Introduction of human pharmaceuticals from wastewater treatment plants into the aquatic environment: A rural perspective

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ABSTRACT

Incomplete removal of pharmaceuticals during wastewater treatment can result in their discharge into the aquatic environment. The discharge of pharmaceuticals in wastewater treatment plant (WWTP) effluents into rivers, lakes and the oceans has led to detectable concentrations of pharmaceuticals in the aquatic environment in many countries. However, to date studies of WWTP discharges into the aquatic environment have largely been confined to areas of relatively high population density, industrial activity or systems impacted on by such areas. In this work, two sites in the far north of Scotland were used to assess whether, and which, pharmaceuticals were being introduced into natural waters in a rural environment with low population density.

Samples from two WWTPs (with differing modes of operation), and one receiving water, the River Thurso, were analysed for the presence of twelve pharmaceuticals (diclofenac, clofibric acid, erythromycin, ibuprofen, mefenamic acid, paracetamol, propranolol, sulfamethoxazole, tamoxifen, trimethoprim and dextropropoxyphene). Ten of the twelve pharmaceuticals investigated were detected in at least one of the 40 WWTP effluent samples. Maximum concentrations ranged from 7 ng L^{-1} (sulfamethoxazole) and 22.8 µg L^{-1} (paracetamol) with diclofenac and mefenamic acid being present in all of samples analysed at concentrations between 24.2 and 927 ng L^{-1} and 11.5 µg L^{-1} and 22.8 µg L^{-1} respectively Additionally, the presence of four pharmaceuticals at ng L^{-1} levels in the River Thurso, into which one of the WWTPs discharges, shows that such discharges result in measurable levels of pharmaceuticals in the environment. This provides direct evidence that, even in rural areas with low population densities, effluents from WWTPs can produce quantifiable levels of human pharmaceutical in the natural aquatic environment. These observations indicate that human pharmaceuticals may considered as contaminants, with potential to influence water-quality, management and conservation not only in urban and industrial regions, but also those more rural in nature.

Keywords: pharmaceuticals, wastewater, rural areas, river water

1. Introduction

Pharmaceuticals are now an important and indispensable part of modern society being employed in human and veterinary medicine, agriculture and aquaculture (Dietrich *et al.* 2002). In the UK alone more than 3,000 active ingredients are licensed for use as human pharmaceuticals while the number of prescriptions and number of individual chemical entities involved increases every year; for example the number of prescription items dispensed in Scotland increased 31.8% between 2004/05 and 2013/14 (NHS 2014).

Following administration, human pharmaceuticals may be excreted, either in their original form or as metabolites, before being introduced into the domestic wastewater system (Andreozzi *et al*. 2003). The incomplete removal of pharmaceuticals during wastewater treatment may result in their discharge into the aquatic environment. The first report of human pharmaceuticals in effluents collected from wastewater treatment plants (WWTPs) identified clofibric acid (a metabolite of the blood lipid regulator clofibrate) at concentrations of between 0.8 and 2 μg L^{-1} (Garrison *et al.* 1976) Subsequent studies have demonstrated the presence of pharmaceuticals in wastewaters and their receiving waters in countries around the world, including Italy (Andreozzi *et al.* 2003; Zuccato *et al.* 2004; Zuccato *et al.* 2008), Japan (Nakada *et al.* 2006), U.S.A. (Brown *et al.* 2006), Germany (Ternes, 1998; Heberer *et al.* 2001), Spain (Carballa *et al.* 2007; Farre *et al.* 2001), Canada (Gagne *et al.* 2006), Sweden (Andreozzi *et al.* 2003; Lindberg *et al.* 2006), England (Hilton *et al.* 2003b) and Wales (Kasprzyk-Hordern *et al*. 2008, Baker and Kasprzyk-Hordern 2013: Barbara *et al*., 2008) . In general these studies have been conducted in wastewater treatment plants located in larges cites which serves a population higher than 10,000 inhabitants in addition to industrial discharge.

The investigation of pharmaceuticals in WWTP influent and effluent samples has also been used to calculate the removal efficiencies during the treatment process. For example, Bendz *et al.* (2005) reported 49% and 97% removal of the antibiotic trimethoprim in two different WWTPs in Germany. Similarly, Jones *et al.* (2007) investigated the removal of paracetamol, mefenamic acid, and ibuprofen in a WWTP located in the south of England. Results indicated that while elimination rates were ~90% for each compound, they were still present at concentrations of several hundred ng L^{-1} in the final effluent discharged into the receiving waters. The consequence is that

pharmaceuticals are being discharged continuously in to the aquatic environment of many countries including Italy (Zuccato *et al.* 2005; Zuccato *et al.* 2005), Romania (Moldovan, 2006), Germany (Ternes, 2001), Japan (Nakada *et al.* 2007), China (Zhao *et al.* 2010, Sui et al., 2012) and Canada (Metcalfe *et al.* 2003). The potential effects of these substances in the aquatic environment have also been investigated (Boxall *et al*. 2004; Corcoran *et al.* 2010; Kools *et al.*2008; Kümmerer 2008; Kümmerer 2009; ; Sanderson et al. 2007; Kemper et al. 2008; Sarmah et al. 2006).

Scotland has a population of approximately 5,100,000 inhabitants within a total area of 77,925 km^2. A considerable proportion of the population live in rural area, with population densities less than 1,000 per km^2. The largest cities in Scotland are Glasgow (3,378 inhabitants per km^2), Dundee (2,412 inhabitants per km^2), Edinburgh (1,843 inhabitants per km^2) and Aberdeen City (1,169 inhabitants per km^2) which have a total combined area of 685 km^2 representing approximately 1 % of the total Scottish land area (The Office for National Statistics, 2013). In these locations domestic, industrial and hospital discharges are potential sources of pharmaceuticals. However, most of Scotland supports a population density of less than 100 inhabitants per km^2. Considering the fact that to date the detection of pharmaceuticals in the environment and in WWTP discharges has been largely limited to areas relatively high population densities or sites impacted on by such areas, there is a need no investigate the presence of these substances in rural areas and in wastewater effluent of WWTPs which serve populations lower than 10,000.

This paper seeks to test the hypothesis that even in rural areas with low population densities, WWTPs can be the source of quantifiable levels of pharmaceuticals in the natural aquatic environment.

Employing an HPLC-MS/MS method for the analysis of twelve pharmaceuticals reported previously we report results from a study conducted in the far North of Scotland which has one of the lowest population densities in Europe. The study was conducted in Caithness situated in the far north of the mainland UK; it has a population of 25,160, an area of 1844 km^2 and this a population density of 14 inhabitants per km^2.This provides an initial assessment of whether (and which) pharmaceuticals may be

introduced into the natural aquatic environment in a rural areas with low population density and low industrial intensity.

2. Materials and Methods

2.1. Chemicals and Standards

Methanol (HPLC-grade) and acetone (glass-distilled grade) were obtained from Rathburn Chemicals Ltd. (Walkerburn, UK); hydrochloric acid (HCl, AR grade), ammonia solution 28–30% (GR for analysis ACR) and acetic acid (GPR grade) from VWR International (Lutterworth, UK); and ammonium acetate (GR for analysis ACR) from Merck Pharmaceuticals (West Drayton, UK). Diclofenac (sodium salt), clofibric acid, erythromycin, ibuprofen, mefenamic acid, paracetamol, (±)-propranolol hydrochloride, sulfamethoxazole, tamoxifen and trimethoprim were obtained from Sigma–Aldrich Co. Ltd. (Dorset, UK). Dextropropoxyphene hydrochloride were obtained from British Pharmacopoeia Commission Laboratory (Teddington, UK). All compounds were of a purity >95%. Milli-Q water was used unless otherwise stated.

Standard solutions were prepared in a 50:50 (V/V) mix of methanol: 10 mM ammonium acetate at pH 6 (pH was regulated using 0.1 M HCl). Standards of concentration <2,000 µg L^{-1} were prepared every three months and those >2000 µg L^{-1} every six months. All standards were stored in the dark at −18 °C. Standard solutions containing 20 mg L^{-1} of individual pharmaceuticals were diluted to give individual standard solutions at concentrations of 250 and 1,000 µg L^{-1} for MS tuning and HPLC optimization. Standard solutions of individual compounds (20 mg L^{-1}) were mixed to obtain mixed standard solutions containing all pharmaceuticals at concentrations of 1, 2, 10, 50, 200, 250 µg L^{-1}, which were employed for calibration.

2.2. Equipment

The HPLC–MS/MS system consisted of an Alliance 2695 HPLC (Waters; Manchester, UK) coupled to a Micromass® Quattro Micro™ detector with electrospray ionization (ESI). Data acquisition and control were carried out using Masslynx™ NT software (Waters; Milford, MA, USA).

Analytes were separated on a 250 mm × 2 mm C_{18} Luna® column (10 µm particle size; Phenomenex; Macclesfield, UK) used in conjunction with a 4.0 mm × 2.0 mm C_{18} guard column (Phenomenex; Macclesfield, UK).

Evaporation of extracts was performed with a turbo-evaporator (Turbo Vap® II, Zymark, Hopkinton, MA, USA) using nitrogen gas (supplied by a nitrogen generator; In House Gas, Killearn, UK) model N_2MaxiFlow 60 L (purity 99%).

Statistical analysis of the data was conducted with the software package STATGRAPHICS Centurion XVI version 16.1.11 64-bits (StatPoint Technologies, Inc. Warrenton, Virginia, USA).

2.3. Sample sites and collection

WWTPs characteristics

Two WWTPs, designated WWTP 1 and 2, in a region of relatively low population density (see above) were selected for this study. WWTP 1 serves a village of approximately 950 inhabitants and additionally treats sewage sludge from septic tanks from a wider area of approximately 1,844 km². Treated effluent is discharged into the River Thurso downstream of the village. The cycle time of this plant is highly dependent on levels of precipitation.

WWTP 2 serves a population of approximately 7,000 inhabitants including the main hospital for the area which serves a population of 35,000. In this WWTP the cycle takes approximately nine hours but is also dependant on levels of precipitation.

Effluent samples for the determination of pharmaceuticals were collected from WWTP1 over five separate five day periods: the 18th to the 22nd of July 2005 (Week 1), the 10th to the 14th of October 2005 (Week 2), the 12th to the 16th of December 2005 (Week 3), the 19th to the 23rd of June 2006 (Week 4) and the 26th to the 30th of June 2006 (Week 5). From WWTP 2 samples were collected over three separate five day periods, corresponding to weeks 1, 3 and 5 above.

River characteristics

In order to investigate possible impacts of the discharge of pharmaceuticals from a WWTP on a natural watercourse, samples were collected from the River Thurso into which the WWTP 1 discharges treated water. The River Thurso runs through the county of Caithness in the northern Highlands of Scotland and drains a predominantly peatland catchment of 412.8 km^2. It flows north through a short section of agricultural land before entering the Pentland Firth at the town of Thurso. The mean river flow is 8,980 L s^{-1} and the average rainfall in the area is 1,057 mm (SEPA, 2007c).

River water samples were collected at four points along the river: 'upstream' (0.05 km from WWTP 1 discharge point); 'downstream 1' (0.1 km from the discharge point), 'downstream 2' (12.80 km from the discharge point), 'downstream 3' (14.70 km from the discharge point). River water samples were collected during weeks 2 (10-14 October 2005) and 4 (19- 23 June 2006) (as specified above) between 9 and 11 a.m.

Sample collection

Prior to collection, sample vessels and collecting apparatus were rinsed three times with the waters being sampled. Samples were collected using a extendable sampling pole and transferred into individual, labelled 2.5 L amber glass bottles and stored in a cool box for transportation to the laboratory. On returning to the laboratory samples were either processed immediately or stored at 5 ºC prior to processing. In all cases samples were processed within five hours of collection.

2.4. Sample extraction and analysis

Samples were processed as described by Nebot et al. (2007). Briefly, two litres of each sample was filtered through a GF/F microfibre filter (Whatman; Middlesex, UK) using a positive pressure filtration system (Vernon Hills, Illinois). After filtration, the pH of the 2.0 L sample was adjusted to 6. Pharmaceuticals were extracted using SPE Strata™X cartridges. The eluate was collected in a glass tube and evaporated to 0.1 mL at 55 °C. The volume was then made up to 0.3 mL with 50:50 mix of methanol: 10 mM

ammonium acetate at pH 6, transferred to amber auto-sampler vials (2 mL, containing 0.5 mL insert vials) and stored at −18 °C prior to analysis by HPLC–MS/MS. Blank and fortified samples (1 μg L^{-1}) were processed with each batch of samples.

Two HPLC methods were used for the analysis of the selected pharmaceuticals. The first (Method A) was used for the analysis of paracetamol, trimethoprim, sulfamethoxazole, propranolol, erythromycin, dextropropoxyphene, tamoxifen, diclofenac and mefenamic acid and the second (Method B) for ibuprofen and clofibric acid. Both methods used water, methanol, ammonium acetate (10 mM, adjusted to pH 6.0 with 0.1 M HCl) and acetic acid (0.87 M) as mobile phase components. The flow rates were 0.17 mL min^{-1} (Method A) and 0.2 mL min^{-1} (Method B). Auto-sampler tray and column heater temperatures were maintained constant during the analysis at 5 and 25 °C respectively. The injection volume was 30 μL.

Positive ionisation was used for Method A and negative for Method B. In both, the following general parameters were applied: Extractor voltage, 2.20 V; radio frequency lens, 0.20 V; source temperature, 120 °C; low mass (LM) 1 resolution, 12.70; high mass (HM) 1 resolution 1, 12.70; ion energy 1, 0.40; entrance, 0.00; exit, 1.00; LM 2 resolution, 13.20; HM 2 resolution 1, 13.20; ion energy 2, 1.00; multiplier, 650.00 V. Method A utilised a capillary voltage of 3.20 kV, desolvation temperature of 400 °C, cone gas flow of 71.00 L h^{-1} and desolvation gas flow of 420.00 L h^{-1}. Method B utilised a capillary voltage of 1.20 kV, desolvation temperature of 200.0 °C, cone gas flow of 30.00 L h^{-1} and desolvation gas flow of 256.00 L h^{-1}.

Table 1 shows the LOD and LOQ of the pharmaceuticals in river and effluent samples following the protocol described above.

3. Results and Discussion

3.1. WWTP Samples

A total of 40 effluent samples, 25 from WWTP 1 and 15 from WWTP 2, were collected during the study. The frequency of detection of each compound along with the mean and maximum concentrations and the percentage residual standard deviation of each are

presented in Table 2 and the mean, maximum and minimum concentrations are shown in Figure 1. The concentrations of the pharmaceuticals detected in each sample are provided as supplemental information.

Pharmaceuticals in WWTP Effluents

With pharmaceuticals being detected in the effluents collected from both WWTPs it is clear that at these locations pharmaceuticals are discharged into the environment by the two plants. While this is the first report of the direct quantification of the discharge such substances into Scottish waters, their presence in the WWTP effluents has been reported in numerous other countries; including Sweden (Bendz *et al.* 2005), Japan (Nakada *et al.* 2007), Spain (Gros *et al.* 2006; Carballa *et al.* 2004), Canada (Gagne *et al.* 2006), Italy (Andreozzi *et al.* 2003), Switzerland (Maurer *et al.* 2007), France (Andreozzi *et al.* 2003; Rabiet *et al.* 2006) and England (Ashton *et al.* 2004; Roberts and Thomas 2006; Hilton and Thomas 2003a).

Over the course of the study the most frequently detected pharmaceuticals were diclofenac, mefenamic acid, ibuprofen, propranolol and trimethoprim with each being found in over 80% of the samples analysed (Table 2). Diclofenac and mefenamic acid were detected in all of the samples analysed with the concentration of diclofenac ranging from 24.2 to 927 ng L^{-1} and those of mefenamic acid from 10.7 to 1,327 ng L^{-1}. (Figure 1). The mean concentrations of diclofenac and mefenamic acid were 172 and 280 ng L^{-1}, respectively. Ibuprofen was detected in all but one of the samples (97%), with a mean concentration of 278 ng L^{-1} and a maximum concentration of 2,026 ng L^{-1}. Propranolol and trimethoprim were detected in 84 and 88% of the samples, respectively. Propranolol had a mean concentration of 142 ng L^{-1} and maximum concentration of 773 ng L^{-1}. Trimethoprim had a mean concentration of 266 ng L^{-1} with maximum concentration of 969 ng L^{-1}.

The next most commonly detected pharmaceuticals were dextropropoxyphene, erythromycin and paracetamol measured in 50 - 75% of all samples analysed. Dextropropoxphene and erythromycin were found in 52 and 55% of samples respectively. The concentration of dextropropoxyphene ranged from below the limit of detection to 203 ng L^{-1}, with a mean value of 34 ng L^{-1}. In the case of erythromycin the

concentration ranged from below the limit of detection to 2.5 µg L^{-1}, with a mean concentration of 381 ng L^{-1}. The concentration of paracetamol ranged from less than the limit of detection to 22.8 µg L^{-1}, the highest concentration of any of the compounds detected.

It may be noted that paracetamol is not commonly included in suites of compounds targetted for analysis WWTP effluents. However concentrations in excess of 0.5 µg L^{-1} have been reported in river water in the UK (Bound and Voulvoulis 2006), suggesting that discharges of the magnitude observed in this work may not be unexpected.

Similar high concentrations of erythromycin have been found in effluents collected from WWTPs in Canada (Miao et al. 2003) and England (Ashton et al. 2004). In contrast the concentration of erythromycin measured in effluents from a Spanish WWTP were only 1% of those found here (Gomez et al. 2006). The concentration of erythromycin in final effluents can be reduced by using ozonation, as reported by Nakada et al. (2007), however UV or sand filtration treatments have been shown to be ineffective in removing this compound (Roberts and Thomas, 2006 and Nakada et al. 2007).

In contrast, of the pharmaceuticals found, the least frequently detected were sulfamethoxazole, which was found in only one sample, and tamoxifen, which was present in four of the 40 effluent samples collected with a maximum concentration of 13.4 ng L^{-1}. Clofibric acid were not detected in any of the effluent samples analysed. In tha case of clofibric acid this is not surprising as it is not dispensed in Scotland. However, it was included in this study as its presence been reported in the North Sea Weigel *et al.* 2002; Buser and Müller 1998), which borders the east coast of the county of Scotland in which this work was conducted.

The concentration of individual pharmaceuticals in the effluents varied considerably. Considering all the data, the %RSDs for the concentration of all compounds were greater than 90%. The highest %RSD was for paracetamol (464%). This highly variable concentration of paracetamol in WWTP effluents has previously been reported by Andreozzi *et al.* (2003) who reported concentrations of ibuprofen ranging from 20 ng L^{-}

[1] to 1.82 μg L^{-1} and diclofenac ranging from 250 ng L^{-1} to 5.45 μg L^{-1}, in effluent samples from France, Italy, Greece and Sweden.

Comparison of WWTPs effluents

Comparing the data from the two WWTPs (S1); with the exception of a single occurrence of paracetamol at a concentration of 22.8 μg L^{-1}, the concentration range of the selected pharmaceuticals in the effluents collected from both were similar; between < LOD and 2,505 ng L^{-1} for effluents collected at WWTP 1 and between < LOD and 1,117 ng L^{-1} for those collected at WWTP 2. While, as mentioned above, diclofenac and mefenamic acid were detected in all the samples from both WWTPs, ibuprofen, trimethoprim and propranolol were additionally detected in all of the samples collected from WWTP 2. Other pharmaceuticals with a high frequency of detection in the individual plants were paracetamol, (92%) in WWTP 1, and erythromycin (93%) in WWTP 2. Tamoxifen was only detected in samples taken from WWTP1 with sulfamethoxazole detected in only a single sample, taken from WWTP 2.

In both WWTPs the pharmaceuticals with the highest mean concentrations (erythromycin and trimethoprim) were not necessarily those that most frequently detected in the overall sample set (i.e. diclofenac and mefenamic acid). The pharmaceutical with the maximum mean concentration in WWTP 1 was erythromycin (422 ng L^{-1}) which was detected in 64% of the effluents. Meanwhile trimethoprim has the maximum mean concentration in WWTP 2 (352 ng L^{-1}) and was detected in all the samples from WWTP 2.

The mean concentrations of the most frequently detected pharmaceuticals (diclofenac, mefenamic acid, ibuprofen, propranolol and trimethoprim) in the discharges from the two WWTPs were compared. Figure 2, indicates statistically significant differences for propanol, trimethoprim and ibuprofen ($\rho < 0.05$; Kolmogorov-Smirnov and Mann-Whitney test) between the two plants. In contrast no significant difference was noted for both diclofenac and mefenamic acid. The mean concentrations of diclofenac, mefenamic acid, propranolol and trimethoprim were 35, 25, 59 and 49% higher,

respectively, in the effluents from WWTP 2 with the mean concentration of ibuprofen in effluents from WWTP 1 being 60% higher than that from WWTP 2.

The higher concentrations of the most frequently detected pharmaceuticals in WWTP2 effluents could be due to the fact that WWTP 2 serves a larger population and includes wastewater from a hospital. However, it may also be due to the differences in the treatment processes employed at the two plants i.e. at WWTP1 influent is initially subjected to primary treatment (grit removal, screening bars and settling tank) before being transferred to a trickling biofilter for further treatment. Part of the effluent (approximately 20%) from the trickling biofilter is transferred back to the settling tank and the remainder sent to a second settling tank where clarifiers are added. Meanwhile, at WWTP 2, the influent receives primary treatment (grit removal, screening and settling) followed by secondary treatment with activated sludge. The plant has two activated sludge tanks which are used in turn in a batch process.

3.2. River Water Samples

Results obtained during the first sampling period (Week 1) for effluents form WWTP 1 and 2 demonstrated the presence of pharmaceuticals and consequently their discharge in to the environment. WWTP 1 discharges its effluent into a local river and WWTP 2 into the North Sea. As monitoring at sea presented logistical issues, a monitoring study was conducted in the River Thurso into which WWTP1 discharges. Results are presented in Table 3.

The river was sampled at a time when lower concentrations of pharmaceuticals may have been expected based on rainfall, temperature and sunlight due to dilution factor or degradation (Castiglioni et al. 2006). In total, 40 river water samples and 8 effluent samples were collected during October 2005 and June 2006.

While clofibric acid, dextropropoxyphene and sulfamethoxazole were not detected in the effluent samples, diclofenac, mefenamic acid and ibuprofen were detected in all effluent samples and in some of the river water samples. Diclofenac was detected in

river water samples collected during both sampling periods but mefenamic acid, paracetamol and ibuprofen only in river water samples collected during one of the sampling periods; mefenamic acid (October 2005), paracetamol (June 2006) and ibuprofen (June 2006). Mefenamic acid was the most frequently detected pharmaceutical and was present in 50% of the river water samples collected during October 2005 with a maximum concentration of 0.5 ng L^{-1}.

This concentration was 86% higher than those measured by Hilton and Thomas (2003b) in England and 67% higher than those measured in the River Ebro in Spain (Gros et al. 2006; Ferreira da Silva et al. 2011). Mefenamic acid is not commonly analysed for, and to date studies involving the analysis of this compound have only being conducted in the U.K. (Thomas and Hilton, 2004; Bound et al. 2006; Hilton and Thomas, 2003b), Spain and Croatia (Gros et al. 2006), China (Zhao et al. 2008) and Japan (Hoshina et al. 2011). Diclofenac was detected in river samples collected during both sampling periods with a maximum concentration of 1.9 ng L^{-1}. The presence of diclofenac in natural water has been shown in most European countries including Spain and Croatia (Gros et al. 2006), UK (Thomas and Hilton, 2004; Bound et al. 2006; Hilton and Thomas, 2003b), Switzerland (Öllers *et al.* 2001) and France (Farré et al. 2001; Rabiet et al. 2006). Paracetamol was detected in 30% of the samples with a maximum concentration of 9.1 ng L^{-1}, detected on the 21st of October 2005. The maximum concentrations of diclofenac and paracetamol were not found in the same sample. Of the pharmaceuticals detected, ibuprofen was the least frequently detected; being found in only one sample collected during the second sampling period.

The linearity of the relationship between pharmaceutical concentrations and the distance from the WWTP was evaluated for the four pharmaceuticals detected in the river. A linear relationship was found for mefenamic acid and paracetamol with 95% confidence but not for diclofenac and ibuprofen. For paracetamol the concentration decreases as we go farther from the discharge point but for mefenamic acid it increased. Mefenamic acid was present in samples collected in the mouth of the river maybe due to the transformation of a conjugate to mefenamic acid when the river water comes into contact with the salt water.

Estimated concentration in the River Thurso

The concentration of the pharmaceuticals expected in the river were estimated based on the concentration of each pharmaceutical in the effluent sample obtained for each sampling day, the mean river flow data and WWTP 1 cumulative flow (24 hour flow) during the sampling days. The river flow and WWTP flow data were obtained by from SEPA (Wheeler, 2007, pers. comm.) and Scottish Water (Geddes, 2007, pers. comm.).

When the concentrations of pharmaceuticals detected in the River Thurso are compared to their respective estimated concentrations (Figure 3) we can see that the concentrations largely correspond. Estimated concentration and maximum measured Whitney and Kolmogorov-Smirnov tests because the data did not show a normal distribution. Significant differences were observed with both tests for ibuprofen, propranolol, trimethoprim but not for diclofenac and mefenamic acid. These results indicated that the concentrations of the most frequently detected pharmaceuticals (diclofenac and mefenamic acid) in effluents collected form WWTP 1 could be used to estimate their concentration in the river.

Further work is also needed to the understand degradation of these compounds under environmentally relevant conditions. For example, the results presented here were obtained at ~58.5 °N. At this latitude day-length and light quality (including UV) is highly seasonal and will thus introduce a seasonal component onto degradation rates. The waters also typically have low nutrient levels, but high levels of dissolved organic carbon which will attenuate light penetration (Markager and Vincent, 2000) and therefore limit degradation rates. These factors will influence half-lives and therefore the potential in-situ ecotoxicological impact of pharmaceuticals.

4. Conclusion

This study is the first to demonstrate the presence of human pharmaceuticals in the effluents arising from WWTPs in Scotland and that these represent direct source of human pharmaceuticals into the Scottish natural waters. The ng L^{-1} and μg L^{-1}

concentrations observed add to the growing international body of evidence on the role of WWTPs in introducing a wide range of biologically active compound into natural waters.

Nine human parmaceuticals (diclofenac, erythromycin, ibuprofen, mefenamic acid, paracetamol, propranolol, sulfamethoxazole, tamoxifen, trimethoprim and dextropropoxyphene) were detected WWTP effluent samples collected from a rural location in the north of Scotland. Maximum concentration ranged from 7 ng L^{-1} (sulfamethoxazole) to 22.8 µg L^{-1} (paracetamol) with diclofenac and mefenamic acid being present in all of samples analysed at concentrations between 24.2 and 927 ng L^{-1} and 11.5 µg L^{-1} and 22.8 µg L^{-1}.

Most work on the presence of pharmaceuticals in waste and natural waters has focused on urban areas i.e. those with high population density whereas this research study has been conducted in an area with low population density . The results of this study illustrate that, even at these relatively low population densities (and low industrial intensity) human pharmaceuticals are present at in quantifiable levels in both WWTPs and receiving waters. Indeed two pharmaceuticals (diclofenac and mefenamic acid) were present in most of the samples investigated.

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Table 1. LOD and LOQ (ng L^{-1}) of the pharmaceuticals for river (r) and effluent (e) samples.

	River		Effluents	
	LODr (ng L^{-1})	LOQr (ng L^{-1})	LODe (ng L^{-1})	LOQe (ng L^{-1})
Paracetamol	3.9	13.1	10.9	36.7
Trimethoprim	0.1	0.3	0.1	0.3
Sulfamethoxazole	0.1	0.4	0.2	0.7
Propranolol	0.1	0.4	0.1	0.4
Erythromycin	0.1	0.3	0.1	0.5
Dextropropoxyphene	0.0	0.1	0.2	0.7
Tamoxifen	0.1	0.3	0.1	0.5
Diclofenac	0.2	0.6	0.2	0.5
Mefenamic acid	0.1	0.3	0.1	0.3
Ibuprofen	1.0	3.2	1.1	3.7
Clofibric acid	4.8	16.1	7.1	23.9

Table 2. Frequency of detection, mean and maximum measured concentrations and percentage residual standard deviation for each pharmaceutical measured in samples collected from two wastewater treatment plants, n=40.

Pharmaceutical	Frequency of Detection (%)	Mean Concentration (ng L^{-1})	Maximum Concentration (ng L^{-1})	%RSD
Dextropropoxyphene	52	34	203	159
Diclofenac	100	172	927	110
Erythromycin	55	381	2505	138
Ibuprofen	98	278	2206	134
Mefenamic acid	100	280	1327	117
Paracetamol	75	762	22782	464
Propranolol	85	142	773	110
Sulfamethoxazole	2.5	0	7	624
Tamoxifen	10	1	13.4	348
Trimethoprim	88	266	969	91
Clofibric acid		0	0	0

Table 3. Concentrations of pharmaceuticals (ng L^{-1}) measured in samples taken from the River Thurso.

Collection Day	Pharmaceutical	Upstream	Downstream	Weir	Harbour	Average	STDEV	RSD (%)
			October sampling period					
10/10/2005	Di	1.9	< LODr	0.5	0.7	0.8	0.8	104
11/10/2005	Di	< LODr	0.5	< LODr	0.7	0.3	0.4	119
12/10/2005	Di	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
13/10/2005	Di	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
14/10/2005	Di	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
10/10/2005	Me	< LODr	< LODr	0.5	0.5	0.3	0.3	115
11/10/2005	Me	< LODr	< LODr	0.3	0.2	0.1	0.2	120
12/10/2005	Me	< LODr	< LODr	0.1	0.1	0.1	0.1	115
13/10/2005	Me	< LODr	< LODr	0.3	< LODr	0.1	0.2	200
14/10/2005	Me	< LODr	0.3	0.3	0.2	0.2	0.1	71
			June sampling period					
19/06/2006	Pa	< LODr	3.9	< LODr	< LODr	1.0	2.0	200
20/06/2006	Pa	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
21/06/2006	Pa	9.1	7.2	< LODr	< LODr	4.1	4.8	117
22/06/2006	Pa	< LODr	9.0	< LODr	< LODr	2.3	4.5	200
23/06/2006	Pa	6.4	6.2	< LODr	< LODr	3.2	3.6	115
19/06/2006	Di	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
20/06/2006	Di	0.3	< LODr	< LODr	< LODr	0.1	0.2	200
21/06/2006	Di	< LODr	< LODr	< LODr	0.8	0.2	0.4	200
22/06/2006	Di	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
23/06/2006	Di	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
19/06/2006	Ib	1.3	< LODr	< LODr	< LODr	0.3	0.7	200
20/06/2006	Ib	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
21/06/2006	Ib	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
22/06/2006	Ib	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A
23/06/2006	Ib	< LODr	< LODr	< LODr	< LODr	< LODr	< LODr	N/A

< LODr: Below limit of detection in fresh water samples (Table 3.10); N/A: Not available; SDTEV: Standard deviation; Pa: paracetamol; Di: diclofenac; Ib: ibuprofen; Me: Mefenamic acid

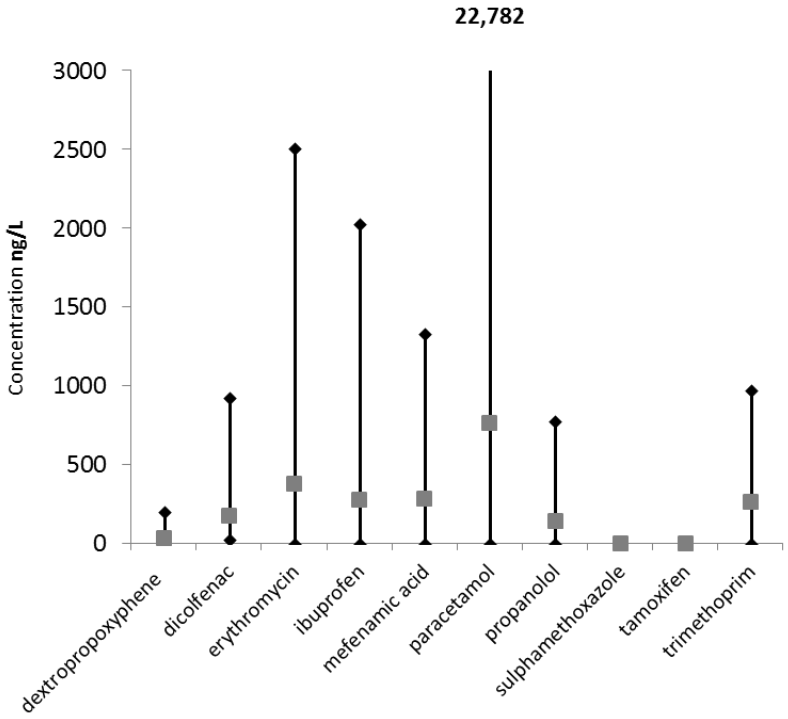
Figure Legends

Figure 1. Range of concentrations for pharmaceuticals detected in effluents from two WWTPs. Bars show maximum and minimum values while 'boxes represent means.

Figure 2. Comparison of the concentrations of the five most frequently detected pharmaceuticals (diclofenac, mefenamic acid, ibuprofen, propranolol and trimethoprim) in the discharges from the two WWTPs. The top and bottom of each box corresponds to the maximum and minimum concentration respectively, with the interior horizontal line indicating the mean. The asterisk signifies a significant difference between the concentrations found in the two plants.

Figure 3. Comparison of the concentrations of individual pharmaceuticals detected in the River Thurso with estimated concentrations based on the concentrations found in WWTP effluents and river flow data.

Figure 1. Range of concentrations for pharmaceuticals detected in effluents from two WWTPs. Bars show maximum and minimum values while 'boxes represent means. The value for paracetamol is off the scale and the maximum value is given on the figure.



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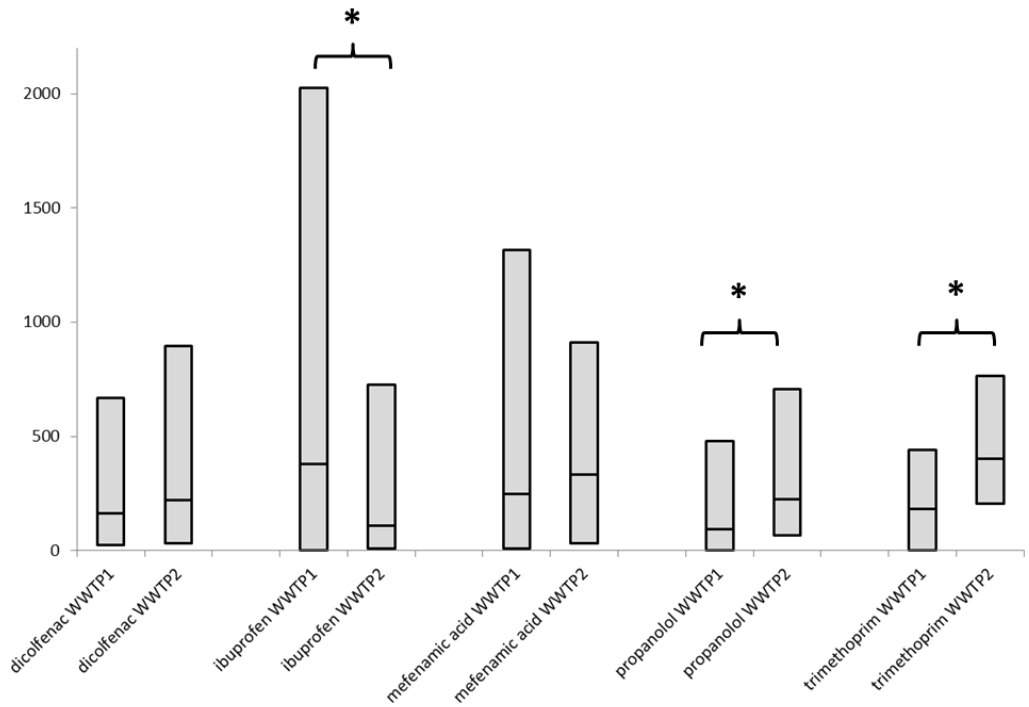

Figure 3. Comparison of the concentrations of individual pharmaceuticals detected in the River Thurso with estimated concentrations based on the concentrations found in WWTP effluents and river flow data.