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Analytical Methods

Interlaboratory comparison of liquid chromatography-tandem mass spectrometry quantification of diarrhetic shellfish toxins in scallop midgut glands

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ABSTRACT

An interlaboratory comparison (ILC) was organized as a measure of the analytical competency in the liquid chromatography-tandem mass spectrometry quantification of okadaic acid (OA) and dinophysistoxin-1 (DTX1) in scallop midgut gland samples. The test sample was prepared using boiled midgut glands of naturally contaminated scallops with DTX1 and its esters by spiking with OA, and homogeneity and stability of this test sample was assessed to be appropriate. Twenty laboratories participated in the ILC based on the Japanese official testing method; they submitted two sets of analytical concentrations of target analytes along with the details of their analytical protocols. For assessing these data, assigned values were established from another ILC where ten participants quantified the target analytes by the standard addition method. The mean analytical results of the former ILC showed good agreement with the assigned values, and the corresponding relative reproducibility standard deviations met the criterion of CODEX STAN 292. Meanwhile, the results of more than half of the participants were out of the uncertainty range of the assigned values; these participants were encouraged to investigate their protocols to improve their analytical capability.

1. Introduction

Diarrhetic shellfish poisoning (DSP) is a sever gastrointestinal illness that was first reported in the late 1970s (Yasumoto, Oshima, & Yamaguchi, 1978). The principal toxins associated with DSP are the okadaic acid (OA) group, which includes OA, dinophysistoxin-1 (DTX1), and dinophysistoxin-2 (DTX2), and their esterified derivatives. DSP is caused by ingestion of bivalve contaminated with these toxins produced by toxic dinoflagellates (Yasumoto, Murata, Lee, & Torigoe, 1989). Therefore, when the algal bloom occurs in bivalve-growing areas, bivalve industries may suffer significant economic damages due to ceasing harvesting bivalves.

For many years, the mouse bioassay has been official testing methods for DSP inspection of bivalve. However, some disadvantages concerning this method have been pointed out. These include poor reproducibility in results between inspection laboratories, poor selectivity for OA group toxins, poor sensitivity, and ethical concern using living animal (European Food Safety Authority, 2008). Thus, many laboratories involved in DSP inspection are required to replace their analytical methods with chemical analysis. In March 2015, in Japan, an analytical method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology was introduced as the official testing method (Ministry of Health, Labour and Welfare, 2015). Here, the requirements of analytical performance for selectivity, trueness, precision, and the limits of quantification are established in the appendix of this notice, and the corresponding validation methods are given in the attachment. In addition, an analytical protocol that had been validated in a single laboratory for samples of the edible part of scallops is provided (hereinafter called “example protocol”); this protocol consists of an extraction of OA group toxins by solvent extraction, hydrolysis of the toxins to OA, DTX1, or DTX2 under alkaline conditions, clean-up by solid phase extraction (SPE), and LC-MS/MS quantification using an external standard method. In some kinds of bivalves including Japanese scallop (*Mizuhopecten yessoensis*), OA group toxins are concentrated in the midgut gland. In such cases, the notice allows using only midgut gland part for the analysis.

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Interlaboratory comparison (ILC) is the collaborative study among different laboratories for testing the same samples and comparing the observed results. One of the principal purposes is to validate a test method. For this purpose, some ILCs of LC-MS/MS quantification of lipophilic toxins in bivalve samples were organized (These, Klemm, Nausch, & Uhlig, 2011; van den Top, Gerssen, McCarron, & van Egmond, 2011). As termed "proficiency testing", in addition, ILC is also used to investigate the ability of the participants to provide accurate results. Thus, laboratories accredited by the ISO/IEC 17025 are encouraged to participate in an appropriate ILC (International Organization for Standardization, 2005). Furthermore, participating in ILC assists participants in locating technical areas that require improvement (Miyashita et al., 2013).

Assigned values in ILC are the best practicable estimate of the true value for target analytes in a test sample. Although consensus values, which are calculated using participants' analytical results, can be used as the assigned values, they may be influenced by the analytical method and/or performance of the participants. Therefore, the uncertainty of this type of assigned value is considered large (International Organization for Standardization, 2010). The use of an appropriate certified reference material (CRM) can potentially provide reliable assigned values (Yarita et al., 2015). However, shellfish CRMs intended to be used for the quality control of DSP inspection are very limited (McCarron, Giddings, & Quilliam, 2011; McCarron, Emteborg, Nulty, et al., 2011; McCarron, Emteborg, Giddings, et al., 2011), and no CRM of midgut gland of Japanese scallop (*Mizuhopecten yessoensis*), which is one of the most important bivalve species in DSP monitoring program in Japan, is available. Another strategy to obtain reliable assigned values is to use an analytical result provided by the national metrology institutes by applying isotope-dilution mass spectrometry (IDMS) (Kim, Ahn, & Mitani, 2011; Sin & Wong, 2015; Yarita, Otake, Aoyagi, Numata, & Takatsu, 2016) because IDMS has potential as a primary method of measurement (Richter, 1997). At present, however, isotopically labeled compounds that can be used as internal standards in IDMS measurements for OA group toxins are not available.

In the present study, we have organized an ILC for quantification of OA group toxins in a scallop midgut gland sample to evaluate the Japanese official testing method and to investigate the analytical performance of the participants. To achieve these objectives, assigned values were obtained from another ILC that was based on the standard addition method. This report summarizes the process and results of these ILCs. Technical concerns observed are also discussed.

2. Experimental

2.1. Test samples

The test samples were prepared by the National Metrology Institute of Japan (NMIJ). Boiled midgut glands of toxic Japanese scallops (*Mizuhopecten yessoensis*), which were contaminated with DTX1 and its esters, and nontoxic scallops were kindly provided by the Aomori Prefecture Scallop Marketing Promotion Association (Aomori, Japan). These raw materials were mixed in an appropriate ratio, homogenized using a food processer, strained using a metal mesh (20 mesh), and mixed with L-ascorbic acid (5% mass fraction) that was used as the antioxidant. Then, OA that had been prepared using a large culture of toxic dinoflagellate *Prorocentrum lima* (Suzuki et al., 2014) from the National Research Institute of Fisheries Science was added to the processed raw materials, and the blend was further mixed. The processed material was bottled in 88 plastic bottles (5 g each) and stored at ca. −30 °C until further use.

2.2. Analytical method for assessment of the test samples

2.2.1. Reagents

National Research Council (NRC) (Ottawa, Canada) CRM-OA and CRM-DTX1 were used for identification and quantification of OA and DTX1 in the homogeneity and stability assessments of the test sample. The NRC CRM-DTX2 was used as a syringe spike. HPLC-grade methanol and LCMS-grade acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). Other reagents were of Pesticide Residue and PCB Analysis grade or of reagent grade and were purchased from Kanto Chemical (Tokyo, Japan). The water used for sample preparation was prepared with an Elga (London, UK) Purelab flex system.

2.2.2. Preparation procedure

The homogeneity and stability of the prepared test sample were assessed by NMIJ. The preparation procedure of the test sample was developed based on the example protocol with some modifications. An outline is as follows. First, the test sample (0.5 g) was weighed in a 50-mL centrifuge tube, homogenized with methanol (9 mL) for 3 min, and centrifuged at 3000 rpm for 10 min; the supernatant was collected. The residue was then re-homogenized with a 9 mL methanol/water mixture (9:1, v/v) for 3 min and centrifuged under the same condition as above; the supernatant was collected again. The residue and the inner wall of the centrifuge tube was rinsed with a 2 mL methanol/water mixture (9:1, v/v), and the rinse solution was collected after centrifuging. All the obtained solutions were combined and weighed, and then a 2.0 mL portion was fractionated and weighed in a screw tube. The solution was spiked with a 250 μL 2.5 mol/L sodium hydroxide solution, allowed to stand at 76 °C for 40 min, cooled to room temperature, and spiked with a 250 μL 2.5 mol/L hydrochloric acid solution. The 250 μL syringe spike solution was weighed and added to the neutralized solution to prevent the analytical bias due to the change in the volume of the sample solution. The sample solution was finally prepared by filtering the obtained solution using a 0.45-μm membrane filter (hydrophilic polyolefin membrane).

2.2.3. LC-MS/MS measurement

Quantification of OA and DTX1 in the sample solution was performed using a Shimadzu (Kyoto, Japan) LCMS-8030 triple quadrupole mass spectrometer with a Shimadzu LC-20A series high performance liquid chromatography (HPLC) apparatus. An Imtakt (Kyoto, Japan) Cadenza CD-C18 column (100 mm × 2 mm I.D., particle size: 3 μm) was used as the separation column. The HPLC apparatus was operated under the following conditions: a mobile phase with eluent A consisting of water with 2 mmol/L ammonium formate and 50 mmol/L formic acid and eluent B consisting of 95% acetonitrile/5% water with 2 mmol/L ammonium formate and 50 mmol/L formic acid (v/v); a linear gradient program with 40% eluent B (0–2 min), from 40% to 100% eluent B (2–14 min), with 100% eluent B (14–20 min), and from 100% to 40% eluent B (20–21 min), equilibration program with 40% eluent B for 4 min; a flow rate of 0.2 mL/min; a column temperature of 40 °C; and an injection volume of 10 μL. In addition, the MS used electrospray ionization (ESI) in negative ion mode with an interface temperature of 350 °C. The MS data were obtained in the selected reaction monitoring mode. The precursor ions, product ions used for quantification, and product ions used for confirmation (m/z) were 803, 255, and 113, respectively, for OA and DTX2 and 817, 255, and 113, respectively, for DTX1.

2.3. Interlaboratory comparison

2.3.1. Interlaboratory comparison based on the Japanese official testing method (JOTM)

The ILC based on the example protocol by the Japanese official testing method (hereinafter called "JOTM") was announced to inspection laboratories, public research institutes, etc. in Japan, and 20 laboratories participated. On Oct. 19, 2015, the test samples (2 bottles) and working instructions were sent to each participant by a delivery company using refrigerated transport. The participants were asked to store these samples at −30 to −20 °C in the dark and to perform