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# Surfactants: non-biodegradable, significant pollutants in sewage treatment plant effluents

# Separation, identification and quantification by liquid chromatography, flow-injection analysis–mass spectrometry and tandem mass spectrometry

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ABSTRACT

Effluents from biological waste water treatment plants contain mainly non-biodegradable polar compounds. Methods for the detection, identification and determination of these hardly or non-eliminatable polar organic compounds are described. Flow-injection analysis (FIA) and liquid chromatographic (LC) separation on an analytical column by mass spectrometric (MS) and tandem mass spectrometric (MS–MS) detection coupled by a thermospray (TSP) interface were performed. The results showed that non-ionic surfactants and their metabolites (primary degradation products) besides linear alkyl benzene sulphonates (LABS) may dominate the range of pollutants. LC–MS confirmed that retention time shifts may occur if waste water extracts are separated on analytical columns. This cannot be recognized by UV detection. The identification of a biochemical degradation product of a non-ionic surfactant was carried out by both FIA–MS–MS and LC–MS–MS. Quantification of this compound was performed by standard addition analysis using FIA–MS or LC–MS in the selected-ion monitoring (SIM) mode. The time required for quantification is 25–30 times higher using LC–MS instead of FIA–MS.

## INTRODUCTION

Normally, surfactants are discharged with waste water after application. In addition to their surface activity, these compounds should have a certain stability towards heat, hydrolysis and/or biochemical degradation according to the different application purposes. Although they are not classed with dangerous compounds within the meaning of Section 7a of the German Federal Water Act, their ecotoxicological potential must not be neglected because of the large amounts produced and applied. Depending on their structure, they have a more or less toxic effect on aquatic life forms such as fish, daphnia and algae [1]. Not only the chronic but also the acute toxicity can be far below 1 mg/l. Some of these compounds are relevant in drinking water because of their high polarity. They have been detected in drinking water produced from river Rhine water [2]. The accumulation of these surfactants in biological sewage sludge is also worth mentioning. Surfactants are partially released from sludge used as fertilizer in agriculture or deposited on a landfill and are able to desorb and to dissolve highly toxic compounds adsorbed in the soil [3]. Together with toxic compounds, the surfactants will then appear in the groundwater [4].

Knowledge about the presence and concentration of such compounds in the environmental compartments water and soil is therefore of great

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importance. For a long time, however, scientists have contented themselves with sum-parameter examination of these surfactants, which were distinguished only between three main types, anionic [5,6], cationic [7] and non-ionic [8,9]. However, interferences between the analytes and the matrix may arise, leading to high or low results. Biochemical degradation products of surfactants, so-called "primary degradation products", with only very small changes in the molecular structure with respect to the precursor compound, cannot be determined by these substance class-specific methods [10,11].

Substance-specific determination of these compounds is, in general, possible only after separation of the matrix compounds. As these compounds are only slightly or non-volatile, either large-scale derivatization reactions are necessary [12] to make gas chromatographic (GC) separation possible, or LC separation methods have to be employed for the non-volatile compounds. As, however, waste water extracts of municipal sewage treatment plants normally contain large numbers of surface-active compounds in each fraction even after preliminary separation, poor chromatographic separations and/or considerable retention-time shifts still result from such separations on analytical columns. If unspecific detectors such as UV, fluorescence, conductivity and refraction index types are employed, the results cannot be interpreted [13]. The application of the mass spectrometer as a specific detector, where separation can be achieved off-line [14] or by selective ionization methods [15], improved the analytical possibilities and led to high sensitivity in such examinations. The ionization methods fast atomic bombardment (FAB), field desorption (FD) and negative field desorption (NFD) did not produce fragments so that structure information could only be obtained after collisionally induced dissociation (CID) by tandem mass spectrometry (MS–MS) [14,16]. The use of MS–MS equipment allowed direct mixture analysis for the examination of complex mixtures [17,18] without chromatographic separation. However, great difficulties still remain during the detection and quantification of surfactants and their primary degradation products in environmental samples, even if these analytical techniques were applied.

In this paper, methods for the qualitative and quantitative determination of polar pollutants having been recognized as relevant (surfactants and their biochemical primary degradation products) in sewage treatment plant influents and effluents are presented. Analytical techniques using the MS–MS function of a tandem mass spectrometer for mixture analysis or performing CID after chromatographic separation on an analytical column should help to solve some of these problems.

## EXPERIMENTAL

### *Materials*

Waste water samples were taken from two different waste water treatment plants in the city of Aachen or from municipal treatment plants in northwest Germany located near the river Rhine. Waste water and sludge for the generation of metabolites were taken from a treatment plant in Aachen (Aachen-Süd).

The primary degradation product (metabolite) of alkanol polyglycol ether surfactants was generated by aeration of the sewage treatment plant influent and of bacterial sludge from this plant. During aeration the mixture was stirred and the degradation process was monitored by flow-injection analysis (FIA)–MS. The separation of this metabolite from the waste water matrix was carried out after $C_{18}$ solid-phase enrichment and elution with hexane, hexane–diethyl ether (1:1, v/v), methanol–water (2:8, v/v) and methanol, the methanol eluate being collected. This procedure yields a mixture of more than 95% purity for the metabolite.

Water pollutants were extracted either using continuous liquid–liquid extraction or solid-phase extraction cartridges from Baker (Deventer, Netherlands). Solid-phase extraction materials were conditioned as prescribed by the manufacturer. Glass-fibre and membrane filters used for the pretreatment of the water samples were obtained from Schleicher & Schüll (Dassel, Germany). Before use, the glass-fibre and membrane filters were heated to 400°C or were treated with ultra-pure water obtained with a Milli-Q system (Millipore, Milford, MA, USA) for 24 h and then washed with 100 ml of the same water. Diethyl ether and methanol used for

the liquid–liquid extraction or desorption of water pollutants from the solid-phase material were Nanograde solvents from Promochem (Wesel, Germany). Acetonitrile, chloroform, dimethyl sulphoxide and methanol used for column-cleaning purposes were of analytical-reagent grade from Merck (Darmstadt, Germany). Nitrogen for drying of solid-phase cartridges was of 99.999% purity (Linde, Germany). All surfactant standards for identification via daughter ion spectral library and for waste water spiking purposes were gifts from the producers (Hüls, Marl; Hoechst, Frankfurt and BASF, Ludwigshafen, Germany) and were of technical grade. Polyethylene glycol (PEG 400) was of technical grade.

GC analyses were done on a DB-17 fused-silica column (J&W Scientific, Folsom, CA, USA) and helium of 99.999% purity (Linde) was used as the carrier gas. LC separations were done on a Nucleosil $C_{18}$ (5 $\mu$m, spherical) column (25 cm × 4.6 mm I.D.) (Chromatography Service Römer). The mobile phase was methanol and acetonitrile (HPLC grade) from Promochem and Milli-Q-purified water. Ammonium acetate for thermospray (TSP) ionization was of analytical-reagent grade from Merck.

*Sampling, sample preparation and handling*

All samples from the waste water treatment plants were taken as grab samples in glass bottles. The bottles were rinsed carefully with several portions of the same water that was subsequently stored in them. The storage temperature was 4°C.

For continuous liquid–liquid extraction, 2 l of waste water were extracted with 300 ml of diethyl ether over a period of 5 h. Liquid–liquid extracts were dried by anhydrous sodium sulphate, filtered and concentrated to 2 ml (influent) or 0.1 ml (effluent) under nitrogen, resulting in a concentration factor of 1000 or 20 000 respectively.

Depending on the degree of pollution, different amounts of water were used for solid-phase extraction. Water samples for FIA and LC–MS analysis were forced through the solid-phase extraction cartridges after passage through a glass-fibre filter. The adsorbed pollutants were desorbed separately. Solvents of different polarities (hexane, hexane–diethyl ether, diethyl ether, water–methanol and methanol) were used for this purpose. All eluates except those with methanol and methanol–water were evaporated to dryness with a stream of nitrogen, and the residue was dissolved in 1 ml of methanol. The samples were rinsed into glass bottles after solid-phase extraction and freeze-drying was applied to enrich non-$C_{18}$-adsorbable compounds. After freeze-drying the samples were dissolved in 1 ml of methanol and used for FIA or LC–MS investigations.

Volumes of 1 or 2 $\mu$l of waste water extracts of influent and effluent, respectively, were injected for GC–MS analysis, and 20 or 100 $\mu$l of solid-phase eluates were injected for qualitative FIA–MS and LC–MS analysis, respectively.

A stock solution containing 12 $\mu$g/$\mu$l was prepared in order to determine the primary degradation product of the non-ionic surfactants. After evaporation of the solvents to dryness, three aliquots (2 ml) of the methanolic eluate of the waste water extract containing the unknown concentration of the metabolite were spiked with 50, 100 or 200 $\mu$l of the stock solution. The samples were diluted to 2 ml. This series of solutions were used to examine the relationship between the peak area and the concentration of the metabolites in both FIA–MS and LC–MS. For quantification by FIA–MS a minimum of five injections was made. The injection volumes for FIA–MS and LC–MS were 20 and 100 $\mu$l per injection, respectively.

*Gas chromatographic system*

A Varian (Darmstadt, Germany) Model 3400 GC system with a fused-silica capillary column was used. The conditions were as follows: carrier gas, helium; linear gas velocity, 15 cm/s; injector temperature, 250°C; transfer line temperature, 250°C; column, DB-17, film thickness 0.25 $\mu$m (30 m × 0.32 mm I.D.).

Combined with GC, electron impact (EI) ionization was applied with an ionization energy of 70 eV. Under these conditions the pressure in the ion source was $8 \cdot 10^{-6}$ Torr (1 Torr = 133.322 Pa) and in the vacuum system of the mass spectrometer $3 \cdot 10^{-2}$ Torr. The electron multiplier was operated at 1200 V with a conver-

sion dynode voltage at 5 kV. The temperature in the ion source was 150°C.

*Liquid chromatographic system*

LC separations coupled with MS, MS–MS and UV detection were achieved with a Waters (Milford, MA, USA) Model 600 MS system. A Waters Model 510 pump was used for post-column addition of 0.1 *M* ammonium acetate solution in the TSP mode. A Waters Model 490 MS UV detector was connected in-line with the TSP interface. The conditions in FIA bypassing the analytical column were as follows: mobile phase I, methanol–water (60:40); mobile phase II, 0.1 *M* ammonium acetate in water. The overall flow-rate was 1.5 ml/min with a ratio of 0.8 ml/min of mobile phase I and 0.7 ml/min of mobile phase II.

The chromatographic separations on the analytical column were carried out after optimization of the conditions by a standardized method, shown in Table I.

The flow-rate for column separation was 1.0 ml/min of mobile phase I. After passing the UV detector, 0.5 ml/min of mobile phase II was added, which resulted in an overall flow-rate of 1.5 ml/min.

The reversed-phase column was cleaned with a mixture of acetonitrile, chloroform, methanol and dimethyl sulphoxide (3:3:3:1, v/v) after finishing analysis and before equilibration for a new separation.

TABLE I

GRADIENT ELUTION SCHEME AND COMPOSITION OF MOBILE PHASE I

Solvent A = acetonitrile; solvent B = water–methanol (80:20, v/v).

| Time (min) | Solvent A (%) | Solvent B (%) |
|---|---|---|
| 0 | 10 | 90 |
| 10 | 30 | 70 |
| 25 | 60 | 40 |
| 35 | 90 | 10 |

*MS and MS–MS systems*

The mass spectrometer was a TSQ 70 combined with a PDP 11/73 data station. The TSP interface was obtained from Finnigan MAT. For coupling the LC system with the mass spectrometer, the conditions for TSP ionization using ammonium acetate were chosen as vaporizer temperature, 90°C and jet block temperature, 250°C. The conditions varied during the analytical separations. Under the above conditions the ion source pressure was 0.5 Torr and the pressure in the vacuum system of the mass spectrometer was $2 \cdot 10^{-5}$ Torr.

The electron multiplier was operated at 1200 V and the conversion dynode at 5 kV. In the MS–MS mode the ion source pressure was also 0.5 Torr. Under CID conditions the pressure in quadrupole 2 (collision cell) normally was 1.3 mTorr or is specified in the captions of the figures. The collision energy was adjusted from −10 to −50 eV. The electron multiplier voltage in quadrupole 3 was 1500 V with a conversion dynode voltage at 5 kV.

GC–MS analysis was performed by scanning at 1 s from 45 to 500 u.

FIA and LC analyses were applied, recording TSP mass spectra scanning from 150 to 1200 u at 1 or 3 s, respectively. FIA bypassing the analytical column with MS detection was performed accumulating 50 scans after injection. The mass spectrum averaging the total ion current from the beginning of the signal up to the end is called the "overview spectrum".

TSP ionization was normally carried out in the positive mode, unless specified otherwise.

For quantification the mass spectrometer was operated in the selected ion monitoring (SIM) mode using a dwell time of 200 ms for each mass.

RESULTS AND DISCUSSION

The elimination of non-polar volatile compounds from municipal waste water in the biological waste water treatment process is successful with an efficiency of more than 95%, as the GC–MS total ion current (TIC) chromatograms (Fig. 1a and b) of a representative treatment plant influent and effluent demonstrate. Regard-

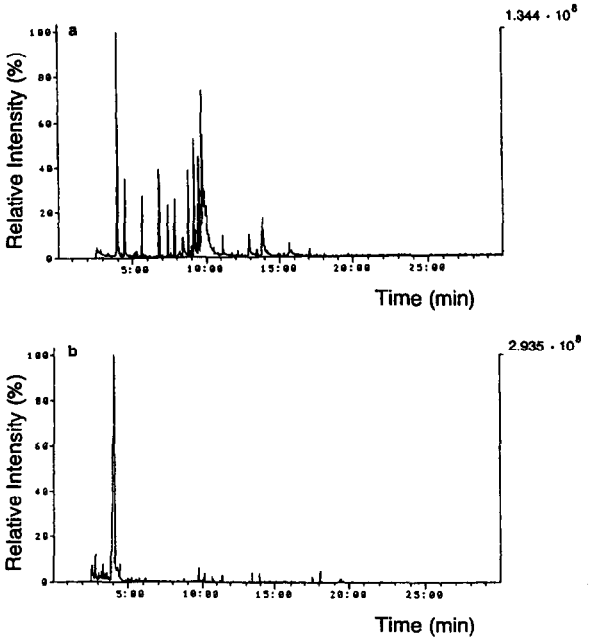



Fig. 1. (a) GC–MS total ion current trace for municipal waste water treatment plant influent. Liquid–liquid extract; solvent, diethyl ether. (b) GC–MS total ion current trace for the effluent of waste water treatment plant as in (a). For concentration factor, see Experimental.

ing the chromatograms the effluent seems to be relatively pure, although a concentration factor of 20 related to the influent extract was chosen. The examination of the influent and effluent by the sum parameter total organic carbon (TOC), however, showed an organic carbon load of 420 and 53 mg/l respectively. The examination of the same waste waters concerning polar organic compounds by means of liquid chromatography coupled with a TSP interface doing flow-injection analysis bypassing the analytical column (FIA–MS) gave mass spectra averaged from a maximum of 50 scans, as shown in Fig. 2a and b. This type of spectrum will be called here and later an "overview spectrum". Recording these spectra, the polar organic compounds existing in the extracts are registered in the form of their molecular and cluster ions, respectively, consisting of molecule and ammonium ion. Normally no fragments are produced in this way (compare the TSP ionization of alkyl ether sulphates [11]) and, consequently, no structure information, but molecular mass information will be obtained. Thus, comparing the spectra shown in Fig. 2a and b and the intensities of the ion currents, it can be recognized that the number of signals, *i.e.*, of the different molecular ions has not decreased. The concentration of compounds in the water, however, has slightly decreased. This means that the elimination mechanisms of the biological treatment process are well able to eliminate non-polar compounds from the liquid phase; however, the efficiency of the waste water treatment process diminishes if polar anthropogenic compounds or those being formed chemically or biochemically during the sewage treatment process dominate the range of pollutants. This is caused by the physical properties of polar waste water compounds: on the one hand they cannot be stripped with air because of their polarity, but on the other hand they cannot be adsorbed at the lipohilic activated sludge. If they are then hardly biodegradable, or degradation is not possible because the compounds first have to be adsorbed on the activated sludge and then absorbed by the cell, they can be detected unchanged and only slightly decreased in the treatment plant effluent. This is especially true for surfactants as they are polar and, as described above, successfully resist any biochemical degradation because of their tasks and the properties connected with them.

Non-ionic surfactants of the polyethylene or polypropylene glycol ether type (Fig. 3a and b) can be clarified at once by their distinct pattern arising in the overview spectra in Fig. 2a and b generated by FIA–MS. Non-ionic surfactants of this type show equidistant signals at $\Delta m/z$ 44 and 58 when polyethylene or polypropylene glycol ether chains, respectively, are present. This finding, however, has to be checked by further examination, via the generation of characteristic daughter ions. In contrast to EI ionization after GC separation, where the ionized compounds are identified by their fragment spectra, unfortunately only molecular ions are typically generated during the TSP ionization process. This means that certain ions have to be selected by mass filtration before decomposing them by CID into fragment ions to permit a positive identification. For this procedure a spectrometer with an

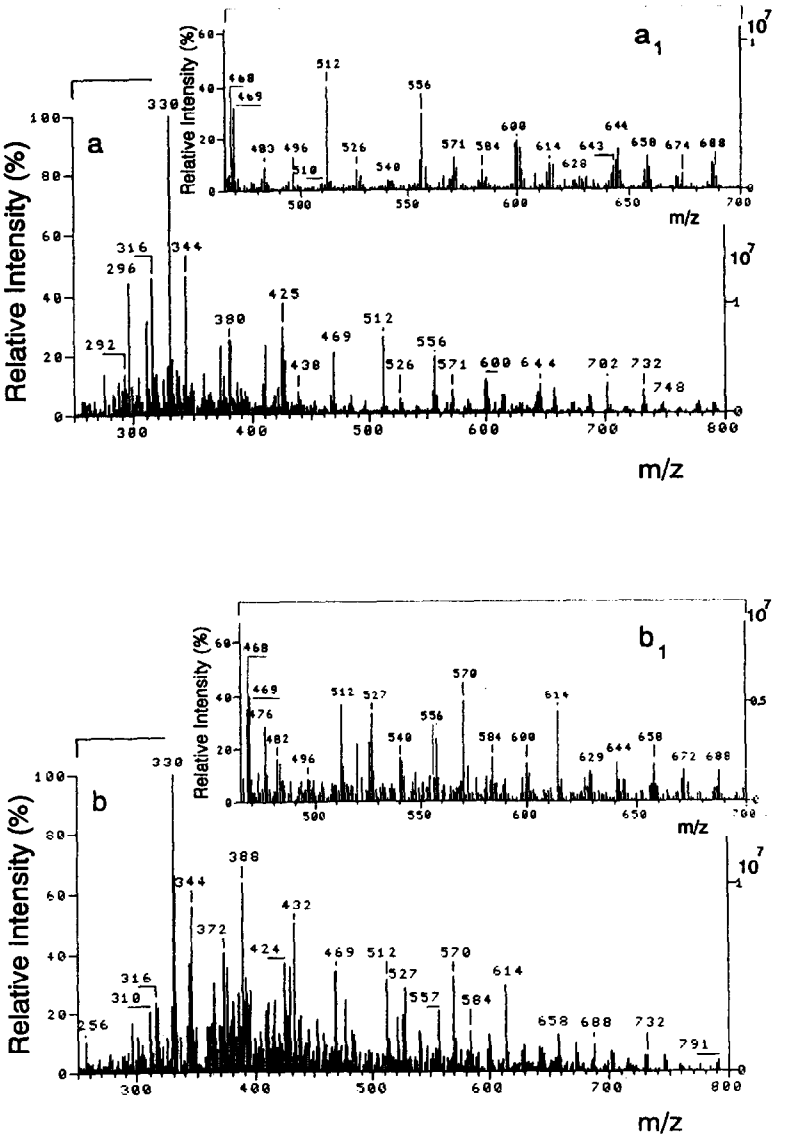
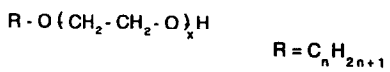



Fig. 2. (a) TSP-MS loop injection trace obtained by bypassing the analytical column (FIA–MS), subsequently called "overview spectrum", for waste water treatment plant influent as in Fig. 1a. ($a_1$) Detail of mass spectrum in (a). (b) FIA–MS overview spectrum of waste water treatment plant effluent as in Fig. 1b. ($b_1$) Detail of mass spectrum in (b). Positive TSP ionization. For FIA conditions, see Experimental. $C_{18}$ solid-phase extract; eluent, methanol.

MS–MS option is necessary. A laboratory-made, computer-aided daughter ion library may be very helpful in this instance [2] if a similar or the same compound is present.

The ions generated by positive ionization at *m/z* 316, 330 and 344 in Fig. 2a and b can be classed at once with an anionic surfactant — linear alkylbenzene sulphonic acid (LABS; see Fig. 3c). This is demonstrated by generating negative daughter ions by CID, resulting in only the fragment at *m/z* 183. This fragment is characteristic of an unbranched alkylbenzene sulphonic acid (ABS) [19]. Negative TSP ionization, shown in Fig. 4, which is the typical ionization method for these waste water compounds, yields only compounds that can be

a

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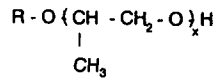


c

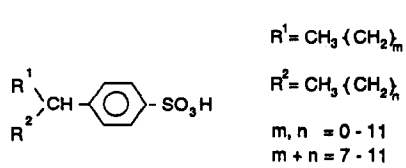


Fig. 3. Formulae of (a, b) non-ionic surfactants and (c) anionic surfactant. (a) Alkanol polyethylene glycol ether; (b) alkanol polypropylene glycol ether; (c) linear alkylbenzene sulphonic acid (LABS).

ionized negatively. In addition to the fragment ion at $m/z$ 183 it confirms again this type of anionic surfactant. In the overview spectrum the signals of the negative LABS ions are at $m/z$ 297, 311 and 325, generating the $[M-1]^-$ ions instead of the ammonium cluster ions $[M+NH_4]^+$ at $m/z$ 316, 330 and 344 in the positive TSP mode.

The striking signals of the ions at $m/z$ 468, 512, 556, etc., up to 732 appearing at $\Delta m/z$ 44 during positive TSP ionization (see Fig. 2a and b) can be classed with non-ionic surfactants of the polyethylene glycol ether type. Here negative ionization is not successful, and therefore they cannot be recognized in Fig. 4. The representative daughter ion spectrum of the ion at $m/z$ 468 choosen by mass filtration is shown in Fig. 5. It contains both the alkyl fragments at $m/z$ 57, 71, 85, 99 and 113 which are characteristic of an alkanol polyglycol ether, and the polyglycol ether fragments at $m/z$ 45, 89, 133 and 177 consisting of 1–4 ethylene glycol units. The fundamental structure of the surfactant, characterized by its daughter ion spectrum, is shown in Fig. 5. This or similar surfactants that differ in a characteristic way in their chromatographic behaviour from alkanol polyethers previously recorded [19,20] were not present in our daughter ion library.

Detailed reports are available that include excellent results concerning the examination of waters and waste waters for polar compounds by means of mixture analysis by MS–MS [2,13,16,18,21–24]. In this mode of operation, however, it is not always possible to obtain all the information necessary for identification and quantification. A chromatographic separation of the same extract that had previously been examined in the FIA–MS mode was carried out to obtain further information serving to confirm the structure proposed in Fig. 5. In parallel, UV traces at different wavelengths were recorded. A $C_{18}$ analytical column and gradient elution according to Table I were chosen. The UV trace at 210 nm and the reconstructed ion current (RIC) of this separation are shown in Fig. 6a and b. Although this waste water extract was highly matrix charged, the chromatographic separation

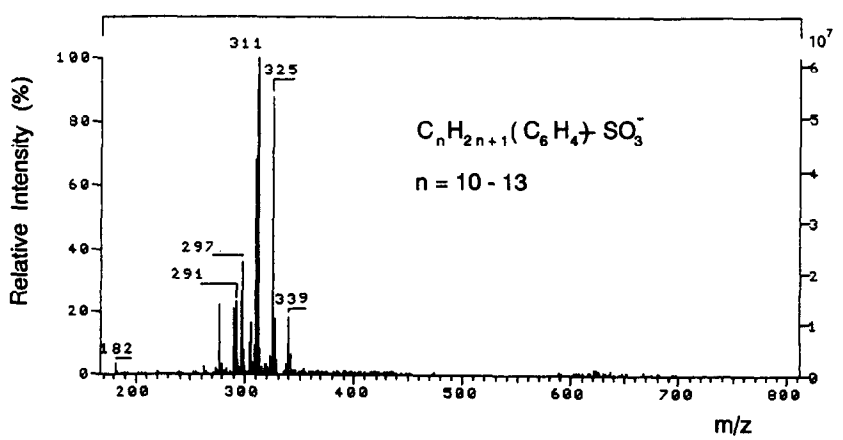


Fig. 4. FIA–overview mass spectrum as in Fig. 2b for extract of waste water treatment plant effluent. Negative TSP ionization.

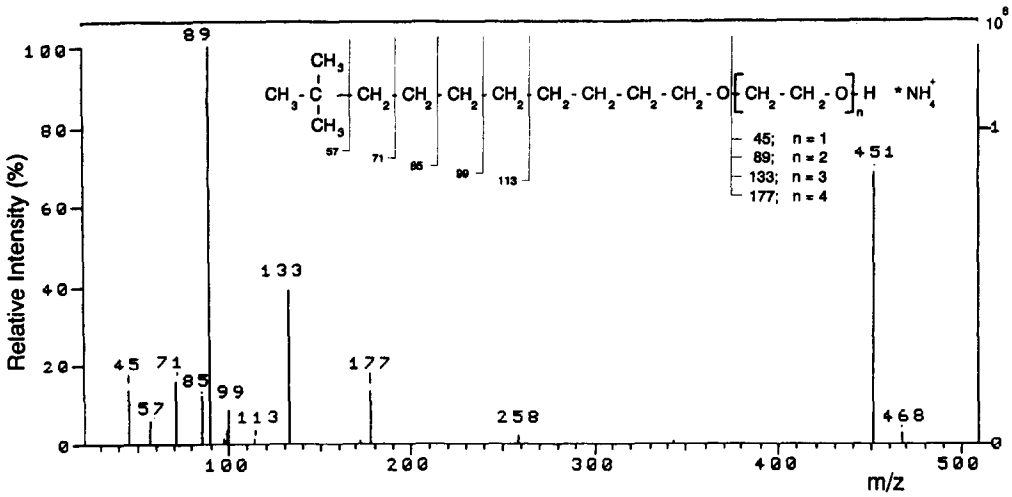


Fig. 5. Daughter ion mass spectrum (FIA–MS–MS) and fragmentation scheme of non-ionic surfactant cluster ion [$m/z$ 468; $C_{12}H_{25}O(CH_2CH_2O)_6H \cdot NH_4^+$] from waste water extract (influent) as in Fig. 2a. Collision energy, −25 eV.

was very successful. As the mass spectra in Fig. 7 show, non-ionic surfactants are hidden under the three peaks marked 1, 2 and 3 in the UV spectrum and in the ion current trace. These surfactants are identical in their fundamental structure, as shown in Fig. 5; only the chain lengths of their polyether residue vary (compare peak 3 with peaks 1 and 2). The reason for the different retention times of the signals was first the number of polyether units in the ether chain. Second, as the daughter ion spectra recorded during chromatographic separation demonstrate, different isomeric surfactant structures are due to this effect. A UV spectrum recorded from 200 to 400 nm shows that these surfactants have an absorption at $\lambda_{max}$ 203 nm, *i.e.*, they differ in their characteristic properties from alkanol propylene glycol ethers, which have an absorption below 190 nm [23].

Additional information about the presence of polyethylene glycols (PEGs) of different chain lengths was obtained during chromatographic separation. Usually these compounds are metabolites of biochemically well degradable non-ionic surfactants and represent the polar primary degradation products of these compounds in the biological waste water treatment process. If non-ionic surfactants are present in the influent of the sewage treatment plant, these metabolites appear in the waste water treatment process in increasing concentration. Owing to the absence of a chromophore in the molecule they cannot be detected by UV spectrophotometry although these polar compounds may have a share of up to 15% of the organic carbon compounds in sewage treatment plant effluents.

Although the chance of a successful analytical separation can be increased during the elution step after enrichment on the solid-phase extraction cartridge by quasi-"selective" elution, new problems may arise. To effect selective elution, the polarity of the solvents and their mixtures used for desorption was increased. The overview spectra (Fig. 8) of the diethyl ether, methanol–water (2:8, v/v) and methanol eluates of the waste water extract, obtained using FIA, show a distinct preseparation depending on the eluent chosen. Further examination of the extract obtained by methanol–water (2:8, v/v) elution, which should consist mainly of polyethylene glycols with 5–16 chain links (see Fig. 8b), leads to the UV trace at 210 nm and the ion current shown in Fig. 9a and b, respectively. Comparing the analytical separation of the eluate produced by "selective" elution (Fig. 9b) with the chromatogram of the total extract of the same waste water (Fig. 6b), it can be seen that an obvious retention time shift of the polyethylene glycols from one sample to another has taken place in spite of identical chromatographic conditions.

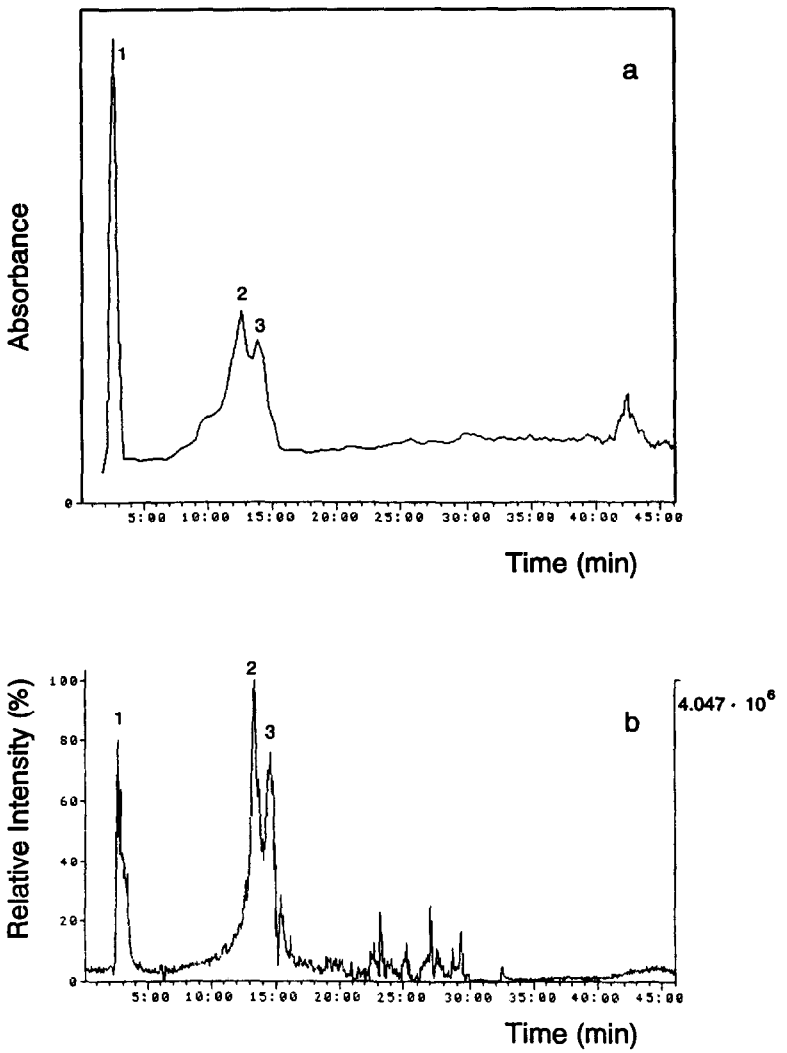


Fig. 6. (a) LC–UV (210 nm) trace for waste water extract as in Fig. 2a (influent). Enrichment and elution as in Fig. 2a; $C_{18}$ column; for chromatographic conditions, see Experimental and Table I. (b) LC–MS total ion current trace of waste water extract in (a). LC conditions as in (a).

This phenomenon of uncontrollable retention time modification of identical samples under the same chromatographic conditions seems to be typical of waste water extracts, especially if they contain surface-active compounds [13]. Constancy of retention times in chromatographic separations of waste water extracts can only be achieved by time-consuming cleaning procedures, as described under Experimental, which have to be carried out between two analyses. These cleaning and equilibration phases are very time and manpower consuming and restrict the sample throughput considerably. Fig. 10 shows a separation, which is reproducible, of a methanolic PEG standard solution without additional matrix compounds under the same chromatographic conditions as before and without intermediate cleaning steps. Possible problems of retention time shifts induced by matrix compounds can be recognized by means of specific detection with a mass spectrometer using mass chromatograms, as demonstrated in Fig. 11. This

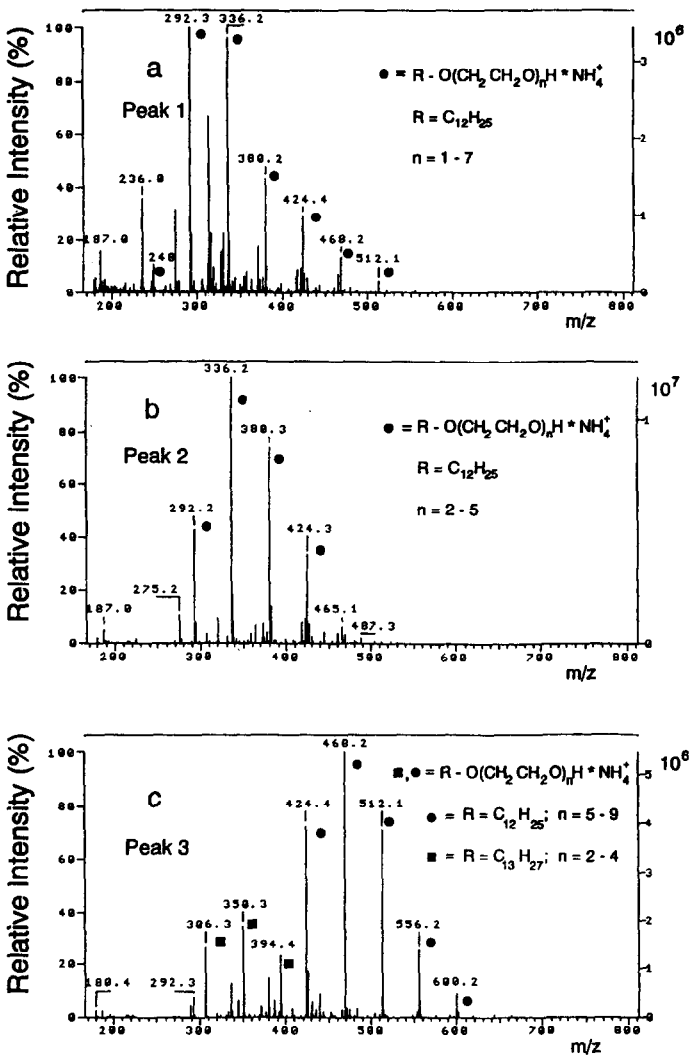


Fig. 7. LC–mass spectra of (a) peak 1, (b) peak 2 and (c) peak 3 in Fig. 6b.

method demonstrates whether a separation such as that shown in Fig. 8b for the matrix-charged waste water extract has been successful or not.

The difficulties during the examination of waste waters for unknown compounds, both by FIA–MS and after LC separation on analytical columns with subsequent MS detection and generation of daughter ions, have been considered earlier. Much greater problems arise, however, if for the anthropogenic compounds to be identified no standard compounds are available for comparison purposes or if corresponding spectra are absent from the daughter ion library. In spite of the fact that these substances are components of common commercially available products, produced in large amounts and inevitably reaching waste waters, so far producers and distributors have made little or no effort to overcome this lack of information. Identification has to be achieved instead by interpreting CID spectra. This very time-consuming procedure becomes especially necessary if hardly degradable polar waste water compounds, in particular surfactants, are submitted to conventional biological treatment processing. Because of their polarity and persistence, the usual elimination

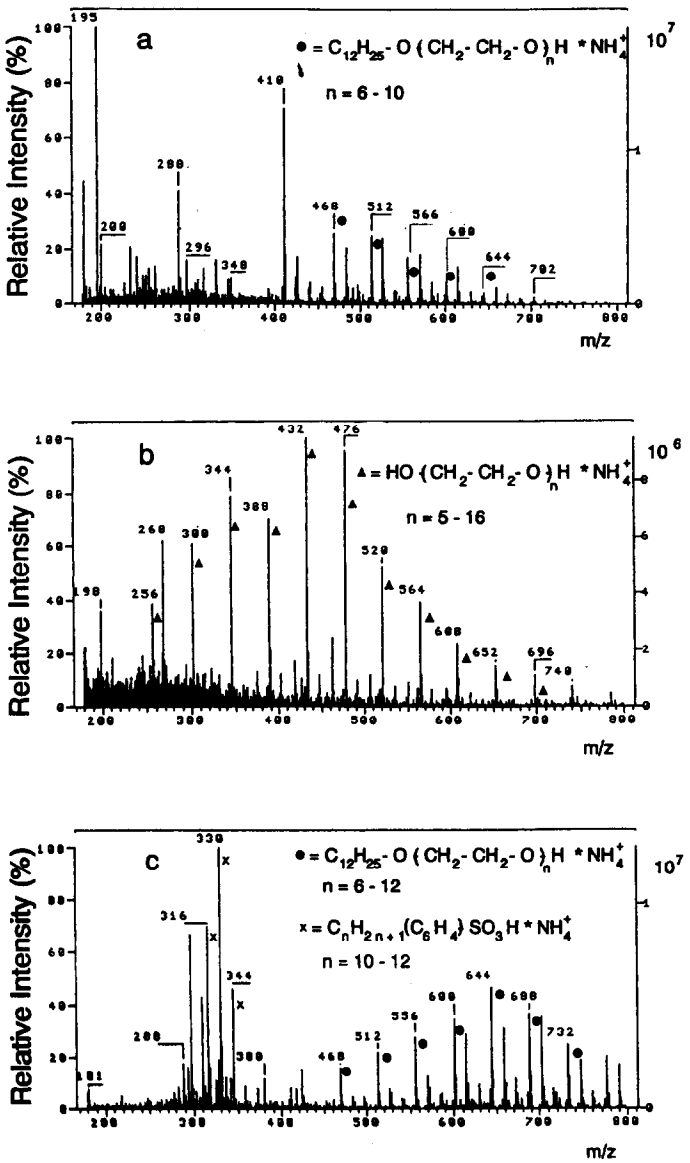


Fig. 8. FIA–overview mass spectra of (a) diethyl ether, (b) methanol–water (2:8, v/v) and (c) methanol eluates. $C_{18}$ solid-phase extract of waste water as in Fig. 6b.

mechanisms of biochemical waste water treatment have no effect. Either the hardly degradable surfactants appear unchanged in the treatment plant effluent or the metabolites of these compounds are present in the water running off. These metabolites cannot be eliminated by stripping or adsorption because biochemical primary degradation has increased the polarity of the molecules. These compounds cannot be detected by substance class-specific determination [5–9,11], but it is possible to determine them by MS [11] coupled with LC. This procedure requires a certain knowledge of the biochemical degradation pathways of the different types of surfactant in order to make a target analysis for the sought compounds in this matrix. Biochemical primary degradation of surfactants during waste water treatment may lead both to non-polar and polar

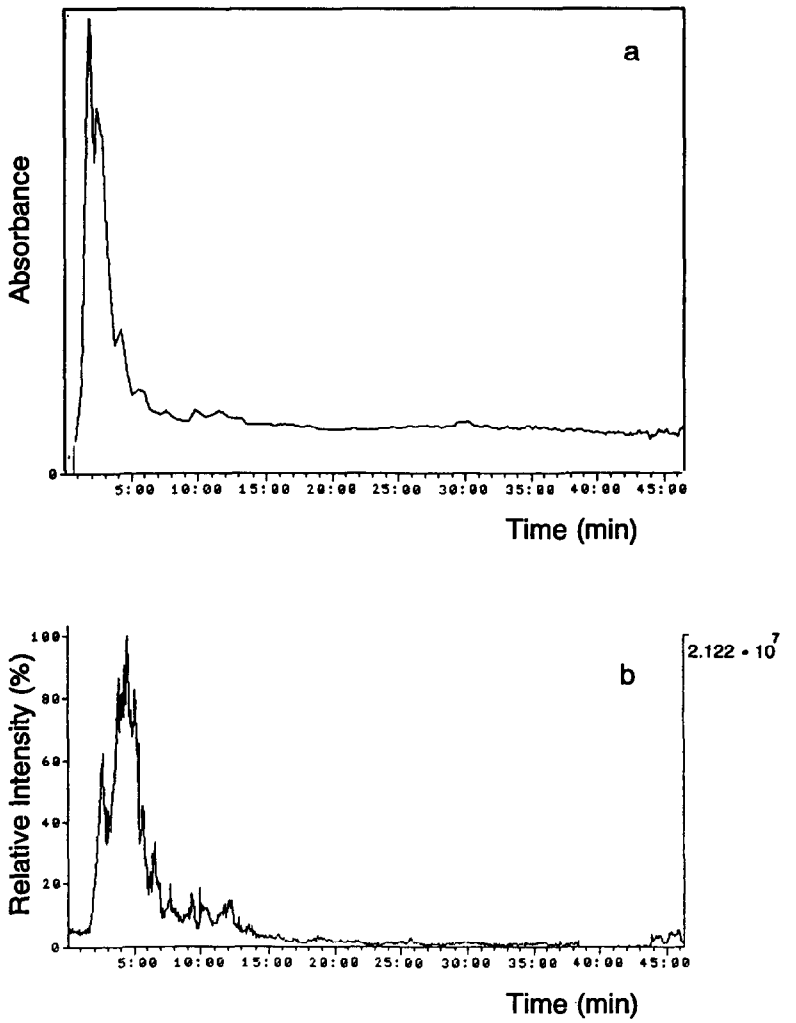


Fig. 9. (a) LC–UV (210 nm) trace for waste water extract. (b) LC–MS total ion current trace for the same extract. Enrichment and elution as in Fig. 8b. $C_{18}$ column; for chromatographic conditions, see Experimental and Table I.

metabolites; the polar degradation products then dissolve in the waste water, whereas the non-polar products are adsorbed at the sludge. One of the best known examples is the degradation of nonyl- and octylphenol ethoxylates because in this instance the biogenic degradation product is more toxic than the original compound [25]. During primary degradation of these compounds the polyethylene glycol chain is cleaved, generating the polar metabolite PEG. Because of the loss of its hydrophilic polyether chain, the aromatic cyclic system with the coupled alkyl residue will then be found as a lipophilic alkylphenol in the activated sludge. Equally spaced signals at $m/z$ 44 or 58 help to identify the metabolites of non-ionic surfactant precursor compounds if the ether chain with polyethylene or polypropylene glycol units remains in the degradation product.

Additional problems arise during the quantification of primary degradation produces both after chromatographic separation on an analytical column and by mixture analysis using MS–MS. The compound to be determined has to be available as a standard that has to be added to the samples in order to obtain reliable concentration data by the standard addition method. Any other procedure, *e.g.*, using compounds

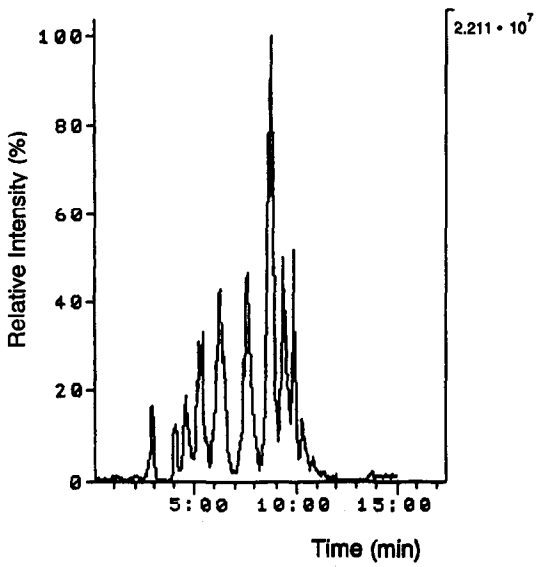


Fig. 10. LC–MS total ion current trace for standard solution of PEG 400. $C_{18}$ column; for chromatographic conditions, see Experimental and Table I.

resembling each other in structure, will provide an estimate. The ion current of the compound to be determined is dependent on its proton affinity. Interferences with matrix compounds will be larger in the FIA–MS mode, but cannot be excluded and even non-ionization can result. To exclude this source of error in our examinations, *i.e.*, for the determination of the primary degradation product of a non-ionic surfactant in the effluent of a sewage treatment plant in Aachen, we first generated in a batch experiment by biochemical degradation the metabolites in as pure a form as possible. These compounds dominate the effluent of the large-scale treatment plant (Fig. 12b) whereas the original compound, an alkanol polyethylene glycol ether, can be detected in the FIA–MS overview spectrum shown in Fig. 12a. By biochemical oxidation of the terminal hydroxyl function of the polyglycol ether, a carboxyl function has been formed that resists further biochemical degradation. Compounds of this type are able to pass through charcoal filters and could be detected in drinking water [26]. Precursor and metabolite compounds were identified by means of their CID spectra

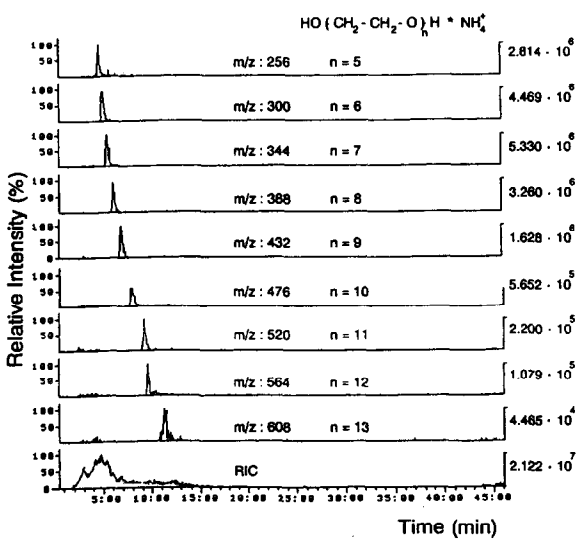


Fig. 11. LC–MS of PEG from total ion current trace for waste water extract in Fig. 8b. Chromatographic conditions as in Fig. 9.

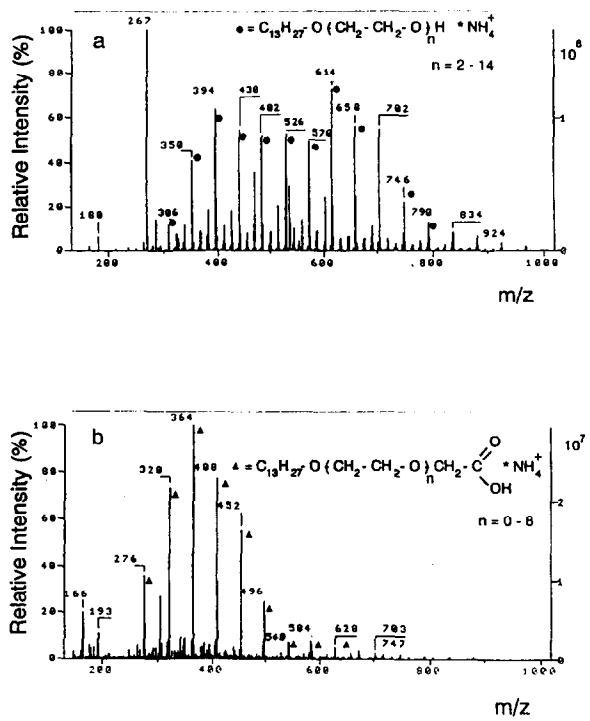


Fig. 12. (a) FIA–overview mass spectrum of waste water treatment plant influent containing surfactant molecules (●). $C_{18}$ solid-phase extract; eluent, diethyl ether; (b) FIA–overview mass spectrum of waste water treatment plant effluent containing metabolite molecules (▲). $C_{18}$ solid-phase extract of effluent; eluent, methanol. Positive TSP ionization; for FIA conditions, see Experimental.

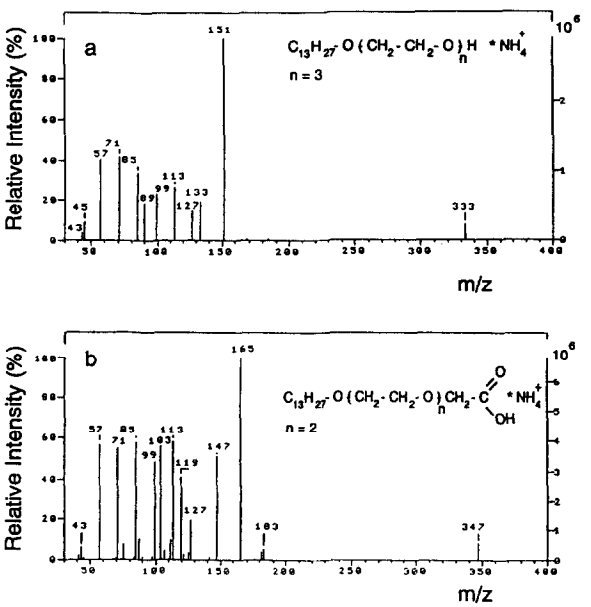


Fig. 13. (a) Daughter ion mass spectrum (FIA–MS–MS) of selected ion ($m/z$ 350) from waste water extract as in Fig. 12a. (b) Daughter ion mass spectrum as in (a) of selected ion ($m/z$ 364) from waste energy extract as in Fig. 12b. For FIA conditions, see Experimental; collision energy, −15 eV.

shown in Fig. 13a and b, respectively. In Fig. 14a and b the structures of the compounds and their fragmentation behaviour under CID conditions are shown.

For the generation of the metabolite standard from the batch experiment it was helpful that a preliminary separation of the original compound present in the treatment plant effluent from the primary degradation product was successfully achieved by selective elution. The original compound was eluted with diethyl ether and the metabolite with methanol from the $C_{18}$ material. The purity of this metabolite mixture was confirmed by FIA–MS and MS–MS.

The chromatographic separation of the metabolite from the total waste water matrix on an analytical column is shown in Fig. 15, which contains the RIC of the separation together with the mass spectrum of peak 1.

After successful chromatographic separation, quantification by the standard addition method with four different concentrations of the metabolite was carried out. The original samples and the three samples spiked with a standard solution of the standards were analysed both in the FIA and after chromatographic separation by SIM recording the ion current of the cluster ions at $m/z$ 320, 364 and 408. These ions are the main components of peak 1 in Fig. 15, representing more than 90% of the area under the TIC. The results of the standard addition analysis showed that the peak area obtained in FIA–MS and LC–MS was linearly related to the metabolite concentration in the chosen range and under the selected conditions. Concentration of 880 and 960 $\mu$g/l of metabolite in the treatment plant effluent were determined by FIA–MS and LC–MS, respectively.

The results agree well; the time required for determination after LC separation, however, was much higher than that in the FIA mode. This

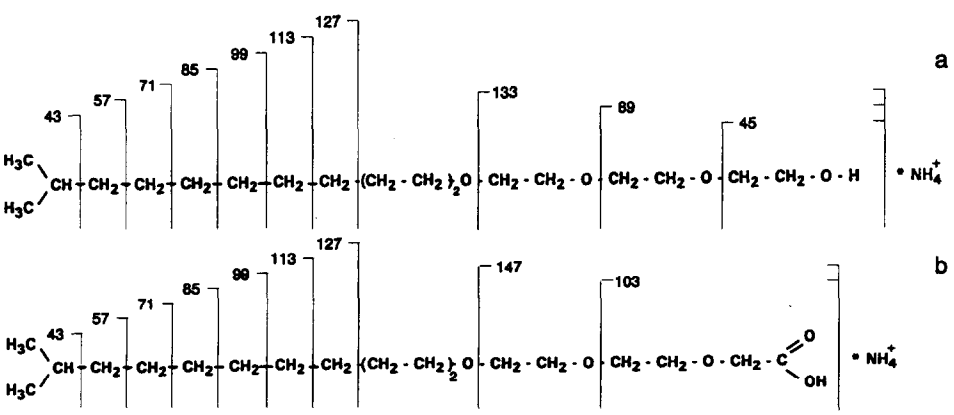


Fig. 14. (a) Structural formula and fragmentation scheme under CID conditions of non-ionic surfactant of alkanol polyethylene glycol ether type. (b) Metabolite (primary degradation product) of non-ionic surfactant in (a).

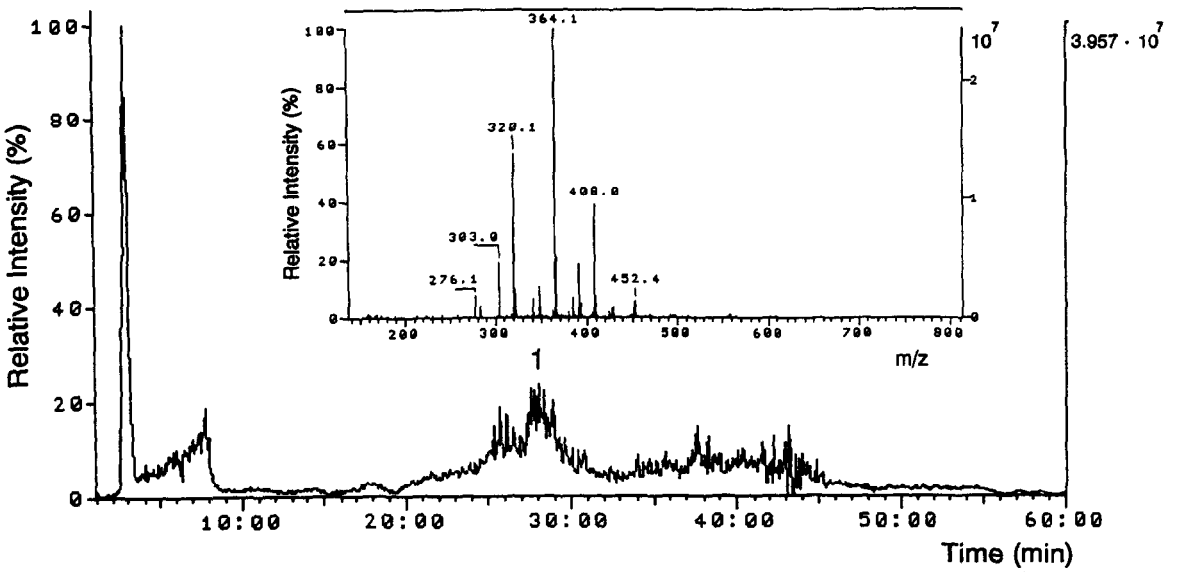


Fig. 15. LC–MS total ion current trace for waste water extract containing the metabolite in Fig. 14b. Inset: LC–mass spectrum of peak 1.

was caused by the prolonged analytical separations (45 min and more) and by the time-consuming cleaning and equilibration procedures (60 min) before each analytical separation. As this cycle of analysis, column cleaning and equilibration could not be done automatically, it was possible to make only three runs at different concentrations on an analytical column per day (8.5 h). In the FIA mode less than 10 min were required to acquire data at one concentration with a minimum of five injections per concentration. As the quantification of one compound by the standard addition method requires a minimum of four different concentrations, about 30 min are necessary for this examination. For quantification in the LC–MS mode 1.5 days are required for the whole procedure, assuming that the mass spectrometer is working well within the whole cycle of quantification. Taking these times into account, LC–MS takes 25–30 times longer than by FIA–MS.

## CONCLUSIONS

The examination of some representative municipal sewage treatment plants, which apply biological treatment processes, showed that in spite of considerable pollutant concentrations in the influents, only small amounts of non-polar organic compounds can be detected in the effluents. While these compounds could be eliminated up to 99%, considerable amounts of both anthropogenic and biogenic polar organic compounds, mainly anionic and non-ionic surfactants, could be detected.

Detection and identification of these surfactants and their biogenic metabolites can be carried out in the FIA–MS or FIA–MS–MS mode and also after chromatographic separation (LC–MS or LC–MS–MS). UV detection is possible only if there is a chromophore in the surfactant molecule. Standard retention time shifts induced by surface-active compounds can be recognized at once by using a mass spectrometer as detector. An exact quantitative determination of each pollutant by FIA–MS or LC–MS, however, requires in both instances that it exists as a standard and makes standard addition necessary. The results of the two methods correspond well. Determination by LC–MS took 25–30 times longer than FIA–MS.

The presence of some of these pollutants in drinking water produced from surface waters [2,26] has to make us think, for the toxicity of the precursor compound or the primary degradation product, respectively, is not always cleared

up. In the future, efforts to avoid or to replace some of these persistent substances will have to be considered. The surface activity of these compounds in combination with their non-biodegradability in water and soil enables them to introduce compounds into the groundwater which are more toxic than themselves.

The analytical possibilities have proven their suitability, now the legislator is challenged.

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