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EVALUATION OF COMBINED SEWER OVERFLOW IMPACTS ON SHORT-TERM PHARMACEUTICAL AND ILLICIT DRUG OCCURRENCE IN A HEAVILY URBANISED TIDAL RIVER CATCHMENT (LONDON, UK)

Kelly Munro,[a] Claudia P.B. Martins,[b] Matthew Loewenthal,[c] Sean Comber,[d] David A. Cowan,[a] Luisa Pereira,[e] and Leon P. Barron[a]*

[a] *Dept. Analytical, Environmental & Forensic Sciences, School of Population Health & Environmental Sciences, Faculty of Life Sciences & Medicine, King's College London, Franklin Wilkins Building, 150 Stamford Street, SE1 9NH, London, UK*

[b] *Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA*

[c] *Environment Agency, National Water Quality Instrumentation Service, Bristol, UK*

[d] *Dept. Environmental Science, Plymouth University, Drake Circus, Plymouth, PL4 8AA, UK*

[e] *Thermo Fisher Scientific, Manor Park, Tudor Road, Runcorn, UK*

*Corresponding author

Dr Leon P. Barron

27 Email. leon.barron@kcl.ac.uk

28 Tel. +44(0)20 7848 3842

Abstract

The occurrence of pharmaceutical and illicit drug residues potentially arising from combined sewer overflows (CSOs) in the Central London portion of the Thames Estuary is presented. Approximately 39 million tonnes of untreated sewage enter the River Thames at 57 CSO points annually. Differential analysis of influents and effluents in a major wastewater treatment plant identified seven potential drug-related CSO markers based on removal rates. Three were present in influent at concentrations >1 µg L^{-1} (caffeine, cocaine and benzoylecgonine). During dry weather, analysis of hourly samples of river water revealed relatively consistent concentrations for most drugs, including CSO markers, over a tidal cycle. River water was monitored over a week in January and July and then daily across six consecutive weeks in November/December 2014. Out of 31 compounds monitored, 27 drug residues were determined in the River Thames and, combined, ranged between ~1,000-3,500 ng L^{-1}. Total drug concentration generally declined during extended periods of drier weather. For CSO markers, short-term increases in caffeine, cocaine and benzoylecgonine concentration were observed ~24 h after CSO events (especially those occurring at low tide) and generally within one order of magnitude. Timings of elevated occurrence also correlated well with ammonium ion and dissolved oxygen data following CSOs. This work also represents an important study of pharmaceutical occurrence before a major 'Super Sewer' infrastructure upgrade in London aiming to reduce CSOs by 95 %.

Keywords: river water monitoring, emerging contaminants, high resolution mass spectrometry, CSOs

1. Introduction

Pharmaceuticals as environmental contaminants have been the focus of much research in the past 20 years. Concentrations, generally in the ng-µg L^{-1} range, have now been reported in most environmental compartments including wastewater [1-3], surface/ground water [4-6], marine water [7-9], solids [10], biota [11] and even in air [12]. However, the primary source of pharmaceutical and illicit drug contamination in the receiving environment has been identified as outputs from wastewater treatment plants (WWTPs), as either treated effluent or via sludge. In the EU, some pharmaceutical compounds have been placed on a 'watch-list' until sufficient evidence on the full extent of their impacts is known [13]. Environmental contamination and effects of illicit drugs have also been reported, albeit on a smaller scale to pharmaceuticals, and the focus for these has been largely on their measurement in untreated wastewater to estimate community consumption patterns [14-16].

As part of the wastewater infrastructure of many developed towns and cities, combined sewers are often used to simultaneously carry storm water and municipal sewage to urban WWTPs. Such sewers are often designed to carry several fold the average dry-weather load, but in extreme cases of runoff, rainfall or snowmelt, capacity can be breached. In these cases, combined sewer overflow (CSO) events occur to avoid back-flooding of streets and homes. Storm flow is normally mixed with treated or untreated wastewater and released directly into a nearby river or water body. Many reports have detailed the resultant changes in water quality [17] and ecosystem impacts [18] arising from faecal matter [19], microbial pathogens [20, 21], priority pollutants [22] and other storm water-related contents [23] .

In London, ~39 million tonnes of untreated sewage is discharged into the river Thames every year on average, but following exceptional wet weather and flooding in 2014, that total rose to 62 million tonnes [24]. London is mostly served by a Victorian combined sewer system built by Sir Joseph Bazalgette following the ‘Great Stink’ of 1858. From 1831 until its completion in 1865, an estimated 40,000 Londoners died from cholera. The expansion of London and an increasing population (>8.3 m) has meant that the system is currently running at approximately 80 % of its capacity, resulting in more frequent breaches with CSOs occurring at least once a week, even at times of light rainfall. London’s sewer system contains 57 CSO vents, 36 of which were assessed as having adverse environmental effects [25, 26]. CSO discharges were found to reduce the dissolved oxygen (DO) levels in river, introduce pathogenic organisms and to cause negative aesthetic changes in the river through the release of sewage, sewage litter, grease and scum directly into the river. A potential solution has been the Thames Tideway Tunnel, or ‘Super Sewer’, currently being built ~66 m under the river over 25 km. This major upgrade will intercept 34 CSOs and reroute sewage to a relief WWTP at Beckton in east London. It is due to be completed by 2023 and aims for an average 95 % reduction in sewage discharged to the river [27].

In comparison to prioritised pollutants, the impact of CSOs containing multiple pharmaceutical residues on receiving waters has received relatively little attention. A recent study by Kay et al. [28], showed that concentrations of five compounds monitored over 18 months in non-tidal rivers did not decrease even 5 km from the nearest WWTP in Northern England, which may potentially influence risk assessments based on models using first-order decay kinetics in

rivers [29]. Repeated sampling was also performed to identify fluctuations across a day, which showed significant variance in measured concentrations and, in some cases, across two-three orders of magnitude. A second study by Benotti and Brownawell near New York City reported concentrations of 12 high-volume pharmaceutical residues in mixed freshwater-saline regions across Jamaica Bay during dry and wet weather conditions [30]. Of these, two compounds had similar or higher concentrations in comparison to dry weather conditions (acetaminophen and nicotine). Despite being a comprehensive spatial study, repeated sampling was not performed to monitor temporal changes at each site. However, this study demonstrated the effect of salinity on pharmaceutical concentrations. Weyrauch et al. showed that compounds with removal efficiencies >95 % during wastewater treatment could result in elevated concentration in river water after CSOs [31]. For example, and though not a pharmaceutical, concentrations of nitrilotriacetic acid in the River Spree increased by 10-fold following a CSO and was well removed by a WWTP in Berlin. Compounds with intermediate removal above ~56 % also showed an increase in some cases, despite dilution with rainwater. Madoux-Humery et al., performed high resolution temporal sampling of sewage outfalls over a year in Canada [32]. Several CSO markers were monitored and *E. coli* was considered the best overall. However, of four pharmaceuticals monitored, carbamazepine was determined to be the best marker of CSOs due to its persistence, specificity for human use, stability and correlation with *E. coli*. Previous work by the same group showed that caffeine was correlated with faecal coliforms [33] and its use as an indicator of wastewater contamination was also shown by other groups in different parts of the world [34-38]. Acetaminophen was also identified as a

129 suitable CSO marker by other groups [38, 39]. In an alternative approach, Fono
130 et al. showed that chirality could be exploited to identify raw sewage discharges
131 and/or CSOs using the ratio of one of the isomers of propranolol to its total
132 concentration [40]. Aside from CSOs, use of drug markers has also recently
133 been proposed to differentiate sewage from manure contamination [41]. Save
134 for a few studies [42-44], the number of pharmaceuticals and especially illicit
135 drugs included is generally small. More comprehensive analytical methods are
136 required to fully identify the scale of CSO impacts more broadly regarding such
137 compounds. Ideally, these should be more tailored to the catchment at the
138 method development stage. The advent of liquid chromatography-high
139 resolution mass spectrometry (LC-HRMS) has enabled a more flexible
140 approach to multi-residue analysis, by allowing targeted, untargeted and
141 suspect screening to be performed on large numbers of compounds, often
142 simultaneously [45-48]. However, reports using such approaches for CSO
143 impact assessment on receiving waters for pharmaceuticals and illicit drugs are
144 few.

145 The aim of this work was to identify fluctuations in drug concentrations
146 in the Central London catchment of the River Thames potentially arising from
147 CSO events. The objectives were (a) to perform a differential quantitative
148 analysis of influent and effluent wastewater to identify CSO-related drug
149 markers, and (b) to monitor fluctuations in general drug occurrence, as well as
150 ammonium and DO in receiving river water during dry and wet weather. In
151 particular, sampling sites were chosen for their location ~25 km away from any
152 main WWTP effluent discharge points. This project focused on quantitative
153 monitoring of a larger number of pharmaceutical and illicit drug compounds than

studied previously (n=31), and measured at high frequency, with an analytical method based on LC-HRMS that was flexibly adapted for the catchment. Also, this work serves as a potential snapshot of drug contamination before a major sewer infrastructure upgrade such as the Thames 'Super Sewer' project.

2. Experimental

2.1 Materials and Reagents

All reagents were of analytical grade or higher. Methanol (MeOH), acetonitrile (MeCN), dichloromethane (DCM) and dimethyldichlorosilane (DMDCS) were purchased from Fisher Scientific (Loughborough, UK). Ammonium acetate and 37 % (w/v) hydrochloric acid solution were sourced from Sigma-Aldrich (Gillingham, Dorset, UK). Ultra-pure water was obtained from a Millipore Milli-Q water purification system with a specific resistance of 18.2 MΩ.cm (Millipore, Bedford, USA). All glassware including stock solution vials and evaporation tubes were silanised to reduce loss of analyte through adsorption to the glass surfaces. Each component was rinsed with a 50:50 (v/v) MeOH/H_2O solution before triplicate rinses with DCM. A 10:90 (v/v) DMDCS/DCM solution was then used to rinse the container followed by triplicate rinses with each of DCM, 50:50 MeOH:H_2O solution and water. A total of 51 pharmaceuticals, illicit drugs and metabolite reference materials were purchased from Sigma Aldrich (Gillingham, UK) for analytical method development and assessment (See Table S1. Stock solutions (1,000 mg L^{-1}) were prepared in MeOH and working standard solutions prepared weekly in ultrapure water or LC mobile phase A. All solutions were stored in silanised amber glass vials at 4 °C in dark conditions.

179

180 *2.2 Sampling sites and procedures*

181 Wastewater influent (immediately after the fine screen) and treated effluent
182 were taken as seven 24-hour composite samples from a major sewage
183 treatment works in London (population equivalent = 3.5 million) from 11-17th
184 March 2014 to identify pharmaceuticals and illicit drug residues potentially
185 indicative of CSO events. A 12-hour diurnal occurrence study was conducted
186 using 13 hourly grab samples (500 mL) taken on Tuesday 12th August 2014, at
187 Gabriel's Pier, London (51°30'31.0" N; 0°06'35.1" W) covering a period from
188 07:00 to 19:00 and collected at ~0.5 m depths. A moderate temperature (16-23
189 ^{o}C), mainly dry day (<1 mm rainfall) was chosen to reflect a normal daily river
190 cycle and free from storm runoff or triggered CSOs. For inter-season
191 occurrence of pharmaceutical and illicit drug CSO marker candidates, samples
192 were taken from two sites, again at ~0.5 m depths each time: Site 1 was at
193 Lambeth Bridge (51°29'42.4"N 0°07'27.8"W) and Site 2 was at Gabriel's Pier
194 (as above). Of 57 vents in total in London, six CSO vents lay in close proximity
195 to Site 2 in both directions, spanning from Westminster Bridge to Blackfriars
196 Bridge. For Site 1, a CSO vent lay within 50 m of the sampling site on the same
197 bank. Following this, a high frequency sampling campaign was conducted by
198 taking grab samples over a 6-week period at 09:00 on weekdays from Site 2
199 from 3rd November-13th December 2014. All samples of wastewater and river
200 water were collected in 500 mL Nalgene bottles, transported immediately to the
201 laboratory (~30-60 min transit time), acidified to < pH 2 with HCl and frozen (-
202 20 °C) until analysis. Tide heights were also recorded at the river sampling site
203 at each timepoint using the local tidal gauge pole. Daily rainfall data for the

sampling site was gathered from the published CEH-GEAR dataset by Tanguy et al. [49].

2.3 Sample pre-treatment and solid phase extraction

Before extraction, samples were thawed and filtered under vacuum using Whatman GF/F 0.7 µm glass microfiber filters. For matrix-matched standards, acidified 100 mL sample aliquots were spiked volumetrically before solid phase extraction (SPE). HyperSep Retain Polar Enhanced Polymer (PEP) cartridges (200 mg x 6 mL) were selected for SPE of river water and wastewater (Thermo Fisher Scientific, Runcorn, UK). Cartridges were conditioned with 4 mL MeOH and 4 mL ultrapure water. Acidified samples (100 mL) were loaded under vacuum at ~5 mL min^{-1} and washed thereafter with 4 mL 5:95 (v/v) MeOH:H_2O. The sorbent was dried under vacuum prior to elution for ~10 min before elution with 4 mL MeOH. Eluted extracts were evaporated to dryness under N_2 at 35 °C and reconstituted in 100 µL of 10 mM ammonium acetate 90:10 water:acetonitrile (mobile phase A) using a positive displacement pipette. The reconstituted samples were then sonicated for ~10 min before being transferred to an amber HPLC vial fitted with a silanised insert for analysis.

2.4 Instrumentation

For LC-HRMS analysis, an Accela ultra-high performance LC system, an HTS-A5 autosampler (at 10 °C) and an Exactive™ (Orbitrap) HRMS detector were used throughout. All separations were performed on a Thermo 150 × 2.1 mm, 2.6 µm Accucore C_{18} analytical column fitted with a matching 10 × 2.1 mm, 2.6 µm Accucore C_{18} guard column. The LC flow rate was 0.4 mL min^{-1}, the

temperature was maintained at 24 ^{o}C and the injection volume was 20 µL. A binary gradient elution profile of 90:10 to 20:80 10 mM ammonium acetate in water:acetonitrile (mobile phase A and B, respectively) was used as follows: 0% B for 2.5 min; 0-30% B from 2.5 to 7.5 min; 30% B from 7.5 to 12.5 min; 30-40% B from 12.5 to 15 min; 40-100% B from 15.0 to 20.0 min; 100% B from 20.0 to 27.5 min. Re-equilibration time was 7.5 min. The Exactive^{TM} HRMS was fitted with a heated electrospray ionisation source (HESI-II). All samples and model solutions were run separately in either positive or negative ionisation mode at 50,000 FWHM with a scan range of m/z 100–1000. Each acquisition cycle comprised of a full-scan without higher energy collisional dissociation (HCD) followed by a full scan with HCD enabled (collision energy: 20 eV; cycle time: ~2 s). Sheath, auxiliary and sweep gas settings were 50, 10 and 0 arbitrary units, respectively. The capillary temperature was 350 ^{o}C; the heater temperature was 300 ^{o}C; and the positive/negative spray voltages were +4.50 kV and −3.00 kV. All acquisition data was processed using Xcalibur v2.0 software. The entire analytical method was validated to ICH guidelines in wastewater and river water (see Tables S2-S4) [50]. Method development details are also presented in the Supplementary Information. For wastewater influent and effluent, the method was found to be quantitative for n=33 and n=38 compounds in untreated influent and treated effluent, respectively. For river water, the method could reliably quantify n=31 compounds at environmentally relevant concentrations.

2.5 Targeted analysis,quantitation and statistical procedures

253 Confirmation of target analyte occurrence in all samples was based on the
254 accurate mass of the protonated/deprotonated precursor ion and its associated
255 major HCD product ion to within 5 ppm mass accuracy, the ratio between these
256 two ions (<30 % to a matrix-matched standard) and a matching
257 chromatographic retention time (t_R) to within 15 s. For 24-h composite
258 influent/effluent wastewater samples, duplicate aliquots were extracted for each
259 day and determined using matrix-matched calibration using a pooled matrix of
260 all samples across the week-long sampling period. Background correction was
261 performed, as needed. Calibration lines were prepared for N ≥5 points,
262 alongside triplicate background-corrected quality control samples (50 ng/L) to
263 allow the accuracy of the method to be monitored. Given that the river was tidal
264 and brackish, significant variance in analyte matrix effects across days was
265 observed for a number of compounds (data not shown), so all drugs were
266 determined in duplicate using 3-point standard addition in each sample
267 separately for added accuracy. Drug occurrence in all samples is reported as
268 the average of duplicates with error bars representing the larger of the two
269 measurements. For temporal occurrence experiments, measured values over
270 each timeframe were averaged and the associated variance expressed as the
271 standard deviation, unless otherwise specified.

272 All statistical treatment of data was performed in Microsoft Excel. For
273 quantitation/calibration, lines-of-best-fit were applied and coefficients of
274 determination (R^2) calculated. For correlations between tide height/rainfall and
275 drug concentration (Figure S4), the Pearson correlation (R) was calculated and
276 significance tested by considering a p-value threshold of 0.05 to reject the null
277 hypothesis. For statistical comparisons of drug removal efficiency from

wastewater, data was first checked for normality and the *p*-value quoted following application of the specified test.

2.6 Suspect screening of wastewater and river water

Suspect screening was performed on wastewater samples only to differentially identify unique drugs/metabolites or those with potentially higher concentrations in influent. Post-acquisition automated peak selection was performed using Thermo TraceFinder™ version 3.1 software which contained a library of HRMS spectra for n=1,492 pesticides, herbicides, fungicides, pharmaceuticals, metabolites and illicit drugs. Following this, predicted t_R for potentially new compounds was performed using a previously developed neural network algorithm (Trajan v6.0, Trajan Software Ltd., Lincolnshire, UK) using reference t_R data for 166 pharmaceuticals, illicit drugs and metabolites measured in influent and effluent wastewater extracts [51]. Compounds were tentatively identified using a t_R window of ±1.3 min and an accurate m/z within 5 ppm of its calculated m/z. Lastly, an 80% fit threshold to theoretical isotope profile was set, with an acceptable intensity threshold deviation for each isotope ion set at 25% of the theoretical value.

2.7 DO, ammonium and conductivity monitoring

Percentage DO, pH, conductivity (as a measure of salinity), and ammonium concentration were taken at 15-minute intervals by the Environment Agency (EA), UK and analysed at three sites (Putney, Brentford and Hammersmith) using YSI6600 systems (Environmental Monitoring Systems, Herts, UK). DO was measured as % saturation using the YSI optical DO Sensor. The Sonde

software automatically compensated for the effect of temperature. River pH was measured using a combination electrode with an Ag/AgCl reference electrode. Ammonium was measured using an YSI ion selective electrode and the reference being provided by the pH combination electrode. Conductivity (µS cm^{-1}) was reported as specific to 25 ^{o}C and was calibrated using a solution of KCl. The YSI6600 sensors were calibrated every 4 weeks following standard EA operating procedures.

3. Results and Discussion

3.1 Differential analysis of influent and effluent wastewaters and identification of candidate CSO markers

To shortlist a selection of CSO-related pharmaceutical and illicit drug markers, differential analysis of influent and effluent wastewaters was performed. Direct analysis of in-sewer CSO samples was not performed due to limited access. Two important criteria were considered. Candidate CSO drug markers were shortlisted where they were: (a) ideally only present in untreated influent wastewater (i.e. high removal efficiency in the WWTP); and (b) remained at measurable and relatively consistent concentrations every day (i.e., minimal seasonal variation or recreational usage patterns should be evident).

All determined drug concentrations are presented in Tables S5 and S6 and summarised in Figure 1. A total of 14 compounds were quantifiable almost every day in untreated influent wastewaters and two of these were unique to it, i.e. diazepam and sulfapyridine, present at 76 ±14 and 184 ±96 ng L^{-1}, respectively, which were both selected as candidates. Prescription drug concentrations were generally consistent across the week in both influent and

effluent (except for sulfapyridine, which was not detected on one day). Both bezafibrate and furosemide were quantifiable in influent at similar concentrations (~400 ng L^{-1}), but less than the lower limit of quantification (LLOQ) in effluent. This corresponded to an >10-fold lower concentration, so both were considered as potential CSO markers. Tramadol exhibited the opposite trend, with significantly higher levels detected in effluent at 1,138 ±106 ng L^{-1} (p =3x10^{-7}, Student's two-tailed t-test), with over a two-fold concentration increase observed between both matrices. Nine other compounds were present at quantifiable levels on a regular basis in effluent. Extensive wastewater monitoring over the past five years as part of the £130 m UK Water Industry Research (UKWIR) Chemical Investigation Programme (CIP) Phase 2 (CIP2) has played a key role in the selection of substances and sites for future controls and remedial measures [52, 53]. It included up to 73 individual determinands across 44 WWTPs from 2015-2017 including data for six pharmaceutically-related compounds for which removal rates could be calculated: diclofenac (42 ±29 %), ibuprofen (98 ±4 %), propranolol (28 ±24 %), carbamazepine (-8 ±35 %), carbamazepine epoxide (30 ±28 %) and fluoxetine (43 ±22 %) [54]. The London-based WWTP studied here was not included within the 44 CIP2 sites. Comparative removal rates for this WWTP could be calculated reliably here for carbamazepine (-61 %, i.e., more concentrated in the effluent) and propranolol (34 %), and an estimation made for fluoxetine (65 %; occurrence was <LLOQ, but >LOD in influent).

For the selected illicit drugs, most were quantifiable during the week except for methylenedioxymethamphetamine (MDMA) and generally increased over the weekend. This was consistent with recreational consumption trends

353 seen previously [15]. Ketamine was eliminated as a candidate CSO marker, as
354 it was present at slightly higher concentrations in effluents than influents (58 ±5
355 and 42 ±9 ng L^{-1}, respectively) and measurements also lay close to the LLOQ.
356 Ketamine has been shown to display partial transformation in sewer transit
357 (<25 %) [55], as well as variable and even negative removal rates following
358 wastewater treatment [56, 57]. Possible reasons for higher concentrations in
359 effluent include residence times below 24 h, as well cleavage of conjugated
360 metabolites and desorption from particulate matter during treatment [58-60].
361 Mephedrone was detected at low levels in all samples and quantifiable at 83
362 ±45 ng L^{-1} in six out of seven influent samples (<LLOQ in effluent). Interestingly,
363 concentrations of cocaine and its metabolite benzoylecgonine remained high in
364 influent wastewater across the week with only a relatively minor increase in
365 occurrence over the weekend (%RSD <10 % for benzoylecgonine and <25 %
366 for cocaine), which is not consistent with many other cities. London is known as
367 one of the highest consumers of cocaine and this result suggested everyday
368 usage [16]. Cocaine was detected at significantly higher levels in influent (p
369 $=3x10^{-5}$; Student's two-tailed t-test) as well as analyte concentrations in effluent
370 at ~30-fold lower levels, which represented >99 % removal efficiency at this
371 WWTP. While WWTP removal performances can differ between sites, similar
372 removal of cocaine and benzoylecgonine from influent has been reported in
373 other parts of UK and globally, even up to 100 % [57, 61]. Given their metabolic
374 linkage, both were given further consideration as CSO markers. In addition to
375 these compounds, caffeine was also detected only in influent. However, its
376 concentration was so high that it lay outside of the quantifiable range when
377 using background corrected matrix-matched standard addition. However,

previous work using stable isotope internal standards showed that caffeine concentration in untreated wastewater from London was quite stable at 23 ±2 µg L^{-1} across a full week [15]. Caffeine has also been shown to be removed almost completely by wastewater treatment processes by both aerobic and anaerobic degradation [57, 62]. Caffeine was therefore retained as a candidate CSO marker and more reliable measurements in river water matrix were possible when present at a diluted concentration. Another compound, salicylic acid, was present at excessively high concentrations to quantify it in influent and was not detected in effluent. However, the poor method performance for this compound, observed in all three matrices assessed, meant it was not suitable for quantitative monitoring and was eliminated for use.

Application of HRMS database searching (TraceFinder) and reference to matching predicted chromatographic retention times resulted in tentative identification of n=32 more drug residues in influent and n=28 more in effluent across the week (Tables S7 and S8). For influent only, two detectable chromatographic peaks were present for four compounds in extracted ion chromatograms within their 1.3 min retention window even at 5 ppm mass accuracy/isotope profile matching (i.e., matching hydrocortisone, salbutamol, testolactone and acetylsalicylic acid, but not confirmed with reference standards). A total of 14 compounds were detected in influent at higher signal intensities than effluent at least once across the week (Figure 2 and Table S9). Eleven compounds were tentatively identified in effluent every day, including nine also present in influent every day. However, two unresolved isomers (quinine and quinidine) were present at markedly higher signal intensities in influent and were used together as a combined signal as potential CSO

markers. It was expected that of the two, quinine was likely to be the dominant compound given its widespread use in tonic waters.

A total of seven target analytes (bezafibrate, benzoylecgonine, caffeine, diazepam, sulfapyridine, cocaine and furosemide) were shortlisted as candidate CSO markers quantitatively. Quinine and quinidine were used together as qualitative CSO markers. For the six-week monitoring study, all other compounds were still included for river water monitoring, even if not considered as potential CSO markers to assess the potential contribution of CSOs in general.

3.2 Diurnal variation in drug concentrations in the River Thames

The river sampling sites in Central London lay within the Thames Estuary, where river levels often change by up to seven metres, twice a day. River flow is relatively small compared with the volume of the tide and therefore, is well mixed. Generally, the entire water mass travels in and out of the estuary with tidal cycles. When CSOs discharge to the river, it takes approximately one month for litter and sewage to exit the estuary to the sea in Winter and up to three months in Summer [24]. River water is also brackish to the top of the estuary at Teddington Lock, which lies west of the city. Previous research has shown that varying salinity, dissolved organic carbon (DOC) and/or suspended particulate matter (SPM) can influence drug concentrations in tidal waters [42, 43]. Therefore, fluctuations in drug concentration were monitored over a tidal cycle on a day free from storm water runoff or CSOs to understand the impact of fresh/saline water changes. From a qualitative perspective, n=24/31 compounds included in the validated method were detected at least once

across the day at Site 2 (Table S10) showing that the selection of compounds was highly relevant to this catchment and benefited greatly from the use of flexible full-scan LC-HRMS-based methods. Of these, n=18 drug residues were quantifiable and n=13 of those determined at all sampled time points. Figure 3(a) shows that four potential CSO marker drugs were quantifiable and remained relatively low in concentration. As perhaps expected, caffeine was present at the highest concentration across the day at 112 ±48 ng L^{-1}, and it presented a minor correlation with tide. No obviously apparent correlation with tide was observed for the other three CSO markers and all remained below ~20 ng L^{-1}. Figure 3 (b)-(d) show the other determined pharmaceutical residues, again most of which showed low and relatively consistent concentration profiles. Tramadol and carbamazepine concentrations were the highest between ~100-300 ng L^{-1} over the 12-hour period. Tramadol occurrence has been linked to hospital effluent contribution to CSOs, but was present at lower concentrations in untreated wastewaters here [63]. Trimethoprim, sulfamethazine, carbamazepine and ketamine were the only obvious cases showing any correlation with tide or water conductivity. These almost doubled in concentration at high tide which was in contrast to observations for pharmaceuticals by some other researchers [42, 43]. Three of London's five WWTPs (Beckton, Riverside and Crossness) discharge treated wastewater into the Thames ~25-30 km to the east of the Central London location (Site 2) and serve a combined population equivalent of ~5.9 million (~71 % of Greater London). The remainder of the population is served mainly by Mogden WWTP, which discharges effluent ~25 km west of Site 2 (~2 million population equivalent). Therefore, concentration rises with high tide are likely due to drug

residues from more treated effluent entering downstream being swept inland towards Site 2. Therefore, and in general, drug residues were not removed from the sampling site by a tidal cycle and concentrations largely remained relatively consistent. This was particularly useful for CSO markers considering that river water conductivity changed from ~650-1,000 µS cm^{-1} across the tidal cycle on this date showing the salt water influx/efflux.

3.3 Inter-season occurrence of pharmaceutical and illicit drug CSO marker candidates

CSOs were categorised into two main types. CSO Type 1 comprised of storm water combined with untreated sewage, which was discharged directly into the river. CSO Type 2 represented heavily diluted storm water that was screened, settled in tanks and mixed with fully treated wastewater at a major WWTP before release to the river. Public notifications of either CSO type corresponded to two monitored sites in London: (a) Hammersmith pumping station (CSO Type 1) and (b) Mogden WWTP (CSO Type 2). Weather in January 2014 was one of the wettest on record since 1910 with ~135 mm rainfall and available data from Hammersmith Pumping Station alone revealed ~1,637,456 m^3 of CSO Type 1 discharge and 2,505,000 m^3 of Type 2 from Mogden WWTP [64]. However, the total volume of either CSO type was likely much higher given that several more pumping stations and CSO vents exist across the Central London catchment. Across 2014, 16 million tonnes of untreated sewage were discharged into the River Thames from just the central London CSO vents covering the two sampling points selected. Three of these (the Hammersmith, Lots Road, and Western Pumping Stations) contributed 11 million tonnes to that total. One Type

1 CSO event occurred during the week sampled in winter on 16th January, 2014 at 21:50 hours, but after a grab sample was taken. However, concentrations of caffeine and benzoylecgonine increased at both Sites 1 and 2 on the following day (Figure 4). Furthermore, at Site 1 increases in concentration were also observed for bezafibrate and cocaine, most likely as it lay so close to a CSO vent, but this trend was not observed at Site 2. Caffeine had the highest concentration overall and reached a maximum of 1,520 ng L^{-1} at Site 1 and ~13 h after this Type 1 CSO. Its high concentration was prolonged in this instance and took roughly two days to return to baseline concentrations. No CSOs occurred during the week of sampling in July, 2014. Only ~44 mm rainfall was recorded for the month with 24,000 m^3 of Type 1 CSO discharge from Hammersmith Pumping Station and no Type 2 CSO discharge from Mogden WWTP. By comparison, caffeine concentrations were much lower in Summer and rarely reached >200 ng L^{-1}. Detection of all other substances was intermittent. Interestingly, baseline concentrations of bezafibrate and benzoylecgonine remained relatively consistent with the January samples, despite recorded rainfall and tidal height differences of >3.5 m across all sampling timepoints. At this time of year, salinity of the river was also much higher and more affected by tide as its freshwater composition was much lower (conductivity of ~600-700 µS in the Winter dates studied versus 900-3,000 µS in Summer)

3.4 Longitudinal daily monitoring of pharmaceutical and illicit drug occurrence in the River Thames over six weeks

Site 2 was selected for a longitudinal occurrence study of all 31 pharmaceuticals given its convenience, reliability and safety of access during bad weather across six weeks in Autumn and Winter, 2014. Furthermore, it represented an equidistant point in the river between the major west and east WWTP discharge points (~25 km in either direction). A total of 27 drug residues were determined in the River Thames (Figure 5). The total (summed) concentration of all compounds monitored varied from ~1-3.5 μg L^{-1}.

Over the course of the study, 13 CSOs were triggered due to heavy rainfall (Table S11). In all, six Type 1 CSOs were recorded over the six-week period, which were most relevant to this study. Of these, four samples were taken within 24 hours following a CSO event. Available Type 1 CSO-related records from the Hammersmith, Lotts Road and Western pumping stations showed that a combined total of 1,883,485 and 204,150 m^3 of untreated sewage mixed with storm water was discharged into the Central London region of the River Thames in November and December months, respectively [64]. Measured total concentrations of illicit drugs and pharmaceuticals decreased in general throughout November and December (Figure 5 and Table S12). Approximately 75 % (~80-90 mm) of the total rainfall fell in the first three weeks. Dilution with freshwater arising from the upper Thames may have been a contributor to this decline, amongst other factors such as changing temporal consumption patterns, varying WWTP performance, changing river water chemistry (e.g., salinity, etc.), molecular stability and biological activity. On the other hand, prolonged elevated concentrations following CSOs could have arisen here where several events occurred in rapid succession, especially in the first three weeks, and which were slowly removed by the tide. The top five

most concentrated compounds on average across the six weeks were caffeine (477 ±313 ng L^{-1}), diazepam (305 ±558 ng L^{-1}), tramadol (220 ±75 ng L^{-1}), carbamazepine (154 ±99 ngL^{-1}) and amitriptyline (102 ±57 ngL^{-1}). Temporal variance in measured concentrations across the 30 sampled days was, as perhaps expected, high and not likely to only include any impact of CSOs, but also changes in community consumption behaviour, illness/disease treatments or seasonal consumption patterns influencing the concentrations in treated wastewater effluents [65]. Where Type 1 CSOs occurred, no readily identifiable spikes in total concentration of all drugs determined were observed within a 24 to 48hour period, nor any correlations with tide height, daily rainfall, or a ratio of both (R^2 <0.1 in all cases). Principal component analysis did not yield any further classification between daily concentrations determined for all 27 compounds (Figure S2). In addition, five out of six Type 1 CSOs were also accompanied by Type 2 CSOs, which may have served to dilute untreated wastewater entering the Thames Tideway further. Some additional interesting observations were made. The illicit drugs ketamine and mephedrone were detected almost every day at 12 ±4 ngL^{-1} and 9 ±2 ng L^{-1}, respectively. The latter was banned in the UK in 2010, but was still determined in wastewater influent, effluent and river water here in 2014. However, despite being present at higher concentrations in influent, its concentration flux did not align with CSOs, likely in part due to recreational use increasing over the weekend.

When focussing on the seven shortlisted candidate CSO markers, some trends became more evident, but were very complex to interpret. Firstly, concentrations of caffeine, cocaine and its metabolite benzoylecgonine in river water showed a correlation with some CSOs. As their concentrations in

552 untreated wastewater was regularly >1 µg L^{-1}, this was perhaps expected over
553 the other four compounds. Elevated concentrations were mainly detected in
554 samples taken on the following day (Figure 6) especially following the two
555 heaviest rainfall events and CSOs on 23rd November and 11th December, 2014,
556 both during the lower portion of incoming flood tidal phases. For the latter date,
557 two CSOs were triggered on the following day at 06:25 (Type 1) and 08:58
558 (Type 2) just before the sample was taken and which enabled subsequent
559 determination of all compounds at higher concentrations, even within 3 hours
560 following a Type 1 discharge. However, neither cocaine nor benzoylecgonine
561 were detected at obviously elevated levels following Type 1 CSOs on the 4th or
562 14th November. On both occasions, the river was at the top of its tidal phase
563 and dilution may have occurred. As before, elevated caffeine concentration
564 following CSOs seemed prolonged over several days in comparison to cocaine,
565 especially after the heaviest rain event on the 22nd/23rd November.
566 Concentrations of diazepam were high across the first two weeks of the
567 campaign and then decreased markedly thereafter and did not correlate with
568 any one CSO event directly. Short-term elevated concentrations may be more
569 prolonged for this compound given its potential for sorption to sediment [66].
570 Following the CSO event on the 4th November, elevated concentrations of
571 sulfamethazine and sulfamethoxazole occurred, and a mild rise in
572 concentration of sulfapyridine over the following 48 h. However, sulfapyridine
573 was not useful to indicate other Type 1 CSO events across the remainder of
574 the campaign. Lastly, furosemide and bezafibrate yielded no apparent trends
575 and were removed from further interpretations.

576 The majority of compounds tentatively identified during suspect
577 screening as being indicative of influent wastewater were not present in river
578 water. However, the combined signal for the stereoisomers quinine/quinidine
579 was detected every day ($[M+H]^+$ m/z 325.1910), but revealed no obvious co-
580 incidence with CSO events (Figure S3). However, achieving chromatographic
581 resolution of both compounds and quantification is still required to fully evaluate
582 their individual value as CSO markers. Furthermore, the use of signal intensities
583 from LC-HRMS analysis was likely subject to variable matrix interference due
584 to the influence of seawater with tide, especially over the first week of the
585 sampling campaign (Figure 7(a)). However, for the majority of the six seeks,
586 conductivity measurements indicated that the river was predominantly
587 composed of freshwater (600-800 µS), mainly arising from influx of upstream
588 sources to Teddington Lock experiencing heavy rainfall and run-off.
589
590 *3.5 Ammonium, pH and %DO*
591 Comparison of drug concentrations with ammonium and %DO data
592 gathered simultaneously from Putney, Hammersmith and Brentford (each
593 ~5-7 km apart) in the west of the city revealed correlations with most Type 1
594 CSOs (Figure 7 (b)-(d)). Interestingly, and despite their distances apart, the
595 changes in ammonium/%DO concentrations at each site aligned well with each
596 other, indicating that CSOs may be triggered across the length of the network
597 simultaneously. However, and in agreement with some of the drug
598 measurements here, poorly discernable changes in ammonium concentration
599 or %DO were observed for Type 1 CSOs on the 4th, 8th or 9th November (only
600 observed clearly at the Brentford site). The pH of the river remained relatively

601 constant over the six weeks (pH = 7.77 ±0.09), and very minor reductions of
602 <0.25 pH units were observed during periods of elevated ammonium
603 concentration.

604 The duration of CSO impacts could be interpreted from ammonium
605 and %DO data (unfortunately, data for CSO duration and discharge volumes
606 were not available for specific dates). Generally, and like CSO drug markers,
607 changes occurred within 24 h after a CSO and returned to normal levels ~24 h
608 later. A mild positive, but statistically significant correlation (R =0.6023;
609 p=0.0049) existed between total concentrations of the three main CSO drug
610 markers determined on the following day with tide height:daily rainfall ratio at
611 the time of sampling (Figure S4). Therefore, it was concluded that there exists
612 a fine balance between tide height/direction, rainfall and time (<24 h here)
613 before an influent wastewater-specific drug can be measured in the river to
614 potentially indicate CSO influx. The Type 1 CSO event on the 23rd of November
615 2014 was the most prominent and prolonged from these data which explains
616 why concentrations of some CSO drug markers increased so markedly. The
617 Putney site is closest by distance to Site 2 chosen for drug monitoring (~11 km).
618 Despite being more central, smaller changes in ammonium and %DO were
619 observed across the six-week period. Therefore, proximity to a local CSO vent
620 will likely affect measurements overall. Ideally, more sites should be monitored
621 across this catchment to more fully understand spatial impacts of
622 pharmaceuticals and illicit drugs from CSOs on receiving waters. However,
623 despite short-lived peaks in concentration, longer term concentrations of
624 pharmaceuticals and illicit drugs in CSO material may decline overall upon

completion of the Thames Tunnel, which aims to reduce annual sewage discharge via CSOs by 95 % [27].

Conclusions

Of 31 compounds monitored quantitatively, 27 pharmaceuticals and illicit drug residues were determined in river water in the Thames Tideway in daily measurements over six weeks. However, occurrence and total concentrations of pharmaceuticals and illicit drugs as a whole showed no short-term correlation with specific CSO events (total concentration lay between ~1.0-3.5 µg L^{-1}). Following differential analysis of influent and effluent wastewater, seven compounds were shortlisted as potentially being influent wastewater specific and three of these were present at concentrations >1,000 ng L^{-1} in influent (i.e. caffeine, cocaine and benzoylecgonine). In river water, these three compounds showed noticeably elevated concentrations ~24-48 h after CSO events following major rainfall events and aligned with ammonium and %DO data. It was found that there existed a fine balance between tide height, direction and rainfall, before any elevated concentrations of these CSO markers were recorded. Therefore, CSO releases should be ideally aligned with the onset of the ebb tidal phase to enable sufficient dilution to occur. However, even with dilution, more research is required to understand the longer-term impacts of CSOs on drug occurrence in receiving waters and particularly any potential improvements following a major infrastructure upgrade such as that planned in London to mitigate them.

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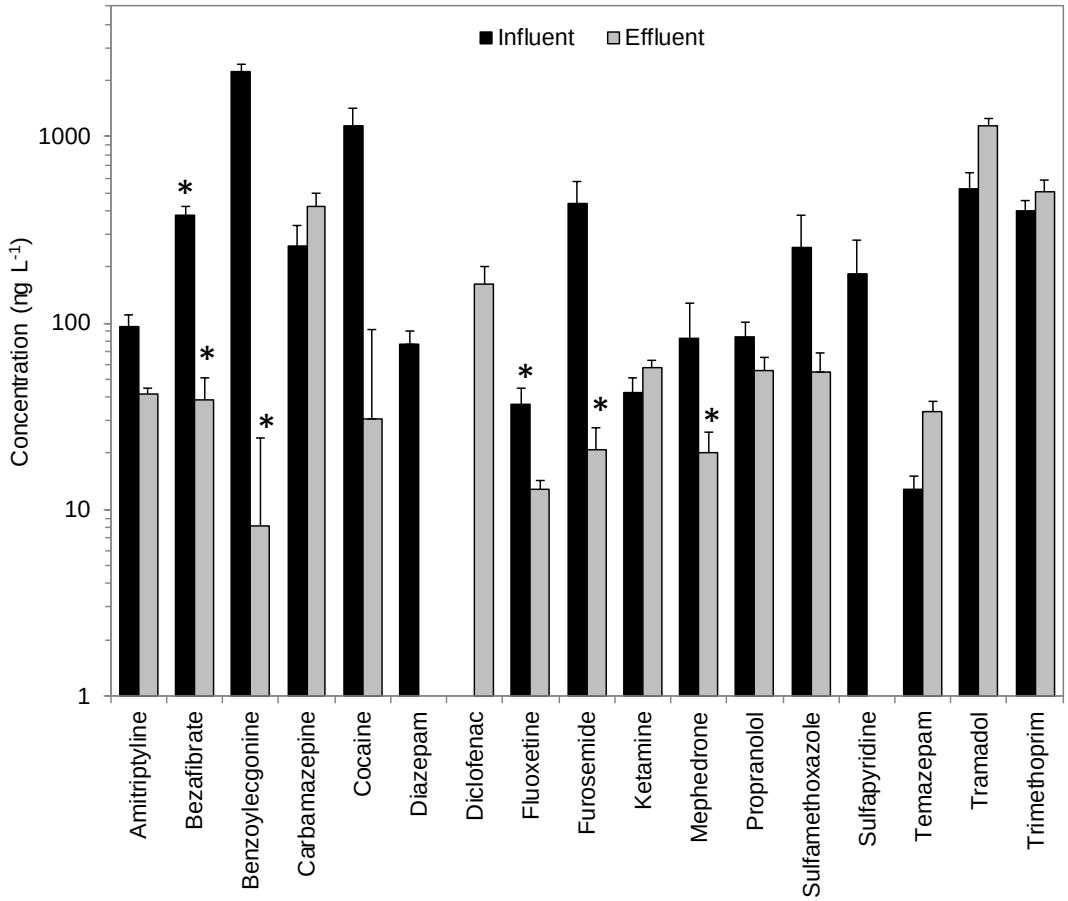

Figure 1. Differential analysis of drug occurrence in untreated influent and treated effluent wastewaters from a major treatment works in London in n=7 consecutive 24-h composite samples in March, 2014. Bars marked with * represent semi-quantitative measurements as values were <LLOQ, but >LOD. Error bars represent the standard deviation of the means of all measurements for each compound across the 7-day period.

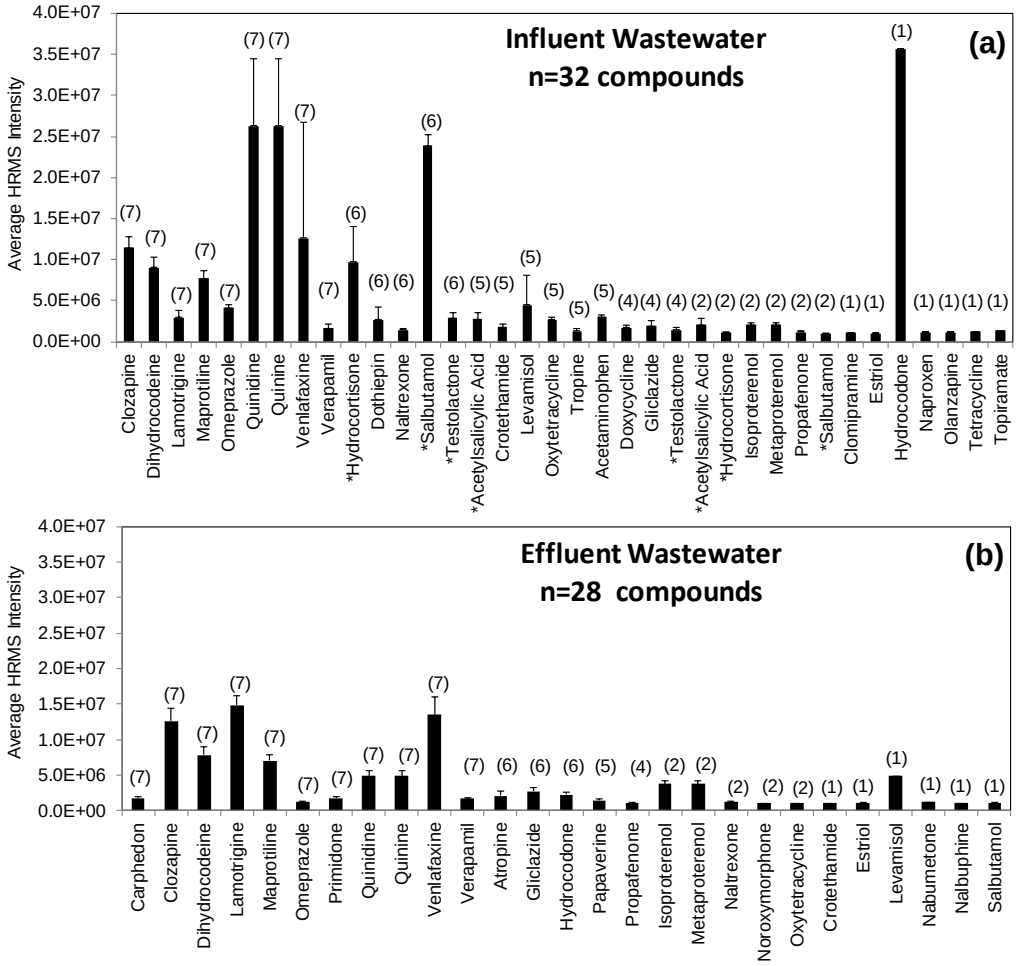

Figure 2. Average signal intensity for each compound tentatively identified by retrospective *in silico* suspect screening in (a) untreated influent and (b) treated effluent wastewaters. Their corresponding occurrence frequency out of 7 days is shown in parenthesis. Bars represent the mean and whiskers represent the standard deviation of that number of daily measurements in (c) and (d). Compounds marked with * represent those where two matching predicted t_R values (±1.30 min threshold) and HRMS signals (δ <5ppm for $[M+H]^+$ or $[M-H]^-$) were obtained.

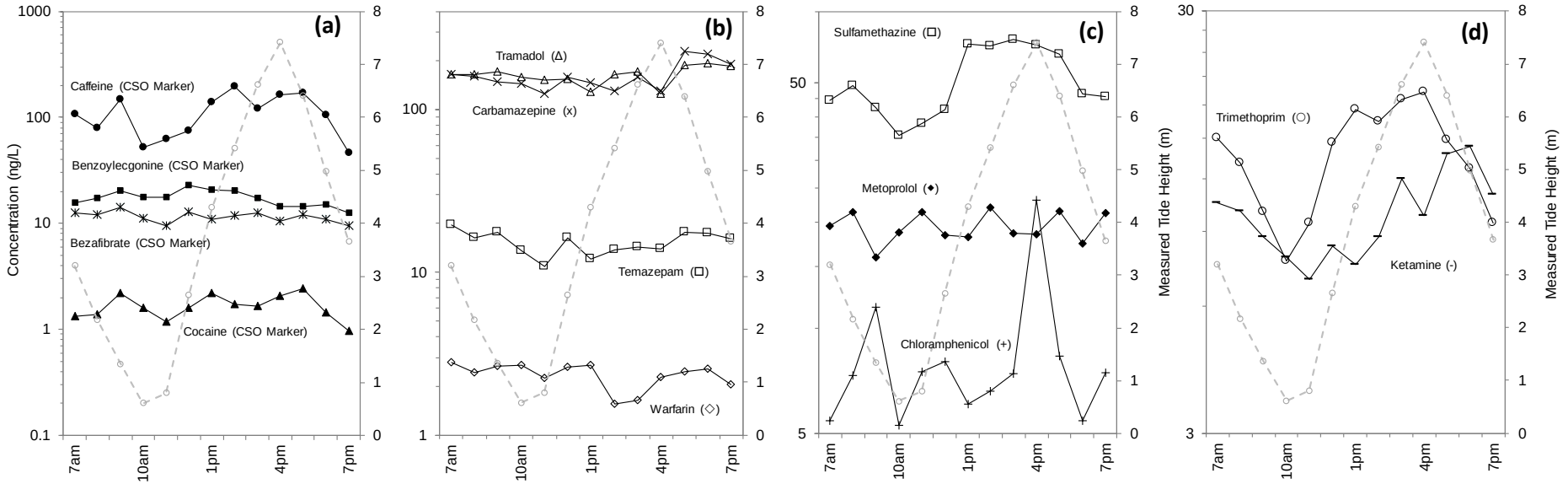

Figure 3. Diurnal variation in (a) CSO marker drug compounds and (b)-(d) all other drug compounds determined above the LLOQ in the River Thames on the 14th August, 2014. Black datapoints represent the mean of n=2 replicate grab sample analyses. Grey dashed lines represent the measured tide height at the time of sampling. No CSOs occurred on this day (<1 mm rainfall).

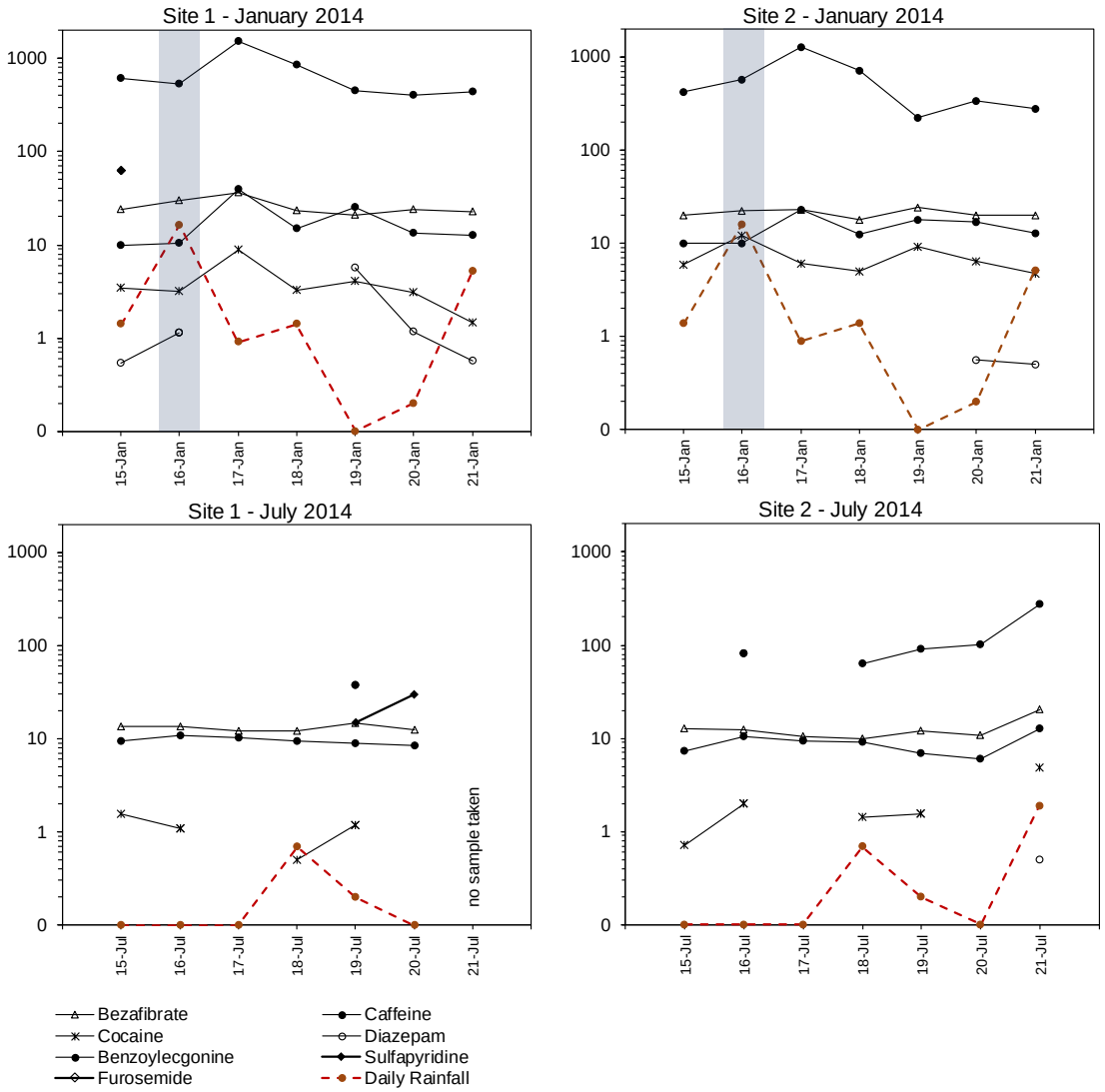

Figure 4. Measured concentrations of seven shortlisted candidate drug CSO markers in samples of Thames River water from two sites in January and July 2014 and overlaid with daily rainfall. A Type 1 CSO occurred on on 17th January, 2014 at 21:50 hours (shaded in grey). Note: No sample was taken from Site 1 on 21st July, 2014. All measurements represent the mean of n=2 replicates.

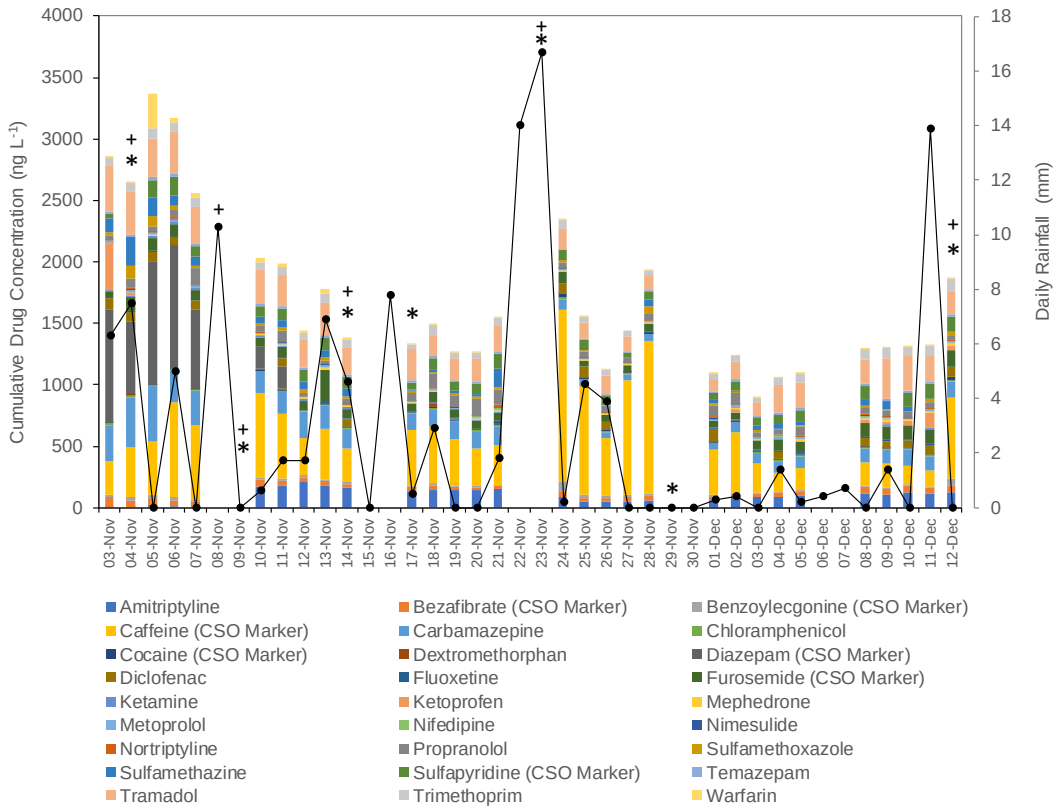

Figure 5. Cumulative concentration of all drug residues determined on weekdays in the River Thames across Nov-Dec, 2014. Dates marked with + are Type 1 CSOs where storm water and untreated sewage were combined and released directly into the river. Dates marked with ∗ represent Type 2 CSO events where storm water was mixed with treated wastewater effluent at a WWTP and then released into the river (where both + and ∗ exist, two such CSOs occurred on the same date, also see Table S11).

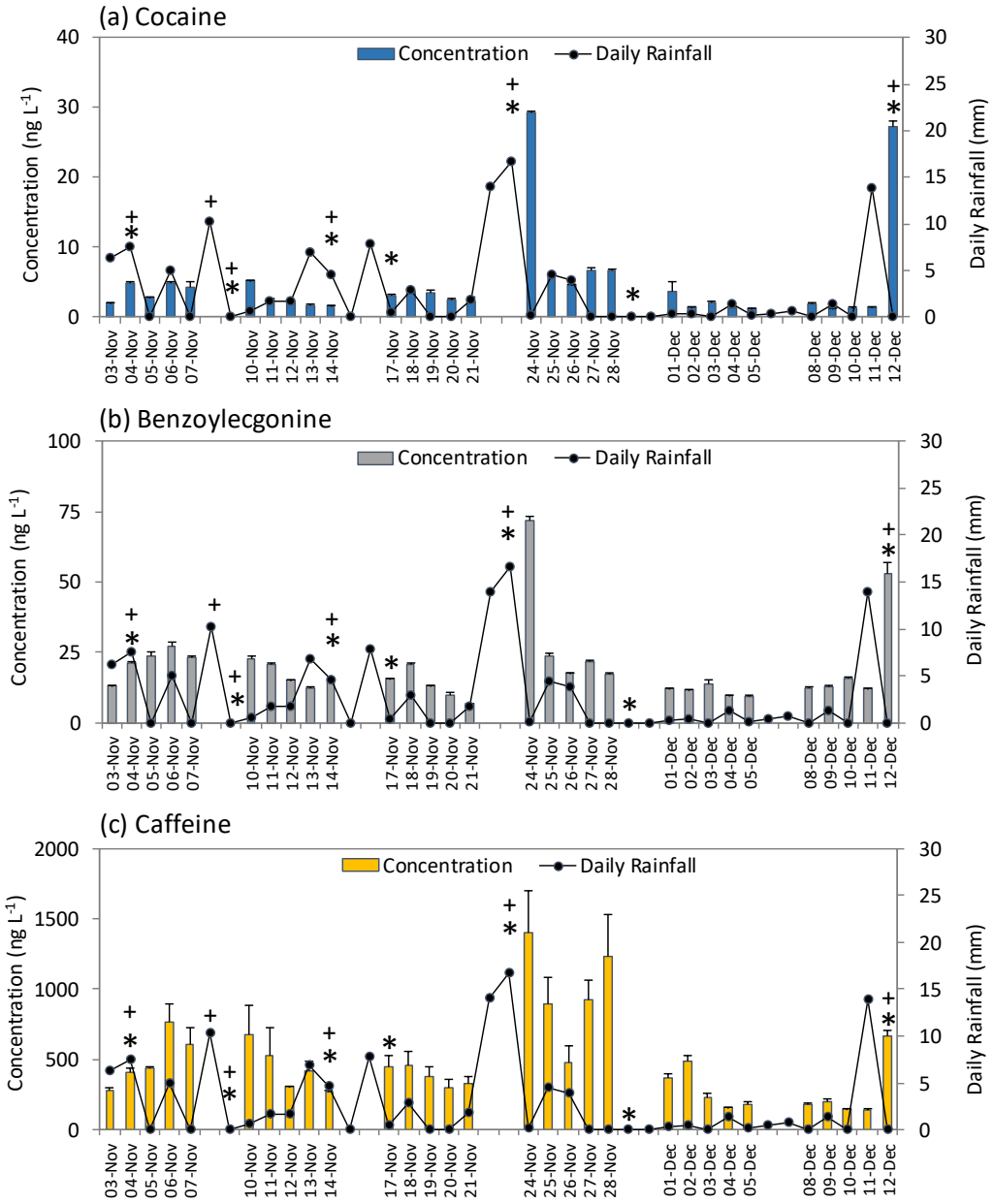

Figure 6. Occurrence of three drug CSO markers in river water from the Thames over six weeks in Nov-Dec, 2014 (overlaid with daily rainfall). Dates marked with + or ∗ are as in Figure 5. Bars represent the mean of two replicates and whiskers represent the maximum value measured.

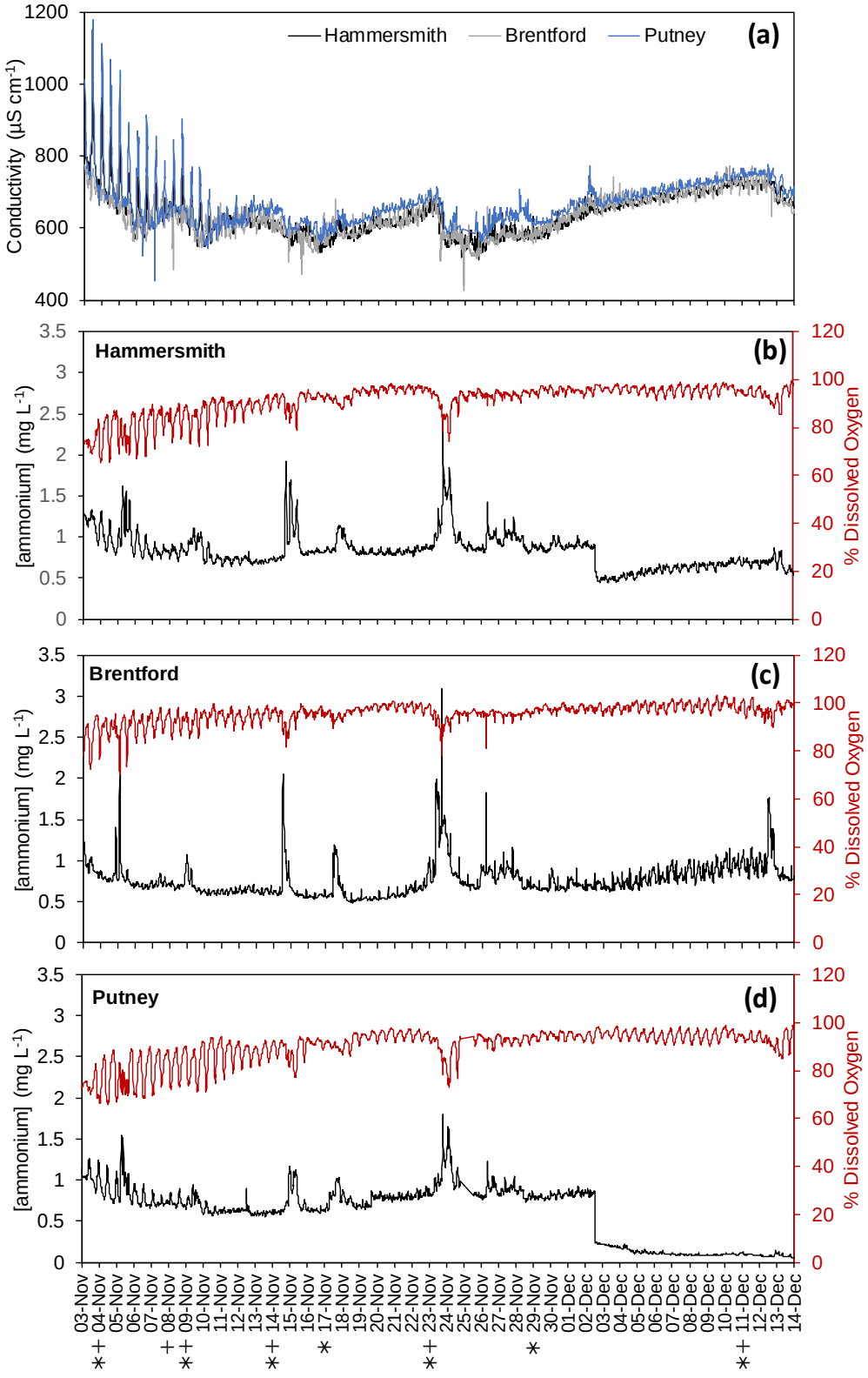

Figure 7. Continuous monitoring data at three sites on the River Thames in Nov-Dec, 2014 for (a) conductivity and (b)-(d) % DO (red)/ammonium concentration (black) at Hammersmith, Brentford and Putney sites, respectively. Data-acquisition frequency =15 min. Dates marked with +/∗ represent CSO Types 1 and/or 2, respectively.