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Niina Vieno

Occurrence of Pharmaceuticals in Finnish Sewage Treatment Plants, Surface Waters, and Their Elimination in Drinking Water Treatment Processes

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Thesis for the degree of Doctor of Technology to be presented with due permission for public examination and criticism in Festia Building, Auditorium Pieni Sali 1, at Tampere University of Technology, on the 8th of June 2007, at 12 noon.

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ABSTRACT

Pharmaceuticals are consumed in high quantities in the modern society and especially in industrialized countries. Residues of the consumed pharmaceuticals are carried to sewage treatment plants and can end up in the aquatic environment via discharges of the treated effluents to surface waters. In the environment, the compounds and there mixtures may cause adverse effects on aquatic organisms or they can be carried to drinking water treatment plants.

In this thesis, two analytical methods were developed to allow the determination of thirteen pharmaceuticals at low ng L^{-1} concentrations in environmental samples. These pharmaceuticals were: ciprofloxacin, norfloxacin and ofloxacin (antibiotics), carbamazepine (antiepileptic), diclofenac, ibuprofen, ketoprofen and naproxen (anti-inflammatories), and bezafibrate (lipid modifying agent). The methods included the following steps: isolation and concentration with solid phase extraction, separation by liquid chromatography and detection with triple quadrupole mass spectrometry. Quantification limits were 0.2–22 ng L^{-1} in drinking water and 3.5–163 ng L^{-1} in raw sewage. Sulfamethoxazole, an antibiotic, was also selected for the study but could only be analyzed at concentrations above 5 µg L^{-1}. Hence, the compound could not be analyzed in the environmental samples. The methods were utilized to study the occurrence of the pharmaceuticals in raw and treated sewages, rivers and drinking waters in Finland. Additionally, the elimination of the pharmaceuticals was studied in different drinking water treatment processes.

The studied pharmaceuticals were detected in the sewage influents at concentrations ranging from <0.02 to 29 µg L^{-1}. The compounds were not fully eliminated in the treatment processes and were measured at concentrations as high as 3.9 µg L^{-1} in the effluent samples. Low sewage temperature as well as the increase of the influent flow rate during heavy rain with a subsequent lowering of the hydraulic retention time hampered the elimination of the pharmaceuticals in the treatment plants.

Sewage effluents were found to be the main source of the studied pharmaceuticals in the sampled rivers. All compounds but norfloxacin were detected above their LOQ concentrations at least in one sample. The concentrations of the compounds were mainly <100 ng L^{-1}. Residuals of pharmaceuticals can be harmful to aquatic organisms at the measured concentrations. Downstream of the treatment plants, lower concentrations of the pharmaceuticals were measured especially in the summer. It was suggested that this was due to more effective phototransformation and biodegradation of the pharmaceuticals in the rivers in the summer than in the winter.

Some of the studied pharmaceuticals were detected at concentrations of 5–8 ngL^{-1} in the drinking water samples. Of the different drinking water treatment techniques, coagulation and sand filtration were inefficient in elimination of pharmaceuticals whereas granular activated carbon filtration and ozonation effectively eliminated the compounds from the raw water. Only ciprofloxacin was not fully eliminated in these unit operations. The effects on humans caused by chronic exposure to low concentrations of pharmaceuticals over a long period of time are unknown. Still, the risk for the consumers is most probably negligible.

TIIVISTELMÄ

Nyky-yhteiskunnassa käytetään suuria määriä erilaisia lääkkeitä. Ihmisten nauttimien lääkkeiden jäämiä kulkeutuu jätevedenpuhdistamoille ja puhdistamoiden purkuvesien mukana lääkeaineet voivat päätyä vesistöihin. Ympäristössä yhdisteet ja niiden seokset voivat aiheuttaa haittaa vesieliöille ja ne voivat myös kulkeutua vesistöjen alajuoksujen vedenottamoille.

Tämän työn tarkoituksena oli tutkia lääkeaineiden esiintymistä Suomen jätevesissä, jokivesissä sekä yhdisteiden poistumista erilaisissa juomavedenkäsittelyprosesseissa. Työssä tutkittiin seuraavia lääkeaineita: siprofloksasiini, norfloksasiini, ofloksasiini (antibiootit), karbamatsepiini (epilepsialääke), diklofenaakki, ibuprofeeni, ketoprofeeni ja naprokseeni (tulehduskipulääke) sekä betsafibraatti (seerumin lipidejä muuntava lääkeaine). Näiden lääkeaineiden analysoimiseksi ympäristönäytteistä kehitettiin kaksi menetelmää, jotka perustuivat näytteiden eristämiseen ja konsentroimiseen käyttäen kiinteäfaasiuuttoa. Konsentroidut näytteet analysoitiin käyttäen nestekromatografiaa ja kolmoiskvadrupoli massaspektrometriä (LC-MS/MS). Juomavedessä yhdisteiden määritysrajat olivat 0.2–22 ng/l ja puhdistamattomassa jätevedessä 3.5–160 ng/l. Tutkimukseen valittiin myös sulfametoksatsoli (antibiootti), mutta sitä pystyttiin analysoimaan ainoastaan >5 µg/l pitoisuuksissa, eikä yhdistettä sen vuoksi voitu analysoida ympäristönäytteistä.

Tutkittavien lääkeaineiden pitoisuudet jätevedenpuhdistamoiden tulevissa jätevesissä vaihtelivat välillä <0,02–29 µg/l. Yhdisteet eivät poistuneet kokonaan puhdistusprosesseissa ja niiden pitoisuudet puhdistetuissa jätevesissä olivat korkeimmillaan 3,9 µg L^{-1}. Jäteveden alhainen lämpötila heikensi lääkeaineiden poistumista. Lisäksi puhdistamolle tulevan jäteveden laimeneminen sadevedellä johti puhdistettavan veden viipymäajan lyhentymiseen laitoksella ja täten myös lääkeaineiden poistumisen heikentymiseen.

Jätevedenpuhdistamoiden havaittiin olevan lääkeaineiden pääasiallinen lähde jokivesistöissä. Kaikkia muita lääkeaineita paitsi norfloksasiinia havaittiin ainakin yhdessä jokivesinäytteessä. Yhdisteiden pitoisuudet jokivedessä olivat suurimmaksi osaksi alle 100 ng/l. Kuitenkin jo näin alhaiset pitoisuudet voivat olla haitallisia vesieliöille. Jokien alajuoksuilla lääkeaineiden pitoisuudet olivat yleensä pienempiä kuin purkupaikan kohdalla. Suurin vähentyminen havaittiin kesällä ja sen oletettiin johtuvan yhdisteiden tehokkaammasta valo- ja biohajoamisesta.

Joitakin lääkeaineita havaittiin juomavesissä hyvin alhaisissa (5–8 ng/l) pitoisuuksissa. Juomavedenkäsittelymenetelmistä kemiallinen saostus ja pikahiekkasuodatus eivät poistaneet lääkeaineita raakavedestä. Aktiivihiilisuodatus ja otsonointi taas poistivat erittäin tehokkaasti lähes kaikkia tutkittuja lääkeaineita. Ainoastaan siprofloksasiini poistui näissä prosesseissa vain osittain. Tällä hetkellä ei tiedetä, aiheutuuko ihmisille haittaa kroonisesta altistumisesta pienille, alle 10 ng/l, pitoisuuksille lääkeaineita. Voidaan kuitenkin olettaa, että riskit kuluttajille ovat erittäin vähäiset.

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Niina Vieno

LIST OF ORIGINAL PUBLICATIONS

(I) Vieno, N., Tuhkanen, T. and Kronberg, L. 2006. Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography – tandem mass spectrometry detection. *Journal of Chromatography A*, **1134**(1-2), 101-111.

(II) Vieno, N., Tuhkanen, T. and Kronberg, L. 2006. Elimination of pharmaceuticals in sewage treatment plants in Finland. *Water Research*, **41**(5), 1001-1012.

(III) Lindqvist, N., Tuhkanen, T., Kronberg, L. 2005. Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. *Water Research*, **39**(11), 2219-2228.

(IV) Vieno, N.M., Tuhkanen, T., Kronberg, L. 2005. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. *Environmental Science and Technology*, **39**(21), 8220-8226.

(V) Vieno, N., Tuhkanen, T., Kronberg, L. 2006. Removal of pharmaceuticals in drinking water treatment: effect of chemical coagulation. *Environmental Technology*, **27**(2), 183-192.

(VI) Vieno, N.M., Härkki, H., Tuhkanen, T. and Kronberg, L. 2007. The occurrence of pharmaceuticals in river water and their elimination in a pilot-scale drinking water treatment plant. *Environmental Science and Technology*, Accepted for publications.

THE AUTHOR´S CONTRIBUTION

Paper I: Niina Vieno planned and performed the experimental work, interpreted the results and wrote the paper.

Paper II: Niina Vieno interpreted the results and wrote the paper. She planned the experimental work and was responsible for the analysis of pharmaceuticals.

Paper III: Niina Vieno interpreted the results and wrote the paper. She planned the experimental work and was responsible for the analysis of the pharmaceuticals.

Paper IV: Niina Vieno planned and performed the experimental work, interpreted the results and wrote the paper.

Paper V: Niina Vieno wrote the paper and was the corresponding author. She planned and performed the experimental work and interpreted the results.

Paper VI: Niina Vieno interpreted the results and wrote the paper. She was responsible for the analysis of pharmaceuticals. MSc Heli Härkki planned and conducted the pilot experiments

ABBREVIATIONS AND SYMBOLS

AC	Activated carbon
ACE	Acebutolol
ACN	Acetonitrile
ALP	Alprenolol
AOP	Advanced oxidization process
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
AS	Activated sludge
ATC	Anatomical Therapeutic Chemical
ATE	Atenolol
CBZ	Carbamazepine
CIP	Ciprofloxacin
DAD	Diode array detector
DCF	Diclofenac
DCM	Dichloromethane
DDD	Defined daily dose
DHA	Dissolved humic acid
DHCBZ	Dihydrocarbamazepine
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DP	Discharge point (of STP effluent)
DS	Downstream (of a STP)
DW	Drinking water
EC50	Effective concentration 50%
ENRO	Enrofloxacin
ESI	Electrospray ionization
ESI–	Negative ESI
ESI+	Positive ESI
EtOAc	Ethyl acetate
FB	Fixed bed reactor
FEN	Fenoprop
GAC	Granular activated carbon
GC	Gas chromatography
GW	Groundwater
HAc	Acetic acid
HFBA	heptafluorobutyric acid
HPLC	High-performance liquid chromatography
HPSEC	High performance size exclusion chromatography
HRT	Hydraulic retention time
IBP	Ibuprofen
IQL	Instrumental quantification limit
I.S.	Internal standard
K_d	Sorption distribution coefficient
KET	Ketoprofen

K_{ow}	Octanol-water partitioning coefficient
LC	Liquid chromatography
LFL	Levofloxacin
LC50	Lethal concentration 50%
LOD	Limit of detection
LOEC	Lowest observed effect concentration
LOQ	Limit of quantification
MEC	Measured environmental concentration
MeOH	Methanol
MET	Metoprolol
MBR	Membrane bioreactor
MRM	Multiple reaction monitoring
MS	Mass spectrometer
MTBE	methyl-*tert*-butylether
MW	Molecular weight
N/DN	Nitrification/ denitrification
NaAc	Sodium acetate
NH_4Ac	Ammonium acetate
NOM	Natural organic matter
NOR	Norfloxacin
NPX	Naproxen
OFL	Ofloxacin
PAC	Powdered activated carbon
pK_a	Acid dissociation constant
PNEC	Predicted no-effect concentration
PS	Primary sludge
Q	Quadrupole
RS	Raw sewage
SMX	Sulfamethoxazole
SOT	Sotalol
SPE	Solid phase extraction
SRT	Solids retention time
SS	Secondary sludge
STP	Sewage treatment plant
SW	Surface water
TEA	Triethylamine
TOC	Total organic carbon
TOF	Time of flight
tQ	Triple quadrupole
UPLC	Ultra-performance liquid chromatography
US	Upstream (of a STP)
UV_{254}	UV absorption at wavelength 254 nm
WHO	World Health Organization

TABLE OF CONTENTS

1 INTRODUCTION

Human pharmaceuticals end up in the environment through human consumption of medicines and disposal of unused medicines via the drain. Consumed pharmaceuticals are excreted through urine or feces as a mixture of parent compounds and metabolites. The compounds are then carried to sewage treatment plants (STPs) where they can be eliminated (e.g. via sorption and biodegradation), be re-formed due to cleavage of the conjugates or pass the process unchanged. The part that survives the treatment process ends up in the aquatic environment. This is considered to be the main route for contamination of the aquatic environment by pharmaceuticals. (Heberer 2002a, Daughton and Ternes 1999, Halling-Sørensen et al. 1998)

The presence of pharmaceuticals in STPs was discovered already in the 1970´s but it was not until the next decade when they were detected in the environment (reviewed in Ternes et al. 2001). Since that, the research in the field has expanded dramatically and almost 100 pharmaceuticals or their metabolites have until now been detected in STPs or in the environment (Kümmerer 2004). Researchers all over the world have began to study the occurrence and fate of pharmaceuticals in different environmental compartments and ecotoxicologists debate whether these sub to low nanogram per liter concentrations are of concern to the aquatic organisms. Even though there is consensus among scientists that acute toxicity is unlikely at the environmentally relevant concentrations (Fent et al. 2006, Jones et al. 2004), there have been studies that report on the adverse effects to the aquatic organisms due to their chronic exposure to pharmaceuticals or mixtures of pharmaceuticals (De Lange et al. 2006, Gagné and André 2006, Thibaut et al. 2006, Flaherty and Dodson 2005). Even though some studies have found pharmaceuticals or their metabolites in drinking water, they are thought to be of minor concern for humans due to their extremely low concentrations (in the range of few ng L^{-1}) compared to the therapeutic doses (in the ranges of milligrams) (Schwab et al. 2005, Webb et al. 2003). Anyway, their presence in drinking water is still a sign of contamination originating from sewage.

In Finland, the research in this field started in the year 2001 in the form of an EU funded project called POSEIDON (EVK1-CT-2000-00047). In this project, participants

from eight countries studied the "Technologies for the removal of pharmaceuticals and personal care products in sewage and drinking water facilities to improve the indirect potable water reuse" (Poseidon 2006). This three-year project was the starting point for this thesis. Originally, only drinking water treatment was to be studied in Finland. However, since there was no data available about the occurrence and elimination of pharmaceuticals in Finnish STPs and surface waters, the thesis expanded to cover also that area. The studied pharmaceuticals belong to the groups of antibacterials, antiepileptics, anti-inflammatories, beta blockers and lipid reducing agents and they are highly consumed in Finland.

1.2 Aims of the thesis

The aims of this thesis were to:

- develop novel analytical methods to quantitatively measure the selected pharmaceuticals in sewage, surface and drinking waters at low ng L^{-1} level (Papers **I** and **III**),
- study the occurrence of the pharmaceuticals in the influents and effluents of Finnish STPs (Papers **I**–**IV**),
- determine the elimination of the pharmaceuticals in Finnish STPs (Papers **II**–**IV**),
- study the seasonality of the elimination of the pharmaceuticals in a STP (Paper **IV**),
- identify the parameters affecting the elimination of the pharmaceuticals in the STPs (Paper **II**),
- study the occurrence of the pharmaceuticals in rivers receiving sewage effluents (Papers **I**, **III** and **IV**),
- study the seasonality of the occurrence and the elimination of the pharmaceuticals in a river (Paper **IV**),
- study the occurrence of the pharmaceuticals in Finnish drinking waters (Paper **III** and partly published first time in this thesis), and
- define the elimination of the pharmaceuticals in drinking water treatment processes: coagulation (Papers **IV**–**IV**), sand filtration (Paper **VI**), granular activated carbon filtration (Papers **IV** and **VI**), ozonation (Paper **VI**) and UV-disinfection (Paper **VI**).

2 LITERATURE REVIEW

2.1 The selected pharmaceuticals

Pharmaceuticals are biologically active compounds that have been developed to treat various diseases. In Finland, there are almost 900 different active substances on the market (National Agency for Medicines 2006). Of these, fourteen were selected on the basis of their high consumption in Finland, low degree of metabolism in humans, and/or frequent detection in the aquatic environment in previous studies. The selected compounds may also be hazardous to the aquatic organisms (see Chapter 4.3.3), but the selection of the compounds was not primarily based on this aspect. There are several ways to classify pharmaceuticals. In this study, Anatomical Therapeutic Chemical (ATC) -classification system introduced by WHO Collaborating Centre for Drug Statistics Methodology (WHO 2006) was used. It is an internationally accepted classification method for drug utilization studies. Based on the ATC-codes, the selected pharmaceuticals were divided into five groups: antibacterials, antiepileptics, anti-inflammatory and antirheumatics, beta blocking agents, and lipid modifying agents (Table 2.1).

Antibacterials were further classified into the fluoroquinolones: ciprofloxacin, norfloxacin and ofloxacin and the sulfonamide, sulfamethoxazole. The fluoroquinolones are used in the treatment of urinary tract infections, respiratory infections, gonorrhea, bacterial prostatitis, cervicitis and anthrax. Sulfamethoxazole is mainly given together with trimethoprim (combination is called co-trimoxazole) to treat pneumonia (especially in AIDS patients) but also urinary tract infections and genital infections. (Rang et al. 2003)

Carbamazepine is one of the most widely used antiepileptic drugs. Beside in the treatment of epilepsy, it is used in the treatment of neuropathic pain (e.g. trigeminal neuralgia) and manic-depressive illness. (Rang et al. 2003)

Diclofenac, ibuprofen, ketoprofen and naproxen are classified as anti-inflammatory and antirheumatics and are also called non-steroidal anti-inflammatory drugs. They are among the most widely used pharmaceuticals worldwide and are often

prescribed for the treatment of rheumatic musculoskeletal complaints. They also have analgesic and antipyretic effects and are thus used in the treatment of various pains (e.g. headache and menstrual pain) as well as to lower a raised temperature. Diclofenac and ketoprofen are also used as a therapeutic agent in various gels and sprays that are used to treat muscle ache. (Rang et al. 2003)

The beta blockers acebutolol, atenolol and metoprolol are used in the treatment of angina, hypertension and dysrhythmias but may also be helpful in the treatment of migraine. Sotalol is mainly used in the prevention of chronic malignant ventricular tachyarrhythmia. (Rang et al. 2003)

A lipid modifying agent, bezafibrate, is used in the treatment of mixed dyslipidaemia which is a risk factor for atheromatous disease. In industrialized countries, atheromatous disease underlies the commonest causes of death or stroke. Bezafibrate reduces the triglyceride level and increases the high density lipoprotein level in the serum. (Rang et al. 2003)

Table 2.1 Names and structures of the selected pharmaceuticals

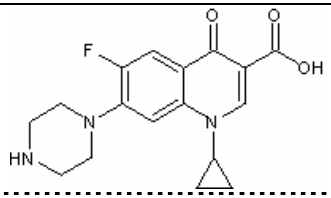
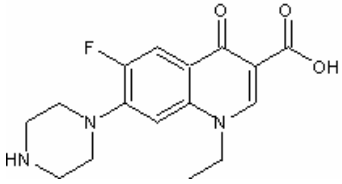
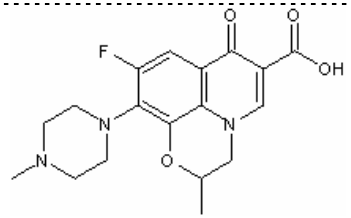
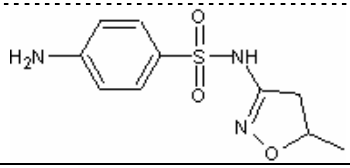
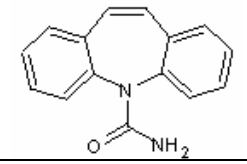
Therapeutic use (ATC-code)	Compound	Molecular structure	CAS number	IUPAC name	Trade names*, 1)	MW (g moL^{-1})
Antibacterials (J01)	Ciprofloxacin (CIP)		85721-33-1	1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid	Cipromed Ciproxin	331.35
	Norfloxacin (NOR)		70458-96-7	1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid	Noroxin	319.34
	Ofloxacin (OFL)		82419-36-1	(±)-9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1, 4-benzoxazine-6-carboxylic acid	Exocin, Tarivid, Oftaquix[a)] Tavanic[a)]	361.38
	Sulfamethoxazole (SMX)		723-46-6	4-Amino-N-(5-methyl-3-isoxazolyl)-benzenesulfonamide	Cotrim[b)]	253.28
Antiepileptics (N03A)	Carbamazepine (CBZ)		298-46-4	5H-Dibenz[b,f]azepine-5-carboxamide	Neurotol Tegretol	236.28

Table 2.1 continues

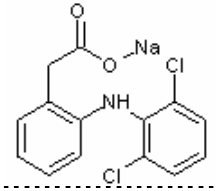
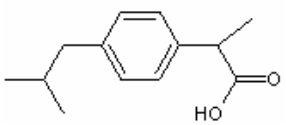
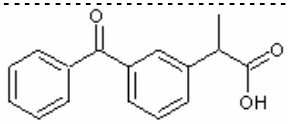
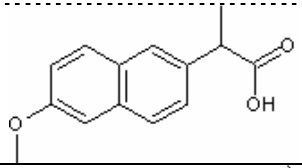
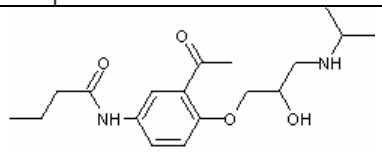
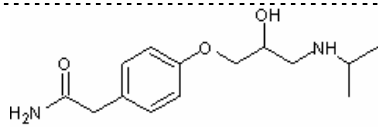
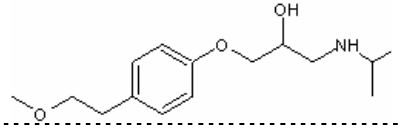
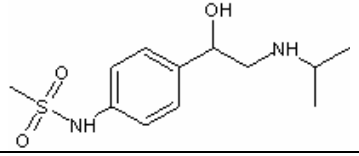
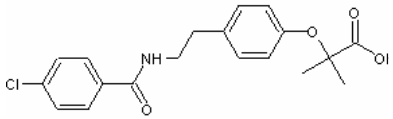
Anti-inflammatory and antirheumatics (M01A)	Diclofenac (DCF)		15307-86-5	2-[(2,6-Dichlorophenyl)amino] benzeneacetic acid monosodium salt	Diclomex Eeze Spray Voltaren	296.16 (sodium salt)
	Ibuprofen (IBP)		15687-27-1	α-Methyl-4-(2-methylpropyl)benzeneacetic acid	Burana Dexit [c)] Ibumax Ibumetin Ibusal Ibuxin	206.29
	Ketoprofen (KET)		22071-15-4	3-Benzoyl-α-methylbenzeneacetic acid	Keto Ketomex Ketorin Orudis	254.28
	Naproxen (NPX)		22204-53-1	(S)-6-Methoxy-α-methyl-2-naphthaleneacetic acid	Alpoxen Miranax Naprometin Pronaxen	230.26
Beta blocking agents (C07A)	Acebutolol (ACE)		37517-30-9	N-[3-Acetyl-4-[2-hydroxy-3-[(1-methylethyl) amino]propoxy]phenyl] butanamide	Diasectral Espesil	336.43
	Atenolol (ATE)		29122-68-7	4-[2-Hydroxy-3-[(1-methylethyl)amino] propoxy]benzeneacetamide	Atenblock Atenol Tenoblock Tenoprin	266.34
	Metoprolol (MET)		56392-17-7	1-[4-(2-Methoxyethyl) phenoxy]-3-[(1-methyl-ethyl)amino]-2-propanol	Logimax Metoprolin Seloken	267.40

Table 2.1 continues

	Sotalol (hydrochloride) (SOT)		959-24-0	N-[4-(1-Hydroxy-2-[(1-methylethyl)amino]ethyl) phenyl]methanesulfonamide	Sotacor Sotalin	308.83
Lipid modifying agents (C10A)	Bezafibrate (BZF)		41859-67-0	2-[4-[2-[(4-Chlorobenzoyl)amino] ethyl]phenoxy]-2-methyl-propanoic acid	Bezalip	361.83

[1)] National Agency for Medicines (2006)
* the medicines on sale in Finland
[a)] therapeutic substance is levofloxacin (the active enantiomer of ofloxacin)
[b)] includes trimethoprim
[c)] therapeutic substance is dexibuprofen (the active enantiomer of ibuprofen)

2.1.1 Physico-chemical properties of the pharmaceuticals

Physico-chemical properties of pharmaceuticals are important in the prediction of their environmental fate and in the development of analytical detection methods. The selected compounds represent only about 2% of all the pharmaceuticals on the market in Finland. Still, the compounds are characterized by wide variation in properties such as water solubility, functional groups, ionic state and partitioning between phases (Table 2.2). A common feature is their low Henry's law constant and preferential partitioning to water rather than to air. In general, the potential of a compound for volatilization can be defined based on Henry's law constant (H_c) and octanol-water distribution coefficient (K_{ow}). The compounds that posses low volatilization potential have $H_c < 10^{-4}$ and $H_c/K_{ow} < 10^{-9}$ (Rogers 1996).

The ability of a compound to partition between water and organic phase is characterized by its K_{ow}. In general, compounds with high affinity for the lipid phase, and thus a high value of K_{ow}, are often characterized by low water solubility. The potential of a compound for sorption to hydrophobic materials and surfaces has been characterized to be low, medium or high if $\log K_{ow}$ is <2.5, 2.5–4.0 or >4.0, respectively (Rogers 1996). In addition, the degree of ionization of a compound affects its K_{ow} value since only the unionized species is partitioned to the lipid phase. The ionization of a compound depends on the pH of the solution. When the solution pH is equal to the pK_a value of a compound, there is a 50:50 mixture of ionized and non-ionized species in the solution. At pH values $pK_a \pm 2$ the other species becomes predominant (Schwarzenbach et al. 2003). K_{ow} value is often used in the estimation of the potential of a compound to sorb to soil and sludge, i.e. the K_d value (Cunningham 2004). This approach is suitable for neutral and hydrophobic compounds that are mainly sorbed by hydrophobic interactions. Most of the studied pharmaceuticals are, however, ionizable and can have ionic, ion pairing and/or complexation reactions with the particles, mineral surfaces and micro-organisms present in soil, sediment and sludge (Cunningham 2004). For them, K_d values should be experimentally determined at different pH values to assess the role of ionic interactions. Sorption of the studied pharmaceuticals to soil, sediment and sludge are discussed in more detail in Chapters 2.3.1, 2.4 and 2.5.

Table 2.2 Physico-chemical properties of the studied pharmaceuticals.

Compound	Henry's law constant (atm m^3 mol^{-1}) [1]	Water solubility (mg L^{-1}) [1]	pK_a [1–8]	$logK_{ow}$ [1]	$logK_d$	
					Sludge [9–14]	Soil and sediment [15–18]
Ciprofloxacin	$5.09 \cdot 10^{-19}$	30 000	3.01, 6.27, 8.87, 10.58	0.28	4.3 AS 3.4 RS	2.6
Norfloxacin	$8.70 \cdot 10^{-19}$	178 000	3.11, 6.26, 8.85, 10.56	-0.13	4.2	-
Ofloxacin	$4.98 \cdot 10^{-20}$	28 300	5.97, 7.65*	-0.39	-	2.49–3.55
Sulfamethoxazole	$6.42 \cdot 10^{-13}$	610	1.85, 5.6	0.89	2.05–2.60 AS	-0.64–1.58
Carbamazepine	$1.08 \cdot 10^{-10}$	17.7	13.9	2.45	0.09 AS <1.3 PS	-0.68–0.72 Sed -0.31–1.57 Soil
Diclofenac	$4.73 \cdot 10^{-12}$	2.37	4.15	4.51	1.2 AS 2.7 PS	-0.26–0.67 Sed -0.35–2.22 Soil
Ibuprofen	$1.50 \cdot 10^{-7}$	21	4.91	3.97	0.85 SS <1.3 PS	-0.74–0.23 Sed
Ketoprofen	$2.12 \cdot 10^{-11}$	51	4.45	3.12	-	-
Naproxen	$3.39 \cdot 10^{-10}$	15.9	4.15	3.18	2.34	0.46 Sed
Acebutolol	$1.34 \cdot 10^{-20}$	259	9.2	1.71	-	-
Atenolol	$1.37 \cdot 10^{-18}$	13 300	9.6	0.16	1.6	-
Metoprolol	$1.40 \cdot 10^{-13}$	4 780	9.7	1.69	~0.5	-
Sotalol	$2.66 \cdot 10^{-14}$	137 000	9.55	0.24	1.6	-
Bezafibrate	$2.12 \cdot 10^{-15}$	0.355	3.61	4.25	-	-

* Similar to the other fluoroquinolones, ofloxacin exhibits four pK_a values, but only two were found in the literature. **References**: [1] SRC PhysProp Database 2006, [2] Schmitt-Kopplin et al. 1999, [3] The Merck Index 2001, [4] Jones et al. 2002, [5] Martínez et al. 1999, [6] SPARC 2006, [7] Bezalip 2003, [8] Qiang and Adams 2004, [9] Golet et al. 2003, [10] Jones et al. 2002 (calculated estimations), [11] Löffler et al. 2005 (experimental), [12] Ternes et al. 2004a, [13] Göbel et al. 2005, [14] Maurer et al. 2007 (experimental), [15] Scheytt et al. 2005, [16] reviewed in Beausse 2004, [17] Drillia et al. 2005, [18] Lin et al. 2006
[AS]= activated sludge, [RS]= raw sewage, [SS]= secondary sludge, [PS]= primary sludge, [Sed]= sediment, -= data not found

2.1.2 Consumption of the pharmaceuticals

The consumption profile of pharmaceuticals in a particular country affects the profile of compounds found in sewage. In Finland, National Agency for Medicines publishes statistics on annual drug consumption. The figures are reported as the amount of defined daily doses (DDD) of a drug per thousand inhabitants. The DDD-values in the reports are based on ATC/DDD classification system by WHO Collaborating Centre for Drug Statistics Methodology (WHO 2006). From the reported figures, the annual consumption of the pharmaceuticals can be calculated using the following formula:

$$\text{Consumption (kg)} = \text{DDD (g)} \times {}^{\text{DDD}}\!/_{1000\,\text{inh}} \times \frac{\text{Population}}{1\,000\,000} \times 366 \qquad (2.1)$$

where DDD is the defined daily dose and DDD/1000 inh is the amount of daily doses consumed per 1000 inhabitants in one year. Table 2.3 compiles the consumption of the studied pharmaceuticals over the years 2002–2005 in Finland. The consumption varies from few hundred to almost 100 000 kilograms. Nowadays, ibuprofen is the most consumed pharmaceutical in Finland and its consumption has increased dramatically over the few years. In the year 2005, altogether 115 tons of the studied pharmaceuticals were consumed in Finland. Out of this amount, about 80% was accounted for ibuprofen. For many of the pharmaceuticals, however, a decreasing trend in their consumption can be observed. This may be due to higher consumption of other drugs in the same therapeutic group. It should also be remembered that the reported figures of DDD/1000 inh are estimates based on the sold amount of pharmaceuticals reported by three major drug wholesale companies in Finland (i.e. KD Tukku, Oriola and Tamro) (National Agency for Medicines 2006).

Table 2.3 Defined daily doses (DDD) and the consumption of the studied pharmaceuticals in Finland (National Agency for Medicines 2006).

Compound	DDD	Consumption (kg yr^{-1})			
	(mg)	Yr. 2002	Yr. 2003	Yr. 2004	Yr. 2005
Ciprofloxacin	1 000 (o) 500 (p)	755	829	803	849
Norfloxacin	800	289	290	291	261
Ofloxacin[a)]	400 (OFL) 500 (LFL)	418	431	411	397
Sulfamethoxazole[b)]	2 000	304	305	268	269
Carbamazepine	1 000	4 940	4 860	4 607	4 354
Diclofenac	100	963	963	927	1 065
Ibuprofen	1 200	70 070	75 430	77 830	94 020
Ketoprofen	150	1 422	1 273	1 118	1 100
Naproxen	500	6 690	6 540	5 980	6 050
Acebutolol	400	1 140	1 029	940	836
Atenolol	75	972	912	863	790
Metoprolol	150	5 400	5 315	5 340	5 270
Sotalol	160	921	747	612	489
Bezafibrate	600	502	446	390	357
Population (10^6 inhabitants)	-	5.206	5.222	5.237	5.255

[a)] Including levofloxacin (LFL)
[b)] Estimated from the consumption figure of a combination drug that includes trimethoprim
o= oral, p= parenteral

Due to the low number of inhabitants in Finland compared to many other countries, the absolute amount of consumed pharmaceuticals is low in Finland (Table 2.4). The per person consumption of pharmaceuticals allows a better way to compare the utilization figures between countries (Table 2.4). By this approach, the consumption of carbamazepine, diclofenac, ketoprofen, atenolol and sotalol in Finland falls into the range reported elsewhere. Lower per person consumption is reported for ciprofloxacin, sulfamethoxazole and bezafibrate in Finland whereas naproxen, metoprolol and especially ibuprofen are consumed at higher amounts in Finland per person compared to other countries.

Table 2.4 Consumption of the studied pharmaceuticals in different countries.

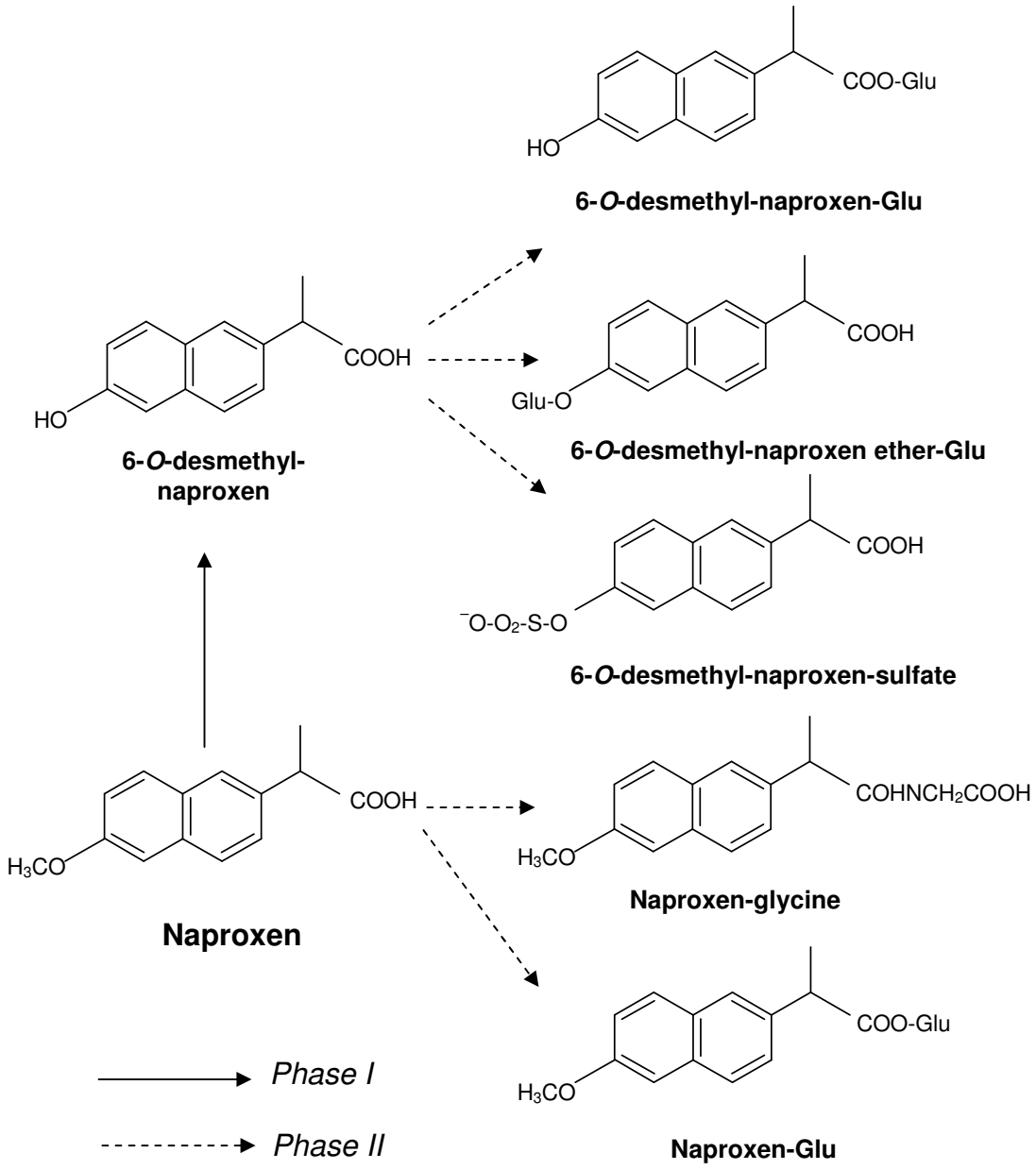
Compound	Consumption in the whole country (= *C* as t year^{-1}) and per person (= *P* as mg inh^{-1} year^{-1})													
	Spain[1] (yr 2003)		Austria[2] (-)		Australia[3] (yr 1998)		Japan[4] (yr 2002)		Germany[5,6] (yr 1995)		USA[7] (yr 2000)		Finland[8] (yr 2005)	
	C	*P*	*C*	*P*	*C*	*P*	*C*	*P*	*C*	*P*	*C*	*P*	*C*	*P*
Ciprofloxacin											132	470	0.85	160
Norfloxacin											2.70	9.5	0.26	50
Ofloxacin													0.40	75
Sulfamethoxazole	12.7	295	0.96	120	7.32	385			76.0*	950	309	1 100	0.27	50
Carbamazepine	20.0	465	6.33	790	10.0	525	162*	1 280	80.0	1 000			4.35	830
Diclofenac	32.3	750	6.14	770	4.39	230			75.0	940			1.07	200
Ibuprofen	2.76	6 400	6.70	835	14.2	745	99.0	780	105.0	1 310	2 300	8 190	94.0	17 890
Ketoprofen					4.44	235	71.0	560					1.10	210
Naproxen					22.9	1 200	33.0*	260					6.05	1 150
Acebutolol													0.84	160
Atenolol					5.19	275							0.79	150
Metoprolol									50.0	625			5.27	1 000
Sotalol					2.10	110							0.49	95
Bezafibrate			4.47	560					30.0	375			0.36	70
Pop. (10^6)	43		8		19		127		80		281		5.3	

References: [1] Carballa 2005, [2] Clara et al. 2005b, [3] Khan and Ongerth 2004, [4] Nakada et al. 2006, [5] Ternes 1998, [6] Hirsch et al. 1999, [7] Schwab et al. 2005, [8] National Agency for Medicines 2006. Pop. = population, * The highest value reported in the reference.

2.1.3 Metabolism of the pharmaceuticals in the human body

After ingestion, pharmaceuticals are subjected to various metabolic reactions in the human body. Drug metabolism involves two steps, known as phase I and phase II reactions. The main reactions in phase I metabolism are oxidation, hydrolysis, hydroxylation, dealkylation and deamination, and the metabolites are usually more hydrophilic and more chemically reactive (Rang et al. 2003). Phase II reactions involve conjugation, i.e. attachment of a substituent group (e.g. glucuronyl, sulfate, methyl, acetyl, glycyl and glutathione) either to the parent molecule or to the phase I metabolite (Rang et al. 2003). As an example of drug metabolism, naproxen phase I and phase II metabolism in the human body is presented in Scheme 2.1.

Pharmaceuticals and their metabolites are eliminated from the human body through urine and feces. Excretion via the lungs only occurs with highly volatile gaseous compounds. The studied pharmaceuticals and their metabolites usually leave the body in urine (Skordi et al. 2004, Dorado et al. 2003, Miao and Metcalfe 2003, Lim et al. 2001, Anderson and Prystowsky 1999, Davies 1998, Miller and Spence 1998, Vree et al. 1994, Wadworth et al. 1991, Todd and Clissold 1990). The fluoroquinolones (4–28% of the dose) and acebutolol (56–63% of the dose) are also eliminated in feces (Lode et al. 1990, Ryan et al. 1985).



Scheme 2.1 The two phases of naproxen metabolization in human body (adapted from Sidelmann et al. 2001). Glu= glucuronide

After ingestion, most of the studied pharmaceuticals are metabolized to some degree in the human body (Table 2.5). Some of the pharmaceuticals are mainly excreted as the parent compound whereas more extensive metabolism occurs for others. Consequently, pharmaceuticals that are mainly excreted unmetabolized will end up in

sewage at higher concentrations than pharmaceuticals undergoing extensive metabolism. The formed metabolites have usually lost the pharmacological activity of the parent compound. In some cases, also the metabolites carry activity. For example, diacetolol, a major metabolite of acebutolol, has pharmacological properties similar to the parent drug (Abernethy et al. 1985). Also, the metabolites of ciprofloxacin have been found to carry antimicrobial activity although lower than the parent compound (Lode et al. 1990). The studied pharmaceuticals are excreted to varying degree as conjugated metabolites. This can affect their concentrations especially in the treated sewage. Namely, in sewage treatment plant, the parent compound can be released via the cleavage of the conjugate subsequently increasing the concentration of the parent compound (Ternes et al. 1999).

Table 2.5 Metabolism of the studied pharmaceuticals in the human body.

Compound	**Excretion (%)**	
	As parent compound	As conjugates of the parent compound
Ciprofloxacin	33% [2]	Data not found
Norfloxacin	22% [2]	Minor portion [1]
Ofloxacin	80% [3]	Minor portion [1]
Sulfamethoxazole	15–30% [4, 5]	0% [5]
Carbamazepine	1–2% [6, 8]	Observed [7]
Diclofenac	2–15% [5, 8]	<1–15% [5, 8]
Ibuprofen	0–15% [5, 9–10]	5 – 18% [6, 9–11]
Ketoprofen	10% [5]	70% [5]
Naproxen	10% [12]	60% [12]
Acebutolol	39% [13]	Data not found
Atenolol	93% [5]	0% [5]
Metoprolol	3–10% [8, 14]	0% [5]
Sotalol	>75% [15]	0% [5]
Bezafibrate	50% [16]	20% [16]

References: [1] Lode et al. 1990, [2] Well et al. 1998, [3] Horstkötter and Blascke 2001, [4] Hirsch et al. 1999, [5] Khan and Ongerth 2004, [6] Miao and Metcalfe 2003, [7] Maggs et al. 1997, [8] Ternes 1998, [9] Davies 1998, [10] Kepp et al. 1997, [11] Tan et al. 1997, [12] Todd and Clissold 1990, [13] Ryan et al. 1985, [14] Lim et al. 2001, [15] Anderson and Prystowsky 1999, [16] Abshagen et al. 1979

2.2 Environmental analysis of pharmaceuticals

Due to the low concentrations (down to few ng L^{-1}) of pharmaceuticals found in the environment, highly sensitive and selective analytical methods are needed for their detection. Sample concentration and clean-up steps are normally required prior the analysis to meet these requirements. The adsorptive methods are the most widely used in the isolation and concentration of environmental samples (Zwiener and Frimmel 2004). Among these, solid phase extraction (SPE) using either disks or cartridges, is the most commonly used. The subsequent analysis is typically performed using gas or liquid chromatography (GC and LC, respectively) combined with mass spectrometry (MS). Nowadays, ever more methods reported in the literature apply LC-MS or LC-MS/MS instead of GC-MS. This is due to the often laborious derivatization procedures that are needed prior the analysis with GC-MS to increase the volatility of the analytes. Table 2.6 presents the analytical methods reported in the literature that are based on SPE, liquid chromatography and mass spectrometry.

Table 2.6 Analytical methods based on solid phase extraction (SPE), liquid chromatography (LC) and mass spectrometry used in the environmental analysis of pharmaceuticals.

Analyzed	**SPE**			**LC separation**		**Detection**	**LOQ**	**Ref.**
compounds*	**Material**	**pH**	**Elution solvent**	**Column**	**Mobile phase**	**method**	**(ng L^{-1})**	
CIP, OFL, SMX, IBP, ATE, BZF	Oasis MCX	2.0	MeOH+MeOH/Ammonia + MeOH/NaOH	C8	ESI+: Aq. formic acid/ACN ESI–: Aq. TEA/ACN	ESI-tQ	1–2 effluent	(1)
CBZ	LiChrolut EN	7.0	MeOH + EtOAc				1.3 effluent	
SMX	Oasis HLB	4.0	MeOH/EtOAc + MeOH	C18	Aq. formic acid/MeOH (1% formic acid)	ESI-tQ	62 influent 11 effluent	(2)
CBZ, DCF, IBP, NPX, BZF	Oasis HLB	8.2	MeOH	C18ec	ESI+: Aq.HFBA/Aq.NH_4Ac/MeOH/ACN ESI–: Aq. NH_4Ac/ACN	ESI-Ion Trap	0.5–25	(3)
SMX	LiChrolut EN	2.5	MeOH/H_2O + MeOH	C18ec	Aq. formic acid/ACN	ESI-tQ	2.5	(4)
ATE, MET, SOT	RP-C_{18}EC (Isolute)	10.5	MeOH	C18ec	H_2O/ACN	ESI-tQ	170–550 effluent	(5)
SMX, DCF, IBP	StrataX (Phenomenex)	3.0	MeOH	C18	Aq. NH_4Ac/MeOH	ESI-tQ	20–50	(6)
SMX	LiChrolut EN +Lichrolut C_{18}	3.0	MeOH	C18	Aq. NH_4Ac/ACN	ESI-tQ	20	(7)
SMX, CIP, OFL	C_2/ENV+ (IST)	3.0	MeOH/TEA	C18	Aq. formic acid/ACN formic acid	ESI-Ion Trap	3–70	(8)
DCF, IBP, KET, NPX, BZF	Oasis MCX	2.0	Acetone	C18	Aq. HAc/ACN	ESI-tQ	*For solid samples.*	(9)
CBZ	Oasis-HLB	7.0	MeOH	C8	ACN/MeOH/Aq.NH_4Ac/Aq.formic acid	ESI-tQ	nr	(10)

OFL, SMX, CBZ, DCF, IBP, KET, NPX, ATE, MET, SOT, BZF	Oasis HLB	nr	MeOH	C18 UPLC	ESI+: Aq.NH_4OH/Aq.HAc/ACN/MeOH ESI–: H_2O/MeOH	ESI-Q-TOF	15–500 influent (LOD)	(11)
CIP	Oasis HLB	2.8	MeOH	C18	Aq. HAc/ACN	ESI-Q	4	(12)
SMX ATE, MET, SOT	Isolut ENV+ PPL Bond-Elut	5 7	ACN + ACN/H_2O/TEA MeOH	C18	Aq. NH_4OH/ACN-MeOH NH_4OH (same elution program for SMX and the beta blockers)	ESI-tQ	6.2 ~8.0	(13)
SMX, CBZ, IBF, DCF, MET, BZF	Oasis-MCX	3.0	MeOH/Ammonia	C18	Aq. NH_4Ac/MeOH NH_4Ac	ESI-Q-TOF	5–10	(14)
CBZ, MET	RP-C_{18} EC	7.5	MeOH	C18	Aq. NH_4Ac/ACN	ESI-tQ	5 tap water 50 effluent	(15, 16)
SMX, CBZ, DCF, IBP, NPX	Oasis-HLB	2.0	MeOH/MTBE	C12	Aq. formic acid/MeOH	APCI or ESI-tQ	1.0	(17)
ACE, ATE, SOT, MET	Oasis-MCX	3.0	DCM/2-propanol/NH_4OH	C8	Aq. formic acid/ACN	ESI	6–9	(18)

* For abbreviations of the compounds, refer to Abbreviations and symbols in the beginning of the thesis. MeOH= methanol, EtOAc= ethyl acetate, Aq= aqueous, TEA= triethylamine, ACN= acetonitrile, MTBE= methyl-*tert*-butylether, HFBA= heptafluorobutyric acid, NH_4Ac= ammonium acetate, HAc= acetic acid, UPLC= ultra-performance liquid chromatography, ESI= electrospray ionization, Q= quadrupole, tQ= triple quadrupole, TOF= time of flight, APCI= atmospheric pressure chemical ionization, LOQ= limit of quantification, LOD= limit of detection, IQL= instrumental quantification limit, EC= end capped, DCM= dichloromethane, effluent= sewage effluent, influent= sewage influent,

References: (1) Castiglioni et al. 2005, (2) Göbel et al. 2004, (3) Hao et al. 2006, (4) Hartig et al. 1999, (5) Hernando et al. 2004, (6) Hilton and Thomas 2003, (7) Hirsch et al. 1998, (8) Lindberg et al. 2004, (9) Löffler and Ternes 2003, (10) Miao and Metcalfe 2003, (11) Petrovic et al. 2006, (12) Reverté et al. 2003, (13) Sacher et al. 2001, (14) Stolker et al. 2004, (15) Ternes 2001, (16) Ternes et al. 1998, (17) Vanderford et al. 2003, (18) Lee et al. 2007

2.2.1 Solid phase extraction

Wide variety of silica or copolymer based SPE materials are nowadays commercially available. The copolymer based sorbent materials (such as LiChrolut EN, Oasis HLB, Oasis MCX, and StrataX in Table 2.6) have become increasingly popular in the environmental analysis of pharmaceuticals. Especially popular are the SPE materials that allow the retention of wide variety of compounds. For example, the copolymer poly(divinylbenzene-co-N-vinylpyrrolidone) (Oasis HLB, Waters) has both hydrophilic and lipophilic retention characteristics (Figure 2.1) and can be used to retain both polar and non-polar compounds.

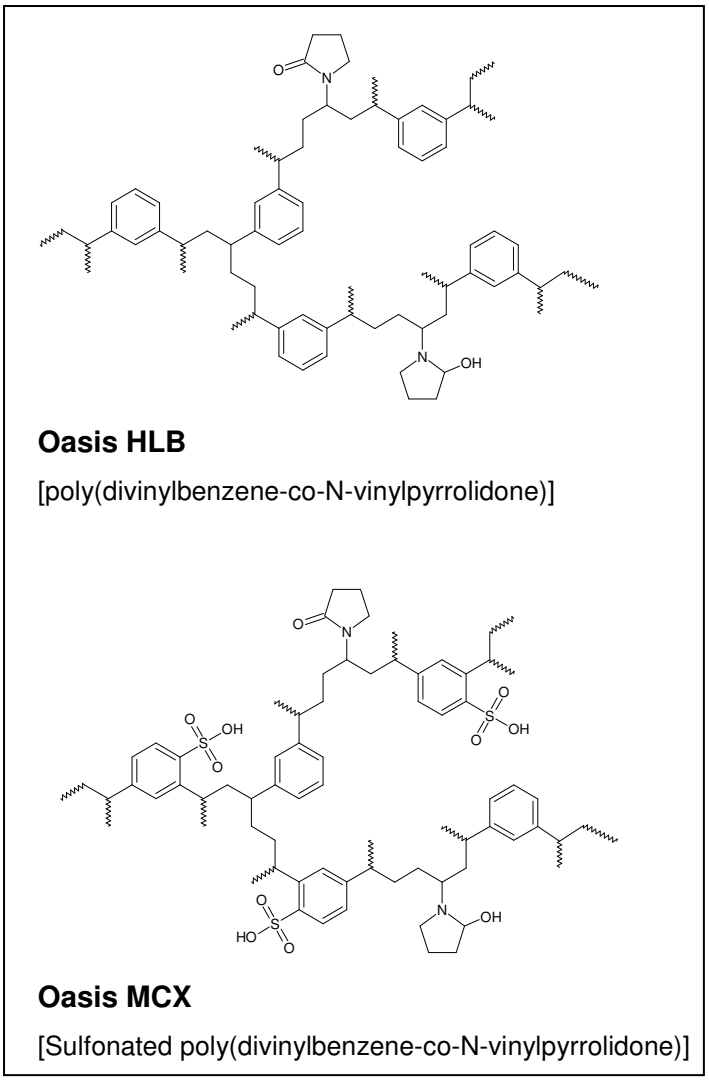


Figure 2.1. The structures of Oasis HLB and MCX solid phase extraction sorbents (adapted from Waters 2003).

In the literature, all the pharmaceuticals studied in this thesis have been successfully retained on the Oasis HLB material (Table 2.6). For more selective sample concentration, SPE materials can be used that allow the retention of a group of compounds with similar physico-chemical properties. For example, Oasis MCX (mixed-mode cation exchange) (Figure 2.1) and MAX (mixed mode anion exchange) materials (both from Waters) have high selectivity for basic and acidic compounds, respectively. The retention of the compounds on the SPE material can be controlled by adjusting the sample pH. Acidic pharmaceuticals (e.g. the anti-inflammatories and bezafibrate) are often extracted at acidic pH where the ionization of the compounds is suppressed (Castiglioni et al. 2005, Kosjek et al. 2005, Lin et al. 2005, Stolker et al. 2004, Hilton and Thomas 2003, Löffler and Ternes 2003, Sacher et al. 2001, Öllers et al. 2001). Due to the same reasons, basic compounds (e.g. the beta blockers) are sometimes extracted at basic pH (Hernando et al. 2004). The adsorbed compounds are normally eluted from the sorbent by a small volume of organic solvent, such as methanol, acetonitrile, ethyl acetate and acetone (Table 2.6). Prior the elution, sorbent material is sometimes washed with few milliliters of water or with water containing low portion of an organic solvent (Hao et al. 2006, Lin et al. 2005, Stolker et al. 2004, Öllers et al. 2001). This results in cleaner extract by removing matrix constituents that can interfere with the analysis of the pharmaceuticals.

2.2.2 Liquid chromatography and mass spectrometry

Most of the methods reported in the literature apply liquid chromatography in the environmental analysis of pharmaceuticals. This is due to the low volatility and high hydrophilicity of most of the pharmaceuticals. Gas chromatographic methods have also been reported (Kosjek et al. 2005, Lin et al. 2005, Huggett et al. 2003, Sacher et al. 2001, Ternes 2001, Öllers et al. 2001), however, derivatization of pharmaceuticals to increase the compound volatility is needed prior the analysis. In LC, reversed phase materials (mostly octadecyl C18-bonded silica) are most often used in the separation of pharmaceuticals (Table 2.6). The separation of the analytes from the matrix components and from each other is especially important when detection methods such as UV or fluorescence are used (Santos et al. 2005, Golet et al. 2001). By using mass spectrometer

(MS), the analytes do not have to be fully separated from each other due to the selectivity of the detection method. Prior the mass spectrometric detection, positive or negative ions of the analytes are produced using atmospheric pressure ionization (API), mainly electrospray ionization (ESI). In quantitative analyses, triple quadrupole mass analyzer is the most widely used (Table 2.6) due to the sensitive and selectivity of the detection method called the multiple reaction monitoring (MRM) (Figure 2.2). In the MRM, compound is first ionized to form precursor ions. The first quadrupole is monitoring the selected precursor ion which is subsequently fragmented in the second quadrupole (also called as a collision cell) to form product ions and finally, the third quadrupole is monitoring one selected product ion. All in all, the transition from precursor to product ion is followed and recorded.

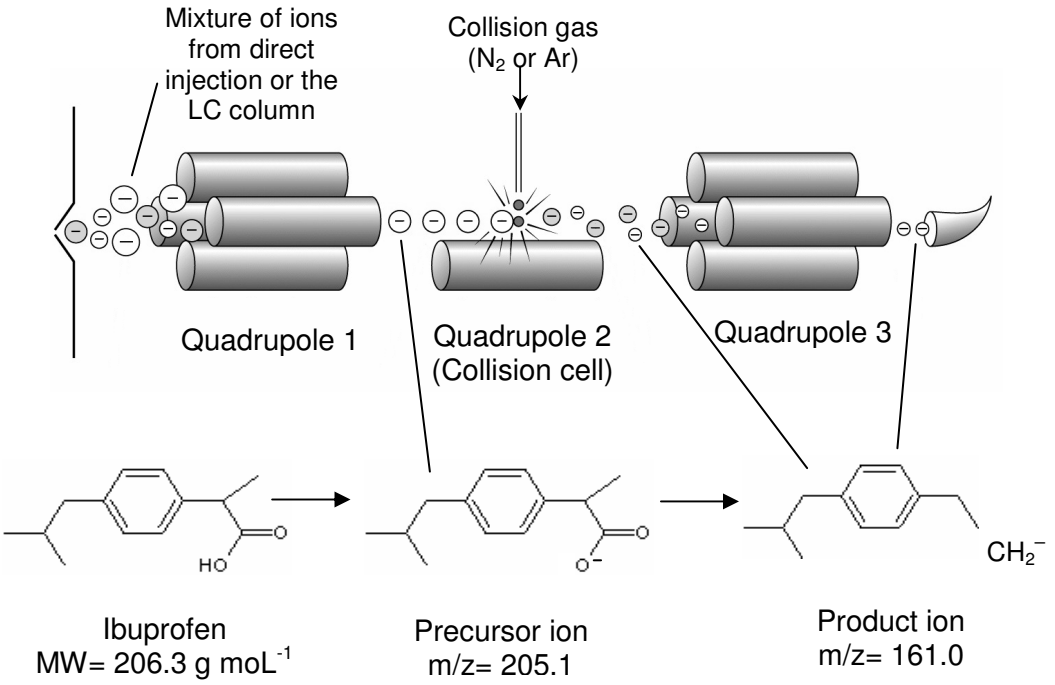


Figure 2.2 The principle of the multiple reaction monitoring (MRM) in triple quadrupole mass spectrometer (modified from Harris 1999, p. 747) and the precursor and product ion structures of ibuprofen in negative electrospray ionization and MRM. m/z= mass to charge ratio

The main disadvantage in the LC-MS/MS analyses is the matrix interference associated with the ionization of the analytes in the API source. This appears either by enhancement or, more frequently, suppression of the ionization of the analytes causing uncertainty in the detection and quantification of the target compounds. Various compounds can cause

ion suppression or enhancement, among these endogenous compounds present in the sample matrix or exogenous compounds introduced to the sample in the pre-treatment or the analysis step (Antignac et al. 2005). Matrix interferences are normally most extensive during the analysis of sewage samples due to the high amount of matrix components in the sample (Petrovic et al. 2006, Hernando et al. 2004). Ion suppression or enhancement can occur at any stage of the chromatographic run, but it is most likely to occur at the beginning (elution of the highly polar and nonretained compounds) and the end of the chromatographic run (elution of the strongly retained compounds) (Antignac et al. 2005, Hernando et al. 2004, Quintana and Reemtsma 2004, Hilton and Thomas 2003). To overcome the problems associated with the matrix interferences, several strategies have been suggested. These are, for example, post-column splitting, that reduces the eluent flow entering the API interface, reduction of the matrix compounds in the SPE extracts by more stringent clean-up steps, adequate separation of the analytes and the matrix compounds in the chromatographic column and use of appropriate internal standards (preferable deuterated or isotopically labeled analogues of the analytes) to compensate for the loss of the analyte signal intensity (Antignac et al. 2005, Kloepfer et al. 2005, Richardson and Ternes 2005).

2.2.3 Quantification methods

To relate the response of the detector to the concentration of the compound, calibration should be performed. The most often used methods are external, internal and standard addition calibration. In external calibration, varying concentrations of the pure analyte are analyzed and the peak responses are plotted versus the concentration (Figure 2.3a). The concentration of the analyte in the unknown sample can then be determined from the calibration curve. External standard calibration does not take into account the loss of the analyte in the sample pretreatment or the variation in the performance of the instrument. Therefore, internal standard calibration is used when intensive sample pretreatment is included in the method. In this approach, a fixed concentration of an internal standard (different than the analyte) is added to the sample to compensate for the losses of the analyte in the sample pretreatment and analysis. Thus, in the calibration curve, the

concentration of the analyte is plotted versus the ratio of the response of the analyte and the response of the internal standard (Figure 2.3b). A proper internal standard should have a retention time close to that of the analyte, should not be present in the original sample, should mimic the analyte in any sample pretreatment steps, should be stable and unreactive with sample or mobile phase and should have a similar detector response to the analyte for the concentrations studied (Snyder et al. 1997). Standard addition method can be used in the case sample blank is not available. In this approach, different weights of the analyte are added to the sample matrix, which contains an unknown concentration of the analyte. The plot of response, obtained for the standard-addition calibration concentrations, is extrapolated to zero concentration to define the concentration of the analyte in the original sample (Figure 2.3c).

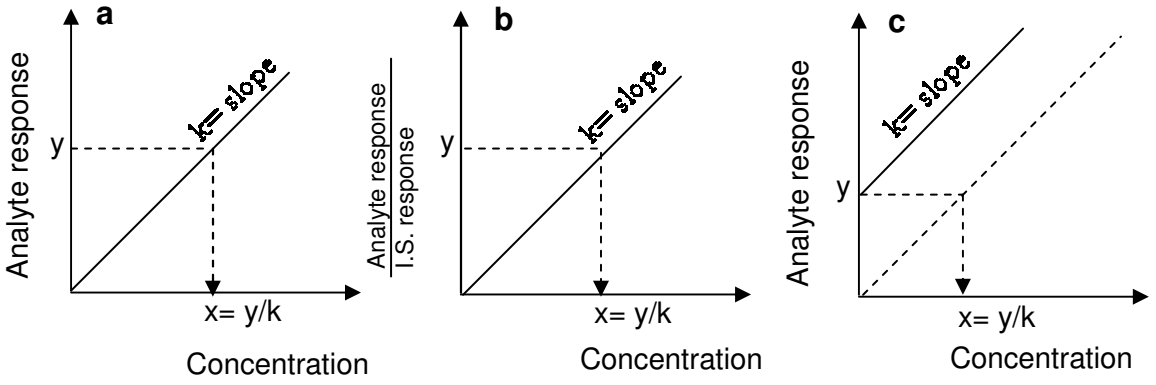


Figure 2.3 Different calibration methods, a) external standard method, b) internal standard (I.S.) method and c) standard addition method.

In the environmental analysis of pharmaceuticals, external standard calibration is sometimes used (Lee et al. 2007, Hernando et al. 2004, Stolker et al. 2004, Reverté et al. 2003, Vanderford et al. 2003, Sacher et al. 2001, Hirsch et al. 1998) but the internal standard method is preferred due to the extensive pretreatment of the samples. Table 2.7 compiles compounds used as internal standards in the quantification of the studied pharmaceuticals when mass spectrometry is used as the detector. The most appropriate internal standard is the deuterated or isotopically labeled analogue of the analyte, due to their similar recoveries in the pretreatment and retention times in the chromatographic run. However, they are often not commercially available and are quite rarely used in the

previously published analytical methods. Sometimes, pharmaceuticals from the same therapeutic group as the analyte are used as internal standards. For example, a veterinary fluoroquinolone, enrofloxacin, has been used as an internal standard for human fluoroquinolones or a beta blocker levobunolol in the calibration of other beta blockers. Also compounds other than pharmaceuticals have been used as internal standards, e.g. herbicides fenoprop and mecoprop and polycyclic aromatic hydrocarbon, anthracene.

Table 2.7 Internal standards used in the quantification of the studied pharmaceuticals.

Compound	**Internal standard**
Ciprofloxacin	Salbumatol-d_3 [1], Enrofloxacin [2]
Norfloxacin	Enrofloxacin [2]
Ofloxacin	Salbumatol-d_3 [1], Enrofloxacin [2]
Sulfamethoxazole	Ibuprofen-d_3 [1], Sulfamethoxazole-d_4 [3], Sulfaphenazole [4], ^{13}C-phenacetin [5], Sulfamethazine [2]
Carbamazepine	Salbumatol-d_3 [1], $^{13}C_6$-sulfamethazine phenyl [6], Dihydrocarbamazepine [7–10], $^{2}H_{10}$-carbamazepine [12]
Diclofenac	$^{13}C_6$-sulfamethazine phenyl [6], ^{13}C-phenacetin [5], Fenoprop [13, 14], Mecoprop-d_3 [10], Mecoprop [15], Anthracene-d_{10} [16]
Ibuprofen	Ibuprofen-d_3 [1], $^{13}C_6$-sulfamethazine phenyl [6], ^{13}C-phenacetin [5], Fenoprop [13, 14], $^{3}H_3$-ibuprofen [12], Mecoprop-d_3 [10], Mecoprop [15], Anthracene-d_{10} [16]
Ketoprofen	Fenoprop [13, 14], Mecoprop-d_3 [10], Mecoprop [15], Anthracene-d_{10} [16]
Naproxen	$^{13}C_6$-sulfamethazine phenyl [6], Fenoprop [13, 14], Mecoprop-d_3 [10], Mecoprop [15], Anthracene-d_{10} [16]
Acebutolol	Data not found
Atenolol	Salbumatol-d_3 [1], $^{2}H_7$-atenolol [12], Levobunolol [17]
Metoprolol	$^{2}H_7$-atenolol [12], Levobunolol [17]
Sotalol	$^{2}H_7$-atenolol [12]
Bezafibrate	Ibuprofen-d_3 [1], $^{13}C_6$-sulfamethazine phenyl [6], Fenoprop [13, 14]

References: 1) Castiglioni et al. 2005, 2) Lindberg et al. 2004, 3) Göbel et al. 2004, 4) Hartig et al. 1999, 5) Hilton and Thomas 2003, 6) Hao et al. 2006, 7) Miao and Metcalfe 2003, 8) Ternes 2001, 9) Ternes et al. 1998, 10) Öllers et al. 2001, 12) Petrovic et al. 2006, 13) Löffler and Ternes 2003, 14) Quintana and Reemtsma 2004, 15) Kosjek et al. 2005, 16) Farré et al. 2001, 17) Nikolai et al. 2006,

2.3 Pharmaceuticals in sewage treatment plants

Pharmaceuticals are excreted to urine and feces and are thus carried to sewage treatment plants. The treatment processes in STPs are designed to remove suspended solids, biodegradable organics, pathogens and nutrients by physical, chemical and biological means (Figure 2.4). They typically consist of preliminary treatment (e.g. screening and grit removal), primary treatment (e.g. fine screening and sedimentation) and secondary treatment (e.g. biological treatment and sedimentation). In some of the STPs, tertiary treatment (e.g. chlorination and biological filter) is applied to further increase the purity of the effluent. STPs mainly differ in their design of the biological treatment unit. The most common secondary treatment in STPs is activated sludge (AS) process either with or without a nitrification/denitrification (N/DN) unit to enhance the nitrogen removal. Also, fixed bed reactors or membrane bioreactors are applied in some STPs.

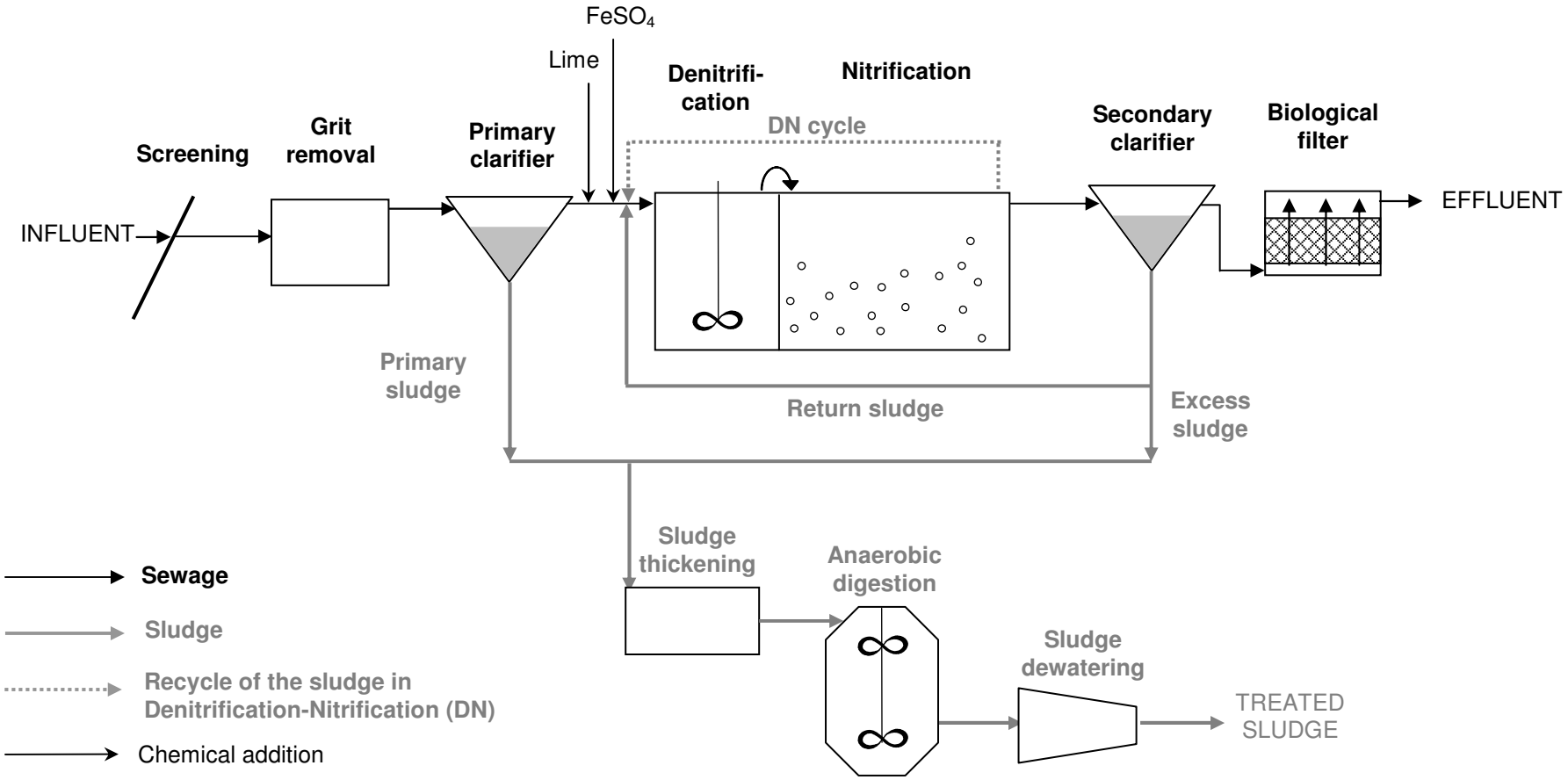


Figure 2.4 Example of a sewage treatment plant.

The ubiquitous occurrence of pharmaceuticals in raw sewage has been confirmed in several studies all over the world (Table 2.8 for the studied pharmaceuticals). The concentrations of the pharmaceuticals in municipal sewage generally vary from low nanograms to few micrograms per liter. In hospital sewage, concentrations of over 100 $\mu g\ L^{-1}$ have been measured due to high per person consumption of pharmaceuticals in hospitals (Lindberg et al. 2004). During the treatment process, the concentrations of pharmaceuticals are typically decreased due to elimination occurring in the process. However, in the previous studies, the entire elimination of the studied pharmaceuticals has rarely occurred in the samples STPs. (Table 2.9). Therefore, concentrations up to several micrograms per liter have still been measured in sewage effluents (Table 2.8). During the treatment process, stripping of the pharmaceuticals is negligible due to the low Henry's law constants of the compounds (Table 2.2) (Carballa 2005, Larsen et al. 2004). The main elimination mechanisms of the pharmaceuticals in the preliminary, primary and secondary treatments of STPs are sorption with the subsequent elimination in sludge separation, and biodegradation. Additional elimination is achieved in the tertiary treatment such as chemical oxidation (ozone and chlorine) and UV-disinfection. These processes are more commonly applied in the drinking water treatment and are therefore discussed in Chapters 2.5.4 and 2.5.5.

Table 2.8 The occurrence of the studied pharmaceuticals in influents and effluents in different countries.

	Location	Influent ($\mu g\ L^{-1}$)	Effluent ($\mu g\ L^{-1}$)	Reference
Ciprofloxacin	Europe[l]		0.03–0.07	Andreozzi et al. 2003
	Italy		0.03–0.51	Castiglioni et al. 2005
	Switzerland		0.05–0.11	Golet et al. 2001
	Switzerland	0.31–0.57	0.06–0.11	Golet et al. 2002
	Sweden[c]	3.60–101		Lindberg et al. 2004
	Sweden	0.09–0.30	0.06[Max]	Lindberg et al. 2005
	Canada	0.58[Mean]	(0.12) 0.40[a]	Miao et al. 2004
	USA		<0.02	Nakata et al. 2005
	Spain		0.60[Mean]	Reverté et al. 2003
	Italy		0.25[Median]	Zuccato et al. 2005
Norfloxacin	Europe[l]		0.03–0.08	Andreozzi et al. 2003
	Switzerland		0.05–0.12	Golet et al. 2001
	Switzerland	0.26–0.55	0.04–0.07	Golet et al. 2002
	Sweden[c]	<0.004		Lindberg et al. 2004
	Sweden	0.07–0.17	0.04[Max]	Lindberg et al. 2005
	Canada		(0.05) 0.11[a]	Miao et al. 2004
	USA		<0.045	Nakata et al. 2005
Ofloxacin	Europe[l]		0.12–0.58	Andreozzi et al. 2003
	Italy		0.15–1.08	Castiglioni et al. 2005
	Sweden[c]	0.20–7.60		Lindberg et al. 2004
	Sweden	0.29[Max]	0.05[Max]	Lindberg et al. 2005
	Canada		(0.09) 0.51[a]	Miao et al. 2004
	USA		0.10	Nakata et al. 2005
	Spain/Croatia	<0.50	<0.50	Petrovic et al. 2006
	Italy		0.60[Median]	Zuccato et al. 2005
Sulfamethoxazole	Europe[l]		(0.05) 0.09[a]	Andreozzi et al. 2003
	Sweden	0.02	0.07	Bendz et al. 2005
	Spain	0.58	0.25	Carballa et al. 2004
	USA		(0.15) 0.59[a]	Glassmeyer et al. 2005
	Germany		2.00	Hartig et al. 1999
	UK		<0.05	Hilton and Thomas 2003
	Germany		(0.40) 2.00[a]	Hirsch et al. 1999
	Sweden[c]	0.40–12.8		Lindberg et al. 2004
	Sweden	0.67[Max]	0.30[Max]	Lindberg et al. 2005
	Canada		(0.24) 0.87[a]	Miao et al. 2004
	Spain/Croatia	(0.45) 0.96[a]	(0.40) 0.80[a]	Petrovic et al. 2006
	South Korea		(0.13) 0.41[b]	Kim et al. 2007
	Germany		0.62±0.05	Ternes et al. 2003
	Italy		0.13[Median]	Zuccato et al. 2005
	Switzerland	(0.43) 0.57[b]	(0.29) 0.86[b]	Göbel et al. 2005
Carbamazepine	Austria	0.45–1.85	0.47–1.62	Clara et al. 2005a
	Europe[l]		0.30–1.20	Andreozzi et al. 2003
	Sweden	1.68	1.18	Bendz et al. 2005
	Spain	<0.07	<0.07	Carballa et al. 2004
	Italy		1.32[Max]	Castiglioni et al. 2005
	USA		(0.08) 0.27[a]	Glassmeyer et al. 2005
	USA		0.06[Max]	Gross et al. 2004

Table 2.8 continues

Carbamazepine	Germany	(1.78) 3.80[a]	(1.63) 5.00[b]	Heberer 2002b
	Taiwan		0.42[Mean]	Lin et al. 2005
	Canada		0.007–0.13	Metcalfe et al. 2003
	Canada	0.36[Mean]	0.25[Mean]	Miao et al. 2005
	Europe[III]		(0.44) 1.2[a]	Paxéus 2004
	Spain/Croatia	(0.40) 0.95[a]	(0.36) 0.60[a]	Petrovic et al. 2006
	Japan	(0.05) 0.27[a]	(0.05) 0.16[a]	Nakada et al. 2006
	France		0.16-0.29	Rabiet et al. 2006
	South Korea		(0.23) 0.73[b]	Kim et al. 2007
	Spain	(0.50) 2.1[b]	(0.43) 0.75[b]	Santos et al. 2005
	Germany		(2.10) 6.30[b]	Ternes 1998
	Germany		2.10±0.05	Ternes et al. 2003
	Switzerland		0.10–0.80	Öllers et al. 2001
	Spain	0.12–0.31	0.11–0.23	Gómez et al. 2006
Diclofenac	Spain	<0.05	<0.05	Carballa et al. 2004
	Austria	0.91–4.11	0.78–3.46	Clara et al. 2005a
	Europe[I]		(0.68) 5.45[a]	Andreozzi et al. 2003
	Sweden	0.16	0.12	Bendz et al. 2005
	Switzerland	4.70–1.92	0.31–0.93	Buser et al. 1998
	Germany	(3.02) 7.10[a]	(2.51) 4.70[b]	Heberer 2002b
	Europe[II]	0.15[Max]	1.43[Max]	Hernando et al. 2006
	UK		0.41–0.46	Hilton and Thomas 2003
	Taiwan		<0.002	Lin et al. 2005
	Canada		0.005–0.36	Metcalfe et al. 2003
	Europe[III]		(0.29) 1.48[a]	Paxéus 2004
	Spain/Croatia	(0.25) 0.50[a]	(0.32) 0.50[a]	Petrovic et al. 2006
	France		0.21–0.49	Rabiet et al. 2006
	South Korea		(0.04) 0.13[b]	Kim et al. 2007
	Spain	<0.93	<0.47	Santos et al. 2005
	Switzerland	0.10–0.53[fig]	0.05–0.56[fig]	Soulet et al. 2002
	Germany		(0.81) 2.10[b]	Ternes 1998
	Germany		1.30±0.10	Ternes et al. 2003
	Switzerland		0.10–0.70	Öllers et al. 2001
	Spain	0.20–3.60	0.14–2.20	Gómez et al. 2006
	USA	0.11	0.09	Yu et al. 2006
	Germany	2.33[n= 1]	1.56[n= 1]	Quintana and Reemtsma 2004
Ibuprofen	USA		<0.003	Boyd et al. 2003
	Italy		<0.001	Castiglioni et al. 2005
	Austria	1.20–3.68	2.40[Max]	Clara et al. 2005a
	Europe[I]		0.02–7.11	Andreozzi et al. 2003
	Sweden	3.59	0.15	Bendz et al. 2005
	Switzerland	0.99–3.30	~0.002–0.08	Buser et al. 1999
	Spain	2.64–5.70	0.91–2.10	Carballa et al. 2004
	USA		0.05–0.43	Gross et al. 2004
	Germany		0.10[Mean]	Heberer 2002b
	Europe[II]	0.86[Max]	6.90[Max]	Hernando et al. 2006
	UK		1.70–3.80	Hilton and Thomas 2003
	UK	3.50–11.50[fig]	4.50[Max, fig]	Kanda et al. 2003
	Taiwan		0.03[Mean]	Lin et al. 2005

Table 2.8 continues

Ibuprofen	Canada		0.08–1.89	Metcalfe et al. 2003
	Europe[III]		(0.11) 1.96[a]	Paxéus 2004
	Spain/Croatia	(0.54) 1.20[a]	(0.27) 1.05[a]	Petrovic et al. 2006
	Japan	(0.67) 1.13[a]	(0.02) 0.07[a]	Nakada et al. 2006
	France		0.020–0.22	Rabiet et al. 2006
	South Korea		(0.07) 0.14[b]	Kim et al. 2007
	Spain	(72.2) 143[b]	(6.40) 10.10[b]	Santos et al. 2005
	Switzerland	0.15–1.00[fig]	0.01–0.30[fig]	Soulet et al. 2002
	Germany		(0.37) 3.40[b]	Ternes 1998
	Germany		0.13±0.03	Ternes et al. 2003
	Italy		0.12[Median]	Zuccato et al. 2005
	Switzerland		0.005–1.50	Öllers et al. 2001
	Spain	34–168	0.24–28	Gómez et al. 2006
	USA	1.90	0.25	Yu et al. 2006
	Germany	5.53[n= 1]	<0.003[n= 1]	Quintana and Reemtsma 2004
Ketoprofen	Europe[I]		(<LOQ) 1.62[a]	Andreozzi et al. 2003
	Sweden	0.94	0.33	Bendz et al. 2005
	USA		0.03[Max]	Gross et al. 2004
	Germany	0.30[Mean]	0.23[Mean]	Heberer 2002b
	Europe[II]	0.13[Max]	<0.075	Hernando et al. 2006
	Taiwan		<0.002	Lin et al. 2005
	Canada		0.01[Max]	Metcalfe et al. 2003
	Spain/Croatia	(0.23) 0.96[a]	(0.20) 0.75[a]	Petrovic et al. 2006
	Japan	(0.21) 0.37[a]	(0.13) 0.22[a]	Nakada et al. 2006
	France		0.02–1.08	Rabiet et al. 2006
	Spain	2.10[Max]	1.76[Max]	Santos et al. 2005
	Switzerland	0.10–0.53[fig]	0.05–0.56[fig]	Soulet et al. 2002
	Germany		(0.20) 0.38[b]	Ternes 1998
	Switzerland		0.20[Max]	Öllers et al. 2001
	USA	1.20	0.28	Yu et al. 2006
	Germany	0.32[n= 1]	0.15	Quintana and Reemtsma 2004
Naproxen	Europe[I]		(1.12) 5.22[a]	Andreozzi et al. 2003
	Sweden	3.65	0.25	Bendz et al. 2005
	USA		0.08–0.11	Boyd et al. 2003
	Spain	1.79–4.60	0.80–2.60	Carballa et al. 2004
	USA		0.17[Max]	Gross et al. 2004
	Germany	0.44[Mean]	0.08[Mean]	Heberer 2002b
	Europe[II]	0.46[Max]	0.63[Max]	Hernando et al. 2006
	Taiwan		0.17[Mean]	Lin et al. 2005
	Canada		0.02–0.52	Metcalfe et al. 2003
	Europe[III]		(0.41) 1.51[a]	Paxéus 2004
	Spain/Croatia	<0.055	<0.055	Petrovic et al. 2006
	Japan	(0.10) 0.23[a]	(0.06) 0.14[a]	Nakada et al. 2006
	France		0.04–0.29	Rabiet et al. 2006
	South Korea		(0.13) 0.48[b]	Kim et al. 2006
	Spain	(5.40) 11.4[b]	(2.00) 3.12[b]	Santos et al. 2005
	Germany		(0.30) 0.52[b]	Ternes 1998
	Switzerland		0.10–3.50	Öllers et al. 2001
	USA	3.20	0.38	Yu et al. 2006

Table 2.8 continues

Naproxen	Germany	0.73[n= 1]	0.26[n= 1]	Quintana and Reemtsma 2004
Acebutolol	Europe[I]		0.13[Max]	Andreozzi et al. 2003
	Canada	0.38[Median]	0.31[Median]	Lee et al. 2007
Atenolol	Sweden	0.03	0.16	Bendz et al. 2005
	Italy		0.03–1.17	Castiglioni et al. 2005
	Europe[III]		(0.19) 0.73[a]	Paxéus 2004
	Spain/Croatia	(0.23) 1.00[a]	(0.28) 1.20[a]	Petrovic et al. 2006
	Germany		0.36±0.01	Ternes et al. 2003
	Italy		0.47[Median]	Zuccato et al. 2005
	Switzerland	2.23[Mean]	0.54[Mean]	Maurer et al. 2007
	Canada	1.65[Median]	0.99[Median]	Lee et al. 2007
Metoprolol	Europe[I]		0.01–0.39	Andreozzi et al. 2003
	Sweden	0.160	0.19	Bendz et al. 2005
	USA		(0.02) 1.20[a]	Huggett et al. 2003
	Europe[III]		(0.08) 0.39[a]	Paxéus 2004
	Spain/Croatia	<0.015	<0.015	Petrovic et al. 2006
	Germany		(0.73) 2.20[b]	Ternes 1998
	Germany		1.70±0.04	Ternes et al. 2003
	Switzerland	0.20[Mean]	0.13[Mean]	Maurer et al. 2007
	Canada	0.27[Median]	0.24[Median]	Lee et al. 2007
Sotalol	Spain/Croatia	<0.05	<0.05	Petrovic et al. 2006
	Germany		1.32±0.14	Ternes et al. 2003
	Switzerland	0.34[Mean]	0.25[Mean]	Maurer et al. 2007
	Canada	0.31[Median]	0.26[Median]	Lee et al. 2007
Bezafibrate	Austria	1.53–7.60	4.80[Max]	Clara et al. 2005a
	Europe[I]		(<LOQ) 1.07[a]	Andreozzi et al. 2003
	Italy		0.0003–0.12	Castiglioni et al. 2005
	Canada		0.01–0.26	Metcalfe et al. 2003
	Spain/Croatia	<0.05	<0.05	Petrovic et al. 2006
	Germany		(2.20) 4.60[a]	Ternes 1998
	Italy		0.055[Median]	Zuccato et al. 2005
	Germany	2.78[n= 1]	0.57[n= 1]	Quintana and Reemtsma 2004

Values with "<" were below the limit of quantification (LOQ) or the limit of detection
[a] (median) maximum, [b] (mean) maximum, [c] hospital sewage (seven samples in 13 h), [fig]= estimated from a figure
[I] Data from France, Greece, Italy and Sweden, [II] Data from Spain, Belgium, Germany and Slovenia,
[III] Data from France, Greece, Italy, Sweden, Denmark

Table 2.9 Elimination of the studied pharmaceuticals in sewage treatment plants (STPs) and the biological processes applied in the STPs.

Compound	Location	Process (number of STPs studied)	Elimination (%)			Reference
			Min	Max	Mean	
Ciprofloxacin	Italy	AS (6)	45^{W}, 53^{S}	78^{W}, 69^{S}		Castiglioni et al. 2006
	Switzerland	AS $^{N/DN}$ (4)	79^{filt}	86^{filt}	82^{filt}	Golet et al. 2002
	Sweden	AS (with or without N/DN) (5)	58^{filt}	$>97^{filt}$	87^{filt}	Lindberg et al. 2005
	Sweden	AS (1)			$78^{filt, n=1}$ $96^{Solids\ included}$	Lindberg et al. 2006
Norfloxacin	Switzerland	AS $^{N/DN}$ (4)	80^{filt}	100^{filt}	82^{filt}	Golet et al. 2002
	Sweden	AS (with or without N/DN) (5)	64^{filt}	100^{filt}	87^{filt}	Lindberg et al. 2005
	Sweden	AS (1)			80^{filt}, $97^{Solids\ included}$	Lindberg et al. 2006
Ofloxacin	Italy	AS (6)	0^{W}, 33^{S}	62^{W}, 66^{S}	43^{W}, 57^{S}	Castiglioni et al. 2006
	Sweden	AS (with or without N/DN) (5)	63^{filt}	100^{filt}	86^{filt}	Lindberg et al. 2005
Sulfamethoxazole	Sweden	AS (1)			$-30^{n=1}$	Bendz et al. 2005
	Spain	AS (1)			$57^{n=1}$	Carballa et al. 2004
	Italy	AS (6)	0^{W}, 71^{S}	84^{W}, 71^{S}	17^{W}, 71^{S}	Castiglioni et al. 2006
	Switzerland	AS $^{N/DN}$ (1), AS $^{N/DN}$/ FB (1)			0^{B}	Joss et al. 2005
	Sweden	AS (with or without N/DN) (5)	0	100	45	Lindberg et al. 2005
Carbamazepine	Austria	AS (1)			$-3^{n=1}$	Clara et al. 2005a
		AS $^{N/DN}$ (3)	-56	14	-26	
		MBR (1)	-13	12	1	
	Sweden	AS (1)			$30^{n=1}$	Bendz et al. 2005
	Italy	AS (6)	0^{W}, 0^{S}	0^{W}, 0^{S}	0^{W}, 0^{S}	Castiglioni et al. 2006
	Switzerland	$AS^{N/DN}$ (1), $AS^{N/ND}$/FB (1)			0^{B}	Joss et al. 2005
	Canada	AS (1)			$29^{n=1}$	Miao et al. 2005
	Europe[I]	STPs with different processes (10)	<10	53		Paxéus 2004
	Japan	AS (5)	-122	77	3	Nakada et al. 2006
	Germany	AS (P^{Chem}) (1)			7	Ternes 1998
	Spain	AS (1)			20±15	Gómez et al. 2006

Table 2.9 continues

Diclofenac	Austria	AS (1)			-7 [n= 1]	Clara et al. 2005a
		AS [N/DN] (3)	-5	63	34	
		MBR (1)	-7	51	23	
	Sweden	AS (1)			22 [n= 1]	Bendz et al. 2005
	Switzerland	nr (3)	5	51	26	Buser et al. 1998
	Germany	nr			17	Heberer 2002b
	Europe[II]	nr			40	Hernando et al. 2006
	Switzerland	AS [N/ND] (1), AS [N/ND]/ FB (1)	20	40		Joss et al. 2005
	Europe[I]	STPs with different processes (10)	<10	80	30	Paxéus 2004
	Germany	AS (P^{Chem}) (1)			69	Ternes 1998
	Spain	AS (1)			59±17	Gómez et al. 2006
	USA	BNR (1)			18	Yu et al. 2006
Ibuprofen	Italy	AS (6)	25[W], 0[S]	72[W], 100[S]	38[W], 93[S]	Castiglioni et al. 2006
	Austria	AS (1)			-4 [n= 1]	Clara et al. 2005a
		AS [N/DN] (3)	92	>99	98	
		MBR (1)	97	99	98	
	Sweden	AS (1)			96 [n= 1]	Bendz et al. 2005
	Switzerland	Not reported (3)	96	100	99	Buser et al. 1999
	Spain	AS (1)	63	67	64	Carballa et al. 2004
	Europe[II]	Not reported	45	75		Hernando et al. 2006
	Switzerland	AS [N/DN] (1), AS [N/DN]/ FB (1)			≥90[B]	Joss et al. 2005
	UK	STPs with different processes (5)	14	100		Kanda et al. 2003
	Europe[I]	STPs with different processes (10)	52	99	90	Paxéus 2004
	Japan	AS (5)	84	98	96	Nakada et al. 2006
	UK	AS [N/DN] (1)	80	91	86	Jones et al. 2007
	Germany	AS (P^{Chem}) (1)			90	Ternes 1998
	Spain	AS (1)			95±7	Gómez et al. 2006
	USA	BNR (1)			87	Yu et al. 2006
Ketoprofen	Sweden	AS (1)			65 [n= 1]	Bendz et al. 2005
	Japan	AS (5)	14	68	45	Nakada et al. 2006
	USA	BNR (1)			77	Yu et al. 2006

Table 2.9 continues

Naproxen	Sweden	AS (1)			93 $^{n=1}$	Bendz et al. 2005
	Spain	AS (1)	43	55	48	Carballa et al. 2004
	Switzerland	AS $^{N/DN}$ (1), AS $^{N/DN}$/ FB (1)	50^{B}	80^{B}		Joss et al. 2005
	Europe^{I}	STPs with different processes (10)	42	93	72	Paxéus 2004
	Japan	AS (5)	-2	83	46	Nakada et al. 2006
	Germany	AS (P^{Chem}) (1)			66	Ternes 1998
	USA	BNR (1)			88	Yu et al. 2006
Acebutolol	Canada	STPs with different processes (7)			19$^{Median, n=7}$	Lee et al. 2007
Atenolol	Sweden	AS (1)			-81 $^{n=1}$	Bendz et al. 2005
	Italy	AS (6)	0^{W}, 36^{S}	21^{W}, 78^{S}	10^{W}, 55^{S}	Castiglioni et al. 2006
	Europe^{I}	STPs with different processes (10)			<10	Paxéus 2004
	Switzerland	Not defined (2)			76	Maurer et al. 2007
	Canada	STPs with different processes (7)			40$^{Median, n=7}$	Lee et al. 2007
Metoprolol	Sweden	AS (1)			-19 $^{n=1}$	Bendz et al. 2005
	Europe^{I}	STPs with different processes (10)			≤10	Paxéus 2004
	Germany	AS (P^{Chem}) (1)			83	Ternes 1998
	Switzerland	Not defined (2)			30	Maurer et al. 2007
	Canada	STPs with different processes (7)			9$^{Median, n=7}$	Lee et al. 2007
Sotalol	Switzerland	Not defined (2)			27	Maurer et al. 2007
	Canada	STPs with different processes (7)			15$^{Median, n=7}$	Lee et al. 2007
Bezafibrate	Austria	AS (1)			36 $^{n=1}$	Clara et al. 2005a
		AS $^{N/DN}$ (3)	35	>99	76	
		MBR (1)	77	96	89	
	Italy	AS (6)	0^{W}, 0^{S}	66^{W}, 98^{S}	15^{W}, 87^{S}	Castiglioni et al. 2006
	Germany	AS (P^{Chem}) (1)			83	Ternes 1998

AS= activated sludge treatment, AS (P^{chem})= activated sludge with simultaneous phosphorous removal by chemical coagulation, N/DN= nitrification/denitrification, MBR= membrane bioreactor, BNR= biological nutrient removal, FB= fixed bed reactor

W= winter, S= summer, ^{filt} filtered sample, ^{B} elimination in the biological treatment, n= number of samples

^{I} Data from France, Greece, Italy, Sweden, Denmark

^{II} Data from Spain, Belgium, Germany and Slovenia

2.3.1 Elimination by sorption

Sorption of pharmaceuticals to sludge can be either absorption or adsorption. In absorption, the compound is removed by hydrophobic interactions between the compound and the lipid fraction of the sewage or the cell membranes of the micro-organisms. The ability of a substance to absorb to sludge is defined by its octanol-water partitioning coefficient (K_{ow}). Charged compounds and uncharged ones with $\log K_{ow} < 2.5$ are assumed to be poorly absorbed to sewage sludge (Rogers 1996). In adsorption, the compound interacts electrostatically with the negatively charged cell membranes of the micro-organisms (Ternes et al. 2004b). Due to the ionic natures of majority of the studied pharmaceuticals, adsorption is their most plausible sorption mechanism.

The ability of a compound to sorb to sludge is described by a sorption constant (K_d). The higher the K_d is the higher is the amount of the compound sorbed. (Cunningham 2004) Sorption to sludge can be considered as a relevant elimination process for compounds with K_d values of >300 L kg^{-1} (i.e. $\log K_d$ >2.48) (Joss et al. 2005). Of the studied pharmaceuticals, ciprofloxacin, norfloxacin, sulfamethoxazole and diclofenac have K_d values above the threshold of 300 L kg^{-1} (Table 2.2). They all have been detected in primary, secondary and/or digested sludges of STPs although mainly at lower concentrations than in the aqueous sewage (Lindberg et al. 2005 and 2006, Drillia et al. 2005, Golet et al. 2003, Göbel et al. 2005, Miao et al. 2005, Ternes et al. 2004a and 2005). Only the fluoroquinolones have been observed to readily sorb to sewage sludge. It has been shown that the majority of the elimination of the fluoroquinolones (in general >80% in STPs, see Table 2.9) occurs due to adsorption via electrostatic interactions of the positively charged amino groups of the compounds and negatively charged surfaces of the micro-organisms (Ternes et al. 2004b, Golet et al. 2003).

2.3.2 Elimination by biodegradation

The biological elimination of pharmaceuticals in STPs could occur by direct metabolization or by co-metabolization. In the former, bacteria use a compound as their primary carbon source whereas in the latter, bacteria break down or partially convert a

compound but do not use it as the primary carbon source (Jones et al. 2007, Ternes et al. 2004b). Due to the low concentrations of pharmaceuticals compared to other organic constituents in sewage, co-metabolization has been considered as the more plausible mechanism (Jones et al. 2007, Quintana et al. 2005, Ternes et al. 2004b). Degradation at these conditions occurs primarily as a first-order reaction, i.e. the rate of degradation is directly proportional to the dissolved concentration of the substance (Joss et al. 2006). Therefore, in STPs with diluted sewages, the rate of biodegradation and elimination of pharmaceuticals is slow (Joss et al. 2006). Dilution of the sewage may be caused by, for example, the rain water entering the plant.

For the majority of the studied pharmaceuticals, biodegradation has been considered to be the primary elimination mechanism in STPs. Carbamazepine, diclofenac and sulfamethoxazole have been concluded to be poorly biodegradable (Joss et al. 2006, Yu et al. 2006, Alexy et al. 2004, Zwiener and Frimmel 2003) and are consequently poorly eliminated in many STPs (see Table 2.8). Ibuprofen has been found to be readily biodegradable (Joss et al. 2006, Yu et al. 2006, Quintana et al. 2005, Zwiener and Frimmel 2003, Buser et al. 1999) and high elimination rates (>90%) have typically been observed in STPs. Naproxen and bezafibrate have been reported to be moderately biodegradable (Joss et al. 2006, Yu et al. 2006, Quintana et al. 2005) and are normally eliminated in STPs by 50–80%. Atenolol, metoprolol and sotalol have been found to be biodegradable in STPs (Maurer et al. 2007). However, the degradation rates are too low for the entire biodegradation of the compounds and therefore, elimination in STPs has been found to be incomplete (Table 2.9).

2.3.3 Factors affecting the elimination of pharmaceuticals in STPs

The elimination of pharmaceuticals in a STP is affected by several factors, such as sewage temperature, a configuration of the treatment process (e.g. redox conditions) and operational parameters of the STP (e.g. hydraulic and solids retention time). It has been noted that the temperature of the raw sewage can have a major influence on the elimination of biodegradable pharmaceuticals in STPs. Castiglioni et al. (2006) noted significantly higher elimination of ibuprofen, atenolol and bezafibrate in a STP during the

warm season compared to the cold season. They suggested that the increased elimination of the pharmaceuticals was due to the more efficient biodegradation of the compounds caused by the enhanced microbial activity at higher sewage temperatures.

Biodegradation of pharmaceuticals may occur under different redox conditions, i.e. under aerobic, anaerobic or anoxic conditions. For example, ibuprofen has been reported to be biodegraded faster in oxic than in anoxic conditions (Zwiener et al. 2002). Therefore, elimination of a wide variety of pharmaceuticals is assumed in processes with both oxic and anoxic reactors, such as the N/DN process.

Hydraulic retention time (HRT) is the residence time of the aqueous sewage in a reactor or cascade of reactors. The water residence time has been reported to affect the elimination of the biodegradable pharmaceuticals ibuprofen, ketoprofen, atenolol, sotalol and metoprolol in STPs (Maurer et al. 2007, Tauxe-Wuersch et al. 2005). Lower elimination of the compounds occurred in the STPs that were operated at shorter HRT. Tauxe-Wuersch et al. (2005) suggested that the more efficient elimination of the pharmaceuticals was due to the higher rate of biodegradation caused by the increased contact time between the micro-organisms and the water to be treated.

The residence time of activated sludge solids in a STP is characterized by a solids retention time (SRT). In conventional AS processes, SRT ranges from 5–15 days whereas in oxidation ditches and N/DN processes, SRTs can exceed 20 days. Increased SRT has been found to result in higher elimination of certain biodegradable pharmaceuticals, e.g. ibuprofen and bezafibrate (Clara et al. 2005b, Kreuzinger et al. 2004). This is thought to be due to enrichment of certain microbial communities who excrete enzymes that are able to break down pharmaceuticals (Ternes et al. 2004b). It has been suggested that SRTs of at least 10 days are necessary to efficiently eliminate biodegradable pharmaceuticals (Clara et al. 2005b). Non-biodegradable pharmaceuticals, such as carbamazepine, are not affected by the SRT (Bernhard et al. 2006, Clara et al. 2005a and 2005b, Kreuzinger et al. 2004). Since long SRTs are applied especially in nitrification/denitrification and oxidation ditch processes, high elimination of biodegradable pharmaceuticals is assumed in these processes (Clara et al. 2005b, Kanda et al. 2003).

2.4 Pharmaceuticals in surface waters

After the sewage treatment, the effluents are discharged to surface waters. In areas where no surface water is available, effluents may also be infiltrated to ground. STP effluents are the main source of pharmaceuticals in the environment (Kümmerer 2004). Due to dilution, the concentrations of pharmaceuticals are typically lower in surface waters than in STP effluents. In previously studied surface waters, the concentrations of the studied pharmaceuticals have typically been below 200 ng L^{-1} (Table 2.10). However, there are places where concentrations of individual compounds have been several µg L^{-1}.

Table 2.10 Occurrence of the studied pharmaceuticals in surface water.

Compound	Country	Type of surface water	Conc. (ng L^{-1})	Reference
Ciprofloxacin	Italy	Rivers	14.4–26.2	Calamari et al. 2003
	Switzerland	River	18^{Max}	Golet et al. 2002
	USA	Rivers	(20) 30[a]	Kolpin et al. 2002
	USA	Rivers $^{low\ flow}$	30	Kolpin et al. 2004
		Rivers $^{normal\ flow}$	<10	
		Rivers $^{high\ flow}$	<10	
	Canada/USA	River/Lake	<19	Nakata et al. 2005
	USA	Streams	<20	Stackelberg et al. 2004
Norfloxacin	Switzerland	River	18^{Max}	Golet et al. 2002
	USA	Rivers	120^{Max}	Kolpin et al. 2002
	USA	Rivers $^{low\ flow}$	30	Kolpin et al. 2004
		Rivers $^{normal\ flow}$	<10	
		Rivers $^{high\ flow}$	<10	
	Canada/USA	River/Lake	<45	Nakata et al. 2005
	USA	Streams	<100	Stackelberg et al. 2004
Ofloxacin	Canada/USA	River/Lake	<8.6	Nakata et al. 2005
Sulfamethoxazole	Sweden	River	10^{Max}	Bendz et al. 2005
	USA	River	768^{Max}	Glassmeyer et al. 2005
	Germany	Rivers	30–85	Hartig et al. 1999
	UK	River	<50	Hilton and Thomas 2003
	Germany	Not reported	(30) 480[a]	Hirsch et al. 1999
	USA	Rivers	(66) 520[a]	Kolpin et al. 2002
	USA	Rivers$^{low\ flow}$	63^{Max}	Kolpin et al. 2004
		Rivers$^{normal\ flow}$	<23	
	South Korea	Rivers$^{high\ flow}$	10	Kim et al. 2007

Table 2.10 continues

Sulfamethoxazole	USA	Rivers/Lakes	(20) 36[b]	Stackelberg et al.
		Streams	4–10[fig]	2004
	Germany	River Elbe [yr 1998]	70[Max]	Wiegel et al. 2004
	UK	River	<0.08–21	Zhang and Zhou 2007
Carbamazepine	Austria	Rivers	23–133	Ahrer et al. 2001
	Sweden	River	500[Max]	Bendz et al. 2005
	USA	River	186[Max]	Glassmeyer et al. 2005
	USA	River/Wetland	<1	Gross et al. 2004
	Canada	River	16[Max]	Hao et al. 2006
	USA	Rivers[low flow]	263[Max]	Kolpin et al. 2004
		Rivers[normal flow]	8[Max]	
		Rivers[high flow]	2[Max]	
	Taiwan	River	<8	Lin et al. 2005
	Canada	River	(185) 650[a]	Metcalfe et al. 2003
		Harbor area	(120) 310[a]	
		Great Lakes	(20) 20	
	France	Rivers	24, 56	Rabiet et al. 2006
	South Korea	Rivers/Lakes	(25) 61[b]	Kim et al. 2007
	USA	Streams	60–1500[fig]	Stackelberg et al. 2004
	Germany	Rivers/Streams	(250) 1100[a]	Ternes 1998
	Germany	River Elbe [yr 1998]	140[Max]	Wiegel et al. 2004
		Rivers and	<20–640[R(Min)]	(*13 datasets*)
		streams (n= 109)	<20–1200[R(Med)]	
			20–7100[R(Max)]	
	Switzerland	Lake	35–60	Öllers et al. 2001
		River	30–250	
	Romania	River	75[Max]	Moldovan 2006
	UK	River	20–650	Zhang and Zhou 2007
Diclofenac	Austria	Rivers	16–36	Ahrer et al. 2001
	Sweden	River	120[Max]	Bendz et al. 2005
	Switzerland	Rivers/Lakes	310[Max]	Buser et al. 1998
	Canada	River	<10	Hao et al. 2006
	Europe*	Rivers	72[Max]	Hernando et al. 2006
	UK	River	91[Max]	Hilton and Thomas 2003
	Slovenia	Rivers	282[Max]	Kosjek et al. 2005
	Taiwan	River	<2	Lin et al. 2005
	Canada	River	(26) 42[a]	Metcalfe et al. 2003
		Harbor area	(194) 194[a]	
		Great Lakes	<5	
	France	Rivers	1.4–33	Rabiet et al. 2006
	South Korea	Rivers/Lakes	(3.0) 6.8[b]	Kim et al. 2007
	Germany	Rivers/Streams	(150) 1200[a]	Ternes 1998
	Germany	River Elbe [yr 1998]	10–50	Wiegel et al. 2004
		River Elbe [yr 2000]	69[Max]	
	Switzerland	Lake	10[Max]	Öllers et al. 2001
	Switzerland	River	20–150	Öllers et al. 2001

Table 2.10 continues

Diclofenac	UK	River	3.2–68	Zhang and Zhou 2007
	Germany	Lake	270[n= 1]	Quintana and Reemtsma 2004
Ibuprofen	Sweden	River	10–22	Bendz et al. 2005
	UK	River	(789) 3080[b]	Bound and Voulvoulis 2006
	USA/Canada	Rivers/Lakes	<2.6	Boyd et al. 2003
	Switzerland	Rivers/Lakes	8[Max]	Buser et al. 1999
	Italy	Rivers	79[Max]	Calamari et al. 2003
	USA	River/Wetland	1–250	Gross et al. 2004
	Canada	River	<25	Hao et al. 2006
	Europe*	Rivers	152[Max]	Hernando et al. 2006
	UK	River	<20	Hilton and Thomas 2003
	USA	Rivers	(200) 1000[a]	Kolpin et al. 2002
	USA	Rivers[low flow]	<18	Kolpin et al. 2004
		Rivers[normal flow]	<18	
		Rivers[high flow]	<18	
	Slovenia	Rivers	<2	Kosjek et al. 2005
	Taiwan	River	<2	Lin et al. 2005
	Canada	River	(141) 790[a]	Metcalfe et al. 2003
		Harbor area	(64) 93[a]	
		Great Lakes	5	
	France	Rivers	0.3–4.5	Rabiet et al. 2006
	South Korea	River/Lakes	(28) 38[b]	Kim et al. 2007
	USA	Streams	<18	Stackelberg et al. 2004
	Germany	Rivers/Streams	(70) 530[a]	Ternes 1998
	Germany	River Elbe[yr 1998]	70[Max]	Wiegel et al. 2004
		River Elbe[yr 2000]	146[Max]	
	Switzerland	Lake	5–15	Öllers et al. 2001
		River	80[Max]	
	Romania	River	115[Max]	Moldovan 2006
	USA	Raw water	5 850	Loraine and Pettigrove 2006
	Germany	Lake	<0.05[n= 1]	Quintana and Reemtsma 2004
Ketoprofen	Sweden	River	70[Max]	Bendz et al. 2005
	USA	River/Wetland	<1	Gross et al. 2004
	Europe*	Rivers	<26	Hernando et al. 2006
	Slovenia	Rivers	<2	Kosjek et al. 2005
	Canada	River	(12) 17[a]	Metcalfe et al. 2003
		Harbor area	(31) 47[a]	
		Great Lakes	(50) 50[a]	
	France	Rivers	2.8–15	Rabiet et al. 2006
		Wells	0.3–3.0	
	Germany	Rivers/Streams	(<10) 120[a]	Ternes 1998
	Switzerland	Lake	<4.5	Öllers et al. 2001
		River	5[Max]	
	Germany	Lake	330[n= 1]	Quintana and Reemtsma 2004

Table 2.10 continues

Naproxen	Sweden	River	250[Max]	Bendz et al. 2005
	USA	River/Lake	22–107	Boyd et al. 2003
	Canada	River	63	Boyd et al. 2003
	USA	River/Wetland	1.0[Max]	Gross et al. 2004
	Canada	River	41[Median]	Hao et al. 2006
	Europe*	Rivers	70[Max]	Hernando et al. 2006
	Slovenia	Rivers	313[Max]	Kosjek et al. 2005
	Taiwan	River	30[Mean]	Lin et al. 2005
	Canada	River	(207) 551[a]	Metcalfe et al. 2003
		Harbor area	(94) 139[a]	
		Great Lakes	<5	
	France	Rivers	7.2–9.1	Rabiet et al. 2006
		Lakes	0.1–0.2	
	South Korea	Rivers and lakes	(11) 18[b]	Kim et al. 2007
	Germany	Rivers/Streams	(70) 390[a]	Ternes 1998
	Germany	River Elbe [yr 2000]	32[Max]	Wiegel et al. 2004
	Switzerland	Lake	10[Max]	Öllers et al. 2001
		River	10–400	
	Germany	Lake	<0.08[n= 1]	Quintana and Reemtsma 2004
Acebutolol	*Data not found*			
Atenolol	Sweden	River	10–60	Bendz et al. 2005
	Italy	Rivers	3–241	Calamari et al. 2003
Metoprolol	Sweden	River	30–70	Bendz et al. 2005
	Germany	Rivers/Streams	(45) 2200[a]	Ternes 1998
	Germany	River Saale	224[Max]	Wiegel et al. 2004
Sotalol	*Data not found*			
Bezafibrate	Austria	Rivers	1.6–12.5	Ahrer et al. 2001
	Italy	Rivers	0.8–57.2	Calamari et al. 2003
	Canada	River	<2	Hao et al. 2006
	Canada	River	(52) 200[a]	Metcalfe et al. 2003
		Harbor area	<10	
		Great Lakes	<10	
	Germany	Rivers/Streams	(350) 3100[a]	Ternes 1998
	Germany	River Elbe 1998	130[Max]	Wiegel et al. 2004
		River Elbe 2000	88[Max]	
	Germany	Lake	847[n= 1]	Quintana and Reemtsma 2004

Values with "<" were below the limit of quantification or the limit of detection
[a](median) maximum, [b]= (mean) maximum, [Max]= maximum value reported, [R(Min)]= range of minimum values, [R(Med)]= range of median values, [R(Max)]= range of maximum values, [fig]= estimated from a figure,
*Data from Spain, Belgium, Germany and Slovenia.

In surface waters, the concentrations of pharmaceuticals in the aqueous phase are lowered due to dilution, sorption, biodegradation and phototransformation. The extent of dilution depends on the portion of sewage effluent in the receiving water and varies significantly between the receiving waters and between seasons. For example, in the study of Kolpin et al. (2004), higher concentrations of pharmaceuticals were measured in

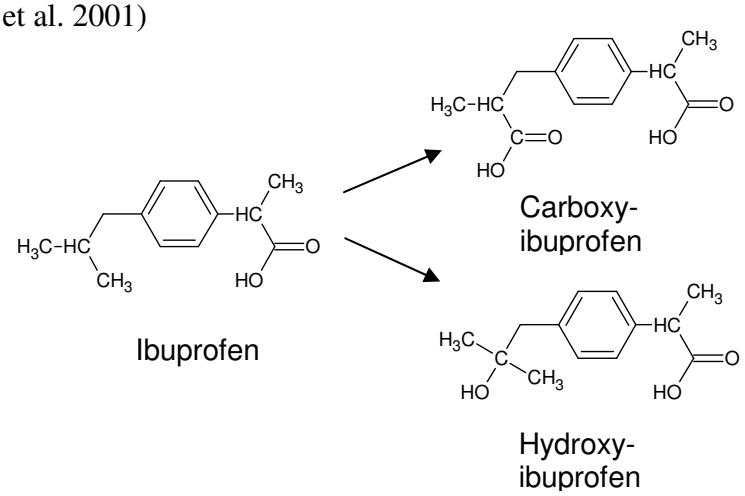
a river during a low flow than during the high flow season due to a lower degree of dilution. Table 2.11 compiles the literature data about the elimination mechanisms (i.e. sorption, biodegradation and phototransformation) of the studied pharmaceuticals in surface waters. Few of the pharmaceuticals are likely to sorb to lake and river sediments. However, the prediction of the sorbed fraction of a compound in different surface waters is difficult because sorption depends on the characteristics of the sediment, e.g. the amount of the organic matter and the particle size (Scheytt et al. 2005). It should also be remembered that sorption eliminates the compounds from the aqueous phase but accumulates them into the sediments.

Table 2.11 The various elimination processes of the studied pharmaceuticals in surface waters.

	Sorption	**Biodegradation**	**Phototransformation**
Ciprofloxacin	(+)[1]	–[5]	(+)[7]
Norfloxacin	dnf	dnf	(+)[A) 6,7]
Ofloxacin	(+)[1]	–[5, 6]	+[3]
Sulfamethoxazole	–[1]	–[5]	+[3]
Carbamazepine	(+)[1,2]	–[2]	(+)[B) 3,4]
Diclofenac	–[9, 18]	–[9]	+[3, 9]
Ibuprofen	–[1, 2, 10] (+)[19]	+[2, 10–12, 17]	–[C) 13, 14]
Ketoprofen	(+)[18, 19]	dnf	+[14]
Naproxen	(+)[10, 18, 19]	–[10], +[17]	+[14, 17]
Acebutolol	dnf	dnf	dnf
Atenolol	(+)[1]	dnf	(+)[A) 4, 15]
Metoprolol	dnf	+[17]	(+)[17]
Sotalol	dnf	dnf	dnf
Bezafibrate	dnf	dnf	(+)[16]

+= the most likely process, (+)= a likely process, – = not a likely process, dnf= data not found
[A)] most significant absorption at wavelengths < 295 nm, [B)] slow indirect photolysis occurs
[C)] fairly slow process (half-lives of 100-500 d) ,
References*:* [1] Castiglioni et al. 2006, [2] Löffler et al. 2005, [3] Andreozzi et al. 2002, [4] Doll and Frimmel 2003, [5] Alexy et al. 2004, [6] Kümmerer et al. 2000, [7] Burhenne et al. 1999, [8] Park et al. 2002, [9] Buser et al. 1998, [10] Lin et al. 2006, [11] Buser et al. 1999, [12] Winkler et al. 2001, [13] Packer et al. 2003, [14] Lin and Reinhard 2005, [15] Zuccato et al. 2005, [16] Cermola et al. 2005, [17] Fono et al. 2006, [18] Antonić and Heath 2007, [19] Rice and Mitra 2007

Of the studied pharmaceuticals, metoprolol, ibuprofen and naproxen have been shown to undergo biodegradation in natural waters (Table 2.11). However, a complete biodegradation may not occur since at least metabolites of ibuprofen (i.e. hydroxy- and carboxy-ibuprofen) have been shown to be formed in a laboratory-scale river biofilm reactor (Scheme 2.2). Additionally, hydroxy-ibuprofen was shown to be significantly more recalcitrant to further biodegradation than ibuprofen and carboxy-ibuprofen. (Winkler et al. 2001)



Scheme 2.2 Biodegradation of ibuprofen in river biofilms (adapted from Winkler et al. 2001)

Another mechanism that lowers the concentrations of the pharmaceuticals in natural waters is the phototransformation. Direct photolysis, where the photon is absorbed by a compound, can occur only if the compound absorbs light at wavelengths above 295 nm (Mill 1999). However, a compound can also be degraded by indirect photolysis via reactions with oxidants that are formed in the reactions between sunlight and so called sensitizers. For example, singlet oxygen and hydroxyl radical that can oxidize compounds are formed in a reaction of sunlight with dissolved organic matter (DOM) and nitrate, respectively (Mill 1999). All pharmaceuticals in surface waters can be phototransformed via the indirect reaction route. For example, ibuprofen does not absorb light at appropriate wavelength but is found to be degraded by indirect photolysis (Packer et al. 2003, Lin et al. 2006). Also, enhanced phototransformation of many pharmaceuticals that undergo direct photolysis has been observed in the presence of nitrate or DOM (Lin and Reinhard 2005, Doll and Frimmel 2003, Andreozzi et al. 2002). Apart from acting as a photosensitizer, DOM can inhibit the phototransformation of

pharmaceuticals since the DOM may absorb UV radiation in a broad range of wavelengths and thus reduce the available energy for the organic compounds in the solution. DOM has been found to inhibit the phototransformation of at least carbamazepine, diclofenac and ketoprofen (Lin and Reinhard 2005, Andreozzi et al. 2002). Phototransformation of pharmaceuticals in the environment produces transformation products, which may be of greater concern than the parent compounds. For example, a toxic, mutagenic and carcinogenic compound, acridine, is formed in the phototransformation of carbamazepine (Chiron et al. 2006).

2.5 Fate of pharmaceuticals in drinking water treatment processes

Drinking water can be contaminated by pharmaceuticals if the raw water source, either ground or surface water, contains these compounds. The occurrence of pharmaceuticals in groundwaters is due to the infiltration of sewage effluent and surface water to the ground, or in the production of artificial groundwater. During the soil passage, some pharmaceuticals are efficiently sorbed to soil particles or biodegraded, whereas some are very persistent (Poseidon 2006, Grünheid et al. 2005, Ternes et al. 2002, Heberer et al. 2001). The occurrence of metoprolol, sotalol, carbamazepine, diclofenac and sulfamethoxazole in ground waters in Europe and the USA (Table 2.12) shows that these compounds are not entirely eliminated during ground water infiltration.

The occurrence of the selected pharmaceuticals in drinking waters has been studied less frequently than in sewage or surface waters. In most studies, concentrations of the pharmaceuticals in drinking waters have been below the detection limits (Table 2.12). Only carbamazepine and ibuprofen have previously been detected in the drinking water (Loraine and Pettigrove 2006, Stackelberg et al. 2004).

Several processes are applied in the production of drinking water (see Figure 2.5). Natural organic matter (NOM), turbidity and micro-organisms are conventionally eliminated from water via chemical coagulation followed by flocculation, sedimentation or flotation, sand filtration, and disinfection. Nowadays, ever more treatment plants apply additional treatment steps to further improve the water purity. These are, for example, activated carbon (AC) filtration, ozonation and UV-disinfection. (Snyder et al. 2003).

Table 2.12 The concentrations (Conc.) of the studied pharmaceuticals in ground and drinking water.

Compound	**Location**	**Water type**	**Conc. (ng L^{-1})**	**Reference**
Ciprofloxacin	Spain	GW	<4	Reverté et al. 2003
	USA	DW	<20	Stackelberg et al. 2004
Norfloxacin	USA	DW	<100	Stackelberg et al. 2004
Ofloxacin	*Data not found*			
Sulfamethoxazole	Germany	GW	470^{Max}	Hirsch et al. 1999
	Germany	GW	410^{Max}	Sacher et al. 2001
	USA	DW	<50	Stackelberg et al. 2004
Carbamazepine	Taiwan	GW and DW	<6	Lin et al. 2005
	France	GW	14–43	Rabiet et al. 2006
	Germany	GW	900^{Max}	Sacher et al. 2001
	USA	DW	258^{Max}	Stackelberg et al. 2004
	USA	DW	$140^{Max, n= 14}$	Stackelberg et al. 2007
Diclofenac	Germany	GW	380^{Max}	Heberer 2002b
	Europe*	DW	<7	Hernando et al. 2006
	Slovenia	DW	<3	Kosjek et al. 2005
	Taiwan	GW and DW	<2	Lin et al. 2005
	France	GW	1.4–2.5	Rabiet et al. 2006
	Germany	GW	590^{Max}	Sacher et al. 2001
Ibuprofen	USA	DW	<2.6	Boyd et al. 2003
	Germany	GW	200^{Max}	Heberer 2002b
	Europe*	DW	<12	Hernando et al. 2006
	Slovenia	DW	<2	Kosjek et al. 2005
	Taiwan	GW and DW	<2	Lin et al. 2005
	France	GW	0.2–0.6	Rabiet et al. 2006
	Germany	GW	<12	Sacher et al. 2001
	USA	DW	<18	Stackelberg et al. 2004
	USA	DW	510–1350	Loraine and Pettigrove 2006
Ketoprofen	Germany	GW	30^{Max}	Heberer 2002b
	Europe*	DW	<26	Hernando et al. 2006
	Slovenia	DW	<2	Kosjek et al. 2005
	Taiwan	GW and DW	<2	Lin et al. 2005
	France	GW	2.8–15	Rabiet et al. 2006
	Germany	GW	<16	Sacher et al. 2001
Naproxen	Slovenia	DW	<5.6	Kosjek et al. 2005
	Taiwan	GW and DW	<1	Lin et al. 2005
	Germany	GW	<13	Sacher et al. 2001
Acebutolol	*Data not found*			
Atenolol	Germany	GW	<8.2	Sacher et al. 2001
Metoprolol	Germany	GW	$>10^{not\ reported}$	Sacher et al. 2001
Sotalol	Germany	GW	560^{Max}	Sacher et al. 2001
Bezafibrate	*Data not found*			

Values with "<" are below the limit of quantification or the limit of detection, GW= ground water, DW= drinking water, ^{Max}= maximum value reported, *Data from Spain, Belgium, Germany and Slovenia

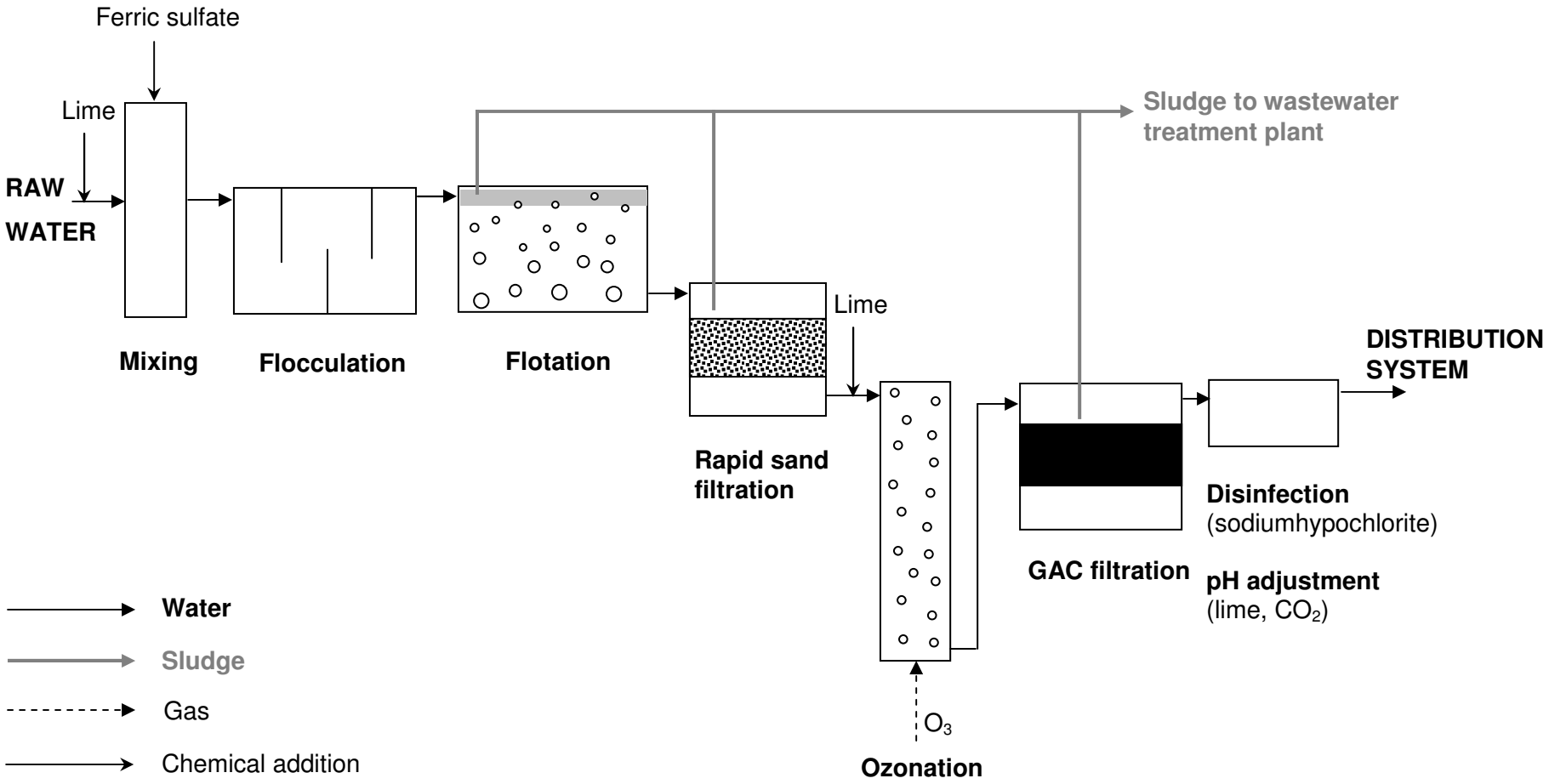


Figure 2.5 Example of a drinking water treatment plant, (GAC= granular activated carbon).

2.5.1 Fate in coagulation/flocculation

Coagulation efficiently reduces the amount of NOM and turbidity in water and is therefore a widely used method in drinking water treatment (Matilainen et al. 2002, Vuorio et al. 1998, Randtke 1988, Collins et al. 1986, Semmens and Ayers 1985). Inorganic particles (such as clays), NOM and micro-organisms in natural waters are negatively charged and remain in dispersed state via electronic repulsion (Gregory and Duan 2001). In contact with water, a coagulant, most frequently a metal salt (aluminum or ferric), hydrolyses and forms either dissolved or precipitated products. For example, for aluminum salts the following processes occur in water (Gregory and Duan 2001):

$$Al^{3+} \rightarrow Al(OH)^{2+} \rightarrow Al(OH)_2^{+} \rightarrow Al(OH)_3\ (solid) \rightarrow Al(OH)_4^{-} \qquad (2.2)$$

Increase of pH causes the equilibrium to be shifted to the right. In the pH range commonly employed in the drinking water treatment (i.e. pH about 5–7), the cationic and the solid hydrolysis products dominate. In coagulation, the constituents in surface water react with the hydroxides of the coagulant via different mechanisms, most importantly charge neutralization and sweep flocculation. In charge neutralization, negative surface charge of the inorganic particles is neutralized by adsorption of cationic hydrolysis products on the particle surface. Dissolved molecules of the NOM can be precipitated as metal-NOM complexes as a result of charge neutralization. In sweep flocculation, the particles, the floc formed by charge neutralization and the metal-NOM complexes bind with the precipitated hydroxide of the coagulant. Coagulation is followed by flocculation, where water is stirred to allow the aggregation of larger flocs that can be removed by sedimentation, flotation or filtration (Gregory and Duan 2001, Huang and Shiu 1996, Semmens and Ayers 1985).

Coagulation is particularly efficient in removing high molecular weight (MW> 1000 Da) compounds, such as humic substances (Matilainen et al. 2002, Vuorio et al. 1998, Randtke 1988, Collins et al. 1986, Semmens and Ayers 1985). The removal of lower molecular weight and more hydrophilic NOM as well as small organic compounds by coagulation has typically been low (Matilainen et al. 2002, Huang and Shiu 1996,

Julien et al. 1994, Collins et al. 1986, Semmens and Ayers 1985). Low molecular weight compounds could be removed by coagulation if they are associated with the particles or the NOM in the raw water, or if they are adsorbed to the floc during the process of coagulation/flocculation (Levebre and Legube 1993, Rebhun et al. 1998). For pharmaceuticals, the studies conducted with distilled or surface waters have concluded that coagulation/flocculation mainly results in less than 10% elimination of the compounds (Stackelberg et al. 2007, Hua et al. 2006, Kim et al. 2007, Westerhoff et al. 2005, Stackelberg et al. 2004, Boyd et al. 2003, Adams et al. 2002, Ternes et al. 2002). Poor elimination in coagulation has been suggested to be due to low hydrophobicity and low molecular weight of pharmaceuticals as well as due to the lack of specific mechanisms for adsorption of the compounds to the floc (Westerhoff et al. 2005, Snyder et al. 2003).

2.5.2 Fate in sand filtration

Sand filtration is applied after the chemical treatment to remove the excess floc from water (called rapid sand filtration) or to remove biodegradable organics in so called slow sand filtration process where biomass grows on the surface of soil particles (Ho et al. 2007, Collins et al. 1986). For example, taste and odor causing compounds, 2-methylisoborneol and geosmin, can be biologically removed in slow sand filtration (Ho et al. 2007). In the rapid sand filtration, a fraction of a high molecular weight NOM has been found to be removed (Collins et al. 1986). The elimination of pharmaceuticals in rapid or slow sand filtration has rarely been studied. In one study, it was reported that carbamazepine was poorly eliminated in rapid sand filtration (Hua et al. 2006).

2.5.3 Fate in activated carbon adsorption

Activated carbon is used either in a form of powdered activated carbon (PAC) or granular activated carbon (GAC). PAC is added to water for a certain contact time and is then removed by coagulation and/or filtration. GAC is placed in bed configuration and water flows through the filter bed. Depending on the placement of the filter in the treatment

train, compounds can either be adsorbed to the AC or be subjected to biodegradation. For example, ozonation prior the GAC filtration produces organic acids that induce the growth of biomass on the surface of the GAC and consequently, the micropollutants can undergo biodegradation during the filtration (Win et al. 2000, Camel and Bermond 1998, Vuorio et al. 1998, Ribas et al. 1997). Adsorption of compounds to the AC occurs via hydrophobic or ion exchange interactions (Snyder et al. 2003). A major portion of the carbon surface is nonpolar or hydrophobic and typically, the hydrophobic interactions between the AC and the adsorbate are more important (Faust and Aly 1983). However, carbon surface also contains functional groups that can take part to the adsorption processes. The adsorption to AC is influenced by several physico-chemical properties of the compounds. Because the hydrophobic bonding to the AC is favored, the adsorption tends to increase with decreasing polarity and increasing K_{ow} value of the adsorbate (Poseidon 2006, Westerhoff et al. 2005). Other factors influencing the adsorption of substances to AC are the particle size, the surface area, and the pore size distribution of the AC (Faust and Aly 1983).

Activated carbon adsorption, either in the form of PAC or GAC, has been found to be a powerful technique in the elimination of pharmaceuticals in drinking water treatment (Stackelberg et al. 2007, Snyder et al. 2007, Kim et al. 2007, Poseidon 2006, Adams et al. 2002, Ternes et al. 2002). The compounds that have high K_{ow} values are generally the most readily adsorbed to the AC (Poseidon 2006, Westerhoff et al. 2005). For example, carbamazepine that has a $\log K_{ow}$ value of 2.45 is more readily adsorbed to the AC than sulfamethoxazole whose $\log K_{ow}$ is 0.89 (Poseidon 2006, Westerhoff et al. 2005). Some pharmaceuticals, such as ibuprofen, diclofenac and naproxen, can carry negative charge in water due to deprotonation of the acid functional groups. Typically, these pharmaceuticals are less removed by the AC that could be expected from their $\log K_{ow}$ values (in this case, $\log K_{ow}$= 3.18–4.51) (Poseidon 2006, Westerhoff et al. 2005). On the contrary, certain pharmaceuticals that have low K_{ow} values but contain heterocyclic nitrogen in their structures have been noted to be well removed by the AC due to specific interactions between the protonated functional groups of the compounds and the functional groups on the surface of the AC (Westerhoff et al. 2005).

Natural waters contain a broad spectrum of substances, such as micropollutants and NOM. In the AC treatment of natural waters, competition occurs between the different water constituents (Newcombe et al. 1997). Due to the presence of NOM in water, higher PAC doses are needed for efficient contaminant elimination in natural waters compared to distilled water (Poseidon 2006, Adams et al. 2002). In the GAC filter, additional drawback is the clogging of the carbon pores by NOM molecules and a subsequent decrease of the surface area of the carbon (Chen et al. 1997, Newcombe et al. 1997). As a result, the adsorption of compounds to GAC decreases with increased operation time and at some point compounds start to breakthrough from the GAC (Snyder et al. 2007, Matilainen et al. 2006, Stackelberg et al. 2004, Matsui et al. 2002). Compounds that are weakly adsorbed to GAC start breakthrough more rapidly than the more strongly adsorbed compounds (Snyder et al. 2007). When the AC is fully exhausted, the effluent concentrations of the contaminants equal the influent concentrations. This was noted by Stackelberg et al. (2004), who reported that carbamazepine, a compound that is readily adsorbable to AC, was not removed in the GAC filter that had been in operation for 3 years. Compounds may also desorb from the GAC if weakly adsorbed compounds are displaced by more strongly adsorbed ones (Snyder et al. 2003, Faust and Aly 1983). In these cases, the concentration of a compound in the effluent can exceed the influent concentration (Faust and Aly 1983). To restore the adsorption capacity of the GAC, it should be regenerated from time to time by, for example, pyrolysis. In the study of Snyder et al. (2007), regular regeneration of a GAC was noted to maintain an efficient elimination of pharmaceuticals in a drinking water treatment plant (Snyder et al. 2007).

2.5.4 Fate in oxidation

2.5.4.1 Ozonation

Ozonation is the most efficient technique in drinking water treatment for the oxidation of both inorganic and organic compounds (von Gunten 2003). It can be used in the removal of iron and manganese, NOM and color, to inactivate micro-organisms and to enhance

the coagulation/flocculation process (Camel and Bermond 1998). The oxidation of compounds occurs via reactions with ozone (O_3) and the hydroxyl radical (•OH), which is formed when ozone decomposes in water. Ozone is a very selective oxidant and particularly reactive toward deprotonated amines, compounds having C=C double bonds, and aromatic rings substituted with electron donor groups, such as OH and NH_2. The aromatics substituted with electron withdrawing groups, such as COOH, NO_2 and Cl, are less reactive towards ozone. (von Gunten 2003, Langlais et al. 1991)

In contrast to ozone, hydroxyl radical is an unselective oxidant and is the strongest oxidant in water. Many compounds not oxidized by ozone can be efficiently transformed by hydroxyl radical oxidation. It is formed when ozone decomposes in water and the formation can be accelerated by increasing the pH or applying so called advanced oxidation processes (AOPs). In AOPs, hydroxyl radical formation is accelerated by the addition of hydrogen peroxide or via UV irradiation. (von Gunten 2003, Langlais et al. 1991)

Ozonation of natural waters is a very complex process. The rate at which ozone is decomposed to hydroxyl radicals is dependant especially on the amount of NOM and the alkalinity of water. Carbonate and bicarbonate ions inhibit the ozone decomposition and thus ozone is fairly stable in water with a high alkalinity. The effect of the NOM on ozonation is not that straightforward. On one hand, NOM may enhance ozone decomposition and accelerate the formation of •OH. On the other hand, NOM may consume the radicals and act as a radical scavenger. As a consequence, NOM normally slows down the oxidation of micropollutants and makes AOP processes fairly inefficient in waters with high amount of NOM (von Gunten 2003).

Many pharmaceuticals can be efficiently oxidized by either ozone or hydroxyl radicals (Dodd et al. 2006, Hua et al. 2006, Westerhoff et al. 2005, Huber et al. 2003 and 2005a, Vogna et al. 2004, Ternes et al. 2003, Andreozzi et al. 2002, Zwiener and Frimmel 2000). Table 2.13 compiles the apparent rate constants of the studied pharmaceuticals with O_3 or •OH. These values can be used to predict the behavior of the compounds in ozonation of natural waters.

Table 2.13 Apparent rate constants of the studied pharmaceuticals with ozone (k_{O3}) and hydroxyl radical (k_{OH}) measured in distilled water.

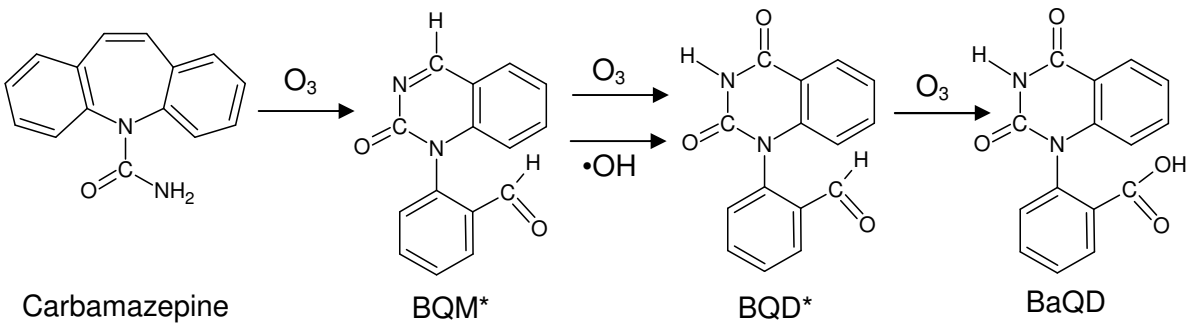
Pharmaceutical	Apparent k_{O3} (10^5 $M^{-1}s^{-1}$)	Apparent k_{OH} (10^9 $M^{-1}s^{-1}$)
Ciprofloxacin	0.004^{++} 0.075^{+} 0.19^{0}	4.1 ± 0.3
Norfloxacin	-	-
Ofloxacin	-	-
Sulfamethoxazole	0.47^{+} 5.7^{0} (pH 7)	5.5
Carbamazepine	~3	8.8 ± 1.2
Acebutolol	-	-
Atenolol	-	-
Metoprolol	-	-
Sotalol	-	-
Diclofenac	~10	7.5 ± 1.5
Ibuprofen	0.000096	7.4 ± 1.2
Ketoprofen	-	-
Naproxen	~2	9.6 ± 0.5
Bezafibrate	0.0059	7.4 ± 1.2

- = data not found
References: Huber et al. 2003, except for naproxen in Huber et al. 2005a and for ciprofloxacin and sulfamethoxazole in Dodd et al. 2006. $^{++}$ for diprotonated species, $^{+}$ for monoprotonated species ^{0} for deprotonated species.

Sulfamethoxazole, carbamazepine, diclofenac and naproxen have been found to be easily oxidized by ozone and they have also been found to be efficiently eliminated in pilot or full scale ozonation of wastewater or drinking water (Boyd et al. 2003, Ternes et al. 2003, Ternes et al. 2002). Ciprofloxacin, bezafibrate and ibuprofen seem to be less prone to react with ozone and especially ibuprofen is mainly oxidized by OH radicals. Its elimination has been found to be higher in water that has low ozone stability (i.e. low alkalinity and high amount of dissolved organic carbon, DOC) due to the extensive formation of OH radicals in this type of water (Huber et al. 2003). For example, in distilled water, 1 mgO_3 L^{-1} oxidized only about 10% of ibuprofen (Zwiener and Frimmel

2000) whereas similar ozone dose resulted in 50% oxidation of ibuprofen in river water that contained 3.2 mg L^{-1} of DOC (Snyder et al. 2006). Due to the same reasons, ibuprofen has been found to be more efficiently eliminated in AOP processes (Huber et al. 2003, Zwiener and Frimmel 2000). For bezafibrate, ozone doses of 1–3 mg L^{-1} has been needed to obtain oxidation of ≥80% in wastewater or drinking water treatment (Huber et al. 2005a, Huber et al. 2003, Ternes et al. 2002). Ciprofloxacin in wastewater could be oxidized by >99% with ozone dose of 3 mg L^{-1} (Dodd et al. 2006). Atenolol, sotalol and metoprolol have been found to be removed in ozonation of STP effluent by ozone doses of 5–10 mg L^{-1} (Ternes et al. 2003). Their elimination has, however, not been assessed in ozonation of drinking water.

An advantage of ozonation is that ozone can achieve the biochemical deactivation of a pharmaceutical by reacting with the moieties of the compound that are responsible for its pharmacological effect (Dodd et al. 2006, Huber et al. 2004). However, it has been noted that in some antibiotics, e.g. ciprofloxacin, ozone does not primarily oxidize the biochemically essential moieties (Dodd et al. 2006). In addition, ozonation can lead to formation of oxidation products whose biochemical actions are unknown. For example, several products have been found to be formed in ozonation of the readily oxidized diclofenac and carbamazepine (Vogna et al. 2004, McDowell et al. 2005). Further, the oxidation products of carbamazepine have been identified (Scheme 2.3) (McDowell et al. 2005). There are no data available on the biological effects of the formed oxidation products.



BQM= 1-(2-Benzaldehyde)-4-hydro-(1*H*,3*H*)-quinazoline-2-one

BQD= 1-(2-Benzaldehyde)-(1*H*,3*H*)-quinazoline-2,4-dione

BaQD= 1-(2-Benzoic acid)-(1*H*,3*H*)-quinazoline-2,4-dione

Scheme 2.3 Reaction pathways proposed by McDowell et al. (2005) for the oxidation of carbamazepine with ozone and hydroxyl radicals. *= The compound has been identified in ozonated water from a German waterworks.

2.5.4.2 Chlorination and chlorine dioxide

Chlorination in the drinking water treatment is used for disinfection and oxidation purposes. There are several chlorine-containing compounds available for disinfection of water. Of these, chlorine (C_2), chlorine dioxide (ClO_2) and sodium hypochlorite (NaOCl) are probably the most commonly used. In addition, chloramines are formed in the reaction of chlorine and ammonia present in water. Chloramines tend to be the least reactive of the disinfectants (Dodd and Huang 2004, Pinkston and Sedlak 2004). An advantage of disinfection by chlorination over the other disinfection methods (UV or ozone) is that some of the chlorine remains dissolved in water thus producing disinfection also in the distribution system. A major disadvantage of chlorination is the formation of mutagenic disinfection by-products (Kronberg et al. 1988, Hemming et al. 1986).

Chlorine dioxide and chlorine can oxidize pharmaceuticals even though not as efficiently as ozone (Huber et al. 2005b). Particularly the compounds containing electron rich functional groups such as amines and phenols react with ClO_2 and chlorine. Of the studied pharmaceuticals, diclofenac and sulfamethoxazole have been found to react readily with chlorine especially at neutral pH (Snyder et al. 2007, Huber et al. 2005b, Westerhoff et al. 2005, Dodd and Huang 2004, Adams et al. 2002). Also naproxen, atenolol and metoprolol may be oxidized by ClO_2 and free chlorine (Boyd et al. 2005,

Huber et al. 2005b, Pinkston and Sedlak 2004). On the contrary, many pharmaceuticals, including carbamazepine, ibuprofen, ketoprofen and bezafibrate have been found not to be appreciably reactive with ClO_2 or free chlorine (Snyder et al. 2007, Huber et al. 2005b, Pinkston and Sedlak 2004). However, in the study of Westerhoff et al. (2005), carbamazepine was eliminated by >90% by free chlorine (dose 3.5–3.8 mg L^{-1}) and ibuprofen by 25–80%.

2.5.5 Fate in UV disinfection

In the drinking water treatment, ultraviolet light at wavelength of 254 nm is used for disinfection. UV light disrupts the DNA of micro-organisms, preventing their replication and inactivates the cells. Disinfection is normally applied as the final treatment since NOM and particles in water decrease the rate of UV disinfection. (Baird and Cann 2005)

It has been demonstrated that UV-radiation (at λ= 254 nm) may induce transformation of some pharmaceuticals, e.g. sulfamethoxazole, carbamazepine, diclofenac, ketoprofen and naproxen (Pereira et al. 2007, Meunier et al. 2006, Adams et al. 2002). However, doses that are needed for the extensive transformation of the pharmaceuticals by UV-light are generally significantly higher than the doses employed for disinfection purposes in the drinking water treatment. For example, Meunier et al. (2006) reported a reduction of only 14 and 29% in the concentrations of sulfamethoxazole and diclofenac, respectively, with UV dose of 400 J m^{-2}, which is a regulatory standard for disinfection in Austria and Germany. In the study by Pereira et al. (2007), similar UV-dose resulted in no elimination of carbamazepine and naproxen in laboratory grade water whereas ketoprofen and ciprofloxacin could be degraded by 80 and 48%, respectively.

3 MATERIALS AND METHODS

A complete description of the materials and methods is presented in the Papers **I**-**VI**. Here, a brief summary is given.

3.1 Sampling of sewage, surface and drinking waters

Water samples were collected from sewage treatment plants, rivers and drinking waters. All the samples were frozen at –18 °C either at the treatment plants or at the laboratory. Information about the samplings is compiled in Table 3.1. Altogether fourteen STPs were sampled for influents and effluents. The samples were collected as 24-h composite samples. The influents of the STPs were sampled prior any treatment had took place and the effluents after all the treatment steps. In Papers **I** and **III**, detailed information of the STPs, such as influent flow rate, inhabitants serviced and treatment processes, is presented.

Grab samples were collected from six rivers (described in Papers **I**, **III** and **IV**) receiving effluents from STPs. The rivers were sampled upstream and downstream of the STPs. Two of the rivers, River Aura and River Vantaa, were more thoroughly studied due to their use or possible use as raw water sources for drinking water production (Papers **II** and **IV**).

Drinking water samples were collected from cities Turku and Vaasa where raw water was pumped from Rivers Aura and Kyrö, respectively (Papers **III** and **IV**, partly unpublished). Both rivers received effluents from several upstream STPs.

Table 3.1 Samplings of drinking, surface and sewage waters conducted in this study.

Type of sample	Sampling place	Sampling time	Sampling procedure	n	Pharmaceuticals analyzed [b)]
Paper I and II					
Sewage influent and effluent	STPs of:				
	Aura	Mar-04	24-h composite	1	Method 1
	Hyvinkää (Kalteva)	Nov-04	sample	1	
	Nurmijärvi (Klaukkala)	"		1	
	Riihimäki	"		1	
	Helsinki [a)]	Nov-05		1	
	Joensuu	"		1	
	Jyväskylä	"		1	
	Lappeenranta	"		1	
	Oulu	"		1	
	Tampere	"		1	
	Turku	"		10	
	Vaasa	"		1	
Tertiary effluent	Effluent from a tertiary biofilter in the STP of Helsinki	Nov-05	24-h composite sample	1	Method 1
Surface waters	River Vantaa	Nov-05	Grab samples	5	Method 1 and 2
Paper III					
Sewage influent and effluent	STPs of:				
	Aura	Sep-03	24-h composite	1	Method 2
	Harjavalta	"	samples	1	
	Helsinki	"		1	
	Seinäjoki	"		1	
	Tampere	"		1	
	Turku	"		1	
	Vaasa	"		1	

Table 3.1 continues

Surface water	River Aura	Sep-03	Grab samples	3	Method 2
	River Kokemäenjoki	"		3	
	River Kyrö*	"		4	
Drinking water	City of Vaasa	Sep-03	Grab samples	1	Method 2
	City of Turku	"	Grab samples	1	
Paper IV					
Sewage influent and effluent	STP of Aura	Mar-04	24-h composite samples	1	Method 2
		May-04		1	
		Aug-04		1	
Surface water	River Aura	Mar-04	Grab samples	8	Method 2
		May-04		8	
		Aug-04		8	
Drinking water	City of Turku	Mar-04	Grab samples	1	Method 2
		May-04		1	
		Aug-04		1	
Paper VI					
Surface water	River Vantaa	Jul-05	Grab samples	1	Method 1 and 2
		Nov-05		1	
		Jan-06		1	
		Mar-06		2	
		Apr-06		1	
Unpublished					
Drinking water	City of Vaasa	May-04	Grab samples	1	Method 2
	City of Turku	Jun-05		1	

[a)] Secondary effluent, [b)] see Table 3.3 for the pharmaceuticals analyzed with Method 1 and 2
* Included River Seinäjoki, a tributary of the River Kyrö, n= number of samples

3.2 Drinking water treatment

3.2.1 Laboratory scale coagulation

The elimination of sulfamethoxazole, carbamazepine, diclofenac, ibuprofen and bezafibrate was studied in laboratory coagulation. Experiments were undertaken on one liter samples with a mini-flocculator (Figure 3.1). Coagulation/ flocculation/ sedimentation studies were performed by spiking pharmaceuticals (at concentrations of 30–40 µg L^{-1}) to Milli-Q water, lake water and model waters made from dissolved humic acid (DHA) (5, 15 and 30 mg L^{-1} measured as dissolved organic carbon) and performing coagulation with aluminum sulfate, $Al_2(SO_4)_3 \cdot 18H_2O$ at pH 6.0 and ferric sulfate, $Fe_2(SO_4)_3$ at pH 4.5. Detailed prescription of the experimental setup is presented in Paper **V**.

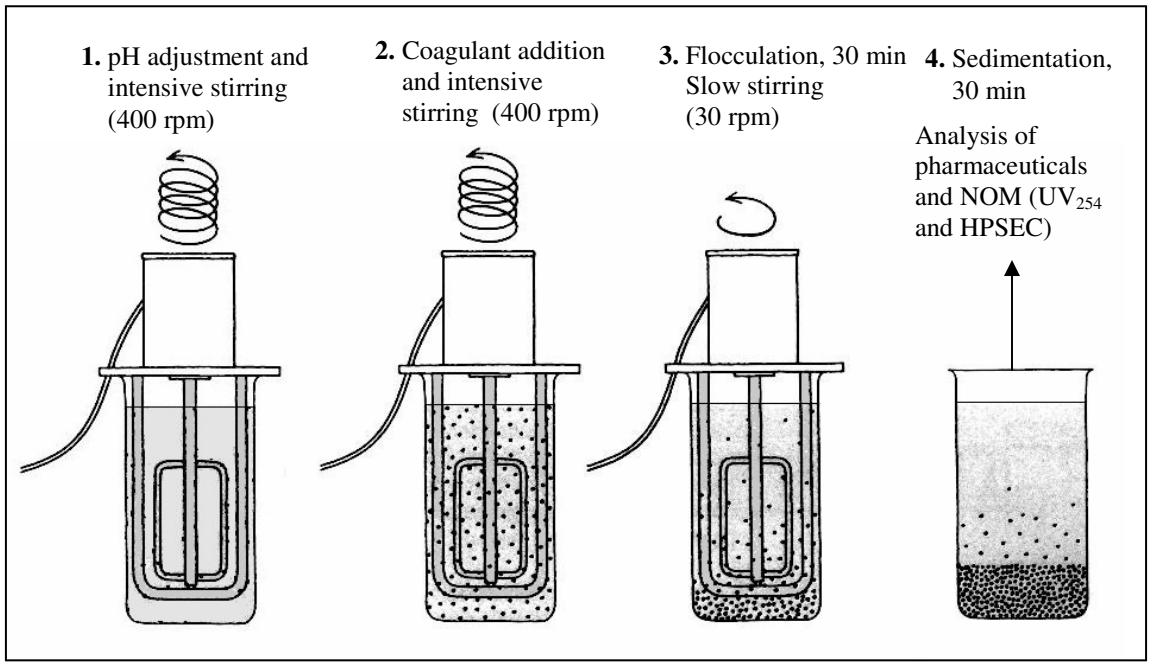


Figure 3.1 The stages of the jar test coagulation. NOM= natural organic matter, UV_{254}= UV absorbance at λ= 254, HPSEC= high-performance size-exclusion chromatography.

3.2.2 Pilot-scale drinking water treatment plant

The elimination of the pharmaceuticals from the water of River Vantaa was studied in a pilot-scale drinking water treatment plant (Paper **VI**). Scheme of the treatment process is presented in Figure 1 of **VI**. The process mimicked the full scale treatment plant of Helsinki City waterworks and consisted of coagulation by ferric sulfate at pH 5 (coagulant dose was dependent on the amount of NOM in the raw water) followed by flocculation, sedimentation and sand filtration. Further, water was ozonated to obtain a residual ozone concentration of 0.3 mg L^{-1} (applied ozone dose was 1 mg L^{-1}, corresponding to 0.2–0.4 mgO_3/mgTOC), filtered in two-stage GAC filtration and finally UV-disinfected (dose of 250 Jm^{-2}). The pilot plant experiments were conducted four times between July 2005 and April 2006. After every unit operation, a 1-L sample was taken and frozen at –18 °C.

3.2.3 Full-scale drinking water treatment plant

The studied drinking water treatment plant (Paper **IV**) supplied Turku City with drinking water. Raw water was pumped from River Aura, which received effluents from three upstream STPs. The treatment process consisted of two stage ferric coagulation along with GAC filtration and chlorination. The first coagulation was conducted at acidic pH and the flocs were removed by flotation. In the second coagulation step, pH was increased, and flotation or sedimentation was used remove the floc. Additionally, chlorine dioxide was added in the second coagulation step for disinfection, and to remove iron and manganese. The dose of ferric salt (around 25–35 mg L^{-1}) was adjusted according to turbidity, and the amount of NOM in the raw water (measured as $KMnO_4$ number). Samples were collected in March, May and August 2004 from the raw water, after the first and second coagulation/flocculation/floc separation, after GAC filtration, and from the purified water. Samples were immediately delivered to the laboratory and frozen at –18 °C.

3.3 Analytical methods

Methods for the analysis of NOM are compiled in Table 3.2. In Paper **VI**, the NOM analyses were made in the laboratory of Helsinki Water.

Table 3.2 Summary of the methods carried out to analyze NOM in water samples.

Parameter	Apparatus	Additional information
Dissolved organic carbon (DOC) (Papers **III, IV, V**)	Shimadzu TOC-5000 and ASI-5000	-
UV absorbance at λ= 254 nm (Paper **V**)	Shimadzu UV-1601 UV-Vis Spectrophotometer	-
High-performance size exclusion chromatography (HPSEC) (Paper **V**)	Hewlett Packard 1100 HPLC	Column: TSKgel G3000SW Eluent: 10 mM NaAc Detection: UV (λ= 254 nm)

NaAc= sodium acetate, DAD= diode array detection

In the laboratory coagulation studies (Paper **V**), pharmaceuticals were analyzed using high-performance liquid chromatography (HPLC, Hewlett Packard 1100) and UV-diode array detection. Samples were chromatographed on a C18 column (Nucleosil 100-5, 4.0 × 250 mm, Agilent Technologies) using isocratic elution with H_3PO_4 (c= 10 mM) and methanol. Details of the method are presented in Paper **V**. The limit of quantification (LOQ) of the method was 5 μg L^{-1} for the studied pharmaceuticals (sulfamethoxazole, carbamazepine, diclofenac, ibuprofen and bezafibrate) and this analytical procedure could not be used in the analysis of the environmental samples. Therefore, two analytical methods consisting of sample concentration by SPE, separation by HPLC and detection by triple quadrupole mass spectrometer were developed. The methods are summarized in Table 3.3 and described in detail in Papers **I** and **III**.

Table 3.3 Summary of the methods used in analysis of pharmaceuticals in the environmental samples.

	Method 1 (Paper I)	**Method 2 (Paper III)**
Analyzed compounds	Fluoroquinolones, beta blockers, carbamazepine	Anti-inflammatories, bezafibrate
Sample volume:		
Sewage influent/ effluent	100 mL/ 300mL	100 mL/ 250 mL
Surface water	500 mL	500 mL
Drinking water	500–1000 mL	500–1000 mL
Sample filtration*	0.45 µm glass fiber filter [a]	0.45 µm glass fiber filter [a]
Extraction pH	10.0 (adjusted with NaOH)	2.0 (adjusted with HCl)
SPE sorbent	Oasis HLB 3cc (60 mg) [b]	Oasis MCX 3cc (60 mg) [b]
Sorbent pre-conditioning	2 mL n-hexane, 2 mL acetone, 10 mL methanol, 10 mL groundwater (pH 2.0 or 10.0)	
Wash	2 mL of 5% methanol in NH_4OH (2% solution)	None
Elution	1 × 4 mL of methanol	1 × 4 mL of acetone
Evaporation	To dryness with N_2 gas	To 100 µL with N_2 gas, addition of 100 µL of methanol, evaporation to 50 µL
The extracted sample	500 µL (480 µL 1% acetic acid, 20 µL methanol)	500 µL (450 µL 10 mM NH_4OH, 50 µL methanol)
HPLC column	Zorbax XDB-C18 (5 µm, 2.1 × 50 mm) [c]	Zorbax Extend-C18 (5 µm, 2.1 × 50 mm) [c]
Injection volume	30 µL	50 µL
Flow rate	250 µL min^{-1}	250 µL min^{-1}
Eluents	A= Acetonitrile, B= 1% acetic acid	A= Acetonitrile, B= 10 mM NH_4OH
Elution program	0 min: 3% A, 97% B. 0→12 min: 3→28% A, 12→17 min: 28→53% A, 17→18 min: 53%A, 18 →19 min: 53→3% A, 19→27 min: 3% A.	0 min: 5% A, 95% B, 0→3 min: 5% A, 3→15 min: 5→35% A, 15→16 min: 35→100% A, 16→21 min: 100% A, 21→22 min: 100→5% A, 22→30 min: 5% A.

* sewage and surface water samples, [a] GF6, Schleicher & Schuell, [b] Waters, [c] Agilent Technologies

The chromatographic separations were carried out with the use of an Agilent 1100 HPLC system (Agilent Technologies). Mass spectrometry was performed using a Quattro Micro triple-quadrupole mass spectrometer (Micromass) equipped with an ESI source. Nitrogen was used as the desolvation and nebulizing gas and argon was used as the collision gas. The mass spectrometer was operated in the MRM mode and the mass transitions as well as the cone voltage and the collision energy were optimized for each analyte by direct infusion of a pure compound to the MS/MS compartment (Table 3.4). Limits of quantifications (LOQs) were determined for each compound in drinking, surface and sewage waters (Table 3.4).

Internal standard (I.S.) method was used in the quantification of the studied pharmaceuticals. Group analogue internal standards were used for the fluoroquinolones (I.S. was enrofloxacin) was and the beta blockers (I.S. was alprenolol). Enrofloxacin is a veterinary fluoroquinolone and has previously been used in the quantification of fluoroquinolones in sewage samples (Lindberg et al. 2004). Alprenolol is a beta blocker that is not subscribed in Finland (National Agency for Medicines 2006) and was not found in any sewage, surface water or ground water samples analyzed. Internal standard for carbamazepine was dihydrocarbamazepine, which has previously been used in many studies (Miao and Metcalfe 2003, Ternes 2001, Ternes et al. 1998, Öllers et al. 2001). Fenoprop, a herbicide, was used as an internal standard for the anti-inflammatories and bezafibrate. It has previously been used in the quantification of these pharmaceuticals (Quintana and Reemtsma 2004, Löffler and Ternes 2003).

Table 3.4 MS/MS parameters using electrospray ionization (ESI) for the studied pharmaceuticals as well as the limit of quantifications of the compounds in drinking water (DW), surface water (SW) and in sewage waters.

	ESI	Cone voltage (V)	Collision energy (eV)	Precursor ion (m/z)	Product ion (m/z)	Limit of quantification (ng L^{-1})			
						DW	SW	Effluent	Influent
Ciprofloxacin	Negative	30	17	331.9	287.9	8.4	24	29	163
Norfloxacin	Negative	28	16	319.8	275.9	7.0	24	24	78
Ofloxacin	Negative	29	18	361.8	317.9	1.6	2.6	5.8	18
Carbamazepine	Negative	29	18	237.0	193.9	0.2	0.5	1.4	3.5
Diclofenac	Positive	16	12	293.8	249.9	1.0	1.0	5.0	5.0
Ibuprofen	Positive	15	8	205.1	161.0	1.0	1.0	5.0	5.0
Ketoprofen	Positive	14	8	253.0	209.0	5.0	5.0	25	25
Naproxen	Positive	15	16	229.0	169.9	5.0	5.0	25	25
Acebutolol	Negative	28	20	336.8	116.0	0.4	0.8	2.1	6.4
Atenolol	Negative	30	23	267.0	144.9	6.5	11.8	21	49
Metoprolol	Negative	25	15	267.9	190.9	22	3.8	9.1	21
Sotalol	Negative	30	23	254.8	132.9	1.6	3.9	5.2	19
Bezafibrate	Positive	21	16	360.0	273.9	1.0	1.0	5.0	5.0
Enrofloxacin	Negative	28	18	359.9	315.9	Not determined for the internal standards.			
DHCBZ	Negative	35	21	239.0	193.9				
Fenoprop	Positive	24	15	266.8	194.8				
Alprenolol	Negative	25	15	249.9	172.9				

DHCBZ= dihydrocarbamazepine

4 RESULTS AND DISCUSSION

In this section, the results of the study are briefly summarized. A more detailed presentation of the results can be found in the Papers **I–VI**.

4.1 Analytical methods for the determination of the pharmaceuticals in the environmental samples

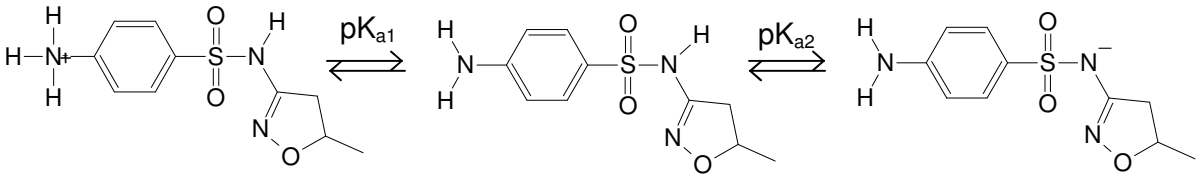
4.1.1 Solid phase extraction

Two methods were developed in order to analyze the studied pharmaceuticals in environmental samples. Both methods applied SPE to the sample concentration. Fluoroquinolones (ciprofloxacin, norfloxacin and ofloxacin), carbamazepine and beta blockers (acebutolol, atenolol, metoprolol and sotalol) were extracted at basic pH onto a sorbent that allowed both hydrophilic and hydrophobic retention (Oasis HLB) (Paper **I**). Oasis MCX sorbent, having both cation exchange and reversed phase properties, was used for the extraction of anti-inflammatories (diclofenac, ibuprofen, ketoprofen and naproxen) and bezafibrate at acidic pH (Paper **III**). Oasis HLB sorbent has been widely used in the analysis of pharmaceuticals due to its ability to retain compounds with various physico-chemical properties (see Table 2.6).

A major contributor to extraction recoveries of the pharmaceuticals was pH, which typically has been one of the most important parameters affecting the SPE extraction efficiencies (Hao et al. 2006, Castiglioni et al. 2005, Santos et al. 2005, Göbel et al. 2004, Reverté et al. 2003, Zhang and Zhou 2007). The anti-inflammatories and bezafibrate were extracted at pH 2.0, at which these compounds are mainly non-ionized (pK_a values 3.61–4.91, see Table 2.2) and consequently better retained by the polymeric phase of the MCX sorbent. For carbamazepine and the fluoroquinolones, pH had no pronounced effect on the retention of the compounds on the HLB sorbent. However, atenolol and sotalol were poorly recovered (<10%) with an extraction pH of 4.0 and could only be recovered to appreciable degree (>60%) at pH 10. Atenolol and sotalol are basic compounds (pK_a values 9.6 and

9.55, respectively) and are thus better retained by the polymeric sorbent in their uncharged forms that prevail at pH 10.

Neither of the isolation methods was suitable for the extraction of sulfamethoxazole and therefore the compound could not be analyzed in the environmental samples. The compound was recovered by >90% on the HLB sorbent with an extraction pH of 4.0. However, at pH 10, which was applied in the Method 1, sulfamethoxazole was recovered only by <10%. This is in accordance with the study of Zhang and Zhou (2007) who noted that sulfamethoxazole was better retained on the Oasis HLB sorbent at low extraction pH. This was most probably caused by the different retention of the various dissociation species of sulfamethoxazole on the HLB sorbent (Scheme 4.1). At pH 4.0, sulfamethoxazole occurs mainly as the non-ionized species which seems to be better retained on the HLB sorbent than the negatively charged form of the compound that prevails at pH 10.



Scheme 4.1 Dissociation of sulfamethoxazole in water at different pH (pK_{a1}= 1.85, pK_{a2}= 5.60).

The pharmaceuticals were chromatographically separated on two reversed phase C18 columns. The anti-inflammatories and bezafibrate were eluted with basic eluent (aqueous NH_4OH and acetonitrile) due to the enhancement of the ion signal produced in the negative ESI. Due to high pH of the eluent (about 10), a column was chosen that provided a long lifetime and good peak shapes at this extreme pH (Figure 4.1b). The ionization of the basic beta blockers could be enhanced by using acidic eluent (aqueous acetic acid and acetonitrile). The column that was used in the separation of the analytes provided sharp peaks and good separation of the compounds (Figure 4.1a).

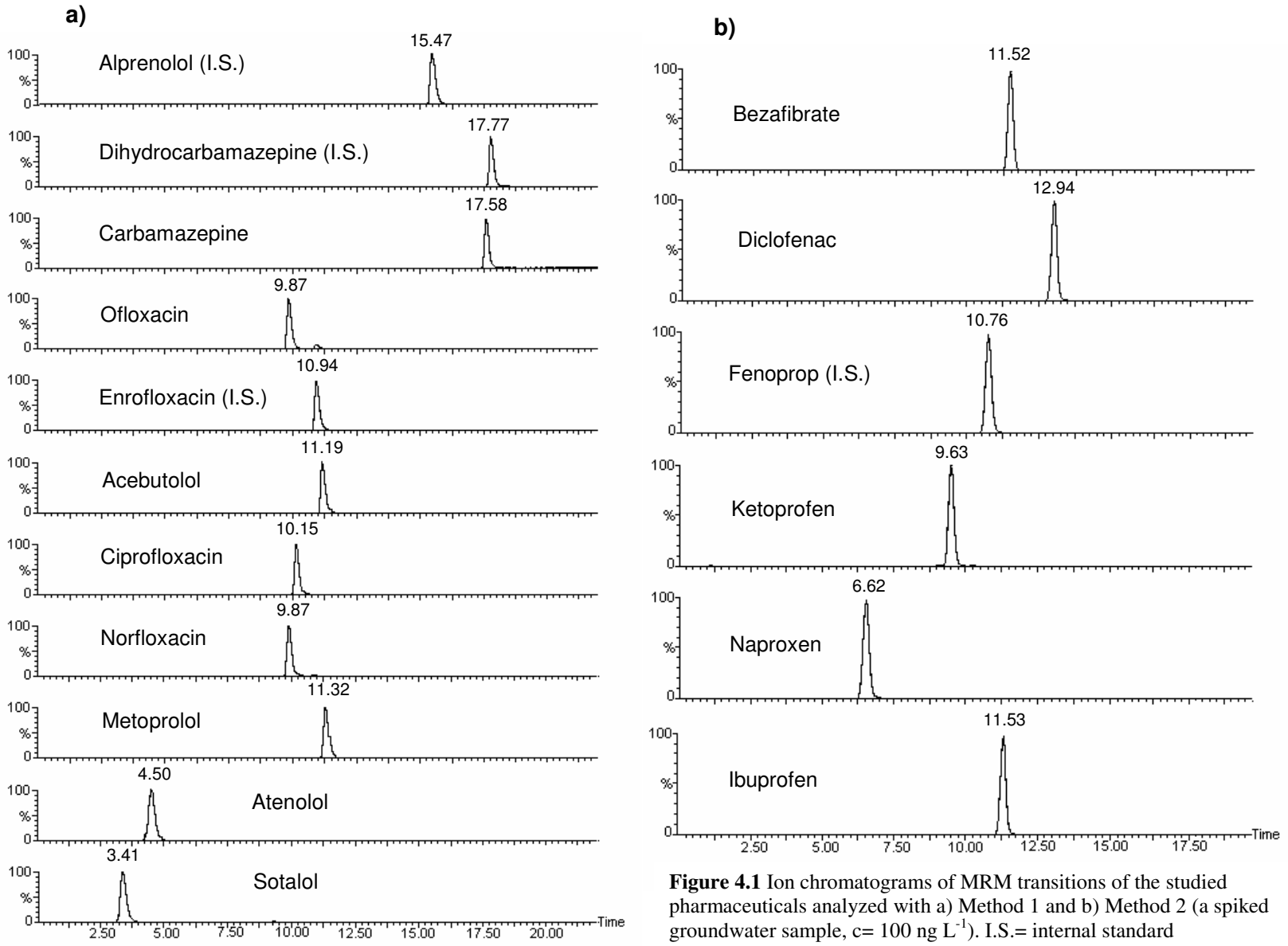


Figure 4.1 Ion chromatograms of MRM transitions of the studied pharmaceuticals analyzed with a) Method 1 and b) Method 2 (a spiked groundwater sample, c= 100 ng L^{-1}). I.S.= internal standard

4.1.2 Matrix effects in LC-MS/MS

ESI followed by triple quadrupole mass spectrometric detection is the most frequently used method to quantitatively determine the presence of pharmaceuticals in environmental samples (see Table 2.6). This technique allows selective and sensitive quantification of pharmaceuticals without the need for derivatization. The major disadvantage relates to the suppression of the analyte signal caused by matrix constituents and consequently, difficulties in analyte quantification (Petrovic et al. 2006, Antignac et al. 2005, Kloepfer et al. 2005, Hernando et al. 2004, Quintana and Reemtsma 2004, Hilton and Thomas 2003). In this study, matrix effects were determined for the compounds analyzed by Method 1 (Paper **I**). Signal suppression was detected in sewage samples for all compounds except the early eluting atenolol and sotalol. Suppression was particularly severe for carbamazepine that eluted at the longest retention time, indicating that the hydrophobic matrix constituents were responsible for the suppression. Late eluting compounds have previously been found to be prone to matrix interferences (Antignac et al. 2005). Hernando et al. (2004) noted suppression of up to 60% for late eluting compounds in sewage influent. They also observed severe ion suppression for compounds eluting at retention times less than 2 minutes in the area where the most polar and unretained compounds elute from the column. Similarly, Hilton and Thomas (2003) found the areas of ion suppression to occur during the first few minutes of the chromatographic run.

4.1.3 Recoveries of the pharmaceuticals

It has been stated that an absolute analytical recovery in sample pre-treatment and analysis should be at least 75% (Snyder et al. 1997). Due to interferences of the matrix components with the analytes in the sample treatment or in the analysis step, pharmaceuticals have typically been recovered at higher rates from ground and drinking waters, than from surface or sewage waters (see Table 4.1). In our study, lower recoveries were noted for the studied pharmaceuticals in sewage compared to drinking water (DW) or surface water (SW) (Table 4.1). In DW and SW, most of the pharmaceuticals were recovered by >75% whereas in sewage, the absolute recoveries were often <75%. For the majority of the compounds, the

internal standards compensated for the losses in sample treatment and analysis. Recoveries relative to the internal standards can be found in Table 4 of **I** and Table 5 of **III**.

Table 4.1 Absolute recoveries (in %) for the studied pharmaceuticals in influent and effluent of sewage treatment plant, drinking water (DW), and surface water (SW) in this thesis and in the literature (Lit.)

	DW		**SW**		**Effluent**		**Influent**		**Ref.**
	Thesis	Lit.*	Thesis	Lit.	Thesis	Lit.	Thesis	Lit.	
Ciprofloxacin	62	(30)	44	(102)	72	(34–97)	32	(61–92)	1–5
Norfloxacin	46	(-)	27	(-)	53	(35–92)	41	(70–91)	2, 4, 5
Ofloxacin	84	(30)	108	(-)	96	(81–85)	76	(72–75)	1, 2, 4, 5
Carbamazepine	105	(65–103)	98	(57–108)	66	(46–95)	66	(91)	6–12
Diclofenac	75	(62–116)	77	(68–80)	64	(68–78)	77	(-)	5, 7, 9, 10–14
Ibuprofen	93	(67–117)	96	(54–110)	46	(83–97)	68	(-)	5, 7, 9, 10–14
Ketoprofen	95	(50–109)	83	(65–104)	69	(77–83)	83	(-)	5, 7, 10–12,14
Naproxen	87	(68–102)	89	(30–105)	81	(90–98)	86	(-)	5, 7, 9,10,12,14
Acebutolol	93	(95)	105	(-)	78	(72)	64	(-)	5, 19
Atenolol	81	(59–98)	90	(49–67)	101	(50– 67)	108	(28–74)	7, 8, 15, 18
Metoprolol	90	(55–98)	104	(43–54)	87	(43–95)	93	(34–104)	5, 7, 8,15,16,18
Sotalol	76	(76–95)	62	(63–81)	94	(52)	66	(18)	7, 8, 15, 19
Bezafibrate	73	(92– 102)	64	(-)	58	(79– 93)	64	(97)	5, 14, 17

* Also includes values reported for distilled or ground water, - = data not found

References: [1] Castiglioni et al. 2005, [2] Lindberg et al. 2004, [3] Reverté et al. 2003, [4] Golet et al. 2001, [5] Andreozzi et al. 2003, [6] Miao and Metcalfe 2003, [7] Sacher et al. 2001, [8] Ternes 2001, [9] Vanderford et al. 2003, [10] Öllers et al. 2001, [11] Santos et al. 2005, [12] Lin et al. 2005, [13] Hilton and Thomas 2003, [14] Löffler and Ternes 2003, [15] Hernando et al. 2004, [16] Ternes et al. 1998, [17] Quintana and Reemtsma 2004, [18] Nikolai et al. 2006, [19] Lee et al. 2007

Pharmaceuticals were recovered at similar yields in this study compared to literature studies (Table 4.1). Of the fluoroquinolones, ofloxacin was efficiently (>75%) recovered from all the different types of water samples. Poor recoveries were, however, observed for ciprofloxacin (32–72%) and norfloxacin (27–53%) in all the sample matrices. The losses could not be compensated by the use of the internal standard enrofloxacin. Previously, Reverté et al. (2003) reported recoveries of 103% for ciprofloxacin using the same SPE sorbent and the same eluents. In contrast to our study, they extracted the samples at pH 2.8 but also concluded that ciprofloxacin could be recovered at high yield at basic pH. Similar

to our study, Andreozzi et al. (2003) found the analysis of fluoroquinolones challenging. They observed high recovery of ofloxacin (85%) and low recoveries of ciprofloxacin and norfloxacin (~35%) in sewage effluent. Interestingly, practically the same analytical method than in Andreozzi et al. (2003) was used in the study of Lindberg et al. (2004) who, in turn, reported recoveries of >60% for all the fluoroquinolones in hospital sewage.

For carbamazepine, higher recoveries have been reported for sewage waters in previous studies (Santos et al. 2005, Miao and Metcalfe 2003). The lower recoveries of carbamazepine in our study were related to matrix interferences in the LC-MS/MS analysis but the loss could be entirely compensated by the internal standard dihydrocarbamazepine.

The beta blockers were recovered to a similar a slightly higher degree in this study than in previously published studies (Hernando et al. 2004, Nikolai et al. 2006, Lee et al. 2007). A drawback of our method was that, for unknown reasons, the internal standard alprenolol could not be detected in the sewage influent samples. Therefore, for these samples, quantification was done using external calibration and the results were corrected with the recoveries. For the other type of water samples alprenolol was used as the internal standard and internal calibration method was used for the quantification. However, due to the high difference in the retention times between the pharmaceuticals and the internal standard and the noted matrix disturbances at longer retention times, a more proper internal standard should be applied in the further analyses of the beta blockers. Other choices could be levobunolol, a beta blocker applied by Nikolai et al. (2006), or the deuterated or isotopically labeled analogues of the compounds.

Anti-inflammatories were mainly recovered at high yields from all the water samples. The internal standard fenoprop compensated for the losses in the sample treatment and analysis for all but ibuprofen in sewage effluent. Anti-inflammatories are among the pharmaceuticals studied the most in the environment and many methods have been developed for their detection (see Table 2.6). They have been recovered by >80% with various sorbents, e.g. Oasis MCX (Stolker et al. 2004, Löffler and Ternes 2003), Oasis HLB (Santos et al. 2005, Vanderford et al. 2003, Öllers et al. 2001) and PPL Bond Elut (Sacher et al. 2001). Bezafibrate was analyzed together with the anti-inflammatories and due to somewhat lower recoveries of the compound compared to the anti-inflammatories, the internal standard fenoprop could not entirely compensate for the recovery losses in the

sample treatment and analysis. In the literature, the recoveries of bezafibrate have been rarely reported. In Quintana and Reemtsma (2004), bezafibrate could be recovered by >90% in sewage samples using Oasis HLB sorbent and an extraction pH of 2. In the study by Löffler and Ternes (2003), SPE concentration similar to ours was applied with a recovery of 102% for bezafibrate in groundwater. Most probably, the differences between the studies arise from the different LC-MS/MS detection methods. In Löffler and Ternes (2003), acidic eluent in the LC and atmospheric pressure chemical ionization prior the MS detection was applied, whereas we used basic eluent and electrospray ionization.

4.2 Pharmaceuticals in the sewage treatment plants

4.2.1 Occurrence of the pharmaceuticals in raw and treated sewages

Fourteen STPs were sampled and altogether 21 paired influent and effluent samples were analyzed for the fluoroquinolones, the beta blockers and carbamazepine, and 13 paired influent and effluent samples for the anti-inflammatories and bezafibrate (Publications **I–IV**). In addition, one sample was collected after a nitrifying biofilter that was applied as a tertiary treatment step at the STP of Helsinki City (Publication **II**). The summary of the concentrations of the pharmaceuticals in the influents and effluents are presented in Table 4.2.

Table 4.2 The occurrence of the studied pharmaceuticals (μg L^{-1}) in Finnish sewage treatment plants (only the liquid phase was analyzed). For the abbreviations of the compounds, refer to Abbreviations and symbols in the beginning of the thesis.

	CIP	**NOR**	**OFL**	**CBZ**	**DCF**	**IBF**	**KET**	**NPX**	**ACE**	**ATE**	**MET**	**SOT**	**BZF**
							Influent						
n	21	21	21	21	13	13	13	13	21	21	21	21	13
n> LOQ	20	20	13	21	13	13	13	13	21	21	21	21	13
Mean	0.60	0.12	0.10	0.35	0.42	16.1	2.06	5.72	0.34	0.80	1.06	0.83	0.97
Median	0.39	0.07	0.07	0.28	0.46	15.2	1.77	4.89	0.27	0.73	1.10	0.72	0.69
Min	<LOQ	<LOQ	<LOQ	0.16	0.23	9.74	1.09	3.57	0.04	0.35	0.46	0.37	0.08
Max	4.23	0.96	0.35	0.82	0.64	28.7	3.46	10.7	1.04	1.71	1.46	3.28	3.20
							Effluent						
n	21	21	21	21	13	13	13	13	21	21	21	21	13
n> LOQ	21	1	21	21	13	12	12	13	21	21	21	21	12
Mean	0.06	<LOQ	0.02	0.72	0.35	0.65	0.37	0.69	0.14	0.33	0.76	0.28	0.24
Median	0.07	<LOQ	0.02	0.50	0.32	0.12	0.32	0.50	0.14	0.29	0.77	0.26	0.15
Min	0.03	<LOQ	0.006	0.29	0.14	<LOQ	<LOQ	0.17	0.03	0.04	0.28	0.13	<LOQ
Max	0.13	0.03	0.11	2.44	0.62	3.91	1.24	1.93	0.26	1.18	1.60	1.12	0.84

n= number of samples analyzed, LOQ= limit of quantification, n> LOQ= number of samples with concentration of the pharmaceutical >LOQ

All pharmaceuticals but the fluoroquinolones were frequently detected in both the influent and the effluent samples. In the influent samples, the mean concentrations of the pharmaceuticals varied from 0.10 µg L^{-1} (norfloxacin) to 16.1 µg L^{-1} (ibuprofen). In the effluent samples, all the mean concentrations were <1 µg L^{-1}. Ibuprofen was the dominating pharmaceutical in the influent samples with a mean concentration of 16.1 µg L^{-1} and a maximum concentration of 28.7 µg L^{-1}. The compound was together with naproxen and metoprolol the major pharmaceutical constituent in the effluent water with mean and maximum concentration of 0.65 and 3.91 µg L^{-1}, respectively.

In general, the concentrations measured in the sewage influents fall into the ranges reported in the literature (compiled in Table 2.8). There is a great variation in concentrations between studies due to different consumption profile of pharmaceuticals in each country. For example, in the year 2005, ibuprofen was consumed in Finland at almost 18 g per person. This was the highest per person consumption figure found for ibuprofen in the literature (see Table 2.4). Therefore, the concentrations of ibuprofen in raw sewages in Finland were generally higher than in other countries. On the contrary, the per person consumption of diclofenac is roughly five times higher in Germany than in Finland and consequently this is reflected in the concentrations of the compound in sewage influents. In Finland, the highest concentration observed for diclofenac in raw sewage was 0.64 µg L^{-1} while in Germany, the compound has been found in concentrations up to 7.1 µg L^{-1} (Heberer 2002b).

4.2.1.1 Calculated versus observed concentrations of the pharmaceuticals

The theoretical concentrations of the pharmaceuticals in sewage influents can be calculated using the information on the consumption and degree of metabolism of the compounds, and the flow rates of the sewage influents. This approach can be applied, for example, by STPs to estimate the concentrations of pharmaceuticals in the influents. They can also be used in the estimation of the quality of the analytical method.

The following equation was used for the calculations:

$$C_{calc} = \frac{A \times P \times e\% \times 10}{365 \times Q} \quad (4.1)$$

Where C_{calc} is the theoretically calculated concentration of a pharmaceutical in the influent of an STP (μg l^{-1}), *A* is the amount of pharmaceuticals used per year per capita (in grams per inhabitant per year), P is the number of inhabitants serviced by the STP and *e%* is the amount of the pharmaceutical excreted unmetabolized by humans (in %) and Q_{inf} is the influent flow rate (m^3 d^{-1}). The highest fraction of the unmetabolized compound excreted by humans (see Table 2.5) was used in the calculations. The calculated and observed concentrations of the pharmaceuticals are presented in Table 4.3.

Table 4.3 The average concentrations of the studied pharmaceuticals with standard deviations (as ng L^{-1}) estimated from the consumption figures ($C_{calculated}$) and the influent concentrations measured in this study ($C_{measured}$).

Compound	***e%****	**$C_{calculated}$**	**$C_{measured}$**
Ciprofloxacin	33%	0.47 ± 0.13	0.60 ± 0.86
Norfloxacin	22%	0.10 ± 0.03	0.12 ± 0.21
Ofloxacin	80%	0.55 ± 0.15	0.10 ± 0.07
Carbamazepine	3%	0.23 ± 0.06	0.35 ± 0.19
Diclofenac	15%	0.28 ± 0.06	0.42 ± 0.12
Ibuprofen	15%	22.5 ± 6.1	16.1 ± 6.3
Ketoprofen	10%	0.24 ± 0.07	2.1 ± 0.7
Naproxen	10%	1.3 ± 0.4	5.7 ± 2.4
Acebutolol	39%	0.58 ± 0.17	0.34 ± 0.25
Atenolol	93%	1.3 ± 0.4	0.80 ± 0.37
Metoprolol	10%	0.9 ± 0.25	1.0 ± 0.4
Sotalol	90%	0.81 ± 0.26	0.83 ± 0.59
Bezafibrate	50%	0.42 ± 0.12	0.97 ± 1.00

*e%= the fraction of the pharmaceuticals excreted unmetabolized by the human body

For majority of the pharmaceuticals, the calculated values were close to the measured ones and thus provide the confidence in the results of the analyses. Ofloxacin was measured at significantly lower concentrations than were predicted by the calculations. This can be due to high fluctuation in human consumption of antibiotics during the year and in different parts of the country. It could also be due to the fact that only the liquid phase of the sewage was analyzed in this study and ofloxacin is known to be excreted partially in feces (Lode et al. 1990). Thus, part of the compound may be associated with the particulate matter of the sewage. Similarly, acebutolol is excreted in feces by about 60% of the consumed dose (Ryan et al. 1985) and the compound was found in the sewage at lower concentrations than was theoretically calculated. On the contrary, some compounds, most notably ketoprofen and naproxen, were measured at significantly higher concentrations than could be theoretically calculated. In the calculations, only the amount of compound that was reported to be excreted unmetabolized was considered. However, 60 and 70% of naproxen and ketoprofen, respectively, are excreted as glucuronides which may be cleaved in the sewer and release the parent compound. All in all, for most of the studied pharmaceuticals, the concentrations in the influents could be fairly well estimated by simple calculations.

4.2.2 Elimination of the pharmaceuticals in the sewage treatment plants

4.2.2.1 Elimination in full scale treatment processes

Lower concentrations of the studied pharmaceuticals were generally observed in the STP effluents compared to the influents. The elimination percentages of the studied pharmaceuticals are compiled in Table 4.4. The eliminations were calculated from the concentrations of the pharmaceuticals in the liquid phases of the influents and effluents.

Table 4.4 The summary of the elimination percentages of the studied pharmaceuticals in Finnish sewage treatment plants (only the liquid phase was considered).

Compound	n	Mean (%)	Median (%)	Range (%)
Ciprofloxacin	21	84	82	71–98
Norfloxacin	21	81	82	51–97
Ofloxacin	21	92	100	-4–100
Carbamazepine	21	-121	-86	-761–(-18)
Diclofenac	13	17	15	-39–60
Ibuprofen	13	95	99	78–100
Ketoprofen	13	82	81	51–100
Naproxen	13	85	91	55–98
Acebutolol	21	47	49	-15–85
Atenolol	21	51	60	-136–97
Metoprolol	21	17	35	-222–77
Sotalol	21	65	66	41–81
Bezafibrate	13	58	61	-11–100

n= number of samples

The fluoroquinolones were efficiently eliminated (>80%) in the studied STPs. High elimination rates have also been reported in the literature (see Table 2.9). The fluoroquinolones are not biodegraded in the STPs (Alexy et al. 2004) but the high elimination rates are due to their ability to sorb to the sludge (Lindberg et al. 2006, Golet et al. 2002b). Even though fluoroquinolones are very hydrophilic compounds with K_{ow} values of 0.41–1.90 they are readily sorbed to sewage sludge via interactions of the positively charged moieties of the fluoroquinolones and the negatively charged cell membranes of the micro-organisms (Ternes et al. 2004b).

No removal of carbamazepine was detected in any of the studied STPs. In fact, the concentration of carbamazepine was, on average, twice as high in the effluent as in the influent samples. In previous studies, the elimination of carbamazepine has generally been low due to the negligible biodegradation in the secondary treatment (Joss et al. 2006) and the low sorption affinity of the compound to both the primary and the activated sludge (Löffler et al. 2005). Some studies have reported elimination rates of >50% (Nakada et al.

2006, Paxéus 2004) but in general, carbamazepine has been eliminated by <10% in STPs (Castiglioni et al. 2006, Joss et al. 2005, Paxéus 2004, Ternes 1998). Similar to our study, the increase of carbamazepine concentration during the sewage treatment has been reported in the studies of Nakada et al. 2006, and Clara et al. 2005a. One explanation suggested is that if the hydraulic retention time is not considered during the sampling, the influent and effluent samples are not directly comparable (Clara et al. 2005a). However, the more plausible explanation would be the cleavage of the glucuronic moiety from carbamazepine-*N*-glucuronide (CBZ-Glu) (identified by Maggs et al. 1997) and the release of the parent compound during the secondary treatment. Activated sludge has been found to have glucuronidase activity and thus the cleavage of the glucuronic acid moiety is possible in STPs (Ternes et al. 1999). In our study, we could detect CBZ-Glu in the influent samples but during the treatment process, the amount had diminished considerably (Paper **II**). It was not possible to quantify CBZ-Glu due to the lack of standard compound. This observation does, however, support the theory that glucuronic acid moiety could be cleaved during the process and the unconjugated carbamazepine could be released resulting in the increased concentrations noted in the effluent samples.

Ibuprofen, ketoprofen and naproxen were the pharmaceuticals that were eliminated to the highest degree in the STPs (in average of 82–95%). High elimination rates have also been observed in other studies (see Table 2.9). The elimination has been found to occur mainly via biodegradation (Joss et al. 2006, Yu et al. 2006, Quintana et al. 2005, Zwiener and Frimmel 2003, Buser et al. 1999). In contrast to high elimination rates of the other anti-inflammatories, diclofenac was eliminated on average of only 17% in the Finnish STPs. Poor elimination of the compound in STPs has been reported in several studies (Yu et al. 2006, Bendz et al. 2005, Clara et al. 2005a, Paxéus 2004, Heberer 2002b, Buser et al. 1998) but elimination rates of >50% have also been observed (Gómez et al. 2006, Clara et al. 2005a, Paxéus 2004, Buser et al. 1998, Ternes 1998). Low elimination of diclofenac has been suggested to be due to a low rate of biodegradation of the compound in STPs (Joss et al. 2006). Diclofenac has been noted to sorb to the sewage sludge, but this has been considered to be a minor elimination route for the compound in STPs (Löffler et al. 2005, Ternes et al. 2005).

In the studied STPs, acebutolol, atenolol and sotalol were moderately eliminated (in average of 47–65%), whereas metoprolol was poorly eliminated (on average of 17%). There are few studies that have reported the elimination of these beta blockers in STPs. Acebutolol has previously been eliminated on average of 19% in Canadian STPs (Lee et al. 2007). Bendz et al. (2005) and Paxéus (2004) concluded that atenolol is not eliminated in sewage treatment whereas Maurer et al. (2007) reported elimination rate of 76% for the compound in Swiss STPs and Lee et al. (2007) on average elimination rate of 40% in Canadian STPs. Castiglioni et al. (2006) reported elimination rates of 0–21% for atenolol in the wintertime while in the summertime, the elimination rates of the compound were 36–78%. The authors suggested that this was due to increased biodegradation of atenolol due to enhanced microbial activity in the STPs during the summer. This is supported by the low degree of sorption of atenolol to sewage sludge and by the reported biodegradability of the compound in STPs (Maurer et al. 2007). Sotalol has been found to be eliminated by 15–27% in STPs (Lee et al. 2007, Maurer et al. 2007) mainly by biodegradation (Maurer et al. 2007). In accordance with the results presented in this thesis, Bendz et al. (2005), Paxéus (2004), Lee et al. (2007), and Maurer et al. (2007) reported ≤30 % elimination of metoprolol in STPs. On the contrary, Ternes (1998) reported that metoprolol is eliminated on average of 83% during sewage treatment.

The elimination of bezafibrate was on average of 58%, which is slightly lower than in some of the previously published studies (Clara et al. 2005a, Ternes 1998). Even though sorption of bezafibrate to sludge has not been evaluated, biodegradation is the most likely elimination mechanism in STPs because in laboratory studies the compound has been found to undergo biodegradation (Joss et al. 2006, Quintana et al. 2005).

4.2.2.2 Elimination in nitrifying biofilter

Tertiary treatment in STPs aims to increase the elimination of organic matter, suspended solids or nutrients. Efficient tertiary treatment is needed at least in the cases where the effluent is infiltrated to the ground instead of discharged to surface waters (Clara et al. 2004). In this study, we collected a sample from the STP of Helsinki City after the nitrifying biofilter that was used as tertiary treatment to increase nitrogen removal (Paper **II**). No

significant elimination of any of the studied pharmaceuticals occurred in this filter. However, it does not exclude the possibility that some pharmaceuticals could be eliminated in the biofilter. In the literature, other tertiary treatments have been studied, for example ozonation, chlorination, UV-treatment and activated carbon adsorption (Nowotny et al. 2007, Huber et al. 2005a, Miao et al. 2005, Pinkston and Sedlak 2004, Ternes et al. 2003). Of these, ozonation and activated carbon adsorption have been suggested to be the most viable tools to reduce the pharmaceutical load into the environment (Nowotny et al. 2007, Huber et al. 2005a, Ternes et al. 2003).

4.2.3 Factors affecting the elimination of the pharmaceuticals in the STPs

Two factors, low sewage temperature and dilution of the raw sewage by rain water were found to affect the elimination of the studied pharmaceuticals in STPs. The effect of the sewage temperature was noted when seasonal variation of the occurrence of the anti-inflammatories and bezafibrate was studied in the STP of Aura Municipality (Paper **IV**). In the influent, the highest concentrations of the pharmaceuticals were measured in the samples collected in the spring and the summer, whereas the samples collected in the autumn and the winter contained a lower concentration of these compounds. In the effluents, the concentrations of the compounds were highest in the winter. This was due to lower elimination of the pharmaceuticals during the winter compared to the other seasons. The compounds were eliminated on average of 61% in the winter (water temperature 7 °C) and 84–87% in other seasons (water temperature 13–21°C). A similar trend was observed by Castiglioni et al. (2006) in Italian STPs where several pharmaceuticals were found to be more efficiently eliminated in the summer (average water temperature 18.6 C) than in the winter (average water temperature 9.7 C). In both cases, low elimination of the pharmaceuticals in the winter was most probably due to the decreased biodegradation of the compounds due to the lower microbial activity at lower water temperatures. In Finnish STPs, sewage temperature can be near 0 °C in the winter (Rantanen et al. 2003), and consequently the elimination of the pharmaceuticals in the STPs can be seriously hampered during the winter months.

Dilution of the raw sewage by rain water was the other factor that affected the elimination of the pharmaceuticals in sewage treatment (Paper **II**). This was observed as a dramatic reduction in the elimination rates of the beta blockers in one of the studied STPs during an event of heavy rain. The dilution of the raw sewage had no effect on the elimination of the fluoroquinolones and carbamazepine. Similar trend has previously been reported by Ternes (1998) for a German STP, where the elimination of several pharmaceuticals was seriously reduced when the rain water entered the plant. Accordingly, in the study by Tauxe-Wuersch et al. (2005), ibuprofen and ketoprofen were not eliminated in a STP during the occasion of heavy rain. In all these cases, the lower elimination rates of the compounds could have been due to a lower biodegradation rate of the pharmaceuticals in the diluted sewage, since the biodegradation rate of many pharmaceuticals has been noted to be directly proportional to the compound concentration (Joss et al. 2006). Shorter HRTs in STPs due to increased sewage volume could have also reduced the elimination rates of the pharmaceuticals due to the decreased contact time between the pharmaceuticals and the micro-organisms (Tauxe-Wuersch et al. 2005). In our study, this is a plausible explanation to the decreased elimination rates since the HRT in the studied STP was reduced to a half (from 20 h to 10 h) during the rain. During the normal operation, HRT in the studied STPs was not found to affect the elimination rates of the beta blockers while Maurer et al. (2007) concluded that atenolol, metoprolol and sotalol could be more efficiently eliminated at longer HRT. In addition, in Tauxe-Wuersch et al. (2005), ibuprofen and ketoprofen were better eliminated in STPs operating at longer HRT.

Other factors that have previously been found to influence on the elimination rates of the pharmaceuticals in STPs are the SRT and the configuration of the treatment plant. The higher elimination of the pharmaceuticals at higher SRT has been suggested to occur due to enrichment of certain microbial communities that excrete enzymes that able to break down pharmaceuticals (Ternes et al. 2004b). Previously, an SRT of at least 10 d has been suggested as necessary to efficiently eliminate the biodegradable pharmaceuticals, e.g. ibuprofen and bezafibrate (Clara et al. 2005b, Kreuzinger et al. 2004). In our study, there was no significant correlation of the elimination rates of the beta blockers, the fluoroquinolones, and carbamazepine with the SRTs applied in the STPs.

The degradation of pharmaceuticals may occur under aerobic, anaerobic or anoxic conditions. Therefore, the plant configuration and especially the type of the biological treatment can significantly affect the elimination of the pharmaceuticals. The plants applying nitrification/denitrification have been suggested to achieve higher elimination of pharmaceuticals (Clara et al. 2005b). This is presumably due to the higher SRTs normally applied in the N/DN processes and due to the differences in redox conditions. Kanda et al. 2003 reported that the elimination of ibuprofen was higher in oxidation ditches or in activated sludge treatment, compared to filter beds, due to higher SRTs applied in the previous processes. In our study, the type of the biological treatment (activated sludge, oxidation ditch or N/DN) was not found to significantly affect the elimination of the studied pharmaceuticals.

4.3 Pharmaceuticals in the rivers

4.3.1 Occurrence of the pharmaceuticals in the rivers

Six rivers were sampled and analyzed for the studied pharmaceuticals (Papers **I, III, IV**). Rivers Kyrö and Aura were raw water sources for the drinking water treatment plants of Vaasa and Turku Cities, respectively. River Vantaa is a reserve water source for the drinking water treatment plant of Helsinki City. During normal operation of the plant, raw water is pumped from Lake Päijänne, which was not found to contain the studied pharmaceuticals (unpublished data). From the rivers, samples were collected upstream the STPs, at the discharge point of sewage effluents, and downstream of the STPs. The results were averaged and compiled in Table 4.5. In the table, the average and the maximum concentrations of the pharmaceuticals measured in all the river water samples is also presented.

Table 4.5 Average concentrations of the studied pharmaceuticals measured in the rivers (ng L^{-1}) upstream of the STPs (US), at the discharge point (DP) of the STP, and downstream of the STPs (DS) (the distance to the STP is reported in parenthesis). The average and maximum concentrations measured in all the river water samples is also presented. For abbreviations of the compounds, refer to Abbreviations and symbols in the beginning of the thesis.

	n	CIP	NOR	OFL	CBZ	ACE	ATE	MET	SOT	DCF	IBP	KET	NPX	BZF
River Aura														
US	4	-	-	-	-	-	-	-	-	4	23	7	29	8
DP (STP A)	4	nd*	nd*	nd *	nd *	nd *	nd *	102*	32*	26	25	13	46	15
DS (1 km)	3	-	-	-	-	-	-	-	-	9	32	11	39	14
DS (5 km)	3	-	-	-	-	-	-	-	-	6	32	8	33	17
DS (8 km)	3	-	-	-	-	-	-	-	-	5	23	8	23	9
DS (14 km)	3	-	-	-	-	-	-	-	-	4	34	9	27	9
DS (23 km)	3	-	-	-	-	-	-	-	-	2	17	5	11	7
DS (32 km)	23	nd*	nd*	nd *	nd*	nd*	nd*	nd*	nd*	2	16	7	11	4
River Kokemäenjoki														
US	1	-	-	-	-	-	-	-	-	0	1	nd	nd	2
DP (STP B)	1	-	-	-	-	-	-	-	-	11	64	26	57	23
DS (10 km)	1	-	-	-	-	-	-	-	-	1	2	nd	nd	1
River Seinäjoki [1)] and Kyrö														
US	1	-	-	-	-	-	-	-	-	nd	6	nd	nd	nd
DP (STP C)	1	-	-	-	-	-	-	-	-	39	11	39	56	6
DS (2 km)	1	-	-	-	-	-	-	-	-	35	14	23	45	4
DS (90 km)	1	-	-	-	-	-	-	-	-	2	2	nd	6	nd

Table 4.5 continues

River Vantaa [2)]														
US	1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
DS (STP D, 8 km)	1	nd	nd	nd	66	8	25	116	52	65	54	68	110	75
DS (STP E, 5 km)	1	nd	nd	nd	26	4	19	35	19	23	16	30	45	31
DS (STP E,60 km)	1	nd	nd	nd	20	2	12	20	15	16	24	23	30	20
DS (Helsinki[a]) [3)]	6	17	nd	nd	51	8	33	67	59	31	35	18	23	11
River Luhtajoki														
DS (STP F, 4 km)	1	25	nd	nd	23	8	nd	38	37	27	37	29	6	9
All the river water samples														
Average	64[4)]	nd	nd	nd	36	5	22	49	39	10	22	11	23	9
Maximum	64[4)]	36	nd	5	80	14	56	116	86	65	90	68	129	75

STPs: A= Aura, B= Harjavalta, C= Seinäjoki, D= Riihimäki, E= Hyvinkää (Kalteva), F= Nurmijärvi (Klaukkala)

n= number of samples, * n= 1, nd= not detected (i.e., <LOQ), - = not analyzed

[a] Site: the drinking water treatment plant of Helsinki (about 60 km downstream of STP E)

[1)] River Seinäjoki is a tributary of River Kyrö, [2)] Sampling sites and STPs are presented in Figure 2 of Paper **I**, [3)] Note that the samples were not collected at the same time as the other samples of River Vantaa, [4)] n= 12 for the fluoroquinolones, carbamazepine and the beta blockers

Concentrations of the studied pharmaceuticals in the upstream samples were generally <LOQ. In a few cases, pharmaceuticals were detected in these samples, most probably due to discharges from STPs further upstream. In the rivers, the concentrations of the pharmaceuticals increased at the sites of effluent discharges and decreased downstream the STPs. Concentrations of the pharmaceuticals in the effluent discharge points resembled the concentrations in the corresponding effluent samples. Thus, the STPs were assumed to be the main source of the studied compounds in the rivers.

The average concentrations of the pharmaceuticals in the rivers ranged from <LOQ for the fluoroquinolones to nearly 50 ng L^{-1} for metoprolol. The highest concentration of 128 ng L^{-1} was measured for naproxen in the River Aura at the discharge point of sewage effluent. Significant seasonal variation of concentrations was observed for the pharmaceuticals. Typically, the compounds were detected at higher concentrations in the winter than in other seasons. However, ibuprofen and carbamazepine were detected at more similar concentrations throughout the year. During the samplings, dilution of the effluent with the river water was higher in the winter than in the summer, and could not explain the higher winter concentrations. Higher concentrations observed in the winter were probably due to higher loads of the pharmaceuticals from the STPs due to decreased elimination rates at low water temperature (discussed in Chapter 4.2.3), and a lower rate of elimination of the pharmaceuticals in the rivers (discussed in Chapter 4.3.2).

In general, the concentrations of the pharmaceuticals in the studied rivers were low compared to the concentrations reported in the literature (see Table 2.10). For example, in Germany, USA, and UK, concentrations of several µg L^{-1} have been measured for many pharmaceuticals (Bound and Voulvoulis 2006, Loraine and Pettigrove 2006, Wiegel et al. 2004, Kolpin et al. 2002, Ternes 1998). However, in most studies, concentrations of pharmaceuticals range from a few to 200 ng L^{-1}, i.e. in the same range as in this study. It should be emphasized that the concentrations of the pharmaceuticals reported here cannot be applied to all surface waters in Finland. Samples were collected from rivers that were known to receive sewage effluents. In general, it is highly unlikely that pharmaceuticals would be detected in lakes where the volume of water is larger and thus dilution more extensive. This is supported by the absence of the studied pharmaceuticals in Lake Päijänne, the second largest lake in Finland (unpublished results).

4.3.2 Elimination of the pharmaceuticals in the rivers

In surface waters, pharmaceuticals can be sorbed and accumulated to sediments and/or undergo transformation processes, mainly biodegradation and phototransformation. In the River Aura, the elimination of the anti-inflammatories and bezafibrate was studied by investigating the attenuation of the compounds in the river at different times throughout the year. The relative concentrations of the studied pharmaceuticals along the River Aura during each sampling month (March, May and August) are presented in Figure 4 of Paper **IV**. The concentrations of all the pharmaceuticals were attenuated in the river but to a varying degree. The lowest degree of attenuation was found for ibuprofen, whereas diclofenac was attenuated almost entirely by the sampling point located 32 km downstream of the discharge point. In this sample, ibuprofen was noted to be the most and diclofenac the least abundant of the studied pharmaceuticals (Figure 4.2).

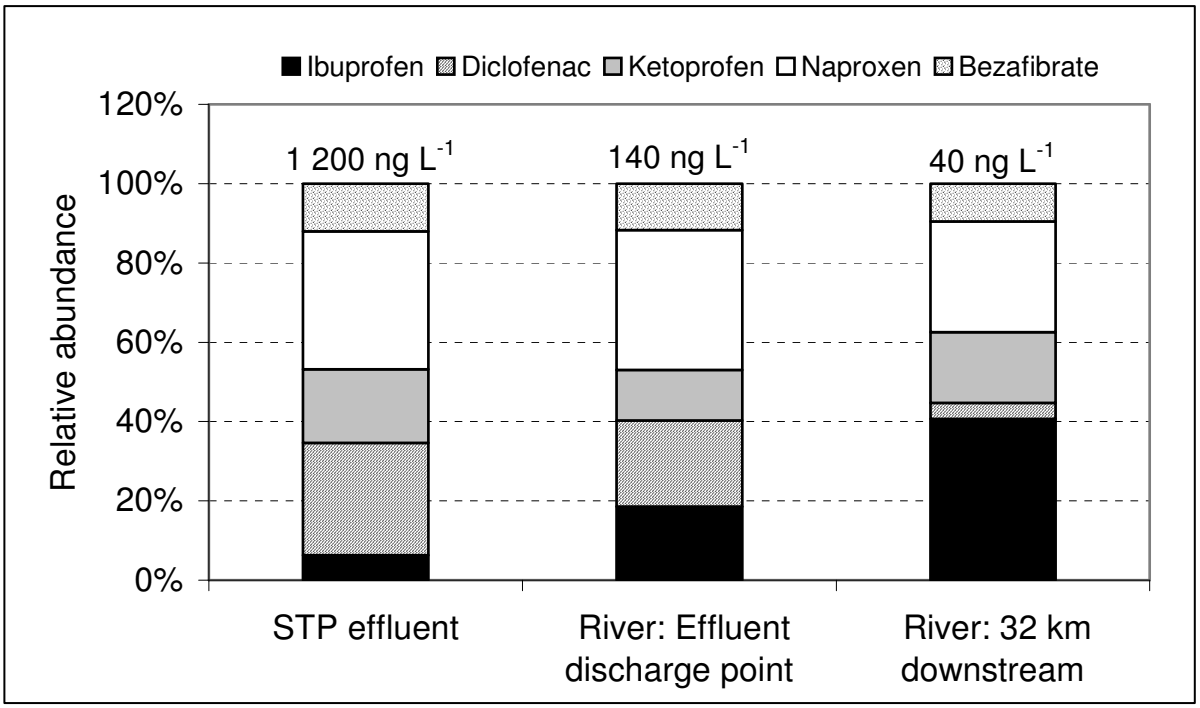


Figure 4.2 The relative abundances of the pharmaceuticals in the samples collected from the effluent of the Aura STP, and in the River Aura at the effluent discharge point and 32 km downstream (average from all the sampling months). Also, the total concentration of the compounds in the collected samples is presented above the columns.

The type of the water system, such as the depth and the residence time of water, plays a major role in the natural attenuation of pharmaceuticals. Biodegradation is assumed to be important in deep rivers and lakes with high water residence time whereas phototransformation is thought to be the primary elimination method in waters with low water residence time, such as shallow rivers (Fono et al. 2006, Lin et al. 2006). For example, in a shallow river ibuprofen has been found to be more persistent than naproxen whereas in a deep river naproxen was the more persistent of the two compounds (Fono et al. 2006, Lin et al. 2006). This was due to the fact that ibuprofen seems to be more rapidly biodegraded in natural waters whereas naproxen undergoes more efficient phototransformation (Fono et al. 2006, Lin et al. 2006, Lin and Reinhard 2005, Löffler et al. 2005, Winkler et al. 2001, Buser et al. 1999). River Aura is a relatively shallow river with water residence time ranging from less than a day to several days. The observed attenuation of the studied pharmaceuticals in the river during different seasons indicates that both biodegradation and phototransformation of the compounds may occur but the phototransformation seems to be the more likely elimination mechanism. Diclofenac and ketoprofen, that have previously been found to undergo rapid phototransformation under environmentally relevant conditions (Lin and Reinhard 2005, Andreozzi et al. 2003, Buser et al. 1998), were attenuated faster in May and August, compared to March. This indicates rapid phototransformation of the compounds in the river during the spring and summer. Ibuprofen was found to be more persistent in the river than the other pharmaceuticals most probably due to its inability to be phototransformed in environmentally relevant conditions (Lin and Reinhard 2005). The similar degree of attenuation for the compound in different sampling months also suggests that effective biodegradation does not occur. Bezafibrate exhibited higher degree of attenuation in the summer suggesting phototransformation and biodegradation to be viable fate processes. The latter mechanism is more likely because bezafibrate does not undergo rapid phototransformation (Cermola et al. 2005). Naproxen has been reported to undergo phototransformation but at about 100 times lower rate than ketoprofen (Lin et al. 2006, Lin and Reinhard 2005). The compound has also been shown to be biodegradable in river water (Fono et al. 2006). The similar attenuation rates observed for the compound at different seasons suggest that neither of these attenuation mechanisms is particularly efficient in the River Aura. However, higher attenuation of naproxen compared to ibuprofen suggests that

naproxen could be sorbed to the sediments. This is supported by the studies where naproxen has been found in river sediments downstream STPs (Antonić and Heath 2007, Rice and Mitra 2007). Ibuprofen has also been found in river sediments (Rice and Mitra 2007) but the affinity for the compounds for sorption to the sediment has been shown to be relatively low (Löffler et al. 2005).

The attenuation of the fluoroquinolones, carbamazepine and the beta blockers was roughly estimated from the concentrations measured in River Vantaa (Paper **VI**). Ciprofloxacin and ofloxacin were rarely detected in the river water due to their low concentrations in the STP effluents and further dilution in the river. For the rest of the compounds, the lowest degree of attenuation was observed for carbamazepine, atenolol and sotalol, whereas metoprolol and acebutolol were attenuated by >60% in the river. Previously, atenolol has been suggested to have a lower persistency in the environment than metoprolol (Bendz et al. 2005). It has also been found to sorb to river sediments (Castiglioni et al. 2006). Metoprolol can undergo both biodegradation and phototransformation with the emphasis on the former at least in a deep river with high water residence time (Fono et al. 2006). The persistence of carbamazepine in the River Vantaa suggests that it does not undergo significant transformation or degradation processes. This is in accordance with the literature, where carbamazepine has been found to undergo only limited phototransformation (Andreozzi et al. 2003, Doll and Frimmel 2003) and has been found to be one of the most persistent pharmaceuticals in the aquatic environment (Löffler et al. 2005, Tixier et al. 2003).

4.3.3 Risk to the aquatic environment

Pharmaceuticals are designed to have biological effects in humans, and in the aquatic environment they may affect organisms having identical or similar target organs, cells or biomolecules (Fent et al. 2006). Using the approach presented in Paper **III**, the risk to the aquatic organisms posed by the studied pharmaceuticals was estimated by dividing the measured environmental concentration (MEC) by the predicted no effect concentrations (PNECs) or with the lowest observed effect concentrations (LOECs) (Table 4.6).

Table 4.6 For the studied pharmaceuticals, the predicted no-effect concentrations (PNEC, ng L^{-1}), the lowest observed effect concentrations (LOEC, ng L^{-1}), the measured environmental concentrations (MEC, maximum in this study, ng L^{-1}), and the risk quotients (MEC divided by the lowest PNEC or LOEC).

Compound	PNEC	LOEC	MEC	Risk quotient
Ciprofloxacin	3 000 [1)] *	-	36	0.01
Ofloxacin	530 [2)] *	-	5	0.01
Carbamazepine	420 [3)]	10 [4)] **	80	8[§]
Diclofenac	116 000 [3)]	1 000 [5, 11)]	65	0.07[§]
Ibuprofen	5 000 [6)]	10 [4)] **	90	9[§]
Ketoprofen	306 000 [7)]	-	68	0.0002
Naproxen	560 [8)]	-	129	0.2
Acebutolol	-	-	14	-
Atenolol	310 000 [9)]	-	58	0.0002
Metoprolol	7 900 [9)]	1 000 [11)]	116	0.12[§]
Sotalol	300 000 [10)] *	-	92	0.0003
Bezafibrate	200 000 [10)] *	-	75	0.0004

References: [1)] Golet et al. 2002, [2)] Isidori et al. 2005a, [3)] Ferrari et al. 2003, [4)] De Lange et al. 2006, [5)] Reviewed in Fent et al. 2006, [6)] Halling-Sørensen et al. 1998, [7)] Estimated with ECOSAR (US EPA 2006), [8)] Isidori et al. 2005b, [9)] Cleuvers 2005, [10)] Hernando et al. 2004, [11)] Triebskorn et al. (2007)
* lowest LC50 or EC50 with assessment factor of 1000, ** behavioral end point,
[§] MEC/LOEC

The PNEC values for the pharmaceuticals have mainly been estimated from acute or chronic studies that have used mortality, growth or reproduction as endpoints. By calculating MEC/PNEC, the risk quotient was <1 for all the studied pharmaceuticals, indicating no risk to the aquatic organisms (TGD 1996). Recently, De Lange et al. (2006) published a paper where the LOEC values for carbamazepine and ibuprofen were assessed using behavioral effects, such as locomotion, as endpoints of the ecotoxic experiments. When considering these values and the MECs reported in this study, risk quotients exceeded one for both carbamazepine and ibuprofen. Thus, there is a possibility for adverse effects for aquatic organisms already at these low ng L^{-1} concentrations of pharmaceuticals. Recently, it has been pointed out that the environmental risk assessment of pharmaceuticals should be focused on studies that find out the chronic toxicity and the subtle effects that

pharmaceuticals and their mixtures cause in the environment (Triebskorn et al. 2007, De Lange et al. 2006, Escher et al. 2006, Fent et al. 2006). It is known that certain pharmaceuticals (e.g. beta blockers) that exhibit the same mode of toxic action in organisms can have additive effects as a mixture (Escher et al. 2006, Cleuvers 2005). A concern is that the mixtures of pharmaceuticals could cause synergistic effects on the aquatic organisms (Flaherty and Dodson 2005). There is also a concern for development and spread of antibiotic resistant bacteria in the environment. Among the different classes of antibiotics, *E. coli* isolated from sewage and sewage sludge showed the least resistance for the fluoroquinolones (Reinthaler et al. 2003). In addition, bacteria isolated from fish organs showed only minor resistance for ciprofloxacin (Pathak and Gopal 2005).

4.4 Occurrence and elimination of the pharmaceuticals in drinking water treatment

4.4.1 Occurrence of the pharmaceuticals in drinking waters

Drinking water samples were collected from cities of Turku and Vaasa. In general, the concentrations were <LOQ in the samples collected from the drinking water treatment plants or from the tap. Occasionally, some pharmaceuticals were observed at low concentrations in the samples. Carbamazepine and metoprolol were once detected in the drinking water of Vaasa (c= 5 and 8 ng L^{-1}, respectively, unpublished results), and ibuprofen and ketoprofen in the drinking water of Turku (c= 8 ng L^{-1}, Paper **IV**). Previously, low concentrations of pharmaceuticals have been detected in some ground and drinking waters in other countries (see Table 2.12). In some cases, the concentrations have exceeded 1 µg L^{-1}, but generally the concentrations are in the range of low ng L^{-1}.

4.4.2 Elimination of the pharmaceuticals in drinking water treatment processes

Elimination of the pharmaceuticals in drinking water treatment processes was studied in the laboratory (coagulation/flocculation, Paper **V**), in a pilot plant (coagulation, sand filtration, ozonation, GAC filtration, UV-disinfection, Paper **VI**), and in a full-scale treatment plant

(coagulation, GAC filtration, chlorination, Paper **IV**). Next, the ability of the different unit operations to eliminate the studied pharmaceuticals is discussed separately.

4.4.2.1 Elimination in coagulation and floc separation

In the laboratory, the elimination of sulfamethoxazole, carbamazepine, diclofenac, ibuprofen and bezafibrate by coagulation was studied in Milli-Q water, lake water, and model DHA waters. All waters were spiked with pharmaceuticals, and coagulated with aluminum and ferric sulfate at pH 6.0 and 4.5, respectively. These were the optimum doses for the removal of NOM. The results are presented in Figures 3–5 of Paper **V**. In Milli-Q and lake water, diclofenac was eliminated by roughly 60 and 30%, respectively, by ferric coagulation and by <10% with aluminum sulfate. The elimination of the other compounds was insignificant from these waters regardless of the coagulant or the coagulant dose. In the coagulation of the synthetic model waters containing 5, 15 and 30 mg L^{-1} of DHA (measured as DOC) none of the pharmaceuticals were eliminated by aluminum sulfate coagulation. On the contrary, the acidic pharmaceuticals diclofenac, ibuprofen and bezafibrate could be eliminated to some degree with ferric coagulation. Elimination rates of 77, 50 and 36% were measured with a DHA dose of 30 mg L^{-1} and a ferric sulfate dose of 350 µmoL Fe^{3+} L^{-1}.

In the pilot plant, raw water collected from the River Vantaa was coagulated with ferric sulfate at pH 5. Pharmaceuticals were not spiked in the raw water, but they occurred in the river water at low ng L^{-1} levels due to effluent discharges from upstream STPs (see Chapter 4.3.1). Coagulation and the subsequent flocculation and sedimentation did not result in significant elimination of the pharmaceuticals. In the subsequent sand filtration, only on average of 8% of the compounds was eliminated.

In the full-scale treatment plant of Turku City, raw water was pumped from the River Aura which received effluents from upstream STPs. Ibuprofen, ketoprofen and naproxen were measured at concentrations >LOQ in the raw water. Two-step coagulation was applied in the treatment plant and the second coagulation involved an addition of chlorine dioxide. The two-stage coagulation decreased the naproxen concentration to <LOQ (5 ng L^{-1}) but was inefficient in removing ibuprofen and ketoprofen.

In accordance with the previous studies, the results obtained here from the laboratory, pilot plant and full-scale treatment plant, confirmed that chemical coagulation at

the conditions normally applied in the drinking water treatment plants, is not a viable tool for elimination of pharmaceuticals from natural waters (Stackelberg et al. 2007, Hua et al. 2006, Kim et al. 2007, Westerhoff et al. 2005, Stackelberg et al. 2004, Boyd et al. 2003, Adams et al. 2002, Ternes et al. 2002). In the coagulation of DHA model waters in the laboratory, some of the compounds could be fairly efficiently eliminated using ferric salt as the coagulant. The conditions in the experiment were, however, not relevant for the treatment plants. Elimination of naproxen in the coagulation of the full-scale treatment plant could have been due to oxidation by ClO_2 that was added in the second coagulation step. ClO_2 has previously been shown to efficiently oxidize naproxen (Pinkston and Sedlak 2004). Ibuprofen and ketoprofen are not readily oxidized by ClO_2 (Huber et al. 2005b, Pinkston and Sedlak 2004) which could explain there persistence in the coagulation step. The poor elimination of the pharmaceuticals in the pilot scale sand filtration was most probably due to too low hydraulic retention time in the filter for sorption or biodegradation of the compounds.

4.4.2.2 Elimination in ozonation

The elimination of all the studied pharmaceuticals was investigated in a pilot plant ozonation system using ozone dose that is typically applied in drinking water treatment (about 1 $mgO_3\ L^{-1}$, corresponding to 0.2–0.4 mgO_3/mgTOC). Ozone was added to the water after the coagulation and the sand filtration. Our results are in accordance with the previous studies where ozonation has been concluded to be one of the most viable techniques to reduce the concentrations of pharmaceuticals from water (Dodd et al. 2006, Hua et al. 2006, Poseidon 2006, Snyder et al. 2006, Huber et al. 2003 and 2005a, Westerhoff et al. 2005, Vogna et al. 2004, Andreozzi et al. 2002, Ternes et al. 2002, Zwiener and Frimmel 2000). Significant reductions in the concentrations of the studied pharmaceuticals were detected after the pilot scale ozonation. The concentrations of carbamazepine, diclofenac, ketoprofen, acebutolol, atenolol, and metoprolol were reduced to <LOQ in every experiment. Only residues of ibuprofen, naproxen, sotalol and bezafibrate were occasionally detected after the ozone treatment. However, the concentrations were significantly reduced during ozonation and, on average, the elimination of the compounds were 92, 75, >96% and >77%,

respectively. Ciprofloxacin was detected twice in the sand filtered water and its concentration was not significantly reduced in the ozonation.

Our results are consistent with the previous studies where carbamazepine, diclofenac and the beta blockers have been reported to be efficiently oxidized at ozone doses of 0.2–0.4 mgO_3/mgTOC (Snyder et al. 2006, Westerhoff et al. 2005, Huber et al. 2003, Ternes et al. 2003, Ternes et al. 2002). All the compounds contain amine functionalities or C=C double bonds in their structures which renders them highly reactive with ozone (von Gunten 2003). To efficiently oxidize bezafibrate, slightly higher ozone doses (i.e. >0.4 mgO_3/mgTOC) has been shown to be needed than was applied in the pilot plant (Huber et al. 2003, Ternes et al. 2002). The incomplete elimination of naproxen occurred at the pilot plant at ozone doses of 0.2–0.3 mgO_3/mgTOC but the compound was eliminated to <LOQ at the dose of 0.4 mgO_3/mgTOC. Previously, naproxen has been reported to be readily oxidized at ozone doses of ≥0.4 mgO_3/mgTOC (Snyder et al. 2006, Westerhoff et al. 2005). Ibuprofen has previously been oxidized by 50–77% in natural waters with ozone doses similar to our study (Snyder et al. 2006, Huber et al. 2003). Incomplete elimination of ibuprofen in ozonation can be explained by its low apparent rate constant with ozone (Huber et al. 2003). This is caused by the –COOH functional group in the structure of ibuprofen which inactivates the aromatic ring towards the attack of ozone (von Gunten 2003). However, decomposition of ozone in water leads to formation of hydroxyl radicals that are very strong and nonselective oxidants (von Gunten 2003). Ibuprofen has been found to be oxidized mainly by these radicals (Huber et al. 2003). Since the formation of •OH is enhanced by the TOC, ibuprofen has been found to be oxidized by higher degree in natural water than in distilled water (Huber et al. 2003). The elimination of ibuprofen can also be enhanced by the addition of hydrogen peroxide that accelerates the ozone decomposition to hydroxyl radicals (Huber et al. 2003, Zwiener and Frimmel 2000).

The reason for the incomplete elimination of ciprofloxacin in ozonation is not fully understood. Previously, ciprofloxacin has been oxidized by 40–65% with ozone doses of 0.2–0.3 mgO_3/mgTOC (Dodd et al. 2006). The lack of oxidation could be caused by several factors, e.g. competition between NOM and ciprofloxacin for the ozone, scavenging of OH radicals by NOM or due to decreased oxidation of the compound at the pH of the experiments. The kinetic constant for the reaction of ciprofloxacin and ozone is highly pH

dependant (Dodd et al. 2006). At low or neutral pH, the ozone rate constants of ciprofloxacin are lower than at higher pH (Table 2.13). The pH of the ozone treated water was 7.5 and at these conditions ciprofloxacin is protonated and consequently the oxidation reaction is hampered.

4.4.2.3 Elimination in granular activated carbon filtration

GAC filtration was studied in the pilot and full-scale treatment plants. In the pilot plant, samples were collected after two consecutive GAC filters in the experiments with and without the preceding ozonation step. In the full-scale treatment plant, GAC filtration followed coagulation and sedimentation.

Previously, activated carbon treatment (PAC or GAC) has been found to efficiently remove pharmaceuticals from water (Stackelberg et al. 2007, Snyder et al. 2007, Kim et al. 2007, Poseidon 2006, Westerhoff et al. 2005, Ternes et al. 2002). Supporting these findings, the concentrations of most of the studied pharmaceuticals were efficiently reduced in the two-stage GAC filtration of the pilot plant. In the experiment conducted without the preceding ozonation, the anti-inflammatories, carbamazepine, acebutolol and metoprolol were all eliminated to <LOQ concentrations in the filtration steps. The two-stage filtration also eliminated ibuprofen and naproxen when they were detectable in the water after ozonation treatment. However, ciprofloxacin was not removed by the GAC filters to appreciable degree. This was probably due to the hydrophilic nature of ciprofloxacin ($\log K_{ow}$= 0.28) and the fact that the affinity of a compound for adsorption decreases with the increasing hydrophilicity of a compound (Snyder et al. 2003). Probably for the same reasons, atenolol and sotalol ($\log K_{ow}$= 0.16 and 0.24, respectively) passed the filtration steps occasionally but their concentrations were mainly reduced in the treatment. In few cases, atenolol, sotalol and bezafibrate reappeared in the samples after the GAC filters. The concentrations were near the LOQ values of the compounds and the reappearance was most probably caused by source variation or fluctuation in the performance of the pilot plant.

In the full-scale treatment plant, ibuprofen and ketoprofen were detected in the post sedimentation water and their elimination could be studied in the GAC filtration. The filtration mainly reduced concentrations of the compounds to <LOQ but in one survey,

incomplete elimination occurred. Elimination of only 33 and 23% were observed for ibuprofen and ketoprofen in the filtration step, respectively. Similar to our findings, poor elimination of carbamazepine was observed in a full scale GAC filtration that had been in operation for 3 years (Stackelberg et al. 2004). The incomplete elimination of pharmaceuticals in the filtration could have been due to saturation of the GAC by NOM and micropollutants, and subsequent breakthrough of the pharmaceuticals from the filter. NOM is known to compete for the adsorption sites in the AC with the micropollutants and also to reduce the surface area of the carbon (Chen et al. 1997, Newcombe et al. 1997). Very hydrophilic compounds can breakthrough the GAC filter already after the treatment of 2000–3000 bed volumes of water (Snyder et al. 2007). Even though several times higher amounts of water can be treated with the GAC filter to efficiently remove more strongly adsorbed compounds, also they start to breakthrough the filter at some point (Snyder et al. 2007). To maintain efficient elimination of pharmaceuticals in full-scale GAC filtration, the carbon should be regularly regenerated or replaced (Snyder et al. 2007).

4.4.2.4 Elimination in UV-disinfection

In the pilot plant, the final step in the treatment train was UV-disinfection (dose of 250 Jm^{-2}). Sotalol, naproxen and ciprofloxacin could be detected in the GAC filtered water and their elimination in this treatment step could be assessed. After the treatment, the concentrations of sotalol and naproxen were reduced to <LOQ (i.e. <1.6 and 5 ng L^{-1}, respectively). Ciprofloxacin was still present after the disinfection at unaltered concentrations. Bezafibrate reappeared after the UV-treatment in one experiment. The compound could be detected also after previous unit operations but it could not be quantified. Therefore, the reappearance was most probably caused by source variation or fluctuation in the performance of the pilot plant. The dose used for disinfection was most probably too low to induce efficient photolytic transformations in bezafibrate and ciprofloxacin. Normally, UV doses in the range of thousands of Jm^{-2} are needed for efficient photolysis of pharmaceuticals (Pereira et al. 2007, Meunier et al. 2006, Adams et al. 2002). However, transformations in some pharmaceuticals that adsorb UV radiation at λ= 254 nm may be induced already at UV doses in the range used for disinfection for compounds (Pereira et al. 2007).

5 CONCLUSIONS

In this thesis, fourteen pharmaceuticals were studied. These pharmaceuticals were chosen on the basis of their reported high consumption rates in Finland, low degree of metabolization in the human body and/or detection at high environmental concentrations in the previous studies. The studied pharmaceuticals and their therapeutic classes were:

Antibiotics:	ciprofloxacin, norfloxacin, ofloxacin and sulfamethoxazole
Antiepileptics:	carbamazepine
Anti-inflammatories:	diclofenac, ibuprofen, ketoprofen, naproxen
Beta blockers:	acebutolol, atenolol, metoprolol, sotalol
Lipid modifiers:	bezafibrate

Two methods were developed to analyze the studied pharmaceuticals in environmental samples. The pharmaceuticals were isolated and concentrated with SPE, chromatographed with HPLC and selectively detected with a triple quadrupole mass spectrometry working in the multiple reaction monitoring mode. The methods allowed the analysis of all the studied pharmaceuticals but sulfamethoxazole at low ng L^{-1} in sewage and in surface and drinking waters.

In the raw sewage, the studied pharmaceuticals were measured at concentrations ranging from <0.02 µg L^{-1} to almost 30 µg L^{-1}. The concentration profile of the pharmaceuticals observed in the sewage influents was similar to consumption profile in Finland, i.e. the pharmaceuticals that were highly consumed were also found at high concentrations in the sewage influents. It was also demonstrated that the concentrations of pharmaceuticals in the raw sewage could be estimated with high precision by simple calculations.

In the effluents, the concentrations of the studied pharmaceuticals ranged from <0.005 to 3.9 µg L^{-1}. This shows that elimination occurred in the treatment processes. The compounds can be divided into four classes on the basis of their average eliminations in the STPs:

(I) Efficient elimination (>80%):	ciprofloxacin, norfloxacin, ofloxacin, ibuprofen, ketoprofen and naproxen
(II) Moderate elimination (40–80%):	acebutolol, atenolol, sotalol and bezafibrate
(II) Poor elimination (<40%):	diclofenac and metoprolol
(IV) No elimination:	carbamazepine

The concentrations of carbamazepine were consistently higher in the effluents than in the influents. This was most probably due to cleavage of a glucuronic conjugate of carbamazepine and the release of the parent compound during the treatment. This was supported by the observation that the amount of carbamazepine-*N*-glucuronide was significantly reduced during the sewage treatment.

The seasonality of the elimination of the anti-inflammatories and bezafibrate was studied in a STP. The elimination of the compounds was lower in the winter than in the summer, most probably due to lower degree of biodegradation caused by low sewage temperature. The effect of the type of the secondary treatment (activated sludge, nitrification/ denitrification or ditch oxidation), as well as the solids and hydraulic retention times applied in the STPs on the elimination of the beta blockers, the fluoroquinolones and carbamazepine was studied. During the normal operation of the STPs, these parameters did not affect the elimination of the compounds. However, the elimination of the beta blockers was decreased in one of the studied STPs during heavy rain most probably due to decreased degree of biodegradation caused by lowering of the hydraulic retention time in the STP. All in all, higher load of pharmaceuticals can be expected to enter the environment in the winter and during rain events.

In the Finnish rivers, the studied pharmaceuticals were generally detected at lower concentrations (mainly at concentrations <100 ng L^{-1}) than in some other countries where concentrations up to several µg L^{-1} have been measured. STPs were shown to be the main source of pharmaceuticals in the studied rivers since the concentrations of the compounds in the rivers increased considerably at the discharge points of the STP effluents. The highest concentrations of most of the pharmaceuticals were measured in the winter. Most probably, this was due to higher load of pharmaceuticals from the STPs to the receiving rivers as well as the hindered phototransformation and biodegradation of pharmaceuticals in the rivers.

Diclofenac, naproxen, ketoprofen, acebutolol and metoprolol showed high degree of attenuation during the river transportation whereas ibuprofen, atenolol, sotalol and carbamazepine were highly persistent in the studied rivers. The risk to the environment posed by the studied pharmaceuticals at ng L^{-1} concentrations can be considered negligible according to ecotoxic studies using mortality, growth or reproduction as endpoints. However, behavioral disturbances in aquatic organisms could be caused already at these low concentrations.

In the laboratory, pilot and full scale studies, coagulation and subsequent sand filtration were inefficient in eliminating the studied pharmaceuticals. Therefore, when the raw water contains pharmaceuticals, drinking water treatment plants cannot be expected to produce pharmaceutical-free water by applying only these conventional treatment steps. Fortunately, the raw waters in Finland that contain pharmaceuticals are limited to a few places where river water receives STP effluents. To efficiently reduce the concentrations of the pharmaceuticals in drinking water treatment, granular activated carbon filtration and ozonation were shown to be needed. Only ciprofloxacin was not eliminated with these techniques but could be detected at similar concentrations after the treatment process as in the raw water (~20 ng L^{-1}). In two of the sampled drinking waters, carbamazepine, ibuprofen, ketoprofen and metoprolol were detected at concentrations of 5–8 ng L^{-1}. In general, the possible amounts of pharmaceuticals obtained via drinking water are 10^6 times smaller in a day than the therapeutic doses of the compounds. The effects caused by chronic exposure to low concentrations of pharmaceuticals over a long period of time are unknown. Still, the risk for the consumers posed by residues of pharmaceuticals in drinking water is most probably negligible.

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