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Determination of typical lipophilic marine toxins in marine sediments from three coastal bays of China using liquid chromatography–tandem mass spectrometry after accelerated solvent extraction

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ABSTRACT

A method based on sample preparation by accelerated solvent extraction and analysis by liquid chromatography–tandem mass spectrometry was validated and used for determination of seven typical lipophilic marine toxins (LMTs) in marine sediment samples collected from three typical coastal bays in China. Satisfactory specificity, reproducibility (RSDs ≤ 14.76%), stability (RSDs ≤ 17.37%), recovery (78.0%–109.0%), and detection limit (3.440 pg/g–61.85 pg/g) of the developed method were achieved. The results obtained from the analysis of samples from Hangzhou Bay revealed okadaic acid as the predominant LMT with concentrations ranging from 186.0 to 280.7 pg/g. Pecenotoxin-2 was quantified in sediment samples from Laizhou Bay at the concentrations from 256.4 to 944.9 pg/g. These results suggested that the proposed method was reliable for determining the typical LMTs in marine sediments and that the sediments obtained from Hangzhou Bay, Laizhou Bay and Jiaozhou Bay were all contaminated by certain amounts of LMTs.

Harmful algal blooms (HABs) occur with increased frequency (Smayda, 2007) as a result of the influences of eutrophication and global climate change. Marine biological toxins, which are produced by the toxic HAB algae, cause remarkable economic losses in the aquaculture sector and pose a risk to human health, which may even lead to death (Granéli and Turner, 2006; Zingone and Oksfeldt Enevoldsen, 2000). Thus far, more than 200 marine HAB toxins (Gerssen et al., 2011) have been found; these toxins can be divided into lipophilic and hydrophilic marine toxins based on their dissolving properties. More than 90% of the toxins are lipophilic marine toxins, which are widely distributed in the world; thus, these toxins may greatly affect human health. Typical LMTs, such as okadaic acid (OA) and its derivatives (DTXs), yessotoxins (YTXs), azaspiracids (AZAs), pectenotoxins (PTXs), and spirolides (SPXs), have been frequently found in seafoods obtained from the United States, Ireland, China, Greece, and Norway (Abraham et al., 2006; Furey et al., 2010; Heil, 2009; Li et al., 2012a; Mouratidou et al., 2006; Torgersen et al., 2005). Human consumption of shellfish that contains high levels of OA and DTXs results in diarrhea, nausea, vomiting, and abdominal pain. YTXs cause adverse effect on cardiac muscle and liver cells (Aune et al., 2002; Terao et al., 1990). AZAs show adverse effects comparable with OA and DTXs that include, nausea, diarrhea, and stomach cramps (Ito et al., 2000). SPXs cause adverse effects on nervous and respiratory systems (Richard et al., 2001), while PTXs is mainly diarrheic and hepatotoxic (Terao et al., 1986). The European Union (EU) demands the monitoring of some typical LMTs, including OA, DTXs, YTXs, AZAs, and PTXs, and limits the maximum permitted levels of these LMTs in edible shellfish tissues to protect consumers (Regulation, 2004). Legal limits for SPXs have not been set. However, the European Food Safety Authority has issued several opinions for each toxin group, which recommends a revision of these legal limits (Alexander et al., 2008).

LMTs present in marine environment have attracted considerable attention during the past decade. Several scholars successively found a series of LMTs in the seawater of Ireland, America, Chile, Australia, China, and other sea areas (Fux et al., 2009; Krock et al., 2009, 2014; Lane et al., 2010; Li et al., 2010; McCarthy et al., 2014; Takahashi et al., 2007). However, few studies on the presence of LMTs in marine sediments have been conducted. Only Mendoza et al. (2008) detected brevetoxin analogs in surficial sediments from sites along the Florida coastline of the United States. Since then, Hitchcock et al. (2012) showed that brevetoxins can persist in marine sediment. Hydrophobic (lipophilic) contaminants are absorbed by organic particulate matter

Abbreviations: LMTs, lipophilic marine toxins; OA, okadaic acid; PTX2, pecenotoxin-2; HAB, harmful algal bloom; LC–ESI-IT–MS/MS, liquid chromatography–electrospray ionization ion trap tandem mass spectrometry; ASE, accelerated solvent extraction; MRM, multiple reaction monitoring; SPE, solid phase extraction.

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(OPM) (Santschi et al., 1999), and OPMs are transferred into marine sediments (Bianchi, 2007). Therefore, sediments become the final sink of LMTs in the ocean. Kuuppo et al. (2006) showed that PTX2 and DTX1 could be deposited in suspended particulate matter, which is a good indication of the above inference. Previous research (Landrum and Fisher, 1999) has shown that the creatures underwater intaking pollutants combined with sediment is the main course of bioaccumulation, especially for low trophic level creatures just like benthic invertebrates, which played a key role in pollutants combined with sediment entering marine food chains by taking small particles. Thus, LMTs in marine sediment may have potential effects on benthonic invertebrates. To the best of our knowledge, no previous research focused on typical LMTs, such as OA, DTXs, PTXs, YTXs, AZAs, and SPXs in marine sediments. Studies on the presence of typical LMTs in marine sediments should be performed given the toxic effects of LMTs on benthonic organisms.

To date, LC–MS/MS has been the most widely used technique in determining LMTs in seafood and seawater because this method provides high sensitivity and selectivity (McCarron et al., 2014; McNabb et al., 2005). However, extraction and determination of LMTs in marine sediment by ASE combined with LC–MS/MS have not been reported. As the property of sediment is different with seafood and seawater, a suitable pretreatment method to efficiently extract LMTs from marine sediments should be developed. Several methods, such as ultrasonic-assisted extraction (Peng et al., 2006; Ternes et al., 2002), Soxhlet extraction (Peng et al., 2006), and pressurized liquid extraction (PLE) (Jelić et al., 2009; Preud'homme and Potin-Gautier, 2003; Vazquez-Roig et al., 2010) have been used to extract organic pollutants in environmental solid samples. Compared with Soxhlet extraction and other preparation methods, pressurized liquid extraction uses high temperature and pressure automate extraction of organic pollutants in a solid matrix, which is a rapid and effective sample extraction technology (Richter et al., 1996). Therefore, PLE has been widely used to extract organic pollutants in sediments; all of the tests performed obtained good results of sample preparation (Bernsmann and Furst, 2004; Hussen et al., 2007; Sporring et al., 2006). Moreover, PLE has become a popular extraction technology because it is accepted as an official US Environmental Protection Agency (EPA) method for persistent organic pollutants in several environmental solid samples.

The present study aimed to develop a robust offline ASE combined with liquid chromatography–electrospray ionization ion trap tandem mass spectrometry (LC–ESI-IT–MS/MS) to simultaneously extract and determine the seven typical LMTs, including OA, YTX, DTX1, AZA1, AZA2, SPX1, and PTX2 in marine sediments. Additionally, the use of ASE and LC–MS/MS was validated and applied to determine the concentration levels of LMTs in authentic marine sediment samples obtained from the coastline of Hangzhou Bay, Laizhou Bay, and Jiaozhou Bay, China.

Four surface sediment samples were collected from Jiaozhou Bay, six surface sediment samples were collected from Laizhou Bay and the other sediment samples were collected from twelve locations along the coastline of Hangzhou Bay, China. All the sediment samples were placed in a refrigerator at −20 °C. Prior to analysis, the sediment samples were freeze-dried in powder and sieved using 2 mm mesh and stored in a refrigerator at 4 °C. Fig. 1 indicates the distribution of sampling sites.

Extractions were performed on a Dionex ASE 100 system (Thermo Co., Sunnyvale, CA, USA) equipped with 34 mL-stainless steel extraction cells. Aliquots of 10.0 ± 0.2 g of dried sediment sample were weighted and transferred into the 34 mL extraction cells. Extraction solvents greatly affect the extraction efficiency of organic pollutants in a solid environmental sample (Krogh et al., 2008). Several studies (Fux et al., 2009; Jørgensen et al., 2005) have shown that methanol exhibits a high extraction rate for various lipophilic toxins in tissues of shellfish; thus, methanol was selected as the extraction solvent in this study. Pressure used in ASE maintains the liquid solvent at the operating temperature and forces the solvent through the sample; thus, target compounds are extracted from the sample. A default pressure value of 1500 psi was selected in the study because pressure adjustments do not significantly affect analyte recovery (Preud'homme and Potin-Gautier, 2003; Vandenburg et al., 1998). According to the standard operating conditions of the US EPA method, the flush volume and purge time were set at 60% and 100 s, respectively.

Due to the spiked sediment material references that are not available, the ASE conditions were optimized using a real marine sediment sample that contained OA to ensure that the optimal ASE parameters were suitable in extracting the typical LMTs in real sediment samples. Extraction temperature and static extraction and extraction times were optimized using a single factor approach that used the relative extraction ratio of OA as the performance indicator.

The temperature used in ASE is a crucial parameter because an optimal temperature is necessary to achieve near complete desorption of contaminants from a solid matrix (Bell et al., 2009). Fig. 2A shows the preliminary experimental result. The extraction efficiency of OA gradually decreased when extraction temperature increased. This result suggests that low temperature was more suitable in extracting OA. Fig. 2B shows the optimization results of extraction temperature. The extraction efficiency of OA in real marine sediments became constant up to 60 °C, and the extraction efficiency decreased when temperatures increased. The decrease in the observed extraction efficiency was possibly due to the degradation of OA at temperatures >60 °C. Thus, 60 °C was chosen as the extraction temperature.

Fig. 3 shows the result of the optimization of static extraction time. The extraction efficiency of OA increased from 2 min to 5 min. Further increase in the static extraction time significantly reduced the OA yield, and 5 min provided the best extraction efficiency. Finally, the effect of extraction time was determined. Table 1 shows that extraction performed twice was sufficient to completely extract the target compounds from the marine sediment sample. Therefore, the optimal ASE conditions were set as follows: extraction temperature of 60 °C, static extraction time of 5 min, extraction pressure of 1500 psi, flush volume equivalent to 60% of the cell volume, N_2 purging time of 100 s, extraction performed twice, and one cycle for each extraction.

To determine the applicability of the optimum ASE conditions in extracting all seven typical LMTs in the marine sediments, the spiked sediment samples with seven typical LMTs were extracted using ultrasonic-assisted extraction as a comparison. The temperature of ultrasonic-assisted extraction was not controlled, and the samples were not stirred during extraction. The samples were extracted 30 min for two times consecutively, and the extracts were combined. The extraction efficiency of the seven typical LMTs in the sediments using ultrasonic-assisted extraction ranged from 51.0% to 87.0% of the ASE efficiency. The RSD of each compound peak area in ultrasonic-assisted extraction ranged from 7.80% to 15.54% and that in ASE ranged from 8.24% to 13.65%. Thus, ASE was an ideal and suitable extraction method for the seven typical LMTs in the marine sediments because of the results of extraction efficiency and method precision. The absolute extraction ratios of ASE for seven typical LMTs in marine sediment are detailed below.

The sediment extractions were measured according to previously described LC–MS/MS method (Li et al., 2014) for the determination of three typical LMTs with slight modification. Briefly, a 1200 series HPLC system (Agilent Technologies, Wilmington, DE, USA) that consists of a vacuum degasser, quaternary pump, and an autosampler, and equipped with a ZORBAX Extend-C18 analytical column (3 mm × 150 mm × 3.5 μm) was used in chromatographic separation. The column was maintained at 22 ± 2 °C. Water was used as mobile phase A and acetonitrile/water (9:1, v: v) was used as mobile phase B. Both mobile phases contained 6.7 mM ammonium hydroxide. Gradient elution started with 20% B, increased to 30% over 15 min, followed by another linear gradient to 47.5% in 20 min, and finally to 100% with a linear increase at 45 min (held for 5 min, decreased to 20% B in 5 min, and held for 8 min to

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