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Mass spectrometric strategies for the analysis of polar industrial chemicals and their by-products in wastewater and surface water

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Abstract

Various mass spectrometric techniques, such as gas chromatography (GC)–mass spectrometry (MS) after derivatization, liquid chromatography–electrospray ionization (LC–ESI) MS and ESI-time of flight (TOF)-MS have been applied to the determination of, in general substituted, polar organic sulfonates. Methods were developed for the rapid quantification of such industrial chemicals in wastewater effluents and surface water, as required following spills leading to unusually high emissions into the river Rhine, Germany. Using these methods, the tonnage of methylsulfamido-antipyrin, an intermediate of the pain reliever metamizole synthesis, and 3-nitro-benzenesulfonate could be directly calculated without time-consuming enrichment or clean-up procedures. Thereby a significant increase in sensitivity was achieved by switching a cation suppressor between LC and MS. But still, the evaluation and identification of more complex chemical structures of formerly unknown substituted and halogenated by-products of 2-chlorotoluene methylsulfon-synthesis in wastewater was only possible through the combined interpretation of the GC– and LC–ESI mass spectra and with the additional information obtained from application of ESI-TOF-MS to the samples.

Keywords: Water analysis; Environmental analysis; Sulfonates; Nitrobenzenesulfonate; Methylsulfamideantipyrin

1. Introduction

Due to their partial to complete resistance to biodegradation during the treatment process, wastewater treatment plants (WWTPs) receiving industrial wastewater release a complex (and ill-defined) mixture of mainly synthetic chemicals into the aquatic environment. Only a minority of the compounds passing through the industrial WWTP, accounting for less than 5% of the total organic carbon (TOC), are characterized. There is a need for more information to enable better evaluation of effluent quality with regard to its effects in surface waters and its potential for indirect potable reuse. Therefore today, not only industries but also managers of urban and domestic WWTPs must apply more effort to the reduction of harmful substances in wastewater.

In cases of accidental spills of polar chemicals from industrial sources into surface waters via industrial WWTPs, fast and reliable analytical methods are required for the detection of the peak pollution in order to devise appropriate measures. Since the infamous pesticide spill occurred at the Sandoz plant, Switzerland in 1988 [1], significant

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progress in the development of fast and reliable analytical methods has been achieved [2–7].

Until the beginning of the 1990s, heavy metals and halogenated, nonpolar pesticides were the focus of interest and subsequently a drastic reduction of emission was achieved by the elimination of the dominant pollution sources. Awareness of the presence of the nonpolar hazardous compounds in wastewater was achieved mainly through the use of gas chromatography (GC)-based techniques. These methods have enabled intense monitoring programs for such compounds, with the result that today, after adoption of appropriate measures, the pollution by these compounds is nowadays less relevant for the industrialized countries. Conversely polar organic industrial chemicals emitted in wastewater discharges have only been recognized for the last few years. Consequently, there is still a considerable lack of knowledge concerning this kind of pollution, and as such this is an important key point of further investigation [8,9].

The problems associated with this group of pollutants, which include organic sulfonates, aromatic acids, amines and nitro-compounds, are strongly related to their polarity, which enables them to bypass natural as well as manmade filtration steps [10]. Many of these polar compounds are also resistant to degradation and have been found in ground and drinking water. In some cases they are even formed during the wastewater treatment process [9,11].

The analytical method for the determination of more polar pollutants, after several preparation and enrichment and derivatization steps has conventionally been GC–mass spectrometry (MS). Meanwhile, liquid chromatography (LC)–electrospray ionization (ESI) MS and LC–ESI-MS–MS methods have developed into the most powerful techniques for the detection of polar water-soluble compounds in aquatic matrices [5,12–16].

Although application of LC–MS has significantly increased the fraction of identified compounds as measured in percent of TOC, the vast majority of polar organic effluent constituents is still unknown and furthermore knowledge regarding their formation, their behavior in the aquatic environment as well as degradation and toxicity is still quite limited. In cases where no reference substances are available, identification can only be achieved by interpretation of the mass spectra [9]. However, such identification by GC–MS as well as LC–ESI-MS analysis can often prove difficult due to overlapping signals which cannot be separated on the GC or HPLC column. In general, the thereby obtained mass spectra are difficult to interpret. These problems can be overcome with the aid of ESI time-of-flight (TOF) MS, which allows the acquisition of fullscan mass spectra with quite efficient sensitivity in the upper ng l^{-1} range and a sufficient accuracy between 50 and 0.1 ppm allowing also the calculation of sum formulas [17]. In combination with in-source fragmentation the method also provides characteristic fragmentions varying with the selected orifice voltage.

In this work I describe both, quantitative methods for the determination of polar target pollutants in waste and surface waters, as well as the identification of so far unknown pollutants using several different LC–MS approaches.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade. Milli-Q water was used in all the experiments. The reference compounds methylsulfamido-antipyrin (MSAAP) and 3-nitro-benzenesulfonate (3-NBS) were provided by Infraserv, Frankfurt, Germany and BASF, Ludwigshafen, Germany, respectively. The purity was greater than 98%.

2.2. Sampling

For investigation of individual industrial spills, 1-h mixed samples from wastewater effluents and 4-h mixed samples from corresponding surface water were collected for a period of 4 days immediately after the event. For other determinations, unless otherwise indicated, 24-h mixed wastewater samples were analyzed.

Mixed samples of river Main water were taken twice following the MSAAP spill (17 December 1997) on the 18 December 1997 (19–21 h) and the 18–19 December 1997 (11–9 h).

Mixed samples of river Rhine water were taken from 5 to 8 March 1998 following the spill with 3-NBS on 5 March 1998.

2.3. Sample preparation

2.3.1. MSAAP

Industrial sewage water and surface water samples (river Rhine and river Main, Germany) were filtered through a glass fiber filter (0.45 μm) and unless otherwise indicated, analyzed without further sample preparation. If deemed necessary, 100 mg of ethylenediaminotetraacetate (EDTA) was added to the 100 ml samples, lyophilization was performed for 8 h and the samples were then redissolved in HPLC eluent [18].

2.3.2. 3-NBS

Surface water samples (100 μl) from the river Rhine, Germany were filtered through a syringe filter (Spartan 13/0.45 RC, Schleicher and Schuell, Dassel, Germany) and analyzed without further sample preparation.

2.3.3. 2-Chlorotoluene methylsulfon (CMST) and related by-products

Industrial sewage water (100 ml) to which 30 g of sodium chloride was added were first extracted at pH 7 with 5 ml methyl-*tert*.-butyl ether (MTBE) in a liquid–liquid extractor at 1200–1300 U min^{-1} for 20–30 min [19]. After cooling at 5 °C for 45–60 min, the organic phase was removed and the extraction procedure repeated. Both MTBE phases were combined and evaporated to 1 ml under a gentle nitrogen stream and an internal standard (heptadecanoic nitrilo acid, final concentration: 1 μg ml^{-1}) was added prior to analysis by GC–MS.

The acidity of the water phase was then adjusted to pH 0.5 by addition of 3.5 *M* sulfuric acid and the extraction described above was repeated twice. The combined acidic extracts were evaporated to dryness under a gentle nitrogen stream and either (i) 500 μl ammonium acetate buffer added for LC–MS analysis or (ii) derivatization performed for GC–MS analyses. GC–MS samples were methylated using 700 μl *n*-hexane and 150 μl diazomethane in diethylether (in excess) at 20 °C, with the reaction being terminated after 60 min by addition of two droplets of acetic acid in acetone (10%, v/v). Internal standard (heptadecanoic nitrilo acid, final concentration: 1 μg ml^{-1}) was added and the extract made up to a final volume of 1 ml with *n*-hexane.

2.4. High-performance liquid chromatography (HPLC) separation

All separations were performed using an LC 200 binary pump (Perkin-Elmer, Norwalk, CT, USA) equipped with a 100-μl injection loop. To assure a flow of 0.25 ml min^{-1} into the ESI-interface, the LC effluent flow (0.5 ml min^{-1}) was split (1:1) by means of a zero dead volume T-piece. The HPLC separation was achieved on a 5 μm; 250×4.6 mm I.D. C_{18} reversed-phase column (Inertsil ODS-2, MZ-Analysentechnik, Mainz, Germany). The column temperature was held at 35 °C.

2.4.1. 3-NBS

The eluent consisted of water–acetone (60:40, v/v) containing 5 m*M* tetrabutyl ammonium hydroxide and adjusted to pH 7 with hydrochloric acid. The sample (100 μl) was separated under isocratic conditions and before being injected into the ESI-MS system passed through a cation exchanger (SUP-LC–MS). For the cation exchange, a Metrohm suppressor module 753 was used, where three small ion exchanger columns were sequentially rotated between the column and the ESI-MS interface between injections. While the first column is integrated into the effluent flow for H^+ cation exchange, the second is regenerated with 25 m*M* sulfuric acid containing 10% (v/v) acetone and the third rinsed with ultra pure water (Milli-Q). To prevent overloading of the micro cation-exchange columns a fresh regenerated unit was rotated into the effluent flow after every run.

2.4.2. MSAAP

Eluent A consisted of 10 m*M* ammonium acetate, adjusted to pH 4.1 with acetic acid; eluent B was acetonitrile. The initial conditions of the gradient program were 100% A, held for 10 min. From 10 to 20 min the eluent A was reduced down to 10%, and this held for 5 min. The solvent composition was

then brought back to 100% over 5 min. Under these conditions MSAAP elutes after 21.9 min.

2.4.3. CMST by-products

Eluent A consisted of 10 m*M* ammonium acetate, adjusted to pH 7.1 with ammonium hydrogencarbonate; eluent B was acetonitrile. The initial conditions of the gradient program were 85% A, held for 2 min and then decreased to 60% over a period of 10 min. From 12 to 20 min the eluent A was reduced down to 5%, which was held for 5 min. This was then brought back to 85% over a period of 5 min.

2.5. Mass spectrometric analysis

2.5.1. GC–MS

Analysis was done with a GC–MS system (Fisons) comprising an AS 800 autosampler, a gas chromatograph 800 and an MD 800 mass-selective detector.

An XTI-5 (Restek, Bellefonte, PA, USA) column (film thickness 0.25 μm, 30 m×0.25 mm I.D.) was used for separation with helium as carrier gas. Injections (2 μl) were made in the splitless mode at 50 °C oven temperature. This temperature was held for 2 min, followed by a 30 °C min^{-1} ramp to 100 °C and than a 4 °C min^{-1} ramp to 265 °C. Finally a 30 °C min^{-1} ramp to 290 °C was used and this temperature held for 10 min. The ion source temperature was 200 °C.

2.5.2. LC–ESI-MS

The analyses were performed on an atmospheric pressure ionization (API) 150 single quadrupole mass spectrometer (Perkin-Elmer Sciex API 150, Thornhill, Canada) equipped with an API source, via a turboionspray interface. The instrument was either run in the negative ion mode at an ionspray voltage applied to the electrospray emitter tip of −3 kV and an orifice voltage of −30 V (3-NBS and CMST by-products) or in the positive ion mode at an ionspray voltage of 4.5 kV and an orifice voltage of 40 V (MSAAP).

The interface temperature was held at 450 °C. Nitrogen grade 5.0 at a flow-rate of 7 l min^{-1} was used as turbo ion spray and curtain gas in the API source and nitrogen (99%), at a flow-rate of 1.48 l min^{-1} as the nebulizing gas.

For the calibration and quantitative analysis of 3-NBS the deprotonated molecular ion at m/z 202, measuring in the range of 201–203 u, was used. For MSAAP the ions m/z 296 $[M–H]^-$ and the most intense fragment at m/z 177 were used.

2.5.3. ESI-TOF-MS

The measurements were performed on an ESI-TOF mass spectrometer Mariner (Perkin-Elmer Biosystems, Foster City, CA, USA) under the following operating parameters: accelerating potential 4 kV; spray tip potential 3.5 kV; path length 1 m; flow-rate 200 μl min^{-1}. The mass axis was externally calibrated using a mixture of peptides in the 300 to 1000 u mass range. Since screening for unknowns, no lock-mass was used. The orifice voltage was −70 V. Spectra were acquired in the mass range from 80 to 500 u at a scan rate of 1 s/spectrum. The HPLC conditions used were as for the LC–ESI-MS analyses.

2.6. Calibration

2.6.1. 3-NBS

A four-point calibration was performed in the range of 1 to 20 μg l^{-1} in ground water. The values obtained in surface water were checked by three standard additions for which a recovery rate of 94% was achieved. The calculated limit of quantitation (LOQ) in surface water without enrichment was 1 μg l^{-1}.

2.6.2. MSAAP

A four-point calibration curve was performed in the range of 0.25 to 2 mg l^{-1} in surface water. Without enrichment the limit of detection (LOD) obtained was 25 μg l^{-1} and the LOQ was 100 μg l^{-1}. With enrichment, an LOQ of 2 μg l^{-1} could be achieved in surface water. In addition to the calibration, the obtained values were also verified by standard addition. Standard addition of 1 mg l^{-1} to an unaffected WWTP sample gave a recovery rate of 96% for the spiked MSAAP.

For verification of the recovery rates, three 100 ml samples of pristine groundwater were spiked with 0.5 μg l^{-1} MSAAP and after addition of 100 mg EDTA lyophilized for 8 h. Two surface water

samples were analyzed in triplicate after lyophilization as described above after reconstitution in 500 μl HPLC buffer; 100 μl being injected into the HPLC system. For the third surface water sample method validation by standard addition of 1 μg was performed.

3. Results and discussion

For many intermediates of chemical syntheses, such as 4-aminoantipyrinsulfonate (4-AAPS) and MSAAP, the main intermediates of the pain reliever metamizole (Fig. 1), there are no analytical methods available for quantification of low concentrations. These compounds are normally directly chemically modified to the final product without being introduced into the environment. However, in 1996 1.5 metric tons of 4-AAPS were introduced into the river Main, Germany during a spill from the manufacturing company. The subsequent persistence of the compound was monitored following development of an ion pair solid-phase extraction procedure by HPLC–diode array detection [20]. However, such cases require fast and reliable analytical data and for this the method was limited due to its time-consuming and unspecific nature.

Therefore, in case of urgent development of an analytical method for MSAAP, which was also introduced into the river Main, Germany, about 1 year later, LC–ESI-MS was chosen due its higher selectivity and sensitivity compared to photometric detectors. Thus MSAAP could be analyzed and quantified directly and without any further sample

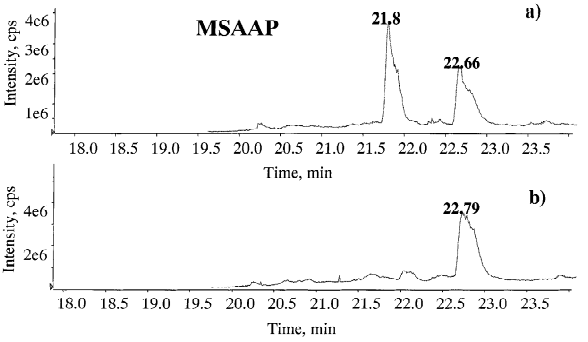


Fig. 2. (+)-LC–ESI-MS chromatograms of MSAAP in the effluent of an industrial WWTP (mixed samples): (a) during the industrial spill (18–19 December 1997); (b) before the industrial spill (17 December 1997); selected ions were m/z 296 $[M-H]^-$ and 177 (fragment ion).

preparation (see Experimental) in the sewage effluent obtained following the spill (Fig. 2a). Under normal circumstances MSAAP is never introduced into the WWTP and consequently also not into the aquatic environment (Fig. 2b). During the spill approx. 100 kg was emitted into the river Main. The concentrations of MSAAP in the river Main, where mainly a dilution occurred, were in the range of 1 and 2.7 $\mu g\ l^{-1}$ and thus much lower compared to the spill of 4-AAPS where values of up to 90 $\mu g\ l^{-1}$ could be detected. Being able to report the data only 1 day after sampling is an enormous improvement compared to the off-line methods previously used for similar analytes.

The developed method, involving direct analysis of the compounds, may well solve the problem of losses known to occur in both enrichment and

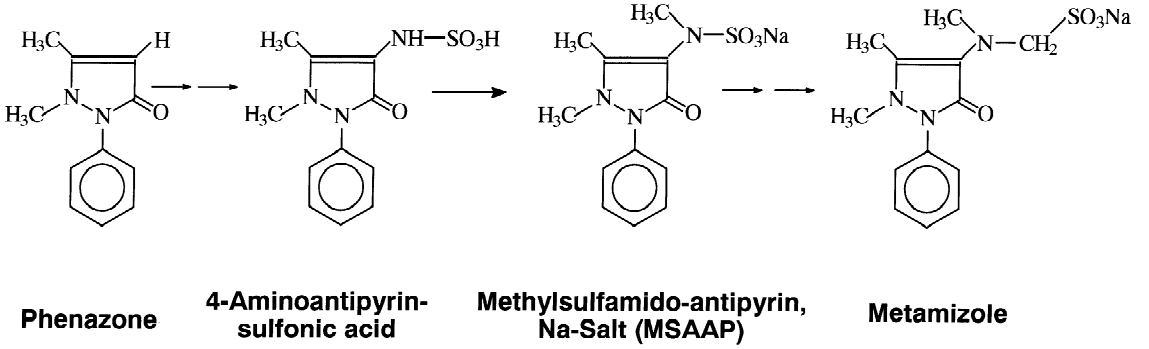


Fig. 1. Simplified scheme of the synthesis of metamizole starting from phenazone via 4-AAPS and MSAAP.

derivatization steps. High recoveries and low standard deviations prove that enrichment after lyophilization is both, an effective and fast preparation method for the subsequent detection via LC–ESI-MS.

During another breakdown in a chemical plant in Germany in 1998, it was estimated that approx. 3 tons of 3-NBS (Ludigol) were emitted via the WWTP into the river Rhine. The emission lasted over a period of 36 h and thus maximum values of 65 μg l^{-1} were calculated for the peak pollution once it reached Mainz, Germany, approximately 100 km downstream of the event. In order to determine the relevance of the river pollution it was extremely important to obtain reliable analytical data as fast as possible, such that the correct amount of 3-NBS being spilled into the river could be calculated.

A problem in using LC–ESI-MS for polar analytes such as 3-NBS is that saline eluents or buffer solutions are mandatory to achieve a sufficient HPLC separation, especially from the early eluting humic acids [21]. When using such eluents, combined with the high salt levels in matrices such as wastewater or lyophilized enriched samples, a high background is likely to occur in the MS chromatogram. Likewise, cluster ions are detected and strong incrustations at the vacuum interface can occur. Thus only if problems due to high amounts of salts or the usage of non-volatile buffers can be overcome, LC–ESI-MS will function as a good tool for this application. One such option is to switch a suppressor between the HPLC and the ESI-MS resulting in cation exchange of H^+ from the effluent and the sample [22–24].

For analysis and in order to get a separation of 3-NBS on an RP-C_{18} column, the use of a non-volatile ion pair reagent such as tetrabutyl ammonium hydroxide is essential and thus a suppressor to be switched between the LC and the MS interface was also required. A sufficient retention time could be achieved for 3-NBS enabling separation from the dominating inorganic salts, mainly chloride and sulfate (Fig. 3). By applying this technique the background noise was reduced to nearly half the magnitude as well as the ionization enhanced, thus allowing the quantification of 3-NBS as an ion-pair directly from the river water with an LOD of 0.5 μg l^{-1} without any sample preparation. This LOD was well within the requirements, with the maximum

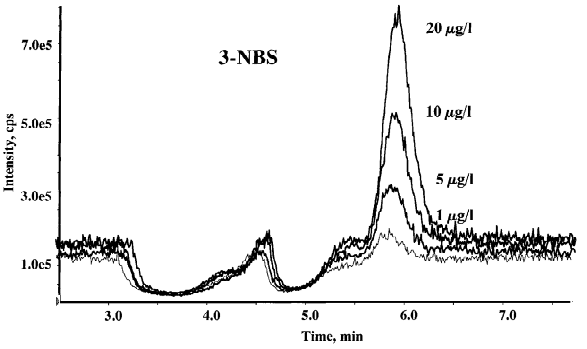


Fig. 3. (−)-SUP-LC–ESI-MS chromatogram of river Rhine samples spiked with different 3-NBS concentrations without any further sample preparation procedure.

concentration measured at the peak of the three day pollution wave being 72 μg l^{-1} (Fig. 4).

Based on the determined concentrations and sampling of representative samples over the entire width of the river, it was possible to calculate exactly the tonnage of 3-NBS being introduced into the river. The figure obtained was 4.6 metric tons, and this data was in good correlation with the estimated values of the producing company [25].

In order to determine the persistence of this compound in the aquatic environment subsequent to the spill, a microbial degradation assay [26] was also conducted. Results from this assay showed good aerobic biodegradability (data not shown). Therefore

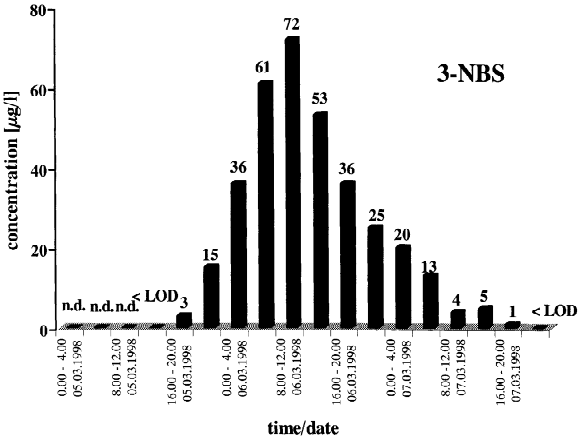


Fig. 4. Measured 3-NBS concentrations in the river Rhine at Mainz, Germany (4-h mixed samples; values in μg/l); n.d.=not detected; LOD=limit of detection.

it is highly likely that biodegradation of 3-NBS also occurs in the river and during bank filtration.

The analysis of complex mixtures as occurring in the effluents of chemical industries requires a systematic strategy in order to get as much as possible information for the organic compounds present. Regarding this aspect, our strategy is thus to apply different mass spectrometric detection methods following sequential liquid–liquid extraction procedures [9]. The analysis method applied was selected according to the pH of the solution. For example, the samples enriched at pH 7 were analyzed by GC–MS (Fig. 5a) and those obtained at pH 0.5 were either methylated and investigated by GC–MS (Fig. 5b) or introduced without further derivatization into the LC–ESI-MS, with analysis in the negative ionization mode. Additionally samples extracted at pH 10 were either derivatized with an excess of trifluoro acetanhydride and analyzed via GC–MS or analyzed by LC–ESI-MS in the positive ionization mode (data not shown). The GC–MS chromatograms obtained for the neutral and acidic fractions differed substantially and with the help of libraries to aid spectra interpretation it could also be determined that there was no overlap of the pollutants being detected (Fig. 5).

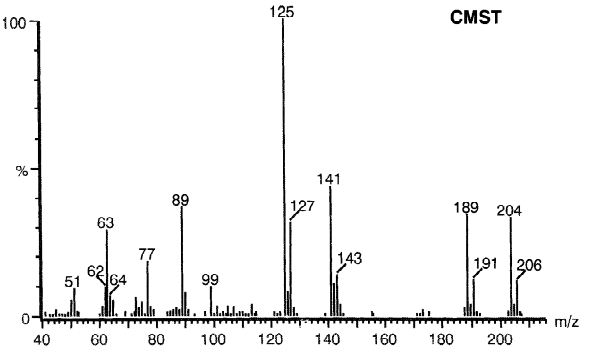


Fig. 6. Electron impact (EI) GC–MS spectrum of 2-chloro toluene methylsulfon (CMST); assignment of masses see Table 1.

In the GC–MS chromatograms of neutral extracts almost all peaks could be assigned to organic micropollutants in the investigated wastewater effluent. The most predominant signal at a retention time of 23.37 min was identified as CMST, an industrial intermediate used in various syntheses. The mass spectrum and structural formula are shown in Fig. 6 and Table 1. In addition to the molecular radical cations at m/z 204 and 206 a series of characteristic fragment ions could also be assigned (Table 1; No. 1a).

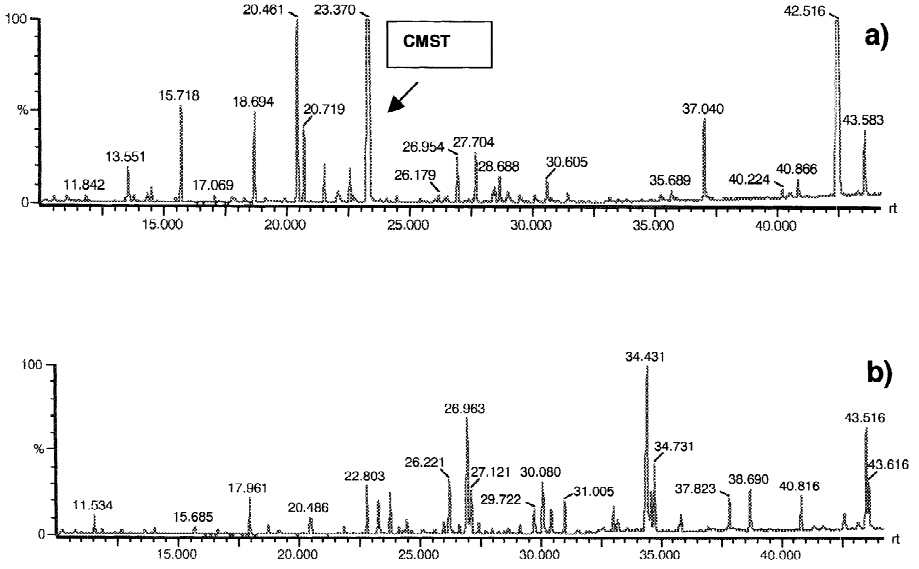
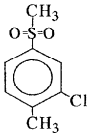
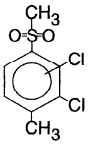
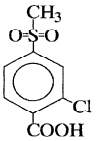
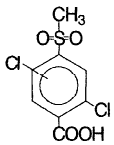
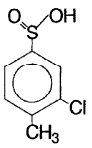
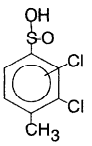
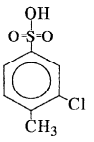
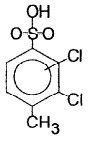


Fig. 5. GC–MS chromatograms of (a) the neutral extracted and (b) the acidic extracted and methylated fraction of an industrial wastewater effluent.

Table 1
Results obtained after comparison of EI-GC–MS after derivatization with diazomethane, LC–ESI-MS and LC–ESI-o-TOF-MS of CMST and identified by-products

Compound*			t_R (min)	m/z (Assignment)	
Name (No.)	Structure	M_r (monoisotopic)		GC–MS	LC–ESI-MS, LC–ESI-o-TOF
2-Chloro toluene methylsulfon (1a)		204	23.37	(a) 204/206 ($M^{+\cdot}$) 189/191 ($M-CH_3^{+\cdot}$) 141/143 ($M-SOCH_3^{+\cdot}$) 125/127 ($M-SO_2CH_3^{+\cdot}$) 89 ($M-SO_2CH_3^{+\cdot}$); –HCl	(a) Not detected
Di-chloro toluene methylsulfon (1b)		238	27.2	(a) 238/240/242($M^{+\cdot}$) 223/225/227 ($M-CH_3^{+\cdot}$) 175/177/179 ($M-SOCH_3^{+\cdot}$) 159/161/163 ($M-SO_2CH_3^{+\cdot}$) 123/125 ($M-SO_2CH_3^{+\cdot}$); –HCl	(a) Not detected
2-Chloro methyl-sulfon benzoate (2a)		234	30.0 13.0	(b) 248/250 ($M^{+\cdot}$) (c) 217/219 ($M-OCH_3^{+\cdot}$) 169/171 ($M-SO_2CH_3^{+\cdot}$)	(b) 233/235 ($[M-H]^-$) (c) 189/191 ($[M-CO_2H]^-$)
Di-chloro methyl-sulfon benzoate (2b)		268		Not detected	Not detected
2-Chloro toluene sulfinate (3a)		190	19.1 5.5	(b) 204/206 ($M^{+\cdot}$) (c) 173/175 ($M-OCH_3^{+\cdot}$) 125/127 ($M-SO_2CH_3^{+\cdot}$)	(b) 189/191 ($[M-H]^-$) (c) 188.9510/190.9487 ($[M-H]^-$) (d)
Di-chloro toluene sulfinate (3b)		224	23.8 13.5	(b) 238/240/242 ($M^{+\cdot}$) (c) 207/209/211 ($M-OCH_3^{+\cdot}$) 175/177/179 ($M-SOCH_3^{+\cdot}$) 159/161/163 ($MSO_2CH_3^{+\cdot}$) 123/125 ($M-SO_2CH_3^{+\cdot}$); –Cl	(b) 223/225/227 ($[M-H]^-$) (c) 159/161/163 ($[M-SO_2H]^-$) 222.9470/224.9448/ 226.9402 ($[M-H]^-$) (d)
2-Chloro toluene sulfonate (4a)		206	5.5	(c) Not detected	205/207 ($[M-H]^-$) (c) 189/191 ($[M-OH]^-$) 205.946/207.943 ($[M-H]^-$) (d)
Di-chloro toluene sulfonate (4b)		240	7.7	(c) Not detected	239/241/243 ($[M-H]^-$) (c) 238.9321/240.9291/ 242.9234 ($[M-H]^-$) (d)

*Position of halogen atoms only tentative. (a) GC–MS without derivatization. (b) GC–MS after derivatization with diazomethane; mass assignment of the methylester. (c) LC–ESI-MS. (d) ESI-o-TOF-MS.

In the GC–MS chromatograms obtained for the acidic fraction after derivatization with diazomethane (Fig. 5b) several signals could be assigned, however the majority of the detected signals could not be interpreted. Quite characteristic was a series of signals all showing an halogen isotopic pattern and the loss of m/z 31 and m/z 79, corresponding to $-OCH_3-$ and $-SO_2CH_3-$ groups, respectively (Table 1). A typical GC–MS spectrum shown in Fig. 7a, gives an additional loss of m/z 63 that can be correlated to the loss of an $-SOCH_3-$ group (Table 1).

Since those spectra could not be completely interpreted and also because no similar spectra were present in the Wiley and NIST-spectral libraries used, the information obtained was compared with the LC–ESI-MS spectra obtained in the negative ionization mode. In these LC–MS chromatograms a series of halogenated signals showing isotope patterns of singly and doubly chlorinated compounds were also observed. Furthermore, some of them showed losses of m/z 64 corresponding to SO_2 (Fig. 7b; Table 1). However, the information obtained still did not enable full characterization of the unknowns and to create possible suggestions for the structural formulas. Consequently, it became clear that the information obtained by dual application of GC–MS and LC–ESI-MS was insufficient for the unequivocal assignment of these contaminants. In order to obtain more diagnostic information the idea then was to apply LC–ESI-TOF method, leading to additional information regarding the elemental composition which can be calculated based on the determined accurate masses [27].

Application of this technique after online LC separation yielded a series of MS spectra with a much higher mass accuracy than usual LC–ESI-MS (Fig. 7c; Table 1). A selective search was performed especially on those signals having the halogen pattern already observed in the GC–MS and LC–ESI-MS spectra. With a mass accuracy in the range between 1 and 40 ppm and the information of the amount of C, O, S and Cl atoms the elemental composition from four of five polar by-products of the CMST-synthesis could be calculated (Tables 1 and 2). One example is shown in Fig. 7c) with the most intense monoisotopic molecular mass at m/z 222.9470. Calculating the corresponding sum formula with a precision between 1 and 50 ppm, there were 20 theoretical possibilities (Table 2). But since the amount of C and Cl atoms could be calculated out of the isotopic pattern, 19 of them could be ruled out. The only possibility left over was the sum formula $C_7H_5O_2SCl_2$, which could be calculated

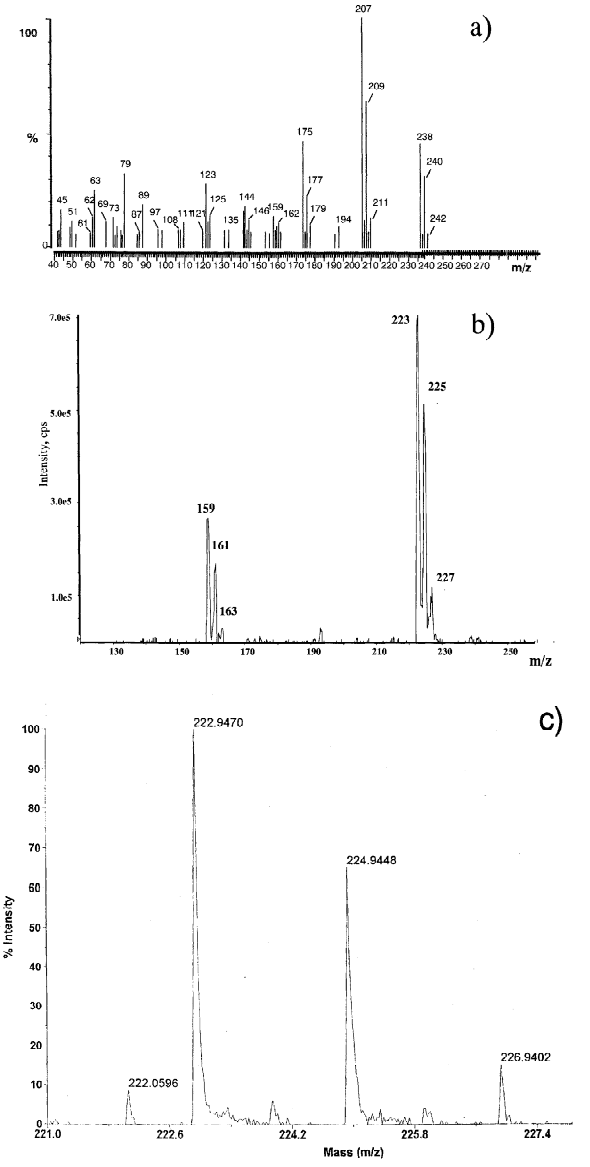


Fig. 7. Comparison of (a) EI-GC–MS after derivatization with diazomethane, (b) (−)-LC–ESI-MS and (c) (−)-LC–ESI-o-TOF-MS spectra of dichloro-toluene-sulfinate; assignment of masses see Table 1.

Table 2
Top 20 hits for m/z 222.94700 and charge 1 with a mass tolerance of 0.01115 u

Calculated mass	mDa	ppm	DBE formula
222.94659	0.40685	1.8	$C_9HN_2OC_{12}$
222.94642	0.58154	2.6	$C_8HN_2O_2PCl$
222.94807	−1.07369	−4.8	$C_6H_6O_3P_2Cl$
222.94825	−1.24838	−5.6	$C_7H_6O_2PCl_2$
222.94430	2.7035 1	12.1	$C_{10}H_4S_2Cl$
222.94979	−2.79043	−12.5	$C_5H_5N_2O_2PSCl$
222.94997	−2.96511	−13.3	$C_6H_5N_2OSCl_2$
222.95044	−3.44117	−15.4	$C_{13}HPCl$
222. 94155	5.45042	24.4	$C_5H_6N_2P_2SCl$
222.94092	6.07547	27.3	$C_{13}SCl$
222.94027	6.72622	30.2	$C_5H_4N_2O_2S_2Cl$
222.95381	−6.81313	−30.6	$C_{10}H_5PSCl$
222.94001	6.99246	31.4	$C_7H_7P_2C_{12}$
222.93873	8.26826	37.1	$C_7H_5O_2SCl_2$
222.93856	8.44295	37.9	$C_6H_5O_3PSCl$
222.93818	8.82238	39.6	$C_8H_2N_2P_2Cl$
222.95649	−9.48922	42.6	$C_7H_5O_4Cl_2$
222.93690	10.09817	45.3	$C_8N_2O_2SCl$
222.95719	−10.1850	45.7	$C_7H_9PS_2Cl$
222.95803	−11.0312	−49.5	$C_SH_4N_2O_4SCl$

with an accuracy of 37.1 ppm. Joining together the information obtained from the fragmentation pattern observed in GC–MS and LC–ESI-MS, this compound must be di-chloro toluene sulfinate. The assignment of the fragments of all postulated compounds is given in Table 1.

Quite interesting for many of the detected compounds is the stability of the aromatic sulfinate group, which is normally known from the literature to be quite unstable. A request to the CMST producing company did not rule out the assignment of these compounds to be produced during the CMST synthesis as by-products. But in order to be totally sure of the structural composition and also for quantitation of these compounds, reference substance must be either synthesized or extracted out of waste water. Since this is quite a demanding and also time-consuming issue, as for example shown for the identification and quantification of bis-ethyl-iso-octanol lactone isomers [28] this will be a task of its own. Anyhow, since the halogenated toluenesulfinates as well as the corresponding benzoic and sulfonic acids (Table 1) were never detected in river water, this might not be of high priority. Our purpose was to demonstrate the usefulness of such a battery of screening methods, allowing also to detect yet unknown presence of organic polar compounds at low concentrations.

4. Conclusion

One of the major limitations in the analysis of polar compounds remains to be the sample preparation step, especially in the complex matrix of wastewater. With the integration of a suppressor module into an LC–MS system, new methods for measurement are possible, further opening the door for the analysis of polar micro pollutants by LC–MS. In comparison with the other detection methods, SUP-LC–MS is also considerably advantageous in terms of sample preparation and analysis time.

With the combined structural information gained from GC–MS spectra, following derivatization to the methylester, and LC–ESI-MS and ESI-TOF-MS spectra it is possible to elucidate the structures of several other previously unknown polar pollutants present.

Even in the industrial wastewater of chemical industries with well documented production lines, less than 10% of the emitted dissolved organic carbon (DOC) is normally known, and as such there

is still a need for analytical methodology that permits identification of new metabolites and by-products of chemical synthesis.

In the case of the evaluation of complex structures of xenobiotics in difficult matrices, parallel application of a range of mass spectrometric methods are required in order to achieve complete structural characterisation.

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