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Detection of DSP Toxins and Azaspiracids in M~~a~~orocc~~c~~an Mussels: Comparison of LC-MS Method with ~~the~~ Commercial Immunoassay Kit

Abstract: Diarrheic shellfish poisoning (DSP) is ~~one of~~a recurrent gastrointestinal illness~~es~~ in M~~a~~orocco, resulting from consumption of ~~the~~ contaminated shellfish. In order ~~T~~to develop a rapid and reliable technique for toxin~~s~~ detection, we have compared the results obtained by a commercial immunoassay-"DSP-Check" kit~~"~~ with those obtained by LC-MS. Both techniques are capable~~able~~ 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~~ non-esterified ~~forms of~~ toxin~~s~~ forms. 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 azaspiracid~~s~~ contamination of shellfish harvested in the coastal areas of M~~a~~orocco. The "DSP-Check" kit was found to be able to quantitatively detect ~~detect quantitatively~~ DSP toxins in all contaminated samples containing only okadaic acid, provided that the parent toxins were within the range of detection and were~~as~~ not in an ester form. A good correlation was observed between the ~~2~~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 ~~consumption~~ with toxigenic dinoflagellates, such as certain species of the genus *Dinophysis* and *Prorocentrum* algae. The European Commission~~EC~~ has ~~subdivided~~ categorized ~~the~~ DSP monitoring into 4four distinct families: dinophysistoxins (OA, DTX1, DTX2, and DTX3) and pectenotoxins (PTX1 and PTX2) at ~~with~~ 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~~ due to background~~the~~ chemical noise ~~background~~. It is also laborious, time-consuming, and, in practice, duplicate or triplicate analyses are ~~carried out~~required in ~~the~~ experiments. Additionally, ~~an~~ alkaline hydrolysis, necessary for simultaneously quantifying ~~simultaneously~~ OA and its ester derivatives in monitoring analyses, as recently proposed by several authors [2], was found to increase the time required for~~of~~ 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

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have been proposed [3]. Interestingly, antibodies against DSP toxins have ~~been developed only against~~only been developed for okadaic acid.

So far, the diarrheic shellfish poisoning parent toxins found in M~~a~~orocc an bivalves are OA and DTX2. The first detection of these toxins on~~in~~ the Mediterranean coast of M~~a~~orocco was in oysters and clams in the~~of~~ Nador's area in 1999 and in m~~M~~ussels in 2003. O~~I~~n 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~~is~~ type of contamination was ~~very important~~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~~is~~ used in the M~~a~~orocc an 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~~Now~~, we report the detection of DSP toxins in mussels collected in Oualidia lagoon by using two methods: a commercial enzyme-linked ~~immunoabsorbent~~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 simultaneously detect ~~simultaneously~~ the esterified and non-esterified ~~forms of~~ toxin~~s~~ forms [5]. The "DSP-check" kit was then compared with the HPLC method for determining its predictive~~ng~~ capabilities for the complex toxin profiles found in M~~a~~orocc an shellfish.