

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	subcatc h-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), anxiolytics (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.

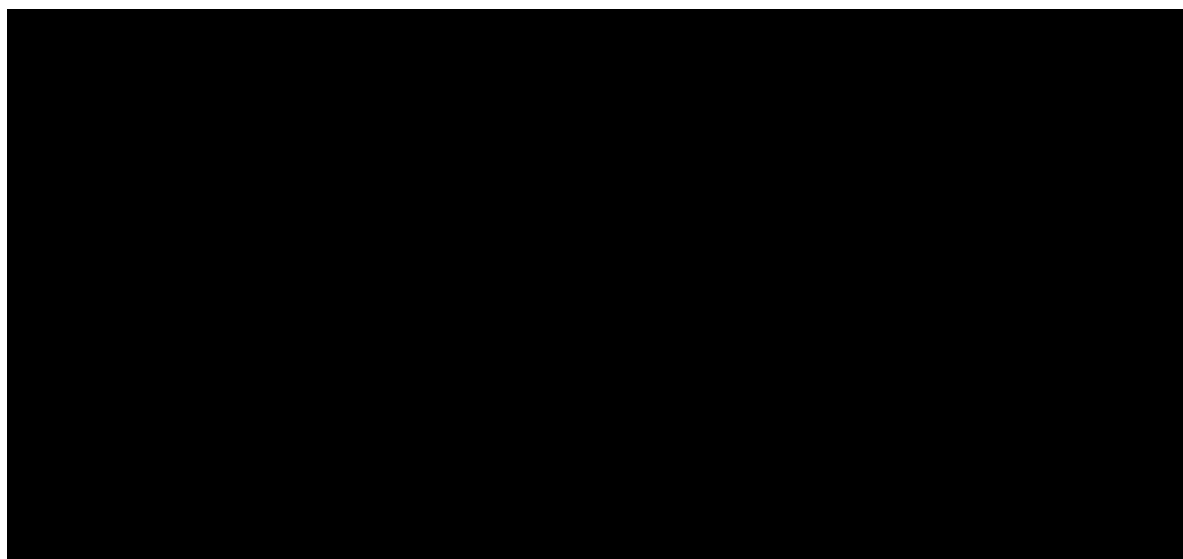


Figure S1. Boxplots derived from the cumulated values of the 10 chemical classes. Boxplots with the same letters do not differ significantly based on Kruskal-Wallis post hoc comparisons ($p < 0.05$). Each box represents the minimum and maximum values of the certain compound groups. The band in the box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

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
Antiepileptics	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	amitriptyllin	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 anesthetic s/psyched elic 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/Anti convulsants	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
Opioids/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

Opioids/morphine derivatives	NSAIDs		Local anesthetics			Hormones/hormon derivatives							Dissociative anesthetics/psychodelic drugs					
	codeine	naproxen	tetracaine	lidocaine	bupivacaine	testosterone	progesterone	levonorgestrel	EE2	E3	E1	bE2	aE2	ketamin	warfarin	verapamil	trimetazidine	propranolol
				8.8			1.2		2.7	3.3	17.0				0.1			0.4
0.6	28.9	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								
				0.3		1.0			1.0	3.4					0.5			
				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							
				9.0		0.8			2.2	0.7								
				1.1					0.9	0.2								
				3.6		1.0			0.9									
				1.0					1.2									
				0.5		0.6			0.5									
				1.4		1.1			0.4									
	2.2			1.5		1.3			0.1	0.5	0.1							
				59.5		0.7		0.3	0.8									
	3.3			141.3	0.1	1.4		0.7	5.4						0.2	0.1		1.3
13.3	5.5			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									
									0.3									
						1.1			0.5	0.2								
									0.2									
				0.3					0.2									

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	<i>7 140</i>	43 217	<i>1 869</i>	<i>4 927</i>
6-monoacetyl- morphine	60 252	<i>925</i>	<i>2 362</i>	<i>800</i>	<i>860</i>
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	<i>1 608</i>	<i>17 517</i>	<i>393</i>	<i>826</i>
atropine	189 271	<i>1 458</i>	<i>3 660</i>	<i>1 160</i>	<i>2 759</i>
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	<i>278</i>	<i>1 747</i>	<i>414</i>	<i>0</i>
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.

	direct injection	weak wash	Solvent/water portion of strong wash solutions		
			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.

	Quantification from	direct injection	1		2	
			Elute 1 by 100 % ACN	Elute 2 by 14% NH ₄ OH/ACN	Elute 1 by 100 % methanol	Elute 2 by 14% NH ₄ OH/ACN
tesztoszteron	Elute 1	10 356	10 112	0	5 105	1 287
levonorgestrel	Elute 1	13 922	9 588	0	2 114	6 048
progesteron	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