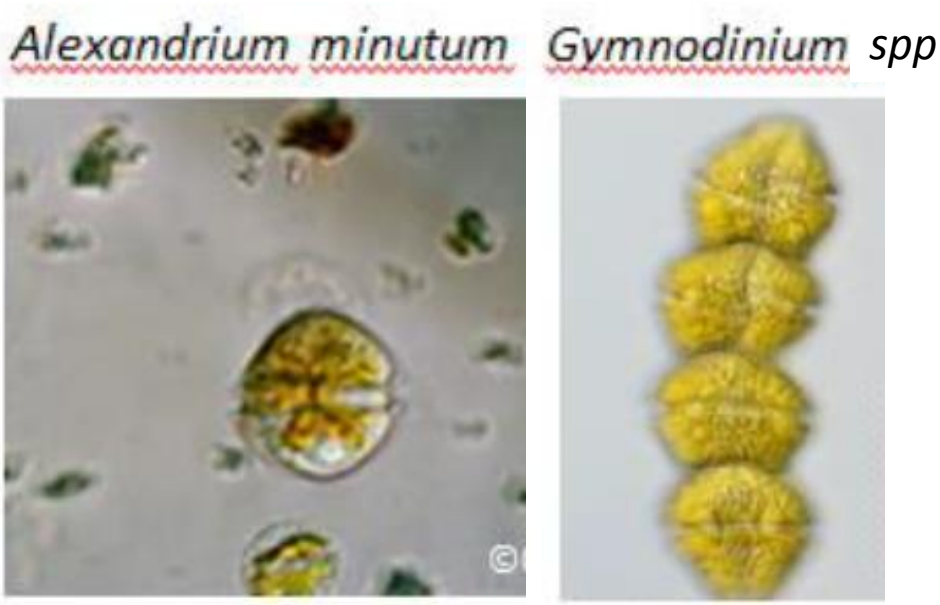


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Development and validation of methods alternative to the biological method for the rapid determination of Marine biotoxin in bivalve molluscs

INTRODUCTION

Paralytic shellfish poisoning (PSP) toxins are highly toxic compounds which are mainly produced by marine dinoflagellates belonging to Alexandrium, Gymnodinium and Pyrodinium genera. The phytoplanktonic toxins can be accumulated in filter feeding shellfish and other seafood. PSP toxins reversibly bind to voltage-gated sodium channels and prevent the passage of sodium ions across the membrane. As a result, the toxins cause paralytic shellfish poisoning (e.g. neurological distress) in human, A dose of about 1 mg of saxitoxin in contaminated shellfish is fatal to human.

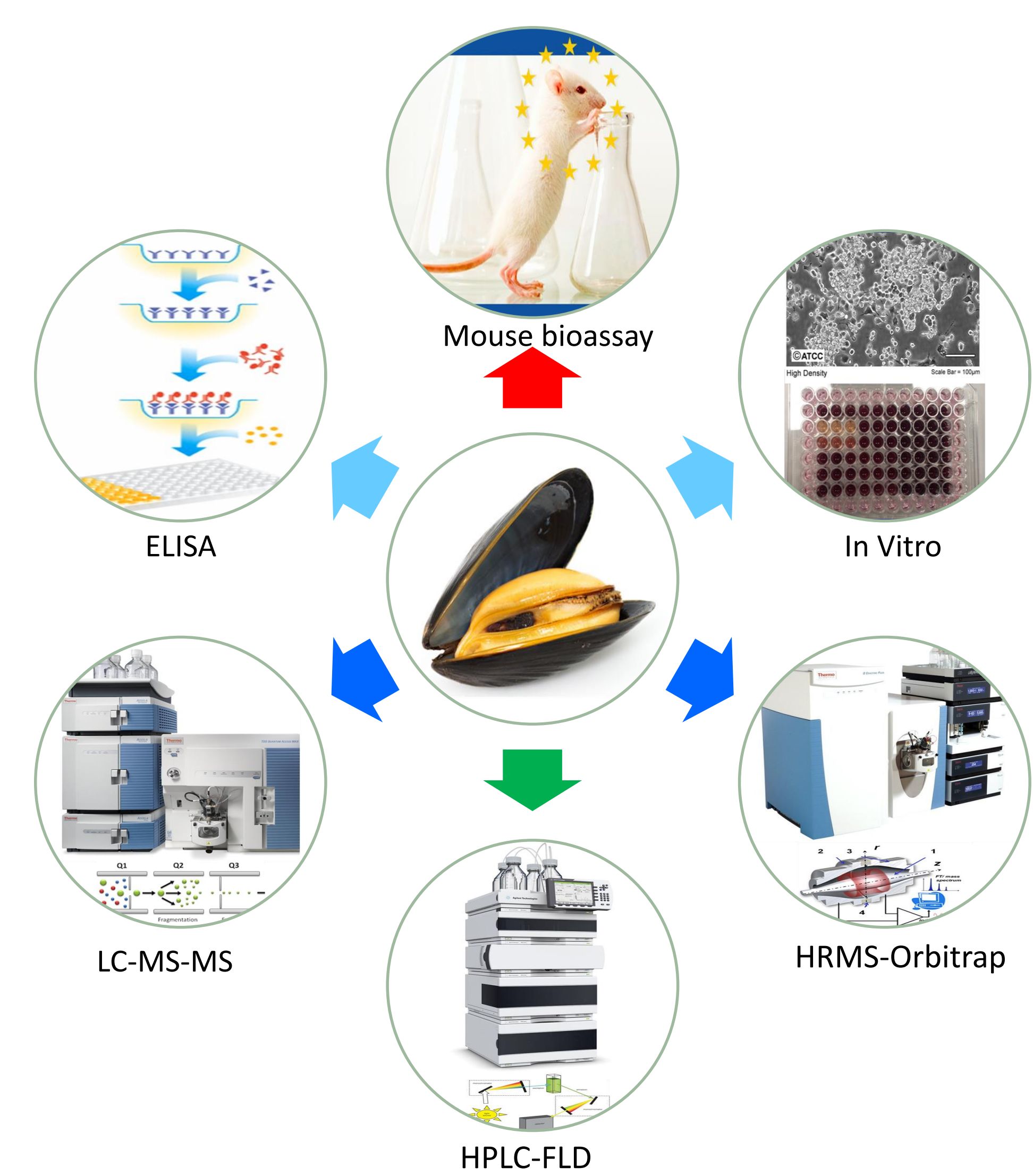


Paralytic shellfish poisoning

The PSP toxins are tetra-hydropurine derivatives and can be categorized into three groups based on the chemical structure of their side chain: *carbamoyl*, *N-sulfocarbamoyl* and *decarbamoyl* groups. Although all the toxins can bind to sodium channels, their binding affinities are different. As a result, they have different toxicities and the carbamoyl group toxins, such as STX and GTX, are known to be the most toxic compounds. In general, the toxicities of individual PSP toxins are expressed in relation to STX. The worldwide regulatory limits for PSP toxins in shellfish are set at 80mg STX eq./100 g meat.

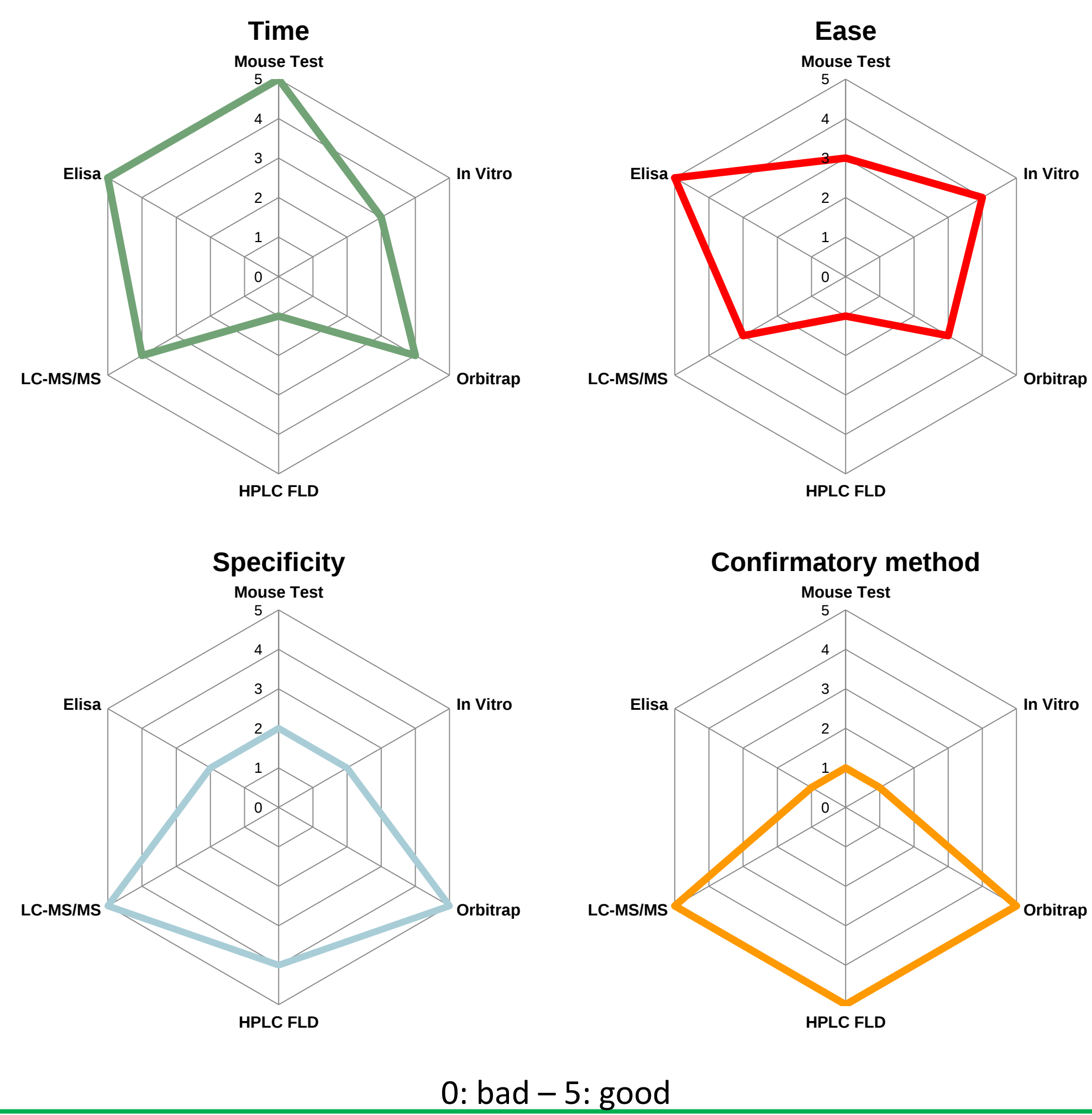
Toxin	R ₁	R ₂	R ₃	R ₄	Toxicity Equivalency Factors	
					Oshima	EFSA
STX	H	H	H		1.00	1.00
GTX2	H	H	OSO ₂ ⁻		0.36	0.40
GTX3	H	OSO ₂ ⁻	H		0.64	0.60
NEO	OH	H	H		0.92	1.00
GTX1	OH	H	OSO ₂ ⁻		0.99	1.00
GTX4	OH	OSO ₂ ⁻	H		0.73	0.70
M2	H	OH	H		-	-
M4	H	OH	OH		-	-
GTX5	H	H	H		0.06	0.10
C1	H	H	OSO ₂ ⁻		0.01	-
C2	H	OSO ₂ ⁻	H		0.10	0.10
C3	OH	H	OSO ₂ ⁻		0.01	-
C4	OH	OSO ₂ ⁻	H		0.06	0.10
GTX6	OH	H	H		0.06	0.10
M1	H	OH	H		-	-
M3	H	OH	OH		-	-
dcSTX	H	H	H		0.51	1.00
dcGTX2	H	H	OSO ₂ ⁻		0.15	0.20
dcGTX3	H	OSO ₂ ⁻	H		0.38	0.40
dcNEO	OH	H	H		-	0.40
dcGTX1	OH	H	OSO ₂ ⁻		-	-
dcGTX4	OH	OSO ₂ ⁻	H		-	-

Analisis Methods



The mouse bioassay (MBA) method has been classified as type IV and therefore cannot be a reference method. The Fluorescence Detection (HPLC-FLD) Lawrence method is currently the *reference method* for PSP analysis however we are studying alternative methods for analysis such as immunoassays, cells assay, and other chemicals methods such as LC-MS/MS method and Orbitrap to obtain faster, quicker and easier method.

Paralytic shellfish poisoning



0: bad – 5: good

Conclusion

Different analytical methods were studied for analysis of samples. The first results showed that in vitro assay could be used as screening method. LC-MS/MS and Orbitrap methods are characterised by high rapidity, sensitivity and can be a reliable alternative for routine and confirming analysis to HPLC-FLD method the only allowable within EU law.

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4. COMMISSION REGULATION (EC) 2074/2005

5. COMMISSION REGULATION (EC) 2017/1980

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