

# UNIVERSITY *of* York

This is a repository copy of *Temporal and spatial variation in pharmaceutical concentrations in an urban river system*.

White Rose Research Online URL for this paper:  
<https://eprints.whiterose.ac.uk/128156/>

Version: Accepted Version

---

## Article:

Burns, Emily E. [orcid.org/0000-0003-4236-6409](https://orcid.org/0000-0003-4236-6409), Carter, Laura J., Kolpin, Dana W et al. (2 more authors) (2018) Temporal and spatial variation in pharmaceutical concentrations in an urban river system. *Water research*. pp. 72-85. ISSN 0043-1354

<https://doi.org/10.1016/j.watres.2018.02.066>

---

## Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

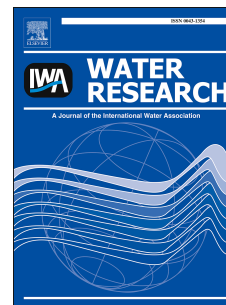
## Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

# Accepted Manuscript

Temporal and spatial variation in pharmaceutical concentrations in an urban river system

Emily E. Burns, Laura J. Carter, Dana W. Kolpin, Jane Thomas-Oates, Alistair B.A. Boxall



PII: S0043-1354(18)30179-9

DOI: [10.1016/j.watres.2018.02.066](https://doi.org/10.1016/j.watres.2018.02.066)

Reference: WR 13618

To appear in: *Water Research*

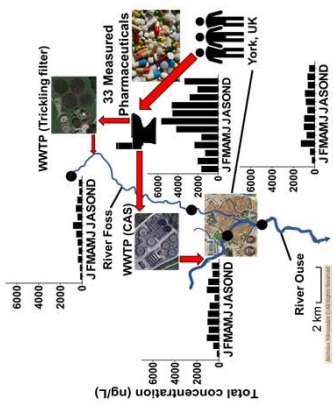
Received Date: 14 December 2017

Revised Date: 27 February 2018

Accepted Date: 28 February 2018

Please cite this article as: Burns, E.E., Carter, L.J., Kolpin, D.W., Thomas-Oates, J., Boxall, A.B.A., Temporal and spatial variation in pharmaceutical concentrations in an urban river system, *Water Research* (2018), doi: 10.1016/j.watres.2018.02.066.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



# Temporal and spatial variation in pharmaceutical concentrations in an urban river system

Emily E. Burns<sup>a</sup>, Laura J. Carter<sup>b</sup>, Dana W. Kolpin<sup>c</sup>, Jane Thomas-Oates<sup>a</sup>, Alistair B.A. Boxall<sup>b</sup>

<sup>a</sup>Chemistry Department, University of York, York, YO10 5DD United Kingdom

<sup>b</sup>Environment Department, University of York, York, YO10 5DD United Kingdom

<sup>c</sup>U.S. Geological Survey, Iowa City, IA, 52242, United States

Corresponding author: Emily Burns ([emily.burns@york.ac.uk](mailto:emily.burns@york.ac.uk))

Other Author emails: Laura Carter ([laura.carter@york.ac.uk](mailto:laura.carter@york.ac.uk)), Dana Kolpin ([dw.kolpin@usgs.gov](mailto:dw.kolpin@usgs.gov)), Jane Thomas-Oates ([jane.thomas-oates@york.ac.uk](mailto:jane.thomas-oates@york.ac.uk)), Alistair Boxall ([alistair.boxall@york.ac.uk](mailto:alistair.boxall@york.ac.uk))

## Abstract

Many studies have quantified pharmaceuticals in the environment, few however, have incorporated detailed temporal and spatial variability due to associated costs in terms of time and materials. Here, we target 33 physico-chemically diverse pharmaceuticals in a spatiotemporal exposure study into the occurrence of pharmaceuticals in the wastewater system and the Rivers Ouse and Foss (two diverse river systems) in the city of York, UK. Removal rates in two of the WWTPs sampled (a carbon activated sludge (CAS) and trickling filter plant) ranged from not eliminated (carbamazepine) to >99% (paracetamol). Data comparisons indicate that pharmaceutical exposures in river systems are highly variable regionally, in part due to variability in prescribing practices, hydrology, wastewater management, and urbanisation and that select annual median pharmaceutical concentrations observed in this study were higher than those previously observed in the European Union and Asia thus far.

30 Significant spatial variability was found between all sites in both river systems, while  
31 seasonal variability was significant for 86% and 50% of compounds in the River Foss  
32 and Ouse, respectively. Seasonal variations in flow, in-stream attenuation, usage and  
33 septic effluent releases are suspected drivers behind some of the observed temporal  
34 exposure variability. When the data were used to evaluate a simple environmental  
35 exposure model for pharmaceuticals, mean ratios of predicted environmental  
36 concentrations (PECs), obtained using the model, to measured environmental  
37 concentrations (MECs) were 0.51 and 0.04 for the River Foss and River Ouse,  
38 respectively. Such PEC/MEC ratios indicate that the model underestimates actual  
39 concentrations in both river systems, but to a much greater extent in the larger River  
40 Ouse.

41 Keywords: LC-MS/MS; surface water; wastewater; seasonal; exposure; predicted  
42 environmental concentration

43

## 44 **1.0 Introduction**

45 Determining pharmaceutical exposures in environmental matrices has become a  
46 substantial area of research since the 1990s (Daughton, 2016). The presence of  
47 pharmaceuticals in freshwater systems has now been documented globally, with  
48 research especially focused in Europe and North America (aus der Beek et al., 2016).  
49 Pharmaceuticals primarily enter the environment through patient use when an  
50 unmetabolised fraction is excreted and subsequently passes through wastewater  
51 treatment plants (WWTPs), which are typically not designed to remove such organic  
52 contaminants (Luo et al., 2014). Consequently, WWTPs are significant sources of  
53 pharmaceuticals to the environment (Lindholm-Lehto et al., 2016). A recent study of  
54 United Kingdom (UK) WWTPs estimated that 13% of effluent discharges could pose  
55 risks to the receiving environment regarding pharmaceutical exposures (Comber et al.,

56 2018). Removal rates are highly variable between treatment types (Kasprzyk-Hordern et  
57 al., 2009; Luo et al., 2014), seasons (Golovko et al., 2014), and even within treatment  
58 plants themselves (Verlicchi et al., 2012). Moreover, removal rates have only been  
59 estimated for a small fraction of the total number of pharmaceuticals in use (Boxall et al.,  
60 2014) and only a few studies have reported WWTP removals in the UK specifically  
61 (Comber et al., 2018; Kasprzyk-Hordern et al., 2009, 2008). WWTP removal rates are  
62 valuable parameters, and their inclusion in occurrence modelling substantially improves  
63 the accuracy of pharmaceutical exposure predictions (Burns et al., 2017; Verlicchi et al.,  
64 2014).

65 The potential for, and extent of, effects posed by pharmaceutical exposure to non-  
66 target organisms, such as fish or invertebrates, is largely unknown (Vasquez et al.,  
67 2014). However, there is mounting evidence that select pharmaceuticals are having  
68 deleterious effects at environmentally relevant (i.e. real-world) concentrations. Examples  
69 of documented effects at environmentally relevant concentrations include  
70 antidepressants causing behavioural changes in fish (fluoxetine) (Mccallum et al., 2017),  
71 disruption during early development (venlafaxine) (Thompson et al., 2017), the  
72 equivalent of human side effects from exposure to the anti-diabetic drug metformin  
73 (Niemuth et al., 2015) or the feminization of wild fish populations downstream of a  
74 pharmaceutical manufacturing facility in France (Sanchez et al., 2011). It is therefore  
75 important to characterise the source and fate of pharmaceuticals in the aquatic  
76 environment to aid in risk assessment as approaches evaluating potential adverse effect  
77 concentrations emerge.

78 To adequately characterise the fate of pharmaceuticals in the environment, robust  
79 monitoring campaigns which include seasonal or year-long sampling covering a range of  
80 compounds at a reasonable spatial resolution are required. However, only a small  
81 number of spatiotemporal exposure studies have been performed that meet these

82 criteria (Baker and Kasprzyk-Hordern, 2013; Daneshvar et al., 2010; Kasprzyk-Hordern  
83 et al., 2008; Paíga et al., 2016). These exposure studies are extremely valuable as they  
84 provide detailed information which can be related back to the myriad of factors (many  
85 varying both seasonally and temporally) that influence environmental concentrations of  
86 pharmaceuticals including hydrology (Kasprzyk-Hordern et al., 2008), WWTP removal  
87 efficiency (Silva et al., 2014), pharmaceutical usage (Sun et al., 2014), and in-stream  
88 removal processes (e.g. biodegradation and sorption to sediment) (Daneshvar et al.,  
89 2010; Camacho-Munoz et al., 2010; Moreno-González et al., 2014). In combination, the  
90 impact of these processes on pharmaceutical exposure and fate is largely unknown but,  
91 if better defined, could improve exposure prediction approaches and offer greater  
92 confidence, in terms of exposure, when evaluating risks that pharmaceuticals may pose  
93 to the environment.

94 Recently, a handful of aqueous rapid pharmaceutical determination high-  
95 performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS)  
96 methods have been developed that achieve comparable limits of detection (LODs) to  
97 those including sample pre-concentration or clean-up (Anumol et al., 2015; Boix et al.,  
98 2015; Campos-Mãnas et al., 2017; Furlong et al., 2014; Oliveira et al., 2015). Such  
99 methods involve utilising larger than normal injection volumes (~100  $\mu\text{L}$ ) to increase the  
100 likelihood of detection (Petrie et al., 2016). Removal of the extraction step reduces  
101 sample preparation time and can increase the number of samples that can be processed  
102 (highly beneficial to large spatiotemporal exposure campaigns). A significant analytical  
103 problem arising during pharmaceutical quantification is matrix effects (typically mass  
104 spectrometric ionisation enhancement or suppression). The presence of background  
105 interferences in “dirty” matrices (e.g. streams, WWTP effluent, etc.) can co-elute with  
106 target analytes and impair quantification past the point of suitability (Petrović et al.,  
107 2005). Several approaches have been attempted to reduce matrix effects including

108 sample pre-concentration and clean-up to help isolate target pharmaceuticals (Van De  
109 Steene et al., 2006). Such pre-concentration, however, is difficult to optimise, time  
110 consuming, costly, and may also concentrate interfering analytes, thus unintentionally  
111 increasing matrix effects (Yu et al., 2012). Matrix interferences have been reported to be  
112 comparatively lower for rapid determination methods than more costly and laborious  
113 sample pre-concentration/clean-up methods (Anumol et al., 2015).

114 In this study, which was performed in the frame of the Innovative Medicines  
115 Initiative iPiE project on intelligent assessment of pharmaceuticals in the environment,  
116 we validate and apply a rapid determination aqueous HPLC-MS/MS method for the  
117 quantification of 33 physico-chemically diverse pharmaceuticals to a year-long surface-  
118 water exposure campaign. Monitoring was conducted during 2016 at 11 sites along the  
119 urbanised and larger River Ouse and smaller, more rural River Foss which converge  
120 within the city of York, UK (Figure 1). The monthly sampling design provided good  
121 temporal resolution while unparalleled spatial resolution was achieved in the two  
122 contrasting river systems. In addition, influent and effluent samples from two of the  
123 WWTPs that serve the city were collected when possible and removal efficiencies  
124 estimated. Predicted exposure concentrations (PECs) were calculated for both rivers  
125 using a simple model and the model was then evaluated against annually averaged  
126 measured environmental concentrations (MECs) calculated from the monthly sampling  
127 data.

## 128 **2.0 Methods**

### 129 2.1 Study area and sample collection

#### 130 *2.1.1 Study Compounds*

131 Study compounds were selected based on those previously detected in the York  
132 river system during an initial scoping study in which 95 pharmaceutical and degradation



133 products were surveyed (Burns et al., 2017). From these results, 32 pharmaceuticals  
134 were selected due to either their known or expected presence. An additional compound,  
135 gabapentin, was also included in the study due to its high usage, resistance to  
136 environmental degradation, and ecotoxic potential (Herrmann et al., 2015).

### 137 *2.1.2 Study Area*

138 The River Ouse and River Foss were chosen for the study, as they flow through  
139 the city of York, UK, and converge downstream of the city centre (Figure 1). The two  
140 rivers represent differing levels of urbanisation and size. To minimise potential variability,  
141 grab water samples were collected from the network of 11 sampling sites in the same  
142 order and on approximately the same day and time each month from January to  
143 December 2016. Site locations were strategically chosen based on their ease of access  
144 and position in relation to WWTP outfalls. Both rivers were sampled with sufficient  
145 spatial resolution to build concentration profiles and increase the probability of detecting  
146 transient pharmaceuticals in the absence of composite sampling techniques. Three  
147 WWTPs serve the city within the sampling network (Figure 1). WWTP A is a trickling  
148 filter plant and serves a population of 18 600, WWTP B is a conventional activated  
149 sludge (CAS) facility serving a population of 27 900, while WWTP C is a surplus  
150 activated sludge (SAS) plant serving a population of 180 500. Sampling site and WWTP  
151 characteristics along with dates of sampling are detailed in Supplemental Material,  
152 Tables S1 and S2.

### 153 *2.1.3 Sample Collection*

154 All samples collected were subject to the same sampling protocol. At each site,  
155 three 1-L field replicates were collected from the centroid of flow (when possible);  
156 sampling sites had been previously determined to be well-mixed, therefore sampling in a  
157 single location was deemed appropriate (Supplemental Material, Figure S1). For each  
158 field replicate, a 10-mL aliquot was drawn into a 24-mL disposable syringe and filtered

159 through a primed 0.7- $\mu\text{m}$  glass-fibre filter (GF/F) (Whatman Inc.) into an amber glass vial  
160 and immediately frozen in the field using dry ice. To demonstrate that field filtration and  
161 collection did not contaminate samples, three field blanks per sampling visit were  
162 collected. HPLC-grade water was brought to the field, filtered and prepared identically to  
163 field samples. Samples were then returned to the laboratory and stored at  $-18^{\circ}\text{C}$  until  
164 analysis which occurred within seven days. The concentration reported for each sample  
165 per site is the median of the three field replicates collected. The filtering of samples in  
166 the field is beneficial as it removes particulates which can extend HPLC column life,  
167 reduce instrument maintenance, as well as remove bacteria associated with particulates  
168 that could facilitate analyte degradation. There is a formal possibility that analytes could  
169 be retained on the filter; however pharmaceutical filtration studies including 26  
170 compounds (acids, bases and amphoteres) ranging in hydrophobicity ( $\log K_{ow}$  -2.3 to  
171 6.3) suggest these losses will be insignificant ( $<5\%$ ) (Mompelat et al., 2013), thus an  
172 assessment of filter losses has not been repeated here.

## 173 2.2 High performance liquid chromatography-tandem mass spectrometry

174 A Thermo Scientific™ TSQ Endura MS operating in multiple reaction monitoring  
175 mode interfaced with an EASY-Max NG™ heated electrospray source operating in  
176 positive mode was used for pharmaceutical detection. Two transitions were monitored  
177 for each analyte and the  $m/z$  and collision energy optimised using the Thermo™ Tune  
178 2.0 software, summarised in Supplemental Material, Table S3. Chromatographic  
179 separation was achieved with a Dionex Ultimate 3000 HPLC (Thermo Scientific™)  
180 equipped with a 100- $\mu\text{L}$  sample injection loop and autosampler maintained at  $4^{\circ}\text{C}$ .  
181 Mobile phase A consisted of HPLC-grade water amended with 12-mL of 1 M formic acid  
182 and 10-mL of 1 M ammonium hydroxide for a total volume of 1-L, and mobile phase B  
183 was 100% methanol (Furlong et al., 2014). The chromatographic conditions and  
184 program are reported in the Supplemental Material Table S4.

185 Internal standard (IS) calibration was used to quantify the pharmaceuticals in the  
186 method described. For reasons of expense and availability, not all pharmaceuticals had  
187 a corresponding isotopically labelled internal standard (ILIS) (Supplemental Material,  
188 Table S3). In these cases, atrazine-d<sub>5</sub> was used and has been previously determined  
189 suitable for this role (Furlong et al., 2014). Samples were fully thawed and a 995- $\mu$ L  
190 aliquot pipetted into a 1.5-mL LC vial and a 5- $\mu$ L spike of IS solution (80 ng/L) added.  
191 Samples were immediately analysed after preparation. Peak detection criteria were in  
192 accordance with Commission Decision (2002/657/EC). Due to analytical complications,  
193 fexofenadine could not be quantified in the April surface-water samples. Further details  
194 of peak qualification and quantitation are provided in the Supplementary Material.

195 The use of ILIS is a good strategy to compensate for matrix effects (Stüber and  
196 Reemtsma, 2004). This is not a perfect solution as matrix effects can still influence  
197 quantification, possibly due to a slight difference in retention time ( $t_R$ ) between the ILIS  
198 and target analyte resulting in differing ionisation efficiencies (Wang et al., 2007).  
199 Therefore, sample matrix spikes were routinely prepared and analysed with all sample  
200 batches to provide an indication of the presence of interferences which cause signal  
201 suppression/enhancement and could impact quantification. In this study, acceptable  
202 matrix recovery was considered to be 70% to 120% in accordance with previously  
203 published methods (Boix et al., 2015; USEPA, 2016; Furlong et al., 2014). Matrix  
204 'recovery' falling outside this range indicates signal suppression/enhancement could be  
205 occurring and samples should quantitatively be interpreted with caution. At least three  
206 matrix spike samples from different sampling sites were prepared per analytical batch to  
207 monitor for matrix effects throughout the sampling campaign as the sample matrices are  
208 heterogenous and likely to vary temporally. Surface-water matrix spikes were prepared  
209 by spiking 20  $\mu$ L of 80 ng/L or 200 ng/L calibration solution into a sample replicate with 5  
210  $\mu$ L of IS solution. The much higher ambient concentration of pharmaceuticals in WWTP

211 influent and effluent required the matrix spike samples to be prepared at a higher  
212 concentration, 4000 ng/L. Matrix recovery was calculated by subtracting the ambient  
213 sample concentration and dividing by the concentration spiked.

214 With each sample batch at least three calibration check samples (CCSs) were  
215 prepared to monitor accuracy throughout the analytical batch (injected every 10  
216 samples). These CCSs were prepared to a concentration of 80 ng/L by pipetting 20  $\mu$ L  
217 of the relevant calibration solution into 975  $\mu$ L of HPLC grade water and spiked with 5  $\mu$ L  
218 of IS solution. At the end of each batch a 4 ng/L calibration solution spike, prepared  
219 similarly, was also injected. The accuracy of these CCSs was required to be within 20%  
220 or affected samples were re-analysed (Furlong et al., 2014; USEPA, 2016).

221 This formed part of a rigorous quality control plan which was followed during  
222 environmental sample analysis using a series of sample matrix spikes, calibration  
223 solution spikes, field blanks, and laboratory blanks randomly dispersed throughout  
224 analytical batches. Further detail of quality control, how these samples were prepared  
225 and results are reported in the Supplementary Material.

### 226 *2.3 Analytical method validation*

227 Method validation included an assessment of precision (inter- and intra-day), limits  
228 of detection, limits of quantification, and recovery from all studied matrices. The methods  
229 and results with which each of these parameters were assessed are reported in the  
230 Supplemental Material.

### 231 *2.4 WWTP removal efficiency*

232 Due to access restrictions, 24 h composite samples for influent and effluent could  
233 only be collected once from WWTP A and B during summer 2016 (Supplementary  
234 Material, Table S2). Only grab samples unsuitable for estimating removals could be  
235 collected from WWTP C. WWTP removal efficiency was estimated, when appropriate,

236 for WWTP A and B based on mean influent and effluent concentrations according to  
237 Equation 1. In this context 'removal' is the change in concentration between influent and  
238 effluent which does not represent true removal, but rather partitioning to the solid phase  
239 and/or the formation of transformation products. Negative removals can occur,  
240 potentially due to sampling limitations (e.g. longer than 24 h hydraulic/sludge retention  
241 time) (Ort et al., 2010), from the conversion of conjugated metabolites back to the parent  
242 compound during treatment (Verlicchi et al. 2012), or desorption from sludge during  
243 secondary treatment (Blair et al., 2015).

$$244 \quad \% \text{ Removal} = \left( 1 - \frac{\text{Effluent}}{\text{Influent}} \right) \times 100 \quad [1]$$

## 245 2.5 Statistical Analysis

246 Data analysis was performed using Graphpad Prism (Graphpad Software, 2017).  
247 To use statistical tests when non-detects were present, data substitution according to  
248 Equation 2 was undertaken. This approach was suggested to be appropriate for left  
249 censoring of up to 40% of a dataset (Antweiler, 2015). If the non-detect frequency for a  
250 compound was greater than 40%, it was not included in statistical testing. To determine  
251 whether significant spatial differences existed between sites, pairwise t-tests were  
252 conducted based on the monthly concentrations (Furlong et al., 2017). To determine  
253 whether any analytes were seasonally variable in each river, concentrations from sites  
254 F3-F4 and O3-O4 were grouped by season and a Friedman's Test followed by a Dunn's  
255 multiple comparisons post hoc test was undertaken. These sites were used in the  
256 seasonality test due to their downstream location in relation to WWTP A and B, as well  
257 as their location in relation to Environment Agency flow gauges (Figure 1) as the flow  
258 recorded at these gauges was not representative of flow conditions at the remaining  
259 study sites (Center for Ecology & Hydrology, 2016).

$$260 \quad \text{Substitution} = \frac{\sqrt{2}}{2} * \text{LOD} \quad [2]$$

## 261 2.6 Predicted environmental concentrations

262 Annual average MECs were compared to PECs to gauge the accuracy of simple  
263 exposure algorithms commonly used for the prioritisation of pharmaceuticals and risk  
264 assessment (Burns et al., 2017). Local annual pharmaceutical usage data were obtained  
265 from the National Health Service Business Authority (National Health Service, 2016),  
266 while wastewater generation was assumed to be 200 L/person-day (European  
267 Medicines Agency, 2006). Experimental WWTP removal rates (Eqn. 1) were used with  
268 river specific dilution factors based on the average flow from sampling days to generate  
269 a PEC for both rivers. PEC calculations were based on the approach suggested by the  
270 European Medicines Agency (2006). Parameters and equations used to predict the  
271 PECs are provided in the Supplemental Material Table S6.

## 272 **3.0 Results & Discussion**

### 273 3.1 Method performance and quality control

274 The method was determined to be sufficiently reproducible as assessed by the relative  
275 standard deviation of multiple injections (n=8) during (5.5 %RSD) and across (7.5  
276 %RSD) analysis days according to USEPA (2016) guidelines and Boix et al. (2015)  
277 where an  $RSD \leq 20\%$  above the LOQ (i.e. 80 ng/L) is desirable. The limits of detections  
278 (LOD) ranged from 0.9 ng/L (carbamazepine) to 12.4 (gabapentin) and an LOD <10 ng/L  
279 was achieved for 91% of analytes (Table S5). There were no quantifiable concentrations  
280 of any of the target pharmaceuticals in field blanks collected routinely throughout the  
281 monitoring campaign. Routine matrix spikes in surface water fell within the acceptable  
282 70 – 120% recovery range for concentrations of 80 and 200 ng/L, indicating that  
283 throughout the sample analysis quantification was not unacceptably impaired due to  
284 matrix effects (Figure 2). Matrix effects were observed in WWTP effluent and influent, a  
285 phenomenon also reported by others, and suggested to be due to the presence of a  
286 greater proportion of chemical species that can affect consistent ionisation in

287 comparison to surface water (Boix et al., 2015; Oliveira et al., 2015). In effluent 13% and  
288 in influent 19% of analytes fell outside the acceptable matrix signal response, identified  
289 in Figure 2 and 3. Signal enhancement was most prominent for diphenhydramine in both  
290 influent and effluent (442% and 375%, respectively), while metformin (214%) and  
291 tramadol (156%) also exhibited significant signal enhancement in influent. In this study,  
292 a slight shift in relative  $t_R$  of the analyte with respect to its ILIS, was observed in WWTP  
293 influent and effluent in comparison to surface water, which, in addition to it containing a  
294 larger number of chemical constituents, could help explain why matrix effects were not  
295 well compensated for all analytes using isotopically labelled internal standards. WWTP  
296 influent and effluent matrix spikes indicate that caution is needed when interpreting  
297 quantitative results and removal efficiencies due to significant matrix effects, while matrix  
298 spikes in surface water indicate that matrix effects are sufficiently compensated for by  
299 the internal standards.

### 300 3.2 Pharmaceuticals in WWTPs

301 The highest summed pharmaceutical concentrations in influent were observed in  
302 samples from WWTP B, while highest summed concentrations in effluent were observed  
303 in samples taken at WWTP A. Paracetamol had the highest concentration in all WWTP  
304 influents, 282, 186 and 117  $\mu\text{g/L}$  at WWTP B, A and C, respectively. In effluent,  
305 gabapentin had the highest concentration (8541 ng/L) at WWTP C followed by  
306 metformin (6111 ng/L) at WWTP A and fexofenadine (2094 ng/L) in effluent at WWTP C.  
307 Seven pharmaceuticals (diphenhydramine, norethisterone, oseltamivir, raloxifene,  
308 sertraline, triamterene and verapamil) were not detected in any WWTP sample. Average  
309 concentration and standard deviation (SD) of WWTP influent and effluent samples are  
310 reported in the Supplemental Data Table S10.

311 In a global review of pharmaceuticals in WWTPs, Verlicchi et al. (2012) reported  
312 influent concentrations for many compounds also observed in the WWTP samples in this

313 study. Codeine, paracetamol, gabapentin, hydrocodone, tramadol, erythromycin,  
314 trimethoprim, diltiazem, atenolol, propranolol, carbamazepine, gabapentin, cimetidine,  
315 and ranitidine influent concentrations all fell within the ranges reported by Verlicchi et al.  
316 (2012), while concentrations of amitriptyline were an order of magnitude lower. A study  
317 of effluents in the European Union (EU) reported average concentrations an order of  
318 magnitude lower than those determined here for tramadol, codeine, citalopram,  
319 fexofenadine, diltiazem, ranitidine, and amitriptyline, while effluent concentrations were  
320 similar for venlafaxine, trimethoprim, carbamazepine, and sulfamethoxazole in the York  
321 samples (Loos et al., 2013).

322 The estimated removal efficiency in each WWTP is presented for all detected  
323 analytes in Figure 3. The median removal efficiency was estimated to be 75% in WWTP  
324 A and 38% in WWTP B. Paracetamol was the analyte most efficiently removed at both  
325 treatment plants (>99%), while removals greater than 75% were reported for gabapentin,  
326 ranitidine, atenolol, sulfamethoxazole, metformin, and codeine. Despite being a trickling  
327 filter plant which might be expected to have poorer pharmaceutical removal than CAS  
328 systems (Kasprzyk-Hordern et al., 2009), WWTP A had similar and even greater  
329 removals for select compounds (i.e. carbamazepine, diltiazem, citalopram, erythromycin,  
330 cimetidine, and ranitidine). In the UK specifically, similar removals were reported  
331 previously (Kasprzyk-Hordern et al., 2009) for trimethoprim, amitriptyline, diltiazem,  
332 cimetidine, gabapentin, and paracetamol, while sulfamethoxazole, erythromycin,  
333 codeine, tramadol, carbamazepine, propranolol and ranitidine were, in general, more  
334 efficiently removed for this study. WWTPs with similar treatment capabilities were also  
335 studied previously in the UK (Kasprzyk-Hordern et al., 2009). In comparison with results  
336 reported here, WWTP removal rates were highly variable despite operating in the same  
337 region and employing similar treatments, a conclusion also observed in other regions  
338 (Verlicchi et al., 2012). The single sampling event in the WWTPs is limited, however



339 these estimates are still useful for comparative purposes. For example, sitagliptin  
340 removal efficiency (25 - 40%) has not been previously reported to the authors'  
341 knowledge. Therefore, while WWTPs are significant sources of pharmaceuticals entering  
342 the environment, analysis of WWTP removal efficiencies (i.e. reduction in parent  
343 pharmaceutical concentration from influent to effluent) as documented in this and  
344 previously published studies, demonstrate that WWTPs are generally decreasing the  
345 aquatic environmental burden by significantly reducing certain parent pharmaceutical  
346 concentrations (not considering degradates or transformation products) for many of the  
347 compounds studied.

### 348 3.3 Pharmaceuticals in Surface Water

349 Of the 33 pharmaceuticals monitored, 21 were detected in all 12 months in  
350 samples from the River Foss. Three compounds, oxazepam, verapamil, and triamterene,  
351 were not detected in any Foss sample. The remaining nine study compounds,  
352 diazepam, diphenhydramine, loratadine, norethisterone, oseltamivir, raloxifene,  
353 sulfamethoxazole, sertraline, and temazepam, were sporadically detected from month to  
354 month in this river. In comparison, ten compounds (carbamazepine, codeine,  
355 fexofenadine, gabapentin, hydrocodone, lidocaine, metformin, paracetamol, tramadol,  
356 and trimethoprim) were detected in all 12 months in the River Ouse samples. Eight  
357 compounds were not detected in any Ouse sample: diazepam, loratadine, oseltamivir,  
358 oxazepam, raloxifene, sulfamethoxazole, triamterene, and verapamil. The highest five  
359 annual median concentrations followed the same trend in both rivers:  
360 metformin>gabapentin>paracetamol>fexofenadine>tramadol, indicating that usage  
361 patterns, WWTP removal and environmental fate for the most prevalent pharmaceuticals  
362 are similar in these two systems. The range, detection frequency and annual median for  
363 each pharmaceutical in both river systems is reported in Tables 1 and 2.

364 Monthly total pharmaceutical concentrations at each sampling site are presented  
365 in Figures 4 and 5. These concentration figures provide a spatiotemporal overview of the  
366 relationship between sampling sites, rivers, and WWTPs serving the city. Monthly  
367 summed concentrations are higher in the River Foss (e.g. above 2000 ng/L) at sites  
368 downstream of the WWTP in comparison to the River Ouse, where most monthly  
369 summed concentrations are below 1000 ng/L despite the WWTPs on the River Ouse  
370 serving a larger population. This is due to greater dilution of discharged effluent in the  
371 Ouse; for example, flow ranged from 9.2 to 233 m<sup>3</sup>/s in the Ouse, compared with 0.0096  
372 to 1.68 m<sup>3</sup>/s in the Foss on sampling days (Figure 1). For the sites immediately  
373 downstream of the WWTPs (O3, O6, and F2), the months with the lowest flows, July and  
374 June, yielded both the most analytes and the highest concentrations. Thus,  
375 concentrations appear to be inversely proportional to flow at site F2, similarly to  
376 observations reported previously (Kolpin et al., 2004). The trend is not continued moving  
377 downstream in the River Foss (sites F3-F5), potentially due to pharmaceutical losses  
378 stemming from dilution or in-stream removal processes such as biodegradation or  
379 sorption to sediment (Moreno-González et al., 2014), or due to pharmaceutical  
380 contributions from domestic septic systems (Carmona et al., 2014), and/or inputs from  
381 combined sewer overflows (CSO) (Phillips et al., 2012). In the Foss, a substantial spike  
382 downstream of F2 in paracetamol (9822 ng/L) was detected in the March sampling along  
383 with less intense spikes from other pharmaceuticals, such as metformin (2592 ng/L).  
384 These observations may be explained by local septic tank effluent entering the river  
385 downstream of the F2 site, captured during the March sampling period. Paracetamol can  
386 be >99% removed and metformin >93%, in conventional water treatment (Figure 3),  
387 therefore the spike in March concentrations might be explained by releases of septic  
388 effluent (James et al., 2016). James et al. (2012) reported paracetamol concentrations of  
389 5000 ng/L at a septic effluent impacted site and identified it as a possible tracer of septic

390 system contamination. Combined sewer overflow (CSO) releases could provide an  
391 alternative explanation for the concentration spike (Philips et al. 2012), as a CSO is  
392 located just upstream of the F3 site. Low rainfall (University of York, 2018) prior to  
393 sampling suggest CSOs would not likely be in operation, therefore septic effluent  
394 releases provide a plausible explanation. Concentrations in the River Ouse varied less  
395 month to month than in the Foss, and a relationship with flow was less clear, with March  
396 and May in general having slightly greater total concentrations. March has also been  
397 reported to have the highest monthly concentration in recent temporal studies (Padhye  
398 et al., 2014; Sun et al., 2014). Sun et al. (2014) suggested March coincided with a spike  
399 in pharmaceutical usage and reduced WWTP removal capacity. This may explain the  
400 slightly higher concentrations observed in the River Ouse at sites upstream of the Foss  
401 confluence (O1-O4), while the spike in May (River Ouse) coincides with decreased river  
402 flow (Figure 1).

403 Metformin, a type II diabetes drug, had the highest annual median concentration  
404 (1117 and 237 ng/L in the Foss and Ouse, respectively), followed by gabapentin (anti-  
405 convulsant) (843 and 230 ng/L, Foss and Ouse, respectively) and paracetamol  
406 (analgesic) (209 and 77.6 ng/L, Foss and Ouse, respectively). This trend is different from  
407 those observed in previous temporal exposure campaigns studying similar compounds  
408 throughout the world. For example in China, Zhang et al. (2015) studied urbanized rivers  
409 and found antibiotics the most frequently detected pharmaceuticals. They did, however,  
410 report atenolol as having one of the highest annual median concentrations (53 ng/L),  
411 which is similar to the median concentration for this compound reported at site F2 (55.4  
412 ng/L) in the current study. In Spain, Camacho-Munoz et al. (2010) reported propranolol  
413 most frequently detected in surface water, with a higher average concentration (80 ng/L)  
414 than observed in this study (20.1 ng/L). In Portugal, Paíga et al. (2016) reported  
415 carbamazepine the most frequently detected pharmaceutical with an annual median of

416 31.7 ng/L, while other similarly studied compounds, citalopram and venlafaxine had  
417 annual median concentrations of 0.86 and 40.1 ng/L, respectively and trimethoprim was  
418 not detected. In the River Foss, the highest annual median concentrations for  
419 carbamazepine, citalopram and venlafaxine was 66 ng/L, 15.4 and 21 ng/L, respectively  
420 while trimethoprim was detected in 100% of samples with an annual median of 30 ng/L.  
421 In Sweden, carbamazepine was also most frequently detected and at a higher annual  
422 mean than observed in York, 204 ng/L versus 66 ng/L in the River Foss, while atenolol  
423 concentration was similar to that reported here (60.2 ng/L, compared to 55.4 ng/L)  
424 (Daneshvar et al., 2010). In a similar temporal study in Wales, tramadol and gabapentin  
425 had the highest annual median concentrations (968 ng/L and 227 ng/L, respectively)  
426 (Kasprzyk-Hordern et al., 2008). Median concentrations of: gabapentin, tramadol,  
427 trimethoprim, paracetamol, carbamazepine, cimetidine and atenolol, in Wales were  
428 higher than we saw in York, while concentrations of diltiazem, atenolol,  
429 sulfamethoxazole, and erythromycin concentrations in the River Foss were lower than  
430 observed in Wales (Kasprzyk-Hordern et al., 2008). These comparisons suggest that  
431 annual pharmaceutical exposures in river systems are highly variable regionally, in part  
432 due to variability in prescribing practices, hydrology, wastewater management, and the  
433 degree of urbanisation. In addition, certain annual median concentrations of  
434 pharmaceuticals observed in this study are higher than those previously observed in the  
435 European Union and Asia.

### 436 *3.3.1 Spatial Trends*

437 The spatial trends for both rivers are presented in Figure 6; significant differences  
438 between a site and the adjacent downstream site are also noted. Spatial trends are  
439 apparent in both rivers, the greatest number of significant differences ( $p < 0.05$ ) were  
440 found between the sites upstream and downstream of the WWTPs (i.e. F1-F2, O3-O4  
441 and O5-O6) (Figure 6). In addition, significance increases were found when comparing

442 to sites further downstream. WWTPs make a significant contribution to pharmaceutical  
443 concentrations in both river systems, however upstream sources of certain  
444 pharmaceuticals exist in both rivers as significance was not achieved for cimetidine in  
445 the Foss and paracetamol, codeine, trimethoprim, and atenolol in the Ouse. There are  
446 WWTPs along the River Nidd (Figure 5) and upstream of sites O1 and F1 (>10 km)  
447 demonstrating that pharmaceuticals from upstream sources are transported into the city.  
448 Concentrations are generally highest immediately downstream of the WWTPs and  
449 decrease moving to downstream sites, evidenced by difference in height (i.e.  
450 concentration) between the bars from each site (Figure 6), similarly to observations in  
451 previous studies (Kasprzyk-Hordern et al., 2008). The decrease in concentrations  
452 moving downstream is variable between compounds indicating that in-stream  
453 attenuation is compound specific. For example, carbamazepine concentrations are  
454 similar between sites downstream of the WWTP in the River Foss (i.e. F2-F5), while  
455 over the same stretch of river concentrations of hydrocodone and citalopram decreases  
456 by 51% and 38%, respectively (Figure 6). In the Ouse, all concentrations decrease  
457 slightly from O3 to O4, however there is a slight increase occurring at O5, likely due to  
458 the confluence with the River Foss and again at O6, which is downstream of WWTP C.

459 In the River Foss, carbamazepine had only a single significant spatial difference  
460 between the site upstream of WWTP A discharge (site F1) and the sites downstream of  
461 the discharge. Carbamazepine has been reported to be resistant to biodegradation and  
462 stable in the environment (Moreno-González et al., 2014). In the River Ouse, all  
463 pharmaceuticals exhibited spatially significant trends. Carbamazepine was significantly  
464 different between each site downstream of WWTP B tested (i.e. O3 to O6). Since this  
465 did not occur in the River Foss over a similar distance, 13.3 km between sites F2 and F5  
466 versus 11 km between sites O3 and O6, and the literature agrees that carbamazepine is  
467 resistant to biotransformation, a combination of dilution (e.g. urban drainage/runoff) and

468 other pharmaceutical sources (i.e. River Foss) moving downstream could be a plausible  
469 explanation.

470 Overall, these results indicate that a wide variety of environmental processes such  
471 as dilution and in-stream degradation are operating to differing extents in neighbouring  
472 rivers leading to different spatial patterns in pharmaceutical concentrations between  
473 sampling sites. For example, the reduction in concentrations moving downstream in the  
474 River Foss may be symptomatic of in-stream removal processes such as photolysis or  
475 microbial degradation (Daneshvar et al., 2010), while fluctuating concentrations in the  
476 River Ouse could be due to a complex dynamic between dilution and other  
477 pharmaceutical sources (i.e. tributaries, urban drainage) while natural removal  
478 processes potentially operating in the Foss may be masked or occur to a lesser extent in  
479 the larger Ouse system.

### 480 *3.3.2 Seasonal Variability*

481 Temporal variability between the seasons (Figure 7) is presented similarly to the  
482 approach for displaying spatial variability between sampling sites (Figure 6). Seasonal  
483 differences in pharmaceutical concentrations exist in the two river systems, especially in  
484 the River Foss. In both rivers, the lowest concentrations correspond with winter, the  
485 season which had the highest average flow (2.7 times higher than the next highest  
486 season, autumn). Conversely, the highest mass loads occur in winter, 1.4 times higher  
487 than the next highest season, spring. Lower concentrations in winter have also been  
488 reported previously (Baker and Kasprzyk-Hordern, 2013; Kasprzyk-Hordern et al., 2008),  
489 however several studies report higher concentrations in winter (Kot-Wasik et al., 2016;  
490 Lindholm-Lehto et al., 2016; Zhang et al., 2015). In addition, the extent of concentration  
491 variability between seasons differs between compounds, which could be due to  
492 seasonal patterns in usage (Sun et al., 2014) or seasonal variability in photodegradation  
493 or biodegradation, of which both processes can peak in summer, thus having a greater

494 impact on more readily biodegradable compounds (Lindholm-Lehto et al., 2016). In  
495 general, autumn was the season with the second highest median concentrations, except  
496 for paracetamol, where highest median values were observed during spring in both  
497 rivers. This could be due to increased usage coinciding with symptomatic treatment of  
498 illnesses more common in spring such as colds (Vatovec et al., 2016) in conjunction with  
499 lower flows than winter. To determine whether concentrations between seasons were  
500 significant, Friedman's test was used for pharmaceuticals with sufficient detections.  
501 Concentrations of 17 compounds (86%) were found to vary significantly by season in the  
502 River Foss, while amitriptyline, codeine, cimetidine, metformin, and ranitidine did not  
503 vary seasonally. Nine compounds (50%) had significant seasonal differences in the  
504 River Ouse, atenolol, carbamazepine, codeine, desvenlafaxine, gabapentin, lidocaine,  
505 ranitidine, sitagliptin, and trimethoprim.

506 The reasons for temporal variations in pharmaceutical concentrations have varied  
507 between studies with several reporting flow as the major driver, observing higher  
508 concentrations during times of low flow (Kasprzyk-Hordern et al., 2008; Kolpin et al.,  
509 2004). Others suggest higher pharmaceutical concentrations in winter months coincide  
510 with higher winter usage patterns (Sun et al., 2014) or decreased biodegradation in  
511 winter (Moreno-González et al., 2014), while others found no significant differences  
512 between sampled seasons (Camacho-Munoz et al., 2010). Due to higher concentrations  
513 coinciding with low-flow months in this study, we also suggest that flow appears to be a  
514 major driver behind the observed seasonal variability in pharmaceutical concentrations  
515 in the current study. The lack of significant seasonal differences found in the River Ouse  
516 could be explained by a lower annual variability in flow on sampling days than the River  
517 Foss (i.e. two orders of magnitude versus three). Further detailed investigation into the  
518 drivers behind the pharmaceutical concentrations observed both temporally and spatially  
519 is required to differentiate between the possible explanations, and could include

520 comparing results with prescription data and flow and estimating the impact of in-stream  
521 losses seasonally in different climates, and in river hydrological properties (e.g. depth  
522 and flow). Such analyses will be facilitated by the detailed pharmaceutical monitoring  
523 data reported in this study.

524

### 525 *3.4 Comparisons of PECs and MECs*

526 The PEC/MEC ratios for each compound for which it was possible to calculate an  
527 annual average MEC are reported in Figure 8. A ratio greater than 1 indicates PECs  
528 were higher than MECs and lower when less than 1. The PECs are severely  
529 underestimated in the Ouse; this may be due to pharmaceuticals being transported from  
530 upstream or problems with sewer connectivity within the sampling network not being  
531 accounted for in the simplistic PEC calculation. Several studies have attempted to gauge  
532 the accuracy of PECs by calculating a ratio with MECs, however the criterion for what  
533 constitutes accurate is variable across studies (Burns et al., 2017). This assessment has  
534 been previously limited to a small number of compounds and based on a limited number  
535 of sampling events not representative of the annual average MEC which the PEC was  
536 designed to predict. In this way, we present novel findings that indicate when an annual  
537 average MECs is calculated, less hydrologically complex river systems where  
538 pharmaceutical sources are limited, PECs characterise annual exposure within a factor 2  
539 for 41% of compounds in this study (average factor 2.8), with no factor greater than 11.  
540 However paracetamol is an exception (underestimated by a factor of 73); the usage  
541 estimate did not incorporate over-the-counter contributions therefore underestimates  
542 were not unexpected (Burns et al., 2017). Conversely, the results from the River Ouse  
543 indicate that major limitations are associated with this predictive approach. All ratios



544 were off by a factor of at least 7 (average 27) and up to 139, which according to studies  
545 characterising the PEC/MEC, is outside an acceptable range (Verlicchi et al., 2014).

546 As the simple exposure model is routinely used for regulatory environmental risk  
547 assessment (ERA) of new pharmaceuticals, our findings have important regulatory  
548 implications. The predictions of exposure, currently being used to assess new  
549 compounds, are likely under- or over-estimating concentrations, depending on the type  
550 of compound. The use of a spatially referenced 'down the drain' hydrological model such  
551 as LF2000-WQX (Williams et al., 2012) or GREAT-ER (Feijtel et al., 1997) would likely  
552 result in improved predictions, as these models have the capacity to incorporate inputs  
553 from upstream sources; this is appropriate, as many rivers in the region pass through  
554 multiple urbanised areas and thus are subject to multiple WWTP inputs. In addition, the  
555 hydrological aspect can incorporate contributions or dilutions from the confluence with  
556 other river systems. Work currently being performed in the iPiE project involves the  
557 development of a spatially resolved model for European surface waters. The high-quality  
558 monitoring data presented in this study will be used to help evaluate this model. Our  
559 work also shows that inputs from other sources, potentially septic effluent, can be very  
560 important for some compounds at certain time of year. The consideration of these direct  
561 inputs in the risk assessment process may therefore be warranted.

## 562 **4.0 Conclusion**

563 A rapid determination HPLC-MS/MS method for 33 pharmaceuticals was validated  
564 and applied in a 12-month spatiotemporal pharmaceutical exposure campaign. WWTP  
565 removal efficiency was found to be similar between CAS and trickling filter technology for  
566 the target pharmaceuticals. Pharmaceutical concentrations in two contrasting river  
567 systems that run through the city of York, UK were found to vary significantly spatially  
568 and temporally, with the greatest variation observed for paracetamol in the River Foss,  
569 ranging from not detected to over 9822 ng/L. Temporal variations in concentration were

570 less frequently observed in the larger River Ouse, potentially due to the lower variability  
571 in flow which could be an important driver behind pharmaceutical concentrations in the  
572 study system. PEC/MEC ratios indicated that compounds in both rivers were generally  
573 underestimated by commonly used simple predictive exposure algorithms. In total, 41%  
574 of PEC/MEC ratios for the River Foss data were within a factor of 2, while for the River  
575 Ouse average ratios indicated predictions were off by a factor of 27. This analytical  
576 method and extensive monitoring results will be instrumental in improving the  
577 understanding of temporal pharmaceutical fate and occurrence in river systems.

### 578 **Acknowledgement**

579 The present work is funded by the European Union's Seventh Framework  
580 Programme for research, technological development and demonstration under grant  
581 agreement no. 608014 (CAPACITIE) and partly supported by the EU/EFPIA Innovative  
582 Medicines Initiative Joint Undertaking (iPiE Grant 115635). The York Centre of Excellence  
583 in Mass Spectrometry was created thanks to a major capital investment through Science  
584 City York, supported by Yorkshire Forward, with funds from the Northern Way Initiative, and  
585 subsequent support from EPSRC (EP/K039660/1; EP/M028127/1). The authors would also  
586 like to thank the U.S. Geological Survey (USGS) Toxic Substances Hydrology Program  
587 for its support of this research and P. Phillips for valuable comments and suggestions to  
588 improve the quality of the paper. Any use of trade, firm, or product names is for  
589 descriptive purposes only and does not imply endorsement by the U.S. Government.

### 591 **References**

- 592 Antweiler, R.C., 2015. Evaluation of statistical treatments of left-censored environmental  
593 data using coincident uncensored data sets. II. Group comparisons. *Environ. Sci.*  
594 *Technol.* 49, 13439–13446.
- 595 Anumol, T., Wu, S., Santos, M., D., Daniels, K.D., Snyder, S.A., 2015. Rapid direct

- 596 injection LC-MS/MS method for analysis of prioritized indicator compounds in  
597 wastewater effluent. *Environ. Sci. Water Res. Technol* 1, 632-643.
- 598 aus der Beek, T., Weber, F.-A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A., Küster,  
599 A., 2016. Pharmaceuticals in the environment-Global occurrences and  
600 perspectives. *Environ. Toxicol. Chem.* 35, 823–835.
- 601 Baker, D.R., Kasprzyk-Hordern, B., 2013. Spatial and temporal occurrence of  
602 pharmaceuticals and illicit drugs in the aqueous environment and during wastewater  
603 treatment: New developments. *Sci. Total Environ.* 454–455, 442–456.
- 604 Blair, B., Nikolaus, A., Hedman, C., Klaper, R., Grundl, T., 2015. Evaluating the  
605 degradation, sorption, and negative mass balances of pharmaceuticals and  
606 personal care products during wastewater treatment. *Chemosphere* 134, 395–401.
- 607 Boix, C., Ibáñez, M., Sancho, J. V, Rambla, J., Aranda, J.L., Ballester, S., Hernández,  
608 F., 2015. Fast determination of 40 drugs in water using large volume direct injection  
609 liquid chromatography–tandem mass spectrometry. *Talanta* 131, 719-727.
- 610 Boxall, A.B.A., Keller, V.D.J., Straub, J.O., Monteiro, S.C., Fussell, R., Williams,  
611 R.J., 2014. Exploiting monitoring data in environmental exposure modelling and risk  
612 assessment of pharmaceuticals. *Environ. Int.* 73, 176–185.
- 613 Burns, E.E., Thomas-Oates, J., Kolpin, D.W., Furlong, E.T., Boxall, A.B., 2017. Are  
614 exposure predictions, used for the prioritization of pharmaceuticals in the  
615 environment, fit for purpose? *Environ. Toxicol. Chem.* 36, 2823-2832.
- 616 Camacho-Muñoz, D., Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2010. Occurrence,  
617 temporal evolution and risk assessment of pharmaceutically active compounds in  
618 Dónana Park (Spain). *J. Hazard. Mater.* 183, 602–608.
- 619 Campos-Mañas, M.C., Plaza-Bolanos, P., Sánchez-Pérez, J.A., Malato, S., Agüera, A.,  
620 2017. Fast determination of pesticides and other contaminants of emerging concern  
621 in treated wastewater using direct injection coupled to highly sensitive ultra-high  
622 performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A*  
623 1507, 84–94.
- 624 Carmona, E., Andreu, V., Picó, Y., 2014. Occurrence of acidic pharmaceuticals and  
625 personal care products in Turia River Basin: From waste to drinking water. *Sci.*  
626 *Total Environ.* 484, 53–63.

- 627 Center for Ecology & Hydrology, 2016. National River Flow Archive. *27009- Ouse*  
628 *Skelton; 27083 – Foss Hungtington*. URL <http://nrfa.ceh.ac.uk/data/search>  
629 (accessed 3.9.17).
- 630 Comber, S., Gardner, M., Sörme, P., Leverett, D., Ellor, B., 2018. Active pharmaceutical  
631 ingredients entering the aquatic environment from wastewater treatment works: A  
632 cause for concern? *Sci. Total. Environ.* 613-614, 538-547.
- 633 European Commission, 2002. Commission Decision 2002 implementing Council  
634 Directive 96/23/EC concerning the performance of analytical methods and the  
635 interpretation of results (2002/657/EC). *Off. J. Eur. Communities*. L221/8-L221/36.
- 636 Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G.A., 2010.  
637 Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals  
638 in a Swedish river–lake system. *Chemosphere* 80, 301–309.
- 639 Daughton, C.G., 2016. Pharmaceuticals and the Environment (PiE): Evolution and  
640 impact of the published literature revealed by bibliometric analysis. *Sci. Total*  
641 *Environ.* 562, 391–426.
- 642 European Medicines Agency, 2006. Guideline on the Environmental Risk Assessment of  
643 Medicinal Products for Human Use. EMEA/CHMP/SWP/4447/00. Committee for  
644 Medicinal Products for Human Use, London, UK.
- 645 Feijtel, T., Boeije, G., Matthies, M., Young, A., Morris, G., Gandolfi, C., Hansen, B., Fox,  
646 K., Holt, M., Koch, V., Schroder, R., Casani, G., Schowanek, D., Rosenblom, J.,  
647 Niessen, H., 1997. Development of a geography-referenced regional exposure  
648 assessment tool for European rivers – GREAT-ER contribution to GREAT-ER #1.  
649 *Chemosphere* 34, 2351–2373.
- 650 Furlong, E.T., Batt, A.L., Glassmeyer, S.T., Noriega, M.C., Kolpin, D.W., Mash, H.,  
651 Schenck, K.M., 2017. Nationwide reconnaissance of contaminants of emerging  
652 concern in source and treated drinking waters of the United States:  
653 Pharmaceuticals. *Sci. Total Environ.* 579, 1629–1642.
- 654 Furlong, E.T., Kanagy, C.J., Kanagy, L.K., Coffey, L.J., Burkhardt, M.R., 2014.  
655 Determination of human-use pharmaceuticals in filtered water by direct aqueous  
656 injection-high-performance liquid chromatography/tandem mass spectrometry: U.S.  
657 Geological Survey Techniques and Methods, Book 5, Laboratory Analysis. p. 49.

- 658 Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R., 2014. Removal and  
659 seasonal variability of selected analgesics/anti-inflammatory, anti-  
660 hypertensive/cardiovascular pharmaceuticals and UV filters in wastewater treatment  
661 plant. *Environ. Sci. Pollut. Res.* 21, 7578–7585.
- 662 Graphpad Software, 2017. Graphpad Prism version 7.00 for Windows, Graphpad  
663 Software, La Jolla California USA, URL [www.graphpad.com](http://www.graphpad.com).
- 664 Herrmann, M., Menz, J., Olsson, O., Kümmerer, K., 2015. Identification of  
665 phototransformation products of the antiepileptic drug gabapentin: Biodegradability  
666 and initial assessment of toxicity. *Water Res.* 85, 11–21.
- 667 James, C.A.; Miller-Schulze, J.P.; Ultican, S.; Gipe, A.D. 2016. Evaluation of  
668 contaminants of emerging concern from septic systems. *Water Res.* 101, 241–251.
- 669 Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of  
670 pharmaceuticals, personal care products, endocrine disruptors and illicit drugs  
671 during wastewater treatment and its impact on the quality of receiving waters. *Water*  
672 *Res.* 43, 363–380.
- 673 Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of  
674 pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in  
675 surface water in South Wales, UK. *Water Res.* 42, 3498–3518.
- 676 Kolpin, D.W., Skopec, M., Meyer, M.T., Furlong, E.T., Zaugg, S.D., 2004. Urban  
677 contribution of pharmaceuticals and other organic wastewater contaminants to  
678 streams during differing flow conditions. *Sci. Total Environ.* 328, 119–130.
- 679 Kot-Wasik, A., Jakimska, A., Śliwka-Kaszyńska, M., 2016. Occurrence and seasonal  
680 variations of 25 pharmaceutical residues in wastewater and drinking water  
681 treatment plants. *Environ. Monit. Assess.* 188, 661.
- 682 Lindholm-Lehto, P.C., Ahkola, H.S.J., Knuutinen, J.S., Herve, S.H., 2016. Widespread  
683 occurrence and seasonal variation of pharmaceuticals in surface waters and  
684 municipal wastewater treatment plants in central Finland. *Environ. Sci. Pollut. Res.*  
685 23, 7985–7997.
- 686 Loos, R., Carvalho, R., António, D.C., Comero, S., Locoro, G., Tavazzi, S., Paracchini,  
687 B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K.,  
688 Haglund, P., Fick, J., Lindberg, R.H., Schwesig, D., Gawlik, B.M., 2013. EU-wide

- 689 monitoring survey on emerging polar organic contaminants in wastewater treatment  
690 plant effluents. *Water Res.* 47, 6475–6487.
- 691 Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C.,  
692 2014. A review on the occurrence of micropollutants in the aquatic environment and  
693 their fate and removal during wastewater treatment. *Sci. Total Environ.* 473–474,  
694 619–641.
- 695 McCallum, E.S., Bose, A.P.H., Warriner, T.R., Balshine, S., 2017. An evaluation of  
696 behavioural endpoints: The pharmaceutical pollutant fluoxetine decreases  
697 aggression across multiple contexts in round goby (*Neogobius melanostomus*).  
698 *Chemosphere* 175, 401-410.
- 699 Mompelat, S., Jaffrezic, A., Jardé, E., Le Bot, B., 2013. Storage of natural water samples  
700 and preservation techniques for pharmaceutical quantification. *Talanta* 109, 31–45.
- 701 Moreno-González, R., Rodríguez-Mozaz, S., Gros, M., Pérez-Cánovas, E., Barceló, D.,  
702 León, V.M., 2014. Input of pharmaceuticals through coastal surface watercourses  
703 into a Mediterranean lagoon (Mar Menor, SE Spain): Sources and seasonal  
704 variations. *Sci. Total Environ.* 490, 59–72.
- 705 National Health Service, 2016. GP practice prescribing presentation-level data: January  
706 to December 2016. URL <https://digital.nhs.uk/article/4214/Prescribing> (accessed  
707 24.2.18).
- 708 Niemuth, N.J., Jordan, R., Crago, J., Blanksma, C., Johnson, R., Klaper, R.D., 2015.  
709 Metformin exposure at environmentally relevant concentrations causes potential  
710 endocrine disruption in adult male fish. *Environ. Toxicol. Chem.* 34, 291–296.
- 711 Oliveira, T.S., Murphy, M., Mendola, N., Wong, V., Carlson, D., Waring, L., 2015.  
712 Characterization of Pharmaceuticals and Personal Care products in hospital effluent  
713 and waste water influent/effluent by direct-injection LC-MS-MS. *Sci. Total Environ.*  
714 518–519, 459–478.
- 715 Ort, C., Lawrence, M.G., Reungoat, J., Mueller, J.F., 2010. Sampling for PPCPs in  
716 wastewater systems: Comparison of different sampling modes and optimization  
717 strategies. *Environ. Sci. Technol.* 44, 6289–6296.
- 718 Padhye, L., Yao, H., Kung'u, F., Huang, C., 2014. Year-long evaluation on the  
719 occurrence and fate of pharmaceuticals, personal care products, and endocrine

- 720 disrupting chemicals in an urban drinking water treatment plant. *Water Res.* 51,  
721 266–276.
- 722 Paíga, P., Santos, L.H.M.L.M., Ramos, S., Jorge, S., Silva, J.G., Delerue-Matos, C.,  
723 2016. Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and  
724 seasonal variation. *Sci. Total Environ.* 573, 164–177.
- 725 Petrie, B., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2016. Multi-residue analysis of  
726 90 emerging contaminants in liquid and solid environmental matrices by ultra-high-  
727 performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. A*  
728 1431, 64–78.
- 729 Petrović, M., Hernando, M.D., Díaz-Cruz, M.S., Barceló, D., 2005. Liquid  
730 chromatography-tandem mass spectrometry for the analysis of pharmaceutical  
731 residues in environmental samples: A review. *J. Chromatogr. A* 1067, 1–14.
- 732 Phillips, P.J., Chalmers, A.T., Gray, J.L., Kolpin, D.W., Foreman, W.T., Wall, G.R., 2012.  
733 Combined Sewer Overflows: An Environmental Source of Hormones and  
734 Wastewater Micropollutants. *Environ. Sci. Technol.* 46, 5336–5343.
- 735 Sanchez, W., Sremski, W., Piccini, B., Palluel, O., Maillot-Maréchal, E., Betoulle, S.,  
736 Jaffal, A., Aït-Aïssa, S., Brion, F., Thybaud, E., Hinfrey, N., Porcher, J.-M., 2011.  
737 Adverse effects in wild fish living downstream from pharmaceutical manufacture  
738 discharges. *Environ. Int.* 37, 1342–1348.
- 739 Silva, L.J.G., Pereira, A.M.P.T., Meisel, L.M., Lino, C.M., Pena, A., 2014. A one-year  
740 follow-up analysis of antidepressants in Portuguese wastewaters: Occurrence and  
741 fate, seasonal influence, and risk assessment. *Sci. Total Environ.* 490, 279–287.
- 742 Stüber, M. Reemtsma, T., 2004. Evaluation of three calibration methods to compensate  
743 matrix effect in environmental analysis with LC-ESI-MS. *Anal. Bioanal. Chem.* 378,  
744 910–196
- 745 Sun, Q., Lv, M., Hu, A., Yang, X., Yu, C.-P., 2014. Seasonal variation in the occurrence  
746 and removal of pharmaceuticals and personal care products in a wastewater  
747 treatment plant in Xiamen, China. *J. Hazard. Mater.* 277, 69–75.
- 748 Thompson, W.A., Arnold, V., Vijayan, M.M., 2017. Venlafaxine in embryos stimulates  
749 neurogenesis and disrupts larval behavior in zebrafish. *Environ. Sci. Technol.* 51,  
750 12889–12897.

- 751 University of York, 2018. Department of Electronics: Data Archive: *March 2016*. URL  
752 <https://weather.elec.york.ac.uk/data/vaisala/archives/0316.txt> (accessed  
753 24/02/2018).
- 754 USEPA, 2016. Method 542: Determination of pharmaceuticals and personal care  
755 products in drinking water by solid phase extraction and liquid chromatography  
756 electrospray ionisation tandem mass spectrometry (LC/ECS-MS/MS). EPA 815-R-  
757 15-012. 35 p.
- 758 Van De Steene, J.C., Mortier, K.A., Lambert, W.E., 2006. Tackling matrix effects during  
759 development of a liquid chromatographic–electrospray ionisation tandem mass  
760 spectrometric analysis of nine basic pharmaceuticals in aqueous environmental  
761 samples. *J. Chromatogr. A* 1123, 71–81.
- 762 Vasquez, M.I., Lambrianides, A., Schneider, M., Kümmerer, K., Fatta-Kassinos, D.,  
763 2014. Environmental side effects of pharmaceutical cocktails: What we know and  
764 what we should know. *J. Hazard. Mater.* 279, 169–189.
- 765 Vatovec, C., Phillips, P., Van Wagoner, E., Scott, T.-M., Furlong, E., 2016. Investigating  
766 dynamic sources of pharmaceuticals: Demographic and seasonal use are more  
767 important than down-the-drain disposal in wastewater effluent in a University City  
768 setting. *Sci. Total Environ.* 572, 906-914.
- 769 Verlicchi, P., Al Aukidy, M., Jelic, A., Petrović, M., Barceló, D., 2014. Comparison of  
770 measured and predicted concentrations of selected pharmaceuticals in wastewater  
771 and surface water: A case study of a catchment area in the Po Valley (Italy). *Sci.*  
772 *Total Environ.* 470–471, 844–854.
- 773 Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical  
774 compounds in urban wastewater: Removal, mass load and environmental risk after  
775 a secondary treatment-A review. *Sci. Total Environ.* 429, 123–155.
- 776 Wang, S., Cyronak, M., Yang, E., 2007. Does a stable isotopically labelled internal  
777 standard always correct analyte response? A matrix effect study on a LC/MS/MS  
778 method for the determination of carvedilol enantiomers in human plasma. *J. Pharm.*  
779 *Biomed. Anal.* 43, 701–707
- 780 Williams, R.J., Churchley, J.H., Kanda, R., Johnson, A.C., 2012. Comparing predicted  
781 against measured steroid estrogen concentrations and the associated risk in two  
782 United Kingdom river catchments. *Environ. Toxicol. Chem.* 31, 892–898.



783 Yu, K., Li, B., Zhang, T., 2012. Direct rapid analysis of multiple PPCPs in municipal  
784 wastewater using ultrahigh performance liquid chromatography–tandem mass  
785 spectrometry without SPE pre-concentration. *Anal. Chim. Acta* 738, 59–68.

786 Zhang, H., Du, M., Jiang, H., Zhang, D., Lin, L., Ye, H., Zhang, X., 2015. Occurrence,  
787 seasonal variation and removal efficiency of antibiotics and their metabolites in  
788 wastewater treatment plants, Jiulongjiang River Basin, South China. *Environ. Sci.*  
789 *Process. Impacts* 17, 225–234.

790

791

792

793

794

**Table 1.** Summary results (ng/L) for the River Foss from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency of detection for each sampling site are reported.

Compound	F1		F2		F3		F4		F5	
	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%
Amitriptyline	n.d.	0	n.d. – 25.7 (10.3)	92	1.2* – 12.2 (5.7)	100	n.d. – 11.2 (2.6)	83	n.d. – 6.4 (2.0*)	75
Atenolol	n.d.	0	18.9 – 100 (55.4)	100	12.3* – 98.2 (43.6)	100	13.7* – 97.8 (34.8)	100	10.1* – 67.0 (21.8)	100
Carbamazepine	n.d. – 11.8 (4.5)	67	19.0 – 195 (45.2)	100	8.7 – 194 (66.0)	100	12.5 – 175 (61.6)	100	11.4 – 193 (36.8)	100
Cimetidine	n.d. – 49.6 (19.8)	83	n.d. – 44.0 (19.9)	92	3.0* – 40.5 (10.6)	100	2.1* – 16.9 (7.3*)	100	n.d. – 11.8 (7.2*)	67
Citalopram	n.d.	0	5.0 – 71.4 (15.4)	100	3.8* – 31.0 (15.3)	100	3.1* – 13.5 (7.8)	100	n.d. – 11.4 (5.9)	83
Codeine	n.d. – 10.8 (5.9*)	83	8.0 – 101 (59.2)	100	11.5 – 84.2 (57.3)	100	12.9 – 97.7 (44.0)	100	12.0 – 64.7 (29.1)	100
Desvenlafaxine	n.d. – 55.8 (16.8)	83	25.8 – 268 (70.0)	100	4.6* – 195 (86.2)	100	11.7 – 170 (77.3)	100	8.5* – 96.4 (44.5)	100
Diazepam	n.d.	0	n.d. – 1.6* (n.d.)	8.3	n.d. – 1.6* (n.d.)	8.3	n.d. – 1.8* (n.d.)	8.3	n.d. – 2.3* (n.d.)	8.3
Diltiazem	n.d. – 4.1 (1.2*)	75	4.7 – 48.7 (16.4)	100	4.7 – 36.0 (14.5)	100	4.4 – 25.0 (10.6)	100	n.d. – 12.7 (5.8)	92
Diphenhydramine	n.d.	0	n.d. – 12.7 (9.5)	67	n.d. – 3.8 (n.d.)	25	n.d. – 1.6* (n.d.)	17	n.d. – 3.4 (n.d.)	8.3
Erythromycin	n.d. – 34.5 (20.2*)	58	26.8 – 242 (90.0)	100	15.0* – 263 (88.8)	100	18.8* – 142 (80.5)	100	14.4 – 116 (45.9)	100
Fexofenadine <sup>1</sup>	n.d. – 104 (24.9)	83	43.8 – 1144 (177)	100	17.2 – 956 (253)	100	27.5 – 638 (166)	100	26.4 – 268 (92.5)	100
Gabapentin	17.4* – 229 (82.7)	100	476 – 1429 (789)	100	260 – 1445 (843)	100	404 – 1183 (768)	100	223 – 1341 (544)	100
Hydrocodone	n.d. – 5.7 (n.d.)	43	11.2 – 91.8 (21.6)	100	6.4 – 60.3 (25.0)	100	6.8 – 43.5 (20.6)	100	5.2 – 22.2 (11.1)	100
Lidocaine	n.d. – 3.9 (2.6*)	58	4.6 – 40.4 (8.2)	100	1.7* – 39.7 (11.8)	100	3.1 – 36.9 (10.4)	100	n.d. – 16.0 (6.1)	92
Loratadine	n.d.	0	n.d.	0	n.d. – 6.46 (n.d.)	8.3	n.d.	0	n.d.	0
Metformin	45.2 – 291 (121)	100	246 – 1783 (856)	100	266 – 2339 (1117)	100	340 – 2595 (888)	100	263 – 1750 (664)	100

**Table 1.** Summary results for the River Foss from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency of detection for each sampling site are reported.

Compound	F1		F2		F3		F4		F5	
	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%
Norethisterone	n.d.	0	n.d. – 7.4* (n.d.)	8.3	n.d.	0	n.d.	0	n.d.	0
Oseltamivir	n.d.	0	n.d. – 8.8* (n.d.)	8.3	n.d.	0	n.d.	0	n.d.	0
Oxazepam	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Paracetamol	n.d. – 119 (60.0)	67	14.3* - 749 (74.4)	100	n.d. – 9822 (97.2)	92	32.0 – 9676 (209)	100	25.0 – 5445 (180)	100
Propranolol	n.d.	0	n.d. – 64.9 (17.8)	92	n.d. – 29.9 (20.1)	92	n.d. – 20.6 (10.0*)	92	n.d. – 18.3 (10.4*)	50
Raloxifene	n.d.	0	n.d.	0	n.d. -7.2*	8.3	n.d. – 7.2*	8.3	n.d.	0
Ranitidine	n.d. – 10.8* (n.d.)	17	n.d. – 69.6 (53.4)	83	6.6* – 74.0 (27.9)	100	n.d. – 60.6 (22.2)	92	n.d. – 30.0 (13.6*)	92
Sertraline	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d. - 21.2 (n.d.)	8.3
Sitagliptin	n.d.	0	16.5 – 121 (35.2)	100	9.3* - 103 (46.5)	100	15.2 – 85.7 (36.9)	100	12.2* – 33.9 (19.5)	100
Sulfamethoxazole	n.d.	0	n.d. – 10.2* (n.d.)	33	n.d. – 33.0 (n.d.)	50	n.d. – 27.5 (n.d.)	42	n.d. – 18.1* (n.d.)	17
Temazepam	n.d.	0	n.d. – 38.2 (12.1)	67	n.d. – 25.0 (16.7)	75	n.d. – 27.8 (15.9)	67	n.d. – 12.6 (7.1*)	58
Tramadol	n.d. – 48.1 (31.2)	75	54.4 – 650 (117)	100	21.0 – 456 (177)	100	34.0 – 368 (169)	100	29.2 – 201 (84.7)	100
Triamterene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Trimethoprim	n.d. – 9.8 (2.5*)	75	13.2 - 76.0 (30.3)	100	10.1- 60.3 (26.4)	100	15.2 – 49.4 (19.8)	100	5.3 – 38.0 (13.8)	100
Venlafaxine	n.d. – 4.3 (2.2*)	42	9.2 – 102 (16.2)	100	2.4* – 82.6 (20.6)	100	5.9 – 37.9 (17.6)	100	2.3* -17.8 (9.2)	100
Verapamil	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0

\*Below LOQ

<sup>1</sup> data for 11 months only available (April 2016 missing).

n.d. No detect

(Med) Median

% Detection frequency (100% = 12 months)



**Table 2.** Summary results (ng/L) for the River Ouse from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency detection for each sampling site are reported.

Compound	O1		O2		O3		O4		O5		O6	
	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%
Oxazepam	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Paracetamol	22.3* – 191 (46.4)	100	15.4* - 202 (51.7)	100	16.8* – 186 (54.5)	100	20.1* – 186 (54.3)	100	22.7 – 369 (77.6)	100	21.2 – 226 (66.9)	100
Propranolol	n.d.	0	n.d.	0	n.d. – 8.3* (n.d.)	33	n.d.	0	n.d.	0	n.d. – 7.6* (n.d.)	8
Raloxifene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Ranitidine	n.d. -10.3* (n.d.)	25	n.d. – 10.5* (n.d.)	25	n.d. – 30.6 (15.1*)	75	n.d. - 13.3* (n.d.)	42	n.d. – 12.0* (n.d.)	25	n.d. – 15.5* (9.2*)	75
Sertraline	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Sitagliptin	n.d. – 10.7 (n.d.)	33	n.d. – 16.2 (9.3*)	75	n.d. – 32.5 (15.0)	92	n.d. – 16.9 (12.0*)	83	n.d. – 15.8 (10.4*)	83	n.d. – 26.5 (18.2)	92
Sulfamethoxazole	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Temazepam	n.d.	0	n.d.	0	n.d. – 7.2* (n.d.)	8	n.d.	0	n.d. – 4.4* (n.d.)	8	n.d. – 4.7* (n.d.)	8
Tramadol	n.d. – 27.0 (19.6)	83	3.9* - 39.9 (19.8)	100	n.d. – 57.2 (34.6)	92	n.d. – 44.8 (28.9)	92	n.d. – 47.4 (27.4)	92	20.7 – 52.4 (40.5)	100
Triamterene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Tramadol	n.d. – 27.0 (19.6)	83	3.9* - 39.9 (19.8)	100	n.d. – 57.2 (34.6)	92	n.d. – 44.8 (28.9)	92	n.d. – 47.4 (27.4)	92	20.7 – 52.4 (40.5)	100
Triamterene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Trimethoprim	n.d. – 19.0 (2.7)	92	2.0* – 8.9 (5.3)	100	2.8* - 19.3 (12.4)	100	n.d. – 11.1 (5.4)	92	2.3* - 12.1 (5.5)	100	7.3 – 22.9 (14.2)	100
Venlafaxine	n.d. – 2.6* (n.d.)	42	n.d. – 5.2 (2.6*)	75	n.d. – 8.5* (4.9)	83	n.d. – 4.3 (2.9*)	75	n.d. – 5.0 (3.1)	75	n.d. – 8.2 (4.5)	83
Verapamil	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0

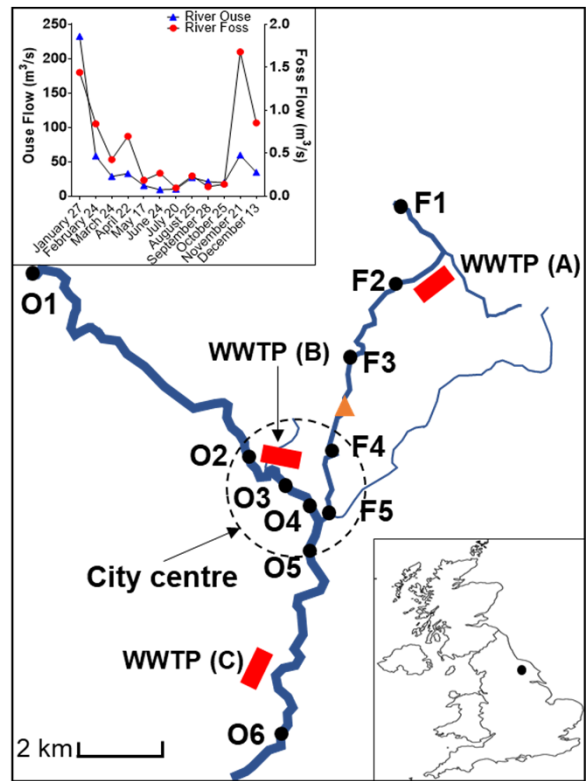
\* Below LOQ

<sup>1</sup> data for 11 months only available (April 2016 missing).

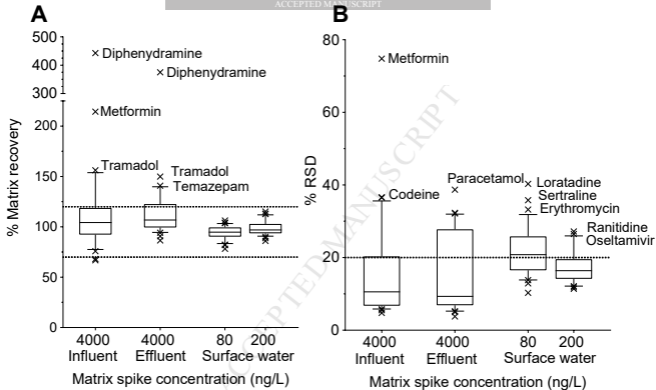
n.d. No detect

(Med) Median

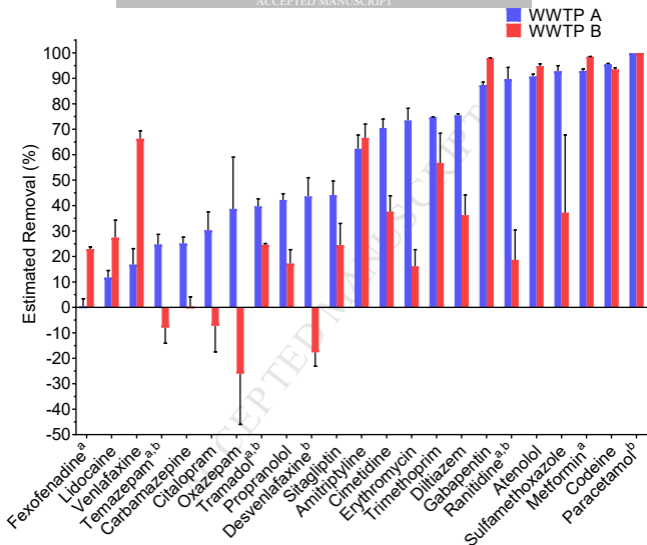
% Detection frequency (100% = 12 months)



**Figure 1.** Map of 11 sampling sites within the sampling network. River flows recorded from a gauge in each river (orange triangle) from each sampling day ( $\text{m}^3/\text{s}$ ) are pictured top left. WWTPs that serve the city (3) are represented by the red rectangles. Sites F1-F5 are along the smaller River Foss, while sites O1-O6 are along the larger River Ouse.

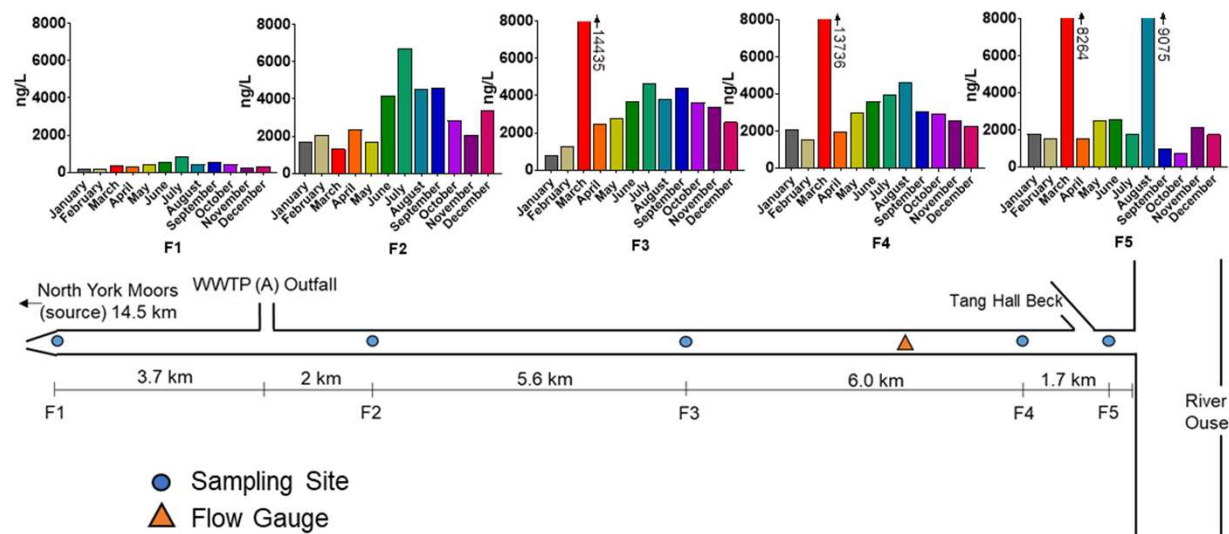


**Figure 2.** A) Routine matrix spikes run alongside environmental samples during the 12-month monitoring campaign in WWTP influent, effluent, surface water and reagent water. The dotted lines represent the 70 – 120% acceptable recovery range. B) %RSD of matrix spike replicates. An RSD below 20% is desirable (depicted with dotted line). The median, 25th and 75th quartiles are presented while the whiskers represent the 10th to 90th percentile, compounds outside this range are depicted with an X.

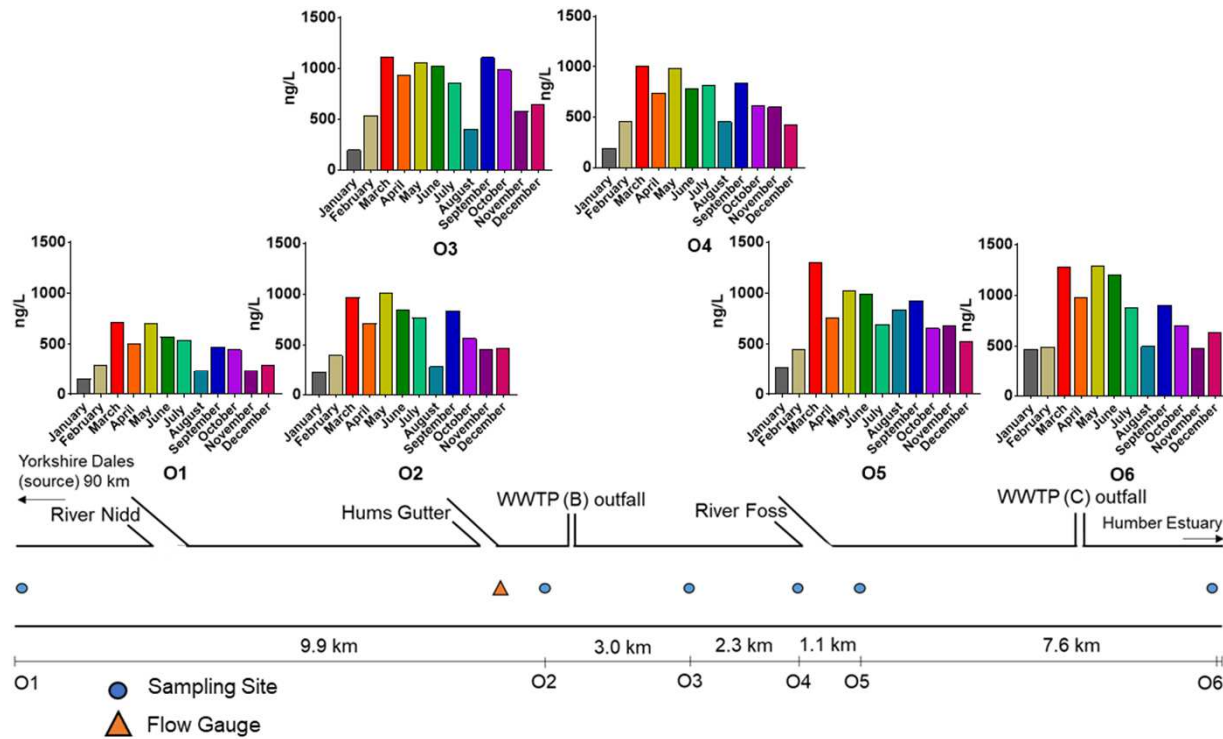


**Figure 3.** Estimated % removal in WWTP A (trickling filter), WWTP B (carbon activated sludge). Hydrocodone not shown, estimated removal in WWTP A -307% and in WWTP B -597%. Matrix recovery was outside the 70 -120% desired range is identified for (a) influent and (b) effluent.

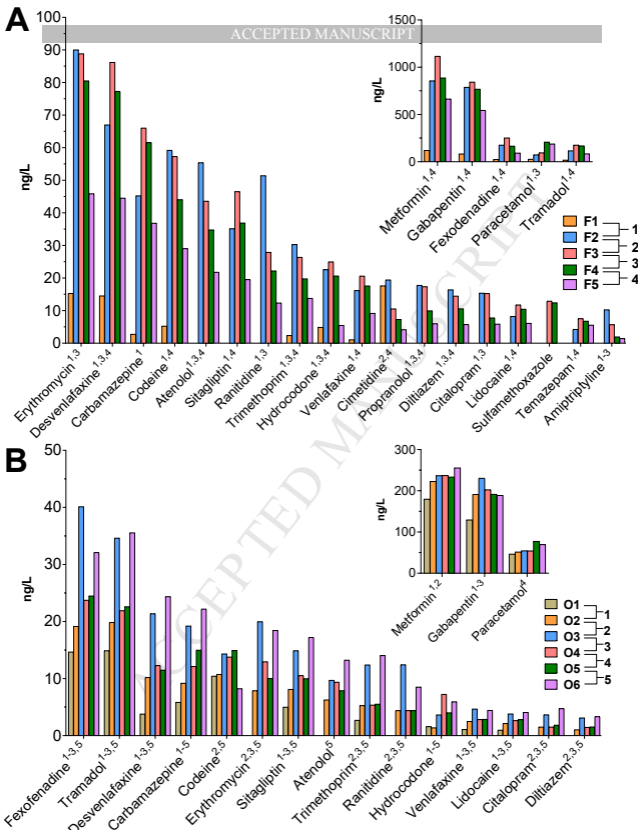


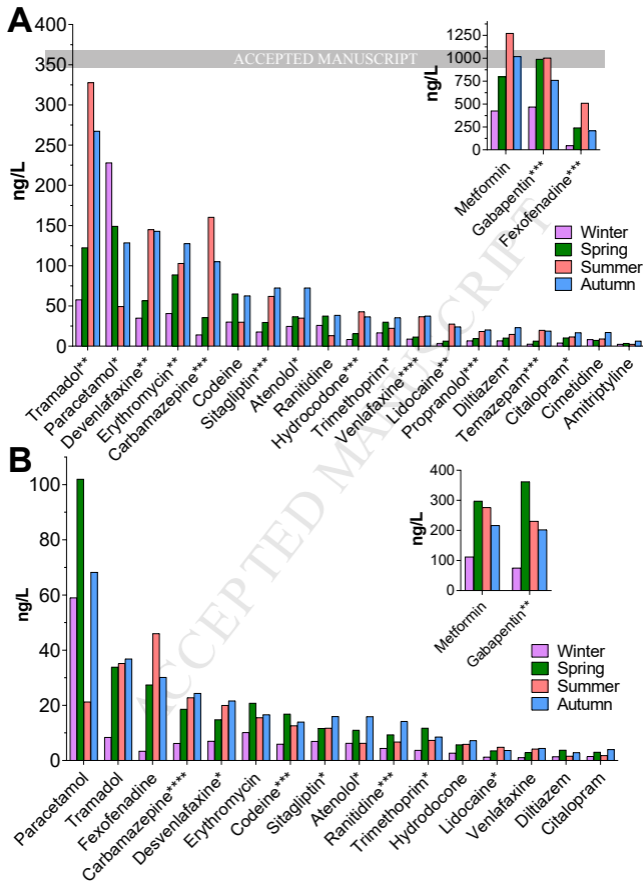


**Figure 4.** Total pharmaceutical concentration (summed) of all detected analytes at each sampling site from each month during 2016 in the River Foss. Sampling locations (blue circles) in relation to Environment Agency Flow gauges (orange triangles) are depicted along the river.

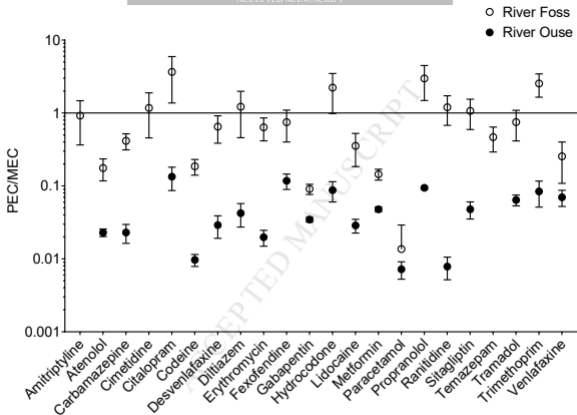


**Figure 5.** Total pharmaceutical concentration (summed) of all detected analytes at each sampling site from each month during 2016 along the River Ouse. Sampling locations (blue circles) in relation to Environment Agency Flow gauges (orange triangles) are depicted along the river.





**Figure 7.** Median seasonal concentration from sites F3-F4 in the River Foss (A) and O3-O4 in the River Ouse (B) for select pharmaceuticals. Temporal variations were tested using Friedman's Test and results are reported for each compound where a significant result was found,  $p < 0.05$  (\*),  $p < 0.01$  (\*\*),  $p < 0.0005$  (\*\*\*),  $p < 0.0001$  (\*\*\*\*).



**Figure 8.** The annual average PEC/MEC ratios are plotted for the River Foss (open circles) and the River Ouse (closed circles). PECs were calculated for each river based on experimental WWTP removals and the average flow from sampling days. PEC/MEC ratios were calculated for site F2-F5 and O3-O6 and averaged, error bars represent the standard deviation.

## Highlights

- 11 sites from two nested river systems sampled monthly for one year.
- Seasonal and spatial variation due to flow, usage and compound stability.
- Removal efficiency estimated for 24 pharmaceuticals in two WWTPs.
- Disagreement between measured concentrations and exposure model predictions.