Polar organic pollutants in the Elbe river
Liquid chromatographic–mass spectrometric and flow-injection analysis–mass spectrometric analyses demonstrating changes in quality and concentration during the unification process in Germany

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Abstract

During the unification process of the two German states from 1989 to 1994, water extracts from the Elbe river were analyzed by gas chromatography–mass spectrometry (GC–MS) and by high-performance liquid chromatography (HPLC) combined with ultraviolet (UV) and/or mass spectrometric detection (MS) in order to monitor the pollutants in one of the most contaminated European rivers. After column chromatography (LC) or flow-injection analysis (FIA) bypassing the analytical column, ionization was performed by a thermospray interface (TSP). Semiquantitative estimations of the pollution of the Elbe were made form the total-ion current traces (TIC) of the extracts. Determination of the total dissolved organic carbon (DOC) indicated a reduction of more than 55% of pollutants in the water phase. The pollutants were identified by tandem mass spectrometry (MS–MS), generating daughter-ion spectra by collision-induced dissociation (CID) using either column chromatography or direct mixture analysis. Compound-specific analyses indicated a reduction in the amount of pollutants as well as a change in their composition. This could be explained by reduced industrial production and by intensified construction of biological sewage treatment plants.

1. Introduction

Today as well as over the last ten years, the production of drinking water from surface waters, i.e. from the Rhine and Elbe rivers, supplies more than 20% of the demand in Germany. While in the 1980s the Rhine water quality was rapidly improved, fewer efforts were made in that respect for the Elbe in the former German Democratic Republic (GDR), existing as an independent state up to 1990, and in former Czechoslovakia where the Elbe has its source.

As biological sewage treatment plants did not exist in these states, municipal and industrial waste water was formerly at most mechanically purified before discharge into the Elbe and its tributaries. Due to these insufficiently treated discharges, the Elbe became one of the most polluted rivers in Europe. Up to the unification of the two German states, the results of investigations on the water quality of the Elbe carried out in the GDR were kept strictly under lock and key. Information on the condition of the Elbe was only available from water samples

taken downstream from river km 474.5, i.e. at Schnackenburg. Here the river left GDR territory and was accessible for sampling.

Our own investigations in 1989 found a dissolved organic carbon content (DOC) in the Elbe water of about 16 mg/l. This confirmed estimations of specialists about the state of pollution of the Elbe. While the spectrum of nonpolar organic pollutants and organo-metallic compounds in this river was subject to numerous investigations [1–3], there was still limited knowledge on the polar organic substances because of a lack of analytical detection methods. Compounds which can be recorded by sum parameter analysis might either be of anthropogenic or biogenic origin. Polar and nonpolar organic pollutants [4] in the Elbe differed considerably from those in other West German rivers. The reasons were, on the one hand, the differing industrial production processes applied in the GDR and Czechoslovakia and the way of life of their inhabitants. On the other hand, a pollutant spectrum generally different form that in the rivers of the Federal Republic of Germany (FRG) had to be expected because of the lack of biological sewage treatment plants.

Though only the results of a few selected samples can be presented here, it is possible to recognize trends. Comparisons with the first results from 1989, i.e. from the time before unification, and further investigations during the years following the unification process by means of LC– and flow-injection analysis (FIA)–MS and –MS–MS allow us to describe the changes in the spectrum of polar organic compounds in the Elbe, which went on for years and years.

2. Experimental

2.1. Materials

Water samples from the Elbe were taken at the beginning of the research work from the Elbe monitoring station at Schnackenburg and additionally in 1994 from the Elbe monitoring station at Magdeburg. Water pollutants were extracted using continuous liquid–liquid or solid-phase extraction. For liquid–liquid extraction,

hexane was used. Solid-phase extraction cartridges were either filled with C_{18} material from Baker (Deventer, Netherlands) or with LiChrolut EN from Merck (Darmstadt, Germany) during the last sampling period. From the beginning of 1994, XAD 2 and XAD 4 (Amberlite) from Serva (Heidelberg, Germany) were additionally used for the enrichment and concentration of pollutants.

Inorganic salts were removed from the water phase by an anionic (Duolite A 102 D col) and a cationic ion exchanger (Duolite C 20 col), both from Merck, before freeze-drying.

Solid-phase extraction materials were conditioned as prescribed by the manufacturers. Glass-fibre and membrane filters used for pretreatment of the water samples were obtained from Schleicher and Schüll (Dassel, Germany) or Sartorius (Göttingen, 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 from Millipore (Milford, MA, USA) for 24 h and then washed with 100 ml of the same water. Hexane for liquid–liquid extraction and hexane, diethyl ether, ethyl acetate and methanol used for desorption of water pollutants from the solid-phase material, and acetone and methanol for cleaning purposes 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. Nitrogen for drying of solid-phase cartridges was of 99.999% purity (Linde, Germany).

LC separations were done on a Nucleosil C_{18} (5 μm, spherical) column (25 cm × 4.6 mm I.D.) (CS, Langerwehe, Germany). The mobile phase was methanol (HPLC grade) from Promochem and Milli-Q-purified water. Ammonium acetate for TSP ionization, stored always at 4°C, was of analytical-reagent grade from Merck.

2.2. Sampling and sample preparation

All samples were taken as composite samples in glass bottles. Prior to use the bottles were rinsed carefully with Nanograde acetone and methanol and dried at 105°C. The bottles were

rinsed directly before use with several small portions of the same water that was subsequently stored in them. The storage temperature was 4°C. The cold bottles from refrigerator storage were placed in an insulated packaging and were then transported by a parcel service within 24 h from the sampling point to the research laboratory.

Depending on the degree of pollution as monitored by DOC, 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. To ensure complete adsorption, the water samples were forced through two cartridges in series. The adsorbed pollutants were desorbed more or less separately. Solvents of different polarities (hexane, hexane–diethyl ether, diethyl ether, water–methanol and methanol) were used for this purpose in combination with C_{18} and LiChrolut material. The pollutants adsorbed by Amberlite were desorbed continuously under reflux, first with 150 ml of methanol and then with 150 ml methanol–ethyl acetate (1:1, v/v). All eluates from the solid-phase extraction except the methanol and methanol–water eluates were evaporated to dryness with a nitrogen stream at 30°C. Mixtures of solvents and pollutants from XAD 2 and 4 desorption were brought to dryness by rotary evaporation. The residues from both desorption procedures were dissolved in methanol and could be used for injection during FIA- and LC–MS analysis.

The samples were put into glass bottles after solid-phase extraction or XAD adsorption, and freeze-drying was applied to enrich non-adsorbable compounds after the removal of inorganic salts using an additional ion-exchange step. After freeze-drying, the compounds were desorbed with methanol from the glass walls of the bottles by ultrasonification. The methanolic solutions can be used for FIA- and LC–MS investigations.

For GC–MS analysis, depending on the degree of pollution, 2 or 6 l of water were extracted with hexane. After drying with anhydrous sodium sulphate, the volume of the extract was reduced to 200 μl by rotary evaporation and in

an nitrogen stream. This concentrate (concentration factor: 10 000 or 30 000) could be used for injection into the GC–MS system.

2.3. Sum parameter analysis

The dissolved organic carbon (DOC) content of the water samples was measured using a Dohrman total carbon analyzer DC 80. After filtration of the pretreated samples using a glass-fibre filter, wet oxidation with an acidic potassium persulphate solution in water assisted by UV radiation was performed.

2.4. 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 μm (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 that in the vacuum system of the mass spectrometer $3 \cdot 10^{-2}$ Torr. The electron multiplier was operated at 1200 V with the conversion dynode voltage at 5 kV. The temperature in the ion source was 150°C.

2.5. 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 (up to the beginning of 1994) and then a Waters 996 photodiode-array detector system in combination with a Millenium 2010 data system (Millipore) 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

Table 1
Gradient elution scheme and composition of mobile phase I

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

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

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 standardized methods, shown in Tables 1 and 2. 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 once a day with acetonitrile–chloroform–methanol–dimethyl sulphoxide (3:3:3:1, v/v).

2.6. MS and MS–MS systems

From 1988 to 1993 a TSQ 70 mass spectrometer (Finnigan MAT, San Jose, CA, USA) combined with a PDP 11/73 data station was used for research work. At the beginning of 1994, the mass spectrometer was upgraded to a TSQ 700 with a DEC 5000/33 data station. The TSP interface was obtained from Finnigan MAT.

Table 2
Gradient elution scheme and composition of mobile phase II

Time (min)	Solvent A (%)	Solvent B (%)
0	20	80
10	100	0

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

After upgrading, the TSP interface was adapted to the new system by Finnigan.

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 these 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, unless otherwise 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 the 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 while accumulating a maximum of 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 positive and negative modes.

For quantification the mass spectrometer was operated in the selected-ion monitoring (SIM) mode using a dwell time of 200 ms for each mass. Estimations on the concentration of the whole pollutant spectrum were made using the total-ion current trace (TIC) after FIA.

3. Results and discussion

Due to the special situation of the two German states, it was still necessary in 1989 to take samples on FRG territory in order to obtain information on the condition of the Elbe. Thus, in 1989, the first samples for these investigations,

carried out at the beginning of an unforeseeable historic change, were taken in Schnackenburg at river km 474.5, where the river enters FRG territory. These samples could serve to determine the state of pollution of the Elbe at that period, with special regard to polar organic compounds.

Screening tests of this 24-h composite sample by GC or LC and FIA–MS, respectively, revealed high concentrations of moderately polar, volatile as well as polar, non-volatile organic pollutants. With 16 mg/l the sum parameter DOC, determined in parallel, was excessive for a surface water serving also for drinking water purposes on GDR territory.

This and the following water samples were analyzed intensively for organic compounds in order to determine especially the polar pollutant spectrum of the Elbe as completely as possible for quantitative assessment and characterization. For this purpose GC–MS, LC–MS and FIA–MS as well as MS–MS analysis with different enrichment procedures were carried out. Fig. 1b shows the GC–MS total-ion current trace of the hexane extract from this composite sample. Many of these pollutants can be identified immediately because of their specific fragmentation pattern or by library search using the NBS library of electron-impact (EI) spectra. These substances, which can be identified without clean-up steps,

are nearly exclusively phthalates of different molar masses in high concentrations. This is proven by the mass trace of the fragment m/z 149, which is characteristic for this class of compounds (Fig. 1a). The majority of the other compounds of this extract, for example tetrabutyl stannane and long-chain hydrocarbons, which can be separated by GC, have an even less polar structure and are therefore not as soluble in water. This is the reason why they were present in the water phase at a considerably lower concentration than the phthalates and should be found in higher concentrations in the river sediment. Due to a lower concentration in the water, they could be detected only after adequate clean-up steps by target analysis, as high phthalate concentrations in the extract predominated in the TICs and prevented the detection of other pollutants during non-targeted screening tests.

At the beginning of our examinations, the polar, non-volatile compounds were obtained by enrichment on C_{18} material and selective elution with solvents and their mixtures of different polarities [5]. Under the conditions of the pre-separation method applied here, their selective elution behaviour in these samples was only poorly distinct. Some of the overview spectra recorded by FIA–MS of the eluates from "selective elution" are shown in Figs. 2a and 2b,
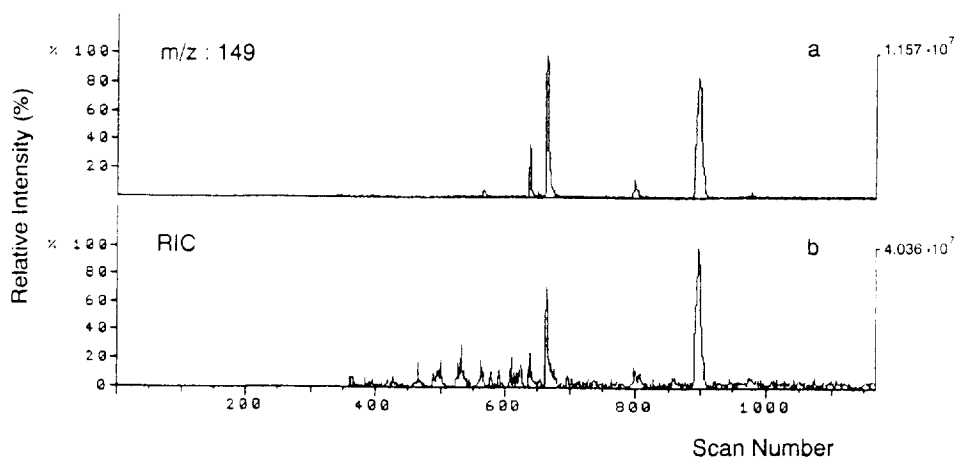

Fig. 1. (a) GC–MS mass trace of fragment ion m/z 149 for water sample no. 1 of the Elbe river. Liquid–liquid extract; solvent, hexane. (b) GC–MS total-ion current trace as in (a). For concentration factor, see Experimental.
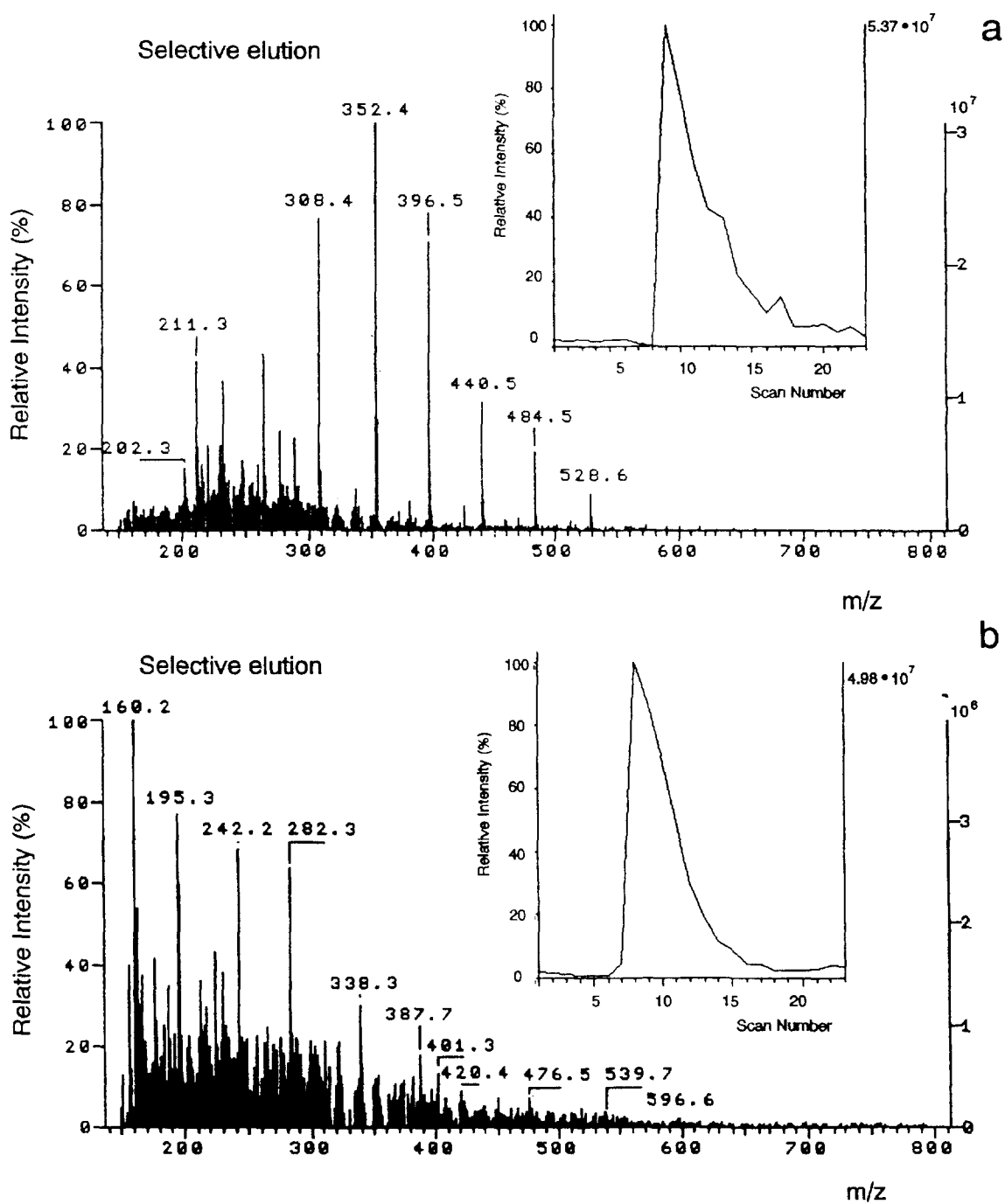
Fig. 2. (a) TSP–MS loop injection spectrum obtained by bypassing the analytical column (FIA–MS), subsequently called "overview spectrum". for water sample no. 1 of Elbe river. C$_{18}$ solid-phase extract; eluent, diethyl ether. (b) FIA–MS overview spectrum as in (a). Eluent. methanol. Positive TSP ionization. For FIA conditions, see Experimental.
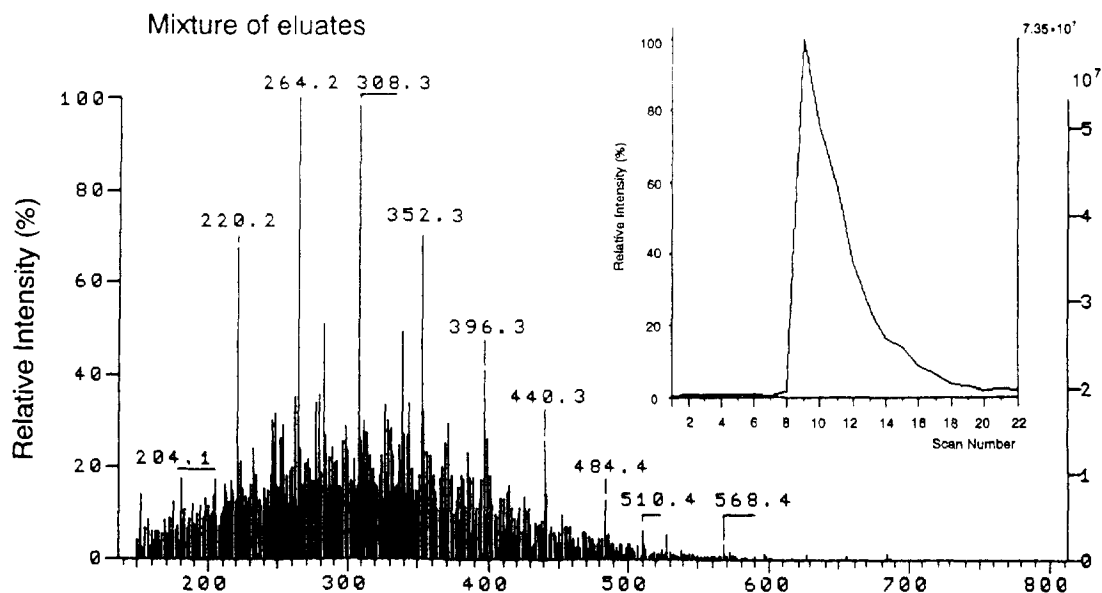
Fig. 3. FIA–MS overview spectrum as in Fig. 2. Mixture of eluates. Eluents, hexane, hexane–ether, ether, water–methanol, methanol.

including their TICs. The overview spectrum in Fig. 3 was recorded from a mixture of all different eluates. The overview spectra here contain their own TIC. which have been inserted into the figures. This method represents a good and quick possibility to make quantitative estimations and to compare the content of pollutants qualitatively. These procedures were possible because the samples to be compared were always handled in the same way and were dissolved in the same solvent.

Examining the compounds of these "selective eluates" by FIA–MS. i.e. bypassing the analytical column without chromatographic separation, but only using the tandem function of the mass spectrometer. some of them can already be identified. This is possible by interpretation of the daughter-ion spectra generated by CID or by comparison of these CID spectra with those contained in our laboratory-made CID library of polar organic water compounds [6].

But also this analytical method has its application limits. Thus FIA–MS analysis with subsequent identification by direct mixture analysis

(MS–MS) is only successful if the mixture consists of a large number of various compounds which differ in their m/z ratio. Otherwise, the CID spectrum would consist of a mixture of at least two differently dissociating ions [7] and therefore could not be interpreted. Due to the complexity even of the C_{18} extracts obtained by "selective" elution and to the application limits of mixture analysis, these extracts were also submitted to chromatographic separation on an RP_{18} phase. Detection followed after TSP ionization by MS and inserted in-line UV detection at 220 nm. The total-ion current trace (TIC) and UV trace are shown in Figs. 4a and 4b. It can be seen that only a small part of the thermospray-active compounds absorbs at 220 nm, i.e. could actually be detected by UV. Although the TIC shows a good total-ion mass trace, only some of the compounds are clearly separated. This can be demonstrated in Fig. 4b, showing the signals of a mixture of compounds hidden under peak 2 (Fig. 4d). These ions are equally spaced with $\Delta m/z$ 44. These compounds are non-ionic surfactants of the alkylpolyglycolether type, as verified
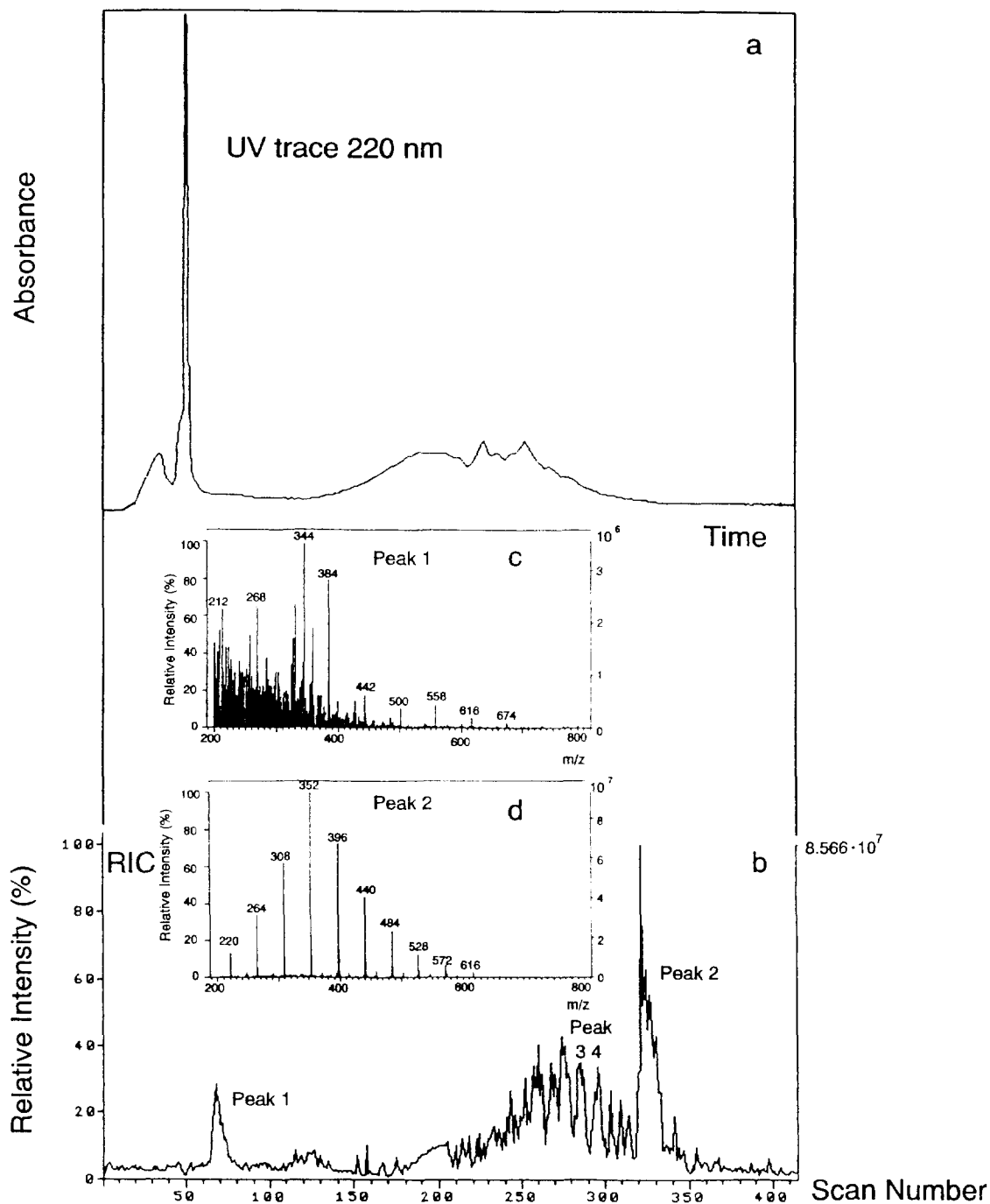
Fig. 4. (a) UV trace (220 nm) for water sample no. 1 of Elbe river. C_{18} solid-phase extract; mixture of eluates as in Fig. 3; for chromatographic conditions. see Experimental and Table 1. (b) LC–MS total-ion current trace of Elbe river extract in (a) and LC mass spectra of (c) peak 1 and of (d) peak 2; LC conditions as in (a). Positive TSP ionization.
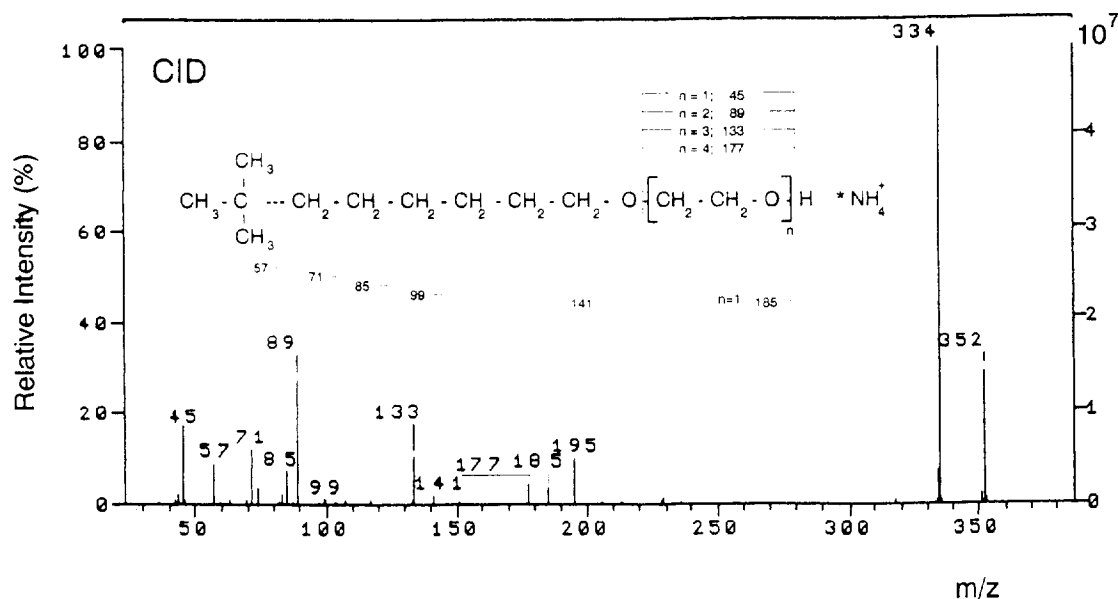
Fig. 5. Daughter-ion mass spectrum (LC–MS–MS) and fragmentation scheme of non-ionic surfactant cluster ion [m/z 352; $C_{10}H_{21}O(CH_2CH_2O)_4H^*NH_4^+$] in peak 2 from Elbe river extract as in Fig. 4b. Collision energy, −20 eV.

by the daughter-ion spectrum of m/z 352 in Fig. 5 [8]. The general empirical formula for this compound is $C_{10}H_{21}O(CH_2CH_2O)_nH$, taken from comparison with daughter-ion spectra in our library. For the ammonium adduct ion with m/z 352, the number n of glycol units is 4. Most signals in the TIC (Fig. 4b) consist of a larger number of very different polar compounds [see Fig. 6a (peak 3 in Fig. 4b) and 6b (peak 4 in Fig. 4b)], such as polyethylene- and poly-propyleneglycol and their biochemical oxidation products [9] as well as phthalates and alkyl benzenesulphonic acid (LABS). LABS was also identified by negative TSP ionization [12]. At the same time a large number of non-striking compounds are hidden under nearly each of these signals (Fig. 4b), which cannot be separated or focussed by the applied column, even under strongly varying chromatographic conditions. On the simplifying assumption that the signal height in the mass spectra is a measure of the concentration of compounds, up to 40% of the thermospray-active compounds of the extracts are hidden behind these matrix compounds.

Another 24-h composite sample of the Elbe, taken in 1990 for comparison purposes at exactly

the same location and on the same day as the earlier water sample, is shown as TIC after LC–MS in Fig. 7b. Comparing the results of this sample 2 with those of sample 1, first estimations during GC–MS, FIA–MS and LC–MS analysis point to reduced concentrations of nonpolar as well as polar compounds. This is confirmed by the sum parameter DOC, which had been measured in parallel as 10 mg/l, i.e. the carbon content is reduced by 40%.

While the ion current of the GC–MS analysis is characterized here also by phthalates, as before in sample 1, the spectrum of compounds which can be recorded by LC–MS has considerably changed. In contrast to sample 1, dominant signals suited straightaway for MS–MS characterization were no longer found in the FIA–MS spectra of the extract obtained by selective elution. Therefore, LC separation was carried out directly with the mixture of all eluates. As separation failed with the gradient elution mentioned in Table 1, it was carried out under the chromatographic conditions listed in Table 2. The UV trace at 220 nm and ion-current trace are shown in Fig. 7. The mass spectrum of the signal in the scan region 759–775 of the TIC can
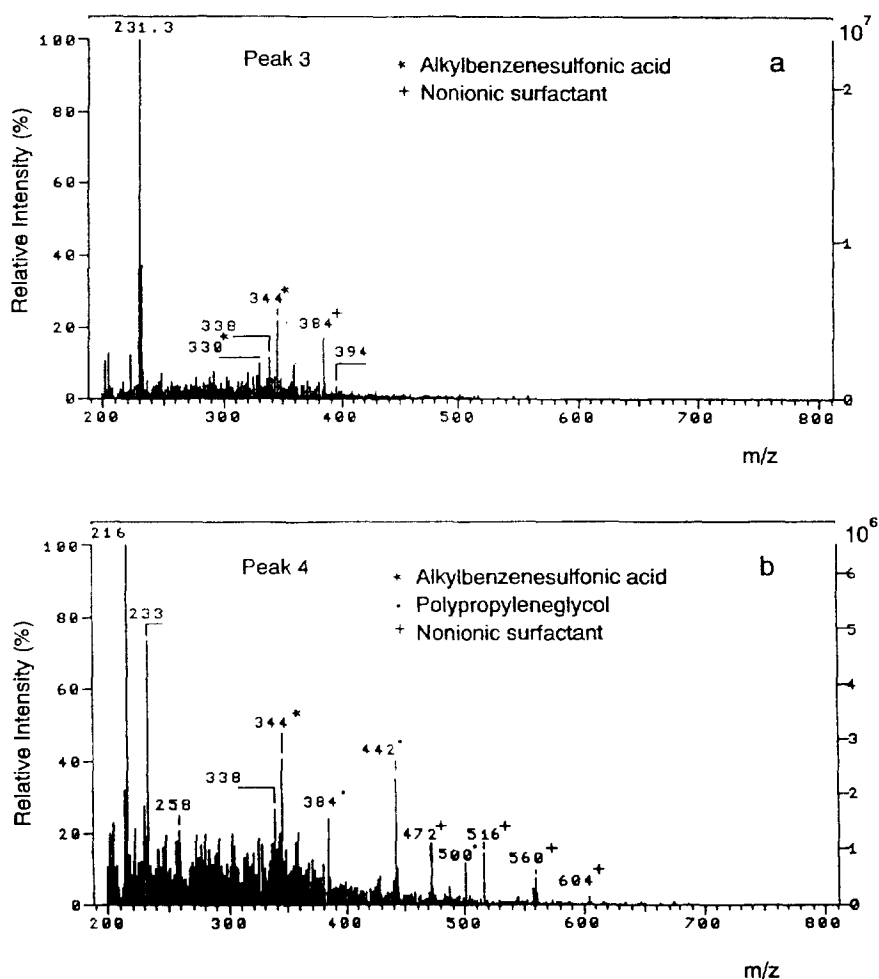
Fig. 6. LC–MS spectra of (a) peak 3 and (b) peak 4 in Fig. 4b.

be identified at once as non-ionic surfactant because of the equidistant signals with $\Delta m/z$ 44 (see Fig. 7c). The daughter-ion spectrum in Fig. 7d allows an unequivocal characterization as nonylphenol ethoxylate. This substance is only rarely detected in surface waters of the FRG. Its application is no longer allowed because of its toxic primary degradation product nonylphenol. The polyglycolether of either anthropogenic or biogenic origin, which could still be observed clearly in Figs. 2 and 3 in the background of the overview spectra of extracts from sample 1, has now almost completely disappeared, as proven by overview spectra.

The examination of another sample from the Elbe (sample 3) taken in 1992, i.e. about three years after first sampling, indicated a further reduction of the pollutant concentration in the river. First proof for this development was the DOC content, being now about 7 mg/l and, thus, more than 55% lower than in sample 1 taken in 1989. Because of the low DOC value, the water volume used for enrichment was tripled for solid-phase as well as hexane extraction. Considerably reduced concentrations of phthalates were detected in the ion-current trace of the GC–MS analysis, so that it was possible to recognize during screening even small quantities

of chloronitrobenzene, chloronitroaniline and hexachlorobenzene in the ion-current trace without special sample preparation.

The observations made for sample 2 apply also to the spectra of this sample 3 generated by FIA–MS; i.e. signals which normally dominate the FIA–MS overview spectra were not found here, except phthalates (m/z 279 and 391) in low concentrations. Therefore, the extract was submitted immediately to LC separation under the same chromatographic conditions as for sample 1. The ion-current trace of the LC separation is

shown in Fig. 8, together with the UV trace. Besides some ions belonging to non-ionic surfactants on a polyethylene or polypropylene glycol basis (see Figs. 8c and 8d), it was not possible to identify other compounds because of the reduced pollutant concentration and, thus, lack of sampling material.

The pollutant content in the 24-h composite sample, taken in 1994 in early summer, was even lower than in all sampling materials from the Elbe analyzed up till then. This sample 4 was taken nearly exactly five years after the first one.
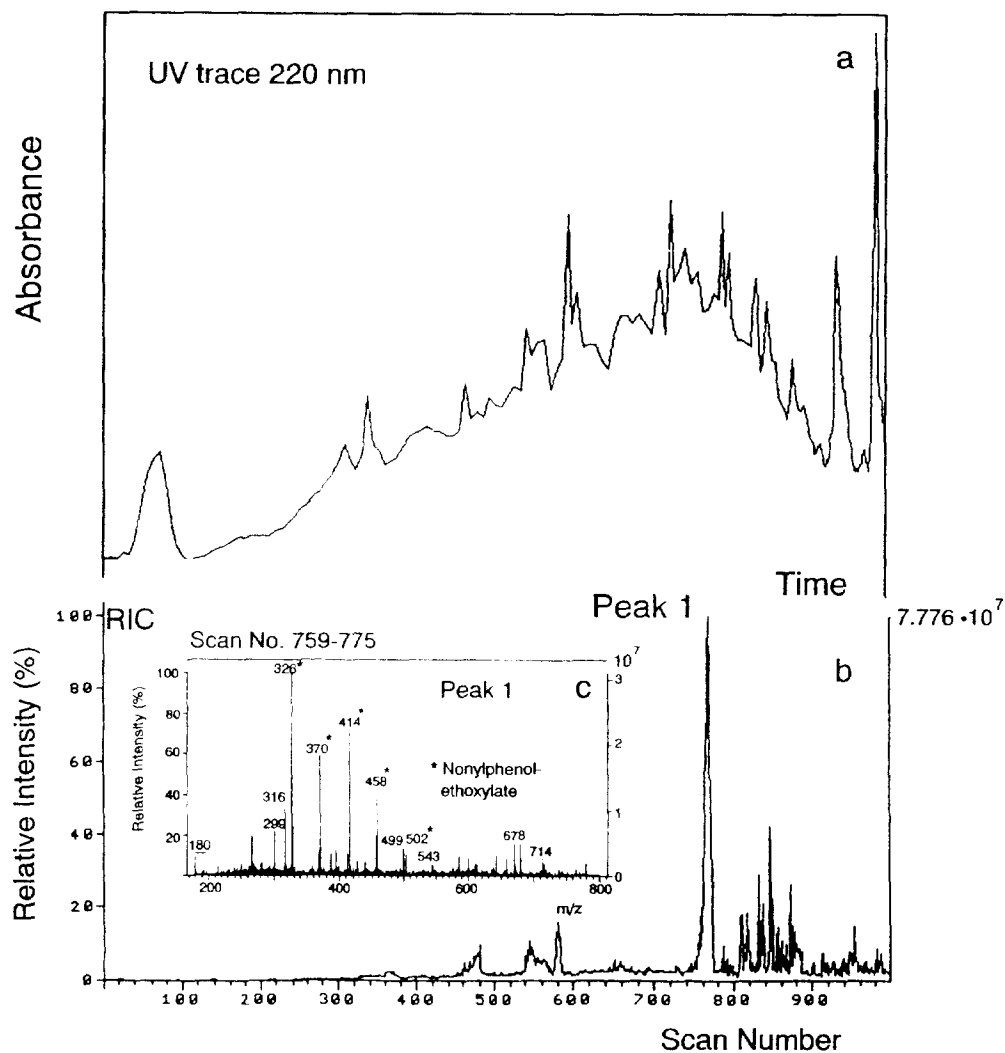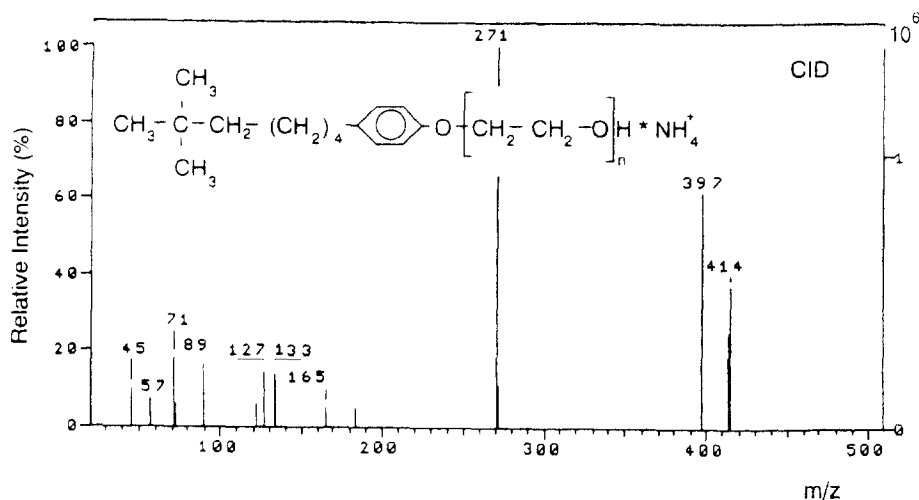

Fig. 7 (continued on p. 134).

Fig. 7. (a) UV trace (220 nm) for water sample no. 2 of Elbe river. C_{18} solid-phase extract; mixture of eluates as in Fig. 3; for chromatographic conditions, see Experimental and Table 2. (b) LC–MS total-ion current trace of Elbe river extract in (a) and LC–MS spectrum of (c) peak 1; LC conditions as in (a). Positive TSP ionization. (d) Daughter-ion mass spectrum (LC–MS–MS) and fragmentation scheme of non-ionic surfactant (nonylphenolethoxylate) cluster ion [m/z 414; $C_9H_{19}(C_6H_4)O$-$(CH_2CH_2O)_4H^*NH_4^+$] in peak 1 from Elbe river extract as in (b). LC conditions as in (a). Collision energy, -25 eV.

It corroborates the trend towards progressive improvement of the water quality which was noted in the earlier samples. So the DOC was with 6 mg/l again lower than in all other tested water samples. Besides hexachlorobenzene, tribromofluoromethane and some phenols, especially phthalates, which are widespread all over the environment, dominate the ion current of the GC–MS analysis. The intensity of the ion current in sample 4 was now only about 20% of that measured in sample 1.

This was nearly the same with the ion-current traces of the extracts obtained by selective elution and examined by FIA–MS, where we tried to compensate for the low concentration of compounds by enrichment of pollutants from a larger water volume. A mass spectrum of the compounds of the whole extract generated by FIA–MS, i.e. without selective elution, shows a Gaussian distribution, and the maximum intensity of the molecule ions is about m/z 300 (Fig. 9), while the ions of the compounds from sample 1 have their maximum at m/z 200.

The chromatographic separation corresponding to LC conditions of sample 1 results in

a TIC which is hardly structured and contains only a few signals (Fig. 10d) and a maximum plot after photodiode-array detection which is not very informative (Fig. 10a). The largest part of the very polar compounds of this extract can be separated only insufficiently under those conditions, except for the ions m/z 257 and 274 (Fig. 10f). This complicates the identification of compounds, as they elute more or less continuously. Thus the specific objective of chromatography, i.e. separation, focussing and, if necessary, identification by CID, is missed. Therefore, we tried to characterize these compounds without preceding LC separation by mixture analysis, using the tandem function of the spectrometer (MS–MS). Even from the confusing mixture in Fig. 9, daughter ions can be generated without problems if the parent ions are accessible to the collision-induced fragmentation process. Besides the parent ions with m/z 257 and 274, which could be separated by LC, we tried to generate daughter ions and to identify the ions with m/z 268 and 270 with their strikingly characteristic isotope pattern. In spite of painstaking optimization of the collision energy and collision gas
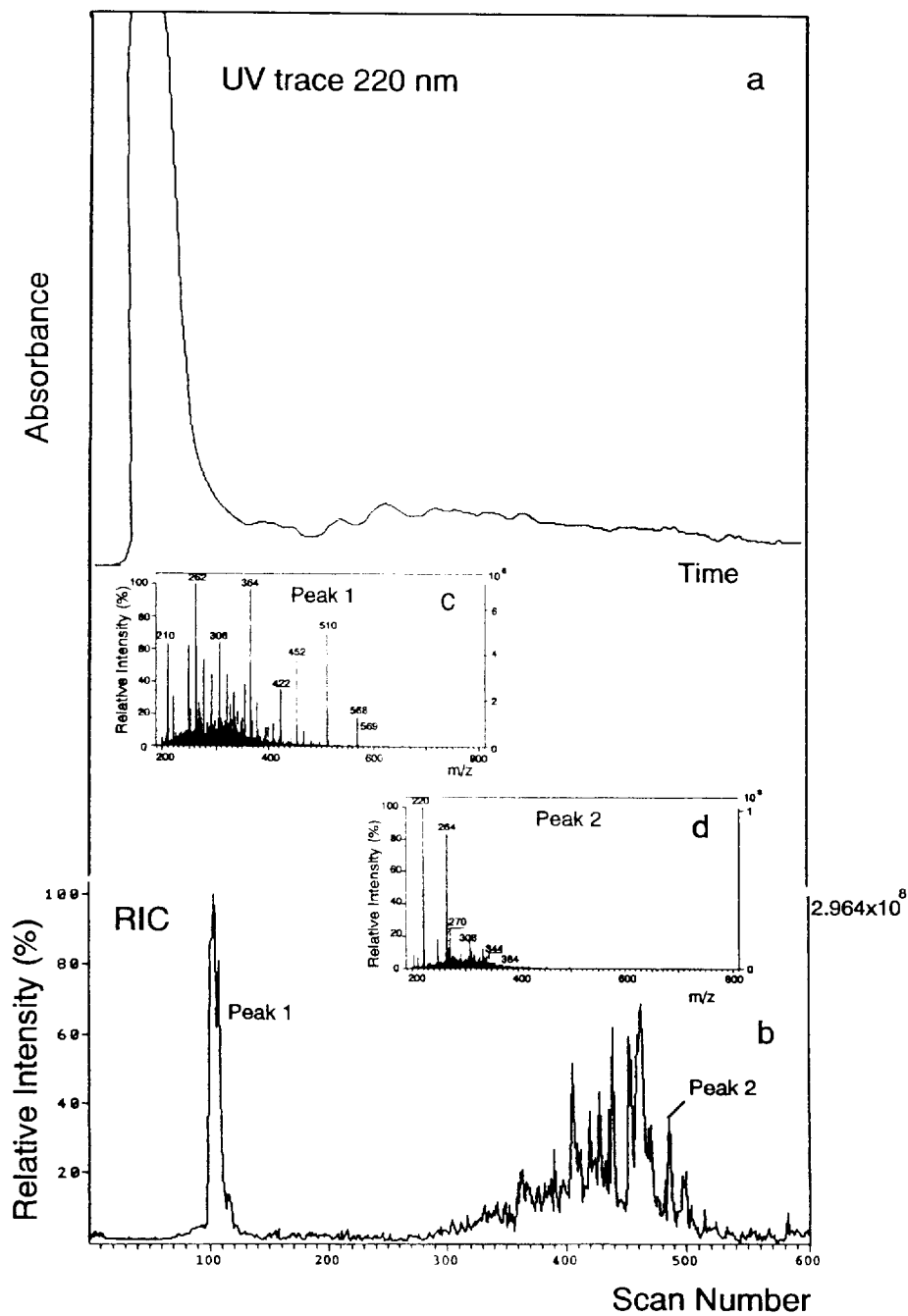
Fig. 8. (a) UV trace (220 nm) for water sample no. 3 of Elbe river. C_{18} solid-phase extract; mixture of eluates as in Fig. 3; for chromatographic conditions, see Experimental and Table 1. (b) LC–MS total-ion current trace of river Elbe extract in (a) and LC–MS spectrum of (c) peak 1 and of (d) peak 2; LC conditions as in (a). Positive TSP ionization.
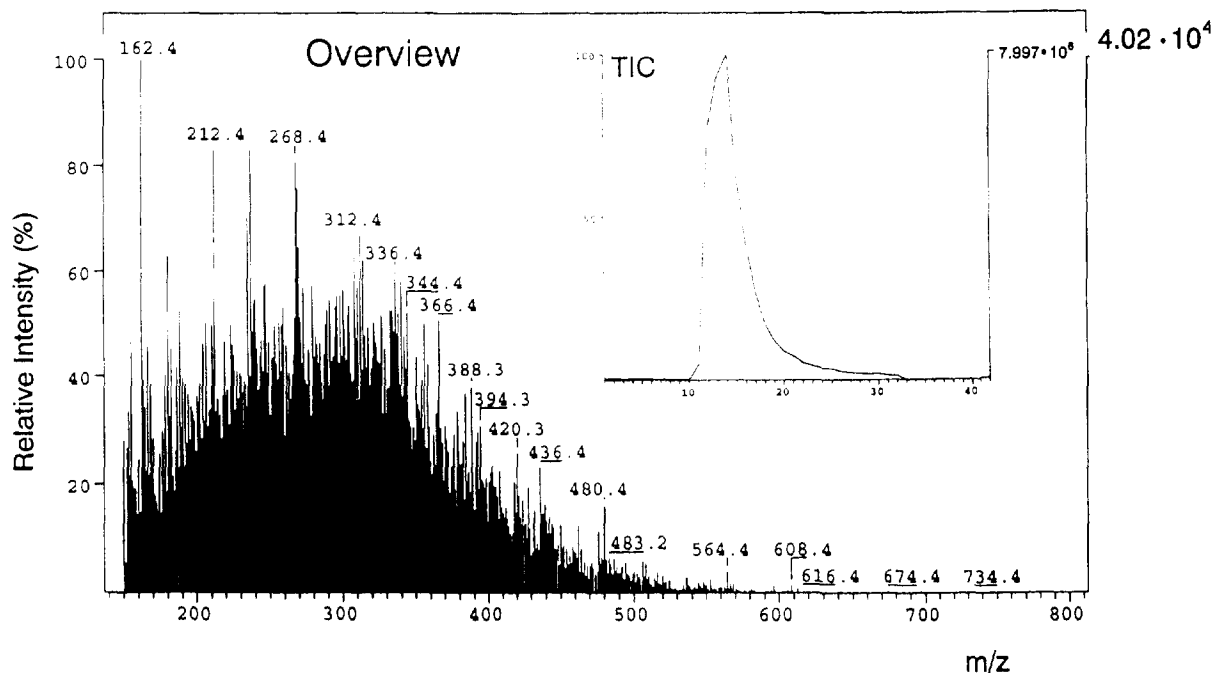
Fig. 9. FIA–MS overview spectrum as in Fig. 2. Mixture of eluates. Eluents, hexane, hexane–ether, ether, water–methanol, methanol.

pressure, the parent ions with m/z 257 and 274 were decomposed into a single daughter ion (m/z 91) only, common to both of them. CID fragmentation of the ions with m/z 268 and 270 resulted in daughter-ion spectra which were very rich in lines and resembled those from electron-impact spectra (Fig. 11).

The concurrence in the daughter ion 91 for both parent ions with m/z 257 and 274 gives rise to the presumption that the same compound is concerned. Ion 257 was formed by the addition of a proton and ion 274 by the addition of an ammonium ion from the ionization agent ammonium acetate to the molecule of molar mass 256. Identification is not possible using a daughter ion spectrum which is so poor in lines.

The daughter-ion spectra of m/z 268 and 270 are rich in lines, which is remarkable for CID spectra. The intensity of the signals of the fragment ions shows that the same compound is concerned, but substituted with different isotopes (see Fig. 11). The different molar masses are caused by the masses of the bromine isotopes

found in the natural environment (^{79}Br/^{81}Br). Fragments containing bromine (for example in the ^{79}Br-substituted molecule: 93, 107, 119, 131, 149, 165, 177, 191, 205, 233 and 251) can be recognized very easily because of the mass difference of 2 u in both CID spectra (compare Figs. 11a and 11b). A final characterization has yet to be done. At present, synthesis of reference substances with the empirical formula $C_{11}H_{23}OBr$ and the molar mass 250/252 is in progress.

4. Conclusions

Sampling from the Elbe, carried out always in early summer in 1989, 1990, 1992 and 1994, and analysis on polar organic compounds showed a clear trend towards ever-decreasing pollutant concentrations. These results were confirmed by sum parameter analysis (DOC) as well as by estimation of the ion-current trace during FIA–
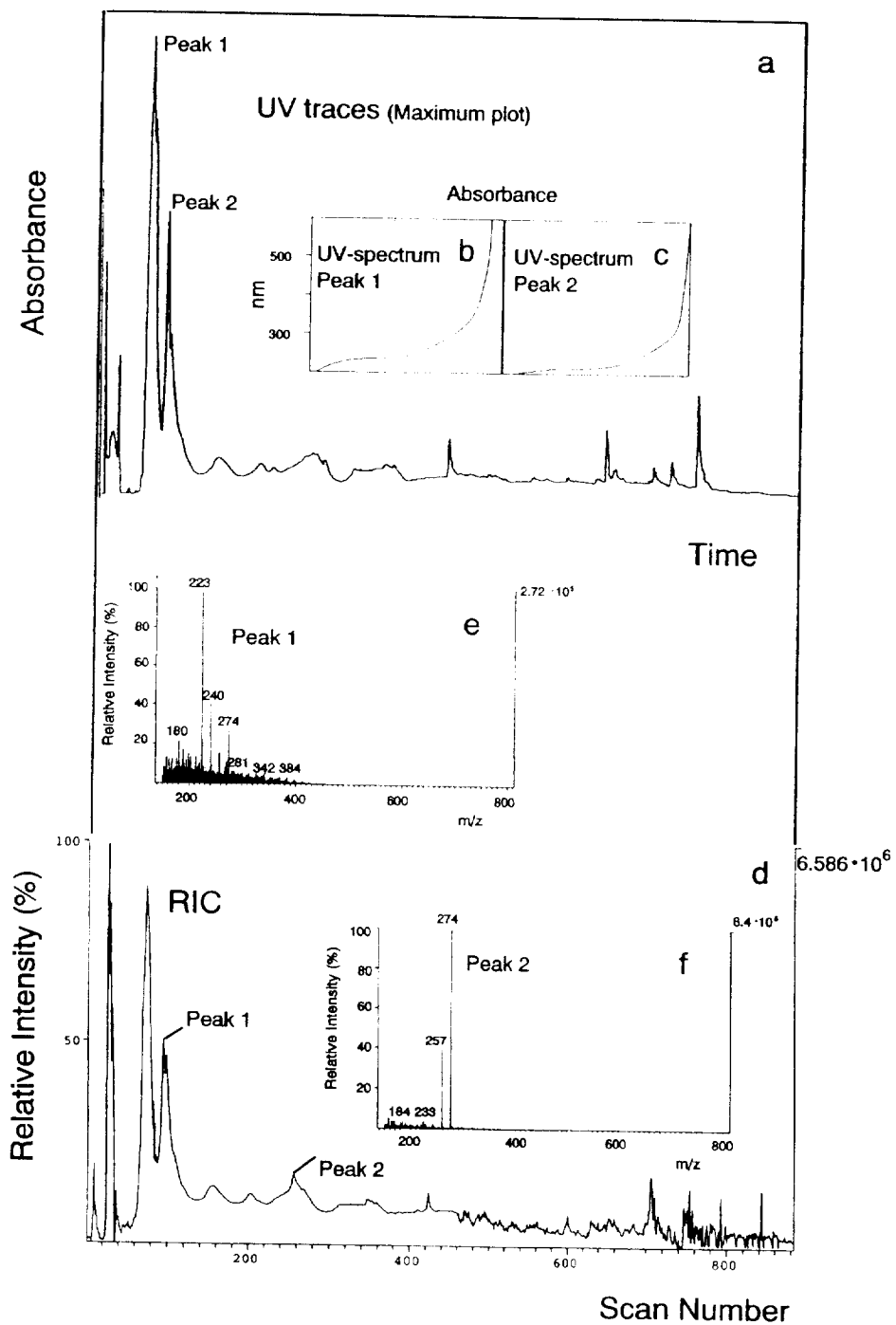
Fig. 10. (a) UV trace (maximum plot) for water sample no. 4 of Elbe river and UV spectra of (b) peak 1 and of (c) peak 2. C_{18} solid-phase extract; mixture of eluates as in Fig. 3; for chromatographic conditions, see Experimental and Table 1. (d) LC–MS total-ion current trace of Elbe river extract in (a) and LC–MS spectra of (e) peak 1 and of (f) peak 2; LC conditions as in (a). Positive TSP ionization.
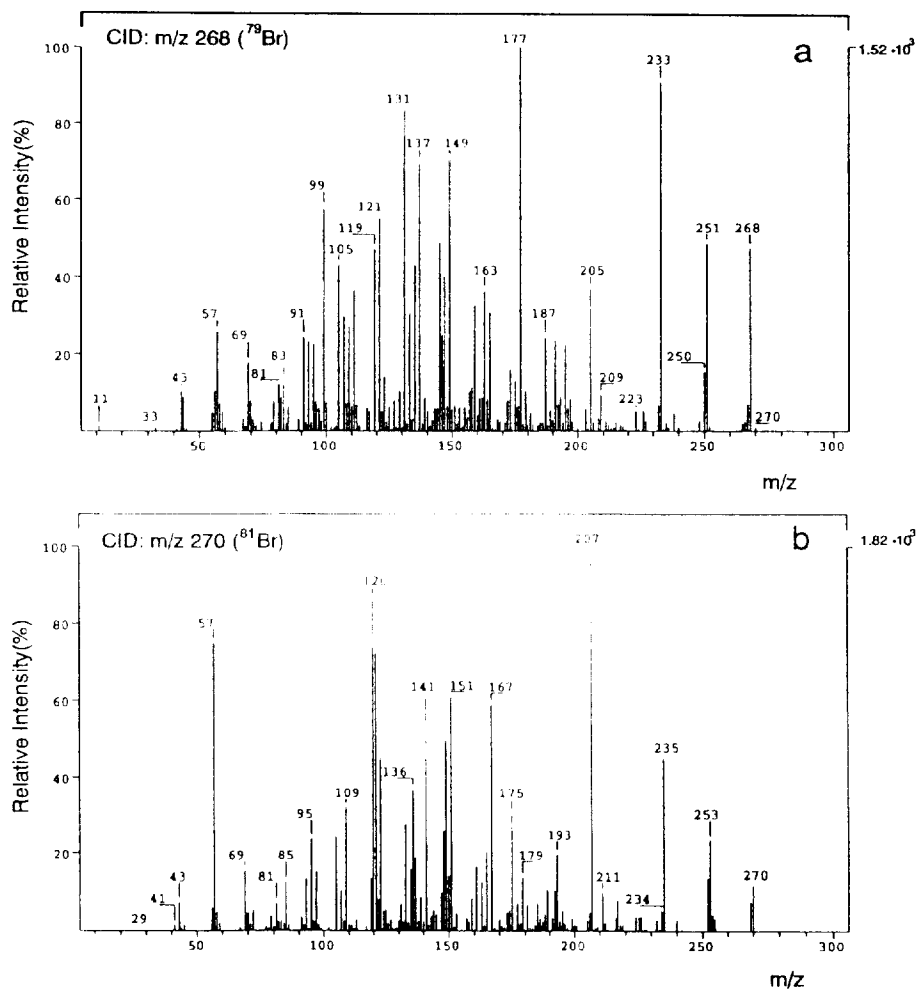
Fig. 11. Daughter-ion mass spectra (FIA–MS–MS) of (a) cluster ion m/z 268 (^{79}Br) and of (b) cluster ion m/z 270 (^{81}Br). Collision energy. −15 eV.

MS and LC–MS analysis. Screening by GC–MS, carried out in parallel, showed the same results.

The pollutant spectrum of the Elbe water, admittedly assessed on the basis of four 24-h composite samples which were taken, however, always at the same seasonal date, showed characteristic changes as to concentration and quality. The quantitative reduction of pollutants is confirmed by the ion-current traces recorded by FIA–MS.

Qualitative changes during the research period can be observed with the help of the overview spectra generated from the TICs which were recorded by FIA–MS. At the end of the investigation period, they contain only a few compounds which rise above the background of the matrix, in contrast to those recorded at the beginning of this investigation (Figs. 2 and 3). This qualitative change of compounds is also demonstrated by the shift of the maximum intensity of ion distribution in the overview spectra up to higher m/z ratios (compare Figs. 2 and 3 with Fig. 9).

By combining C_{18} material and freeze-drying for enrichment and concentration, respectively, of adsorbable or non-adsorbable pollutants, it

was possible to compare the results of the samples. By this, it was proven that the concentration of individual compounds has become more and more equalized during the sampling period.

The qualitative change in the compounds is shown by the fact that, on the one hand, as already mentioned, the appearance of the overview spectra recorded during FIA–MS analysis of the C_{18} extracts has changed. On the other hand, approved liquid chromatographic separation conditions, as applied for the successful LC–MS separation of the extracts of samples 1 and 2, could now separate only insufficiently the extracts from samples 3 and 4. With few exceptions, the compounds of these samples do not elute as sharp signals, but continuously. This shows that the polarity of enriched substances has increased from year to year, as always the same C_{18} column with constant separation efficiency, ensured by standards, had been used.

Direct mixture analysis using FIA–MS and FIA–MS–MS solves part of the difficulty and enables detection and characterization of selected thermospray-active compounds [6,8,10–12]. Direct mixture analysis using the MS–MS option can be applied successfully even in such cases where no dominant signals are observed, but pollutants in similar concentrations are present.

By application of other adsorption materials during the last sampling period, such as LiChrolut EN, XAD 2 or 4, no improvement – except for LiChrolut EN – could be found by comparison of the intensity of the different TICs, i.e. only slightly increased adsorption of polar organic compounds related to the C_{18} material applied could be observed. In parallel, compounds different in character from the C_{18}-adsorbable compounds could be adsorbed and should be examined in future.

Unfortunately, the stability of the pollutant mixture from solid-phase extraction, stored in methanolic solution at $-30°C$, is limited. Many compounds are already decomposed after 2–3 months, and thus it is no longer possible to compare extracts from old and new samples directly in one run. At present, the stability of sampling material at storage temperatures of $-86°C$ and at liquid nitrogen temperature is being examined.

The reduction of the concentration of pollutants in the Elbe and their change during the research period of five years can only partly be explained by the increased number of biological sewage treatment plants used to improve the treatment of waste waters discharged into the Elbe. Since the start of the German unification process the discharge of industrial waste waters has also continuously decreased, because of a massive reduction of industrial capacity in the former GDR [13].

As a result of these changes, the concentration of pollutants has decreased, and the pollutant spectrum has changed dramatically. In spite of increased efforts made towards waste water treatment along the Elbe and its tributaries, the concentrations of organic pollutants will hardly change in the future. Other research work [2] suggests that the DOC content will remain as high as that found in the last sample, because the Elbe has normally rather high contents of dissolved organic carbon as matrix. Most of the biogenic compounds hidden behind the DOC are polar, which will continue to complicate the detection of anthropogenic substances.

Acknowledgements

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