Supporting Information for

Fate of Pharmaceuticals and Their Transformation Products in Four Small European Rivers Receiving Treated Wastewater

Zhe Li[*], *Anna Sobek*, *Michael Radke*[*,1]

Department of Environmental Science and Analytical Chemistry (ACES), Stockholm University, 10691 Stockholm, Sweden

*** Corresponding authors:**

Zhe Li

Department of Environmental Science and Analytical Chemistry (ACES), Stockholm University, 10691 Stockholm, Sweden

Phone: +46 8 674 7188

E-mail: zhe.li@aces.su.se

Michael Radke

Institute for Hygiene and Environment, Marckmannstraße 129b, 20539 Hamburg, Germany

Phone: +49 40428 45 3778; Fax: +49 40427 97 3778

E-mail: michael.radke@hu.hamburg.de

Contents:

[1] Present address: Institute for Hygiene and Environment, Marckmannstraße 129b, 20539 Hamburg, Germany

Chemicals and reagents

All PCs and TPs (purity >98%) except tramadol were purchased from Sigma-Aldrich (Steinheim, Germany) and Toronto Research Chemicals Inc. (North York, Canada). A tramadol stock solution was obtained by grinding one commercial tablet (nominal tramadol content: 50 mg) into powder, which was dissolved in methanol followed by filtration (0.45 µm PTFE syringe filter; Chromacol, Partille, Sweden). The physical-chemical properties of all analytes are provided as Table S1 in the Supporting Information. Isotope-substituted internal standards were purchased from Toronto Research Chemicals Inc. and CDN Isotopes (Pointe-Claire, Canada). LC/MS-grade acetic acid, formic acid and sulfuric acid were purchased from Sigma-Aldrich. LC/MS-grade acetonitrile and methanol were purchased from VWR (Stockholm, Sweden). Milli-Q water was produced by a Milli-Q Integral Water Purification System (Merck Millipore, Solna, Sweden).

Table S1. Physical-chemical properties of the investigated parent compounds (PCs) and transformation products (TPs). Compounds marked with an asterisk are TPs of the PC above.

chemical	*molecular formula*	*molecular weight* [a] *(g mol^{-1})*	*log K_{OW}* [b]	*log D_{OW}* [c]	*pKa* [b]
acetaminophen	$C_8H_9NO_2$	151.16	0.91	0.91	9.46
acesulfame	$C_4H_5NO_4S$	163.15	-0.55	-1.20	3.02
bezafibrate	$C_{19}H_{20}ClNO_4$	361.82	3.99	0.90	3.83
*4-chlorobenzoic acid**	$C_7H_5ClO_2$	156.57	2.23	-0.88	4.07
bicalutamide	$C_{18}H_{14}F_4N_2O_4S$	430.37	2.71	2.71	11.95
carbamazepine	$C_{15}H_{12}N_2O$	236.27	2.77	2.77	15.96
*carbamazepine-10,11-epoxide**	$C_{15}H_{12}N_2O_2$	252.27	2.58	2.58	5.13
chlorthalidone	$C_{14}H_{11}ClN_2O_4S$	338.77	1.60	1.60	8.58
clofibric acid	$C_{10}H_{11}ClO_3$	214.65	2.90	-0.27	3.37
diclofenac	$C_{14}H_{11}Cl_2NO_2$	296.15	4.26	1.20	4.00
*4'-hydroxydiclofenac**	$C_{14}H_{11}Cl_2NO_3$	312.15	3.96	0.77	3.76
fluconazole	$C_{13}H_{12}F_2N_6O$	306.27	0.56	0.56	12.71
furosemide	$C_{12}H_{11}ClN_2O_5S$	330.74	1.75	-1.45	4.25
*saluamine**	$C_7H_7ClN_2O_4S$	249.98	0.66	-2.42	4.38
glimepiride	$C_{24}H_{34}N_4O_5S$	490.62	3.12	2.38	4.32
hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	297.74	-0.58	-0.58	9.09
*chlorothiazide**	$C_7H_6ClN_3O_4S_2$	295.72	-0.44	-0.44	9.10
*4-amino-6-chloro-1,3-benzenedisulfonamide**	$C_6H_8ClN_3O_4S_2$	285.73	-1.04	-1.04	9.19
ibuprofen	$C_{13}H_{18}O_2$	206.29	3.84	0.95	4.85
*2-hydroxyibuprofen**	$C_{13}H_{18}O_3$	222.28	2.37	-0.53	4.63
*carboxyibuprofen**	$C_{13}H_{16}O_4$	236.26	2.78	-3.30	3.97
ketoprofen	$C_{16}H_{14}O_3$	254.28	3.61	0.51	3.88
metoprolol	$C_{15}H_{25}NO_3$	267.36	1.76	0.14	9.67
*metoprolol acid**	$C_{14}H_{21}NO_4$	267.32	-1.24	-0.44	3.54
*α-hydroxymetoprolol**	$C_{15}H_{25}NO_4$	283.36	0.84	-0.79	9.67
naproxen	$C_{14}H_{14}O_3$	230.26	2.99	-0.16	4.19
propranolol	$C_{16}H_{21}NO_2$	259.34	2.58	0.95	9.67
*1-naphthol**	$C_{10}H_8O$	144.17	2.66	2.66	9.60
sotalol	$C_{12}H_{20}N_2O_3S$	272.36	-0.40	-1.62	9.43
sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.28	0.79	0.09	6.16
tramadol	$C_{16}H_{25}NO_2$	263.38	2.45	1.31	9.23

[a] Molecular weights refer to the dissociated molecule and not to the corresponding salts in case of ionic species. [b] K_{OW} and pKa values were collected from Chemicalize (http://www.chemicalize.org/); [c] D_{OW} values represent the pH dependent *n*-octanol-water partition coefficients of ionizable compounds and were calculated at a pH of 7.5, which represents the average pH of of the studied rivers.

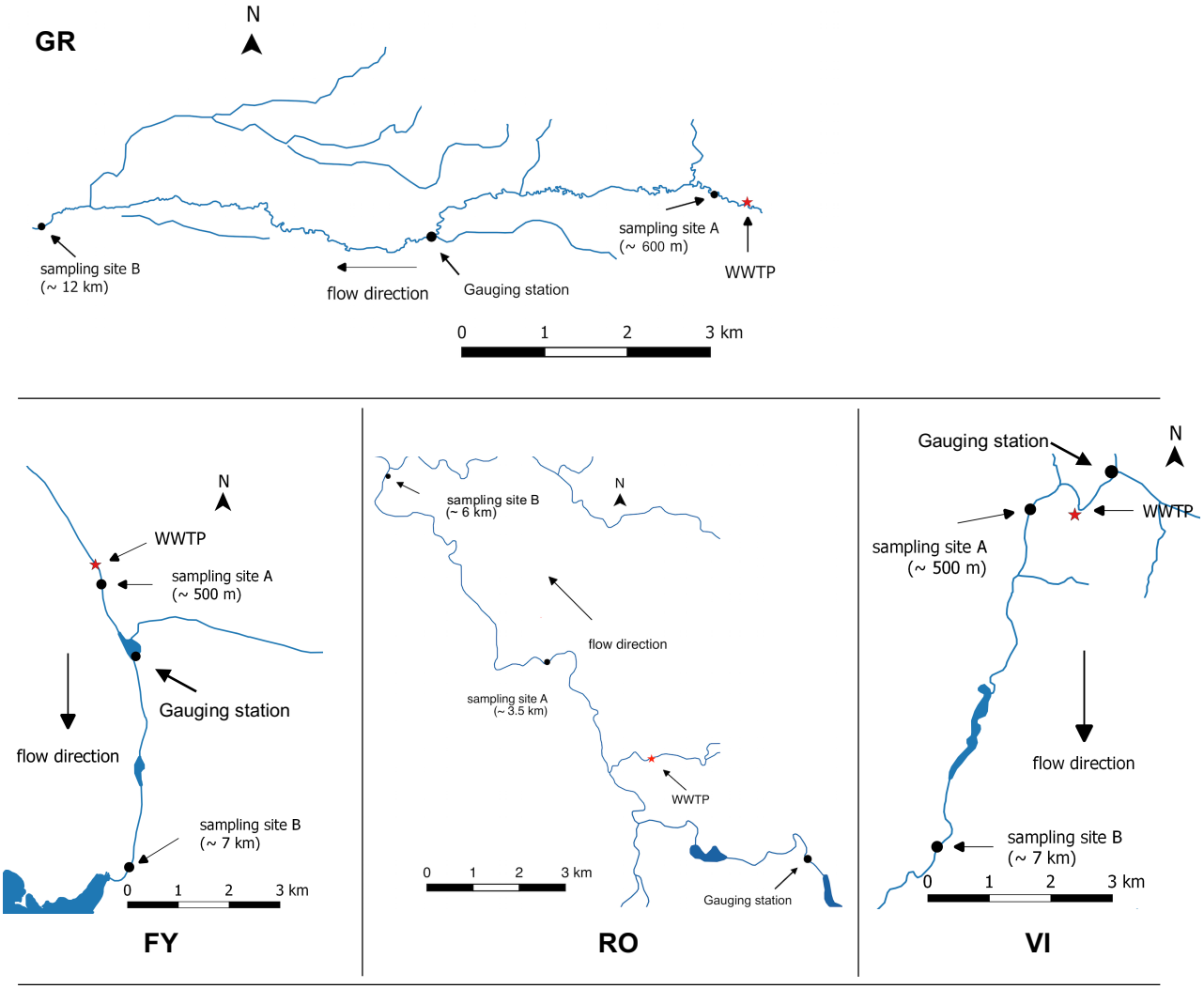

Figure S1. Maps of the studied rivers (GR, FY, RO and VI) showing the WWTPs, sampling sites (A and B) and gauging stations (for river FY, discharge record was from historical monitoring data). Numbers in parenthesis indicate the distance from the WWTPs. The distance shown at sampling site A is the distance from the WWTP outlet, the distance shown at sampling site B is the distance to sampling site A.

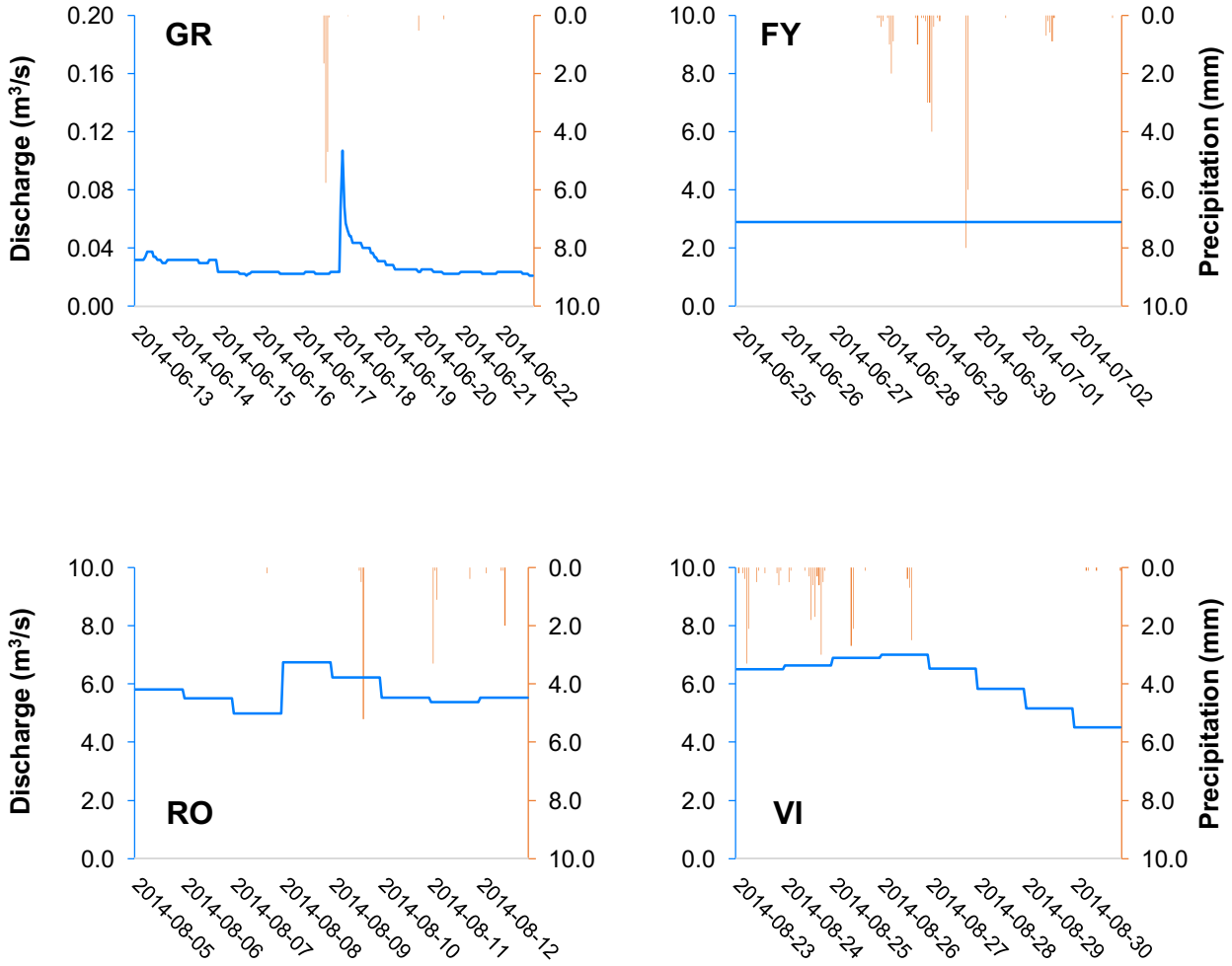

Figure S2. Discharge and precipitation of the four studied rivers during the sampling period. Discharge of FY was average historical data in June 2013 extracted from http://vattenweb.smhi.se/hydronu/ as the gauging station was not operational during the sampling campaign. Hourly discharge data for GR was obtained from www.hnd.bayern.de, while daily discharge data for RO and VI were obtained from the Swedish Water Archive (SVAR).

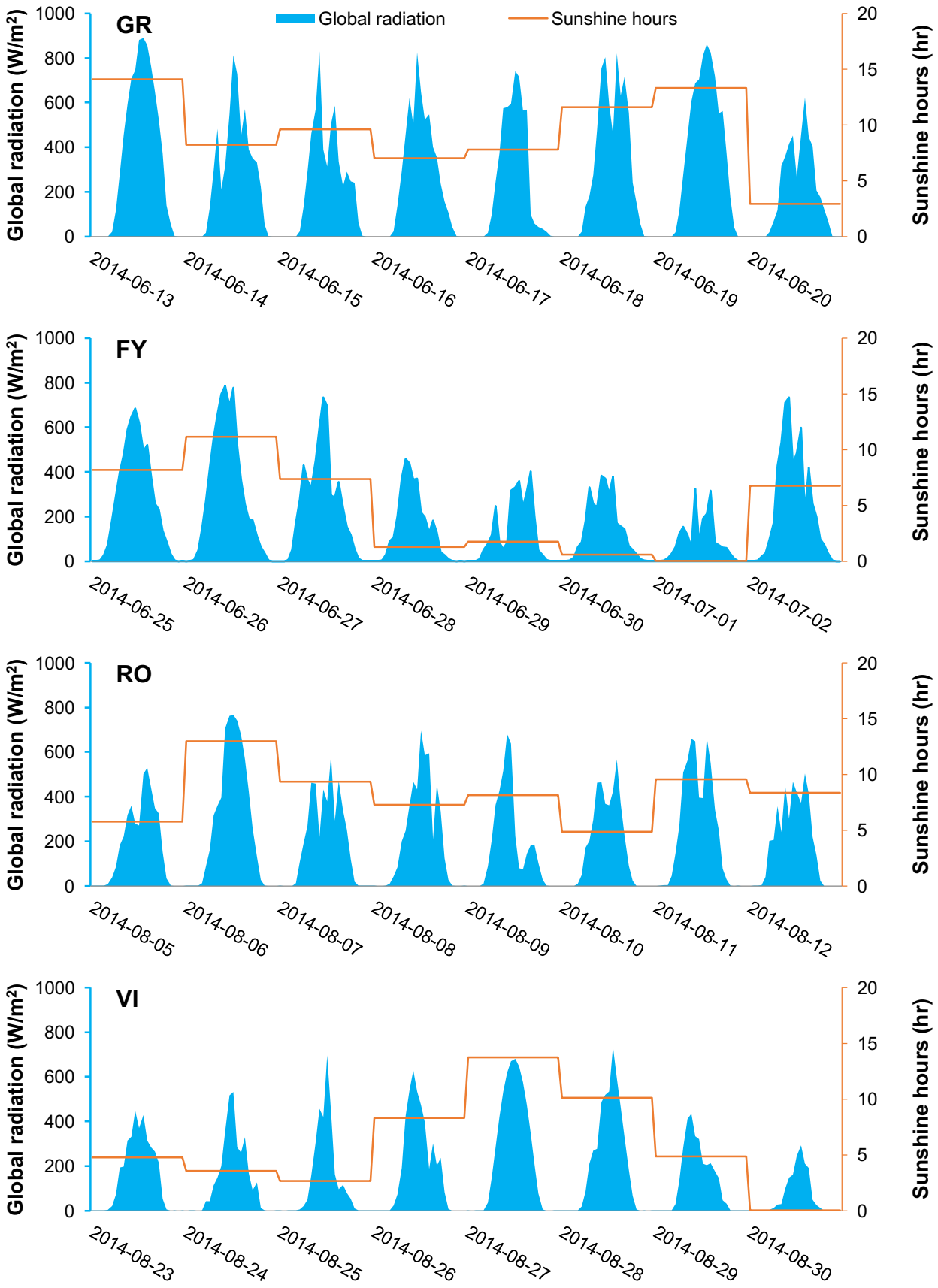

Figure S3. Global radiation (W/m^2) and sunshine hours (hr) for the studied rivers during the sampling period. The total sunshine hours at the four rivers are: 74.5 hours at GR, 37.3 hours at FY, 66.7 hours at RO and 48.1 hours at VI. Global radiation data and sunshine hours for GR were obtained from the German Weather Service, station Nürnberg; data for Swedish rivers was collected from the Swedish Meteorological and Hydrological Institute.

Table S2. Wastewater discharge and proportion in the rivers during the sampling period, distance for complete mixing of WWTP effluent and river water calculated according to USGS,[1] and routine parameters (temperature, electric conductivity (k), pH, concentrations of dissolved oxygen (DO) and total organic carbon (TOC), and UV absorbance at 254 nm) in each of the four rivers. [a]

Parameter	***GR***	***FY***	***RO***	***VI***
discharge of WWTP effluent ($m^3 s^{-1}$)	0.025	0.59	0.039	0.37
percentage of WWTP effluent in river [b]	80%	20%	1%	7%
average depth (m)	0.15	3	4	4
average width (m)	3	20	15	25
distance for complete mixing between WWTP effluent and river water (m)	293	109	80	133
T (ºC)	15.5 / 15.2	16.8 / 17.0	20.3 / 19.7	16.3 / 15.9
k ($\mu S\ cm^{-1}$)	n.a.	650 / 530	220 / 200	124 / 132
pH	7.9 / 7.8	7.8 / 8.1	7.3 / 7.0	6.8 / 7.4
DO ($mg\ L^{-1}$)	n.a.	11.2 / 10.8	7.5 / 7.2	8.3 / 8.0
TOC ($mg\ L^{-1}$)	6.1 / 5.9	11.2 / 11.9	14.4 / 15.3	7.9 / 8.4
UV absorbance at 254 nm (-)	0.12 / 0.13	1.15 / 1.16	0.60 / 0.55	0.29 / 0.28

[a] Figures before and after slashes represent the average values at site A and B during the sampling period, respectively; n.a.: not analyzed. [b] Percentage of WWTP effluent for river FY: the average discharge of June 2013 was used to estimate the proportion of WWTP effluent as gauging station was not operational during the sampling period.

Analytical methods

UHPLC method: a linear gradient from 100% A to 95% B in 3.7 min at a flow rate 0.4 mL min^{-1}, maintained for 0.5 min, followed by an increased flow rate to 0.6 mL min^{-1} within 0.1 min, maintained for 2.0 min, then the flow rate decreased back to 0.4 mL min^{-1} within 0.1 min followed by a linear gradient first to 20% A within 0.2 min then to 100% A within 0.4 min. Details on UHPLC column and solvent composition are available in the manuscript.

Table S3. Details on assignment of internal standards to target compounds, the electrospray ionization (ESI) mode, precursor-product ion transition used for quantification, selected instrumental parameters, and method quantification limit (MQL). Compounds marked with an asterisk are transformation products of the parent compound above.

analyte	*internal standard* [a]	*ionization mode*	*precursor > product ion (m/z)* [b]	*cone voltage (V)*	*collision energy (eV)*	*MQL (ng L^{-1})*
acetaminophen	acetaminophen-d_4	ESI+	152.1 > 110	42	14	0.04
acesulfame	acesulfame- d_4	ESI-	162 > 82	29	14	0.04
bezafibrate	bezafibrate-d_4	ESI-	360 > 274	15	15	0.04
*4-chlorobenzoic acid**	benzoic acid-d_5	ESI-	155 > 111	38	12	0.04
bicalutamide	bicalutamide-d_4	ESI-	429 > 185	2	44	0.04
carbamazepine	carbamazepine-d_{10}	ESI+	237 > 192	35	19	0.04
*carbamazepine-10,11-epoxide**	carbamazepine-d_{10}	ESI+	253 > 236	26	12	0.04
chlorthalidone	furosemide-d_5	ESI-	337 > 190	18	14	0.04
clofibric acid	clofibric acid-d_4	ESI-	213 > 127	35	15	0.04
diclofenac	diclofenac-$^{13}C_6$	ESI-	294 > 250	15	11	0.04
*4'-hydroxydiclofenac**	naproxen-d_3	ESI-	310 > 266	10	12	0.04
fluconazole	fluconazole-d_4	ESI+	307 > 169	30	20	0.04
furosemide	furosemide-d_5	ESI-	329 > 205	45	21	0.04
*saluamine**	furosemide-d_5	ESI-	249 > 205	18	12	0.04
glimepiride	glimepiride-d_5	ESI+	491 > 352	36	12	0.04
hydrochlorothiazide	hydrochlorothiazide-^{13}C-d_2	ESI-	296 > 269	45	18	0.04
*chlorothiazide**	hydrochlorothiazide-^{13}C-d_2	ESI-	294 > 214	72	28	0.04
*4-amino-6-chloro-1,3-benzenedisulfonamide**	hydrochlorothiazide-^{13}C-d_2	ESI-	284 > 78	62	24	0.04
ibuprofen	ibuprofen-d_3	ESI-	205 > 161	30	8	2.0
*2-hydroxyibuprofen**	2-hydroxyibuprofen-d_6	ESI-	221 > 177	20	8	0.4
*carboxyibuprofen**	carboxyibuprofen-d_3	ESI-	235 > 191	16	8	4.0
ketoprofen	ketoprofen-^{13}C-d_3	ESI+	255 > 209	42	14	0.04
metoprolol	metoprolol-d_7	ESI+	268 > 116	50	17	0.04
*α-hydroxymetoprolol**	metoprolol-d_7	ESI+	268 > 145	2	24	0.04
metoprolol acid *	metoprolol acid-d_5	ESI+	284 > 116	80	18	0.04
naproxen	naproxen-d_3	ESI-	229 > 170	50	15	1.0
propranolol	propranolol-d_7	ESI+	260 > 116	40	18	0.04
*1-naphthol**	naproxen-d_3	ESI-	143 > 115	36	24	0.2
sotalol	sotalol-d_6	ESI+	273 > 255	30	12	0.04
sulfamethoxazole	sulfamethoxazole-d_4	ESI+	254 > 156	30	14	0.04
tramadol	tramadol-d_6	ESI+	264 > 58	2	10	0.04

[a] TPs for which no corresponding isotope-substituted IS was available were quantified using the isotope-substituted compounds that were most similar in terms of retention time and molecular structure. [b] for the majority of the analytes there was at least one more ion transition that was used for confirmation.

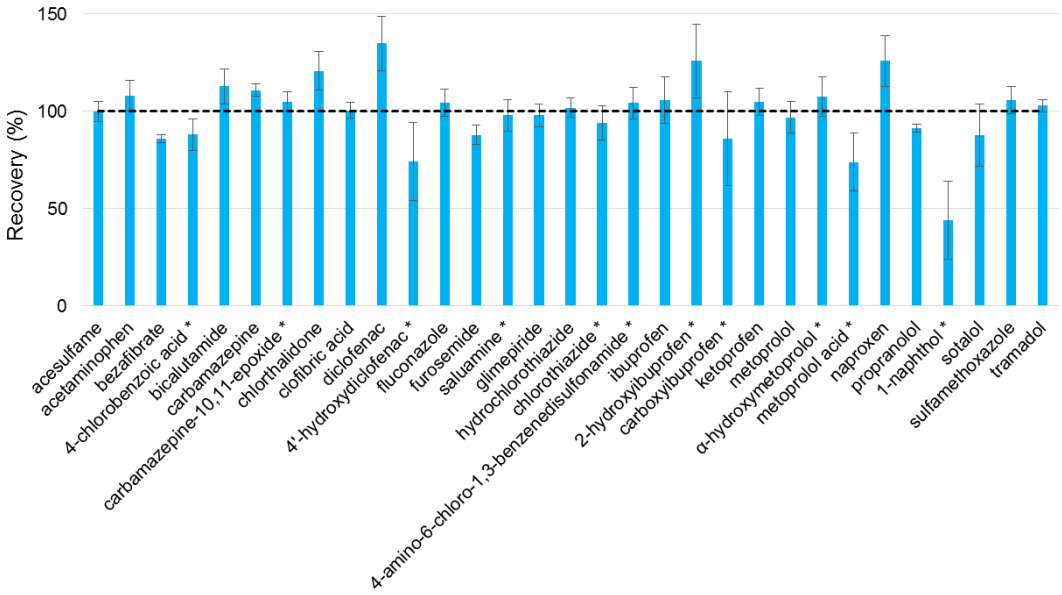

Figure S4. Recoveries (relative to internal standards) of parent compounds and transformation products spiked to river water (spike level 10 µg L^{-1}). Error bars represent the standard deviation of triplicate samples. Compounds marked with an asterisk are transformation products. The dashed line represents a recovery of 100%.

Table S4. Concentrations (ng L^{-1}) of all analytes at sites A and B in the four rivers (active sampling). Compounds marked with an asterisk are transformation products of the parent compound above.

compound	***GR_A***	***GR_B***	***FY_A***	***FY_B***	***RO_A***	***RO_B***	***VI_A***	***VI_B***
acesulfame	6400	4820	900	450	170	210	190	180
acetaminophen	7.4	0.2	170	83	17	11	20	12
bezafibrate	110	14	25	2.5	4.6	4.5	1.6	1.3
*4-chlorobenzoic acid**	88	33	54	16	16	39	5.9	11
bicalutamide	14	6.3	27	13	3.1	2.9	3.1	2.4
carbamazepine	310	230	83	42	4.9	5.9	15	14
*carbamazepine-10,11-epoxide**	80	60	22	10	1.3	1.5	3.8	3.7
chlorthalidone	5.9	3.1	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
clofibric acid	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
diclofenac	490	150	150	49	42	44	47	39
*4'-hydroxydiclofenac**	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
fluconazole	19	14	32	16	1.0	1.2	1.8	1.7
furosemide	72	5.6	150	30	36	26	42	22
*saluamine**	18	7.7	38	17	<0.04	<0.04	<0.04	<0.04
glimepiride	0.54	0.26	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
hydrochlorothiazide	470	220	140	54	3.2	3.0	19	15
*chlorothiazide**	28	19	8.5	4.1	0.31	0.38	1.1	1.0
*4-amino-6-chloro-benzenedisulfonamide**	67	33	34	16	0.9	1.0	3.4	2.9
ibuprofen	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
*2-hydroxyibuprofen**	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
*carboxyibuprofen**	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0	<4.0
ketoprofen	<0.04	<0.04	21	1.8	<0.04	<0.04	5.8	3.4
metoprolol	510	160	2000	810	10	10	36	33
*α-hydroxymetoprolol**	19	1.9	49	23	0.29	0.30	1.2	1.0
*metoprolol acid**	890	400	550	300	52	73	190	180
naproxen	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
propranolol	7.1	1.4	18	6.5	0.72	0.61	2.5	2.3
*1-naphthol**	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
sotalol	120	59	70	35	3.0	3.5	10	7.7
sulfamethoxazole	60	36	19	9.4	0.23	0.28	1.2	1.1
tramadol	140	79	130	58	10	11	16	14

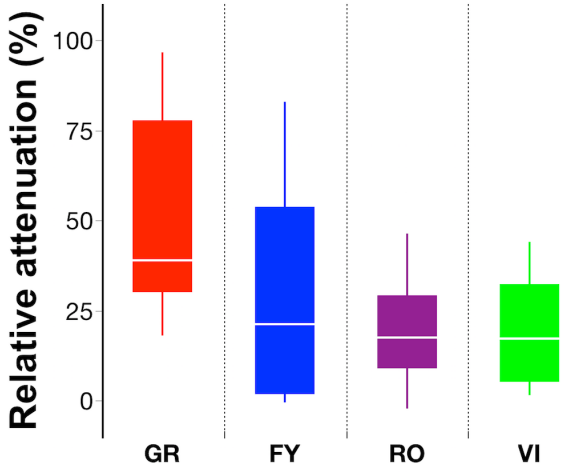

Figure S5. Box-whisker plots of the overall relative attenuation (Att_x) of the 14 parent compounds that were attenuated between sites A and B in at least one of the studied rivers (based on active sampling). Boxes represent the interquartile ranges of all values. The horizontal lines within the boxes represent the median values. Upper and lower ends of the whiskers represent the maximum and minimum values, respectively.

Table S5. Comparison of the Att_x values of all 16 detected parent compounds determined from samples collected with active and passive sampling. [a]

compound	*GR*		*FY*		*RO*		*VI*	
	active	**passive**	**active**	**passive**	**active**	**passive**	**active**	**passive**
acesulfame	-2.2	-0.5	0.1	0.3	-2.9	2.0	0.8	-1.0
acetaminophen	96	99	2.4	1.5	46	42	37	29
bezafibrate	83	86	80	78	17	16	17	19
bicalutamide	39	50	3.7	4.9	22	15	18	32
carbamazepine	-1.1	-0.9	-1.2	1.2	0.0	1.2	2.6	1.6
chlorthalidone	29	30	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
diclofenac	58	63	35	32	13	10	12	11
furosemide	89	86	60	53	40	35	44	36
glimepiride	35	38	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
hydrochlorothiazide	36	38	23	30	21	18	18	20
ketoprofen	n.a.	n.a.	83	80	n.a.	n.a.	38	35
metoprolol	57	60	19	17	16*	-9.1*	1.8*	-10*
propranolol	73	78	28	30	29*	-2.3*	1.7*	-6.8*
sotalol	33	32	0.0	-1.0	2.8*	-6.5*	20*	-5.0*
sulfamethoxazole	18	14	1.0	0.1	-1.5*	-11.3*	4.0*	-8.8*
tramadol	23	25	11	11	8.8*	-9.9*	7.4*	-12*

[a] Values marked with an asterisk indicate Att_x values calculated from the two sampling approaches were significantly different at 95% confidence level; negative values indicate higher concentrations at site B compared to site A; n.a. indicates Att_x values cannot be calculated due to concentrations <MQL.

Table S6. Comparison of the Att_x values (%) in river GR for the pharmaceuticals in common to both the present study and Kunkel and Radke (2012).[2]

compound	*this study*	*Kunkel and Radke (2012)*
bezafibrate	83	63
diclofenac	58	69
ibuprofen	n.a. [a]	n.a. [a]
metoprolol	57	68
naproxen	n.a. [a]	50
propranolol	73	70
sotalol	33	42
sulfamethoxazole	18	26

[a] n.a.: Att_x could not be calculated as the compound was not detected.

Table S7. Molar concentration ratio of parent compounds (PC) to transformation products (TP) at site A in the four rivers (active sampling).

PC / TP	*GR*	*FY*	*RO*	*VI*
bezafibrate / 4-chlorobenzoic acid	0.54	0.20	0.12	0.12
carbamazepine / carbamazepine-10,11-epoxide	4.1	4.1	4.0	4.2
furosemide / saluamine	3.0	3.0	n.a. [a]	n.a. [a]
hydrochlorothiazide / chlorothiazide	17	16	10	17
hydrochlorothiazide / 4-amino-6-chloro-benzenedisulfonamide	6.7	4.0	3.4	5.3
metoprolol / α-hydroxymetoprolol	28	43	36	31
metoprolol / metoprolol acid	0.56	3.6	0.20	0.19

[a] n.a.: not applicable as saluamine was not detected at either sampling site in rivers RO and VI.

Reference:

(1) Kilpatrick, F. A.; Cobb, E. D. Measurement of discharge using tracers; 1985; pp 33–52.

(2) Kunkel, U.; Radke, M. Fate of pharmaceuticals in rivers: Deriving a benchmark dataset at favorable attenuation conditions. *Water Res.* **2012**, *46* (17), 5551–5565.