Occurrence and fate of chiral and achiral drugs in estuarine water: a case study of the Clyde estuary, Scotland. [Dataset]

PETRIE, B. and MOFFAT, C.F.

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Occurrence and fate of chiral and achiral drugs in estuarine water – a case study of the Clyde Estuary, Scotland

Bruce Petrie*, Colin F. Moffat

School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, AB10 7GJ, UK

*Email: b.r.petrie@rgu.ac.uk

The Supplementary Information contains details on the methods used in the microcosm study and the corresponding results and discussion. Five tables are also included which detail the instrumental precision and accuracy, method performance data, drug degradation rates and half-lives in microcosm studies, replicate data of fish analysis and dissolved oxygen, pH, and temperature data from microcosms.

1. Materials and methods

1.1. Microcosm study

The water sample used in the microcosm study was kept cool (but not frozen) for 72 hours before use. Four 2 L vessels of water were prepared in borosilicate Duran bottles. Two vessels were treated with 1 g L^{-1} NaN_3 to inhibit microbial activity (abiotic conditions). One biotic and one abiotic vessel were kept in a cold room maintained at 4 °C in the dark. The other biotic and abiotic vessels were kept in an All-Round Toxkit Incubator (Microbiotests, Gent, Belgium) under light provided by light emitting diode lamps. The contents of the various vessels were continually mixed on a magnetic stirrer. This achieved a water temperature of 8 °C in all vessels (Table S5). Once this temperature was reached, each vessel was spiked at 1 µg L^{-1} with individual drug enantiomers using 100 µL of a 20 µg mL^{-1} mixed analyte solution in 50:50 water: methanol (achiral analytes were at 40 µg mL^{-1} and were therefore spiked at 2 µg L^{-1}). Each microcosm was sampled (25 mL) in triplicate at the following intervals: 0, 0.33, 1, 1.33, 4, 5, 7, 8, 11 and 14 days. Samples were then extracted as described in Section 2.3.1. Drug degradation was fitted to the first-order exponential degradation model using eq (1):

$$C_t = C_0 e^{-kt} \quad (1)$$

Here, C_t is the drug concentration at time t (days (d)) and C_0 is the drug concentration at the start of the study (0 d), and k is the degradation rate constant (1/d). Drug half-life ($t_{1/2}$) was calculated according to eq (2):

$$t_{1/2} = \frac{\ln(2)}{k} \quad (2)$$

Water temperature, pH, and dissolved oxygen concentration were also monitored (Table S5).

Method detection limits (MDLs) and method quantitation limits (MQLs) of the entire SPE-enantioselective LC-MS/MS method was calculated using Eq (3) and (4)[1]:

$$MDL = \frac{(IDLx100)}{(Rec\ x\ CF)} \quad (3)$$

$$MQL = \frac{(IQLx100)}{(Rec\ x\ CF)} \quad (4)$$

Here *IDL* is the instrument detection limit (ng L^{-1}), *IQL* is the instrument quantitation limit (ng L^{-1}). These were the lowest concentrations which gave a signal to noise ratio of ≥3 and ≥10 respectively. *Rec* is the recovery from spiked matrix (%) and *CF* is the SPE sample concentration factor.

2. Results and discussion

2.1. Microcosm study

Under biotic conditions paracetamol had a $t_{1/2}$ value of 31.7 and 5.5 days in light and dark conditions respectively (Table S3). A cloudy appearance developed in the light microcosm after a few days. This was not observed in the dark biotic microcosm suggesting differences in the changes in the microbial community under light and dark conditions. This could account for differences in degradation observed. In abiotic microcosms no degradation of paracetamol or indeed any of the drugs was observed under either light or dark conditions. Microcosm studies by Benotti and Brownawell[2] found paracetamol $t_{1/2}$ values in the range 1.2-11.0 days in estuarine and coastal waters from inside and outside of Jamaica Bay, New York, USA, albeit under slightly different conditions (15 °C and aeration). The mean dissolved oxygen concentrations in this study, provided by continuous mixing of the water, were 11.2-11.6 mg L^{-1} at 8 °C.

No degradation of carbamazepine, carbamazepine-10,11-epoxide, caffeine, or *(-)*-cotinine was observed during 14 days under light or dark conditions (Table S3). Carbamazepine is well-known for its recalcitrant nature in the environment, including marine waters.[2,3] Cotinine has also previously been found to be resistant to degradation in marine water microcosms.[2] However, Benotti and Brownawell[2] found caffeine $t_{1/2}$ values to range from 3.5 days to >100 days depending on the sampling location of the water.

Most of the studied chiral drugs, including amphetamine, methamphetamine, atenolol, sotalol, bisoprolol, acebutolol, metoprolol, venlafaxine and desmethylvenlafaxine, did not show any degradation (or enantioselectivity) in biotic microcosms (Table S3). On the other hand, both chlorpheniramine and propranolol degraded in the light microcosm, but without enantioselective changes. Interestingly, the degradation of chlorpheniramine was lower in the dark microcosm and no

degradation of propranolol was noted (Table S3). This is opposite to what was observed for paracetamol which exhibited greater degradation in the dark microcosm.

Both fluoxetine and citalopram degraded enantioselectively in the light microcosm (Table S3). The $t_{1/2}$ values of *S(+)*-fluoxetine and *R(-)*-fluoxetine were 10.4 and 19.1 days, respectively. Previous research has found fluoxetine to be susceptible to degradation in marine waters with $t_{1/2}$ values ranging from 5.9 to 9.8 days.[2] The initial EF value of 0.51 reduced to 0.37 over 14 days due to the slower degradation of *R(-)*-fluoxetine. This follows the same enantioselectivity of fluoxetine observed in activated sludge microcosms.[4,5] On the other hand, enrichment of *S(+)*-fluoxetine has been observed in freshwater microcosms.[5] This demonstrates that enantiomer enrichment can proceed in either direction depending on the environmental compartment or associated microbial community. However, the microcosm data could not be compared to environmental concentrations of fluoxetine in the Clyde Estuary owing to fluoxetine being present below the MQL in the estuarine waters (Table 1). Nevertheless, our study is the first to demonstrate the enantioselective degradation of fluoxetine or indeed any drug in estuarine water.

Citalopram degradation was slower with no appreciable degradation of *R(-)*-citalopram observed. The $t_{1/2}$ value of *S(+)*-citalopram was 37.1 days (Table S3). The initial EF value of 0.50 reduced to 0.44 over 14 days. A reduction in EF has previously been observed in activated sludge microcosms, however in freshwater microcosms no enantioselectivity was observed.[5] In the monitoring study of the Clyde Estuary, no clear change in citalopram EF was observed down the estuary. It is considered that the enantioselective changes observed within the microcosm studies were too slow to have an appreciable effect on citalopram EF values within the estuary. However, it should be noted that the findings of the microcosm and estuary monitoring study will not be directly comparable as the estuary will be subject to other wastewater inputs between sampling locations that could influence the EF values.

Table S1. Precision and accuracy of triplicate injection of quality control samples prepared in solvent (methanol)

Analyte	Accuracy (%)			Precision (%)		
	5 ng mL^{-1}	50 ng mL^{-1}	250 ng mL^{-1}	5 ng mL^{-1}	50 ng mL^{-1}	250 ng mL^{-1}
Pain killer						
Paracetamol[a]	105	100	101	3	3	2
Anti-convulsant						
Carbamazepine[a]	100	98	97	2	3	1
Carbamazepine epoxide[a]	93	96	92	4	5	2
Stimulant						
Caffeine[a]	106	102	102	3	2	2
Cotinine[a]	99	101	98	4	4	5
S(+)-amphetamine	97	103	99	1	2	2
R(-)-amphetamine	99	100	98	3	2	2
S(+)-methamphetamine	103	97	100	1	2	1
R(-)-methamphetamine	102	97	102	1	2	1
R/S(±)-MDMA[a]	95	94	97	1	3	4
Antihistamine						
S(+)-chlorpheniramine	98	99	100	3	3	2
R(-)-chlorpheniramine	97	99	101	3	2	2
β-blocker/agonist						
Salbutamol-E1	95	105	101	2	5	3
Salbutamol-E2	96	104	103	2	4	3
S(-)-propranolol	104	103	98	4	6	5
R(+)-propranolol	102	104	99	4	6	5
S(-)-atenolol	94	96	96	5	4	2
R(+)-atenolol	94	93	98	5	4	3
Sotalol-E1	106	98	97	6	8	2
Sotalol-E2	107	99	96	5	7	2
Bisoprolol-E1	101	99	101	1	2	1
Bisoprolol-E2	102	99	100	1	1	1
Acebutolol-E1	98	101	101	1	3	2
Acebutolol-E2	97	100	101	1	2	2
Metoprolol-E1	103	96	96	1	2	3
Metoprolol-E2	103	97	96	1	1	3
Antidepressant						
S(+)-fluoxetine	98	102	104	2	2	3
R(-)-fluoxetine	101	100	102	3	1	2
R(-)-citalopram	98	99	97	2	2	4
S(+)-citalopram	96	102	101	2	3	4
Venlafaxine-E1	94	99	102	3	3	1
Venlafaxine-E2	95	99	98	2	3	1
Desmethylvenlafaxine-E1	93	98	99	3	2	1
Desmethylvenlafaxine-E2	96	100	96	3	2	1

Key: MDMA, 3,4-methylenedioxymethamphetamine; [a]10, 100 and 500 ng mL^{-1}

Table S2. Results of blank extractions and method recovery and trueness from spiked environmental samples (n=3).

Analyte	Water samples[a]			Fish				
				Blank extraction[e] (ng g^{-1})	Muscle[b]		Liver[c]	
	Blank extraction[d] (ng L^{-1})	Method recovery[f] (%)	Method trueness[g] (%)		Method recovery[f] (%)	Method trueness[g] (%)	Method recovery[f] (%)	Method trueness[g] (%)
Pain killer								
Paracetamol	<MQL	16±1	99±5	-	-	-	-	-
Anti-convulsant								
Carbamazepine	ND	28±2	100±2	ND	12±4	115±11	9±1	99±6
Carbamazepine-10,11-epoxide	ND	39±1	123±9	ND	12±3	114±6	8±2	94±10
Stimulant								
Caffeine	<MQL	54±1	98±9	-	-	-	-	-
(-)-cotinine	<MQL	45±1	109±1	-	-	-	-	-
R/S(±)-MDMA	ND	33±11	104±1	ND	5±1	101±9	18±10	107±3
S(+)-amphetamine	ND	6±1	88±16	ND	3±4	70±25	3±1	105±13
R(-)-amphetamine	ND	6±2	90±15	ND	3±3	68±28	3±2	108±2
S(+)-methamphetamine	ND	3±1	104±4	ND	1±0	85±27	5±3	111±13
R(-)-methamphetamine	ND	21±9	89±2	ND	57±42	89±11	63±43	103±4
Anti-histamine								
S(+)-chlorpheniramine	ND	65±9	97±1	ND	30±9	106±3	50±14	93±14
R(-)-chlorpheniramine	ND	64±8	100±2	ND	27±4	97±10	42±14	103±7
β-blocker/agonist								
Salbutamol-E1	ND	2±2	80±6	-	-	-	-	-
Salbutamol-E2	ND	2±2	69±7	-	-	-	-	-
S(-)-propranolol	ND	15±4	93±6	ND	45±7	106±3	44±4	105±8
R(+)-propranolol	ND	15±4	83±11	ND	40±5	106±7	42±12	102±8
S(-)-atenolol	ND	32±2	123±4	-	-	-	-	-
R(+)-atenolol	ND	43±3	117±3	-	-	-	-	-
Sotalol-E1	ND	73±2	106±3	-	-	-	-	-
Sotalol-E2	ND	79±2	111±3	-	-	-	-	-
Bisoprolol-E1	ND	66±5	104±2	ND	85±4	116±5	69±11	106±10
Bisoprolol-E2	ND	64±5	100±1	ND	57±8	109±16	36±7	103±8
Acebutolol-E1	ND	51±1	125±1	ND	39±1	105±9	44±14	103±5
Acebutolol-E2	ND	50±1	122±1	ND	42±5	104±4	47±2	103±4
Metoprolol-E1	ND	56±3	102±1	ND	33±2	112±6	40±11	109±1
Metoprolol-E2	ND	56±3	110±3	ND	22±5	113±20	31±4	100±13
Antidepressant								
S(+)-fluoxetine	ND	1±1	89±7	ND	36±2	105±4	41±10	107±7
R(-)-fluoxetine	ND	7±5	90±4	ND	16±14	107±4	12±12	106±5
R(-)-citalopram	ND	74±7	111±0	ND	48±6	106±1	49±6	100±4
S(+)-citalopram	ND	74±8	103±1	ND	55±12	103±4	52±12	103±13
Venlafaxine-E1	ND	85±2	96±2	ND	34±14	86±25	38±8	96±3

Venlafaxine-E2	ND	91±3	100±2	ND	28±20	90±8	37±10	98±4
Desmethylvenlafaxine-E1	ND	85±3	105±2	ND	23±3	59±4	26±7	65±4
Desmethylvenlafaxine-E2	ND	91±0	111±4	ND	19±11	67±10	25±8	67±16

Key: MQL, method quantitation limit; ND, not detected; MDMA, 3,4-methylenedioxymethamphetamine; -, not recovered from fish sample

[a]500mL water sample collected from Dunoon spiked at 100 ng L^{-1} (200 ng L^{-1} for achiral analytes and MDMA) [b]Muscle (1.0 g) spiked at 2.5 ng g^{-1} (5 ng g^{-1} for carbamazepine and carbamazepine-10,11-epoxide) [c]Liver (0.5 g) spiked at 5 ng g^{-1} (10 ng g^{-1} for carbamazepine and carbamazepine-10,11-epoxide) [d]500 mL ultra-pure water [e]0.5 g diatomaceous earth [f]$Method\ recovery\ (\%) = \frac{(PA_S - PA_U)}{PA_{STD}}\ x\ 100$ where PA_S is peak area of the extracted spiked sample, PA_U is the peak area of the unspiked sample and PA_{STD} is the peak area of a corresponding standard solution assuming 100 % recovery through the extraction process [g]$Trueness\ (\%) = \frac{(Conc_S - Conc_U)}{Spike}\ x\ 100$ where $Conc_S$ is the determined concentration of the spiked sample, $Conc_U$ is the concentration of the unspiked sample and *Spike* is the spiked concentration.

Table S3. Degradation rates and half-lives of a range of drugs determined using laboratory microcosm studies

Analyte	Light and biotic conditions					Dark and biotic conditions				
	k (d^{-1})	r^2	$t_{1/2}$ (d)	Day 0 EF	Day 14 EF	k (d^{-1})	r^2	$t_{1/2}$ (d)	Day 0 EF	Day 14 EF
Pain killer										
Paracetamol	0.022	0.640	31.7	NR	NR	0.126	0.932	5.5	NR	NR
Anti-convulsant										
Carbamazepine	-	-	>100	NR	NR	-	-	>100	NR	NR
Carbamazepine-10,11-epoxide	-	-	>100	NR	NR	-	-	>100	NR	NR
Stimulant										
Caffeine	-	-	>100	NR	NR	-	-	>100	NR	NR
(-)-cotinine	-	-	>100	-	-	-	-	>100	-	-
R/S(±)-MDMA	-	-	>100	-	-	-	-	>100	-	-
S(+)-amphetamine	-	-	>100	0.50	0.49	-	-	>100	0.49	0.48
R(-)-amphetamine	-	-	>100			-	-	>100		
S(+)-methamphetamine	-	-	>100	0.51	0.52	-	-	>100	0.51	0.51
R(-)-methamphetamine	-	-	>100			-	-	>100		
Anti-histamine										
S(+)-chlorpheniramine	0.036	0.726	19.5	0.51	0.52	0.011	0.523	60.8	0.50	0.51
R(-)-chlorpheniramine	0.038	0.760	18.4			0.014	0.614	49.9		
β-blocker/agonist										
Salbutamol-E1	-	-	>100	0.47	0.49	-	-	>100	0.48	0.47
Salbutamol-E2	-	-	>100			-	-	>100		
S(-)-propranolol	0.010	0.613	69.3	0.51	0.52	-	-	>100	0.51	0.52
R(+)-propranolol	0.008	0.694	82.5			-	-	>100		
S(-)-atenolol	-	-	>100	0.51	0.49	-	-	>100	0.52	0.51
R(+)-atenolol	-	-	>100			-	-	>100		
Sotalol-E1	-	-	>100	0.53	0.53	-	-	>100	0.52	0.52
Sotalol-E2	-	-	>100			-	-	>100		
Bisoprolol-E1	-	-	>100	0.51	0.51	-	-	>100	0.51	0.51
Bisoprolol-E2	-	-	>100			-	-	>100		
Acebutolol-E1	-	-	>100	0.46	0.46	-	-	>100	0.47	0.46
Acebutolol-E2	-	-	>100			-	-	>100		
Metoprolol-E1	-	-	>100	0.51	0.52	-	-	>100	0.52	0.51
Metoprolol-E2	-	-	>100			-	-	>100		
Anti-depressant										
S(+)-fluoxetine	0.067	0.740	10.4	0.51	0.37	-	-	>100	0.51	0.51
R(-)-fluoxetine	0.036	0.749	19.1			-	-	>100		
R(-)-citalopram	-	-	>100	0.50	0.44	-	-	>100	0.49	0.50
S(+)-citalopram	0.019	0.643	37.1			-	-	>100		
Venlafaxine-E1	-	-	>100	0.49	0.51	-	-	>100	0.50	0.50
Venlafaxine-E2	-	-	>100			-	-	>100		
Desmethylvenlafaxine-E1	-	-	>100	0.51	0.52	-	-	>100	0.53	0.52
Desmethylvenlafaxine-E2	-	-	>100			-	-	>100		

Key: k, degradation rate constant; $t_{1/2}$, half-life; EF, enantiomeric fraction; MDMA, 3,4-methylenedioxymethamphetamine; NR, not relevant; -, unable to determine

Note: analytes with low k values <0.00693 d^{-1} were reported as $t_{1/2}$ values >100 d for comparative purposes[2]

Table S4. Replicate data of drug enantiomers detected in *Platichthys flesus* from the inner Clyde Estuary

Analyte	Muscle tissue (ng g^{-1} ww)					Liver tissue (ng g^{-1} ww)				
	R1	***R2***	***R3***	***R4***	***R5***	***R1***	***R2***	***R3***	***R4***	***R5***
S(-)-propranolol	ND	ND	ND	ND	ND	<MQL	<MQL	<MQL	<MQL	<MQL
R(+)-propranolol	ND	ND	ND	ND	ND	<MQL	<MQL	<MQL	<MQL	<MQL
S(+)-fluoxetine	0.30	0.37	0.29	0.29	0.31	2.78	2.29	2.73	2.96	2.78
R(-)-fluoxetine	0.18	0.23	0.17	0.20	0.17	0.86	0.85	0.94	1.03	1.01
R(-)-citalopram	0.27	0.26	0.23	0.25	0.26	1.50	1.30	1.75	1.54	1.40
S(+)-citalopram	0.11	0.09	0.12	0.11	0.10	0.37	0.26	0.36	0.32	0.33
Venlafaxine-E1	0.24	0.29	0.25	0.25	0.25	1.23	1.12	1.18	1.21	1.27
Venlafaxine-E2	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL

Key: ww, wet weight; *R*, replicate; MQL, method quantitation limit; ND, not detected;

Table S5. Dissolved oxygen, pH and temperature of water microcosms

Measured parameter	Light conditions		Dark conditions	
	Biotic	**Abiotic**	**Biotic**	**Abiotic**
Dissolved oxygen (mg L^{-1})	11.6±0.4	11.2±0.3	11.4±0.2	11.3±0.4
pH	7.8±0.2	7.9±0.1	7.1±0.1	7.9±0.2
Temperature (°C)	8±0	8±0	8±0	8±0

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