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Spatiotemporal variations of pharmacologically active compounds in surface waters of a summer holiday destination

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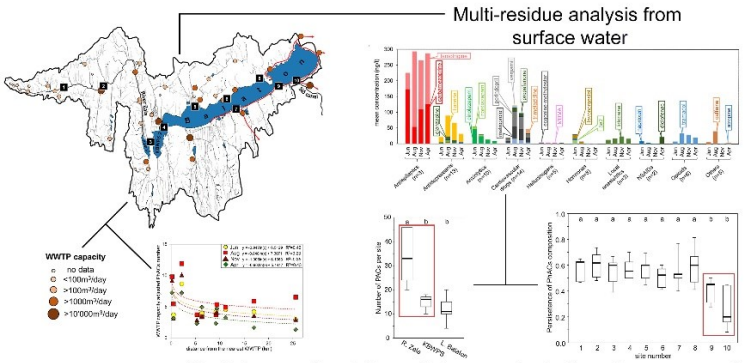

Spatiotemporal variations of pharmacologically active compounds

Abstract

The release of pharmacologically active compounds (PhACs) into aquatic ecosystems poses an environmental risk resulting in a chronic exposure of non-target organisms. A great variety of PhACs, of generally low concentrations, and the complicated sample preparation, makes circumstantial the accurate detection and quantification. Additionally, there is little information published about the spatiotemporal variation of the PhAC load in a larger catchment area utilised for touristic purposes. In addition to the natural biotic and abiotic changes, the seasonal variation of tourism also has a dramatic impact on water quality and the natural ecosystem in larger catchment areas. Therefore, our aim was to develop a reliable solid-phase extraction (SPE)-supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS) method for simultaneous multi-residue analysis of drugs to reveal the spatiotemporal changes in the PhAC contaminations in the waters of an important touristic region, the catchment area of the largest shallow lake in Central Europe, Lake Balaton (Hungary). The environmental application of the developed method revealed 69 out of the traced 134 chemical compounds, including 15 PhACs, which were detected from natural waters for the first time. Wastewater treatment plant (WWTP) loads have a major role in the PhAC contamination of the studied area; at the same time, the mass tourism-induced PhAC contamination was also detectable. Furthermore, the impact of tourism was indicated by elevated concentrations of recreational substances (e.g.,

caffeine and illicit drugs) in the touristic season affecting the water quality of this important summer holiday destination.

Keywords: Shallow lake, Environmental monitoring, Mass Spectrometry, Solid Phase Extraction, Pharmaceuticals, Multi-residue analysis

1. Introduction

Efficient sewage treatment plays a key role in preserving freshwaters in appropriate condition (Goel, 2006). However, the wastewater treatment technology used today is still not able to eliminate all kinds of pollutants. Several PhACs, which are excreted and entered into the sewage system, are not eliminated completely by WWTP, therefore, these contaminants appear in the recipient natural waters (Postigo et al., 2010). The concentration of these pollutants is generally low (ng/l to μg/l range) (Silva et al., 2012), therefore, only the more recently developed analytical techniques (e.g. liquid chromatography coupled mass spectrometry) are sensitive enough for their detection and exact quantification in environmental samples (Bianchi et al., 2018). The reason could be that while the effects of certain pollutants, such as heavy metals and pesticides (Dean et al., 1972), have been analysed for a long time, the importance of PhACs has just been recognised in the last decades (Daughton and Ternes, 1999). Besides the low concentration levels, the large number and the great structural variety of the potentially detectable PhACs makes it difficult to determine the contamination level of the recipient freshwaters (Kasprzyk-Hordern, 2010; Kolpin et al., 2002). Because of the abovementioned features, usually a limited number of components are surveyed in environmental samples (Cantwell et al., 2018; Gomez et al., 2007; Musolff et al., 2009; Roberts and Thomas, 2006). In addition, there are only a

few recently published notes about the spatiotemporal distribution of PhACs in larger drainage systems (Carpenter and Helbling, 2018; Guzel et al., 2018; Lindholm-Lehto et al., 2015).

Chemical pollutants, like PhACs, of surface water poses a threat to the aquatic environment, with effects such as acute and chronic toxicity in aquatic organisms, accumulation of pollutants in the ecosystem and loss of habitats and biodiversity, and also pose a threat to human health. According to the Environmental Quality Standards Directive (Directive 2008/105/EC) for determination of anthropogenic chemical pollutions at a global level, a new Watch List (WL) is needed to provide high-quality monitoring information on the concentrations of polluting substances in the aquatic environment. The surface water WL supports the identification of priority substances for regulation under the Water Framework Directive. The first WL was established in 2013 under the Directive 2008/105/EC (as amended by Directive 2013/39/EU), collecting four PhACs (E1, E2, EE2 and diclofenac). After, the WL was modified based on the Joint Research Center (JRC) Technical Report in 2015 (EU Commission JRC 2015) and in April 2018 (EU Commission JRC 2018), additionally, the latter provided several recommendations for the second WL. New candidate WL substances should be selected among substances posing a potential risk for the environment, but for which there is not enough good quality monitoring data to confirm this risk. Therefore, there is a continuing need for the development and optimisation of sensitive analytical techniques to detect and measure environmental substances.

Environmentally high throughput analysis is an analytical challenge when the criteria are optimal sample preparation for more types of drugs together with an analytical system with appropriate capacity. Multi-residue analysis achieves simultaneous drug screening. The most commonly used sample preparation method for the multi-residue analysis is solid phase extraction (SPE). One of the greatest challenges with multi-residue analysis is the selection of

sorbent able to give acceptable recoveries for all compounds characterised by different physicochemical properties (Baker and Kasprzyk-Hordern, 2011). The number of processable molecules can be increased by using mixed stationary phases via SPE procedures.

Besides the sample preparation, there is the limitation of past analytical systems used in determining the number of detectable molecules. Nowadays, by using an analytical method based on supercritical fluid chromatography/tandem mass spectrometry (SFC-MS/MS), we can achieve detection of some pesticides (e.g., dinotefuran, fenbuconazole, isofenphos-methyl) and their metabolites in environmental samples (e.g., honey, fruits, vegetables, cereals, soil and water) (Granby et al., 2004; Hernandez et al., 2011; Kamel, 2010). Application of carbon dioxide (CO_2), as supercritical fluid has many benefits. It is non-toxic, and has low viscosity and high diffusivity, contributing greatly to improving the separation efficiency and reducing the organic solvents utilisation (Chen et al., 2015; 2016; Tao et al., 2018). The MS/MS is able to overcome the traditional incompatibility, offering high resolution and narrow peaks (Chen et al., 2016). Summarised, this technique provides a rapid, efficient, sensitive, reliable and environmentally friendly solution for detection of several pesticide compounds in environment samples (Chen et al., 2015; Tao et al., 2018). Moreover, it is also important to mention that SFC-MS/MS can be a powerful tool in the simultaneous analysis of a wide range of compounds (multi-residue analysis) in difficult matrices that require high sensitivity and rapid screening capacity. For example, 441 pesticide compounds were determined simultaneous in a food sample by applying the SFC-MS/MS analytical method (Fujito et al., 2017). In addition, the instrumental improvements have led to the emergence of ultrahigh performance supercritical fluid based chromatography (UHPSFC) that merges the advantages of SFC and ultrahigh performance liquid chromatography technology. Also UHPSFC-MS/MS analytical methods have already been developed for the simultaneous analysis of several PhACs in environmental matrices. This novel technique was

well-suited for the simultaneous analysis of 23 veterinary and human PhACs in wastewater samples (Camacho-Munoz et al., 2016).

Beyond the traditional economic sectors (agriculture, industry), tourism has become an important water utiliser in the last decades (Gossling et al., 2012). At the same time, the growing tourism industry frequently has a negative impact on natural ecosystems (Hadwen et al., 2005; Katircioglu, 2014; Mihalic, 2000). In addition, to the elevated macronutrient (N, P) intake through the increased PhACs and personal care products load, tourism may have a potentially negative effect on the quality of surface waters (Gonzalez-Alonso et al., 2017; Mandaric et al., 2017). These tourism-generated negative effects may occur seasonally. Seasonal changes induced by anthropogenic factors were also observed earlier using physico-chemical variables (Barakat et al., 2016; Vega et al., 1998), microbiological indicators (Lenart-Boron et al., 2016) and physicochemical parameters of water and bacterial water quality indicators (Bojarczuk et al., 2018).

Therefore, our aim was to develop a fast and reliable SPE-SFC-MS/MS method suitable for multi-residue analysis of drugs in environmental samples. In this study, we focused on the psychoactive drug contamination, which can be classified into the alkaloid, antiepileptic, antipsychotic/antidepressant, anxiolytic, dissociative anesthetic/psychedelic, narcotic/sedative/anticonvulsant, opioid/morphine derivatives and stimulant/hallucinogen groups. Furthermore, other widely used and chronically administered drug groups, such as cardiovascular drugs, hormone/hormone derivatives, local anesthetic and nonsteroidal anti-inflammatory drugs (NSAIDs) were also investigated. Additionally, our other aim was to reveal the spatiotemporal changes in the PhACs concentration in the waters of an important summer holiday destination, the catchment area of Lake Balaton (Hungary) using our fast and reliable developed method.

2. Materials and methods

136 2.1 Study area

137 Our study was carried out on the catchment area of Lake Balaton, Hungary (Table S1,
138 Fig. 1), which is one of the largest (A: 594 km^2, mean depth: 3.2 m, V: ~1.8 km^3) freshwater
139 shallow lakes in Central Europe (Istvánovics et al., 2007). The lake and its catchment area can be
140 characterised by diverse flora and fauna (Istvanovics et al., 2008; Palffy et al., 2013; Specziar et
141 al., 2009). Its largest tributary is the River Zala, which empties into the westernmost basin of the
142 lake. Its mean discharge of 8 m^3/s supplies almost 50% of the lake's total surface water input. The
143 only outflow of the lake is the artificial Sió canal, situated in the eastern basin of the lake at
144 Siófok, joining the Balaton catchment (A: 5775 km^2) to the Danubian River Network (Fig.1). The
145 human population shows uneven spatial and temporal distribution in this area. While the largest
146 town in the catchment area (~60,000 inhabitants), Zalaegerszeg, is located on the riverbank of
147 River Zala about 80 river kms from the Lake. Two-thirds of the total permanent inhabitants
148 (~380,000) of the catchment, are distributed at the near-coastal area of the lake ("Lake Balaton
149 Resort Area" LBRA, see: URL1). There is no considerable industrial activity in the catchment
150 area of the lake, therefore, this region is characterised as a barely contaminated area by industrial
151 pollutants (e.g., heavy metals) (Nguyen et al., 2005). The LBRA is an internationally important
152 tourist attraction and recreation center visited by about 2,000,000 tourists a year. The number of
153 guest-nights, which exceeds the 6',400,000 per year, is unevenly distributed, and weighted to two
154 summer months (July and August), mostly to the southern shoreline of the eastern basin of the
155 Lake, at the area of Zamárdi and Siófok (Horvath, 2011).

156 The increased, but uncoordinated utilisation of the catchment's environmental resources
157 caused massive eutrophication of the lake at the end of the 1970s (Hatvani et al., 2014; Puczkó
158 and Rátz, 2000). For this reason, in the early 1980s, a regional nutrient load control strategy was
159 worked out for Lake Balaton. Among others, 1) a "filtering" shallow wetland (mean depth ~1.2

m) was reconstructed in two "phases" at the estuary of River Zala (Kis Balaton Water Protection System (KBWPS) 1 and 2 (Fig. 1A)) (Tatrai et al., 2000) and 2) several WWTPs were built and the larger existing WWTPs (e.g., in Zalaegerszeg and Keszthely) were expanded with tertiary treatment (chemical P precipitation). Nowadays, more than 40 WWTPs can be found in the catchment of Lake Balaton (Fig.1A). Their capacity varies between 2 and 50,000 m^3/day. The largest WWTP is situated to the city of Zalaegerszeg. Here, we have to note that the cleared wastewater intake exceeds the 30% of the mean discharge of the recipient Zala section (URL2). 3) To minimise the direct treated sewage load into the lake, a sewage transfer duct system was constructed at the southern and eastern near-coastal area, which collects and draws most of the purified communal sewage away from the Lake Balaton catchment (Fig.1A). At the same time, the WWTPs situated away from the lake, empty their outflow into the tributaries of the lake.

2.2 Sample collection and on-site hydrophysico-chemical parameter recordings

Designation of sampling sites (Fig. 1A) was based on earlier screening (Avar et al., 2016a), where the main sources of contamination and contaminated locations were determined on the catchment area of Lake Balaton. All water samples were collected in June (summer), August (summer), and November (autumn) of 2017, and in April (spring) of 2018 within one day from 10 sampling sites. Six sites were designated on the littoral region of the lake, two on the area of the KBWPS and two on River Zala, upstream and downstream of the municipal WWTP of Zalaegerszeg (Table S1, Fig. 1A).

All samples were collected in amber silanised glass bottles (2 L) with Teflon faced caps (Thermo Fisher Scientific). The oxygen saturation, conductivity, pH and temperature were measured during the collections (Table S1) using Voltcraft DO100 oxygen meter and HANNA HI98129 multimeter. To further protect sample preparation, the samples were transported back to the laboratory in a dark and iced cool box within 4 hours.

184 2.3 Chemicals, reagents and materials

185 All analytes and internal standards (IS) were of high purity available (>97%). Analyte
186 names and CAS numbers are shown in Table S3. Solvents and additives to solid phase extraction
187 and SFC-MS/MS analysis were all of LC–MS quality and purchased from Scharlab, with the
188 exception of ammonium solution (20% in water) and formic acid (100%), which were purchased
189 from VWR. The IS were dissolved in methanol (MeOH) or acetonitrile (ACN) at a concentration
190 of 1 or 0.1 g/L: Citalopram-d6 (#C-090, Sigma-Aldrich), Carbamazepine-d10 (#C-094, Sigma-
191 Aldrich), 13C3-E2 (#13E2-122, Lipomed AG) and N-ethyl-oxazepam (#OXA-325, Lipomed
192 AG). Individual stock solutions were purchased or prepared from solid substance in either ACN
193 or MeOH at a concentration of 1 or 0.1 g/L and stored in the dark at −20°C. Mixed standard
194 solutions were prepared at 10 mg/L in MeOH and diluted as necessary to prepare working
195 solutions on a daily basis.

196 2.4 Sample preparation, SPE and derivatisation

197 One liter of each samples was acidified with 100% formic acid (compatible with all tested
198 sorbent types) to pH 3.5–4.0. All IS were added to samples before filtration, the final
199 concentration was 5 ng/L to each IS (Citalopram-d6, Carbamazepine-d10, E2-13C3 and N-ethyl-
200 oxazepam) and were used for the quantification of samples. After spiking, samples were vacuum
201 filtered, first through a GF/A 1.6 µm glass microfibre filter (#1820-047, Whatman), and
202 subsequently, through a GF/F 0.7 µm glass microfibre filter (#516-0345, VWR). Samples were
203 stored in the dark at 4°C and extracted within 20 hours, thereby, the sample was fully prepared
204 within 24 hours from the sampling.

205 The SPE of samples was carried out with AutoTrace 280 automata SPE system (Thermo
206 Scientific). Nitrogen gas stream was utilised for the evaporation of SPE extracts. The method was
207 optimised through several preliminary experiments involving the following variables: type (Strata

208 X, X-CW, C8, C18E) and amount (100–500 mg) of sorbent, sample volume (0.5–2 L),
209 solvent/water portion of washing solutions (10–50%), elution (MeOH, ACN, 1–14%
210 NH_4OH/ACN) and evaporation conditions (0.2–1 bar, 30–50°C) (see Supplementary information
211 "SPE optimisation" part).
212 The final SPE procedure was as follows. Initially, the Strata X-CW (33 µm, 200 mg/6mL,
213 #8B-S035-FCH, Phenomenex) column was conditioned with MeOH (3 mL) and equilibrated with
214 0.1% HCOOH/H_2O (3 mL, pH 4), both at a flow rate of 10 mL/min. Acidified water samples
215 (1000 mL) were passed through the X-CW cartridge at a rate of 15 mL/min. Immediately
216 following loading, cartridges were washed with 0.1% HCOOH/H_2O (6 mL, pH 4) and 20% ACN/
217 HCOOH/H_2O both at a flow rate of 10 mL/min. The syringe of SPE automata was washed with 6
218 mL of ACN, then the cartridges were dried with N_2 gas for 2 min to eliminate the aqueous
219 residues. The elution was performed by two steps to reach the optimal recovery of all analytes.
220 Firstly, for the restoration of optimal condition, the dried cartridge was soaked with 1 mL ACN
221 for 1 min, followed by the first elution with 100% ACN (4 mL) at a flow rate of 5 mL/min into
222 sample tube (Eluate1). The second elution was applied with 7% NH_4OH/ACN (5 mL) at a flow
223 rate of 5 mL/min into a new sample tube (Eluate2). Then both eluates were evaporated to dryness
224 by nitrogen gas stream (35°C, 0.5 bar) and reconstituted with ACN (500-500 µL) induced by
225 ultrasound and vortex mixing. To maximum recovery, deactivated vials with PTFE septa
226 (Waters) 300 µL reconstituted samples were transferred. The basic, and some amphoteric, drugs
227 were analysed from Eluate2. Furthermore, the acidic, neutral and some amphoteric drugs were
228 measured from Eluate1 (Table S3). Derivatisation of steroid agents was performed to reach the
229 appropriate sensitivity. To the remainder of the 200 µL reconstituted sample (Eluate1), 160 µL
230 Na_2CO_3-t (0.1 M in water) and 20 µL dansyl-chloride (40 mM in ACN, #39220-1G-F, Sigma-
231 Aldrich) were added. These mixtures were incubated in a thermomixer (65°C, 300 rpm) for 10

232 min. After the incubation, the mixtures were centrifuged (20,000 rpm, 20°C) for 5 min, followed
233 by adding 80 µL toluene and vortex mixed. All samples were centrifuged again (20,000 rpm, 20
234 °C) for 5 min before being transferred to maximum recovery deactivated vials with PTFE septa
235 (Waters).
236 For quantitative analysis, five-point calibration curves were used in each external
237 standard. The waters were spiked with the standards, on which the total sample preparation
238 method was applied. The limit of detection (LOD) and limit of quantification (LOQ) were also
239 determined (Table S3) by analysis of spiked and fully prepared water samples.
240 2.5 SFC-MS/MS analysis
241 Measurements were performed by an ACQUITY UPC2 supercritical fluid
242 chromatography system (Waters) coupled with a Xevo TQ-S Triple Quadrupole Mass
243 Spectrometer (Waters). Data were recorded by MassLynx software (V4.1 SCN950) and evaluated
244 by TargetLynx XS software.
245 Separation of compounds was performed on a 3.0 mm x 100 mm, 1.7 µm particle size,
246 ACQUITY UPC2 BEH analytical column (#186007607, Waters). Chromatography was
247 performed at 45°C and the injected volume was 2 µL. The flow rate of the mobile phase was 1.2
248 mL/min. For the analysis of hormones and hormone derivatives from Eluate1 using 13C3-E2
249 internal standard, the mobile phase consisted of a mixture of carbon dioxide (A) and 5 mM
250 ammonium hydroxide in MeOH (B). The following gradient profile was used: 100% A at 0 min,
251 87.5% A at 0.5 min and 77.5% A at 4 min. For the analysis of acidic or neutral and some
252 amphoteric drugs from Eluate1 using Carbamazepine-d10 internal standard, the mobile phase
253 consisted of a mixture of carbon dioxide (A) and 30 mM ammonium hydroxide and 15 mM acetic
254 acid in methanol (B). The following gradient profile was used: 99.9% A in the beginning and
255 72.5% A at 5.5 min. For the analysis of basic and some amphoteric drugs from Eluate2 using

Citalopram-d6 and N-ethyl-oxazepam internal standards, the mobile phase consisted of a mixture of carbon dioxide (A) and 30 mM ammonium hydroxide and 15 mM acetic acid in MeOH (B). The following gradient profile was used: 99.9% A at 0 min. and 65.0% A at 7 min. A pre-equilibration period lasting 2 min was applied before each injection. Constant 200 bar back pressure was used to maintain the supercritical state.

To sustain a suitable electrospray, an additional solution consisting of 5 mM ammonium hydroxide in MeOH was applied with a flow rate of 0.1 mL/min. This makeup solvent was delivered by a Waters 515 HPLC Pump.

The MS measurement was performed in positive ion mode (except for some antiepileptics). The ESI source was operated with a spray voltage of 3 kV in both positive and negative ion modes; cone voltage was 30 V. The source was set at 150°C. Both desolvation and cone gases were nitrogen delivered at 300 and 150 L/min, respectively. Desolvation gas was tempered at 300°C. The collision gas was argon with a flow rate of 0.13 mL/min.
MS/MS experiments were performed in MRM (multiple reaction monitoring) mode with an isolation window of 0.4 m/z. The utilised precursor-product ion transitions with the related collision energies in Table S3 were indicated.

Peak detection and quantification was achieved using TargetLynx XS software (Waters). The observed ions (mass in m/z) were accepted and quantified if they were within the following limits: appropriate MS1 mass, retention time, MS2 masses, fragmentation pattern and IS correction.

2.6 Data analysis and presentation

The frequency of occurrences and mean concentrations of the observed PhACs are indicated on bar charts. Principal Component Analysis (PCA) was made using concentration data of the PhACs grouped into 10 chemical classes to present the sample sites detachments in the

different study periods. Number of PhACs by sampling sites, periods and areas are indicated on boxplots. The persistence of pharmaceutical composition was calculated using the Jaccard similarity index. Similarity computations were made for each possible combination (n=6, June-August, June-November, June-April, August-November, August-April, November-April) for each site's data. In this case, the similarity values ranged between 0 and 1, where 0 indicates that the drug composition of the samples collected from the same site are absolutely different, and 1 indicates that the compared samples have identical composition. These results are presented on boxplots as well. All pairwise comparisons were tested for significance by Kruskall-Wallis nonparametric tests. Regression analyses were made to reveal the role of WWTPs in the PhACs pollution of the studied system. Numbers of the indicated PhACs per sites were presented as a function of its distance from the nearest "upstream" WWTP which load empties into the inflowing rivers or into the lake. In this case the WWTPs which loads are transferred beyond the border of the watershed were not considered (Fig.1).

Hydrographic distances for each sample site from the nearest "upstream" WWTP, were measured using Google Earth software (Gorelick et al., 2017). The coordinates, distances and capacity of the nearest WWTPs are presented in Table S2. To decrease the effect of the differences in WWTP capacities, the number of the indicated PhACs per site in each sample period, were adjusted by the logarithm of capacity (m^3/day) of the nearest WWTP. Additionally, covariance analyses were made to test the equality and homogeneity of regression slopes. All computations were executed using PAST statistical software (Hammer et al., 2001).

3. Results and discussion

3.1 Analytical processes

3.1.1 SPE optimisation

In this work, several sorbents were investigated, among them were polymer and silica-based sorbents capable of non-polar and/or ion-exchange interactions (see section 2.3), with the aim of achieving one sorbent extraction for all PhACs. The Strata X-CW was found to give the highest recoveries for the majority of PhACs from those investigated. The Strata X-CW, as mixed mode SPE, has lipophilic surface property like a generally used C18 sorbent, but it also has the ability to bind the basic PhACs selectively due to its weak acidic character (Musile et al., 2018). The acidic and neutral PhACs can be eluted selectively by organic solvents, meanwhile the basic PhACs are retained. Finally, the elution of basic PhACs provides visibly cleaner extracts in comparison to the phases with single interaction mode (Tolgyesi et al., 2018).

The optimal applied adsorbent amount to SPE was also tested because this parameter seriously influences the final recoveries. If the used cartridge contains less adsorbent, the overload is a real problem, but with use of internal standards, the lost amount can be controlled. However, use of too high amount of adsorbent might lead to incomplete elution (Fontanals et al., 2017). Based on these data, 200 mg adsorbent was used considering that the type of matrix is surface water.

Two wash steps were applied to remove matrix, provide cleaner extracts and improve signal to noise ratio (S/N); firstly, acidified water followed by acidified ACN-water mixture. The acidified water did not result in any loss of investigated PhACs. However, acidified ACN-water mixture resulted in the breakthrough of less lipophilic compounds (e.g., levetiracetam, amphetamine) and, subsequently, lower recoveries of these compounds. In spite of all this, due to the significantly cleaner extracts provided by this second washing step, it was concluded that the washing step should remain.

The use of mixed stationary phases and selective elutions phase by phase increased the number of detectable PhACs and achieved the multi-residue analysis. The first step (organic

phase-ACN) ensured the selective elution of acidic and neutral compounds, including hormones, which required further derivatisation. Consequently, the effectivity of derivatisation was increased since the dansyl-chloride can also react with primary- and secondary-amine bases, which are retained on the SPE column. The second step provided the appropriate elution of other PhACs.

Deuterated and isotope labeled IS were added prior to SPE extraction in order to minimalise the matrix effects and compensate for losses or enhancement of compounds during the sample preparation procedure. The average absolute SPE recovery (to 5 ng/L spiked ultra-high quality water) was 76.5%.

3.1.2 Quantification and method validation

Concentrations of compounds were calculated using the standard calibration curve for the water spiked with compounds before extraction, which were constructed using a detector response defined as the ratio of the peak ion (the specific product ion of the highest intensity as qualifier ion) to the base peak ion of the related internal standard. The mean correlation coefficient (R^2) of the calibration curves was typically higher than 0.95 and showed linearity in the range of 0.1–1000 ng/L for the majority of PhACs. The average method accuracy was 89.4%. The method used achieved simultaneous quantitative analysis of 134 drugs, where the LOD and LOQ values (Table S3) were 0.01–80.00 and 0.05–200.00 ng/L concentration range (mean 2.70 and 8.26 ng/L), respectively. In addition, the proposed analytical method offers rapid analysis applying only one extraction with low limits of quantification, thus overcoming the drawbacks of previously published procedures (Martin et al., 2011). Based on these data, the SPE-SFC-MS/MS method was suitable to the multi-residue analysis of the freshwater samples.

3.2 Environmental application

The on-site measurements of hydrophysico-chemical parameters during the sample collections did not show discrepancies from the seasonal averages (Table S1). Therefore, considerable occasional local (sewage) pollution could not be detected on the sampled sites in the sampling periods.

Altogether, 69 out of 134 PhACs were revealed from all samples (Table 1). In Fig. 2A, the detected PhACs were ranked by their frequency of occurrence (FO). All detected PhACs, according to their physiological effect, were grouped into 10 chemical classes. Cumulated values of the classes were collected per sites on boxplots (Fig. S1) and the distribution of PhACs in the classes per periods are presented on bar charts (Fig. 2B).

The number of PhACs per chemical class showed considerable differences. Cardiovascular drugs showed the highest diversity (n=14), at the same time, only two NSAIDs were detected (Table 1, Fig. 2B). According to the authors' knowledge, 15 PhACs (lacosamide, metoclopramide, procyclidine, buspirone, cinolazepam, practolol, propafenone, trimetazidine, dibutylon, bupivacaine, tetracaine, ethylmorphine, 3-Cl-ephedrine, atropine, and atracurium) have been described from natural waters for the first time from 69 detected PhACs (Table 1).

Out of the indicated pharmaceuticals only the antiepileptic carbamazepine (CBZ) (av.: 126.0 ng/L) appeared from all sites in each sampling period (FO: 100%). Besides the CBZ, there were other five PhACs with FO beyond 95%. These most frequent pollutants were the antiepileptic lamotrigine (FO: 98%, av.: 129.2 ng/L), the opioid tramadol (FO: 98%, av.: 31.8 ng/L), the antipsychotic tiapride (FO: 95%, av.: 65.5 ng/L), perindopril which is a cardiovascular drug (FO: 95%, av.: 45.8 ng/L) and the hormone E1 (FO: 95%, av.: 1.8 ng/L). More than five PhACs were detected in more than half of the samples, at the same time 11 pharmaceuticals were indicated from only single samples. Our results did not show any trend in the FO of the different chemical classes (Fig. 2A).

According to the published data, the CBZ is frequently recorded in high concentration in several countries. In European surface waters: 75 and 294 ng/L in Austria, 70 and 370 ng/L in Finland (Lindholm-Lehto et al., 2015; Vieno et al., 2006), 78 and 800 ng/L in France, 25 and 110 ng/L in Germany, and 30 and 150 ng/L in Switzerland; median and maximum concentration of CBZ were measured, respectively (Ternes et al., 2004). Moreover, in river samples of the Baltic Sea region, 138 ng/L mean CBZ was also found (UNESCO and HELCOM, 2017). A recent study shows that CBZ is the most frequent PhAC in Turkish environmental samples (Guzel et al., 2018). The incomplete CBZ biodegradation and the insufficient capacity of soil microbes to transform it might explain the persistent CBZ appearance in environmental waters (Martinez-Hernandez et al., 2016). Lamotrigine also occurred frequently in South African surface water in 190 and 586 ng/L mean and maximum concentration, respectively (Wood et al., 2017). Presumably, these contamination levels were provoked by persistent contaminations and consumption habits of drug-users as well as intensive use and chronic administration. Furthermore, the background of the persistent contaminations might be the high amount of drug content per tablet (200–500 mg per tablets).

A further problem might be the low and variable elimination efficacy of WWTP. Gurke et al (2015) notes lamotrigine is not eliminated, but concentrated in the studied WWTP, therefore, its concentration was increased in its outflow (Gurke et al., 2015). The tramadol is also detected in surface waters in some European countries, e.g., Estonia and Finland, and the highest tramadol concentration (256 ng/L) was measured in river water (UNESCO and HELCOM, 2017). In Germany, the concentrations of tramadol found in surface waters ranged from <LOQ to 381 ng/L (Rua-Gomez and Puttmann, 2012). Presumably, these contamination levels were provoked by persistent contaminations and low removal rates of WWTPs, which is approximately 3% (UNESCO and HELCOM, 2017).

EU regulation put three estrogenic compounds (E1, E2 and EE2) on the WL of emerging pollutants in 2013 (Directive 2013/39/EU); maximum acceptable LOD have been established for them. These limits are 0.035 ng/L for EE2 and 0.4 ng/L for E1 and E2, which were also included in the EU Commission Implementing Decision 2018/840. Our method (SPE-SFC-MS/MS) with dansyl derivatisation is also appropriate (LOD 0.01 ng/L for E1, E2 and EE2) for monitoring studies. The E1 shows the highest occurrence (97%) inside the hormones/hormone derivatives group. The contamination levels of E2, EE2 and progestogens are similar to the earlier screening, which partially investigates the catchment area of Lake Balaton (Avar et al., 2016a; Avar et al., 2016b).

As Fig. 3A indicates, the number of detected PhACs ranged between 4 and 46 per sample. Their number was significantly highest on Site 2 (av.±SD: 44.3±5.7), followed by Site 1 (av.±SD: 26.3±5.6), which is also significantly different ($p<0.05$) from the other eight sampling sites (Fig. 3A). The highest number of PhACs (66) was detected in the Zala catchment, followed by the Lake Balaton (42) and KBWPS sites (29) (Table 2). However, the mean number of detected PhACs were highest in the Zala catchment (av.±SD: 34.3±10.0, min: 20, max: 46), while higher values were detected in the KBWPS (av.±SD: 15.4±2.7, min: 10, max: 18) than in the lake (av.±SD: 12.1±4.0, min: 4, max: 20) (Fig. 3B). These results indicate that the KBWPS receive larger, but less variable, PhACs loads than the sites situated next to the lake. Furthermore, these observations show that although the KBWPS receives the outflow water of several WWTPs (see: Fig. 1), this wetland area not only reduces the macronutrient (N, P) load to the lake (Hatvani et al., 2011; Kiedrzynska et al., 2008; Kovacs et al., 2011; Tatrai et al., 2000), but may play an important role in the PhACs load management, as well. Many other works also suggest that natural and artificial wetlands can be responsible for the decrease of PhACs contamination (Auvinen et al., 2017; Breitholtz et al., 2012; Hijosa-Valsero et al., 2016; Li et al., 2014;

McEachran et al., 2018; Zhang et al., 2013). Presumably, the longer retention time (up to 30 days) and the relatively shallow water, which is favourable for the UV induced degradation (Aullo-Maestro et al., 2017), and the elevated microbial activity could provide the similar degradation of PhACs in the KBWPS. However, additional studies are needed to clearly explore this observed effect in detail.

Not only the spatial, but the temporal distribution of the detected PhACs, showed a high level of variation. The number of detected pharmaceuticals per sampling period fluctuated between 43 and 55 (48.75±4.9). The average number of detected PhACs per site was significantly ($p<0.05$) lower in the April 2018 time period than in August 2017 time period (Table 2 and Fig. 3C).

Although the result of the PCA analysis showed that most of the PhACs groups appeared in highest concentrations at Site 2 (Fig. 3D), the PhACs composition showed a considerable level of variation. On average, the persistence of the sample composition varied about 0.5–0.6, which means that 50–60% of the detected PhACs were identical in two randomly chosen samples, which originated from the same site. The highest average persistence level was indicated in Site 2 (0.594±0.1), where the highest number of PhACs were detected during our study. This result can be attributed to the fact that this area has a large, and more or less permanent, population which causes diverse, but more permanent PhACs loads into the recipient River Zala.

The two eastern-most sites showed the significantly lower persistence values (0.402±0.09 and 0.256±0.15, respectively) (Fig. 3E). These results can be explained by the fact that some hallucinogens (e.g., ecgonine-methylester, MDMA) and some PhACs classified into the "Others" group (e.g., ketamine, caffeine), appeared only in the summer (touristic season) period (Table S4, Fig. 3D). And, whereas the wastewater of this area is collected and drained outside of the catchment (Fig. 1), these PhACs are more likely to enter the pond by a direct load (urine and

446 waste) and/or the precipitation washes these components from the shoreline of the lake.
447 Therefore, in this case, the area is a summer holiday destination, so the elevated number and
448 concentration of mostly recreational substances (e.g., caffeine and illicit drugs) were indicated,
449 especially in the summer period at the shoreline of the lake. These assumptions are refined by the
450 results of regression and covariance analyses (Fig. 4). The adjusted number of PhACs per site
451 show decreasing trends away from the WWTPs in each sampling period. Results of covariance
452 analyses showed that no significant differences can be detected on the equality and homogeneity
453 of regression slopes for data from the four sampling periods ($F=1.829$, $p=0.152$, $F=0.202$ and
454 $p=0.894$, respectively). Therefore, it seems to be a permanent trend, which is only slightly
455 modified by the effect of tourism in the summer period. Our results indicate that the WWTPs may
456 be the most important sources of PhAC pollution in the studied water system. At the same time,
457 they show that through the periodically increased direct PhACs load, tourism may have a major
458 detectable impact effect on the quality of surface waters. Moreover, the shallow lakes, due to
459 their limited puffer capacity (caused by their low volume and the relatively long shoreline), seem
460 to be particularly at risk by PhACs exposure.

461 **4. Conclusion**

462 The new method was appropriate for simultaneous detection of multiple PhACs
463 characterised by highly different concentrations and chemical composition. Therefore, -the use of
464 mixed phase SPE makes the sample preparation easier and helps to reveal the effects of
465 contamination of PhACs in environmental samples. Results of our field investigations showed
466 that PhACs were detectable in each site, while their distribution and concentration represented
467 considerable spatiotemporal variations. On the sites characterised by permanent and dense
468 populations, PhACs used in human medicine (antiepileptics and cardiovascular drugs) dominated
469 the samples. While those sites exposed to mass tourism on average, recorded lower but more

variable PhACs contamination. Some significant seasonal outlier values of recreational substances (e.g., caffeine and illicit drugs) were indicated in these sites, presumably due to their direct load (e.g., urine) in the summer touristic period.

To summarise: the WWTP loads have a possible major role on the PhAC contamination of the studied area. At the same time, the mass tourism induced PhAC contamination was also detectable. Moreover, the interventions initially aimed to reduce the impact of macro-pollutants (P, N) on the lake, but reduce its PhAC contamination in the recipient surface waters, as well. Here we have to note, that via the sewage transfer duct system, the pollution is only shifted through the border of the area to be protected. The optimal solution would be to improve the PhACs elimination technology in the WWTPs in parallel with the application of quaternary treatment on the effluent water, such as the retention of WWTP effluent in constructed wetlands for a shorter period before release into natural surface waters.

Authors' contribution

The study was design by MG, PZ and TP, and analytical methods were developed by MG, MM and ZZ. The water collection was performed by MG and TP and the experimental work was performed by ME, KM, FI. The manuscript was written by MG and TP with feedback from PZ.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figures and figure legends

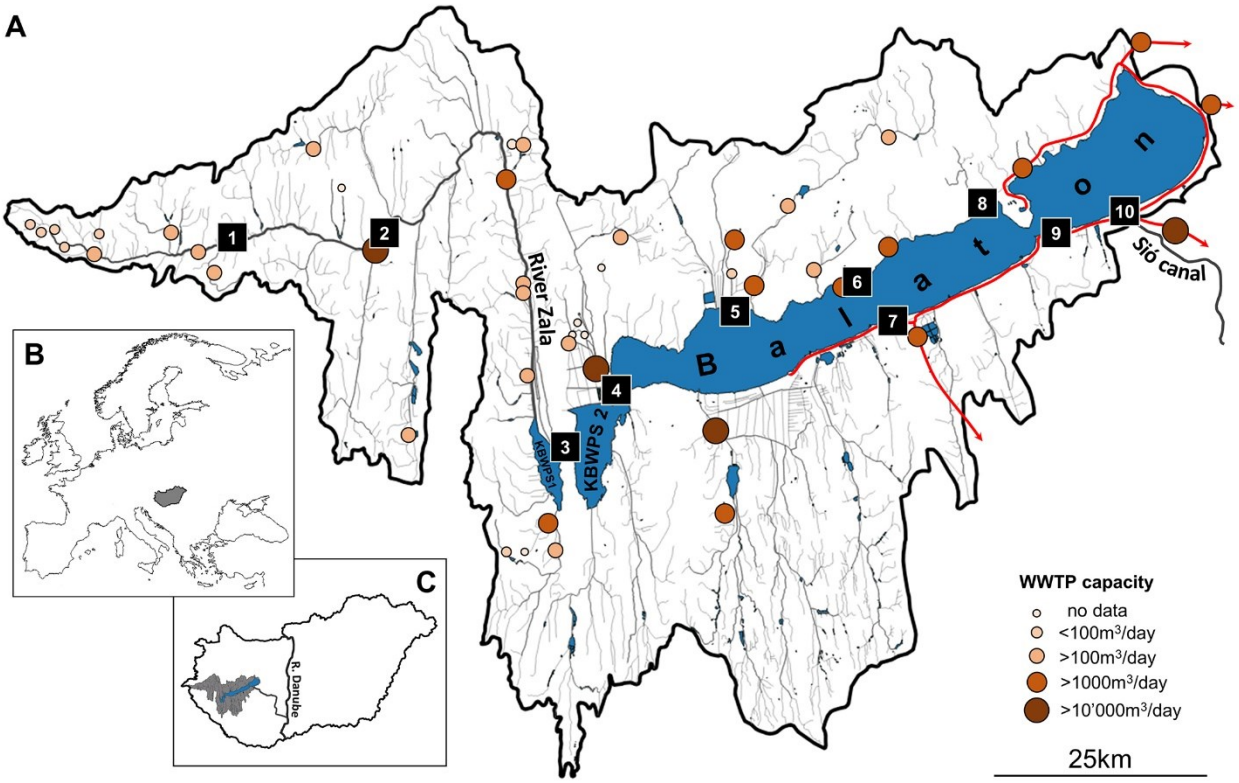


Fig. 1. Distribution of the ten sampling sites in the studied drainage system (A). Black solid line is the border of the Balaton catchment. Numbered rectangles identify sampling sites. Brown circles in different sizes and colours show WWTPs with different capacities. Red line is sewage transfer duct system. Red arrows show the direction of wastewater disposal. Geographic position of Hungary in Europe and the Balaton catchment's position in Hungary are indicated in the inserts B and C, respectively.

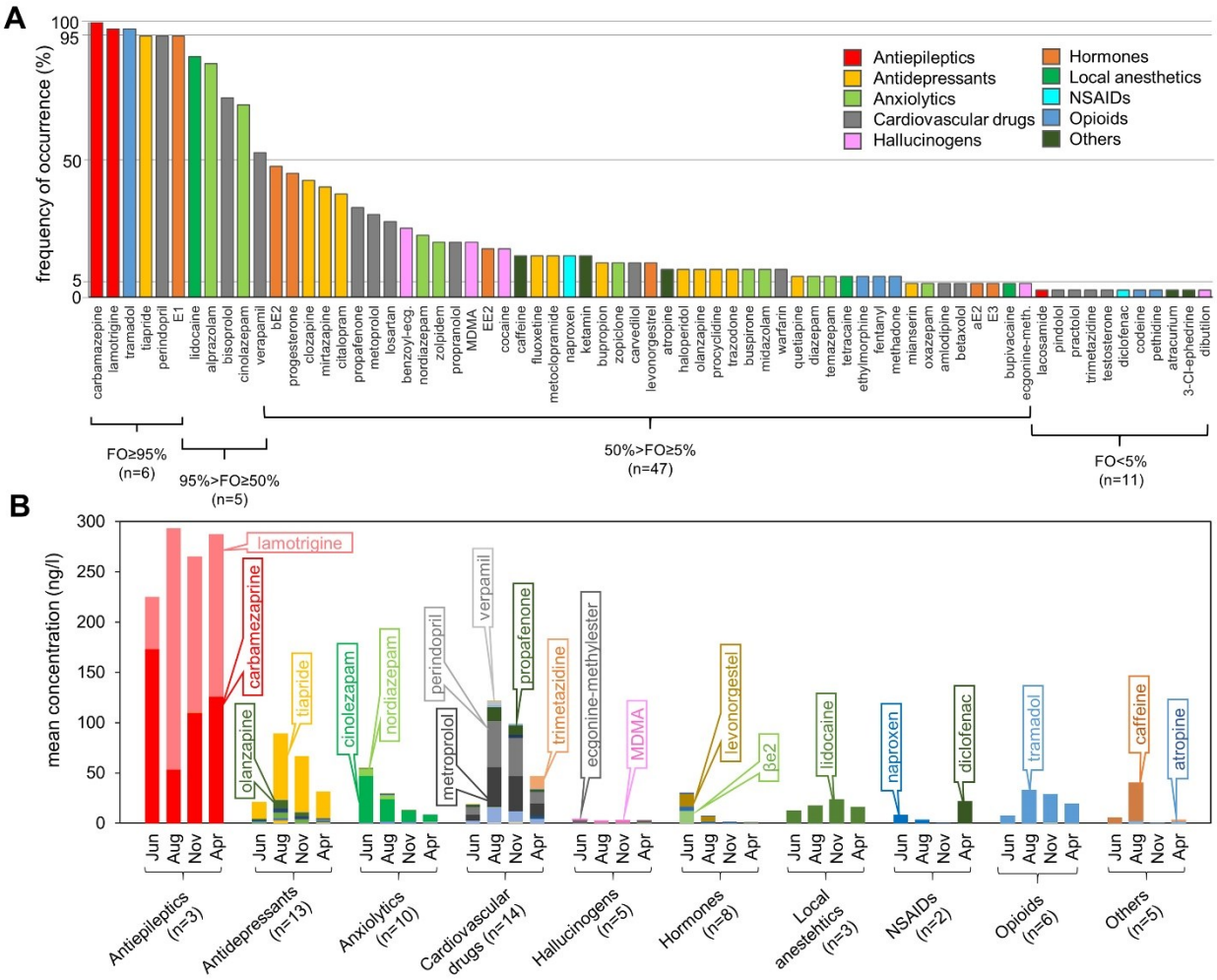
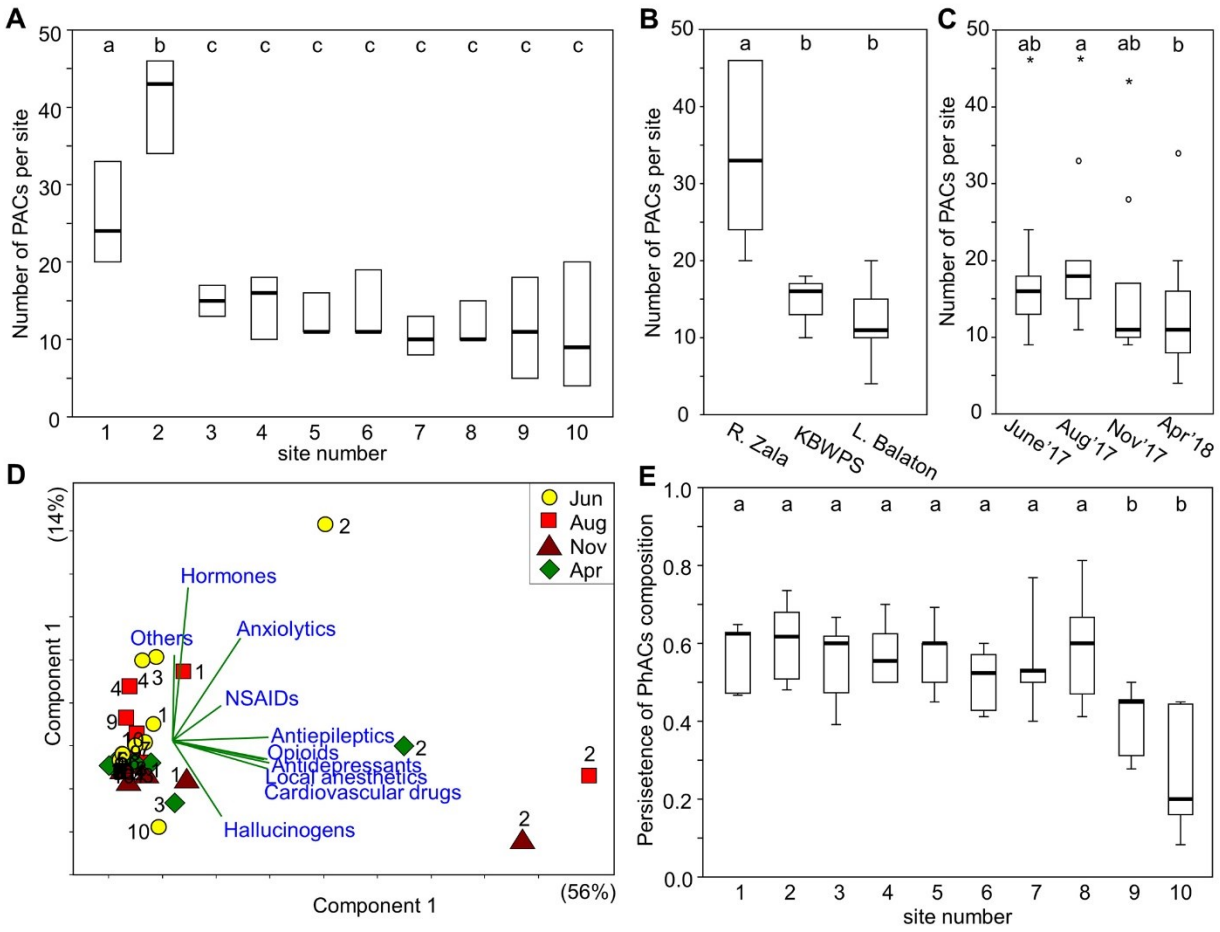


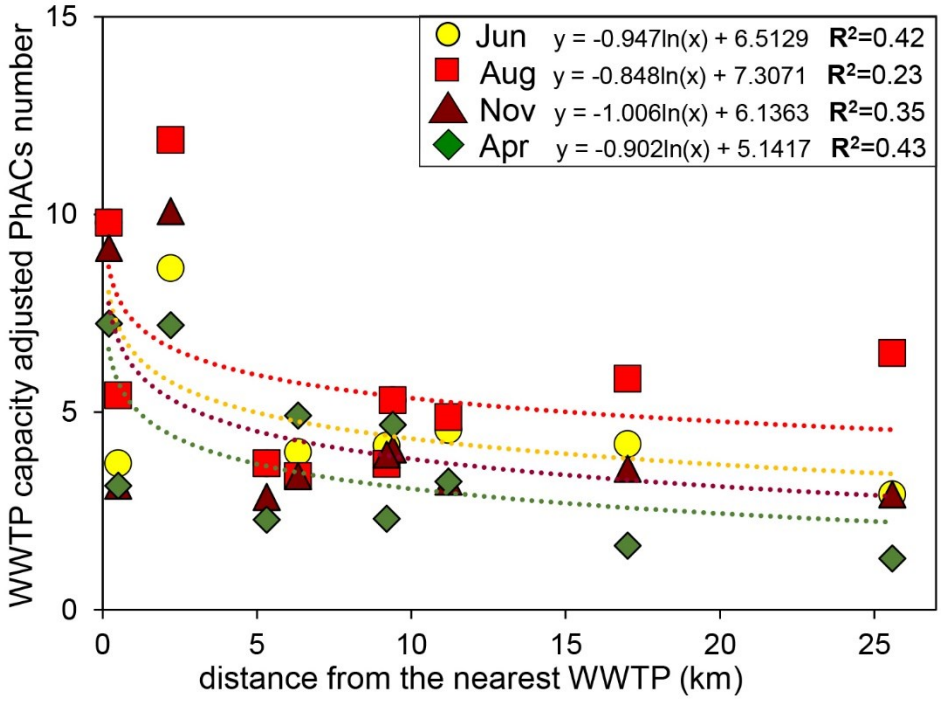
Fig. 2. Frequency of occurrences (A) and mean concentrations (B) of the 69 recorded PhACs. In the latter case, PhACs were classified into 10 chemical classes, and the 2-3 most characteristic drugs were named per class (B).



774

775 **Fig. 3.** Spatio-temporal distribution characteristics of the detected PhACs in the study area.
776 PhACs number per site (n=4 for each site) (A), per subcatchment (n=8 for River Zala, n=8 for
777 KBWPS, n=24 for Lake Balaton) (B) and per sampling period (n=10 for each period) (C). PCA
778 plots of the recorded drug concentrations on the sample sites in the four study periods, site
779 numbers correspond with Fig. 1. (D). Persistence of pharmaceutical compositions for each sample
780 site (n=6). Persistence is expressed by the Jaccard similarity values. In the case of subfigures A,
781 B, C and E boxplots are used to present the data distribution, where each box represents the 25%
782 and 75% quartiles of the dataset, the band in the box is the median. The whiskers are drawn from
783 the top of the box up to the largest data point less than 1.5 times the box height from the box (the
784 "upper inner fence") and, similarly, below the box. Values outside the inner fences are shown as

785 circles, values greater than 3 times the box height from the box (the "outer fences") are shown as
786 stars. Boxplots marked with the same letters do not differ significantly based on nonparametric
787 Kruskal-Wallis pairwise comparisons ($p<0.05$).



788

789 **Fig. 4.** Regression plot of the adjusted PhACs number and the sample sites' distance from the
790 nearest WWTP. Note, in this case, the number of PhACs is adjusted by the logarithm of capacity
791 (m^3/day) of the nearest WWTP. For more details see text and Table S2. The regression equations
792 and coefficient of determinations (R2) are also indicated on the plot.

793

Table 1. Average concentrations, frequency of occurrences (FO), and min max values of the detected PhACs in the catchment system of Lake Balaton in the June 2017 – April 2018 time period. The FO columns shows sum incidence per survey per compounds in 10 sample sites. PhACs recorded from surface waters at the first time are indicated by asterisk (*).

Chemical class	**Average concentration [ng/L]**				**Frequency of occurrence [%]**				**MIN [ng/L]**				**MAX [ng/L]**			
PhACs	*Jun*	*Aug*	*Nov*	*Apr*	*Jun*	*Aug*	*Nov*	*Apr*	*Jun*	*Aug*	*Nov*	*Apr*	*Jun*	*Aug*	*Nov*	*Apr*
Antiepileptics																
carbamazepine	173.1	53.2	109.6	126.0	100	100	100	100	18.3	4.7	6.1	21.2	659.2	230.6	804.6	397.3
lacosamide*	-	-	9.3	-	0	0	10	0	-	-	9.3	-	-	-	9.3	-
lamotrigine	57.8	240.2	154.8	161.7	90	100	100	100	5.7	15.5	17.1	16.4	319.3	1734.8	1102.4	1078.7
Antidepressants																
bupropion	1.1	14.7	-	4.6	10	20	0	20	1.1	2.0	-	2.6	1.1	27.5	-	6.6
citalopram	1.2	4.2	4.3	6.6	40	50	30	30	0.1	0.1	0.2	0.9	3.8	17.7	11.1	16.5
clozapine	1.8	9.3	12.5	9.9	70	60	30	10	0.1	0.2	0.4	9.9	10.3	54.3	35.9	9.9
fluoxetine	1.8	-	-	-	60	0	0	0	0.5	-	-	-	3.4	-	-	-
haloperidol	0.2	0.4	0.3	-	10	20	10	0	0.2	0.0	0.3	-	0.2	0.8	0.3	-
metoclopramide*	1.0	5.8	3.7	10.1	10	20	20	10	1.0	0.4	0.6	10.1	1.0	11.2	6.8	10.1
mianserin	-	0.4	-	-	0	20	0	0	-	0.3	-	-	-	0.6	-	-
mirtazapine	0.8	8.2	5.5	1.6	60	50	40	10	0.2	0.2	0.2	1.6	3.2	38.8	19.9	1.6
olanzapine	11.9	82.8	19.7	-	10	10	20	0	11.9	82.8	11.8	-	11.9	82.8	27.6	-
procyclidine*	0.5	2.4	1.5	2.4	10	10	10	10	0.5	2.4	1.5	2.4	0.5	2.4	1.5	2.4

quetiapine	0.1	0.1	0.4	-	10	10	10	0	0.1	0.1	0.4	-	0.1	0.1	0.4	-
tiapride	16.1	66.5	55.5	33.1	100	100	100	80	0.2	1.1	0.6	0.1	133.1	566.1	432.0	217.3
trazodone	0.1	0.4	0.4	-	10	20	10	0	0.1	0.1	0.4	-	0.1	0.7	0.4	-
Anxiolytics																
alprazolam	0.6	1.7	1.4	1.2	70	100	100	70	0.1	0.2	0.2	0.1	2.2	12.6	8.4	5.3
buspirone*	-	0.1	-	3.2	0	20	0	20	-	0.1	-	0.5	-	0.1	-	5.9
cinolazepam*	58.4	36.8	14.6	10.9	80	60	80	60	1.6	0.7	0.4	1.4	357.3	197.0	84.5	53.9
diazepam	-	-	0.1	1.1	0	0	10	20	-	-	0.1	0.3	-	-	0.1	2.0
midazolam	0.1	0.4	0.3	1.7	10	10	10	10	0.1	0.4	0.3	1.7	0.1	0.4	0.3	1.7
nordiazepam	17.5	13.8	0.9	-	40	30	20	0	1.4	1.1	0.4	-	60.5	38.0	1.4	-
oxazepam	-	9.8	-	1.8	0	10	0	10	-	9.8	-	1.8	-	9.8	-	1.8
temazepam	7.9	4.0	-	1.0	10	10	0	10	7.9	4.0	-	1.0	7.9	4.0	-	1.0
zolpidem	0.1	0.6	0.5	0.5	20	20	20	20	0.0	0.1	0.1	0.2	0.3	1.2	1.0	0.8
zopiclone	0.8	2.2	1.3	-	10	20	20	0	0.8	0.3	0.3	-	0.8	4.1	2.2	-
Cardiovascular drugs																
amlodipine	-	7.3	8.9	-	0	10	10	0	-	7.3	8.9	-	-	7.3	8.9	-
betaxolol	-	2.8	3.4	-	0	10	10	0	-	2.8	3.4	-	-	2.8	3.4	-
bisoprolol	3.9	14.6	11.0	14.4	60	100	100	30	0.7	0.5	1.0	0.5	10.0	72.2	84.4	36.1
carvedilol	0.4	2.2	2.0	-	20	20	10	0	0.3	1.9	2.0	-	0.5	2.5	2.0	-
losartan	0.2	0.5	1.3	6.4	10	20	30	50	0.2	0.3	0.1	0.1	0.2	0.8	3.0	24.8
metoprolol	31.2	134.3	116.1	30.8	20	30	30	40	8.1	5.1	3.9	1.2	54.4	367.3	312.2	104.2
perindopril	7.3	46.1	38.2	14.0	100	100	100	80	0.1	0.8	0.5	0.8	52.4	386.0	283.1	86.0

pindolol	-	0.5	-	-	0	10	0	0	-	0.5	-	-	-	0.5	-	-
practolol*	-	-	28.1	-	0	0	10	0	-	-	28.1	-	-	-	28.1	-
propafenone*	8.6	34.1	23.3	13.7	30	40	40	20	0.8	0.7	0.6	5.4	18.2	116.3	86.9	22.0
propranolol	1.0	10.6	8.3	5.7	30	20	20	10	0.0	1.5	1.3	5.7	2.6	19.7	15.4	5.7
trimetazidine*	-	-	-	122.2	0	0	0	10	-	-	-	122.2	-	-	-	122.2
verapamil	0.2	4.7	0.8	1.2	70	100	20	20	0.1	0.1	0.1	0.9	0.5	27.1	1.5	1.4
warfarin	3.7	2.0	0.2	0.7	10	10	10	10	3.7	2.0	0.2	0.7	3.7	2.0	0.2	0.7
Hallucinogens																
benzoyl-ecgonine	0.1	1.1	1.2	1.6	10	50	20	20	0.1	0.2	0.5	1.2	0.1	2.3	1.9	1.9
cocaine	-	0.1	0.1	0.9	0	40	10	20	-	0.1	0.1	0.6	-	0.2	0.1	1.2
dibutylon (N.N-dimethyl-butylone)*	-	-	-	1.1	0	0	0	10	-	-	-	1.1	-	-	-	1.1
ecgonine-methylester	34.8	-	-	21.6	10	0	0	10	34.8	-	-	21.6	34.8	-	-	21.6
MDMA (Ecstasy)	3.2	7.5	10.6	2.7	10	30	30	10	3.2	2.9	0.5	2.7	3.2	10.3	26.8	2.7
Hormones																
aE2	-	0.4	0.2	-	0	10	10	0	-	0.4	0.2	-	-	0.4	0.2	-
bE2	12.4	0.3	-	0.2	100	40	0	50	2.9	0.1	-	0.2	19.6	0.7	-	0.3
E1	4.1	1.6	1.0	0.5	90	100	100	90	0.4	0.4	0.2	0.2	10.5	7.5	5.4	1.1
E3	0.1	0.1	-	-	10	10	0	0	0.1	0.1	-	-	0.1	0.1	-	-
EE2	2.2	0.6	0.4	-	30	10	30	0	1.8	0.6	0.2	-	2.7	0.6	0.7	-
levonorgestrel	41.9	49.4	-	1.9	30	10	0	10	38.2	49.4	-	1.9	44.7	49.4	-	1.9
progesterone	1.2	0.9	1.0	0.8	70	70	20	20	0.7	0.6	0.7	0.6	2.3	1.3	1.4	1.1
testosterone	-	-	-	1.1	0	0	0	10	-	-	-	1.1	-	-	-	1.1

Local anesthetics																
bupivacaine*	-	0.1	0.1	-	0	10	10	0	-	0.1	0.1	-	-	0.1	0.1	-
lidocaine	14.2	17.5	23.7	27.1	90	100	100	60	0.3	0.5	0.1	0.3	42.2	114.8	141.3	87.0
tetracaine*	0.5	-	-	-	30	0	0	0	0.1	-	-	-	1.2	-	-	-
NSAIDs																
diclofenac	-	-	-	221.4	0	0	0	10	-	-	-	221.4	-	-	-	221.4
naproxen	42.5	11.7	3.3	-	20	30	10	0	28.9	2.2	3.3	-	56.1	28.1	3.3	-
Opiods																
codeine	-	-	5.5	-	0	0	10	0	-	-	5.5	-	-	-	5.5	-
ethylmorphine*	0.6	17.5	13.3	-	10	10	10	0	0.6	17.5	13.3	-	0.6	17.5	13.3	-
fentanyl	0.2	1.1	0.8	-	10	10	10	0	0.2	1.1	0.8	-	0.2	1.1	0.8	-
methadone	0.0	0.1	-	0.6	10	10	0	10	0.0	0.1	-	0.6	0.0	0.1	-	0.6
pethidine	0.1	-	-	-	10	0	0	0	0.1	-	-	-	0.1	-	-	-
tramadol	8.5	32.1	27.3	19.5	90	100	100	100	0.2	0.7	0.3	0.2	64.7	279.9	217.2	161.4
Others																
3-Cl-ephedrine*	-	-	-	0.2	0	0	0	10	-	-	-	0.2	-	-	-	0.2
atropine*	-	0.3	-	10.6	0	20	0	20	-	0.2	-	2.2	-	0.4	-	18.9
atracurium*	-	-	0.3	-	0	0	10	0	-	-	0.3	-	-	-	0.3	-
caffeine	56.7	77.3	-	-	10	50	0	0	56.7	15.6	-	-	56.7	138.7	-	-
ketamin	1.6	6.3	5.4	15.9	10	30	10	10	1.6	2.5	5.4	15.9	1.6	8.8	5.4	15.9

Table 2. Number of detected PACs per sample sites, areas and periods, and for the whole study.

Nº	Location	site name	jun. 2017	aug. 2017	nov. 2017	apr. 2018	Σ area	Σwhole study
1.	R. Zala	Zalalövő-Budafa	24	33	28	20		
2.	R. Zala	Zalaegerszeg	46	46	43	34		
	ΣZala		***46***	***48***	***47***	***37***	**66**	
3.	KBWPS	Balatonhídvég	17	17	13	15		
4.	KBWPS	Balatonberény	18	16	17	10		
	ΣKBWPS		***20***	***22***	***18***	***15***	**29**	
5.	L. Balaton	Szigliget	13	11	11	16		
6.	L. Balaton	Révfülöp	13	19	11	11		
7.	L. Balaton	Balatonlelle	13	13	10	8		
8.	L. Balaton	Tihany-Sajkod	14	15	10	10		
9.	L. Balaton	Zamárdi	16	18	11	5		
10.	L. Balaton	Siófok	9	20	9	4		
	ΣBalaton		***24***	***27***	***14***	***24***	**42**	
	Σperiods		**49**	**55**	**48**	**43**		**69**

Supplementary information

Spatiotemporal variations of pharmacologically active compounds in surface waters of a summer holiday destination

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Table S1. Details of sample locations and hydrophysico-chemical parameters of water in each sample site from the June 2017 through April 2018 time period.

site code	subcatch-ment	threat	Location	Coordinates	sampling date	pH	O_2 cc. [mg/L]	conductivity [µS/cm]	T [°C]
Site 1	River Zala	WWTP	Zalalövő-Budafa	46.85024, 16.62694	2017.06.26	8.3	9.3	269	15.9
					2017.08.10	8.3	6.6	362	18.4
					2017.11.02	7.6	11.9	386	9.0
					2018.04.18	7.6	11.7	336	12.8
Site 2	River Zala	WWTP	Zalaegerszeg	46.85234, 16.8608	2017.06.26	8.2	12.3	605	16.4
					2017.08.10	8.4	10.2	760	21.1
					2017.11.02	7.5	11.6	700	10.7
					2018.04.18	7.9	12.0	632	14.7
Site 3	KBWPS	WWTP	Balaton-hídvég	46.63706, 17.18347	2017.06.26	8.5	12.0	480	21.0
					2017.08.10	9.4	9.2	310	30.3
					2017.11.02	7.6	8.7	709	9.7
					2018.04.18	7.5	9.8	830	18.3
Site 4	KBWPS	WWTP	Balaton-szentgyörgy	46.70243, 17.25866	2017.06.26	7.9	9.9	674	19.0
					2017.08.10	8.2	6.2	770	29.1
					2017.11.02	7.4	11.1	793	8.4
					2018.04.18	7.8	12.0	875	17.2
Site 5	Lake Balaton	tourism/ WWTP	Szigliget	46.78541, 17.4349	2017.06.26	8.4	10.0	779	18.9
					2017.08.10	9.0	4.1	778	24.5
					2017.11.02	7.5	7.2	792	8.0
					2018.04.18	8.1	5.9	873	14.4
Site 6	Lake Balaton	WWTP/ tourism	Révfülöp	46.82411, 17.60672	2017.06.26	8.3	9.1	773	19.5
					2017.08.10	8.4	3.4	830	24.2
					2017.11.02	7.9	10.0	841	8.6
					2018.04.18	8.1	9.9	794	14.6
Site 7	Lake Balaton	tourism	Balatonlelle	46.79708, 17.72528	2017.06.26	8.9	9.7	725	23.6
					2017.08.10	8.4	7.0	764	26.7
					2017.11.02	8.0	14.0	794	9.7
					2018.04.18	8.2	7.8	820	14.1
Site 8	Lake Balaton	tourism	Tihany (Sajkod)	46.90339, 17.85037	2017.06.26	8.6	9.8	845	20.2
					2017.08.10	9.5	9.8	784	28.5
					2017.11.02	7.9	10.1	700	8.7
					2018.04.18	8.3	8.0	860	13.5
Site 9	Lake Balaton	tourism	Zamárdi	46.88525, 17.93139	2017.06.26	9.0	9.1	753	22.9
					2017.08.10	8.5	7.1	828	25.7
					2017.11.02	8.2	10.0	800	9.8
					2018.04.18	8.3	7.9	836	13.8
Site 10	Lake Balaton	tourism	Siófok	46.91102, 18.04604	2017.06.26	9.0	9.8	772	24.2
					2017.08.10	8.4	6.7	867	25.4
					2017.11.02	8.2	14.0	818	10.9
					2018.04.18	8.4	7.3	850	14.0

Table S2. Geographic position, capacity and distance of the nearest WWTPs from the study sites.

site code	nearest WWTP		
	coordinate	distance (km)	capacity (m^3/day)
Site 1	46.843087, 16.611164	2.2	600
Site 2	46.853176, 16.858007	0.2	50000
Site 3	46.546116, 17.138125	9.4	1600
Site 4	46.736630, 17.236070	9.2	21500
Site 5	46.823104, 17.481717	6.3	1800
Site 6	46.824482, 17.604031	0.5	3200
Site 7	46.824482, 17.604031	5.3	3200
Site 8	46.877235, 17.713468	11.2	1200
Site 9	46.877235, 17.713468	17.0	1200
Site 10	46.877235, 17.713468	25.6	1200

Spatial occurrence per PhAC groups

Antiepileptics (n=3) usually appear in elevated concentrations compared to the other chemical classes. Its highest value was recorded at Site2, while the mean concentration shows a decreasing trend to the east. In the lake, only Site 6 shows elevated values. This site is situated beside one of the WWTPs that empty their outflow into the lake. The antidepressants (n=13), anxiolitics (n=10) and cardiovascular drugs (n=14) show similar trends. The highest values were detected at Site 2. Hormones and their derivatives (n=8) show some outlier values on the sites situated next to the River Zala and KBWPS. Considerably lower values were detected on the lake's shoreline. In the case of local anesthetics (n=3), permanent high values were recorded at Sites 1 and 2. At Sites 7 and 9, outlier values were detected. NSAIDs (n=2) show elevated values at the first two sites. The opioids (n=6) were indicated on permanent high concentration at the Site 2. Hallucinogens (n=5) were recorded continuously at the first two sites. At the other sites, these PhACs appeared as outlier values during the summer period. PhACs classified into the "Others" category (n=5) appear on the Sites 1 and 2 permanently. At Sites 4, 6, 9 and 10, some outlier values were detected in summer, mostly due to the elevated ketamine and caffeine concentrations.

Figure S1. Boxplots derived from the cumulated values of the 10 chemical classes. Boxplots with the same letters do not differ significantly based on Kruskal-Wallis post hoc comparisons ($p<0.05$). Each box represents the minimum and maximum values of the certain compound groups. The band in the box is the median. Numbers on the X axis correspond with the site numbers indicated on Fig. 1.

53 **Table S3** Validation parameters of PhACs

Chemical class	PhACs' name	Ionization mode	Precursor ion [m/z]	Quantifier ion [m/z]	CE [V]	Product ion [m/z]	CE [V]	Product ion [m/z]	CE [V]	Product ion [m/z]	CE [V]	Product ion [m/z]	CE [V]	t_R [min]	LOD [ng/L]	LOQ [ng/L]	Linearity range [ng/L]	R^2	Measured Eluate
Alkaloids	atropine	positive	290.00	102.95	45	123.95	25	92.95	30	260.00	20	76.95	40	5.100	0.01	0.05	0.05-100	0.9836	2
	caffeine	positive	194.95	137.95	20	109.95	20	92.95	20	82.95	25			2.370	5.00	10.00	10-20000	0.9862	1
	drotaverin	positive	398.15	354.18	30	370.22	25	326.15	35	282.12	40	340.15	35	3.420	0.07	0.10	0.1-200	0.9959	2
	papaverin	positive	340.00	202.00	25	324.15	30	296.00	30	170.95	35	280.00	45	3.150	0.03	0.10	0.1-200	0.9805	2
	scopolamine	positive	304.15	137.95	25	155.95	15	120.95	20	109.95	25	102.95	35	3.280	0.01	0.05	0.05-100	0.9937	2
	theophyllin	positive	180.95	123.95	15	95.95	20	68.95	25	41.95	25			2.640	5.00	10.00	10-20000	0.9927	1
Antiepi-leptics	carbamazepine	positive	237.10	194.10	20	179.05	30	165.05	35	152.10	35	220.10	15	3.030	0.02	0.10	0.05-100	0.9952	1
	lacosamide	positive	251.12	108.10	10	91.10	10	116.08	15	219.12	6	74.10	25	2.700	0.10	0.50	0.5-1000	0.9488	1
	lamotrigine	positive	256.02	211.00	25	144.95	35	109.00	45	159.00	25	187.00	25	4.270	1.15	5.00	5-10000	0.9792	2
	levetiracetam	positive	171.15	126.05	15	154.10	6	69.05	25					2.790	80.00	200.00	200-400000	0.9786	2
Antipsychotics/Antidepressants	amitriptillin	positive	278.00	233.00	15	190.95	20	116.95	20	104.95	20	90.95	20	3.470	0.03	0.10	0.1-200	0.9850	2
	aripirazol	positive	448.20	285.12	25	218.15	25	176.05	30	146.05	45	98.10	35	4.010	0.05	0.10	0.1-200	0.9988	2
	bupropion	positive	240.00	183.95	10	165.95	20	138.95	25	130.95	25	102.95	35	1.760	0.15	0.50	0.5-1000	0.9418	2
	chlorpromazine	positive	319.15	85.95	20	246.00	20	238.00	20	57.95	20			3.600	0.10	0.50	0.5-1000	0.9949	2
	citalopram	positive	325.15	108.95	25	280.00	15	262.00	20	234.00	25	165.95	25	4.060	0.03	0.10	0.1-200	0.9981	2
	clozapine	positive	327.15	270.00	25	296.00	25	227.00	25	191.95	40	83.95	20	4.260	0.03	0.10	0.1-200	0.9914	2
	cyproheptadine	positive	288.00	190.95	30	95.95	25	195.95	20	215.00	40	109.95	20	3.560	0.15	0.50	0.5-1000	0.9927	2
	droperidol	positive	380.15	164.95	25	193.95	15	122.95	45	94.95	60			3.710	0.01	0.10	0.1-200	0.9785	1
	duloxetine	positive	298.00	267.00	15	239.00	25	122.95	20	182.95	20	156.95	25	3.910	1.00	5.00	5-10000	0.9095	2
	fluoxetine	positive	310.15	147.95	5	259.00	15	251.00	20	290.00	10	43.95	5	3.530	0.01	0.50	0.5-1000	0.9882	2
	haloperidol	positive	376.15	164.95	20	206.00	25	193.95	20	358.15	20	122.95	40	4.030	0.01	0.10	0.1-200	0.9872	2
	mCPP	positive	196.95	154.05	20	119.08	20	118.10	30	111.02	30	104.05	30	3.580	0.50	5.00	5-10000	0.9839	2
	metoclopramide	positive	300.15	227.00	20	183.95	30	140.95	45	112.95	55	89.95	45	5.620	0.01	0.20	0.2-400	0.9822	2
	mianserin	positive	265.00	208.00	20	90.95	40	192.95	35	57.95	40	117.95	40	2.740	0.02	0.10	0.1-200	0.9849	1
	mirtazapine	positive	266.00	194.95	25	223.00	20	209.00	20	235.00	20	71.95	20	3.380	0.01	0.10	0.1-200	0.9635	1
	olanzapine	positive	313.15	256.00	20	282.00	20	213.00	30	197.95	40	83.90	20	4.610	1.00	5.00	5-10000	0.9542	2
	paliperidone	positive	427.20	207.00	25	109.95	40	178.95	40	164.95	40	81.95	40	4.380	0.03	0.10	0.1-200	0.9853	2
	paroxetine	positive	330.15	191.95	20	150.95	20	122.95	25	108.95	30	69.95	25	4.040	0.50	5.00	5-10000	0.9846	2
	procyclidine	positive	288.00	83.95	20	94.95	25	90.95	40	270.00	15	55.95	40	3.690	0.02	0.20	0.2-400	0.9535	2
	quetiapine	positive	384.15	253.00	20	221.00	35	279.00	25	210.00	35	157.95	20	3.700	0.05	0.10	0.1-200	0.9813	2
	risperidone	positive	411.20	190.95	30	162.95	45	109.95	50	81.95	50	68.95	50	4.490	0.05	0.10	0.1-200	0.9203	2
	sertraline	positive	306.15	158.95	20	196.95	15	275.00	10	128.95	20	90.95	20	3.390	3.00	5.00	5-10000	0.9827	2
	tiapride	positive	329.15	256.00	20	213.00	30	133.95	45	176.95	35	241.00	30	5.040	0.01	0.10	0.1-200	0.9786	2
	trazodone	positive	372.15	175.95	20	147.95	30	132.95	35	119.95	50	77.95	50	3.120	0.01	0.05	0.05-100	0.9794	2
Anxiolytics	7-aminoflunitrazepam	positive	284.00	134.95	25	256.00	20	236.00	25	227.00	25	264.00	20	3.740	0.03	0.10	0.1-200	0.9567	2
	alprazolam	positive	309.15	281.00	25	205.00	40	274.00	25	241.00	25	164.95	30	3.660	0.01	0.10	0.1-200	0.9963	2
	buspirone	positive	386.15	121.95	30	222.00	25	149.95	25	265.00	25	108.95	35	3.110	0.01	0.10	0.1-200	0.9732	2

	chlordiazepoxide	positive	300.15	227.00	25	282.00	25	255.00	20	241.00	20	283.00	15	3.240	0.15	0.50	0.5-1000	0.9805	2
	cinolazepam	positive	358.15	312.15	20	340.15	15	272.00	35	245.00	40	210.00	45	2.670	0.03	0.10	0.1-200	0.9887	1
	clonazepam	positive	316.15	270.00	25	214.00	40	241.00	35	207.00	30	150.95	55	2.930	0.02	0.10	0.1-200	0.9791	1
	diazepam	positive	285.00	192.95	30	153.95	25	222.00	25	257.00	20	228.00	25	2.210	0.01	0.10	0.1-200	0.9979	2
	diclazepam	positive	319.15	227.00	30	262.00	25	256.00	25	291.00	20	153.95	30	2.340	0.15	0.50	0.5-1000	0.9943	1
	flumazenil	positive	304.15	258.00	15	161.95	35	229.00	25	217.00	25			2.730	0.01	0.10	0.1-200	0.9869	1
	meprobamate	positive	219.00	158.12	10	97.15	15	69.12	15	55.10	20			2.980	1.50	5.00	5-10000	0.9917	1
	midazolam	positive	326.15	291.00	25	249.00	35	244.00	25	128.95	40			3.220	0.01	0.10	0.1-200	0.9969	2
	nitrazepam	positive	282.00	236.00	25	207.00	35	179.95	35	151.95	55	189.95	40	2.890	0.03	0.10	0.1-200	0.9650	1
	nordiazepam	positive	271.00	139.95	25	226.00	25	208.00	25	164.95	25	243.00	20	2.740	0.02	0.10	0.1-200	0.9701	1
	oxazepam	positive	287.00	241.00	20	269.00	15	231.00	20	162.95	35	103.95	30	3.030	0.05	0.10	0.1-200	0.9918	1
	temazepam	positive	301.15	255.00	20	283.00	15	228.00	20	192.95	35	176.95	35	2.330	0.05	0.10	0.1-200	0.9972	1
	zolpidem	positive	308.15	235.00	35	263.00	25	221.00	35	91.95	50	248.00	35	3.420	0.01	0.01	0.1-200	0.9481	2
	zopiclone	positive	389.15	245.00	20	217.00	30	345.15	8	138.95	40			3.450	0.05	0.10	0.1-200	0.9847	2
Cardiovascular drugs	acenocoumarol	positive	354.15	163.05	15	279.08	30	249.08	35	296.05	20	121.05	35	3.450	0.05	0.10	0.1-200	0.9873	1
	amiodarone	positive	646.50	100.12	30	86.10	30	276.12	35	201.12	30	159.05	60	2.830	1.50	5.00	5-10000	0.9887	2
	amlodipine	positive	409.20	238.05	10	294.10	10	220.05	25	206.05	25	170.05	30	4.300	0.30	5.00	5-10000	0.9982	2
	betaxolol	positive	308.15	115.95	20	97.95	20	176.95	20	160.95	20	158.95	20	3.840	0.05	0.50	0.5-1000	0.9946	2
	bisoprolol	positive	326.15	115.95	15	73.95	25	97.95	25	146.95	20			3.890	0.01	0.50	0.5-1000	0.9919	2
	carvedilol	positive	407.20	224.00	20	222.00	20	283.00	20	179.95	20	99.95	25	4.850	0.02	0.10	0.1-200	0.9844	2
	cloranolol	positive	292.00	236.00	15	218.00	20	201.00	20	174.95	25	144.95	40	3.380	0.03	0.10	0.1-200	0.9896	2
	esmolol	positive	296.00	144.95	25	219.00	20	254.00	15	115.95	20	97.95	20	3.770	0.05	0.10	0.1-200	0.9985	2
	losartan	positive	423.20	207.00	20	179.95	35	235.00	20	377.15	15	405.20	10	5.380	0.02	0.10	0.1-200	0.9941	2
	metoprolol	positive	268.00	115.95	20	158.95	20	190.95	15	97.95	20	132.95	25	3.790	0.02	0.10	0.1-200	0.9768	2
	nebivolol	positive	406.20	150.95	30	176.95	25	388.15	20	122.95	40	102.95	50	4.080	1.00	5.00	5-10000	0.9952	2
	perindopril	positive	369.15	171.95	20	295.00	15	169.95	20	97.95	30	71.95	25	4.040	0.02	0.10	0.1-200	0.9966	2
	pindolol	positive	249.00	115.95	15	145.95	20	133.95	25	171.95	15	97.90	20	4.610	0.05	0.20	0.2-400	0.9426	2
	practolol	positive	267.00	189.95	15	225.00	15	177.95	20	163.95	20	147.95	20	5.110	0.05	0.50	0.5-1000	0.9426	2
	prajmaline	positive	369.15	157.95	40	327.15	30	143.95	45	130.95	45	121.95	35	5.350	5.00	20.00	20-40000	0.9801	2
	propafenone	positive	342.15	115.95	20	265.00	20	324.15	15	97.95	20	71.95	25	4.010	0.05	0.50	0.5-1000	0.9944	2
	propranolol	positive	260.00	115.95	15	182.95	15	156.95	20	154.95	25	97.95	15	3.830	0.01	0.10	0.1-200	0.9959	1
	trimetazidine	positive	267.00	180.95	15	165.95	25	150.95	35	135.95	30	90.95	35	4.360	1.00	20.00	20-40000	0.9866	2
	verapamil	positive	455.20	164.95	25	260.00	30	176.95	35	303.15	25	149.95	40	3.600	0.01	0.05	0.05-100	0.9923	2
	warfarin	positive	309.15	162.95	15	251.00	20	291.00	10	146.95	15	120.95	40	2.790	0.03	0.10	0.1-200	0.9606	1
e anesthetics/psychedelic drugs	deschloro ketamine	positive	204.00	172.95	10	185.95	15	144.95	15	154.95	20	90.95	25	2.420	10.00	20.00	20-40000	0.8817	2
	ketamin	positive	238.00	124.95	30	220.00	15	207.00	15	178.95	15	162.95	20	2.160	0.20	0.50	0.5-1000	0.9942	2
	N-ethylketamine	positive	252.00	178.95	15	234.00	15	207.00	15	124.95	25	162.95	20	1.840	0.05	0.50	0.5-1000	0.9729	2
	norketamin	positive	224.00	124.95	20	188.95	15	178.95	15	207.00	10	66.95	20	2.290	0.10	5.00	5-10000	0.9169	2
Hormones/hormon derivatives	aE2 (dansyl)	positive	506.20	170.95	35	114.95	70	155.95	55	425.20	30	440.20	25	2.140	0.01	0.05	0.05-100	0.9786	1 after derivatization
	bE2 (dansyl)	positive	506.20	170.95	35	114.95	70	155.95	55	425.20	30	440.20	25	2.210	0.01	0.05	0.05-100	0.9813	1 after derivatization
	drospirenone	positive	367.20	105.00	35	97.05	20	131.05	30	239.15	15	349.20	15	2.050	0.25	1.00	1-2000	0.9793	1 after derivatization
	E1 (dansyl)	positive	504.20	170.95	35	114.95	70	155.95	55	425.20	30	440.20	25	1.760	0.01	0.05	0.05-100	0.9858	1 after derivatization

	E3 (dansyl)	positive	522.20	170.95	35	114.95	70	155.95	55	425.20	30	440.20	25	2.850	0.01	0.05	0.05-100	0.9884	1 after derivatization
	EE2 (dansyl)	positive	530.20	170.95	35	114.95	70	155.95	55	425.20	30	440.20	25	2.090	0.01	0.05	0.05-100	0.9782	1 after derivatization
	levonorgestrel	positive	313.20	109.05	25	90.05	40	245.20	15	277.20	15	295.20	15	1.720	0.50	1.00	1-2000	0.9904	1 after derivatization
	progesterone	positive	315.20	109.05	20	97.05	20	215.00	20	279.20	15	297.20	15	1.520	0.05	0.50	0.5-1000	0.9578	1 after derivatization
	testosterone	positive	289.20	109.05	20	97.05	20	253.20	15	271.20	15			1.840	0.15	0.50	0.5-1000	0.9754	1 after derivatization
Local anesthetics	benzocaine	positive	165.95	119.95	15	137.95	10	93.95	15	91.95	25	76.95	25	1.830	5.00	20.00	20-40000	0.9718	1
	bupivacaine	positive	289.00	140.12	20	98.15	35	84.10	40					2.310	0.01	0.10	0.1-200	0.9966	2
	lidocaine	positive	235.00	86.10	15	58.10	30							1.920	0.05	0.10	0.1-200	0.9948	2
	nitracaine	positive	309.15	149.95	25	236.00	15	141.95	20	85.95	25	68.95	20	1.050	0.01	0.10	0.1-200	0.9565	2
	ropivacain	positive	275.00	125.95	20	83.95	35	97.95	35	149.95	20	55.95	40	2.340	0.01	0.10	0.1-200	0.9976	2
	tetracaine	positive	265.00	175.95	15	220.00	15	119.95	35	91.95	35	71.95	20	3.240	0.03	0.10	0.1-200	0.8162	2
Narcotics/Sedatives/Anticonvulsants	amobarbital	negative	225.00	181.95	10	137.95	15	84.95	15	41.95	15			1.800	0.05	10.00	10-20000	0.9958	1
	barbital	negative	182.95	139.95	10	41.95	15	84.95	10	95.95	10	118.95	15	1.860	50.00	10.00	10-20000	0.8033	1
	butobarbital	negative	211.00	167.95	10	123.95	15	84.95	15	41.95	15			1.810	1.00	10.00	10-20000	0.9677	1
	phenobarbital	negative	231.00	187.95	10	84.95	10	41.95	15	143.95	15			2.160	50.00	10.00	10-20000	0.9310	1
	phenytoin	negative	251.00	208.00	15	179.95	15	101.95	20	76.95	25			2.660	1.00	10.00	10-20000	0.9883	1
NSAIDs	diclofenac	positive	296.00	215.08	20	250.00	10	278.00	10	151.05	55			2.710	0.10	0.50	0.5-1000	0.9579	1
	fenacetin	positive	179.85	109.95	20	137.95	15	151.95	15	92.95	25	64.95	30	2.690	0.05	0.50	0.5-1000	0.9975	2
	metamizol	positive	218.00	186.95	10	158.95	10	124.95	10	96.95	15	55.95	15	3.760	15.00	200.00	200-400000	0.9584	1
	naproxen	positive	231.00	184.95	15			169.95	25	152.95	30	140.95	40	2.390	0.02	0.10	0.1-200	0.9854	1
	paracetamol	positive	151.95	110.05	15	93.05	20	82.10	25	43.08	20			3.610	3.50	20.00	20-40000	0.9337	1
Opiods/morphine derivatives	6-monoacetylmorphine	positive	328.15	164.95	40	268.00	20	211.00	25	192.95	25	271.00	20	4.350	0.03	0.50	0.5-1000	0.9858	2
	codeine	positive	300.15	215.00	25	243.00	20	225.00	25	282.00	20	164.95	35	4.240	0.10	5.00	5-10000	0.9911	2
	embutramide	positive	294.00	120.95	25	134.95	20	148.95	20	190.95	15	208.00	15	2.990	0.05	0.10	0.1-200	0.9938	2
	ethylmorphine	positive	314.15	229.00	25	257.00	20	239.00	25	296.00	20	164.95	35	4.210	0.15	0.50	0.5-1000	0.9899	2
	fentanyl	positive	337.15	187.95	20	104.95	35	78.95	55	216.00	20			2.960	0.05	0.10	0.1-200	0.9876	1
	methadone	positive	310.15	265.00	15	104.95	25	223.00	20	219.00	20	158.95	20	3.790	0.01	0.02	0.02-40	0.9933	2
	morphine	positive	286.00	201.00	25	164.95	35	211.00	25	152.95	40	156.95	35	5.130	2.00	5.00	5-10000	0.9729	2
	nalbuphine	positive	358.15	340.15	20	254.00	30	211.00	30	200.00	30	184.95	35	3.450	0.25	0.50	0.5-1000	0.9845	2
	oxycodone	positive	316.15	298.00	20	256.00	25	241.00	25	212.00	40	186.95	25	3.400	0.10	5.00	5-10000	0.9271	2
	pethidine	positive	248.00	220.00	20	202.00	15	173.95	20	130.95	30	69.95	30	3.070	0.02	0.10	0.1-200	0.9927	2
	tramadol	positive	264.00	57.95	10	246.00	10							3.120	0.01	0.10	0.1-200	0.9984	2
Others	3-Cl-ephedrine	positive	200.00	181.95	15	166.95	20	115.95	25	114.95	25	55.95	20	3.990	2.00	8.00	8-16000	0.9722	2
	atracurium	positive	358.15	206.00	20	188.95	25	150.95	25	327.15	20	106.95	45	4.110	0.03	0.10	0.1-200	0.9844	2
	ephedrine	positive	165.95	147.95	10	132.95	20	116.95	20	114.95	25	90.95	25	4.020	20.00	80.00	80-160000	0.9887	2
/Hallucinogens and their metabolite	4-FA	positive	153.95	108.95	15	136.95	10	82.95	30					3.360	5.00	8.00	8-16000	0.9647	2
	amphetamine	positive	135.95	118.95	10	90.95	15							3.410	5.00	80.00	80-160000	0.9656	2
	benzoyl ecgonine	positive	290.00	167.95	15	272.00	15	149.95	20	118.95	25	104.95	25	5.990	0.01	0.10	0.1-200	0.9972	2
	cocaine	positive	304.15	181.95	20	149.95	25	90.95	30	104.95	30			2.440	0.01	0.05	0.05-100	0.9825	2

dibutylon (N,N-dimethylbutylone)	positive	236.00	190.95	15	148.95	25	160.95	20	162.95	20	132.95	25	1.880	0.10	0.20	0.2-400	0.9841	2
dipentylone (N,N-dimethylpentylone)	positive	250.00	205.00	15	174.95	20	148.95	25	134.95	25	99.95	20	1.810	0.03	0.20	0.2-400	0.9815	2
ecgonine methylester	positive	200.00	181.95	15	149.95	20	107.95	25	81.95	25			2.840	5.00	20.00	20-40000	0.9665	2
MBDB	positive	208.00	134.95	15	176.95	10	146.95	15	118.95	20	71.95	15	3.210	0.50	2.00	2-4000	0.9860	2
MDA	positive	179.95	162.95	10	134.95	15	132.95	15	104.95	20	78.95	25	3.530	25.00	80.00	80-160000	0.9878	2
MDAI	positive	177.95	160.95	10	130.95	15	102.95	25	76.95	30			3.740	20.00	80.00	80-160000	0.9756	2
MDEA (MDE)	positive	208.00	162.95	15	134.95	20	132.95	20	104.95	25	78.95	30	3.560	0.20	2.00	2-4000	0.9746	2
MDMA (Ecstasy)	positive	193.95	162.95	10	134.95	20	132.95	20	104.95	25	78.95	30	3.720	0.15	2.00	2-4000	0.9693	2
MDPHP	positive	290.00	134.95	25	188.95	20	148.95	25	139.95	25	219.00	15	2.210	0.10	0.20	0.2-400	0.9937	2
MDPV	positive	276.00	125.95	25	205.00	15	174.95	20	134.95	25	148.95	25	2.230	0.03	0.20	0.2-400	0.9959	2
methamphetamine	positive	149.95	90.95	15	118.95	10	64.95	25					3.620	1.00	2.00	2-4000	0.8866	2
methylone	positive	208.00	159.95	15	189.95	10	146.95	20	131.95	25	116.95	30	2.850	5.00	8.00	8-16000	0.9141	2
MTXA (MXE)	positive	248.00	203.00	15	174.95	20	120.95	25	66.95	25	90.95	40	2.160	1.00	5.00	5-10000	0.9656	2
N-ethylpentylone	positive	250.00	202.00	15	232.00	15	205.00	15	188.95	25	173.95	30	2.190	0.10	0.20	0.2-400	0.9470	2
N-methyl-bk-MMDA-2 (6-MeO-methylone)	positive	238.00	189.95	15	220.00	10	57.95	10	207.00	10	161.95	25	3.620	1.00	2.00	2-4000	0.9081	2
sibutramine	positive	280.00	124.95	25	178.95	15	152.95	15	138.95	15	108.95	15	1.370	20.00	80.00	80-160000	0.7985	2

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Table S4. Measured data from all periods and sites.

Chemical class	PACs' name	Jun'17										Aug'17									
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Alkaloids	atropine														0.2			0.4			
	coffeine		56.7									62.5			138.7		15.6			79.8	89.9
Antiepileptics	carbamazepine	308.6	659.2	349.1	167.1	68.8	68.3	28.0	44.6	18.3	19.4	81.1	230.6	99.0	16.8	17.1	46.3	12.2	11.0	4.7	13.3
	lacosamide																				
	lamotrigine	16.2	319.3	106.2	44.1	7.1	5.7	7.0	8.6	6.0		64.7	1734.8	305.5	26.4	28.3	162.2	29.9	15.5	17.6	17.0
Antipsychotics/Antidepressants	bupropion		1.1									2.0	27.5								
	citalopram	0.6	3.8		0.1					0.1		2.6	17.7		0.3		0.1				0.2
	clozapine	0.1	10.3	0.6	0.2		0.4		0.2		0.5	0.2	54.3	0.5			0.3		0.6	0.2	
	fluoxetine		1.0	2.7	3.4	0.5		1.7		1.4											
	haloperidol		0.2									0.0	0.8								
	metoclopramide		1.0									0.4	11.2								
	mianserin												0.6	0.3							
	mirtazapine	0.3	3.2	0.2		0.4			0.2		0.5	1.1	38.8	0.3			0.2		0.5		
	olanzapine		11.9										82.8								
	procyclidine		0.5										2.4								
	quetiapine										0.1							0.1			
	tiapride	12.2	133.1	6.1	4.3	2.5	0.5	0.7	0.8	0.6	0.2	44.1	566.1	12.8	8.4	6.7	14.4	2.3	6.2	3.0	1.1
	trazodone		0.1									0.1	0.7								

Chemical class	PACs' name	Nov'17										Apr'18									
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Alkaloids	atropine											18.9					2.2				
	coffeine																				
Antiepileptics	carbamazepine	70.2	804.6	80.1	80.5	15.5	15.9	6.5	8.7	7.8	6.1	145.9	397.3	257.2	207.6	77.5	44.4	44.1	37.9	27.0	21.2
	lacosamide		9.3																		
	lamotrigine	80.5	1102.4	176.3	67.8	22.1	22.0	17.1	18.6	22.0	19.7	21.7	1078.7	205.2	183.4	18.6	16.4	23.5	19.3	33.4	16.6
Antipsychotics/Antidepressants	bupropion											2.6							6.6		
	citalopram	1.7	11.1		0.2							0.9	16.5						2.4		
	clozapine		35.9	1.1	0.4								9.9								
	fluoxetine																				
	haloperidol		0.3																		
	metoclopramide	0.6	6.8										10.1								
	mianserin																				
	mirtazapine	1.6	19.9		0.4					0.2			1.6								
	olanzapine		27.6				11.8														
	procyclidine		1.5										2.4								
	quetiapine		0.4																		
	tiapride	56.5	432.0	26.0	18.7	11.8	4.6	1.4	2.4	1.5	0.6	12.4	217.3	17.0	4.1	12.0	0.4	1.3	0.1		
	trazodone		0.4																		

Cardiovascular drugs										Anxiolytics									
propafenone	practolol	pindolol	perindopril	metoprolol	losartan	carvedilol	bisoprolol	betaxolol	amlodipine	zopiclone	zolpidem	temazepam	oxazepam	nordiazepam	midazolam	diazepam	cinolazepam	buspirone	alprazolam
6.6			9.1	8.1		0.3	3.7				0.0			3.0			18.4		0.6
18.2			52.4	54.4	0.2	0.5	10.0			0.8	0.3	7.9		60.5	0.1		357.3		2.2
			6.3											5.2			49.2		0.7
			1.7				0.7										20.2		0.4
			0.9														5.8		0.1
			1.2											1.4			13.0		0.1
			0.3				6.3												
			0.5				0.7										2.2		
			0.4				2.1										1.6		0.1
0.8			0.1																
18.3			38.3	30.6	0.3	1.9	34.0			0.3	0.1			2.2					1.7
116.3		0.5	386.0	367.3	0.8	2.5	72.2	2.8	7.3	4.1	1.2	4.0	9.8	38.0	0.4		197.0		12.6
			8.8				0.6							1.1			10.6		0.9
			2.7				0.9										2.0		0.2
			1.9				0.9												0.2
			17.7	5.1			2.9										9.9	0.1	0.9
			0.8				0.5										0.7		0.2
1.0			1.2				2.9										0.7		0.2
0.7			2.2				16.7												0.2
			0.9				14.6											0.1	0.2
5.0			34.4	32.4	0.8		8.3			0.3	0.1						8.7		1.6
86.9	28.1		283.1	312.2	3.0	2.0	84.4	3.4	8.9	2.2	1.0				0.3	0.1	84.5		8.4
0.6			39.2				1.0							1.4			11.1		1.6
0.9			17.0	3.9	0.1		2.4										8.1		0.6
			1.6				3.1										1.2		0.2
			3.8				1.5										2.0		0.3
			0.6				2.8										0.4		0.3
			0.5				1.5										0.5		0.2
			1.0				1.4							0.4					0.2
			0.5				3.4												0.2
5.4			11.5	16.6	4.7		6.6										3.5	0.5	0.7
22.0			85.9	104.2	24.8		36.1				0.8	1.0	1.8			1.9	53.9		5.3
			4.7	1.4	1.4												2.9		1.0
			4.2														1.4		0.5
			2.1		0.8						0.2				1.7	0.3	2.1	5.9	0.7
			1.9	1.2	0.1		0.5										1.7		
			0.8																
			0.8																0.2
																			0.1

Opiods/morphine derivatives		NSAIDs		Local anesthetics			Hormones/hormon derivatives								Dissociative anesthetics/psychedelic drugs				
ethylmorphine	codeine	naproxen	diclofenac	tetracaine	lidocaine	bupivacaine	testosterone	progesterone	levonorgestrel	EE2	E3	E1	bE2	aE2	ketamin	warfarin	verapamil	trimetazidine	propranolol
		28.9			8.8			1.2		2.7		3.3	17.0				0.1		0.4
0.6		56.1		0.1	41.2			1.1	42.7	1.8		10.5	19.3		1.6	3.7	0.4		2.6
					2.9			1.3	44.7			1.6	19.1				0.1		
					4.2			2.3	38.2	2.1		9.8	18.0				0.2		
					0.4							5.5	4.0						0.0
					0.5			0.9				3.6	2.9						
				1.2	42.2			0.7				1.0	19.6				0.5		
					0.3			1.0				1.0	3.4				0.1		
				0.2	27.8						0.1	0.4	17.0				0.1		
													3.7						
		4.8			35.1			1.0	49.4			1.3	0.2				1.6		1.5
17.5		28.1			114.8	0.1		0.8				7.5			7.5	2.0	4.4		19.7
					7.6					0.6		0.5		0.4			2.2		
					9.0			0.8				2.2	0.7				1.5		
					1.1							0.9	0.2				1.3		
					3.6			1.0				0.9					0.4		
					1.0							1.2					0.1		
					0.5			0.6				0.5					2.0		
		2.2			1.4			1.1				0.4			2.5		27.1		
					1.5			1.3			0.1	0.5	0.1		8.8		6.7		
		3.3			59.5			0.7		0.3		0.8		0.2		0.2	0.1		1.3
13.3	5.5				141.3	0.1		1.4		0.7		5.4			5.4		1.5		15.4
					19.0							1.0							
					14.1							1.3							
					1.8					0.2		0.4							
					0.5							0.3							
					0.1							0.2							
					0.3							0.3							
					0.2							0.2							
					0.4							0.2							
					35.4							1.1	0.2						
			221.4		87.0							0.2	0.2		15.9	0.7	0.9	122.2	5.7
					22.2			0.6	1.9			0.5	0.2						
					11.6							0.8	0.3						
					5.8							0.5					1.4		
												0.3							
							1.1					0.5	0.2						
								1.1				0.2							
					0.3							0.2							

	fentanyl		0.2										1.1										0.8																		
	methadone		0.0										0.1																						0.6						
	pethidine		0.1																																						
	tramadol	5.5	64.7	1.7	2.8	0.3	0.5	0.3	0.3	0.2		24.2	279.9	4.0	2.3	1.5	6.1	0.7	0.7	0.8	1.0	32.3	217.2	10.8	8.4	1.5	0.8	0.4	0.4	0.6	0.3	12.9	161.4	8.0	6.2	3.0	0.9	1.0	0.8	0.4	0.2
Others	atracurium																						0.3																		
	3-Cl-ephedrine																																0.2								
Stimulants/Hallucinogens and their metabolites	benzoyl-ecgonine		0.1									0.6	1.4				0.2			0.8	2.3	0.5	1.9									1.2	1.9								
	cocaine											0.1	0.1							0.2	0.1	0.1										1.2	0.6								
	dibutilon (N.N-dimethyl-butylone)																																1.1								
	ecgonine-methylester										34.8																							21.6							
	MDMA (Ecstasy)		3.2									2.9	10.3								9.2	4.5	26.8		0.5								2.7								

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SPE optimization

The SPE of samples was carried out with AutoTrace 280 automata SPE system (Thermo Scientific). The method was optimised through several preliminary experiments involving the following variables:

a) Sorbent type

The Strata X, X-CW, C8, C18E and Strata Screening C were tested by the recommended protocol of the manufacturer. One-one L mixed standard solution was used in each experiment, where the final concentration was 100 ng/L to each PhACs (59). This group of PhACs represented the structure and chemical type of later-investigated PhACs (134). Finally, to further the experiment, the Strata X-CW was used because it ensured the highest number of detectable PhACs and appropriate linearity for quantification.

Table S5. Test of different sorbent types. The "Best" line represents the number of best results (bold) in each sorbent type. The "Not appropriate" line shows how many PhACs were not available (italics and shaded in gray) for that sorbent type. Data are presented in the areas under the curves.

	STRATA X-CW	STRATA X	STRATA SCREEN-C	STRATA C18E	STRATA C8
3-Cl-ephedrine	**1 446 783**	*7 140*	43 217	*1 869*	*4 927*
6-monoacetyl-morphine	**60 252**	*925*	*2 362*	*800*	*860*
7-amino-flunitrazepam	17 372	16 409	**42 505**	2 323	9 544
acenocoumarol	84 246	84 168	16 685	100 798	**115 730**
alprazolam	137 914	159 704	91 190	191 841	**229 987**
amlodipine	2 962	4 346	836	4 247	**5 256**
amphetamine	**211 254**	*1 608*	*17 517*	*393*	*826*
atropine	**189 271**	*1 458*	*3 660*	*1 160*	*2 759*
benzoyl-ecgonine	**3 217**	1 688	1 122	771	549
bisoprolol	**614 551**	25 490	40 827	153 343	357 589
buspirone	203 738	79 796	184 231	147 695	**254 220**
C13E2	109 575	124 074	45 477	130 692	**142 152**
carbamazepine	253 149	**327 269**	48 936	314 422	270 497
carbamazepineD10	318 468	**340 950**	35 289	201 234	308 038
citalopram	**375 950**	241 198	91 789	399 347	374 217
citalopramD6	337 716	261 777	77 147	371 938	**379 002**
clonazepam	58 314	67 957	17 810	**90 343**	84 232
clozapine	127 621	45 914	56 688	79 110	**129 985**
codeine	**49 687**	*278*	*1 747*	*414*	*0*
diazepam	264 690	305 271	189 218	247 351	**344 319**
diclofenac	368 809	406 291	126 437	403 336	**419 216**
droperidol	**512 767**	277 364	67 838	436 059	505 807
drospirenone	49 125	56 112	29 760	**67 045**	66 358

EE2	196 882	192 547	111 895	**278 246**	252 875
ethyl-morphine	**46 521**	*357*	*2 662*	*548*	*243*
fenacetin	**109 527**	207 097	*37*	*1 419*	20 727
fluoxetine	4 816	4 850	3 663	6 952	**10 802**
haloperidol	669 455	361 784	163 362	773 299	**688 078**
ketamine	21 633	*83*	*4 853*	*369*	*471*
lamotrigine	2 150	2 200	*367*	**5 558**	*362*
levonorgestrel	16 063	14 971	11 444	28 733	**28 827**
losartan	15 107	31 479	4 586	**45 879**	36 181
MD-a-PVP	**1 686 664**	*35 713*	483 141	239 954	1 603 660
MDMA	**623 336**	*619*	*53 863*	*1 149*	*2 555*
metamphetamine	**20 965**	*0*	*2 366*	*30*	*3*
methadone	250 799	191 356	139 606	300 963	**304 974**
metoprolol	**62 544**	*2 123*	*0*	*5 117*	*7 409*
mianserin	66 959	40 745	88 754	114 683	**128 989**
midazolam	18 151	25 912	15 267	28 417	**55 795**
mirtazapine	**98 381**	26 013	44 506	27 148	42 305
morphine	**2 423**	2 312	*0*	*0*	*0*
nordiazepam	316 013	379 287	149 144	300 873	**408 052**
norketamin	**34 959**	*15*	*7 073*	*48*	*349*
perindopril	**184 606**	56 540	*7 673*	71 525	83 772
procyclidine	536 474	370 826	331 100	635 945	**749 384**
progesterone	99 265	106 505	116 803	140 334	**164 596**
propafenone	**209 445**	94 437	27 160	122 490	145 918
quetiapine	89 155	45 871	36 466	79 099	**102 322**
risperidone	**471 775**	206 097	*32 885*	269 980	245 386
scopolamine	**199 322**	*568*	*6 396*	*0*	*288*
sertraline	20 751	14 109	14 059	26 224	**27 997**
testosterone	65 749	71 749	36 251	98 603	**96 243**
tiapride	**397 032**	*33 713*	*17 164*	69 042	*9 582*
tramadol	**433 257**	*19 227*	69 611	61 629	69 725
trazodone	287 441	145 679	155 734	278 356	**331 104**
verapamil	**722 611**	435 938	162 580	545 988	563 105
warfarin	280 654	255 223	68 765	268 844	**287 245**
zolpidem	395 781	32 551	155 453	197 508	**446 053**
zopiclone	**30 123**	*317*	3 456	*2 643*	10 247
Best	**26**	**2**	**1**	**5**	**23**
Not appropriate	**0**	**16**	**17**	**15**	**15**

b) Solvent/water portion of strong wash solutions

The lipophilic hormones and hormone derivatives supplemented with some PhACs, which are representative of different pKa (acid dissociation constant) values, were used in this test. One-one L mixed standard solution was used in each experiment, where the final concentration was 100 ng/L to each PhAC (13). Higher than 20% acetonitrile portion caused significant loss of analytes. Finally, to further the experiment, the 20% acetonitrile portion was used to reach the appropriate washing.

Table S6. Test of different strong wash solutions. Undesirable elutions are shaded in gray. Data are presented in the area under the curve.

			Solvent/water portion of strong wash solutions		
	direct injection	weak wash	10% ACN	25% ACN	50% ACN
testosterone	9 405	0	0	0	3 743
drospirenone	23 978	0	0	0	4 210
progesterone	94 124	0	0	0	3 944
levonorgestrel	12 929	0	0	0	2 766
E1	291 263	0	0	0	65 126
aE2	233 464	0	0	0	74 484
bE2	221 860	0	0	0	73 227
E3	231 994	0	0	10 898	124 533
carbamazepine	1 021 555	0	0	3 589	66 369
sertralin	112 360	0	0	0	0
citalopram	685 649	0	0	0	0
fluoxetine	1 417	0	0	0	0
alprazolam	6 036 095	0	0	0	767

c) Composition of eluting solution

The lipophilic hormones and hormone derivatives supplemented with some PhACs representing the different pKa (acid dissociation constant) values were used in this test. One-one L mixed standard solution was used in each experiment, where the final concentration was 100 ng/L to each PhAC (27). Methanol and acetonitrile were tested as elution solvent. The acetonitrile ensured better elution to the lipophilic PhACs and not eluted the basic PhACs. Finally, in further experiments, the appropriate elutions, 100% acetonitrile and 14% NH4OH/ACNs were used as Elute 1 and Elute 2, respectively.

Table S7. Test of different eluting solvents. Undesirable elutions are shaded in gray. Data are presented in the area under the curve.

			1		2	
	Quantification from	direct injection	Elute 1 by 100 % ACN	Elute 2 by 14% NH_4OH/ACN	Elute 1 by 100 % methanol	Elute 2 by 14% NH_4OH/ACN
tesztoszteron	Elute 1	10 356	10 112	0	5 105	1 287
levonorgesztrel	Elute 1	13 922	9 588	0	2 114	6 048
progeszteron	Elute 1	114 215	69 909	2 517	42 687	2 607
drospirenon	Elute 1	25 192	18 287	0	11 508	0
EE2	Elute 1	343 365	323 507	0	127 687	89 197
E1	Elute 1	526 735	515 805	0	200 851	0
aE2	Elute 1	301 330	351 552	0	165 776	56 008
bE2	Elute 1	282 789	1 240 508	0	151 598	0
E3	Elute 1	334 879	326 178	0	132 876	215 241
meprobamate	Elute 1	16 855	12 408	50	12 800	0
carbamazepine	Elute 1	1 450 032	1 300 001	158 071	1 260 565	22 341
lacosamide	Elute 1	22 010	16 000	1 205	151 087	0
mianserin	Elute 1	710 000	601 000	0	125 009	40 521
mirtazapine	Elute 1	2 000 146	2 066 080	0	370 080	18 332
clonazepam	Elute 1	300 026	109 251	21 325	280 040	2 354

bupropion	Elute 2	42 049	0	40 554	0	26 025
lamotrigine	Elute 2	65 043	0	44 023	0	21 055
diazepam	Elute 2	312 155	128 025	286 007	158 408	14 007
cyproheptadine	Elute 2	678 001	0	581 452	0	73 869
sertraline	Elute 2	236 009	0	212 111	0	10 008
zolpidem	Elute 2	4 195 090	1 012	3 339 011	8 761	567 335
alprazolam	Elute 2	2 600 000	29 080	1 805 328	49 162	235 887
fluoxetine	Elute 2	65 887	0	62 460	0	5 100
citalopram	Elute 2	4 000 564	4 156	3 036 069	15 254	447 089
midazolam	Elute 2	2 456 037	0	2 260 127	8 478	254 342
trazodone	Elute 2	3 300 000	0	3 482 486	5 321	550 114
risperidone	Elute 2	12 887 569	0	6 336 889	22 231	880 055