Science of the Total Environment 511 (2015) 153–160

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Pharmaceuticals in the freshwater invertebrate, *Gammarus pulex*, determined using pulverised liquid extraction, solid phase extraction and liquid chromatography–tandem mass spectrometry

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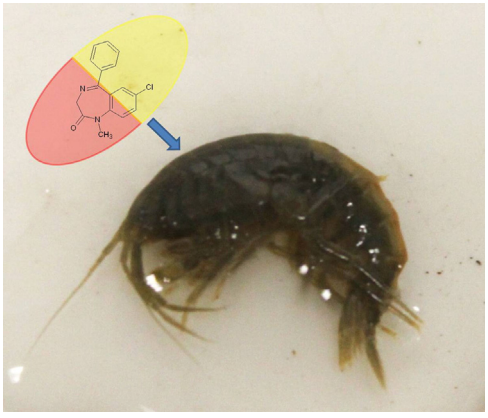
HIGHLIGHTS

- Analytical method considerations for small invertebrates are presented.
- Application of method to screening of *G. pulex* for 29 pharmaceuticals
- Method validated for quantitation of 10 pharmaceuticals in *G. pulex*
- Six pharmaceutical residues were determined up to 36 ng g^{-1} in *G. pulex*.
- Five identified compounds in *G. pulex* are present in river water up to 344 ng/L.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form 12 December 2014
Accepted 13 December 2014
Available online xxxx

Editor: D. Barcelo

Keywords:
Gammarus pulex
Environmental analysis
Emerging contaminants
Occurrence

ABSTRACT

The development, characterisation and application of a new analytical method for multi-residue PPCP determination in the freshwater amphipod, *Gammarus pulex* are presented. Analysis was performed using pulverised liquid extraction (PuLE), solid phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Qualitative method performance offered excellent limits of detection at <20 ng g^{-1} for 18 out of 29 compounds. For quantitative application, linearity and precision were considered acceptable for 10 compounds across the ng-μg g^{-1} range ($R^2 \geq 0.99$; ≤20% relative standard deviation respectively). The method was applied to the analysis of *G. pulex* and river water sourced from six tributaries of the River Thames. Carbamazepine, diazepam, nimesulide, trimethoprim and warfarin were determined in *G. pulex* samples at low ng g^{-1} (dry weight) concentrations across these sites. Temazepam and diclofenac were also detected, but were not quantifiable. Six pharmaceuticals were quantified in surface waters across the eight sites at concentrations ranging from 3 to 344 ng L^{-1}. The possibility for confirmatory detection and subsequent quantification of pharmaceutical residues in benthic organisms such as *G. pulex* will enable further understanding on the susceptibility and ecological effects of PPCPs in the aquatic environment.

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http://dx.doi.org/10.1016/j.scitotenv.2014.12.034
0048-9697/

1. Introduction

The continuous influx of pharmaceuticals and personal care products (PPCPs) into the aquatic environment via wastewater treatment plant (WWTP) effluent is driving research into the field of ecotoxicology due to a rising concern for the health of biota residing in contaminated waters. Numerous monitoring studies have been carried out to assess the extent of PPCP contamination in wastewater effluent and impacted surface waters such as rivers, lakes and seawater with PPCP residues detected up to μg L^{-1} concentrations (Ashton et al., 2004; Behera et al., 2011; Brown et al., 2006; Carmona et al., 2014; Kosma et al., 2010; McEneff et al., 2014; Roberts and Thomas, 2006; Thomas and Hilton, 2004; Vazquez-Roig et al., 2013). The release of pharmaceuticals at low μg L^{-1} concentrations has been shown to impact on the quality of the surrounding aquatic environment in Europe and America (Corcoran et al., 2010; Huerta et al., 2012). However the paradigm of transient exposure to temporal flow makes the environmental risk assessment complex. Internal concentrations are clearly the key to better understanding (and therefore prediction) of risk (Rand-Weaver et al., 2013). The exposure of wild-caught and caged biota to contaminated surface waters over extended periods of time have revealed the potential for PPCP uptake and subsequent adverse chronic effects (Dodder et al., 2014; Gatidou et al., 2010; Huerta et al., 2013; Subedi et al., 2012). It is widely believed that bioaccumulation of contaminants occurs through passive diffusion where the hydrophobicity of the compound (log*P*) largely describes their permeability through membranes (Hamelink and Spacie, 1977; McKim et al., 1985). However, due to their ability to ionise and undergo various transformation processes, there is mounting evidence to support carrier mediated transport of PPCPs through facilitated diffusion and active transport (Dobson and Kell, 2008; Schultz et al., 2010). PPCP occurrence data in aquatic biota is of particular importance as results may highlight highly bioaccumulative compounds that may direct the attention of future risk assessment and management strategies for PPCPs. Furthermore, and given that several thousand pharmaceutical compounds currently exist on the market, this represents a significant challenge. It is of interest to enable discovery of PPCPs in the environment, which might not otherwise be predicted using simple log*P*-based approaches and analytical methods to detect these are urgently required to aid in prioritisation efforts.

Aside from localised monitoring programmes, the only international body in the EU to recognise PPCPs as an emerging environmental concern was, until recently, the Oslo–Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) Commission. Pharmaceuticals remained outside the scope for regulation and formal monitoring under the European Water Framework Directive (WFD). However, following the results of numerous European monitoring studies at a national level, the list of priority pollutants has recently been revised with the addition of a 'watch list' of new compounds. This list includes the anti-inflammatory, diclofenac and the hormones, 17α-ethinylestradiol and 17β-estradiol, which are not subject to regulation, but are instead closely monitored in EU surface waters for possible future addition to the priority list (Commission, 2012).

Gammarus pulex has many attributes for use in biomonitoring studies. It is a freshwater benthic dwelling detritivore which has an important role in freshwater food chains as a food source for other invertebrates, fish and birds (Friberg et al., 1994; Maltby et al., 2002). *G. pulex* is widely distributed in freshwater rivers and tributaries across Europe and can be collected in large numbers using simple kick sampling techniques. More importantly *G. pulex* has already been used as a model organism for assessing both the adverse effects (De Lange et al., 2006, 2009) and uptake potential of PPCPs (Meredith-Williams et al., 2012) as well as other common pollutants (Ashauer et al., 2012; Nyman et al., 2012). The main disadvantage of using *G. pulex* in biomonitoring is their small size which poses a significant analytical challenge for multi-residue screening. A trade-off exists between achieving suitable method sensitivity and using the minimum number of specimens to make a single measurement. Very few methods exist for PPCP residue analysis of such small species in the aquatic environment. It is generally accepted that liquid or gas chromatography coupled to mass spectrometry offers the sensitivity and selectivity required. It has been successfully applied to the analysis of other smaller invertebrate species such as *Chironomus tentans* and *Hyallela azteca* (Dussault et al., 2009; Klosterhaus et al., 2013). However, robust analytical methods to determine PPCP residue occurrence in *G. pulex* are still lacking.

This paper presents, for the first time, the occurrence and relative distribution of PPCPs in surface waters and *G. pulex* collected from several tributaries located in the greater London catchment area. The aim of this study was to evaluate the extent of contamination in surface waters flowing into the River Thames and to investigate the potential for the crustacean, *G. pulex*, to be utilised in future monitoring studies as an indicator for PPCP pollution.

2. Materials and methods

2.1. Reagents, chemicals and consumables

HPLC grade methanol, acetonitrile, acetone, ethyl acetate, dichloromethane and dimethyldichlorosiloxane were purchased from Fischer Scientific (Loughborough, UK). Analytical grade ammonium acetate was sourced from Sigma-Aldrich (Dorset, UK). Propranolol hydrochloride, ketoprofen, diclofenac sodium salt, bezafibrate, warfarin, flurbiprofen, indomethacin, ibuprofen sodium salt, meclofenamic acid sodium salt, gemfibrozil, atenolol, sulfamethoxazole, sulfamethazine sodium salt, furosemide, carbamazepine, nimesulide, (±)-metoprolol (+)-tartrate salt, triclocarban, cimetidine, ranitidine, antipyrin, temazepam, diazepam, fluoxetine, nifedipine and mefenamic acid were all obtained from Sigma-Aldrich (Steinheim, Germany). Trimethoprim, caffeine, and naproxen were ordered from Fluka (Buchs, Switzerland). Stable isotope-labelled standards including carbamazepine-d_{10}, propranolol-d_7, temazepam-d_5 and diazepam-d_5 were ordered from Sigma-Aldrich. Trimethoprim-d_3 and warfarin-d_5 were ordered from QMX Laboratories (Essex, UK). All pharmaceuticals were of a purity of ≥97%. Ultra-pure water was obtained from a Millipore Milli-Q water purification system with a specific resistance of 18.3 M Ω cm or greater (Millipore, Bedford, MA, USA). Stock solutions (1 mg mL^{-1}) were prepared in methanol and stored in silanised amber vials (40 mL). Working solutions were prepared daily in ultra-pure water, as required. All solutions were stored at 4 °C and in the dark for optimum stability.

2.2. Sample collection and preparation

G. pulex and surface waters were sourced from eight tributaries of the River Thames, UK. These were spread across the greater London catchment area and included the River Wandle (Sites 1 and 2), the River Quaggy (Site 3), the River Ravenstone (Site 4), the River Cray (Sites 5 and 6), the River Darent (Site 7) and Beverley Brook (Site 8). The specific locations of the selected sites are shown in Fig. 1. Adult specimens were collected in September 2012 via the kick sampling netting method and weighed >5 mg (wet weight). Samples were transported back to the laboratory in Nalgene™ flasks containing 500 mL of freshwater obtained from each corresponding sampling site. A bulk sample of *G. pulex* from Site 1 was used in all analytical method optimisation experiments and was taken 6 months prior to samples from the same site used for analyte reporting. *G. pulex* were wiped free of debris, rinsed immediately with ultra-pure water (n = 3) and gently blotted dry before freezing at −20 °C. A separate 1 L grab sample of surface water at each site was also collected and transported back to the laboratory in 500 mL Nalgene™ flasks. Water samples were also frozen at −20 °C until analysis. All glassware was washed in HPLC-grade solvents prior to use and on a monthly basis silanised by washing each vessel with 10% (*v*/*v*) dichlorodimethylsilane

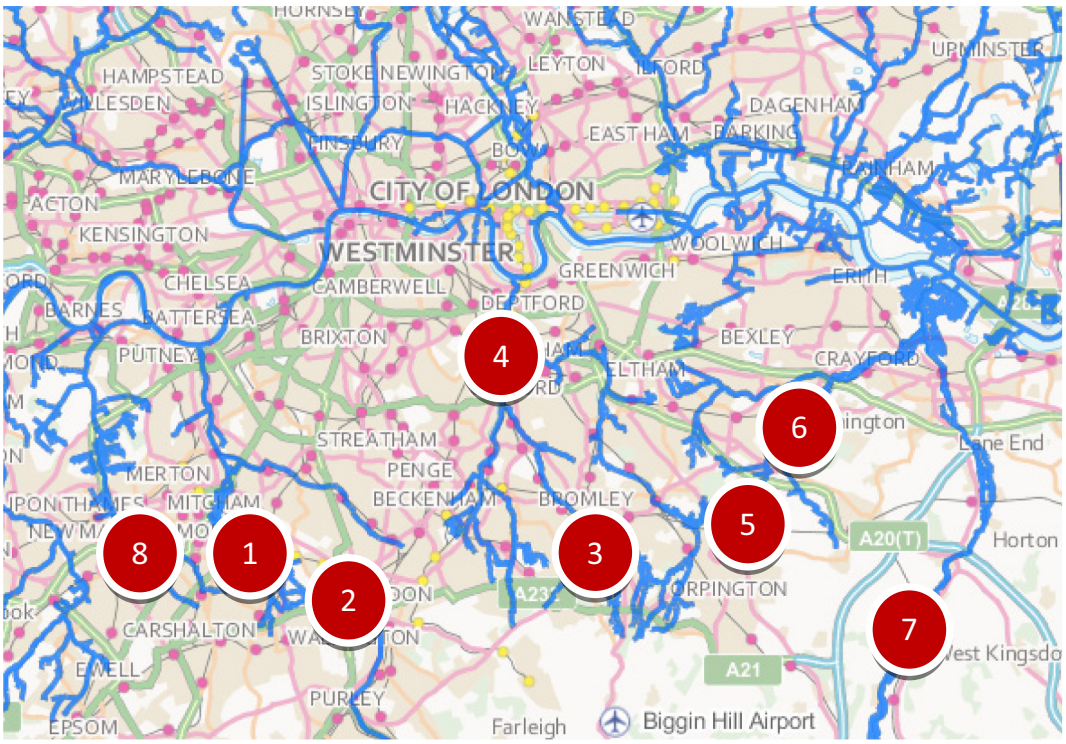

Fig. 1. Locations from which samples of *G. pulex* and surface waters were collected. Sites 1 and 2 — River Wandle, Site 3 — River Quaggy, Site 4 — River Ravensbourne, Sites 5 and 6 — River Cray, Site 7 — River Darent, and Site 8 — Beverley Brook. Map used with permission and contains Environment Agency information (© Environment Agency and database right).

solution in dichloromethane (n = 3) and followed by a sequence of triplicate rinses with each of dichloromethane, methanol and ultrapure water respectively.

2.3. Sample extraction and clean-up

Prior to extraction, frozen *G. pulex* samples were freeze-dried at −50 °C under vacuum for 24 h and ground into a coarse material using a clean pestle and mortar. Pulverised liquid extraction (PuLE) was performed on an Ultra-Turrax® tube driver (IKA, Staufen, Germany). The tube driver was used with an extraction vessel for sample homogenisation and extraction. The contents of the extraction tube were agitated and pulverised at a set rate by means of a rotor and glass beads located inside the tube. For each analysis, freeze-dried composite sample material from each sampling site (0.1 g) was transferred to a 20 mL extraction tube (IKA) with any necessary spiking carried out directly onto the solid matrix using a 100 μL volume of an appropriate working solution followed by 5 mL of acetonitrile. Two glass beads (diameter = 5 mm) were then added to the extraction tube to enable further pulverisation of the sample and the tube agitated at 2500 rotations per minute (rpm) for 5 min (optimised). Following extraction and settling, an aliquot of the supernatant (4.5 mL) was diluted to 100 mL with 10 mM ammonium acetate in ultra-pure water (pH 6.5). Solid phase extraction (SPE) was then carried out as in previously published work on a similar selection of compounds (Barron et al., 2008) on the diluted sample using Oasis HLB cartridges (6 mL, 200 mg, Waters Corp., Hertfordshire, UK). Before loading of the sample, SPE cartridges were first conditioned with 6 mL of methanol and 6 mL of ultra-pure water. After sample extraction, cartridges were then washed with 1 mL ultra-pure water and dried for ~30 min under a vacuum. Sample extracts were eluted with 10 mL of 50:50 (*v*/*v*) ethyl acetate:acetone and dried under pure nitrogen (1.0 bar) and heated at 30 °C using a TurboVap (Biotage, Uppsala, Sweden). Extract residues were reconstituted in 0.5 mL 90:10 (*v*/*v*) 10 mM ammonium acetate in water:acetonitrile. Surface water samples (100 mL) were adjusted to pH 6.5 with ammonium acetate (1 mL of a 1 M solution). Water samples then underwent SPE and reconstitution as described above. Any necessary spiking or liquid volume measurements were carried out using positive displacement pipettes (Gilson Microman, Villiers-le-Bel, France).

2.4. Instrumental conditions

A previously published chromatographic method for the analysis of PPCPs in soil and sludge was adapted and applied to a biological matrix (*G. pulex*) (Barron et al., 2008). Briefly, liquid chromatography (LC) was performed on an Agilent 1100 series LC system (Agilent Technologies, Cheshire, UK) using a Waters SunFire C_{18} column (3.5 μm, 2.1 mm × 150 mm, Waters Corp., Milford, MA, USA) with a KrudKatcher™ Ultra guard column (0.1 mm ID, 0.5 μm filter, Phenomenex, Macclesfield, UK) at a flow rate of 0.2 mL min^{-1} and an injection volume of 20 μL. Mobile phases were 90:10 (*v*/*v*) 10 mM ammonium acetate in water:acetonitrile (A) and 20:80 (*v*/*v*) 10 mM ammonium acetate in water:acetonitrile (B). The profile followed a linear ramp of mobile phase B which increased to 10% at 5 min, 35% at 28 min, 40% at 35 min, 50% at 40 min and 100% at 55 min and was held for a further 7.5 min before returning to initial conditions. Re-equilibration time was 12.5 min resulting in an overall run time of 75 min. Detection and quantification was carried out with a Waters Quattro triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an atmospheric pressure interface–electrospray ionisation (API–ESI) source. Mass spectrometric (MS) analysis was carried out in selected reaction monitoring (SRM) mode using positive–negative ionisation polarity switching. A scan rate of 0.03 min was utilised with a minimum of 15 points per peak measuring ±0.5 mass units for all transitions monitored. Confirmation of the selected compounds was achieved using both retention time and two transitions (MSn^2 fragment ions where possible) with the most intense fragment ion selected for analyte quantification (Table 1). MS conditions as well as all SRM transitions are summarised in Tables S1 and S2 of the supplementary information and were determined by

direct infusion using a syringe pump which delivered 300 mL h^{-1} of analyte solution.

2.5. Method performance characteristics and quality control

For this study, method performance characteristics are presented for *G. pulex* only. Matrix-matched calibration curves were generated for biota to assess method performance. Linearity was determined by measuring the peak area at concentrations from 0.01 to 10 μg g^{-1} for the *G. pulex* ($n \geq 5$ for each compound). Limits of detection (LODs) were determined as the lowest concentration of analyte which produced a signal-to-noise (S/N) ratio of 3:1. Limits of quantification (LOQs) were determined as that analyte concentration to give an S/N ratio of 10:1. Both LOD and LOQ were calculated using the S/N ratios of low concentration spiked samples and precision checked at this level for $n = 6$ replicates to ensure satisfactory performance. Instrumental retention time and method precision (intra-day) experiments were performed for $n = 6$ replicate injections of a biotic sample spiked at 1 μg g^{-1}. Method accuracy (intra-day) was determined using a biotic sample spiked at 20 ng g^{-1} for all compounds except for sulfamethazine, metoprolol, propranolol, nimesulide and nifedipine, where the biotic sample was spiked at 75 ng g^{-1} ($n \geq 3$). Recovery was determined by comparing spiked samples at 1 μg g^{-1} in *G. pulex* ($n = 6$ and which was also used to assess mid-range method precision) to sample extracts spiked post-extraction ($n = 3$) at the expected final concentration. Control samples were also analysed for background correction purposes, where necessary. The measurement of ion suppression or enhancement in ESI–MS involved the comparison of sample extracts spiked post-extraction to a 100 ng g^{-1} working solution mixture ($n = 3$). The target analytes in both surface waters and biota were quantified based on their peak areas relative to that of an isotopically-labelled internal standard or, where unavailable, by external matrix-matched calibration. Relative recovery in *G. pulex* was measured following the analysis of spiked biotic samples (analytes and the internal standard at 100 ng g^{-1} each) by comparing the analyte peak areas to that of the internal standard ($n = 12$).

Mobile phase A was injected between samples from each site as well as between matrix-matched standards and controls to minimise the possibility of carry over. Direct infusion of a propranolol standard (1 μg mL^{-1}) was carried out before each batch analysis to ensure that the MS was operating satisfactorily. None of the targeted analytes were detected in any solvents, reagents or ultra-pure water used in this study.

3. Results and discussion

3.1. Optimisation of analytical methods for G. pulex

The presence of pharmaceuticals in aquatic organisms, particularly molluscs, has been previously investigated using extraction techniques such as pressurised liquid extraction (PLE) or ultrasound-assisted solvent extraction which involve the use of high temperatures, pressures and/or relatively large volumes of organic solvent (Cueva-Mestanza et al., 2008; McEneff et al., 2013). Other studies have utilised PuLE as a simple and fast extraction method for the quantification of pharmaceuticals in solid dosage forms (Kok and Debets, 2001). Due to the complexity of biological tissues (and especially here where keratinous material was present), PuLE was used here to simultaneously blend and extract all material before SPE. For this purpose, a specially designed extraction vial was used and was equipped with a rotor for agitation of the sample, liquid and two glass beads. The beads were required for satisfactory agitation and pulverisation of the *G. pulex* material, but also were considered valuable to potentially confer flexibility in the future for extraction of whole tissues/specimens where necessary. Sorption to glass beads was briefly investigated here by performing the extraction procedure on a mixed 1 μg mL^{-1} standard solution of all analytes prepared in the extraction solvent. The use of silanised glass beads did not yield higher analyte recoveries than those

Table 1
Method performance characteristics for *G. pulex* analysis.

Compound	$t_R \pm$ SD[a] (min) $n = 6$	SRM transitions	ESI Mode	Range (ng g^{-1})	R^{2b} $n \geq 5$	LOD (ng g^{-1}) $n = 6$	LOQ ± SD (ng g^{-1}) $n = 6$	Intra-day accuracy ± SD (%) $n \geq 3$	Absolute recovery & intra-day precision (%) $n = 6$	Ion suppression (%) $n = 6$
Sulfamethazine	11.9 ± 0.1	(279 → 186) (279 → 124)	(+)	25–10,000	0.9987	4	15 ± 6[d]	95 ± 3[f]	41 ± 2	11 ± 2
Trimethoprim	16.8 ± 0.1	(291 → 230) (291 → 123)	(+)	10–10,000	0.9987	2	5 ± 4[c]	126 ± 31[g]	65 ± 1	13 ± 6
Metoprolol	19.7 ± 0.1	(268 → 116) (268 → 159)	(+)	10–10,000	0.9992	1	4 ± 1[d]	100 ± 6[f]	71 ± 5	11 ± 2
Propranolol	30.9 ± 0.5	(260 → 116) (260 → 183)	(+)	50–10,000	0.9952	13	61 ± 9[e]	81 ± 8[f]	52 ± 11	56 ± 4
Carbamazepine	31.3 ± 0.1	(237 → 194)	(+)	25–10,000	0.9983	2	6 ± 1[c]	124 ± 6[g]	69 ± 3	9 ± 2
Warfarin	22.7 ± 0.1	(307 → 161) (307 → 250)	(−)	50–10,000	0.9973	2	5 ± 1[c]	102 ± 9[g]	71 ± 3	28 ± 4
Temazepam	40.2 ± 0.1	(301 → 255) (301 → 283)	(+)	10–10,000	0.993	2	6 ± 1[c]	100 ± 3[g]	85 ± 1	11 ± 3
Diazepam	46.8 ± 0.1	(285 → 153) (285 → 193)	(+)	10–10,000	0.9991	2	5 ± 2[c]	176 ± 28[g]	89 ± 2	43 ± 3
Nimesulide	44.0 ± 0.1	(307 → 229) (307 → 79)	(−)	25–10,000	0.9945	3	13 ± 2[c]	97 ± 7[f]	87 ± 4	35 ± 3
Nifedipine	44.4 ± 0.1	(347 → 315) (347 → 271)	(+)	10–10,000	0.9985	1	4 ± 1[d]	104 ± 6[f]	70 ± 1	39 ± 4

[a] SD = standard deviation.
[b] $n > 5$ concentration data points each performed in triplicate.
[c] Signal to noise ratio of 10:1 from 20 ng g^{-1} spiked matrix-matched sample.
[d] Signal to noise ratio of 10:1 from 75 ng g^{-1} spiked matrix-matched sample.
[e] Signal to noise ratio of 10:1 from 60 ng g^{-1} spiked matrix-matched sample.
[f] Using a 75 ng g^{-1} spiked sample ($n = 3$) and determined by matrix matched calibration ($n = 3$) in triplicate.
[g] Using a 20 ng g^{-1} spiked sample ($n = 4$) and determined by the relevant isotopically-labelled internal standard.

achieved using unsilanised glass beads. Therefore, glass bead silanisation was not required (see Table S2).

The extraction of pharmaceuticals from aqueous samples is generally performed by SPE and HLB-type SPE cartridges enable the extraction of both polar and non-polar compounds from both water and biological extracts (Baker and Kasprzyk-Hordern, 2011a; Barron et al., 2008; Gomez et al., 2006; Weigel et al., 2004). The effect of the sample pH prior to SPE was investigated at pH 2, 7 and 10 (n = 3). A sample pH of 7 yielded the highest recoveries and lowest variability for the selected analytes. Sample reconstitution volumes of 0.1 mL and 0.5 mL were also investigated. As shown in Table S4, analyte recovery using a reconstitution volume of 0.5 mL was higher overall than that of 0.1 mL. In particular, compounds such as diazepam, gemfibrozil, triclocarban, ibuprofen, diclofenac and nimesulide showed moderately higher recoveries when reconstituted to 0.5 mL. Solubility of a dried matrix residue may have been a limiting factor at the reduced reconstitution solvent volume. Furthermore, matrix suppression effects in ESI–MS are likely to increase with a more concentrated sample extract (as was observed here). Although a thorough sample clean-up method was developed, final extracts of the *G. pulex* contained an immiscible red-pigmented liquid. A likely identity for this contaminant is astaxanthin, a relatively non-polar carotenoid pigment found in crustaceans (Johnson and An, 1991), but further analysis would be required to confirm this. Attempts to remove this substance from the ground *G. pulex* sample via liquid–liquid extraction (using a 50:50 (*v*/*v*) solution of acetonitrile with hydrochloric acid at pH 2 and hexane) did not prove successful. Additional approaches such as centrifugation and neutral alumina addition were also investigated (the latter of which is often used to remove pigments), but the substance remained in the sample extract and analyte recoveries did not improve (data not shown). Therefore, a 0.5 mL reconstitution volume in 90:10 (*v*/*v*) 10 mM ammonium acetate in water:acetonitrile was considered optimised.

In line with many other studies, LC–MS/MS was the chosen analytical technique for the confirmatory detection and subsequent determination of PPCPs in surface waters and *G. pulex*. A 10 mM ammonium acetate solution in a mixture of acetonitrile and water was selected again as a suitable mobile phase for reversed-phase separations in line with previously published work (Barron et al., 2008). The mobile phase gradients and column temperature (20, 30, 40 and 45 °C) were further optimised to allow better separation of more PPCPs. A column oven temperature of 45 °C offered the best separation of all compounds. For mass spectrometry, direct infusion was carried out initially in full scan mode to determine the most abundant precursor ion for each analyte and to optimise MS parameters for the best signal response. Compounds yielded $[M + H]^+$, $[M - H]^-$ or $[M\text{-}COOH]^-$ precursor ions in positive or negative polarity ESI–MS mode. Furthermore, SRM performed under positive–negative switching mode yielded MS/MS data for 29 analytes (Table S2). Secondary fragment ion transitions were observed for 21 of these compounds.

3.2. Method performance characteristics

Few validated multi-residue methods exist for PPCP determination in biota due to their complexity, variability, and potential to cause analytical matrix effects. Therefore, a larger number of compounds were included in the method development process originally as it was expected that some would not meet acceptable method performance criteria for quantitative analysis of small biotic samples. Those considered acceptable are presented in Table 1 (data from the full method performance experiment are shown in Tables S4 and S5). Method performance in surface waters is not presented here, but the method was deemed suitable for semi-quantitative purposes as it was based on previously published work (Lacey et al., 2012; Lacey et al., 2008). Instrumental retention time precision in *G. pulex* matrix was <0.5% for all analytes except for propranolol which was 1.7% (n = 6). For method linearity, correlation coefficients of $R^2 > 0.98$ (n ≥ 5 data points) were achieved for triplicate experiments, again in *G. pulex* matrix, over the calibration range for 18 compounds. Twelve compounds achieved unacceptable correlation coefficients and were included for qualitative purposes only (Table S5). Following the assessment of linearity, limits of quantitation (LOQs) of the method were determined for 23 analytes and lay between 4 and 687 ng g^{-1}. Intra-day method reproducibility was <30% for 20 analytes at 1 μg/g. Mean absolute recoveries of 28 analytes ranged from 4 to 89% (n = 6) with eight compounds displaying absolute recoveries >70%. Overall, it was observed that compounds

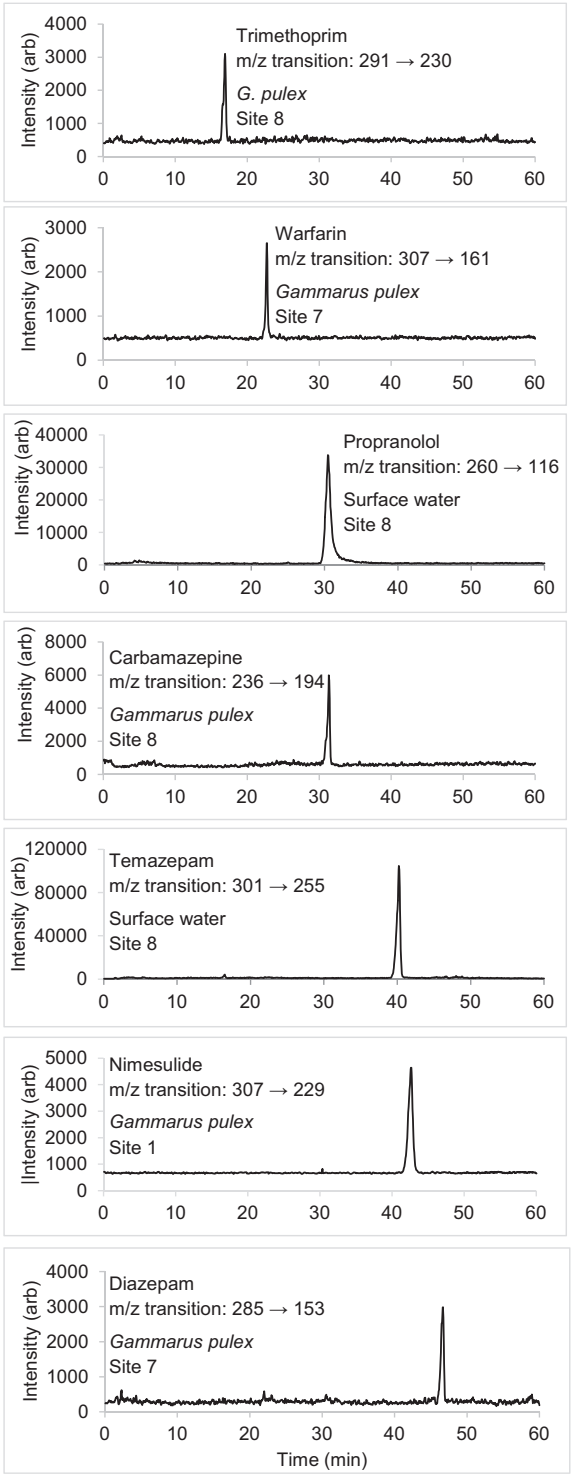

Fig. 2. Example extracted ion chromatograms of PPCPs detected in surface waters and *G. pulex* sampled from each of the eight selected sites.

determined in negative ESI–MS mode demonstrated lower recoveries than compounds detected in positive ESI–MS mode. Significant signal suppression was observed in *G. pulex* matrix as per Table S4 and signals for 6 of 29 compounds were suppressed greater than 50% in comparison to a standard mixture. The precision and recovery of this method in *G. pulex* correlates to data reported by Klosterhaus et al. (2013) for the analysis of PPCPs in mussels from San Francisco Bay. Other similar studies carried out on mussel and fish tissues, and other complex matrices such as biosolids and sludge, have shown similar method performance for PPCPs (Dodder et al., 2014; McEneff et al., 2013). Overall, and of the 29 PPCPs initially included in method development and performance characterisation, 10 compounds (carbamazepine, trimethoprim, warfarin, diazepam, temazepam, propranolol, nifedipine, nimesulide, sulfamethazine and metoprolol) showed acceptable method performance for quantification purposes. Precision was also maintained for these compounds when measured near the LOQ across n = 6 replicates.

3.3. Application to G. pulex and surface waters in tributaries of the River Thames, UK

The developed methods were applied to the identification of pharmaceutical residues in water and biotic samples. Surface water samples (500 mL) and *G. pulex* (n ≈ 60–100 specimens) were used for replicate analysis across the selected sites. As the surface water and biotic samples were collected by grab sampling, the results presented represent pharmaceutical concentrations present at that point in time. Example extracted ion chromatograms for compounds quantified in surface waters and *G. pulex*, are shown in Fig. 2.

Of the 29 compounds included in the analytical method for qualitative screening of invertebrate tissues, a total of 6 compounds were quantified in *G. pulex* (Table 2). Diclofenac was also detected at all sites but was not quantifiable due to method performance limitations (Table S5). Due to the lack of information regarding pharmaceutical uptake in *G. pulex*, similar occurrence studies on other species were used for comparison. Five compounds were quantified across the eight sites at concentrations ≤36 ng g^{-1} dry weight (Table 2). Carbamazepine was the most frequently detected compound, which is perhaps unsurprising as this compound has been reported in several solid and biological matrices (Barron et al., 2008; Huerta et al., 2013). Site 7 was the most contaminated of all sites. Diazepam was quantifiable across four sites at concentrations ≤9 ng g^{-1} dry weight. Kwon and co-workers detected several PPCP residues including carbamazepine and diazepam in fish livers and the latter was determined at concentrations up to 110 ng g^{-1} wet weight (Kwon et al., 2009). If hydrophobicity is to be considered a reliable quantity for bioaccumulation of PPCPs, then this may be explained given their moderate hydrophobicity relative to other PPCPs (log*P* = 2.45 and 2.86, respectively) (Barron et al., 2009). However, Meredith-Williams et al. recently studied bioconcentration of diazepam and carbamazepine in *G. pulex*, both of which were found to be minimal with BCF values of 38 and 7, respectively. As these compounds have relatively higher log*P* values in comparison to most other pharmaceuticals, it suggests that uptake models based on hydrophobicity may not accurately represent the potential for a compound to bioconcentrate in invertebrates. Tanoue et al. recently presented an analytical method for the determination of 17 intermediate-polarity PPCPs (log*P* = 1.40–5.74) and its application to biological tissue from Japanese fish and birds (Tanoue et al., 2014). Up to 13 PPCPs were determined across fish plasma (0.03–22 ng mL^{-1}), the brain and liver tissue (1–910 and 0.11–16 ng g^{-1} wet weight respectively) and all 17 were detected in the surrounding aquatic environment (at 3–871 ng L^{-1}). McEneff et al. investigated pharmaceutical concentrations in marine bivalves residing in effluent-contaminated seawater. The antibiotic, trimethoprim, measured highest at concentrations up to 9 ng g^{-1} dry weight and carbamazepine was also detected, but below its LOQ (McEneff et al., 2014). Here, trimethoprim was only quantifiable at Site 8 at 5 ng g^{-1} dry weight. Nimesulide was quantified at slightly higher concentrations on average. This coincides with the results from surface water analysis where nimesulide was detected in samples from Sites 1 to 4 and 7. Nimesulide was banned in the Republic of Ireland in 2007 due to risks associated with hepatic failure and it is not available on the UK market as a pharmaceutical for human consumption. However, sources of input into the environment still exist via veterinary routes and in addition this compound has also been found in selected food supplements (Lacey et al., 2012; MHRA, 2014).

Adverse effects such as increased oxidative stress and tissue lesions have been observed following the exposure of species such as fish and molluscs to environmentally relevant PPCP concentrations. A study carried out by De Lange et al. (2006) investigated the behavioural responses of *G. pulex* when exposed to environmentally relevant concentrations (from 0.1 ng L^{-1} up to 1 mg L^{-1}) of the pharmaceuticals carbamazepine, fluoxetine and ibuprofen (De Lange et al., 2006). The lowest observed effect concentration (LOEC) for fluoxetine was reported as 100 ng L^{-1} and the LOEC for ibuprofen and carbamazepine was measured at even lower concentrations at 10 ng L^{-1}. However, it was noted that the reduced activity observed in *G. pulex* exposed to carbamazepine (≥10 ng L^{-1}) was not significantly different to the control exposure. Carbamazepine was measured at concentrations up to 344 ng L^{-1} in the surface waters from Site 8. Further work is still required to establish reliable LOEC levels for pharmaceuticals both in isolation and when present as a mixture (Arnold et al., 2014; Brooks, 2014). Indeed, with ongoing extensive debate in the literature questioning the validity and reproducibility of experiments revealing effects on bespoke endpoints (Sumpter et al., 2014), it is clearly essential to have the tools available to measure both the exposure concentrations (water) and internal concentrations in order to attribute cause and effect.

With respect to surface waters, six PPCPs were detected at quantifiable levels across all sites at concentrations ranging from 3 to 344 ng L^{-1} (Table 3). Carbamazepine and trimethoprim measured highest at concentrations of 344 ng L^{-1} and 289 ng L^{-1}, respectively. From the eight sites, all of the selected analytes measured at their highest concentration at Site 8, with the exception of propranolol which measured at concentrations >250 ng L^{-1} at Site 5. An effluent outfall pipe lay in close proximity to Site 8, and potentially higher concentrations of pharmaceutical contamination in river water were

Table 2
Pharmaceutical residues (ng g^{-1}) detected in *Gammarus pulex* material sampled from eight sites located on the tributaries of the River Thames, UK. Note: diclofenac was detected at all sites but was not quantifiable based on method performance limitations.

Compounds	Site 1 n = 2	Site 2 n = 3	Site 3 n = 2	Site 4 n = 3	Site 5 n = 3	Site 6 n = 2	Site 7 n = 3	Site 8 n = 2
Carbamazepine	–	<LOQ	–	ND–< LOQ	<LOQ	–	ND– < LOQ	6
Diazepam	ND, 6	ND–8	–	ND–6	–	–	ND–9	–
Temazepam	–	–	–	–	–	–	<LOQ	ND– < LOQ
Trimethoprim	–	–	–	–	–	–	ND– < LOQ	5
Warfarin	–	–	–	–	–	–	ND–7	–
Nimesulide[a]	13, 36	ND– < LOQ	–	ND– < LOQ			ND–16	

ND — not detected, all other compounds marked with '–'were also not detected.
[a] Quantified by three-point standard addition calibration.

Table 3
Pharmaceutical residues (ng L^{-1}) detected in surface water sampled from eight sites located on the tributaries of the River Thames, UK.

Compounds	log*P* (Barron et al., 2009)	Site 1 n = 3	Site 2 n = 3	Site 3 n = 3	Site 4 n = 3	Site 5 n = 3	Site 6 n = 3	Site 7 n = 3	Site 8 n = 3
Carbamazepine	2.45	12–27	10–62	13–17	20–156	6–149	8–9	6–53	320–344
Diazepam	2.86	ND	ND–<LOQ	ND–3	ND–<LOQ	ND–<LOQ	ND–<LOQ	<LOQ	4
Propranolol	3.48	ND–<LOQ	<LOQ–59	8–22	<LOQ–11	ND–253	21–52	5–23	98–119
Temazepam	2.19	ND–<LOQ	<LOQ–2	<LOQ	5–6	ND–<LOQ	ND	ND–LOQ	60–67
Trimethoprim	0.91	<LOQ–8	ND–<LOQ	4–10	<LOQ–48	ND–41	6–16	<LOQ–9	263–289
Warfarin	2.60	ND–<LOQ	ND–53	<LOQ–12	ND–<LOQ	ND–9	11–14	ND–LOQ	15–29

ND — not detected.

measured as a result. However, this did not translate across to elevated concentrations measured in *G. pulex* (highest internal concentrations measured at Site 7). The Beverley Brook tributary was previously classed as having 'poor ecological status' by the Environment Agency in 2012 for failures in surrounding ecology, water chemistry and morphology standards (Ehmann, 2013). River water samples from Site 5 were also found to contain relatively high concentrations of carbamazepine, propranolol and trimethoprim. Besides direct input from WWTPs, other sources of pharmaceutical contamination include sewage from the numerous combined sewer overflows serving this river catchment area which overflow during periods of heavy rainfall and interestingly, have been shown to intermittently discharge sewage into surface waters even without precipitation (Buerge et al., 2006). The Environment Agency (England) has reported poor status for two groundwater bodies which predominantly supply the river flow through Sites 5, 6 and 7 (Gorman, 2013). Although most of the PPCPs were detected at all three of these sites, surface water samples from the River Cray (Sites 6 and 7) were found to contain relatively low residue concentrations overall. Diazepam was the least detected compound in water and measured concentrations were 3 and 4 ng L^{-1} at Sites 3 and 8, respectively.

As expected from this 'snapshot' collection method, there was poor correlation between water and *G. pulex* measured concentrations (compare Tables 2 and 3). Temazepam and propranolol were both measured in water (propranolol in 7 of the 8 sites), but neither were detected within any *G. pulex* despite having higher Log*P* values than trimethoprim which was measured in these organisms. This would further support the hypothesis that for invertebrates, uptake is driven by more than hydrophobicity alone. Intuitively one would predict a constant state of flux in the uptake and depuration of compounds as the flow of water and external exposure changes, and the poor correlation could support that. Therefore, measuring concentrations within the organism is likely to be more relevant (and important) for understanding risk than external water alone.

Several monitoring studies have been carried out in freshwater throughout the UK. A study carried out on the River Taff and the River Ely in Wales consistently detected the presence of carbamazepine at concentrations up to 684 ng L^{-1}. Several recent monitoring studies have detected the presence of propranolol and trimethoprim in surface waters across the UK at concentrations up to 40 ng L^{-1} and 215 ng L^{-1}, respectively (Ashton et al., 2004; Baker and Kasprzyk-Hordern, 2011b; Roberts and Thomas, 2006). The benzodiazepines, temazepam and diazepam have also been reported in river water measuring at concentrations of 53 ng L^{-1} and 1 ng L^{-1}, respectively (Baker and Kasprzyk-Hordern, 2011a). All six selected pharmaceuticals detected here have also been determined in river waters around Europe and Asia at concentrations measuring up to 1 μg L^{-1} in countries such as South Korea, Spain and France (Feitosa-Felizzola and Chiron, 2009; Silva et al., 2011; Yoon et al., 2010).

4. Conclusions

For the first time, the occurrence of six pharmaceuticals was reported from measured internal concentrations in the river shrimp, *G. pulex*, and its surrounding waters. An analytical method involving PuLE, SPE and LC–MS/MS was optimised and applied to surface waters and *G. pulex* samples collected from eight sites along several tributaries of the River Thames. Five pharmaceuticals detected in the freshwater samples were also found to occur in exposed *G. pulex* at concentrations up to 36 ng g^{-1} dry weight, although direct correlation with water concentrations at individual sites was not possible. Carbamazepine and trimethoprim measured highest in river water at concentrations up to 344 ng L^{-1} and 289 ng L^{-1}, respectively. These findings provide new knowledge on the occurrence of pharmaceutical residues in a key aquatic invertebrate, and tools to further investigate their potential effects via internal concentration measurement.

Acknowledgements

This work was conducted with funding from the Biotechnology and Biological Sciences Research Council (BBSRC) CASE industrial scholarship scheme (Reference BB/K501177/1) and AstraZeneca Global SHE research programme. AstraZeneca is a biopharmaceutical company specialising in the discovery, development, manufacturing and marketing of prescription medicines, including some products measured here. Funding bodies played no role in the design of the study or decision to publish. The authors declare no financial conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.12.034.

References

Arnold, K.E., Brown, A.R., Ankley, G.T., Sumpter, J.P., 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Philos. Trans. R. Soc. B Biol. Sci. 369.

Ashauer, R., Hintermeister, A., O'Connor, I., Elumelu, M., Hollender, J., Escher, B.I., 2012. Significance of xenobiotic metabolism for bioaccumulation kinetics of organic chemicals in *Gammarus pulex*. Environ. Sci. Technol. 46, 3498–3508.

Ashton, D., Hilton, M., Thomas, K.V., 2004. Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Sci. Total Environ. 333, 167–184.

Baker, D.R., Kasprzyk-Hordern, B., 2011a. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography–positive electrospray ionisation tandem mass spectrometry. J. Chromatogr. A 1218, 1620–1631.

Baker, D.R., Kasprzyk-Hordern, B., 2011b. Multi-residue determination of the sorption of illicit drugs and pharmaceuticals to wastewater suspended particulate matter using pressurised liquid extraction, solid phase extraction and liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1218, 7901–7913.

Barron, L., Tobin, J., Paull, B., 2008. Multi-residue determination of pharmaceuticals in sludge and sludge enriched soils using pressurized liquid extraction, solid phase extraction and liquid chromatography with tandem mass spectrometry. J. Environ. Monit. 10, 353–361.

Barron, L., Havel, J., Purcell, M., Szpak, M., Kelleher, B., Paull, B., 2009. Predicting sorption of pharmaceuticals and personal care products onto soil and digested sludge using artificial neural networks. Analyst 134, 663–670.

Behera, S.K., Kim, H.W., Oh, J.E., Park, H.S., 2011. Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Sci. Total Environ. 409, 4351–4360.

Brooks, B.W., 2014. Fish on Prozac (and Zoloft): ten years later. Aquat. Toxicol. 151, 61–67.

Brown, K.D., Kulis, J., Thomson, B., Chapman, T.H., Mawhinney, D.B., 2006. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. Sci. Total Environ. 366, 772–783.

Buerge, I.J., Poiger, T., Müller, M.D., Buser, H.-R., 2006. Combined sewer overflows to surface waters detected by the anthropogenic marker caffeine. Environ. Sci. Technol. 40, 4096–4102.
Carmona, E., Andreu, V., Pico, Y., 2014. Occurrence of acidic pharmaceuticals and personal care products in Tuna River Basin: from waste to drinking water. Sci. Total Environ. 484, 53–63.
Commission E, 2012. Commission Proposal on Priority Substances (COM(2011)876). p. 2014.
Corcoran, J., Winter, M.J., Tyler, C.R., 2010. Pharmaceuticals in the aquatic environment: a critical review of the evidence for health effects in fish. Crit. Rev. Toxicol. 40, 287–304.
Cueva-Mestanza, R., Torres-Padrón, M., Sosa-Ferrera, Z., Santana-Rodríguez, J., 2008. Microwave-assisted micellar extraction coupled with solid-phase extraction for preconcentration of pharmaceuticals in molluscs prior to determination by HPLC. Biomed. Chromatogr. 22, 1115–1122.
De Lange, H.J., Noordoven, W., Murk, A.J., Lürling, M., Peeters, E.T.H.M., 2006. Behavioural responses of *Gammarus pulex* (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquat. Toxicol. 78, 209–216.
De Lange, H.J., Peeters, E.T.H.M., Lürling, M., 2009. Changes in ventilation and locomotion of Gammarus pulex (Crustacea, Amphipoda) in response to low concentrations of pharmaceuticals. Human and Ecological Risk Assessment 15 (1), 111–120.
Dobson, P.D., Kell, D.B., 2008. Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? Nat. Rev. Drug Discov. 7, 205–220.
Dodder, N.G., Maruya, K.A., Lee Ferguson, P., Grace, R., Klosterhaus, S., La Guardia, M.J., et al., 2014. Occurrence of contaminants of emerging concern in mussels (Mytilus spp.) along the California coast and the influence of land use, storm water discharge, and treated wastewater effluent. Mar. Pollut. Bull. 81, 340–346.
Dussault, È.B., Balakrishnan, V.K., Solomon, K.R., Sibley, P.K., 2009. Matrix effects on mass spectrometric determinations of four pharmaceuticals and personal care products in water, sediments, and biota. Can. J. Chem. 87, 662–672.
Ehmann, P., 2013. Beverley Brook Information Pack 2014. Environment Agency.
Feitosa-Felizzola, J., Chiron, S., 2009. Occurrence and distribution of selected antibiotics in a small Mediterranean stream (Arc River, Southern France). J. Hydrol. 364, 50–57.
Friberg, N., Andersen, T.H., Hansen, H.O., Iversen, T.M., Jacobsen, D., Krøjgaard, L., et al., 1994. The effect of brown trout (*Salmo Trutta* L.) on stream invertebrate drift, with special reference to *Gammarus pulex* L. Hydrobiologia 294, 105–110.
Gatidou, G., Vassalou, E., Thomaidis, N.S., 2010. Bioconcentration of selected endocrine disrupting compounds in the Mediterranean mussel, *Mytilus galloprovincialis*. Mar. Pollut. Bull. 60, 2111–2116.
Gomez, M.J., Petrovic, M., Fernandez-Alba, A.R., Barcelo, D., 2006. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters. J. Chromatogr. A 1114, 224–233.
Gorman, A., 2013. Water Body Action Plan 2014. Environment Agency.
Hamelink, J.L., Spacie, A., 1977. Fish and chemicals: the process of accumulation. Annu. Rev. Pharmacol. Toxicol. 17, 167–177.
Huerta, B., Rodriguez-Mozaz, S., Barcelo, D., 2012. Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications. Anal. Bioanal. Chem. 404, 2611–2624.
Huerta, B., Jakimska, A., Gros, M., Rodriguez-Mozaz, S., Barcelo, D., 2013. Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1288, 63–72.
Johnson, E.A., An, G.-H., 1991. Astaxanthin from microbial sources. Crit. Rev. Biotechnol. 11, 297–326.
Klosterhaus, S.L., Grace, R., Hamilton, M.C., Yee, D., 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. Environ. Int. 54, 92–99.
Kok, S.J., Debets, A.J.J., 2001. Fast sample preparation for analysis of tablets and capsules: the ball-mill extraction method. J. Pharm. Biomed. Anal. 26, 599–604.
Kosma, C.I., Lambropoulou, D.A., Albanis, T.A., 2010. Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece. J. Hazard. Mater. 179, 804–817.
Kwon, J.-W., Armbrust, K.L., Vidal-Dorsch, D., Bay, S.M., Xia, K., 2009. Determination of 17 alpha-ethynylestradiol, carbamazepine, diazepam, simvastatin, and oxybenzone in fish livers. J. AOAC Int. 92, 359–369.
Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A., Tobin, J.M., 2008. An LC–MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. Talanta 75, 1089–1097.
Lacey, C., Basha, S., Morrissey, A., Tobin, J., 2012. Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. Environ. Monit. Assess. 184, 1049–1062.
Maltby, L., Clayton, S.A., Wood, R.M., McLoughlin, N., 2002. Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness, and relevance. Environ. Toxicol. Chem. 21, 361–368.
McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., 2013. The determination of pharmaceutical residues in cooked and uncooked marine bivalves using pressurised liquid extraction, solid-phase extraction and liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 405, 9509–9521.
McEneff, G., Barron, L., Kelleher, B., Paull, B., Quinn, B., 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. Sci. Total Environ. 476–477, 317–326.
McKim, J., Schmieder, P., Veith, G., 1985. Absorption dynamics of organic chemical transport across trout gills as related to octanol–water partition coefficient. Toxicol. Appl. Pharmacol. 77, 1–10.
Meredith-Williams, M., Carter, L.J., Fussell, R., Raffaelli, D., Ashauer, R., Boxall, A.B.A., 2012. Uptake and depuration of pharmaceuticals in aquatic invertebrates. Environ. Pollut. 165, 250–258.
MHRA, 2014. Medicines and Healthcare Products Regulatory Agency. p. 2014.
Nyman, A.-M., Schirmer, K., Ashauer, R., 2012. Toxicokinetic–toxicodynamic modelling of survival of *Gammarus pulex* in multiple pulse exposures to propiconazole: model assumptions, calibration data requirements and predictive power. Ecotoxicology 21, 1828–1840.
Rand-Weaver, M., Margiotta-Casaluci, L., Patel, A., Panter, G.H., Owen, S.F., Sumpter, J.P., 2013. The read-across hypothesis and environmental risk assessment of pharmaceuticals. Environ. Sci. Technol. 47, 11384–11395.
Roberts, P.H., Thomas, K.V., 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci. Total Environ. 356, 143–153.
Schultz, M.M., Furlong, E.T., Kolpin, D.W., Werner, S.L., Schoenfuss, H.L., Barber, L.B., et al., 2010. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue. Environ. Sci. Technol. 44, 1918–1925.
Silva, B.F.d, Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85, 1331–1339.
Subedi, B., Du, B., Chambliss, C.K., Koschorreck, J., Rudel, H., Quack, M., et al., 2012. Occurrence of pharmaceuticals and personal care products in German fish tissue: a national study. Environ. Sci. Technol. 46, 9047–9054.
Sumpter, J.P., Donnachie, R.L., Johnson, A.C., 2014. The apparently very variable potency of the anti-depressant fluoxetine. Aquat. Toxicol. 151, 57–60.
Tanoue, R., Nomiyama, K., Nakamura, H., Hayashi, T., Kim, J.-W., Isobe, T., et al., 2014. Simultaneous determination of polar pharmaceuticals and personal care products in biological organs and tissues. J. Chromatogr. A 1355, 193–205.
Thomas, K.V., Hilton, M.J., 2004. The occurrence of selected human pharmaceutical compounds in UK estuaries. Mar. Pollut. Bull. 49, 436–444.
Vazquez-Roig, P., Blasco, C., Pico, Y., 2013. Advances in the analysis of legal and illegal drugs in the aquatic environment. Trac-Trends Anal. Chem. 50, 65–77.
Weigel, S., Kallenborn, R., Hühnerfuss, H., 2004. Simultaneous solid-phase extraction of acidic, neutral and basic pharmaceuticals from aqueous samples at ambient (neutral) pH and their determination by gas chromatography–mass spectrometry. J. Chromatogr. A 1023, 183–195.
Yoon, Y., Ryu, J., Oh, J., Choi, B.-G., Snyder, S.A., 2010. Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea). Sci. Total Environ. 408, 636–643.