The use of liquid chromatography-atmospheric pressure ionization-mass spectrometry in water analysis – Part I: Achievements

Thorsten Reemtsma*
Department of Water Quality Control, Technical University of Berlin, Sekr. KF 4, Strasse des 17 Juni 135, 10623 Berlin, Germany

Liquid chromatography-mass spectrometry using atmospheric pressure ionization is dramatically changing the analytical methods, potential and tasks of water analysis. The present status in applying this technique to organic as well as inorganic water constituents and its use in the context of wastewater treatment and drinking water preparation is reviewed. Separation techniques such as reversed-phase HPLC, ion chromatography, capillary electrophoresis and size-exclusion chromatography are also considered. LC-MS is well established to detect industrial mass chemicals such as dyes, aromatic sulfonates, surfactants and complexing agents but also trace compounds such as drugs, endocrine-disrupting compounds, toxins, phenols and haloacetic acids. The applications of LC-MS are now expanding towards the analysis of inorganic compounds such as oxo-anion. The success in characterizing natural organic matter by LC-MS is still limited, but an interesting potential is seen in detecting organometallic complexes as well as non-covalently bound adducts following electrospray ionization.

Keywords: Liquid chromatography-mass spectrometry; Water analysis; Wastewater treatment; Drinking water preparation

1. Introduction

With the fundamental development of liquid chromatography-atmospheric pressure ionization-mass spectrometry (LC-API-MS) in the 1980s, and its broad instrumental implementation in the 1990s unforeseen analytical capabilities became available. Its introduction into environmental analysis began comparatively late, but since then tremendous progress has been made and a wide variety of compound classes has been determined in water by means of LC-MS using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI).

This development is reflected in the growing number of publications dealing with LC-MS in water analysis and a parallel reduction in the number of papers dealing with the chemical derivatization of polar analytes to make them amenable to analysis by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography with ultraviolet (UV) and fluorescence detection, respectively. The literature database built for this review records less than two entries per year for the period 1980 to 1990. For 1995 there are 12 entries and some 80 entries per year for 1999 and 2000. Thus, the last two years account for approximately 50% of all literature published to date on LC-MS in water analysis.

This change in analytical methodology for water analysis is accompanied by a gradual shift in the perception of the tasks of water quality control: from the traditional focus on environmental contaminants of limited polarity that have long been accessible by GC-MS, towards more polar com-
pounds that are relevant for the water cycle and, thus, for the potential of water reuse and for which LC-MS is the appropriate tool for analysis. Additionally, it is widely recognized that every treatment process not only removes compounds from water but may also generate new, unknown and often more polar substances.

This review is applications oriented. Numerous excellent reviews and books cover the development of LC-MS and the interface techniques [1–4], the processes underlying ion generation and transfer into the gas phase namely in the electrospray process [5–7] as well as specialties of the mass measuring methods [8]. These reviews provide a deeper understanding of the technique and are helpful in solving also application problems.

Moreover, this review confines itself to applications in explicit aquatic compartments such as surface water, river and seawater, effluents and groundwater. Detection in aqueous matrices such as cell or body liquids, urine, aqueous extracts or beverages is not considered, although similar methods and compounds are involved.

While scanning the literature different incentives to develop LC-MS methods became obvious, that can be categorized as follows:

- to perform quantitative analyses faster, more conveniently, cheaper and/or at lower concentrations than previously possible;
- to analyze compounds that could not be detected before or to identify novel substances;
- to do it by LC-MS because no one had reported it before.

This review focuses on those studies falling into the first two categories.

Finally several compound classes covered here have been the subject of specific reviews, among them dyes [9], surfactants [10,11] and pesticides [12,13] and the reader may find more detailed information on specific aspects therein.

2. Chromatographic separation systems

2.1. Reversed-phase HPLC

Coupling of mass spectrometry with reversed-phase liquid chromatography (RPLC) is by far the most important and widely used combination in LC-MS. As the use of LC-MS in water analysis is directed towards polar compounds, the portion of water in the elution system is often high. However, pure water should be avoided, as this extends the time required for equilibrating the column and may suppress the sensitivity of detection. In critical cases post-column addition of organic solvent is used to improve sensitivity.

The selection of inorganic or organic modifiers to increase retardation of polar compounds in RPLC-MS is restricted to volatile compounds. Though API interfaces are now quite robust and can tolerate involatile constituents in considerable amounts, one should still avoid adding involatile modifiers in order to maintain the sensitivity and integrity of the system. The addition of ammonium acetate to increase the ionic strength of the eluent is most common in LC-MS and replaces the hydrogenphosphates traditionally employed in many RPLC methods.

When ionic compounds are to be analyzed, ion-pair formation is often desirable and the modifier selection depends upon the properties of the analytes: if anions are to be detected in the negative mode, ammonium acetate is often added to increase retention with the ammonium cation, likely forming an ion pair with the anionic analyte. For detection of cations in the positive mode, acidification with a volatile acid such as acetic or formic acid or trifluoroacetic acid is advisable.

For more polar ionic compounds these additives may be insufficient. A typical example is the anionic naphthalenesulfonates: with ammonium acetate only monosulfonates are sufficiently retained [14], while triethylamine (TrEA) can be used for slightly more polar compounds [15]. The more hydrophobic tributylamine (TrBA), finally, allows even trisulfonates to be retained [16]. This example illustrates that the lack of hydrophobicity of ionic analytes may be compensated for by using a more hydrophobic ion-pairing agent. However, sufficient volatility of the amine must be ensured.

Ion-pair chromatography with cations to separate and analyze anions is of special importance, as natural ionic compounds tend to be anionic. Moreover, surfaces in aquatic systems tend to be anionic, making it less likely that cationic compounds remain in the aqueous phase for longer periods of time or over longer migration distances, respectively.

Nevertheless, some important classes of xenobiotic compounds are cationic, such as certain pesticides and cationic surfactants. To retard cations in
RPLC volatile anionic ion-pairing agents such as perfluorinated low molecular weight carboxylic acids [17] or perfluorinated fatty acids [18] may be used.

Ion exchange devices (supressor) have been repeatedly positioned between the chromatographic column and the MS interface. They can be used to remove involatile eluent constituents such as tetraalkylammonium ions [19,20] or phosphate [21] or to reduce adduct formation, namely with alkaline cations in the ESI-positive mode [22] or in the negative mode if compounds with several acidic groups are to be analyzed [20]. Some operational problems may occur when using suppressor modules: those with ion exchange columns have generally limited exchange capacity and in membrane systems the membrane may rupture due to the back pressure of the interface [23]. It should be kept in mind that cations can be removed when anions are analyzed and vice versa. Thus, we can either remove cations or anions.

2.2. Ion chromatography

For even more polar ionic compounds of low molecular mass or in cases where ion-pair chromatography is not desirable, ion chromatography (IC) with a weak cation exchanger has been used. A typical field of application is the analysis of small anions such as oxo-anions and volatile organic acids [24] or complexing agents [20]. The post-column use of an ion suppressor may be required to remove non-volatile buffer constituents or to suppress the formation of sodium adducts, that may significantly depress sensitivity [20,23,25,26]. However, IC-MS appears still as a specialty in water analysis rather than as routine practice.

2.3. Capillary electrophoresis

Considerable success has been made in developing capillary electrophoresis (CE)-MS methods in environmental analysis [27]. This is also true for water analysis, where there are a number of publications concerning CE-MS analysis of dyes [28], aromatic sulfonates [29], phenols [30], complexing agents [31] and carboxylic acids [32,33]. Despite this progress, CE-MS is not routine in water analysis. A recent paper concludes that ‘future work to improve stability of the system’ is required [29]. This limited stability may partly be due to the variable electrolyte matrix of waters, namely of effluents, which affects the electroosmotic flow through the capillary and, thus, the analyte separation. More sophisticated clean-up steps may be required to eliminate this disturbance. A second drawback is the generally limited concentration sensitivity of all detection methods coupled to CE owing to the very small sample volumes applied. The mass spectrometer inherently suited to detect narrow electrophoretic peaks is the time-of-flight (TOF)-MS, as it records a spectrum within microseconds [34].

A very potent field of application in which CE is attractive is the separation of enantiomers which is comparatively easily obtained with cyclodextrin systems. This has already been applied to the CE-MS analysis of chiral pesticides [35].

2.4. Size-exclusion chromatography

Size-exclusion chromatography (SEC) is rarely used in environmental analysis and this is even more pronounced for SEC-MS. This technique is well established for polymer analysis [36] and it was, correspondingly, used in the field of water analysis to characterize dissolved organic matter (see Section 3.9) [37,38].

3. Organic analytes

3.1. Dyes

The removal of colored substances from effluents is an important task of wastewater treatment [39] and investigations on the decolorization of textile dyes in spent dyeing and rinsing baths have been going on for a long time. Dyes were among those compounds employed to illustrate the very early applications of electrospray ionization [40,41]. Techniques are now well established [9] and frequently used to analyze dyes in effluents, with special emphasis on sulfonated azo dyes [9,42-44] and dye metabolites formed in various treatments [45-49].

While anion chromatography was used in a very early study [50], RPLC without [42] or with the addition of modifiers such as ammonium acetate [45,46] or phosphoric acid [49] is the standard separation method. Detection of these anionic species is best performed by ESI-MS in the negative mode; however, more in-source fragmentation of the alkali cations was observed when using APCI [43]. A common feature of sulfonated dyes is the
formation of multiply-charged molecular anions with variable numbers of sodium [51]. By adding an alkylamine such as diethylamine to the dye solution, amine adducts can be detected at low cone voltages; their maximum number corresponds to the number of sulfonate groups minus one [52] (Fig. 1). As these adducts dissociate into the sulfonic acid and the amine at elevated cone voltages, the addition of alkylamines to the eluent while analyzing sulfonated dyes helps to suppress the formation of multiply-charged alkali cations [51,52]. This is favorable in terms of sensitivity, clarity of spectra and the fragmentation behavior in collision-induced dissociation (CID), as the alkali cations of sulfonated dyes show only weak fragmentation.

The addition of a volatile amine to the eluent in LC-MS, however, evokes an ion-pairing effect and increases retardation on the reversed-phase column; this corresponds to the chromatographic approach now being used for naphthalene sulfonate analysis (see Section 3.2). Besides the above-mentioned positive effects of alkylamines on the electrospay process, ion-pair chromatography allows the retention and detection of more polar degradation products of dyes [47,48].

Using a triple-quadrupole mass spectrometer, a parent ion scan of \( m/z \) 80 (SO\(_3^-\)) detected all sulfonated dyes present in the mixture analyzed [41]. This fragment is characteristic for all sulfonated aromatic compounds. Cleavage of the azo bond can be induced and helps to confirm dye structures [42]. For those dyes bearing a carboxylate group, decarboxylation can be observed by in-source fragmentation [43] as well as by CID [42].

### 3.2. Aromatic sulfonates

The last thorough review on detection methods available for aromatic sulfonates from aqueous samples dates back to 1995 [53]. At that time HPLC with UV and fluorescence detection was the most commonly used approach, while the use of particle beam-MS [50,54] and ESI-MS (ion-spray) [55] as detectors was documented but not used in practice. Since then the situation has changed drastically and HPLC-ESI-MS has been established as the standard method to analyze various aromatic sulfonates from effluents [15,16,56], surface water and groundwater [14] by either selected ion recording (SIR) [14,15,56] or multiple reaction monitoring (MRM) detection [16].

Common to the extraction and chromatography of polar aromatic sulfonates is the addition of ion-pairing agents, traditionally tetraalkylammonium cations [53]. Due to their virtual involatility and their tendency to form stable anionic clusters with disulfonates [14], these agents are not suited for LC-MS. If ammonium acetate is used instead, only monosulfonates are sufficiently retained [14], while the situation gradually improves with TrEA.
The effect of alkylamines of different alkyl chain lengths was studied in more detail. As illustrated in Fig. 2, TrBA is a much stronger ion-pairing agent, with strong retardation also of disulfonated naphthalenes, even when gradient elution starts with a significant portion of methanol. This is advantageous with respect to sensitivity and analysis time.

As noted above, alkylamines influence the ionization of sulfonates in ESI in that they form clusters which may dissociate at elevated cone voltages. Though we have not detected clusters with TrBA, its concentration significantly influenced the sensitivity of ESI-MS detection. For monosulfonates the sensitivity generally decreases with increasing TrBA concentration in the eluent, while the intensity of the monoanions of di- and trisulfonates initially increases by adding TrBA. It is reasonable to assume that the protonation of one sulfonate group upon dissociation of a TrBA–sulfonate cluster accounts for this effect. A similar effect of alkylamine addition on the sensitivity of ESI-MS detection was recently reported for haloacetic acids. Thus, the concentration of alkylamines should be kept as low as acceptable for the chromatographic retention.

Detection of these and other sulfonates is generally performed by ESI-MS in the negative mode. In SIR the molecular anions are selected for detection, while MRM uses the loss of 64 amu (M-SO₂⁻) or 80 amu (M-SO₃⁻) from the parent anion to detect mono- and disulfonates respectively. The selectivity of MS, namely of MS/MS, allows the use of ion-pair extraction for concentrating aromatic sulfonates from aqueous samples without interferences from humic material, as in UV detection.

Volatile ion-pairing agents are now more frequently used. Recently, TrBA was used to separate sulfophthalic acid and some derivatives, while dibutylamine was used for analyzing bile acids with LC-MS.

### 3.3. Anionic surfactants

Methods to analyze surfactants with LC-MS, namely household detergents and their metabolites, have rapidly emerged. LC-MS provides access to polar metabolites and biodegradation intermediates of surfactants, some of which eluded from previous investigations based on GC-MS after derivatization. Probably, not all findings concerning the pathway and the extent of surfactant biodegradation that have previously been accepted as common knowledge may survive this development.

In the field of anionic surfactants, most work was directed to linear alkylbenzene sulfonates (LAS) as this is still the most widely consumed group of anionic surfactants.

#### 3.3.1. Linear alkylbenzene sulfonates

Several studies on the detection of LAS in raw and treated sewage, together with its biodegradation intermediates, the sulfophenylcarboxylates (SPC), and the byproduct dialkyl tetraline sulfonates (DATS) in laboratory degradation experiments and sewage treatments, surface waters and coastal waters with ESI-MS in the negative mode have been published.

Separation of LAS according to the alkyl chain length by RPLC is straightforward, while retention of SPC requires additives such as TrEA or tetraethylammonium (TEA) acetate. In the latter case a suppressor must be coupled between the LC and the MS to remove this involatile cationic additive. Alternatively, SPC were methylated prior to LC-MS analysis. In all cases, the positional isomers of LAS coelute. Using TrEA or TEA the SPC and LAS can be determined together, with the SPC eluting before the LAS (Fig. 3).

For quantification with a single MS the molecular anions of SPC and LAS are used; at higher cone voltages the styrene-4-sulfonate fragment (m/z 504)
can be detected to confirm the peak assignment \[61,64\]. It may, thus, be necessary to perform two analyses, for confirmation and quantification purposes. Astonishingly, MRM detection has not yet been applied to this task.

The response factors for the molecular anions of the alkyl homologs can vary by a factor of six for LAS and a factor of three for SPC \[64\]. Thus, well-described tenside mixtures and pure SPC alkyl homologs must be available for calibration prior to any quantitative analysis of LAS and SPC by LC-MS. If so, the results of quantitative analyses can be quite comparable in different laboratories \[65\].

Following solid-phase extraction (SPE), limits of detection (LODs) of 3–6 ng/l for LAS and 50–1800 ng/l for SPC from 250 ml sample volumes were reported \[64\]. However, as sensitivity increases LAS determinations at low concentrations can be jeopardized by the ubiquitous presence of LAS in the laboratory environment and, in fact, this may finally determine the LOD.

As surfactants and their biodegradation intermediates and stable metabolites are frequent constituents in raw sewage and treated effluents, methods for the rapid determination of these compounds by flow-injection MS have been developed. For these purposes, tandem MS is essential \[11,59,66\].

### 3.3.2. Others

The potential of LC-ESI-MS to analyze alkylether-sulfates was recognized very early \[67\] and it was applied to raw and treated municipal wastewater as well as to river waters. The analytes were separated by RPLC with ammonium acetate and detected by their molecular anions in SIR with a LOD of 10 ng/l from 200 ml sample volumes extracted. Secondary alkane sulfonates may be determined under similar conditions as the LAS \[65\].

### 3.4. Non-ionic surfactants

In the case of non-ionic surfactants, alcohol ethoxylates (AEO) and alkylphenol ethoxylates (APEO) are most widely used. Due to the lack of a chromophore, AEO were analyzed comparatively early by LC-thermospray-MS \[68\]. Nowadays, ESI and APCI are the ionization methods of choice.

#### 3.4.1. Alkylphenol ethoxylates

However, the most interesting option of LC-MS is the detection of metabolites and biodegradation intermediates of these surfactants. This is of special importance in the case of APEO, since one of its stable biodegradation products, the alkylphenols, have been shown to couple to the estrogen receptor in in vitro tests. Note that the properties of these...
compounds differ widely, from the quite polar parent compounds such as APEO with up to 50 ethoxy units and molecular masses exceeding 2000 AMU [69] (Fig. 4) to the comparatively hydrophobic alkylphenols (AP) and very polar alkylphenol ethoxy monocarboxylates (APEC) and the dicarboxylates of low molecular mass (CAPEC).

Correspondingly, different detection methods are also used: a positive mode in ESI-MS [70]-[73] or APCI [69] is used to analyze for APEO, while its carboxylated biodegradation products (CAPEC and APEC) as well as the alkylphenols are best detected in negative mode ESI by their molecular anions [71]-[73]. As CAPEC bear two carboxylate groups, the (M-2H+Na)$^+$ ion may occur when sodium is present [73]. When using the ESI-positive mode, the sodium adducts of APEO usually dominate; by adding trifluoroacetic acid (TFA) or acetic acid the molecular cations predominate [73], whereas adding ammonium acetate leads to the ammonium adducts [71]. APCI obviates sodium adduct formation [74]. Brominated NPEO, formed in wastewater chlorination processes, are easily detectable due to the characteristic bromine isotope pattern reflected in the molecular anions [72].

In all studies RPLC was used for the separation of APEO and its metabolites. In this mode the homologs coelute and they can only be distinguished by mass spectrometry. However, using normal phase LC a separation of APEO along the ethoxy homologs is achieved [75]. Recently, the use of normal phase separation coupled to APCI-MS was proposed [74]. Intermediate resolution can be obtained by using a C1-reversed-phase column which also provides separation according to the number of ethoxy groups [76].

For MRM detection of NPEO cations an aromatic fragment (m/z 133) and an ethoxylated fragment (m/z 121) may be employed [74]. A characteristic loss of the ethoxylate side chain from the molecular anion of CAPEC provides a specific MRM transition [73].

In other studies, flow-injection analysis (FIA)-MS using parent ion scans was employed to follow the removal of APEO in wastewater treatment plants [11,49]. This very time-saving approach can only provide semi-quantitative data.

To allow for a mass balancing of the microbial removal and metabolism of APEO in sewage treatment works and to provide the data necessary for a risk assessment, reliable quantitation of the parent compounds as well as of their stable metabolites is essential. This is complicated by the fact that technical APEO formulations are mixtures of different alkyl chain structures as well as of ethoxy homologs; moreover, polyethylene glycols (PEG) may remain in the formulation from the production process. As shown for the quantitation of APEO by ESI in the positive mode, the signal intensity of the sodium adducts dramatically decreases with the number of ethoxy groups falling below six [70]. The processes involved in the electrospray ionization of PEG homologs have been described and discussed in detail [5]. The same phenomenon may arise with the metabolites (APEC and CAPEC) but it is not recognized as no pure reference compounds of all relevant ethoxy homologs are available. Thus, we can conclude that most quantitative data provided by LC-MS are accompanied by more or less, and usually unknown, uncertainty.

### 3.4.2. Alcohol ethoxylates

Besides APEO, LC-MS has been used to analyze other ethoxylated non-ionics such as AEO from wastewaters [68,77] and its carboxylated metabolites from degradation experiments [78] first by thermospray-MS and now by either ESI-[77,78] or APCI-MS [77] after RPLC separation. PEG have also been analyzed using ESI in the positive mode [79,80]. Similar to the APEO, the response factors for the AEO ethoxy homologs obtained by detecting the molecular cation strongly increases with the number of ethoxy groups and this dependence differed for different alkyl homologs [77] (Fig. 5).
It is thus essential to have well-analyzed technical mixtures in hand before performing quantitative analysis of any ethoxylates with LC-MS. Recently, a derivatization of AEO prior to the LC-MS analysis was proposed \[81\]: as this puts a permanent positive load onto the analytes, some of response problems are avoided by this approach.

3.4.3. Others

New surfactants based on sugars and fatty alcohols have reached the market in the last years. For two of these groups, the alkylpolyglycosides and alkylglucamide, LC-MS methods have been developed \[82^{–}84\]. The tentative identification of a fluorinated non-ionic surfactant using LC-MS with APCI and ESI was reported \[85\], but no systematic investigation of this group is yet published.

3.5. Complexing agents

The analysis of metal complexing agents such as ethylenediaminotetraacetic acid (EDTA), nitrilotriacetic acid (NTA) or the various aminophosphonic acids, all of which are extremely polar and some of which are poorly biodegradable, is an interesting application of LC-MS. Some methods, specifically for EDTA, have been developed.

EDTA can be analyzed by gradient anion exchange chromatography coupled to ESI-MS \[20\] if a suppressor is coupled between the column and the MS interface for cation exchange. The suppressor avoids the formation of a series of sodium adducts, which are formed in the electrospray process from all polyanionic compounds in the presence of sodium. With this method, free EDTA was detected in µg/l concentrations from directly injected samples together with calcium and iron adducts by the \(m/z\) 291 trace \[20\].

These data show that EDTA–metal complexes can endure the chromatography and they may even survive the electrospray process \[86\] (Fig. 6); this corresponds to other stable complexes (see Section 4.2). Correspondingly ESI-MS can be used to detect a wide variety of metal–EDTA complexes at the 1–2 µM level in the positive mode as molecular cations. Thus, ESI-MS allows EDTA speciation analysis \[86\]. If total EDTA concentrations are to be determined, the free and all complexes species may be converted into one species, Ni–EDTA \[31\]. After extraction the Ni–EDTA complex was determined by CE-ESI-MS \[31\] with a LOD of 0.15 µg/l.

3.6. Drugs and diagnostic agents

The detection and analysis of drugs and their metabolites within biological fluids in pharmacokinetic studies was one of the first applications of LC-MS in the 1980s (i.e. \[87,88\]) and it is still one of the major areas of application. Now that drugs and their environmental fate have attracted the attention of aquatic chemists and the public, methods are being adapted or developed for detecting these compounds in water samples.

Generally, separation of drugs is accomplished by RPLC and detection performed by ESI-MS in the positive mode \[89^{–}92\]. The low concentrations encountered in surface water and treated effluents required enrichment by SPE \[89^{–}91\] or freeze drying \[89\]. In all cases MRM detection from the molecular cations was required to provide sufficient selectivity and to avoid false positive findings.

With this concept a variety of non-ionic drugs \[90\], of sulfonamides \[91\] and other antibiotics \[89\] have been detected in wastewaters and surface waters. Sulfonamides show class characteristic fragments of \(m/z\) 92, 108 and 156 that can be used for parent ion scanning and a substance-specific fragment corresponding to the amide group \[91\]. After the respective enrichment steps, LODs in the ng/l range were obtained for the investigated com-

---

Fig. 5. Dependence of response factors (arbitrary units) on the number of EO groups. The steepest curve was for \(C_{10}^{\text{EO}}\), the shallowest for \(C_{16}^{\text{EO}}\). Reproduced from \[77\] with permission from Elsevier Science.
The aerobic biodegradation of different drugs in batch tests with initial concentrations in the μg/l range was followed after directly injecting samples into an LC-MS system [92]. The number of publications in which LC-MS was applied to drug detection in water is very limited compared to the methods available for drug analysis and their metabolites within other matrices. However, one can expect this number to grow rapidly in the near future.

Methods for the detection of some very polar triiodinated benzenes involved in X-ray diagnostics have been developed, as some of these compounds cause elevated levels of organohalogens (AOX) in treated municipal wastewaters. With these methods triiodinated X-ray contrast agents and some potential metabolites were determined in raw and treated sewage, in surface water, bank filtrate and raw drinking waters [93,94].

Owing to their high polarity ion-pair chromatography [93] or protonation with TFA [94] is required to enhance retention in RPLC. They can be detected by ESI-MS in the positive mode; MRM detection uses the CID fragmentation of the molecular cations by elimination of HI, which is characteristic for aromatic iodinated compounds, as well as the cleavage of side chains [93]. LODs ranging from 10 to 50 ng/l are reported for sample volumes of 1 l extracted. Quantitation may be improved by standard addition [94].

### 3.7. Estrogens and xenoestrogens

Natural and synthetic estrogens are amenable to GC-MS directly [95] or after silylation [96]. Using LC-MS would add the potential to detect estrogen conjugates such as glucuronids and sulfates. The detection of a variety of sterols, among them 17β-
estradiol by LC-MS with ESI and APCI has been systematically studied [97]. In the aquatic environment, natural and synthetic estrogens may be of relevance at concentration levels down to below 1 ng/l and any novel procedure should be able to cope with this concentration.

Methods to detect a set of four estrogens (estrone, 17β-estradiol, estriol and 17α-ethinylestradiol) from raw and treated municipal wastewater were developed [98,99] and extended to a total number of 10 estrogens and progestogens [100]. All studies rely on enrichment by SPE with either carbon [98,99] or C18 [100] and separation by RPLC. Detection methods for the estrogens are quite variable and range from APCI in the positive mode [98] to ESI in the negative mode [99,100]. For the progestogens APCI in the positive mode was used [100]. Considering the high enrichment factors required to achieve sufficiently low detection limits in water, MRM was essential to maintain sufficient selectivity [98,99]. No attempt to analyze the estrogen conjugates has yet been published.

Different mobile phase additives may be selected for the keto-, the keto-enone- and for the hydroxy-sterols to keep highest sensitivity. Using APCI losses of one and two water molecules from the molecular cation were frequently observed, while sodium adducts were most prominent in ESI mode [97].

The LODs reported a range from 2–500 ng/l from 0.5 l of sample using SIR [100] down to 0.5–1 ng/l from 1 l sample volume extracted and detected by MRM [98]. In another study limits of quantification of 80–200 ng/l from 0.4 l by MRM detection were obtained [99]. Analysis of the prominent xenosterrogenic compounds bisphenol A and nonylphenol is treated in the context of phenol analysis (see Section 3.11).

3.8. Haloacetic acids

Haloacetic acids are among the byproducts formed after chlorine disinfection of drinking water. Quality control of drinking waters calls for methods to detect the individual acids in the μg/l range. Several LC-MS approaches have been developed to detect the nine possible chloro- and bromoacetic acids. Liquid chromatographic separation on a polar polymer phase [101] or after ion pairing with dibutylamine [157] was used and the haloacetic acids detected by ESI-MS. Alternatively, a nonaqueous CE-APCI-MS was developed [32]. Some of the methods required extraction of the analytes [32], while others use large volume injection (900 μl) with LODs of about 0.02–0.1 μg/l [157].

Three different approaches have been developed to separate the haloacetic acids from the sample matrix by means other than chromatography: high field asymmetric waveform ion mobility spectrometry (FAIMS) was used as a mass filter and with an ESI-FAIMS-MS system six haloacetic acids were analyzed after direct sample injection with a LOD of about 1 μg/l [102] (Fig. 7). The haloacetic acids have also been coupled with perfluorohexanoic acid to form organic complexes, which are detected in the higher m/z-range with less interferences from the sample matrix [103]. This again makes LC separation in principle superfluous. In this example, liquid–liquid extraction was performed and standard addition had to be used to account for matrix effects [103]. Finally, the high resolution of a TOF-MS allowed the separation of the haloacetic acids from isobaric anionic interferences [104] and the high sensitivity of TOF instruments resulted in a LOD of ca. 50 μg/l by direct injection.

3.9. Natural organic matter

Natural organic matter dissolved (DOM) in water tends to be anionic and high molecular mass com-

![Fig. 7. (a) ESI-MS and (b) ESI-FAIMS-MS spectrum of a haloacetic acid standard solution of the CCl₃⁻ anion (from trichloroacetic acid (TCAA)) and the anion of monobromoacetic acid (MBAA). Reprinted with permission from [102]. Copyright (1999) American Chemical Society.](image-url)
pounds make up a significant portion of it. API-MS should thus be suited to detect and to provide information on this DOM fraction. Considering the important role that dissolved organic matter plays in aquatic environments as well as in several treatment processes a novel technique to reinforce the arsenal of traditional investigation techniques for DOM would be highly welcomed.

First reports concerning the analysis of DOM are now available. ESI-MS was used to analyze groundwater acids [105,106] and a fulvic acid [107] after infusion. The traditional way to separate humic materials is SEC. Consequently, SEC-MS was recently used to characterize humic and fulvic acids [37,38]. The spectra obtained are very complex and difficult to interpret in terms of structural elements (Fig. 8). Owing to the complex nature of DOM, in which compounds with virtually any mass can be found, quasi-continuous spectra with low diagnostic value are obtained in the single MS mode. Average molecular masses may be derived from these spectra, but their appearance strongly depends upon the selected MS conditions [37,38]. Despite the occurrence of multiply-charged ions in ESI-MS, lower average molecular masses were recorded by APCI-MS [38]. More structural information may be obtained by using MS/MS methods (see also [108]).

Natural organic matter, however, does not only consist of humic and fulvic acids. Low molecular weight aromatic and aliphatic carboxylic acids are usually present and can be detected by APCI [109] or ESI [110] in the negative mode from surface and groundwater. Formic acid [110] instead of ammonium acetate [109] as mobile phase additives in RPLC separation resulted in a lower background spectrum, thus allowing for MS detection down to m/z 50 in the scanning mode. Formic acid addition may, however, decrease the sensitivity of the ESI-MS detection [111]. The molecular anion can be detected for all components but aliphatic carboxylic acids show lower sensitivity than aromatic carboxylates due to their lower gas phase acidity [109]. Aromatic di- and tricarboxylic acids and hydroxy acids may exhibit intensive signals corresponding to decarboxylation and elimination of H₂O [48,110]. It was also noted that the relative intensity of the dianion of aliphatic dicarboxylic acids can be influenced by the organic solvent used for chromatography, with the monoanion being favored when acetonitrile is used and the dianion with methanol [112].

Oligosaccharides have also been detected in seawater and sediment porewater as their lithium adducts using LC-ESI-MS in the positive mode [113]. This approach may help to analyze DOM in raw and treated wastewaters.

It is well documented in biological mass spectrometry that ESI-MS can not only be used to analyze large macromolecules, but also to detect organic aggregates of non-covalent interaction ranging up to micelles [114]. Such aggregates not only occur inside biological cells but also in aqueous environment: aggregates with humic acids are believed to facilitate the transport of hydrophobic contaminants through the vadose zone and they may also alter a contaminant’s bioavailability or toxicity. It is therefore of interest to detect and characterize such aggregates by spectrometric means. As in biological applications the ESI-MS may be suited

![Fig. 8. ESI mass spectra of Suwanee River fulvic acid in the (left) positive and (right) negative mode. Reprinted with permission from [115]. Copyright (2000) American Chemical Society.](image-url)
to detect such non-covalent interactions of dissolved species in aqueous samples.

In an alternate approach the interaction of a contaminant with humic acids was detected by SEC-APCI-MS [115]; while SEC was used to fractionate the humic acids, APCI broke down the complexes between the humic acids and the target contaminant, which was then detected by MRM.

Certainly, further method development in both the separation and the mass spectrometric analysis with emphasis on MS/MS methods is required before the potential of LC-MS to analyze natural organic matter and aggregates can be truly evaluated.

### 3.10. Toxins

In recent years it was recognized that toxins generated during blooms of blue-green algae may severely impair the quality of surface waters and their use for humans and other animals. This recently initiated the development of LC-MS methods to determine toxins in water. Meanwhile, methods to detect microcystins by single MS [116] and ion-trap MS [117] and anatoxin-a [18] from water samples were developed.

The cyclic heptapeptidic microcystins were separated by RPLC and detected by ESI-MS in the positive mode [116,117]. For the chromatographic separation of the low molecular mass amine anatoxin-a, ion-pair chromatography with perfluorinated fatty acids was tested and detection performed by ESI-MS in the positive mode. Though sufficient retention and peak shape could be obtained by this approach, the ion pairing suppressed ion formation in the ESI process. Finally derivatization prior to the chromatography was employed and resulted in a LOD of 2 ng/l after SPE of 250 ml of freshwater [18].

Microcystins may be detected as molecular cations [116] and its fragmentation either by the loss of a neutral fragment of 134 AMU [116] or the formation of a fragment of \( m/z 135 \) [117] that characterizes the amino acid (Adda) side chain of the microcystins. Owing to the low concentrations of toxins in water micro-HPLC systems were used by two of the authors [116,117] and coupled to on-line extraction [116].

Further method development for toxin detection by LC-MS from water can be expected in the near future as this application will likely become an important task of water quality control.

### 3.11. Phenols

A series of nitro- and chlorophenols was defined as priority pollutants, for which LC-MS methods for detection from water samples have been developed [118–120].

Following SPE, in some cases on-line [118,119], the phenols are routinely separated by RPLC. Acetic acid addition may enhance chromatographic retention [118,120] but it suppresses sensitivity [119]. APCI and ESI in the negative mode have been used and the molecular anions are obtained as the base peak in all applications. Although the sensitivity of detection using ESI can be significantly enhanced by post-column addition of diethylamine [120], APCI appears to be the more sensitive ionization method for most phenols [120]. Detection limits of 0.02–20 ng injected onto the column [120] and of 0.1–2.5 \( \mu g/l \) after extracting 100 ml of river water [118] were reported. CE-ESI-MS has also been employed to detect a total number of 11 priority phenols [30].

Other phenols have attracted attention in recent years, namely bisphenol A and the octyl- and nonylphenols due to their ability to induce estrogenic effects in a variety of in vitro test systems. Consequently, LC-MS methods for the detection of these compounds appeared with either off-line [121] or on-line SPE [122]. Chromatographic separation and principles of detection correspond to those used for the priority phenols and detection limits fell into the low \( \mu g/l \) range.

These results show that it is feasible to analyze phenols by LC-MS. However, detection limits do not yet compete with those provided by GC. A more impressive and promising potential of LC-MS may lie in the analysis of polyphenols [123] but applications to water analysis have not been reported.

### 3.12. Pesticides

Pesticide residue analysis is perhaps the most frequent application of LC-MS in water analysis (and also in other areas). Correspondingly, the potential of LC-MS for this purpose was recognized early [124] and methods for a wide variety of compounds have been developed [125–127]. Since then, a plethora of methods using LC-MS for virtually all kinds of pesticides has been published. This area has been the subject of several reviews [4,12,13].
Some recent trends in pesticide analysis using LC-MS can be recognized, and are believed to be relevant for future applications in the whole field of water analysis and are therefore outlined here.

As recently pointed out, most pesticide residues found in groundwaters tend to be metabolites of the parent compounds applied [128]. Therefore, LC-MS will become even more attractive in pesticide residue analysis, as polar metabolites are even less amenable to GC-MS analysis. Parallel to this development it is expected that the use of LC-MS in environmental analysis will generally move away from the focus on monitoring of priority pollutants towards an intensified identification and detection of transformation products in the environment, as well as in technical treatment processes.

In the literature on pesticide analysis a clear shift from single MS to MS/MS methods is evident and the transition from SIR to MRM detection appears to be accompanied by decreasing detection limits [129–136].

Another development is the growing awareness of matrix effects that may severely compromise quantitative data generated by LC-MS. Several studies now clearly address this aspect. Experimental evidence for signal suppression by coextracted humic material was obtained [132,133]. The matrix effects may vary considerably from sample to sample and from sampling to sampling [132,133]. A ‘dual precolumn’ extraction with the first used for trapping humic material was shown to reduce the depressive effect of humic materials [132], but it requires analytes that chemically are sufficiently different from the matrix components. Alternatively, an LC–LC coupling prior to MS/MS may be used for an enhanced clean-up [138,139] (Fig. 9).

If the matrix components are not removed, standard addition can be used to compensate for the effects [133]. The obstacles of matrix effects and strategies that have been developed to cope with them are discussed in more detail elsewhere (section 3.3 in [108]).

4. Inorganic analytes

LC-MS has long been a domain of organic analysis but in recent years its potential for inorganic analysis is developing.

4.1. Oxo-anions

Several IC-MS approaches have been developed to analyze oxo-anions such as bromate [25],

![Fig. 9. Scheme of the dual column approach. GP, gradient pump; IP, isocratic pump; IV, injection valve; HV, high-pressure valve; C-1 and C-2, first and second chromatographic column; SP, syringe pump. Reprint from [138] with permission from Elsevier Science.](image)

![Fig. 10. Chromatograms of oxyhalides in suppressed IC mode. (A) Before and (B) after removal of sulfate, chloride and hydrogen carbonate; 1, iodate; 2, bromate; 3, chlorate; and 4, nitrate. Reprint from [26] with permission from Elsevier Science.](image)
together with chlorate, iodate and bromide and chloride [24,26], or methylphosphonates [23]. Recently, methods for the detection of perchlorate from drinking water by FIA-MS with [140] and without extraction [141] have been published. Electro-spray ionization is required for these applications. Detection of the oxo-anions was more sensitive when strong cations from the buffer are exchanged against protons; an increase in sensitivity of a factor of about 10 was achieved [26]. Despite the selectivity of MS detection, bromate analysis in drinking water was hampered by larger amounts of chloride and sulfate, which deteriorated the peak shape and the detector response and a pretreatment of the samples to remove these anions was necessary [24] (Fig. 10).

FIA-MS analysis of perchlorate suffers from the strong chemical noise in this low mass region. The selectivity of MS–MS detection improves the situation [141]. An alternative approach is to couple the anion with an organic cation to detect this complex in the higher mass region with significantly less chemical background [142]. The ion pair, finally, amenable to solvent extraction prior to FIA-MS analysis, which lowers the LOD to 100 ng/l [140].

A variety of metals was detected by FIA-ESI-MS after formation of complexes with organic ligands [143], but this approach was certainly not intended to replace established methods of metal analysis.

4.2. Organometallic complexes

A very interesting option of LC-ESI-MS is to analyze organometallic complexes [144]. Here LC-MS has proven to be a complementary technique to LC ion-coupled plasma MS [145], as it allows identification of the organic compounds masked by the various signals obtained for one element in LC-ICP-MS. This potential has very recently been utilized to analyze organic species of arsenic [146,147] and selenium [148] with either reversed-phase or ion-pair chromatography. LC-MS may also be used [149]. Tributyltin and triphenyltin and related compounds, which have antiestrogenic potential, are amenable to MS [150] and LC-APCI-MS analysis [151–153]. For the di- and tri-substituted tin compounds LODs in the μg/l range were obtained [151,153], but sensitivity of the mono-substituted tin compounds is much lower [153]. More background information is provided in a review of 1996 [154]. Other organometallic species such as zinc pyrithione [155] have also been analyzed by LC-MS.

![Fig. 11. ESI mass spectrum of two series of gallotannins (L and L*) (a) before and (b) after the addition of 400 µM copper nitrate at pH 6, showing the formation of a series of copper(I) complexes. Reprint from [156] with permission from Elsevier Science.](image-url)
While various stable organometallic compounds are also amenable to GC-MS or GC with atomic emission detection, the analysis of complexes of larger and more polar natural organic molecules with metals is an exclusive domain of ESI-MS. This technique was recently employed to analyze metal complexes of tannins [156] (Fig. 11); since these are weak complexes rather than covalently bound structures, each LC eluent system would interfere with the complex formation [156] and a suitable LC separation was difficult to develop.

The limited stability of many organometallic complexes together with their dependency on the water chemistry and their low concentration in natural environments are severe obstacles in investigating these compounds in surface waters. Low concentrations require an enrichment step but this as well as a subsequent chromatographic separation are likely to alter metal speciation. No way out of these problems is presently visible and the future potential of LC-MS in the field of heavy metal speciation in natural waters is thus unclear. Present studies may be restricted to more clearly defined situations with elevated concentrations of both metals and organic ligands.

5. Conclusions

Reviewing the available literature makes it obvious that much has been achieved recently in applying LC-MS to water analysis. A wide variety of polar analytes is readily accessible by LC-MS and the latest methods provide more sensitive and selective procedures. One can expect rapid progress in both the number of compounds for which methods are developed and the sensitivity of detection. With volatile organic additives and by the use of suppressors, LC eluents can be tailor made for introduction into the MS interface.

To date, efforts in water analysis have focused on the detection of parent compounds, while the analysis of metabolites and transformation products is yet limited to only a few compound classes such as some surfactants and pesticides. But LC-MS is ideally suited for the latter task and our understanding of water treatment processes would greatly benefit from this application. With ESI non-covalently bound conjugates of either metals with organics or organics with organics become detectable. This aspect of speciation is of considerable importance in water analysis, but it is yet debatable whether suitable methods for conjugate detection from aquatic samples using LC-ESI-MS can be developed. A similar conclusion can be drawn with respect to the use of LC-MS in characterizing natural organic matter.

While the advantages of LC-MS in analyzing water for organic compounds are already obvious, with inorganic water constituents, its potential as a substitute for existing methods cannot yet be fully evaluated.

The use of LC-MS for qualitative and quantitative analyses of aqueous samples will continue to grow. At the same time, more attention has to be given to quality assurance and control. This is the scope of Part II of this review [108].

References