Environmental Pollution 220 (2017) 1447–1455

Contents lists available at ScienceDirect

Environmental Pollution

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

Widespread, routine occurrence of pharmaceuticals in sewage effluent, combined sewer overflows and receiving waters☆

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ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form
27 October 2016
Accepted 28 October 2016
Available online 6 November 2016

Keywords:
Pharmaceuticals
Water quality
Effluent
Pollution
Emerging contaminants

ABSTRACT

Research addressing the occurrence, fate and effects of pharmaceuticals in the aquatic environment has expanded rapidly over the past two decades, primarily due to the development of improved chemical analysis methods. Significant research gaps still remain, however, including a lack of longer term, repeated monitoring of rivers, determination of temporal and spatial changes in pharmaceutical concentrations, and inputs from sources other than wastewater treatment plants (WWTPs), such as combined sewer overflows (CSOs). In addressing these gaps it was found that the five pharmaceuticals studied were routinely (51–94% of the time) present in effluents and receiving waters at concentrations ranging from single ng to μg L^{-1}. Mean concentrations were in the tens to hundreds ng L^{-1} range and CSOs appear to be a significant source of pharmaceuticals to water courses in addition to WWTPs. Receiving water concentrations varied throughout the day although there were no pronounced peaks at particular times. Similarly, concentrations varied throughout the year although no consistent patterns were observed. No dissipation of the study compounds was found over a 5 km length of river despite no other known inputs to the river. In conclusion, pharmaceuticals are routinely present in semi-rural and urban rivers and require management alongside more traditional pollutants.

1. Introduction

An increasing global population is placing great strain on over 65% of the Earth's rivers with chemical pollution one of the main causes of degradation and biodiversity loss in aquatic ecosystems (Vorosmarty et al., 2010). In chemical pollution research there has been an increasing focus on emerging contaminants over recent decades (Daughton and Ternes, 1999) which enter the aquatic environment following excretion or disposal to the sewer system and passage through wastewater treatment plants (WWTPs) (Kolpin et al., 2002). It is now widely considered that WWTP effluent is the dominant route by which pharmaceuticals enter the aquatic environment (Heberer, 2002; Daughton, 2004; Jones et al., 2005; Tambosi et al., 2010). Although the likelihood of human health impacts due to pharmaceuticals in the environment is low their presence in continually discharged effluent is a major ecological concern due to the potential for effects on aquatic organisms at trace concentrations (Daughton, 2001; Cleuvers, 2003, 2004; Fent et al., 2006; Caliman and Gavrilescu, 2009; Kümmerer, 2009; Santos et al., 2010).

Research into pharmaceutical pollution is expanding largely due to increased concern over potential adverse effects and advancements in the analytical techniques necessary to detect such compounds at trace concentrations (Daughton, 2001; Williams, 2005). Although chemical analysis methods have been improved greatly, there remains a dearth of research which uses these to quantify the occurrence of pharmaceuticals throughout river catchments over periods of time, as has been done for other chemicals such as metals, nutrients (Neal et al., 2012) and pesticides (Bundschuh et al., 2014). Very little work has been conducted in large parts of Africa, Asia, the Middle East and South America and even within those countries with a relatively high level of research the number of studies undertaken remains very small compared to other groups of chemicals (Hughes et al., 2013). Of 155 published pharmaceutical monitoring studies 80% were reported to have been carried out in the US and Europe (Hughes et al., 2013). Furthermore,

☆ This paper has been recommended for acceptance by Dr. Chen Da.

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http://dx.doi.org/10.1016/j.envpol.2016.10.087
0269-7491/

where research has been undertaken, because only a limited number of studies exist, spatial bias exists in pharmaceutical occurrence datasets. For example, UK pharmaceutical pollution monitoring is heavily clustered around south east England and parts of south Wales with very few studies in central, western and northern England or Scotland where large urban areas exist. Where studies are present they often rely on non-repeated sampling and have typically provided very few details on the adopted sampling regime, making it difficult to draw conclusions about the reliability or representativeness of the data presented (Hughes et al., 2013).

In addition to WWTPs, combined sewer overflows (CSOs) and misconnections to storm water drains, which could lead to the discharge of untreated sewage effluent to receiving waters, have been identified as potential sources of pharmaceutical pollution. Despite this, there are very few studies which attempt to examine the contribution they make to overall pharmaceutical loads in rivers (Boyd et al., 2004; Kolpin et al., 2004). This is of concern as it has been hypothesised that such non-WWTP point sources may actually be the key contributor of high pharmaceutical concentrations in reaches far from WWTP effluent outfalls and where dissipation has not been found to occur downstream of WWTPs (Ellis, 2006). Residual low levels of pharmaceuticals have been detected tens or hundreds of kilometres downstream of WWTP outfalls (Waiser et al., 2011), demonstrating the potential for widespread, catchment-level impacts. This presents a pressing research need given the assumption in many risk assessment models of first-order, in-stream decay of pharmaceuticals (Schowanek et al., 2001). Only 16% of monitoring studies included in a recent critical review paper collected samples more than 1 km downstream of WWTP outfalls, indicating a tendency for research to focus on the effluent dominated reaches immediately downstream (Hughes et al., 2013). This is understandable given the likelihood that these areas are most affected by pharmaceutical pollution but this often leaves long reaches of catchments with little or no pharmaceutical monitoring data available.

Research examining temporal variation in pharmaceutical concentrations in receiving waters is also rare, despite some evidence demonstrating high degrees of variation over hourly and daily periods (Kanda et al., 2003) as well as seasons (Lindholm-Lehto et al., 2016; Papageorgiou et al., 2016). Given the tendency towards non-repeated grab sampling of receiving waters it is unlikely that such variation has so far been adequately captured in existing monitoring datasets and they may therefore currently give an inaccurate description of the occurrence of pharmaceuticals in rivers (Ort and Gujer, 2006; Ort et al., 2010a, b).

Given the highlighted research gaps, the aims of the current study were to: carry out repeated sampling of river reaches throughout an eighteen month period for five pharmaceuticals; monitor the chemicals' presence in WWTP and CSO effluent as well as their receiving waters; undertake diurnal monitoring of pharmaceuticals in the receiving waters of WWTPs; and examine dissipation of the study compounds over a 5 km river reach.

2. Methods

2.1. Study area and sampling sites

The Aire and Calder catchments, West Yorkshire, UK, are ideal for studying the occurrence of pharmaceuticals in rivers given the 105 WWTPs that discharge effluent into them (Fig. 1). The catchments are heavily urbanised in the lower reaches with the West Yorkshire Urban Area being one of the ten most populous areas of the UK and being home to around 1.5 million people (Pointer, 2005). There are also a number of smaller towns and villages in the semi-rural and rural upland parts of the catchments. In addition to the WWTPs there are estimated to be 70 CSOs spread across the entire catchment area (Environment Agency, 2010). The total catchment areas of the Aire and Calder above the tidal limit are 1932 and 899 km^2 respectively. Mean annual discharges in the downstream reaches of the catchments are 36 and 19 $m^3\ s^{-1}$ (Carter et al., 2006). Seven WWTPs (supplementary material S1 for treatment techniques and populations served) were monitored monthly for eighteen months and five CSOs were sampled during periods of intensive rainfall which caused them to discharge. More spatially intensive reach monitoring, below one of the WWTPs (Knostrop), was undertaken on seven occasions to look at pharmaceutical dissipation downstream of specific WWTP discharges. This was done over a 5 km length of river; the distance to the next WWTW downstream where more effluent would have entered the river. Diurnal sampling was undertaken on two occasions at Garforth and Oulton WWTPs with samples being collected every 3 h at each.

2.2. Sample collection procedure

Grab samples of effluent (WWTP and CSO) and receiving channel water (0.8 L) for all field surveys were collected in 1 L amber silanised glass bottles with Teflon® lined caps (Fisher Scientific, Leicestershire, UK) and kept chilled in the dark during transit. Samples of WWTP effluent and receiving waters were collected at the start of each month at the same time of day to minimise errors associated with diurnal fluctuations in pharmaceutical concentrations (Kanda et al., 2003). Receiving water samples were collected at a point of five times the stream width downstream of the effluent outfall to allow for mixing (Morris, 2013). Samples were collected from the centre of the stream at 50% depth in-line with established guidelines (USGS, 2006) where possible, or otherwise, due to the size of the channel and bank topography, at the bankside (downstream of Heaton Lodge, Horbury Junction, Oulton/Lemonroyd, and Knostrop WWTP). CSO samples were collected during storm events when the CSOs were discharging to streams and the release of untreated effluent could have an impact on them. All apparatus and glassware used during sample collection and preparation was thoroughly washed with 100% methanol (1 x) and de-ionised water (3 x) prior to each use to remove potential contamination. On return to the laboratory, samples were stored in the dark at 4 °C and extracted within 48 h.

2.3. Study compounds

Five study compounds (Table 1) were chosen based on risk quotients (RQ) (ratio of predicted or maximum environmental concentration to predicted no effect concentration) produced in previously published studies (Jones et al., 2002; Thompson, 2006; Yamamoto et al., 2009). A RQ $\geq$ 1 indicates the potential for impacts on aquatic organisms so this was used as the basis for selection and the number of compounds limited to five to focus on high risk substances.

2.4. Analysis of receiving water and effluent samples

Pharmaceutical standards were used to create working and stock solutions in dilution series for calibration of analytical instruments. All pharmaceuticals were supplied by Sigma-Aldrich Company Ltd. (Dorset, UK) and were of the highest purity available (>99%). Individual stock standard solutions (1000 ng L^{-1}) were prepared on a weight basis in 100% methanol and stored in the dark at −20 °C until used. A fresh working mixture solution of all pharmaceuticals was prepared by appropriate dilution of the individual stocks in methanol-water (20:80, v/v) immediately before

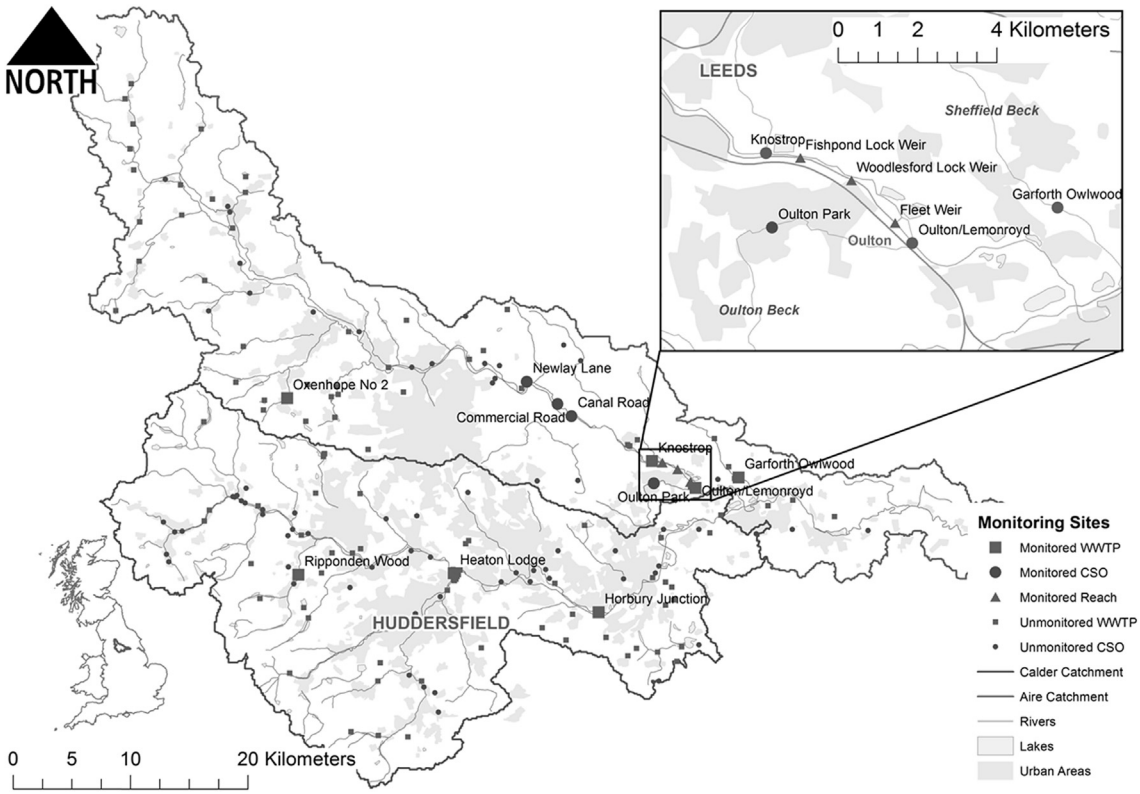
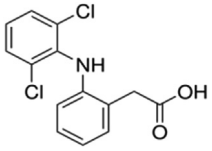
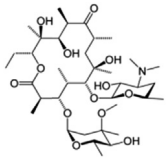
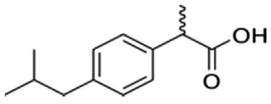
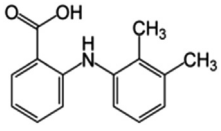
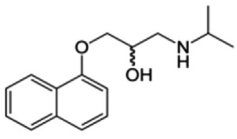


Fig. 1. Map of wastewater treatment plants (WWTPs), combined sewer overflows (CSOs) and river reach sampling sites. (Based on unpublished data supplied by the Environment Agency and Yorkshire Water).

Table 1
Structure and properties of the five study compounds. Risk quotients from Jones et al. (2002), Thompson (2006) and Yamamoto et al. (2009). Physicochemical data from Ternes (1998), Kasprzyk-Hordern et al. (2007), NIH (undated), Knox et al. (2010) and USEPA (2014).

Compound	Therapeutic use	Structure	Physico-chemical properties and risk quotients
Diclofenac	Non-steroidal anti-inflammatory drug (NSAID)		MW = 296.15 Solubility (mg L^{-1}) = 2430 pK_a = 4.0 log K_{OW} = 4.02 RQ_{min} = 0.01 RQ_{max} = 1.13
Erythromycin	Macrolide antibiotic		MW = 733.95 Solubility (mg L^{-1}) = 1.44 pK_a = 8.9 log K_{OW} = 2.48 RQ_{min} = 0.01 RQ_{max} = 1.25
Ibuprofen	NSAID		MW = 206.29 Solubility (mg L^{-1}) = 21 pK_a = 4.91 log K_{OW} = 3.79 RQ_{min} = 0.55 RQ_{max} = 4.20
Mefenamic acid	NSAID		MW = 241.29 Solubility (mg L^{-1}) = 20 pK_a = 4.2 log K_{OW} = 5.12 RQ_{min} = 1.03 RQ_{max} = 8.31
Propranolol	Non-selective beta-blocker		MW = 259.35 Solubility (mg L^{-1}) = 31 pK_a = 9.4 log K_{OW} = 2.60 RQ_{min} = 0.49 RQ_{max} = 4.25

each analytical run and used as working standard solutions. Working standards were also stored in the dark at −20 °C between uses. HPLC-grade methanol was supplied by Fisher Scientific UK Limited (Loughborough, UK) and deionised water by a Purite Select HP160/BP/IT deioniser. The SPE procedure and subsequent analysis by HPLC and Q-TOF MS/MS followed an established method (Petrovic et al., 2006). 800 mL of unfiltered sample was measured for SPE using a 20-position Waters vacuum extraction manifold in conjunction with Oasis HLB SPE cartridges (6 cc, 150 mg; Waters Corporation, Milford, MA, USA). First the SPE cartridges were conditioned with 5 mL 100% methanol followed by 5 mL de-ionised water at a flow rate of 1 mL min^{-1}. The 800 mL river and effluent samples were then loaded onto the SPE cartridge at a flow rate of 10 mL min^{-1} during which care was taken not to let the sorbent material dry out. The cartridges were then rinsed with 5 mL de-ionised water at a flow rate of 1 mL min^{-1} prior to being thoroughly air dried under vacuum for 15 min to remove excess water. Elution of the cartridges was performed with 2 × 4 mL of 100% methanol at a flow rate of 1 mL min^{-1} directly into glass centrifuge tubes. The eluent was evaporated to dryness under vacuum centrifugation and reconstituted in 0.5 mL of methanol: water (20:80, v/v). Sample extracts were stored in the dark at −20 °C prior to analysis which was performed using an Ultimate 3000 nanoLC system (Dionex) and 2.1 × 100 mm Acclaim RSLC 120 C18 2.2 μm particle silica column. In positive ion (PI) mode a solvent system of 5 mM ammonium acetate/acetic acid (pH 2.4; buffer A) and methanol-acetonitrile (2:1, v/v, buffer B) was used at a flow rate of 0.3 mL min^{-1} and the column held at a constant temperature (30 °C). In negative ion (NI) mode a solvent system of 2 mM ammonium acetate (buffer A) and 2 mM ammonium acetate in methanol-water (95:5, v/v, buffer B) was used at a flow rate of 0.3 mL min^{-1} and the column held at 30 °C. After injection, the gradient was held at 5% B for 5 min followed by 5–80% B over 20 min. A 15 min column wash at 95% B and an equilibration of 5 min at 5% B was performed between each sample injection. The LC eluent was directly infused into the Z-spray electrospray source of a quadrupole-ion mobility-orthogonal time of flight (Q-TOF) mass spectrometer (Synapt HDMS, Waters UK). Electrospray desolvation temperature was 150 °C and desolvation gas was 500 L h^{-1}, capillary and cone voltages were 3.2 kV and 30 V respectively. Backing pressure was 2.1 bar, the argon pressure in the trap and transfer region of the IMS cell was 2.17 mbar. MS/MS analysis was performed only on the target pharmaceutical compounds when their intensity was greater than 10 counts per second and their retention time was within ±1 min of that of the corresponding standards. Trap collision energy used for each compound was optimised individually between 15 and 30 V. The lockspray frequency was set to one 1 s scan every 10 s. For PI mode a 5 μM solution of Glu fibrinopeptide in methanol/0.1% formic acid (50:50, v/v) was used as the lock mass, in NI mode the *m/z* 276 peak of a 2 ng μL^{-1} of sodium iodide in methanol water (50:50, v/v) was used as the lock mass.

All data analysis was performed in MassLynx™ (v4.1, Waters Ltd.). Positive identification of the target pharmaceuticals was based on accurate mass measurement of the parent ion within an error of ±20 ppm, accurate mass of at least one product ion, also within an error of ±20 ppm, and LC retention time of the target analyte with reference to that of a standard (±5%). Quantitation was performed by constructing extracted ion chromatograms (XICs) of the protonated (PI mode) and deprotonated (NI mode) ion using a 20 mDa window centred on the theoretical *m/z* of the compound and taking the area of the resulting peak using MassLynx's inbuilt integration algorithms.

Reproducibility of the method was tested with repeated injections of a standard solution both sequentially and day-to-day. Instrumental detection limits (IDLs) were estimated using a standard solution in dilution series until reaching a concentration yielding a signal: noise (S: N) ratio of three. Linear dynamic ranges were determined for each compound by injecting a dilution series of the working standard mixture solutions across a wide concentration range (2–1000 ng L^{-1}). Calibration curves of peak area (from XICs) vs. Concentration were created using linear regression analysis and the linear range that gave good fit ($r^2 > 0.95$) was established for each compound.

SPE recovery and signal suppression due to matrix effects were evaluated by spiking known concentrations (20, 100 and 200 ng L^{-1}) of all of the study compounds into 3 replicates each of deionised water and river water. As matrix effects using this method have already been characterised well for the study compounds (Petrovic et al., 2006), and our results were in good agreement with these for deionised and river water, further matrix effects tests in WWTP and CSO effluent were not repeated. Based on this previous work it was assumed that signal suppression in WWTP effluent would be between 0 and 10% for all of the compounds other than erythromycin (25%). In CSO effluent (based on WWTW influent data in Petrovic et al. (2006)) this would be between 10 and 20% and 40% for erythromycin. The data presented here for WWTP and CSO effluent are, thus, likely to be slight underestimates of actual concentrations. Method performance data are provided in the supplementary material (S2).

3. Results

A total of 320 samples were collected, comprising 121 WWTP effluent, 185 receiving water and 14 CSO samples. The HPLC-MS/MS method resulted in a quantitation limit of 5 ng L^{-1} for all study compounds except ibuprofen (25 ng L^{-1}).

3.1. Monthly sampling

A total of 121 WWTP effluent (Table 2) and 125 receiving water (Table 3) samples were collected during the monthly sampling campaign. For diclofenac, erythromycin and ibuprofen, concentrations were usually in the order of hundreds of ng L^{-1} although some an order of magnitude higher (μg L^{-1}) were measured and ibuprofen was often present at the highest concentration of the three compounds. In contrast, mefenamic acid and propranolol were detected at single and tens of ng L^{-1}. Detection frequencies ranged between 51 and 94%. Although only 14 CSO samples were collected, all five pharmaceutical compounds were present and detection frequencies and concentrations were in the same range as for WWTP effluent and receiving waters (Table 4). The only exception was ibuprofen for which mean and maximum CSO concentrations were an order of magnitude higher than in WWTP effluent and receiving waters. Flow conditions had little impact on detection frequencies although significant differences did exist in concentrations across flow categories with receiving water concentrations measured in high flows being lower (supplementary material S3). Nevertheless, concentrations were higher in medium flows than low flows and so it may be that relatively high concentrations are transient and higher mean concentrations were detected in flow conditions that were sampled more frequently, medium flows being most prevalent and high flows the least.

3.2. Diurnal sampling

Concentrations of all five pharmaceutical compounds sampled in the receiving waters of Garforth and Oulton WWTPs during diurnal sampling varied by up to a factor of four within the same day although changes were usually much smaller than this and no

Table 2
Pharmaceutical concentrations (ng L^{-1}) measured in the effluent of seven wastewater treatment plants (WWTPs) in the River Aire and Calder catchments, UK, during an eighteen month monitoring campaign. Max = maximum, SD = standard deviation, nd = not detected, na = not applicable.

WWTP	n	Diclofenac			Erythromycin			Ibuprofen			Mefenamic acid			Propranolol		
		Max	Mean	SD	Max	Mean	SD	Max	Mean	SD	Max	Mean	SD	Max	Mean	SD
Garforth	17	2830	590	887	1757	394	476	3593	780	829	30	11	8	1464	37	39
Heaton Lodge	17	1302	157	318	586	249	279	949	320	285	91	32	326	20	11	66
Horbury Junction	17	601	173	197	793	200	251	3645	1147	864	15	5	5	35	13	10
Knostrop	17	806	273	266	466	185	302	863	302	223	35	8	9	41	16	13
Oulton	18	994	231	238	1106	249	288	4617	794	1035	33	7	8	36	14	12
Oxenhope	17	401	61	94	1857	399	480	1068	363	319	nd	nd	na	35	15	11
Ripponden	18	411	111	148	782	240	258	2364	524	586	108	1078	na	1	10	5
All sites		2830	228	415	1857	274	348	4617	604	719	108	28	208	146	16	18
Detection frequency (%)		91			78			51			91			94		

Five effluent samples could not be collected due to some of the sites being inaccessible due to flooding on one sampling occasion, therefore 121 samples were collected rather than 126.

Table 3
Pharmaceutical concentrations (ng L^{-1}) measured in the receiving waters of seven wastewater treatment plants (WWTPs) in the River Aire and Calder catchments, UK, during an eighteen month monitoring campaign. Max = maximum, SD = standard deviation, na = not applicable.

WWTP	n	Diclofenac			Erythromycin			Ibuprofen			Mefenamic acid			Propranolol		
		Max	Mean	SD	Max	Mean	SD	Max	Mean	SD	Max	Mean	SD	Max	Mean	SD
Garforth	18	2991	649	958	1378	403	307	2960	812	849	41	11	11	44	19	12
Heaton Lodge	18	284	88	87	413	143	127	1409	319	367	53	22	20	22	5	6
Horbury Junction	18	456	103	122	318	121	108	2172	415	569	97	24	29	21.8 7	4	2
Knostrop	18	937	323	307	845	230	254	4838	720	1091	39	12	11	165	30	38
Oulton	18	592	187	170	451	138	132	2770	457	639	23	11	8	25	8	17
Oxenhope	18	124	39	38	132	49	40	205	100	57	9	9	na	6	3	1
Ripponden	17	76	25	18	174	34	43	358	124	97	15	6	6	6	3	1
All sites		2991	202	438	1378	160	217	4838	421	680	97	11	16	165	10	20
Detection frequency (%)		93			94			93			66			70		

One receiving water sample could not be collected due to the site being inaccessible at the time, therefore 125 samples were collected rather than 126.

Table 4
Pharmaceutical concentrations (ng L^{-1}) measured in combined sewer overflow (CSO) effluent in the River Aire and Calder catchments, UK. nd = not detected.

CSO	n	Diclofenac		Erythromycin		Ibuprofen		Mefenamic acid		Propranolol	
		Max	Mean	Max	Mean	Max	Mean	Max	Mean	Max	Mean
Newlay Lane	1	161	161	1603	1603	76	76	nd	nd	nd	nd
Canal Road	2	274	187	nd	nd	278	143	35	19	nd	nd
Commercial Road	4	175	74	727	256	10,060	2734	19	5	18	10
Oulton Beck	7	1805	388	243	98	14,231	2207	nd	nd	29	11
All sites		1805	203	1603	489	14,231	1290	35	6	29	5
Detection frequency (%)		86		64		100		21		36	

obvious patterns were evident across the 24 h period (Fig. 2).

3.3. River Aire reach monitoring

Concentrations of pharmaceuticals during monitoring of a 5 km length of the River Aire were of the order observed in the monthly monitoring. The data indicate no appreciable reduction in pharmaceutical concentrations along the reach (Fig. 3). The apparent downstream increase in concentrations in some cases should be treated with caution and is likely to be caused by higher effluent concentrations at the upstream sites leading to signal suppression during LC-MS analysis. There was considerable variation in the pharmaceutical concentrations measured at the same sites on different dates although the lack of dissipation was consistent. The lack of any consistent patterns across space and time as well as the absence of a significant relationship with flow conditions across diurnal sampling events (see supplementary material S4) make this difficult to explain and it is likely that a range of factors contributed to this variation which are discussed in Section 4.4.

4. Discussion

The dataset presented here contributes to the knowledge base of pharmaceutical occurrence in sewage effluents and rivers and details how frequently they are present, the general concentrations at which a range of compounds occur, changes over time, and dissipation in rivers downstream of effluent sources.

4.1. Frequency of detection

The routine detection of the five study pharmaceuticals demonstrates their widespread presence in river systems and pseudo-persistence due to the continuous release of sewage effluent. Pharmaceuticals should therefore be considered as ubiquitous pollutants in any freshwater ecosystem receiving sewage effluent. This finding is supported by similarly high detection frequencies where pharmaceuticals have been monitored in other studies (Hughes et al., 2013). Other studies have also reported high detection frequencies for the compounds monitored in the current

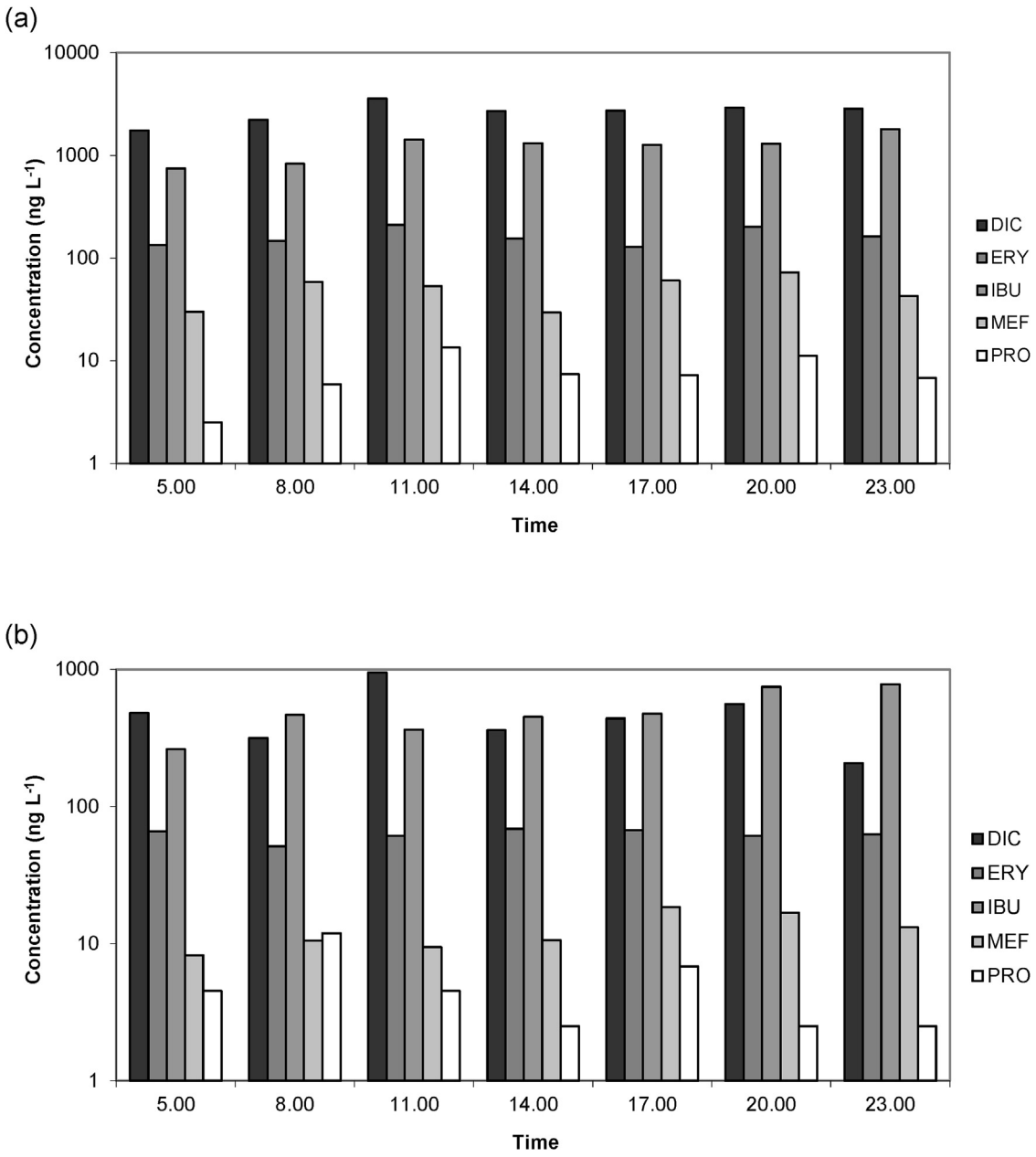


Fig. 2. Mean (of two sampling occasions) pharmaceutical concentrations measured in the receiving waters of Garforth (a) and Oulton (b) wastewater treatments plants, UK, during diurnal sampling events.

study. These ranged from 5 to 100% for diclofenac (Zhou et al., 2009), 3–100% for erythromycin (Kolpin et al., 2004; Kasprzyk-Hordern et al., 2009), 1–100% for ibuprofen (Bound and Voulvoulis, 2006; Focazio et al., 2008), 14–88% for mefenamic acid (Kasprzyk-Hordern et al., 2009; López-Roldán et al., 2010) and 37–100% for propranolol (Ashton et al., 2004). Whilst diclofenac was detected at a similar frequency in sewage effluent and river water, erythromycin and ibuprofen were measured less frequently in effluent than receiving waters at our sites, potentially due to matrix effects or release from unmonitored effluent sources upstream (see Fig. 1) These include some of the largest WWTPs in Yorkshire, such as Esholt (River Aire) and Deighton (River Calder) which serve population equivalents of 760,000 and 680,000 respectively (Yorkshire Water, pers. comm.). Conversely, mefenamic acid and propranolol were detected less in receiving waters than effluent, potentially indicating more rapid dissipation in the environment than the other study compounds.

4.2. Pharmaceutical concentrations in effluent and receiving waters

More comprehensive monitoring than that undertaken in previous studies in the UK has identified concentrations of the study compounds in treated effluent and receiving waters of the same order of magnitude as those previously reported (Petrie et al., 2015). This provides greater certainty that the substances monitored are present in sewage effluent and receiving waters in the range of single nanograms to micrograms per litre. Mean receiving water concentrations were also very similar to global means reported by Hughes et al. (2013) although peak concentrations are lower than global maxima, due predominantly to the higher

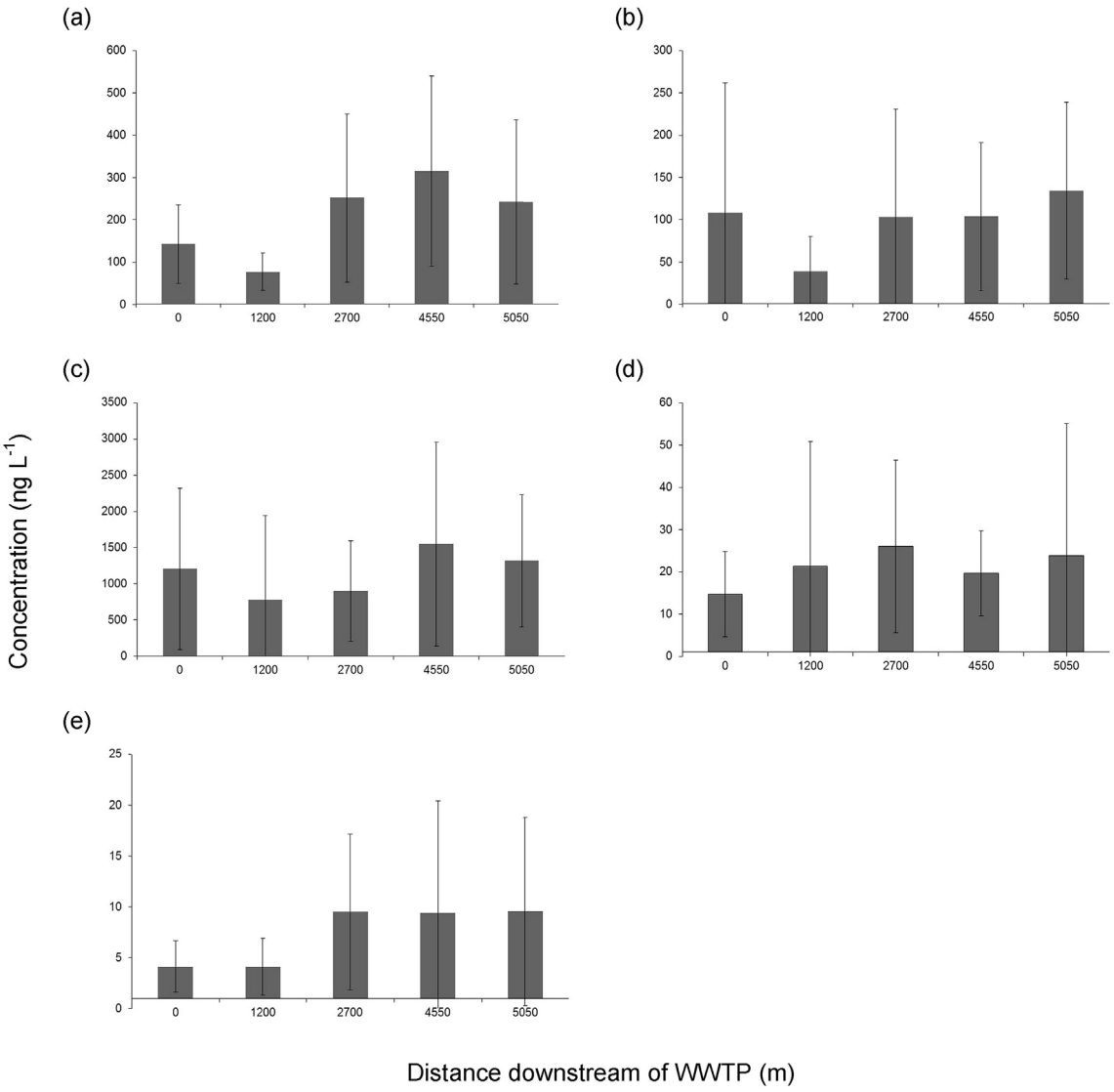


Fig. 3. Concentration profiles for pharmaceuticals over a 5 km length of the River Aire, UK, downstream of Knostrop wastewater treatment plant (WWTP). Values are means of seven replicates and error bars represent ±1 standard deviation. a = diclofenac, b = erythromycin, c = ibuprofen, d = mefenamic acid, e = propranolol.

concentrations detected in Chinese rivers. Logically, mean sewage effluent concentrations were greater than those in receiving waters, presumably due to some dilution and removal by biotic and abiotic processes. Nevertheless, peak concentrations for three of the five study compounds were actually higher in the receiving water than they were in effluent. This could be due to signal suppression in effluent samples or pharmaceuticals being discharged from upstream WWTPs and unknown sources such as septic tanks and foul water misconnections to surface water drains. Overall peak and mean concentrations were of the same order of magnitude in effluent and receiving waters. This indicates that significant dilution does not occur in river systems which receive large volumes of effluent, such as the Aire and Calder (Jürgens et al., 2002), where extensive lengths of river carry concentrations of pharmaceuticals and do not have the capacity to dilute downstream inputs.

Although the CSO dataset is small (n = 14), frequent detection indicates that raw sewage from sewer overflows is a significant source of pharmaceuticals to rivers. Moreover, given that concentrations in this raw effluent, which logically will be the same as WWTP influent, were of the same order as in WWTP effluent it can be hypothesised that the study compounds are not removed to any significant extent by WWTPs used in this study. Existing research shows that pharmaceutical elimination rates in WWTPs can vary greatly depending on a range of factors including concentrations in raw sewage inputs and conditions in particular WWTPs (e.g. Heberer, 2002; Carballa et al., 2004; Jones et al., 2007; Vieno and Sillanpää, 2014).

4.3. Temporal variations in pharmaceutical pollution

Very little work has been undertaken on the temporal variation of pharmaceutical concentrations in rivers (Papageorgiou et al., 2016) although Kanda et al. (2003) found ibuprofen concentrations to peak in the middle of the day. This contrasts with the current work where no obvious pattern was found. Moreover, no consistent temporal pattern was found during the monthly sampling of WWTP effluents and receiving waters (supplementary material S5). Winter peaks may occur due to increased pharmaceutical consumption, higher treatment efficiencies in WWTPs, and reduced degradation in surface waters (MacLeod and Wong, 2010; Sui et al., 2011; Valcárcel et al., 2013; Lindholm-Lehto et al., 2016). Conversely, some compounds have occurred in

higher concentrations during the summer months, possibly due to greater usage at this time of year (Lindholm-Lehto et al., 2016; Papageorgiou et al., 2016).

4.4. Downstream profiles of pharmaceutical pollution

The consistent presence of the study compounds along a 5 km reach of river suggests that in-stream dissipation along reaches of several kilometres is negligible for the study compounds and that exposure of freshwater ecosystems to pharmaceutical pollution will occur well beyond the locality of WWTP discharges. The in-stream fate of pharmaceuticals is poorly understood, with a limited number of laboratory studies having been undertaken and even fewer in the field (Radke et al., 2010). In agreement with our results, Radke et al. (2010) measured just 5–15% dissipation of pharmaceuticals along a 14 km length of German river. In contrast, however, Gross et al. (2004) demonstrated significant removal (up to 97%) of ibuprofen along a 11 km stretch of a shallow Californian river due to reduced flow, biodegradation and microbial transformation (Lin et al., 2006) and greater light penetration in the shallow river (Moss, 1988). Similarly, Fono et al. (2006) measured high in-stream removal of ibuprofen along a >100 km reach of a Texan river. The results presented here and elsewhere highlight the need for caution when making assumptions about in-stream pharmaceutical removal with rates likely to depend on site specific factors, individual compound properties, interaction with sediments, latitude, season, weather, flow, turbidity, hyporheic exchange, microbial communities and dissolved organic carbon concentrations (Kunkel and Radke, 2011). Nevertheless, the compounds studied here had a range of physicochemical properties but showed no appreciable difference in downstream fate. This is not necessarily surprising given that, despite the fact that degradation half-lives may vary between days and weeks (Kunkel and Radke, 2012), even the shortest of these will allow significant transport in a river. Moreover, suitable conditions (e.g. high suspended solids concentration, contact with bed sediments) for sorption may not exist even for hydrophobic compounds.

5. Conclusions

Despite much research on pharmaceuticals in the environment during the last two decades there remain significant knowledge gaps, including a lack of repeat sampling, quantification of spatial and temporal patterns of pollution (e.g. in-stream dissipation, seasonal and diurnal variation), and little monitoring of some potential sources (e.g. CSOs). The current study found that the five pharmaceuticals monitored are ubiquitous in rivers (present in 51–94% of samples) throughout urban and even semi-rural rivers. Our results support the findings of other papers that pharmaceuticals are routinely present in UK rivers at single ng to μg L^{-1} concentrations. Mean concentrations were typically in the range of tens to hundreds of ng L^{-1}. Although there is little legislation regulating the presence of pharmaceuticals in rivers, the mean measured concentration for diclofenac exceeded the proposed EQS limit of 100 ng L^{-1} by a factor of two. The scale of the pharmaceutical pollution problem is highlighted by the fact that only 6% of samples monitored for pesticides in the UK exceed the same threshold (Environment Agency, 2009). Pharmaceuticals must, therefore, be seen as important environmental pollutants which should be added to and regulated under existing policies (e.g. European Commission, 2011). Concentrations varied over time although no consistent patterns were found. Future monitoring strategies should move towards robust sampling programmes similar to those employed when monitoring more traditional pollutants (Ort et al., 2010a). CSOs may be as relevant as WWTPs as sources of drugs in rivers when they are discharging and must be studied more and potentially managed better to limit the release of untreated sewage to rivers. Of great importance is the observation that pharmaceutical concentrations did not decline over a river length of 5 km even though there were no known sources of pharmaceuticals below the WWTP. Future research efforts must be made to understand why this phenomenon has been observed in this study and others. This understanding then needs to be incorporated into environmental risk assessment approaches.

Acknowledgements

Stephen Hughes was in receipt of a Natural Environment Research Council PhD studentship (NE/G523771/1).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.10.087.

References

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 (1–3), 167–184.
Bound, J.P., Voulvoulis, N., 2006. Predicted and measured concentrations for selected pharmaceuticals in UK rivers: implications for risk assessment. Water Res. 40 (15), 2885–2892.
Boyd, G.R., Palmeri, J.M., Zhang, S., Grimm, D.A., 2004. Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA. Sci. Total Environ. 333 (1–3), 137–148.
Bundschuh, M., Goedkoop, W., Kreuger, J., 2014. Evaluation of pesticide monitoring strategies in agricultural streams based on the toxic-unit concept – experiences from long-term measurements. Sci. Total Environ. 484, 84–91.
Caliman, F.A., Gavrilescu, M., 2009. Pharmaceuticals, personal care products and endocrine disrupting agents in the environment – a review. Clean. – Soil, Air, Water 37 (4–5), 277–303.
Carballa, M., Omil, F., Lema, J.M., Llompart, M., García-Jares, C., Rodríguez, I., Gomez, M., Ternes, T., 2004. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res. 38 (12), 2918–2926.
Carter, J., Walling, D.E., Owens, P.N., Leeks, G.J.L., 2006. Spatial and temporal variability in the concentration and speciation of metals in suspended sediment transported by the River Aire, Yorkshire, UK. Hydrol. Process. 20, 3007–3027.
Cleuvers, M., 2003. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol. Lett. 142 (3), 185–194.
Cleuvers, M., 2004. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicol. Environ. Saf. 59 (3), 309–315.
Daughton, C.G., 2001. Pharmaceuticals and personal care products in the environment: overarching issues and overview. In: Daughton, C.G., Jones-Lepp, T. (Eds.), Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues: American Chemical Society (ACS) Symposium Series 791. ACS Publications Division, Washington D. C., pp. 2–38
Daughton, C.G., 2004. PPCPs in the environment: future research - beginning with the end always in mind. In: Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks. Springer, K. Kümmerer, Berlin, pp. 463–495.
Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107, 907–938.
Environment Agency, 2009. Prioritisation of Pharmaceuticals of Potential Environmental Concern (EA R&D Technical Report).
Environment Agency, 2010. Notice of Determination of Applications for Consent for Discharges of Storm Sewage and Sewage in an Emergency. S. R. Hughes.
European Commission, 2011. Diclofenac EQS Dossier. Report of the Common Implementation Strategy for the Water Framework Directive.
Ellis, J.B., 2006. Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environ. Pollut. 144 (1), 184–189.
Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquat. Toxicol. 76 (2), 122–159.
Focazio, M.J., Kolpin, D.W., Barnes, K.K., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Barber, L.B., Thurman, M.E., 2008. A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States — II) Untreated drinking water sources. Sci. Total Environ. 402 (2–3), 201–216.
Fono, L.J., Kolodziej, E.P., Sedlak, D.L., 2006. Attenuation of wastewater-derived contaminants in an effluent-dominated river. Environ. Sci. Technol. 40 (23), 7257–7262.
Gross, B., Montgomery-Brown, J., Naumann, A., Reinhard, M., 2004. Occurrence and

fate of pharmaceuticals and alkylphenol ethoxylate metabolites in an effluent-dominated river and wetland. Environ. Toxicol. Chem. 23 (9), 2074–2083.
Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol. Lett. 131 (1–2), 5–17.
Hughes, S.R., Kay, P., Brown, L.E., 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. 47 (2), 661–677.
Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2005. Human pharmaceuticals in wastewater treatment processes. Crit. Rev. Environ. Sci. Technol. 35 (4), 401–427.
Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2002. Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. Water Res. 36 (20), 5013–5022.
Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2007. The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilising activated sludge treatment. Environ. Pollut. 145 (3), 738–744.
Jürgens, M.D., Holthaus, K.I.E., Johnson, A.C., Smith, J.J.L., Hetheridge, M., Williams, R.J., 2002. The potential for estradiol and ethinylestradiol degradation in English rivers. Environ. Toxicol. Chem. 21 (3), 480–488.
Kanda, R., Griffin, P., James, H.A., Fothergill, J., 2003. Pharmaceuticals and personal care products in sewage treatment works. J. Environ. Monit. 5 (5), 823–830.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2007. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography-positive electrospray ionisation tandem mass spectrometry. J. Chromatogr. A 1161 (1–2), 132–145.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43 (2), 363–380.
Knox, C., Law, V., Jewison, T., Liu, P., Ly, S., Frolkis, A., Pon, A., Banco, K., Mak, C., Neveu, V., Djoumbou, Y., 2010. DrugBank 3.0: a comprehensive resource for 'Omics' research on drugs. Nucleic Acids Res. 39 (Suppl. 1), D1035–D1041.
Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. Streams, 1999-2000: a national reconnaissance. Environ. Sci. Technol. 36 (6), 1202–1211.
Kolpin, D.W., Skopec, M., Meyer, M.T., Furlong, E.T., Zaugg, S.D., 2004. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Sci. Total Environ. 328 (1–3), 119–130.
Kümmerer, K., 2009. The presence of pharmaceuticals in the environment due to human use - present knowledge and future challenges. J. Environ. Manag. 90 (8), 2354–2366.
Kunkel, U., Radke, M., 2011. Reactive tracer test to evaluate the fate of pharmaceuticals in rivers. Environ. Sci. Technol. 45 (15), 6296–6302.
Kunkel, U., Radke, M., 2012. Fate of pharmaceuticals in rivers: deriving a benchmark dataset at favourable attenuation conditions. Water Res 46 (17), 5551–5565.
Lin, A.Y.C., Plumlee, M.H., Reinhard, M., 2006. Natural attenuation of pharmaceuticals and alkylphenol polyethoxylate metabolites during river transport: photochemical and biological transformation. Environ. Toxicol. Chem. 25 (6), 1458–1464.
Lindholm-Lehto, P.C., Ahkola, H.S., Knuutinen, J.S., Herve, S.H., 2016. Widespread occurrence and seasonal variation of pharmaceuticals in surface waters and municipal wastewater treatment plants in central Finland. Environ. Sci. Pollut. Res. 23 (8), 7985–7997.
López-Roldán, R., López de Alda, M., Gros, M., Petrovic, M., Martín-Alonso, J., Barceló, D., 2010. Advanced monitoring of pharmaceuticals and estrogens in the Llobregat River basin (Spain) by liquid chromatography–triple quadrupole-tandem mass spectrometry in combination with ultra performance liquid chromatography–time of flight-mass spectrometry. Chemosphere 80 (11), 1337–1344.
MacLeod, S.L., Wong, C.S., 2010. Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments. Water Res. 44 (2), 533–544.
Morris, C., 2013. Mixing Zones 101. Office of Science and Technology, Washington DC.
Moss, B., 1988. Ecology of Fresh Waters: Man and Medium. Blackwell Scientific Publications, Oxford.
Neal, C., Bowes, M., Jarvie, H.P., Scholefield, P., Leeks, G., Neal, M., Rowland, P., Wickham, H., Harman, S., Armstrong, L., Sleep, D., Lawlor, A., Davies, C.E., 2012. Lowland river water quality: a new UK data resource for process and environmental management analysis. Hydrol. Process. 26 (6), 949–960.
NIH, (undated). ChemIDplus database. Available at: http://chem.sis.nlm.nih.gov/chemidplus/. Last accessed 20/01/15.
Ort, C., Gujer, W., 2006. Sampling for representative micropollutant loads in sewer systems. Water Sci. Technol. 54 (6), 169–176.
Ort, C., Lawrence, M.G., Reungoat, J., Mueller, J.F., 2010b. Sampling for PPCPs in wastewater systems: comparison of different sampling modes and optimization strategies. Environ. Sci. Technol. 44 (16), 6289–6296.
Ort, C., Lawrence, M.G., Rieckermann, J.R., Joss, A., 2010a. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions Valid? A critical review. Environ. Sci. Technol. 44 (16), 6024–6035.
Papageorgiou, M., Kosma, C., Lambropoulou, D., 2016. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. Sci. Total Environ. 543, 547–569.
Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. Water Res. 72, 3–27.
Petrovic, M., Gros, M., Barcelo, D., 2006. Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. J. Chromatogr. A 1124 (1–2), 68–81.
Pointer, G., 2005. Focus on People and Migration - chapter 3: the UK's major urban areas. Office Natl. Statistics 45–60.
Radke, M., Ulrich, H., Wurm, C., Kunkel, U., 2010. Dynamics and attenuation of acidic pharmaceuticals along a river stretch. Environ. Sci. Technol. 44 (8), 2968–2974.
Santos, L.H.M.L.M., Araújo, A.N., Fachini, A., Pena, A., Delerue-Matos, C., Montenegro, M.C.B.S.M., 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard. Mater. 175 (1–3), 45–95.
Schowanek, D., Fox, K., Holt, M., Schroeder, F.R., Koch, V., Cassani, G., Matthies, M., Boeije, G., Vanrolleghem, P., Young, A., Morris, G., 2001. GREAT-ER: a new tool for management and risk assessment of chemicals in river basins Contribution to GREAT-ER No. 10. Water Sci. Technol. 43 (2), 179–185.
Sui, Q., Huang, J., Deng, S., Chen, W., Yu, G., 2011. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ. Sci. Technol. 45 (8), 3341–3348.
Tambosi, J.L., Yamanaka, L.Y., José, H.J., Moreira, R.F.P.M., Schröder, H.F., 2010. Recent research data on the removal of pharmaceuticals from sewage treatment plants (STP). Quimica Nova 33, 411–420.
Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32 (11), 3245–3260.
Thompson, A., 2006. The Fate and Removal of Pharmaceuticals during Sewage Treatment. PhD thesis. Cranfield University.
USEPA, 2014. ECOSAR. Available at. http://www.epa.gov/oppt/newchems/tools/21ecosar.htm. Last accessed: 20/01/15.
USGS, 2006. Collection of Water Samples (Ver. 2.0): U.S. Geological Survey Techniques of Water-resources Investigations. Book 9, Chapter A4.
Valcárcel, Y., Alonso, S.G., Rodríguez-Gil, J.L., Castaño, A., Montero, J.C., Criado-Alvarez, J.J., Mirón, I.J., Catalá, M., 2013. Seasonal variation of pharmaceutically active compounds in surface (Tagus River) and tap water (Central Spain). Environ. Sci. Pollut. Res. 20 (3), 1396–1412.
Vieno, N., Sillanpää, M., 2014. Fate of diclofenac in a municipal wastewater treatment plant – a review. Environ. Int. 69, 28–39.
Vorosmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. Nature 467 (7315), 555–561.
Waiser, M.J., Humphries, D., Tumber, V., Holm, J., 2011. Effluent-dominated streams. Part 2: presence and possible effects of pharmaceuticals and personal care products in Wascana Creek, Saskatchewan, Canada. Environ. Toxicol. Chem. 30 (2), 508–519.
Williams, R.T., 2005. In: Williams, R.T. (Ed.), Human Health and Pharmaceuticals in the Environment - an Introduction, in Human Pharmaceuticals: Assessing the Impacts on Aquatic Ecosystems. SETAC Press, Pensacola, FL., pp. 1–46
Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y., Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., Sekizawa, J., 2009. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: laboratory photolysis, biodegradation, and sorption experiments. Water Res. 43 (2), 351–362.
Zhou, J.L., Zhang, Z.L., Banks, E., Grover, D., Jiang, J.Q., 2009. Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. J. Hazard. Mater. 166 (2–3), 655–661.