Assessment of human pharmaceuticals in drinking water catchments, tap and drinking fountain waters

André M.P.T. Pereira [1,*], Liliana J.G. Silva [1], Célia S.M. Laranjeiro [1], Angelina Pena [1]

[1] *LAQV, REQUIMTE, Laboratory of Bromatology and Pharmacognosy, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de St[a] Comba, 3000-548 Coimbra, Portugal*

***Corresponding author:**

LAQV, REQUIMTE, Group of Bromatology, Pharmacognosy and Analytical Sciences, Faculty of Pharmacy, University of Coimbra

Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.

Tel: +351239488400

Fax : +351239827126

E-mail addresses:

amptpereira@gmail.com (A.M.P.T. Pereira), ljgsilva@hotmail.com (L.J.G. Silva), celialaranjeiro@gmail.com (C.S.M. Laranjeiro), cmlino@ci.uc.pt (C. Lino), apena@ci.uc.pt (A. Pena)

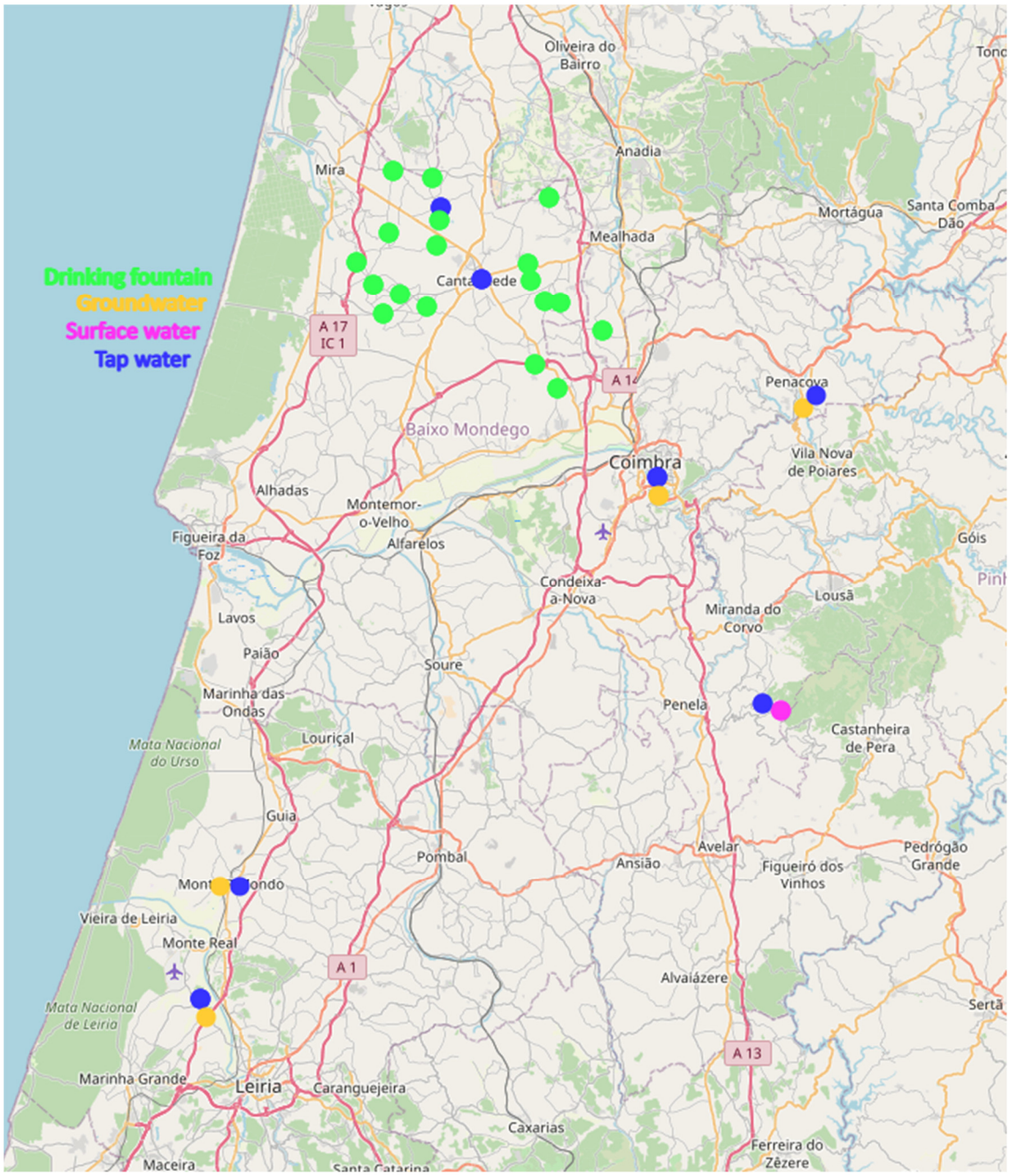

Figure S1. Sampling location.

Table S1. Sampling site and type of water

Sampling number	Sampling site	Type of water	pH
G13	Franciscas	Drinking foutain	7,4
G2	Fervença	Drinking foutain	6,13
G5	Cadima	Drinking foutain	6,95
G7	Fonte da Azenha (Fontinha)	Drinking foutain	7,12
G16	Corticeiro de Cima	Drinking foutain	6,2
G4	Olheiro (Sanguinheira)	Drinking foutain	6,98
G15	Cordinhã	Drinking foutain	6,92
G18	Come Vacas (Cordinhã)	Drinking foutain	6,43
G19	Amieiras (São Caetano)	Drinking foutain	6,29
G20	Iséu (Ançã)	Drinking foutain	6,8
G3	Casal do Netos	Drinking foutain	6,32
G17	Corgo Enchieiro	Drinking foutain	5,66
G12	Ferraria (Barcouço)	Drinking foutain	5,64
G10	Sete-fontes	Drinking foutain	6,18
G14	Freixial (Venda Nova do Bolho)	Drinking foutain	6,04
G1	Porto-Sobreiro	Drinking foutain	5,22
G11	Porto de Carros (Murtede)	Drinking foutain	7,15
G6	Ançã	Drinking foutain	6,79
G8	Febres	Tap water	7,15
G9	Cantanhede	Tap water	7,2
M1 - 9	Boavista WTP water cathment 1	Groundwater	6.62
M1 - 10	Boavista WTP water cathment 2	Groundwater	6.73
M1 - 3	Boavista WTP treated water	Tap water	6.76
M1 - 1	Ronqueira WTP water catchment	Groundwater	6.48
M1 - 4	Ronqueira WTP treated water	Tap water	6.64
M1 - 5	Louçainha WTP water catchment	Surface water	6.46
M1 - 8	Louçainha treated water	Tap water	7.11
M1 - 11	Amor WTP water catchment	Groundwater	6.89
M1 - 6	Amor WTP treated water	Tap water	6.72
M1 - 2	Paúl WTP water catchment	Groundwater	6.16
M1 - 7	Paúl WTP treated water	Tap water	6.71
M2 - 9	Boavista WTP water cathment 1	Groundwater	6.75
M2 - 10	Boavista WTP water cathment 2	Groundwater	6.80
M2 - 3	Boavista WTP treated water	Tap water	6.79
M2 - 1	Ronqueira WTP water catchment	Groundwater	6.64
M2 - 4	Ronqueira WTP treated water	Tap water	6.65
M2 - 5	Louçainha WTP water catchment	Surface water	6.51
M2 - 8	Louçainha treated water	Tap water	7.16
M2 - 11	Amor WTP water catchment	Groundwater	6.99

M2 - 6	Amor WTP treated water	Tap water	7.05
M2 - 2	Paúl WTP water catchment	Groundwater	5.70
M2 - 7	Paúl WTP treated water	Tap water	6.28
M3 - 9	Boavista WTP water cathment 1	Groundwater	6.78
M3 - 10	Boavista WTP water cathment 2	Groundwater	6.81
M3 - 3	Boavista WTP treated water	Tap water	6.86
M3 - 1	Ronqueira WTP water catchment	Groundwater	6.71
M3 - 4	Ronqueira WTP treated water	Tap water	6.56
M3 - 5	Louçainha WTP water catchment	Surface water	6.41
M3 - 8	Louçainha treated water	Tap water	6.45
M3 - 11	Amor WTP water catchment	Groundwater	6.76
M3 - 6	Amor WTP treated water	Tap water	6.89
M3 - 2	Paúl WTP water catchment	Groundwater	5.60
M3 - 7	Paúl WTP treated water	Tap water	7.56
M4 - 9	Boavista WTP water cathment 1	Groundwater	6.74
M4 - 10	Boavista WTP water cathment 2	Groundwater	6.71
M4 - 3	Boavista WTP treated water	Tap water	6.85
M4 - 1	Ronqueira WTP water catchment	Groundwater	6.70
M4 - 4	Ronqueira WTP treated water	Tap water	6.48
M4 - 5	Louçainha WTP water catchment	Surface water	6.56
M4 - 8	Louçainha treated water	Tap water	7.35
M4 - 11	Amor WTP water catchment	Groundwater	6.75
M4 - 6	Amor WTP treated water	Tap water	6.90
M4 - 2	Paúl WTP water catchment	Groundwater	5.81
M4 - 7	Paúl WTP treated water	Tap water	7.73
M5 - 9	Boavista WTP water cathment 1	Groundwater	6.75
M5 - 10	Boavista WTP water cathment 2	Groundwater	6.67
M5 - 3	Boavista WTP treated water	Tap water	6.96
M5 - 1	Ronqueira WTP water catchment	Groundwater	6.73
M5 - 4	Ronqueira WTP treated water	Tap water	6.75
M5 - 5	Louçainha WTP water catchment	Surface water	6.44
M5 - 8	Louçainha treated water	Tap water	7.30
M5 - 11	Amor WTP water catchment	Groundwater	6.83
M5 - 6	Amor WTP treated water	Tap water	6.79
M5 - 2	Paúl WTP water catchment	Groundwater	5.68
M5 - 7	Paúl WTP treated water	Tap water	7.88
M6 - 9	Boavista WTP water cathment 1	Groundwater	6.68
M6 - 10	Boavista WTP water cathment 2	Groundwater	6.69
M6 - 3	Boavista WTP treated water	Tap water	6.95
M6 - 1	Ronqueira WTP water catchment	Groundwater	6.67
M6 - 4	Ronqueira WTP treated water	Tap water	9.48
M6 - 5	Louçainha WTP water catchment	Surface water	6.36
M6 - 8	Louçainha treated water	Tap water	7.42

M6 - 11	Amor WTP water catchment	Groundwater	6.93
M6 - 6	Amor WTP treated water	Tap water	6.65
M6 - 2	Paúl WTP water catchment	Groundwater	6.40
M6 - 7	Paúl WTP treated water	Tap water	7.47
M7 - 9	Boavista WTP water cathment 1	Groundwater	6.94
M7 - 10	Boavista WTP water cathment 2	Groundwater	6.77
M7 - 3	Boavista WTP treated water	Tap water	6.98
M7 - 1	Ronqueira WTP water catchment	Groundwater	6.48
M7 - 4	Ronqueira WTP treated water	Tap water	6.66
M7 - 5	Louçainha WTP water catchment	Surface water	6.43
M7 - 8	Louçainha treated water	Tap water	7.41
M7 - 11	Amor WTP water catchment	Groundwater	7.35
M7 - 6	Amor WTP treated water	Tap water	6.59
M7 - 2	Paúl WTP water catchment	Groundwater	5.81
M7 - 7	Paúl WTP treated water	Tap water	7.55

Table S2. Gradient elution scheme.

TIME	***%A***	***%B***
0.00	90	10
3.00	90	10
3.10	55	45
5.00	55	45
8.00	15	85
9.00	15	85
9.10	5	95
14.00	5	95
14.10	90	10
30.00	90	10

Table S3. Target compounds organized by therapeutic groups and their internal standards.

Group	*Target compound (C/M)*							*Internal Standard (IS)*				
	Compound		*MW*	*ESI-MS2*				*Compound*	*MW*	*ESI-MS2*		
				RT range (min)	*ESI mode*	*MRM transitions (m/z) Precursor ion > Product ion*				*ESI mode*	*MRM transitions (m/z) Precursor ion > Product ion*	
Antibiotics	Ciprofloxacin (CIP)	*C*	331	14.05-14.15	PI	m/z 332 (|M+H|+) > m/z 314 (|M+H-H2O|+)	*QP*	Ciprofloxacin D8 (CIP-D8)	339	PI	m/z 340 (|M+H|+) > m/z 322 (|M+H-H2O|+)	*QP*
						m/z 332 (|M+H|+) > m/z 288 (|M+H-CO2|+)	*CP*				m/z 340 (|M+H|+) > m/z 296 (|M+H-CO2|+)	*CP*
	Erythromycin (ERY)	*C*	733	17.09-17.20	PI	m/z 734 (|M+H|+) > m/z 576 (|M+H-C8H14O3|+)	*QP*	Azithromycin D3 (AZI-D3)	751	PI	m/z 752 (|M+H|+) > m/z 594 (|M+H-C8H14O3|+)	*QP*
						m/z 734 (|M+H|+) > m/z 716 (|M+H-H2O|+)	*CP*					
	Azithromycin (AZI)	*C*	748	17.30-17.55	PI	m/z 749 (|M+H|+) > m/z 591 (|M+H-C8H14O3|+)	*QP*					
						m/z 749 (|M+H|+) > m/z 573 (|M+H-C8H16O4|+)	*CP*				m/z 752 (|M+H|+) > m/z576 (|M+H-C8H16O4|+)	*CP*
	Clarithromycin (CLA)	*C*	747	17.54-17.63	PI	m/z 748 (|M+H|+) > m/z 590 (|M+H-C8H14O3|+)	*QP*					
						m/z 748 (|M+H|+) > m/z 558 (|M+H-C8H14NO3-H2O|+)	*CP*					
Anti-inflammatory and/or analgesics	Diclofenac (DIC)	*C*	295	8.40-8.60	PI	m/z 296 (|M+H|+) > m/z 278 (|M+H-H2O|+)	*QP*	Diclofenac 13C6 (DIC-13C6)	301	PI	m/z 302 (|M+H|+) > m/z 284 (|M+H-H2O|+)	*QP*
						m/z 296 (|M+H|+) > m/z 250 (|M+H-CH2O2|+)	*CP*				m/z 302 (|M+H|+) > m/z 256 (|M+H-CH2O2|+)	*CP*
	4-hidroxy-diclofenac (4-OH-DIC)	*M*	311	8.80-8.90	PI	m/z 312 (|M+H|+) > m/z 294 (|M+H-H2O|+)	*QP*	4-hidroxy-diclofenac 13C6 (4-OH-DIC-13C6)	317	PI	m/z 318 (|M+H|+) > m/z 300 (|M+H-H2O|+)	*QP*
						m/z 312 (|M+H|+) > m/z 250 (|M+H-H2O-CO2|+)	*CP*				m/z 318 (|M+H|+) > m/z 256 (|M+H-H2O-CO2|+)	*CP*
	Naproxen (NAP)	*C*	230	7.15-7.30	PI	m/z 231 (|M+H|+) > m/z 185 (|M+H-CH2O2|+)	*QP*		209	PI	m/z 210 (|M+H|+) > m/z 164 (|M+H-CH2O2|+)	*QP*

Group	*Target compound (C/M)*							*Internal Standard (IS)*				
	Compound		*MW*	*ESI-MS2*				*Compound*	*MW*	*ESI-MS2*		
				RT range (min)	*ESI mode*	*MRM transitions (m/z) Precursor ion > Product ion*				*ESI mode*	*MRM transitions (m/z) Precursor ion > Product ion*	
						m/z 231 (|M+H|+) > m/z 213 (|M+H-H2O|+)	*CP*	Ibuprofen D3 (IBP-D3)				
	Ibuprofen (IBU)	*C*	206	6.50-6.67	PI	m/z 207 (|M+H|+) > m/z 161 (|M+HCH2O2|+)	*QP*				m/z 210 (|M+H|+) > m/z 192 (|M+H-H2O|+)	*CP*
						m/z 207 (|M+H|+) > m/z 189 (|M+H-H2O|+)	*CP*					
	Paracetamol (PARA)	*C*	151	5.95-6.10	PI	m/z 152 (|M+H|+) > m/z 134 (|M+H-H2O|+)	*QP*	Acetaminophen D4 (ACE-D4)	155	PI	m/z 156 (|M+H|+) > m/z 138 (|M+H-H2O|+)	*QP*
						m/z 152 (|M+H|+) > m/z 110 (|M+H-C2H2O|+)	*CP*					
Psychiatric drugs	Citalopram (CIT)	*C*	324	10.70-10.82	PI	m/z 325 (|M+H|+) > m/z 262 (|M+H-C2H9NO|+)	*QP*	Citalopram D6 (CIT- D6)	330	PI	m/z 331 (|M+H|+) > m/z 262 (|M+H-C2H3D6NO|+)	*QP*
						m/z 325 (|M+H|+) > m/z 234 (|M+H-C4H13NO|+)	*CP*				m/z 331 (|M+H|+) > m/z 234 (|M+H-C4H7D6NO|+)	*CP*
	Fluoxetine (FLU)	*C*	309	11.55-11.64	PI	m/z 310 (|M+H|+) > m/z 148 (|M+H-C7H5F3O|+)	*QP*	Fluoxetine D5 (FLU- D5)	314	PI	m/z 315 (|M+H|+) > m/z 153 (|M+H-C7H5F3O|+)	*QP*
						m/z 310 (|M+H|+) > m/z 117 (|M+H-C8H10F3NO|+)	*CP*				m/z 315 (|M+H|+) > m/z 122 (|M+H-C8H10F3NO|+)	*CP*
	Sertraline (SER)	*C*	305	13.71-13.83	PI	m/z 306 (|M+H|+) > m/z 275 (|M+H-CH5N|+)	*QP*	Sertraline D3 (SER-D3)	308	PI	m/z 309 (|M+H|+) > m/z 275 (|M+H-CH2D3N|+)	*QP*
						m/z 306 (|M+H|+) > m/z 159 (|M+H-C10H13N|+)	*CP*				m/z 309 (|M+H|+) > m/z 159 (|M+H-C10H10D3N|+)	*CP*
Lipid regulators	Gemfibrozil (GEM)	*C*	250	15.48-15.57	PI	m/z 251 (|M+H|+) > m/z 121 (|M+H-C7H14O2|+)	*QP*	Gemfibrozil D6 (GFZ-D6)	256	PI	m/z 257 (|M+H|+) > m/z 121 (|M+H-C7H8D6O2|+)	*QP*
						m/z 251 (|M+H|+) > m/z 233 (|M+H-H2O|+)	*CP*				m/z 257 (|M+H|+) > m/z 239 (|M+H-H2O|+)	*CP*

Group	*Target compound (C/M)*							*Internal Standard (IS)*				
	Compound		*MW*	*ESI-MS2*				*Compound*	*MW*	*ESI-MS2*		
				RT range (min)	*ESI mode*	*MRM transitions (m/z) Precursor ion > Product ion*				*ESI mode*	*MRM transitions (m/z) Precursor ion > Product ion*	
	Bezafibrate (BEZ)	*C*	361	16.00-16.28	PI	m/z 362 (|M+H|+) > m/z 316 (|M+H-CH2O2|+)	*QP*	Bezafibrate D4 (BZF-D4)	365	PI	m/z 366 (|M+H|+) > m/z 320 (|M+H-CH2O2|+)	*QP*
						m/z 362 (|M+H|+) > m/z 276 (|M+H-C4H6O2|+)	*CP*				m/z 366 (|M+H|+) > m/z 280 (|M+H-C4H6O2|+)	*CP*
Hormones	Estrone (E1)	*C*	270	8.95-9.21	NI	m/z 269 (|M-H|-) > m/z 145 (|M-H-C6H12O|-)	*QP*	Estradiol D5 (E2-D5)	277	NI	m/z 276 (|M-H|-) > m/z 185 (|M-H-C5H9D3O|-)	*QP*
						m/z 269 (|M-H|-) > m/z 159 (|M-H-C7H10O|-)	*CP*					
	17β-estradiol (E2)	*C*	272	9.27-9.39	NI	m/z 271 (|M-H|-) > m/z 183 (|M-H-C5H12O|-)	*QP*					
						m/z 271 (|M-H|-) > m/z 145 (|M-H-C8H14O|-)	*CP*				m/z 276 (|M-H|-) > m/z 147 (|M-H-C8H11D3O|-)	*CP*
	17α-ethinylestradiol (EE2)	*C*	296	9.81-9.95	NI	m/z 295 (|M-H|-) > m/z 185 (|M-H-C7H10O|-)	*QP*					
						m/z 295 (|M-H|-) > m/z 159 (|M-H-C9H12O|-)	*CP*					
Antiepileptics	Carbamazepine (CAR)	*C*	236	15.22-15.35	PI	m/z 237 (|M+H|+) > m/z 194 (|M+H-CHNO|+)	*QP*	Carbamazepine D10 (CBZ- D10)	246	PI	m/z 247 (|M+H|+) > m/z 204 (|M+H-CHNO|+)	*QP*
						m/z 237 (|M+H|+) > m/z 220 (|M+H-NH3|+)	*CP*				m/z 247 (|M+H|+) > m/z 230 (|M+H-NH3|+)	*CP*

C: compound; M: metabolite; ESI: electrospray ionization; PI: positive ionization mode; NI: negative ionization mode; RT: retention time; MW: Molecular weight; MRM: Multiple Reaction Monitoring; QP: quantification purpose; CP: confirmation purpose

Table S4. Analytical quality control for the quantification of each pharmaceutical in water.

Therapeutic group / compound	Matrix Matched Linearity (R^2)	MDL (ng L^{-1})	MQL (ng L^{-1})	ME (%)	Recovery (%)			RSD within-day (%)			RSD between-day (%)		
					20 ng L^{-1}	60 ng L^{-1}	125 ng L^{-1}	20 ng L^{-1}	60 ng L^{-1}	125 ng L^{-1}	20 ng L^{-1}	60 ng L^{-1}	125 ng L^{-1}
ANTIB													
AZI	0.9995	3.29	9.97	100	99.52	96.06	98.62	0.20	4.40	0.84	0.07	4.15	0.48
CIP	0.9988	5.26	15.93	100.96	99.12	99.28	98.63	0.42	0.67	1.26	0.44	0.24	0.45
CLA	0.9999	1.51	4.58	98.77	98.57	94.03	98.59	0.67	4.33	1.40	0.58	6.85	1.30
ERY	0.9991	4.49	13.60	98.84	99.02	98.17	99.24	0.72	1.59	0.56	0.30	0.55	0.32
LIP REG													
BEZ	0.9998	2.26	6.84	100.00	99.37	99.24	98.44	0.76	0.93	1.89	1.80	0.54	0.90
GEM	0.9994	3.78	11.46	94.02	99.78	96.84	98.75	0.71	4.88	1.17	1.18	0.70	0.72
ANTIEPI													
CAR	0.9992	4.22	12.80	101.27	97.99	95.90	97.86	0.89	2.87	1.72	1.16	3.63	1.67
SSRIS													
CIT	1.0000	1.13	3.41	100.00	98.60	98.74	97.51	0.66	1.26	2.03	1.52	0.42	2.01
FLU	0.9999	1.84	5.59	100.61	99.50	96.98	99.20	0.74	3.12	0.78	1.24	3.05	0.71
SER	0.9996	3.04	9.22	99.44	99.88	97.03	99.06	0.57	3.99	0.60	1.21	0.76	0.40
ANTI-INF													
DIC	0.9991	4.61	13.97	101.96	98.33	98.47	98.85	1.10	1.58	0.91	1.48	0.90	1.12
4-OH-DIC	0.9990	4.92	14.92	99.33	97.97	98.40	98.44	1.34	2.25	1.58	1.31	0.57	1.22
IBU	0.9994	3.66	11.09	93.55	97.66	99.40	99.17	1.45	1.79	1.10	0.51	0.66	0.59
NAP	0.9992	4.38	13.27	101.27	97.66	96.41	97.50	0.84	5.16	1.37	2.05	1.58	3.10

Therapeutic group / compound	Matrix Matched Linearity (R^2)	MDL (ng L^{-1})	MQL (ng L^{-1})	ME (%)	Recovery (%)			RSD within-day (%)			RSD between-day (%)		
					20 ng L^{-1}	60 ng L^{-1}	125 ng L^{-1}	20 ng L^{-1}	60 ng L^{-1}	125 ng L^{-1}	20 ng L^{-1}	60 ng L^{-1}	125 ng L^{-1}
PARA	0.9995	3.57	10.82	99.20	99.14	97.22	98.10	0.56	3.32	2.27	1.15	1.34	1.34
HORM													
E1	0.9987	5.45	16.53	100.68	98.90	99.68	97.88	0.99	0.98	0.99	0.50	1.16	0.74
E2	0.9991	4.56	13.82	102.68	96.68	95.16	96.65	0.92	2.76	1.98	2.21	5.01	0.94
EE2	0.9990	4.94	14.98	99.44	99.07	96.86	97.45	0.37	5.47	1.47	0.60	3.60	0.86

MDL – Method detection limit; MQL – Method quantiication limit; ME – Matriz effect; RSD – Relative standard deviation.

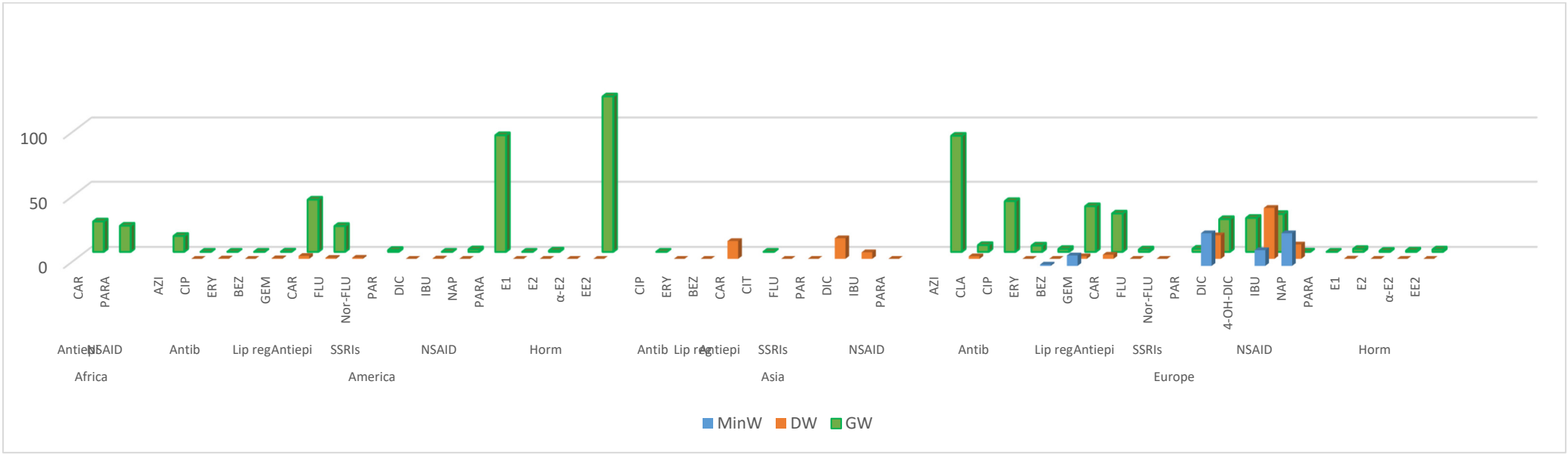

Figure S2. Median concentrations for each pharmaceutical in mineral, drinking and groundwaters by continent.

CIP – Ciprofloxacin; ERY – Erythromycin; AZI – Azithromycin; CLA – Clarithromycin; GEM – Gemfibrozil; BEZ - Bezafibrate (BEZ)), antiepileptics (Carbamazepine (CAR)), selective serotonin reuptake inhibitors (SSRIs) (Citalopram (CIT), Fluoxetine (FLU), Sertraline (SER)), non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, still referred to only as NSAIDs, (Diclofenac (DIC), 4-hidroxy-diclofenac (4-OH-DIC), Naproxen (NAP), Ibuprofen (IBU), Paracetamol (PARA)) and hormones (Es-trone (E1), 17β-estradiol (E2) and 17α-ethinylestradiol (EE2)Anx—anxiolytics; Antib - Antibiotics; Antiepi - Antiepileptics; DW – Drinking water; GW – Groundwater; Horm—hormones; Lip reg - Lipid regulators; MinW – Mineral water; NSAIDs - Non-steroidal anti-inflammatory drugs; SSRIs – Selective serotonin reuptake inhibitors.