

Focal Point: Environmental Analytical Chemistry

ILMAC Congress: October 13, 1999

Trace Determinations of Emerging Water Pollutants: Endocrine Disruptors, Pharmaceuticals, and Specialty Chemicals*

Environmental analysis aims to identify and quantify contaminants found in trace quantities in various compartments of the environment. The objective of this one-day meeting was to review significant new developments and applications made in the aquatic environment including wastewaters, natural waters (rivers, lakes, ground water) and drinking water.

An important issue which has emerged over the last few years is the potential risk caused by endocrine-disrupting environmental chemicals. The challenge for the analytical chemist is to carry out real-world measurements to provide a reliable basis for exposure and risk assessment. The determination of pharmaceuticals and their metabolites was the focus of the morning session, chaired by Dr. Suter.

The afternoon session was then devoted to hydrophilic organic pollutants originating from the use of different specialty chemicals. State-of-the-art analytical techniques were discussed, with the emphasis on surfactants, surfactant metabolites, and concrete admixtures (Chairman: Prof. Giger). Abstracts by the authors are given below.

Keywords: Analytical chemistry · Environmental pollution · ILMAC · Trace determination

Novel Methods for the Determination of Pharmaceuticals in the Aquatic Environment using LC-Electrospray Tandem MS

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To date only a few investigations have been published about the assessment of the environmental relevance of pharmaceuticals, although tons of these compounds have been used every year over the past decades. In Germany, for example, up to 100 tons of individual drugs are prescribed annually. In general, no analytical methods are available for environmental matrices that allow limits of quantification (LOQ) down to the ng/l range. Therefore, in our laboratory, analytical procedures were elaborated for a total of 84 pharmaceuticals and metabolites, enabling the simultaneous determination of polar drug residues belonging to different medicinal classes such as lipid regulators, antibiotics, estrogens, and metabolites in both sewage and drinking water. Several methods were used for the determination of drugs and their metabolites in the lower ng/l range using solid-phase extraction, derivatization, detection and confirmation by GC/MS and GC/MS/MS or specifically LC-electrospray/MS/MS. A first multi-method which has been developed, allows the determination of betablockers and β_2 -sympathomimetics as well as

neutral drugs like diazepam or carbamazepine at concentrations in the ng/l range after a joint solid-phase extraction detection using detection by GC/MS or LC-electrospray/MS/MS. A second multi-method allows determination down to the lower ng/l range of acidic drugs possessing one carboxylic moiety and additionally in some cases one or two hydroxy groups. The third method emphasizes the efficiency of LC-electrospray tandem MS detection, because 18 antibiotics can be analyzed simultaneously after lyophilization without any further cleanup step. Finally, contrast media were determined after SPE using LC-electrospray tandem MS detection down to 10 ng/l.

36 of 55 pharmaceuticals and five of nine metabolites were quantified in at least one sewage treatment plant (STP) effluent. In general, the removal rates in the investigated municipal STP exceeded 60%. In 40 German rivers and streams, 31 pharmaceuticals and five metabolites were quantified in at least one sample. Highest median values were detected for bezafibrate with 0.35 $\mu\text{g/l}$ and carbamazepine with 0.25 $\mu\text{g/l}$. In groundwater samples taken close to the banks of streams, relatively high concentrations of pharmaceuticals up to 2.4 $\mu\text{g/l}$ were occasionally detected. In drinking water, only 9 of 65 pharmaceuticals analyzed were found in very few samples and without exception in the lower ng/l range.

Immunochemical Methods for Quantifying Hormones in Polluted Waters

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Quantification of hormones in polluted waters is a formidable task that requires the separation and detection of a moderately hydrophobic analyte in a matrix that contains high concentra-

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tions of natural organic matter (NOM). Immunochemical techniques provide an approach for analyzing hormones and other pharmaceuticals that takes advantage of highly selective binding by antibodies. Immunochemistry can be used to separate an analyte from potentially interfering NOM (*i.e.* immunoaffinity) or it can be used to quantify organic compounds (*i.e.* ELISA or RIA). We have used solid-phase extraction followed by HPLC cleanup and ELISA to study the fate of 17β -estradiol, ethinyl estradiol, and testosterone in wastewater treatment plants and polluted surface waters. With the exception of samples from a wastewater treatment plant that uses reverse osmosis, concentrations of hormones were always more than an order of magnitude higher than the method detection limit. Our results indicate that hormone removal from wastewater is a function of the sophistication of the treatment process. Concentrations of hormones in samples from secondary wastewater treatment plants are always higher than those detected in plants using effluent filtration, which, in turn, are always higher than plants equipped with reverse osmosis. Analyses of samples from an effluent-dominated stream indicate a relatively fast loss of hormones after discharge. Immunoaffinity cleanup can be used to separate hormones from NOM prior to GC/MS analysis. However, satisfactory results also can be obtained without immunoaffinity cleanup by using negative chemical ionization GC/MS/MS.

Chemical Analyses of Estrogenic Hormones and Bisphenol-A in Environmental Samples

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An analytical procedure was developed for routine analyses of estrogenic hormones (17β -estradiol, 17α -estradiol, estrone, and 17α -ethinylestradiol) in surface water and wastewater in concentrations below 1 ng/l. The method consists of disk extraction, column and HPLC cleanup, SIL-A derivatization and analysis by GC/MS/MS. The recovery was 89–112% with a limit of detection of 0.3–0.8 ng/l. This method can also be applied for the analysis of hormone glucuronides by pretreating the sample with β -glucuronidase and for the analysis of the xeno-estrogenic bisphenol-A (detection limit 10 ng/l, recovery 117%). The occurrence of the estrogenic hormones, their glucuronides, and bisphenol-A was studied in wastewater and in marine and freshwater surface water in The Netherlands. The data show that estrogenic hormones (mainly estrone and 17β -estradiol) are present at low concentrations (up to 6 ng/l) at some locations in surface water and in domestic effluents of wastewater treatment plants. In untreated wastewater, concentrations were higher by approximately a factor 10, while the contraceptive 17α -ethinylestradiol was also observed occasionally. Hormone glucuronides were not detected in either surface water or wastewater. Bisphenol-A was detected at all surface water locations (up to 150 ng/l) and in treated and untreated wastewater. Highest concentrations (over 2 μ g/l) were observed in untreated industrial wastewater.

Pharmaceuticals in the Aquatic Environment

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The occurrence of pharmaceutical compounds in the aquatic environment has become a topic of substantial scientific and public concern. Little is known about the risk of low levels of drugs in the environment, since current risk assessment methodologies do not address pharmaceuticals properly. It is an unanswered question whether the occurrence of antibiotics in the environment contributes to the increasing resistance of microorganisms towards antibiotics.

The fate of human and veterinary drugs differs quite significantly. Human pharmaceuticals have to pass a sewage treatment plant (STP) prior to entering surface and ground waters. Veterinary drugs on the other hand will contaminate soil and ground water *via* manure disposal. Surface waters can be polluted with drugs after a rain event, by run-off from fields treated with digested sludge or livestock slurries. Additionally, transport of drugs *via* bank infiltration from highly contaminated surface waters into groundwater is also a possibility, as well as the infiltration from wastewaters directly from leaks and rain overflows.

The annual consumption of antibiotics in Switzerland in 1997 was in the order of 80 tons. 28 tons were used in human medicine, of which one fifth was consumed in hospitals, while the rest was consumed in private households. The consumption in human medicine has remained more or less constant since 1992. The main group of antibiotics used in human medicine is the penicillins (β -lactam). Macrolides, cephalosporines (β -lactams), and fluoroquinolones are additional important groups.

Details about use in veterinary medicine are more difficult to obtain. In intensive livestock production low doses of antimicrobial additives are often used as growth promoters to increase yield. The veterinarian use of antibiotics makes up 65% of the total antibiotic consumption in Switzerland. Total consumption of antibiotics was reduced from 125 tons in 1992 to 80 tons in 1997. The cause was the strong reduction of the feed additives from 91 to 36 tons. In the same time period, the consumption of the therapeutic antibiotic use has doubled with a strong shift from feed additives to therapeutic use. The use of antibiotics as feed additives has been forbidden in Switzerland since January 1999. In veterinary medicine, the more important antibiotics are again the β -lactams and chloramphenicol, in addition to tetracyclines and sulfonamides. β -Lactam antibiotics are not expected to be persistent in the aquatic environment since the lactam bond hydrolyses in aqueous medium.

Macrolides were found in concentrations ranging from 0.02 (roxithromycin) to 11 μ g/l (erythromycin- H_2O , a metabolite of erythromycin), in the effluent of STPs using LC/MS as the analytical method. Fluoroquinolone (FQ) antibiotics, are important broad-spectrum antibiotics licensed for the use in both humans and animals. Field studies were undertaken to follow the daily input variations of human antibiotics into surface waters. The leading FQ in human medicinal use, ciprofloxacin, was measured in concentrations of 70–80 ng/l in 24 h composite samples of the effluent of a municipal STP, using HPLC with fluorescence detection. These investigations show that ciprofloxacin is eliminated only to 70–80% in this WTP and can reach surface waters. In combination with other fluoroquinolone antibiotics, ciprofloxacin might effect some of the most susceptible organisms.

Analytical Determination of Surfactants in Products, Wastewater, and Water Samples

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A short overview was given about recent developments in surfactant analysis by HPLC. In the case of surfactant analysis, it is necessary to distinguish between different types of samples and concentration ranges:

- g/l range - product analysis,
- mg/l range - wastewater analysis, biotests,
- µg/l range - environmental analysis.

The analytical procedures were presented using examples covering each concentration range.

Furthermore, 'surfactant' describes the physical property to decrease surface tension and is only a generic term for chemicals with different behavior in chemical analysis. The choice of the analytical procedure depends on the properties of the surfactant (*e.g.* preconcentration, separation or detection method) and on the question to be answered (*e.g.* separation of homologous compounds). HPLC methods for six different types of surfactants (anionic, cationic, non-ionic) were presented.

Determination of Nonylphenol Ethoxylate Metabolites in Biomatrices

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Nonylphenol ethoxylates (NPEO) belong to the group of non-ionic surfactants. After use they are discharged into municipal and industrial wastewaters. Due to the persistence, the toxicity and the estrogenic activity of their biodegradation products, especially nonylphenol (NP), NPEOs are ecotoxicologically rather questionable. For this reason their occurrence and fate in different environmental compartments have been extensively investigated over the last two decades. Data on NP in biomatrices, however, is almost non-existent. Therefore, we developed an off-line LC-GC method for the quantitative determination of NP in biomatrices such as food. The analyte was isolated from liquid and solid samples spiked with 4-n-NP as the internal standard by a combination of steam distillation and continuous liquid-liquid extraction. After cleanup of the raw extracts on normal-phase HPLC, the NP content was finally analyzed by GC-MS equipped with a large volume injector (LVI) allowing injection of more than 100 µl sample volumes. Current results on dairy products show increasing NP concentrations with rising fat content.

NP consists of a complex mixture of different isomers leading to a typical peak pattern after gas chromatographic separation. Several articles have been published with mass spectra of the NP isomers and proposals for their structures. Based on these studies we drew up a list of 30 NP isomers which we have had synthesized in an external laboratory. Elucidation of the NP isomer structures by GC-MS analyses of technical NP and the first completed 15 NP isomers using a 100 m capillary column was presented.

Fate of LAS Co-Products and Nonylphenolethoxylates in Activated-Sludge Sewage Treatment Plants

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After soaps, linear alkylbenzenesulfonates (LAS) are the most widely used surfactants. Major co-products in commercial LAS mixtures are dialkyltetralinsulfonates (DATS) and the so-called iso-LAS (single methyl branch on the alkyl chain). At present, the possible accumulation in the environment of breakdown products of DATS and iso-LAS is the subject of heated debate. Part of this study has been devoted to following the fate of LAS co-products in five Roman activated-sludge sewage treatment plants (STPs) and determining nature and abundance of persistent metabolites leaving the plants. Over a period of eight months, influent and effluent samples of STPs were collected monthly and analyzed. The analytical procedure involves solid-phase extraction of the analytes in both influent (50 ml) and effluent samples (200 ml) followed by LC-electrospray (ES)-MS analysis. Although the MS instrumentation was equipped with a single quadrupole, confirmatory ions were obtained by decomposing quasi-molecular ions in the intermediate region of the MS, thus obtaining so-called in-source CID spectra. Field data indicated that LAS were extensively mineralized during activated-sludge treatment and that sulphophenylalkylcarboxylated intermediates exiting the plant primarily originate from the biotransformation of iso-LAS. Over eight months of monitoring, efficiencies of the STPs at removing LAS, co-products and related metabolites were estimated. These data highlight that, like LAS, their co-products undergo extensive primary biodegradation. Removal efficiencies of iso-LAS and DATS intermediates (DATSI) averaged 65 and 46%, respectively. These figures indicate that bacteria are unable to mineralize a remarkable fraction of those breakdown products with a large steric hindrance. DATSI amounted to more than 50% of the total analyte amount leaving the plants. Interestingly, almost 13% of the metabolites were dicarboxylated species.

Nonylphenolpolyethoxylates (A₉PEs) are one of the most widely used class of surfactants. Recent findings on the weakly estrogenic effect of some of their breakdown products have raised concern about the fate of A₉PEs in the environment. Initial biotransformation of A₉PEs occurs by ethoxy-chain shortening, mainly producing A₉PE₂, which is then oxidized to A₉PE₂C. From this point onward the metabolic pathway of A₉PEs is largely in the dark. We shed light on the fate of the multi-branched alkyl side chain by a laboratory biodegradation test. After a five day lag time, the alkyl chain of many but not all of the previously formed A₉PE₂C isomers was carboxylated and shortened with simultaneous formation of a new family of metabolites (CAPECs) with molecular weights regularly spaced by 14 Da. CAPECs were shown to be very recalcitrant species, as their concentrations did not vary significantly, even after six months from the beginning of the biodegradation test. To ascertain the occurrence and abundance of CAPEC in STPs, influent and effluents were taken monthly and analyzed over a period of eight months. The A₉PEI removal efficiency averaged 56% (n = 36). Data showed that CAPECs, as a total, were much more abundant than other intermediates of A₉PEs. Among CAPECs, those with six and eight residual alkanoyl groups, *i.e.* CA₆PEC and CA₈PEC, were the most prominent homologues (> 80%). Almost 85% of CAPEC formed during activated-sludge treatment had zero or one residual ethoxy group.

Quantitative Trace Determination of Sulfonated Naphthalene-Formaldehyde Condensates in Aqueous Environmental Samples by HPLC with Fluorescence Detection

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Unknown compounds which have been frequently observed as a regular peak pattern in the HPLC/FLD analysis of aqueous environmental samples for monomeric naphthalene sulfonates, have now been identified for the first time as sulfonated naphthalene-formaldehyde condensates (SNFC).

SNFC are anthropogenic and xenobiotic compounds which are used in many industrial applications. Examples are their use as concrete plasticizers, tanning agents, auxiliaries for paper manufacturing and dispersants for dyes and pesticides. Until recently, SNFCs could not be analyzed in environmental samples, both due to the lack of reference compounds and because sufficiently sensitive analytical methods were not available. This presentation dealt with the development of an analytical method consisting of solid-phase extraction (SPE) and HPLC/FLD.

The preparation of calibration standards was the key for the qualitative and especially for the quantitative determination of SNFC residues in natural waters, wastewaters, and landfill leachates. The reference compounds were prepared by organic synthesis and by semi-preparative isolation of components from a technical product. The structural model of the SNFC was confirmed by ^1H NMR and LC-MS and the concentration of SNFC in the standard solution was determined by ICP-OES.

In most of the investigated aqueous environmental samples, SNFC could be detected on the $\mu\text{g/l}$ scale. While the homologue patterns differ from product to product and from sample to sample, the isomer pattern within each degree of condensation remains constant. In environmental samples, condensates of up to four 2-naphthalene-sulfonate monomer units usually occur. While the monomer 2-naphthalene sulfonate is readily degradable under aerobic conditions, the present results suggest a high persistence of SNFC.

Analysis of Aromatic Sulfonates and Polysulfonates in Industrial Effluents by HPLC-MS/MS

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Aromatic sulfonates are widely used in industrial production processes as dispersants, wetting agents, dyes, and in chemical production as intermediates. Their removal in wastewater treatment is hampered by their limited biodegradability and high polarity and may, thus, require oxidative treatment processes.

We have developed analytical methods for the analysis of aromatic sulfonates in industrial effluents by LC-MS/MS, in order to quantitatively determine aromatic sulfonates, for which reference material is available, and to follow the degradation and transformation processes in wastewater treatment.

A novel chromatography method with a volatile ion-pairing agent was developed that makes ion-pair chromatography compatible with MS detection and provides sufficient retention also for the very polar polysulfonated compounds. With selected

reaction monitoring in the MS/MS mode, detection limits ranging from 0.1 to 5 $\mu\text{g/l}$ are obtained for various aromatic sulfonates from directly injected samples. Full scan analyses in single-MS mode and product-ion scanning and accurate mass measurements help to identify transformation products formed during wastewater treatment.

The methods were applied to different wastewaters and examples were presented, such as the transformation of azo dyes in anaerobic/aerobic treatment of a dyehouse wastewater or naphthalene sulfonates in ozonation processes.