Comparison of MBA and HPLC Post-column Oxidation Methods for the Quantification of Paralytic Shellfish Poisoning Toxins

Hongsik Yu[1], Keun Sik Lim[1], Ki Cheol Song[2], Ka Jeong Lee[1], Mi Ae Lee[1] and Ji Hoe Kim[1]*

[1]Food Safety Division, National Fisheries Research and Development Institute, Busan 619-705, Korea
[2]West Sea Fisheries Institute, National Fisheries Research and Development Institute, Incheon 400-420, Korea

Abstract

The mouse bioassay and high performance liquid chromatography (HPLC) post-column oxidation method are different methods of quantifying paralytic shellfish poisoning toxins. In this study, we compared their ability to accurately quantify the toxicity levels in two types of field sample (oysters and mussels) with different toxin profiles for routine regulatory monitoring. A total of 72 samples were analyzed by both methods, 44 of which gave negative results, with readings under the limit of detection of the mouse bioassay (40 μg/100 g saxitoxin [STX] eq). In 14 oysters, the major toxin components were gonyautoxin (GTX) 1, -2, -3, -4, -5, decarbamoylgonyautoxin-2 (dcGTX2), and decarbamoylsaxitoxin (dcSTX), while 14 mussels tested positive for dcSTX, GTX2, -3, -4, -5, dcGTX2, neosaxitoxin (NEO), STX, and dcSTX. When the results obtained by both methods were compared in two matrices, a better correlation ($r^2 = 0.9478$) was obtained for mussels than for oysters ($r^2 = 0.8244$). Additional studies are therefore needed in oysters to investigate the differences in the results obtained by both methods. Importantly, some samples with toxin levels around the legal limit gave inconsistent results using HPLC-based techniques, which could have a strong economic impact due to enforced harvest area closure. It should therefore be determined if all paralytic shellfish poisoning toxins can be quantified accurately by HPLC, and if the uncertainties of the method lead to doubts regarding regulatory limits.

Key words: Mouse bioassay, Post-column oxidation method, PSP toxins, Regulatory limits

Introduction

Paralytic shellfish poisoning (PSP) is caused by the consumption of shellfish containing neurotoxins produced by naturally occurring phytoplankton groups such as *Alexandrium* spp. and *Gymnodinium* spp. Importantly, these toxins cannot be destroyed by common processing steps, including cooking (Diener et al., 2006). One example of such a neurotoxin is saxitoxin (STX), 57 analogues of which were reported recently (Wiese et al., 2010). The health standards of most countries dictate that live bivalve mollusks for human consumption must not contain total PSP toxin levels exceeding 80 μg/ 100 g STX eq.

PSP toxins block excitation currents in nerve and muscle cells, ultimately resulting in paralysis and death (Luckas et al., 2004). Testing shellfish for the presence of PSP family toxins is therefore critical for both consumers and the shellfish industry. The mouse bioassay (MBA) has been the preferred testing method worldwide for over 50 years, and is the official method of AOAC International (Association of Official Analytical Chemists, 2005). The time from exposure to death in the MBA is used to estimate the amount of toxin present in shellfish, and the assay has a detection limit of 40 μg/100 g STX eq. MBA has the additional advantages of reporting the total toxicity of samples in a short period of time, and it does not require specific instruments. Although the MBA is a reli-

*Corresponding Author
E-mail: yhspknu@korea.kr

able method, there is ethical pressure to eliminate testing with animals (Balls et al., 1995; Hess et al., 2006). In addition, the MBA provides no toxin profiles, and is subject to considerable variability (Park et al., 1986).

Alternative methods to reduce the use of MBA testing for PSP toxins in a regulatory environment are therefore becoming desirable. The LC-FLD method was successful in an inter-laboratory study (Association of Official Analytical Chemists, 2006), and was accepted by the Association of Official Analytical Chemists (AOAC) as the first analytical alternative to the MBA. However, this method was overly time-consuming for the routine regulatory monitoring of shellfish for PSP toxins, and cannot distinguish between isomeric toxins that may exhibit significantly different toxicities. Recently, a new high performance liquid chromatography post-column oxidation method (HPLC PCOX) was published; this involves in shorter run times, and demonstrated potential as an alternative to the MBA in our previous study (Song et al., 2013). The aim of this study was to compare the MBA and HPLC PCOX methods, and evaluate their ability to accurately assess the toxicity levels of two types of field sample with different toxin profiles for routine regulatory monitoring.

Materials and Methods

HPLC equipment

A Finnigan Surveyor Plus HPLC system, equipped with a Finnigan Surveyor FL Plus Detector (Thermo Electron, San Jose, CA, USA), was operated at an excitation wavelength of 330 nm and an emission wavelength of 390 nm. The Post-column Derivatization (Pickering Laboratories, Mountain View, USA) was capable of maintaining temperature at 85°C. The chromatographic columns used were Zorbax Bonus RP 4.6×150 mm, 3.5 μm (Agilent, Santa Clara, CA, USA) for the analysis of GTXs & STXs, and Phenosphere-NEXT 4.6×250 mm, 5 μm (Phemomenex, Torrance, USA) for the analysis of C toxins.

Reagents

All solvents and reagents were of HPLC grade, and all mobile phase and post-column reagents were filtered through a 0.45-μm membrane before use. The mobile phases used were as follows: 1) HPLC mobile phases for GTXs and STXs—solvent A, 11 mM heptane sulfonate and 5.5 mM phosphoric acid solution adjusted to pH 7.1 with ammonium hydroxide; solvent B, 11 mM heptane sulfonate, 16.5 mM phosphoric acid, 11.5% acetonitrile solution adjusted to pH 7.1 with ammonium hydroxide. 2) HPLC mobile phases for C toxins—solvent C, 2 mM tetrabutyl ammonium phosphate solution adjusted to pH 5.8 with 1% ammonium hydroxide; solvent D, 2 mM tetrabutyl ammonium phosphate solution adjusted to pH 5.8 with 4%

acetonitrile. 3) Post-column oxidant—a 100 mM phosphoric acid, 5 mM periodic acid solution adjusted to pH 7.8 with 5 M sodium hydroxide. 4) Post-column acid—0.75 M nitric acid.

Standards

Certified reference materials were purchased from the Institute for Marine Biosciences, National Research Council (NRC) of Canada. The following standards were used: C1 (N-sulfocarbamoylgonyautoxin-C1), C2 (N-sulfocarbamoylgonyautoxin-C2), dcGTX2 (decarbamoylgonyautoxin-2), dcGTX3 (decarbamoylgonyautoxin-3), dcSTX (decarbamoyl saxitoxin), GTX1 (gonyautoxin-1), GTX2 (gonyautoxin-2), GTX3 (gonyautoxin-3), GTX4 (gonyautoxin-4), GTX5 (gonyautoxin-5), NEO (neosaxitoxin), and STX (saxitoxin). All individual stock solutions were prepared following the NRC instructions. Two working solutions were then prepared, the first containing dcGTX2, dcGTX3, dcSTX, GTX1, GTX2, GTX3, GTX4, GTX5, NEO, and STX, and the second containing C1 and C2.

Sample preparation

Farmed oysters *Crassostrea gigas* and mussels *Mytilus galloprovincialis* that were harvested from coastal regions of Tongyeong City and Geoje City during the spring of 2010, as part of the marine biotoxin control program of the National Fisheries Research and Development Institute, were analyzed in this study. On receipt, the outside of the shellfish were cleaned with fresh water, and they were then shucked into a No. 10 sieve, and drained for 5 min. PSP toxins were extracted from 100-g samples of homogenized shellfish tissue following the AOAC MBA method 959.08 (Association of Official Analytical Chemists, 2005) using 0.1 M HCl. To deproteinate the samples for high performance liquid chromatography (HPLC) analysis, 25 μL 30% (w/v) trichloroacetic acid (TCA) were added to 500 μL shellfish extract in a microcentrifuge tube, which was then mixed in a vortex mixer and centrifuged at 16,000 *g* for 5 min. Twenty microliters of 1.0 M NaOH were added to neutralize the solution, which was mixed and then centrifuged at 16,000 *g* for 5 min. Finally, the solution was filtered through a 0.2 μm syringe filter into an autosampler vial in preparation for LC analysis.

HPLC post-column oxidation analysis

For the analysis of GTXs and STXs, the LC system was equilibrated for a minimum of 20 min at a column oven temperature of 40°C with a 100% solvent A flow at 0.8 mL/min. For the analysis of C toxins, the LC system was equilibrated for a minimum of 20 min at a column oven temperature of 15°C, with 100% solvent C flowing at 0.8 mL/min. Aliquots of working solutions or sample extracts (10 μL for GTXs and STXs, and 5 μL for C toxins) were injected into the HPLC sys-

tem, and separated using the gradient conditions shown in Table 1. Separated toxins were derivatized using a post-column reaction module (with an oxidant flow rate of 0.4 mL/min, an acid flow rate of 0.4 mL/min, a reaction oven temperature of 85°C, and a reaction coil of 5 m × 0.50 mm id). The derivatives were then detected by florescence.

The amount (µmoles of STXeq) of each toxin in the sample extracts was calculated using the linear regression of the calibration graph, and the specific relative toxicity of each individual PSP toxin was determined (Table 2). To compare the data with MBA results, the total toxicity of the samples was calculated by totaling the µg/100 g STXeq for each toxin using following equation (Song et al., 2013):

$$\mu g/100 \text{ g STX eq} = \sum [\mu M \times 0.3722 \times (Fvol/Ext.vol) \times (10/Wt) \times ReTx].$$

Where µM = concentration in the extract; 0.3722 = mg STX/µmole; Fvol = final volume of the deproteinated extract (560 µL); Ext.vol = volume of crude extract (500 µL); Wt = sample weight; and ReTx = relative toxicity to STX.

Table 1. Step gradient conditions for toxins analysis and post-column oxidation

Toxin	Time (min)	Solvent (%)	Flow rate (mL/min)		
			LC	Oxidant	Acid
GTXs/	0	B[*] 0	0.8	0.4	0.4
STXs	7.9	0	0.8	0.4	0.4
	8	100	0.8	0.4	0.4
	18.5	100	0.8	0.4	0.4
	18.6	0	0.8	0.4	0.4
	24	0	0.8	0.4	0.4
C	0	D[†] 0	0.8	0.4	0.4
toxins	8	0	0.8	0.4	0.4
	15	100	0.8	0.4	0.4
	16	100	0.8	0.4	0.4
	19	0	0.8	0.4	0.4
	24	0	0.8	0.4	0.4

GTX, gonyautoxin; STX, saxitoxin.
[*,†]Solvents for elution of each toxin group by step gradient.

Table 2. Relative toxicity of paralytic shellfish toxins

Toxin	ReTx[*]	Toxin	ReTx
Saxitoxin	1.0000	GTX5	0.0644
NEO	0.9243	dcSTX	0.5131
GTX1	0.9940	dcGTX2	0.1538
GTX2	0.3592	dcGTX3	0.3766
GTX3	0.6379	C1	0.0060
GTX4	0.7261	C2	0.0963

ReTX, relative toxicity to saxitoxin; GTX, gonyautoxin; NEO, neosaxitoxin; dcSTX, decarbamoylsaxitoxin; dcGTX, decarbamoylgonyautoxin; C1, N-sulfocarbamoylgonyautoxin-C1; C2, N-sulfocarbamoylgonyautoxin-C2.
[*]Relative toxicity.

Mouse bioassay

One milliliter of HCl extract was intraperitoneally injected into 17-23 g mice following the AOAC Official Method 959.08 (Association of Official Analytical Chemists, 2005), and the death times were recorded. The concentrations of PSP toxins were then calculated, and presented as µg/100 g STX eq sample using Sommer's Table.

Results and Discussion

Comparison of the HPLC PCOX and MBA methods

The HPLC PCOX method used an extraction procedure similar to that of the MBA. Toxins were separated using ion-pair chromatography on a reverse-phase column, oxidized with periodate/phosphate buffer, and detected using fluorescence (Rourke et al, 2008). This method showed high sample throughput and was rapid, suggesting it to be a viable alternative to the MBA for routine monitoring, consistent with our previous single laboratory validation study (Song et al., 2013).

To compare the HPLC PCOX with MBA, we tested field samples using different matrices. A total of 72 samples of mussels and oysters were analyzed by both methods. Forty-four samples gave a negative result, which was under the limit of detection (LOD) of the MBA (40 µg/100 g STX eq). Table 3 lists the 28 samples that gave results above the MBA LOD, including the description of the shellfish species, the scientific name, results obtained by both methods, and the toxin composition. The major toxins in the oysters were GTX1, -2, -3, -4, -5, dcGTX2, and dcSTX, whereas GTX2, -3, -4, -5, dcGTX2, NEO, STX, and dcSTX were detected in mussels. C toxins were not detected in any of the samples.

In 8 of 14 oysters (sample codes 2, 3, 4, 5, 6, 10, 11, and 13), a large positive or negative bias (relative standard deviations greater than 15%) was obtained using the MBA or HPLC PCOX (Fig. 1). A comparison of the results of both methods for oysters indicated a linear correlation of $r^2 = 0.8244$ (Fig. 2). However a negative bias of the HPLC PCOX method was obtained in two mussels (sample codes 20 and 21) (Fig. 3). A comparison of the results of both methods for mussels indicated a better linear correlation of $r^2 = 0.9478$ (Fig. 4). When the results obtained by both methods were compared in two matrices, a good correlation for mussels was obtained, but a relatively poor correlation for oysters. Turner et al. (2010) previously analyzed some oyster samples using HPLC PCOX, an electrophysiological assay, and a hydrophilic interaction liquid chromatography with tandem mass spectrometric detection. The results obtained using the other methods showed a good correlation, suggesting that the difference may be due to either an under- or overestimation by MBA and/or the HPLC method. Turner et al. (2012) reported that the presence of some metals (particularly zinc, which is present in oyster ma-
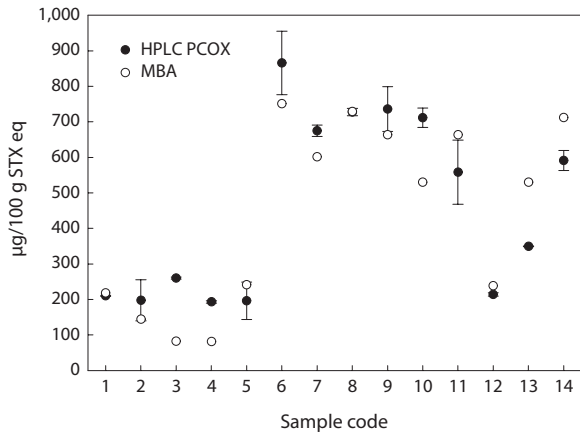
Fig. 1. Paralytic shellfish poisoning toxin concentrations in oyster obtained by mouse bioassay and high performance liquid chromatography post-column oxidation (HPLC PCOX) methods. STX, saxitoxin; MBA, mouse bioassay.
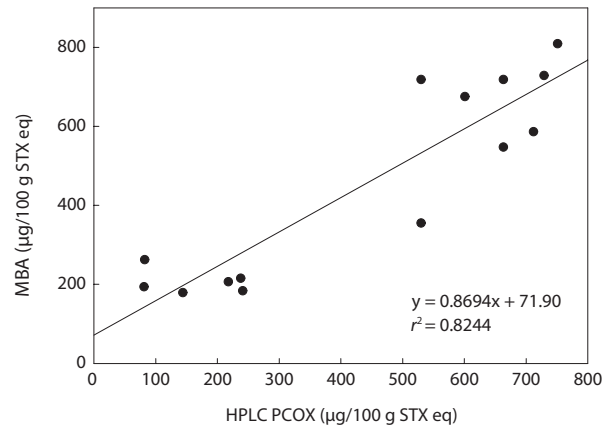

Fig. 2. Correlation between mouse bioassay and high performance liquid chromatography post-column oxidation (HPLC PCOX) method for analysis of paralytic shellfish toxins in oyster (n = 14). STX, saxitoxin; MBA, mouse bioassay.

Table 3. Sample information and toxin profile

Sample	Toxin concentration (µg/100 g STX eq)			Toxin composition
	Code	MBA AOAC	HPLC PCOX	
Fresh oyster *Crassostrea gigas*	1	218	206	GTX1,2,3,4,5; dcGTX2; dcSTX
	2	144	179	GTX1,2,3,4,5; dcGTX2; dcSTX
	3	82	262	GTX1,2,3,4,5; dcGTX2; STX; dcSTX
	4	81	194	GTX1,2,3,4,5; dcGTX2; dcSTX
	5	241	183	GTX1,2,3,4,5; dcGTX2; STX; dcSTX
	6	751	809	GTX2,3,5; dcGTX2; dcSTX
	7	601	675	GTX2,3,4,5; dcGTX2; dcSTX
	8	729	728	GTX2,3,4,5; dcGTX2; dcSTX
	9	663	718	GTX2,3,5; dcGTX2; STX; dcSTX
	10	530	718	GTX2,3,4,5; dcGTX2; dcSTX
	11	663	547	GTX2,3,5; dcGTX2,3; dcSTX
	12	238	215	GTX1,3,4,5; dcGTX2; dcSTX
	13	530	355	GTX1,2,3,4,5; dcGTX2,3; dcGTX3; dcSTX
	14	712	586	GTX1,2,3,4,5; dcGTX2,3; dcSTX
Fresh mussel *Mytilus galloprovincialis*	15	75	66	GTX2,3,4,5; dcGTX3; NEO; STX; dcSTX
	16	77	104	GTX1,2,3,4,5; dcGTX2,3; NEO; STX; dcSTX
	17	167	182	GTX2,3,5; NEO; STX; dcSTX
	18	284	287	GTX2,3,5; dcGTX2; NEO; STX; dcSTX
	19	476	356	GTX2,3,4,5; NEO; STX; dcSTX
	20	591	504	GTX2,3,5; dcGTX2; NEO; STX; dcSTX
	21	794	550	GTX2,3,4,5; dcGTX2,3; NEO; STX; dcSTX
	22	789	756	STX; dcSTX
	23	467	454	GTX5; STX; dcSTX
	24	239	234	GTX2,3,4,5; dcGTX2; NEO; STX; dcSTX
	25	210	207	GTX2,3,5; dcGTX2; NEO; STX; dcSTX
	26	123	90	GTX2,3,5; NEO; STX; dcSTX
	27	58	47	GTX2,3,4,5; dcGTX2; STX; dcSTX
	28	50	54	GTX2,3,4,5; NEO; STX; dcSTX

STX, saxitoxin; MBA AOAC, mouse bioassay Association of Official Analytical Chemists; HPLC PCOX, high performance liquid chromatography post-column oxidation method; GTX, gonyautoxin; dcGTX, decarbamoylgonyautoxin; dcSTX, decarbamoylsaxitoxin; NEO, neosaxitoxin.
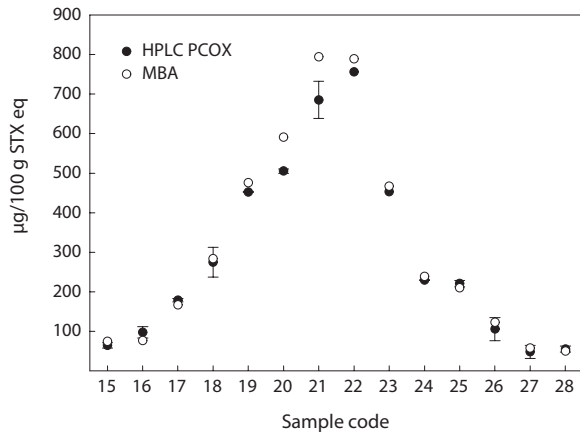
Fig. 3. Paralytic shellfish toxins concentrations in mussel obtained by mouse bioassay and high performance liquid chromatography post-column oxidation (HPLC PCOX) methods. STX, saxitoxin; MBA, mouse bioassay.
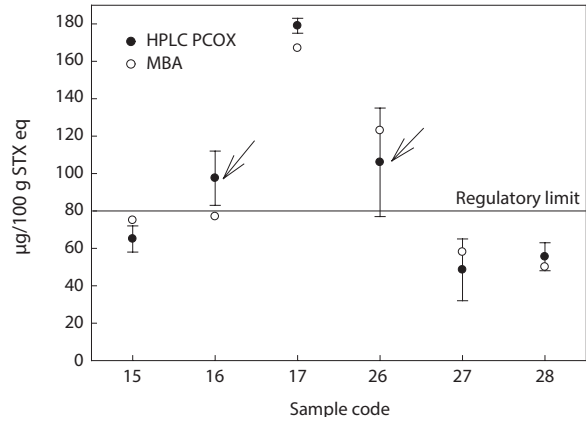

Fig. 5. Paralytic shellfish toxins toxin concentrations in mussel obtained by mouse bioassay and high performance liquid chromatography post-column oxidation (HPLC PCOX) methods (samples with results <200 μg/100 g saxitoxin [STX] eq by both method). Line presents regulatory limit in Korea. MBA, mouse bioassay.
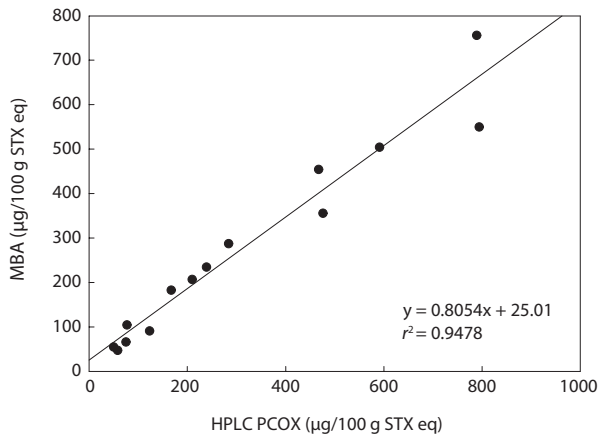

Fig. 4. Correlation between mouse bioassay and high performance liquid chromatography post-column oxidation (HPLC PCOX) method for analysis of paralytic shellfish toxins in mussel ($n = 14$). STX, saxitoxin; MBA, mouse bioassay.

Performance of both methods in measuring samples with toxicity around the regulatory limit

Fig. 5 shows the detection of PSP toxins at low levels using both methods. The regulatory limit in Korea (80 μg/100 g STX eq) (Korea Food and Drug Administration, 2013) is also shown in the Figure. Arrows indicate samples that gave inconsistent results concerning the regulatory limit, for example those that were above or below the official limit, depending on the analysis method. Importantly, the measurement of some samples using HPLC PCOX would have an economic impact because they would lead to a ban on harvesting. In cases, such as these, that are close to the regulatory limit, the uncertainty of both methods should therefore be considered before decisions allowing or banning the harvesting of shellfish are made. In addition, whether all relevant PSP toxins can be detected by the HPLC PCOX method, and whether the uncertainty of both methods would lead to doubts over the regulatory limits, should be assessed. Investigations of the occurrence of phytoplankton and long-term and short-term monitoring of each specific area would also be required, and precautionary steps should be taken if necessary. However, this is not possible using samples from an unknown harvesting area (origin). When this occurs, methods that assess the toxicity of the samples rather than the presence of toxins may be more appropriate (Ben-Gigirey et al., 2012).

Adoption of a new method

When considering adoption of a new method into routine monitoring programs, it is important to note that HPLC methods are currently in use, and the number of laboratories using

trices) could cause MBA to underestimate toxin levels, while the presence of metals had no effect on the performance of the HPLC method. Additional toxins (for example neurotoxins, including carbamate and organophosphate insecticides) also elicited a positive response in the MBA (Rourke et al., 2008). Furthermore, MBA performance can be variable when high toxin concentrations are present, which can lead to unexpected results (Botana et al., 1996). Additional studies using oysters are needed to investigate the differences in the data obtained by the different methods.

these methods is increasing slowly. The HPLC PCOX technique has been used in a Norwegian monitoring program since 2003 (DeGrasse et al., 2011). In addition, the method was adopted by the Canadian monitoring program as a screening tool in 2009, with samples then confirmed by MBA (DeGrasse et al., 2011). The decision regarding which method should be implemented in different regulatory environments may be more of a practical issue than one of scientific merit. In many cases, the availability of equipment and the prevalence of specific toxin components will be the determining factors for any given official control laboratory. Finally, the level of validation or approval of each method may determine whether specific regulatory laboratories implement one method over the other. For example, US National Shellfish Sanitation Program approval determined the method implemented as a regulatory tool in the United States (US Food and Drug Administration, 2009).

Acknowledgements

References

Association of Official Analytical Chemists. 2005. AOAC Official Method 959.08. Paralytic shellfish poison. Biological method. In: AOAC Official Methods of Analysis. 18th ed. Truckses MW, ed. AOAC International, Gaithersburg, MD, US, pp. 79-80.

Association of Official Analytical Chemists. 2006. Paralytic shellfish poisoning toxins in shellfish. Prechromatographic oxidation and liquid chromatography with fluorescence detection. First action 2005, official method 2005.06. In: Official Methods of Analysis of AOAC International. Horwitz W and Latimer GW, eds. AOAC International, Gaithersburg, MD, US, p. 83.

Balls MB, Goldburg AM, Fentem JH, Broadhead CL, Burch RL, Festing MFW, Frazier JM, Hendricksen CFM, Jennings M, van der Kamp MDO, Morton DB, Rowan AX, Russell C, Russell WMS, Spielmann H, Stephens ML, Stokes WS, Straughan DW, Yager JD, Zurlo JD and van Zutphen BFM. 1995. The three Rs: the way forward: the report and recommendation of ECVAM Workshop 11. Altem Lab Anim 23, 883-866.

Ben-Gigirey B, Rodriguez-Velasco ML and Gago-Martinez A. 2012. Interlaboratory study for the extension of the validation of 2005.06 AOAC Official Method for dc-GTX-2,3. J AOAC 95, 1-13.

Botana LM, Rodriguez-Vieytes M, Alfonso A and Louzao MC. 1996. Phycotoxins: paralytic shellfish poisoning and diarrhetic shellfish poisoning. In: Handbook of Food Analysis. Nollet LML, ed. Marcel Dekker Inc., New York, US, pp. 1147-1169.

DeGrasse SL, van de Riet J, Hatfield R and Turner A. 2011. Pre- versus post-column oxidation liquid chromatography fluorescence detection of paralytic shellfish toxins. Toxicon 57, 619-624. http://dx.doi.org/10.1016/j.toxicon.2010.12.017.

Diener M, Erler K, Hiller S, Christian B and Luckas B. 2006. Determination of paralytic shellfish poisoning (PSP) toxins in dietary supplements by application of a new HPLC/FD method. Eur Food Res Technol 224, 147-151. http://dx.doi.org/10.1007/s00217-006-0302-4.

Hess P, Grune B, Anderson DB, Aune T, Botana LM, Caricato P, van Egmond HP, Halder M, Hall S, Lawrence JF, Moffat C, Poletti R, Richmond J, Rossini GP, Seamer C and Vilageliu JS. 2006. Three Rs approaches in marine biotoxin testing: the report and recommendations of a Joint ECVAM/DG SANCO Workshop (ECVAM Workshop 54). Altem Lab Aanim 34, 193-224.

Korea Food and Drug Administration. 2013. Korean Food Standard. Chapter 2. Korea Food and Drug Administration, Cheongwon, KR, pp. 2-26.

Luckas B, Hummert C and Oshima Y. 2004. Analytical methods for paralytic shellfish poisons. In: Manual on Harmful Marine Microalgae. Hallegraeff GM, Anderson DM and Cembella AD, eds. UNESCO Publishing, Paris, FR, pp. 191-209.

Park DL, Adams WN, Graham SL and Jackson RC. 1986. Variability of mouse bioassay for determination of paralytic shellfish poisoning toxins. J Assoc Off Anal Chem 69, 547-550.

Rourke WA, Murphy CJ, Pitcher G, van de Riet JM, Burns G, Thomas KM and Quilliam MA. 2008. Rapid postcolumn methodology for determination of paralytic shellfish toxins in shellfish tissue. J AOAC Int 91, 589-597.

Song KC, Lee KJ, Yu HS, Mok JS, Kim JH, Lim KS, Lee MA and Kim MH. 2013. Intra-laboratory validation of an HPLC post-column oxidation method for the analysis of PSP toxins in oysters and mussels. Korean J Food Sci Technol 45, 241-247. http://dx.doi.org/10.9721/KJFST.2013.45.2.241.

Turner AD, Hatfield RG, Rapkova-Dhanji M, Norton DM, Algoet M and Lees DN. 2010. Single-laboratory validation of a refined AOAC HPLC method 2005.06 for oysters, cockles and clams in U.K. shellfish. J AOAC Int 93, 1482-1493.

Turner AD, Dhanji-Rapkova M, Algoet M, Suarez-Isla BA, Cordova M, Caceres C, Murphy CJ, Casey M and Lees DN. 2012. Investigations into matrix components affecting the performance of the official bioassay reference method for quantitation of paralytic shellfish poisoning toxins in oysters. Toxicon 59, 215-230. http://dx.doi.org/10.1016/j.toxicon.2011.11.013.

US Food and Drug Administration. 2009. Guide for the Control of Molluscan Shellfish, 2007 revision. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington DC, US.

Wiese M, D'Agostino PM, Mihali TK, Moffitt MC and Neilan BA. 2010. Neurotoxic alkaloids: saxitoxin and its analogs. Mar Drugs 8, 2185-2211. http://dx.doi.org/10.3390/md8072185.