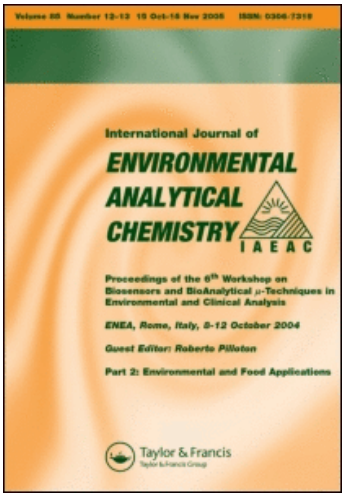

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ANALYSIS OF ACIDIC DRUGS IN SWISS WASTEWATERS

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Five acidic drugs (clofibric acid, ibuprofen, ketoprofen, mefenamic acid and diclofenac) were chosen in order to determine their behavior in a sewage treatment plant (STP). An analytical method using solid phase extraction (SPE) and a gas chromatograph coupled with a mass spectrometer (GC-MS) was used. The results show that four pharmaceuticals (clofibric acid, ketoprofen, mefenamic acid and diclofenac) are not well removed by treatment in Swiss STPs. Maximum concentration in the effluent was determined for mefenamic acid up to 1.0 μg/L. This component seems to be relevant in Swiss STPs effluents and we can expect its presence in surface waters.

Keywords: Nonsteroidal anti-inflammatory drugs; Lipid regulating agent metabolite; Wastewater; Sewage treatment plant

INTRODUCTION

Among anthropogenic substances capable of being harmful to the environment, drugs and their metabolites are of growing interest. Indeed, regarding their biological activity they could induce unknown effects on aquatic fauna [1]. Recent studies have shown that some pharmaceuticals are present in the aquatic medium at concentrations of about one hundred ng/L [2–5]. Although these concentrations are too low to cause acute toxicity, they can induce chronic long term effects.

In Switzerland, statistics about the quantities of drugs consumed are not available. In order to have an indication for the most sold pharmaceuticals, we conducted a survey among chemists concerning the drugs they sell the most frequently. The results of this survey are presented in Table I. We chose four substance (Table II) according to their ability to be analyzed by the same method: ibuprofen, ketoprofen, mefenamic acid and diclofenac. Although acetaminophen and salicylic acid are among the most sold, we did not focus our interest on these substances because they are known to be entirely

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TABLE I List of most sold pharmaceuticals in Swiss pharmacies (Vaud canton)

Substance	*Application*
Acetaminophen	Analgesic
Amoxicilline	Antibacterial
n-acetylcysteine	Mucolytic
Mefenamic acid	Antiinflammatory
Acetyl salicylic acid	Analgesic
Zolpidem	Hypnotic
Carbocystein	Mucolytic, expectorant
Omeprazol	Antiulcerative
Tyrothrycine	Topical antibacterial
Phenylephrine HCl	Mydriatic, decongestant
Diclofenac	Antiinflammatory
Ibuprofen	Antiinflammatory
Estradiol, hormones	Contraceptive
Oxazepam	Anxiolytic
Ascorbic acid	Vitamin
Xylometazoline	Adregenic (vasoconstrictor)
Hexetidine	Antifungal
Codeine	Narcotic analgesic, antitussive
Clarithromycine	Antibiotic
Enalapril	Antihypertensive

TABLE II Substances chosen and some related names of medicine on sale

Active substances or metabolites	*Synonyms*	*Medicines on sale in Switzerland*
Clofibric acid	2-(4)-chlorophenoxy-2-methyl propionic acid	Clofibrat Tripharma®, Lipo-Merz® retard, Duolip®
Ibuprofen	2-(4-isobutylphenyl)-propionic acid	Algifor®, Dolocyl®, Ecoprofen®, etc.
Ketoprofen	2-(meta-benzoylphenyl)-propionic acid	Fastum®
Mefenamic acid	*N*-(2,3-xylyl)anthranilic acid	Méfé-basan, Ponstan®, Spiralgin®, etc.
Diclofenac	2-[(2,6-dichlorophenyl)amino] benzeneacetic acid	Inflama®, Voltaren, Olfen®, etc.

degraded in sewage treatment plants (STP) [6]. Moreover, we added clofibric acid because it is one of the oldest drugs detected in the environment and it was found to be ubiquitous in surface water [7].

Clofibric acid is a metabolite of clofibrate, etofibrate and etofyllinclofibrate which are drugs used as blood lipid regulators. It is excreted in the urine, predominantly in the form of a glucuronide conjugate [8]. It has already been detected in STP effluents at concentrations up to 1600 ng/L [4]. In the aquatic environment, it was detected in German river waters at concentrations up to 550 ng/L [6] and in Swiss lakes at concentrations up to 9 ng/L [9]. It was even found in samples of ground water wells at a drinking water treatment plant (up to 7300 ng/L) [5].

Ibuprofen, ketoprofen, mefenamic acid and diclofenac are nonsteroidal anti-inflammatory drugs (NSAIDs). They are largely used for their analgesic, anti-inflammatory and antipyretic properties in a growing number of applications.

Ibuprofen is one the most important pharmaceuticals in term of quantities consumed. It is rapidly excreted in the urine (60%) and in feces (40%) mainly as metabolites and their conjugates. About 4% is excreted in urine as unchanged form. Diclofenac is mainly used as sodium salt. It is excreted primarily in the form of metabolites, mainly in the urine (55–65%) but also in the bile. Ketoprofen is metabolized mainly by conjugation with glucuronic acid, and is excreted in the urine (85%). Over 50% of a dose of mefenamic acid may be recovered in the urine, mainly as conjugated metabolites [10].

Some of these compounds were already detected in German surface waters [6] with maximum concentrations as follow: 530 ng/L (ibuprofen), 1200 ng/L (diclofenac), 120 ng/L (ketoprofen). To our knowledge, no study has been conducted on mefenamic acid in STP or in the environment. The objectives of the present one are to determine, with a semiquantitative method, in which proportions these products are removed by some Swiss sewage treatment plants (STP) and to evaluate the quantities discharged in the effluents.

EXPERIMENTAL

Reagents and Solvents

Acetone, *n*-hexane, methanol (super purity solvent, Romil), toluene (GR, Merck). Pentafluorobenzyl bromide, 2-(4-chlorphenoxy)propionic acid (Aldrich), Ibuprofen, ketoprofen (USP XXIII, Sigma), suprofen, diclofenac, clofibric acid, mefenamic acid (Sigma). Stock solutions of all compounds were prepared in methanol.

Analytical Method

Solid Phase Extraction

One liter of the sample was filtered (0.45 μm) and the pH was adjusted to 2 with concentrated HCL solution. Subsequent extraction of solid matter retained by the 0.45 μm filter with diethyl ether did not show any presence of analytes of interest. Extraction was effected by percolation through a reverse phase packed tube (Supelclean ENVI-18, 1 g, 6 mL) at a flow rate of approximately 3 mL/min by applying a low vacuum. The solid phase was previously conditioned by flushing with 3 mL acetone, followed by 3 mL methanol and 3 mL of water adjusted to $pH < 2$. After drying the solid phase for one hour under vacuum, the analytes were eluted with 6 mL of methanol. The methanol extract was dried under a gentle stream of nitrogen.

Derivatization

Derivatization was performed as described by Heberer [11], at 90°C for one hour using 400 μL of pentafluorobenzyl bromide (2% in toluene) and 4 μL of triethylamine.

Clean-up

The derivatized extract was passed through a small silica column (0.7 g of silica gel 60, 35–70 mesh ASTM, Merck; 5 mm i.d. Pasteur pipette). The analytes were eluted with 15 mL of toluene. The eluate was dried under a gentle stream of nitrogen to a

volume of 100 µL and corrected for quantification with 2-(4-chlorphenoxy)propionic acid (CPP) as internal standard. The internal standard CCP is chosen seeing that it is a propionic acid, as most of studied chemicals. Then, gas chromatograph coupled with a mass spectrometer (GC-MS) was used.

GC-MS Parameters

Separation and detection of the analytes was achieved using a GC-MS system: a Hewlett-Packard HP 5890 gas chromatograph coupled with a HP 5971 A mass spectrometer. The gas chromatograph was equipped with a 50 m × 0.2 mm i.d. × 0.33 µm DB-5 capillary column and a 5 m deactivated fused silica 250 precolumn. The head pressure was 30 psi helium and the linear velocity was 23.2 cm/s.

GC injection parameters: 1 µL stainless (purge delay time: 1 min); injection port: 210°C; 100°C for 1 min; 30°C/min to 150°C; 150°C isothermal 1 min; 7.5°C/min to 215°C isothermal 20 min; 15°C/min to 290°C; 290°C isothermal 35 min.

MS parameters: transfer line temperature: 250°C; EI mode, electron energy: 70 eV. For SIM mode, three characteristic ions were selected for each compound (Table III) and scanned using corresponding time windows with a dwell time of 150 ms per ion. The repeatability standard deviation of the instrument is smaller than 10% for each compound analyzed.

Determination of Recoveries and Detection Limits

One liter of STP influent or effluent was spiked with 1 mL of a standard solution (1 µg/mL in methanol) of the five compounds. SPE extraction, clean up, derivatisation and detection by GC-MS were performed as described above. Recoveries (Table IV) were determined by comparison with nonspiked samples using a six points calibration curve.

TABLE III GC/MS data of the PFB derivatives

Compound	*Retention time* (min)	*Characteristic ions (relative abundance)*	*Time window* (min)
CPP (internal standard)	29.0	128 (40), 155 (100), 380 (29)	24.0–31.0
Clofibric acid	29.4	128 (100), 130 (33), 394 (3)	24.0–31.0
Ibuprofen	31.9	118 (33), 161 (100), 386 (12)	31.0–34.0
Ketoprofen	51.0	105 (70), 209 (100), 434 (8)	34.0–51.4
Mefenamic acid	51.7	194 (60), 223 (100), 421 (70)	51.4–53.0
Diclofenac	54.2	216 (100), 214 (35), 477 (7)	38.5–43.33

TABLE IV Average recoveries of acidic pharmaceuticals in STP influents and effluents

Substance	*Recovery ± relative standard deviation* (%)	
	Influent (n = 3)	*Effluent (n = 3)*
Clofibric acid	46 ± 2	44 ± 4
Ibuprofen	31 ± 8	45 ± 9
Ketoprofen	133 ± 16	143 ± 12
Mefenamic acid	35 ± 10	76 ± 8
Diclofenac	55 ± 16	54 ± 13

TABLE V Limits of detection and quantification

Substance	*Limits of detection GC-MS* mg/L (S/N = 3)	*Limit of quantification GC-MS* mg/L (S/N = 10)	*Limit of quantification enrichment procedure* ng/L
Clofibric acid	0.007	0.02	2
Ibuprofen	0.004	0.01	1
Ketoprofen	0.23	0.8	80
Mefenamic acid	0.06	0.2	20
Diclofenac	0.05	0.2	20

We observe a great variability in recovery measures as described by Heberer [12] for clofibric acid in wastewater. This variability can be due to the dissolved organic matter that can interfere with the adsorption of pharmaceuticals on the solid phase used for extraction. Ketoprofen shows a very high recovery. This difference with other substances may be due to interferences during the detection.

Due to the low recoveries and to avoid a high degree of uncertainty, the concentrations are not corrected for recoveries. Thereby, the presented results are semi-quantitative.

Limits of detection were calculated as three times the background signal and the limits of quantification as ten times the background signal (Table V).

Sampling of Influents and Effluents of STPs

Random samples of influent and effluent of 3 municipal sewage treatment plants were taken in order to analyze acidic pharmaceuticals (see Fig. 1 for location). A flow proportional automatic sampler carried out sampling over 24 h. All the STPs discharge their effluents into the lake Geneva and their characteristics are shown in Table VI. Each one collects domestic wastewater. In addition, the plants of Nyon and Aubonne collect waters from pharmaceutical industries. Every plant consisted of a physical and a biological treatment stage. In the plant of Nyon an additional physico-chemical treatment is used before the biological treatment.

RESULTS AND DISCUSSION

Mass Spectra

To our knowledge, only the spectrum of clofibric acid PFB ester was published [11]. The spectrum we obtained is similar to the published one. The spectra of other compounds are shown in Fig. 2. The mass $m/z = 181$ is common in all spectra. It is characteristic of the pentafluorobenzyl ester part of the compounds and its intensity is generally high.

Occurrence of Acidic Drugs in STP Waters

Clofibric Acid

As it is shown in Fig. 3a only a small quantity of clofibric acid is eliminated from water by the STP. Moreover, for two cases out of six, the concentration in the effluent is higher than the concentration in the influent. Such a result was already described by

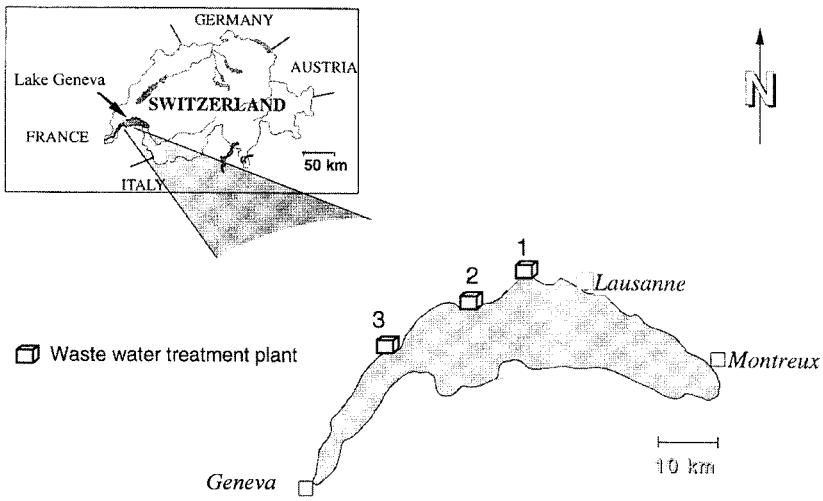

FIGURE 1 Map of Lake Geneva with the location of wastewater treatment plants, 1: Morges, 2: Aubonne, 3: Nyon.

TABLE VI Characteristics of the sewage treatment plants

Location	*Type of sewage*	*Equivalents inhabitants*	*Average effluent flow rate* (m^3/24 h)
Aubonne	Domestic pharmaceutical plant hospital	3800	1300
Morges	Domestic hospital	32 000	12 500
Nyon	Domestic pharmaceutical plant	40 000	8500

Garrison *et al.* [13] in domestic wastewater in USA. They found concentrations of clofibric acid in raw wastewater of 0 and 0.8 µg/L and in activated sludge effluent of 1 and 2 µg/L. The authors suggest that clofibric acid could have been adsorbed on activated sludge particulate matter from earlier raw sewage effluents and partially desorbed during the sampling. Regarding our results on 24 h sampling, it seems that the efficiency of removal is dependent on the functioning of the STP, which varies from day to day. Removal efficiencies range from approximately 0 to 40% and the maximum concentration in the effluent reaches 0.2 µg/L. Another explanation could be that during 24 h the biodegradation of clofibric acid could be higher in sampling bottles of the influent than in the effluent water.

The higher concentration in effluents could also be explained by the time delays of the water moving through the plants. So influent and effluent samples were never exactly the same parcel of water.

Nonsteroidal Anti-inflammatory Drugs

Ibuprofen is well eliminated by the STP process (Fig. 3b) except in one case (Aubonne 30/11/99) corresponding probably to a STP dysfunction, since same results are found

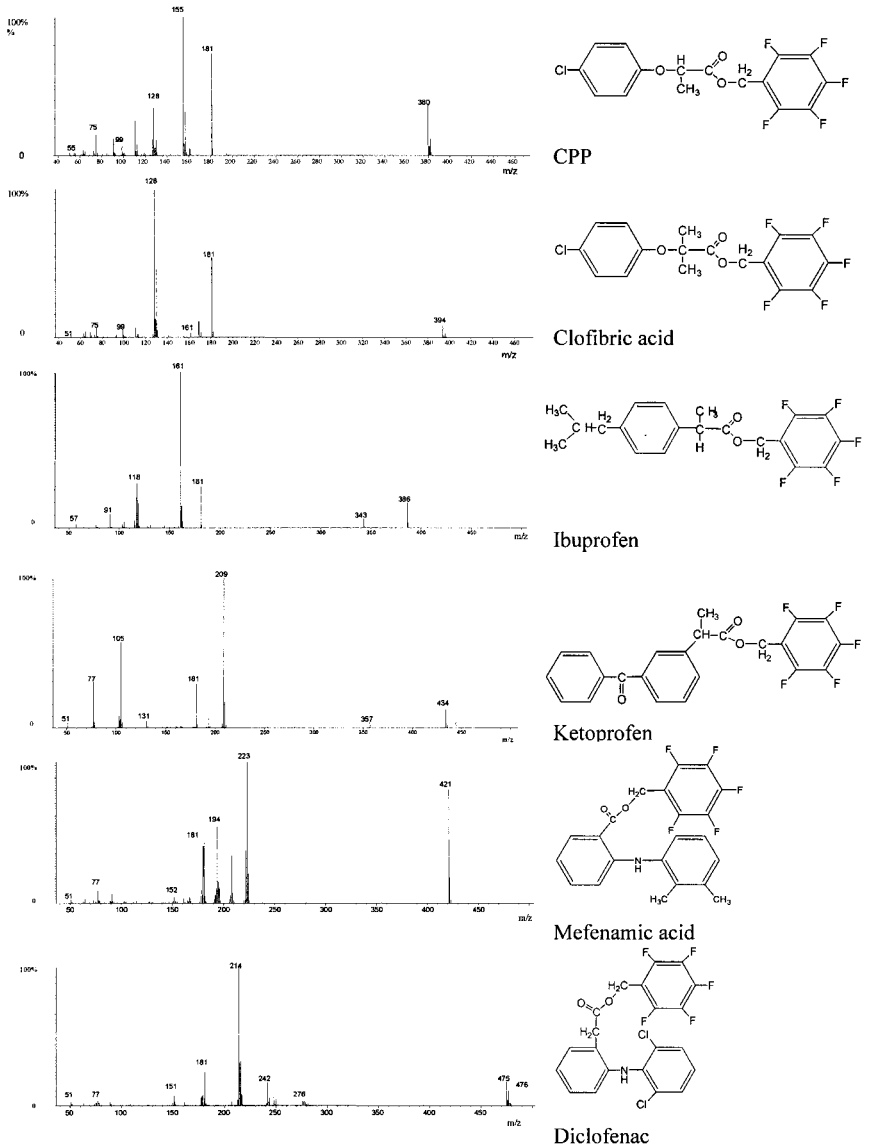

FIGURE 2 Mass spectra and structural formula of PFB esters of the indicated drugs.

for two other substances (mefenamic acid and diclofenac). This is in agreement with the observation made by Ternes [6] for a German sewage treatment plant.

Our results show that concentrations of Ibuprofen in Swiss STP effluent vary between 0.01 and 0.3 µg/L, which is lower than the data published in the literature [6].

Removal of mefenamic acid depends on the type of treatment (Fig. 3d). The STP of Nyon is more efficient than the STP of Morges in which we observed a maximal concentration of 1.0 µg/L. One explanation is that the plant of Nyon functioned for a long time in a stable way whereas the STP of Morges was in way of stabilization.

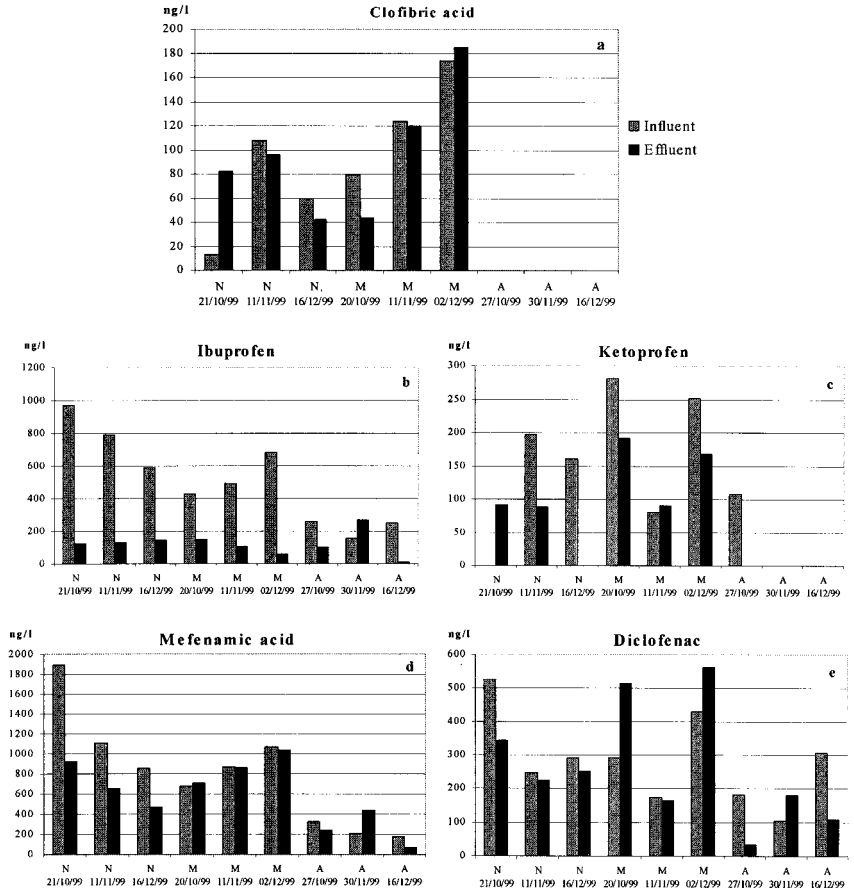

FIGURE 3 Concentrations of acidic drugs in wastewater STP. N: Nyon; M: Morges; A: Aubonne.

The STP of Aubonne is efficient, excepted for the 30 November sample, which cause problems for other substances (see above).

As mefenamic acid, the behavior of diclofenac seems to depend on the STP (Fig. 3e). Globally the efficiency of removal is bad. The maximal concentration in the effluent reaches 0.6 µg/L.

The results of ketoprofen show a great variability (Fig. 3c), which could be explained by the detection interferences. The quantities of that product discharged in STP effluent are weak compared to other drugs; the maximum concentration is 0.2 µg/L.

CONCLUSION

These results show that the analyzed drugs are not removed by the sewage treatment plants except for ibuprofen. However, the quantities of this drug in the influents are so high that the levels detected in the outflows are significant. On the contrary, although clofibric acid and ketoprofen are persistent in STPs, as the inputs seem to

be low the quantities rejected are weak. Substances that seem to pose a problem are mefenamic acid and diclofenac. They are not removed by the STP and the quantities discharged are important. Studies on the behavior of diclofenac in surface water show that it is rapidly degraded by photodegradation in multiple products [14]. No data concerning the occurrence, fate and the ecotoxicity of mefenamic acid exist. This product is one of the most sold in Switzerland and as the removal in STP is insufficient, we can suppose that it will be found in surface waters.

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