species entering the Mediterranean Sea due to the "Lessepsian migration" caused by creating the artificial Suez Canal[7)]. Problems due to pufferfish consumption occur in many countries along the Mediterranean coast[8-11)]. Besides, the increase in seawater temperature due to global warming is a rising concern as warming may create suitable habitats for pufferfish globally; thus, TTX poisoning incidents may become a worldwide concern[12)]. The globally found pufferfish species containing TTX are shown in Table 1. Recently, TTX and its analogs have been identified[13,14)]; however, their extraction and analysis methods used in various studies differ. Thus, it is challenging to compare the contents of TTX and its analogs in different pufferfish species. TTX contents in pufferfish may vary by species, season, and habitat[7,9,11,15-17)].

Tetrodonic acid (TDA), 4-epiTTX, and anhydroTTX were first identified as pufferfish TTX analogs in 1985[18)]. Since then, many studies have been performed for identifying TTX analogs. Moreover, toxicity caused TTX analogs may vary, with few analogs exhibiting higher toxicity than TTX. Additionally, there is a lack of systemic approaches comparing TTX content in pufferfish species found worldwide.

To ensure safe consumption of pufferfish, it is necessary

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Table 1. Global report of tetrodotoxin content in pufferfish

Species	Area	References
Takifugu pardalis	Japan	(65)
Takifugu poecilonotus	Japan	(66)
Takifugu vermicularis	Japan	(66)
Arothron firmamentum	Japan	(66)
Tetraodon nigroviridis	Japan	(16)
Chelonodon patoca	Okinawa, Japan	(67)
Fugu niphobles	Korea	(16)
Tetraodon nigroviridis	Thailand	(68)
Tetraodon steindachneri	Thailand	(69)
Tetraodon oblongus	Bangladesh	(24)
Arothron hispidus	Philippines	(70)
Arothron mappa	Philippines	(70)
Arothron manilensis	Philippines	(70)
Arothron nigropunctatus	Philippines	(70)
Arothron stellatus	Philippines	(70)
Arothron reticularis	Philippines	(70)
Sphoeroides spengleri	USA	(71)
Sphoeroides testudineus	USA	(71)
Sphoeroides marmoratus	Portugal	(36)
Lagocephalus sceleratus	Greece, Spain, Turkey	(7, 9, 17, 23)
Lagocephalus suzensis	Turkey	(9)

to establish an analytical method with high accuracy and sensitivity to monitor the toxins found in pufferfish species (i.e., TTX analogs). TTX content has conventionally been measured using mouse bioassay (MBA)[19]. However, MBA can only measure the effects of pufferfish toxins and cannot determine TTX content alone. Also, MBA has ethical issues. To address this issue, instrumental analysis methods such as high-performance liquid chromatography coupled with a fluorescence detector (HPLC-FLD) have been proposed for TTX analysis[20]. Liquid chromatography-mass spectrometry (LC-MS) is the commonly used method for analyzing TTX analogs recently[21,22]. However, the currently available studies have reported variations of sample preparation and analysis methods[13,16,17,23-26].

We investigated different sample preparation methods for LC-MS analysis of TTX and its analogs to address these issues. In this study, we also reviewed various LC-MS methods applied in TTX analysis. TTX contents in different pufferfish species and the difference in TTX content depending on the habitats and seasonal variations have been reported.

TTX overview

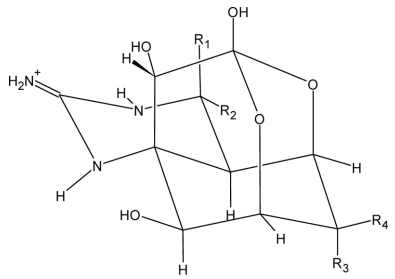
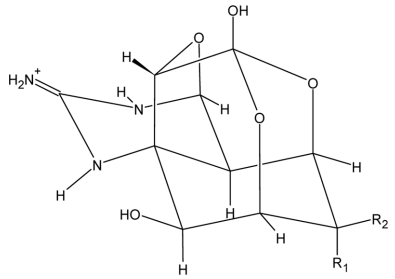
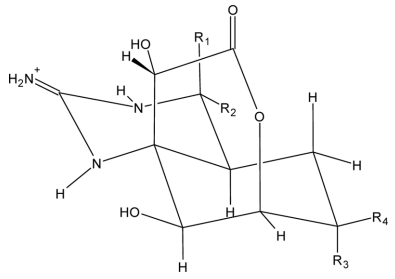
TTX is a potent and old marine toxin[27]. In 1910, Dr. Tahara successfully isolated TTX from pufferfish ovaries and named it "tetrodotoxin" after the order Tetraodontiformes[28]. TTX structure was discovered by several research groups in the 1960s[29-31]. TTX is a colorless and odorless non-protein molecule with a low molecular weight of 319.27 g/mol. Structurally, it has a highly oxygenated carbon backbone comprising a 2,4-dioxaadamantane structure containing five hydroxyl groups that are connected to a single guanidinium moiety[32]. The structures of TTX and its analogs are shown in Table 2.

TTX strongly and selectively blocks voltage-gated sodium channels without affecting other receptors and ion channel systems[33]. It blocks these channels outside the nerve membranes, causing paralysis, and high exposure to TTX may lead to death[33]. In humans, the median lethal dose (or MDL_{50}) of TTX is predicted to be about 10,000 MU[34], which is equivalent to about 2 mg of TTX[35]. Additionally, TTX is thermostable and generally does not degrade by cooking, and hence, TTX poisoning incidents have been occurring historically[27]. Approximately 30 TTX analogs have been reported to date, and the degree of toxicity of these analogs depends on their structures. For example, hydroxyl analogs are more toxic than TTX; however, deoxy analogs are less toxic than TTX[23,36-38]. Additionally, the effective dose (ED_{50}) of 11-oxo TTX was smaller than the ED_{50} of TTX[38]. Thus, TTX analog composition can also considerably affect toxicity. The type and severity of poisoning symptoms in humans depend on toxin amounts consumed, age, and health. The initial symptoms start with paresthesia of the lips, tongue, and pharynx as well as gastrointestinal symptoms such as vomiting and diarrhea, followed by systemic sensory abnormalities and quadriplegia. In severe cases, extreme hypotension and seizures, cranial nerve dysfunction, and respiratory failure can be observed, potentially leading to death[39]. Death usually occurs 4-6 hrs after exposure to TTX. Currently, there is no fundamental cure or antidote for TTX[40]. TTX mediates its toxic effects by blocking the voltage-gated sodium channels in neurons and causing paralysis. Thus, it can be expected to function as a painkiller. Research is being conducted to assess TTX for treating pain, such as cancer pain[41-43].

Traditional TTX analysis methods in pufferfish

Mouse bioassay (MBA) is the first and most frequently used method to analyze TTX in marine organisms[44]. However, MBA alone cannot identify TTX analogs. Other marine toxins, such as saxitoxin (STX), may block voltage-gated sodium channels and exhibit symptoms similar to TTX poisoning. Owing to this similarity, TTX poisoning cannot be

Table 2. Structures of tetrodotoxin (TTX) and TTX analogs found in pufferfish

Structure	Compound	R_1	R_2	R_3	R_4	$[M+H]^+$ (*m/z*)
	TTX	H	OH	OH	CH_2OH	320
	4-epiTTX	OH	H	OH	CH_2OH	320
	11-norTTX-6(S)-ol	H	OH	OH	H	290
	11-norTTX-6(R)-ol	H	OH	H	OH	290
	6,11-dideoxyTTX	H	OH	H	CH_3	288
	11-oxoTTX	H	OH	OH	$CH(OH)_2$	336
	4,9-anhydroTTX	OH	CH_2OH			302
	5-deoxyTTX	H	OH	OH	CH_2OH	304
	5,6,11-trideoxyTTX	H	OH	H	CH_3	272

differentiated from STX poisoning using MBA[21]. Moreover, MBA is expensive and labor-intensive and is associated with ethical problems. To address these issues, immunological methods using enzyme-linked immunosorbent assay (ELISA) and competitive inhibition enzymatic immunoassay (CIEIA) have been proposed for TTX analysis[45,46]. However, the use of ELISA has several issues such as variability. Although CIEIA has better accuracy and repeatability than ELISA, the antibody used is too expensive[21,47]. Additionally, neither assay can detect TTX analogs. The initial instrumental analysis of TTX was performed using gas chromatography-mass spectrometry (GC-MS) with good sensitivy[48,49]. As TTX is non-volatile, it needs to be derivatized for use in GC-MS. This proves to be a major disadvantage, as derivatization is time-consuming[21].

The analysis of TTX analogs in pufferfish samples using HPLC-FLD was first developed in 1985[50]. This method helps identify TTX and its analogs (i.e., 11-oxoTTX, 4-epiTTX, and 4, 9-anhydroTTX). However, the HPLC-FLD method is not suitable for routine sample analysis because of changes in the fluorescence intensity of TTX analogs and the interference of background signals of the matrices. The fluorescence intensity of 6-epiTTX and 11-norTTX-6 (R)-ol was 20 and 100 times higher than that of TTX, while that of 11-deoxyTTX were 100 times lower than that of TTX[51,52].

LC-MS methods for TTX analog analysis

Recently, LC-MS has been widely used for TTX analysis in pufferfish samples, as TTX analogs can also be identified and quantified with high accuracy and specificity using the method[22]. Table 3 shows various sample preparation methods for LC-MS analysis. For sample preparation in most cases, pufferfish samples are homogenized and extracted using 0.1−2% acetic acid-containing heated water [7,16,17,20,23,36]. Then, solid-phase extraction (SPE) using C_{18} cartridges and filtration are performed together for further clean-up before LC-MS analysis. Hydrophilic interaction liquid chromatography (HILIC) SPE must be performed in gravity flow as vacuum interferes with the TTX recovery rate[53]. Recently, accelerated solvent extraction has been used in the extraction of TTX from pufferfish[54]. This method has a high recovery rate, but it is associated with long prepration time because of an added lyophilization process[54].

Table 3. Different pufferfish sample preparation techniques for the analysis of tetrodotoxin and its analogs

Sample	Sample matrix	Sample preparation	References
Lagocephalus sceleratus	Whole body	Homogenization, lyophilization, accelerated solvent extraction with acetic acid, sonication, centrifugation, and filtration	(54)
	Ovary	Extraction with acetic acid, SPE[1)] with acetic acid and ethanol, evaporation, and ultrafiltration	(13)
Fugu poecilonotus, *F. pardalis*	Ovary	Extraction with AcOH, treat charcoal column with AcOH:EtOH:H_2O, evaporation, and ultrafiltration	(25)
Diodon holocanthus, D. hystrix, Ostracion nasus, A. immaculatus, Arothron manilensis, A. stellatus, Chelonodon patoca, L. inermis, L. lunaris, L. sceleratus, L. spadiceus, Tetraodon nigroviridis, Torquigener pallimaculatus, Xenopterus naritus	Liver, muscle, and skin	Homogenization, extraction with acetic acid, ultrasonication, centrifugation, and filtration	(72)
L. sceleratus	GI tract[2)], muscle, skin, and liver	Homogenization, extraction with acetic acid, heating, cooling, sonication, centrifugation, SPE with acetic acid/methanol solution, evaporation, and filtration	(23)
	Ovary, liver, muscle, and skin	Homogenization, extraction with acetic acid, heating, cooling, SPE with acetic acid, and filtration	(26)
T. rubripes	Ovary, liver, muscle, and skin	Homogenization, extraction with acetic acid, heating, cooling, centrifugation, and SPE with acetic acid	(20)
L. sceleratus	Gonads (only in females), liver, skin, and muscle	Double extraction with acetic acid, evaporation, and filtration	(11)
Sphoeroides marmoratus, *L. lagocephalus*	Gonads, liver, digestive tract, and muscle (without skin)	Extraction with acetic acid, heating, cooling, centrifugation, an SPE with NH_3	(36)
L. sceleratus	Gonads, GI tract, liver, muscle, and skin	Extraction with acetic acid, heating, evaporation, and filtration	(17)
Torquigener flavimaculosus	Gonads, intestines, dorsal muscle, whole upper and lateral skin from head to tail	Extraction with acetic acid and methanol, homogenization, sonication, centrifugation, SPE with methanol, evaporation, and filtration	(10)
L. sceleratus, *L. spadiceus* *L. suezensis*	Gonads, intestines, dorsal muscle, whole upper and lateral skin from head to tail	Extraction with acetic acid, homogenization, sonication, centrifugation, SPE with acetic acid, evaporation, and filtration	(73)
T. oblongus	Testis/ovary, viscera, liver, muscle, and skin	Homogenization, extraction with acetic acid, centrifugation, and filtration	(24)
F. niphobles, *T. nigroviridis*, *T. biocellatus*	*F. niphobles* – ovary/testis, liver, intestine, dorsal skin, and dorsal muscle *T. nigroviridis*, *T. biocellatus* – whole body	Homogenization, extraction with acetic acid, heating, centrifugation, SPE with acetic acid/EtOH/H_2O, evaporation, and centrifugation	(16)
L. sceleratus	Dorsal muscle, gonads, whole upper and lateral skin from head to tail, GI tract, and liver	Extraction with acetic acid, homogenization, ultrasonication, centrifugation, SPE with methanol, evaporation, and filtration	(9)
L. sceleratus	Muscle, liver, gonad, kidney, Intestine, and skin	Homogenization, extraction with acetic acid, heating, defatting, and ultrafiltration	(7)

[1)]GI tract: gastrointestinal tract.
[2)]SPE: solid-phase extraction.

Table 4. Different LC-MS methods for tetrodotoxin analysis in pufferfish

Instrument	Stationary phase	Mobile phase	Gradient	m/z	LOD[1] (ppm)	Matrix	References
UHPLC-QqQ[2]	C_{18} (250×4.6 mm, 5 μm)	A: 0.1% formic acid in water B: 0.1% formic acid in methanol	Gradient	320/162, 320/256, 320/302	0.03-0.08 μg/g	Ovary, liver, muscle, and skin	(26)
UHPLC-QqQ	Supelco C_{18} column (4.6×250 mm; 5 μm)	A: 20 mM heptafluorobutyric acid, 20 mM ammonium hydroxide and 10 mM ammonium formate (pH 4.0 with formic acid) with 1% acetonitrile B: 20 mM heptafluorobutyric acid, 20 mM ammonium hydroxide and 10 mM ammonium formate (pH 4.0 with formic acid) with 5% acetonitrile	Gradient	320/302	0.01 μg/g	Dorsal muscle, gonads, whole upper and lateral skin from head to tail, GI tract[3] and liver	(9)
UHPLC-QqQ	Develosil C30 UG-5 column (250×4.6 mm)	1 vol% acetonitrile, 20 mM ammonium heptafluorobutyrate, and 10 mM ammonium formate (pH 4.0)	Isocratic	320/162	0.0002 μg/mL	Ovary	(25)
UHPLC- Quadrupole	Shodex RSpak NN-414 column (150×4.6 mm, 5 μm)	20 mM ammonium acetate–methanol (75 : 25)	Isocratic	320/302	0.1 μg/g	Ovary, liver, muscle, and skin	(20)
UHPLC-qTOF [3]	Poroshell 120 Hilic (3.0×50 mm; 2.7 mm) column	A : 20 mM ammonium acetate in distilled water B : 20 mM ammonium acetate in acetonitrile	Gradient	320/302 320/284	0.3 μg/g	Gonads, intestines, dorsal muscle, whole upper and lateral skin from head to tail	(10)
UHPLC-qTOF	Poroshell 120 Hilic (3.0×50 mm; 2.7 mm) column	A : 20 mM ammonium acetate in distilled water B : 20 mM ammonium acetate in acetonitrile	Gradient	320/302	0.3 μg/g	Gonads, intestines, dorsal muscle, whole upper and lateral skin from head to tail	(73)
UHPLC- qTRAP[4]	Sunfire C_{18} column (250×4.6 mm;5 μm) with a guard cartridge (10×4.6 mm) XBridge™ Amide column (150×2.1 mm; 3.5 μm) with a guard cartridge (10×2.1 mm)	A: 1% acetonitrile; 20 mM heptafluorobutyric acid; 20 mM ammonium hydroxide and 10 mM ammonium formate (pH 4.0 with formic acid) B: the same mixture but with 5% acetonitrile	Gradient	320/302	0.016 μg/mL	Gonads, GI tract, liver, muscle, and skin	(17)
UHPLC-QqQ	Acquity UPLC BEH HILIC column (100×2.1 mm; 1.7 μm)	A: 5% acetonitrile (ACN) B: 95% ACN containing 1% acetic acid (pH 3.5)	Gradient	319.92/ 302.00, 319.92/ 161.80	0.00007 μg/mL	Muscle	(54)
UHPLC-QqQ	150×2.1 mm inner diameter, ZIC-HILIC column	A: 10 mM ammonium formate and 10 mM formic acid in water B: 5 mM ammonium formate and 2 mM formic acid in acetonitrile/water (80/20, v/v)	Gradient	320/162	0.05 μg/g	Liver, muscle, and skin	(72)
UHPLC-QqQ[1]	Atlantis HILIC Silia column (100×2.1 mm, 3 μm)	10 mmol/L ammonium formate (adjusted to pH 3.5 with formic acid) - acetonitrile (22:78, v/v)	Isocratic	320/302	0.0001 μg/mL	Ovary	(13)

Table 4. (Continued) Different LC-MS methods for tetrodotoxin analysis in pufferfish

Instrument	Stationary phase	Mobile phase	Gradient	m/z	LOD[1] (ppm)	Matrix	References
UHPLC-QqQ	SeQuant ZIC HILIC column (150×2.1 mm, 5 μm)	A: ammonium acetate (5 mM) and acetic acid (0.01%) in water B: ammonium acetate (5 mM) and acetic acid (0.01%) in 90:10 acetonitrile: water	Gradient	320/302, 320/162	0.01 μg/mL	GI tract[2], muscle, skin, and liver	(23)
UHPLC-QqQ	ZIC-HILIC column (150×2.1 mm; 5 μm) with corresponding guard column (20×2.1 mm)	A: 10 mM ammonium formate and 10 mM formic acid in water B: 80% acetonitrile and 20% water with a final concentration of 5 mM ammonium formate and 2 mM formic acid	Gradient	320/302	0.00009 μg/g	Testis/ovary, viscera, liver, muscle, and skin	(24)
UHPLC-QqQ	HILIC XBridge Amide column	A: water B: acetonitrile/water	Gradient	320.1/302.1 320.1/162.2	0.05 μg/g	Gonads (only in females), liver, skin, and muscle	(11)
UHPLC-QqQ	1.7 μm, 2.1×150 mm; Waters Acquity Glycan BEH Amide UPLC column	A: water/formic acid/NH_4OH B: acetonitrile/water/formic acid	Gradient	320.1/302.1	0.005 μg/g	Gonads, liver, digestive tract, and muscle (without skin)	(36)
UHPLC-QqQ	TSKgel Amide-80 column (150×2.0 mm, 5 μm)	Aqueous solution containing 16 mM ammonium formate buffer (pH 5.5) and acetonitrile (3:7, v/v)	Isocratic	320/162	0.16 μg/g	*F. niphobles* – ovary/ testis, liver, intestine, dorsal skin, and dorsal muscle *T. nigroviridis*, *T. biocellatus* – whole body	(16)
UHPLC-QqQ	TSKgel Amide-80 column (2.0 internal diameter × 150 mm, particle size 3 μm)	16 mM ammonium formate bufferr (pH 5.5) and acetonitrile (4:6, v/v)	Isocratic	320/162	0.0071 μg/g	Muscle, liver, gonad, kidney, intestine, and skin	(7)

[1] LOD: limit of detection.
[2] UHPLC-QqQ: ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry.
[3] GI tract: gastrointestinal tract.
[4] UHPLC-qTOF: ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry.
[5] UHPLC- qTRAP: ultra-high-performance liquid chromatography coupled with triple quadrupole ion-trap mass spectrometry.

Various LC-MS methods used for TTX analysis are shown in Table 4. Reverse-phase chromatography has been the conventionally used method for TTX analysis[9,17,25,26,53,55,56]. However, this method cannot separate all analogs of TTX. In addition, TTX analogs are highly polar in nature and are rapidly eluted in reverse-phase separation, resulting in poor separation of TTX analogs. Thus, normal-phase separation is a preferred option for TTX analogs to ensure better separation[21]. HILIC columns have a hydrophilic stationary phase that can separate hydrophilic substances such as TTX analogs and are reported to have a lower background noise. These columns also have better resolution and sensitivity for TTX analog analysis than C_{18} columns[24,57]. Ammonium formate or ammonium acetate in water with organic phases (i.e., acetonitrile or methanol) is widely used as the mobile phase for TTX analysis. Moreover, both acetonitrile and methanol facilitate similar separation of TTX analogs; however, owing to the low cost of methanol, the latter is a preferred choice for the process[53].

TTX contents in pufferfish analyzed by LC-MS

TTX is the most characteristic toxin among pufferfish toxins. Although there are many studies on the accumulation of TTX in pufferfish, little is known about the biosynthesis of TTX. Interestingly, pufferfish lose their TTX capacity when cultured[58,59]. Therefore, it has been hypothesized that TTX in pufferfish is derived from the food chain and not from an endogenous factor[58].

Recent studies have used LC-MS for identifying and quantifying TTX in pufferfish[13,17,23,24]. For identification, the fragmentation pattern of TTX has been propsed in positive ionization mode of LC-MS. The precursor ion of TTX ion at *m/z* 320 is fragmented to *m/z* 302 (loss of one water molecule), *m/z* 284 (loss of three water molecules), *m/z* 256, *m/z* 178, and *m/z* 162 fragment ions[21].

Table 5 shows the distribution of TTX in various organs of pufferfish samples analyzed by LC-MS. TTX contents in pufferfish differ greatly by organs. The liver and ovaries of pufferfish have the highest TTX contents, followed by the intestine and skin[11,20,24]. The muscles and testicles usually contain trace amounts or do not contain TTX, except for those in *Lagocephalus lunaris* and *Chelonodon patoca*[58]. During the spawning period, TTX is transferred from the liver to the ovaries, which results in increased TTX in the ovaries of female pufferfish[60,61]. In contrast, TTX in male pufferfish is distributed from the liver to the skin during spawning period, and hence, the skin in male pufferfish has high TTX content[61]. Additionally, TTX contents are generally higher in females and male pufferfish during the maturation/spawning period than usual contents[61].

TTX analog contents in pufferfish analyzed by LC-MS

Table 6 shows TTX analog levels in pufferfish determined using LC-MS. TTX analogs can be identified and quantified using ultra-HPLC coupled with triple quadrupole or ion-trap mass spectrometry[11,16,23,25,36]. Few TTX analogs that have been identified in pufferfish include tetodonic acid (TDA); 4-epiTTX; anhydroTTX; 11-deoxyTTX; 11-norTTX-6,6-diol; 11-norTTX-6(R)-ol; 11-norTTX-6(S)-ol; 5-deoxyTTX; and 6,11-dideoxyTTX-5,6,11-trideoxyTTX[18,62-64]. In 2001, Shoji et al. identified TTX analogs in pufferfish by LC-MS and obtained characteristic fragmentation ion spectra of TTX and its analogs in positive ionization mode of electrospray ionization tandem mass spectrometry (MS/MS)[25]. Authentic TTX, TTX analogs, and semi-purified TTX mixture, have been used to quantify TTX and analogs in LC-MS/MS[15-17,23,36].

Major TTX analogs found in pufferfish and their composition vary across different species. In all tissues of *Fugu niphobles* and two *Tetraodon* spp. in Korea and *F. niphobles* in Japan, 5,6,11-trideoxyTTX and 6,11-dideoxyTTX were identified as the major TTX analogs, and 5-deoxyTTX and 11-deoxyTTX were the minor analogs[16]. Up to 8 TTX analogs have been found in *Lagocephalus sceleratus* in the Aegean Sea, with 5,6,11-trideoxyTTX being the major TTX analog[17]. Moreover, *L. sceleratus* in the Aegean Sea had a higher level of 11-deoxyTTX and 11-norTTX-6(S)-ol than TTX[11,17]. In the Atlantic *Sphoeroides marmoratus*, TTX analogs such as 4,9-anhydroTTX and 4-epiTTX and three deoxy analogs 5-deoxyTTX, 6,11-dideoxyTTX, and 11-deoxyTTX were identified[36].

Conclusion

TTX is one of the oldest and most potent marine toxins as well as a non-protein, low molecular weight neurotoxin that blocks sodium channels in humans. Many methods have been used for TTX analysis; however, LC-MS is being studied for identifying and quantifying TTX analogs due to their respective disadvantages. In LC-MS analysis, HILIC column use, in particular, results in better separation of TTX analogs than reverse-phase column (e.g., C_{18} column) use. In pufferfish, LC-MS using HILIC columns revealed TTX, with the liver and ovary having the highest toxin levels, followed by the intestine and skin, muscle, and testis. Differences in TTX levels were noted based on species, seasonal variations, and organ. TTX analog levels varies with species and region. And certain TTX analogs were present in high levels, with few having higher toxicity than TTX. However, little is known about the TTX and its analog composition various species pufferfish. In terms of food safety, further research is required monitor TTX and its analog composition in pufferfish.

Table 5. Tetrodotoxin contents in pufferfish samples

Sample	Area	Sex	Liver (μg/g)	Muscle (μg/g)	Skin (μg/g)	Gonad (μg/g)	Intestine (μg/g)	Device	References
F. niphobles	Tongyeong bay, Korea	F	1.28-7.66	0.96-2.55	24.90-29.69	41.19-83.97	2.55-21.39	UHPLC-QqQ[1)]	(16)
		M	2.87-9.90	2.55-5.11	12.13-62.90	0.32-3.51	0.96-2.23		
L. sceleratus	Aegean Sea		<LOQ[2)]-0.07	<LOQ-0.40	0.02-0.18		13.52	UHPLC- QqQ	(23)
S. marmoratus.	Northeastern Atlantic Ocean	F	1.4	0.5		7.4	4.0	UHPLC- QqQ	(36)
		M	5.4	1.7		5.7	15.0		
T. oblongus	Bay of Bengal	F	45.71	1.64	25.35	356		UHPLC- QqQ	(24)
L. sceleratus	Mediterranean Sea	F	2.3	0.7	1.2	21.8		UHPLC- QqQ	(11)
F. pardalis	Miyagi Prefecture, Japan	F	7.66-60.34			8.62-94.50		UHPLC- QqQ	(15)
		M	1.60-5.43			6-24			
L. sceleratus	Northeastern Mediterranean Sea	F	NA[3)]-46.18	0-2.83	0.60-3.43	0.58-52.07	0.07-7.64	UHPLC- QqQ	(9)
		M	NA-0.40	0-0.44	0.13-1.31	0.43-30.7	0.15-0.84		
L. sceleratus	Marmaris Bay and Iskenderun Bay, Turkey	F	0.33-1.61	0.10-0.62	0.35-1.28	1.65-80.0	0.62-1.12	UHPLC- QqQ	(7)
		M	0.12-0.84	0.07-1.46	0.10-2.08	0.19-1.59	0.13-1.70		
T. flavimaculosus	Northeastern Mediterranean Sea	F	11.62-106.80	42.07-75.04	35.19-139.72	41.49-100.71	12.59-55.24	UHPLC-qTOF[4)]	(10)
		M	7.04-85.63	15.88-86.07	33.95-139.88	5.03-61.05	14.89-86.30		
L. sceleratus	Northeastern Mediterranean Sea	F	1.03-21.1	0.70-5.12	2.20-11.8	4.15-35.6	0.79-12.5	UHPLC-qTOF MS	(73)
		M	1.70-12.3	1.17-4.90	2.90-5.02	0.69-11.8	2.29-3.00		
L. suzensis		F	<LOQ-0.82	<LOQ-1.26	<LOQ-1.89	<LOQ-2.02	<LOQ-1.91		
		M	<LOQ-1.44	<LOQ-1.44	<LOQ-3.09	<LOQ-1.50	<LOQ-13.34		
T. pardalis	no information	F	757	5.3	8.5	327		UHPLC-Quadrupole	(20)
L. sceleratus	Aegean Sea		<LOQ-44.15	<LOQ-3.47	<LOQ-1.4	0.47-46.3	<LOQ-37.60	UHPLC- qTRAP[5)]	(17)

[1)] UHPLC-QqQ: Ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry.
[2)] LOQ: Limit of quantification.
[3)] NA: Not analyzed.
[4)] UHPLC-qTOF: Ultra- high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry.
[5)] UHPLC- qTRAP: Ultra-high-performance liquid chromatography coupled with triple quadrupole ion-trap mass spectrometry.

Table 6. Tetrodotoxin analog contents in pufferfish samples

Sample	Area	Sex	Matrix	Toxin content (μg/g)								References
				4-epiTTX	4,9-anhydroTTX	5-deoxy TTX	11-dexoy TTX	6,11-dideoxyTTX	5,6,11-trideoxyTTX	11-norTTX-6(R)-ol	11-norTTX-6(S)-ol	
F. niphobles	Tongyeong bay, Korea	F	Liver	<0.16-1.92	1.51-11.75	<0.15-0.91	<0.15-0.30	0.29-6.89	0.81-4.34			(16)
			Skin	2.55-3.19	6.33-9.04	0.91-3.04	0.91-2.43	1.72-3.73	29.84-42.05			
			Muscle	<0.16-0.32	<0.15-0.30	<0.15	<0.15	0.29-1.72	1.36-1.90			
			Gonad	3.19-8.30	9.04-25.00	1.22-6.69	2.43-11.83	22.12-89.05	78.40-163.58			
			Intestine	0.32-5.11	2.11-33.14	0.30-1.22	<0.15-3.64	3.16-41.65	6.78-19.26			
		M	Liver	0.32-0.96	2.71-4.52	<0.15-0.61	<0.15-0.30	<0.14-16.95	2.71-4.88			
			Skin	0.64-2.87	1.81-7.83	0.30-2.13	0.30-2.73	0.57-8.04	10.58-41.50			
			Muscle	0.32-0.96	<0.15-0.30	<0.15	<0.15	<0.14-1.44	<0.14-3.80			
			Gonad	<0.16-0.32	<0.15-0.90	<0.15	<0.15	<0.14-2.59	0.27-3.53			
			Intestine	<0.16-0.32	0.60-1.81	<0.15	<0.15	0.57-8.04	1.36-1.90			
T. nigroviridis			Whole body	<0.16-1.60	<0.15-14.16	<0.15-2.13	<0.15-2.12	<0.14-2.30	<0.14-40.96			
T. biocellatus			Whole body	0.64-5.11	1.21-12.35	<0.15-0.61	<0.15-5.16	<0.14-5.17	5.70-54.80			
F. pardalis	Miyagi Prefecture, Japan	F	Liver	1-5	16-90	<0.4-2	<0.4-20		8-68			(15)
			Gonad	1-5	8-27	<0.4-2	<0.4-25		35-226			
		M	Liver	<0.4	<0.4-8	<0.4	<0.4		3-22			
			Gonad	<0.4-1	<0.4-5	<0.4	<0.4		10-18			
S. marmoratus	Northeastern Atlantic Ocean	F	Liver	0.1	0.0	0.2	0.4	0.0				(36)
			Muscle	0.0	0.0	0.2	0.2	0.0				
			Gonad	1.0	0.5	4.4	5.6	1.8				
			GI tract[1)]	0.4	0.0	0.4	0.8	< LOQ[2)]				
		M	Liver	0.5	0.3	2.3	3.1	0.9				
			Muscle	0.2	0.1	0.6	1.0	0.2				
			Gonad	0.7	0.3	3.2	4.0	1.3				
			GI tract	1.2	0.3	2.7	4.3	1.3				
L. sceleratus	Aegean Sea		Liver	< LOQ	0.85	< LOQ	< LOQ-2.25		1.92	< LOQ	< LOQ-1.10	(23)
			Skin			< LOQ	< LOQ		< LOQ		< LOQ	
			Muscle	< LOQ			< LOQ		< LOQ		< LOQ	
			GI tract	0.46	2.43	0.02	6.65		4.62	< LOQ	4.75	

Table 6. (Continued) Tetrodotoxin analog contents in pufferfish samples

Sample	Area	Sex	Matrix	Toxin content (μg/g)								References
				4-epiTTX	4,9-anhydroTTX	5-deoxy TTX	11-dexoy TTX	6,11-dideoxyTTX	5,6,11-trideoxyTTX	11-norTTX-6(R)-ol	11-norTTX-6(S)-ol	
L. sceleratus	Mediterranean Sea	F	Liver	0.7	0.2	ND	0.2	0.2	12.4	0.3	1.3	(11)
			Muscle	0.3	0.1	ND	0.1	0.1	1.2	0.2	1.1	
			Skin	0.3	ND	ND	0.1	ND	1.8	0.1	0.6	
			Gonad	4.3	0.5	0.5	1.1	0.4	94.3	1.1	16.3	
L. sceleratus	Aegean Sea		Liver	0-7.55	0-6.60	< LOD[3]-13.40	< LOQ-92.00		< LOQ-602.50	0-20.10	0.41-182.25	(17)
			Muscle	0-0.41	0-< LOQ	< LOD-1.15	< LOQ-8.85		< LOQ-45.60	0-1.41	< LOD-6.40	
			Skin	0-<LOD	0-<LOD	0-0.47	< LOQ-3.55		< LOD-56.50	0-1.08	0-3.32	
			Gonad	0-6.15	0-5.55	0.37-20.40	1.11-230.88		< LOQ-4847.50	0.41-22.35	0.43-221.88	
			GI tract	0-5.15	0-2.65	0-12.65	< LOQ-72.50		< LOD-359.75	0-10.75	0-70.50	

[1] GI tract: gastrointestinal tract.
[2] LOQ: limit of quantification.
[3] LOD: limit of detection.

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국문요약

테트로도톡신(Tetrodotoxin)은 나트륨 통로를 차단하는 신경독소로 가장 오래되고 강력한 해양독소 중 하나이다. 전통적으로 아시아에서 복어 섭취로 인한 테트로도톡신 중독사고가 많이 발생했었다. 최근 아열대 복어 종과 그 잡종 섭취로 인한 테트로도톡신 중독사고가 아시아 외 지역에서도 산발적으로 발생하고 있다. 그러나 복어 종별 테트로도톡신 및 유사체의 함량과 기관 내 분포에 대해서는 잘 알려져 있지 않다. 이 총설에서는 식품공전 복어독(테트로도톡신) 분석법인 쥐를 이용한 생물학적 정량법, 효소면역측정법, 고압 액체 크로마토그래피-형광검출기, 가스 크로마토그래피-질량분석기를 이용한 분석법의 장단점을 비교하였다. 또한 최근 주로 사용되는 액체 크로마토그래피-질량분석기를 이용한 테트로도톡신과 테트로도톡신 유사체 분석법을 소개하고 이에 사용된 전처리법, 컬럼 및 이동상을 비교하였다. 또한 복어 종별 테트로도톡신과 그 유사체 함량과 다양한 기관에서의 분포를 정리하였다. 이 총설은 테트로도톡신 분석법과 다양한 복어 종의 테트로도톡신과 유사체 함량 및 기관에서의 분포를 이해하는데 도움을 줄 수 있을 것이다.

Conflict of interests

The authors declare no potential conflict of interest.

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