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Multi-residue analysis of emerging pollutants in benthic invertebrates by modified micro-quick-easy-cheap-efficient-rugged-safe extraction and nanoliquid chromatography–nanospray–tandem mass spectrometry analysis

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ABSTRACT

Aquatic ecosystems are continuously contaminated by agricultural and industrial sources. Although the consequences of this pollution are gradually becoming visible, their potential impacts on aquatic ecosystems are poorly known, particularly regarding the risk of bioaccumulation in different trophic levels. To establish a causality relationship between bioaccumulation and disease, experiments on biotic matrices must be performed. In this context, a multi-residue method for the analysis of 35 emerging pollutants in three benthic invertebrates (*Potamopyrgus antipodarum*, *Gammarus fossarum*, and *Chironomus riparius*) has been developed. Because the variation in response of each individual must be taken into account in ecotoxicological studies, the entire analytical chain was miniaturised, thereby reducing the required sample size to a minimum of one individual and scaling the method accordingly. A new extraction strategy based on a modified, optimised and miniaturised "QuEChERS" approach is reported. The procedure involves salting out liquid–liquid extraction of approximately 10–20 mg of matrix followed by nano-liquid chromatography–nano electospray ionisation coupled with tandem mass spectrometry. The validated analytical procedure exhibited recoveries between 40 and 98% for all the target compounds and enabled the determination of pollutants on an individual scale in the ng g^{-1} concentration. The method was subsequently applied to determine the levels of target analytes in several encaged organisms which were exposed upstream and downstream of an effluent discharge. The results highlighted a bioaccumulation of certain targeted emerging pollutants in three freshwater invertebrates, as well as inter-species differences. 18 out of 35 compounds were detected and eight were quantified. The highest concentrations were measured for ibuprofen in *G. fossarum*, reaching up to 105 ng g^{-1}.

1. Introduction

The aquatic ecosystem is the result of a balance between the natural environment and the organisms and plants that inhabit it. This balance can be permanently modified by the influx of excessive amounts of substances generated from human activities. In addition to conventional priority pollutants, new unregulated pollutants, known as "emerging pollutants," are causing major environmental concerns [1–3]. These pollutants include, among others, pharmaceuticals, personal care products, alkyphenols and plasticisers. There is still insufficient information to assess the risk of these compounds on aquatic ecosystems, particularly regarding the risk of bioaccumalation in the first trophic levels. This lack of data could be partly explained by the lack of suitable analytical methods for small organisms, such as benthic invertebrates. In this context, the researchers sought to determine how best to assess contamination of benthic invertebrates used to evaluate the state of the ecosystem. Filtering organisms, such as molluscs, as well as amphipods and aquatic larvae, are used in monitoring programs as bio-indicators of pollution due to their capability of accumulating higher concentrations of contaminants [4–8]. Moreover, these organisms hold an important position in the aquatic food chain,

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being a major food source for other invertebrates and vertebrates, such as fishes. In addition, these organisms are easy to culture and have a short lifecycle. Thus, *Potamopyrgus antipodarum*, *Gammarus fossarum* and *Chironomus riparius* are used extensively as sentinel species to assess acute and sublethal toxicities of contaminated sediments and water [9–14]. Studies on the occurrence or levels of hydrophobic contaminants are frequently performed on aquatic vertebrates or macro-invertebrates; however, the number of studies that refer to their presence in benthic invertebrates is quite limited. Moreover, the methodologies described only focus on the specific analysis of limited chemical families of pollutants [15]. Nevertheless, the use of multi-residue, inter-family analysis to detect and quantify different chemical families in the same organism is essential to obtain a global view of aquatic pollution. Previously, the identification and quantification of emerging contaminants in biota have been reported using liquid (LC) [16–18] or gas chromatography (GC) [19–21] coupled with mass spectrometry (MS) because they provide high selectivity and the specificity required for these types of studies.

However, prior to LC analysis, sample preparation is required not only to extract the targeted compounds from these complex matrices but also to remove certain substances that may interfere with the detection of the pollutants of interest, reduce the separation efficiency, or shorten the LC column life [22]. The extraction techniques employed are common procedures based on pressurised liquid extraction [23], sonication [24], and microwave assisted extraction [25,26]. However, the disadvantages of these sample preparations include the use of large amounts of organic solvent and the long time required for the preparation of each sample. Furthermore, an efficient clean-up to remove interferences is mandatory to avoid the presence of interfering substances, especially lipids. Solid phase extraction (SPE) [19,26,27] is the most common purification technique. In 2003, a new extraction procedure, known as QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe), was developed by Anastassiades et al. [28] and was first used to extract pesticides from food matrices, such as fruits and vegetables. This method is based on a salting-out extraction with an organic solvent followed by a dispersive solid phase extraction (dSPE). For 10 years, the two steps of this method have been optimised and adjusted several times and have recently been used for the extraction of various compounds (veterinary drugs, pesticides, pharmaceuticals) in different complex matrices, such as animal tissues [29,30], honeybees [31] and fishes [32,33]. Therefore, this technology, which requires low solvent consumption, is of great interest because of its favourable toxicological, environmental and economic aspects.

In the last decade, a great deal of attention has been paid to developing miniaturised analytical systems that can offer reliable separation and higher sensitivity with the use of smaller volumes of both solvent and samples [34–38]. Nanoliquid chromatography (NanoLC) introduced in 1988 by Karlsson and Novotny [39] is a chromatographic technique which uses capillary columns of internal diameter (ID) below 100 μm. Downscaling from conventional LC columns of 4.6 mm ID to 100 μm ID would then result in a gain in sensitivity of nearly 2000 [40] which can be ascribed to both a reduction of analyte dilution [41] and an increase in efficiency [42]. The coupling of the NanoLC system with mass spectrometry can also be used to increase the sensitivity. Indeed, using an electrospray ionisation interface as a continuous flow ionisation technique enhances the ionisation process [43–45]. The largest application for NanoLC is proteomic analysis [46–51]. However, this analytical technology has not been so widely applied in other important fields such as environmental field analysis.

The aim of this study was to develop an innovative, rapid, robust and sensitive analytical method for trace analysis of emerging pollutants in three benthic invertebrates (*P. antipodarum*, *G. fossarum* and *C. riparius*) based on a modified, optimised and miniaturised QuEChERS extraction followed by nanoLC–MS/MS analysis. This method increases sensitivity and reduces the required initial sample amount: only one individual is used for gastropods and amphipods and a pool of 10 mg wet weight for insect larvae. A list of 35 compounds (Table 1) was chosen based on scientific criteria (occurrence and persistence in the environment, presence of different prioritisation lists and physico-chemical properties) [1,2,52–55]. This list contains a majority of pharmaceuticals covering several families of human drugs and including anticonvulsants, non-steroidal anti-inflammatory drugs, anticancer drugs, antibiotics, antidepressants and local anaesthetics. Seven hormones belonging to the chemical class of steroids and formed naturally by humans and wildlife or produced synthetically were selected since their ability to impair wildlife has been demonstrated [56–58]. To the list were added substances that are

Table 1
Selected compounds and their CAS numbers, molecular formulae and molecular weights (MW).

Compound (abbreviation)	CAS	Formula	MW ($g\,mol^{-1}$)
Pharmaceuticals and their metabolites			
Atenolol (Ate)	29122-68-7	$C_{14}H_{22}N_2O_3$	266.3
Amitriptyline (Ami)	50-48-6	$C_{20}H_{23}N$	277.4
Bezafibrate (Beza)	41859-67-0	$C_{19}H_{20}ClNO_4$	361.8
Carbamazepine (Carba)	298-46-4	$C_{15}H_{12}N_2O$	236.3
Oxazepam (Oxa)	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.7
Tamoxifen (Tamo)	10540-29-1	$C_{26}H_{29}NO$	371.5
4-Hydroxytamoxifen	68047-06-3	$C_{26}H_{29}NO_2$	387.5
Nicardipine (Nicar)	55985-32-5	$C_{26}H_{29}N_3O_6$	479.5
Cyclophosphamide (Cyclo)	50-18-0	$C_7H_{15}Cl_2N_2O_2P$	261.1
Desloratadine (Deslo)	100643-71-8	$C_{19}H_{19}ClN_2$	310.8
Econazole (Eco)	27220-47-9	$C_{18}H_{15}Cl_3N_2O$	381.7
Ritonavir (Rito)	155213-67-5	$C_{37}H_{48}N_6O_5S_2$	720.9
Mifepristone (Mife)	84371-65-3	$C_{29}H_{35}NO_2$	429.6
Diclofenac (Diclo)	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.1
Ketoprofen (Keto)	22071-15-4	$C_{16}H_{14}O_3$	254.3
Ibuprofen (Ibu)	15687-27-1	$C_{13}H_{18}O_2$	206.3
Roxithromycin (Roxi)	80214-83-1	$C_{41}H_{76}N_2O_{15}$	837.0
Lidocaine (Lido)	137-58-6	$C_{14}H_{22}N_2O$	234.3
Prednisolone (Predni)	50-24-8	$C_{21}H_{28}O_5$	360.4
Pantoprazole (Panto)	102625-70-7	$C_{16}H_{15}F_2N_3O_4S$	383.4
Pesticides			
Diuron (Diu)	330-54-1	$C_9H_{10}Cl_2N_2O$	233.1
Spinosad (Spin)	131929-60-7	$C_{41}H_{65}NO_{10}$	731.9
Hormones			
Testosterone (Testo)	58-22-0	$C_{19}H_{28}O_2$	288.4
Estrone (E1)	53-16-7	$C_{18}H_{22}O_2$	270.4
17α-ethynylestradiol (EE2)	57-63-6	$C_{20}H_{24}O_2$	296.4
17α-estradiol (αE2)	57-91-0	$C_{18}H_{24}O_2$	272.4
17β-estradiol (βE2)	50-28-2	$C_{18}H_{24}O_2$	272.4
Norethindrone (Nore)	68-22-4	$C_{20}H_{26}O_2$	298.4
Levonogestrel (Levo)	797-63-7	$C_{21}H_{28}O_2$	312.4
Plasticizer			
Bisphenol A (BPA)	80-05-7	$C_{15}H_{16}O_2$	228.3
Alkylphenols			
4-ter-octylphenol (ToP)	140-66-9	$C_{14}H_{22}O$	206.3
4-ter-nonylphenol (TnP)	84852-15-3	$C_{15}H_{24}O$	220.3
Perfluorinated compounds			
Perfluorooctanoic acid (PFOA)	335-67-1	$C_8HF_{15}O_2$	414.1
Perfluorooctanesulfonic acid (PFOS)	1763-23-1	$C_8HF_{17}O_3S$	500.1
UV filter			
4-methylbenzylidene camphor (4MBC)	36861-47-9	$C_{18}H_{22}O$	254.4