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Passive sampling of wastewater as a tool for the long-term monitoring of community exposure: Illicit and prescription drug trends as a proof of concept

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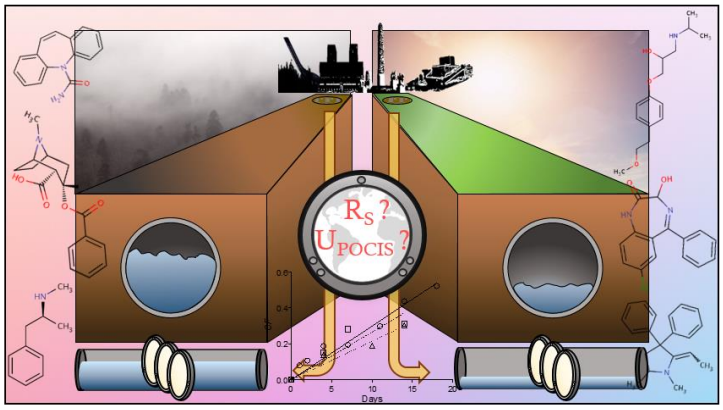

Abstract

A passive sampling device, the Polar Organic Chemical Integrative Sampler (POCIS), was calibrated in-situ over a 4-week period in Oslo (Norway) for 10 illicit drugs and pharmaceuticals with the goal of developing an approach for monitoring long-term wastewater drug loads. The calibrations were performed in triplicate using three different overlapping calibration sets under changing environmental conditions that allowed the uncertainty of the sampling rates to be evaluated. All 10 compounds exhibited linear uptake kinetics and provided sampling rates of between 0.023 and 0.192 L d^{-1}. POCIS were deployed for consecutive 2-week periods during 2012 and 2013 and the calculated time-weighted average (TWA) concentrations used to define different drug use trends. The relative uncertainty related to the POCIS data was approximately 40 % and, except for citalopram, 85% of all the long-term measurements of pharmaceuticals were within the confidence interval levels calculated to evaluate the effects of changing environmental conditions on the TWA estimations. POCIS was demonstrated to be sufficiently robust to provide reliable annual drug use estimates with a smaller number of samplers (n=24) than recommended for active sampling (n=56) within an acceptable level of sample size related uncertainty < 10 %. POCIS is demonstrated to be a valuable and reliable tool for the long-term monitoring of certain drugs and pharmaceuticals within a defined population.

- Keywords

POCIS, Community level drug use, Wastewater epidemiology, In-situ calibration, Long-term monitoring

1. Introduction

Monitoring drug use has traditionally been performed by questionnaire-based surveys and police statistics. Estimating population drug use through the analysis of wastewater samples has been established as an approach for monitoring patterns of community drug use (Castiglioni et al. 2014). Wastewater-based epidemiology (WBE) studies for drugs have provided valuable information, showing spatial and temporal differences across different countries (Ort et al. 2014b, Thomas et al. 2012). More recently, WBE results have been also compared with other sources of information confirming its potential as a complementary approach for obtaining a more accurate picture of the drug use situation (Baz-Lomba et al. 2016b, Been et al. , Zuccato et al.). Furthermore, WBE has recently been applied to assess the community level exposure of humans to a range of environmental stressors (Gracia-Lor et al. 2016, Rousis et al. 2016, Rousis et al. 2017)as well as their combined response to such stressors(Ryu et al. 2016). The generation of community level exposure data that can be compared with other complementary sources of data has a clear potential within environmental epidemiology.

Despite good agreement with the other sources of data, it is possible that wastewater data may be typified by low temporal representativeness and high spatial variability due to the use of different substances and spatial and temporal trends in availability. WBE results therefore need to be carefully interpreted (Baz-Lomba et al. 2016b). Thus far, the temporal coverage of most of the WBE studies performed has been typically limited to a one-week sampling program (Ort et al. 2014b). In order to more accurately estimate the representative mean annual substance use by WBE a recent study recommended the use of stratified random sampling schemes (typically 56 samples per year) (Ort et al. 2014a). Furthermore, different sampling strategies based on the sampling frequency or composite sampling mode have been evaluated in order to decrease the sampling uncertainty (Ort et al. 2010). However, increasing the sampling frequency implies an additional costs together with the power and space requirements of an automated sampling device and such a frequency may still prove inadequate

66 in certain circumstances such as the short-term changes in use patterns or variations in concentrations
67 associated with other external factors such as precipitation (Ort et al. 2014a).

68 Passive sampling devices (PSD) are an alternative sampling tool to overcome some of the above-
69 mentioned issues. PSD have been demonstrated as a good alternative for the monitoring of drugs and
70 other micropollutants in wastewater providing time-integrated estimates that compensate for
71 fluctuating concentrations (Harman et al. 2011b, Kaserzon et al. 2014). PSD may also decrease the limits
72 of quantification compared with traditional sampling and be used as a screening tool for the detection
73 of emerging compounds present at very low concentrations (Alvarez et al. 2014). Furthermore, the use
74 of PSD over a specified period can be performed without human intervention, without any power
75 requirements and at low cost. For example, the annual monitoring of drugs in wastewater can be
76 performed with as few as 26 PSD (Harman et al. 2011b).

77 The polar organic chemical integrative sampler (POCIS) has thus far been applied for the analysis of over
78 300 polar organic substances in water (Harman et al. 2012, Morin et al. 2012). This includes a number
79 of pharmaceuticals and illicit drugs, as well as other polar contaminants such as pesticides (Gonzalez-
80 Rey et al. 2015, Jones-Lepp et al. 2004, Metcalfe et al. 2011). The physicochemical properties of the
81 compounds will determine whether they accumulate in the sampler based on the different solute-
82 solvent-sorbent interactions (i.e. the version of POCIS presented in this study has a good selectivity for
83 compounds with log K_{ow} approximately between 2 and 4) (Harman et al. 2011b). The theory and
84 modelling of chemical uptake by POCIS have been explained in detail elsewhere (Alvarez et al. 2004,
85 Huckins et al. 1993, Vermeirssen et al. 2012). There are however few studies that consider modelling
86 uptake rates for real in situ environmental exposures. Depending on sampler design, PSD can be used
87 in either equilibrium or time-weighted average (TWA) modes to give concentrations of the desired
88 analyte. In contrast to PSDs for hydrophobic compounds, where sampling rates (R_S) can be modelled by
89 physicochemical properties such as molecular weight, R_S for POCIS must first be calibrated
90 experimentally. Laboratory generated R_S can vary significantly between different studies depending on

91 the different calibration methods and conditions used and standardization of the different calibration
92 methods has been recommended in order to reduce these discrepancies (Harman et al. 2012, Morin et
93 al. 2012). Furthermore, R_S generated in the laboratory under controlled exposure conditions may not
94 be representative of the actual values under different and variable environmental conditions that can
95 lead to biased data when calculating TWA concentrations (Miller et al. 2016).

96 One of the primary uncertainties associated with the calculation of POCIS R_S, which in turn
97 fundamentally affects the reliability of POCIS derived TWA concentrations, is the influence of
98 environmental factors, such as the rate of water flow (Kaserzon et al. 2013, Li et al. 2010b), temperature
99 (Li et al. 2010a), pH (Li et al. 2011) and biofouling (Harman et al. 2009). Different approaches have been
100 proposed, such as the use of external R_S corrections (Alvarez et al. 2007), the performance reference
101 compounds (PRC) approach used for hydrophobic PSD (Huckins et al. 2002) and more recently the
102 development of the diffusive gradient in thin-film sampler for organics (Challis et al. 2016). All of these
103 approaches have challenges, but a comprehensive method for relating the uptake in POCIS to
104 environmental factors remains elusive (Harman et al. 2012). In-situ calibration of POCIS has been
105 proposed as an alternative strategy to generate more reliable and constant R_S for a specific site, however
106 only a few papers have published in-situ R_S values (Harman et al. 2011b, Jacquet et al. 2012, Mazzella
107 et al. 2010, Zhang et al. 2008). However, in-situ calibration is also not without its challenges; largely due
108 to the extra costs and the need for more extensive water sampling, compared with laboratory methods.
109 An overall lack of understanding of the sorption phenomena for different compounds means that it is
110 prudent to consider POCIS derived water concentration data as semi-quantitative (Harman et al. 2011a,
111 Miège et al. 2015). Dalton and colleagues (Dalton et al. 2014) have described the variability of in-situ R_s
112 associated with environmental factors (Morin et al. 2012) and more recently, Poulier and colleagues
113 (Poulier et al. 2014) estimated that the uncertainty related to their POCIS data for several pesticides
114 might be as high as 138%.

115 The aim of the current study was to evaluate whether POCIS are a suitable and cost-effective alternative
116 to grab sampling for the long-term monitoring of substance use and potentially exposure at community
117 level through WBE. The in-situ R_S was determined for a number of pharmaceuticals (atenolol,
118 citalopram, carbamazepine, oxazepam, metoprolol, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
119 (EDDP) and morphine) and illicit drugs (cocaine, benzoylecgonine and methamphetamine) in POCIS to
120 estimate substance use trends over 2012 and 2013. The reproducibility of R_S was estimated by
121 overlapping three in-situ calibrations to mitigate the potential confounding effects and impact of
122 different environmental conditions. The accuracy of the POCIS R_S was evaluated by assessing two
123 different uncertainty levels, taking into account the coefficient of variation of the three R_S calculated
124 during the three different calibration periods and the repeatability for each of the triplicates deployed
125 during subsequent long-term (2-yr) monitoring.

126 ## 2. Materials and methods

127 ### *Chemicals, materials and POCIS samplers*

128 Information on chemicals, materials and POCIS samplers is provided in the Supporting Information.

129 ### *Wastewater and POCIS extraction and analysis*

130 Information on wastewater and POCIS extraction and analysis is provided in the Supporting Information.

131 ### *Quality Assurance*

132 Information on quality assurance is provided in the Supporting Information.

133 ### *In situ calibration study design*

134 All samples were collected at the VEAS wastewater treatment plant (WWTP) in Oslo (Norway). VEAS
135 treats sewage for approximately 600 000 people of which the city contributes about 70.5 % and the
136 adjoining areas representing the other 29.5% (8% from Asker and 21.5 % from Bærum, see Figure S1).

137 The total length of the sewer line is 42.3 km and the mean residence time in the sewer system is 5 hours
138 (see www.veas.nu for further details).

139 The in situ calibration took place during February 2014. The calibration experiment was performed over
140 a 4-week period using triplicate POCIS deployed for varying periods of time and in three different sets
141 for a total of 54 POCIS including 3 blanks (Figure 1). POCIS were immersed in wastewater in a
142 perpendicular direction to the wastewater flow. The main set consisted of 10 POCIS triplicates while the
143 two supporting sets consisted of 4 and 3 triplicates. The rationale for overlapping calibration periods
144 was to study the reproducibility of determining the R_S under changing environmental conditions. These
145 data also provided valuable information to determine confidence intervals for the TWA based on the
146 uncertainty associated in the R_S calculation.

147 An ISCO Avalanche Portable Refrigerated Sampler (Lincoln, NE, USA) was used to collect a total of 96
148 wastewater samples during the course of the calibration, three daily samples (8-hour composites,
149 comprising of 15 mL/15 min) from Monday to Thursday and four daily samples (6-hour composites)
150 from Friday to Sunday.

151 Wastewater samples and POCIS for both the calibration and long-term monitoring were collected in the
152 same location from an overflow channel following a sedimentation tank. Flow rate, temperature and
153 pH were provided by VEAS WWTP (All data in SI). The mean flow rate measured with an hourly
154 measurement resolution was 16,369 m^3 $hour^{-1}$ and the range between the flow rate for dry days and
155 rainfall days was 7698 - 33,184 m^3 $hour^{-1}$. The mean flow data for the three calibration sets considering
156 only the first 14 days of each set was 23,340, 23,751 and 21,882 m^3 $hour^{-1}$ respectively. The water
157 temperature was stable during the calibration with a mean value of 7.1 °C while the mean pH was 7.5.

158 *POCIS sampling rate calculation*

159 The accumulation of target compounds in the receiving phase of PSD follows first order kinetics with an
160 initial linear regime, followed by curvilinear and equilibrium stages (Morin et al. 2012). Thus the overall
161 accumulation of a chemical in a passive sampler can be described by

1) $C_s = K_{sw} C_w [1 - e^{-k_e t}]$

where C_s is the concentration of the given compound in the sampler, C_w the average concentration in the wastewater or TWA, K_{sw} the sampler-water partition coefficient, k_e the elimination rate constant of the pollutant from the receiving phase and t the exposure time. The time to reach half of the equilibrium concentration ($t_{1/2}$) corresponding to the limit between the kinetic regime and the curvilinear regime can be estimated from the first order curves fitted to calibration data to corroborate their linearity during the exposure time.

The mechanisms controlling the uptake of chemicals by POCIS are complex and remain only superficially described. A range of sorbate-sorbent interactions are possible and interactions with the PES membrane are also compound specific, although these appear to be related to hydrophobicity (Vermeirssen et al. 2012). Thus there is some evidence of bi-phasic uptake in POCIS (Fauvelle et al. 2014). In addition, the adsorption of chemicals to POCIS sorbents is a surface phenomenon that can be competitive. For these reasons the above equation, although regularly applied, may be invalid for use with POCIS. Therefore, for simplicity POCIS is often considered as an infinite sink for contaminants with uptake in the linear phase. The relation between C_s and C_w can be expressed by

2) $R_s = \frac{C_s M_s}{C_w t}$

Where R_S is the sampling rate and M_S is the mass of the sorbent. POCIS uptake was described as concentration factors (CF), by dividing by the water concentrations (C_s/C_w), to normalize for fluctuating concentrations in wastewater:

3) $CF = \frac{C_S}{C_W} = \frac{R_S t}{M_S}$

Long-term measurements and TWA calculations

POCIS (n=3) were replaced every two weeks from December 29th of 2011 to January 3rd of 2014 for a total of 49 measurements. Sampling periods remained stable during the two year-long period with a 15

185 day average, with some exceptions largely for practical reasons such as summer and Christmas holidays.
186 As mentioned previously, environmental conditions affect POCIS R_S and therefore the accuracy of
187 subsequent TWA concentrations. Wastewater flow rate, temperature and pH were recorded during the
188 two year-long study showing seasonal differences.

189 Average R_S obtained from the 3 in-situ calibrations (14 day data) were used to estimate the average
190 water concentration of the target compounds during each exposure time. The concentration obtained
191 in the POCIS extract was used to calculate the TWA (C_w) by using the eq. 2. TWA concentrations were
192 normalized to the median concentrations.

193 *Pharmaceutical sales data. Comparison between predicted environmental trends and WBE.*

194 The environmental mass loads for atenolol, citalopram, carbamazepine, oxazepam and metoprolol in
195 wastewater were estimated from the per capita monthly sales data from 2012 to 2014 obtained from
196 the Norwegian Institute of Public Health (FHI) (Norwegian Drug Wholesales statistics; FHI, Oslo,
197 Norway). These data are gathered from the same catchment area connected to the sewer system under
198 investigation. The consumption of these pharmaceuticals is relatively stable as the general population
199 regularly uses them. Furthermore, these compounds are present in wastewater at detectable and
200 quantifiable concentrations. The monthly turnover by dosage was multiplied by the defined daily dose
201 (DDD), considered as the average maintenance dose per day in milligrams for a drug used for its main
202 indication in adults. Correction factors for excretion, degradation and the ratio purchase/consumption
203 were not considered since are expected to remain constant during study within the same catchment.

204 These predicted environmental pharmaceutical trends were calculated only to estimate their variability
205 during the study from 2012 to 2014. This information was then compared with the wastewater samples
206 and POCIS results. In parallel, the uncertainty related with the sample size for both sampling modes was
207 also compared via calculating the standard error of the mean (SEM) using equation 4 as described
208 elsewhere (Ort et al. 2014a). The coefficients of variation (CV) were calculated using the population-
209 normalized loads (mg/day/1000 inh.) for the 28 wastewater samples daily analysed during the in-situ

calibration and the 49 TWA concentrations (ng L^{-1}) determined with POCIS for the long-term monitoring, where *n* is the number of samples. The average flow rates during the exposure of POCIS were not applied to the TWA concentrations in order to avoid a deviation on the standard error.

4) $U = \frac{CV}{\sqrt{n}}$

TWA confidence intervals

The study of the confidence intervals associated with POCIS data was carried out as described elsewhere (Poulier et al. 2014). The variations in R_S due to environmental conditions during POCIS exposure are considered the primary contributor to the overall uncertainty associated with the POCIS data and were considered to be within a factor of two (Harman et al. 2012, Morin et al. 2012). The accuracy of the R_S herein was evaluated as the coefficient of variation of the three in-situ calibrations intending to capture the variations during the three different sampling periods. Despite the fact that the three in-situ sampling sets were performed during the same season, the water flow rates varied substantially providing a realistic measure of the reproducibility of R_S. The evaluation of the repeatability on POCIS triplicates following the two-week exposure was also included as a contributor of uncertainty by calculating the relative standard deviation (RSD) of all the POCIS triplicates exposed in wastewater.

The overall equation is described below, where % accuracy are the upper and lower limits of the accuracy range calculated in the in-situ calibration, *k* is the coverage factor that produces an expanded uncertainty to an approximate level of confidence (in this study *k*=2, leading to a confidence interval of about 95%), % RSD is the repeatability and U_{POCIS} the POCIS data uncertainty:

5) $\%Accuracy_{low} - k\ x\ \%\ RSD \leq U_{POCIS} \leq\ \%Accuracy_{up} + k\ x\ \%\ RSD$

Two different diagnosis levels based on uncertainty data were calculated for POCIS. The % RSD based on the repeatability was the same for both, while the first level used the % accuracy obtained from the in-situ R_S calculation and the second level used two predefined levels of -50% and + 100% based on the assumption that R_S values vary within a two-fold range(Harman et al. 2012). Finally, the confidence

234 interval levels were evaluated using TWA concentrations for the 5 pharmaceuticals included in this
235 study.

236 ## 3. Results and discussion

237 *Sample rates: Three in situ calibrations in one*

238 All of the target compounds were detected and quantified in both POCIS and wastewater samples with
239 the exception of methamphetamine in POCIS-Set 1 (after 18 days of exposure), which suffered from
240 high ion suppression due to the complexity of the matrix. Water concentrations for all of the target
241 compounds were mainly influenced by the wastewater flow rates due to the heavy periods of rain during
242 calibration. When comparing the water flow-corrected loads, cocaine, and its main urinary metabolite
243 benzoylecgonine, showed an increase during the weekend while methamphetamine and the other
244 pharmaceuticals presented a more stable trend, in agreement with previously reported data (Baz-
245 Lomba et al. 2016b, Ort et al. 2014b, Salvatore et al. 2015). Wastewater and POCIS concentrations are
246 presented in Figure S2.

247 Accumulation curves obtained for each of the 10 target compounds were assessed for the three time
248 periods. R_S were calculated as the slope of the linear part of the fitted curves that were forced through
249 the origin (Figure 2). No lag in uptake was observed. Half-time ($t_{1/2}$) to equilibrium values were estimated
250 where possible from the fitted non-linear curves and complementary R_S were also calculated from the
251 linear portion of these curves (Figure S3). Citalopram, oxazepam and EDDP showed very similar curves
252 when using non-linear or linear models. Carbamazepine showed curvilinear accumulation kinetics
253 especially for the set 1 (28 days) with a $t_{1/2}$ *of 6.7 days.* For the other compounds the linear model
254 appeared to better fit the data, therefore this was used for R_S calculation and it was not possible to
255 estimate $t_{1/2}$. It is possible to overestimate the linear part of the curve through using the linear fit model,
256 however results obtained using the curvilinear model appear to be somewhat ambiguous. Fouling was
257 not removed from the samplers during the exposure periods as has been performed in previous studies

(Harman et al. 2011b) . Therefore, the amount of fouling accumulated on POCIS was considerably high after three or four weeks, which may reduce uptake and cause the apparent non-linearity.

In-situ sampling rates were calculated over the three different exposure times (Table 1). The average CV for the three different in-situ calibration sets was higher using all of the measurements than when just using the results from the first 14 days. Using a linear fit for the in-situ calibration during the first 14 days, the average CV for all the studied compounds was 17.1 %. Atenolol and morphine had the best reproducibility during the three different calibration exposures with a coefficient of variation of 10.1% whereas benzoylecgonine showed a CV of 26.3 %. POCIS R_S ranged from the lowest values presented by morphine, methamphetamine and EDDP with 0.023, 0.026 and 0.027 L d^{-1} respectively, to 0.192 L d^{-1} for metoprolol. When compared with the results reported by Harman et al. (Harman et al. 2011c), a study performed at the same WWTP in 2010, the R_S seem to be approximately half for all of the compounds except for methamphetamine for which R_S is about a fifth lower. In general, the results obtained herein compare well to in-situ R_S reported in previous studies (Fedorova et al. 2014, Morin et al. 2012). As suggested elsewhere (Alvarez et al. 2004), the mass transfer of compounds into PSD is mainly controlled by the aqueous boundary layer, implying a correlation between sampling rates and the water flow velocity and turbulence, although results in the literature are somewhat ambiguous for POCIS (Harman et al. 2012, Morin et al. 2012).

Two heavy rainfall events occurred during the course of the calibration and the average wastewater flow rates varied considerably during the calibration period. For example, during the first 4 days of the in-situ calibration the wastewater average flow rate was 284,298 m^3 day^{-1} while during the first 4 days of the second in-situ calibration it was 677,410 m^3 day^{-1}. Concurrently, the wastewater average concentrations for metoprolol for example, were 362 ng L^{-1} during the first 4 days of the first calibration and 145 ng L^{-1} for the second set (Figure S2). The heavy rain increased the water flow rates “diluting” the water concentrations, but the mass loads in wastewater (C_w x Q_w) for the pharmaceuticals remained stable. Despite the water flow rate more than doubling, the mass adsorbed in POCIS decreased from

235 ng POCIS^{-1} in the first in-situ calibration set to 134 ng POCIS^{-1} in the second set. This shows that POCIS was able to capture the lower concentrations, but that sampling rates were not significantly affected by the increased flow rates. This may be due to the increasing volume of wastewater not translating into an equivalent increase in laminar flow that might reduce the aqueous boundary and increase uptake. Furthermore, the heavy rainfall events prevented the assessment of the competitive sorption/dissipation of the target compounds by interfering substances.

Li et al. (Li et al. 2010b) observed an increase in POCIS sampling rates for most of the pharmaceuticals, personal care products (PPCPs) and endocrine disrupting substances (EDS) evaluated in their study when flow velocities increased from 2.6 to 37 cm s^{-1}. Certain compounds, such as atenolol, appeared not to be influenced by the changes in flow rate, whereas other compounds, such as carbamazepine and citalopram, exhibited greater uptake in POCIS when the flow rate increased. Kaserzon and colleagues (Kaserzon et al. 2013) also found that the dependence of sampling rates on the flow rate was analyte specific. Furthermore, those experiments were performed under very stable wastewater concentrations. Therefore when POCIS are exposed to extreme fluctuations in flow rate and concentrations, as in the current study, the expected uncertainty should be higher.

Annual TWA concentrations

All of the target substances were detected at quantifiable levels in all of the POCIS samples (*n*=147) deployed during 2012 and 2013. Metoprolol, oxazepam and carbamazepine showed the highest average concentrations detected in POCIS with concentrations of 1560, 928 and 434 ng POCIS^{-1} respectively. Morphine showed the lowest levels with an average concentration of 80 ng POCIS^{-1} while, in contrast to what is normally reported for wastewater samples, the average concentration of cocaine in POCIS was higher than benzoylecgonine (271 and 164 ng POCIS^{-1} respectively).

TWA concentrations for pharmaceuticals were generally stable with certain exceptions. Atenolol, citalopram and carbamazepine showed the highest TWA concentrations in February 2012. Oxazepam and metoprolol showed the highest TWA concentration during September and October 2012. Also all

of the measurements performed during January and March 2013 were noticeably higher for all of the pharmaceuticals. All the aforementioned events concurred with dry seasons during which the water flow rates were considerably lower than average (Figure S4). The fact that the water flow rates during the in-situ calibration were extremely high could result in an underestimation of the R_S and therefore an overestimation of the TWA concentrations during low flow rate seasons. When the turbulent conditions are very high, the aqueous boundary layer may thin-out to the point that it is no longer the limiting barrier to solute transport (Alvarez et al. 2004). Under such conditions the controlling factor for uptake into POCIS is poorly defined but is likely to be compound specific and may be either the membrane or the boundary layer surrounding the sorbent surface.

Wastewater temperature during the in-situ calibration was stable (7.1 ± 0.6 °C) while the annual mean during 2012 and 2013 shows a broader variability (11.1 ± 2.9 °C). VEAS WWTP provided 41 pH measurements during the studied period with a very stable pH average of 7.5 (CV = 1.6%) (both temperature and pH information is presented in Figure S5).

Wastewater temperature fluctuations during the year can also influence the uncertainty and therefore decreasing the accuracy of TWA concentrations. Assuming that consumption of the studied pharmaceuticals was stable during the period of investigation, the fact that there are large increases in POCIS accumulations during certain periods (e.g. February 2012 or January 2013) (Figure 3), or do not occur for certain compounds such as cocaine and morphine (Figure S6), lead us to hypothesize that these fluctuations are due to compound-dependent physicochemical properties or other unknown factors. Direct disposal of pharmaceuticals into the sewer system may be one reason for these fluctuations. Recently, Petrie et al. (Petrie et al. 2016) confirmed the direct disposal of fluoxetine into wastewater by comparing its metabolite profiling with enantioselective analysis and differentiating between consumed and non-consumed drugs, similar to a previous study where the direct disposal of MDMA was identified in wastewater through comparing its enantiomeric ratio (Emke et al. 2014).

Data variability and TWA confidence intervals

333 The predicted environmental trends estimated from the pharmaceutical sales data from 2012 and 2013
334 was used as a complementary information to evaluate the TWA concentrations measured in POCIS
335 (Table 2). The sales trends for the five pharmaceuticals were very stable during the two-year study.
336 Carbamazepine sales loads showed the highest variability (CV = 13.9 %) while oxazepam was the most
337 stable (CV = 7.3 %), all within an acceptable level of uncertainty below 3 %, confirming the stability of
338 sales during the monitoring period.

339 The variability of the population-normalized loads for the consecutive 28 wastewater samples collected
340 in February 2014 was also low, showing a good agreement with previous publications (Ort et al. 2014a).
341 Metoprolol population-normalized loads showed the highest variability for composite samples (CV =
342 37.9 %) while citalopram showed the lowest variability (CV = 13.6%). The uncertainty levels related with
343 the sample size for the 28 samples were all below 10 %, confirming good agreement with sales data
344 although these results must be interpreted carefully since sampling on consecutive days cannot account
345 for seasonal variations during the rest of the year. Ort et al. (Ort et al. 2014a) estimated that the relative
346 error for the annual mean estimation of cocaine consumption was approximately 60 % when using 7
347 consecutive wastewater samples, mainly due to the temporal variations linked with the types of drug
348 use. Furthermore, they suggested that using 56 stratified wastewater samples per year the uncertainty
349 for most of the substances and locations is approximately 10 %.

350 POCIS TWA concentrations showed higher variability during the sampling campaign with a CV ranging
351 from 47 % for oxazepam to 26 % for atenolol in 2012 and 35 % for carbamazepine to 27 % also for
352 atenolol in 2013. The uncertainty due to the sample size for the five pharmaceuticals was lower than 10
353 %. In this case the variability is higher than sales loads and composite samples, but still at a very good
354 level considering that the annual TWA concentrations might be influenced by drastic changes in flow
355 rates due to the different environmental conditions and, especially in Oslo, where there are known to
356 be large shifts in the city´s population during the summer vacation in July and Christmas
357 holidays(Norway 2014).

358 Concurrently, the uncertainty of POCIS TWA concentrations was assessed by calculating two different
359 confidence intervals based on: 1) The accuracy of POCIS R_S during the in-situ calibration and the POCIS
360 triplicates repeatability during the 2-year monitoring. 2) The assumption that R_S values vary within a
361 two-fold range (Harman et al. 2012). Atenolol showed the highest R_S accuracy levels during the in-situ
362 calibration with a CV of 10.1 % while metoprolol showed the highest variance with 27.4 %. The average
363 CV calculated from the 49 deployments of POCIS triplicates ranged from 15.6 % for citalopram to 19.1
364 % for metoprolol. The accuracy and repeatability of the POCIS R_S for the selected pharmaceuticals are
365 shown in Table S1 and were used in equation 4 to define two different uncertainty levels. The
366 uncertainty estimated for level 1 using the experimental data obtained from the in-situ calibration was
367 lower than that calculated for the level 2 (R_S values vary within a two-fold range). In both cases atenolol
368 had the lowest uncertainty range (± 35.4%) while metoprolol showed the highest (43.1%), due to the
369 higher variability during the in-situ calibration (Table 2).

370 Figure 3 shows the normalized TWA concentrations for the selected pharmaceuticals within the
371 different uncertainty ranges. Citalopram showed the biggest variations during the two-year monitoring
372 period, presenting 2 measurements outside of the level 2 (red dotted line) and 9 measurements outside
373 of level 1 (green dashed line). For the remaining pharmaceuticals, atenolol, carbamazepine, oxazepam
374 and metoprolol showed no or a single data point outside of level 2 and 4, 5, 4 and 2 outside of level 1
375 respectively. With the exception of citalopram, 85% of all the measurements where within level 1 of
376 uncertainty.

377 *POCIS annual estimations*

378 The annual mass loads in 2012 and 2013 for atenolol, citalopram, carbamazepine, oxazepam and
379 metoprolol, using the median of the TWA concentrations and the annual flow rate average shown in
380 Table 2 are in agreement with wastewater grab samples analysed in the same WWTP during the last
381 years (Baz-Lomba et al. 2016a, Baz-Lomba et al. 2016b). Cocaine and benzoylecgonine were present at
382 stable concentrations over the two-years. The cocaine mass loads reported in wastewater, based on a

week-long sampling during the last 3 years(Ort et al. 2014b) showed that the average loads in Oslo were 96, 70 and 271 mg/day/1000 inhabitants during 2012-2014 respectively. More recently, and also for a 1-week sampling campaign in Oslo in 2015, we have reported cocaine average mass loads of 152 mg/day/1000 inhabitants (EMCDDA 2015). The previously mentioned mass loads were calculated for benzoylecgonine. When comparing these results with those presented herein using the benzoylecgonine median concentration and the total annual wastewater average flow rate, the average cocaine mass load during 2012 and 2013 in Oslo estimated from POCIS was 120 mg/day/1000 inhabitants which agrees well with active sample measurements in wastewater in Oslo during the last four years.

Methamphetamine showed a decreasing trend from an average of 645 ng L^{-1} in 2012 to 363 ng L^{-1} in 2013 in good agreement with previous reports on methamphetamine trends in Norway (Bramness et al. 2015). The reported methamphetamine mass loads during 2012-2014 were 169, 108 and 237 mg/day/1000 inhabitants respectively while in 2015 the weekly average was 172 mg/day/1000 inhabitants. The methamphetamine mass loads average during 2012 and 2013 measured in POCIS was 263 mg/day/1000 inhabitants, showing a good agreement with the aforementioned wastewater results. Finally, morphine showed a very small variability across the 49 POCIS measurements with a TWA median concentration of 234 ng L^{-1} while EDDP had two big peaks in October 2012 and February 2013 and a TWA median concentration of 278 ng L^{-1} (Figure S6).

4. Conclusions

POCIS TWA concentrations have been shown as a good complementary tool for the monitoring of certain pharmaceuticals and drugs present in wastewater when performing in-situ calibration. The poor knowledge of modelling uptake and the use of proper exposure corrections are the main issues related with the estimation of POCIS TWA concentrations and were solved by performing three overlapped in-situ calibrations under different environmental conditions. Furthermore, in-situ data allowed the determination of the R_S accuracy and POCIS uncertainty

The reliability of this procedure was tested by comparing the POCIS TWA annual concentrations trends for certain pharmaceuticals with those from their sales data within the same catchment area. The annual variability of the POCIS TWA concentrations for the five pharmaceuticals ranged between 25.9 to 46.7 % with uncertainty levels around 40 % (lower than previous publications (Miège et al. 2012, Poulier et al. 2014)) while pharmaceuticals sales data confirmed a very stable consumption trend over time. In addition, TWA concentrations for the other five drugs were measured and compared with previously reported concentrations in wastewater showing good agreement within similar levels of uncertainty. TWA concentrations for the target pharmaceuticals were found to be within an acceptable level of uncertainty demonstrating that POCIS can be a valuable tool for the widespread and long-term application of WBE.

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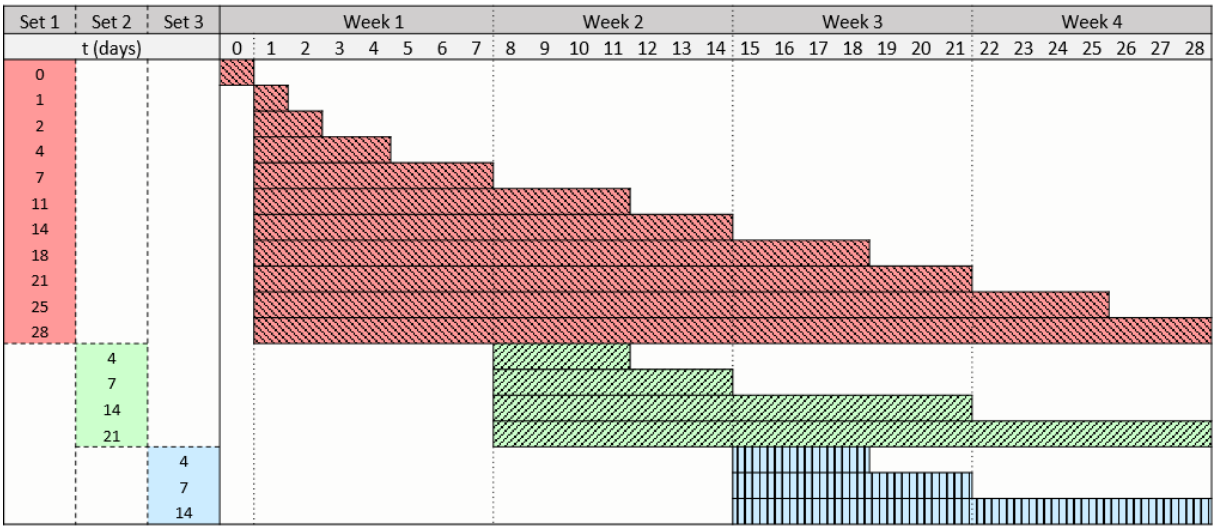

Figure 1. Distribution of POCIS deployments during the in-situ calibration. Number of exposure days during which POCIS were deployed at VEAS WWTP (Oslo, Norway) in February 2014.

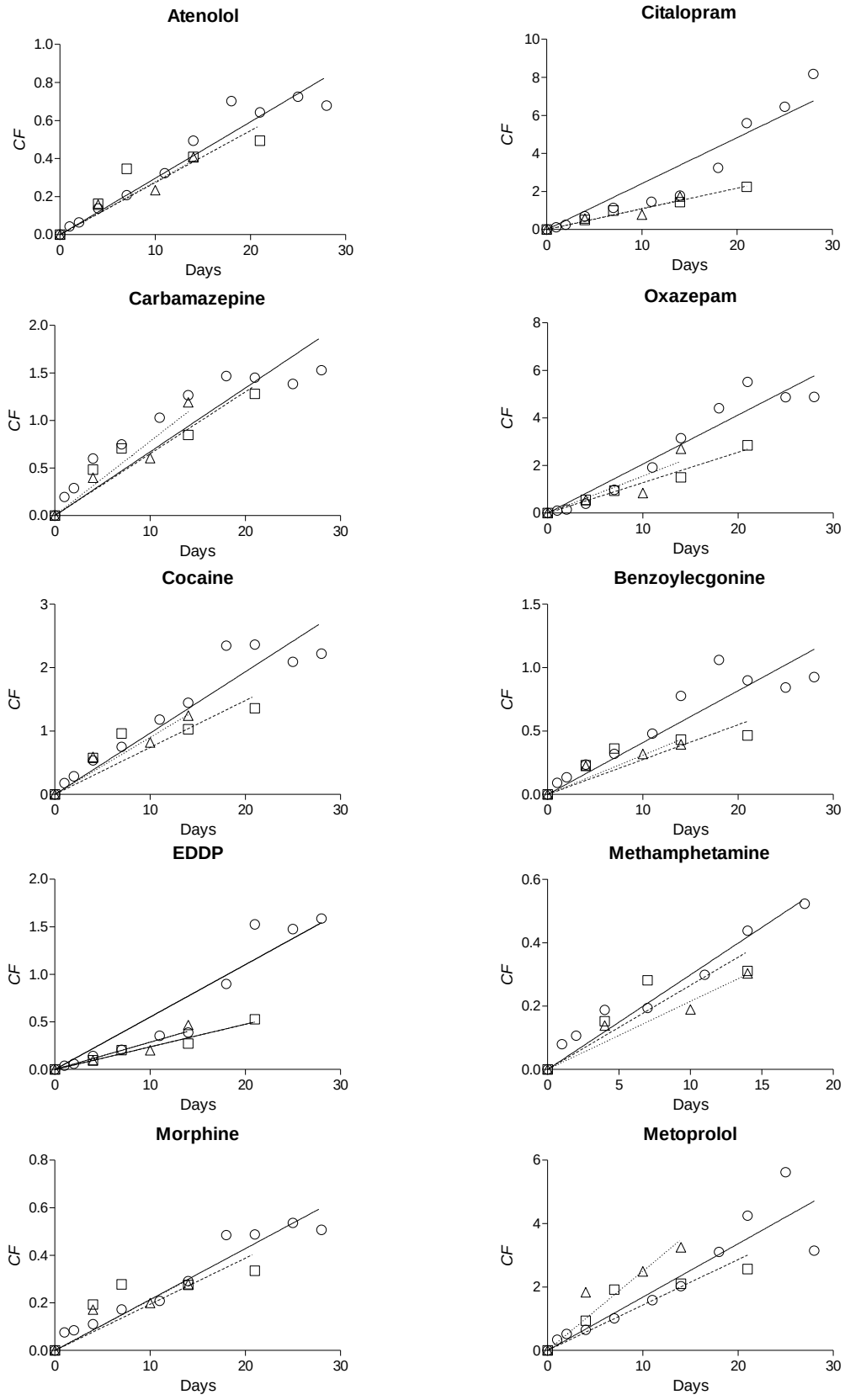

Figure 1. Linear fits in POCIS obtained for each of the 10 target compounds assessed over three time periods in, concentration factor vs time. Set-1: 28 days (solid line, circles); Set-2: 21 days (dashed line, squares); Set-3: 14 days (dotted line, triangles).

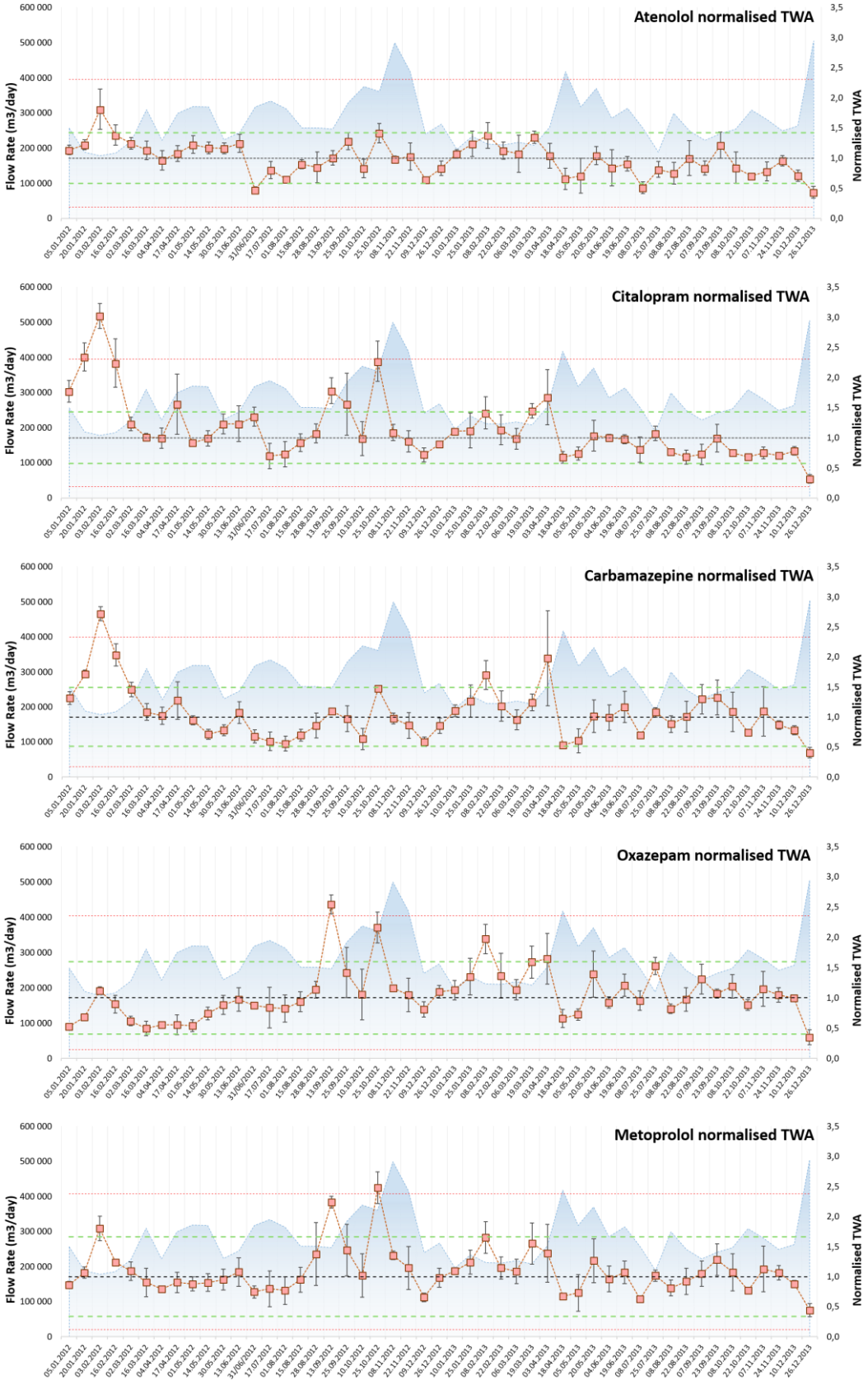

Figure 1. Uncertainty ranges and normalized time-weighted average concentrations in POCIS (n=3) for atenolol, citalopram, carbamazepine, oxazepam and metoprolol (right axis). Wastewater flow rate (m^3 L^{-1}) is represented in the background (left axis). Dates (x axis) represent the mid-point of the exposure time.

Table 1. Sampling rates (R_S) in L·day^{-1} for the three different in-situ sets (C1, C2 and C3), average value and coefficient of variation using a linear model during the whole exposure time and only the first 14 days.

Rs - Linear (C1=28 d; C2=21 d; C3=14 d)					
	C1	C2	C3	Av.	CV (%)
Atenolol	0.030	0.027	0.028	0.028	4.3
Citalopram	0.242	0.109	0.111	0.154	49.6
Carbamazepine	0.067	0.065	0.078	0.070	9.9
Oxazepam	0.206	0.128	0.156	0.163	24.3
Cocaine	0.097	0.074	0.090	0.087	13.4
Benzoylecgonine	0.041	0.027	0.031	0.033	21.1
EDDP	0.055	0.024	0.029	0.036	47.0
Methamphetamine	0.030	0.027	0.021	0.026	16.3
Morphine	0.021	0.019	0.021	0.021	5.4
Metoprolol	0.168	0.143	0.250	0.187	29.7
Av.	**0.096**	**0.064**	**0.081**	**0.080**	**22.1**
Rs - Linear 14 d					
Atenolol	0.033	0.034	0.028	0.031	10.1
Citalopram	0.136	0.112	0.111	0.119	12.0
Carbamazepine	0.097	0.072	0.078	0.082	15.8
Oxazepam	0.191	0.114	0.156	0.154	25.0
Cocaine	0.107	0.090	0.090	0.096	10.4
Benzoylecgonine	0.051	0.036	0.031	0.039	26.3
EDDP	0.030	0.021	0.029	0.027	17.0
Methamphetamine	0.031	0.027	0.021	0.026	17.4
Morphine	0.021	0.025	0.021	0.023	10.1
Metoprolol	0.147	0.179	0.250	0.192	27.4
Av.	**0.084**	**0.071**	**0.081**	**0.079**	**17.1**

Table 1. Pharmaceuticals variability and uncertainty due to sample size (in brackets). presented as coefficient of variation (CV = standard deviation divided by mean) and standard error of the mean (SEM = coefficient of variation divided by the square root of the number of samples). for the 24 monthly sales loads data during 2012 and 2013, the 28 consecutive wastewater samples studied during the in-situ calibration and the 49 TWA concentrations estimated with POCIS during 2012 and 2013 (left). Estimated uncertainty levels associated with POCIS data using both, the in-situ accuracy levels and a predefined value (center). Estimated population-normalized loads using wastewater samples from 2014 and POCIS from 2012 and 2013 (right).

	Variability (Uncertainty); CV (SEM)				Confidence Intervals			Estimated Mean Value (mg/day/1000 inhabitants)		
	Sales loads	WW loads	POCIS [TWA]		U_{POCIS} (in-situ)	U_{POCIS} ($R_s \leq 2$)		WW loads	[TWA] POCIS	
Compound	n=24	n=28	2012 (n=24)	2013 (n=25)	%	$\%_{MIN}$	$\%_{MAX}$	Feb 2014 (n=28)	2012 (n=24)	2013 (n=25)
Atenolol	12.7 % (2.6 %)	15.2 % (2.9 %)	25.9 % (5.3 %)	27.4 % (5.5 %)	±35.4	-81.4	131.4	166.3	231.6	182.2
Citalopram	10.7 % (2.2 %)	13.6 % (2.6 %)	43.5 % (6.2 %)	33.5 % (4.8 %)	±37.6	-81.2	131.2	54.6	75.4	57.2
Carbamazepine	13.9 % (2.8 %)	15.7 % (3.0 %)	44.6 % (6.4 %)	35.1 % (5.0 %)	±38.6	-82.8	132.8	263.6	163.8	164.0
Oxazepam	7.3 % (1.5 %)	27.0 % (5.1 %)	46.7 % (6.7 %)	32.2 % (4.6 %)	±40.5	-84.8	134.8	189.7	163.9	197.1
Metoprolol	12.7 % (2.6 %)	37.9 % (7.2 %)	37.9 % (5.4 %)	28.6 % (4.1 %)	±43.1	-88.2	138.2	129.5	257.1	252.8

Supporting Information

Passive sampling of wastewater as a tool for the long-term monitoring of community exposure: Illicit and prescription drug trends as a proof of concept

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Chemicals, materials and POCIS samplers

Atenolol, citalopram, carbamazepine, oxazepam, morphine, metoprolol, methadone, EDDP, benzoylecgonine and methamphetamine were purchased from Nerliens Meszansky (Oslo, Norway) as solutions in methanol (MeOH) or acetonitrile (ACN) at concentrations of 1 mg mL^{-1}. Deuterated standard analogs for each target compound (except for citalopram, morphine and EDDP for which buprenorphine-d4, atenolol-d7 and atomoxetine-d7 were used respectively) were purchased from Nerliens Meszansky as solutions of 100 ng mL^{-1} in MeOH or ACN and were used as surrogate isotope labelled internal standards (ILIS) for quantification.

Ultrapure water was obtained by purifying demineralized water in an Elga Maxima Ultrapure Water purification system (Elga, Lane End, UK). Ammonium formate (for mass spectroscopy, ≥99.0%), HPLC-grade formic acid (eluent additive for LC-MS) and UHPLC-grade water, MeOH and ACN (Fluka for HPLC) were acquired from Sigma-Aldrich, (Oslo, Norway).

The pharmaceutical version of the POCIS was constructed in-house as previously described by Harman et al. [1]. Briefly, 220 ±5 mg of Oasis HLB sorbent (Waters, Milford, MA, USA) was sandwiched between two polyethersulphone membranes (Pall Supor 100 Membrane Disc Filters, 0.1 µm pore size, 90 mm diameter; VWR, Oslo, Norway) clamped between two steel rings providing an exposure area of ~24 cm^2 on each side. Assembled POCIS were kept in individual foil lined bags (to prevent cross-contamination) and stored at -20°C prior to and following deployment in wastewater to prevent biodegradation of the analytes.

Wastewater and POCIS extraction and analysis

The method used for the extraction and analysis of the target compounds in wastewater has been described elsewhere [2]. Briefly, 50 ng of the ILIS solution mix was spiked into wastewater (100 mL) and then extracted using a fully automatable solid phase extraction (SPE) system (Horizon Technology, Salem, NH, USA) with HLB extraction disks (47 mm, I.D.; Horizon Technology, City, Country). 5 µL of the final eluent (evaporated to 100 µL and reconstituted to 400 µL with water) was injected into a LC-QTOF system. The compounds were chromatographically separated on a Waters Acquity UPLC system (Milford, MA, USA) fitted with a Acquity UPLC HSS C18 column (2.1 x 150 mm, particle size 1.8 µm) (Waters, Milford, MA, USA). A Xevo G2-S Q-TOF mass spectrometer (Waters, Milford, MA USA) was used in positive ESI mode for acquisition using MS^e, that allows both precursor and product ion data to be simultaneously acquired during a single run. The data processing took place using UNIFI screening platform (Waters Corporation, Milford MA, USA).

Following deployment, POCIS were defrosted and the HLB sorbent transferred into an empty solid phase extraction cartridge (6 mL). Two cartridge volumes of 90:10 water:methanol were used to wash the sorbent and 100 ng of the isotopic labelled internal standards (ILIS) solution mix was added. The analytes were eluted using 5 mL of 5% ammonium hydroxide in methanol and 5 mL of 5% acetic acid in methanol. The final eluent was dried to approximately 0.1 mL under a stream of nitrogen (35°C) and reconstituted into 1.5 mL of H_2O/MeOH (80/20, v/v). Finally, an aliquot was centrifuged at 20.000 x g for 2 minutes and 5 µL of the supernatant were injected into the UHPLC-QTOF using the same method described above [2].

Quality Assurance

The influence of the wastewater matrix on POCIS extracts was evaluated using the corresponding ILIS for each of the studied compounds. The final eluate was split into two fractions, evaporated, and then reconstituted into 500 µL of H_2O/MeOH (80/20, v/v). Different dilution factors were tested by adding different volumes (250, 500, 750 and 1000 µL) of ultrapure water, resulting in a considerable reduction in ion suppression (Figure S7). A higher dilution factor would probably decrease the ion suppression, but a higher volume also involves a higher amount of ILIS. A compromise for ion suppression and dilution factor was found by diluting the eluent with 1 mL of ultrapure water. All of the studied compounds were recovered from the HLB sorbent at a satisfactory range of between 72 and 118% [2]. The selection of the target compounds was based on two factors: frequency of occurrence in wastewater and at concentrations above LOQ. Blank POCIS were analyzed for both in-situ calibration (n=3) and long-term sampling (n=3/new batch) and none of the target compounds were detected.

The stability of analytes on POCIS during the deployment has not been assessed in this study due to the complexity of performing such a study. However, the possible loss of the analyte is assumed to be corrected by performing the in-situ calibration. Only Carlson et al. [3] studied the stability of 24 pharmaceuticals stored on POCIS concluding that the losses were smaller than the variability associated with the use an application of POCIS. Therefore, further research is needed for the better understanding of both stability and competitive sorption of analytes during the deployment in wastewater.

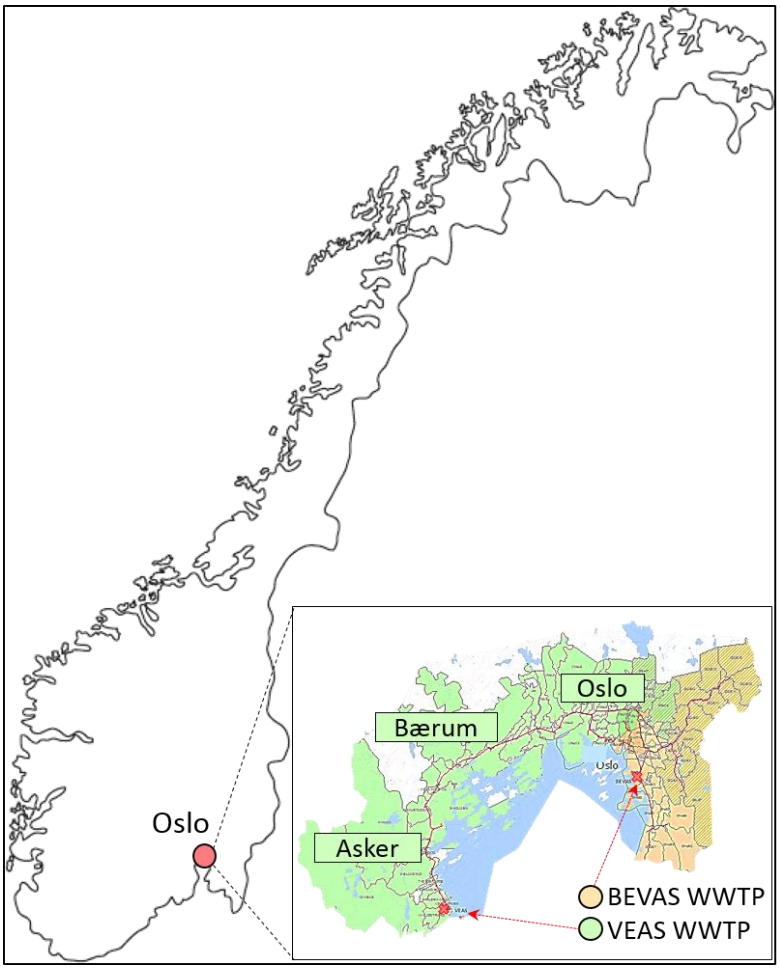

Figure S1. Sewer system distribution in Oslo. Green area represents VEAS wastewater catchment area whereas the orange area represents BEVAS wastewater catchment area.

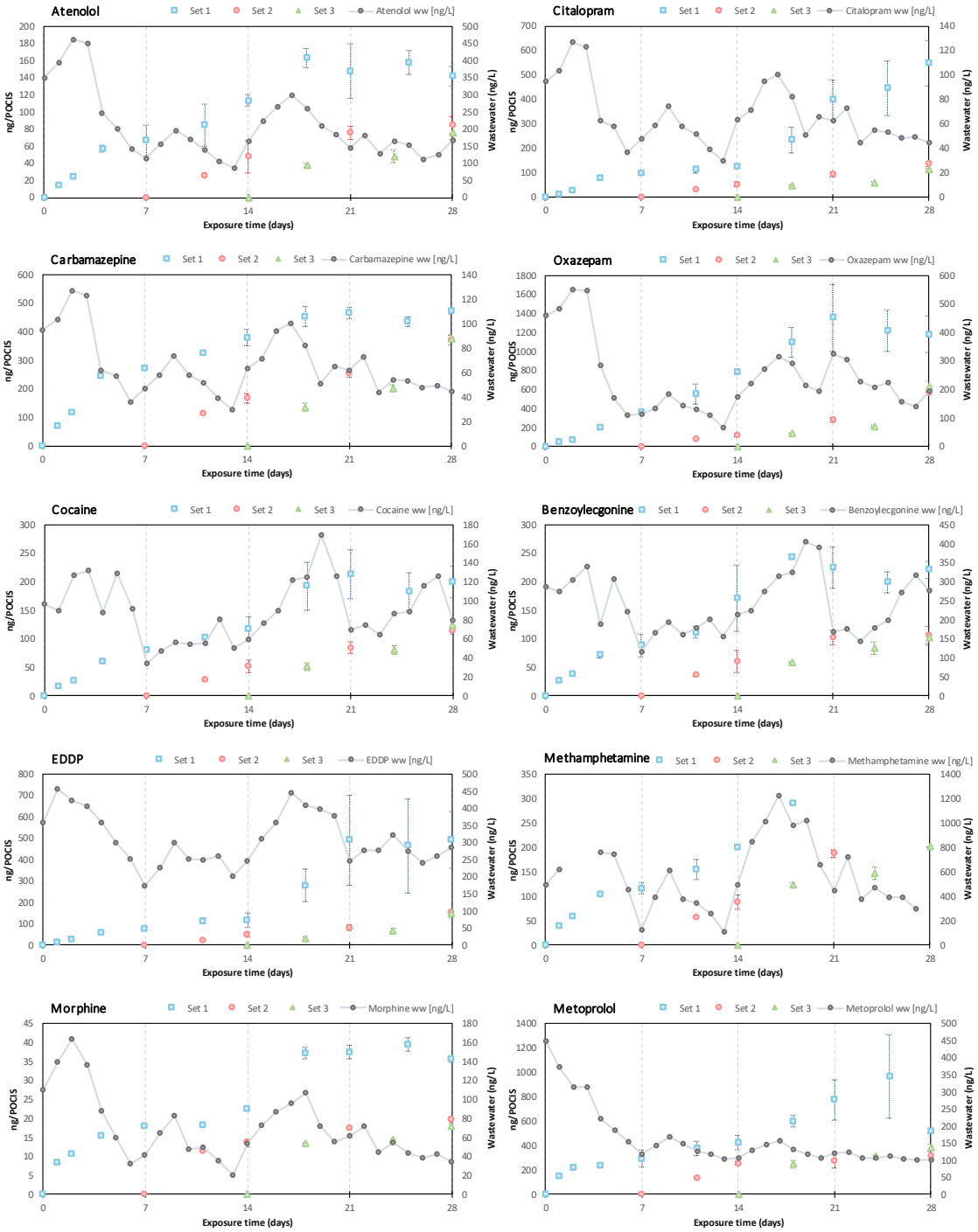

Figure S2. In-situ calibration data. Set 1, 2 and 3 represent the POCIS uptake for each of the target compounds displayed in blue, red and green respectively. Black line shows the different compound concentrations in wastewater.

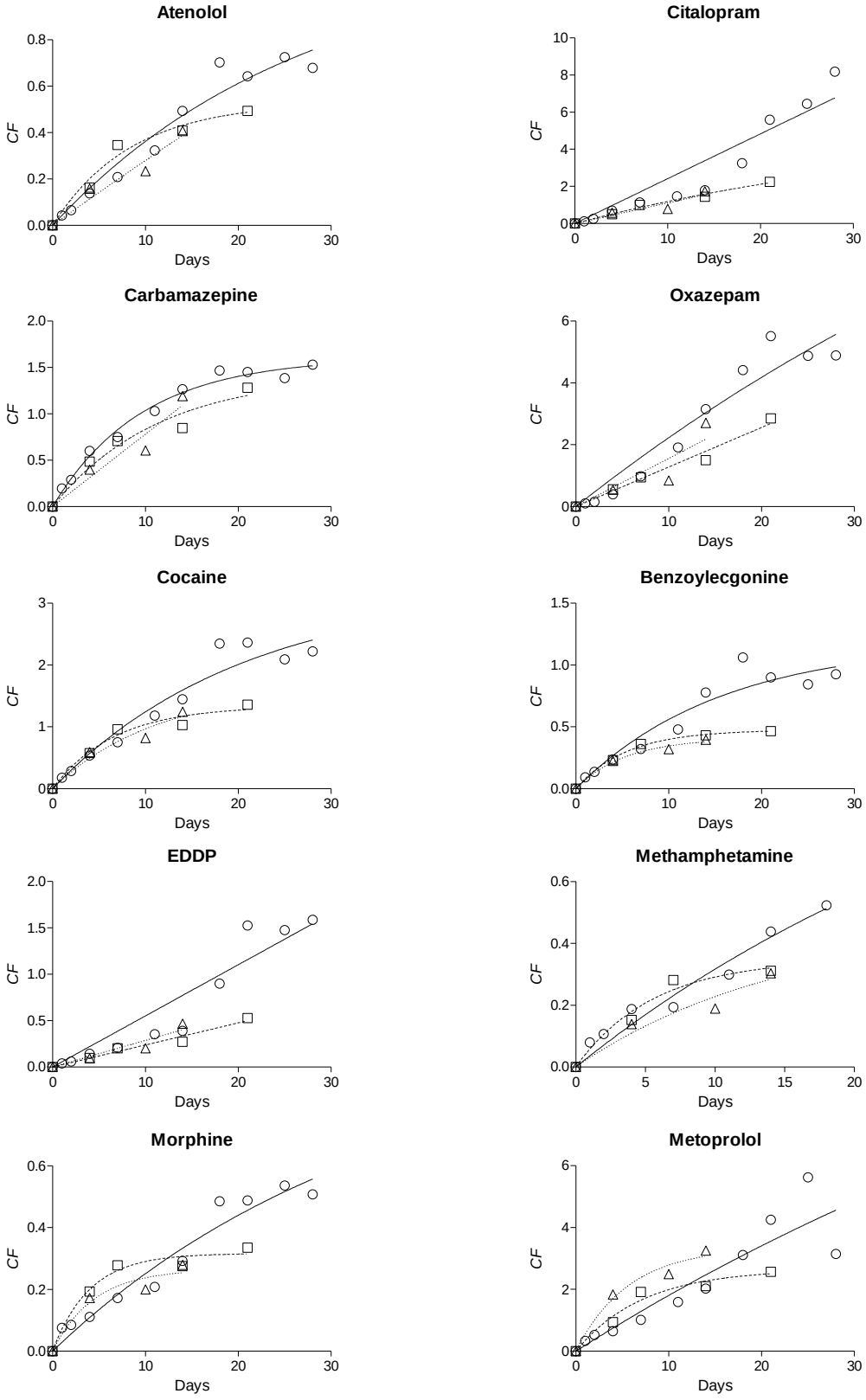

Figure S3. Non-linear fits in POCIS obtained for each of the 10 target compounds assessed over three time periods in, concentration factor vs time. Set-1: 28 days (solid line, circles); Set-2: 21 days (dashed line, squares); Set-3: 14 days (dotted line, triangles).

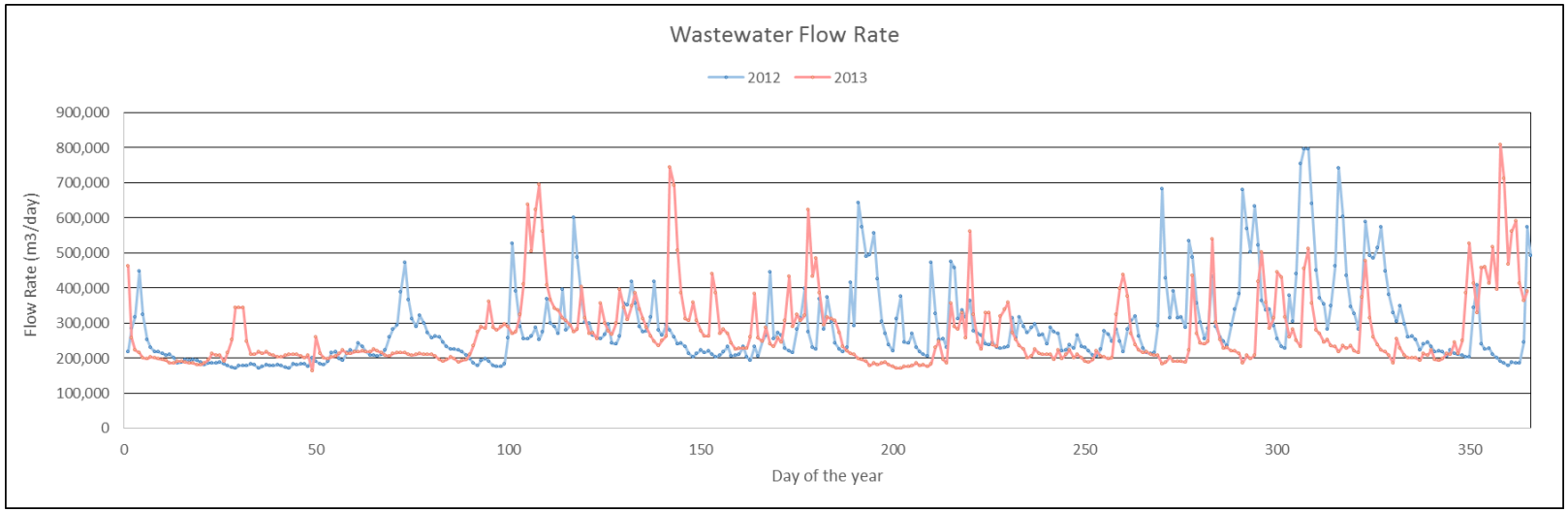

Figure S4. Wastewater flow (m3 day^{-1}) rate at VEAS WWTP during the monitoring study.

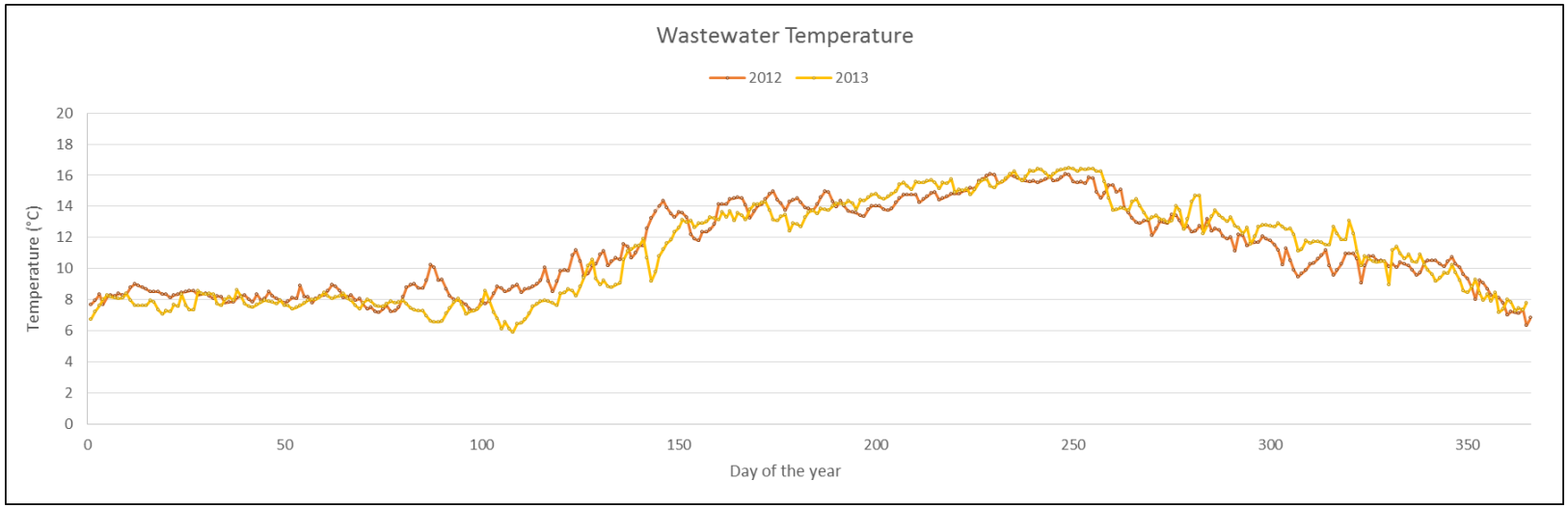

Figure S5. Wastewater temperature (°C) at VEAS WWTP during the monitoring study.

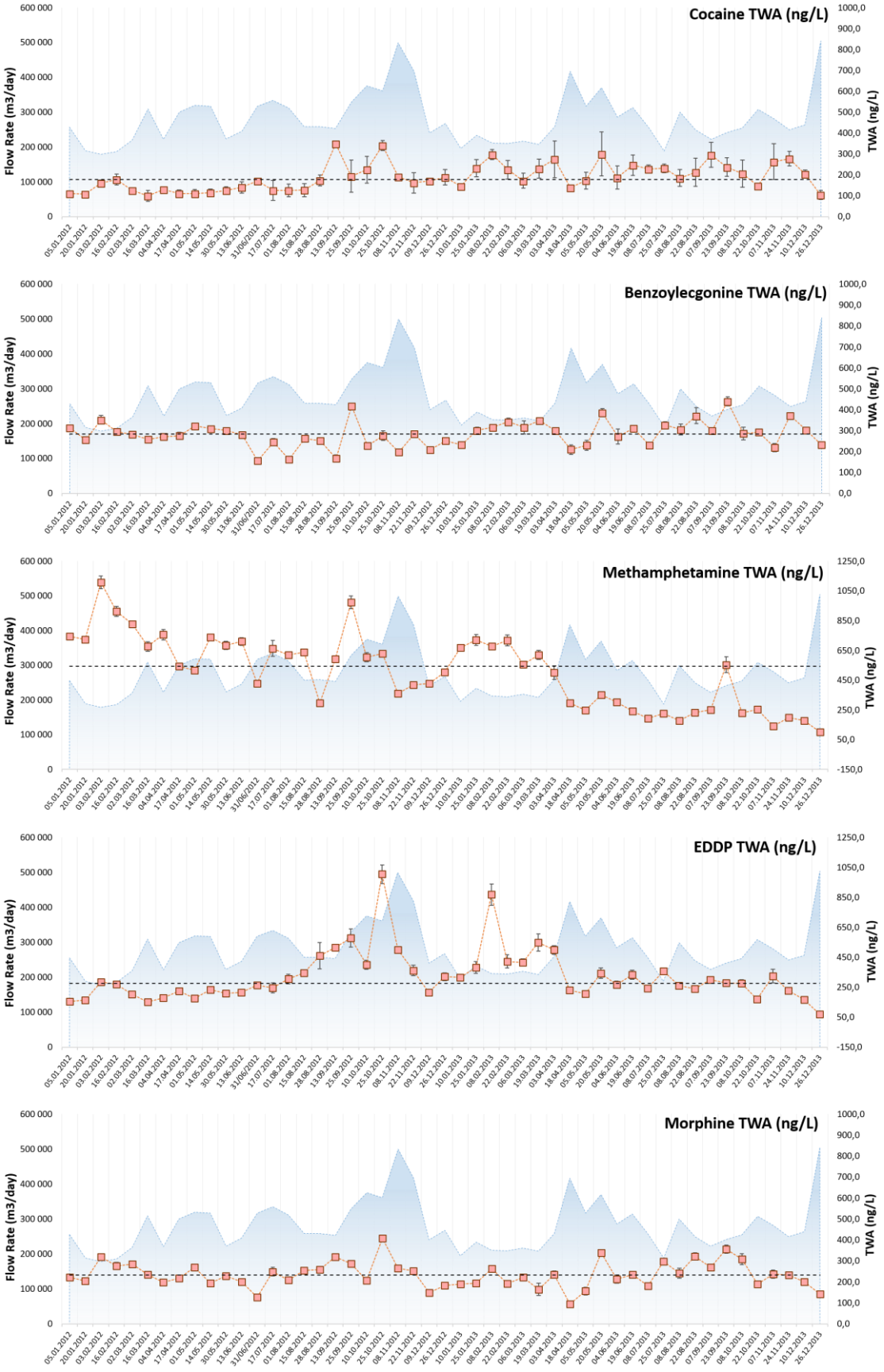

Figure S6. Time-weighted average concentrations (ng L^{-1}) in POCIS (n=3) for cocaine, benzoylecgonine, methamphetamine, EDDP and morphine (right axis). Wastewater flow rate (m^3 d^{-1}) is represented in the background (left axis).

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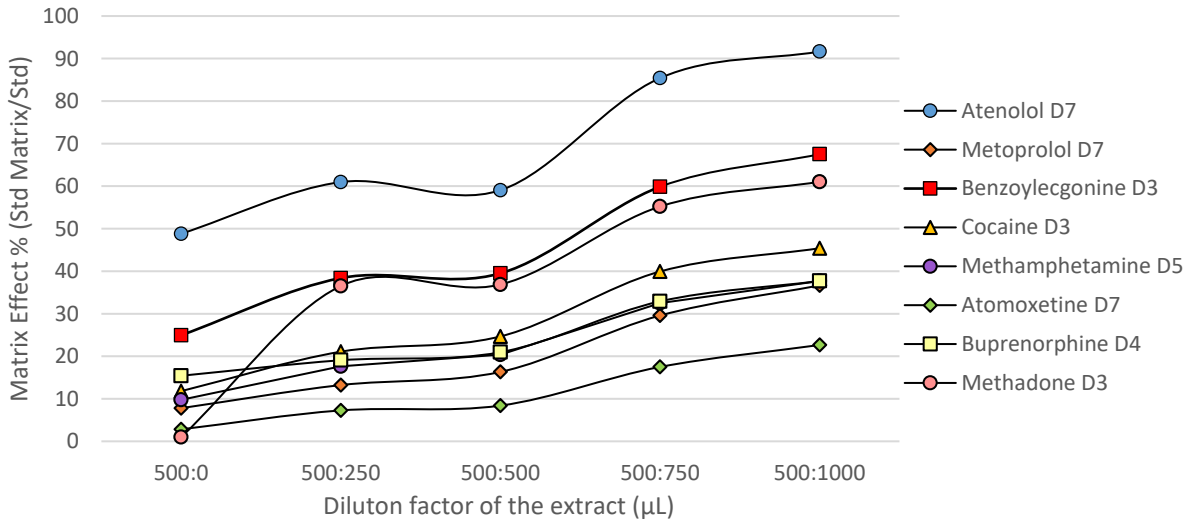

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113 Figure S7. Influence of the dilution factor of the POCIS eluent on the matrix effect (extract water).

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115 Table S1. POCIS repeatability and R_S accuracy for the selected pharmaceuticals.

	Long-term repeatability	In-situ accuracy
	POCIS (n = 49 triplicates)	Rs (n=3)
	Average CV	CV
Atenolol	15.7 ± 8.4	10.1
Citalopram	15.6 ± 8.2	12.0
Carbamazepine	16.4 ± 9.2	15.8
Oxazepam	17.4 ± 10.4	25.0
Metoprolol	19.1 ± 10.8	27.4

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117 References:

118 [1] C. Harman, M. Reid, K.V. Thomas, Environ Sci Technol 45 (2011) 5676.
119 [2] J.A. Baz-Lomba, M.J. Reid, K.V. Thomas, Analytica Chimica Acta 914 (2016) 81.
120 [3] J.C. Carlson, J.K. Challis, M.L. Hanson, C.S. Wong, Environ Toxicol Chem 32 (2013) 337.

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