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

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- 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.

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

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

1.0 Introduction

Determining pharmaceutical exposures in environmental matrices has become a substantial area of research since the 1990s (Daughton, 2016). The presence of pharmaceuticals in freshwater systems has now been documented globally, with research especially focused in Europe and North America (aus der Beek et al., 2016). Pharmaceuticals primarily enter the environment through patient use when an unmetabolised fraction is excreted and subsequently passes through wastewater treatment plants (WWTPs), which are typically not designed to remove such organic contaminants (Luo et al., 2014). Consequently, WWTPs are significant sources of pharmaceuticals to the environment (Lindholm-Lehto et al., 2016). A recent study of

United Kingdom (UK) WWTPs estimated that 13% of effluent discharges could pose risks to the receiving environment regarding pharmaceutical exposures (Comber et al., 2018). Removal rates are highly variable between treatment types (Kasprzyk-Hordern et al., 2009; Luo et al., 2014), seasons (Golovko et al., 2014), and even within treatment plants themselves (Verlicchi et al., 2012). Moreover, removal rates have only been estimated for a small fraction of the total number of pharmaceuticals in use (Boxall et al., 2014) and only a few studies have reported WWTP removals in the UK specifically (Comber et al., 2018; Kasprzyk-Hordern et al., 2009, 2008). WWTP removal rates are valuable parameters, and their inclusion in occurrence modelling substantially improves the accuracy of pharmaceutical exposure predictions (Burns et al., 2017; Verlicchi et al., 2014).

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

To adequately characterise the fate of pharmaceuticals in the environment, robust monitoring campaigns which include seasonal or year-long sampling covering a range of compounds at a reasonable spatial resolution are required. However, only a small number

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

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

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

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

2.0 Methods

2.1 Study area and sample collection

2.1.1 Study Compounds

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

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

2.1.2 Study Area

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

2.1.3 Sample Collection

All samples collected were subject to the same sampling protocol. At each site, three 1-L field replicates were collected from the centroid of flow (when possible); sampling sites had been previously determined to be well-mixed, therefore sampling in a single location was deemed appropriate (Supplementary Material, Figure S1). For each field replicate, a 10-mL aliquot was drawn into a 24-mL disposable syringe and filtered through a primed 0.7-µm glass-fibre filter (GF/F) (Whatman Inc.) into an amber glass vial and immediately

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

172 2.2 High performance liquid chromatography-tandem mass spectrometry

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

184 Internal standard (IS) calibration was used to quantify the pharmaceuticals in the
185 method described. For reasons of expense and availability, not all pharmaceuticals had a
186 corresponding isotopically labelled internal standard (ILIS) (Supplementary Material,
187 Table S3). In these cases, atrazine-d_5 was used and has been previously determined
188 suitable for this role (Furlong et al., 2014). Samples were fully thawed and a 995-µL aliquot
189 pipetted into a 1.5-mL LC vial and a 5-µL spike of IS solution (80 ng/L) added. Samples
190 were immediately analysed after preparation. Peak detection criteria were in accordance
191 with Commission Decision 2002/657/EC (European Commission, 2002). Due to analytical
192 complications, fexofenadine could not be quantified in the April surface-water samples.
193 Further details of peak qualification and quantitation are provided in the Supplementary
194 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 by
209 spiking 20 µL of 80 ng/L or 200 ng/L calibration solution into a sample replicate with 5 µL

of IS solution. The much higher ambient concentration of pharmaceuticals in WWTP influent and effluent required the matrix spike samples to be prepared at a higher concentration, 4000 ng/L. Matrix recovery was calculated by subtracting the ambient sample concentration and dividing by the concentration spiked.

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

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

2.3 Analytical method validation

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

2.4 WWTP removal efficiency

Due to access restrictions, 24 h composite samples for influent and effluent could only be collected once from WWTP A and B during summer 2016 (Supplementary Material, Table S2). Only grab samples which are unsuitable for estimating removals could

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

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

2.5 Statistical Analysis

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

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

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 or 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), while
266 wastewater generation was assumed to be 200 L/person·day (European Medicines
267 Agency, 2006). Experimental WWTP removal rates (Eqn. 1) were used with river specific
268 dilution factors based on the average flow from sampling days to generate a PEC for both
269 rivers. PEC calculations were based on the approach suggested by the European
270 Medicines Agency (2006). Parameters and equations used to predict the PECs are
271 provided in the Supplementary 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 %RSD)
276 analysis days according to USEPA (2016) guidelines and Boix et al. (2015) where an
277 RSD≤ 20% above the LOQ (i.e. 80 ng/L) is desirable. The limits of detections (LOD)
278 ranged from 0.9 ng/L (carbamazepine) to 12.4 ng/L (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 70
282 – 120% recovery range for concentrations of 80 and 200 ng/L, indicating that throughout
283 the sample analysis quantification was not unacceptably impaired due to matrix effects
284 (Figure 2). Matrix effects were observed in WWTP effluent and influent, a phenomenon

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

299 3.2 Pharmaceuticals in WWTPs

300 The highest summed pharmaceutical concentrations in influent were observed in
301 samples from WWTP B, while highest summed concentrations in effluent were observed
302 in samples taken at WWTP A. Paracetamol had the highest concentration in all WWTP
303 influents, 282319, 185878 and 116810 ng/L at WWTP B, A and C, respectively. In effluent,
304 gabapentin had the highest concentration (8541 ng/L) at WWTP C followed by metformin
305 (6111 ng/L) at WWTP A and fexofenadine (2094 ng/L) in effluent at WWTP C. Seven
306 pharmaceuticals (diphenhydramine, norethisterone, oseltamivir, raloxifene, sertraline,
307 triamterene and verapamil) were not detected in any WWTP sample. Average
308 concentration and standard deviation (SD) of WWTP influent and effluent samples are
309 reported in the Supplementary Data Table S10.

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

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

336 employing similar treatments, a conclusion also observed in other regions (Verlicchi et al.,
337 2012). The single sampling event in the WWTPs is limited, however these estimates are
338 still useful for comparative purposes. For example, sitagliptin removal efficiency (25 - 40%)
339 has not been previously reported to the authors' knowledge. Therefore, while WWTPs are
340 significant sources of pharmaceuticals entering the environment, analysis of WWTP
341 removal efficiencies (i.e. reduction in parent pharmaceutical concentration from influent to
342 effluent) as documented in this and previously published studies, demonstrate that
343 WWTPs are generally decreasing the aquatic environmental burden by significantly
344 reducing certain parent pharmaceutical concentrations (not considering degradates or
345 transformation products) for many of the compounds studied.

346 3.3 Pharmaceuticals in Surface Water

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

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

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

Metformin, a type II diabetes drug, had the highest annual median concentration (1117 and 237 ng/L in the Foss and Ouse, respectively), followed by gabapentin (anti-convulsant) (843 and 230 ng/L, Foss and Ouse, respectively) and paracetamol (analgesic) (209 and 77.6 ng/L, Foss and Ouse, respectively). This trend is different from those observed in previous temporal exposure campaigns studying similar compounds throughout the world. For example in China, Zhang et al. (2015) studied urbanized rivers and found antibiotics to be the most frequently detected pharmaceuticals. They did, however, report atenolol as having one of the highest annual median concentrations (53 ng/L), which is similar to the median concentration for this compound reported at site F2 (55.4 ng/L) in the current study. In Spain, Camacho-Munoz et al. (2010) reported propranolol most frequently detected in surface water, with a higher average concentration (80 ng/L) than observed in this study (20.1 ng/L). In Portugal, Paíga et al. (2016) reported carbamazepine the most frequently detected pharmaceutical with an annual median of 31.7 ng/L, while other similarly studied compounds, citalopram and venlafaxine had

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

433 3.3.1 Spatial Trends

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

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

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

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

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

3.3.2 Seasonal Variability

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

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

501 The reasons for temporal variations in pharmaceutical concentrations have varied
502 between studies with several reporting flow as the major driver, observing higher
503 concentrations during times of low flow (Kasprzyk-Hordern et al., 2008; Kolpin et al.,
504 2004). Others suggest higher pharmaceutical concentrations in winter months coincide
505 with higher winter usage patterns (Sun et al., 2014) or decreased biodegradation in winter
506 (Moreno-González et al., 2014), while others found no significant differences between
507 sampled seasons (Camacho-Munoz et al., 2010). Due to higher concentrations coinciding
508 with low-flow months in this study, we also suggest that flow appears to be a major driver
509 behind the observed seasonal variability in pharmaceutical concentrations in the current
510 study. The lack of significant seasonal differences found in the River Ouse could be
511 explained by a lower annual variability in flow on sampling days than the River Foss (i.e.
512 two orders of magnitude versus three). Further detailed investigation into the drivers
513 behind the pharmaceutical concentrations observed both temporally and spatially is
514 required to differentiate between the possible explanations, and could include comparing
515 results with prescription data and flow and estimating the impact of in-stream losses
516 seasonally in different climates, and in river hydrological properties (e.g. depth and flow).

517 Such analyses will be facilitated by the detailed pharmaceutical monitoring data reported
518 in this study.

519

520 3.4 Comparisons of PECs and MECs

521 The PEC/MEC ratios for each compound for which it was possible to calculate an
522 annual average MEC are reported in Figure 8. A ratio greater than 1 indicates PECs were
523 higher than MECs and lower when less than 1. The PECs are severely underestimated in
524 the Ouse; this may be due to pharmaceuticals being transported from upstream or
525 problems with sewer connectivity within the sampling network not being accounted for in
526 the simplistic PEC calculation. Several studies have attempted to gauge the accuracy of
527 PECs by calculating a ratio with MECs, however the criterion for what constitutes accurate
528 is variable across studies (Burns et al., 2017). This assessment has been previously
529 limited to a small number of compounds and based on a limited number of sampling
530 events not representative of the annual average MEC which the PEC was designed to
531 predict. In this way, we present novel findings that indicate when an annual average MECs
532 is calculated, less hydrologically complex river systems where pharmaceutical sources
533 are limited, PECs characterise annual exposure within a factor 2 for 41% of compounds
534 in this study (average factor 2.8), with no factor greater than 11. However paracetamol is
535 an exception (underestimated by a factor of 73); the usage estimate did not incorporate
536 over-the-counter contributions therefore underestimates were not unexpected (Burns et
537 al., 2017). Conversely, the results from the River Ouse indicate that major limitations are
538 associated with this predictive approach. All ratios were off by a factor of at least 7
539 (average 27) and up to 139, which according to studies characterising the PEC/MEC, is
540 outside an acceptable range (Verlicchi et al., 2014).

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

4.0 Conclusion

A rapid determination HPLC-MS/MS method for 33 pharmaceuticals was validated and applied in a 12-month spatiotemporal pharmaceutical exposure campaign. WWTP removal efficiency was found to be similar between CAS and trickling filter technology for the target pharmaceuticals. Pharmaceutical concentrations in two contrasting river systems that run through the city of York, UK were found to vary significantly spatially and temporally, with the greatest variation observed for paracetamol in the River Foss, ranging from not detected to over 9822 ng/L. Temporal variations in concentration were less frequently observed in the larger River Ouse, potentially due to the lower variability in flow which could be an important driver behind pharmaceutical concentrations in the study

567 system. PEC/MEC ratios indicated that compounds in both rivers were generally
568 underestimated by commonly used simple predictive exposure algorithms. In total, 41%
569 of PEC/MEC ratios for the River Foss data were within a factor of 2, while for the River
570 Ouse average ratios indicated predictions were off by a factor of 27. This analytical method
571 and extensive monitoring results will be instrumental in improving the understanding of
572 temporal pharmaceutical fate and occurrence in river systems.

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585

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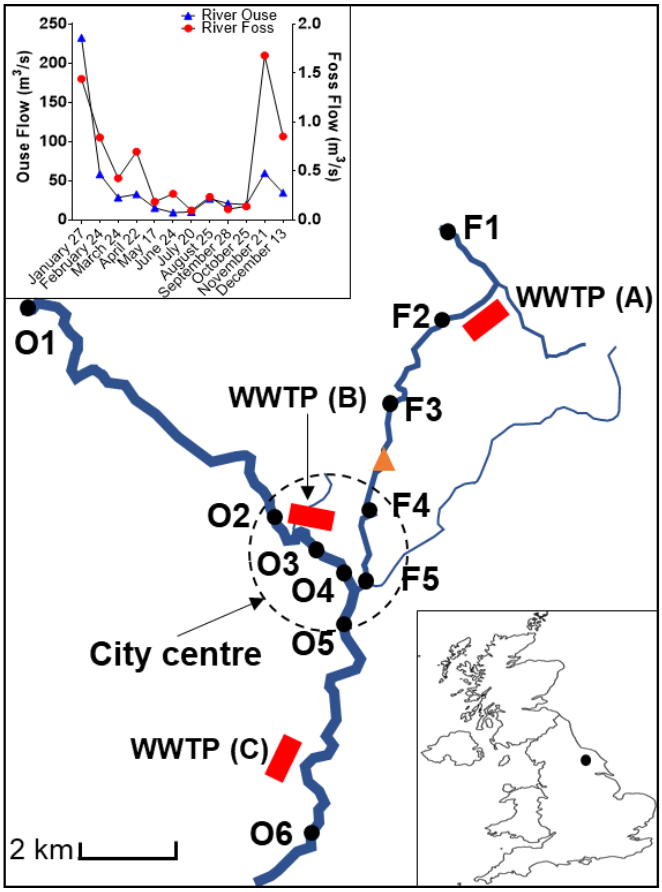

Figure 1. Map of 11 sampling sites within the sampling network. River flows recorded from a gauge in each river (orange triangle) from each sampling day (m^3/s) are pictured top left. WWTPs that serve the city (3) are represented by the red rectangles. Sites F1-F5 are along the smaller River Foss, while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

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[1]	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

[1] 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)	%
Amitriptyline	n.d.	0	n.d.	0	n.d. – 2.7 (n.d.)	17	n.d. -1.2* (n.d.)	17	n.d. – 1.5* (n.d.)	8	n.d. -2.5 (n.d.)	17
Atenolol	n.d.	0	n.d. – 22.0 (11.1*)	58	n.d. – 19.5 (10.7*)	67	n.d. – 16.9* (10.2*)	75	n.d. – 20.4 (10.4*)	67	n.d. – 18.8 (13.6*)	92
Carbamazepine	1.0* – 14.0 (5.8)	100	1.1* - 34.8 (9.2)	100	1.4* - 54.4 (19.2)	100	1.1* - 31.4 (12.1)	100	1.7* - 33.9 (15.0)	100	7.9 – 48.0 (23.4)	100
Cimetidine	n.d. – 2.3* (n.d.)	8	n.d. – 2.4* (n.d.)	8	n.d. - 5.7* (n.d.)	33	n.d. – 2.9* (n.d.)	17	n.d.	0	n.d. – 3.7 (n.d.)	42
Citalopram	n.d. - 3.3* (n.d.)	8	n.d. – 3.7* (n.d.)	33	n.d. – 7.0 (4.0*)	75	n.d. – 3.2* (n.d.)	50	n.d. – 4.0* (2.2*)	67	n.d. – 7.2 (4.8)	83
Codeine	n.d. – 13.5 (10.5*)	92	3.3 – 17.1 (10.7)	100	3.0* – 20.5 (14.3)	100	3.5* – 17.5 (13.8)	100	4.5* – 17.4 (14.9)	100	6.4* - 17.8 (8.8)	100
Desvenlafaxine	n.d. – 14.8 (n.d.)	50	n.d. – 27.5 (11.3)	75	n.d. – 46.8 (21.5)	83	n.d. -31.0 (14.2)	83	n.d. – 28.8 (15.2)	75	12.3 – 40.1 (26.8)	100
Diazepam	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Diltiazem	n.d. – 1.6* (n.d.)	25	n.d. – 2.5 (n.d.)	50	n.d. – 8.0 (3.6)	92	n.d. – 6.4 (1.8*)	67	n.d. – 3.7 (1.8*)	75	n.d. – 4.3 (3.7)	92
Diphenhydramine	n.d.	0	n.d. – 1.7* (n.d.)	8	n.d.- 2.9 (n.d.)	25	n.d.	0	n.d.- 4.8 (n.d.)	8	n.d. - 2.2* (n.d.)	8
Erythromycin	n.d.	0	n.d. – 17.3* (n.d.)	33	n.d. – 31.1 (21.3*)	92	n.d. – 20.3* (15.3*)	67	n.d. – 21.7* (n.d.)	50	n.d. – 33.9 (21.3*)	83
Fexofenadine[1]	n.d. – 41.7 (17.9)	83	n.d. – 48.7 (24.1)	83	n.d. – 77.8 (46.1)	92	n.d. – 68.2 (25.8)	83	n.d. – 44.0 (29.2)	92	7.4* – 98.5 (33.4)	100
Gabapentin	28.1* -242 (130)	100	39.4 – 351 (191)	100	24.5* - 429 (230)	100	30.0* - 369 (202)	100	33.8* - 364 (192)	100	39.5 – 450 (208)	100
Hydrocodone	n.d. – 2.9 (n.d.)	50	n.d. – 5.7 (3.6)	83	n.d. – 14.9 (7.8)	92	n.d. – 8.0 (4.0)	92	n.d. – 6.9 (4.0)	92	2.2 – 10.7 (6.0)	100
Lidocaine	n.d. – 4.1 (n.d.)	50	n.d. – 5.0 (2.7*)	83	n.d. – 6.5 (3.7)	92	n.d. – 5.4 (2.8)	83	n.d. – 5.6 (3.1)	83	1.6* – 8.8 (4.1)	100
Loratadine	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Metformin	52.5 – 323 (180)	100	63.4 – 431 (223)	100	60.6 – 422 (237)	100	60.2 – 422 (237)	100	73.6 – 445 (233)	100	142 – 483 (276)	100

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)	%
Norethisterone	n.d.	0	n.d. -7.7 (n.d.)	8	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Oseltamivir	n.d.	0	n.d.	0	n.d.	0	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	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

[1] data for 11 months only available (April 2016 missing).

n.d. No detect

(Med) Median

% Detection frequency (100% = 12 months)

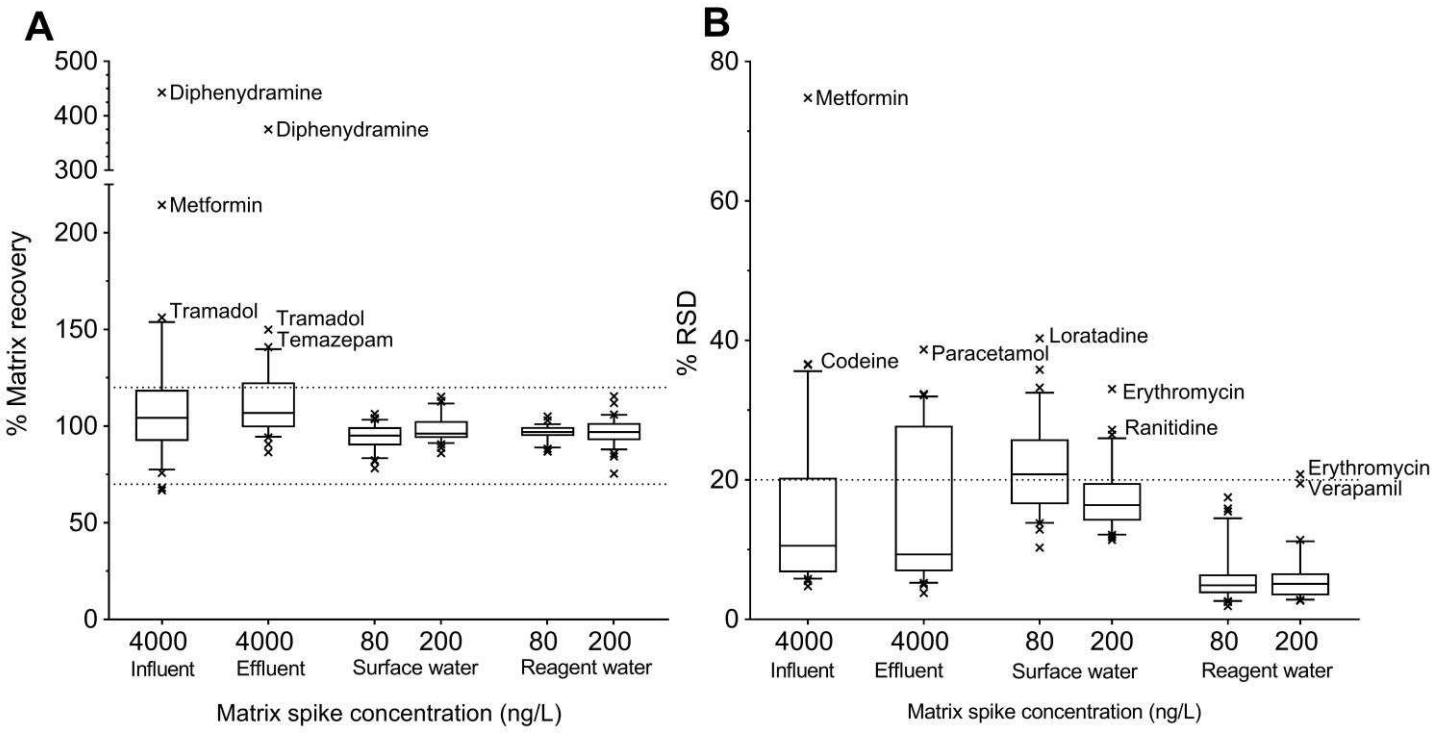

Figure 2. A) Routine matrix spikes run alongside environmental samples during the 12 month monitoring campaign in WWTP influent, effluent, surface water and reagent water. The dotted lines represent the 70 – 120% acceptable recovery range. B) %RSD of matrix spike replicates. An RSD below 20% is desirable (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.

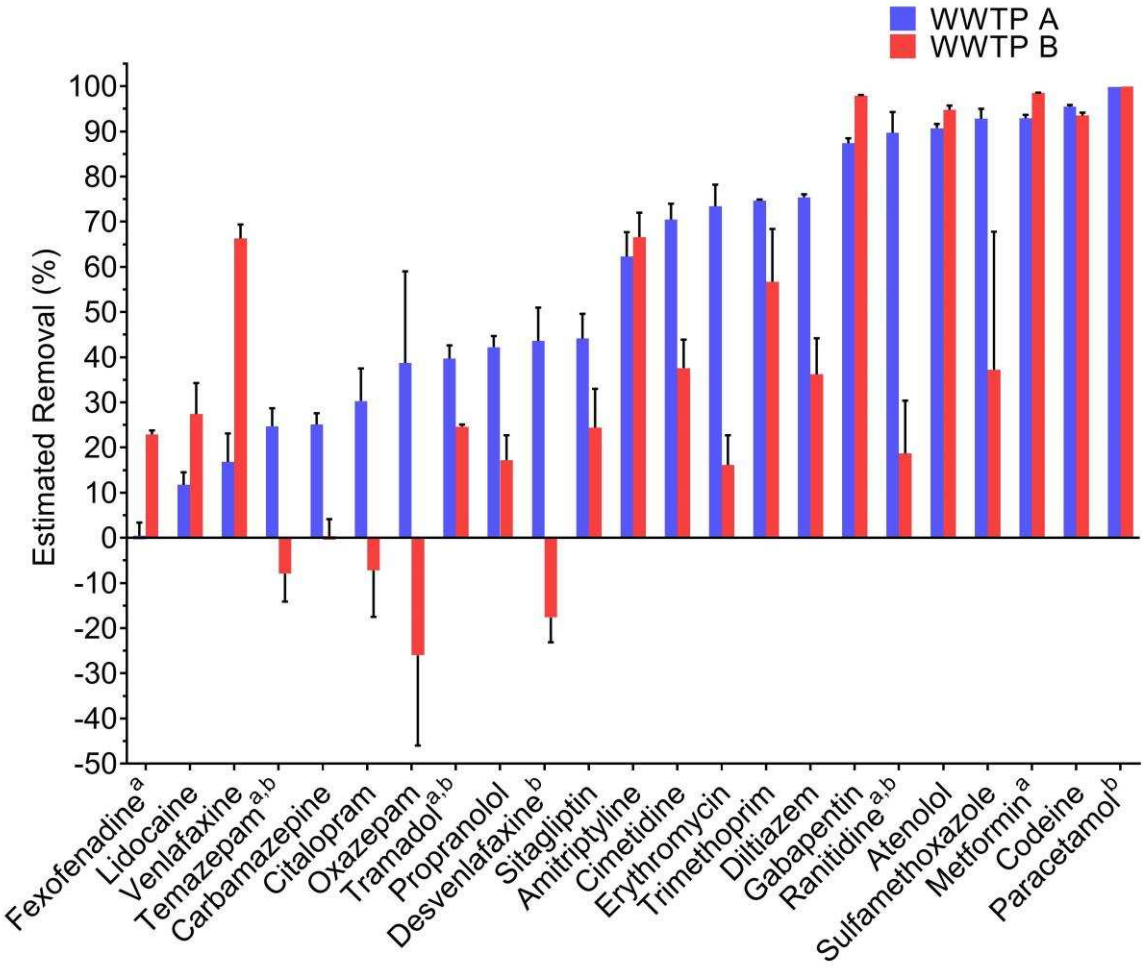

Figure 3. Estimated % removal in WWTP A (trickling filter), WWTP B (carbon activated sludge). Hydrocodone not shown, estimated removal in WWTP A -307% and in WWTP B -597%. Matrix recovery outside the 70 – 120% desired range is identified with an (a) for influent and (b) for effluent.

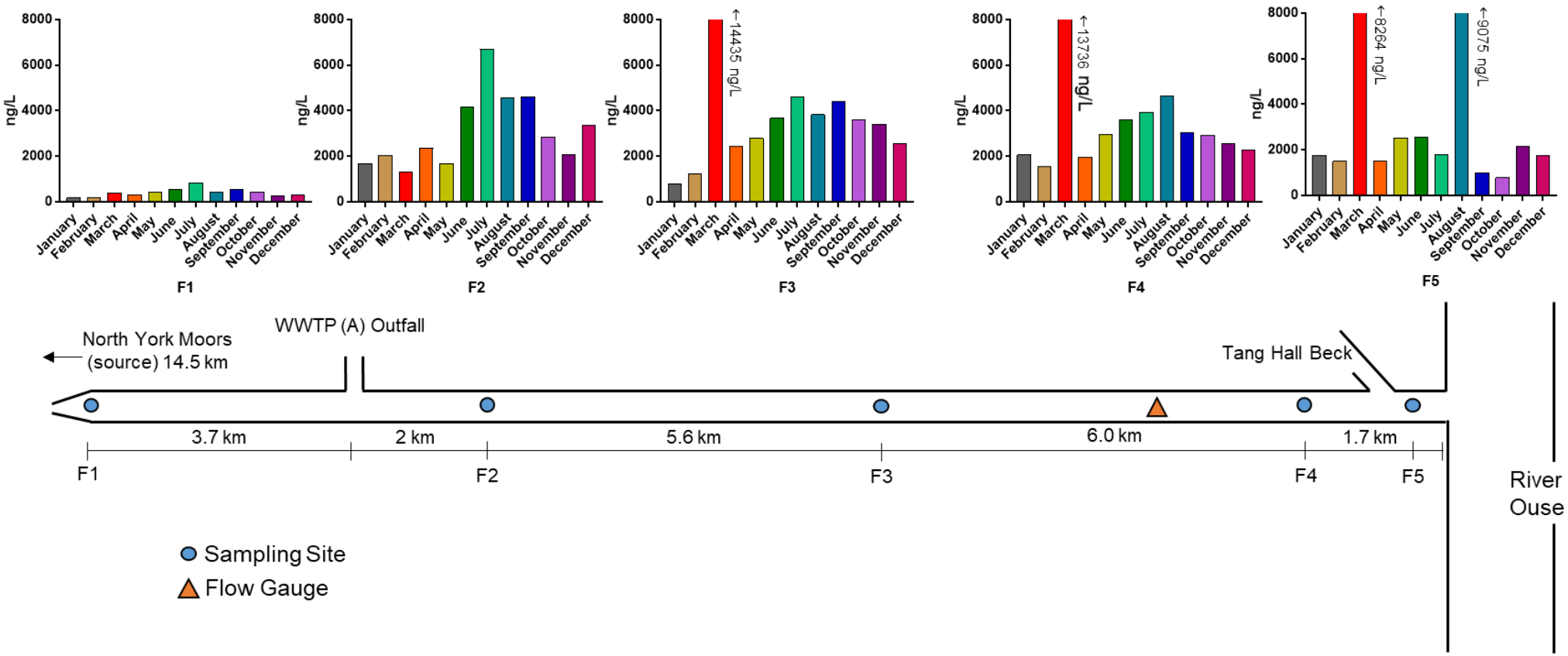

Figure 4. Total pharmaceutical concentration (summed) of all detected analytes at each sampling site from each month during 2016 in the River Foss. Sampling locations (blue circles) in relation to Environment Agency Flow gauges (orange triangles) are depicted along the river.

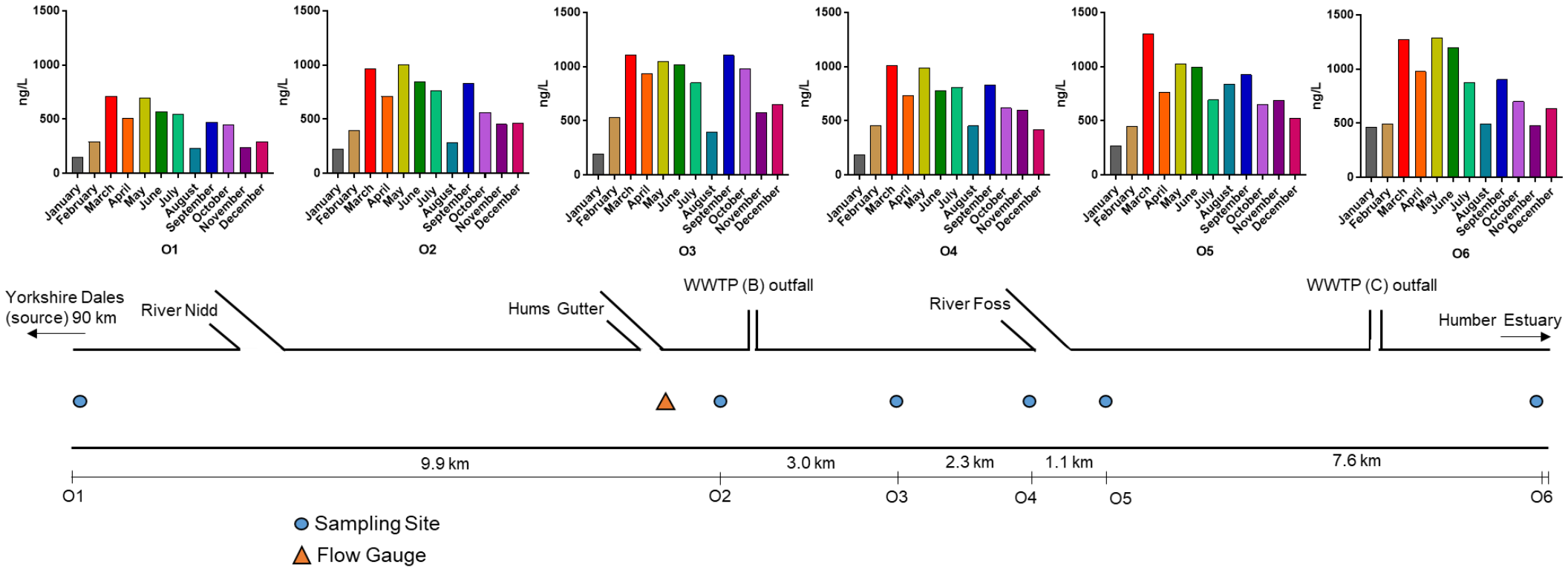

Figure 5. Total pharmaceutical concentration (summed) of all detected analytes at each sampling site from each month during 2016 along the River Ouse. Sampling locations (blue circles) in relation to Environment Agency Flow gauges (orange triangles) are depicted along the river.

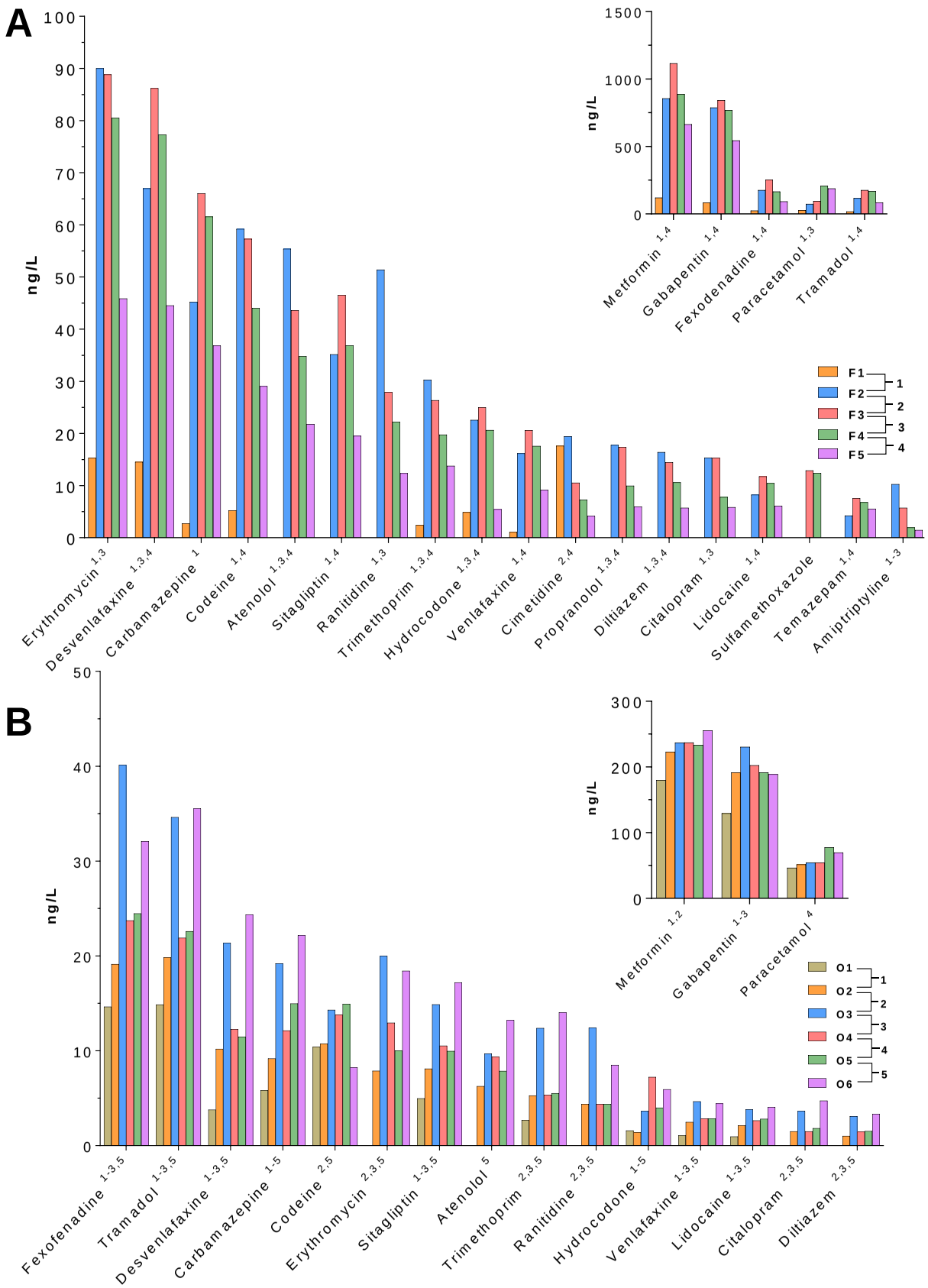

Figure 6. Annual median concentration from all sampled sites in (A) the River Foss and (B) River Ouse. Pairwise t-tests were conducted between neighbouring sites and significant differences are denoted by the corresponding number. Sites F1-F2, O1-O2 =1; F2-F3, O2-O3 =2; F3-F4, O3-O4 =3; F4-F5, O4-O5 =4; O5-O6 =5.

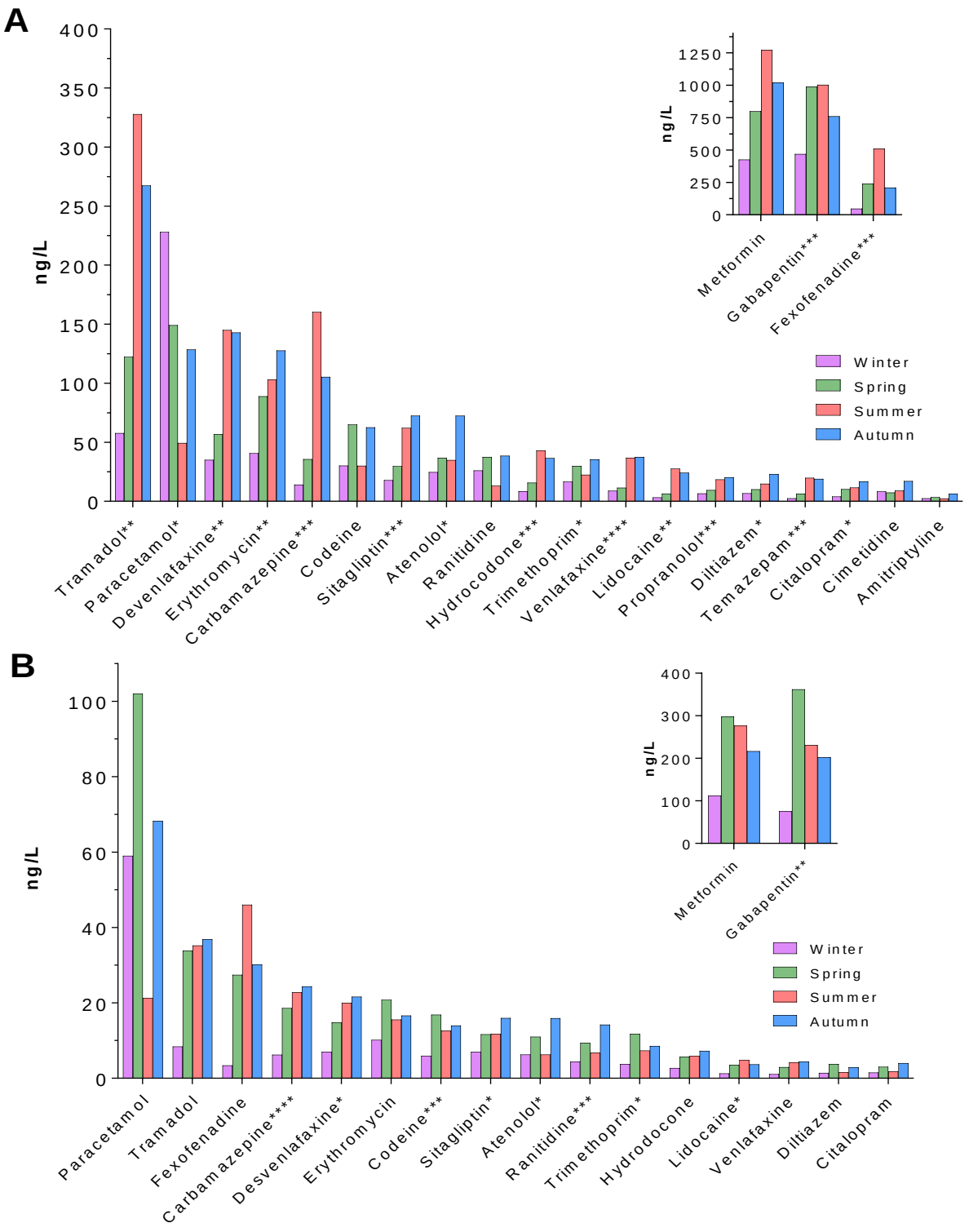

Figure 7. Median seasonal concentration from sites F3-F4 in the River Foss (A) and O3-O4 in the River Ouse (B) for select pharmaceuticals. Temporal variations were tested using Friedman's Test and results are reported for each compound where a significant result was found, $p<0.05$ (*), $p<0.01$ (**),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

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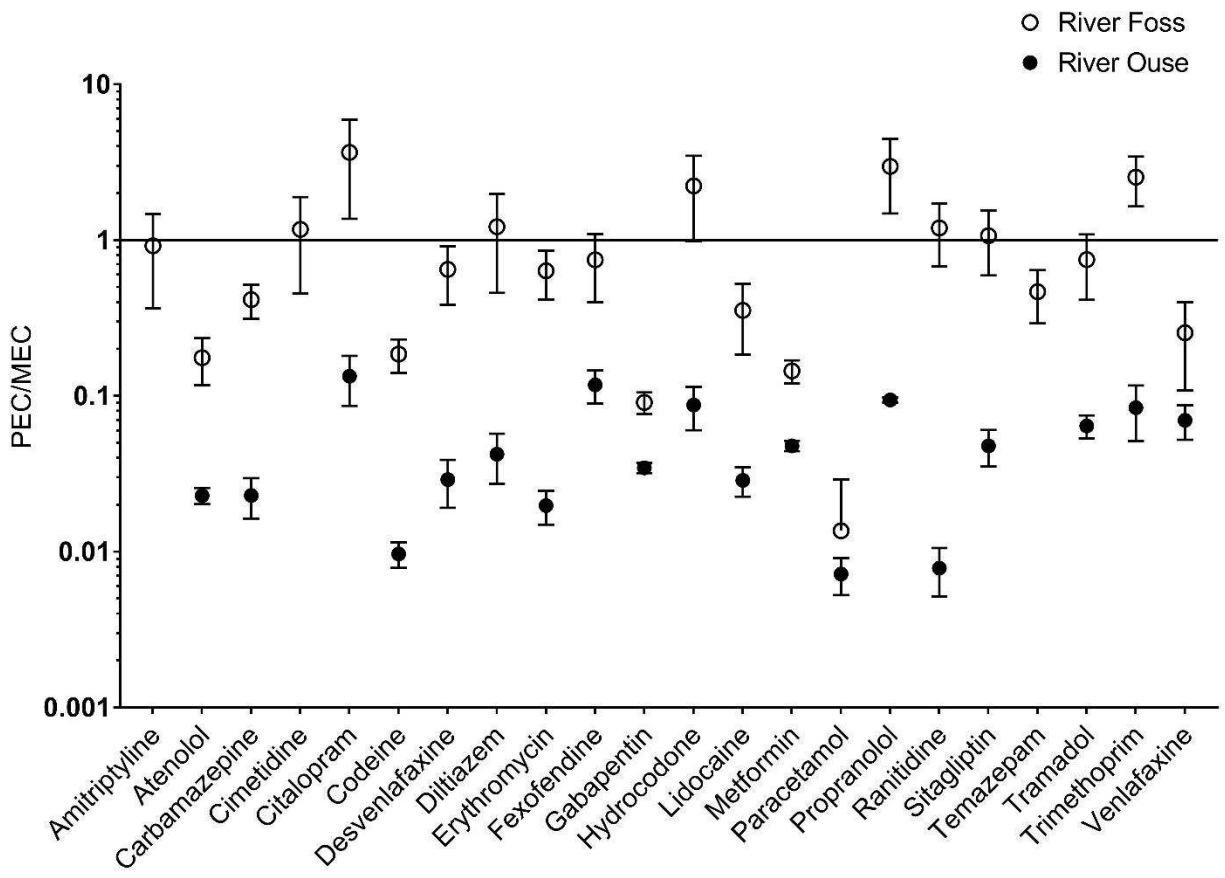

Figure 8. The annual average PEC/MEC ratios are plotted for the River Foss (open circles) and the River Ouse (closed circles). PECs were calculated for each river based on experimental WWTP removals and the average flow from sampling days. PEC/MEC ratios were calculated for site F2-F5 and O3-O6 and averaged,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