

## Fate of drugs during wastewater treatment

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### Abstract

Recent trends for the determination of pharmaceutical drugs in wastewaters focus on the development of rapid multi-residue methods. These encompass a large number of drugs (up to 90) of varying physicochemical composition (hydrophobicity, molecular weight etc) at ng l<sup>-1</sup> concentrations in the aqueous phase of complex heterogeneous matrices. These are well suited for drug determination in secondary effluents which contain detectable concentrations of pharmaceuticals. Drugs are not routinely monitored for in particulate phases of wastewaters despite being essential for fate determination, particularly in secondary processes receiving relatively high concentrations of suspended solids. Secondary effluents tend to contain multiple drugs, often above their proposed legislative targets for consent. Thus, tertiary processing may be considered to enhance drug removal and provide additional environmental protection. However, current analytical methods do not enable the efficacy of tertiary processes to be fully ascertained due to the inherently lower drug concentrations achieved. Existing method optimisation is required to lower detection capabilities for tertiary process monitoring. This would aid the understanding of breakdown reaction completeness and the criticality between parent drug and degradation product concentrations. Numerous degradation products are formed by biological and chemical processes which can exhibit toxicity. The complimentary use of biological assays here would improve understanding of the synergistic toxicological effect of multiple drugs and their degradation products at low concentration. Additionally, tertiary processes receive secondary effluents comprising comparatively high concentrations of dissolved organics (e.g., colloids). However, knowledge of drug behaviour in the charged colloidal fraction of wastewater and its impact to treatment is limited. Monitoring and understanding here is needed to develop tertiary process diagnosis and optimisation.

**Key words:** Pharmaceutical drug; 17 $\alpha$ -ethinylestradiol; metabolite; analytical method; liquid chromatography; mass spectrometry; activated sludge; sand filter; ozone; activated carbon

## 1. Introduction

It is well established that wastewater contains a diverse range of anthropogenic compounds [1] and that their quantitative analysis poses numerous problems analytically, both in terms of extraction efficiency [2] and interference from co-extractives [3]. Through medical use large numbers of drugs are now included in this group of determinants [4-7]. A clear trend in legislation has been a lowering of acceptable environmental concentrations (e.g., 17 $\alpha$ -ethinylestradiol (EE2)) and analytical method development has become an iterative process to achieve ever lower detection limits [8-9] as further potential environmental health issues emerge [10]. A growing understanding of the possible environmental impact to aquatic ecosystems has led to the classification of diclofenac and the steroid estrogen EE2 as priority hazardous chemicals [11-12]. These drugs have proposed environmental quality standards (EQS) of 100 ng l<sup>-1</sup> and 0.035 ng l<sup>-1</sup> respectively, and require monitoring to ensure compliance to 'good' water status [11-12]. The effects of EE2, which results in intersex in male fish, are the most studied of the drugs in the environment [13]. Concentrations of EE2 below 1 ng l<sup>-1</sup> are known to cause intersex and vitellogenin induction in male fish during laboratory studies. The environmental impact of other drugs and mixtures is less clear. However, it has been demonstrated that a mixture of acetaminophen, carbamazepine, gemfibrozil and venlafaxine (in the low  $\mu$ g l<sup>-1</sup> range) had a significant impact to fish embryo development in the short term [14]. The chronic impact of drugs (i.e., ecological and evolutionary), either individually or as mixtures, remains unknown [15]. Without such information it is desirable to limit entry of these chemicals into the aquatic environment. A total of 12 drugs of varying therapeutic class (ibuprofen, diclofenac, naproxen, ketoprofen, carbamazepine, bezafibrate, propranolol, fluoxetine, EE2, ofloxacin, erythromycin and oxytetracycline) are highlighted in this review to represent a variety of physicochemical compositions (e.g., molecular size, hydrophobicity) (Table 1). This includes those most studied in the literature [7, 17-18] and those in national studies (i.e., the UK Chemical Investigations Programme (CIP)) [19]. The CIP found diclofenac, propranolol, fluoxetine, EE2, erythromycin and oxytetracycline above their indicative legislative targets for consent (10 ng l<sup>-1</sup> for those not listed as priority hazardous substances) in over 50 % of effluents studied [19]. Consequently, a variety of drugs have been observed in surface waters in the ng l<sup>-1</sup> range (Table 2). This underpins the need to understand drug fate during wastewater treatment for process/strategy optimisation.

Determining drug fate during wastewater treatment relies on the application of robust analytical methodologies. These require the ability to simultaneously determine a number of drugs, of differing physicochemical properties, within environmental matrices comprised of comparatively high concentrations of complex bulk organics [23]. The high complexity of environmental matrices underlines the analytical challenge posed. Drugs and their

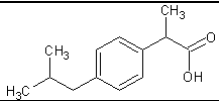
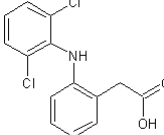
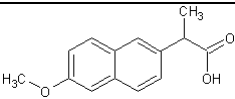
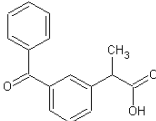
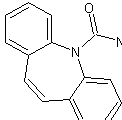
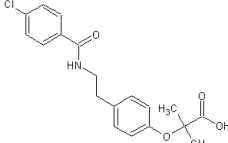
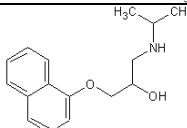
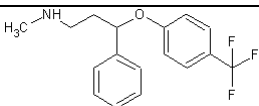
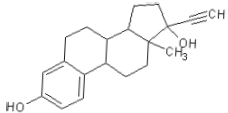
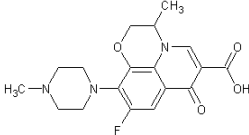
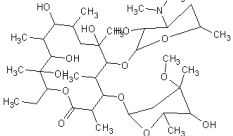
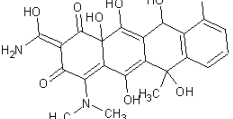
**Table 1. Physicochemical properties of pharmaceutical drugs present in crude wastewaters and secondary effluents [16]**

Drug	MW / g mol <sup>-1</sup>	Water solubility / mg l <sup>-1</sup>	Henry's law / atm m <sup>3</sup> mol <sup>-1</sup>	VP / mm Hg	pKa	Log K <sub>ow</sub>	Log K <sub>oc</sub>
Ibuprofen	206.30	21.0	1.52.10 <sup>-7</sup>	1.86.10 <sup>-4</sup>	4.91	3.79-3.97	2.35
Diclofenac	296.15	2.4	4.73.10 <sup>-12</sup>	2.18.10 <sup>-6</sup>	4.15	4.02-4.51	2.61
Naproxen	230.26	15.9	3.39.10 <sup>-10</sup>	1.27.10 <sup>-6</sup>	4.15	3.10-3.18	1.97
Ketoprofen	254.28	51.0	2.12.10 <sup>-11</sup>	6.81.10 <sup>-6</sup>	4.45	3.00-3.12	2.08
Carbamazepine	236.27	112	1.08.10 <sup>-10</sup>	8.80.10 <sup>-8</sup>	-	2.25-2.45	2.23
Bezafibrate	361.82	7.9	2.12.10 <sup>-15</sup>	6.12.10 <sup>-11</sup>	-	4.25	2.31
Propranolol	259.35	61.7	7.98.10 <sup>-13</sup>	9.44.10 <sup>-8</sup>	9.42	2.60-3.48	2.45
Fluoxetine	309.33	60.3	8.90.10 <sup>-8</sup>	2.52.10 <sup>-5</sup>	-	4.05-4.65	4.97
EE2	296.40	11.3	7.94.10 <sup>-12</sup>	1.95.10 <sup>-9</sup>	-	3.67-4.12	4.65
Ofloxacin	361.37	2.8.10 <sup>4</sup>	4.98.10 <sup>-20</sup>	9.84.10 <sup>-13</sup>	-	<0	1.09
Erythromycin	733.94	0.5	5.42.10 <sup>-29</sup>	2.12.10 <sup>-25</sup>	8.88	2.48-3.06	2.75
Oxytetracycline	460.43	313	1.70.10 <sup>-25</sup>	9.05.10 <sup>-23</sup>	3.27	<0	1.87

KEY: MW, molecular weight; VP, vapour pressure; pKa, acid dissociation constant; Log K<sub>ow</sub>, octanol-water coefficient; Log K<sub>oc</sub>, organic carbon-water coefficient

metabolites were first reported in wastewater effluents in the 1970's [24]. Hignite *et al* [24] measured chlorophenoxyisobutyrate and salicylic acid in wastewater effluents at relatively high mean concentrations of 7 µg l<sup>-1</sup> and 29 µg l<sup>-1</sup>, respectively. Ion exchange chromatography was used for extraction followed by gas chromatography-mass spectrometry (GC-MS). Numerous other drugs have been given significant attention recently due to the development of sophisticated analytical methodologies [17-18, 25]. Extraction media enabling simultaneous retention of acidic, basic and neutral species of varying polarities has aided this. Furthermore, the coupling of liquid chromatography (LC), and particularly ultra-performance liquid chromatography (UPLC) to MS has seen significant reductions in sample pre-treatment requirements and instrument analysis time [23]. Other than analytical difficulties, the variety of physicochemical behaviour (e.g., hydrophobicity) exhibited by drugs results in significant differences in their fate and removal during wastewater treatment [6, 26]. Tertiary treatment processes are being considered to enhance hazardous chemical removal to levels which comply with proposed EQS's [27], as are the analytical methods capable of supporting their diagnosis and optimisation for this critical group of emerging chemicals. This review addresses recent analytical trends for drug determination in environmental matrices used to facilitate fate studies. Analytical requirements for further fate evaluation and tertiary process selection/optimisation are also discussed.

**Table 2. Recently reported occurrence of pharmaceutical drugs in surface waters**

Drug of interest	Class	Chemical structure	Proposed legislative target / ng l <sup>-1</sup>	Surface water <sup>a</sup> / ng l <sup>-1</sup>	Location
Ibuprofen	NSAID		10 <sup>b</sup>	<0.3-56 <6.4-542 21-2,796	UK Mainland Europe North America
Diclofenac	NSAID		100 <sup>c</sup>	<0.5-261 <12-154 17-42	UK Mainland Europe North America
Naproxen	NSAID		-	<0.3-55 <3.1-109 22	UK Mainland Europe North America
Ketoprofen	NSAID		-	<0.5-4 <15-517 -	UK Mainland Europe North America
Carbamazepine	Anti-epileptic		-	0.5-495 <1.5-54 1-1,238	UK Mainland Europe North America
Bezafibrate	Lipid regulator		-	<10-66 <2.0-26 -	UK Mainland Europe North America
Propranolol	Beta blocker		10 <sup>b</sup>	<0.5-27 <0.4-39 53	UK Mainland Europe North America
Fluoxetine	Anti-depressant		10 <sup>b</sup>	- <7.4-24 <1.3-65	UK Mainland Europe North America
EE2	Contraceptive		0.035 <sup>c</sup>	- - -	UK Mainland Europe North America
Ofloxacin	Antibiotic		10 <sup>b</sup>	- 4.8-105 -	UK Mainland Europe North America
Erythromycin	Antibiotic		10 <sup>b</sup>	<0.5-20 <28-52 2-438	UK Mainland Europe North America
Oxytetracycline	Antibiotic		10 <sup>b</sup>	- <12-37 -	UK Mainland Europe North America

<sup>a</sup>occurrence data taken from: UK-[20] Mainland Europe-[17] North America-[21-22], <sup>b</sup>UK Chemicals Investigation Programme [19], <sup>c</sup>[12]

KEY: NSAID, non steroidal anti-inflammatory drug

## 2. Analytical strategies for fate evaluation

Prior to laboratory work, the first step for drug determination in wastewaters is sampling. This process is fundamental to any strategy for monitoring, and careful consideration of sampling equipment, sample handling and types of samples is needed. It is beyond the scope of this review to examine further; however an excellent overview of sampling strategies has been given by Ort *et al* [28-29]. Nevertheless, it is important to highlight that to fully understand fate during wastewater treatment, determination in both aqueous and particulate phases of wastewaters is essential [9, 30].

Trace determination of drugs in aqueous wastewater fractions (typically 0.45  $\mu\text{m}$  filtered) requires an enrichment step followed by chromatographic separation and mass spectrometry (MS) detection. Sample pre-concentration and clean up commonly involves solid phase extraction (SPE), and can be undertaken off-line (using extraction systems not linked to analytical equipment) or on-line, where extraction and quantification are automated and linked together. Off-line analysis tends to use up to 1 litre of sample, and Gros *et al* [31] investigated the efficacy of various SPE sorbents (Oasis HLB, Oasis MCX, Isolute C18 and Isolute ENV+) for the simultaneous extraction of 13 pharmaceutical drugs of varying physicochemical composition. The Oasis HLB sorbent (polystyrene-devinylbenzene-N-vinylpyrrolidone terpolymer) [32] (without pH adjustment) exhibited superior performance, utilising both hydrophilic and lipophilic retention mechanisms. Consequently, a full suite of drugs (up to 90) can be extracted simultaneously off-line using this sorbent [17-18, 25, 33]. The introduction of fully automated methods further reduces sample processing restrictions [34-35]. At present these are emerging techniques whose use are not widespread. Their reproducibility and ability to use smaller total sample volumes mean they will supersede traditional labour intensive SPE protocols in commercial laboratories in the future. Drug determination in the particulate phase of wastewaters is not routinely monitored but those methods which do, use ultrasonic solvent extraction (USE) [36] or most commonly, accelerated solvent extraction (ASE) [30, 37-40]. The application of pressure enables the use of extraction solvents (eg methanol) at temperatures much higher than their boiling point, increasing solubility and mass transfer [41]. Following extraction the solvent can be diluted in water to <5 % (v/v) and subjected to SPE as an aqueous fraction [38-39].

Gas chromatography-mass spectrometry is well established for quantification of chemicals in environmental samples, achieving method quantitation limits (MQLs)  $\leq 10 \text{ ng l}^{-1}$  for some drugs [42-43]. However, derivatization of more polar drugs with toxic chemicals is required prior to analysis to improve volatility, thermal stability and sensitivity of detection, increasing cost and time of sample preparation. A further disadvantage is the run time required for

analysis; often up to 1 hour per sample [42-43]. This has been a rate limiting step of such research in the past, severely restricting sample numbers which can be analysed. Despite these limitations, methods report the ability to simultaneously measure  $\geq 63$  drugs of varying therapeutic class from a single injection by GC-MS following derivatization [44-45]. The use of LC coupled to tandem MS detection (MS/MS) improves sample throughput without the need for additional sample preparation [46]. Furthermore, the introduction of UPLC offers additional reductions in run times, whilst improving sensitivity over conventional LC [23]. For UPLC, run times are generally less than 10 minutes with MQLs  $< 100 \text{ ng l}^{-1}$  for most drugs [17-18, 25]. However, a well known problem of environmental sample analysis by LC-MS, with electrospray ionisation (ESI) source particularly, is the quenching influence (ionisation suppression) of sample matrix on analyte signal strength [23, 47]. The commercial availability of deuterated surrogates now offers substantial improvements in minimizing the impact of matrix interferences, improving accuracy of analysis and up to 50 isotopic labelled standards are used in some multi-residue methods [35].

The selection of MS/MS detector type is critical for analysis type (i.e., qualitative or quantitative). Quantitative analysis commonly employs a triple quadrupole (QqQ) due to its high sensitivity. The use of hybrid detectors such as quadrupole time of flight (QqTOF) offers the ability to screen and identify unknown degradation products/metabolites. Its full scan sensitivity, high selectivity and specificity enable structural elucidation of non-target species [32]. This is a significant advance in the determination of drug fate where degradation products in both biological and chemical processes are formed; enabling degradation pathways to be identified. However, data processing can be time consuming due to the lack of searchable libraries, often requiring manual spectral interpretation [32, 46]. A further disadvantage of TOF detection is it generally offers lower sensitivity (3 to 5 times) than conventional MS/MS such as QqQ [48]. Thus, the ever increasing requirement to reduce MQLs for trace analysis confines its use to qualitative fate evaluation. Alternatively, hybrid linear ion trap (LIT) Orbitrap instrumentation offers high sensitivity for environmental quantitation (as offered by QqQ) and the ability to perform accurate mass determinations for drug identification (as offered by TOF) [46]. Although Orbitrap technology has been used to screen environmental samples for unknowns [49], unequivocal confirmation of degradation products observed by non-target MS/MS screening requires a complementary analytical technique or use of analytical reference standards [50]. However, as degradation products are often not known, there is a subsequent lack of standards for these. Further opportunities are offered by Fourier transform-MS which demonstrates unrivalled mass accuracy, rapid data collection, good dynamic range and, high sensitivity and resolution. However, the high cost of such instrumentation limits its widespread application.

Overall, recent trends for drug determination in wastewaters tend to use a single stage Oasis HLB off-line SPE followed by UPLC with detection by QqQ [17, 25, 33] or LIT [18] (Table 3). These methods are well suited for the determination of multiple drugs in the aqueous phase of crude wastewaters and secondary effluents where their concentrations are relatively high. However, monitoring EE2 at environmentally relevant levels requires devoted clean up protocols which can be laborious due to its inherently lower concentrations [23].

Nevertheless, there is a lack of particulate phase analysis undertaken and the concentrations present in tertiary effluents continue to pose an analytical challenge.

### **3. Drug removal by conventional wastewater treatment; the current problem**

Activated sludge is an extensively implemented secondary wastewater treatment process, effective for carbonaceous material removal and can be adapted for nutrient removal.

Removals of many drugs (and other chemicals of anthropogenic origin) are also observed [6-7]. Removal of drugs and other hazardous chemicals during treatment is attributed to biological degradation and sorption onto biomass [52-54]. Pharmaceutical drugs have low vapour pressures and pKa's ranging from 3 to 10 (Table 1) restricting any removal by volatilization. The relative resistance to biodegradation and/or sorption of some drugs makes enhancing removal by such processes difficult. Ibuprofen, diclofenac and carbamazepine encompass extremities in susceptibility to biodegradation and sorption. These represent drugs reasonably amenable to sorption and biodegradation (ibuprofen), sorption only (diclofenac) and neither sorption or biodegradation (carbamazepine), respectively. Consequently, removal differs between one another during activated sludge treatment [6-7]. Solids retention time (SRT) (which is proportional to the food: micro-organism (F:M) ratio) is the simplest way of manipulating existing activated sludge operation in the short term and, is considered critical to the removal of non-drug derived hazardous chemicals [6-7, 27]. An increased SRT (>10 days) is often cited as the condition required to achieve greatest hazardous chemical removal [6-7] but has little effect to removals of these drugs (Figure 1, Table 4). Generally, diclofenac is removed by  $\leq 50\%$  and any carbamazepine removal is negligible. Negative drug removals are also observed during treatment and are considered attributable to the deconjugation of metabolites present in the crude stream [26]. Conjugates and intermediate chemicals tend to go undetermined by current analytical approaches. Parent drugs are often above their legislative targets for consent in secondary effluents despite accounting for typical dilution ratios in the environment [19]. Source control would limit drug entry into wastewater, similar to what has been achieved with nonylphenol [23]. Without the availability of substitute drugs, less persistent and with a less burdensome environmental impact, this will not be achievable. However, the possibility of separate treatment of urine

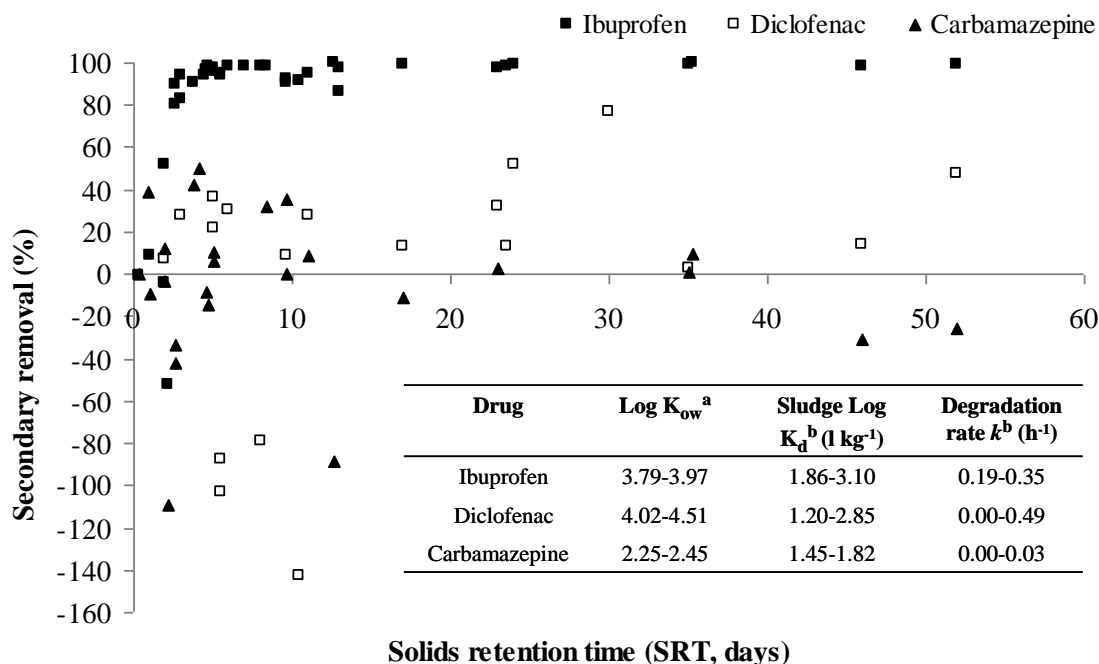
**Table 3. Recently validated LC-MS/MS methods applied for the quantitation of drugs in environmental matrices**

No. of drugs	Sample	SPE	Chromatography	Detector	Run time / min <sup>-1</sup>	SE recovery / %	SE MQL / ng l <sup>-1</sup>	No. of drugs quantified in methods application				Method benefits	Method limitations	Ref.
								Crude WW	SE	TE	SW			
81	Aq.	Oasis HLB	UPLC	QqLIT	4 <sup>a</sup> 7 <sup>b</sup>	22-146	0.6-51	57 <sup>c</sup>	59	28	45	√	XX	[18]
47	Aq.	Oasis HLB	UPLC	QqQ	10	49-127	0.8-170	-	37	-	31	√	X	[25]
74	Aq.	Oasis HLB	UPLC	QqQ	8 <sup>a</sup> 5 <sup>b</sup>	0-174	0.1-378	-	-	-	73	√	X XX	[17]
90	Aq.	Oasis HLB	HPLC	QqQ	25	5-246	0.1-78	-	63	-	-	√	X	[33]
33 <sup>d</sup>	Aq.	Oasis HLB + 3 sorbents	HPLC	QqQ	36 <sup>e</sup>	64-166	2.3-186	-	-	-	16	√√ √√√	X	[35]
87	Aq.	TurboFlow column	HPLC	QqQ	22 <sup>e</sup>	12-345	0.1-164	-	-	-	44	√ √√ √√√	X XX	[34]
14	Part.	Oasis HLB	HPLC	QqQ	-	70-120	0.6-19 <sup>f,g</sup>	-	11 <sup>g</sup>	-	-	√√√√	X	[40]
60	Part.	Oasis MCX	UPLC	QqQ	20	7-142 <sup>c</sup>	0.1-20 <sup>f,g</sup>	30 <sup>f</sup>	-	-	-	√	X	[30]
5	Aq. Part.	MIP	HPLC	QqQ	25	95-105 77-91	4-12 4-10 <sup>f,i</sup>	-	5 <sup>j</sup>	-	4 <sup>f,i</sup>	√√√√	X XXX	[51]
1 <sup>k</sup> (EE2)	Aq. Part.	C18, NH <sup>2</sup> Silica, NH <sup>2</sup>	UPLC	QqQ	9	96 97	0.06 2.96 <sup>f</sup>	1	1	-	-	√√√√ √√√√√	X XXXX	[23]

<sup>a</sup>positive ionisation mode <sup>b</sup>negative ionisation mode <sup>c</sup>application is not a sequential train of processes <sup>d</sup>includes additional 55 chemicals in method <sup>e</sup>includes on-line SPE time <sup>f</sup>ng g<sup>-1</sup> <sup>g</sup>de-watered sludge <sup>h</sup>settled sewage <sup>i</sup>sediment <sup>j</sup>aqueous only <sup>k</sup>includes 4 natural estrogens in method

Key: MQL, method quantitation limit; SPE, solid phase extraction; SE, secondary effluent, WW, wastewater; TE, tertiary effluent; SW, surface water; Aq., aqueous; Part., particulate; QqLIT, linear ion trap; QqQ, triple quadrupole; MIP, molecularly imprinted polymer; √, high number of drugs varying in physio-chemical composition; √√, includes some known degradation products; √√√, fully automated, small sample requirements; √√√√, high recoveries for all drugs measured; √√√√√, very low MQL; X, application to real samples limited; XX, numerous drugs reported <MQL; XXX, limited to drugs of specific physio-chemical composition; XXXX, extensive sample pre-treatment required





**Figure 1. Removals of ibuprofen (■, n=36), diclofenac (□, n=19) and carbamazepine (▲, n=25) by activated sludge operating at varying solids retention time. Ibuprofen, diclofenac and carbamazepine represent drugs reasonably amenable to sorption and biodegradation, sorption only and neither sorption or biodegradation, respectively. Inset, octanol-water coefficients, partition coefficients and degradation rate constants – <sup>a</sup>[16] <sup>b</sup>[53]**  
(removal data obtained from: [6-7, 26, 55-62] – Table 4, no data reported as <MDL)

streams may be an effective solution in some circumstances [63]. Additionally, membrane bioreactor systems generally achieve greater drug removals than conventional secondary processes [32, 60-61]. Without their application, the remaining alternative to enhance drug removal is the addition of a tertiary process or processes to an existing conventional secondary treatment asset (e.g., activated sludge). This requires a process not excessively space consuming and can treat secondary effluents at relatively short contact times. Some available options suiting these criteria include; biofiltration (sand or trickling filters), chemical (ozone) and adsorption (granular activated carbon) processes. The fate and removal of drugs differ substantially between these systems.

#### 4. Drug fate and removal in tertiary processes

##### 4.1. Biologically active sand filters

Drug removal by biofiltration processes (tertiary sand or trickling filters) is by physical and biological mechanisms. Total removals by sand filters vary from 2 % for carbamazepine to >95 % for ibuprofen [64] (Figure 2, Table 5), similar to those achieved by activated sludge. Despite treating different wastewaters of differing composition, activated sludge and tertiary sand filters essentially rely on the same mechanisms. Sand filters depend on a fixed biofilm

**Table 4. Removal of ibuprofen, diclofenac and carbamazepine in activated sludge at varying SRT**

Drug	Flow rate / m <sup>3</sup> d <sup>-1</sup>	SRT/ d <sup>-1</sup>	HRT/ h <sup>-1</sup>	MLSS / mg l <sup>-1</sup>	COD / mg l <sup>-1</sup>	SS conc. / ng l <sup>-1</sup>	SE conc. / ng l <sup>-1</sup>	Removal / %	Ref.
Ibuprofen	-	0.3	-	-	-	-	-	-1.0	[6]
	-	1	-	-	-	-	-	9.0	[6]
	626,000	1.9	7	-	-	50,700	24,600	51.5	[55]
	-	2	1.9	4,000	-	2,300	2,400	-4.2	[7]
	47,860	2.2	13	-	-	14,200	21,700	-52.8	[55]
	185,000	2.7	12	-	-	27,900	5,400	80.6	[55]
	585,667	2.7	14	-	-	58,200	6,200	89.3	[55]
	3,967	3	10	3,030	113*	-	-	94.0	[26]
	22,000	3	12	-	508.2	13,355- 17,585	1,420- 6,056	82.5	[61]
	645,000	3.8	8.6	-	-	909	86.7	90.5	[58]
	1,984	4.5	15	2,482	113*	-	-	94.0	[26]
	409,000	4.6	8.0	-	-	593	21.0	96.4	[58]
	125,248	4.7	13	-	-	21,800	300	98.6	[55]
	1,199,000	5.0	7.1	-	-	578	14.3	97.5	[58]
	-	5.0	-	-	-	-	-	96.0	[6]
	5,506	5.5	15	2,836	205*	-	-	95.0	[26]
	15,300	5.5	15	1,743	154*	-	-	94.0	[26]
	19,260	6	22	2,084	128*	-	-	98.0	[26]
	125,000	7	12	2,000	-	1,966	40.0	98.0	[60]
	20,000	6-10	35	-	510-680	5,700	88.5	98.4	[62]
	210,000	8.4	8.9	-	-	595	8.0	98.7	[58]
	68,498	9.6	15	-	-	27,300	2,700	90.1	[55]
	-	9.6	-	-	-	-	-	92.0	[6]
	17,994	10.5	13	2,105	51*	-	-	91.0	[26]
	-	10-12	7.3	-	-	-	-	91-99	[56]
	366,898	12.6	23	-	-	39,100	50.0	>99.9	[55]
	60,000	12-14	22	5,000- 6,000	-	6,242	194	97.0	[57]
	11,783	13	13.5	2,740	143-160	-	-	86.0	[59]
	-	17.0	-	-	-	-	-	99.0	[6]
	-	22-24	16.8	-	-	-	-	96-98	[56]
-	23.6	-	-	-	-	-	98.0	[6]	
-	24.0	-	-	-	-	-	99.0	[6]	
-	35.0	-	-	-	-	-	99.0	[6]	
5,074	35.3	27	-	-	58,900	50.0	>99.9	[55]	
-	46	28.8	3,100	-	1,200	24.0	98.0	[7]	
-	52	326	4,000	-	2,448	20.0	99.2	[7]	
Diclofenac	-	1.0	-	-	-	-	-	0.0	[6]
	-	2	1.9	4,000	-	1,400	1,300	7.1	[7]
	3,967	3	10	3,030	113*	-	-	28.0	[26]
	105,300	5	16	2,450	127*	-	-	22.0	[26]
	-	5.0	-	-	-	-	-	36.0	[6]
	5,506	5.5	15	2,836	205*	-	-	-88.0	[26]
	15,300	5.5	15	1,743	154*	-	-	-103	[26]
	19,260	6	22	2,084	128*	-	-	30.0	[26]
	20,000	6-10	35	-	510-680	100	485	-79.4	[62]
	-	9.6	-	-	-	-	-	9.0	[6]
	17,994	10.5	13	2,105	51*	-	-	-143.0	[26]
	-	10-12	7.3	-	-	-	-	22-33	[56]
	-	17.0	-	-	-	-	-	13.0	[6]
	-	22-24	16.8	-	-	-	-	30-34	[56]
	-	23.6	-	-	-	-	-	13.0	[6]
	-	24.0	-	-	-	-	-	52.0	[6]
	4,554	30	23	4,554	178*	-	-	77.0	[26]
	-	35.0	-	-	-	-	-	3.0	[6]
	-	46	28.8	3,100	-	905	780	13.8	[7]
	-	52	326	4,000	-	3,190	1,680	47.3	[7]

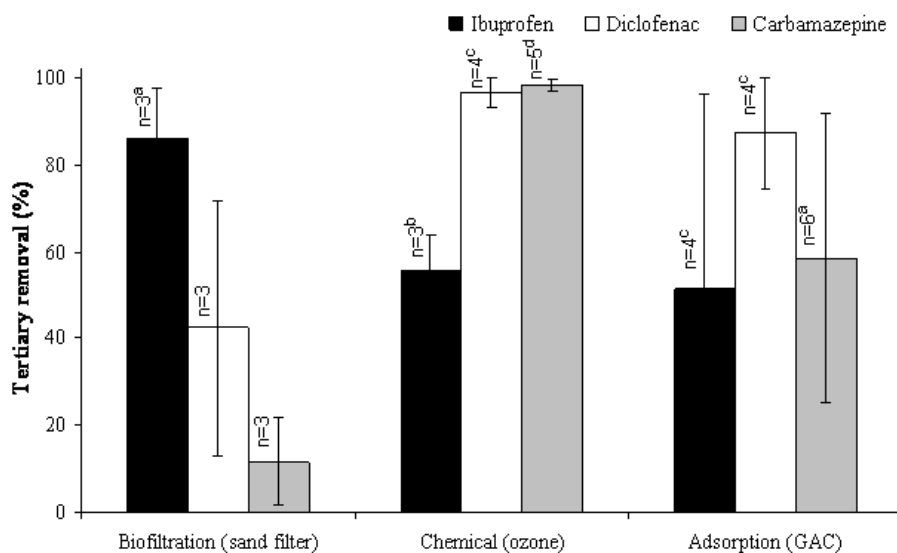
**Table 4 (continued)**

Drug	Flow rate / m <sup>3</sup> d <sup>-1</sup>	SRT/ d <sup>-1</sup>	HRT/ h <sup>-1</sup>	MLSS / mg l <sup>-1</sup>	COD / mg l <sup>-1</sup>	SS conc. / ng l <sup>-1</sup>	SE conc. / ng l <sup>-1</sup>	Removal / %	Ref.
Carbamazepine	-	0.3	-	-	-	-	-	0.0	[6]
	17,364	0.9	12	-	-	1,300	800	38.5	[55]
	-	1.0	-	-	-	-	-	-9.0	[6]
	626,000	1.9	7	-	-	800	700	12.5	[55]
	-	2	1.9	4,000	-	670	690	-3.0	[7]
	47,860	2.2	13	-	-	1,100	2,300	-109.1	[55]
	185,000	2.7	12	-	-	1,200	1,700	-41.7	[55]
	585,667	2.7	14	-	-	600	800	-33.3	[55]
	645,000	3.8	8.6	-	-	55.9	32.1	42.6	[58]
	6,243	4.1	14	-	-	1,000	500	50.0	[55]
	409,000	4.6	8.0	-	-	43.1	46.9	-8.6	[58]
	125,248	4.7	13	-	-	700	800	-14.3	[55]
	1,199,000	5.0	7.1	-	-	50.5	45.4	10.2	[58]
	-	5.0	-	-	-	-	-	6.0	[6]
	210,000	8.4	8.9	-	-	173	117	32.4	[58]
	68,498	9.6	15	-	-	700	700	0.0	[55]
	-	9.6	-	-	-	-	-	9.0	[6]
	-	10-12	7.3	-	-	-	-	0-18	[56]
	366,898	12.6	23	-	-	900	1,700	-88.9	[55]
	-	17.0	-	-	-	-	-	-11.0	[6]
-	22-24	16.8	-	-	-	-	0-5	[56]	
-	35.0	-	-	-	-	-	1.0	[6]	
5,074	35.3	27	-	-	1,000	900	10.0	[55]	
-	46	28.8	3,100	-	325	426	-31.1	[7]	
-	52	326	4,000	-	704	952	-26.1	[7]	

SRT, solids retention time; HRT, hydraulic retention time; MLSS, mixed liquor suspended solids;

COD, chemical oxygen demand; SS, settled sewage; SE, secondary effluent

\*Biological oxygen demand



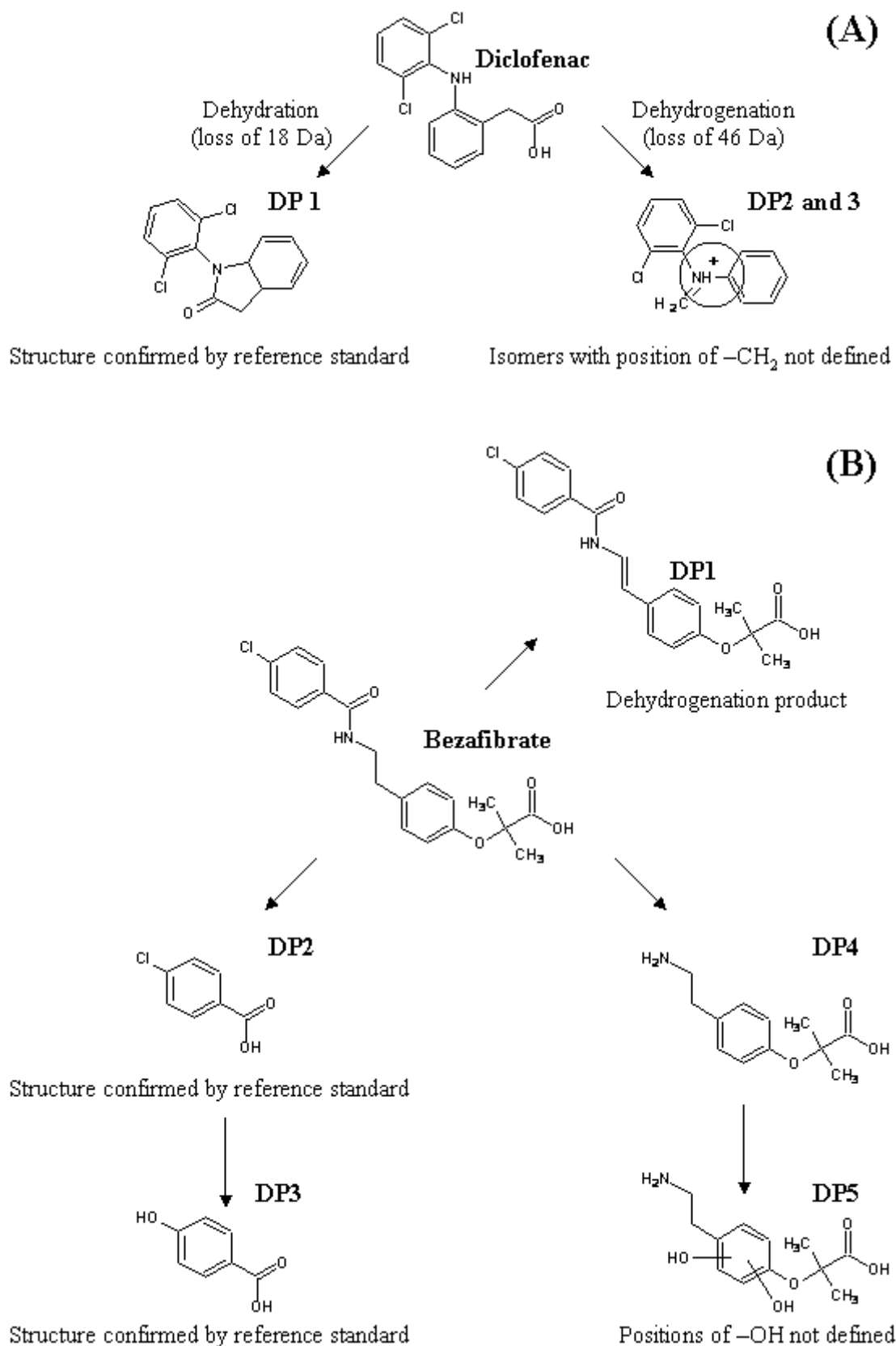
**Figure 2. Removals of ibuprofen, diclofenac and carbamazepine reported in the literature for biofiltration, chemical and adsorption processes. Ibuprofen, diclofenac and carbamazepine represent drugs reasonably amenable to sorption and biodegradation, sorption only and neither sorption or biodegradation, respectively (removal data obtained from: [64-75] - Table 5, 6 and 7)**

<sup>a</sup>1/3 removed <MQL <sup>b</sup>2/3 removed <MQL <sup>c</sup>3/4 removed <MQL <sup>d</sup>4/5 removed <MQL

**Table 5. Removal of drugs from environmental waters by biofiltration processes**

Drug	Process	Temp. °C	HRT h <sup>-1</sup>	Wastewater type	Upfront process	SE. conc. / ng l <sup>-1</sup>	TE conc. / ng l <sup>-1</sup>	Removal / %	Ref.
Ibuprofen	SF	-	4-6	Municipal	-	11,700 <sup>a</sup>	1,170	90	[67]
	SF	-	0.3	River	-	276	<14	>95	[64]
	SF	-	1	Municipal	ASP	-	-	73 <sup>b</sup>	[68]
Diclofenac	SF	-	4-6	Municipal	-	820 <sup>a</sup>	197	76	[67]
	SF	-	0.3	River	-	252	181	28	[64]
	SF	22	2	Municipal	-	-	-	23	[73]
Carbamazepine	SF	-	4-6	Municipal	-	2,060 <sup>a</sup>	1,833	11	[67]
	SF	-	0.3	River	-	85	84	2	[64]
	SF	-	1	Municipal	ASP	-	-	22 <sup>b</sup>	[68]
Naproxen	SF	-	4-6	Municipal	-	1,570 <sup>a</sup>	314	80	[67]
	SF	-	0.3	River	-	170	24	86	[64]
	SF	-	1	Municipal	ASP	-	-	32 <sup>b</sup>	[68]
Ketoprofen	SF	-	1	Municipal	ASP	-	-	16 <sup>b</sup>	[68]
EE2	SF	-	0.3	River	-	316	246	22	[64]
	SF	18	-	Municipal	ASP	-	0.2	82	[76]
	SF	-	-	Municipal	ASP	-	-	7	[77]
	SF	-	-	Municipal	OD	-	-	9	[77]
	SF	22-25	-	Municipal	-	109	64	41	[78]
Erythromycin	SF	-	0.3	River	-	104	75	28	[64]
	SF	19-22	0.4	Municipal	ASP	-	-	20	[79]

comprised of a diverse community of micro-organisms embedded within a matrix of extracellular polymeric substances (EPS) consisting of proteins, nucleic acids, polysaccharides and amphiphilic polymeric compounds [81]. The composition of EPS shifts with biofilm age [81], and is known to influence EE2 uptake [54]. The high tendency of some drugs to partition to solid organic matter, similar to biofilms has been confirmed by ASE followed by LC-MS/MS analysis [30, 38-40]. Those hydrophobic drugs with a comparatively high log  $K_{ow}$  (>4) (e.g., diclofenac and fluoxetine) are considered to have a tendency to sorb to solid organic surfaces such as biofilms [82-83]. However, sorption cannot be predicted by hydrophobicity alone [83-84]; other properties such as molecular weight and ionic speciation are of known importance [83], as is the nature of other dissolved species with which they may interact [85]. Extracellular polymeric substances offer both anionic and cationic functional groups for the exchange of charged species [83]. At a pH typical of municipal wastewaters (e.g., 7-8), EPS is negatively charged [83] with pKa's generally ranging from 6.2 to 10.1 [86]. Those drugs whose pKa is <7 (e.g., ibuprofen, diclofenac,



**Figure 3. Biological degradation products (DP) of diclofenac (A, adapted from [90]) and the proposed biotransformation pathway of bezafibrate (B, adapted from [50])**

naproxen, ketoprofen and oxytetracycline) (Table 1) will themselves be negatively charged and repulsion with the biofilm may restrict sorption. Removal will also be influenced by biofilm porosity. Drugs whose molecular size is comparatively large (e.g., erythromycin), will have a reduced rate of mass transfer between the liquid medium and the biofilm, limiting partitioning [83]. Drugs which are comparatively smaller ( $<300 \text{ g mol}^{-1}$ ) and relatively hydrophobic in nature ( $\log K_{ow}$ 's  $>3$ ) (e.g., ibuprofen, diclofenac, naproxen, ketoprofen, propranolol and EE2, Table 1) are expected to partition well within the biofilm matrix.

Sorption is also considered to be an intermediate step in biodegradation [52, 54]. Assuming similar behaviour to EE2 and other hazardous chemicals in biological processes, drug biodegradation is likely to be mediated on the surface and/or within intact bacterial cells [52, 54, 87]. Free ammonia mono-oxygenase enzymes released by lysis extracellularly are not likely to be involved in biodegradation [52, 87]. Gaulke *et al* [88] observed that EE2 removal in nitrifying batch studies was not by nitrifying bacteria activity, dismissing the hypothesis that nitrification augments EE2 removals [89]. The synthesis of nitro-EE2 confirmed that EE2 is nitrated at high ammonia feed concentrations caused by the high production of nitrates; EE2 removal here is an artefact of laboratory scale investigation. Biodegradation at environmentally representative conditions is by heterotrophic micro-organisms, capable of scavenging a broad range of organic material [87]. Interestingly, differences in biodegradation are observed for drugs which sorb similarly to biomass (e.g., ibuprofen and diclofenac, Figure 1) suggesting chemical structure controls susceptibility to biological attack. The structure of ibuprofen is comparatively simpler than that of diclofenac (i.e., single aromatic ring and non-halogenated, Table 2) which may aid its biodegradation. Drugs of increasing structural complexity and elemental diversity such as antibiotics (e.g., ofloxacin, erythromycin and oxytetracycline) may be less favourable to biodegradation especially considering their possible toxicity to bacteria. However, 'biodegradation' here is not indicative of complete mineralisation. Drugs rich in functional groups provide more possible sites for biological attack, inducing a change to the parent structure. Kosjek *et al* [90] investigated the degradation products of diclofenac, utilising Oasis HLB SPE and UPLC separations to ensure adequate sensitivity required by QqTOF. In full scan mode the total ion chromatogram (TIC) was screened and a protonated compound was then selected for further product ion scans [90]. Accurate mass measurements and in-source fragmentation enabled chemical structure elucidation of three biotransformation products (Figure 3A). Similarly Helbing *et al* [50] used LIT-Orbitrap MS to identify five degradation products of bezafibrate (Figure 3B). The dehydrogenation product (DP1) is structurally similar to the parent drug indicating it may behave similarly in the environment.

#### 4.2. Chemical oxidation

Titanium dioxide photocatalysis [91] and Fenton chemistry (i.e., catalytic oxidation of hydrogen peroxide) [92] have been applied to water treatment however ozone is the most well established and studied of the chemical processes for drug removal. Ozone treatment enhances the removal of all drugs including carbamazepine where removals of  $\geq 96\%$  are observed [65, 70-71, 73-74] (Figure 2, Table 6). However, typical ozone doses applied during water treatment often do not enable full mineralization of drugs [93], likely to be caused by the clouding influence of the matrix. Wastewaters contain relatively high concentrations of bulk organics which can shield targeted chemicals from removal, quenching the ozone dose. Furthermore, Huber *et al* [93] observed that following removal of EE2 from clean water by a high ozone dose, a slow re-appearance of the drug (0.1-0.5 % of the initial concentration) occurred. It is hypothesized that some EE2 is in the form of hydroperoxides which are not readily reactive to ozone. This could be greater in wastewater where clouding will reduce reaction kinetics and this will hinder the complete mineralisation of the parent drug. Degradation by ozonation can occur selectively by direct ozone attack itself and non-selectively by hydroxyl radicals formed upon ozone decay [93]. Ozone reacts rapidly with phenols at neutral or basic pH [94] therefore it will readily attack the phenolate anion of EE2 and oxytetracycline. It also selectively attacks amines and double bonds of aliphatic chemicals [95]. The chemical structure of all the drugs considered here (except ibuprofen) are highly susceptible to direct ozone attack (Table 2). Hydroxyl radicals are less selective and react with a range of chemical functional groups. The non-selective behaviour of the hydroxyl radicals can induce complex reaction pathways [93]. Numerous authors observed high removals of various drugs by ozone treatment, often to concentrations below their MQLs [65, 70-71]. However, complete removal of the parent drug does not necessarily represent removal of toxicity. Structurally similar degradation products of potential toxicity can be formed and remain undetected using conventional MS/MS (i.e., QqQ). A large number of degradation products for various drugs have been observed [93, 96]. Non-target screening of ozone treated water enabled determination of 17 degradation products of diclofenac [96] (Figure 4). The majority of these products are structurally similar to the parent drug indicating similar behaviour in the receiving environment. Again these were identified by Oasis HLB extraction and QqTOF detection. Accurate mass spectra were collected at mass to charge ( $m/z$ ) ratios  $>50$  encompassing all degradation products of notable size.

#### 4.3. Adsorption by activated carbon

Activated carbon often contained in a packed bed or filter is a highly porous medium offering a large internal surface area for sorption to take place. Performance is dependant on activated carbon properties (e.g., pore size, surface charge) and solute characteristics (e.g., shape, size)

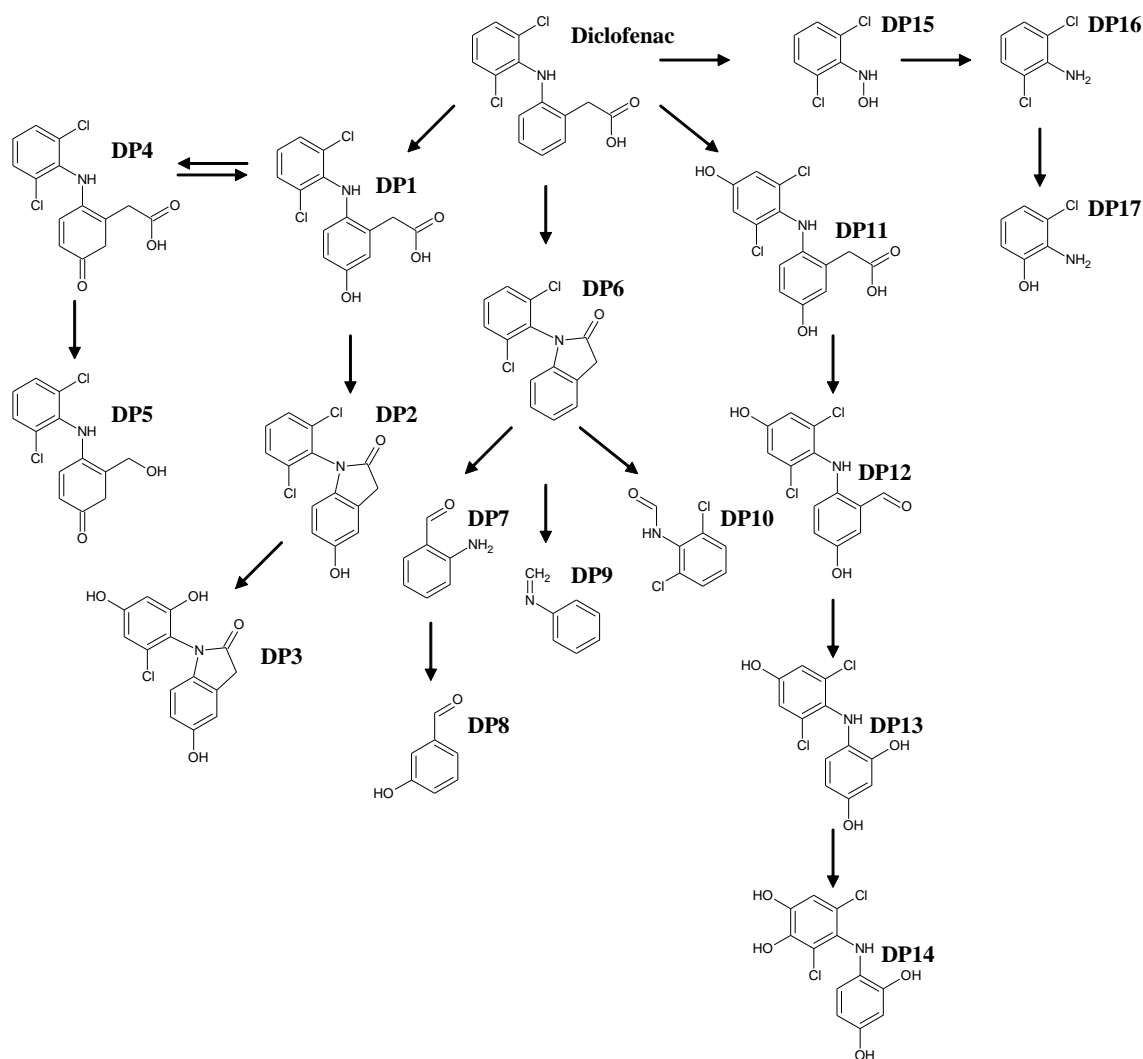
**Table 6. Removal of drugs from environmental waters by ozone treatment**

Drug	Process	Chemical dose	HRT h <sup>-1</sup>	Wastewater type	Upfront process	SE. conc. / ng l <sup>-1</sup>	TE conc. / ng l <sup>-1</sup>	Removal / %	Ref.
Ibuprofen	Ozone	10-15 mg l <sup>-1</sup>	0.3	Municipal	ASP	130	<50	>62	[65]
	Ozone	2 mg l <sup>-1</sup>	0.2	Surface waters	-	-	-	40-77	[75]
	Ozone	3 mg l <sup>-1</sup>	0.5	Municipal	ASP + SF	-	-	>46 <sup>a</sup>	[68]
Diclofenac	Ozone	0.6 g O <sub>3</sub> g DOC <sub>0</sub> <sup>-1</sup>	-	Municipal	ASP	2,000	<10	>99	[71]
	Ozone	3 mg l <sup>-1</sup>	0.3	Municipal	-	-	-	92	[73]
	Ozone	50 µM	<0.1	Municipal	-	433	<1	>99	[70]
	Ozone	10-15 mg l <sup>-1</sup>	0.3	Municipal	ASP	1,300	<50	>96	[65]
Carbamazepine	Ozone	0.6 g O <sub>3</sub> g DOC <sub>0</sub> <sup>-1</sup>	-	Municipal	ASP	900	<1	>99	[71]
	Ozone	3 mg l <sup>-1</sup>	0.3	Municipal	-	-	-	96	[73]
	Ozone	130 µM	0.1	Municipal	-	106	<1	>99	[70]
	Ozone	1 mg l <sup>-1</sup>	-	Municipal	GAC	67	1	99	[74]
	Ozone	10-15 mg l <sup>-1</sup>	0.3	Municipal	ASP	2,100	<50	>98	[65]
Naproxen	Ozone	3 mg l <sup>-1</sup>	0.5	Municipal	ASP + SF	-	-	>99 <sup>a</sup>	[68]
	Ozone	10-15 mg l <sup>-1</sup>	0.3	Municipal	ASP	100	<50	>50	[65]
Bezafibrate	Ozone	0.6 g O <sub>3</sub> g DOC <sub>0</sub> <sup>-1</sup>	-	Municipal	ASP	1,500	345	77	[71]
	Ozone	340 µM	0.3	Municipal	-	115	4	97	[70]
	Ozone	2 mg l <sup>-1</sup>	0.2	Surface waters	-	-	-	>98	[75]
Fluoxetine	Ozone	50 µM	<0.1	Municipal	-	17	<2	>88	[70]
Ketoprofen	Ozone	3 mg l <sup>-1</sup>	0.5	Municipal	ASP + SF	-	-	73 <sup>a</sup>	[68]

[69]. Preferential attraction to the activated carbon surface is by hydrogen bonding and London forces creating a strong binding affinity. Even moderately hydrophobic chemicals ( $\log K_{ow} > 2$ ) have a high propensity to removal [95]. The availability of some drugs as anions causes them to be attracted to the carbon surface. As a result substantial removals of hydrophobic and hydrophilic drugs have been observed by granular activated carbon (GAC) [66, 73-74] (Figure 2, Table 7). Carbamazepine removals up to 97 % have been achieved [73]. Despite the very hydrophilic nature of oxytetracycline (Table 1), its large molecular size is likely to be entrapped within the highly porous structure of the activated carbon. At full-scale treatment processes low removals have been observed for some drugs; ibuprofen (16 %) [69], carbamazepine (16-23 %) [69, 72] and propranolol (17 %) [72] (Table 7). The quality of the secondary effluent will have a significant influence on the performance of GAC through the competition for available sorption sites [69]. The frequency of replacement/regeneration of the activated carbon medium is another controlling factor to its success, especially whilst treating wastewaters comprising relatively high concentrations of



bulk organics. This may account for large variations in drug removals observed between processes. Chiu *et al* [97] demonstrated the possibility of *in situ* catalytic regeneration of GAC using iron nano-catalysts. This could provide an effective means of regeneration in the future, limiting variations in performance currently observed.



**Figure 4. Degradation intermediates formed by ozone treatment of diclofenac and proposed degradation pathways (adapted from [96])**

## 5. Future trends

Secondary effluents typically demand analytical MQLs in the low  $\text{ng l}^{-1}$  range to determine most drugs. Reported analytical methods are well suited for determining drugs in the aqueous phase of secondary effluents. To demonstrate, no data in Figure 1 (and Table 4) was reported below MQL ( $n=80$ ). Despite proposed legislative targets being applied to the aqueous phase

**Table 7. Removal of drugs from environmental waters by adsorption processes**

Drug	Process	HRT h <sup>-1</sup>	Wastewater type	Upfront process	SE. conc. / ng l <sup>-1</sup>	TE conc. / ng l <sup>-1</sup>	Removal / %	Ref.
Ibuprofen	GAC	0.3	Municipal	ASP	64	<10	>84	[74]
	GAC	-	Raw water	-	23	<1	>96	[66]
	GAC	-	Surface water	-	1.1	<1	>10	[69]
	GAC	-	-	SF	8,760	7,325	16	[69]
Diclofenac	GAC	0.3	Municipal	ASP	99	<10	>90	[74]
	GAC	-	Municipal	ASP	-	-	>98	[72]
	BAC	2	Municipal	MBR	-	-	92	[73]
	GAC	-	-	SF	3.2	<1	>69	[69]
Carbamazepine	GAC	0.3	Municipal	ASP	250	67	73	[74]
	GAC	-	Municipal	ASP	-	-	23	[72]
	BAC	2	Municipal	MBR	-	-	97	[73]
	GAC	-	Raw water	-	8	<1	>87	[66]
	GAC	-	Surface water	-	2.2	<1	>55	[69]
	GAC	-	-	SF	199	168	16	[69]
Propranolol	GAC	-	Municipal	ASP	-	-	17	[72]
EE2	GAC	-	Municipal	ASP	-	-	>43	[72]
Erythromycin	GAC	0.3	Municipal	ASP	270	28	90	[74]
	BAC	2	Municipal	MBR	-	-	92	[73]

HRT, hydraulic retention time; SE, secondary effluent; TE, tertiary effluent; ASP, activated sludge plant; GAC, granular activated carbon; BAC, biologically activated carbon; MBR, membrane bioreactor

of wastewaters (i.e., a pre-filtered sample); suspended solids can provide a pathway to their release into the environment [30]. Their determination in the particulate phase is also essential for fate evaluation. Suspended solids are ubiquitous to wastewaters and can vary spatially and temporally. Particulate bound drugs often go undetermined (Table 3), owing to the complexity of the matrix and the additional analytical requirements it demands. The proposed requirement to undertake particulate phase analysis to determine drug fate is most pertinent to secondary processes which receive relatively high concentrations of suspended solids. The relatively hydrophobic nature of some drugs can cause them to partition well to solids. For example crude wastewaters can contain >50 % of fluoxetine bound to particulates [30]. Monitoring here enables complete process mass balances to be determined, aiding fate and performance understanding. Particulate fate understanding may indicate a clouding influence during treatment which limits removal. Activated sludge sorption and biodegradation may be restricted for drugs associated with particulates in the receiving wastewater. This could lead to conventional process optimisation to enhance drug removal. For example, the use of micro-screens in place of conventional primary sedimentation tanks

could enhance particulates removal from the crude stream. However, there is a substantial gap between drug concentrations achieved by the current operations of existing secondary assets and proposed legislative targets [19] (Table 2, Table 4). Therefore there is an expectant need for tertiary treatment technologies to target these specific chemicals.

Tertiary treatment processes enhance drug removal, significantly reducing effluent concentrations. To fully ascertain tertiary process performance, analytical methods require MQLs  $<10 \text{ ng l}^{-1}$  [64, 70-71, 74], and ideally  $<1 \text{ ng l}^{-1}$  [66, 69-71] (Tables A2-4). This poses a further analytical challenge as such concentrations cannot be ascertained for the majority of drugs with current MQLs. To illustrate, concentrations of the representative drugs; ibuprofen, diclofenac and carbamazepine in sand filtration, ozone and activated carbon treated effluents were reported below MQL in 49 % of cases (n=35) (Figure 2). Despite these being below proposed legislative targets for most drugs (Table 2), monitoring at these concentrations is needed as the cumulative toxicological effect of drugs is not known. This could result in a future reduction in legislative requirements. To illustrate, the previous EE2 predicted no effect concentration (PNEC) in the UK was  $0.1 \text{ ng l}^{-1}$  [98]. The proposed EQS is now  $0.035 \text{ ng l}^{-1}$  following its classification as a priority hazardous chemical [12]. This has created a serious analytical burden as such concentrations are now beyond current analytical capabilities [23]. Lowering current MQLs is also needed to assess breakdown reaction completeness. The first stage of this is to determine parent drug removal. Further investigation of specific breakdown mechanisms to understand the criticality between parent drug final concentration, and degradation product production is needed. Methodologies to quantify the full range degradation products will be restricted in the short term by the lack of unique reference standards available for these. However, the identification of numerous degradation products in both biological and chemical processes has brought attention to their presence and created a demand for their commercial availability (Figures 3-4). The complimentary use of biological assays would improve understanding of the synergistic toxicological effect of multiple drugs and their degradation products at low concentration. Process design and operation must integrate the removal of these intermediates which can be of greater concern than the parent chemical due to their subsequent transformation to more toxic chemicals in the environment [47].

Lowering drug MQLs requires existing analytical method optimisation. The low recoveries ( $<50 \%$ ) typically stipulated prior to internal standard correction [25], can be improved to reduce the achievable MQL. Baker and Kasprzyk-Hodern [99] gave an excellent account of sample preparations parameters which can influence recovery. For example drugs can be adsorbed onto glassware surfaces during handling and processing. Using silanized SPE

extract vials gave recoveries six times higher than non-silanized vials for some drugs. All glassware used during sample collection and processing requires silanization to ensure maximum recoveries. Silanization of glassware is not mentioned in the procedures of most reported analytical methods [17-18, 25, 33]. Improving chromatographic separations could also significantly increase detection capabilities. Incorporating a large number of drugs into a single short UPLC run (<10 minutes) results in a number of co-eluting peaks [17-18, 25]. Despite the use of mass scanning windows which typically range from 0.3 to 2 minutes in length for UPLC separations [17-18], sensitivity can be lost whilst simultaneously scanning for a number of transitions registered at the same time [17]. To demonstrate, Gros *et al* [18] reports up to 10 drugs co-eluting within a 0.1 minute time period with scanning windows of 0.5 minutes. Thus, only monitoring for one drug (of most criticality) in this time period could increase sensitivity and notably reduce the MQL.

Tertiary processes receive secondary effluents comprising comparatively high concentrations of dissolved organics (e.g., colloids). However, knowledge of drug behaviour in the charged colloidal fraction of wastewater is limited. Shen *et al* [100] successfully showed humic acid, a small molecular weight charged species could effectively retain the hormone, estrone in solution. This can restrict sorption in tertiary processes characterised by very short contact times. Furthermore, the complexity of the colloidal fraction and its interaction with the drugs could also lead to incomplete breakdown reactions in both biological and chemical processes. A fractionation step during sample pre-treatment to separate dissolved colloids by molecular weight will aid this. It is postulated that drugs will preferentially be in specific size fractions. This is likely to vary between drugs due to the range of physicochemical behaviour they exhibit. Understanding drug fate in the colloidal fraction of wastewater is essential for tertiary process selection, diagnosis and optimisation.

## **6. Conclusion**

Advances in both quantitative and qualitative determinations of pharmaceutical drugs have aided the understanding of their occurrence and fate during wastewater treatment. A robust understanding of tertiary process performance is now needed by improving analytical focus. An appropriate treatment strategy could then be implemented to ensure adequate protection of the aquatic environment is achieved.

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