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Review

Liquid chromatography–mass spectrometry and strategies for trace-level analysis of polar organic pollutants

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Abstract

Liquid chromatography–mass spectrometry using atmospheric pressure ionization (LC–API-MS) has drastically changed the analytical methods used to detect polar pollutants in water. The present status of application of this technique to organic water constituents is reviewed. The selection of the appropriate LC conditions, whether reversed-phase liquid chromatography, ion-pair chromatography, capillary electrophoresis or ion chromatography, and of the most sensitive ionization mode, electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI), depends upon the polarity and acidity of the analytes. Strongly acidic compounds such as aromatic sulfonates, sulfonated dyes, haloacetic acids, linear alkylbenzene sulfonates, aliphatic sulfonates and sulfates and complexing agents, weakly acidic compounds such as carboxylates and phenols, neutral compound classes, namely alkylphenol ethoxylates, alcohol ethoxylates and polycyclic aromatic hydrocarbons and the basic toxins, quaternary ammonium compounds and organometallic compounds are considered. The selection of the mass spectrometer depends upon the analytical task: triple-quadrupole mass spectrometers are highly suited for sensitive quantitation and for qualitative analyses, ion traps are especially suited for structure elucidation, whereas time-of-flight mass spectrometers and quadrupole time-of-flight mass spectrometers with their higher mass resolution are ideal for the determination of molecular formulas of unknown compounds and for screening purposes. While large steps have already been made, future efforts with respect to water analysis may be directed at fine-tuning the methodical arsenal for increased sensitivity and selectivity and to extend LC–MS application to transformation products.

Keywords: Reviews; Water analysis; Atmospheric pressure ionization; Pollutants, polar

Contents

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1. Introduction

The broad instrumental implementation of liquid chromatography–atmospheric pressure ionization mass spectrometry (LC–API-MS) in the 1990s made unforeseen analytical capabilities available for water analysis. With these instruments at hand it became possible to set our sights on highly polar compounds that travel along the water cycle.

Volume 773 of the *Journal of Chromatography A*, published in 1996, was a milestone in this respect. This volume was dedicated to the analysis of organic pollutants in water, and GC after derivatization and HPLC with UV or fluorescence detection were the major detection methods reported for a wide range of water pollutants. Several papers had already outlined the future benefits expected from LC–API-MS in the analysis of polar water constituents. Only two of the papers in Volume 773 of the *Journal of Chromatography A* presented results obtained using this novel technique for the analysis of pesticides [1] and tensides [2]. This situation has changed rapidly and will continue to do so. LC–MS methods for the detection of a wide variety of compound classes in water have been developed.

Numerous excellent reviews and books accompanied the development of LC–MS and cover the interface techniques [3–6], the processes underlying ion generation and transfer into the gas phase, namely the electrospray process [7–9], as well as the particulars of the mass measuring methods [10]. These reviews provide a deeper understanding of the technique and will, thereby, help to solve application problems.

With respect to water contaminants, several compound classes have been the subject of specific reviews, among them dyes [11], surfactants [12,13] and pesticides [14,15]. A first attempt to summarize the achievements and obstacles of LC–MS in water analysis for a broad range of compounds was made two years ago [16,17]. The last two years have, again, seen rapid development, and several fields of application are now a matter of separate review in this volume of the *Journal of Chromatography A*, such as endocrinically active compounds [18]. Also, for pesticides, a deep and comprehensive review appeared recently [19], therefore these compound classes are not explicitly considered here.

This review covers a wide range of compound classes and in summarizing the procedures developed

to analyze these compound classes by LC–MS, we outline some common principles that have evolved in the analytical methods for trace organics in water and that may be useful for the future extension of LC–MS to new compound classes.

Although the detection limit can indicate the suitability of an LC–MS method for trace analysis of contaminants, detection limits are only rarely reported in this review. This is due to the fact that the criteria used for setting the detection limit are highly variable in the literature and that detection limits are continuously decreasing with the new generations of mass spectrometers.

We are becoming increasingly aware that the analysis of aqueous samples for polar microcontaminants must not be restricted to the compounds initially used and released into the aquatic environment. Rather, polar metabolites may be generated that occur in higher concentrations and frequency than their parent compound. Thus, this review covers equally the primary pollutants and their transformation products, whenever possible. The use of LC–MS in environmental analysis is moving away from a focus on the monitoring of priority pollutants towards an intensified identification and detection of transformation products. It will be shown that today's LC–MS instruments and the methods already established are powerful tools to proceed in this direction.

2. Initial considerations

Optimizing a LC–API-MS combination for a certain analytical task requires making the correct choice for each of the three components, the LC, the API and the MS. Moreover, one has to take into consideration that the selection of one component cannot be made independently from the other two components of the system. Based on the available experience with the use of LC–API-MS for different kinds of analytes and different analytical tasks, this section outlines general guidelines for selecting an appropriate combination of LC, API and MS.

Obviously, the selection of two of the components, the LC and the API mode, is determined by the physico-chemical properties of the analytes of interest, their polarity and acidity. The selection of the third component, the most suitable MS detector, should be made by considering the question the analyst has and the answer he would like to obtain.

2.1. Ionization mode

Most API mass spectrometers offer two interfaces, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), both of which can be operated in positive and negative ion mode. Often, an appropriate selection for a given analyte can be made by considering that ESI transfers ions from solution into the gas phase, whereas APCI ionizes in the gas phase. As a rule of thumb, analytes occurring as ions in solution may be best analyzed by ESI, while non-ionic analytes may be well suited for APCI. Thurman and co-workers [20] attempted to rationalize the selection of either ESI or APCI and, based on a comparison of 75 pesticides, they developed a so-called ionization continuum diagram (Fig. 1). This diagram nicely illustrates the relationship between compound acidity and appropriate

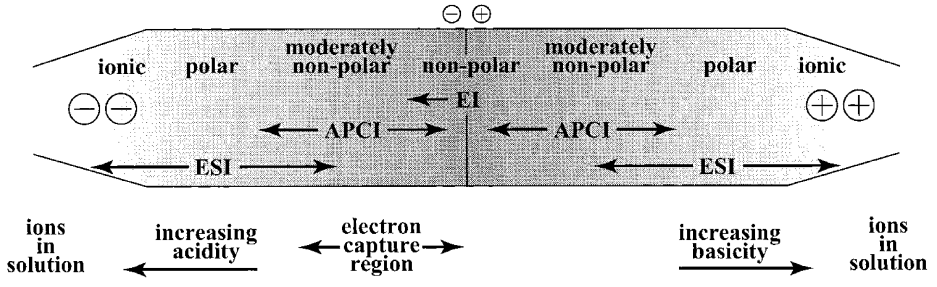

Fig. 1. Ionization continuum diagram of Thurman and co-workers, showing the interrelationship between analyte properties and the appropriate API mode. Reproduced, with permission, from Ref. [20]. 2001 ©American Chemical Society.

ionization modes. Only recently, a third API interface became commercially available, the atmospheric pressure photoionization interface (APPI) [21,22]. This may be suited for non-ionic compounds, for which APCI is proposed in Fig. 1.

The selection of either positive or negative ionization mode may have a strong impact on the signal intensity, the extent of adduct formation and the chemical noise encountered. Thus, the mode appearing best suited in the beginning need not necessarily be the most sensitive mode at the end. For amphoteric analytes the ionization mode may also influence the fragmentation occurring upon collision-induced dissociation (CID) when using MS–MS, as different molecular regions are ionized and sensitized for a subsequent fragmentation, depending on whether positive or negative ion mode was used.

2.2. Chromatography

Contrary to the selection of the ionization mode, elaboration of the appropriate LC conditions must consider the properties of the whole analyte molecule. A diagram that gives an overview of the chromatographic techniques applied in LC–MS similar to the ionization continuum diagram (Fig. 1) has to consider the acidity of certain functional groups and the polarity of the whole molecule (Fig. 2).

Reversed-phase liquid chromatography (RPLC) is by far the most important and widely used chromatographic technique in LC–MS. As the use of LC–MS in water analysis is directed towards polar compounds, the proportion of water in the elution system is often high. Retardation of weakly acidic or basic compounds may be improved by suppressing their dissociation either by adding a volatile acid or a volatile base (ammonium acetate). For very acidic or basic compounds the formation of ion-pairs with organic counter-ions is often desirable to increase retardation in RPLC (Fig. 2). Counter-ions of increasing hydrophobicity should be used to increase the retardation of analyte ions of increasing polarity. However, the counter-ions must be sufficiently volatile to avoid deposition in the interface.

Ion-pairing for acidic compounds is of particular importance, as many acidic compound classes occur in the aquatic environment. The ammonium cation is the weakest ion-pairing agent and is provided as ammonium acetate. When it becomes too weak, tri- or dialkylamines may be used for acidic compounds. For the rare cationic compound classes, volatile perfluorinated organic acids can be used as organic counter-ions [23–26].

It is a common phenomenon of coupled systems that some freedom of choice is lost. In the case of LC–API-MS the selection of an organic or inorganic modifier may substantially influence the ionization process in the API interface. For example, suppressing the dissociation of ionic molecules for enhanced chromatographic retention may reduce the sensitivity of ESI-MS detection. However, this is difficult to predict, since the pH at the surface of an electrospray droplet may differ significantly from the pH in the eluent solution [20,21].

For very 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 volatile organic acids [27] or complexing agents [28]. However, IC-MS is still a specialty in water analysis rather than routine practice. The post-column use of a suppressor (ion-

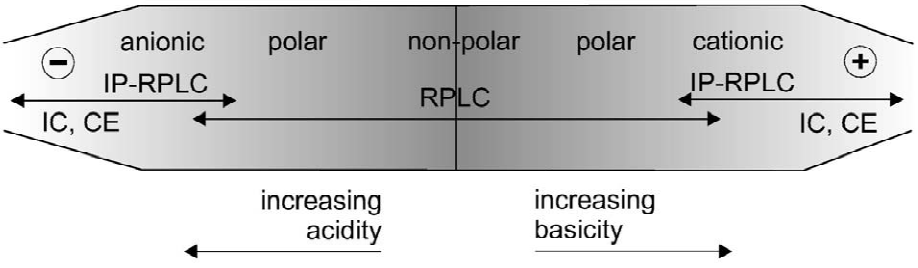

Fig. 2. Diagram showing the interrelationship between analyte properties and the appropriate chromatographic separation method.

exchange device) may be required to remove non-volatile ionic eluent constituents or to suppress undesirable adduct formation [28–31].

Another separation technique for ionic analytes that can be coupled to MS is capillary electrophoresis (CE) [32]. A number of publications on the CE–MS analysis of dyes [33], aromatic sulfonates [34], phenols [35], complexing agents [36] and carboxylic acids [37,38] have been published. Nevertheless, CE–MS has not found recognition in the practice of water analysis.

A combination of Figs. 1 and 2 may provide rapid access to an initial LC–API combination that may be suited to begin method development.

2.3. Mass spectrometry

As outlined above, the selection of a mass spectrometer should depend upon the question to be answered. However, the selection of the MS detector is often driven by availability.

A *quantitative analysis* of target compounds requires selective and sensitive detection over a wide concentration range. Quadrupole mass spectrometers, namely triple-quadrupole mass spectrometers with their ability to perform multiple reaction monitoring, are best suited in this respect. Their large dynamic range is also favorable for quantitative analyses.

The situation is more complicated when *qualitative analysis* has to be performed, either in identifying a certain chromatographic signal or detecting a larger number of previously undetected compounds (screening). For detecting and identifying unknown water constituents, triple-quadrupole mass spectrometers provide different options, the product ion, the parent ion and the neutral loss mode. However, MS–MS fragmentation may be limited and insufficient to perform full structure elucidation. Moreover, the mass resolution of quadrupole MS is not usually high enough to allow for the determination of the molecular formula of an unknown compound. With some additional effort in tuning and calibration the widely distributed quadrupole mass spectrometers can also be used to derive exact masses in LC–MS as a basis for molecular formula calculation [39]. Ion-trap mass spectrometers with their high sensitivity in the scanning mode and the ability to perform MS^n experiments are well suited for many identification purposes. However, their resolution is comparable to the quadrupoles.

Higher resolution for identification purposes is more readily provided by the orthogonal accelerating time-of-flight (oaTOF) mass spectrometers. Their resolving power may be high enough to provide a molecular formula and to confirm or deny a suggested structure [40,41]. Moreover, the ability of a TOF-MS to provide full scan spectra together with high sensitivity makes this instrument an interesting choice for qualitative analysis. Even more useful in terms of qualitative analysis is a quadrupole-TOF combination (qTOF) as it allows MS–MS experiments to be performed, providing more structural information, and the selection of a parent ion to be analyzed by TOF-MS, which adds selectivity. When CE is used for separation and very narrow peaks are obtained, TOF-MS is the ideal detector since these instruments allow full scans to be performed in microseconds [42].

Moreover, TOF instruments are well suited for screening purposes. It has been shown that the higher mass resolution provided by TOF-MS allows the unambiguous detection of certain pesticides in river water, even when they are accompanied by isobaric compounds [40]. Even more resolving power is provided by Fourier transform ion cyclotron resonance (FTICR) mass spectrometers, but these instruments are usually out of reach for environmental applications.

3. Strongly acidic compounds

3.1. Aromatic sulfonates

Because of their acidity, the extraction and chromatography of polar aromatic sulfonates are usually based on ion-pair formation, traditionally with tetraalkylammonium cations [43]. However, tetraalkylammonium cations are not suited for LC–MS coupling, as they are virtually involatile and tend to form adducts that complicate MS detection [44]. If ammonium acetate is used as a volatile ion-pairing agent, only monosulfonates are sufficiently retained [44]. Retention of these and other strongly acidic and polar analytes can be fine tuned by selecting di- or trialkylamines with an appropriate number of ali-

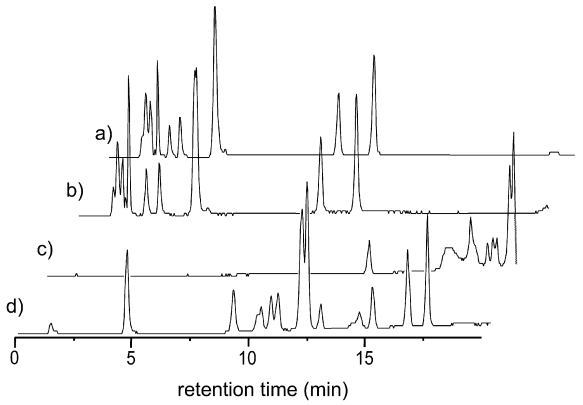

Fig. 3. Retention of naphthalene sulfonates in RPLC with an eluent of 10% methanol and 2.5 m*M* of different ion-pairing agents: (a) triethylamine, (b) dimethylbutylamine, (c) tributylamine and (d) tributylamine with 30% methanol. Reprinted from Ref. [16], with permission from Elsevier Science.

phatic carbons (Fig. 3). Triethylamine can be used for monosulfonated naphthalenes [45], whereas strong retention even of trisulfonated naphthalenes has been obtained with tributylamine (TrBA) [46]. Recently, the dihexylammonium cation (with the same number of aliphatic carbons as TrBA) has been used for this purpose [47]. IP-RPLC with TrBA is also suited for the LC–MS analysis of the degradation products of dyes [48]. However, the alkylamines used for ion-pairing need to be volatile, which limits the number of carbons they can bear.

Contrary to the tetraalkylammonium counter-ions the di- and trialkylamines do not tend to form adducts in modern API interfaces. Instead, they can act as H donors in their ammonium form and may then influence the ionization process of sulfonates in ESI [49] by decreasing the sensitivity of detection [46]. In the same way, these amines diminish the risk of sodium adduct formation and of multiple charging. Due to their suppression effect, the concentration of alkylamines should be kept as low as acceptable for chromatographic retention.

The detection of strongly acidic sulfonates is performed by ESI-MS in the negative ion mode. In SIR the molecular anions or, in the case of polysulfonated compounds, the dianions are selected for detection, while MRM uses a loss of 64 amu [$M-SO_2$] or 80 amu [$M-SO_3$] from the parent anions [46]. It is still unclear which structural elements govern whether SO_2 or SO_3 is preferentially eliminated. The selectivity of MS, especially of MS–MS, allows the use of ion-pair extraction for the enrichment of aromatic sulfonates from aqueous samples without interference from humic material, as in UV detection [50].

Sulfonated naphthalene formaldehyde condensates (SNFC) are another important class of aromatic sulfonates released into the environment and IP-RPLC with tetraalkylammonium cations and fluorescence detection is a sensitive method to detect these compounds [51]. No convincing LC–MS method with volatile alkylamines has yet been developed, as problems occur in the chromatographic and mass spectrometric separation [52].

3.2. Sulfonated dyes

Sulfonated dyes were among the first compounds to illustrate the benefits of ESI-MS [53,54]. The analysis of dyes by LC–MS is a mature field (reviewed, for example, in Ref. [11]) and is frequently used to analyse dyes with special emphasis on sulfonated azo dyes [11,47,55–57].

While anion chromatography was used in a very early study [58] and CE has also been employed [33], RPLC with the addition of modifiers such as ammonium acetate [56,59,60] or acid [55,61] is the standard separation method for sulfonated azo dyes. Polysulfonated dyes may become too hydrophilic for conventional RPLC and IP-RPLC may be required [47].

Detection of these anionic species is best performed by ESI-MS in the negative ion mode. A common feature of polysulfonated dyes is the formation of multiply charged molecular anions with variable numbers of sodium [49]. The addition of di- or trialkylammonium cations to the eluent helps to suppress the formation of multiply charged alkali cations [49,62]. This is favorable in terms of sensitivity, clarity of the 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.

Degradation products and by-products of dyes are of lower molecular mass and more polar than the parent compounds. Thus, ion-pairing is mandatory to enhance retention of these compounds. Dye metabolites formed from azo dyes [39,60] or sulfonated phthalocyanine dyes [48] have been analyzed by IP-RPLC.

Using a triple-quadrupole mass spectrometer, a parent ion scan of m/z 80 (SO_3^-) allows the detection of all sulfonated dyes present in the mixture analyzed [54]. The intensity of this fragment may, however, be low [63]. Cleavage of the azo bond can be induced and helps to confirm the dye structures [55]. A recent study reported that *ortho*-hydroxy azo dyes that are subjected to a hydrazo–azo tautomery have two fragmentation pathways for the azo bond [63]. The azo form is preferentially split at the C–N bond, while homolytic cleavage of the azo bond occurs for the hydrazo form of these dyes (Fig. 4). For those dyes also bearing a carboxylate group, decarboxylation is observed by in-source fragmentation [56] as well as by CID [55].

3.3. Haloacetic acids

As haloacetic acids (HAAs) are very acidic, with pK_a values of 0.7–2.9, the chromatographic retention and separation of the nine possible chloro- and bromoacetic acids in RPLC cannot be obtained by an acidified eluent. Instead, ion-pairing with dibutylamine [5] or triethylamine [64] needs to be used and ESI in negative ion mode is best suited for detection. As mentioned above, an organic cation may decrease the sensitivity of detection [5]. Retention can also be achieved on a polar polymer phase [65]. With increasing halogenation, the decarboxylation of HAA becomes increasingly facile; thus, decarboxylated anions may become the base peak when analyzing trihalogenated acids [66,67]. As an alternative to LC–ESI-MS, a non-aqueous CE–APCI-MS procedure has been developed [37].

Three different approaches have been developed to separate HAAs from the sample matrix by means other than chromatography: flow-injection ESI-MS was used to determine HAA adducts with per-

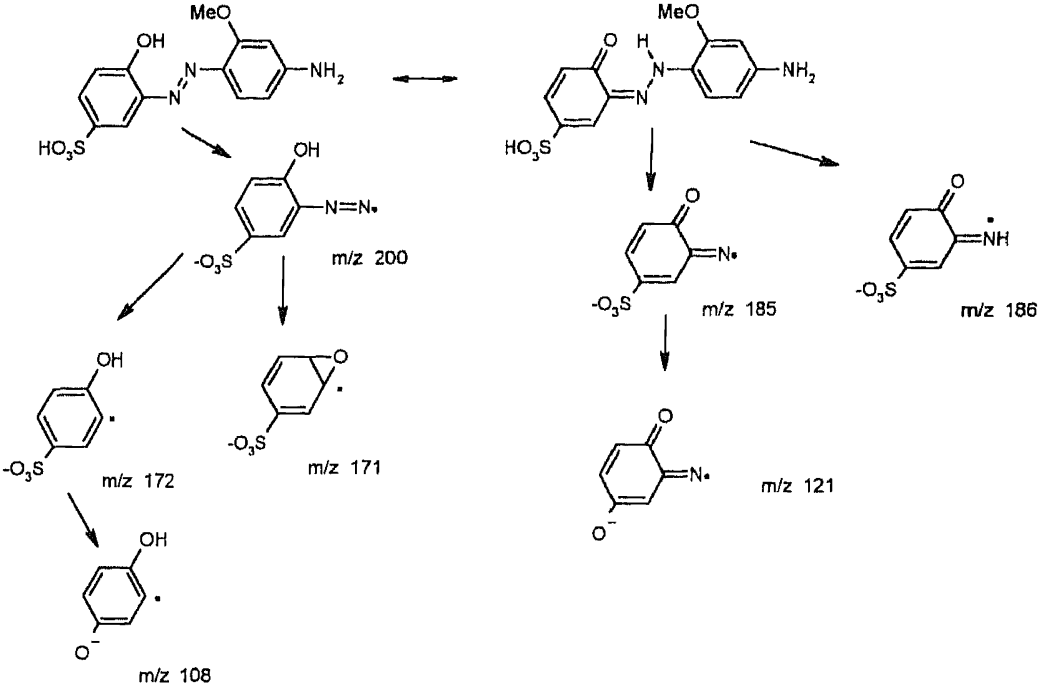

Fig. 4. Scheme of fragment formation from the azo (left) and the hydrazo (right) form of a model dye. Reproduced, with permission, from Ref. [63]. 1998 ©John Wiley and Sons Limited.

fluoroheptanoic acid. These adducts are detected in the higher m/z range with less interferences from the sample matrix [68]. Liquid–liquid extraction was performed and standard addition had to be used to account for matrix effects [68]. Separation of HAAs from isobaric anionic interferences can also be accomplished by the increased resolution of a time-of-flight (TOF) MS [69], but LODs were not sufficient for the direct analysis of drinking waters. The most sensitive approach appears to be ESI high field asymmetric waveform ion mobility spectrometry MS (ESI-FAIMS–MS). In this instrumental configuration the analytes are effectively separated in the drift region of the IMS and all nine haloacetic acids were detected with LODs of 5–40 ng/L without any preconcentration [67]. Compared with a previous study of the same group [70], this corresponds to a sensitivity increase of about two orders of magnitude. However, these kinds of instruments are not commercially available.

3.4. Linear alkylbenzene sulfonates

Methods to analyze anionic 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 escaped from previous investigations based on GC–MS after derivatization.

Most work has been directed towards linear alkylbenzene sulfonates (LAS), as this is still the most widely consumed group of anionic surfactants. Several studies on the detection of LAS in raw and treated sewage [71] have been published, together with their biodegradation intermediates, the sulfophenyl carboxylates (SPC), and the by-products, dialkyl tetraline sulfonates, in laboratory degradation experiments [72,73], and these compounds have also been detected in sewage treatment [74], in surface waters [75] and in coastal waters [76]. As with all sulfonates, ESI-MS detection in the negative ion mode is most suitable.

Although the sulfonate group of LAS is quite acidic, the long hydrophobic alkyl chain provides sufficient retention in RPLC so that IP-RPLC need not be used. In RPLC, LAS mixtures are separated according to their alkyl chain length [72,75]. However, the more polar carboxylated degradation products, the sulfophenyl carboxylates (SPC), require additives such as triethylamine (TrEA) [75–77] or tetraethylammonium (TEA) acetate [73] for sufficient retention (Fig. 5). In the latter case, a suppressor must be coupled between the LC and the MS to remove this involatile cationic additive [73]. IP-RPLC with TrEA is also applicable for the analysis of branched alkylbenzene sulfonates [78]. Alternatively, SPC can be methylated to reduce their polarity prior to LC–MS analysis [72]. In all cases, the positional isomers of LAS coelute. Using TrEA [76,77] or TEA [73], SPC and LAS can be analyzed together, with the SPC eluting before the LAS (Fig. 5).

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 183) can be detected to confirm the peak assignment [73,76]. 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 homologues can vary by a factor of 6 for LAS and a factor of 3 for SPC [76]. Thus, well-described tenside mixtures and pure SPC alkyl homologues must be available for calibration prior to any quantitative analysis of LAS and SPC by LC–MS.

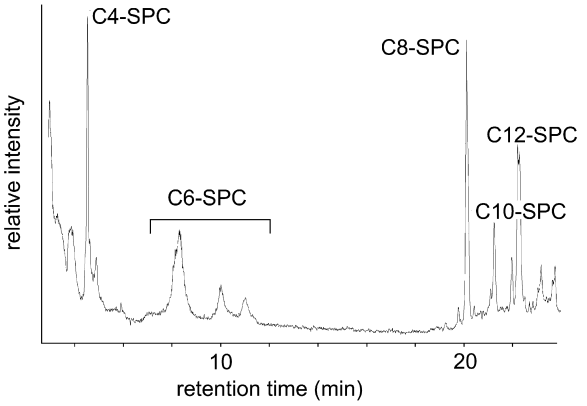

Fig. 5. Separation of sulfophenyl carboxylates (SPC) by IP-RPLC and ESI-MS detection in the negative ion mode. The LAS elute at 27.5 min (time window not shown). Reprinted, with permission, from Ref. [77]. ©SETAC, Pensacola, FL, USA.

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 [13,71,79], but only semi-quantitative results can be obtained by flow-injection MS, as the unresolved matrix can interfere with the ionization of the analytes of interest.

3.5. Aliphatic sulfonates and sulfates

The potential of LC–ESI-MS to analyze alkylether sulfates was recognized very early [80] 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. Secondary alkane sulfonates may be analyzed under similar conditions as the LAS [81].

For alkylphenol ethoxysulfonates mixed-mode RP/anion-exchange chromatography with ammonium acetate as buffer has been used, which provided separation according to the number of ethoxylate units [82]. Extraction from seawater was performed by graphitized carbon cartridges. Surprisingly, ESI in the positive ion mode was used and no comparison was made with the negative ion mode [82].

Ethane sulfonates may be formed from chloracetanilide herbicides in soil if enzymatic activation proceeds via glutathione. As these compounds are more polar and, thus, more mobile in the soil/water system than the respective parent herbicide, ethanesulfonates are more frequently found in groundwaters [83]. The use of ESI in the negative ion mode is clear from the acidity of the sulfonate group, but as these ethanesulfonates are less polar than the aromatic sulfonates considered above, ion-pairing is not required. Instead, conventional RPLC with an acidified eluent may be used [84,85].

3.6. Complexing agents

Complexing agents such as ethylenediaminotetraacetic acid (EDTA) are strongly acidic compounds and chromatographic retention is thus not an easy task and fully satisfying methods are not within reach. Due to its complexing capacity, a variable amount of EDTA in a sample occurs complexed with various dissolved cations. Changing the ionic strength or the pH of an aqueous sample may alter the speciation of EDTA in solution.

For qualitative analysis, aqueous samples may be infused into an ESI-MS, as many EDTA–metal complexes survive the electrospray process [86] (Fig. 6). Indeed, infusion ESI-MS may be the only procedure allowing for the detection of unaltered EDTA speciation. Chromatographic separation of metal–EDTA complexes can be achieved by anion-exchange chromatography [28,87] but due to the chemical differences between the eluent and the aqueous sample the speciation of EDTA may be substantially altered and weak complexes may be destroyed [87]. The use of a suppressor (cation exchanger) post-column strongly improves the detection limits [87] and avoids the formation of sodium adducts in the interface [28], but again this may disintegrate many of the metal–EDTA complexes. Electrospray ionization is indicated, either in the negative mode to detect the monoanions of the complexes [36,87], or free EDTA [28] or protonated species in the positive mode [86].

If the total EDTA concentration is to be determined, the free and all complexed species may be converted into one species, e.g. Ni–EDTA [36]. After extraction, the Ni–EDTA complex can be determined by CE–ESI-MS–MS by monitoring the loss of water from the monoanion of the Ni–EDTA complex (m/z 347>329) [36]. For other chelating agents such as nitrilotriacetic acid (NTA) and aminophosphonic acids, LC–MS methods have not yet been developed.

4. Weakly acidic compounds

4.1. Carboxylates

There is a wide range of natural carboxylic acids that may be detected in aqueous samples, such as resin acids [88] and small carboxylic acids [38]. Important anthropogenic carboxylates are acidic pesticides such as chlorophenoxy acids [89] and a variety of acidic pharmaceuticals [90,91].

Moreover, carboxylic acids occur as intermediates

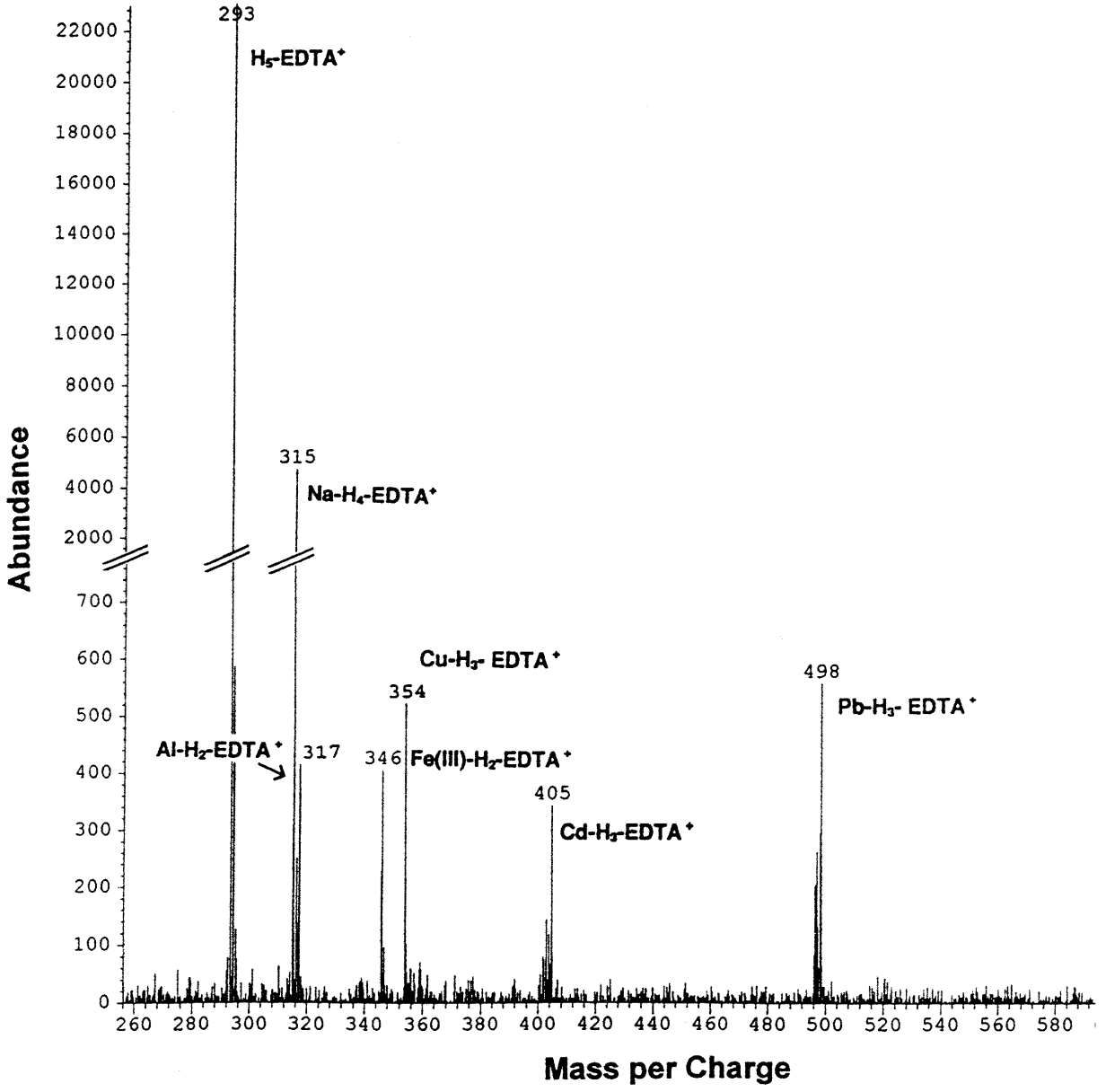

Fig. 6. Positive ion mode ESI spectrum of a Na_2EDTA solution containing Al, Fe(III), Cu, Cd and Pb ions. Reproduced from Ref. [86], with permission from the American Society of Agronomy.

in the degradation of many compound classes. For example, SPC are formed from LAS (Section 3.4), and alkylphenol ethoxycarboxylates (APEC) and dicarboxylates (CAPEC) are formed from non-ionic alkylphenol ethoxylates (Section 5.1). Aromatic carboxylic acids are formed in the aerobic degradation of polycyclic aromatic hydrocarbons (PAHs) (Section 5.4), and oxanilic acid derivatives can be generated microbially from chloracetanilide herbicides [84,85] and succinates in the anaerobic degradation of BTX compounds [92]. The introduction of a carboxylic acid moiety into the parent

molecule renders the compound more hydrophilic, and the potential of LC–MS to detect, identify and quantify such degradation products is one of the major achievements in water analysis.

Most of the degradation products mentioned above are considered in the section dedicated to their parent compound but, nevertheless, some principles are outlined here that are common to all these carboxylates. Carboxylates may cover a wide range of polarity, from moderately polar resin acids to very polar low-molecular-mass carboxylic acids. Chromatographic separation methods for carboxylates thus also vary. For the less or moderately polar classes such as resin acids [88], naphthalene carboxylates [93], chlorophenoxy acids [89], oxanilic acid derivatives of herbicides [84,85], acidic pharmaceuticals [90,91] or succinates [94], the addition of ammonium acetate or formic or acetic acid as modifier is sufficient to attain retention in RPLC. The more polar classes such as bile acid conjugates require the addition of an organic counter-ion such as dibutylamine to perform IP-RPLC [95]. The highly polar low-molecular-mass carboxylates may be separated by CE [38] or IC [27].

Due to their acidity, ESI-MS in negative ion mode is applied to most of these carboxylates [38,84,85,88–91,93,94]. In the case of phenolic acids, APCI in the negative ion mode can be used [96].

4.2. Phenols

Several phenols, namely nitro- and chlorophenols, have been classified as priority pollutants and this has promoted the development of LC–MS methods for these contaminants [97–99]. In addition, phenolic compounds are generated in the degradation of many aromatic hydrocarbons (hydrolylated PAH) and they occur as plant polyphenols and steroids.

Following SPE, in some cases on-line [97,98], the phenols are routinely separated by RPLC. For the more polar and more acidic dinitrophenols, chromatographic retention may become poor. The addition of acetic acid enhances retention [97,99], but it suppresses the sensitivity of detection [98,100]. Thus, any inorganic additive in the eluent system should be avoided. ESI was found to be superior for the detection of the most acidic phenols, the dinitrophenols and pentachlorophenol [100], while for the majority of priority phenols, APCI was more sensitive [99,100]. The sensitivity of detection using ESI may be enhanced by the post-column addition of diethylamine [99].

CE–ESI-MS has also been employed to detect a total number of 11 priority phenols [35]. For the analysis of the more polar and more acidic trinitrophenols, ion-pairing with TrBA is required to enhance retention in RPLC. Due to their acidity, ESI is superior to APCI [101].

Bisphenol A and the octyl- and nonylphenols have attracted attention in recent years, due to their estrogenic properties. Consequently, LC–MS methods for the detection of these compounds have appeared with either off-line [102] or on-line SPE [103]. Similar to the less acidic priority phenols, RPLC with APCI-MS is used. The same approach is also suited to the analysis of chlorinated bisphenol derivatives [104].

Plant phenols are ubiquitous in nature and LC–MS is highly suited for the analysis of these compounds [105,106]. In a very detailed study it was shown that flavonoids are detected most sensitively by ESI-MS in the negative ionization mode with ammonium acetate added to the eluent [21]. But since flavonoids approach the non-ionic compounds discussed below, APCI can also be used and positive as well as negative ionization modes are applicable [21]. Recently, the application of LC–MS for the detection of flavonoid monomers and oligomers as well as of hydroxyphenolic acids and their glycosides in wastewater has been reported [107]. MRM helped to distinguish between the oligomers that could not be resolved chromatographically (Fig. 7).

Recently, the occurrence of steroid estrogens in wastewater and surface waters has attracted attention. All these compounds are of limited polarity and only weakly acidic. Retention in, and separation by, RPLC is not difficult to achieve. Separation of the more polar conjugates such as glucuronides and sulfates has been performed with TEA as ion-pairing agent [108]. ESI- and APCI-MS may be used for detection, but sensitivities remain limited. When using the positive ion mode in ESI, sodium adducts are primarily detected [109], whereas the molecular anions are detected in the negative ion mode [110–113]. When detection is based on the sodium ad-

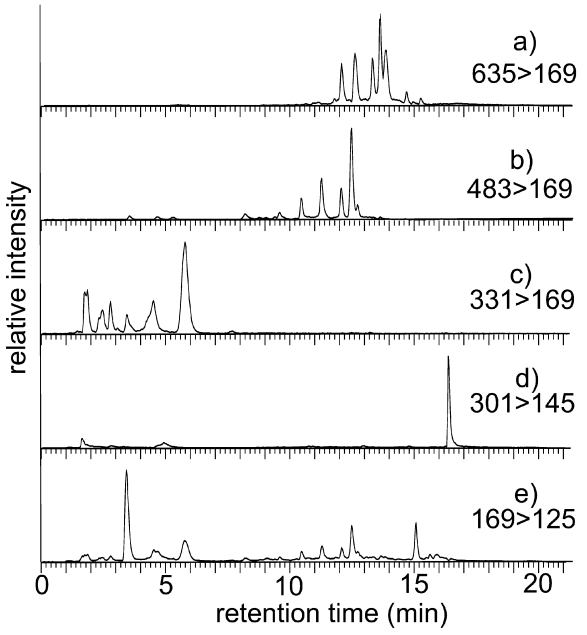

Fig. 7. Chromatograms and transitions used for the MRM detection of chestnut tannins: (a) trigalloyl glucose, (b) digalloyl glucose, (c) monogalloyl glucose, and the monomers (d) elagic acid and (e) gallic acid. Redrawn from Ref. [107].

ducts, as in ESI positive mode, the use of proton donors such as acetic acid or ammonium acetate as mobile phase additives may strongly decrease the sensitivity of detection for many steroids [109].

All studies rely on enrichment by SPE with either carbon [112,114], C_{18} [111,113] or polymeric phases [108]. Considering the high enrichment factors required to achieve sufficiently low detection limits in water, MRM is essential to maintain sufficient selectivity [108,112,114]. This is less critical when immunoaffinity extraction is used [110].

5. Neutral compounds

5.1. Alkylphenol ethoxylates

Due to concern about the potential endocrine effects of APEO degradation products, interest in sensitive analytical procedures for APEO has drastically increased. Because technical APEO products consist of a mixture of ethoxy homologues and alkyl isomers, their quantitative analysis is quite challenging.

RPLC has mostly been used for the LC–MS analysis of APEO. Since the hydrophobic part of APEO is identical for all components, all homologues often coelute in RPLC separation [115–117] and distinction between homologues is only made by means of MS detection. This coelution has often been claimed to be an advantage, since it would increase the sensitivity of detection. As a matter of fact, quantitation is severely compromised by coelution, because the response factors of the homologues vary substantially, with a notoriously poor sensitivity for the monoethoxylate (e.g. NP1EO).

Separation according to the number of ethoxy units has been obtained on a C_8 column [118]. Considerable progress was recently achieved by using so-called mixed-mode chromatography, which makes use of size-exclusion and reversed-phase mechanisms for a full resolution of the homologues [119]. The higher homologues, which are more polar and have a large molecular mass, elute before the lower homologues (Fig. 8a). Chromatographic separation of the homologues allows selection of the optimal cone voltage for each of the homologues and, thus, increased sensitivity (Fig. 8b) [119]. Classical normal phase chromatography can also be used, but it requires long equilibration times [120,121] and a polar modifier may have to be added post-column to support ionization of APEO [121].

Because of the non-ionic character of APEO, one would expect APCI to be most suited [122]. Instead, ESI is the most frequently used ionization technique [115,123–125], based on the strong affinity of polyethoxylates for coordination with sodium cations. This formation of (APEO–Na) cations can be viewed as derivatization of these non-ionic compounds, which makes them well suited for ESI-MS detection in the positive ion mode. However, a decreasing number of ethoxy groups per molecule reduces the affinity for alkaline cations and results in decreasing sensitivity for the lower APEO homologues.

If the more selective MRM detection is to be used, it is advisable to suppress the formation of sodium adducts, as these fragment poorly. Instead, ammonium adducts formed by adding ammonium acetate [118,124] are well suited for MRM detection as they

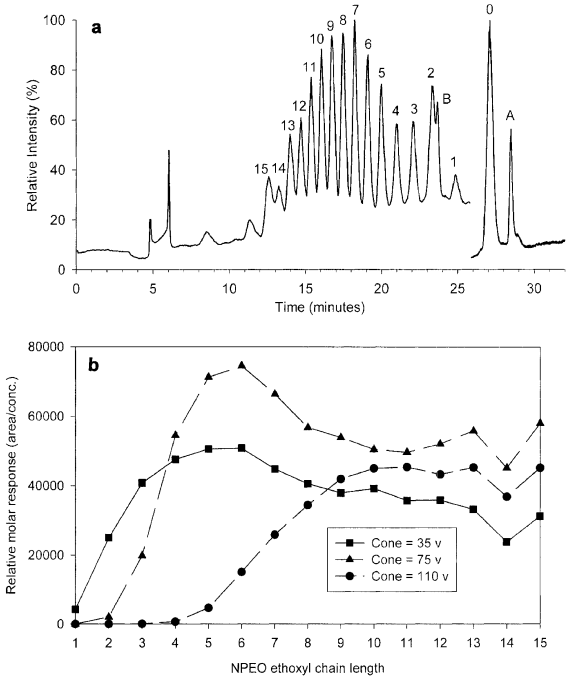

Fig. 8. (a) Mixed-mode separation of a NPEO mixture based on size-exclusion and reversed-phase mechanisms with ESI-MS detection. Peak numbering corresponds to the number of ethoxy groups; A, B: internal standards n-NP and n-NP3EO; 0–25 min, negative ion mode; 25–33 min, positive ion mode. (b) Relative response factors of NPEO homologues at three different cone voltages. Reprinted from Ref. [119], with permission from Elsevier Science.

fragment easily. Using this detection mode provides markedly decreased detection limits compared with the SIR detection of sodium adducts [118]. Acidification of the eluent by adding TFA or acetic acid [125] leads to the formation of pure molecular cations, but should result in reduced sensitivity.

Halogenated NPEO and metabolites that may eventually be formed in wastewater chlorination processes are detectable by the characteristic isotope patterns reflected in the molecular anions [115,117].

Quantitative analysis of mono- and dicarboxylated (APEC and CAPEC) metabolites of APEO, together with the final alkylphenols, is most important for environmental fate studies. Due to their acidic character, all these biodegradation products are best detected in negative mode ESI by their molecular anions [115,124,125] or by MRM [118]. As CAPEC bear two carboxylate groups, the $[M-2H+Na]^-$ anion may occur when sodium is present [125]. Due to the lack of appropriate standards, quantitation of these metabolites becomes even more difficult than that of the parent mixture and no convincing quantitation strategy has yet been published.

In other studies, not LC–MS, but FIA-MS using parent-ion scans has been employed to follow the removal of APEO in wastewater treatment plants [13,61]. This very time-saving approach can only provide semi-quantitative data.

5.2. Alcohol ethoxylates

Due to the lack of a chromophore, alcohol ethoxylates (AEO) were analyzed comparatively early by LC–thermospray-MS [126]. Separation of AEO by RPLC strongly depends upon the organic modifier used: with a water–methanol gradient, AEO are separated according to the length of the hydrophobic alkyl chain [123,127,128], whereas a water–acetonitrile gradient also provides separation according to the number of ethoxy groups [94,127]. Similar to other non-ionic compounds, many different detection methods have been used, such as APCI in the positive ion mode [127,128], and ESI in the positive ion mode [123] and in the negative ion mode [94].

Polyethylene glycols have also been analyzed using ESI in the positive mode [129,130]. Similar to APEO, the response factors for the AEO ethoxy homologues obtained by detecting the molecular cation strongly increase with the number of ethoxy groups. It is thus necessary to have a series of homologues available for calibration. Recently, derivatization of AEO prior to LC–MS analysis was proposed [131]: as this places a permanent positive charge on the analytes, some of the response problems are avoided with this approach.

5.3. Other non-ionic and amphoteric surfactants

Alkylpolyglycosides, surfactants based on sugars and fatty alcohols, have a hydrophobic alkyl chain of eight to 16 carbons that mediates retardation on RPLC [132,133]. ESI and also APCI have been used to determine these non-ionic compounds.

Alkyl glucamides have been separated by RPLC

and detected by ESI-MS. Similar to the ethoxy groups of APEO and AEO, the glycosidic ring of alkyl glycamides exhibits strong affinity towards alkaline elements. The highest signal intensity is thus found for the sodium adducts detected by ESI-MS in the positive mode, but the negative mode with detection of the molecular anions is more reproducible due to reduced chemical noise [134].

The amphoteric cocamidopropylbetaine surfactants have been separated by RPLC after the addition of either acetic acid [94] or TrEA [135]. In principle, ESI in positive or negative ion mode can be applied [94,135]. A wide variety of adducts have been observed in ESI-MS analysis, such as sodium adduct formation, di- and trimerization and multiply charged products may also occur. ESI in the positive ion mode provides more sensitivity [94,135], but using the negative ion mode may yield more reproducible quantitative results, since the extent of adduct formation is reduced and less variable than with positive ionization [135]. Quantification is further complicated by the different response factors for the alkyl chain homologues [94]. The neutral loss of dimethyl glycine (103 amu) has been observed in positive ion CID experiments [94].

5.4. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are not only non-ionic, but also non-polar. While this facilitates RPLC, it strongly impedes ionization and fragmentation in API-MS. Nevertheless, methods for the LC–MS analysis of PAH have been developed. For detection with ESI-MS, adducts with the tropylium cation are well suited, which may be formed by post-column addition of a tropylium solution [136]. The more rigid APCI allows PAH to be determined in the positive ionization mode without derivatization [137]. The recently developed atmospheric pressure photoionization (APPI) may also contribute to the LC–MS analysis of PAH [22]. Ionization in this interface appears to be highly influenced by the eluent composition and chemical transformations may even occur (Fig. 9).

However, it is more reliable to use LC–MS for the determination of the degradation products of PAH, which are more polar and, thus, more readily ionize and fragment. Among these compounds are hydroxylated (phenols) and carboxylated PAH and quinones [138]. The acidic compounds may be detected by ESI-MS in the negative mode [93]. However, con-

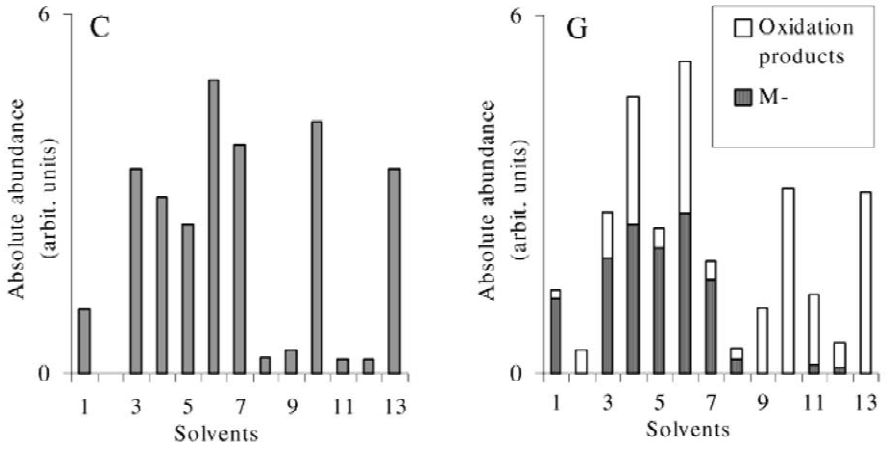

Fig. 9. Influence of the solvent system on the signal intensities for 2-naphthol (C, left) and 1,4-naphthoquinone (G, right) using APPI in the negative ion mode. The solvents tested range from hexane (1) and water (3) to water–methanol (6) and water–acetonitrile (7) to water–methanol buffers (8–10) and water–acetonitrile buffers (11–13). The open bar represents the sum of the variety of oxidation products formed during the ionization process from naphthoquinone. Reproduced, with permission, from Ref. [22]. 2002 ©American Chemical Society.

vincing advantages compared with GC–MS methods have not been reported, and the chromatographic resolution is much more limited [138].

5.5. Derivatization

It has often been argued that using LC–MS rather than GC–MS avoids tedious derivatization procedures for many classes of analytes. However, in a growing number of publications, analytes are derivatized, although LC–MS is used for analysis. Different intentions are associated with this approach.

(i) The sensitivity of detection can be increased for compounds that are difficult to ionize by introducing an ionic functional group. Adduct formation of APEO with sodium that is added to the eluent can also be interpreted as derivatization. For PAH, which are very difficult to ionize, tropylium cation [136] or Ag^+ (as silver nitrate) [139] has been added post-column to the eluent to yield cationic adducts well suited for ESI-MS analysis [136]. The silver adducts dissociate upon CID, leaving the PAH cation behind [139]. Non-ionic AEO have been derivatized with a 2-fluoro-*N*-methylpyridinium salt [131], and ethinylestradiol has been dansylated [140]. Both reactions introduce a basic nitrogen, well suited for more sensitive detection by ESI in the positive ion mode [131,140]. Aldehydes have been detected by LC–MS using either APCI or ESI in the negative ion mode after derivatization with 2,4-dinitrophenylhydrazine [141–143].

(ii) A compound class can be labelled by a uniform fragmentation of the group introduced by derivatization in MS–MS analyses. This labelling can increase sensitivity and may also be suited for screening for unknown members of a predefined class of compounds by precursor ion scanning.

(iii) Derivatizations have also been proposed for some very polar compounds. Here, not the ionization, but the chromatographic retention (and extractability) should be improved by derivatization. The large fluorenyl fluoroformate was used, which renders the very polar amitrole [144] or the amine anatoxin-a [25] less polar and more readily retained in RPLC. This avoids the use of organic fluorinated acids as counter-ions, which were found to suppress ESI-MS detection [25]. Non-ionic aliphatic aldehydes have been reacted with 2,4-dinitrophenylhydrazine to improve their extractability from water and to increases retention in RPLC [143].

6. Basic compounds

6.1. Toxins

Considerable progress has been made in analyzing the toxins generated during blooms of blue-green algae, and methods to detect microcystins by single MS [145] and ion trap-MS [146], and anatoxin-a [25] from water samples, have been developed.

The cyclic heptapeptidic microcystins are amphoteric compounds. Their detection is based on the basic amido groups rather than on the acidic carboxylic acids, since ESI-MS in the positive ion mode is used [145–147]. Microcystins may be detected as molecular cations [145,147] and by fragmentation either by the loss of a neutral fragment of 134 amu [145] or the formation of a fragment of m/z 135 [146], which characterizes the amino acid (Adda) side chain of microcystins. Owing to the low concentrations of toxins in water, micro-HPLC systems are used, which provide a higher sensitivity [145–147], and are coupled to on-line micro-SPE [145]. In one study, a four-step derivatization procedure was developed to distinguish between total microcystine content and total normal microcystine content [148].

For the chromatographic separation of the low-molecular-mass amine anatoxin-a IR-RPLC with pentafluoropropionic acid has been developed. Detection by ESI in the positive ion mode lacks sensitivity due to the organic counter-ion. After derivatization with fluorenyl fluoroformate the toxin is less polar and can be retained in RPLC without the addition of an ion-pairing agent [25].

6.2. Quaternary ammonium compounds

The analysis of most quaternary ammonium compounds by LC methods has always been difficult; the lack of UV absorbance required post-column ion-pair extraction into an organic solvent [149,150] or non-

specific conductivity detection [151]. LC–MS methods are now available that use ESI in the positive ion mode.

Quaternary ammonium compounds such as dialkyl dimethyl ammonium or benzyl alkyl dimethyl ammonium salts are used as cationic surfactants and as anti-bacterial agents. The retention of these compounds in RPLC is no problem due to the benzyl and long-chain alkyl substituents. However, obtaining a good peak shape without tailing has not always been successful. Acidification reduces the tailing to some extent and, thus, most eluents are acidified with formic acid [152,153].

However, increasing acid concentrations may severely reduce the sensitivity of detection for basic nitrogenous compounds with ESI in the positive ion mode [154]. This may be unexpected, as it is not clear why an increasing proton concentration suppresses the ionization of the ammonium cation. But an increasing amount of acid added to the eluent carries increasing amounts of the free anion of this acid with it. At elevated concentrations this anion may inadvertantly form ion-pairs with nitrogenous cations, which are non-ionic in solution and, thus, less effectively transferred into the mass spectrometer [154].

Alternatively, a non-aqueous LC–MS procedure with ternary mixtures of acetonitrile, methanol and chloroform has been used for the analysis of quaternary ammonium compounds, including some biologically less resistant ‘‘modern’’ ethyl esters [155].

If quaternary ammonium compounds become more polar and retention in RPLC is difficult to obtain, ion-pair formation with volatile organic anions can increase retention. For this purpose, hexafluorobutyric acid [23,24] and pentafluoropropionic acid [25] have been used.

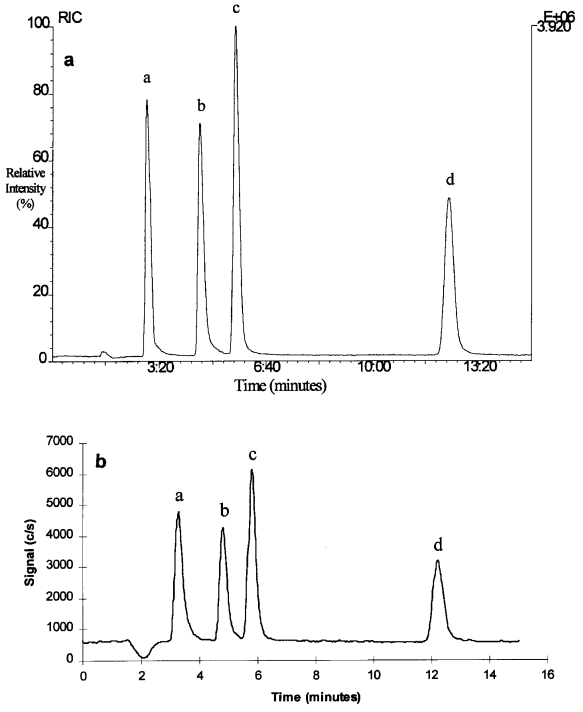

Fig. 10. Top: LC–APCI-MS chromatogram for a mixture of alkyltin compounds: (a) diphenyltin, (b) dibutyltin, (c) triphenyltin and (d) tributyltin (50 mg/L concentration). Bottom: LC–ICP-MS trace (10 μg/L concentration). Reprinted from Ref. [161], with permission from Elsevier Science.

6.3. Organometallic compounds

A very interesting application for LC–ESI-MS is in the analysis of organometallic complexes [156]. Here, LC–MS has been demonstrated to be a complementary, but less sensitive, technique to LC–ion coupled plasma-MS [157], as it allows the identification of the organic compounds that present the various signals obtained for one element in LC–ICP-MS (Fig. 10).

LC–MS methods for the analysis of tributyltin and triphenyltin and related compounds have been developed and are based on the RPLC separation of these compounds, combined with detection by ESI [158–160] or APCI [161,162] in the positive ion mode. A series of adducts may be generated when APCI in the positive ion mode is used [161].

Although acidic in nature, organic species of arsenic [163,164] and selenium [165] have also been detected in the positive ion mode. Here, reversed-phase or ion-pair chromatography [165] or ion chromatography [164,166] was employed. Other organometallic species such as zinc pyrithione [167] have also been analyzed by LC–MS using ESI in the positive ion mode.

7. Aspects of target analysis

7.1. Matrix effects

The high selectivity and low chemical noise usually experienced when using LC–API-MS, namely in MRM mode, weakens the awareness that the target analyte is only an extreme minority of the total amount of sample injected onto the analytical column. This so-called matrix may affect the ionization of the analytes of interest and may result in erroneous quantitation by LC–MS.

There is growing awareness and experimental evidence that matrix effects can severely compromise quantitative data generated by LC–MS (Fig. 11). A typical problem is the coextracted humic material in the trace analysis of acidic pesticides from ground and surface waters [89,168–171]. But, in general, any coeluting organic compound can interfere with the ionization of the target analyte [172–174]. With regard to quantitative analysis from sampling campaigns in the field, one has to be aware that the matrix may vary considerably from one sampling point to another and from one sampling date to the next [168,169].

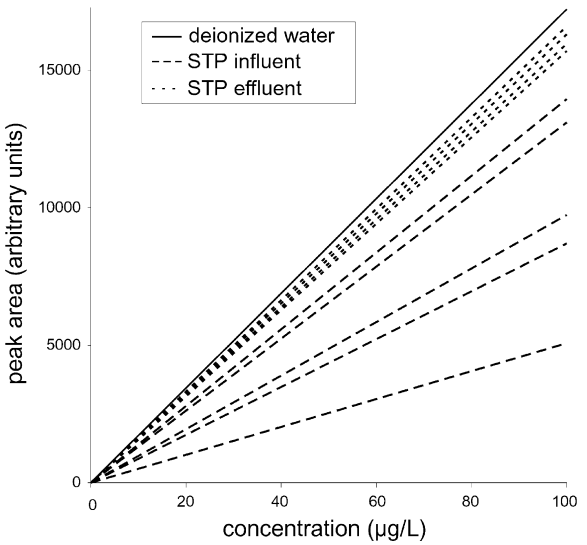

Fig. 11. Calibration curves for naphthalene-2,6-disulfonate using IP-RPLC and ESI in the negative ion mode. Curves were obtained by four-point standard addition into deionized water, raw waste water (STP influent) and treated wastewater (STP effluent). Note that the curves for the two waste water matrices differ in both slope (response factor) and variability. Redrawn from Ref. [193].

The special problem of matrix effects in LC–MS stems from the fact that the sample matrix may also be subjected to chromatographic separation, resulting in a different, and in each case unknown, matrix for each of the analytes in a multi-component analysis [175]. Thus, one internal standard cannot compensate for these effects, but a chemically similar and coeluting standard compound is required for each analyte. An approach common to other fields in which LC–MS is used is the addition of a ^{13}C-marked standard, but isotopically labelled compounds are often not available for environmentally relevant analytes.

Several approaches to *compensate* for matrix effects by standard addition are available.

(i) Quantification by standard addition into each sample and with each analyte investigated. This, however, leads to three to four times greater sample numbers and, although it can *correct* for sensitivity losses by matrix compounds, this approach cannot *avoid* the loss of sensitivity.

(ii) If one is confident of obtaining a uniform matrix within a series of samples, calibration can be performed by standard addition into only one sample of the series [174]. This calibration can be applied to the whole series of samples. However, before this approach can be applied, one has to ensure that the matrix of all samples of a series is sufficiently homogenous.

(iii) An instrumental approach to compensate for matrix effects is provided by the so-called echo peak calibration [174]. A standard mixture of all analytes is injected shortly after the sample into the same chromatographic run. Standard signals reach the detector with a delay of one peak width after the respective sample signal. Provided that the matrix peak is broad, which is often the case due to column overloading, the standard signal can be used as an internal standard to compensate for the sensitivity differences due to matrix effects or sensitivity fluctuations [174].

A more fundamental approach would be to *remove* the disturbing matrix components prior to LC–MS analysis of the target analytes. This strategy can only

succeed when the physico-chemical properties of the analytes and the matrix components are sufficiently different from each other.

(i) Improved sample clean-up during SPE. This may be obtained by using a more selective sorbent or a more elaborate elution procedure in SPE. Alternatively, a two-step extraction procedure may be employed, in which the first extraction removes the matrix from the aqueous sample, while the second step extracts the target analytes. For example, acidic humic and fulvic acids have been removed by extraction at pH 1 and the weakly basic triazine analytes were extracted after neutralization [171]. Analogously, a hydrophobic matrix may be extracted by a first SPE with C_{18} material under neutral conditions, while polar acidic analytes remain in the aqueous phase for a second extraction with a polar polymeric phase at acidic pH.

(ii) Improved clean-up during chromatography. Interfering matrix components may be separated from a target analyte by using a precolumn in the chromatographic system [89,172,176]. The first clean-up column may be packed with restricted access material (RAM) to exclude humic substances from interaction with the reversed-phase sorbent [89,177,178] (Fig. 12). When the initial extraction is also performed on-line, this results in a ‘‘dual precolumn’’ extraction: the first may be used for trapping humic material and the second for analyte enrichment [169]. As these approaches require considerably more complex chromatographic systems, they may be attractive primarily for repeated routine analyses of large sample series (Fig. 13).

(iii) Two-dimensional chromatography. Contrary to the methods described in (ii), the first column is not only used for trapping the matrix or the analytes, but for chromatographic separation of the sample mixture, from which the so-called heart-cut is selected for a second chromatographic separation [172]. Since only a narrow time window of the first LC separation is analyzed further, this technique is hardly applicable to multicomponent analyses.

It was recently pointed out that signal suppression by a coeluting matrix can be considerably reduced by directing lower flow volumes into the ESI source [179]. A nanosplitting device was constructed for this purpose. A broader range of applications also on

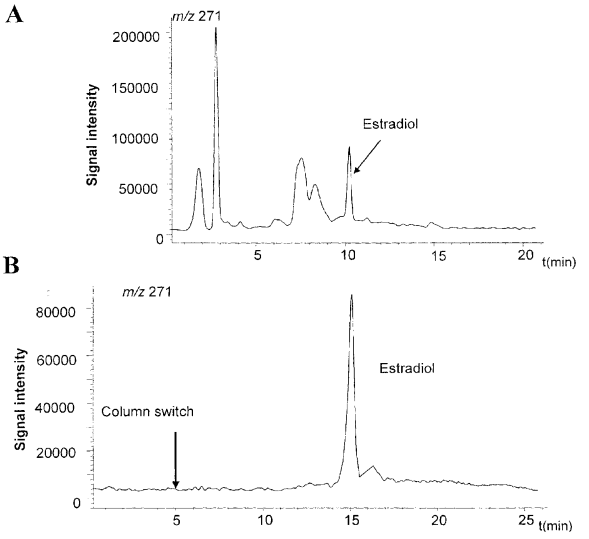

Fig. 12. Removal of sample matrix by LC–LC with a RAM precolumn. Chromatogram of m/z 271 (estradiol) of a sediment extract using LC–ESI-MS in the negative ion mode. (a) Without a RAM precolumn to exclude humic acids. (b) With a RAM column. Reprinted from Ref. [178], with permission from Elsevier Science.

other instruments is required to fully evaluate this approach.

On one hand, these strategies to eliminate sample matrix are laborious, but they may be necessary to obtain reliable quantitative data. On the other hand, the following comparison with GC–MS procedures may ease the situation to a certain degree. The analytical uncertainty of a method is determined by the sum of all of its steps. Since significantly less sample manipulation is usually required when LC–MS analysis is used as compared with procedures based on GC–MS, a LC–MS method may even be able to compete with a GC–MS method when API-MS detection is subject to a larger error due to matrix effects.

7.2. Avoidance of false-positive findings

Besides taking measures to ensure that quantitation is correct, one also has to ensure that the detected signal truly belongs to the suspected target analyte. Indeed, the selectivity of LC–MS detection may be overestimated, as the complexity of en-

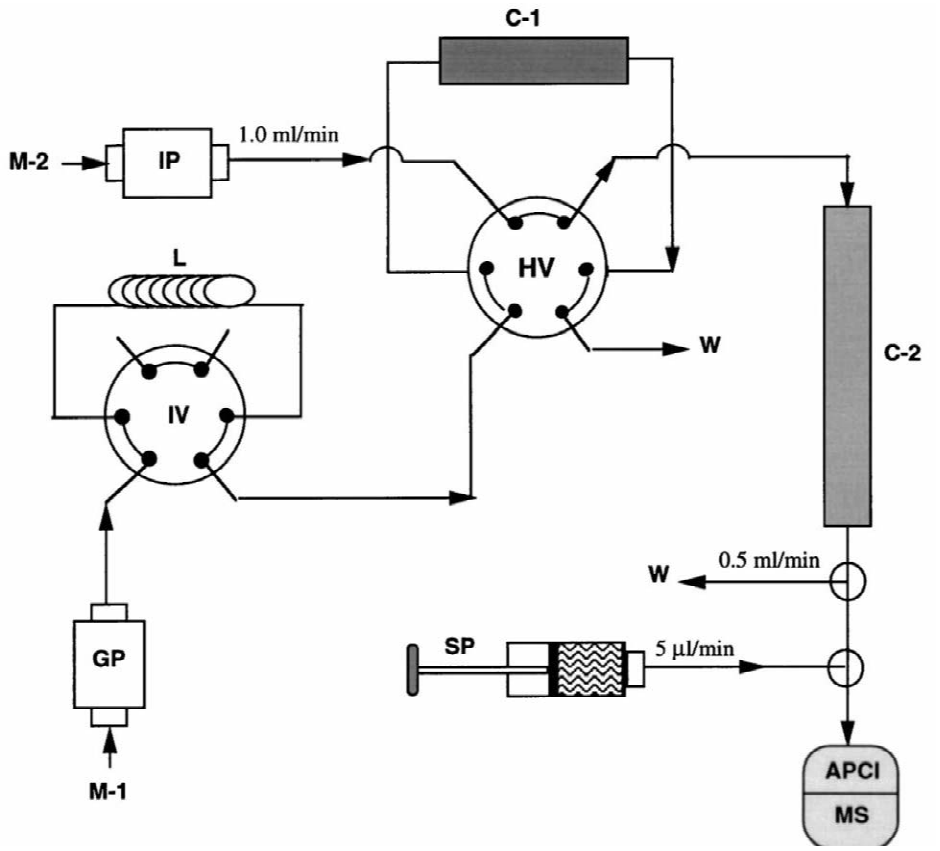

Fig. 13. Scheme of the dual-column approach. GP, gradient pump; IP, isocratic pump; IV, injection valve; HV, high-pressure valve; C-1 and C-2, first and second chromatographic columns; SP, syringe pump. C-1 may be used for analyte trapping from a large volume injection (20 mL), while C-2 is used for chromatographic separation. Reprinted from Ref. [176], with permission from Elsevier Science.

vironmental samples often exceeds the selectivity of MS detection. This may result in false-positive findings, especially when low resolution single mass spectrometric detection by quadrupole or ion-trap MS is used: (i) owing to the limited resolution, one cannot be sure that the ion detected has the suggested molecular formula, and (ii) the single MS approach cannot ensure that the detected ion is truly the molecular ion or fragment ion of the compound one wanted to detect. The uncertainty increases (i) when a larger number of analytes is detected with very limited chromatographic separation, (ii) when isolated substances are to be detected as patterns of analytes, for example a series of homologues are generally detected more safely, or (iii) when complex samples with a contribution from very different and unknown sources are analyzed. The risk of false-positive findings is much smaller when TOF-MS with its higher resolution is employed [40].

Different criteria have been developed to avoid false-positive findings. It may be requested that the retention time be within 1% of the retention time of the standard compound, that the molecular ion and two fragment ions are present and that the intensity ratio of the fragments to the molecular ion is within 20% of the standard value [180]. Criteria reported to be used in the Netherlands are a maximum of 0.2% deviation in retention time, and three diagnostic ions with a maximum 50% variation in intensity ratio as compared to the standard [181].

The criteria established in a commission decision of the European Union may become most important

Table 1
Number of identification points rewarded for a range of LC–MS techniques according to the European Commission [182]

Technique	Number of ions	Identification points
LC–MS (LR)	*n*	*n*
LC–MS–MS (LR)	One precursor, two daughters	4
LC–MS–MS (LR)	Two precursors, one daughter each	5
LC–MS–MS–MS (LR)	One precursor, one daughter, two granddaughters	5.5
LC–MS (HR)	*n*	2*n*

LR, low resolution; HR, high resolution, typically greater than 10 000 at 10% valley. Note that this is usually not met by TOF instruments. *n*, an integer.

in Europe [182]. This directive proposes a system of identification points and three points are required to confirm a positive finding (Table 1). Additionally, the deviation of the relative intensity of the recorded ions must not exceed 50–20% (for relative intensities of <10–50%) that of the standard compound and the retention time must not deviate more than ±2.5%. For MRM analysis, the selection of one precursor ion and the recording of two product ions at low resolution would result in four points and would, thus, be sufficient for a safe positive finding (Table 1).

In light of these quality criteria for residue analysis, many positive findings previously reported in the literature become questionable since these strict confirmation criteria have rarely been applied. In many cases, only the occurrence of one ion corresponding to the nominal mass of the expected analyte ion was considered sufficient.

8. Aspects of qualitative analysis

8.1. Screening

Because of the broadness that LC–API-MS provides with respect to the physical properties of the analytes to be detected, this technique appears to be attractive for the non-target screening of water samples. This would allow the detection of new contaminants that were not recognized before and that may then become the object of target analysis. However, performing a LC–MS analysis requires initial decisions concerning the chromatographic separation, ionization techniques and modes of ionization, which already limit the broadness of the detection.

The limited resolution of quadrupole and ion-trap mass spectrometers would limit their use for screening purposes, as they do not allow the identification of completely unknown compounds. However, triple-quadrupole mass spectrometers can be used to determine compounds with common characteristic substructures by either neutral loss or precursor ion scans. With this approach, novel metabolites of a precursor may be determined, as long as the substructure used for MS detection is retained [183]. However, the sensitivity of detection decreases drastically with a scanning mass spectrometer. Compared with the MRM mode widely used in target analysis, the sensitivity is orders of magnitude lower if one of the quadrupoles is scanning (precursor ion scan) or even if both are scanning (neutral loss scan) (Fig. 14).

Due to its higher resolution and good sensitivity over the full mass range, TOF-MS appears to be advantageous for screening purposes. The use of a quadrupole TOF can be even more useful, as it allows the recording of product ion spectra for further identification [184]. The molecular mass of an unknown compound can be compared with the molecular mass of known environmentally relevant compounds. Furthermore, the product ion spectrum can be compared with product ion spectra stored in a library [184]. This second part of the identification process stands or falls with the availability and

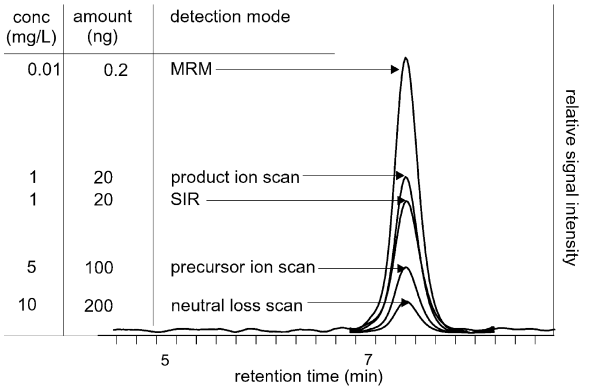

Fig. 14. Comparison of the sensitivity of different MS and MS–MS detection modes. Naphthalene-1,5-disulfonate was analyzed and detected with ESI in the negative ion mode. Redrawn from Ref. [192].

applicability of libraries of product ion spectra generated by CID (see below).

8.2. Libraries for identification

The benefits of spectral libraries in LC–API-MS are still a matter of debate. Due to the strong influence of instrumental as well as operational parameters on collision-induced fragmentation processes in API-MS, this approach is far less straightforward than in GC–MS. The first studies on the influence of operational conditions [185] and on attempts to standardize them have recently been published [186–188]. If model compounds are used to standardize fragmentation conditions and if spectra are generated at different levels of fragmentation, model libraries are applicable with different instruments [187–189]. However, this work was directed at in-source fragmentation, which is not applicable to complex environmental samples.

True MS–MS libraries, in which spectra of collision-induced dissociations of selected precursor ions are compiled, would be of more use. For ion-trap mass spectrometers, such libraries have been created using the so-called wideband excitation [190]. Since the intensity of fragment ions is highly variable among different instruments, a novel approach of library formation does not use the intensities of fragments at all. Instead, only the fragment masses together with the isotopic signals are considered and data originating from very different LC–MS–MS systems and from GC–MS with chemical ionization are combined [191].

Different approaches have to be used to compile LC–MS libraries and the success of any of these approaches is not fully clear. However, growing and merged libraries of polar compounds would be of great help in identifying novel water constituents and to distinguish between compounds of natural and anthropogenic origin.

9. Conclusions

As summarized in this review, much has been achieved in applying LC–MS to the analysis of polar organic pollutants in the last few years. Some general trends concerning the selection of an appropriate LC–API-MS combination for a certain analytical task can be outlined:

- Considering the ionic status of the analyte allows preselection of the ionization mode, while the polarity of the total molecule must be considered for choosing the appropriate LC method.
- For very polar and ionic analytes, MS detection is straightforward. Three methods have been developed for chromatographic separation: IP-RPLC, IC and CE. Of these, IP-RPLC is the most robust and is expected to be more widely used in the future.
- With decreasing charge and polarity, LC separation becomes easier, but finding the appropriate MS mode becomes more difficult. ESI and APCI in both positive and negative ion mode have to be considered and compared, and the method providing the highest signal intensity with standards need not be the most sensitive method with real samples. The negative ion mode is usually more selective and is less prone to adduct formation than the positive ion mode.
- In certain cases, derivatization may be helpful to enhance retention and extractability, to increase the sensitivity of MS detection and to make the fragmentation behavior of a structurally heterogenous class of compounds uniform.
- For the LC–API-MS combination, most instrumental development can be expected for the

third component, the MS. New hyphenation techniques may provide new measurement options and the ongoing increase in the sensitivity of detection will allow more direct analyses of water samples with less need for preconcentration.

The door to the analysis of polar water constituents, whether anthropogenic or biogenic, is wide open and much is to be discovered using the various LC–API-MS combinations.

Nomenclature

APCI	atmospheric pressure chemical ionization
APEC	alkylphenylethoxy carboxylates
APEO	alkylphenol ethoxylates
API	atmospheric pressure ionization
APPI	atmospheric pressure photoionization
CAPEC	carboxylates alkylphenolethoxy carboxylates
CE	capillary electrophoresis
CID	collision-induced dissociation
EDTA	ethylenediaminetetraacetate
ESI	electrospray ionization
FTICR	Fourier transform ion cyclotron resonance
HAA	haloacetic acid
IC	ion chromatography
IMS	ion mobility spectroscopy
LAS	linear alkylbenzene sulfonates
MRM	multiple reaction monitoring
NPEO	nonylphenol ethoxylates
PAH	polycyclic aromatic hydrocarbon
RAM	restricted access material
SIR	selected ion recording
SPC	sulfophenyl carboxylates
SPE	solid-phase extraction
TEA	tetraethylammonium cation
TOF	time of flight
TrBA	tributylamine
TrEA	triethylamine

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