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Methods to Reduce Matrix Effect Corresponding to Different Methods

Sun Yuanxin

Institute for Advanced Study, Shenzhen University, 518060, China

2021300087@email.szu.edu.cn

Abstract. In this paper, LC-MS / MS is the main technology used to detect the toxins that cause diarrhea shellfish poisoning in shellfish toxins, including okadaic acid (OA) or its analogues, the dynophysistoxins (DTXds), spectenotoxin (PTX), yessotoxin and its derivatives (YTX) and azaspiracid (AZA). It is mainly found that when analyzing drugs in biological samples based on LC-MS / MS, Some extracts in the sample may affect the ionization efficiency of the target compound and thus affect the detection results, especially phospholipids. Based on the efforts made and innovative methods provided by other experimental methods that use this method to analyze other substances to solve or mitigate matrix effects, this paper proposes a potential solution to provide a reference way to solve matrix effects caused by phospholipids in DSP toxin detection, that is, three-step microelement can be used in the process.

Keywords: LC-MS/MS, matrix effect, OA

1. Introduction

1.1. DSP and OA

The research of marine biotoxin is mainly driven by the human food poisoning events of bivalves. According to the poisoning symptoms, it can be divided into diarrhea shellfish poisoning (DSP) mainly causing diarrhea symptoms, paralytic shellfish poisoning (PSP) with peripheral neuromuscular system paralysis as the initial symptom, neurotoxic shellfish poisoning (NSP) mainly characterized by paralysis, and amnesia shellfish poisoning (ASP) that can cause long-term damage to memory function[1]. Although these toxins were initially produced by specific species of microalgae, due to the enrichment of shellfish, these toxins really act on the human body through shellfish rather than microalgae. DSP is a poisonous disease caused by the consumption of shellfish infected with okadaic acid (OA) or its equivalent, dynophysistinins (DTXs). It also includes spectenotoxin (PTX), azspiracid (AZA) and yessotoxin and its derivatives (YTX). flagellates of the genera proorocentrum and Dinophysis produce both OA and DTXs, two marine lipophilic phytotoxins. DSP is a severe gastrointestinal poisoning with The main symptoms are nausea, diarrhea, abdominal cramps and vomiting. To date, no deaths have been associated with the chronic toxicity of these toxins. The structure formula of okadaic acid can be seen in the figure 1.

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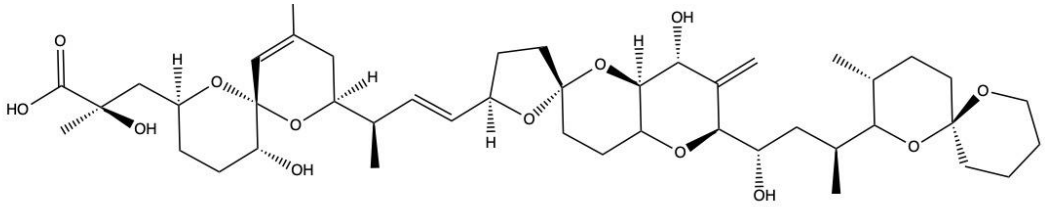

Figure 1. The structural formula of okadaic acid.

1.2. Hazards and solutions

Historically, there have been cases of diarrhoeal shellfish poisoning in various countries. The earliest recorded case of diarrhoeal shellfish poisoning in China occurred in May 2011and broke out in Zhejiang Province. This outbreak was mainly caused by the consumption of mussels enriched with a large amount of DSP toxins, and the scholars detected Okadaic acid (OA) and Dinophysistoxin-1 (DTX-1) in contaminated mussels. In consideration of public health emergencies, the government took measures to ban the sale of mussels and destroy 750 kg of suspected mussels in Zhejiang Province to prevent further outbreak of poisoning incidents. And the contaminated mussels were pointed out to be the seasonal result of the flowering of harmful phytoplankton. Most governments have taken measures to stop the sale of mussels, announce the public, and conduct testing after the outbreak of the disease. Nevertheless, shellfish poisoning, mainly diarrhea shellfish poisoning (DSP), is still occurred every year. And this has a great economic impact on the shellfish farming and fishing industry.

2. Technology and its shortcomings

2.1. Some existing detection technologies

Data monitoring and bio-analysis related to diarrhea shellfish poisoning in shellfish production have been developed for decades[2]. DSP toxin monitoring was carried out through DSP rat bio-assay (1990-1996), which was later replaced by more accurate small mouse bio-assay. Later, chemical analysis methods such as LC-MS and LC-MS/MS were widely used and gradually replaced mouse bio-assay. Due to their faster and more cost-effective scheme design, these chemical analysis methods were also called the main and model methods for monitoring diarrhoeal shellfish toxins in the world. And a tissue culture assay was also proposed to detect the presence of OA and related chemicals in mussels by culturing BGM kidney cells to reflect the toxicological characteristics of okadaic acid in 1997. This method is not only easy to implement, fast and economical, but also reduces the use of organisms such as mice in the laboratory. Although the use of Chromatography-mass spectrometry is a widely accepted and advised technology for the identification of toxins in shellfish, there are still some drawbacks. First, LC-MS/MS and LC-MS are very expensive. For farmers in low-risk areas, in order to fulfill the government's requirement for regular marine biotoxin testing to ensure that toxin levels are within the standard range. Frequent and expensive testing will increase their financial burden. Second, this test method is not instant. For people in remote areas, it is necessary to transport the samples to a special laboratory for professional operation, which also costs high freight. Moreover, due to the complexity of the experiment, the instrument needs complex performance maintenance, and the test cycle is very long. The contract laboratory may require 2–7 days to get the results, which could cost the persons who capture and sell mussels time and money and put consumers at risk.

The functional protein phosphatase inhibitory activity (PPIA) assay; the lateral flow analysis (LFA) rapid test and the antibody-based enzyme-linked immunosorbent assay (ELISA[3]) test are three commercially available rapid assays[4] with the advantages of effective assay efficiency and cost savings. ELISA, like the other detection technology EIA developed by another scientific research group in the same period, took enzyme as the principle of immunoassay and was invented in the 1960s and put into use in the 1970s and 1980s.However, according to the experimental data, the reactivity of ELISA

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and LFAS to DSP homologue DTX-2 is poor. When the content of DTX-3 is high, false negative results may be generated and there is no hydrolysis step taken. When the DSP is below the maximum limit, LFA will also produce some false positive results, but this depends on the toxin profile, geographical area and shellfish species involved.Some scholars further analyzed and compared these rapid detection methods, which showed that the shortcomings of some rapid detection kits were obvious, including large differences in results and even wrong results. It can be seen that many existing detection methods are unable to achieve both accuracy and efficiency.In this situation, Chromatography-mass spectrometry is still the most widely accepted and recognized detection method at present.

2.2. Matrix effect

When analyzing drugs in biological samples by LC-MS based methods, some co extracts of the samples may have an impact on the ionization efficiency of the target compounds, which can be observed from the instrument response, and the signals of the compounds will be enhanced or suppressed. This phenomenon is called matrix effect. Matrix effect is the main influencing factor of LC-MS/MS in detecting the presence of toxins that cause diarrhea shellfish poisoning (DSP). During LC-MS/MS analysis, many substances in biological samples may have matrix effects. These matrix effect substances exist in the finally extracted samples and are eluted together with the compounds or (and) internal standards in the chromatographic system. However, even if these co eluting substances appear, the matrix effect does not necessarily affect the analysis results, because the size of the matrix effect depends on the type of atmospheric pressure ionization (API) which is used in the method (APCI is less susceptible to matrix effect than ESI), the relative concentration of interfering substances, and the size of the counteraction effect of the calibration standard or the isosteric turbulence internal standard. However, in practical application, how to reduce the possible impact of matrix effect on the detection results should be paid attention to. In particular, the measurement of the toxins contained in mussels samples is mostly aimed at avoiding the harm to human health caused by the toxins contained in locally collected mussels through routine testing and judging the causes of poisoning after cases are found, so as to understand the treatment methods. Therefore, it is necessary to reduce the matrix effect caused by the extraction of mussels samples during LC-MS/MS testing.

Experiments have confirmed that in the detection of OA, the matrix strength of the extract is directly proportional to the matrix effect. The re matrix effect of diluted raw mussel and heat-treated mussel extracts was significantly reduced. However, this method is not applicable to all shellfish toxins that may cause DSP, such as AZA1 and PTX2[5],[6].

The most commonly used matrix effect evaluation method is to compare the responses of extracted samples and pure solutions in the standard analytical method. The absolute matrix effect of compounds is defined as the matrix effect factor (MF), which is calculated by comparing the responses of the analytes in the absence and presence of matrix components without considering the recovery rate.

$$MF = \frac{B}{A} \times 100\%$$

A: response of the blank matrix after extraction to the substance to be tested
B: response of the substance to be tested in the pure solution

Substances causing matrix effects can be divided into endogenous and exogenous according to their sources. Exogenous main disturbances mainly refer to foreign substances introduced during sample preparation or sample processing. Endogenous main disturbances usually refer to some organic substances (like proteins, fats, phospholipids [7], etc.) and inorganic substances existing in biological samples. Phospholipids, in particular, are the mainly reason of matrix effect in the detection of biological organism samples. Because the detected substance, the toxin that causes DSP, is lipophilic, it is necessary to select the reverse phase (RP) mode, which makes it easier for phospholipids to elute together with compounds and becomes a source of interference. Most experiments used liquid extraction (LLE) or protein precipitation (PPT) instead of solid-phase extraction (SPE) and stable SIL-IS when using UHPLC-MS/MS and LC-MS/MS for biological analysis. PPT is faster than other technologies,

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but less effective because it cannot solve the matrix effect caused by endogenous phospholipids. For SPE, although it has been applied to many analytical experiments, the effect is not ideal because it has the characteristics of cumbersome steps and long time, and can't be based on SIL-IS.Some methods to eliminate or minimize the impact of matrix effects are summarized in table 1.

2.3. Reference to the application in blood detection

Table 1. In the process of developing biological analysis methods, four different methods can be used to eliminate or minimize the impact of matrix effects on the quantification of analytes.

Early methods to avoid the matrix effect	Simple description
Mass analyzer interfaces investigation	For the most part, ESI interfaces are much more sensitive to matrix effects than APCI. The various behaviors of these two interfaces can be used to explain the bad ionisation mechanism.
Chromatography optimization	Several ways: improve the resolution between analytes and inhibitors, the mobile phase or the temperature of the column, increase the capacity factor, change the selectivity of the column.
Sample-preparation optimization	Primarily used to reduce the matrixal effect of phospholipids and remove other endogenous compounds.
Selection of suitable internal standards(IS)	Can offset the loss or gain of response to the analyte by keeping the analyte called ratio IS constant.Stable isotope labeled ISDs (SIL-ISs) are considered as the most ideal IS.

LC-MS / MS is not only applied to detect the content of marine toxins in mussel samples, but also applied in many fields. In other fields, it should be a potential reference for alleviating the matrix effect caused by phospholipids in the process of measuring marine biological endotoxins[8]. In this article, it mainly refers to the detection of lipophilic toxins that cause DSP. For example, it was found that the combination of specific eluents and reverse-phase extraction cartridges inhibited the matrix effect due to phospholipids [9]. And some researchers have used a method to detect aripiprazole (ARI), a quinoline derivative, in plasma to improve efficiency, prevent drug interaction or reduce side effects: three-step microelement solid phase extraction (SPE, oasis preme HLB 96 well - Evolution plate) [10]. This method effectively eliminates the vast majority of plasma phospholipids and protein precipitation, the possible influence of these substances on the test results is largely avoided.

It only includes three steps: loading, washing and elution. Without evaporation and recombination. The specific operation is to add an appropriate amount of it and formic acid to the plasma sample and place it on the μElution Plate. Then, cleaning was performed with a methanol solution in MilliQ water and 2% ammonia. SPE treatment was performed at each step using a vacuum of 5 to 15 mm Hg. Prepare an acetonitrile/methanol/buffer (ammonium formate, 5 mM, pH 4.0) solution, elute at a ratio of 8:1:1, and collect in 1 ml of a 96-well plate. Then, 5L of eluent was injected into the LC-MS/MS system. Finally, SPE was selected to validate the sample preparation method. This method can be faster, easier to operate and more convenient for biological analysis of large sample size, so it is very suitable for the actual needs that require a large number of toxins in mussels. This method adopts an effective phospholipid removal method, which can be used when analyzing DSP toxins to determine whether matrix effects will be reduced.

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In the past studies, there have been methods such as diluting samples, selecting alkaline environments, and using MeOH: ACN (1:1) to better balance the resolution in the elution process to try to reduce the impact of matrix effect on the results in the process of analyzing toxins contained in marine organisms, but no scholar has tried whether this method has a constructive role in the above studies. Therefore, this may be another effective method to solve the matrix effect caused by phosphate in lipophilic toxin mass spectrometry, using LC-MS / MS operation.

3. Conclusion

LC-MS and LC-MS/MS are regarded as an important part of the alternative methods of animal tests in the determination of lipophilic shellfish toxins, and are now the main recommended detection methods by major international organizations and academic organizations. After all, compared with biological experiments, including toxicology research methods such as mouse experiments and rat experiments, chemical methods provide a more convenient and accurate choice. However, these technologies are prone to matrix effects. Matrix effect is caused by endogenous or exogenous matrix substances competing with target compounds for charge and neutralizing ionization process during sample ionization in mass spectrometry ion source, which will greatly affect the final results of sample detection, which should be considered by scholars when developing and validating methods. At the same time, in the detection of endotoxin in bivalve organisms, considering the complexity of the structure of mussels, the impact of foreign substances that may be carried in the pre-treatment of samples on the detection results, and the current situation that the amount of samples is generally large in the detection of shellfish toxins, Fully understanding the causes of matrix effect can facilitate better experimental planning and find relevant methods that can effectively solve or mitigate the impact of these endogenous or exogenous substances on the test results.

Due to the threat of mussel poisoning in various countries to the local food industry and people's life safety, and the frequent and large demand for detection of related toxins, this article mainly focuses on the innovative three-step microelement used by other scholars in detecting aripiprazole (ARI) in plasma, and proposes the potential possibility of applying this method to the detection of related mussel toxins causing DSP. This new attempt may provide a more rapid, simple method for biological analysis in related fields, which is easy to apply to large sample sizes. At the same time, the phospholipid removal method used in this method has a good recovery rate and shows a good performance of reducing matrix effect in the detection of plasma. It has an objective prospect to introduce this method into LC-MS / MS used in the detection of shellfish toxins.

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