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The QTrap approach in "emerging" marine lipophilic toxins analysis

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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 Gymnodimines - GYMs) are considered "emerging" toxins worldwide not yet regulated.

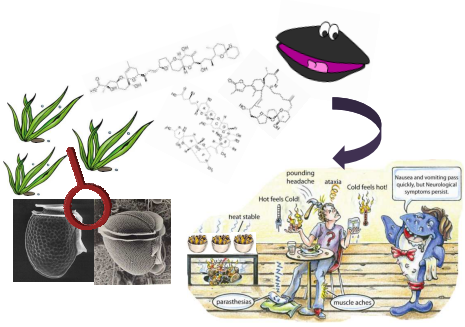

Fig.1 Mechanism of MLTs accumulation in mussels and along the food chain causing shellfish poisoning.

This work aims to investigate AZAs and CIs in mussels by hybrid triple-quadrupole/linear ion trap mass spectrometry. The Istituto Zooprofilattico Sperimentale dell 'Umbria e delle Marche (IZSUM) is responsible for marine biotoxins monitoring in mussels harvested along the Marche coast (mid-Adriatic Sea). Since 2012 the IZSUM adopted the official LC-MS/MS method for the determination of regulated MLTs in bivalve molluscs [1]. This official protocol was properly modified and implemented to allow also the analysis of CIs.

MATERIALS AND METHODS

Samples from breeding sites along the Marche coast (mid-Adriatic sea - Italy) were analysed using the modified LC-MS/MS protocol for marine lipophilic toxins (Fig.2).
Standards materials of **13-desMeC SPX**, **13,19-didesMeC SPX** and **AZA2** were purchased from the NRC Institute for Marine Biosciences (Halifax, NS, Canada), **GYM A** from CIFGA (Lugo, Spain).

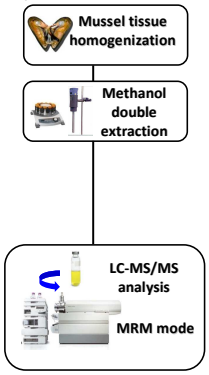

Fig.2 Official LC-MS/MS protocol

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).

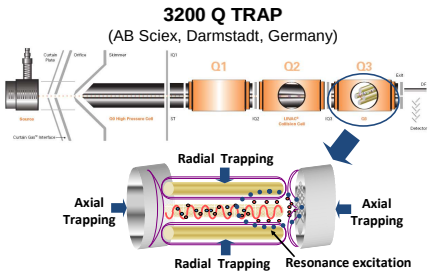

Fig.3 Schematics of Linear Ion Trap (LIT).

Tab.1 MS parameters for MRM (Multiple Reaction Monitoring) and EPI (Enhanced Product Ion) experiments. (CE-Collision Energy)

Analytes	MRM ESI +	CE (eV)	EPI m/z	CE (eV)
13-desMeC SPX	692.5 > 444.2 692.5 > 164.3	40 45	100-695	55
13,19-didesMeC SPX	678.5 >430.5 678.5 > 164.3	40 45	100-680	55
GYM A	508.2> 490.2 508.2 > 162.2	40 55	100-510	45
AZA2	856.5 > 838.5 856.5 > 820.5	55 55	200-860	70

RESULTS AND DISCUSSION

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

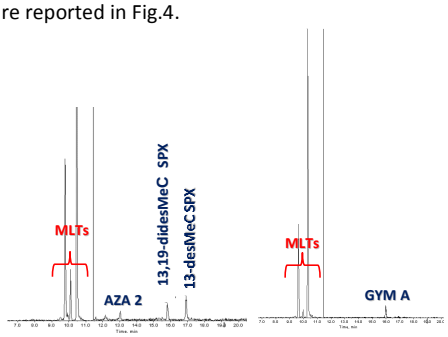

Fig.4 Typical LC-MS/MS chromatograms of mid-Adriatic sea mussel extracts.

Traces 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.

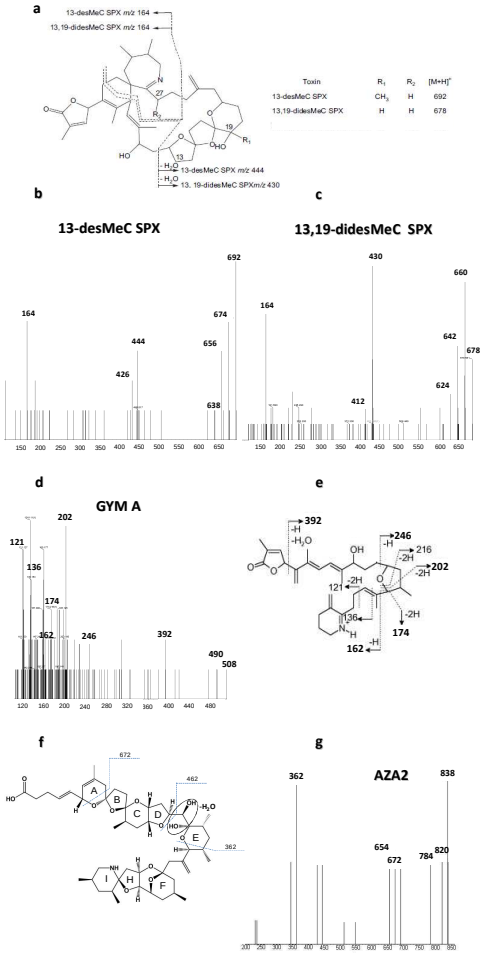

Fig.5 Fragmentation pathways of 13-desMeC SPX , 13,19-didesMeC SPX (a), GYM A (e), AZA 2 (f). EPI spectra of mussel extracts for 13-desMeC SPX (b), 13,19-didesMeC SPX (c), GYM A (d), AZA2 (g).

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

[1] AESAN Agencia Española de Securidad Alimentaria y Nutrición. EU-Harmonised Standard Operating Procedure for determination of Lipophilic marine biotoxins in molluscs by LC-MS/MS vers.5 (2015).
[2] S. Bacchiocchi, M. Siracusa, A. Ruzzi, S. Gorbi, M. Ercolessi, M.A. Cosentino, P. Ammazzalorso and R. Orletti, *Toxicon* **108** (2015) 115-125.

Acknowledgements

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