INVESTIGATION OF THE TOXIN PROFILE OF GREEK MYTILUS GALLOPROVINCIALIS BY LIQUID CHROMATOGRAPHY –MASS SPECTROMETRY

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ABSTRACT

Samples of Mytilus galloprovincialis, were harvested from five different locations in Thermaiokos gulf, Greece after harmful algae bloom. All of the mussel samples were found positive by mouse bioassay for diarrhetic shellfish poisoning (DSP) toxins. Liquid chromatography (LC) coupled with mass spectrometry (MS) was used to search for the following lipophilic toxins: okadaic acid (OA), dinophysistoxins (DTXs), pectenotoxins (PTXs), azaspiracids (AZAs) and yessotoxins (YTXs). In order to investigate the presence of okadaic acid esters, alkaline hydrolysis was performed for all the samples, and LC-MS analyses were carried out on the samples before and after hydrolysis. Hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS) analyses were also carried out to investigate the presence of domoic acid and paralytic shellfish poisoning (PSP) toxins at trace levels. All of the samples were found to be contaminated only with okadaic acid at levels 0.10-0.20 µg/g.

Keywords:  Okadaic acid; DSP; LC-MS; HILIC-MS; Greece; Mytilus galloprovincialis
INTRODUCTION
Shellfish can be vectors of phytoplanktonic toxins accumulated in their organisms by filter feeding mechanisms. Among the phycotoxin-related toxic phenomena, diarrhetic shellfish poisoning (DSP) is a severe human gastrointestinal illness associated with consumption of toxic shellfish that have been feeding on dinoflagellates such as *Dinophysis* sp., *Prorocentrum* sp. and *Protoceratium reticulatum*. DSP represents a serious threat to both public health and shellfish industry.

In February 2004, a DSP outbreak associated with *Dinophysis* sp. occurred in Greece and caused serious threat to public health as well as severe economic losses for shellfish industry.

In the present work, we report on the extensive analysis of samples of *Mytilus galloprovincialis* harvested during this event from five different locations in Thermaikos Gulf. The study was carried out using the comprehensive approach based on LC-MS which allowed to investigate the presence of okadaic acid together with a wide range of toxins distributed in European Seas.

MATERIALS AND METHODS
The mussel samples were extracted according to the official protocols for DSP, ASP and PSP toxins (Gazzetta Ufficiale della Repubblica Italiana, 2002).

Mass spectral experiments were performed by using an API-2000 triple quadrupole mass spectrometer equipped with a turbo-ionspray source (Applied Biosystems, Thornhill, ON, Canada), coupled to an Agilent (Palo Alto, CA, USA) model 1100 LC. Individual OA, AZA1-3, PTX2, PTX2sa, PTX11 and YTX standard solutions were used as reference standards. Selected ion monitoring (SIM) experiments were carried out either in positive or negative ion modes by selecting specific ions. MRM experiments were carried out for OA by selecting the following groups of transitions (822 > 751, 822 > 769, 822 > 787) and (803 > 255, 803 > 563), in positive and negative ion modes, respectively. OA was quantitatively determined in mussels samples by direct comparison to individual standard solutions of OA at similar concentrations injected in the same experimental conditions.

HILIC-MS analyses for PSP (Dell’Aversano et al., 2005) and ASP (Ciminiello et al., 2005) toxins were performed using a 250 × 2.00 mm column packed with 5μm Tosohaas TSK-GEL Amide-80 material at room temperature.

RESULTS AND DISCUSSION
The DSP and PSP crude extracts were tested by mouse bioassay and were found positive only for DSP toxins, with various levels of toxicity among samples from different sites.

No peak corresponding to DTXs, PTXs, AZAs or YTXs was detected throughout the analyses of the mussel extracts. On the contrary, a peak eluting at 8.88 min was detected in all the analyzed extracts for the m/z 805 and m/z 822 traces. The retention time as well as ion ratios of the above peaks matched perfectly those of the pseudo-molecular and adduct ion of an authentic sample of okadaic acid injected in the same experimental conditions, respectively. A peak at the same retention time was also present in negative ion mode for the m/z 803 trace, [M-H] of OA, providing further support for the identity of the toxin. Final confirmation for such a result was provided by multiple reaction monitoring (MRM) experiments which were carried out in both positive and negative ion modes.

In order to fully investigate the toxin profile of Greek mussels, the presence of PSP and ASP toxins, at levels even below the regulatory limits, was also investigated. No peak corresponding to DA or the above PSP toxins was detected in the chromatogram.
CONCLUSIONS

For the first time a complete analysis of the toxin profile of Greek mussels has been carried out by using LC-MS technique. The obtained results shows that okadaic acid was the only toxin contaminating Greek mussels at that time and its concentration in mussel tissues could fully account for toxicity detected by mouse bioassay. This indicates that mollusks contamination due to OA, previously reported by Mouratidou et al. (2004), was not an occasional phenomenon but it persists in time.

At the moment, OA represents the only serious hazard for public health in Greece while the presence of other toxins, widely distributed in European Seas, can be excluded.

REFERENCES


GAZZETTA UFFICIALE DELLA REPUBBLICA ITALIANA 16 July 2002, n. 65; Decreto Ministeriale 16 May 2002, n. 16.

