Supplementary information

Blank and duplicate samples for QA:

The following study blanks were used to check the method:

- Sample bottle blank. Ultra-pure water direct from the dispenser was added to an NLS glass bottle to test for potential contamination. This was run through the system and onto the cartridge, replicating the procedure for other samples.

- Peristaltic pump method blank. A solution of Virkon was passed through the peristaltic pump tubing and HDPE tubing (plus PE connector) to clean them, the tubing was run dry followed by ultra-pure water to rinse. A glass beaker was used. A sample of ultra-pure water was then pumped into an NLS bottle through the clean tubing to test for contamination.

- Post-sampling peristaltic pump blank. After sampling the tidal Thames (15 samples) one litre of ultra-pure water was used to rinse the peristaltic pump tubing, PE connectors and HDPE rigid tubing. After this the in-field procedure was followed, the sampling beaker was rinsed three times and then filled with 600ml of water before filling a clean NLS bottle. This was performed to quantify potential carry-over between samples as we were unable to wash the tubing between samples.

All compounds detected within the blanks were found to be below the limit of detection (LoD) except mepronil (0.0024 µg/l) and carboxin (0.014 µg/l) which were detected when testing the field peristaltic tubing after sampling. Concentrations of carboxin within the river samples were between <LoD and 0.011µg/l and mepronil was not detected in any of the other samples (see Table 1).

Duplicate samples were processed to check the repeatability of the method. For this two samples from the same sample bottle were processed through separate SPE cartridges. Duplicates were processed for sites 1, 12 and 31 and are reported separately on all graphs to show the duplicates variability.

In addition site 8 was sampled on two separate occasions to check the variability within the river over time and in different flow conditions (see section 4.8.1).

Analysis

Extraction from the SPE cartridge was done using dichloromethane. 0.1% Formic acid in methanol: acetonitrile at the Environment Agency's Starcross laboratories prior to analysis.

A Ultra- High-Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) liquid chromatography/mass spectrometry (LC/MS) method was used (Agilent Q-TOF model 6545) to

screen the samples for more polar organic compounds. An isotopically labelled internal standard Carbutamide-d^9 (CAS 1246820-50-7) was added to each of the pre-conditioned SPE cartridges to assess instrument performance.

The LCMS screen method utilises a target reference library for compound identification. This unique library has been developed by analysing pure reference standards for each compound entry at a concentration of 0.1µg/l, the response factor obtained is used to create a single point calibration curve. Estimate of concentration is based on quant ion response and that of the internal standard. In general, results reported will be within a factor of 2 of the actual value. Detection limits are compound specific and are typically between 0.001-0.1 µg/L (Table 1). Compound identification is by Find by Formula (FbF). Target compounds are measured by mass accuracy, isotopic abundance, isotopic spacing and retention time using the Agilent Mass Hunter 'Qualative Analysis' software. Each score contributes to an overall match score. In addition, an internal AQC containing 9 target compounds is analysed with each sample batch, at a concentration of 0.01µg/l to correct for any loss of compound during the sample preparation or injection stages. The results are semi-quantitative as fully quantitative analysis is not practical due to the large number of compounds in the library and the requirement to use a set of standards.

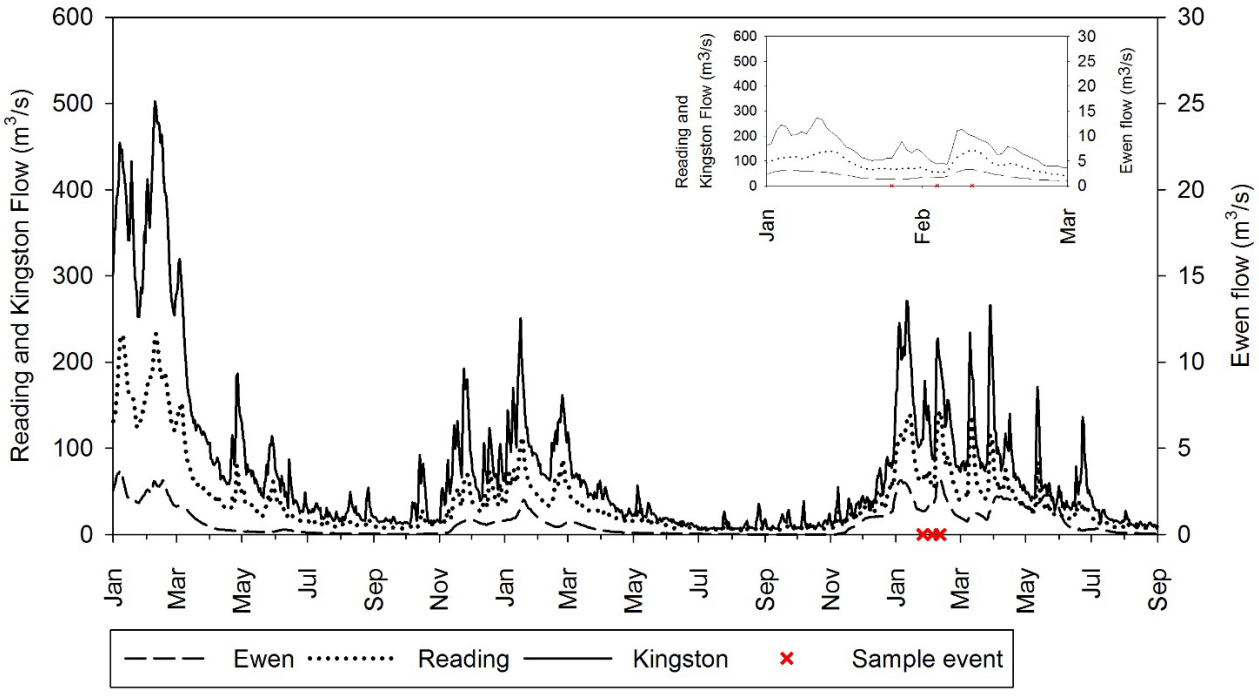
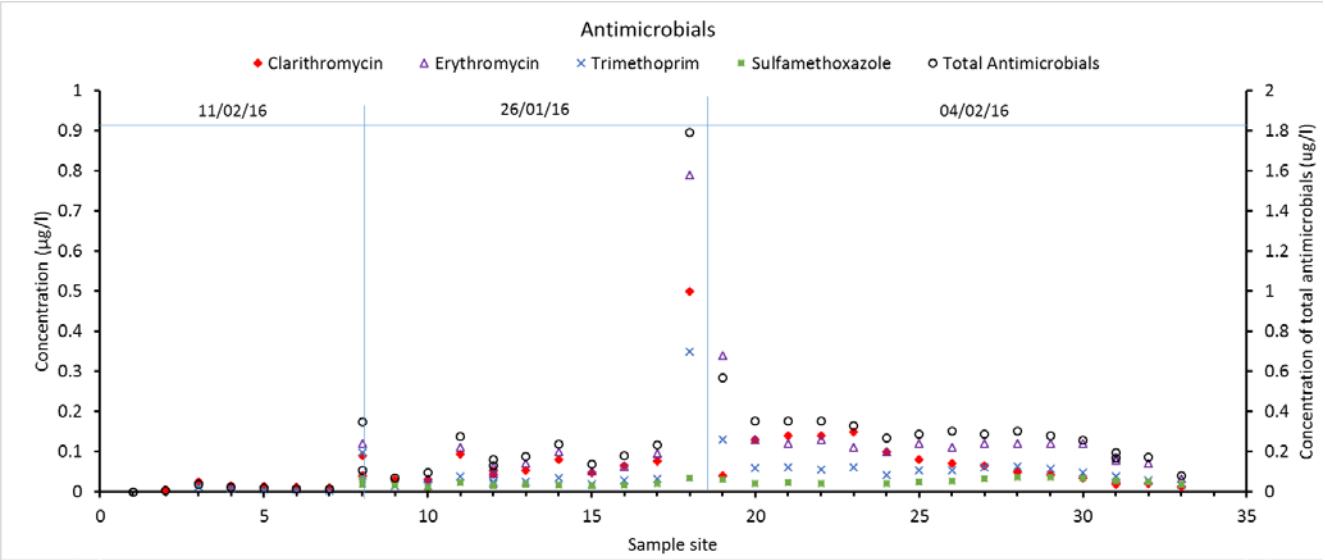


Figure S1. Gauged daily flow in the Thames at Ewen, Reading and Kingston. Sampling events shown.

a)



b)

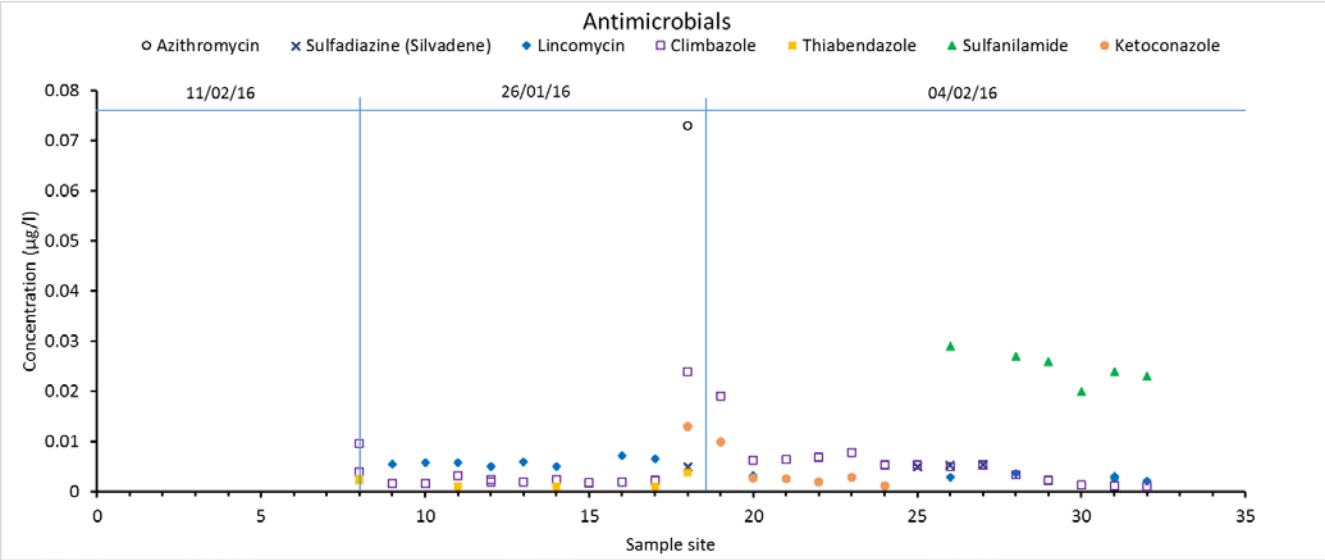


Figure S2. Antimicrobials detected along the River Thames; a) Changes in antimicrobial compounds clarithromycin, erythromycin, trimethoprim, sulfamethoxazole (primary y-axis) and total antimicrobials (secondary y axis). b) Changes in antimicrobials azithromycin, thiabendazole, sulphanilamide, sulfadiazine, lincomycin, climbazole. Site 1 is at the source of the Thames, site 33 is the last Thames estuary sample (figure 1). Sample dates are included and vertical lines represent the different sampling rounds.

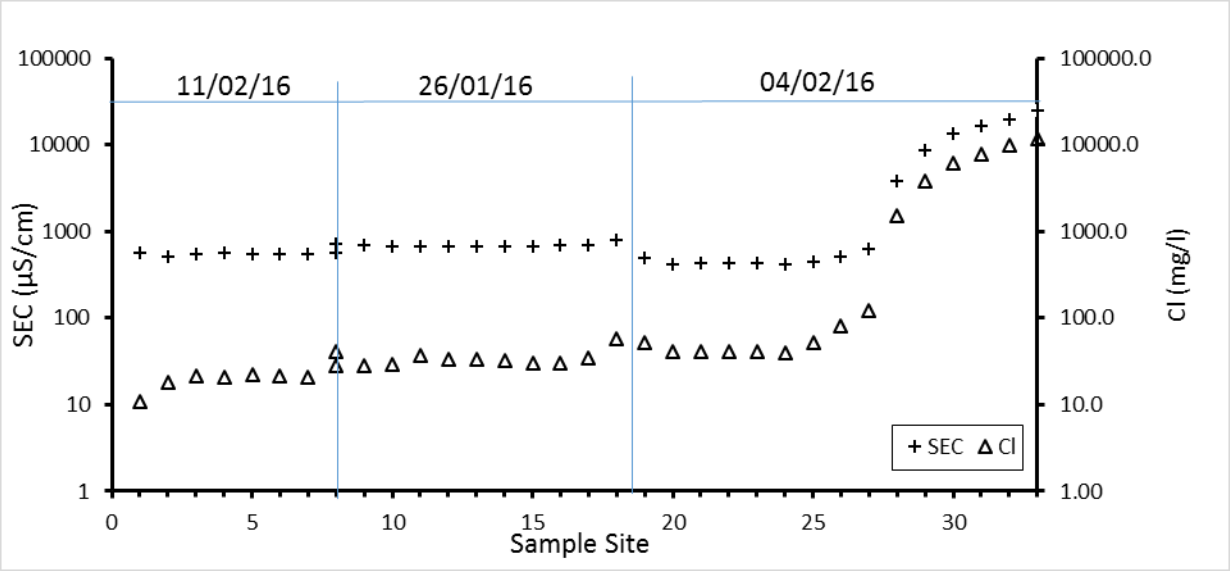
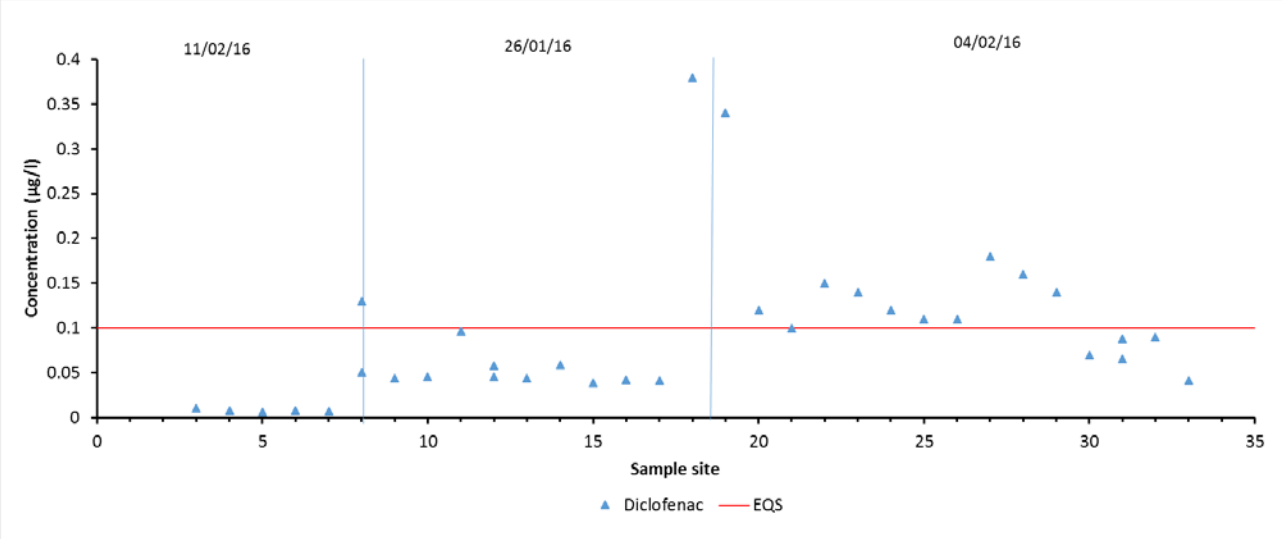


Figure S3. Change in SEC and Cl in the River Thames. SEC and concentration of Cl (log scale). Sample dates are included and vertical lines represent the different sampling rounds.

a)



b)

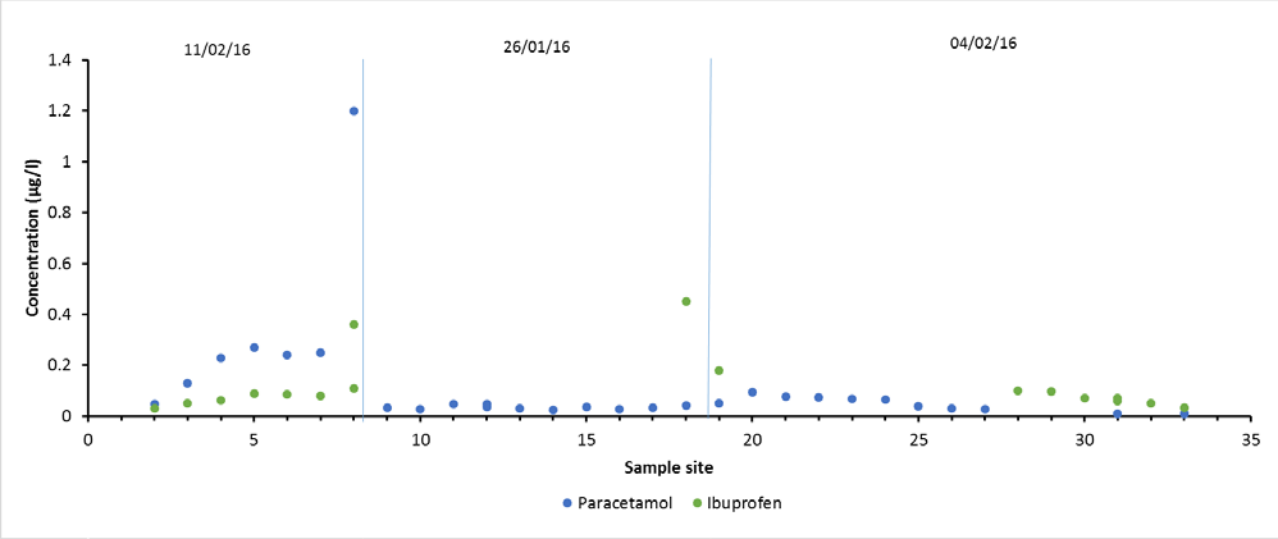
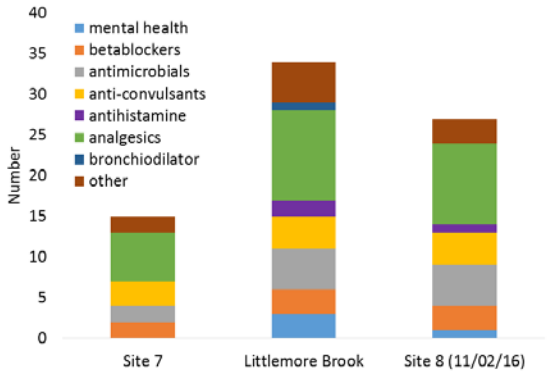
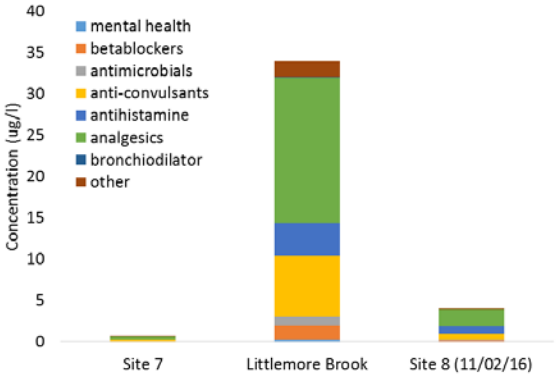


Figure S4. a) Changes in diclofenac concentration from source to sea. The proposed Environmental Quality Standard (EQS) is shown as a red line. b) Changes in paracetamol and ibuprofen concentration from source to sea. Note: Site 1 is at the source of the Thames, site 33 is the last Thames estuary sample. Note: Sample dates are included and vertical lines represent the different sampling rounds.

a)



b)



c)

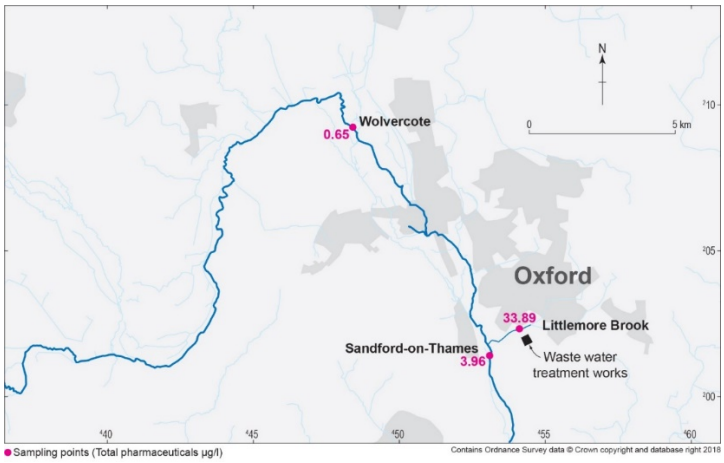


Figure S5. a) concentrations of MOs (by major group) above the LoD in the River Thames sample at Wolvercote (site 7) before the input from the Littlemore Brook; Littlemore Brook after input of effluent from the main Oxford area WWTW; River Thames sample at Sandford-on-Thames (site 8) after the input from the Littlemore Brook. b) total number of MOs in the samples from the Littlemore Brook, the River Thames at Wolvercote (site 7) and Sandford-on-Thames (site 8). All samples taken 11/02/16. c) Total concentration of pharmaceuticals (µg/l) in the Littlemore Brook site and samples upstream (site 7) and downstream (site 8) of the confluence.

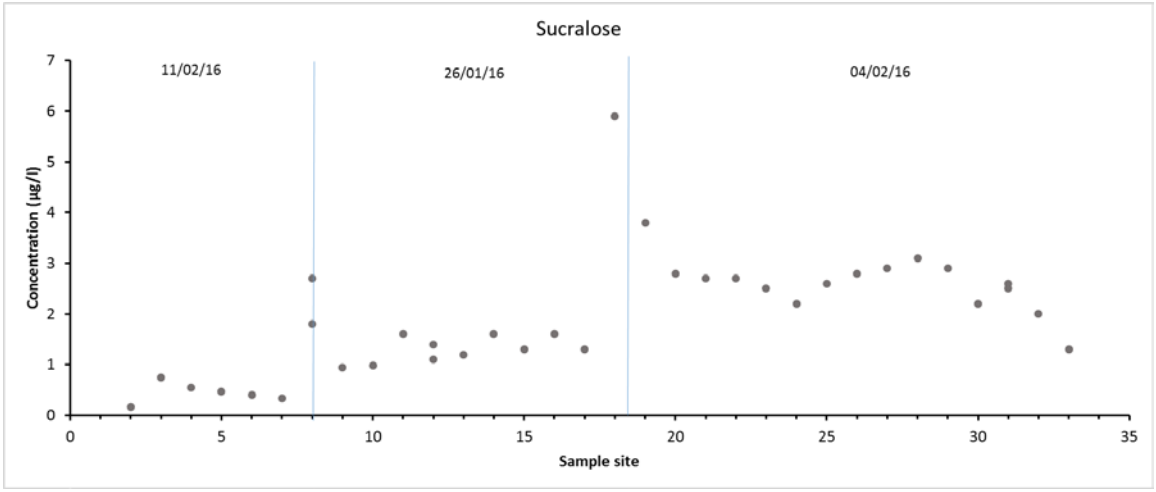


Figure S6. Variation in sucralose concentration from source to sea. Site 1 is at the source of the Thames, site 33 is the last Thames estuary sample. Note: Sample dates are included and vertical lines represent the different sampling rounds.

Statistical analysis

Testing repeatability of analysis

Prior to undertaking the Thames sampling campaign the validity of the method and the stability of the compounds on the cartridges up to 4 weeks after processing in the field lab, was checked by undertaking a replicate duplicate trial (White et al 2017). The results of the trial were statistically analysed (table S1a). It must be noted that the number of compounds has increased since 2014 and to highlight the stability and reproducibility all compounds detected are shown in the table.

During the Thames sampling campaign 3 sites were duplicated from the same bottle by using two separate SPE cartridges to investigate possible errors within the procedure. The duplicate samples (n=2) from site 31 were statistically analysed to look at variability within the duplicate (Table S1). Site 31 was chosen as it contained the highest number of detected compounds.

Table S1. a) results of the statistical analysis of the 2014 duplicate replicate trial on the cartridge method. b) Results of the statistical analysis of the 2016 Thames duplicate sample from site 31. Note RSD% is relative standard deviation expressed as a percentage.

a

Compound detected 2014	LOD (µg/l)	number of samples	Mean (µg/l)	SD	RSD%
Amitriptyline	0.005	8	0.0634	0.0099	15.60
Atenolol	0.001	8	0.3063	0.0362	11.83
Azoxystrobin	0.001	8	0.0039	0.0006	14.30
Boscalid	0.005	8	0.0049	0.0008	17.12
Carbamazepine	0.001	8	0.2838	0.0151	5.31
Carbetamide	0.001	8	0.0656	0.0090	13.77
Climbazole	0.001	8	0.0606	0.0078	12.81
Clozapine	0.001	8	0.0994	0.0068	6.82
Codeine	0.001	8	0.6875	0.0459	6.68
Diclofenac		8	0.5950	0.1180	19.83
Diuron	0.001	8	0.0054	0.0006	11.46
Flufenacet	0.001	8	0.0056	0.0005	9.20
Imidacloprid	0.001	8	0.0501	0.0036	7.19
Lamotrigine	0.001	8	0.4300	0.0239	5.56
Lidocaine	0.001	8	0.1475	0.0071	4.79
Oxazepam	0.001	8	0.0488	0.0023	4.75
Propiconazole	0.001	8	0.0093	0.0012	12.59
Propyzamide	0.001	8	0.0062	0.0013	20.23
Sulfamethoxazole	0.005	8	0.0719	0.0046	6.37
Thiabendazole	0.001	8	0.0040	0.0000	0.00
Trimethoprim	0.001	8	0.5400	0.0131	2.42

b

Compound detected (2016 site 31)	Number of samples	mean (µg/l)	SD	RSD%
Sucralose	2	2.550	0.071	2.77
Atenolol	2	0.065	0.002	3.29
Carbamazepine	2	0.075	0.003	3.77
Cetirizine	2	0.990	0.014	1.43
Clarithromycin	2	0.021	0.002	10.35
Climbazole	2	0.001	0.000	6.15

Clopidol	2	0.001	0.000	16.97
Codeine	2	0.071	0.001	1.00
Dextrorphan (Levorphanol - d form)	2	0.010	0.000	0.73
Diclofenac	2	0.077	0.016	21.26
Erythromycin	2	0.081	0.004	4.39
Furosemide	2	0.080	0.001	0.89
Gabapentin	2	0.545	0.007	1.30
Hydrocodone	2	0.018	0.001	7.86
Ibuprofen	2	0.066	0.009	14.03
Lamotrigine	2	0.090	0.003	3.14
Levamisole	2	0.051	0.000	0.00
Lidocaine	2	0.037	0.000	0.00
Lincomycin	2	0.003	0.000	12.86
Morphine	2	0.011	0.000	0.00
Naproxen	2	0.034	0.003	8.32
Oxcarbazepine	2	0.024	0.002	9.03
Propranolol	2	0.011	0.001	6.73
Salbutamol	2	0.008	0.000	3.77
Sotalol	2	0.071	0.002	3.01
Sulfamethoxazole	2	0.028	0.001	5.05
Tramadol	2	0.145	0.007	4.88
Trimethoprim	2	0.038	0.001	1.89

Spearman's rank correlation

To check the validity of sucralose as a tracer for pharmaceuticals a Spearman's rank correlation, a non-parametric measure of rank correlation, was run and the result was checked for statistical significance. The same statistical test was run to see if there was any correlation between SEC or chloride with selected pharmaceutical concentration to test behaviour of pharmaceuticals in the tidal zone. The pharmaceuticals were selected if they had less than 25% of samples sites with detections below the corresponding LoD, the remaining samples with data below LoD were given a concentration of zero prior to ranking.

As there are two major sources of SEC and Cl, WWTW effluent and salinity within the tidal river, a further test was run on the 16 sites within the tidal area of the river.

Table S2. Spearman's Rank correlation and P value for the relationship between sucralose, SEC and Cl with selected pharmaceuticals and total pharmaceutical sub groups for all samples. In red are p values >0.05.

	Sucralose		SEC		Cl		
Compound	Correlation Coefficient	P Value	Correlation Coefficient	P Value	Correlation Coefficient	P Value	Number of Samples
Cl			0.42	0.00994			

sucralose			0.035	0.835	0.765	2E-07	37
atenolol	0.968	2E-07	0.107	0.527	0.757	2E-07	37
carbamazepine	0.980	2E-07	0.0804	0.634	0.759	2E-07	37
cetrizine	0.964	2E-07	-0.00901	0.957	0.694	2E-07	37
clarithromycin	0.674	2.23E-06	-0.26	0.12	0.328	0.0472	37
codeine	0.863	2E-07	-0.0449	0.79	0.531	0.000791	37
diclofenac	0.949	2E-07	-0.0437	0.796	0.690	4.78E-08	37
gabapentin	0.968	2E-07	-0.0467	0.782	0.697	2E-07	37
hydrocodone	0.963	2E-07	0.077	0.648	0.734	2E-07	37
lamotrigine	0.969	2E-07	0.092	0.586	0.730	2E-07	37
levamisole	0.924	2E-07	0.172	0.306	0.789	2E-07	37
lidocaine	0.944	2E-07	0.23	0.17	0.848	2E-07	37
morphine	0.853	2E-07	-0.0612	0.717	0.544	0.000552	37
naproxen	0.420	0.00989	-0.525	0.000915	0.0384	0.82	37
oxcarbazepine	0.965	2E-07	0.0357	0.832	0.702	2E-07	37
paracetamol	-0.173	0.305	-0.612	5.95E-05	-0.323	0.0509	37
propanolol	0.922	2E-07	-0.143	0.398	0.593	0.000122	37
sotalol	0.964	2E-07	-0.00718	0.966	0.686	5.14E-07	37
sulfamethoxazole	0.875	2E-07	0.335	0.0426	0.800	2E-07	37
tramadol	0.917	2E-07	0.03	0.858	0.598	0.000103	37
trimethoprim	0.968	2E-07	-0.0378	0.823	0.68	1.3E-06	37
total conc pharmaceuticals	0.953	2E-07	0.0241	0.886	0.686	4.61E-07	37
total AMs	0.928	2E-07	-0.157	0.352	0.601	9.14E-05	37
total antihistamines	0.964	2E-07	-0.00901	0.957	0.694	2E-07	37
total conc anticonvulsants	0.980	2E-07	-0.0183	0.914	0.709	2E-07	37
total conc analgesics	0.781	2E-07	-0.194	0.247	0.401	0.0143	37
betablockers	0.976	2E-07	0.0533	0.752	0.730	2E-07	37

Table S3. Spearman's Rank correlation and P value for the relationship between sucralose, SEC and Cl with selected pharmaceuticals and total pharmaceutical sub groups for the tidal Thames (site 19 to 33). In red are p values <0.05.

	SEC		Cl		
Compound	Correlation Coefficient	P Value	Correlation Coefficient	P Value	Number of Samples
Cl	0.973	0.0000002			16
atenolol	0.00888	0.969	0.0192	0.935	16
carbamazepine	-0.192	0.469	-0.159	0.548	16
cetrizine	-0.396	0.124	-0.424	0.0988	16
clarithromycin	-0.912	0.0000002	-0.912	0.0000002	16
codeine	-0.754	0.000372	-0.741	0.000644	16
diclofenac	-0.523	0.0364	-0.563	0.0221	16
gabapentin	-0.518	0.0389	-0.491	0.0517	16
hydrocodone	-0.118	0.656	-0.147	0.578	16
lamotrigine	-0.137	0.601	-0.12	0.648	16
levamisole	0.234	0.372	0.177	0.505	16
lidocaine	0.328	0.211	0.277	0.292	16
morphine	-0.767	0.000114	-0.743	0.000644	16
naproxen	-0.832	0.0000002	-0.821	0.0000002	16
oxcarbazepine	-0.323	0.215	-0.369	0.153	16
paracetamol	-0.872	0.0000002	-0.848	0.0000002	16
propanlol	-0.747	0.0005	-0.719	0.0013	16
sotalol	-0.3	0.252	-0.281	0.287	16
sucralose	-0.317	0.224	-0.3	0.252	16
sulfamethoxazole	0.407	0.112	0.407	0.115	16
tramadol	-0.267	0.308	-0.291	0.267	16
trimeoprim	-0.535	0.0317	-0.549	0.0266	16
total conc pharmaceuticals	-0.455	0.0735	-0.469	0.0637	16
total AMs	-0.761	0.000207	-0.78	0.0000002	16
total antihistamines	-0.396	0.124	-0.424	0.0988	16
total conc anticonvulsants	-0.454	0.0756	-0.434	0.0889	16
total conc analgesics	-0.632	0.00834	-0.631	0.00834	16
betablockers	-0.214	0.416	-0.199	0.449	16