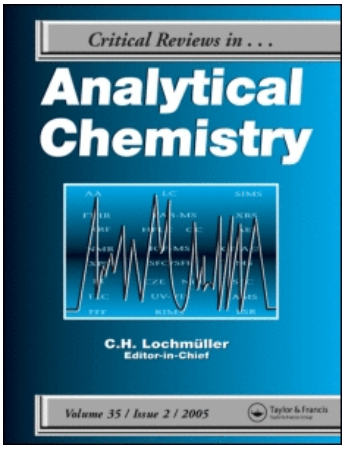

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Fate and Analysis of Pharmaceutical Residues in the Aquatic Environment

Jolanta Dębska[a]; Agata Kot-Wasik[a]; Jacek Namieśnik[a]
[a] Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, Gdańsk-Wrzeszcz, Poland

Online publication date: 10 August 2010

To cite this Article Dębska, Jolanta , Kot-Wasik, Agata and Namieśnik, Jacek(2004) 'Fate and Analysis of Pharmaceutical Residues in the Aquatic Environment', Critical Reviews in Analytical Chemistry, 34: 1, 51 — 67
To link to this Article: DOI: 10.1080/10408340490273753
URL: http://dx.doi.org/10.1080/10408340490273753

Critical Reviews in Analytical Chemistry, 34:51–67, 2004

ISSN: 1040-8347 print
DOI: 10.1080/10408340490273753

Fate and Analysis of Pharmaceutical Residues in the Aquatic Environment

Jolanta Dębska, Agata Kot-Wasik, and Jacek Namieśnik
Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology, Gdańsk-Wrzeszcz, Poland

The presence of pharmaceuticals in different compartments of the environment is a new challenge not only for technologists of water and wastewater treatment but also for analytical chemists involved in development of new analytical methods. Many drugs are not completely degraded in the human body. They are often excreted after only slight transformation or in unchanged form, mainly as polar molecules (clofibric acid, primidone). It has been proved in many studies that substances of pharmaceutical origin are not eliminated in the process of water treatment; their biodegradation in the environment is also difficult (1). Detailed chemical analysis of water is necessary for the safe use of water resources. It is important to identify all pollutants present in water and, hence, to fully evaluate water quality and predict effect on humans. The continuous improvement of analytical techniques makes it possible to identify a wider spectrum of components and improve detection limits. A brief review of input by different sources, and fate and analysis of pharmaceuticals, parapharmaceuticals, and their metabolites in environment is presented.

Keywords analysis, aquatic ecosystem, emission sources, fate, pharmaceutical residues

In the past several years, one of the primary tasks of environmental chemistry has been detection, determination, and fate studies of pharmaceuticals in different compartments of the environment, particularly in water ecosystems (2, 3). An important role is played in this area by analytical chemists who develop and introduce to analytical practice procedures for determination of traces of a wide spectrum of compounds in samples of complex matrices.

The presence of different drugs in the environment results from manufacturing of medical formulations and the impact of the pharmaceutical industry on the environment (4); discharge of large quantities of expired drugs (without treatment) by households (small scale) as well as with hospital wastewater and wastes (much larger scale); and excretion of residues of drugs and their metabolites by animals and humans (5, 6).

Studies on metabolism and fate of pharmaceuticals in human and animal bodies have shown that a large fraction is excreted with feces and urine, and therefore the drugs are present in municipal wastewater (7–10). Compounds of the group are biodegradation resistant and are not completely eliminated in the process of wastewater treatment (11–14). They are present in the environment both in unchanged form and as metabolites.

Application of large amounts of antibiotics, hormones, analgesic and sedative drugs, and different disinfection preparations as well as difficulty in their complete inactivation in water treatment is a serious problem (15–18). The use of water polluted with pharmaceutical residues and their metabolites disturbs balance in the body and enhances dangerous resistance to drugs; developers of new antibiotics must combat this problem (19–22). The first reviews published on this subject have dealt with environmental and analytical problems related to pharmaceutical residues (23–28). Figure 1 presents presence and paths of pharmaceutical residues in the environment.

The Department of Analytical Chemistry constitutes the Centre of Excellence in Environmental Analysis and Monitoring which is a research project supported by the European Commission under the Fifth Framework Programme and contributing to the implementation of the Key Action Sustainable Management and Quality of Water within the Energy, Environment and Sustainable Development (Contract no.: EVK1-CT-2002-80010). The authors acknowledge this generous support.

This article was completed within the framework of a research project funded by the Polish Committee for the Scientific Research (Research Project no. 8 T07G 006 21). The authors thank Bogdan Zygmunt for language corrections on the manuscript, and Dr. Żaneta Polkowska for her contribution in literature search.

Address correspondence to Jolanta Dębska, 11/12 Narutowicz Str., PL 80-952 Gdańsk-Wrzeszcz, Poland. E-mail: chemanal@pg.gda.pl

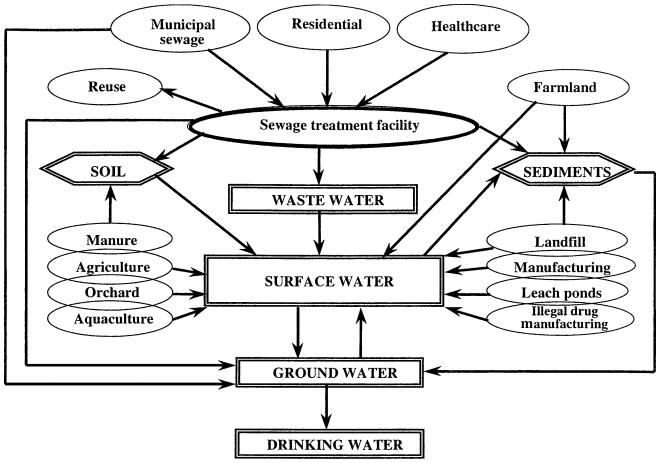

FIG. 1. Occurrence and dispersion of components of pharmaceuticals and parapharmaceuticals (39–43).

Many different pharmaceuticals have been detected in drinking water (29–31). Underground waters can also be polluted with substances of pharmaceutical origin as a result of surface water infiltration and leaching by waste sites. Substances of pharmaceutical origin are monitored in water environments by many research centers (23, 32–36). However, the most important problem with monitoring is lack of appropriate analytical procedures for quantitative determination of residues of active pharmaceutical components and their metabolites (37, 38).

CLASSIFICATION OF MEDICINAL SUBSTANCES

Development of civilization has been accompanied by an increase in the number of diseases that trouble mankind. This has been a driving force for continuous improvement of available drugs, and development and manufacturing of new pharmaceutical formulations.

The description by Galen of Pergamon, a Roman doctor of Greek origin (ca. 130–200 A.D.), of available drugs (as many as several hundred) formed the basis of pharmacy. Nowadays the number of drugs in a wide sense (i.e., all substances or formulations applied by people for therapeutic, preventive, and diagnostic purposes) is enormous and difficult to precisely evaluate for many reasons. The number is estimated to reach 200,000 on a global scale; there are about 5,000–10,000 formulations on the markets of particular countries.

The Polish pharmaceutical industry consists of 274 manufacturers, mostly small or very small producers. There are 10 large plants producing synthetic drugs and 10 plants producing drugs of natural origin. Although this is only small fraction of Polish industrial activity (with respect to the amount as well as the value of products), it employs 25,000 workers and manufactures 2,500 products valued at U.S. $2.5 billion.

The large diversity of the medicinal substances used in present-day pharmacology makes classification necessary. Table 1 presents classification of drugs according to pharmacological activity and brief characteristics of particular classes (45).

THE MOST POPULAR DRUGS

In Poland, many drugs can be purchased without a prescription; this influences sales to a high degree. According to the literature, each Pole buys 29 drugs a year on average (49). The available data on the drugs most often sold in the Tricity, Poland (common name for the three neighboring cities of Gdańsk, Sopot, and Gdymia), drugstores are presented in Table 2. Those most often applied are analgesics, antipyretics, and antibiotics. They are used as remedies for different problems, in different doses, and have different side effects. Some are multicomponent formulations.

The seriousness of the problem of pharmaceutical overuse by people in Poland and in other countries can be seen from the intensive discussion about it in many periodicals. The data show that in Europe the largest quantities of pharmaceuticals are applied by the French; the Polish are second on the list. To a high degree this can be related to availability of drugs. In Germany, 35% of all drugs are purchased without prescription, in Poland 31%; the situation is more strongly controlled in Spain where 94% of drugs must be prescribed (49).

Figure 2 presents the purchase (as diagrams) of drugs by a statistical inhabitant in selected countries. The Polish belong to leading group in this respect. In Europe, only the French buy more drugs than the Polish. Every third pharmaceutical in Germany can be purchased without prescription; in Spain, every nineteenth. The buying structure in Poland is closer to the German model than to the Spanish one. The drugs available without prescription are not harmless. Applied regularly in large doses, they can damage internal organs of the human body. Analgesics with a plus sign (+) contain phenacetin, which can damage mucosa and cause bleeding in alimentary track. Applied for a long time in large doses, they can cause serious kidney and liver damage. This can also lead to serious allergy, cardiac failure, and respiratory problems.

Paracetamol is less harmful than tablets with a plus sign; however, if used for a long time, it causes the same effects—first

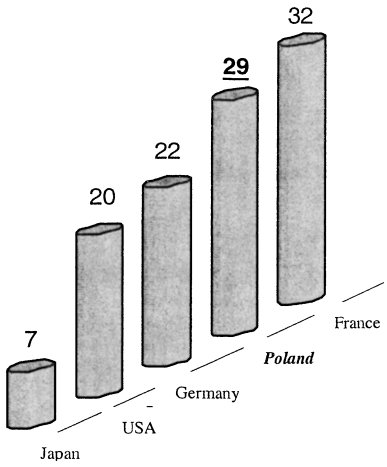

FIG. 2. The number of packages of pharmaceuticals bought by a statistical inhabitant in selected countries.

TABLE 1
Characteristics of the Main Classes of Drugs

No.	Drug group	Application	Additional information
1.	Drugs applied in diseases of respiratory system	Antitussive drugs, antiasthmatic drugs, expectorants. Used to help remove retained secretion or foreign body from respiratory track	Some available without prescription
2.	Drugs used in diseases of alimentary track	Drugs stimulating gastric secretion, drugs applied in chronic peptic ulcer disease, hepatopathy and cholepathy, also laxatives, antidiarrheal agents, antiemetics	Often accessible without prescription (especially laxatives, antidiarrheal agents, and antiemetics)
3.	Drugs producing effects on nervous system	Neurotransmitters, neurohormone, psychetropic drugs, sedatives, hypnagogues, antiepileptic drugs, analgesics, antipyretic drugs, anti-inflammatory drugs, general anesthetics (46)	Often applied in hospitals
4.	Drugs producing effects on circulatory system and affecting blood coagulability	Cardiac drugs, agents used in coronary heart disease, hypotensive blood agents, vasodilators, drugs affecting blood coagulability, blood substitutes	—
5.	Diuretics	Applied in chromic and acute renal failure, hepatic cirrhosis, pulmonary edema Diuretics of sulfonamide class, drug sparing potassium, xanthic diuretics, osmotic diuretics	Often used in hypertension treatment, poisoning, to cause forced diuresis
6.	Autocoids	Histaminic receptor (H_1 and H_2) blocking drugs, drugs belonging to agonists and antagonists of serotoninergic receptors (47)	—
7.	Drugs affecting peripheral nervous system	Sympathomimetic and sympatholytic agents, parasympathomimetic and parasympatholytic agents, drugs affecting vegetative system ganglia, muscle relaxant, local anesthetics	Also applied in chronic diseases (migraine, asthma) and in ophthalmology
8.	Hormones	Substances regulating functions of internal organs by stimulating and inhibiting biochemical processes. Produced in the body, major part is secreted by endocrine glands directly to blood. In health care, also synthetic analogues of hormones are used. This group of compounds includes estrogens (*e.g.*, ethinyl estradiol) and gestagens (*e.g.*, ethynodiol) which are basic oral contraceptives (48)	Though availability of these drugs is controlled by physicians, the amount used increases from year to year. In Great Britain, the state program is presently implemented; it is based on sale of contraceptive tablets without prescription. Similarly the Noruegian government decided to distribute free contaceptive tablets among school-age girls
9.	Vitamins	Biologically active organic substances are delivered to the body with food. Some can be produced in the body directly from precursors (provitamins). Some vitamins are soluble in fat (e.g., A, D, E, K)	The human body is not able to produce other vitamins (, A, E, C, B_1, B_2, B_6, B_{12}, folic acid, pantothenic acid; these vitamins can be supplied with food only or as mineral-vitamin formulations

(Continued on next page)

TABLE 1
Characteristics of the Main Classes of Drugs *(Continued)*

No.	Drugs group	Application	Additional information
		while some are soluble in water (e.g., C, B, folic acid). Vitamin deficiencies cause of avitaminosis. The human body is not able to produce vitamins or produces them in insufficient quantities. Sunlight (ultraviolet radiation) stimulates formation of vitamin D; in the alimentary track are bacteria which produce vitamin K and biotin	
10.	Drugs affecting pathogenic microorganisms	Disinfectants, sulfonamides, derivatives of nitrofuran and chinolon, antibiotics, antitubercular drugs, leprostatic agents, antifungal and antiviral agents Some find wide application in surgery for disinfection of skin, hands, and surgical instruments. They are used to disinfect drinking water and water used for some other purposes. They are effective in therapy of many bacterial and mycotic infections	These drugs are often abused despite a prescription being necessary to purchase them. Moreover, these compounds are often components of household chemistry
11.	Anticarcinogenic drugs	Mainly chemotherapeutics of high cytostatic activity, but also of considerable toxicity leading to unwanted symptoms	Treatment of malignant diseases is one of the most difficult tasks of contemporary medicine. Alarming increases in tumor incidences, especially in industrialized countries make it necessary to develop new methods of malignant disease control. The present health care systems possess over 90 drugs used to control such diseases
12.	Immunomodulating drugs	They act on immune body processes inhibiting immune reactions (immunosuppresive drugs) or stimulating immunological reactions (immunoregulatory agents). This group includes formulations containing antigens in the form of weakened or killed microorganisms (vaccines)	—

of all damage of liver and kidney. It is a component of many medicines available without prescription and advertised in mass media. Calcipirin, polopirin, and aspirin applied regularly for a long time can damage liver, kidney, and mucosa of alimentary track. Application of large doses of some vitamins can also be harmful and even carcinogenic. Lorafen and relanium are psychotropic drugs available by prescription only, but often prescribed. They have strong drug addictive properties. Their overuse can be fatal. Applied for a long time, relanium can irreversibly damage brain and cause memory atrophy.

PHARMACEUTICAL PATHS IN THE ENVIRONMENT

The amounts of the drugs sold evidence their prevailing use; hence, the risk of presence of substances of pharmaceutical origin in the environment (50, 51). Pharmaceuticals' release to and dispersion in wastewater as well their fate in treatment plants can be quite different. A majority of drugs is excreted from the human body in unchanged or metabolite form; those unused are simply eliminated and enter wastewater treatment plants in a nonmetabolized form.

Before active substances are absorbed by the body, they must be released from the application form (tablets, pills, suppositories). Then they are absorbed in the body from the alimentary track (on oral or rectal application), hypodermic injections, and intramuscular tissues (on tissue application). Drugs can also permeate through skin or mucosa other than the mucous membrane of alimentary track (e.g., in the vagina and conjunctival sac). Vapors, gases, and small solid particles applied in the form of aerosols or suspended matter can be absorbed through lungs.

TABLE 2
Drugs Most Often Sold in the Tricity, Poland, Drugstores

Drugstores	Aspirin[a]	Amoksiklav[b]	Augmentin[b]	Biseptol[b]	Doxycycline[b]	Duomox[b]	Duracef[b]	Etopiryna[a]	Ibuprom[a]	Paracetamol[a]	Polfilin[c]	Polopirin[a]	Pyralgin[a]	Ranigast[d]	Rutinoscorbin[d]	Analgesic[a]	Zyrtec[e]
1	+*		+			+			+			+*		+	+*	+*	+
2	+*	+			+	+		+		+*			+	+		+*	+
3			+			+		+*	+*	+			+	+	+		
4		+			+		+	+	+		+	+	+		+*		+
5	+		+			+			+	+		+*			+*	+*	+
6	+*	+			+	+			+	+*	+			+			+
7	+*					+				+*		+		+		+*	+
8	+*				+			+*		+				+	+	+*	
9		+				+		+*	+					+	+*	+	
10	+				+					+*	+		+				+
11	+*					+*		+*		+		+	+				
12	+			+				+	+*						+*		
13	+*					+		+*	+		+				+		
14					+		+	+		+		+	+	+	+*	+*	
15				+		+		+*		+*	+		+	+			+

Drugstores 1–9, Gdańsk; drugstores 10, 11, Sopot; drugstores 12–15, Gdynia. +, drugs sold every day; +* drugs sold in large amounts.
[a]Analgesic, anti-inflammatory drugs.
[b]Antibiotics, antibacterial drugs.
[c]Psychotropic.
[d]Vitamins.
[e]Autocoids.

Most drugs undergo biotransformation to more hydrophilic products since only such compounds can be excreted by kidneys—the main path of drug removal from the body. Compounds that are only sparingly soluble in body fluids and not absorbed in the alimentary track, and also water-soluble electrolytes, are excreted in a nonchanged form. Biotransformation that takes place mainly in the liver leads to loss of pharmacological activity of the drug. The activity of enzymes in the process depends on sex (more drugs are metabolized faster by men), age, physiological state, disease, and the presence of enzymatic inhibitors in the body that block metabolism of drugs and enzymatic inductors that enhance biotransformation of drugs.

Some drugs (e.g., phenylbutazone, morphine, and nicotine) have the capability of self-induction or enhancing their own metabolism on long-term application (this is the main reason for tolerance to drugs). The metabolites formed are generally inactive. However, some show pharmacological activity; paracetamol or phenacetin metabolites can be examples of this (52).

Chemical reactions in the process of biotransformation are grouped into two stages. To the first-stage reaction are classified the reactions of oxidation, reduction, and hydrolysis. The compounds formed are metabolites with polar groups reactive in the second stage. Secondary processes are included among the second-stage reactions termed "reactions of conjugation" or "coupling with endogenic compounds." The second-stage products are generally soluble in water; they are easily excreted with urine. The first-stage metabolites sometimes show activity of an original drug, but second-stage ones are generally inactive. The drugs are excreted from the body via kidneys with urine, liver with bile (to a lower degree with saliva), intestines, lungs (volatile compounds), or skin with perspiration.

A majority of pharmaceuticals is excreted from the body through kidneys, percolating through glomeruli or selectively through spiral tubules. Only a peptide-bonded fraction can be excreted through glomerulis. Excretion intensity depends on kidney blood flow and permeability of glomerulis. Tubule excretion is based on active transport of some substances to urine—in such a way, penicillins, many sulfonamides, salicylates, phenylbutazone, and others are excreted. This kind of excretion can be blocked by specific inhibitors (e.g., probenecid blocks penicillin excretion).

A successively important step in the fate of pharmaceuticals is connected with wastewater treatment plants. In the process of wastewater treatment, pharmaceuticals and their metabolites:

- can be mineralized by microorganisms to CO_2 and H_2O (e.g., aspirin);
- can remain in treated wastewater at lower or higher concentration, depending on lipophilicity and other properties (e.g., ionic bonds); part of a substance will be trapped in sludge. If the sludge is used as fertilizer, pharmaceuticals can be dispersed over arable land;
- can be neither trapped nor degraded in wastewater treatment plants and easily be transported to water environments.

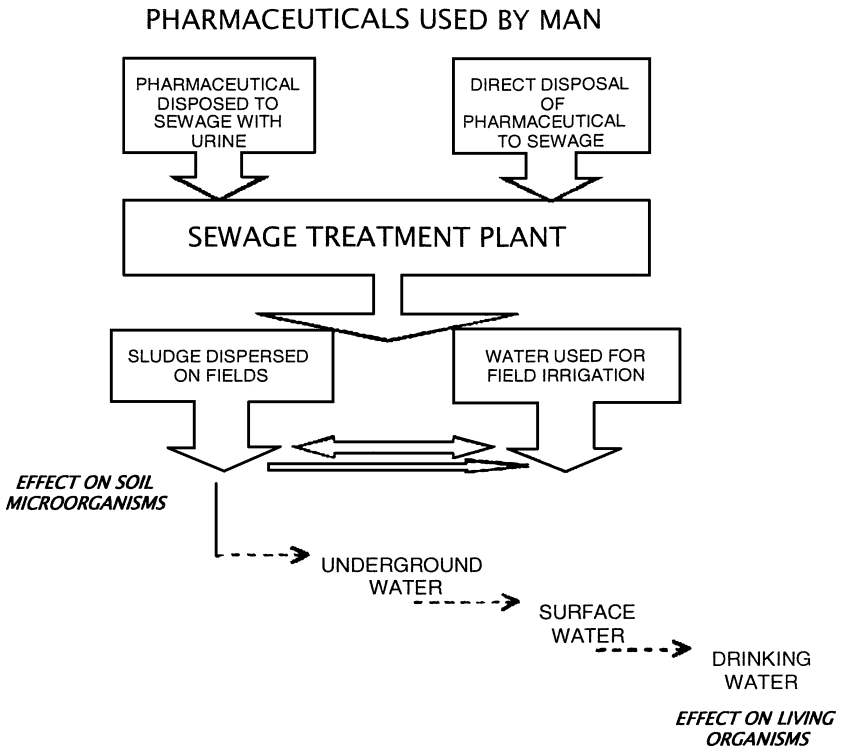

FIG. 3. Predicted paths of pharmaceuticals present in treated wastewater (53, 54).

An unknown part of pharmaceuticals unused by humans goes to sewers as a surplus of medical substances. On reaching wastewater treatment plants, their fate will be identical to those excreted from organisms. The only difference is that wastewater will not contain pharmaceutical metabolites. Predicted paths of pharmaceuticals present in treated wastewater are shown in Figure 3.

DETERMINATION OF RESIDUES OF PHARMACEUTICALS AND THEIR METABOLITES IN ENVIRONMENTAL SAMPLES

Modern analytical methods allow determination of complex substances at very low concentration levels (ppb, ppt) (55). However, all the while intensive research is being conducted to improve the existing methods and develop new methods which could give the best information on water pollution (56, 57). Due to the studies of many different researchers, 25 different pharmaceuticals were determined in environmental samples in 1996; the number increased to 68 in 1999.

Progress in this area is possible thanks to development and introduction to practice of increasingly new analytical procedures that permit researchers to determine trace and ultratrace analytes in samples of a complex matrix. It must be stressed, however, that the most important step is sample preparation (58) and methodological achievements in this area play a crucial role in solving problems of obtaining knowledge on:

- concentration level of pharmaceuticals and their metabolites in different compartments of the environment;
- dispersion of pharmaceuticals in the environment;
- metabolism of drugs;
- efficacy of utilization processes and treatment of wastewater containing pharmaceuticals.

Table 3 presents the techniques of sample preparation for determination of substances of pharmaceutical origin and their metabolites. The concentration ranges of the compounds in different samples are also given.

Due to the high matrix complexity and low level of analyte concentration, a sample preparation step is necessary and very important (82–84). The isolation and enrichment techniques most often used in the case of analytes of pharmaceutical origin belong to solid phase extraction (SPE); solid phase microextraction (SPME); and liquid/liquid extraction (LLE). Supercritical fluid extraction (SFE) was also applied to isolate selected pharmaceuticals from water (diazepam, oxazepam, testosterone, naproxen, indomethacin, ketoprofen, tolmetin)—recoveries were at the level of 70–90% (85).

Determination of pharmaceuticals and parapharmaceuticals in environmental samples requires very sensitive and selective techniques of final analysis (86). At present chromatographic techniques, especially high-performance liquid chromatography (HPLC) and gas chromatography (GC), are mainly used.

The indispensable part of an analytical instrument is an appropriate detector allowing detection of active pharmaceuticals at very low concentration. The detection system most often used for the purpose is mass spectrometry (MS) in the case of HPLC as well as GC, less often flame ionization detection in GC, and diode array detection and fluorescence detection in HPLC.

The Known Techniques of Sample Preparation and Final Analysis of Pharmaceutical Residues in Environmental Samples

Each analytical procedure consists of many interdependent operations which should be precisely planned if the final result is to reflect the real content of a determined substance in the sample. The collected samples are subjected to successive operations in situ or in the laboratory. The operations are aimed at (58): sample preservation, isolation and/or enrichment of analytes, transport and storage of samples or extracts, and clean-up and possible reduction of extract volumes. Figure 4 presents a scheme of steps of the analytical procedure for determination of pharmaceutical residues.

No doubt the final determination and identification are generally conducted with the use of GC/MS as well as LC/MS in different configurations and modifications (87–93). Depending on sample type and analyte characteristics, different procedures and reagents in the extraction step were used. Short

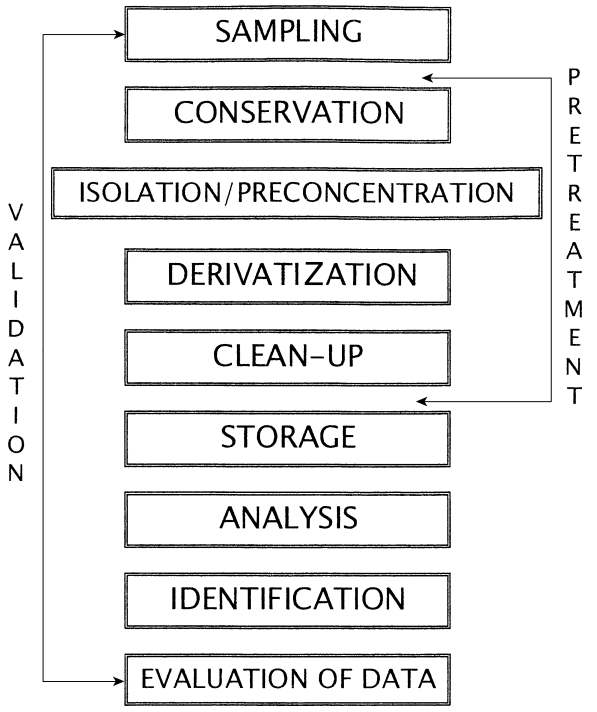

FIG. 4. Steps of analytical procedure for determination of pharmaceutical residues.

TABLE 3

Literature Data on the Presence of Residue of Substances of Pharmaceutical Origin in Environmental Samples and Techniques of Sample Preparation and Final Analysis

Compound	Formula	Therapeutic use	Environmental occurrence	Sample preparation	Chromatographic technique	Detection	Concentration in the environment	Place and year of sampling	Reference
Ciprofloxacin	$C_{17}H_{19}O_3N_3F$	Antibiotic	Surface water	SPE	HPLC	FLD MS/MS	0.294–0.405 μg/dm^3	Switzerland 2000	(59)
Chloramphenicol	$C_{11}H_{12}O_5N_2Cl_2$	Antibiotic	Wastewater	SPE	GC HPLC	MS MS/MS	max–0.56 μg/dm^3	Germany 1996–98	(60)
Diazepam	$C_{16}H_{13}ON_2Cl$	Psychiatric	Surface water	SPE	HPLC	MS/MS	0.88 μg/dm^3	Germany 2000	(61)
			Wastewater	SPE	GC HPLC	MS MS/MS	max–0.04 μg/dm^3	Germany 1996–98	(60)
Diclofenac	$C_{14}H_{13}O_2N$	Analgesic/anti-inflammatory	Surface water	SPE	GC	MS MS/MS	0.05 μg/dm^3	Germany 1996–98	(60)
			Urine	SPE	HPLC	—	0.05 μg/dm^3	—	(62)
			Surface water	LLE SPE	HPLC	CE-MS MS	0.3 μg/dm^3	Japan 1999–2001	(63)
			Surface water	SPE	GC	MS	10 μg/dm^3	United States 1999–2001	(64)
			Wastewater	SPE	HPLC	ESI-MS	5–20 ng/dm^3	Canada 2001	(65)
			Surface water Groundwater	SPE	—	—	0.03–0.2 μg/dm^3 0.4–0.9 μg/dm^3	Germany 2001	(66)
			Seawater	LLE	GC	MS	6.2 ng/dm^3	North Sea 2001	(67)
			Drinking water Surface water	SPE	GC	MS	0.4–0.9 μg/dm^3 0.03–0.2 μg/dm^3	Germany 2001	(66)
			Surface water	LLE	GC	MS	12 ng/dm^3	Switzerland 1998	(68)
Erythromycin	$C_{37}H_{67}O_{13}N$	Antibiotic	Water samples	SPE	HPLC	MS	0.62 μg/dm^3	Germany 1997–1998	(69)
17β-estradiol	$C_{17}H_{23}O_2$	Estrogen	Sediment samples	LLE SPE	HPLC GC	MS	0.02 μg/dm^3	Germany 2001	(70)
			Sediments and river water	SPE	HPLC	DAD MS UV	0.02–0.05 μg/dm^3	Spain 2000	(71)
			Wastewater	SPE	HPLC	UV	0.02 μg/dm^3	—	(72)
			Drinking and surface water	SPE SPME	GC	FID MS	—	Spain 2001	(73)
Estrol	$C_{18}H_{24}O_3$	Estrogen	Sediments and river water	SPE	HPLC	DAD MS UV	0.02–0.05 μg/dm^3	Canada 2000	(33)
			Wastewater	SPE	HPLC	UV	0.02 μg/dm^3	—	(72)
			Drinking and surface water	SPE SPME	HPLC	MS	—	Spain 2001	(73)

Estron	$C_{18}H_{22}O_2$	Estrogen	Raw sewage	LLE SPE	HPLC GC	MS	0.02 μg/dm^3	Germany 2001	(70)
			Surface water	SPE	GC	MS	0.01 μg/dm^3	United States 2000	(56)
			River water and sediment	SPE	HPLC	DAD MS UV	0.02–0.05 μg/dm^3	Spain 2000	(71)
17α-Ethinyl estradiol	$C_{20}H_{26}O_2$	Estrogen	Wastewater	SPE	HPLC	UV	0.02 μg/dm^3	—	(72)
			Raw sewage	LLE SPE	HPLC GC	MS	0.09 μg/dm^3	Germany 2001	(70)
Hydroxyibuprofen[a]	$C_{13}H_{19}O_3$	Analgesic/anti-inflammatory	River water, urine, wastewater	SPE	GC	MS	6.7 μg/dm^3	Germany 1997	(74)
Ibuprofen	$C_{13}H_{18}O_2$	Analgesic/anti-inflammatory	Water samples	SPE	GC	MS MS/MS	0.05 μg/dm^3	Germany 1996–98	(60)
			Urine	SPE	HPLC	—	0.05 μg/dm^3	—	(62)
			Surface water	LLE SPE	HPLC	CE-MS MS	0.6 μg/dm^3	Austria 1999–2000	(63)
			Surface water	SPE	GC	MS	5 μg/dm^3	United States 1999–2001	(64)
			Wastewater	SPE	HPLC	ESI-MS	5–20 ng/dm^3	Canada 2001	(65)
			Seawater	LLE	GC	MS	0.6 ng/dm^3	North Sea 2001	(67)
			River water	SPE	GC	FID	0.087 μg/dm^3	Germany 2000	(75)
			Surface water	SPE	GC	MS	0.1–1.0 μg/dm^3	Switzerland 1998	(76)
Carbamazepine	$C_{15}H_{12}ON_2$	Analgesic; antiepileptic	Surface water	SPE	HPLC GC	MS	0.005 μg/dm^3	Germany 1999	(77)
Carboxyibuprofen[a]	$C_{13}H_{16}O_4$	Analgesic/anti-inflammatory	River water	SPE	GC	MS	0.34 μg/dm^3	Germany 1997	(74)
Caffeine	$C_8H_{10}N_4O_2$	Psychomotor stimulants	Surface water	SPE	HPLC	MS-ESI	—	United States 1999–2000	(78)
			Surface water	LLE	GC	MS	0.016 μg/dm^3	North Sea 2001	(67)
			Surface water	SPE	HPLC	MS/MS	1.9 μg/dm^3	Germany 2000	(61)
Acetylsalicylic acid	$C_9H_8O_4$	Analgesic/anti-inflammatory	Waste	SPE	GC HPLC	MS MS/MS	0.38 μg/dm^3	Germany 1996–98	(60)
			Surface water				0.10 μg/dm^3		
Clofibric acid[a]	$C_{10}H_9O_3$	Lipid-lowering agent	Surface water	SPE	—	—	0.03–0.2 μg/dm^3	Germany 2001	(79)
			Groundwater				0.4– 0.9 μg/dm^3		

(Continued on next page)

TABLE 3

Literature Data on the Presence of Residue of Substances of Pharmaceutical Origin in Environmental Samples and Techniques of Sample Preparation and Final Analysis *(Continued)*

Compound	Formula	Therapeutic use	Environmental occurrence	Sample preparation	Chromatographic technique	Detection	Concentration in the environment	Place and year of sampling	Reference
			Surface water	LLE	GC	MS	0.013 μg/dm^3	North Sea 2001	(67)
			Surface water	SPE	GC	FID	0.049 μg/dm^3	Germany 2001	(66)
			Surface water	SPE	GC	MS	0.005 μg/dm^3	United States 2000	(56)
Salicylic acid[a]	$C_7H_6O_3$	—	Waste	SPE	GC HPLC	MS MS/MS	max–0.14 μg/dm^3	Germany 1996–98	(60)
			Surface water	SPE	HPLC	MS	15–56 ng/dm^3	Spain 1997	(79)
Naproxen	$C_{14}H_{14}O_3$	Analgesic/ anti-inflammatory	Wastewater	SPE	HPLC	ESI-MS	5–20 ng/dm^3	Canada 2001	(65)
			Surface water	SPE	GC	MS MS/MS	max–0.39 μg/dm^3	Germany 1996–1998	(60)
			Surface water	LLE SPE	HPLC	CE-MS MS	0.5 μg/dm^3	Austria 1999–2001	(63)
			Surface water	SPE	GC	MS	10 μg/dm^3	United States 1999–2001	(64)
			Surface water	SPE	GC	MS	0.005 μg/dm^3	United States 2000	(56)
Nonylphenol	$C_{15}H_{24}O$	Estrogen	Drinking and surface water	SPE	GC	FID MS	—	Spain 2001	(73)
			Surface water	SPE	HPLC	MS-ESI	—	United States 1999–2000	(78)
			Wastewater	SPE	HPLC	UV	0.05 μg/dm^3	—	(72)
			Wastewater	SPE	GC	MS	0.1– 253 μg/dm^3	United States 1999–2000	(33)
Norfloxacin	$C_{16}H_{18}O_3N_3F$	Antibiotic	Surface water	SPE	HPLC	FID MS/MS	45–120 ng/dm^3	Switzerland 2000	(59)
Primidone	$C_{12}H_{14}O_2N_2$	Antiepileptic	Surface water	SPE	HPLC GC	MS	0.005 μg/dm^3	Germany 1999	(77)
Sulfadiazine	$C_{10}H_{10}O_2N_4S$	Sulfonamide	Seawater	LLE SPE	HPLC	APCI-MS	2.5 μg/dm^3	Denmark 2001	(80)
Sulfomethoxazole	$C_{10}H_{11}O_3N_3S$	Sulfonamide	Surface water	SPE	HPLC	MS	30–85 ng/dm^3	Germany 1998	(81)
			Surface water	SPE	HPLC	MS	0.48 μg/dm^3	Germany 1997–1998	(69)
Sulfonamides	$C_6H_7O_2N_2SR$	Sulfonamide	Surface water	SPE	HPLC	MS	0.07–15 μg/dm^3	United States 1999–2001	(78)
Trimethoprim	$C_{14}H_{18}O_3N_4$	Antibacterial	Seawater	LLE SPE	HPLC	APCI-MS	2.5 μg/dm^3	Denmark 2001	(80)

—No data; SPE, solid phase extraction; LLE, liquid liquid extraction; GC, gas chromatography; MS, mass spectrometry; FID, flame ionization detection; ESI-MS, electrosprayionization-mass spectrometry; HPLC, high performance liquid Chromatography; APCI-MS, atmospheric pressure-mass spectrometry; CE-MS, capillary electrophoresis-mass spec-trometry.

[a]Metabolite.

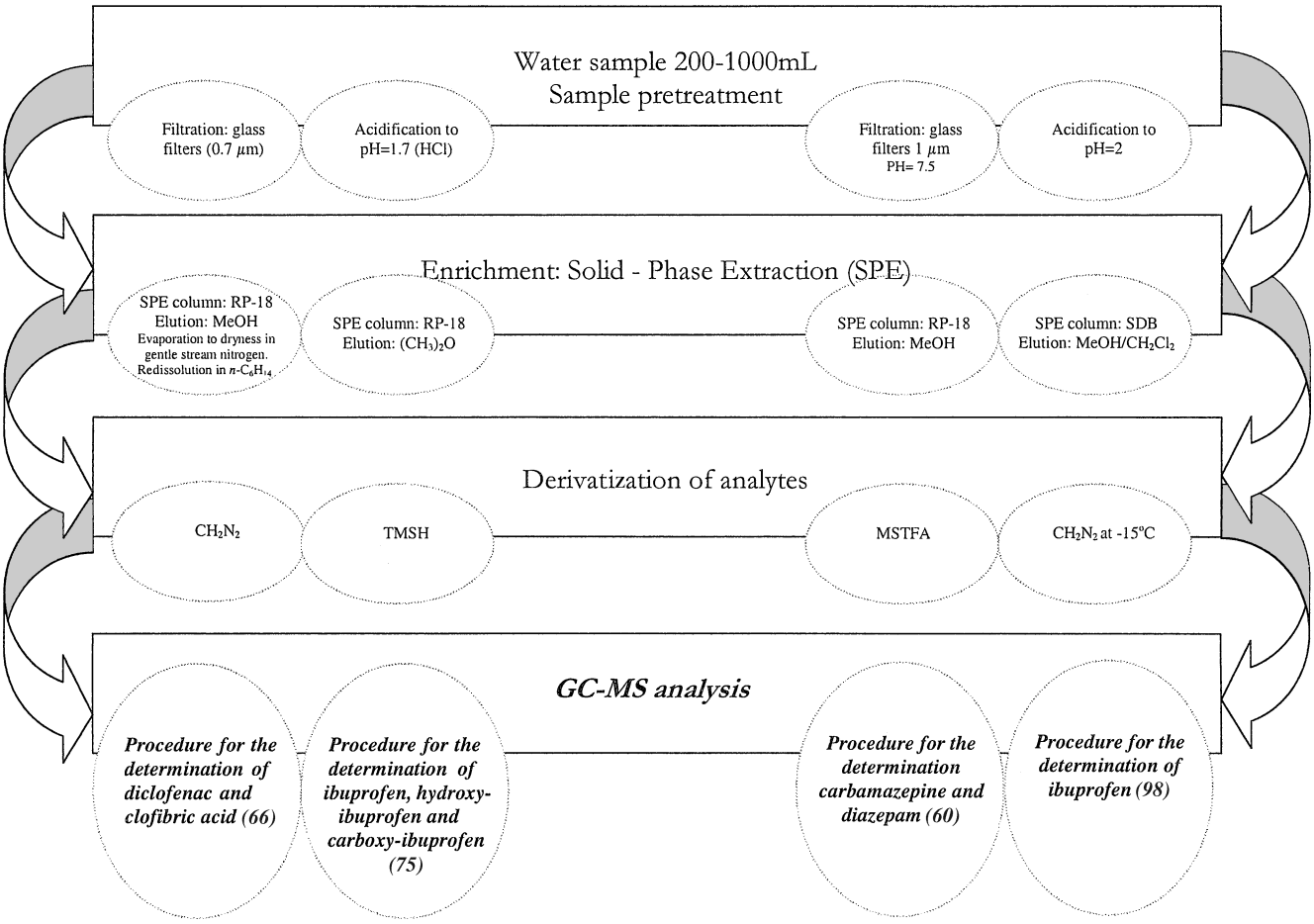

FIG. 5. Schematic representation of the analytical procedures employed for determination of residues of pharmaceuticals with the use of gas chromatography/mass spectrometry (GC/MS).

characteristics of the methods applied in analytical laboratories around the world for the determination of substances of pharmaceutical origin in aqueous samples are presented below.

Preparation of Aqueous Samples for Determination of Substances of Pharmacological Origin with the Use of GC/MS

Gas chromatography coupled with mass spectrometry is widely used in analysis of organic environmental pollutants (94–97). The reason is the increasing requirements of reliability of the results. The mass spectrometer permits researchers to obtain qualitative information on the compounds separated and ensures specificity of final analysis. Generally, two modes of operation of MS as a GC detector are applied: full scanning and selective ion monitoring (SIM).

GC/MS was employed to determine such substances of pharmaceutical origin as:

- chloramphenicol
- diazepam
- diclofenac
- hydroxyibuprofen
- ibuprofen
- carbamazepine
- carboxyibuprofen
- acetylsalicylic acid
- salicylic acid
- metoprolol
- naproxen
- primidone

Figure 5 presents a schematic diagram of procedures of aqueous sample preparation for determination of nonsteroidal anti-inflammatory drugs (NSAIDs) with the use of GC for final analysis.

Aqueous Sample Preparation for Determination of Substances of Pharmaceutical Origin with the Use of HPLC-MS

High-performance liquid chromatography is an increasingly popular technique for final separation and qualitative and quantitative determination of analytes isolated from environmental samples (99–102). Liquid chromatography coupled with

MS was employed to determine the following substances of pharmaceutical origin:

- naproxen
- ibuprofen
- diclofenac
- diazepam
- carbamazepine
- antibiotics (penicillins, tetracyclines, sulfonamides, macrolide antibiotics)
- norfloxacin and ciprofloxacin

Figure 6 presents schematically different techniques of aqueous sample preparation for final analysis of NSAIDs and antibiotics by LC. As can be concluded from the scheme, multistep procedures of aqueous sample preparation are laborious and time consuming. The variety of operations and steps leads to final results that can be characterized by large error. Therefore validation or testing of applicability of the procedures proposed is crucial. Matrix reference materials are usually used for this purpose.

As mentioned above, the problems of determination of pharmaceutical residues in the environment have been developing for a few years and no reference material has been prepared which could be applied in these studies. Therefore, validation becomes a real challenge. The literature data have shown that, due to cycling of pollutants including pharmaceutical residues among different environmental compartments, bottom sediments are an integral part of the aqueous ecosystem and they have been analyzed as well as aqueous samples (103–105). Undoubtedly the next object of interest to analytical chemists will be biota samples since, only then, will it be possible to determine the cycling of these pollutants in a given ecosystem.

OCCURRENCE OF PHARMACEUTICAL RESIDUES IN DIFFERENT PARTS BIOSPHERE (DRAIN WATER, SURFACE WATER, SEDIMENTS)

The information on detection of pharmaceuticals and their metabolites in environmental samples has been increasing (106, 107). Available data are presented in Table 4.

SUMMARY

The rapid increase in interest about problems of emission, dispersion, and transformation of pharmaceutical residues in the environment has become one of the most important challenges for analytical chemists and also the driving force behind development of new analytical procedures.

The main attention of analytical chemists is focused on:

- possibility of determination of a wide spectrum of substances of pharmaceutical origin at low levels of concentration in one analytical cycle;

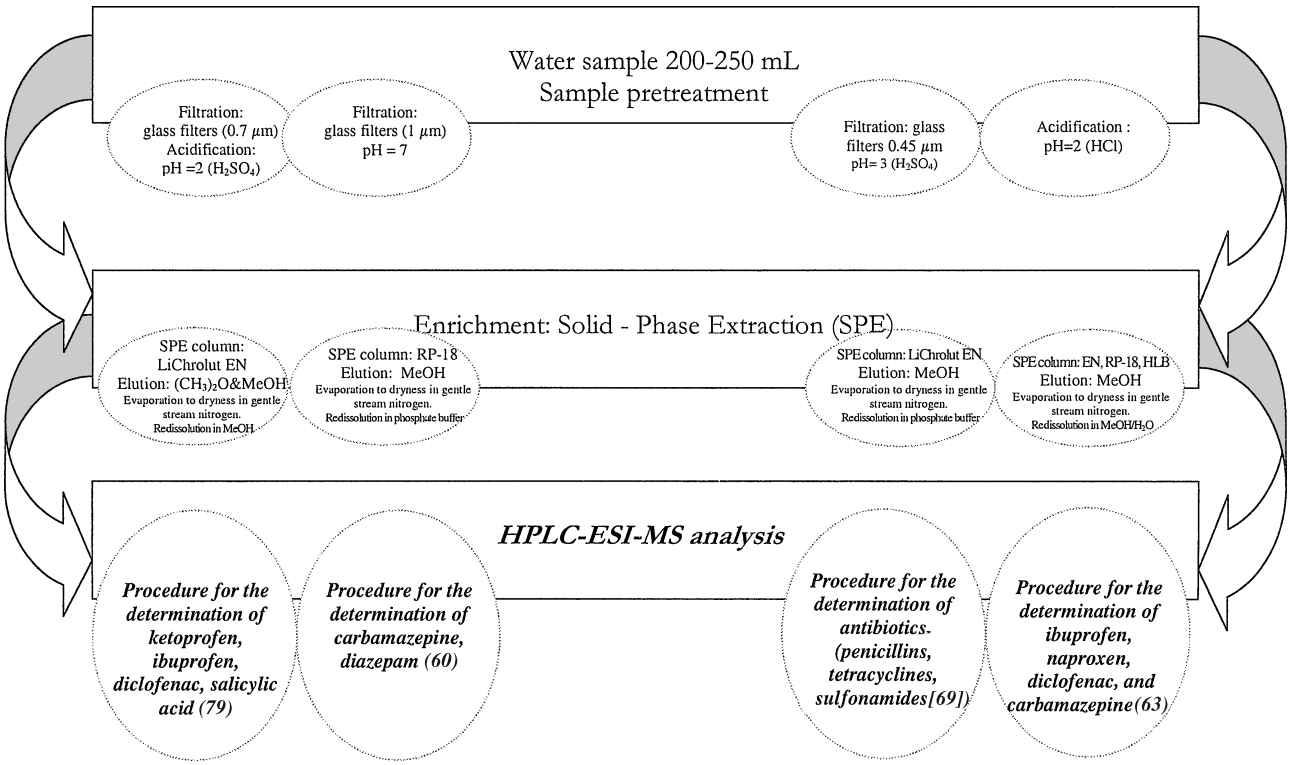

FIG. 6. Schematic representation of the analytical procedures used to determine residues of pharmaceuticals in aqueous samples with the use of high-performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS).

TABLE 4
Occurrence of Pharmaceuticals in the Environment

Analyte	Concentration	Place (town, region, country)	Year	Reference
		Wastewater (μg/l)		
Ketoprofen	0.38	Germany	1996	(108)
Ibuprofen	3.4	Germany	1996	(108)
2,5-Dihydroxybenzoic acid	0.59	Germany	1996	(108)
Salicylic acid	0.14	Germany	1996	(108)
Carbamazepine	0.27–1.47	Stuttgart, Germany	1999	(109)
Amantadine				
Diclofenac				
Primidone				
4-acetylaminoantipyrine				
NSAIDs	0.1–1	Rio de Janeiro, Brazil	1999	(110)
Indomethacin	0.95	Rio de Janeiro, Brazil	1999	(110)
Ibuprofen	0.37	California, USA	2000	(111)
Carbamazepine	2.1	California, USA	2000	(111)
17β-Estradiol	0.002	California, USA	2000	(111)
Estrogens	0.003	Michigan, USA	2000	(111)
Ibuprofen	2.8 (influent)	Spain	2001	(112)
	0.9 (effluent)			
Naproxen	3.6 (influent)	Spain	2001	(112)
	1.9 (effluent)			
Ciprofloxacin	0.25–0.41	Switzerland	2001	(113)
Norfloxacin	0.27–0.37	Switzerland	2001	(113)
Glutaraldehyde	170–3700	Rouen, France	2002	(114)
Ibuprofen	9.9 (influent)	Spain	2002	(112)
	2.15 (effluent)			
Naproxen	4.8 (influent)	Spain	2002	(112)
	2.7 (effluent)			
Clofibric acid	0.005 (effluent)	Greece	2002	(115)
Propyphenazone	0.2 (influent)	Greece	2002	(115)
Diclofenac	0.56 (influent)	Greece	2002	(116)
	0.01–0.36 (effluent)			
Clofibric acid	0.23	USA	2002	(117)
Sunscreen agents	8.2–9.9	Greece	2002	(118)
Naproxen	—	Maur, Switzerland	2003	(119)
Ibuprofen	—	Maur, Switzerland	2003	(119)
17β-Estradiol	0.0003–0.0025	Japan	2003	(120)
Estron	0.0025-0.034	Japan	2003	(120)
		Surface water (ng/l)		
Diclofenac	90	Men River, Germany	1998	(108)
Bezafibrate	100	Men River, Germany	1998	(108)
Clofibric acid	30	Men River, Germany	1998	(108)
Ibuprofen	20	Men River, Germany	1998	(108)
Gemifibrozil	20	Men River, Germany	1998	(108)
NSAIDs	20–40	Rio de Janeiro, Brazil	1999	(108)
Ethinyl estradiol	0.04	Tiber River, Italy	2000	(107)
Estron	1.5	Tiber River, Italy	2000	(107)
Ibuprofen	200–400	Leipzig, Germany	2000	(121)
Steroids	1–13	River Danube, Germany	2000	(122)

(Continued on next page)

TABLE 4
Occurrence of Pharmaceuticals in the Environment *(Continued)*

Analyte	Concentration	Place (town, region, country)	Year	Reference
Ibuprofen	5–15	Lakes, Switzerland	2000	(119)
	80	Rivers, Switzerland		
Naproxen	10	Lakes, Switzerland	2000	(119)
	10–400	Rivers, Switzerland		
Diclofenac	10	Lakes, Switzerland	2000	(119)
	20–150	Rivers, Switzerland		
Carbamazepine	35–60	Lakes, Switzerland	2000	(119)
	30–250	Rivers, Switzerland		
Estron	0.2–17	Thames River, Great Britain	2001	(123)
Codeine	17–123	Lake Mead, USA	2001	(124)
Carbamazepine	14–35	Lake Mead, USA	2001	(124)
Diazepam	3–62	Lake Mead, USA	2001	(124)
Primidone	11–130	Lake Mead, USA	2001	(124)
Trimethoprim	300–710	USA	2001	(87)
Testosterone	210	USA	2001	(87)
Progesterone	200	USA	2001	(87)
Bisphenol A	16–500	Germany	2001	(125)
4-Nonylphenol	6–135	Germany	2001	(125)
cis-Androsterone	210	USA	2001	(87)
Sotalol	560	Germany	2001	(126)
Phenazone	25	Germany	2001	(126)
Diclofenac	590	Germany	2001	(126)
Carbamazepine	900	Germany	2001	(126)
Sulfamethoxazole	410	Germany	2001	(126)
Androstenodione	127	Fenholloway River, USA	2001	(127)
Chlortetracycline	690	USA	2001	(87)
Ciprofloxacin	30	USA	2001	(87)
Lincomycin	730	USA	2001	(87)
Oxytetracycline	340	USA	2001	(87)
17β-Ethinyl estradiol	830	USA	2001	(87)
Tetracycline	110	USA	2001	(87)
Caffeine	24.1–41.2	Miami River, Florida, USA	2002	(128)
Caffeine	7.69–11.9	Biscayne Bay, Florida, USA	2002	(128)
Norfloxacin	5–12	Switzerland	2002	(113)
Ciprofloxacin	5–12	Switzerland	2002	(113)
Ibuprofen, naproxen	—	Aabach Moenchaltorf River, Aa Uster River, Switzerland	2003	(119)
		Sewage sludge (mg/kg of dm)		
Ciprofloxacin	0.27–2.42	Untreated raw sludge, Switzerland	2002	(113, 129)
Norfloxacin	0.27–2.37	Untreated raw sludge, Switzerland	2002	(113, 129)
Ciprofloxacin	2.1–2.4	Sludge-treated soil, Switzerland	2002	(113, 129)
Norfloxacin	2–2.4	Sludge-treated soil, Switzerland	2002	(113, 129)

- limitation of the number of laborious and time-consuming operations composing given analytical procedures;
- validation of analytical procedures;
- possibility of immunoanalysis applications (130) that could reduce the number of operations in the sample preparation step in order to eliminate, or at least reduce, the danger of false results. The analytical results would then be the basis of conclusions on occurrence and stability of pharmaceuticals in the environment. This could be an important guide for developers of pharmaceuticals at least as potent as those available now, but more friendly to the environment.

REFERENCES

1. C. Zwiener, T. Glauner, and F. H. Frimmel, *J. High Res. Chromatogr.* 23(7–8) (2000):474–478.
2. F. Stuer-Lauridsen, M. Birkved, L. P. Hansen, H.-C. Holten Lützhøft, and B. Halling-Sørensen, *Chemosphere* 40 (2000):783–793.
3. E. Zuccato, D. Calamarim, M. Natangelo, and R. Fanelli, *Lancet* 355 (2000):1789.
4. D. K. F. Meijer and J. Wilting, *Eur. J. Pharm. Biopharm.* 43 (1997):243–252.
5. H.-J. Guchelaar, L. Chandi, O. Schouten, and W. A. van den Brand, *Fresenius J. Anal. Chem.* 363 (1999):700–705.
6. M. Y. Haller, S. R. Müller, C. S. McArdell, A. C. Alder, and M. J.-F. Suter, *J. Chromatogr.* A952 (2002):111–120.
7. C. G. Daughton, *ACS Symp. Ser.* 791 (2001):348–364.
8. P. P. Fong, *ACS Symp. Ser*. 791 (2001):264–281.
9. D. B. Huggett, I. A. Khan, C. M. Foran, and D. Schlenk, *Environ. Pollut.* 121 (2003):199–205.
10. J. S. Teeter and R. D. Meyerhoff, *Environ. Toxicol. Chem.* 21 (2002):729–736.
11. R. Andreozzi, R. Marotta, G. Pinto, and A. Pollio, *Wat. Res.* 36 (2002):2869–2877.
12. H. F. Schröder, *Waste Management* 19 (1999):111–123.
13. R. Velagaleti and M. Gill, *ACS Symp. Ser.* 791 (2001):333–345.
14. C. Zwiener, T. Glauner, and F. H. Frimmel, *J. High Res. Chromatogr.* 23(7–8) (2000):474–478.
15. W.-H. Ding and C.-C. Chiang, *Rapid Commun. Mass Spectrom.* 17 (2003):56–63.
16. T. W. Federle, S. K. Kaiser, and B. A. Nuck, *Environ. Toxicol. Chem.* 21 (2002):1330–1337.
17. T. Heberer, A. These, and U. A. Grosch, *ACS Symp. Ser.* 791 (2001):142–155.
18. R. I. Holtey-Weber, H. A. Färber, and H. F. Schöler, *Vom Wasser* 92 (1999):1–9.
19. K. Holmström, S. Gräslund, A. Wahlström, S. Poungshompoo, B.-E. Bengtsson, and N. Kautsky, *Int. J. Food Sci. Tech.* 38 (2003):255–266.
20. R. Hirsch, T. A. Ternes, K. Haberer, and K.-L. Kratz, *Sci. Total Environ.* 225 (1999):109–118.
21. B. Halling-Sørensen, H. C. Holten Lützhøft, H. R. Andersen, and F. Ingerslev, *J. Antimicrob. Chemother.* 46 (2000):53–58.
22. T. Schwartz, W. Kohnen, B. Jansen, and U. Obst, *FEMS Microbiol. Ecol.* 43 (2003):325–335.
23. B. Halling-Sørensen, S. Nors Nielsen, P. F. Lanzky, F. Ingerslev, H. C. Holten Lützhøft, and S. E. Jørgensen, *Chemosphere* 36 (1998):357–393.
24. T. Heberer, *Toxicol. Lett.* 131 (2002):5–17.
25. M. J. López de Alda and D. Barceló, *Fresenius J. Anal. Chem.* 371 (2001):437–447.
26. M. J. López de Alda, S. Díaz-Cruz, M. Petrovic, and D. Barceló, *J. Chromatogr.* A1000 (2003):503–526.
27. M. Petrovic, E. Eljarrat, M. J. Lopez de Alda, and D. Barcelo, *J. Chromatogr.* A974 (2002):23–51.
28. S. D. Richardson, *Anal. Chem.* 74 (2002):2719–2742.
29. C. G. Daughton, *ACS Symp. Ser*. 791 (2001):2–38.
30. T. Heberer, *J. Hydrol.* 266 (2002):175–189.
31. K. Reddersen, T. Heberer, and U. Dünnbier, *Chemosphere* 49 (2002):539–544.
32. J. V. Holm, K. Rügge, P. L. Bjerg, T. H. Christensen, *Environ. Sci. Technol.* 29 (1995):1415–1420.
33. H.-B. Lee, T. E. Peart, G. Gris, and J. Chan, *Water Quality Research Journal of Canada* 37 (2002):459–472.
34. T. Scheytt, S. Grams, E. Rejman-Rasinski, T. Heberer, and H. J. Stan, *ACS Symp. Ser.* 791 (2001):84–99.
35. F. Sacher, S. Gabriel, M. Metzinger, A. Stretz, M. Wenz, F. T. Lange, H.-J. Brauch, and I. Blankenhorn, *Vom Wasser* 99 (2002):183–196.
36. R. Velagaleti and M. Gill, *ACS Symp. Ser.* 791 (2001):320–332.
37. S. A. Snyder, T. L. Keith, D. A. Verbrugge, E. M. Snyder, T. S. Gross, K. Kannan, and J. P. Giesy, *Environ. Sci. Technol.* 33 (1999):2814–2820.
38. R. A. Schnick, *Aquaculture* 196 (2001):277–288.
39. A. C. Alder, C. S. McArdell, E. M. Golet, S. Ibric, E. Molnar, N. S. Nipales, and W. Giger, *ACS Symp. Ser.* 791 (2001):56–69.
40. G. R. Boyd and D. A. Grimm, *Ann. N.Y. Acad. Sci.* 948 (2001):80–89.
41. S. Gräslund and B.-E. Bengtsson, *Sci. Total Environ.* 280 (2001):93–131.
42. S. Gräslund, K. Holmström, and A. Wahlström, *Mar. Pollut. Bull.* 46 (2003):81–90.
43. R. Velagaleti, P. K. Burns, M. Gill, and J. Prothro, *Environ. Health Perspect.* 110 (2002):213–220.
44. K. Gibiński, Farmacja i przemysł farmaceutyczny (in Polish), http://biblioteka.slam.katowice.pl/Biu301.htm
45. A. Zejc and M. Gorczyca, *Chemia Leków* (Warsaw: PZWL, 1998).
46. M. R. Garavito, *Świat Nauki* 7 (1999):80.
47. M. Gergov, J. N. Robson, I. Ojanperä, O. P. Heinonen, and E. Vuori, *Forensic Sci. Int.* 121 (2001):108–115.
48. C. W. Chen, NATO Science Series, Series 1, Life and Behavioral Sciences, Vol. 340, Chyczewski et al. (Eds.) (Amsterdam: IOS Press, 2002), 37–48.
49. Drug Institute in Warsaw, private information.
50. R. E. Alcock, A. Sweetman, and K. C. Jones, *Chemosphere* 38 (1999):2247–2262.
51. O. A. H. Jones, N. Voulvoulis, and J. N. Lester, *Wat. Res.* 36 (2002):5013–5022.
52. J. A. Hinson, N. R. Pumford, and S. D. Nelson, *Drug Met. Rev.* 26 (1994):395–412.
53. S. E. Jorgensen and B. Halling-Sørensen, *Chemosphere* 40 (2000):691–699.
54. C. G. Daughton and A. T. Ternes, *Environ. Health Perspect.* 107 (1999):907–938.
55. M. Petrovic, D. Barcelo, A. Diaz, and F. Ventura, *J. Am. Soc. Mass Spectrom.* 14 (2003):516–527.
56. A. C. Hogenboom, W. M. A. Niessen, and U. A. T. Brinkman, *J. Chromatogr.* A794 (1998):201–210.
57. I. Coille, S. Reder, S. Bucher, and G. Gauglitz, *Biomol. Eng.* 18 (2002):273–280.
58. M. Śliwka-Kaszyńska, A. Kot-Wasik, and J. Namieśnik, *Crit. Rev. Env. Sci. Technol.* 33 (2003):31–44.
59. E. M. Golet, A. C. Alder, A. Hartmann, T. Ternes, and W. Giger, *Anal. Chem.* 73 (2001):3632–3638.
60. T. A. Ternes, *Trends Anal. Chem.* 20 (2001):419–434.
61. T. Ternes, M. Bonerz, and T. Schmidt, *J. Chromatogr.* A938 (2001):175–185.

62. T. Hirai, S. Matsumoto, and I. Kishi, *J. Chromatogr.* B692 (1997):375–388.
63. W. Ahrer, E. Scherwenk, and W. Buchberger, *J. Chromatogr.* A910 (2001):69–78.
64. S. Ollers, H. P. Singer, P. Fassler, and S. R. Muller, *J. Chromatogr.* A911 (2001):225–234.
65. X.-S. Miao, B. G. Koenig, and C. D. Metcalfe, *J. Chromatogr.* A952 (2002):131–138.
66. T. A. Ternes, M. Meisenhiemer, D. Mcdowell, F. Sacher, H.-J. Brauch, B. Haist-Gulde, G. Preuss, U. Wilme, and N. Zulei-Seibert, *Environ. Sci. Technol.* 36 (2002):3855–3863.
67. S. Weigel, J. Kuhlmann, and H. Hühnerfuss, *Sci. Total Environ.* 295 (2002):131–141.
68. H. R. Buser, M. D. Müller, and N. Theobald, *Environ. Sci. Technol.* 32 (1998):188–192.
69. R. Hirsch, T. A. Ternes, K. Haberer, A. Mehlich, F. Ballwanz, and K.-L. Kratz, *J. Chromatogr.* A815 (1998):213–223.
70. T. A. Ternes, H. Andersen, D. Gilberg, and M. Bonerz, *Anal. Chem.* 74 (2002):3498–3504.
71. M. J. López de Alda and D. Barceló, *J. Chromatogr.* A938 (2001):145–153.
72. A. Diaz and F. Ventura, *Anal. Chem.* 74 (2002):3869–3876.
73. J. A. McLachlan, L. J. Guillette, Jr. T. Iguchi, and W. A. Toscano Jr., *Ann. N.Y. Acad. Sci.* 948 (2001):153.
74. M. Stumpf, T. A. Ternes, K. Haberer, and W. Baumann, *Vom Wasser* 91 (1998):291–303.
75. M. Winkler, J. R. Lawrence, and T. R. Neu, *Wat. Res.* 35 (2001):3197–3205.
76. H. R. Buser, T. Poiger, and M. D. Müller, *Environ. Sci. Technol.* 32 (1998):3449–3456.
77. M. Meisenhiemer and T. A. Ternes, *Vom Wasser* 94 (2000):203–212.
78. M. E. Lindsey, M. Meyer, and E. M. Thurman, *Anal. Chem.* 73 (2001):4640–4646.
79. M. Farré, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, and D. Barceló, *J. Chromatogr.* A938 (2001):187–197.
80. L. K. Sørensen and H. Hansen, *J. Liq. Chromatogr. R. T.* 25 (2002):1063–1075.
81. C. Hartig, T. Storm, and M. Jekel, *J. Chromatogr.* A854 (1999):163–173.
82. J. Klimeš, J. Sochor, and J. Kříž, *Il Farmaco* 57 (2002):117–122.
83. A. Kunkel, S. Günter, and H. Wätzig, *J. Chromatogr.* A768 (1997):125–133.
84. Y. Guang-Guo, R. S. Kookana, and Z. Chen, *J. Environ. Sci. Health* B37 (2002):225–234.
85. B. R. Simmons and J. T. Stewart, *J. Chromatogr.* B688 (1997):291–302.
86. D. W. Kolpin, E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton, *Environ. Sci. Technol.* 36 (2002):1202–1211.
87. F. Ariese, W. H. O. Ernst, and D. T. H. M. Sijm, *Environ. Toxicol. Pharmacol.* 10 (2001):65–80.
88. E. Gelpi, *J. Chromatogr.* A703 (1995):59–80.
89. R. Jeannot, H. Sabik, E. Sauvard, T. Dagnac, and K. Dohrendorf, *J. Chromatogr.* A974 (2002):143–159.
90. Y. Kim, S. Park, J. Park, and W. Lee, *J. Chromatogr.* A689 (1995):170–174.
91. M. T. Meyer, J. E. Bumgarner, J. L. Varns, J. V. Daughtridge, E. M. Thurman, and K. A. Hostetler, *Sci. Total Environ.* 248 (2000):181–187.
92. T. A. Ternes, G. Brenner-Weiß, T. Eggert, J. Müller, F. Kirschhöfer, M. Nusser, R.-D. Wilken, and U. Obst, *Vom Wasser* 93 (1999):255–263.
93. T. A. Ternes, R. Hirsch, J. Mueller, and K. Haberer, *Fresenius J. Anal. Chem.* 362 (1998):329–340.
94. R. J. W. Meesters and Y. H. F. Schröder, *Anal. Chem.* 74 (2002):3566–3574.
95. T. Murahashi, S. Sasaki, and T. Nakajima, *J. Health Sci.* 49 (2003):72–75.
96. D. C. McAvoy, B. Schatowitz, M. Jacob, A. Hauk, and W. S. Eckhoff, *Environ. Toxicol. Chem.* 21 (2002):1323–1329.
97. A. Zafra, M. Olmo, B. Suárez, E. Hontoria, A. Navalón, and J. L. Vílchez, *Wat. Res.* 37 (2003):735–742.
98. H. R. Buser, T. Poiger, and M. D. Müller, *Environ. Sci. Technol.* 33 (1999):2529–2535.
99. D. Lauden and A. Handley, *Anal. Chem.* 72 (2000):3922–3926.
100. R. A. Muljono, F. L. Darsono, J. J. C. Scheffer, and R. Verpoorte, *J. Chromatogr.* A927 (2001):39–45.
101. T. Matsuya, N. Hoshino, M. Ogasawara, T. Harita, and S. Arao, *Environ. Sci.* 9 (2002):393–404.
102. D. C. Mays, D. E. Sharp, C. A. Beach, R. A. Kershaw, J. R. Blanchine, and N. Gerber, *J. Chromatogr.* 311 (1984):301–309.
103. R. Delepee, D. Maume, B. Le Bizec, and H. Pouliquen, *J. Chromatogr.* B748 (2000):369–381.
104. H. Hektoen, J. A. Berge, V. Hormazabal, and M. Yndestad, *Aquaculture* 133 (1995):175–184.
105. M. J. López de Alda, S. Díaz-Cruz, M. Petrovic, D. Barceló, Analysis of Selected Emerging Pollutants (Steroid Sex Hormones, Drugs and Alkylphenolic Surfactants) in the Aquatic Environment by LC-MS and LC-MS-MS, in *New Horizons and Challenges in Environmental Analysis and Monitoring*, ed. J. Namieśnik, W. Chrzanowski, and P. Żmijewska (Gdańsk: Centre of Excellence in Environmental Analysis and Monitoring, 2003), ch. 11.
106. W. Ahrer and W. Buchberger, *Am. Lab.* 12 (2001):31–35.
107. C. Baronti, R. Curini, G. D'Ascenzo, A. Corcia, A. Gentili, and R. Samperi, *Environ. Sci. Technol.* 34 (2000):5059–5066.
108. T. A. Ternes, *Wat. Res.* 32 (1998):3245–3260.
109. E. Möhle, S. Horvath, W. Merz, and J. W. Metzger, *Vom Wasser* 92 (1999):207–223.
110. M. Stumpf, T. A. Ternes, R. D. Wilken, S. W. Rodrigues, and W. Baumann, *Sci. Total Environ.* 225 (1999):135–141.
111. D. L. Sedlak, J. L. Gray, and K. E. Pinkston, *Environ. Sci. Technol.* 1 (2000):509–515.
112. I. Rodrıguez, J. B. Quintana, J. Carpinteiro, A. M. Carro, R. A. Lorenzo, and R. Cela, *J. Chromatogr.* A985 (2003):265–274.
113. E. M. Golet, A. C. Alder, and W. Giger, *Environ. Sci. Technol.* 36 (2002):3645–3651.
114. B. Jolibois, M. Guerbet, and S. Vassal, *Arch. Environ. Contam. Toxicol.* 42 (2002):137–144.
115. V. Koutsouba, T. Heberer, B. Fuhrmann, K. Schmidt-Baumler, D. Tsipi, and A. Hiskia, *Chemosphere* 51 (2003):69–75.
116. K. Kümmerer, *Chemosphere* 45 (2001):957–969.
117. D. B. Patterson, W. C. Brumley, V. Kelliher, and P. L. Ferguson, *Am. Lab.* 34 (2002):20–28.

118. D. A. Lambropoulou, D. L. Giokas, V. A. Sakkas, T. A. Albanis, and M. I. Karayannis, *J. Chromatogr.* A967 (2002):243–253.
119. C. Tixier, H. Singer, S. Oellers, and S. R. Müller, *Environ. Sci. Technol.* 37 (2003):1061–1068.
120. T. Isobe, H. Shiraishi, M. Yasuda, A. Shinoda, H. Suzuki, and M. Morita, *J. Chromatogr.* A984 (2003):195–202.
121. M. Moeder, S. Schrader, M. Winkler, and P. Popp, *J. Chromatogr.* A873 (2000):95–106.
122. M. H. Kuch and K. Ballschmiter, *Environ. Sci. Technol.* 35 (2001):3201–3206.
123. X.-Y. Xiao, D. V. McCalley, and J. McEvoy, *J. Chromatogr.* A923 (2001):195–204.
124. S. A. Snyder and K. L. Kelly, *ACS Symp. Ser.* 791 (2001):116–139.
125. M. H. Kuch and K. Ballschmiter, *Fresenius J. Anal. Chem.* 366 (2000):392–395.
126. F. Sacher, F. T. Lange, and H. J. Brauch, *J. Chromatogr.* A938 (2001):199–210.
127. E. J. Durhan, C. Lambright, V. Wilson, B. C. Butterworth, D. W. Kuehl, E. F. Orlando, L. J. Guillette Jr., L. E. Gray, and G. T. Ankley, *Environ. Toxicol. Chem.* 21 (2002):1973–1976.
128. P. R. Gardinali and X. Zhao, *Environ. Int.* 28 (2002):521–528.
129. E. M. Golet, A. Strehler, A. C. Alder, and W. Giger, *Anal. Chem.* 74 (2002):5455–5462.
130. F. Valentini, D. Compagnone, A. Gentili, and G. Palleschi, *Analyst* 127 (2002):1333–1337.