Supplementary Information (SI) for Environmental Science: Processes & Impacts.

Electronic Supplementary Material

Enantiomeric fraction evaluation for assessing septic tanks as a pathway for chiral pharmaceuticals entering rivers

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Contents

List of tables

List of figures

S1 General and chemical information

Table S1: General and chemical information of target analytes.

Class	Pharmaceutical	Cas No.	Mol. Formula	Mol. Weight (g mol^{-1})	Solubility (mg L^{-1})	Log K_{OW}	pKa (most acidic)	pKa (most basic)	Supplier
Analgesics	(±)-Hydroxyibuprofen	51146-55-5	$C_{13}H_{18}O_3$	222.28	-	2.29 [c]	4.63 [d]	-	Sigma Aldrich
	(+)-Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.27	15.9[a]	3.18 [a]	4.19 [e]	-4.8 [e]	Sigma Aldrich
	(-)-Naproxen	23979-41-1							Sigma Aldrich
Antibiotics	(±)-α-Hydroxytrimethoprim	29606-06-2	$C1_4H_{18}N_4O_4$	306.32	-	-	-	-	LGC standards
Anticoagulants	(±)-Warfarin	81-81-2	$C_{19}H_{16}O_4$	308.33	17 [a]	2.7 [a]	5.56 [e]	-6.9 [e]	Sigma Aldrich
Antidepressants	(±)-Citalopram	59729-32-7	$C_{20}H_{21}FN_2O$	324.40	31.1 [b]	3.76 [a]	-	9.78 [a]	Sigma Aldrich
	(±)-Desmethylcitalopram	144025-14-9	$C_{19}H_{19}FN_2O$	310.37	-	3.53 [c]	-	10.54 [d]	LGC standards
	(±)-Desmethylvenlafaxine	93413-62-8	$C_{16}H_{25}NO_2$	263.38	-	2.69 [d]	10.04 [b]	9.33 [b]	Sigma Aldrich
	(±)-Fluoxetine	56296-78-7	$C_{17}H_{18}F_3NO$	309.33	60.3 [b]	4.05 [a]	-	9.8 [e]	LGC standards
	(±)-Venlafaxine	99300-78-4	$C_{17}H_{27}N_1O_2$	277.41	267 [b]	3.28 [b]	14.42 [e]	8.91 [e]	Sigma Aldrich
Anti-fungals	(±)-Climbazole	38083-17-9	$C_{15}H_{17}ClN_2O_2$	292.76	-	3.76 [c]	18.87 [e]	6.49 [e]	TCI
Antihistamines	(±)-Chlorpheniramine	113-92-8	$C_{16}H_{19}ClN_2$	274.79	5500 [a]	3.38 [a]	-	9.13 [a]	Sigma Aldrich
Antiulcer	(±)-Lansoprazole	103577-45-3	$C_{16}H_{14}F3N_3O_2S$	369.36	0.97 [a]	3.68 [c]	9.35 [e]	4.16 [e]	TCI
	(±)-Omeprazole	73590-58-6	$C_{17}H_{19}N_3O_3S$	345.52	359 [a]	2.23 [a]	9.29 [e]	4.77 [e]	Sigma Aldrich
Benzodiazepines	(±)-Lorazepam	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	321.16	80 [a]	2.39 [a]	10.61 [e]	-2.2 [e]	Sigma Aldrich
	(±)-Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.71	179 [b]	2.24 [a]	10.61 [e]	-1.5 [e]	Sigma Aldrich
	(±)-Temazepam	846-50-4	$C_{16}H_{13}ClN_2O_2$	300.75	164 [a]	2.19 [a]	10.68 [e]	-1.4 [e]	Sigma Aldrich
Betablockers	(±)-Acebutolol	34381-68-5	$C_{18}H_{28}N_2O_4$	336.43	259 [a]	1.71 [a]	13.91 [e]	9.65 [e]	Sigma Aldrich
	(+)-Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$	266.34	13300 [a]	0.16 [a]	14.08 [e]	9.67 [e]	Sigma Aldrich
	(-)-Atenolol	93379-54-5							Sigma Aldrich
	(±)-Bisoprolol	104344-23-2	$C_{18}H_{31}NO_4$	325.44	2240 [b]	2.2 [a]	14.09 [e]	9.67 [e]	Sigma Aldrich
	(±)-Metoprolol	56392-17-7	$C_{15}H_{25}NO_3$	267.37	4770 [b]	2.15 [a]	14.09 [e]	9.67 [e]	Sigma Aldrich
	(±)-Propranolol	318-98-9	$C_{16}H_{21}NO_2$	259.35	228 [e]	3.48 [a]	14.09 [e]	9.67 [e]	Sigma Aldrich
	(±)-Salbutamol	18559-94-9	$C_{13}H_{21}NO_3$	239.31	14100 [a]	1.4 [a]	10.12 [e]	9.4 [e]	Sigma Aldrich
	(±)-Sotalol	959-24-0	$C_{12}H_{20}N_2O_3S$	272.36	-	0.24 [c]	10.07 [e]	9.43 [e]	Sigma Aldrich
Chemotherapeutic	(±)-Ifosfamide	3778-73-2	$C_7H_{15}Cl_2N_2O_2P$	261.09	3780 [a]	0.86 [a]	14.64 [e]	-	Sigma Aldrich
Wastewater discharge marker	(-)-Cotinine	486-56-6	$C_{10}H_{12}N_2O$	176.22	999000 [b]	1.37 [d]	-	4.79 [d]	Sigma Aldrich

[a] Drugbank[1], [b] Proctor et al.[2], [c] ChemSpider[3], [d] ChEMBL[4], [e] Drugbank using ChemAxon[1]

Table S2: CAS Number and supplier for deuterated surrogates.

Compound	CAS	supplier
(±)-Acebutolol-d_5 hydrochloride	1189500-68-2	TRC
(±)-Atenolol-d_7	1202864-50-3	Analab
(±)-Bisoprolol-d_5	1189881-87-5	TRC
(±)-Chlorpheniramine-d_6 solution	129806-45-7	Sigma Aldrich
(±)-Citalopram-d_6 solution	1190003-26-9	Sigma Aldrich
(±)-Cotinine-d_3 solution	110952-70-0	Sigma Aldrich
(±)-Fluoxetine-d_6 solution	1173020-43-3	Sigma Aldrich
(±)-Metoprolol-d_7 (+)-tartrate	2378803-75-7	Sigma Aldrich
(±)-Naproxen-d_3	958293-79-3	Sigma Aldrich
(±)-Propranolol-d_7 solution	1613439-56-7	Sigma Aldrich
(±)-Salbutamol-d_3	1219798-60-3	LGC standards
(±)-Sotalol-d_6 hydrochloride	1246820-85-8	LGC standards
(±)-Temazepam-d_5 solution	136765-51-0	Sigma Aldrich
(±)-Venlafaxine-d_6 solution	1062606-12-5	Sigma Aldrich
(±)-Oxazepam-d_5 solution	65854-78-6	Sigma Aldrich

S2 Analytical methods

Table S3: MS/MS detection parameters (precursor ion, cone voltage (CV), quantifier and qualifier ions with collision energies (CE)) for studied pharmaceuticals.

Class	Pharmaceutical	Precursor ion /m/z	CV /V	Quantifier ion	CE /eV	Qualifier ion	CE /eV
Analgesics	(±)-Hydroxyibuprofen	240.2	25	205.2	12	163.2	16
	(±)-Naproxen	231.2	10	185.2	12	170.2	23
Antibiotics	(±)-α-Hydroxytrimethoprim	307.2	22	289.2	14	274.2	20
Anticoagulants	(±)-Warfarin	309.1	32	163.1	14	251.2	19
Antidepressants	(±)-Citalopram	325.2	24	262.2	20	116.1	25
	(±)-Citalopram-d_6	331.2	24	109.1	31	-	-
	(±)-Desmethylcitalopram	311.2	22	109.1	20	262.2	17
	(±)-Desmethylvenlafaxine	264.3	29	246.3	12	107.1	30
	(±)-Fluoxetine	310.2	34	44.1	10	148.1	10
	(±)-Venlafaxine	278.3	36	260.3	10	215.2	16
	(±)-Venlafaxine-d_6	284.3	34	266.3	12	-	-
Anti-fungals	(±)-Climbazole	293.1	23	69.2	21	41.2	26
Antihistamines	(±)-Chlorpheniramine	275.2	30	230.1	18	167.1	43

	(±)-Chlorpheniramine-d_6	281.1	26	230.1	16	-	-
Antiulcer	(±)-Lansoprazole	370.1	29	252.1	11	119.2	20
	(±)-Omeprazole	346.2	21	198.1	11	180.1	23
Benzodiazepines	(±)-Lorazepam	321.1	25	275.1	22	303.1	16
	(±)-Oxazepam	287.1	26	241.1	25	269.1	17
	(±)-Oxazepam-d_5	292.1	26	246.2	25	-	-
	(±)-Temazepam	301.1	24	255.2	21	283.2	14
	(±)-Temazepam-d_5	306.1	24	260.2	21	-	-
Betablockers	(±)-Acebutolol	337.3	20	116.2	18	319.3	16
	(±)-Acebutolol-d_5	342.3	19	121.2	23	-	-
	(±)-Atenolol	267.3	38	145.1	30	190.1	16
	(±)-Atenolol-d_7	274.2	23	145.1	24	-	-
	(±)-Bisoprolol	326.3	20	116.2	16	222.2	10
	(±)-Bisoprolol-d_5	331.2	23	121.2	17	-	-
	(±)-Metoprolol	268.2	30	159.1	22	191.2	17
	(±)-Metoprolol-d_7	275.3	29	123.2	18	-	-
	(±)-Propranolol	260.2	50	116.1	16	183.1	18
	(±)-Propranolol-d_7	267.1	22	189.2	18	-	-
	(±)-Salbutamol	240.2	27	148.1	20	166.1	12
	(±)-Salbutamol-d_3	243.0	21	151.2	21	-	-
	(±)-Sotalol	273.2	25	133.2	28	213.2	17
	(±)-Sotalol-d_6	279.2	24	214.1	17	-	-
Chemotherapeutic	(±)-Ifosfamide	261.1	15	92.1	23	154.0	18
Wastewater discharge marker	(±)-Cotinine	177.1	34	80.1	19	98.1	21
	(±)-Cotinine-d_3	180.2	13	80.1	22	-	-

S3 Sampling

The nominal dilution of the septic tank discharge into the river was calculated from the flow of the receiving river per day (f_{river}) and the calculated flow of the septic tank effluent per day (f_{ST}) following equation S1.

$$dilution = \frac{(f_{\text{river}} - f_{\text{ST}})}{f_{\text{ST}}} \quad (\text{S1})$$

The flow of the river was determined through the SEPA Time series data service (API)[5] and is included in Table S5. For ST 3 and ST 4 no suitable station with daily or hourly river flow data was available, and the mean flow of the river (f_{mean}) was used instead.[6] The flow of the septic tank effluent per day (Table S5) was calculated by multiplying the population equivalents (PE) by the mean daily discharge per person per day (0.7252 m^3 day^{-1}) (equation S2) following industry practice.[6]

$$f_{\text{ST}} = \text{PE} \cdot 0.7252\ \text{m}^3\ \text{day}^{-1} \quad (\text{S2})$$

Table S4: Selected septic tanks (STs) with the respective population equivalents (PE) contributing to the ST, ST volume (V), emptying frequency, location in Scotland, the receiving river and dilution factors. The dilution factor is calculated from the mean river flow over two hours at sampling time[a] or from the mean flow of the river[b] and the flow of the STs following industry practice. Monthly dilution factors are presented in Table S5.

ST	PE	V / m^3	Emptying frequency / weeks	Location	Receiving River	Mean dilution factor and observed range
ST 1	308	75	8	Central Belt	Clyde	3189[a] (580 – 11990)
ST 2	314	75	52	Central Belt	Clyde	3257[a] (592 – 12244)
ST 3	475	75	8	Central Belt	Small tributary to Clyde	96[b]
ST 4	314	100	17	North-West Highlands	Black Water	4808[b]
ST 5	217	225	26	North-West Highlands	Glass	18148[a] (4276 – 35476)

[a] Mean calculated from observed dilution factors during sampling, [b] No daily/hourly river flow data available: Mean calculated from historic mean daily flow.

Table S5: Sampling dates of septic tank (ST) 1 – 5 wastewater and receiving surface water with ST outlet temperature (T_{outlet}), mean air temperature (T_{air}), rain, ST flow (f_{ST}), river flows (f_{river} and f_{mean}) and dilution factors. Rain is the mean of the total rain per day from the day of wastewater sampling and the two days prior.[5] The dilution factor is calculated from the mean daily river flow over two hours at sampling time (f_{river}) received from SEPA.[5] The mean dilution factor was calculated from the historic mean daily flow of the river (f_{mean}) available.[6]

Month	**Septic Tank**	**Wastewater**	**River**	**T_{Outlet} (°C)**	**T_{air} (°C)**	**Rain (mm day^{-1})**	**f_{st} (m^3 day^{-1})**	**f_{river} (m^3 day^{-1})**	**Dilution factor**	**f_{mean} (m^3 day^{-1})**	**Mean dilution factor**
October 2021	ST 1	13/10/2021	-	-	13	0.67	228	$3.5 \cdot 10^5$	1546	9.91	5439
	ST 2	13/10/2021	-	-	13	0.67	223	$3.5 \cdot 10^5$	1579	7.48	2895
	ST 3	13/10/2021	-	-	13	0.13	344	-	-	0.378	96
	ST 4	13/10/2021	-	14	13	3.7	228	-	-	12.7	4808
	ST 5	13/10/2021	-	16	13	3.4	157	$2.7 \cdot 10^6$	17039	29.8	16336
November 2021	ST 1	10/11/2021	10/11/2021	-	7.5	3.1	228	$7.5 \cdot 10^5$	3300	9.91	5439
	ST 2	10/11/2021	10/11/2021	-	7.7	3.1	223	$7.5 \cdot 10^5$	3370	7.48	2895
	ST 3	10/11/2021	10/11/2021	-	7.7	2.0	344	-	-	0.378	96
	ST 4	10/11/2021	11/11/2021	10	7.6	1.1	228	-	-	12.7	4808
	ST 5	10/11/2021	11/11/2021	9.9	7.6	2.1	157	$5.2 \cdot 10^6$	32965	29.8	16336
December 2021	ST 1	14/12/2021	-	-	7.7	2.1	228	$1.1 \cdot 10^6$	4738	9.91	5439
	ST 2	14/12/2021	-	-	7.5	2.1	223	$1.1 \cdot 10^6$	4839	7.48	2895
	ST 3	14/12/2021	-	-	7.5	0.033	344	-	-	0.378	96
	ST 4	14/12/2021	-	8.7	9.0	0.13	228	-	-	12.7	4808
	ST 5	14/12/2021	-	7.7	9.0	2.9	157	$4.3 \cdot 10^6$	27334	29.8	16336
January 2022	ST 1	11/01/2022	-	-	5.2	2.1	228	$1.2 \cdot 10^6$	5102	9.91	5439
	ST 2	11/01/2022	-	-	5.3	2.1	223	$1.2 \cdot 10^6$	5211	7.48	2895
	ST 3	11/01/2022	-	-	5.3	1.2	344	-	-	0.378	96
	ST 4	11/01/2022	-	6.2	5.5	0.80	228	-	-	12.7	4808
	ST 5	11/01/2022	-	5.9	5.5	3.3	157	$2.8 \cdot 10^6$	17773	29.8	16336
February 2022	ST 1	17/02/2022	17/02/2022	-	5.0	10	228	$2.7 \cdot 10^6$	11990	9.91	5439
	ST 2	17/02/2022	17/02/2022	-	4.7	10	223	$2.7 \cdot 10^6$	12244	7.48	2895
	ST 3	17/02/2022	17/02/2022	-	4.7	6.3	344	-	-	0.378	96
	ST 4	17/02/2022	18/02/2022	4.2	2.7	15	228	-	-	12.7	4808
	ST 5	17/02/2022	18/02/2022	3.5	2.7	9.9	157	$5.6 \cdot 10^6$	35476	29.8	16336
March 2022	ST 1	15/03/2022	-	-	6.5	5.3	228	$1.5 \cdot 10^6$	6691	9.91	5439
	ST 2	15/03/2022	-	-	7.0	5.3	223	$1.5 \cdot 10^6$	6833	7.48	2895
	ST 3	15/03/2022	-	-	7.0	2.7	344	-	-	0.378	96
	ST 4	15/03/2022	-	7.0	6.9	0.33	228	-	-	12.7	4808
	ST 5	15/03/2022	-	5.8	6.9	1.3	157	$1.8 \cdot 10^6$	11380	29.8	16336
April 2022	ST 1	19/04/2022	-	-	8.4	2.9	228	$2.7 \cdot 10^5$	1194	9.91	5439
	ST 2	19/04/2022	-	-	8.4	2.9	223	$2.7 \cdot 10^5$	1220	7.48	2895
	ST 3	19/04/2022	-	-	8.4	1.1	344	-	-	0.378	96
	ST 4	19/04/2022	-	9.3	9.5	0.73	228	-	-	12.7	4808
	ST 5	19/04/2022	-	9.6	9.5	0.67	157	$1.9 \cdot 10^6$	11896	29.8	16336
May 2022	ST 1	17/05/2022	17/05/2022	-	13	4.1	228	$2.1 \cdot 10^5$	908	9.91	5439
	ST 2	17/05/2022	17/05/2022	-	13	4.1	223	$2.1 \cdot 10^5$	927	7.48	2895
	ST 3	17/05/2022	17/05/2022	-	13	2.1	344	-	-	0.378	96
	ST 4	-	18/05/2022	-	14	3.7	228	-	-	12.7	4808

	ST 5	-	18/05/2022	-	14	2.6	157	$1.8 \cdot 10^6$	11448	29.8	16336
June 2022	ST 1	14/06/2022	-	-	13	1.8	228	$1.5 \cdot 10^5$	650	9.91	5439
	ST 2	14/06/2022	-	-	14	1.8	223	$1.5 \cdot 10^5$	664	7.48	2895
	ST 3	14/06/2022	-	-	14	1.7	344	-	-	0.378	96
	ST 4	14/06/2022	-	13	13	0.13	228	-	-	12.7	4808
	ST 5	14/06/2022	-	13	13	0.067	157	$3.7 \cdot 10^6$	23810	29.8	16336
July 2022	ST 1	19/07/2022	-	-	21	0	228	$1.3 \cdot 10^5$	580	9.91	5439
	ST 2	19/07/2022	-	-	22	0	223	$1.3 \cdot 10^5$	592	7.48	2895
	ST 3	19/07/2022	-	-	22	0.5	344	-	-	0.378	96
	ST 4	19/07/2022	-	16	18	2.2	228	-	-	12.7	4808
	ST 5	19/07/2022	-	16	18	1.7	157	$2.5 \cdot 10^6$	16069	29.8	16336
August 2022	ST 1	23/08/2022	23/08/2022	-	17	4.3	228	$1.6 \cdot 10^5$	685	9.91	5439
	ST 2	23/08/2022	23/08/2022	-	17	4.3	223	$1.6 \cdot 10^5$	700	7.48	2895
	ST 3	23/08/2022	23/08/2022	-	17	5.7	344	-	-	0.378	96
	ST 4	16/08/2022	24/08/2022	16	12	0.13	228	-	-	12.7	4808
	ST 5	16/08/2022	24/08/2022	15	12	0.47	157	$6.7 \cdot 10^5$	4276	29.8	16336
September 2022	ST 1	20/09/2022	-	-	15	1.7	228	$2.0 \cdot 10^5$	882	9.91	5439
	ST 2	20/09/2022	-	-	14	1.7	223	$2.0 \cdot 10^5$	900	7.48	2895
	ST 3	20/09/2022	-	-	14	0.033	344	-	-	0.378	96
	ST 4	27/09/2022	-	-	9.1	0.13	228	-	-	12.7	4808
	ST 5	27/09/2022	-	-	9.1	3.7	157	$1.3 \cdot 10^6$	8306	29.8	16336

S4 Quality Control

Table S6: Class, pharmaceutical, column, calibration method, chromatographic resolution (R_s), and instrument detection (IDL) and quantification limits (IQL). Enantiomers were assigned using enantiopure standards or following the literature when the same stationary phase and mobile phase was used, *E1* and *E2* were used when assignment was not possible (N/A).

Class	RT /min	Pharmaceutical	Enantiomer assignment	Method	Calibration	R_s	*IDL* / µg L^{-1}	*IQL* / µg L^{-1}
Analgesics	3.3	*E1*-Hydroxyibuprofen	N/A	IG-U	external	0.67	0.25	0.38
	3.5	*E2*-Hydroxyibuprofen	N/A	IG-U	external		0.25	0.38
	4.4	*R*(-)-Naproxen	Standard	IG-U	external	1.2	0.080	0.32
	4.9	*S*(+)-Naproxen	Standard	IG-U	external		0.075	0.30
Antibiotics	14.0	*E1*-α-Hydroxytrimethoprim	N/A	Chiral-V	external	1.4	0.025	0.10
	18.5	*E2*-α-Hydroxytrimethoprim	N/A	Chiral-V	external		0.18	0.38
Anticoagulants	5.1	*E1*-Warfarin	N/A	IG-U	external	3.3	0.025	0.050
	8.5	*E2*-Warfarin	N/A	IG-U	external		0.025	0.050
Antidepressants	15.4	*R*(-)-Citalopram	McKenzie et al.[7]	Chiral-V	*R*(-)-Citalopram-d_6	0.54	$6.3 \cdot 10^{-3}$	0.013
	17.0	*S*(+)-Citalopram	McKenzie et al.[7]	Chiral-V	*S*(+)-Citalopram-d_6		0.013	0.025
	14.7	*R*(-)-Desmethylcitalopram	Evans et al.[8]	Chiral-V	external	1.6	0.10	0.75
	20.6	*S*(+)-Desmethylcitalopram	Evans et al.[8]	Chiral-V	external		0.18	0.75
	7.1	*S*(+)-Desmethylvenlafaxine	Evans et al.[8]	Chiral-V	external	0.86	0.019	0.038
	8.0	*R*(-)-Desmethylvenlafaxine	Evans et al.[8]	Chiral-V	external		0.025	0.050
	10.2	*S*(+)-Fluoxetine	McKenzie et al.[7]	Chiral-V	*S*(+)-Fluoxetine-d_6	2.4	0.25	0.75
	13.3	*R*(-)-Fluoxetine	McKenzie et al.[7]	Chiral-V	*R*(-)-Fluoxetine-d_6		0.38	1.75
	7.8	*S*(+)-Venlafaxine	Evans et al.[8]	Chiral-V	*S*(+)-Venlafaxine-d_6	0.53	0.13	0.75
	8.5	*R*(-)-Venlafaxine	Evans et al.[8]	Chiral-V	*R*(-)-Venlafaxine-d_6		0.075	0.15
Anti-fungals	5.3	*E1*-Climbazole	N/A	IG-U	external	1.5	0.025	0.050
	6.8	*E2*-Climbazole	N/A	IG-U	external		0.025	0.050
Antihistamines	14.2	*S*(+)-Chlorpheniramine	McKenzie et al.[7]	Chiral-V	*S*(+)-Chlorpheniramine-d_6	0.67	0.025	0.050
	15.7	*R*(-)-Chlorpheniramine	McKenzie et al.[7]	Chiral-V	*R*(-)-Chlorpheniramine-d_6		0.025	0.050
Antiulcer	4.5	*E1*-Lansoprazole	N/A	IG-U	external	1.5	0.025	0.050
	5.4	*E2*-Lansoprazole	N/A	IG-U	external		0.025	0.050
	15.3	*S*(-)-Omeprazole	Petrie and Camacho-Muñoz[9]	IG-U	external	3.5	0.025	0.075
	23.0	*R*(+)-Omeprazole	Petrie and Camacho-Muñoz[9]	IG-U	external		0.025	0.075
Benzodiazepines	3.8	*E1*-Lorazepam	N/A	IG-U	external	1.1	0.38	0.50
	4.4	*E2*-Lorazepam	N/A	IG-U	external		0.38	0.50
	4.6	*E1*-Oxazepam	N/A	IG-U	*E1*-Oxazepam-d_5	2.2	0.19	0.38
	6.0	*E2*-Oxazepam	N/A	IG-U	*E2*-Oxazepam-d_5		0.25	0.38
	13.4	*E1*-Temazepam	N/A	IG-U	*E1*-Temazepam-d_5	2.9	0.050	0.25

	18.1	*E2*-Temazepam	N/A	IG-U	*E2*-Temazepam-d_5		0.050	0.15
Betablockers	8.8	*E1*-Acebutolol	N/A	Chiral-V	*E1*-Acebutolol-d_5	0.60	0.025	0.050
	9.9	*E2*-Acebutolol	N/A	Chiral-V	*E2*-Acebutolol-d_5		0.025	0.050
	11.3	*R*(+)-Atenolol	McKenzie et al.[7]	Chiral-V	*R*(+)-Atenolol-d_7	0.65	0.038	0.20
	12.4	*S*(-)-Atenolol	McKenzie et al.[7]	Chiral-V	*S*(-)-Atenolol-d_7		0.038	0.20
	6.0	*E1*-Bisoprolol	N/A	Chiral-V	*E1*-Bisoprolol-d_5	0.59	0.013	0.025
	6.4	*E2*-Bisoprolol	N/A	Chiral-V	*E2*-Bisoprolol-d_5		0.013	0.025
	6.4	*S*(-)-Metoprolol	S. Evans et al.[8]	Chiral-V	*S*(-)-Metoprolol-d_7	0.70	0.025	0.15
	6.9	*R*(+)-Metoprolol	S. Evans et al.[8]	Chiral-V	*R*(+)-Metoprolol-d_7		0.038	0.23
	7.9	*S*(-)-Propranolol	McKenzie et al.[7]	Chiral-V	*E1*-Propranolol-d_7	0.98	0.025	0.10
	8.9	*R*(+)-Propranolol	McKenzie et al.[7]	Chiral-V	*E2*-Propranolol-d_7		0.038	0.10
	5.2	*E1*-Salbutamol	N/A	Chiral-V	*E1*-Salbutamol-d_3	0.63	$6.3 \cdot 10^{-3}$	0.025
	5.9	*E2*-Salbutamol	N/A	Chiral-V	*E2*-Salbutamol-d_3		$7.3 \cdot 10^{-3}$	0.025
	8.2	*E1*-Sotalol	N/A	Chiral-V	*E1*-Sotalol-d_6	1.3	0.013	0.038
	9.5	*E2*-Sotalol	N/A	Chiral-V	*E2*-Sotalol-d_6		0.025	0.050
Chemotherapeutic	3.5	*E1*-Ifosfamide	N/A	IG-U	external	1.2	0.025	0.050
	4.1	*E2*-Ifosfamide	N/A	IG-U	external		0.025	0.050
Wastewater discharge marker	4.3	*S*(-)-Cotinine	Standard	IG-U	*S*(-)-Cotinine-d_3	2.0	0.013	0.025
	5.3	*R*(+)-Cotinine	Standard	IG-U	*R*(+)-Cotinine-d_3		0.013	0.025

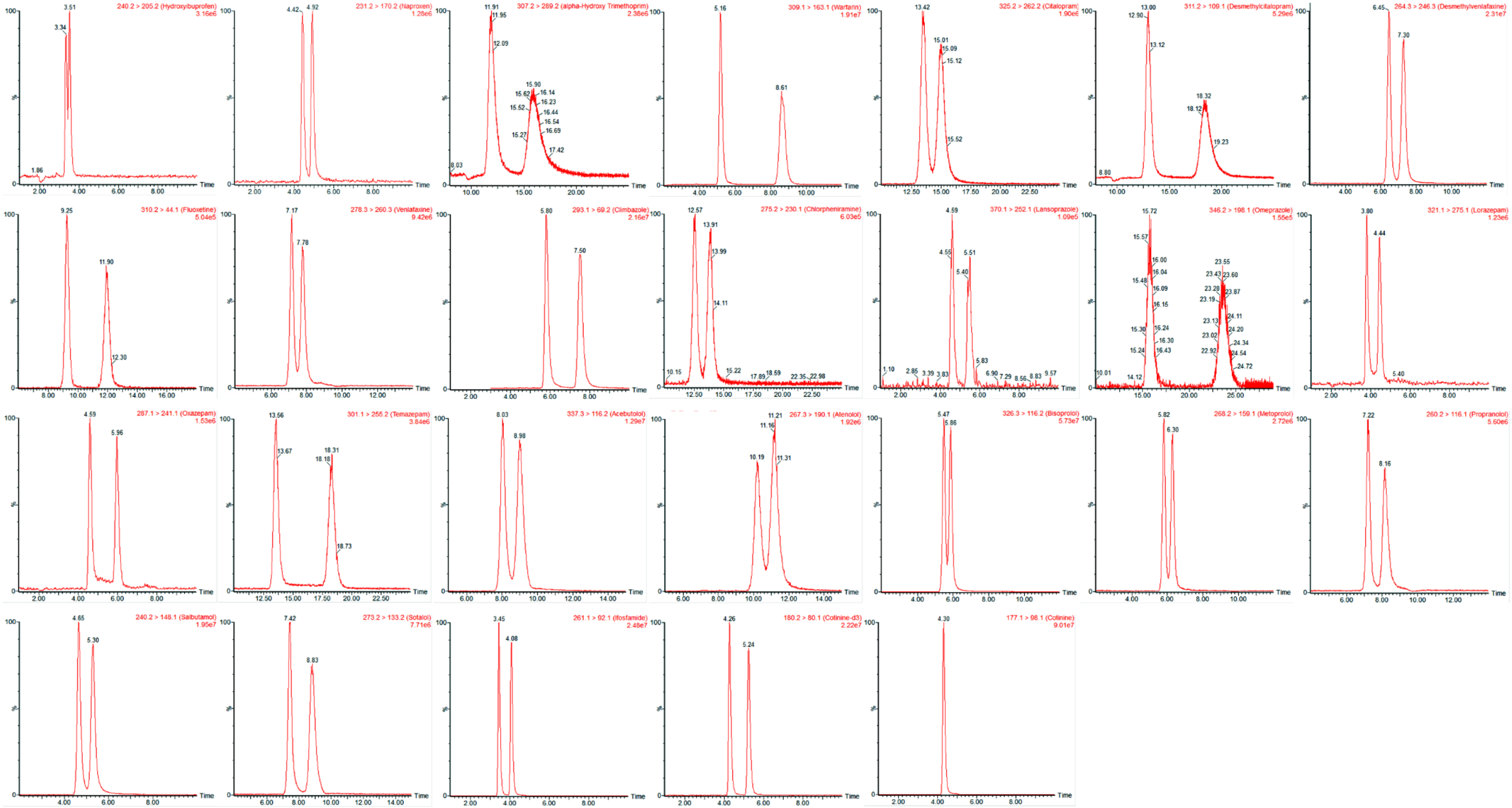

Figure S1: Chromatograms of a 10 µg L^{-1} QC standard, cotinine is only commercially available as the *S*(-)-enantiomer (last chromatogram), hence the chromatogram of (±)-cotinine-d_3 is included to show enantiomer separation.

Table S7: Enantiomeric fractions (EFs) in quality control standards (10 and 50 µg L^{-1}), and spiked influent, effluent and river water samples (10 µg L^{-1}) with numbers of samples used (n, n = 1 for each month). Influent and effluent samples with the pharmaceutical present were not used for quality control.

Class	Pharmaceutical	QC standards	n	Influent	n	Effluent	n	River	n
Analgesics	(±)-2-Hydroxyibuprofen	0.510 ± 0.0450	14	/	0	/	0	0.538 ± 0.0313	6
	(±)-Naproxen	0.500 ± 0.00864	14	/	0	/	0	0.560 ± 0.0253	7
Antibiotics	(±)-α-Hydroxytrimethoprim	0.499 ± 0.021	11	0.495 ± 0.0290	9	0.497 ± 0.0275	8	0.500 ± 0.0146	8
Anticoagulants	(±)-Warfarin	0.501 ± 0.00928	14	0.497 ± 0.0266	11	0.498 ± 0.0494	11	0.500 ± 0.0339	8
Antidepressants	(±)-Citalopram	0.506 ± 0.0130	11	/	0	0.483 ± 0.0746	2	0.505 ± 0.0121	8
	(±)-Desmethylcitalopram	0.506 ± 0.0276	13	0.489	2	0.499 ± 0.0115	8	0.488 ± 0.0497	6
	(±)-Desmethylvenlafaxine	0.499 ± 0.00714	14	/	0	0.468	1	0.494 ± 0.00662	8
	(±)-Fluoxetine	0.502 ± 0.0182	13	0.486 ± 0.125	4	0.516 ± 0.0789	5	/	0
	(±)-Venlafaxine	0.514 ± 0.0257	13	0.519 ± 0.0139	2	0.505 ± 0.0161	2	0.494 ± 0.0232	8
Anti-fungals	(±)-Climbazole	0.499 ± 0.00429	14	0.500 ± 0.0230	8	0.501 ± 0.0366	8	0.500 ± 0.0207	8
Antihistamines	(±)-Chlorpheniramine	0.501 ± 0.0249	10	0.499 ± 0.00742	8	0.484 ± 0.0217	7	0.504 ± 0.0108	8
Antiulcer	(±)-Lansoprazole	0.490 ± 0.0425	11	0.504 ± 0.0136	4	0.484 ± 0.0188	5	0.501 ± 0.00929	7
	(±)-Omeprazole	0.501 ± 0.0190	11	0.493 ± 0.00979	2	0.517 ± 0.0370	2	0.479 ± 0.0244	7
Benzodiazepines	(±)-Lorazepam	0.502 ± 0.0165	14	0.500 ± 0.0492	11	0.498 ± 0.0369	11	0.500 ± 0.0473	8
	(±)-Oxazepam	0.509 ± 0.0388	14	0.492 ± 0.0245	8	0.494 ± 0.0265	7	0.488 ± 0.0355	8
	(±)-Temazepam	0.514 ± 0.0339	14	0.489 ± 0.0278	6	0.486 ± 0.0134	3	0.498 ± 0.00453	8
Betablockers	(±)-Acebutolol	0.488 ± 0.0165	14	0.438 ± 0.0330	9	0.439 ± 0.0334	9	0.455 ± 0.0496	8
	(±)-Atenolol	0.501 ± 0.0105	14	0.494	1	0.545 ± 0.0329	2	0.505 ± 0.0133	8
	(±)-Bisoprolol	0.500 ± 0.00599	12	0.493 ± 0.0185	8	0.495 ± 0.0176	9	0.492 ± 0.00879	8
	(±)-Metoprolol	0.491 ± 0.00574	14	0.491 ± 0.0147	10	0.487 ± 0.00671	8	0.494 ± 0.00664	8
	(±)-Propranolol	0.507 ± 0.0173	13	0.524 ± 0.00418	2	0.550 ± 0.0587	4	0.481 ± 0.0530	8
	(±)-Salbutamol	0.494 ± 0.00801	14	0.503 ± 0.0374	9	0.499 ± 0.0221	10	0.499 ± 0.0119	8
	(±)-Sotalol	0.497 ± 0.0114	14	0.506 ± 0.0303	10	0.514 ± 0.0162	9	0.506 ± 0.00397	8
Chemotherapeutic	(±)-Ifosfamide	0.501 ± 0.00755	14	0.425 ± 0.0523	11	0.500 ± 0.0393	10	0.499 ± 0.0556	8
Wastewater discharge marker	(-)-Cotinine	0.000	14	/	0	/	0	/	0

S5 Quality Control: River water microcosms

In the river water calibrations, the ratios of the peak area against the peak area of the internal standard (area ratio, *ar*) or the peak area (*A*), when no deuterated surrogate was available, were plotted against the standard concentrations (c) for each analyte. A linear regression model (equation S3) was fitted, where m was the slope of the calibration line and b was the intercept with the y-axis.

$$ar = m \cdot c + b \text{ or } A = m \cdot c + b \quad \text{(S3)}$$

The coefficient of determination (R^2) was calculated (Table S8). Method detection (MDL) and quantification limits (MQL) were determined. Absolute (Abs. REC) and for analytes with deuterated surrogates corrected (Corr. REC) were calculated following equation S4 and S5 from peak areas (A) and area ratios (ar) of spiked and unspiked (US) samples and standards (std), respectively.

$$abs.REC = \frac{(A_{\text{spiked-}} - A_{\text{US}})}{A_{\text{std}}} \cdot 100\,\% \quad \text{(S4)}$$

$$corr.REC = \frac{(ar_{\text{spiked-}} - ar_{\text{US}})}{ar_{\text{std}}} \cdot 100\,\% \quad \text{(S5)}$$

Precision, the relative standard deviation of the replicates was calculated for every concentration above the MQL. Accuracies were determined from the percentage deviation of the standards from the calibration curve. Therefore, concentrations (c_{calc}) were calculated from the area ratios (ar) following subtraction of the calculated concentration (c_0) of the blank using equation S6.

$$c_{\text{calc}} = \frac{(\text{ar} - \text{b})}{\text{m}} - c_0 \quad \text{(S6)}$$

Accuracy was then calculated from the ratio of the calculated and standard concentration (c_{std}) according to equation S7.

$$\text{accuracy} = \frac{c_{\text{calc}}}{c_{\text{std}}} \cdot 100\,\% \quad \text{(S7)}$$

Table S8: Calibrations prepared in river water from river A and B with correlation coefficient (R^2), precision (pres.) and accuracy with relative standard deviation, and absolute (abs.) and corrected (corr.) recoveries (REC), and method detection (MDL) and quantification limits (MQL) for the microcosms.

Class	**Pharmaceutical**	**River A**			**River B**			**Microcosms (River A and B)**			
		R²	**Accuracy /%**	**Pres. /%**	***R²***	**Accuracy /%**	***Pres.* /%**	***Abs. REC /%***	***Corr. REC /%***	***MDL* / µg L⁻¹**	***MQL* / µg L⁻¹**
Analgesics	*E1*-Hydroxyibuprofen	0.997	95 ± 16	8.9	0.997	103 ± 9.1	5.6	89	-	0.28	0.42
	E2-Hydroxyibuprofen	0.999	98 ± 8.1	4.1	0.994	101 ± 17	3.7	114	-	0.22	0.33
	S(+)-Naproxen	> 0.999	95 ± 10	6.6	0.999	105 ± 15	6.8	103	-	0.073	0.29
Antibiotics	*E1*-α-Hydroxytrimethoprim	> 0.999	96 ± 8.0	3.8	0.999	106 ± 13	4.1	102	-	0.024	0.17

	E2-α-Hydroxytrimethoprim	> 0.999	97 ± 4.0	3.9	> 0.999	103 ± 12	8.8	100	-	0.17	0.37
Anticoagulants	*E1*-Warfarin	> 0.999	105 ± 13	1.5	0.995	101 ± 17	0.70	100	-	0.025	0.62
	E2-Warfarin	> 0.999	100 ± 5.2	2.7	0.995	102 ± 18	1.1	99	-	0.025	0.53
Antidepressants	*R*(-)-Citalopram	0.999	98 ± 15	1.4	0.993	100 ± 15	1.6	100	84	0.013	0.63
	S(+)-Citalopram	0.998	98 ± 14	1.3	0.994	100 ± 13	1.8	100	84	0.025	0.75
	R(-)-Desmethylcitalopram	0.999	98 ± 12	1.5	0.994	93 ± 7.3	1.7	81	-	0.12	0.93
	S(+)-Desmethylcitalopram	0.999	97 ± 17	2.8	0.996	95 ± 5.6	1.0	82	-	0.21	0.91
	S(+)-Desmethylvenlafaxine	> 0.999	103 ± 9.1	0.78	0.999	103 ± 12	2.0	101	-	0.019	0.14
	R(-)-Desmethylvenlafaxine	> 0.999	103 ± 8.9	1.1	0.999	100 ± 16	2.6	101	-	0.025	0.15
	R(-)-Fluoxetine	0.992	110 ± 17	1.8	0.999	101 ± 6.4	2.8	3	98	7.5	22
	S(+)-Fluoxetine	0.992	110 ± 17	4.3	0.999	107 ± 11	5.6	4	84	9.4	44
	S(+)-Venlafaxine	> 0.999	90 ± 14	1.3	0.993	95 ± 7.7	2.3	100	101	0.12	0.75
	R(-)-Venlafaxine	> 0.999	100 ± 6.7	3.1	> 0.999	98 ± 16	1.5	100	101	0.075	0.15
Anti-fungals	*E1*-Climbazole	> 0.999	97 ± 10	1.8	0.996	103 ± 19	1.5	88	-	0.028	0.62
	E2-Climbazole	> 0.999	96 ± 11	1.1	0.996	105 ± 20	2.1	89	-	0.028	0.62
Antihistamines	*R*(-)-Chlorpheniramine	0.994	119 ± 16	0.68	0.993	97 ± 12	2.2	90	85	0.028	0.69
	S(+)-Chlorpheniramine	0.998	97 ± 15	0.93	0.994	97 ± 11	0.88	90	85	0.028	0.70
Antiulcer	*E1*-Lansoprazole	> 0.999	95 ± 10	1.9	0.997	107 ± 20	1.1	96	-	0.026	0.31
	E2-Lansoprazole	> 0.999	93 ± 13	2.0	0.998	107 ± 19	1.2	98	-	0.025	0.30
	R(+)-Omeprazole	> 0.999	94 ± 12	0.73	0.995	102 ± 17	0.73	100	-	0.025	0.55
	S(-)-Omeprazole	> 0.999	95 ± 10	1.0	0.996	102 ± 16	1.1	99	-	0.025	0.56
Benzodiazepines	*E1*-Lorazepam	> 0.999	93 ± 11	6.3	0.993	101 ± 12	6.2	96	96	0.39	0.52
	E2-Lorazepam	> 0.999	94 ± 10	4.4	0.993	101 ± 15	5.8	96	99	0.39	0.52
	E1-Oxazepam	0.999	100 ± 8.3	8.4	0.994	102 ± 14	4.4	97	95	0.19	0.39
	E2-Oxazepam	> 0.999	99 ± 9.4	5.9	0.994	104 ± 14	4.8	101	99	0.25	0.37
	E1-Temazepam	> 0.999	97 ± 13	3.6	0.997	99 ± 7.9	1.9	99	99	0.050	0.63
	E2-Temazepam	> 0.999	94 ± 15	4.1	0.998	103 ± 14	1.9	99	103	0.051	0.38
Betablockers	*E1*-Acebutolol	> 0.999	96 ± 11	1.6	0.994	98 ± 10	1.3	101	101	0.025	0.52
	E2-Acebutolol	> 0.999	96 ± 12	1.7	0.994	98 ± 10	2.4	96	104	0.026	0.29
	R(+)-Atenolol	> 0.999	103 ± 7.2	1.4	0.993	95 ± 8.6	1.6	93	100	0.040	0.59
	S(-)-Atenolol	> 0.999	101 ± 6.2	3.9	0.994	95 ± 7.2	1.2	99	98	0.038	0.53
	E1-Bisoprolol	> 0.999	96 ± 12	2.5	> 0.999	99 ± 15	2.6	91	102	0.014	0.055
	E2-Bisoprolol	> 0.999	94 ± 14	1.7	0.994	98 ± 11	2.1	99	102	0.013	0.55
	S(-)-Metoprolol	> 0.999	99 ± 3.6	2.7	0.991	97 ± 10	2.6	96	98	0.026	0.55
	R(+)-Metoprolol	> 0.999	96 ± 10	3.0	0.995	96 ± 8.5	2.0	101	102	0.037	0.54
	R(+)-Propranolol	> 0.999	98 ± 9.0	3.5	0.993	95 ± 7.7	1.5	90	99	0.028	0.61
	S(-)-Propranolol	> 0.999	98 ± 9.0	1.6	0.995	96 ± 6.9	1.5	89	98	0.042	0.62
	E1-Salbutamol	> 0.999	105 ± 10	1.9	0.996	109 ± 20	2.0	75	98	0.013	0.74
	E2-Salbutamol	> 0.999	96 ± 12	3.3	0.996	101 ± 9.0	0.99	80	87	0.013	0.64
	E1-Sotalol	> 0.999	101 ± 2.8	3.8	> 0.999	97 ± 13	3.9	98	98	0.013	0.038

	E2-Sotalol	> 0.999	97 ± 6.6	4.0	0.999	100 ± 16	4.6	97	99	0.026	0.052
Chemotherapeutic	*E1*-Ifosfamide	> 0.999	101 ± 4.3	1.2	0.992	102 ± 20	1.8	98	-	0.026	0.64
	E2-Ifosfamide	> 0.999	101 ± 4.1	1.1	0.993	101 ± 19	1.2	98	-	0.026	0.64
Wastewater discharge marker	*S*(-)-Cotinine	> 0.999	102 ± 6.4	0.94	0.993	96 ± 9.3	2.2	99	98	0.013	1.3

S6 Results

Table S9: ST influent and effluent concentrations (25th percentile, mean, 75th percentile, maximum) in µg L^{-1} with numbers of samples ≥ MQL (n).

Class	**Pharmaceutical**	**Influent**					**Effluent**				
		25th	Mean	75th	Max	n	25th	Mean	75th	Max	n
Analgesics	*E1*-Hydroxyibuprofen	1.3	8.8	13	63	58	2.0	6.8	9.6	29	58
	E2-Hydroxyibuprofen	3.7	40	40	340	58	8.6	29	39	124	58
	R(-)-Naproxen	$9.8 \cdot 10^{-3}$	0.12	0.065	1.6	22	0.011	0.029	0.031	0.21	28
	S(+)-Naproxen	0.46	11	9.9	234	58	2.3	6.9	9.0	34	58
Antibiotics	*E1*-α-Hydroxytrimethoprim	0.014	0.14	0.063	1.3	12	0.019	0.033	0.046	0.10	18
	E2-α-Hydroxytrimethoprim	$5.6 \cdot 10^{-3}$	0.039	0.021	0.33	12	$6.1 \cdot 10^{-3}$	0.013	0.018	0.037	18
Anticoagulants	*E1*-Warfarin	0.014	0.073	0.044	0.45	11	$8.3 \cdot 10^{-3}$	0.021	0.025	0.071	14
	E2-Warfarin	0.012	0.077	0.053	0.39	7	$6.8 \cdot 10^{-3}$	0.015	0.018	0.033	9
Antidepressants	*R*(-)-Citalopram	0.010	0.50	0.080	22	52	0.018	0.088	0.13	0.39	50
	S(+)-Citalopram	$8.6 \cdot 10^{-3}$	0.35	0.057	15	52	0.013	0.057	0.082	0.27	50
	R(-)-Desmethylcitalopram	0.012	0.065	0.10	0.49	39	0.013	0.046	0.074	0.20	44
	S(+)-Desmethylcitalopram	0.021	0.083	0.12	0.66	39	0.011	0.044	0.070	0.17	44
	S(+)-Desmethylvenlafaxine	0.031	0.20	0.24	1.4	51	0.041	0.22	0.17	3.6	53
	R(-)-Desmethylvenlafaxine	0.044	0.40	0.49	3.2	51	0.076	0.41	0.32	5.5	53
	R(-)-Fluoxetine	0.013	0.026	0.023	0.14	23	0.020	0.040	0.046	0.17	24
	S(+)-Fluoxetine	0.037	0.074	0.063	0.36	23	0.061	0.10	0.11	0.32	24
	S(+)-Venlafaxine	0.074	0.88	0.55	14	52	0.12	0.46	0.49	5.5	53
	R(-)-Venlafaxine	0.041	0.65	0.34	11	52	0.068	0.32	0.34	3.4	53
Anti-fungals	*E1*-Climbazole	$6.7 \cdot 10^{-3}$	0.040	0.059	0.11	3	0.037	0.11	0.085	0.39	6
	E2-Climbazole	$6.3 \cdot 10^{-3}$	0.028	0.040	0.071	3	0.039	0.11	0.11	0.35	6
Antihistamines	*S*(+)-Chlorpheniramine	$3.9 \cdot 10^{-3}$	0.058	0.028	0.62	39	0.012	0.072	0.073	0.46	41
	R(-)-Chlorpheniramine	$3.2 \cdot 10^{-3}$	0.053	0.026	0.59	39	0.011	0.059	0.078	0.36	41
Antiulcer	*E1*-Lansoprazole	0.069	1.9	1.8	12	10	0.22	2.6	1.3	21	16
	E2-Lansoprazole	0.078	2.0	1.9	13	10	0.20	2.9	1.4	24	16
	R(+)-Omeprazole	0.096	1.8	1.3	12	21	0.13	1.5	1.2	16	29
	S(-)-Omeprazole	0.13	2.8	1.3	31	21	0.14	2.5	1.3	33	30
Benzodiazepines	*E1*-Lorazepam	< MQL	< MQL	< MQL	< MQL	0	< MQL	< MQL	< MQL	< MQL	0

	E2-Lorazepam	< MQL	< MQL	< MQL	< MQL	0	< MQL	< MQL	< MQL	< MQL	0
	E1-Oxazepam	0.014	0.048	0.033	0.38	17	0.016	0.040	0.053	0.15	21
	E2-Oxazepam	0.011	0.048	0.031	0.40	17	0.017	0.041	0.060	0.16	21
	E1-Temazepam	0.013	0.14	0.078	0.89	23	0.016	0.12	0.16	0.77	28
	E2-Temazepam	0.013	0.17	0.11	1.1	23	0.020	0.14	0.16	0.95	28
Betablockers	*E1*-Acebutolol	$1.4 \cdot 10^{-3}$	$2.6 \cdot 10^{-3}$	$3.7 \cdot 10^{-3}$	$4.9 \cdot 10^{-3}$	2	0.019	0.023	0.026	0.029	2
	E2-Acebutolol	$1.3 \cdot 10^{-3}$	$2.0 \cdot 10^{-3}$	$2.8 \cdot 10^{-3}$	$3.6 \cdot 10^{-3}$	2	0.020	0.025	0.030	0.035	2
	R(+)-Atenolol	0.13	1.2	0.85	15	52	0.26	0.72	0.86	3.0	53
	S(-)-Atenolol	0.11	1.0	0.85	9.7	52	0.23	0.72	0.74	3.2	53
	E1-Bisoprolol	0.016	0.12	0.17	0.94	51	0.016	0.069	0.095	0.24	52
	E2-Bisoprolol	0.015	0.12	0.15	1.1	51	0.016	0.069	0.090	0.24	52
	S(-)-Metoprolol	0.014	0.089	0.14	0.27	8	0.019	0.12	0.11	0.59	12
	R(+)-Metoprolol	0.012	0.087	0.15	0.23	8	0.019	0.13	0.12	0.70	12
	R(+)-Propranolol	0.024	0.19	0.27	1.1	49	0.048	0.17	0.21	0.79	49
	S(-)-Propranolol	0.028	0.21	0.23	1.1	49	0.067	0.18	0.22	0.62	49
	E1-Salbutamol	$2.4 \cdot 10^{-3}$	0.014	0.023	0.084	40	$3.2 \cdot 10^{-3}$	0.011	0.015	0.057	49
	E2-Salbutamol	$4.5 \cdot 10^{-3}$	0.026	0.035	0.16	40	$5.5 \cdot 10^{-3}$	0.022	0.028	0.16	49
	E1-Sotalol	$8.2 \cdot 10^{-3}$	0.039	0.042	0.16	7	$1.7 \cdot 10^{-3}$	0.065	0.054	0.47	20
	E2-Sotalol	$9.2 \cdot 10^{-3}$	0.037	0.040	0.14	6	$2.2 \cdot 10^{-3}$	0.063	0.053	0.46	20
Chemotherapeutic	*E1*-Ifosfamide	< MQL	< MQL	< MQL	< MQL	0	$3.9 \cdot 10^{-3}$	$3.9 \cdot 10^{-3}$	$3.9 \cdot 10^{-3}$	$3.9 \cdot 10^{-3}$	1
	E2-Ifosfamide	< MQL	< MQL	< MQL	< MQL	0	$5.6 \cdot 10^{-3}$	$5.6 \cdot 10^{-3}$	$5.6 \cdot 10^{-3}$	$5.6 \cdot 10^{-3}$	1
Wastewater discharge marker	*R*(+)-Cotinine	$1.6 \cdot 10^{-3}$	0.041	0.040	0.57	57	$2.6 \cdot 10^{-3}$	0.030	0.029	0.22	58
	S(-)-Cotinine	0.27	2.2	3	9.9	58	0.46	1.8	2.7	6.8	58

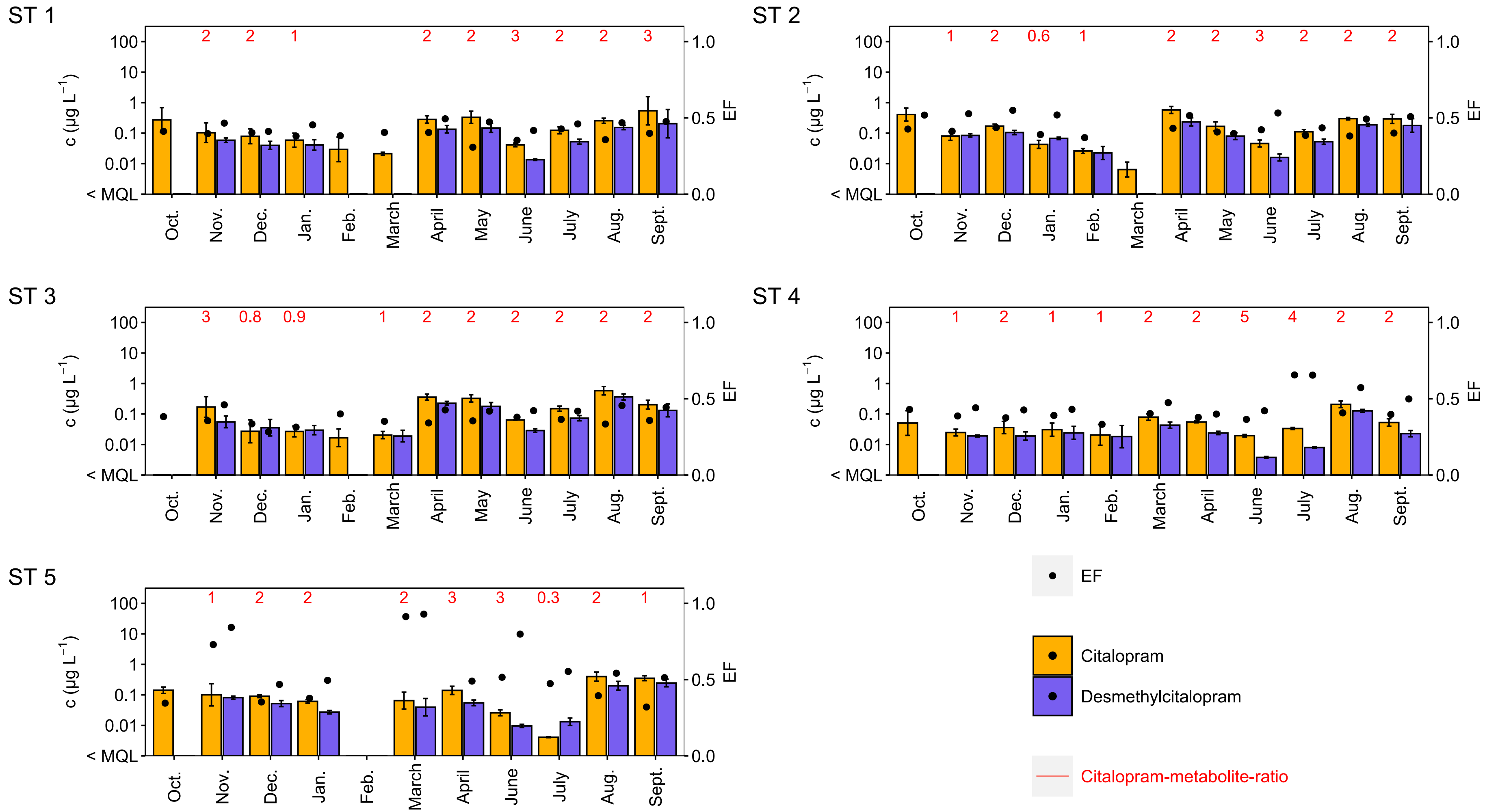

Figure S2: Effluent concentrations (c in ug L^{-1}, logarithmic scale) of citalopram and desmethylcitalopram in ST 1 – 5 with enantiomeric fractions and citalopram-metabolite-ratios. Concentrations are also shown when < MQL in the enantioselective method and no EFs could be calculated. ST 4 and 5 could not be sampled in May.

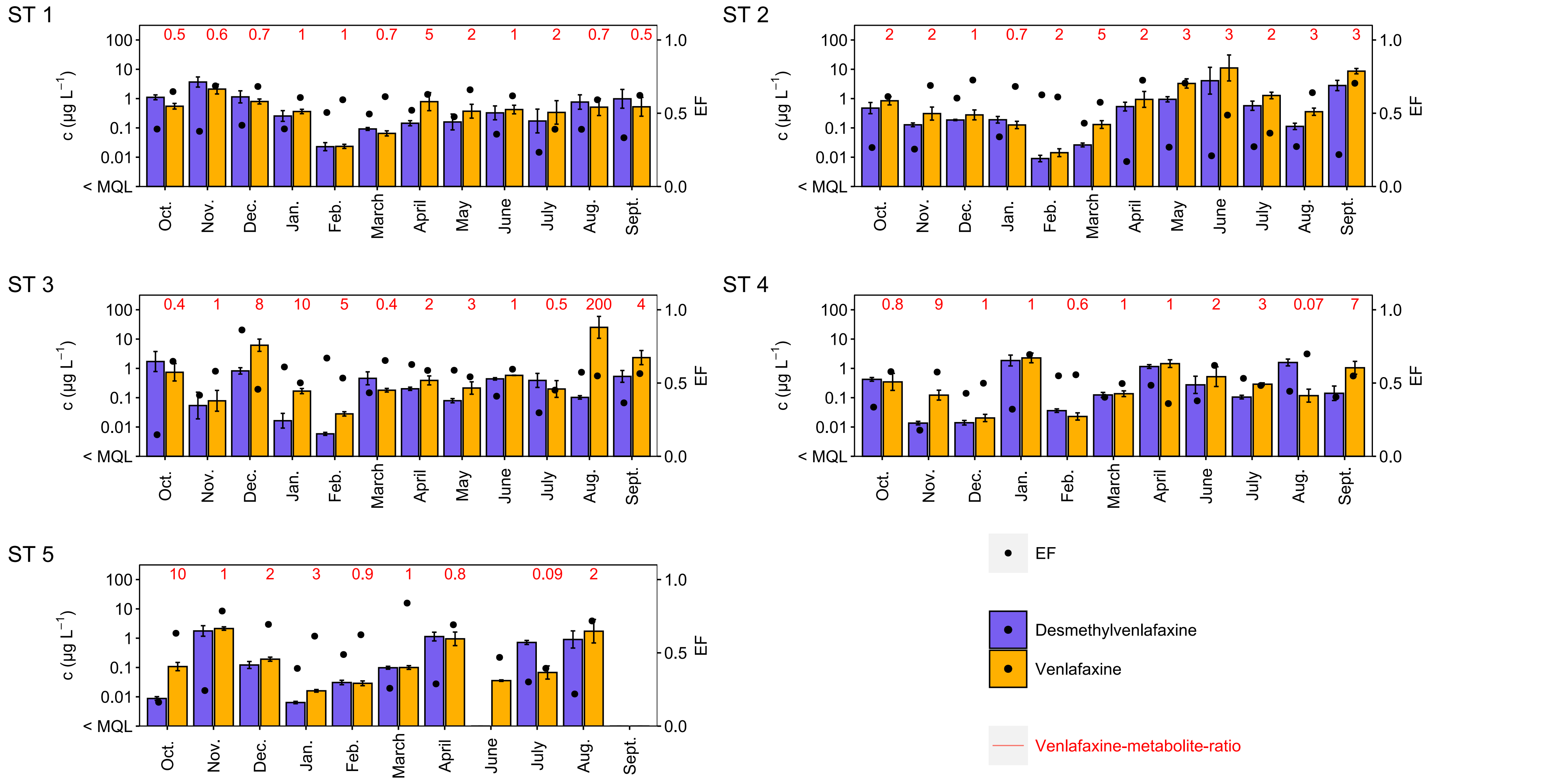

Figure S3: Influent concentrations (c in ug L^{-1}, logarithmic scale) of venlafaxine and desmethylvenlafaxine in ST 1 – 5 with enantiomeric fractions and venlafaxine-metabolite-ratios. ST 4 and 5 could not be sampled in May.

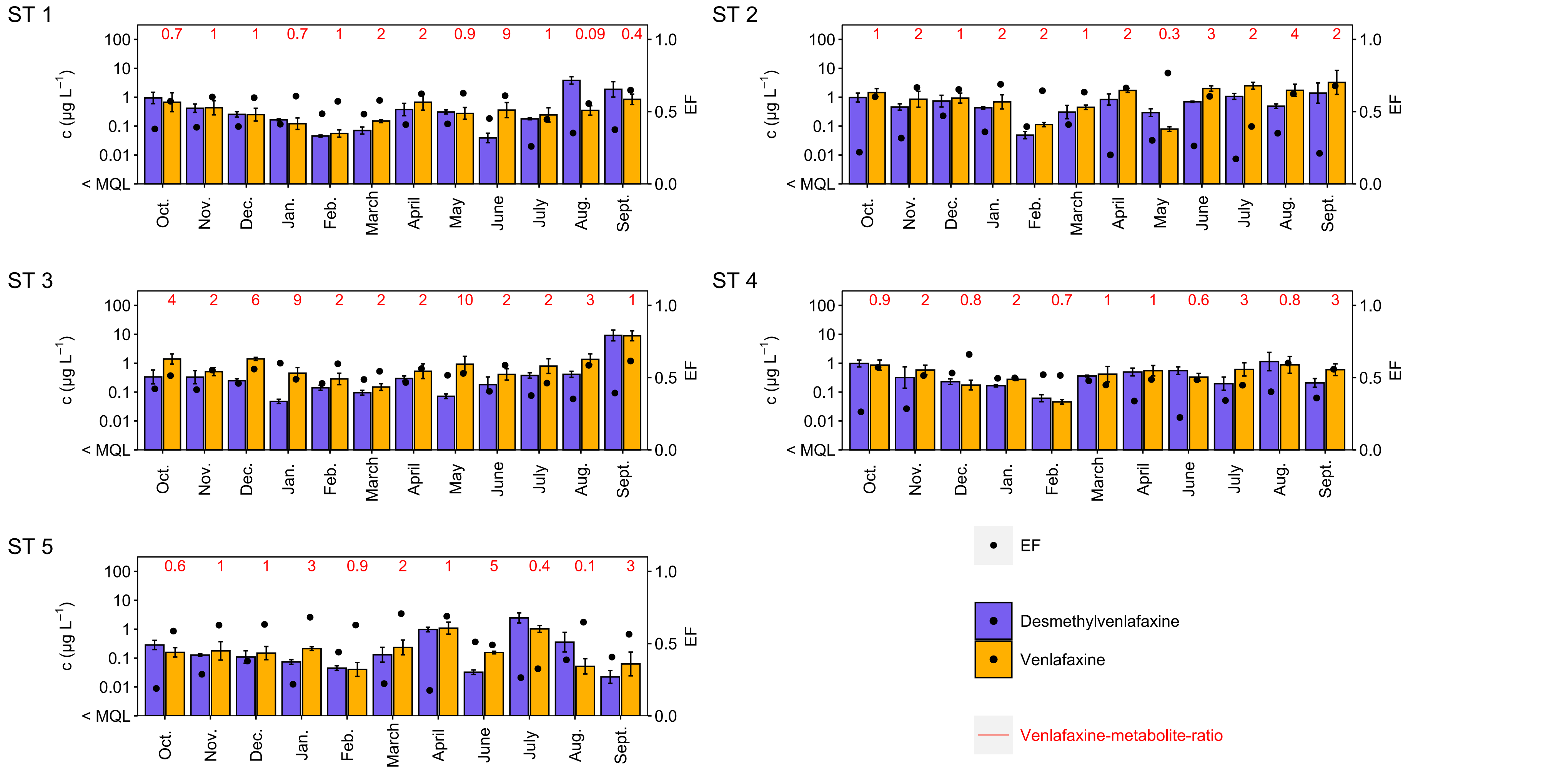

Figure S4: Effluent concentrations (c in ug L^{-1}, logarithmic scale) of venlafaxine and desmethylvenlafaxine in ST 1 – 5 with enantiomeric fractions and venlafaxine-metabolite-ratios. ST 4 and 5 could not be sampled in May.

Table S10: Class, Pharmaceutical, predicted no-effect concentration (PNEC) and how it was determined, e.g., lowest available enantiospecific PNEC or half of the lowest available PNEC of the racemic mixture.[10] Risk quotients were calculated from the PNEC and measured concentrations in rivers.

Class	**Pharmaceutical**	**PNEC / µg L^{-1}**	**Source**	**RQ**
Analgesics	*E1*-Hydroxyibuprofen	3.9	Half of racemic	< MDL – $7.4 \cdot 10^{-3}$
	E2-Hydroxyibuprofen	3.9	Half of racemic	< MDL – $4.2 \cdot 10^{-3}$
	R(-)-Naproxen	1.7	PNEC of S(+)	< MDL – $3.6 \cdot 10^{-4}$
	S(+)-Naproxen	1.7	Enantiospecific	< MDL – 0.056
Antibiotics	*E1*-α-Hydroxytrimethoprim	0.14	Half of racemic	< MDL
	E2-α-Hydroxytrimethoprim	0.14	Half of racemic	< MDL
Anticoagulants	*E1*-Warfarin	0.38	Half of racemic	< MDL
	E2-Warfarin	0.38	Half of racemic	< MDL
Antidepressants	*R*(-)-Citalopram	8.0	Half of racemic	< MDL – $4.2 \cdot 10^{-4}$
	S(+)-Citalopram	2.7	Enantiospecific	< MDL – $6.6 \cdot 10^{-4}$
	R(-)-Desmethylcitalopram	0.25	Half of racemic	< MDL
	S(+)-Desmethylcitalopram	0.25	Half of racemic	< MDL
	S(+)-Desmethylvenlafaxine	3.3	Half of racemic	< MDL – $6.7 \cdot 10^{-4}$
	R(-)-Desmethylvenlafaxine	3.3	Half of racemic	< MDL – $5.6 \cdot 10^{-4}$
	S(+)-Fluoxetine	0.050	Half of racemic	< MDL
	R(-)-Fluoxetine	0.050	Half of racemic	< MDL
	S(+)-Venlafaxine	0.44	Half of racemic	< MDL – $2.1 \cdot 10^{-4}$
	R(-)-Venlafaxine	0.44	Half of racemic	< MDL – $7.0 \cdot 10^{-4}$
Anti-fungals	*E1*-Climbazole	0.056	Half of racemic	< MDL
	E2-Climbazole	0.056	Half of racemic	< MDL
Antihistamines	*S*(+)-Chlorpheniramine	0.33	Enantiospecific	< MDL – $4.5 \cdot 10^{-3}$
	R(-)-Chlorpheniramine	0.78	Half of racemic	< MDL – $1.1 \cdot 10^{-3}$
Antiulcer	*E1*-Lansoprazole	0.24	Half of racemic	< MDL
	E2-Lansoprazole	0.24	Half of racemic	< MDL
	S(-)-Omeprazole	100	Enantiospecific	< MDL
	R(+)-Omeprazole	9.1	Half of racemic	< MDL
Benzodiazepines	*E1*-Lorazepam	0.048	Half of racemic	< MDL – 0.25
	E2-Lorazepam	0.048	Half of racemic	< MDL – 0.23
	E1-Oxazepam	0.19	Half of racemic	< MDL
	E2-Oxazepam	0.19	Half of racemic	< MDL
	E1-Temazepam	0.035	Half of racemic	< MDL
	E2-Temazepam	0.035	Half of racemic	< MDL
Betablockers	*E1*-Acebutolol	1.5	Half of racemic	< MDL
	E2-Acebutolol	1.5	Half of racemic	< MDL
	R(+)-Atenolol	75	Half of racemic	< MDL – $2.2 \cdot 10^{-5}$
	S(-)-Atenolol	75	Half of racemic	< MDL – $2.3 \cdot 10^{-5}$
	E1-Bisoprolol	46	Half of racemic	< MDL – $1.0 \cdot 10^{-3}$
	E2-Bisoprolol	46	Half of racemic	< MDL – $1.0 \cdot 10^{-3}$
	S(-)-Metoprolol	4.3	Half of racemic	< MDL
	R(+)-Metoprolol	4.3	Half of racemic	< MDL
	S(-)-Propranolol	0.10	Half of racemic	< MDL – 0.32
	R(+)-Propranolol	0.10	Half of racemic	< MDL – 0.29
	E1-Salbutamol	500	Half of racemic	< MDL
	E2-Salbutamol	500	Half of racemic	< MDL
	E1-Sotalol	3.3	Half of racemic	< MDL
	E2-Sotalol	3.3	Half of racemic	< MDL
Chemotherapeutic	*E1*-Ifosfamide	3.5	Half of racemic	< MDL
	E2-Ifosfamide	3.5	Half of racemic	< MDL
Wastewater discharge marker	*S*(-)-Cotinine	4.7	Half of racemic	< MDL – $4.5 \cdot 10^{-3}$
	R(+)-Cotinine	4.7	Half of racemic	< MDL – $2.6 \cdot 10^{-5}$

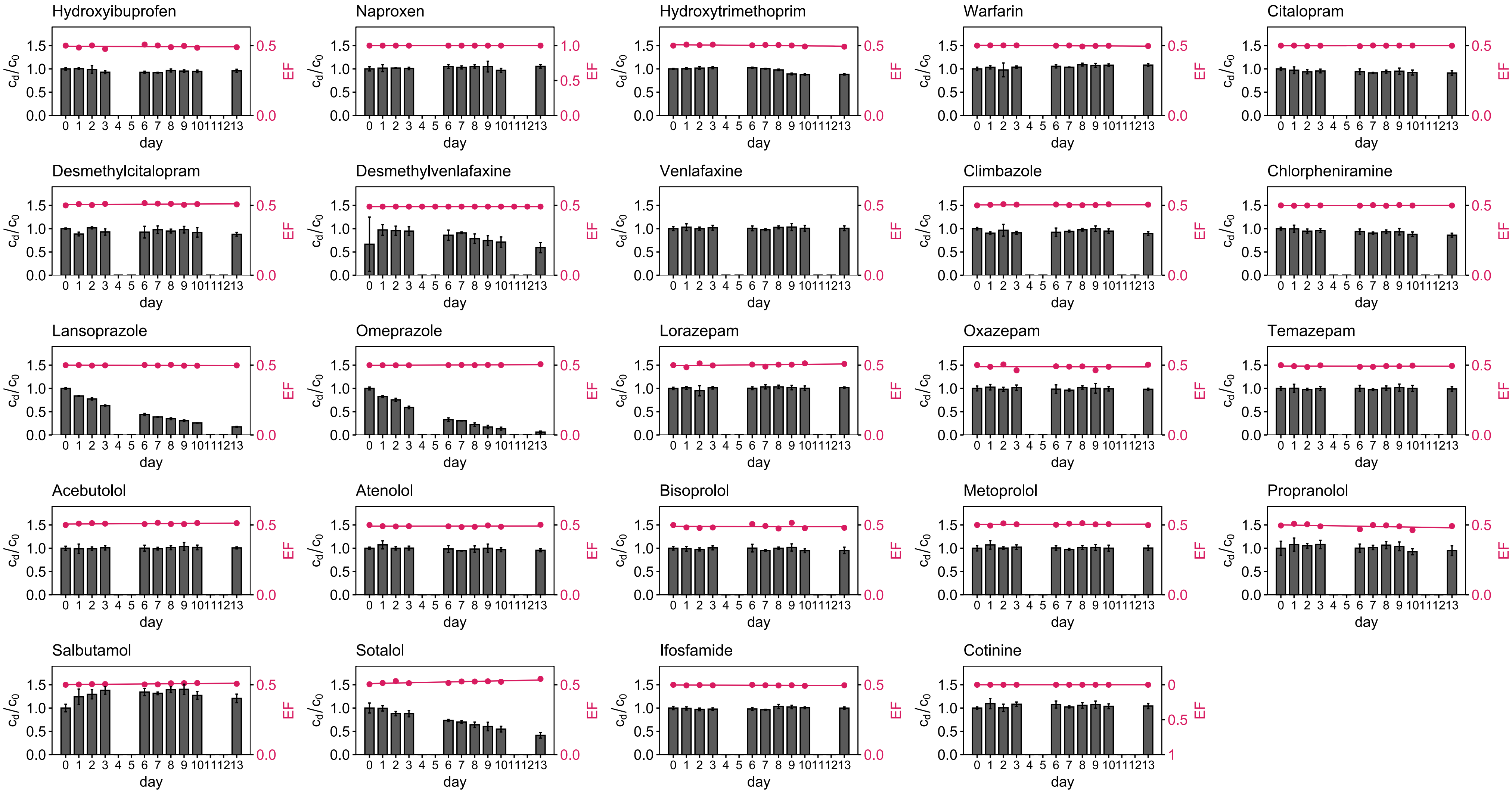

Figure S5: Enantiomeric fraction (EF) and relative concentration, the concentration at a specific day (c_d) divided by the concentration at the start of the experiment (c_0), in biotic mixed-compound river A microcosms (triplicate).

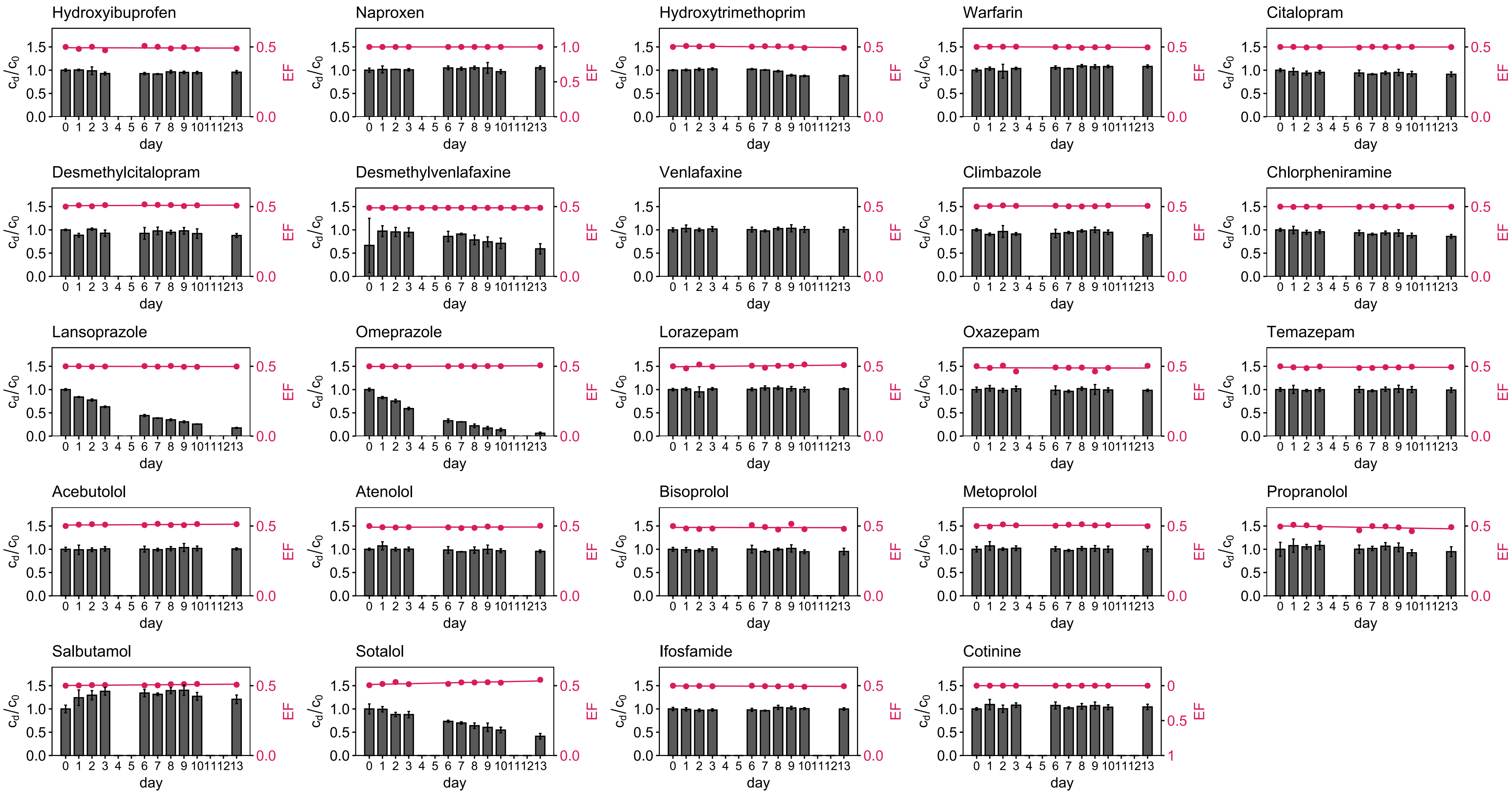

Figure S6: Enantiomeric fraction (EF) and relative concentration, the concentration at a specific day (c_d) divided by the concentration at the start of the experiment (c_0), in abiotic mixed-compound river A microcosms (triplicate). NaN_3 reduces the sensitivity in the Chiral-V method slightly and impacts the peak separation for venlafaxine. Hence only non-enantioselective degradation was investigated.

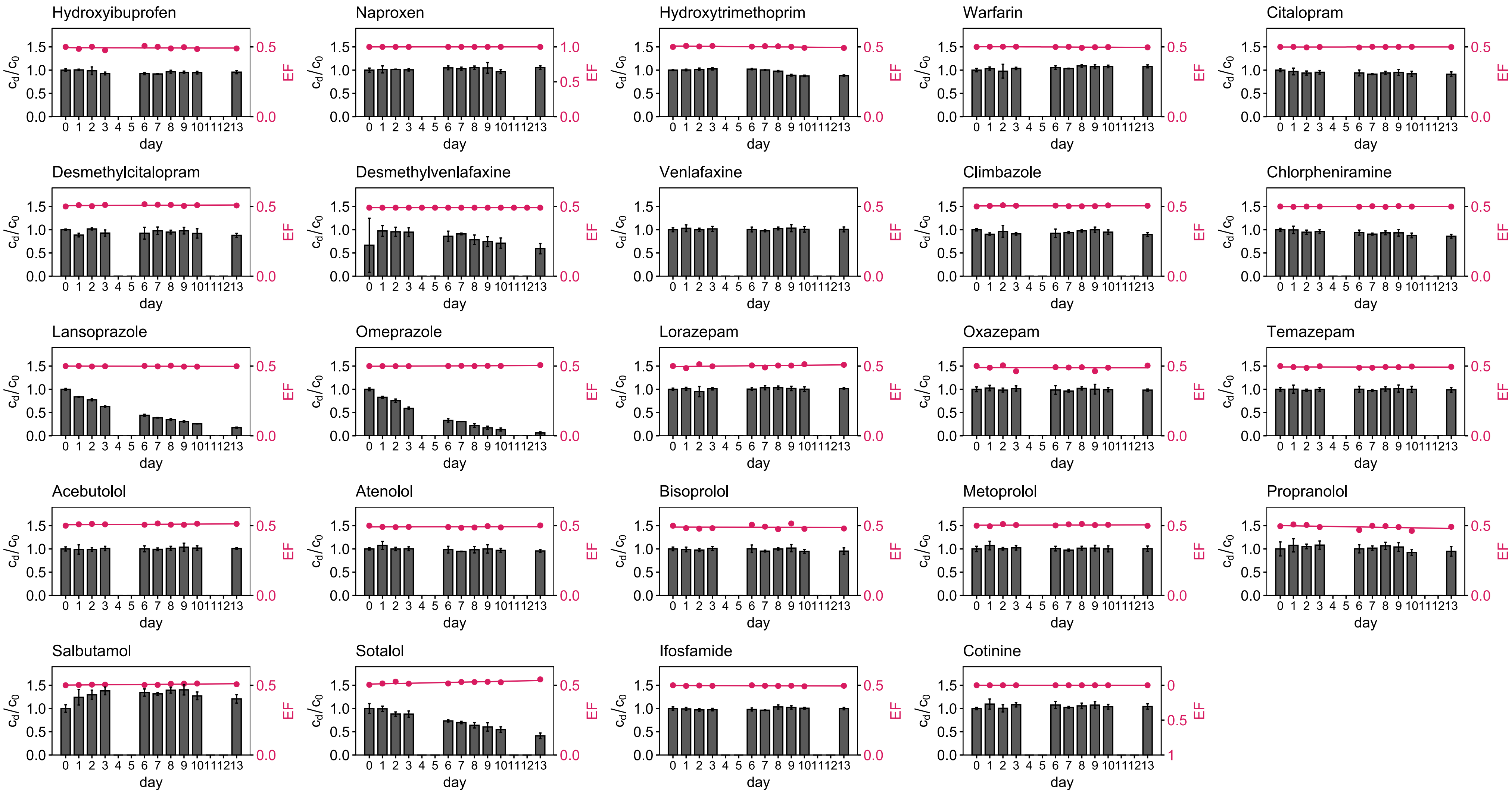

Figure S7: Enantiomeric fraction (EF) and relative concentration, the concentration at a specific day (c_d) divided by the concentration at the start of the experiment (c_0), in abiotic mixed-compound river B microcosms (triplicate). NaN_3 reduces the sensitivity in the Chiral-V method slightly and impacts the peak separation for venlafaxine. Hence only non-enantioselective degradation was investigated.

Table S11: Linear correlation coefficient (R^2) for the degradation of pharmaceuticals in biotic and abiotic river A and B microcosms following equation (2), and degradation constant (k) and half-life ($t_{1/2}$) for pharmaceuticals degraded following the first-order exponential degradation model ($R^2 \geq 0.7$). Enantiomeric fractions at the start and end of the experiment (EF_0, EF_{13}) for all pharmaceuticals. Fluoxetine was < MQL and excluded.

Pharmaceutical	**Biotic A**					**Abiotic A**					**Biotic B**					**Abiotic B**				
	R^2	*k*	$t_{1/2}$	EF_0	EF_{13}	R^2	*k*	$t_{1/2}$	EF_0	EF_{13}	R^2	*k*	$t_{1/2}$	EF_0	EF_{13}	R^2	*k*	$t_{1/2}$	EF_0	EF_{13}
E1-Hydroxyibuprofen	0.510	-	-	0.50	0.49	0.529	-	-	0.50	0.48	0.185	-	-	0.50	0.52	0.242	-	-	0.50	0.49
E2-Hydroxyibuprofen	0.603	-	-			0.530	-	-			0.006	-	-			0.157	-	-		
S(+)-Naproxen	0.722	0.016	43	1.00	1.00	0.605	-	-	1.00	1.00	0.715	0.010	71	1.00	1.00	0.116	-	-	1.00	1.00
E1-Hydroxytrimethoprim	0.097	-	-	0.50	0.47	0.021	-	-	0.50	0.48	0.929	0.030	23	0.50	0.47	0.629	-	-	0.50	0.49
E2-Hydroxytrimethoprim	0.289	-	-			0.507	-	-			0.789	0.019	37			0.617	-	-		
E1-Warfarin	0.696	-	-	0.50	0.50	0.227	-	-	0.50	0.50	0.430	-	-	0.50	0.50	0.630	-	-	0.50	0.50
E2-Warfarin	0.692	-	-			0.193	-	-			0.490	-	-			0.665	-	-		
R(-)-Citalopram	0.349	-	-	0.50	0.49	0.467	-	-	0.50	0.50	0.273	-	-	0.50	0.50	0.649	-	-	0.50	0.50
S(+)-Citalopram	0.432	-	-			0.490	-	-			0.111	-	-			0.560	-	-		
R(-)-Desmethylcitalopram	0.276	-	-	0.50	0.52	0.605	-	-	0.50	0.50	0.749	0.013	53	0.50	0.51	0.154	-	-	0.50	0.51
S(+)-Desmethylcitalopram	0.394	-	-			0.611	-	-			0.559	-	-			0.130	-	-		
S(+)-Desmethylvenlafaxine	0.929	0.023	30	0.50	0.50	0.940	0.036	19	0.50	0.50	0.929	0.009	78	0.50	0.50	0.879	0.036	19	0.51	0.59
R(-)-Desmethylvenlafaxine	0.925	0.023	30			0.935	0.033	21			0.975	0.010	69			0.770	0.047	15		
S(+)-Venlafaxine	0.169	-	-	0.50	0.50	0.213	-	-	/ [a]	/ [a]	0.469	-	-	0.50	0.50	0.001	-	-	/ [a]	/ [a]
R(-)-Venlafaxine	0.188	-	-								0.320	-	-				-	-		
E1-Climbazole	0.174	-	-	0.50	0.50	$5.2 \cdot 10^{-5}$	-	-	0.50	0.50	0.339	-	-	0.50	0.51	0.016	-	-	0.50	0.51
E2-Climbazole	0.155	-	-			0.002	-	-			0.464	-	-			0.019	-	-		
S(+)-Chlorpheniramine	0.781	0.024	29	0.50	0.51	0.724	0.021	33	0.50	0.51	0.853	0.012	56	0.50	0.50	0.851	0.011	65	0.50	0.50
R(-)-Chlorpheniramine	0.804	0.023	31			0.722	0.019	36			0.828	0.012	57			0.846	0.010	67		
E1-Lansoprazole	0.999	0.150	4.6	0.50	0.50	0.996	0.150	4.6	0.50	0.50	0.985	0.177	3.9	0.50	0.48	0.998	0.132	5.3	0.50	0.50
E2-Lansoprazole	0.999	0.150	4.6			0.995	0.151	4.6			0.985	0.172	4.0			0.998	0.131	5.3		
E1-Omeprazole	0.998	0.097	7.1	0.50	0.50	0.994	0.126	5.5	0.50	0.50	0.977	0.112	6.2	0.50	0.50	0.987	0.212	3.3	0.50	0.51
E2-Omeprazole	0.998	0.098	7.1			0.994	0.126	5.5			0.977	0.112	6.2			0.987	0.210	3.3		
E1-Lorazepam	0.403	-	-	0.50	0.50	0.314	-	-	0.50	0.50	0.070	-	-	0.50	0.50	0.682	-	-	0.50	0.51
E2-Lorazepam	0.428	-	-			0.193	-	-			0.593	-	-			0.005	-	-		
E1-Oxazepam	0.348	-	-	0.50	0.51	0.347	-	-	0.50	0.51	0.002	-	-	0.50	0.52	0.077	-	-	0.50	0.50
E2-Oxazepam	0.195	-	-			0.569	-	-			0.416	-	-			0.017	-	-		
E1-Temazepam	0.102	-	-	0.50	0.51	0.222	-	-	0.50	0.50	0.155	-	-	0.50	0.50	$6.6 \cdot 10^{-5}$	-	-	0.50	0.49
E2-Temazepam	0.189	-	-			0.230	-	-			0.260	-	-			0.018	-	-		
E1-Acebutolol	0.220	-	-	0.50	0.51	0.277	-	-	0.50	0.52	0.269	-	-	0.50	0.50	0.645	-	-	0.50	0.51
E2-Acebutolol	0.266	-	-			$1.1 \cdot 10^{-4}$	-	-			0.122	-	-			0.052	-	-		
R(+)-Atenolol	0.328	-	-	0.50	0.51	0.212	-	-	0.50	0.49	0.675	-	-	0.50	0.50	0.392	-	-	0.50	0.50

S(-)-Atenolol	0.304	-	-			0.340	-	-			0.774	0.011	61			0.477	-	-		
E1-Bisoprolol	0.661	-	-	0.50	0.49	0.448	-	-	0.50	0.46	0.557	-	-	0.50	0.51	0.074	-	-	0.50	0.48
E2-Bisoprolol	0.646	-	-			0.394	-	-			0.671	-	-			0.162	-	-		
S(-)-Metoprolol	0.745	0.020	34	0.50	0.49	0.379	-	-	0.50	0.51	0.718	0.010	69	0.50	0.50	0.105	-	-	0.50	0.50
R(+)-Metoprolol	0.761	0.025	28			0.191	-	-			0.686	-	-			0.109	-	-		
S(-)-Propranolol	0.146	-	-	0.50	0.51	0.186	-	-	0.50	0.47	0.869	0.014	48	0.50	0.50	0.331	-	-	0.50	0.49
R(+)-Propranolol	0.361	-	-			0.004	-	-			0.821	0.014	49			0.118	-	-		
E1-Salbutamol	0.196	-	-	0.50	0.49	0.600	-	-	0.50	0.51	0.224	-	-	0.50	0.48	0.167	-	-	0.50	0.51
E2-Salbutamol	0.382	-	-			0.427	-	-			0.241	-	-			0.099	-	-		
E1-Sotalol	0.365	-	-	0.50	0.50	0.762	0.016	43	0.50	0.51	0.708	0.011	63	0.50	0.50	0.977	0.062	11	0.50	0.54
E2-Sotalol	0.367	-	-			0.698	-	-			0.756	0.011	65			0.968	0.070	9.9		
E1-Ifosfamide	0.559	-	-	0.50	0.50	0.465	-	-	0.50	0.50	$2.0 \cdot 10^{-5}$	-	-	0.50	0.49	0.099	-	-	0.50	0.50
E2-Ifosfamide	0.468	-	-			0.435	-	-			0.243	-	-			0.198	-	-		
S(-)-Cotinine	0.330	-	-	0.00	0.00	0.359	-	-	0.00	0.00	0.131	-	-	0.00	0.00	0.014	-	-	0.00	0.00

[a] NaN_3 reduces the sensitivity in the Chiral-V method slightly and impacts the peak separation for venlafaxine. Hence only non-enantioselective degradation was investigated.

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