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Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves

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ABSTRACT

A fast, simple and robust method has been developed for the simultaneous determination of 23 pharmaceuticals (including some major metabolites) in bivalve mollusks. The analytes belong to eight different therapeutic groups: antibiotics, psychiatric drugs, analgesics/anti-inflammatories, tranquilizer, calcium channel blockers, diuretic, and prostatic hyperplasia. The method is based on pressurized liquid extraction (PLE) followed by solid phase extraction clean-up (SPE), and ultra performance liquid chromatography–triple quadrupole mass spectrometry (UHPL–MS/MS) for the identification and quantification of the target analytes. It has been developed and validated in three different species of bivalves: *Crassostrea gigas* (Pacific oyster), *Mytilus galloprovincialis* (Mediterranean mussel), and *Chamelea gallina* (striped venus clam). The majority of the compounds were extracted with a recovery between 40 and 115%. The developed analytical method allowed the determination of the compounds in the lower ng/g concentration levels. The relative standard deviation was under 12% for the intra-day and 20% inter-day analyses, respectively. Finally, the method was applied to oyster, clam and mussel samples collected from the Ebro delta, Spain. The most ubiquitous compounds detected were the psychiatric drug venlanfaxine and the antibiotic azithromycin, with the highest concentrations found in mussel (2.7 ng/g dw) and oyster (3.0 ng/g dw), respectively. To the best of our knowledge, this is the first time that azithromycin has been reported in environmental samples of marine biota.

1. Introduction

Pharmaceuticals are considered “pseudo-persistent” contaminants since their high transformation/removal rates are compensated by their continuous introduction into the environment through human activities. The main sources of pharmaceuticals release into the aquatic system are waste water treatment plants effluents, agricultural runoff and aquaculture facilities. They provide an incessant loading of pharmaceuticals into the aquatic system and as a result they have been detected at a nanogram to microgram/litre range in wastewater, groundwater, surface and marine waters [1–6].

Pharmaceuticals are designed to target specific metabolic and molecular pathways in humans and animals. They can act at very low concentrations which raises the concern about their potential to cause adverse effects on wildlife, like for instance the well-known feminization of male fish due to estrogens exposure [7]. Besides, the ingestion of contaminated seafood may have undesirable effects on consumers’ health like allergies or development of bacterial resistant, which translate into a much bigger problem for consumers’ health when dealing with infections [8].

In order to protect public health and on the basis of the scientific assessment of the safety of pharmaceuticals, The European Community has set maximum residue limits (MRLs) for a variety of these chemicals in foodstuffs of animal origin including all food producing species [9]. Shellfish, like bivalves mollusks, are food commodities consumed worldwide and, like any other type of food, can contain harmful contaminants such as pharmaceuticals among others. Moreover, bivalves are excellent sentinel organisms for monitoring of contamination from environmental waters [10,11]. These sessile and long-lived organisms filter large quantities of surface water for feeding and breathing, being particularly susceptible to environmental stressors.

In Order to study and evaluate the fate, effects, environmental and human risks posed by organic micropollutants such as pharmaceuticals in aquatic ecosystems, information regarding their presence in marine organisms and more particular in species that are important in terms of human consumption like bivalves is

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urgently needed [12]. For this purpose, analytical methods that can be applied to real complex matrices, such as mollusks, in a fast and simple manner need to be developed allowing an easy implementation in other laboratories for routine analysis. Besides, the most significant pharmaceutical families, according to their potential effects, need to be included in order to establish seafood quality control and simultaneous monitoring of contaminated areas through bioindicator species.

In the last 10 years attention has been mainly focused in fish as indicator organism [13–16] and several methods have been published for the extraction of pharmaceuticals from this matrix usually involving long purification stages [17,18]. However, analytical methods for the extraction of these micropollutants from bivalves are less widespread, and they have commonly been developed just in mussels (Table 1). Bivalves are rich in complex biological components, mainly lipids and proteins, which could interfere with the analysis and with the low concentrations at which the target analytes are usually present, and therefore appropriate extraction and clean-up methodologies need to be developed. Different extraction techniques have been used for previous authors in mollusks (Table 1) including QuEChERs (quick, easy, cheap, effective, rugged, and safe) [19], pressurized liquid extraction (PLE) [12,20], and microwave-assisted solvent extraction (MASE), either with the addition of a surfactant concentration [21] (micellar extraction) or with enzymatic extraction [8]. Solid phase extraction (SPE) has been the selected clean-up stage by excellence normally on Oasis HLB cartridges although Strata-X was also used by some authors [12,20](Table 1). In the case of more complex matrices such as fish, gel permeation chromatography (GPC) has also been used for purification purposes but it is long and time consuming [17,18]. The detection techniques previously employed include high performance liquid chromatography (HPLC) coupled to either ultraviolet (UV), diode array (DAD) and fluorescence (FL) detectors, or mass spectrometry (MS). Recently Martinez-Bueno et al. [19] has used liquid chromatography coupled to high resolution mass spectrometry (HRMS) allowing the identification of the main transformation products of two anticonvulsants (carbamazepine and oxcarbazepine) in marine mussels. Later on the same method has been modified and applied to venlafaxine and its metabolites [22] in the same organism. Other target species, like arthropods, have also been subjected to the study of venlafaxine biotransformation products [23]. The relevance of metabolites and transformation products when studying bioaccumulation of pharmaceuticals in organisms has been previously pointed

Table 1
Analytical methods applied in the determination of pharmaceuticals in bivalves.

Pharmaceuticals	Therapeutic family	Organism	Method			Reference
			Extraction	Clean-up	Detection	
Carbamazepine	Psychiatric drugs	*Mytilus galloprovincialis*	Agitation, centrifugation, and microwave-assisted micellar extraction	SPE on Oasis HLB	HPLC–UV	[21]
Clofibric acid, Bezafibrate	Lipid regulators					
Ibuprofen, Ketoprofen, naproxen	Analgesics/anti-inflammatories					
Fluoxetine	Psychiatric drugs	*Dreissena polymorpha*	Bed shaker and centrifugation	–	LC–MS/MS	[52]
Carbamazepine	Psychiatric drugs	*Dreissena polymorpha*	Agitation, centrifugation, and microwave-assisted micellar extraction (according to Cueva-Meztanza)	SPE on Oasis HLB	LC–MS/MS	[53]
Bezafibrate	Lipid regulators					
Ibuprofen	Analgesics/anti-inflammatories					
Sulfonamides, tetracyclines, penicillin, amphenicols	Antibiotics	*Mytilus* spp.	Enzymatic microwave assissted extraction	Centrifugation	HPLC–DAD/FL	[8]
Atenolol, propanolol	β-Blocker	*Mytilus edulis*	Pressurized liquid extraction	SPE on Strata-X	U–HPLC–MS	[12]
Paracetamol, salicylic acid, ketoprofen, diclofenac	Analgesics/anti-inflammatories					
Trimethoprim, ofloxacin, chloramphenicol	Antibiotics					
Carbamazepine	Psychiatric drugs					
Clorfibric acid	Lipid regulators					
Carbamazepine, oxcarbazepine and metabolites	Psychiatric drugs	*Mytilus galloprovincialis*	QuEChERS	Centrifugation	HRMS	[19]
Diclofenac, mefenamic acid	Analgesics/anti-inflammatories	*Mytilus* spp.	Pressurized liquid extraction	SPE on Strata-X	LC–MS/MS	[20]
Trimethoprim	Antibiotics					
Carbamazepine	Psychiatric drugs					
Gemfibrozil	Lipid regulators					
Quinolones, sulfonamides, macrolides	Antibiotics	*Crassostrea talienwhanensis* *Chlamys farreri* *Amussium* *Scapharca subcrenata* *Meretrix merhjgntrix* *Mactra veneriformis* *Mactra chinesis* *Mya arenaria* *Neverita didyma* *Rapama venosa* *Mytilus edulis*	Pressurized liquid extraction	SPE on Oasis HLB	LC–MS/MS	[41]