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# Bivalves as sentinel species to Follow-up the eco-exposome: The sample preparation as a major challenge

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In recent decades, coastal waters have been subjected to a variety of contaminants from urban, industrial and agricultural activities. Numerous persistent chemicals that reach the estuarine environment *via* rivers, wastewater treatment plants and watershed leaching accumulate in seawater and/or aquatic organisms. The Pertuis-Charentais area, in southwest France, is one of them. This area is a transition zone between the Atlantic Ocean and the estuaries of the rivers Sèvre, Charente and Seudre. This region produces 22% of French shellfish production and France is the second largest producer of bivalves in Europe (Diallo et al., 2022). The mortality observed since 2008 in mussels and oysters, sentinel species of environmental quality, suggests a deterioration in water quality, but the precise causes are not yet identified. In this context, the AMPHIBIE project, funded by the ANR JCJC "*AlimOmic*", aims to characterise the contamination by pesticides, veterinary drugs, pharmaceuticals and plastic additives on a large scale in bivalves through active biomonitoring for one year. For this purpose, a comprehensive screening method based on LC-HRMS was developed and validated. Different extraction and purification procedures (QuEChERS/QuPPe) were optimised with pooled seafood samples (bivalves, fishes and crustaceans) using a representative mixture of 300 contaminants (pesticides and veterinary drugs) from different families, with very distinct and large physicochemical properties. To simplify the optimisation of the extraction and purification procedures, the design of experiments (DoE) was used (Taguchi orthogonal array). The screening method was validated according to the EU SANTE/11312/2021 guideline, by extending the number of contaminants up to 850 (pesticides and veterinary drugs). For each contaminant, the screening detection limits (SDLs) and the limits of identification (LOI) were established in seafood samples. The method was also tested for the analysis of other organic contaminants such as pharmaceutical to demonstrate its versatility by suspect screening in non-spike seafood samples.

Finally, the validated methods were applied to biomonitoring real samples of mussels and oysters collected at different times in different sites (with different mortality rates) in southwestern France to try to find markers of exposure and effect of high mortality of bivalves.

**Keywords:** Extraction/Purification, UHPLC-HRMS-MS, Contaminants, Seafood, Design of experiments

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