Detection of DSP Toxins and Azaspiracids in Moroccan Mussels: Comparison of LC-MS Method with the Commercial Immunoassay Kit

Abstract: Diarrheic shellfish poisoning (DSP) is one of the recurrent gastrointestinal illnesses in Morocco, resulting from consumption of contaminated shellfish. In order to develop a rapid and reliable technique for toxins detection, we have compared the results obtained by a commercial immunoassay—“DSP-Check” kit—with those obtained by LC-MS. Both techniques are capable of detecting the toxins in the whole flesh extract, which was subjected to prior alkaline hydrolysis in order to simultaneously detect simultaneously the esterified and non-esterified forms of toxins. The LC-MS method was found to be able to detect a high level of okadaic acid (OA), a low level of dinophysistoxin-2 (DTX2), and surprisingly, traces of azaspiracids-2 (AZA2) in mussels. This is the first report of a survey carried out for azaspiracids contamination of shellfish harvested in the coastal areas of Morocco. The “DSP-Check” kit was found to be able to quantitatively detect DSP toxins in all contaminated samples containing only okadaic acid, provided that the parent toxins were within the range of detection and were not in an ester form. A good correlation was observed between the two methods when appropriate dilutions were performed.
The immunoassay kit appeared to be more sensitive, specific and faster than LC-MS for determination of DSP in total shellfish extract.

**Keywords**: Diarrheic shellfish poisoning (DSP), Okadaic acid, LC/MS, ELISA, Dinophysistoxin 2, *Dinophysis spp.*, azaspiracids toxins.

### 1. Introduction

Diarrheic shellfish poisoning (DSP) is a severe gastrointestinal illness caused by consumption of contaminated seafood with toxigenic dinoflagellates, such as certain species of the genus *Dinophysis* and *Prorocentrum* algae. The European Commission has subdivided the DSP monitoring into four distinct families: dinophysistoxins (OA, DTX1, DTX2, and DTX3) and pectenotoxins (PTX1 and PTX2) at a maximum limit of 160 µg/kg, yessotoxins (YTXs) at a maximum limit of 1 mg/kg level and azaspiracids (AZA1-3) at a maximum limit of 160 µg/kg of shellfish meat.

Highly sensitive methods are required to detect DSP toxins at low concentrations. The HPLC method used by Lee *et al.* [1], despite using the highly fluorescent reagent 9-anthryldiazomethane (ADAM), is not sufficiently sensitive to detect very sensitive for detecting toxins at very low levels because of background chemical noise. It is also laborious, time-consuming, and, in practice, duplicate or triplicate analyses are required in the experiments. Additionally, alkaline hydrolysis, necessary for simultaneously quantifying OA and its ester derivatives in monitoring analyses, as recently proposed by several authors [2], was found to increase the time required for sample preparation. Consequently, several biochemical (phosphatase inhibition assays and enzyme linked immunosorbent assays) and biological (tissue culture assays) methods for detecting DSP toxins with a higher sample throughput...
have been proposed [3]. Interestingly, antibodies against DSP toxins have been developed only against okadaic acid.

So far, the diarrheic shellfish poisoning parent toxins found in Moroccan bivalves are OA and DTX2. The first detection of these toxins in the Mediterranean coast of Morocco was in oysters and clams in 1999 and in mussel in 2003. On the Atlantic coast, the first detection was in clams, also in 1999, and then in mussel and oyster samples in 2000 and 2002, respectively. In 2003, the presence of this type of contamination was significant, and DSP was detected in mussel, clam and oyster samples along the Atlantic littoral from El Jadida to Dakhla, Morocco.

Currently, mouse bioassays are used in the Moroccan monitoring program. However, the introduction of a rapid, selective and quantitative assay is very important for the proper risk management of this recurrent toxicity. In this study, we report the detection of DSP toxins in mussels collected in Oualidia lagoon by using two methods: a commercial enzyme-linked immunosorbent assay (ELISA) [4], and liquid chromatography-mass spectrometry (LC-MS). Both techniques employed the same whole fresh final extract, subjected to prior alkaline hydrolysis in order to detect the esterified and non-esterified forms of toxins simultaneously [5]. The “DSP-check” kit was then compared with the HPLC method for determining its predicting capabilities for the complex toxin profiles found in Moroccan shellfish.