CHAPTER 1
INTRODUCTION
WATER

Water is a ubiquitous chemical substance that is composed of hydrogen and oxygen and is essential for all known forms of life. Water on Earth moves continually through a cycle of evaporation or transpiration (evapotranspiration), precipitation, and runoff, usually reaching the sea. Over land, evaporation and transpiration contribute to the precipitation.

Clean, fresh drinking water is essential to human and other life forms. There is a clear correlation between access to safe water and GDP per capita. However, some observers have estimated that by 2025 more than half of the world population will be facing water-based vulnerability (Kulshreshtha 1998). Water plays an important role in the world economy, as it functions as a solvent for a wide variety of chemical substances and facilitates industrial cooling and transportation. Approximately 70% of freshwater is consumed by agriculture (Baroni et al. 2007).

WATER QUALITY

Water quality is one of the most relevant topics in efforts towards environmental and sustainable development. Still in many countries surface water is one of the most important sources of drinking water. Water intake is crucial to our survival. For example, drinking ample amounts of water has been tied to general good health. Also, water can be a specific antidote to some of the more troubling and inconvenient health problems, such as obesity and many types of cancer. Water has the potential to be one of the most useful and cost-effective medicinal substances available.

Unfortunately, dangerous chemicals, organic materials, and bacteria contaminate much of the water we drink. When combined with these elements, water, crucial to our survival as it is, can present a significant health risk. Despite several governmental efforts to clean, purify, and provide safer sources of water, dangerous contaminants continue to be present in our drinking water. These contaminants, many of which are undetectable by sight or taste, can lead to diseases ranging from asthma to the debilitating Parkinson’s disease (www.historyofwaterfilters.com).
In order to understand drinking water contamination, it is necessary to first understand from where our drinking water comes. For most urban residents, relying upon municipal water systems, drinking water comes from two major sources: groundwater and surface water.

Each source of water has a unique set of contaminants; groundwater stores pesticide chemicals and nitrate while surface water contains most bacteria and other microorganisms. Because of the interconnectedness of groundwater and surface water, these contaminants may be shared between the two sources. Neither water source can ever be entirely free from water contaminants. Due to the cycle of water (hydrology), the two sources of drinking water feed each other, sharing contaminants.

Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either does not support a human use, like serving as drinking water, and/or undergoes a marked shift in its ability to support its constituent biotic communities, such as fish. Natural phenomena such as volcanoes, algal blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water.

FACTORS INFLUENCING WATER QUALITY

Water pollution is the contamination of water bodies such as lakes, rivers, oceans, and groundwater. All water pollution affects organisms and plants that live in these water bodies and in almost all cases the effect is damaging not only to individual species and populations but also to the natural biological communities. It occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful constituents.

Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases, (Pink 2006; West 2006) and that it accounts for the deaths of more than 14,000 people daily (West 2006). Some 90% of China's cities suffer from some degree of water pollution, (Chinadaily.com.cn 2005) and nearly 500 million people lack access to safe drinking water (The New York Times 2007). In addition to the acute problems of water pollution in developing countries, industrialized countries continue to struggle with
pollution problems as well. In the most recent national report on water quality in the United States, 45% of assessed stream miles, 47% of assessed lake acres, and 32% of assessed bay and estuarine square miles were classified as polluted (EPA 2007).

Water pollution has many causes and characteristics. Point source pollution refers to contaminants that enter a waterway through a discrete conveyance, such as a pipe or ditch. Examples of sources in this category include discharges from a sewage treatment plant, a factory, a city storm drain or from a construction site. Non – point source (NPS) pollution refers to diffuse contamination that does not originate from a single discrete source. NPS pollution is often accumulative effect of small amounts of contaminants gathered from a large area. The leaching out of nitrogen compounds from agricultural land which has been fertilized is a typical example. Nutrient runoff in storm water from "sheet flow" over an agricultural field or a forest are also cited as examples of NPS pollution. Contaminated storm water washed off of parking lots, roads and highways, called urban runoff, is sometimes included under the category of NPS pollution. Often, this runoff is typically channeled into storm drain systems and discharged through pipes to local surface waters, and is a point source. However where such water is not channeled and drains directly to ground it is a non – point source.

The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens, and physical or sensory changes such as elevated temperature and discoloration.

Oxygen – depleting substances may be natural materials, such as plant matter (e.g. leaves and grass) as well as man – made chemicals. Other natural and anthropogenic substances may cause turbidity (cloudiness) which blocks light and disrupts plant growth, and clogs the gills of some fish species (EPA 2005).

Many of the chemical substances are toxic. Pathogens can produce waterborne diseases in either human or animal hosts. Alteration of water's physical chemistry includes acidity (change in pH), electrical conductivity, temperature, and eutrophication.
PHARMACEUTICALS IN WATER

The focus of environmental research has recently extended beyond classic environmental pollutants, such as PCBs, dioxins, and pesticides, to pharmaceuticals and personal care products (PPCPs), which enter the environment mainly via regular domestic use (Daughton and Ternes 1999) and other sources.

During the past three decades, research on the impact of chemical pollution has focused almost exclusively on the conventional 'priority' pollutants [i.e. persistent organic pollutants (POPs)] and this has been extensively reviewed recently (Birkett and Lester 2002). Today, these compounds are less relevant for many first world countries because emissions have been substantially reduced through the adoption of appropriate legal measures and the elimination of many of the dominant pollution sources. The focus has consequently shifted to compounds present in lower concentrations but which nevertheless might have the ability to cause harm (Larsen et al. 2004). One of the interesting characteristics of many of the chemicals that might cause this type of pollution is that they do not need to be persistent in the environment to cause negative effects (Ayscough et al. 2000). This is because their high transformation and removal rates can be offset by their continuous introduction into the environment, often through sewage treatment works (Suter and Giger 2001). This is one reason why there is an increasing consensus that this kind of contamination might require legislative action sooner rather than later (Petrovic et al. 2003a; Hilton et al. 2003).

The issue of pharmaceuticals (and their metabolites) in the environment, notably the aquatic compartment, has been a growth area in environmental chemistry for several years (Jones et al. 2001). To date, most of the published literature has addressed the occurrence of drugs in sewage effluent and receiving waters. Although, the risks associated with exposure to drugs are probably most significant with regard to the natural environment; the public's concern is more focused on human exposure. This is especially important in areas that practice indirect water reuse, where sewage effluent is released to streams and rivers that are in turn used as a source of raw water for the production of potable supplies for communities living downstream (van Dijk - Looijaard and van Genderen 2000).
A few studies have documented the occurrence of such organic compounds in drinking water supplies. Exceptions include documentation of low-level concentrations of such compounds in plant-scale studies of drinking water supplies (Loraine and Pettigrove 2006; Petrovic et al. 2003b; Adams et al. 2002; Ternes et al. 2002; Reddersen et al. 2002; Heberer and Stan 1997) and evaluation of their fate in laboratory-scale simulations of drinking-water treatment (DWT) processes (Mompelat et al. 2009; Westerhoff et al. 2005; Huber et al. 2005; Pinkston and Sedlak 2004; Zwiener and Frimmel 2000).

It is often anticipated that pharmaceuticals are easily biodegradable in the environment, since they are transformed to some extent in humans. First findings of drugs in the aquatic environment were reported in the 1970s (Tabak and Brunch 1970; Garrison et al. 1976).

Some investigations showed the existence of drugs in public-owned treatment works effluents. They have been mainly carried out in the UK in the eighties (e.g. Aherne et al. 1990). The concentrations measured in surface waters and STP effluents were in the ng L\(^{-1}\) range. Similar substances were detected in effluents from sewage treatment plants as well as in the aquatic system, e.g. in small creeks and big river such as river Rhine, Elbe, Neckar, Danube, Po, and other (Ternes 1998; Zuccato et al. 2001) as well as lakes (e.g. Lake constane, Swiss Lakes) (Poiger et al. 2001), in ground water (Herberer et al. 1995) as well as the North Sea and The Adriatic Sea (Buser and Muller 1998; Zuccato et al. 2001). Emissions from a landfill containing remainders from pharmaceuticals production were also reported (Holm et al. 1995).

The detected compounds include a wide variety such as hormones, lipid regulators, pain killers, antibiotics, anti-cancer drugs and other cytotoxic compounds, anti-epileptic as well as those regulating blood pressure (Ayscough et al. 2000). Tetracycline is one of the most important antibiotics used in agriculture. It was detected in topsoil (Hamscher et al. 2000) in high concentration (20 mg kg\(^{-1}\) soil). This concentration is twice as high as the PEC (Predicted Environmental Concentration) set as a trigger value by the EU for the need of further investigations. Evidence of a wide variety of different active substances in the aquatic environment...
as well as in liquid manure and in the soil also shows that the active substances are at least not completely eliminated in sewage treatment or in the environment (Kummerer 2001a). Drugs and disinfectants are applied, in contrast to many other chemical substances, because of their specific biological effect. Drugs used in veterinary medicine and husbandry for therapy as well as for prophylactic use and as growth promoters have been assessed (Montforts 2001). Up to now there is not sufficient data available on the occurrence, fate and effects of drugs in the environment and the risks for humans and the environment possibly connected with it (Halling – Sorensen et al. 1998; Stuer – Lauridsen et al. 2000; Kummerer 2001b).

According to present knowledge, for risk assessment most pharmaceuticals can be handled like pesticides. The mode of action should be taken into consideration when assessing effect of pharmaceuticals against organisms with standard tests. Some groups of compounds need special attention (Kummerer 2001b): Cytostatic agents and immunosuppressive drugs, because of their frequently evident carcinogenic, mutagenic or embryotoxic properties as well as other genotoxic compounds (e.g. some antibiotics); antibiotics and disinfectants, because of their pronounced bacterial toxicity and their potential of fostering resistance; Hormones, because of their high efficiency / low effect threshold; Chlorophenols, chlorine – releasing reagents such as sodium hypochlorite, dichloroisocyanuric acid and others used as disinfectants and as bleaching agents or diagnostics such as organic iodine – containing X – ray contrast media because they contribute to the absorbable organic halogen compounds (AOX); these are very often not biodegradable and spread widely in the aquatic environment and / or enter the food web; Heavy metals, e.g., as part of disinfectants and preservatives containing mercury, cytostatic agents containing platinum or MRI contrast media containing gadolinium, as they are not degradable and highly toxic in some oxidation states.

Other groups of drugs, for instance analgesics or sedatives, are also of interest. Barbiturates were reported to influence DDT – metabolism in fish. They also may modulate behavior and predator – prey relations by lowering swimming velocity and influencing reaction times (Kummerer 2001a).
TYPES OF DRUGS PRESENT IN WATER

During and after treatment, humans and animals excrete a combination of intact and metabolized pharmaceuticals. Consequently, many bioactive compounds enter wastewaters and the receiving water bodies without any test for specific environmental effects. In addition, the chemicals that compose personal care products also number in the thousands. The world’s people consume enormous quantities of skin care products, dental care products, soaps, sunscreen agents, and hair styling products to name just a few. In the early 1990’s annual production of these products exceeded 550, 000 metric tons for Germany alone (Daughton and Ternes 1999). Fragrances (e.g., nitro- and polycyclic – musks), UV blockers (e.g., methylbenzylidene camphor), and preservatives (e.g., parabens and isothiazolin derivatives) are included in personal care formulations, for chemical and biological stabilization (Ternes et al. 2004a). Unlike pharmaceuticals, personal care products do not have to pass through the human body. They enter the wastewater after their regular use during showering or bathing. The environmental fate of many cosmetic ingredients, such as preservatives and hair colorants, has not been studied, although considerable persistence and bioaccumulation potential have been reported (Geyer et al. 2000).

The occurrence of the Pharmaceutically active compounds (PhACs) in the aquatic environment has been investigated in several studies in Austria, Brazil, Canada, Croatia, England, Germany, Greece, Italy, Spain, Switzerland, The Netherlands and the U.S. More than 80 PhACs from various prescription classes have been detected up to the μg L⁻¹ level in sewage, surface and groundwater.

Analgesics and anti – inflammatory drugs

Most analgesics also have anti – inflammatory and antipyretic properties. Large amounts of pain killers are prescribed in human medical care but often they are sold at much higher quantities without prescription as so – called ‘over – the – counter’ (OTC) drugs.

Acetaminophen (paracetamol) and acetylsalicylic acid (aspirin) are the two most popular pain killers mainly sold as OTC drugs. As a pro – drug, aspirin is,
however, easily degraded by deacetylation into its more active form salicylic acid and into two other metabolites namely ortho - hydroxyhippuric acid and the hydroxylated metabolite gentisic acid. Ternes (1998), Ternes et al. (1998) detected salicylic acid, ortho - hydroxyhippuric acid and gentisic acid in sewage influent samples at concentrations up to 54, 6.8, and 4.6μg L⁻¹ respectively. Ternes et al. (1998) observed that all three compounds were efficiently removed by the municipal STPs and only salicylic acid was detected at very low concentrations in sewage effluents and also in rivers. Heberer (2002a) also reported average concentrations of only 0.04μg L⁻¹ for salicylic acid in sewage effluents. But in this study, the average influent concentrations of 0.34μg L⁻¹ were relatively low, too. On the other hand, much higher concentrations of salicylic acid up to 13μg L⁻¹ were detected in sewage effluents in Greece and Spain (Farre’ et al. 2001; Heberer et al. 2001a). Residues of salicylic acid do not necessarily have to derive from aspirin. Other sources such as the use of salicylic acid as keratolytic, dermatice, and preservative of food or its natural formation are even more likely to be responsible for the occurrence of this compound in the aquatic environment (Heberer 2002a).

Approximately, 75 tons of the prescription drug diclofenac were annually sold in Germany (Ternes 2001a). In long - term monitoring investigations of sewage and surface water samples from Berlin, Germany, Heberer et al. (2002b) identified diclofenac as one of the most important PhAC present in the water – cycle. Average concentrations of 3.02 and 2.51μg L⁻¹ were detected in the influents and effluents of the municipal STPs, respectively. This low removal rate of only 17% demonstrates the persistence of diclofenac in the STPs and was also reported by Buser et al. (1998b), Stumpf et al. (1999), Zwiener et al. (2000), whereas Ternes (1998) reported a removal of 69% for diclofenac in the STPs. Diclofenac was also frequently detected at concentrations up to the μg L⁻¹ level in investigations of sewage effluents and surface waters in Austria, Brazil, Germany, Greece, Spain, Switzerland, and the U.S. (Buser et al. 1998a; Heberer et al. 1997, 2001a; Ternes 1998; Stumpf et al. 1999; Ahrer et al. 2001; Farre et al. 2001; Ollers et al. 2001; Heberer 2002a). However, Buser et al. (1998b) also observed a significant elimination of diclofenac in the water of a natural lake in Switzerland presuming a possible photolytic degradation of the residues. In laboratory experiments with spiked lake water, Buser
et al. (1998b) confirmed a rapid and extensive photodegradation of diclofenac by sunlight. They also characterized several photoproducts but these could not be detected under natural conditions. Results from surface water monitoring in Berlin, Germany, also indicate a possible photodegradation of diclofenac. But seasonal differences of the concentrations of diclofenac are also due to more extensive application of such drugs during the winter period (Heberer et al. 2002a) because the cold and humid weather causes an increase of rheumatic diseases. In general, the reduction of diclofenac by natural photolytic degradation will also depend on some additional key parameters such as eutrophic conditions, degree of solid or particulate matter or the depth of the watercourse. Under recharge conditions, diclofenac has also been detected in ground water samples (Heberer et al. 1997; Sacher et al. 2001).

First results from laboratory column experiments (Scheytt et al. 2001) and from observations (Brauch et al. 2000) and field experiments (Heberer et al. 2001b) on bank filtration indicate a significant sorption and an efficient attenuation of diclofenac residues in the subsoil (Verstraeten et al. 2002). To date, diclofenac was only sporadically found at trace - level concentrations in raw or treated drinking water (Brauch et al. 2000; Heberer et al. 2001a, b; Ternes 2001a). Zwiener and Frimmel (2000) have shown that diclofenac can be removed from drinking water by ozonation. Together with several other PhACs, diclofenac was also efficiently removed from surface or municipal sewage effluents using membrane filtration (Heberer et al. 2002b).

In Austria, Brazil, Germany, and Switzerland, ibuprofen is found in sewage effluents and rivers, usually at concentrations much lower than those observed for diclofenac (Heberer et al. 1997; Heberer et al. 2002b, Ternes 1998; Buser et al. 1999; Stumpf et al. 1999; Ollers et al. 2001). In Spain, Farre' et al. (2001) detected 1.5, 0.87, and 85µg L\(^{-1}\) of ibuprofen in sewage effluent samples. In the same study, it was also found at relatively high concentrations of up to 2.7µg L\(^{-1}\) in Spanish surface waters. Ibuprofen is degraded in the human body to its principal metabolites hydroxyl- and carboxy-ibuprofen and to carboxy-hydratropic acid (Stumpf et al. 1998; Buser et al. 1999) which are found together with ibuprofen in raw sewage. Stumpf et al. (1998) observed a significant removal of ibuprofen and especially of carboxy-ibuprofen during sewage treatment, whereas the concentrations of
hydroxyl – ibuprofen in the sewage effluents (median: 0.92 µg L\(^{-1}\)) were almost similar to those in the influents. Thus, hydroxyl – ibuprofen was found in 12 German surface waters at much higher concentrations (median: 0.34 µg L\(^{-1}\)) than ibuprofen or carboxy – ibuprofen (median: 0.02 µg L\(^{-1}\), respectively) (Stumpf et al. 1998).

Several other analgesics namely 4 – aminoan - tiyrine, aminophenazone, codeine, fenoprofen, hydrocodone, indometacine, ketoprofen, mefenamic acid, naproxen, phenazone and propyphenazone have also been detected in sewage and surface water samples (Heberer et al. 1997, 2001a; Heberer et al. 2002b, Ternes, 1998; Stumpf et al. 1998; Stumpf et al. 1999; Ahrer et al. 2001; Farre et al. 2001; Ollers et al. 2001; Ternes et al. 2001; Heberer, 2002a, Kolpin et al. 2002).

Under recharge conditions or at land fill leachates several analgesics namely diclofenac, ibuprofen, ketoprofen, phenazone, propyphenazone, gentisic acid or N – methylphenacetin (both metabolites), have also been detected in groundwater samples in Croatia, Denmark and Germany (Holm et al. 1995; Heberer et al. 1997, 2001b; Ahel and Jelicic 2001; Sacher et al. 2001; Reddersen et al. 2002). In Germany, diclofenac, ibuprofen, and phenazone residues have been detected at trace – level concentrations in a few drinking water samples (Heberer et al. 2001a; Ternes 2001). In laboratory experiments, propyphenazone was adsorbed at sediments but there is also some evidence that it might be remobilized by particle transport (Scheytt et al. 2001). In the field experiments on bank filtration, propyphenazone was not totally removed. It was detected in the shallow wells and also reached the water supply wells (Heberer et al. 2001b).

In river Taff and River Ely, UK, paracetamol and tramadol have been quantified at concentrations exceeding single µg L\(^{-1}\). Also codeine was found at relatively high concentrations reaching 0.5 µg L\(^{-1}\). The other anti – inflammatory / analgesics such as ibuprofen, diclofenac, ketoprofen, naproxen, mefenamic acid, aspirin and its metabolite salicylic acid were quantified at levels of less than 10 ng L\(^{-1}\) to hundreds ng L\(^{-1}\) (Barbara et al. 2008).

Ketoprofen, naproxen, ibuprofen, diclofenac, mefenamic acid, paracetamol and aspirin were also analysed and determined at similar levels by several research
groups (Gros et al. 2006; Moldovan 2006; Vieno et al. 2005; Lindqvist et al. 2005; Bendz et al. 2005; Calamari et al. 2003; Kolpin et al. 2002, 2004; Glassmeyer et al. 2005). However, lower levels for naproxen, ibuprofen, diclofenac were obtained in surface water by Vanderford et al. (2003) and higher levels were obtained for ibuprofen by Roberts and Thomas (2006). Codeine (natural opiate) and tramadol (synthetic opioid) were not widely studied, although similar concentrations of codeine were observed by Glassmeyer et al. (2005) and Hummel et al. (2006).

Antibacterial drugs

Several studies have been carried out in Germany (Steeger – Hartmann et al. 1997; Hirsch et al. 1999), Switzerland (Alder et al. 2001; Golet et al. 2001), and the U.S. (Lindsey et al. 2001; Kolpin et al. 2002) to investigate the occurrence and fate of antibacterial drugs in Sewage Treatment Plants (STPs) or surface waters. Macrolide antibiotics (clarithromycin, dehydro – erythromycin [metabolite of erythromycin], roxithromycin, lincomycin), sulfonamides (sulfamethoxazole, sulfadimethoxine, sulfamethazine, and sulfathiazole), chloroquinolones (cipro oxacin, nor oxacin, and enro oxacin), chloramphenicol, tylosin and trimethoprim have been found up to the low µg L⁻¹ level in sewage and surface water samples. In their monitoring investigations of various sewage, surface and groundwater samples in Germany, Hirsch et al. (1999) did not detect penicillins or tetracyclines. This result is no surprise as penicillins are easily hydrolyzed and tetracyclines readily precipitate with cations such as calcium and accumulate in sewage sludge’s or sediments (Daughton and Ternes 1999; Stuer – Lauridsen et al. 2000). Nevertheless, Lindsey et al. (2001), Kolpin et al. (2002) also detected tetracycline drugs (chlorotetracycline, oxytetracycline, and tetracycline) in U.S. surface water samples. Golet et al. (2001) analyzed fluoroquinolone antibiotics in primary and tertiary wastewater effluents in Switzerland. In these samples, ciprofloxacin and norfloxacin occurred at concentrations between 249 to 405ng L⁻¹ and from 45 to 120ng L⁻¹, respectively. Antibiotics have also been identified at high concentrations in hospital effluents (Hartmann et al. 1998; Alder et al. 2001). Thus, Hartmann et al. (1998) detected between 3 and 87µg L⁻¹ of the fluoroquinolone antibiotic ciprofloxacin in hospital effluents. Sacher et al. (2001) reported the occurrence of sulfamethoxazole
(up to 410ngL\(^{-1}\)) and dehydroerythromycin (up to 49ngL\(^{-1}\)) in groundwater samples in Baden – Wurttemberg, Germany. Sulfamethoxazole and sulfamethazine have also been detected at low concentrations in a few groundwater samples in the U.S. and Germany (Hartig et al. 1999; Hirsch et al. 1999; Lindsey et al. 2001). Holm et al. (1995) found residues of different sulfonamides at high concentrations in groundwater samples collected down gradient of a land fill in Grinsted, Denmark.

Among antibacterial drugs studied, trimethoprim, erythromycin – H\(_2\)O and amoxicillin were found at high concentrations exceeding on an average 70ngL\(^{-1}\) and reaching 300ngL\(^{-1}\) during dry weather conditions in river Taff and River Ely, UK. All antibacterial drugs were found at similar levels in the two rivers studied. Their concentrations were found to be at the highest levels at sampling points just after a discharge of treated wastewater effluent into river water. (Barbara et al. 2008).

Similar levels of concentrations for erythromycin, trimethoprim and sulfamethoxazole were obtained by several research groups (Vanderford et al. 2003; Gros et al. 2006; Roberts and Thomas 2006; Bendz et al. 2005; Hirsch et al. 1999; Calamari et al. 2003; Kolpin et al. 2002, 2004; Glassmeyer et al. 2005). Trimethoprim, erythromycin – H\(_2\)O and amoxicillin were found in 100% of the samples at all sampling points located after WWTP discharge in the Taff River. Similar results were obtained for trimethoprim in the Ely River. A strong correlation was observed between the amount of pharmaceutical dispensed in Wales, its excretion as an unchanged drug and the concentration level in surface water. For example, amoxicillin as a pharmaceutical with the highest consumption (almost 9900kg per year) and high excretion as an unchanged drug (60–80%) was therefore expected to be present in surface water at concentrations higher than sulfamethoxazole (consumption: only 115kg per year; excretion as an unchanged drug: only 30%). Of the three antibiotics present in surface water at the highest concentrations (trimethoprim, erythromycin – H\(_2\)O and amoxicillin), only the level of amoxicillin was found to have decreased significantly downstream of treated wastewater discharge (100% decrease 25km downstream of sampling point). In the case of trimethoprim and erythromycin– H\(_2\)O, only a 56 and 12% reduction in their
respective concentration after 25 km of river flow in the River Taff was observed (Barbara et al. 2008).

Antiepileptic drugs

The antiepileptic drug carbamazepine has frequently been detected in municipal sewage and surface water samples (Ternes 1998; Heberer et al. 2001a; Heberer et al. 2002b; Ahrer et al. 2001; Ollers et al. 2001; Heberer 2002a). Investigations of influent and effluent samples from different municipal STPs have shown that it is not significantly removed (less than 10%) during sewage treatment (Ternes 1998; Heberer 2002a). Thus, carbamazepine has been detected at concentrations up to 1075 ng L⁻¹ in surface water samples in Berlin, Germany (Heberer et al. 2002b). Primidone, another antiepileptic drug, has also been detected in samples from municipal sewage influents and effluents and in surface waters (up to 635 ng L⁻¹) in Germany (Heberer et al. 2001a; Heberer et al. 2002b, Heberer 2002a). Different field studies have shown that carbamazepine (Brauch et al. 2000; Heberer et al. 2001b) and primidone (Heberer et al. 2001b) are not attenuated during bank infiltration. Both compounds have been detected in the shallow wells and water supply wells of a transect build to study the behavior of PhACs during bank filtration (Heberer et al. 2001b). This also explains, why carbamazepine has been detected in a number of groundwater samples at a maximum concentration up to 1.1 µg L⁻¹ (Seiler et al. 1999; Sacher et al. 2001; Ternes 2001a) and was also found with a concentration of 30 ng L⁻¹ in drinking water (Ternes 2001a). Carbamazepine and gabapentin were found to be ubiquitous (present in 100% of samples collected at sampling points downstream of WWTP discharge) and persistent in the river water.

Their removal in the River Taff accounted for approximately 50% of 27 km downstream of the treated wastewater discharge. Despite similar quantities of both drugs dispensed in the Welsh community in 2006, gabapentin was found at much higher concentrations of up to 1 µg L⁻¹ in both the River Taff and Ely than carbamazepine (maximum concentration 684 ng L⁻¹ determined in the River Ely during dry weather conditions). This can be explained by the fact that gabapentin is excreted by the human body in unchanged form; while carbamazepine is excreted in approximately 3% as an unchanged compound and therefore its environmental
concentrations are much higher. Poor or no removal of both pharmaceuticals was observed after sewage treatment (Barbara et al. 2008). Similar observations in relation to carbamazepine were made by others (Vanderford et al. 2003; Vieno et al. 2006; Gros et al. 2006; Moldovan 2006; Bendz et al. 2005; Kolpin et al. 2002, 2004; Glassmeyer et al. 2005).

**Beta – adrenoceptor blocking agents**

Several beta-blockers (metoprolol, propanolol, betaxolol, bisoprolol, and nadolol) have been detected in municipal sewage effluents up to the μgL⁻¹ level (Hirsch et al. 1998; Ternes 1998). Only metoprolol, propanolol, and bisoprolol have also been found at relatively low concentrations in surface water samples (Hirsch et al. 1998; Ternes 1998). As far as beta – blockers are concerned; Hirsch et al. (1998) did not recognize any relevance for groundwater recharge or drinking water supply. However, Sacher et al. (2001) also reported the detection of sotalol at maximum concentrations of 560ng L⁻¹ in three groundwater samples from Baden – Wurttemberg, Germany.

In case of surface water in South Wales, UK, only atenolol was found to be present at levels exceeding 100ngL⁻¹. This was expected due to its higher dispersion (over 2300kg per year) and its high excretion rates as an unchanged drug (50%) when compared with the other beta – blockers studied (e.g., propanolol: only 463kg dispensed in Wales in 2006; excretion as an unchanged drug of <0.5%). Despite low concentrations, all β – blockers were found to be present in 100% of the analysed samples collected at sampling points located after treated wastewater discharge over the 10 month sampling regime, with limited decrease of concentration levels with the distance from wastewater discharge point (50%, 11% and 60% degradation of propranolol, metoprolol and atenolol, respectively, 25km downstream of sampling point). The above outcome conforms that some β – blocker such as atenolol and propranolol also belong to the group of pharmaceuticals that are persistent and ubiquitous in the aqueous environment. Comparable results were reported by the other researchers (Calamari et al. 2003; Gros et al. 2006; Bendz et al. 2005; Kolpin et al. 2002, 2004;
Glassmeyer et al. (2005). However, much higher or lower levels of $\beta$-blockers were observed by Vieno et al. (2006) and Roberts and Thomas (2006).

**Lipid regulating agents**

The first incidental findings of the drug metabolite clofibric acid (2-(4-chlorophenoxy)-2-methyl propionic acid) in the aquatic environment in Germany and Switzerland (Heberer et al. 1997; Buser et al. 1998a) probably initiated most of the investigations on PhACs. However, the first detections of clofibric acid, the active metabolite of the blood lipid regulators clofibrate, etofyllin clofibrate, and etofibrate, in samples from STPs in the U.S. have already been reported in the 1970s (Garrison et al. 1976; Hignite and Azarnoff 1977). In Germany, it was found at concentrations up to 4μgL⁻¹ in groundwater samples collected from former sewage irrigation fields near Berlin (Heberer et al. 1997). Underneath the sewage farm areas, it could even be found in samples from the fourth or fifth groundwater aquifer down to a depth of 125m. Up to 270ngL⁻¹ of clofibric acid have been detected in Berlin drinking water samples (Heberer et al. 1997). Buser et al. (1998a) detected clofibric acid at the low ngL⁻¹ range in Swiss lakes from populated areas and also in the North Sea. Clofibric acid was identified as refractory contaminant in several investigations of municipal sewage influents and effluents (Ternes 1998; Stumpf et al. 1999; Heberer et al. 2002b). Zwiener et al. (2000) carried out biodegradation studies using a pilot sewage plant and biofilm reactors operated under oxic or anoxic conditions. In spiking experiments with synthetic sewage water they confirmed the persistence of clofibric acid under anoxic and oxic conditions, as well. Meanwhile, a number of findings in sewage, surface, and groundwater have been reported for clofibric acid from Austria, Brazil and Germany (Heberer and Stan 1997; Heberer et al. 1997, 2001a; Ternes 1998, 2001; Stumpf et al. 1999; Ahrer et al. 2001; Ollers et al. 2001; Heberer 2002a) Bezaflurate, gemfibrozil, and fenofibric acid, the metabolite of fenofibrate, have also been detected up to the μgL⁻¹ level in sewage effluents and surface water samples (Ternes 1998; Stumpf et al. 1999; Ahrer et al. 2001; Farre et al. 2001; Heberer et al. 2001b; Heberer et al. 2002b). Bezaflurate and gemfibrozil have also been found in ground water samples at maximum concentrations of 190 and 340ng L⁻¹, respectively (Ternes 2001; Heberer 2002a). In laboratory experiments
using soil columns (Scheytt et al. 2001), clofibric acid did not show any significant sorption. It leached almost tracer like through the soil columns without retardation. This observation was also confirmed in several studies on bank filtration where clofibric acid was reaching the water supply wells without being removed in the subsoil (Heberer et al. 2001b; Verstraeten et al. 2002). On the other hand, bezafibrate was found to be easily attenuated during bank filtration (Heberer et al. 2001b).

Lipid regulating agents were sporadically detected in River Taff at concentrations not exceeding 100ngL\(^{-1}\). Concentration of clofibric acid (main metabolite of clofibrate) were found to increase in the aquatic environment in UK. (Kasprzyk-Horden 2008) Similarly to the other discussed groups of pharmaceuticals: bezafibrate, clofibric acid were found at comparable levels in surface water around the world (Gros et al. 2006; Vieno et al. 2005; Lindqvist et al. 2005; Calamari et al. 2003; Kolpin et al. 2002, 2004; Glassmeyer et al. 2005).

**H2 – receptor antagonists**

Ranitidine was found at lower concentrations in Italy, Spain and the USA (Gros et al. 2006, Calamari et al. 2003; Kolpin et al. 2002, 2004; Glassmeyer et al. 2005). However, the other H2 – receptor antagonists have rarely been reported.

**Contrast Media**

Iodinated X-ray contrast media, applied at high amounts mostly in hospitals but also in practical surgeries, have been identified by Gartiser et al. (1996) as the main contributors to the loads of total adsorbable organic halogens (AOX) in clinical wastewaters. Oleksy-Frenzel et al. (2000) used adsorbable organic iodine (AOI) detection and measured high concentrations up to 130\(\mu gL^{-1}\) of organic iodine compounds in the influent and effluent of a municipal treatment plant in Berlin and up to 10mgL\(^{-1}\) hospital wastewater observing no degradation or only minor attenuation during sewage purification. They assumed that AOI contamination of the aquatic environment was primarily due to the presence of iodinated X-ray contrast media. This has, however, not yet fully been confirmed by individual component identification. Putschew et al. (2001) identified 39% of the AOI detected in sewage effluents as contrast agents.
In Berlin, Germany, high AOI values of more than 10 μg L\(^{-1}\) have not only been measured in sewage and surface waters but also in bank filtrate and raw drinking water samples (Putschew et al. 2000). In surface waters between 18 and 33% of the AOI could be identified as being iodinated contrast media, whereas in bank filtrate and raw drinking water samples only between 3.4 and 25% of the AOI were identified (Putschew et al. 2000; Putschew and Jekel 2001). Although many of the postulated metabolites have also been analyzed, only one of these compounds was also identified in the samples (Putschew et al. 2001). Thus, it was assumed that the majority of the AOI consists of several other, still unknown, metabolites of iodinated contrast media (Putschew et al. 2000). The five X-ray contrast agents diatrizoate, iohexol, iopamidol, iopromide, and iomeprol were found at or up to μg L\(^{-1}\) concentrations in municipal sewage effluents and in surface water samples (Ternes and Hirsch 2000; Putschew et al. 2001). Iothalamic and ioxithalamic acid have sometimes also been detected at ng L\(^{-1}\) concentrations in influents and effluents of STPs and in surface waters (Ternes and Hirsch 2000). Generally, the loads of the X-ray contrast media are significantly increased on weekdays, because X-ray examinations are performed in hospitals and radiological practices predominately from Monday to Friday (Ternes and Hirsch 2000). Ternes and Hirsch (2000) stated that compared to the other drug residues, the iodinated X-ray contrast media exhibited generally higher maximum levels in STP effluents. Nevertheless, considering their high maximum levels, the average contamination was not as high as it was expected. Thus, the median concentrations of the X-ray contrast media of 50.75 μg L\(^{-1}\) measured by Ternes and Hirsch (2000) are at least more than one order of magnitude lower than the corresponding maximum concentration levels. In all countries with a developed medical care system, it can be expected that X-ray contrast media are present at appreciable quantities in the sewage effluents and hence lead to a contamination of receiving waters. Iodinated contrast agents are very persistent in the aquatic environment and also easily leach into the groundwater aquifers. Thus, diatrizoate, iopromide, iopamidol, and amidotrizoic acid were detected up to μg L\(^{-1}\) level in groundwater and bank filtrate samples (Ternes and Hirsch 2000; Putschew et al. 2000; Sacher et al. 2001). Iothalamic acid and
ioxithalamic acid have also been detected in a few samples at low ngL\(^{-1}\) concentrations by Ternes and Hirsch (2000).

Ternes (2001), Putschew et al. (2000) also reported positive findings of diatrizoate, iopromide, and iopamidol in drinking water or raw water used for drinking water production. The rare earth element Gadolinium (Gd), used in the form of organic complexes in magnetic resonance imaging (MRI), is also consecutively discharged via hospital effluents and public sewage systems into the receiving surface waters (Kummerer 2001). It was detected in hospital effluents at concentrations between a few and up to 100μgL\(^{-1}\) (Kummerer and Helmers 2000). In rivers influenced by STP discharges, Gd has been found at concentrations of about 0.2μgL\(^{-1}\), significantly higher than the natural background value of approximately 0.001μgL\(^{-1}\) (Bau and Dulsk 1996; Kummerer and Helmers 2000).

Cytostatic drugs

The word cytostatic describes the way some anti-cancer drugs work. Most drugs that are used to treat cancer kill the cancer cells. The word 'cytotoxic' means toxic to cells, or cell-killing. Cytostatics are frequently used in chemotherapy. Thus, residues of cytostatic drugs almost exclusively originate from hospital applications and may occur in hospital sewage at concentrations up to the low μgL\(^{-1}\) level (Steger - Hartmann et al. 1997). In effluents from those municipal STP's receiving and purifying hospital effluents, cytostatic drugs have been found at trace concentrations mostly at the low ngL\(^{-1}\) level (Steger - Hartmann et al. 1996; Kummerer et al. 1997; Ternes 1998). Steger - Hartmann et al. (1996) detected ifosfamide and cyclophosphamide in sewage samples from a university hospital at concentrations of 24 and 146ngL\(^{-1}\), respectively. Kummerer et al. (1997) found ifosfamide at mean concentrations of 109ngL\(^{-1}\) in effluents from an oncologic hospital. In the influents and effluents of the receiving municipal STP, it was measured at mean concentrations between 6.2 and 9.3ng L\(^{-1}\) without observing any significant reduction during sewage treatment. In four out of 16 effluent samples from German STPs, Ternes (1998) detected cyclophosphamide at maximum concentrations of 20ng L\(^{-1}\). Ifosfamide was only detected in two samples but in one of these samples with a concentration of 2.9μgL\(^{-1}\). Until now, cytostatics have not been detected in surface...
waters but Kummerer et al. (1997) calculated a predicted environmental concentration (PEC) of 0.8ng L⁻¹ for ifosfamide in German surface waters. Due to their high pharmacological potency, such compounds often exhibit carcinogenic, mutagenic or embryotoxic properties. Thus, further investigations on their occurrence and fate may be interesting regarding their risk potential for humans and the environment (Kummerer 2001).

**Oral contraceptives (17α-ethinylestradiol and mestranol)**

 Synthetic steroids are frequently prescribed as oral contraceptives but because of their high pharmacological potency the total amounts annually sold are relatively low. Ternes et al. (1999b) estimated the annual prescriptions of 17α-ethinylestradiol in Germany at only 50kg per year. Thus, synthetic steroid hormones such as the estrogens 17α-ethinylestradiol (EE2) and mestranol can only appear at trace level concentrations at the low ngL⁻¹ range in the sewage effluents. This presumption was confirmed by results from several investigations of STPs in Brazil, Canada, Germany, England, Italy, The Netherlands and the U.S. (Desbrow et al. 1998; Belfroid et al. 1999; Ternes et al. 1999a; Baronti et al. 2000; Kuch and Ballschmiter 2000; Johnson et al. 2000; Adler et al. 2001; Huang and Sedlak 2001; Xiao et al. 2001; Heberer 2002a). Mestranol has only sporadically been detected in sewage effluents at concentrations up to 4ngL⁻¹ (Ternes et al. 1999a). The median concentration of EE2 in sewage effluents in Germany, England, The Netherlands and the U.S. reported by these authors is approximately between 1 and 3ngL⁻¹ or even lower (below the analytical detection limit). Canadian sewage effluent samples contained EE2 at a median concentration of 9ngL⁻¹ (Ternes et al. 1999a). In recent investigations of six activated sludge STPs in Rome area, Italy, Baronti et al. (2000) determined average concentrations of 3.0ng L⁻¹ for EE2 in sewage influent samples. The median sewage effluent concentration of EE2 was 0.45μgL⁻¹ Baronti et al. (2000) calculated a removal rate of 85% for EE2 and concluded that activated sludge treatment efficiently removed EE2. However, Ternes et al. (1999b) did not observe a significant reduction of EE2 concentrations in aerobic batch experiments containing diluted slurry of activated sludge from a STP near Frankfurt (Germany). In the same experiment, mestranol was rapidly eliminated. On the basis of the daily human
excretion of conjugated estrogens, Baronti et al. (2000) presumed that deconjugation of estrogens preferentially occurs in sewers. However, in investigations of influents and effluents from German STP’s, Adler et al. (2001) observed that conjugated steroids contributed up to 50% of the total steroid concentration. Analysis of a water sample from the Tiber river (Italy) downstream of small towns whose sewages are treated by percolating filter STPs or directly discharged into the river revealed the presence of EE2 at 0.04ng L⁻¹ (Baronti et al. 2000). In general, EE2 was only detected in a few surface water samples at maximum concentrations up to 4.3ngL⁻¹ (Belfroid et al. 1999; Adler et al. 2001) but most of the samples were below the limits of detection (Belfroid et al. 1999; Huang and Sedlak 2001; Adler et al. 2001; Xiao et al. 2001). Although the detected concentrations are very low, they may still be important for the aquatic environment because ‘in vitro’ studies have shown that exposure of fishes to only 0.1ng L⁻¹ of EE2 (Purdom et al. 1994) may provoke feminization in some species of male wild fishes. Due to their physico – chemical properties, estrogenic steroids should be adsorbed in aquatic sediments. Thus, it seems unlikely that they will leach through the subsoil and should therefore; also not appear in groundwater aquifers. Nevertheless, Adler et al. (2001) reported several positive detections of EE2 in ground and drinking water in Germany. They determined EE2 in groundwater and in raw and purified drinking water at maximum upto concentrations of 2.4ngL⁻¹.

Illicit drugs

Two illicit drugs amphetamine, cocaine and its main metabolite benzoylecgonine were found in the River Taff, UK at low levels of single ngL⁻¹ (Kasprzyk – Hordern 2008). Similar levels of the studied illicit drugs were found in Italy (Zuccato et al. 2008). Their presence in the River Taff is again strongly associated with the discharge of treated wastewater effluent. Benzoylecgonine, on the other hand, was often found in the River Taff at levels 10 times higher than the parent compound. Both amphetamine and benzoylecgonine were found with 100% frequency in river water downstream of WWTP, which indicates both high, steady consumption of illicit drugs in the region and a new environmental problem as already indicated by Zuccato et al. (2008). Amphetamine was present at very low...
concentrations and its presence varied at different sampling points (from 13% to 83% frequency). Benzoylecgonine was again quantified at the highest concentrations reaching 50ngL$^{-1}$ (Kasprzyk – Hordern 2008)

**Personal care products and endocrine disruptors**

Benzophenone – 4 (sunscreen agent), methylparaben (preservative), 4 – chloroxylenol (disinfectant/ antiseptic) and 4 – tert – octylphenol were found at concentrations exceeding 100ngL$^{-1}$ in rivers of UK. Concentrations of 4 – tert – octylphenol were found to be much higher in the River Ely than the River Taff because Coslech WWTP discharging treated wastewater effluent into the River Ely treats (as opposed to Cilfynydd WWTP) both communal and industrial wastewater. Triclosan was found at concentrations not exceeding 50ngL$^{-1}$ (Kasprzyk – Hordern, 2008). Similar results were obtained by other researchers (Vanderford et al. 2003; Moldovan 2006; Bendz et al. 2005; Kolpin et al. 2002, 2004; Glassmeyer et al. 2005).

**Other pharmaceuticals**

The bronchodilator drugs ($\beta_2$ – sympathomimetics) salbutamol (albuterol in the U.S.) and terbutaline, and in a few cases clenbuterol and fenoterol were reported by Ternes (1998) to occur at concentrations < 20ngL$^{-1}$ in municipal sewage effluents. For all four compounds, the median sewage effluent concentrations were below the detection limits. In surface waters, only sporadic detections have been reported (Hirsch et al. 1998; Ternes 1998). In investigations of STP effluents and surface waters, Ternes et al. (2001) detected the tranquilizer diazepam, the antidiabetic drug glibenclamide, and the calcium influx inhibitor nifedipine. All three compounds were only found in a few samples at maximum concentrations clearly below 100ngL$^{-1}$. In terms of surface water investigations in the U.S., commissioned by the U.S. Geological Survey, Kolpin et al. 2002 detected low ngL$^{-1}$ concentrations of several other drugs such as the histamine H$2$ – receptor antagonists cimetidine and ranitidine, the calcium ion influx inhibitor diltiazem, the angiotensin converting enzyme inhibitor enalaprilat, the nifedipine metabolite dehydronifedipine, the antidiabetic drug metformin, and the antidepressant fluoxetine. Eckel et al. (1993) detected...
pentobarbital at a concentration of 1µg L\(^{-1}\) in groundwater from a landfill in Florida, USA. In groundwater samples near Reno (Nevada, USA), Seiler et al. (1999) identified residues of the antidiabetic drug chlorpropamide and the anticonvulsant phenytoin. 5, 5'-Diallylbarbituric acid was found together with several other pharmaceuticals and drug intermediates in groundwater from a landfill in Grinsted, Denmark (Holm et al. 1995).

Low concentrations or levels below the detectable limit of bendrofluamide, digoxin, its metabolite digoxigenin and salbutamol were found in surface water of UK. Furosemide as well as valsartan and diltiazem were commonly found in both River Taff and River Ely, UK at levels usually not exceeding 100ng L\(^{-1}\) (Kasprzyk-Hordern 2008).

**SOURCES OF DRUGS IN WATER**

The most significant entry route for drugs into aquatic environments is their release from wastewater treatment plants. Hence different human derived residues and drugs have been reported in different matrices like sewage, ground water, surface water and marine water. Naturally occurring hormones like \(\beta\)-estradiol and synthetic steroids like ethynylestradiol are regularly excreted in urine, making their way into natural environments via wastewater treatment plant effluents. Similarly, substantial amounts of medication are also excreted unmodified which travel via urine and feces into wastewater. As a result, these compounds are commonly detected at elevated level in wastewater influents (Singh et al. 2010).

There is no data available about the total worldwide use of pharmaceuticals. The consumption and application of pharmaceuticals may vary considerably from country to country (Verbrugh and de Neeling 2003; Goossens et al. 2005, 2007; Schuster et al. 2008). If there are legislative changes imposed on the health system it may happen that some compounds are not used any more or others gain more importance, e.g., for economical reasons. According to United Nations' figures, 2.3% of Japanese women of reproductive age take a contraceptive pill containing ethinylestradiol as the main active compound, compared to 16% in North America and up to 59% in Europe (United Nations 2004). Some Pharmaceuticals are sold over
the counter without prescription in some countries, while in other they are only available by prescription. Some antibiotics such as streptomycins are used in the growing of fruits (pomology) while others are used in bee - keeping. Again, the situation may vary from country to country. In Germany, the use of these antibiotics for this purpose has been banned. Antimicrobials are among the most widely used pharmaceutical compounds in animals (Boxall et al. 2003, 2004; Sarmah et al. 2006). These drugs are used in animals husbandry for veterinary purposes, or as growth promoters (particularly in large – scale animals farming and intensive livestock treatment).

Manufacture

Because of good manufacturing practice (GMP) regulations (required for the manufacturing of pharmaceuticals) and the frequently high economic value of the active substances, the amount of emissions occurring during manufacturing has been thought to be negligible. Indeed, such emissions are assumed to be low in Europe and North America. However, manufacturers have not yet published data with regards to this. It has only recently been found that in Asian countries concentrations for single compounds up to several mg L\(^{-1}\) can be found in effluents (Larsson et al. 2007; Li et al. 2008a, b). A study was done in southern part of India, taking samples from a common effluent treatment plant near Patancheru near Hyderabad (Fick et al. 2009). About 21 pharma compounds were detected. However, even in Norway the input from a local manufacturer was high (Thomas 2008).

Hospitals

As to be expected, pharmaceuticals are present in hospital wastewater (Brown et al. 2006; Steger – Hartmann et al. 1996; Kummerer and Helmers 1997; Hartmann et al. 1999; Kummerer 2001a, b; Gomez et al. 2006; Seifrtova et al. 2008; Schuster et al. 2008). The concentrations of pharmaceuticals in hospital wastewater are higher than in municipal sewage. However, the total substance flow is much lower because of the much lower share of effluent from hospitals in municipal effluent in developed countries. The dilution of hospitals wastewater by municipal wastewater is by much more than a factor of 100 (Kummerer and Helmers 1997, 2000).
Private Households

Outdated medicines or their remainders are sometimes disposed off down household drains. In accordance with EU legislation, the discarding of unused drugs via household waste has been permitted since 1994. A recently conducted poll has found that 17.7% of those surveyed get rid of excess and outdated pill by pouring them into the toilet, and about 20% do the same with liquid pharmaceuticals (Gotz and Keil 2007). A survey carried out in the UK investigating the household disposal of unused and expired pharmaceuticals interviewed members of 400 households, predominantly from south-eastern England, and was the basis for a conceptual model to assess the pathways of human pharmaceuticals into the environment. The model demonstrated that the disposal of unused pharmaceuticals, either by household waste or via the sink or toilet, may be a prominent route that requires greater attention (Bound and Voulvoulis 2005). More than half of the patients surveyed in a study conducted in the US reported storing unused and expired medications in their homes, and more than half had pushed them down a toilet. Only 22.9% reported returning medication to a pharmacy for disposal. Less than 20% had ever been given advice about medication disposal by a health care provider (Seehusen and Edwards 2006).

In a study performed in Kuwait (Abahussain et al. 2006) almost half of the respondents (45.4%) obtained medicines by prescription more than three times a year and almost all had unwanted medicines in their homes. The reasons for possessing unused medication were mostly due to a change of medication by the doctor (48.9%), or self-discontinuation (25.8%). Their most common method of disposal was to throw unwanted medicines in the trash (76.5%) or flush them down the drain (11.2%). The results of this study suggest that there is a role for patient education on the proper disposal of unused and expired medications in all countries. In some countries take-back systems are already in place (Niquille and Bugnon 2008). In the EU and the US (http://www.whitehousedrugpolicy.gov/drugfact/factsht/proper_disposal.html) it is legal to throw unused, unneeded or expired drugs in the trash. If the trash is incinerated this is probably the most effective and environmentally sound way to
handle the problem. If the waste is land filled it is a bad solution which only postpones the problem. The APIs will probably show up after some years in the effluent of the land fill. The US FDA advises without an additional explanation that some drugs be pushed down the toilet instead of throwing the trash http://www.whitehousedrugpolicy.gov/drugfact/actsht/proper_disposal.html), which is surprising as the APIs will directly end up in STPs. We could not trace any policy for unused drugs in India.

Landfills

If disposed off along with household waste, compounds end up on landfill sites where they can enter the landfill effluent (Eckel et al. 1993; Holm et al. 1995; Ahel and Jelicic 2001; Metzger 2004). If there is no collection of the effluent, this may be a source for contamination of surface water or ground water. The contribution to this from disposed, unused drugs is not known in many countries, just as the amount released during manufacturing remains unknown.

POLLUTION DUE TO PHARMACUETICALS IN WATER

Ground Water Pollution

A few references may be found in the literature concerning findings of metabolites originating from medical substances in ground water. A landfill in Florida which received wastes from the Jackson Naval Air Station in 1968 and 1969 including wastes from the naval base hospital, has contaminated a nearby shallow ground water (Eckel et al. 1993). The authors reported the presence and persistence of pentobarbital, meprobanate and phensuximide in the 21 years old anaerobic aground water plume. Holm et al. (1995) describes finding and distributions of organic compounds originating from waste from the pharmaceutical industry in the down gradient of a landfill. The authors reported findings of e.g., different sulfonamides (concentrations up to 5mg L\(^{-1}\)), propylphenazone (1, 2 – dihydro – 1, 5 dimethyl – 4 – (1 – methylethyl) – 2 – phenyl – 3 H – pyrazol – 3 – one) (concentrations upto 4mg L\(^{-1}\)), 5, 5 – Diallylbarbituric acid (concentrations up to 0.2mg L\(^{-1}\)). All these medical substances which have been used for treatment of humans in the period
between 1940's and 1970's. As a common practice in that period, waste from pharmaceutical industries was disposed off at landfills with no leachate collection systems. The chemicals may have entered the surrounding aquifers as part of the leachates (Holm et al. 1995). Contamination of tap water by clofibric acid was observed in the samples taken from different districts of Berlin, all containing clofibric acid in concentration between 10 and 165ngL⁻¹. Clofibric acid was additionally detected in all surface water samples taken from the Berlin area. Clofibric acid was also found in samples of surface waters taken from several rivers in other areas of Germany. These findings support the hypothesis that clofibric acid is a substance of considerable persistence in the environment and that regular therapeutic use is the source of clofibric acid which is carried by sewage effluents into the aquatic system. (Halling – Sorensen et al. 1998)

**River Water Pollution**

Watts *et al.* (1983) reported the presence of several antibiotics (erythromycin, sulphamethoxazole, tetracyclines) and the rophylline, in river water samples. They used field desorption mass spectrometry and high performance liquid chromatography. Aherne *et al.* (1985) has used immunoassay techniques for the detection of methotrexate, progesterone, noretheisterone and ethinyloestradiol in various river and potable water samples. Detection limit of between 5 and 10ng L⁻¹ were achieved. Several findings of antineoplastic agents (chemotherapeutics) are found in treated hospital waste water effluents (Steger – Hartmann *et al.* 1996; Aherne *et al.* 1990). This indicates that genotoxic agents might find way to the receiving waters.

**Sediment Pollution**

Several investigations describe finding of antibiotics in sediment cores from medication in fish farms (Bjorklund *et al.* 1990; Coyne *et al.* 1994; Weston *et al.* 1994; Samuelsen *et al.* 1992). Oxytetracycline, an antibiotic agent, was found in concentrations varying between 0.1 and 4.9mg Kg⁻¹ dry matter (Jacobsen and Berglind 1988).
Soil Pollution

Chlortetracyclines were found in soil amended with poultry manure (Warman and Thomas 1981). It was demonstrated that drug metabolites excreted by medicated livestock (e.g., as glucuronides) are decomposed by bacterial action in the liquid manure and reconverted into the active drugs. Due to the application of manure to agricultural soils, multiple drug resistance developed in livestock micro flora, even in the intestinal flora of untreated pigs. Thus, multiple-resistant strains found their way into the food chain. Shore et al. (1988) presented findings of testosterone and estrogen, used as growth promoters, in chicken manure.

TOXIC EFFECTS

Pharmaceuticals have adverse effects on living organisms. These chemicals in the environment may have endocrine-disrupting effects in living organisms, including humans. The incidence of endocrine-related diseases and adverse physiological effect in wildlife is increasing, and there are indications that changes in the reproductive health of humans, including declining male fertility, birth defects and breast and testicular cancer, could be linked to exposure to endocrine disrupting chemicals (Nikolaou et al. 2007). When assessing environmental and health risks from exposure to chemical pollutants, it is important to clearly distinguish between humans and ecosystems in terms of both exposure and effects. The effects of mg L\(^{-1}\) or lower concentrations on ecosystem can range from changes in gene expression to changes in population structure, although little evidence exists for such adverse effects from most pharmaceuticals. However, two extraordinary examples do exist. The best known was the dramatic decrease in vulture populations in India and Pakistan (95% in 3 years), where vultures that fed on carcasses of cattle treated with diclofenac died from renal failure because they were unable to excrete the drug. The other case involved the deliberate dosing of an entire experimental lake with low levels of the active ingredient in birth control pills, ethinyl estradiol. Within the first year, fathead minnows showed evidence of responses at the cellular and tissue levels and declines in the population; by the second year, the first population had collapsed completely (Rodriguez–Mozaz and Weinberg 2010).
Micro-organisms

Ibuprofen, 2-(4-isobutylphenyl) propionic acid, has analgesic, anti-inflammatory and antipyretic properties (Reynolds 1989), and is taken orally to treat mild to moderate pain of rheumatism and other musculo-skeletal disorder. Sanyal et al. (1993), drew to attention the potential antimicrobial activity of Ibuprofen in terms of Minimum Inhibitory Concentration (MIC values), against certain dermatophyte fungi. Anti-fungal activity of ibuprofen was enhanced by lowered pH. These authors also noted that *Staphylococcus aureus* was susceptible to ibuprofen. Elvers and Wright (1995) showed that Ibuprofen inhibited growth of the Gram-positive bacteria, but that two Gram-negative species were unaffected. Growth of *Staphylococcus aureus* was suppressed by ibuprofen concentrations greater than 150µg mL⁻¹ at initial pH 7. At pH 6, such concentration prevented growth. The antibacterial activity of ibuprofen was affected by pH, being more effective at values below pH 7.

Phytoplankton

Streptomycin prevented growth of six blue-green algae species in an investigation performed by Harrass et al. (1985), at concentrations (0.09 to 0.86mg L⁻¹) substantially lower than needed to prevent growth of 7 of 8 green algae tested. *Chlorella vulgaris*, *Scenedesmus obliquus* and *Ulothrix sp.* grew in active streptomycin concentrations less than 21mg L⁻¹, while *Chlamydomonas reinhardtii* growth was prevented at concentration of 0.66mg L⁻¹. Algal growth in sublethal concentration of streptomycin was slowed or delayed, and the maximum density attained by several species was decreased. Result published by Lanzky and Halling-Sorensen (1997) showed that *Chlorella sp.* are sensitive (EC₁₀ = 2.03mg L⁻¹ and EC₅₀ = 12.5mg L⁻¹) to metronidazole.

Plants

Antibiotics chlortetracycline and oxytetracycline effects on plants vary from species to species (Batchelder 1981; 1982). The most sensitive plant species in the Batchelder study was pinto beans when they were grown on sandy loam soil.
Crustaceans/copepods

The acute toxicity of furazolidone, 3-[(5-Nitrofurfurylidene) amino] - 2-oxazolidinone, which are largely used in medicated fish feed, have been investigated by Macri et al. (1988). The authors found a significant toxicity of the compound on *Daphnia Magna*, while *Artemia salina* proved to be the less sensitive. Migliore et al. (1997) showed the toxicity of several agricultural antibiotics to *Artemia*. Acute toxicity studies of four antibiotics; aninosidine, bacitracin, erythromycin and lincomycin, all used as food additive or mass therapy in intensive farming, on *Daphnia magna* Straus have been performed by Dojmi di Delupis et al. (1992). EC_{50} values after 48 hours were found in the range of 30mg L^{-1} to 500mg L^{-1} with Bacitracin as the most potent.

It was found that the calanoid copepods *Temora turbinate*, if raised in pharmaceutical waste concentrations above 1mg L^{-1}, resulted in smaller adult size, reduced egg production rate and an abnormal growth pattern (Lee and Arnold 1983).

Crustaceans/amphipod

Lee and Arnold (1983) studied the toxic effects of ocean - dumped pharmaceutical wastes on the marine amphipod *Amphitoe valida*. The toxic effects increased with increasing duration of exposure to waste concentration. Amphipods chronically exposed to waste concentration above 1% had lower survival rates and reduced fecundity when compared to control groups. The parent amphipods exposed to 3% waste had 100% mortality after three weeks, while those exposed to less than 2% waste were able to survive over 2 months. Larvae of the amphipod survived shorter periods than the parents. No offspring were recorded for amphipods exposed to 3% waste (Lee and Arnold 1983).

Fish

Relatively less details are outlined in the literature concerning the effects of medical substances on fish species. Fewer studies have documented the effect of drugs like salicylate, acetaminophen, and ibuprofen on fish. These are endocrine
disrupters in fish and have the potential to impair the adaptive cortisol response to stressors. (Gravel and Vijayan 2006). Researchers have uncovered new environmental effects, such as feminization or masculinization by hormones or structurally related compounds (xenoesterogens) that exhibited effects on fish down to 1ng L^{-1} (Lange et al. 2001, Routledge et al. 1998)

Mosquito Larvea

Macri et al. (1988) showed that furazolidone had a significant toxic effect on the mosquito larvae Culex pipiens Larvac.

Insects

The potential of animal excreted residues of anthelmitics to adversely affect the development and survival of non – target organisms, important in the process of dung degradation and nutrient cycling, was first recognized in the 1970's. Whereas drugs such as piperazine, thiabendazole and levamisole had little or no effect on dung beetle breeding, formulations of coumaphos, dichlorvos and phenothiazine adversely affected their survival and reproduction for at least 4 to 5 days after treatment (Blume et al. 1976). Phenothiazine was also implicated in deleterious changes in the botanical compositions of pastures (Southcott 1980).

The 1980’s saw the introduction of a new class of compounds known as macrocyclic lactones. Comprising of the avermectin (doramectin, abamectin and avermectin) and the milbemycins (moxidectin), these drugs are excreted in faeces of treated livestock, partly as unaltered drug. No data are available on the insecticidal properties of doramectin residues in dung, but effects of faecal residues of abamectin and avermectin are reported on a wide range of arthropods. In the late 80’s Wall and Strong (1987) discovered that avermectin, an antiparasitic drug for cattle treatment, had an effect on dung degrading insects and delay in degradation of pats from cattle treatment was observed. The environmental aspects and effects of avermectin have also been investigated by e.g. Sommer et al. (1992); Sommer and Overgaard Nielsen (1992) and Holter (1993). Results show that the duration of effects after treatment of
Avermectin on dung degrading organisms is depended on factors like species, temperature, soil composition and type of livestock.

**Resistance development**

Antibacterial resistance is a threat to the efficacy of antibacterial substances. Since it is generally agreed that the extent of usage of antibacterial substances is closely related to the development of antibacterial resistance, it is important to investigate this feature. The development of resistance to antimicrobial agents by many bacterial pathogens has compromised traditional therapeutic regimens, making treatment of infections more difficult. Three factors have contributed to the development and spread of resistance: mutation in common genes that extend their spectrum of resistance, transfer of resistance genes among diverse micro-organisms, and increases in selective pressures that enhance the development of resistant organisms. Resistance to kanamycin and neomycin in the bacterial assemblage of a coastal plain stream of South Carolina, US, was detected by growth of colonies on media containing antibiotics (Leff et al. 1993). Attrassi et al. (1993) reported resistance of bacterial flora to some antibiotics from water, mussels and sediments sampled at three marine sites, localised in Marocco. Resistance to penicillin and ampicillin common to all sites polluted or not, was frequent. However bacterial resistance to erythromycin, tobramycin, chloramphenicol and tetracycline was limited to polluted sites by slaughter house effluents or sewage. Multi-resistance was frequent: more than 55% of strains resisted at least one antibiotic and more than 20% carried plasmids. Antibiotic resistance in sediment bacteria was often found in locations with fish farms. Several papers e.g. Samuelsen et al. (1992); Husevag et al. (1991); Sandaa et al. (1992); Nygaard et al. (1992), have all reported findings of sediment bacteria resistant to various antibiotics used as feed additives in fish farms.

**Genotoxicity**

In recent years there has been an increasing interest in the genotoxicological effects connected to the spreading of genotoxins in the environment. The attention has been focused on the aquatic environment especially testing of surface water samples used as drinking water, waste water and sludge samples. Several researchers also
report on bacterial mutagens in the urine of patients in therapeutic treatment with medical substances e.g. tinidazole, prescribed against protozoal infections, (Espinosa – Aquirre et al. 1996), metronidazole (Connor et al. 1977) and general studies (Monteith et al. 1987).

Giuliana et al. (1996) analysed the genotoxic potential of 800 native (unconcentrated) waste water samples from a hospital with the umuc test. Genotoxic activity was found in 13% of the samples. The highest genotoxic activity occurred in the morning hours, but genotoxic potential without growth inhibition of the bacteria monitored as OD600, in the same way as antineoplastic drugs like mitomycin C or cisplatin. 4% of the genotoxic waste water samples showed combined cytotoxic and genotoxic activities as seen in control experiments using glutaraldehyde containing disinfectants and certain antibiotics. Due to the fact that the samples were unconcentrated a considerable amount of genotoxic substances is released to the environment especially during morning hours.

FATE AND EFFECT OF DRUGS IN WATER

In the beginning research was focused on the analysis of these micro-pollutants. However, it is important to stress that in the few examples in which we really know that drugs have effects on the environment (estrogens and their effects on fish and the effects of diclofenac on vultures), biological effects studies preceded chemical analyses. This is important to stress in order to demonstrate that chemical analyses following up biological effect data may also be an efficient way to find the most problematic chemicals. Later, research into their fate and (eco-) toxic effects came into the foreground. Currently, risk assessment and risk management issues are gaining momentum. However, in the context of India, even chemical analysis data is not available.

Small molecules and biopharmaceuticals

Pharmaceutically active compounds (sometimes called active pharmaceutical ingredients or APIs) are complex molecules with different functionalities and physico – chemical and biological properties. They are
developed and used because of their more or less specific biological activity. Most of them are polar compounds. The molecular weights of the chemical molecules range typically from 200 to 500/1000 Da. Such APIs are called "small molecules". These are the ones which are currently being researched and detected in the environment. They are part of the compounds called "micro-pollutants" because they are often found in the μgL\(^{-1}\) or ngL\(^{-1}\) range in the aquatic environment. Some medicines contain molecules based on proteins ("biopharmaceuticals").

Biopharmaceuticals may be defined as medical drugs produced using biotechnology by means other than direct extraction from a native (i.e. non-engineered) biological source. Examples are proteins (including antibodies) and nucleic acids. The first and best-known example was recombinant human insulin. Biopharmaceuticals are not typically regarded as biopharmaceuticals by the industry. Not all of the naturally occurring compounds which are used as drugs are biopharmaceuticals. For example estrogen is not regarded as a biopharmaceutical.

The environmental relevance of biopharmaceuticals is not yet clear and they are not the focus of environmental research and risk management. One view is that they are not relevant because they are closely related to natural products and are therefore expected to be quickly biodegraded or are denatured, i.e. inactivated in the environment. The other view is that naturally occurring compounds are not in every case easily biodegraded and modified natural compounds even less so. Structurally related compounds such as plasmids have been found in the environment (Schluter et al. 2007; Kummerer 2009a).

Furthermore, it is known that the protein structures known as prions are very stable. Besides the active substances, formulations may also incorporate adjuvants and in some instances pigments and dyes. They are often of minor importance for the environment. Some medicines contain endocrine disrupting chemicals as adjuvants, e.g. Di-n-butylphthalat (DBP) (Koch et al. 2005).

Structure Matters

Pharmaceuticals and disinfectants can be classified according to their purpose and biological activity (e.g. antibiotics, analgesics, anti-neoplastics, anti...
inflammatory substances, antibiotics, anti-histamines, X-ray contrast media, surface disinfectants, etc.). The classification of small molecule APIs by their chemical structure is used mainly for the active substances within subgroups of medicines, e.g. within the group of antibiotics or subgroups within the antibiotics such as β-lactams, cephalosporins, penicillins or quinolones. In this case one may expect that the compounds can be treated as groups with respect to chemical behavior. However, even smaller changes in the chemical structure may have a significant impact on solubility and polarity as well as other properties that govern their environmental fate to some extent. Other classifications refer to the mode of action (MOA), e.g. anti-metabolites or alkylating agents within the group of cytotoxics / anti-neoplasties. In the case of classification according to MOA, chemical structures of molecules within the same group can be very different and therefore their environmental fate can differ too. In this case, compounds cannot be handled as a group with respect to environmental issues.

A closely related chemical structure may be accompanied by an identical or at least a similar mode of action (e.g. β-lactam antibiotics). However, as the example of anti-neoplastics shows, it might also be very different: alkylating, anti-metabolic, mitosis-inhibiting or intercalating substances can, but need not necessarily, belong to different chemical classes. Compared to most bulk chemicals, pharmaceutically active compounds are often complex molecules with special properties, e.g. dependence of the octanol-water partition coefficient (K<sub>ow</sub>) on pH (Cunningham 2008). APIs often have basic or acidic functionalities, sometimes even within the same molecule.

Under environmental conditions molecules can be neutral, cationic, anionic, or zwitterionic. The pKa values ¼ log 10 K<sub>a</sub> (where K<sub>a</sub> is the acid dissociation constant) of ciprofloxacin are 6.16 and 8.63. At a pH of 7.04, the iso-electric point of ciprofloxacin, the molecule carries both a negative and a positive charge, i.e. it is neutral as an entity despite the charges within the molecule. The log K<sub>ow</sub> of ciprofloxacin at pH 7.04 is calculated to be about 1.74 and was experimentally determined to be 0.28 (Meylan and Howard 1995). Other compounds such as ceftazidime are inner salts, i.e. they are already zwitter ions and can additionally
from other zwitterions. This makes their environmental behavior even more complex. Not only are different pharmaceuticals of special interest with respect to the compounds themselves, but also because of the differences in their occurrence, their fate and their effects on humans or on other target organisms such as bacteria or parasites, and on non-target organisms in the environment.

Parent compounds, metabolites, transformation products and their elimination

In recent years it has been learnt that not only are the APIs themselves important, but also the molecules resulting from these parent compounds due to structural changes taking place in the environment. A chemical can undergo different structural changes by a variety of biotic and non-biotic processes after its introduction into the environment. Structural transformations may also be a result of effluent treatment (Ravina et al. 2002; Schroder 2002; Zuhlke et al. 2004; Lee et al. 2007; Trautwein et al. 2008; Mendez–Arriaga et al. 2008).

Many pharmaceuticals are bio-transformed by organisms such as bacteria and fungi in the environment (Haiß and Kummerer 2006; Groning et al. 2007). Nomenclature used by different authors is often somewhat confusing (Langin et al. 2008). For example, the term biodegradation is very often used. However, primary degradation, partial degradation and full mineralization are only rarely differentiated. With the advent of pharmaceuticals as environmental contaminants the situation got even more complicated. Many pharmaceuticals undergo a structural change in the body of humans and animals, respectively. This could be due to micro-organisms in the gut or by human enzymes such as cytochromes. Metabolites are the result of such a process. However, the naming and meaning of "metabolite" in publications is somewhat confusing. The term metabolite is used for compounds resulting from the structural change of pharmaceuticals within the human body, not differentiating biochemical processes performed by human enzymes from the ones due to bacterial activity in the alimentary system and the ones present on skin or non-biotic processes such as hydrolysis in the stomach. The term is also used for molecules resulting from structural change by fungi and bacteria in the environment and sometimes even for structural changes that are the
result of non-biotic processes such as oxidation, hydrolysis and photolysis (e.g. Mendez–Arriaga et al. 2008) in different environmental compartments such as surface water, soil or sewage treatment. As with metabolism, the chemical structure of the active molecules can be changed by biotransformation, biodegradation, and non-biotic transformation such as photo transformation and hydrolysis. Such a structural change results in a change in their physico-chemical and pharmaceutical properties. It is normally assumed that metabolism and other transformation processes of APIs leads to decreased toxicity. In some cases however, metabolism leads to more active compounds (e.g. in the case of prodrugs). The same has been found for photo transformation and other oxidizing processes (Burhenne et al. 1997).

The proper and adequate use of the terms related to the fate of organic chemicals in the environment is advised in order to prevent confusion in the assessment of the fate and risks connected to the presence of these molecules in the environment (Kummerer 2009a).

METHODS OF ANALYSIS OF DRUGS PRESENT IN WATER

Quantitative evaluation of the fate and behaviour of pharmaceutical compounds in the aquatic environment requires sensitive and reliable analytical methods with detection limits in the lower ngL⁻¹ range (Gomes et al. 2004). In the past, the analytical determination of pharmaceuticals has been mainly limited to biological samples such as blood, tissue and urine (Neill et al. 1991). A simple adaptation of these methods to environmental studies is not generally appropriate because the therapeutic dose of pharmaceuticals is usually much higher than the levels found in the environment and elevated levels of potentially interfering compounds such as humic substance often have to be separated out. This also means that until relatively recently, few analytical methods to detect these compounds in environmental samples at relevant concentrations had been developed (Lai et al. 2002).

Analytical procedures for the determination of pharmaceuticals in aqueous samples utilize both gas and liquid chromatography after extraction and clean up
procedures (Sacher et al. 2001; Oilers et al. 2001; Lindsey et al. 2001). A detailed review of analytical methods has recently been undertaken. (Ternes 2001b). However environmental studies frequently require data on the distribution of contaminants within several phases.

The application of advanced measurement technologies (e.g., gas chromatography with mass spectrometry (GC – MS) and GC with tandem MS (GC – MS²) or liquid chromatography with MS (LC – MS) and LC with tandem MS (LC – MS²)) to environmental analysis has allowed the determination of a broader range of compounds, including pharmaceuticals, and has therefore permitted more comprehensive assessment of environmental contaminants. LC – MS² is becoming more commonly used in pharmaceuticals analysis because of its high sensitivity and its ability to confirm compounds (as compared with conventional LC with ultraviolet (UV) or fluorimetric detection). LC – MS² allows separation and detection of compounds having the same molecular mass but different product ions, even if they co-elute. MS² detection is therefore preferred for increased analytical sensitivity and selectivity in complex matrices, such as wastewaters (Diaz – Cruz and Barcelo 2005). From the various studies reviewed by Fatta et al. 2007, it is concluded that GC – MS was used in 17 studies, LC – MS² in 12, high-performance LC with diode-array detection (HPLC – DAD) in two, HPLC – fluorescence in two, and GC – MS² and LC – MS in one each.

As mentioned by Petrovic et al. (2002), both GC – MS and LC – MS methods have some drawbacks. Prior to GC – MS analysis, derivatization of polar pharmaceuticals is necessary, performed using highly toxic and carcinogenic diazomethane or, less frequently, acid anhydrides, benzyl halides and alkylchloroformates. This step can also affect the accuracy of the method. Ternes (2001b) directly compared GC – MS and LC – electrospray ionization (ESI) – MS², and showed that only LC – (ESI) – MS² allows the analysis of extreme polar compounds (e.g., β – blockers atenolol and sotalol) due to an incomplete derivatization of the functional groups.
Farre et al. (2007) compared LC – (ESI) – MS and GC – MS, (after derivatization with BF$_3$ – MeOH) for monitoring some acidic and very polar analgesics (salicylic acid, ketoprofen, naproxen, diclofenac, ibuprofen and gem brozil) in surface water and wastewater. The results showed a good correlation between methods, except for gem brozil, for which derivatization was not completely achieved in some samples. In general, the limits of detection (LODs) achieved so far with LC – MS$^2$ methods are slightly higher than those obtained with GC – MS methods (Diaz – Cruz and Barcelo 2005); however, LC – MS methodology showed advantages in terms of versatility and sample preparation being less complicated (i.e. derivatization is not needed).

SAMPLE PREPARATION

The sample preparation procedure is an important step in such analyses due to lower concentration of target compound and complex nature of matrix. In the case of pharmaceuticals containing acidic groups in their structure and existing largely in their ionized form at neutral pH, acidification of water samples is necessary (Renew 2004). The presence of natural organic matter in the samples may reduce the extraction efficiencies. In general, the water samples are filtered through 0.45μm or 0.2μm glass – fiber filters. Several techniques have been developed and optimized, with SPE being the most frequently used. Also solid – phase microextraction (SPME), liquid – phase microextraction (LPME) and lyophilization have been applied (Hirsch et al. 1998; Ternes 2001b; Kolpin et al. 2002).

Of the 32 studies reviewed by Fatta et al. (2007), sample extraction of water and wastewater was achieved using SPE in 28, SPME in two (in one study both SPE and SPME were applied), LPME in one and lyophilization in two. SPE sorbents in the form of commercially available cartridges (e.g., ENV+, Oasis HLB, Strata – X, Lichrolut C18, and Lichrolut EN) have been assessed for pre – concentration as well as for clean – up of pharmaceuticals in water samples. These were employed most because they give better recovery of both polar and non – polar compounds and have greater capacity than alkyl bonded silicas. However, these are very costly.
SPE is typically performed manually, but there are some significant disadvantages with this approach:

- manual (off-line) SPE is time-consuming as well as labor intensive and costly, which compromises productivity;
- exposure to hazardous or infectious matrices (such as sewage) involves safety issues; and,
- the recovery of the analyte can vary from batch to batch, causing reproducibility problems.
- by automating the process, these problems can be eliminated, with the following benefits:
  - direct injection of untreated samples;
  - automatic sample clean-up and/or analyte enrichment;
  - elimination of conventional manual sample pretreatment steps;
  - faster procedures;
  - methods are less prone to errors, resulting in better reproducibility;
  - reduction of health risks; and,
  - samples can be run unattended (e.g., overnight or over the weekend).

A review on the current aspects and future prospects for automating SPE was published by Rossi and Zhang (2000). However, only a few studies have so far used automated procedures for extraction (e.g., accelerated solvent extraction (ASE) (Richter et al. 1996), on-line coupled continuous flow liquid membrane extraction (CFLME) with a C18 pre-column system (Liu et al. 2003), or sequential injection analysis (SIA) with a lab-at-valve (LAV) approach for on line liquid-liquid micro-extraction (Burakham et al. 2005). These studies focused on the determination of organic pollutants (e.g., polyaromatic hydrocarbons (PAHs) and bisphenol A).

PRE-CONCENTRATION TECHNIQUE

Despite the current availability of advanced detection instrumentation, the rapid, accurate and perhaps simultaneous determination of a large number of pharmaceuticals in complex environmental matrices continues to constitute a major
and fascinating challenge for researchers not only because of the diversity of chemical properties of the pharmaceutical compounds, but also because of the generally low concentrations (usually $\mu$g L$^{-1}$ or ng L$^{-1}$ levels) and the complexity of matrices. Further improvements are needed in order to lower LODs. Moreover, increasing and intensive scientific research is needed to assess the impact of pharmaceuticals, in order to establish limits for their presence in waste-water discharges or drinking water. Current knowledge concerning their impacts on the environment refers to information on individual compounds, and this needs to be extended to more complex environmental mixtures with the help of improved analytical methods. For this reason, probably most of the analytical procedure reported in literature make use of SPE followed by GC – MS or LC – MS.

However, these techniques are relatively expensive and not easily available at several places. Need of the hour; therefore, is to use simpler techniques which however, require higher concentration of target drug molecules. Therefore, prior to the instrumental analysis, attention has to be paid to the sample preparation and enrichment procedure.

Solid Phase Extraction (SPE) is the method of choice for sample enrichment / pre-concentration in environmental analytical chemistry. There are several solid phases on which the sample can be concentrated. (Batt and Aga 2005; Psillakis et al. 2003).

INSTRUMENTAL TECHNIQUES USED IN THE PRESENT WORK

Spectroscopy

Spectroscopy was originally the study of the interaction between radiation and matter as a function of wavelength ($\lambda$). In fact, historically, spectroscopy referred to the use of visible light dispersed according to its wavelength, e.g. by a prism. Later the concept was expanded greatly to comprise any measurement of a quantity as a function of either wavelength or frequency. Thus it also can refer to a response to an alternating field or varying frequency ($v$). A further extension of the scope of the definition added energy ($E$) as a variable, once the very close relationship $E = hv$ for photons was realized ($h$ is the Planck constant). A plot of the response as a function of...
wavelength or more commonly frequency is referred to as a spectrum; and also spectral linewidth.

Spectrometry

Spectrometry is the spectroscopic technique used to assess the concentration or amount of a given species. In this case, the instrument that performs such measurements is a spectrometer or spectrograph. Spectroscopy/spectrometry is often used in physical and analytical chemistry for the identification of substances through the light emitted from or absorbed by them.

The type of spectroscopy depends on the physical quantity measured. Normally, the quantity that is measured is an intensity, either of energy absorbed or produced. Electromagnetic spectroscopy involves interactions of matter with electromagnetic radiation, such as light.

Most spectroscopic methods are differentiated as either atomic or molecular based on whether or not they apply to atoms or molecules. Along with that distinction, they can be classified on the nature of their interaction. Absorption spectroscopy uses the range of the electromagnetic spectra in which a substance absorbs. This includes atomic absorption spectroscopy and various molecular techniques, such as infrared, ultraviolet-visible and microwave spectroscopy.

Most organic compounds and many inorganic ions and complexes absorb radiation in the UV – visible region (180 – 780nm). A plot of this absorption of a compound against wavelength is called an absorption spectrum. The shape of the absorption spectrum is a characteristic of a particular compound or class of compounds. Absorption spectrometry is a non-destructive technique and is extremely sensitive and is therefore, ideal for the characterisation of small amounts of precious compounds. The most important application of the technique is as a means of measuring concentration.

Trace compounds can be measured in presence of other components if there is sufficient difference in their absorption spectra. The technique is best suited for dilute
solutions. Solubility in a suitable solvent is a prerequisite for the accurate measurement of a particular sample.

A very popular application is the monitoring of the effluent from HPLC columns. The concentration of a particular component can be followed by measuring the absorbance at a suitable wavelength, or compounds eluted from the column can be identified by making rapid spectral scans. Absorbance measurements are also the most popular means of following the kinetics of reaction systems since they do not interfere with the progress of the reaction in any way. Another important application of rapid absorption measurement is in the clinical field, where colorimetric assays have been worked out for many biologically important compounds.

Absorption of radiation

When UV-visible radiation encounters a molecule or a molecular ion, an interaction between the radiation and the latter may take place. This absorption is very specific and results in an attenuation of the radiation and an increase in the energy of electrons of the molecule. This may be regarded as the promotion of one the outer electrons from a ‘ground state’ energy level into one of higher energy level. These levels are separated by discrete energy increment, $E$, which is determined by the nature of the molecule and only parcels of radiation of energy $E$ can be absorbed. This parcel of radiation is termed as quantum and its energy is related to the frequency and wavelength of radiation by

$$E = hv = \frac{hc}{\lambda}$$

Where, $h$ is the plank's constant ($6.63 \times 10^{-34}$ J s), $c$ is the velocity of light ($2.998 \times 10^8$ m s$^{-1}$) and $\lambda$ is wavelength.

According to molecular orbital theory, the absorbed wavelength is used for the transfer of an electron from a bonding or non-bonding molecular orbital to anti-bonding molecular orbital. The various electronic transitions involved in the UV-visible region for organic compounds are $\sigma - \sigma^*$, $n - \sigma^*$, $\pi - \pi^*$, $n - \pi^*$ in decreasing order of energy requirement. The energy required for $\sigma - \sigma^*$ and $n - \sigma^*$ transitions are relatively high with absorption in the vacuum ultraviolet region. Conjugation causes
delocalization of \( \pi \) electrons, thus making them more mobile and easy to excite. Greater the conjugation with the chromophores, the easier it is to excite the electrons and higher is the wavelength i.e. in the visible region and the compound appears coloured.

Radiation of a particular wavelength, which is characteristic of the molecule, is absorbed by it. The amount of radiation absorbed will be proportional to the amount (concentration) of the absorbing species, when the pathlength is constant.

According to Beer – Lambert law,

\[
A = \varepsilon \cdot b \cdot c
\]

Where,

- \( A \) = absorbance
- \( \varepsilon \) = Molar extinction coefficient (L mol\(^{-1}\) cm\(^{-1}\))
- \( b \) = Pathlength of the cell (cm)
- \( c \) = Molar concentration of solution (mol. L\(^{-1}\))

\( \varepsilon \) is a characteristic constant for a given absorbing species and the path length \( b \) is a constant for a given set of experimental conditions.

Therefore, absorbance is directly proportional to its concentration. This is the basis of quantitative measurement in spectrophotometry. A calibration curve of concentration of analyte versus absorbance is constructed by using standard solutions for the concentration range obeying Beer’s – Lambert’s law and from the slope of the curve the concentration of the unknown is calculated.

The amount of the individual compounds present in mixture can be derived by solving the equation (1) and (2), which are obtained adding Beer – Lambert’s law. (\( A = \varepsilon \cdot b \cdot c \)), for two component mixture of \( X \) and \( Y \), the mixtures absorbance, \( A_m \) is

\[
(A_m)_{\lambda_1} = (\varepsilon_x)_{\lambda_1} b C_x + (\varepsilon_y)_{\lambda_1} b C_y \quad ------(1)
\]

Where \( \lambda_1 \) is the wavelength at which the absorbance of component \( X \) is measured.

\[
(A_m)_{\lambda_2} = (\varepsilon_x)_{\lambda_2} b C_x + (\varepsilon_y)_{\lambda_2} b C_y \quad ------(2)
\]
Where $\lambda_i$ is the wavelength at which the absorbance of component Y is measured.

$\varepsilon$ is absorptivity (L g$^{-1}$ cm$^{-1}$); its value is determined for each compound at both wavelengths. b is path length (cm) and C the concentration (M).

**Chromatography**

Chromatography is one of the most powerful and versatile analytical techniques available to the modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in a mixture in one single analytical run. Its versatility comes from its capacity to handle a wide range in complexity: from a single substance to a multi component mixture containing widely different chemical species. Another aspect of versatility is that the analysis can be carried out on a very costly complex instrument and on the other hand on a simple inexpensive thin layer plate.

The word chromatography is derived from greek letters *Chromos* meaning colour and *graph* meaning writing. Although colour has little to do with modern chromatography, the name has persisted, and despite its irrelevance, is still used to describe all separation techniques that employ a mobile phase consisting of different solvents and stationary phase involving suitable adsorbents. In a classical manner it can be defined as “A separation process that is achieved by distribution of substances between two phases i.e. a stationary phase and a mobile phase”. The resolved compound can be further determined by suitable detector system.

There are ways of classifying different chromatographic methods based on physical state of phases used or mechanism controlling separation or kind of techniques used and or sample development. One such classification is presented in Table 1.1. This classification is based on nature of mobile phase which can be gas or liquid yielding gas chromatography or liquid chromatography, with solid or liquid as stationary phase. We have gas – liquid, liquid – liquid or liquid – solid chromatography. The later addition is supercritical fluid (SFC) chromatography. In gas chromatography, stationary phase is nonvolatile and is coated or bonded to porous support, while inert gas is used as mobile phase. The separation occurs due to difference in vapour pressure of components. In liquid – liquid partition
Chromatography stationary phase is liquid coated on porous support (Table 1.1). While mobile phase is second liquid which is immiscible with the first phase. In Gas Solid chromatography, the separation occurs due to difference or equilibrium distribution of difference in adsorption on the stationary phase. GSC i.e. gas-solid chromatography uses solid stationary phase (e.g. granulated activated charcoal GAC) and gas mobile phase. The use of supercritical fluid as mobile phase leads to newer methods. This new technique has gained importance very recently.

Table 1.1. Classification of chromatographic methods

<table>
<thead>
<tr>
<th>S.No</th>
<th>Mobile Phase</th>
<th>Stationary Phase</th>
<th>Mechanism</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gas</td>
<td>Liquid (GLC)</td>
<td>Sorption</td>
<td>Column</td>
</tr>
<tr>
<td>2.</td>
<td>Gas</td>
<td>Solid (GSC)</td>
<td>Adsorption</td>
<td>Open bed</td>
</tr>
<tr>
<td>3.</td>
<td>Liquid</td>
<td>Liquid (LLC)</td>
<td>Partition</td>
<td>Column</td>
</tr>
<tr>
<td>4.</td>
<td>Liquid</td>
<td>Solid (LSC)</td>
<td>Adsorption</td>
<td>Batch</td>
</tr>
<tr>
<td>5.</td>
<td>Supercritical fluid</td>
<td>Liquid (SFLC)</td>
<td>Gas – Liquid</td>
<td>Column</td>
</tr>
<tr>
<td>6.</td>
<td>Supercritical fluid</td>
<td>Solid (SFSC)</td>
<td>Gas - Solid</td>
<td>Column</td>
</tr>
</tbody>
</table>

The classification based on a technique is one which involves use of either a column (Table 1.3) or open bed. The column may be packed column or capillary column also called as open tubular columns. The paper and thin layer techniques are examples of plane bed chromatography. Flow of mobile phase is due to capillary wetting. Open bed chromatography is restricted to use of liquid as mobile phase.

Table 1.2. Alternative way of classification on basis of mechanism

<table>
<thead>
<tr>
<th>S.No</th>
<th>Kind</th>
<th>Adsorption</th>
<th>Partition</th>
<th>Exclusion</th>
<th>Ion Exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Technique</td>
<td>Adsorption Chromatography</td>
<td>Gas Chromatography</td>
<td>Exclusion Chromatography</td>
<td>Ion Exchange Chromatography</td>
</tr>
<tr>
<td>4.</td>
<td>Mode</td>
<td>Gas – Solid Chromatography</td>
<td>Liquid – Liquid partition</td>
<td>-</td>
<td>Inorganic Exchangers</td>
</tr>
</tbody>
</table>
Table 1.3. Classification based on separation mechanism

<table>
<thead>
<tr>
<th>S.No</th>
<th>Kind</th>
<th>Adsorption</th>
<th>Partition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adsorption Chromatography</td>
<td>Columnar method</td>
<td>LC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gas – Solid Chromatography</td>
<td>GSC</td>
</tr>
<tr>
<td>2</td>
<td>Partition Chromatography</td>
<td>Liquid – Liquid partition</td>
<td>LLPC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paper</td>
<td>PC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thin layer</td>
<td>TLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gas Liquid Chromatography</td>
<td>GLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reversed phase partition</td>
<td>RPPC</td>
</tr>
<tr>
<td>3</td>
<td>Ion Exchange</td>
<td>Cation exchange</td>
<td>CEC</td>
</tr>
<tr>
<td></td>
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<td>Anion exchange</td>
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<td>Ion chromatography</td>
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<td>4</td>
<td>Exclusion Chromatography</td>
<td>Gel – permeation</td>
<td>GPC</td>
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<td>Ion exclusion</td>
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<td>Molecular sieve / Gel Filtration</td>
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<td>5</td>
<td>Electrochromatography</td>
<td>Zone electrophoresis</td>
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<td>Boundary layer method</td>
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<td>Curtain chromatography</td>
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<td>Capillary electrophoresis</td>
<td>CZE</td>
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The planar chromatographic technique is simple, flexible and best for characterization. Column chromatography has better capacity, ease of operation and excellent efficiency. It is used in preparative and quantitative work. However, the most logical way of classification is based upon the mechanism of retention of solute or analyte in the stationary phase. It forms the backbone of chromatographic separations (Table 1.3.). The processes involved are either, the sorption by adsorption, partition and exclusion e.g. GLC, LLPC involve partition, while adsorption deals with sample fixation on stationary support involving some forces like London forces, dipole induced dipole interaction or the molecular interaction. The distribution ratio is most important factor in the consideration of partition chromatography. While exclusion mechanism is based upon segregation of species by taking the advantage of the difference in size and geometry of the molecules or species as seen in Gel permeation chromatography. The stationary phase is porous medium. Small species permeate faster in comparison to large size particles e.g. protein or polymers. More than one mechanism of retention is usually considered best. It is briefly summarized in Table 1.3.
It is interesting to note that HPLC i.e. high performance liquid chromatography or IC - ion chromatography are both highly instrumental techniques, do not figure in the above classification. The reason is simple; they are techniques and not a class of chromatography. For instance, we can use HPLC for any four kinds of chromatography described in Table 1.3(1 – 4).

Thus, chromatography methods have a few things in common. There is a stationary support in the form of a column packed with inert material, then a phase moving down the column, called the mobile phase and the phase which usually adheres to the stationary support called the stationary phase. Separation is feasible on account of a differential migration front; developed by exploring differences in adsorbility, partition coefficient, exchange potential, molecular size or ionic mobility. Further, most terms used while describing chromatographic methods are common to all the method.

Specifically, if the stationary phase is a liquid, depending on the polarity of the mobile phase in comparison with the stationary phase it may be either a normal phase HPLC or a reverse phase HPLC. The stationary phase is packed in a column and the mobile phase is pumped at a constant flow rate or at a constant pressure by means of a pump. The solute is introduced at the head of the column. The mobile phase carries the solute on to the column, where separation actually occurs. The separated components are carried further to the detector, which in turn is connected to an integrator or a data station. The components are detected by the detector and recorded in the form of a chromatogram (Khopkar 2008).

Mass spectrometry

The mass spectrometer is an instrument that sorts out charged gas molecules or ions according to their weight or mass. This technique has nothing to do with spectroscopy; however, the name spectrometer was chosen because of the similarity between photographic records and optical lines in spectra. As mass spectrum is obtained by converting the compound of a sample into rapidly moving ions ( + ve in nature) and resolving them on the basis of their mass to charge (m/e) ratio. The ionisation process produces positives particles, the mass distribution of which is
characteristic of the parent compound. In elucidation of molecular structure, mass spectras are useful for determining molecular weight. Since the ion current at various mass settings is proportional to concentration, quantitative analysis can also be easily carried out. The sample is bombarded with a beam of electrons which in turn produces ion molecules or ionic fragments of the original species; such charged fragments can be separated according to their mass.

**Qualitative analysis with mass spectrometry**

The identification of an unknown compound is possible by mass spectrometry. This is possible by calibration with a known compound like mercury vapour (m/e = 198 – 204) or perfluorokerosine (PFK) with peaks CF3 (69), C3F3 (93), CC4F3 (131). The mass spectrometer is thus useful for determination of molecular weight or molecular formula, or identification of a compound from the fragmentation patterns.

In determination of molecular weight, a volatile compound is essential as the molecular ion peak has to be identified as (M + 1) peak with chemical ionisation with methane but peaks due to impurities should be ignored. The molecular weight determined by the mass spectrometer may not be the same as that calculated form atomic weights if the parent compound contains certain elements with high isotopic abundances.

With the use of very efficient microprocessor the huge data obtained can be quickly processed. Further, for GC – MS, libraries of mass spectra of compounds using electron ionization are available which are used to identify the compounds.

**STATISTICAL METHODS IN ANALYTICAL CHEMISTRY**

Analytical science accumulates enormous amounts of data out of different measurements. This data has no value unless one examines how much of it is reliable and how much is reproducible. Statistics, a branch of mathematics, deals with the presentation and analysis of numerical data. The application of many statistical methods does not require the services of a ‘statistician’ or a ‘mathematician’ to convert chemical data into useful information.
Exploratory data analysis

Exploratory data analysis is a term used to describe a group of techniques (largely graphical in nature) that sheds light on the structure of the data. Without this knowledge a researcher, or anyone else, cannot be sure that he is using the correct form of statistical evaluation.

Summary statistics

Summary statistics are used to make sense for large amount of data. Typically, the mean, sample standard deviation, range, confidence intervals, quantities and measures for skewness and spread of the distribution are reported.

The mean

The mean, $\bar{x}$ can be shown to be best estimate of the true value; it is calculated as the arithmetic mean of $n$ observations

$$\bar{x} = \frac{\sum x_i}{n}$$

The median

This is the value or observation, which subdivides the numerical, ordered data into two halves. If the number of observation ($n$) is odd, $(n - 1) / 2$ observations are smaller than the median and the next higher value is reported as the median. If $n$ is even, then average of the middle two observation is reported.

The most useful characteristics of the median is the small influence exerted on it by extreme values, that is, its robust nature.

Standard deviation

The standard deviation is a measure of the spread of data (dispersion) about the mean. It is the positive square root of the variance.

$$SD = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$
Standard error

The standard error is the standard deviation of an estimate. Thus, the standard error of the mean (SEM) is the standard deviation of the mean. If \( s \) is the standard deviation of a sample then the SEM is given by the formula.

\[
SEM = \frac{SD}{\sqrt{n}}
\]

Coefficient of variation

The ratio of the standard deviation to the mean is the coefficient of variation (CV). It is expressed as a percentage.

\[
CV = 100 \left( \frac{SD}{x} \right)
\]

Regression and calibration

Calibration is fundamental to achieving consistency of measurement. Often calibration involves establishing the relationship between an instrument response and one or more reference values. Once the relationship between input value and the response value is established, the calibration model is used in reverse that is to predict a value from an instrumental response.

In statistics, the term regression is used to describe a group of methods that summaries the degree of association between one variable and another variable. The most common statistical method used to do this is least – square regression, which works by finding the “best curve” through the data that minimises the sums of square of the residuals. There are a number of least – square regression models, for example, linear, logarithmic, exponential and power.

Statistics of the straight line

The general equation of straight line can be expressed as

\[
y = bx + a
\]

where,
y is the dependent variable
a is the y intercept
b is the slope of the line
x is the independent variable

\[
b = \frac{\sum x_i y_i - \sum x_i \sum y_i / n}{\sum (x_i - x)^2}
\]

Using the method of calculus, the slope and intercept of the line are determined.

Objectives in a simple linear regression analysis

Various inferences and estimates may be made from regression line:

- Estimation of variability associated with the slope (b) and intercept (a)
- Test whether there is any association between the two variables x and y.
- Estimate the variability associated with a given y value.
- Make prediction about y value for a given x value within the range studied.

Error analysis for linear regression

The various numeric tools to determine how well a regression equation fits the data are as follows

\[
a = \bar{y} - b \bar{x}
\]

- The standard error of Y estimates ($S_{yx}$)
It is measure of the certainty with which the independent values in the sample can be used to predict the dependent values. That is, $S_{yx}$ is an estimate of the error in a single value for y calculated using the regression equation. $S_{yx}$ is the standard deviation of the difference observed and calculated values and is calculated with the equation:

\[
S_{yx} = \frac{\sqrt{\sum (y_i - y_{i(x)})^2}}{(N - 2)}
\]

Where $y_i(x_i)$ is the value predicted from the curve, $y_i$ is the observed value and $(N - 2)$ is the degree of freedom.
• Coefficient of determination \( (r^2) \)

It is a measure of the Goodness – of – Fit for a straight line regression. The value of \( r \) square can range from 0 to 1. If the relationship between two variables is strong, the \( r \) square value will be close to 1. The square root of \( r \) square is called correlation coefficient.

The following equation can be used for manually determining the sample correlation coefficient \( (r) \)

\[
r = \frac{\sum x_1 y_1 - \sum x \sum y_1 / n}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}}
\]

• The standard error of coefficient

It is an estimate of the standard deviation of the coefficient of an independent variable in an equation. Generally, the larger the standard error of coefficient in relation to the corresponding X coefficient, the less certain that one can be about prediction. The equation used:

\[
S_b = \sqrt{\frac{S^2}{\sum (x_i - \bar{x})^2}}
\]

Weighted linear regression

In analytical science it is often found that the precision changes with concentration. In particular the standard deviation of the data is proportional to the magnitude of the value being measured. When this relation is observed weighted linear regression is used for obtaining the regression line. The potential benefit of weighed regression analysis is improved quantification at low concentration.

Polynomial regression

When error statistics or a scatter diagram show that a linear characterization of the association between observed value of two variables appears ineffectual, an equation of higher order may be investigated to see if it improves the fit. This equation will be of the general form.

\[
Y (x) = a_0 + a_1 X + a_2 X^2 + \ldots + a_n X^n
\]
Polynomial curve fitting is an extension of the fitting techniques described for linear regression analysis.

Calculations used for the present study

**Determination of concentration of drug by UV-Visible spectrometry**

The concentration is determined by the respective equation of standard curve, by graphical extrapolation method.

**Determination of concentration of drug by HPLC**

The concentration is determined by the respective equation of standard curve.

\[
\text{Concentration of drug} = \frac{(\text{Peak area} - \text{y intercept})}{\text{Slope}}
\]

**Determination of amount of drug present in the solution**

\[
\text{Amount of drug in milligrams} = \frac{\text{Concentration of drug solution in ppm}}{\text{Volume of drug solution}} \times 1000
\]

**Determination of Amount of drug adsorbed by adsorbent**

Amount of drug adsorbed = (Amount of drug present in initial drug solution) – (Amount of drug present in the solution which is collected after passing through the adsorbent)

**Determination of percentage of drug adsorbed**

\[
\text{Percentage of drug adsorbed} = \frac{\text{Amount of drug adsorbed}}{\text{Amount of drug present in initial drug solution}} \times 100
\]

**Determination of amount of drug recovered**

Amount of drug recovered = (Amount of drug adsorbed) – (Amount of drug present in the recovery solvent)
**Determination of percentage of drug recovered**

\[
\text{Percentage of drug recovered} = \frac{\text{Amount of drug adsorbed}}{\text{Amount of drug present in initial drug solution}} \times 100
\]

**Determination of pre – concentration factor (PF)**

\[
\text{Pre – concentration factor (PF)} = \frac{\text{Concentration of drug in recovered solvent in ppm}}{\text{Concentration of initial drug solution in ppm}}
\]
AIM OF THE WORK

It is, thus clear from the foregoing discussion that determination of pharma compounds in water system has not been undertaken as an study in INDIA. Further, most researchers have employed expensive GC – MS and LC – MS² methods for the purpose which are rather expensive and not easily available in all laboratories. Therefore, the present study was undertaken with the following objectives.

- To develop simple, accurate and cost effective techniques to determine such low concentration drugs present in aquatic environment. For our pre-concentration studies we have selected five drugs of different categories: Aspirin, Paracetamol, Esomeprazole Magnesium, Fenofibrate, and Venlafaxin HCl.

- To develop and validate a HPLC method for simultaneous determination of Esomerazole Magnesium, Fenofibrate and Venlafaxine HCl.

- To develop a LC – MS method for the simultaneous determination of Esomerazole Magnesium, Fenofibrate and Venlafaxine HCl in environmental water samples.

- To study the effect of metal ions and metal complex loaded activated charcoal in presence of oxygen to remove drugs from waste water.