INTRODUCTION

Marine lipophilic toxins (MLTs) are compounds produced by secondary metabolism of marine microalgae; they often accumulate in mussels representing a risk to consumers health (Fig.1). Okadaic acid, dinophysistoxins, pectenotoxins, yessotoxins and azaspiracids (AZAs) are regulated MLTs. AZAs have never been reported in Mediterranean seafood until now. Cyclic imines - CIs (including Spirolides -SPXs and Gymnodimins - GYMs) are considered “emerging” toxins worldwide not yet regulated.

The QTrap approach in "emerging" marine lipophilic toxins analysis

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The LC-MS/MS analysis is accomplished in alkaline conditions. Two types of experiments were conducted: a Multiple Reaction Monitoring (MRM) and an Enhanced Product Ion (EPI) scan using the Linear Ion Trap (LIT) (Fig.3). In MRM mode two transitions were selected for each molecule to allow reliable quantification and identification. EPI spectra were obtained with a scan speed of 1.000 Da/s and a dynamic fill time for optimal MS/MS quality (Table 1).

The QTrap approach in the analysis of AZA2 were for the first time detected in Mediterranean seafood by IZSUM researchers as already reported in Toxicon [2]. Two SPX analogues (13-desMeC SPX and 13,19-didesMeC SPX) and GYM A (never detected before in Italy) were measured with a maximum concentrations of 25 μg/kg (sum of the 2 analogues) and of 12 μg/kg respectively.

RESULTS AND DISCUSSION

Toxin profiles of mid-Adriatic sea mussel extracts are reported in Fig.4.

Fig.5 Fragmentation pathways of 13-desMeC SPX , 13,19-didesMeC SPX (a), GYM A (e), AZA 2 (f). EPI spectra of mussel extracts for 13-desMeC SPX(b), 13,19-didesMeC SPX (c), GYM A (d), AZA2 (g).

The 13-desMeC SPX, 13,19-didesMeC SPX, GYM A, AZA2 fragmentation pathways and the respective EPI spectra of the mussel extracts analysed are reported in Fig. 5. The spectra show water losses and characteristic fragments for the monitored molecules. EPI enables the MLTs identity confirmation by comparison of the sample fragmentation pattern with literature data and standards mass spectra. This approach proved to be useful for the investigation of the emerging marine toxins in mid-Adriatic sea.

BIBLIOGRAPHY


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