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

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Despite its enormous potential for the determination of polar compounds in water, the application of liquid chromatography-atmospheric pressure ionization-mass spectrometry (LC-API-MS) has some characteristic obstacles. Qualitative analysis of completely unknown compounds suffers from the limited resolution of quadrupole and ion-trap MS and the limited fragmentation encountered in collision-induced dissociation. Quantitation of target analytes in complex samples may be impaired by matrix effects, especially when using electrospray ionization. This review summarizes characteristic problems when using LC-MS for the qualitative and quantitative analysis of organic compounds in water. It outlines how some of the problems may be reduced by improved instrumentation (e.g. tandem MS or time-of-flight-MS), improved chromatographic separation or by measures taken before or after the LC-MS analysis. Because of the complexity of environmental samples, more effort in quality assurance and control is required. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The potential of atmospheric pressure ionization (API) techniques to transfer polar analytes separated by liquid chromatography (LC) into a mass spectrometer (MS), together with the high selectivity and low chemical noise provided by MS and MS/MS detection, has stimulated the development of water analysis and altered its tasks. The present status in this ongoing development was reviewed in Part I [1]. As far as organic contaminants are concerned, many of the compounds determined in LC-API-MS analysis do not fall into the lists of traditional priority pollutants.

Rather, many are ‘novel pollutants’ of high polarity, among them the primary degradation products of well-known contaminants. Moreover, electrospray ionization has shown some potential to transfer metals with their organic ligands and organic non-covalently bound conjugates into the MS, making them amenable to detection and identification. This can add significantly to our understanding of environmental as well as technical transformation processes. Knowledge of a compound’s metabolism and speciation is important in environmental chemistry, as both aspects markedly influence the properties and the fate of a contaminant in the aquatic system.

The high selectivity and the low chemical noise usually experienced when using LC-API-MS to detect target compounds in water samples is, however, not only fascinating but also misleading:

• In qualitative analysis this selectivity may be overestimated, as the complexity of environmental samples often exceeds the selectivity of
the MS detection. This results in false positive findings if no stringent criteria for a positive detection are applied.

- The selective detection weakens the awareness that the sample matrix is still present behind the target analytes. This matrix may affect the ionization of the target analytes and, thus, the quantitation by LC-MS.

After reviewing the achievements of LC-MS in water analysis [1], this second part of the review outlines the obstacles encountered when using LC-MS for the qualitative and quantitative analysis of waters. The present concepts and technical developments to answer these problems are described and discussed.

2. Aspects of qualitative analysis

2.1. Target analysis

From a review of the current literature [1] it is obvious that most detections of organic contaminants in water samples rely on the use of single-quadrupole mass spectrometers. Historically, this was the first instrument specifically designed for coupling with liquid chromatography and also the first to become commercially available. Triple-quadrupole mass spectrometers have long been too expensive for a non-commercial application in environmental analysis.

For two reasons we must critically reevaluate any positive findings of compounds in water by single MS reported thus far, as an unknown number of false positive results may be among them: (a) owing to the limited resolution of quadrupole MS one cannot be sure that the ion detected had the suggested molecular formula and (b) the single MS approach cannot ensure that the detected ion was truly the molecular ion or fragment ion of the compound one wanted to detect. The uncertainty is especially large in those cases (a) in which a larger number of analytes was detected in a very short period of time with very limited chromatographic separation, (b) when isolated substances were to be detected, as patterns of analytes, e.g. a series of homologues, are generally detected more reliably or (c) if complex samples with a contribution from very different and unknown sources were analyzed.

Some researchers tried to compensate the lack of selectivity of single MS by performing two analyses: one on an ion for quantitation, usually the molecular ion, and a second analysis under conditions that induce fragmentation in the MS interface to detect one or two more or less specific fragments for confirmation purposes. However, besides the larger expense in performing two instead of one analysis this approach is only a gradual and insufficient improvement.

Fortunately, the situation has begun to change recently (section 3.12 of [1]), as ion-trap mass spectrometers were introduced and triple-quadrupole mass spectrometers became less expensive. The selectivity of multiple reaction monitoring (MRM) in the MS-MS mode as compared to a selected ion recording (SIR) detection with single MS is still impressive (Fig. 1). However, false positive findings can occur even when using MRM detection. Thorough criteria have been developed to avoid false positive findings in residue analysis based on LC-MS:

- In some cases the retention time must be within 1% of the retention time of the standard compound, a molecular ion and two fragment ions should be present and the intensity ratio of the fragments to the molecular ion should be within 20% of the standard value [2].

![Fig. 1. Selectivity of SRM (front) as compared to SIR (back) of (a) mercaptobenzothiazole (SIR: m/z 166, SRM: m/z 166 > 134) and (b) mercaptobenzoxazole (SIR: m/z 150, SRM: m/z 150 > 58) from a wastewater sample.](image-url)
In another case only 0.2% deviation in retention time but 50% variation in the intensity ratio is allowed [3].

In the draft version of a revision of criteria for positive findings in animals and animal products (revision of Council Directive 96/23/EC) a system of identification points is proposed and three points are required for a positive detection. Using one precursor ion and two daughters a number of four points is earned in case that the intensity ratio is within an intensity dependent limit of 25–50%, depending on the intensity ratio of the fragments [4].

It appears necessary to establish these quality standards for the positive detection of compounds on a broad basis throughout the field of water analysis. The multitude of potential emission sources and our generally limited knowledge of them, and the corresponding complexity of environmental samples are further arguments in this direction.

The following example may illustrate the benefits of these quality criteria (Fig. 2). We applied MRM detection of two transitions of the molecular ion to detect the analyte benzothiazole in a given time window from a complex wastewater. Nevertheless, two signals were obtained that even met the required intensity ratio of the fragments and, thus, four quality criteria were fulfilled by both signals. It was the fifth criterion, the retention time, that finally allowed us to exclude the wrong signal (5.4 min) against the right one (6.05 min). However, the retention time can only be a strong criterion if a good and stable chromatographic separation of the analytes is obtained. The steeper the elution gradient and the shorter the analysis time, the weaker gets the significance of the retention time criterion and the higher becomes the risk of false positive results. Thus, this example may illustrate that we still need a good chromatography in water analysis, even when using MRM detection.

A second approach of increasing the selectivity and avoiding false positive finding is the use of a time-of-flight-mass spectrometer (TOF-MS). It was shown that the higher mass resolution provided by these instruments allows unambiguous detection of certain pesticides from river water even when they are accompanied by isobaric compounds [5].

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Fig. 2. Detection of benzothiazole by two MRM transitions (T1 and T2) from a standard solution (left) and a wastewater sample (right). Both signals in the sample show up in both MRM traces with T1/T2 intensity ratios corresponding to the standard. The retention time allowed exclusion of the first peak from the sample.
Today, TOF-MS instruments are not widely used and their importance in the future of water analysis remains open (see also Section 2.3).

2.2. Compound identification

The potential of LC-MS to detect polar compounds makes this technique ideally suited for the analysis of polar metabolites and transformation products formed in natural as well as technical processes. This is one of the most stimulating aspect of LC-MS as far as water treatment processes are concerned, as the analysis is no longer restricted to detect the removal or persistence of the parent compound but can provide information on the intermediates and products of a treatment. This contributes significantly to our understanding of the chemical processes underlying a water treatment process. Correspondingly, LC-MS has been used to identify photolysis products of pesticides [6], the transformation of pesticides [7,8], surfactants [9] or dyes [10–12] in advanced oxidation processes applied to wastewaters as well as the products formed from pesticides upon disinfection of groundwater [13,14].

As far as microbial wastewater treatment is concerned, the transformation of a wide variety of industrial and municipal wastewater constituents such as nonylphenol ethoxylates [15–17], linear alkylbenzene sulfonates [18,19], alcohol ethoxylates [20], corrosion inhibitors [21], dyes [22,23] and pesticides [24] has been studied.

Despite these examples, the success of LC-MS in the identification of completely unknown compounds is comparatively limited in many cases. The routinely employed mass resolution of quadrupole and ion-trap systems does not provide sufficiently exact masses for deriving the molecular formula of the ion detected and fragmentation spectra obtained by collision-induced dissociation (CID) do often not carry sufficient information to propose a structure of the suspected ion. Chemical synthesis of the suspected metabolites may still be the only way to succeed with their identification in this situation [25].

In general the application of independent spectroscopic techniques such as NMR is mandatory to distinguish between structural isomers, as their fragmentation is often similar and usually not predictable. An $^1$H-NMR analysis of water constituents can nowadays be performed on-line with LC but the application of this technique will remain limited in water analysis [26,27] as costs of this instrumentation are extremely high.
However, by using LC-TOF mass spectrometers, the detected $m/z$-value of an analyte may be sufficiently exact to provide a molecular formula and to confirm or deny a suggested structure [5,28]. Moreover, the ability of TOF-MS to provide full scan spectra together with a high sensitivity makes it an interesting choice for qualitative analysis.

It was recently shown that the widely distributed quadrupole mass spectrometers can also be used to derive exact masses in LC-MS as a basis for molecular formula calculation [29]. Fig. 4 shows the example of a compound formed in anaerobic treatment of an azo dye that autoxidizes upon contact with air. Together with the exact mass determination of fragment ions and the information provided by isotope signals, this approach can substantially narrow down the number of potential structures of an unknown metabolite.

A basic procedure in GC-MS analysis to identify an unknown compound is the comparison of its electron-impact mass spectrum obtained at an ionization energy of 70 eV with those compiled in spectral libraries. Due to the strong influence of instrumental as well as operational parameters upon the in-source or collision-induced fragmentation processes in API-MS, this approach is far less straightforward in LC-MS. Initial studies on the extent of the influence of operational conditions [30] and on attempts of standardizing them have recently been published [31].

Of course, one can compile and use a spectral library under fixed conditions on one instrument and this has been done for a variety of drugs [32,33]. The library may then be used in another laboratory on the same instrument model under similar measuring conditions [32]. However we do not have generally applicable libraries to hand nor can we expect these in the near future. Finally, an LC-MS library would have to contain a considerably larger number of spectra to obtain the same impact factor as libraries for GC-MS, since LC-MS is able to detect a larger number of different compounds.

### 2.3. Screening

The broadness that LC-API-MS provides with respect to the physical properties of analytes makes this technique attractive for non-targeted screening of water samples. However one has to consider that the different operational conditions that can be used – different chromatographic separation systems, different ionization techniques (electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)) and modes of operation (positive and negative) – require some initial decisions that themselves reduce the broadness of detection. Moreover, non-targeted screening by LC-MS is limited by all the factors that hamper the identification of completely unknown compounds (Section 2.2). Correspondingly, there are no reports to be found in the literature that describe an unambiguous success of a non-targeted screening of water samples by LC-MS. Again, TOF-MS has certain advantages which may make this instrument attractive in screening for unknown compounds.

### Table 1

<table>
<thead>
<tr>
<th>Compound class</th>
<th>Ionization</th>
<th>Product ($m/z$)</th>
<th>Loss (amu)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic sulfonates</td>
<td>ESI neg.</td>
<td>80 (SO$_3^-$)</td>
<td>64 (SO$_2$)</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80 (SO$_3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatic carboxylates</td>
<td>ESI neg.</td>
<td>44 (CO$_2^-$)</td>
<td></td>
<td>[50]</td>
</tr>
<tr>
<td>Aliphatic sulfates</td>
<td>ESI neg.</td>
<td>97 (HSO$_4^-$)</td>
<td></td>
<td>[51]</td>
</tr>
<tr>
<td>Aliphatic carboxylates</td>
<td>ESI neg.</td>
<td>59 (CH$_3$COO$^-$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphates</td>
<td>ESI neg.</td>
<td>97 (H$_3$PO$_4$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>ESI pos.</td>
<td>156</td>
<td></td>
<td>[51]</td>
</tr>
<tr>
<td>Dihydroxyphenyl comp.</td>
<td>ESI neg.</td>
<td>108</td>
<td></td>
<td>[52]</td>
</tr>
<tr>
<td>Phosphorylated peptides</td>
<td>ESI neg.</td>
<td>63 (PO$_2^-$)</td>
<td></td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79 (PO$_3^-$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene glycols</td>
<td>APCI pos.</td>
<td>89 (C$_2$H$_4$OH$^+$)</td>
<td></td>
<td>[54]</td>
</tr>
<tr>
<td>Glycosylated peptides</td>
<td>ESI pos.</td>
<td>204 (HexNAc$^+$)</td>
<td></td>
<td>[53]</td>
</tr>
<tr>
<td>Amides</td>
<td>ESI pos.</td>
<td>H$_2$NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfated glycosides</td>
<td>ESI neg.</td>
<td>120 (NaHSO$_4$)</td>
<td></td>
<td>[55]</td>
</tr>
<tr>
<td>Various glycosides</td>
<td>ESI neg.</td>
<td>162 (Glu)</td>
<td></td>
<td>[56,57]</td>
</tr>
</tbody>
</table>
More success can be expected when LC-MS is used to screen for a limited number of previously selected compounds or compound classes [34]. This strategy may also be combined with biological test systems to preselect fractions of analytical interest and was applied to industrial effluents [35]. In these effect-related approaches using LC-MS only those compounds have been determined for which LC-MS methods had been established beforehand, so their use for screening purposes remains limited [36].

In contrast, a very promising strategy based on LC-MS is screening for compounds with selected functional groups. As mentioned before (section 3 of [1]), a wide variety of functional groups exhibit characteristic fragmentation properties which can be used to detect all compounds with a specific moiety by different MS-MS approaches. Neutral loss scans can indicate all substances losing a specific non-ionic fragment and parent ion scans provide the molecular mass of all compounds that lose a specific ionic fragment. Many characteristic fragmentations and fragments can be found in the literature. Some of those with potential relevance in the field of water analysis are compiled in Table 1.

For example, the use of a fragment with m/z 80 (SO₃⁻) to specifically detect aromatic sulfonated compounds was proposed more than a decade ago [37] and was finally used to detect naphthalene sulfonates by LC-MS from groundwater and leachates [38] (Fig. 5). Due to the selectivity of these detection methods an LC separation of the analytes may be superfluous and samples can be analyzed by FIA-MS. This concept has frequently been used to detect residual surfactants in treated effluents and other water samples [39–41], while other transitions mentioned in Table 1 have not yet been applied to water analysis. For compound classes that lack a characteristic and sufficiently intensive fragmentation, derivatization may be useful to introduce a characteristic fragment into the molecule.

The attraction of functional group screening is that it transforms the MS into a detector that is specific for a certain class of organic compounds. No other instrumentation can provide this potential in similar broadness. Unfortunately, the sensitivity markedly decreases when a triple-quadrupole MS is operated in the parent ion or neutral loss mode.

3. Aspects of quantitative analysis

3.1. Instrumental aspects

Using a triple-quadrupole instrument results in a general lowering of the detection limits as compared to a single-quadrupole instrument, owing to the significant decrease in the chemical noise (Fig. 6). With further increasing sensitivity, analytical artifacts and blank values are becoming an issue of increasing importance. For some omnipresent analytes such as LAS, the detection limit is already often governed by the blank values rather than by the sensitivity of the MS detection.

For quadrupole instruments an inverse interrelationship exists between the sensitivity of detection, the speed of chromatography and the reliability of detection. A high sensitivity requires a long collection time for each ion to be detected, while a fast chromatography requires a short measuring time, as a number of about 15 data points is necessary to adequately represent a chromatographic peak; this limits the time that can be spent recording one transition. It becomes even shorter if more than one compound has to be recorded in a given time span. Finally, the call for two rather than one transition to be detected for each compound (Section 2.1) further halves the available collection time. A
very fast chromatography is thus not always desirable, for quality reasons (see Section 2.1) as well as for sensitivity reasons.

The situation is completely different when a TOF-MS is used, as these instruments record a spectrum very fast and do not filter out any ions prior to detection. Therefore, the portion of ions detected from those that have entered the high-vacuum region is much higher and the signal-to-noise ratio is also much higher, giving very good sensitivity in a full scan operation compared to a quadrupole MS. But the sensitivity is reported to be not as good as with a triple-quadrupole instrument in the MRM mode [28], when nominal mass chromatograms are used. If full use is made of the resolution power of the TOF-MS and narrow mass range chromatograms (±0.1) are extracted, detection limits can compete with those of MRM detection, when detection was chemical noise limited [28], a likely situation in water analysis. At the same time, no spectral information is lost. A drawback of using TOF-MS for quantitative analysis is the narrow dynamic range that covers two orders of magnitude only. After all, the main strength of the TOF-MS instruments appears to lie in the qualitative analysis and in screening applications (Section 2).

### 3.2. Calibration

Besides detection limits the quality of the calibration curve is an important criterion for evaluating the quality of a quantitation method. Linear calibration curves appear to be a fetish in quantitative analysis and, thus, a linear calibration is often claimed in the LC-MS literature.

On the other hand it is generally accepted that for each given analyte the ‘linear dynamic range’ is followed by a ‘level-off’ region in which signal intensity no longer increases, with a region of transition found between the two extremes [42]. Unfortunately, neither the extent of the linear dynamic range, nor that of the transition zone or of the level-off region can be predicted. Hence, one should be aware that the linear calibration curve is the exception of the more general case of a quadratic calibration curve.

Indeed, we often find a quadratic calibration curve to fit the data more closely when the dilution series of a standard covers several orders of magnitude. A linear calibration curve can often fit reasonably, if only a small scale of the concentration up to a maximum of two orders of magnitude is covered, but this is often not sufficient in water analysis. When the calibration curve is sufficiently steep, there should be no problem in using a quadratic function for calculating an analyte’s concentration.

### 3.3. Matrix effects

It was pointed out earlier (section 3.12 of [1]) that a sample matrix may severely affect quantitation by LC-MS. The growing awareness of this problem implies that it has been ignored for too long in water analysis. Humic materials have gained special attention in pesticide analysis [43–46] as these are characteristic matrix constituents in ground- and surface waters, but in general any other coeluting organic compound can interfere with the ionization of the target analyte.
The special problem of matrix effects in LC-MS stems from the fact that the sample matrix may also be subjected to the chromatographic separation, resulting in a different and in each case unknown matrix for each of the analytes in a multicomponent analysis. 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 these compounds are usually not available in environmental analysis.

In other studies matrix effects were shown to continue over a certain time of the chromatographic separation; this was interpreted as being indicative of an overloading of the column by sample matrix, resulting in an extreme tailing of these compounds [47].

Generally, two ways to compensate for matrix effects are:

- Quantification by standard addition into each sample and with each analyte investigated. This, however, results in three to four analyses of each sample instead of one analysis and each sample has to be calibrated and quantified separately. Nevertheless, this approach provides reliable and firm quantitative data (Fig. 7). Though standard addition can correct for sensitivity losses by matrix compounds, it cannot avoid a loss of sensitivity.

- If one is confident of having a uniform matrix within a series of samples, calibration can be performed by standard addition of only one sample of a series and applied to the whole series. However, a series of environmental samples often consists of a collection gained in time or/and space. Contrary to many biological fluids it is thus hardly conceivable that only the analyte concentrations but not the matrix will vary. Therefore, for a significant portion of a sample series by standard addition, the matrix effects must be very similar before using this simplified approach.

A more basic approach would be to remove the disturbing matrix components prior to the ionization process. In all cases, however, this requires analytes that are chemically sufficiently different from the matrix components. Again, two strategies are available:

- Improved sample clean-up. This may be obtained by using a more selective sorbent or elution procedure in solid-phase extraction (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 aims at extracting the target analytes. For example, acidic humic and fulvic acids may be removed by extraction at pH 1, while the weakly basic triazine analytes are extracted after neutralization [46]. Analogously, a hydrophobic matrix may be extracted by an initial SPE with C18 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. A two-step extraction can also be performed online: a ‘dual precolumn’ extraction with the first one used for trapping humic material was shown to reduce the depressive effect of humic materials [44].

- Improved chromatography. Interfering matrix components may be separated from a target analyte by improving the chromatographic separation. However, this may not be obtainable if a complex matrix is present and a variety of target analytes with differing physico-chemical properties has to be quantified. An LC-LC-coupling prior to MS/MS may then be used [47,48].

![Fig. 7. Matrix effects as determined by standard addition for naphthalene-2,6-disulfonic acid in raw and biologically treated industrial wastewater after direct injection into the HPLC system and detection by MRM.](image-url)
Both strategies to eliminate sample matrix are laborious and sophisticated and they revive techniques that have previously been developed for the less selective ultraviolet (UV) and fluorescence detection. When LC-MS was introduced into water analysis it was hoped that these clean-up procedures would become obsolete (‘dilute and shoot’). Most annoyingly, the matrix effects occurring in the API process (namely when using ESI) may force us to apply these techniques to LC-MS also.

4. Conclusions

Though LC-MS has provided unparalleled analytical capabilities to environmental analysts, including water analysts, strong obstacles have meanwhile been recognized. In non-targeted analysis the information obtained by a quadrupole or ion-trap mass spectrometer in conjunction with API is often not sufficient in terms of mass resolution and fragmentation to identify completely unknown compounds. The same is true in non-targeted screening analyses. To date, the benefit of mass spectral libraries has been limited, as is their transferability from one instrument to another. Owing to its higher mass resolution, TOF-MS, especially when preceded by a quadrupole MS, should be able to contribute significantly to both tasks of qualitative analysis of water constituents. Based on the fact that many functional groups show characteristic fragmentations or form characteristic fragments, a tandem mass spectrometer may be used as a compound class-specific detector.

LC-MS is most commonly employed to detect and to quantify target analytes and this is certainly the most potent field for this instrumentation. For both applications, however, measures of quality assurance and control should be applied more widely and on a regular basis. To avoid false positive findings, stringent criteria, such as those proposed in residue analysis, have to be applied in water analysis and MS/MS is recommended to confirm positive findings.

The same is true with respect to quantitative analysis using LC-API-MS, where the awareness of potential matrix effects is increasing. Some strategies are available to partially compensate matrix effects. However, it may be necessary to revive sample clean-up methods, previously developed for use with UV and fluorescence detection, that are able to remove the disturbing matrix.

References