Coupled Liquid Separation and Spectrometric Detection of Organic Compounds Containing Hetero-atoms

BY

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Abstract

This thesis exemplifies the strength in the combination of inductively coupled plasma (ICP) spectrometry and electrospray ionization tandem mass spectrometry ESI-MS/MS as detection techniques for liquid chromatography (LC) in the search for and investigation of compounds that bind or can bind hetero-atoms. Furthermore, some aspects involved in the coupling of LC and ICP spectrometry and quantification without identical standards have been studied.

The importance of using a separation step in combination with ICP spectrometry was shown for urine and blood plasma samples from patients treated with boron neutron capture therapy. In addition to the carrier molecule used in the therapy, one major and a few possible minor metabolites were found in the urine samples. One fragment mass of the major metabolite was obtained with LC-ESI-MS/MS. Liquid chromatography coupled to ICP-MS was also shown to be a valuable tool for fingerprinting metal-binding compounds in complex matrices, such as siderophores (iron-complexing compounds) in soil. The presence of at least two siderophores in a field soil solution sample could be revealed by LC-ICP-MS. Their identities could thereafter be determined by LC-ESI-MS/MS.

The non-UV-absorbing α-carboranylalanine could be quantified in relation to its degradation products by LC-ICP-AES, which provided information about the mechanism behind the degradation. Moreover, LC-ICP spectrometry was shown to provide an accurate quantification of biomolecules (bias < 10%) when evaluated from external calibration graphs based on inorganic elemental standards.

Finally, the causes of the large decrease in boron signal seen when adding acetonitrile to the LC mobile phase in LC-ICP-MS was investigated in some detail. Space charge effects might explain a large part of the depression from carbon species on the boron signal.

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PAPERS INCLUDED IN THE THESIS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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Paper not included in the thesis:

Author contribution
I was responsible for planning, carrying out the experiments and writing Papers I and III–IV. I was also accountable for a major part of the analysis of the results in Papers I and IV. Paper II was carried out in collaboration with My Moberg. In Paper V I took some part in the experimental planning and in the discussion of the results.
## Contents

1 Introduction .................................................................................................................. 1  

2 Speciation analysis ......................................................................................................... 2  
   2.1 Speciation analysis with plasma spectrometry ..................................... 3  
      2.1.1 The ICP technique ................................................................. 4  
      2.1.2 Mass analysers coupled to ICP ............................................. 7  
      2.1.3 LC in speciation analysis with ICP spectrometric detection ...... 9  
   2.2 Speciation analysis with electrospray ionization mass spectrometry 10  
   2.3 A comparison of ICP-MS and ESI-MS/(MS) as detector principles in liquid separation techniques .................................................. 12  
   2.4 Selected analytical applications of combined LC-ICP spectrometry and (LC-)ESI-MS/MS .............................................................................. 15  
      2.4.1 Investigation of a possible metabolite of \( p \)-boronophenylalanine in urine and plasma samples ......................................................... 16  
      2.4.2 Siderophore mapping ............................................................... 18  

3 Aspects of on-line ICP spectrometry in chemical analysis ......................................... 20  
   3.1 Organic solvents ................................................................................. 20  
      3.1.1 Factors influencing the performance of the plasma 20  
      3.1.2 Factors influencing the sensitivity .............................................. 22  
   3.2 Low flow rates .................................................................................... 25  
      3.2.1 Low-flow nebulizers ................................................................... 26  
   3.3 Quantification ..................................................................................... 29  
      3.3.1 Quantification without identical standards ................................. 29  
      3.3.2 Generic detection ........................................................................ 30  
      3.3.3 Accuracy and precision ............................................................... 32  

4 Concluding remarks and future aspects ...................................................................... 33  

5 Acknowledgements ............................................................................................ 34  

6 Summary in Swedish: Kopplad vätskeseparation och spektrometrisk detektion av organiska föreningar innehållande hetero-atomer .............................. 35  
   6.1 Kort om ICP-spektrometri och ESI-MS ............................................. 35  
   6.2 Aspekter på kopplingen av LC och ICP-spektrometri respektive ESI-MS .......................................................... 36  
   6.3 Kvantifiering med LC-ICP-MS .......................................................... 38  
   6.4 Slutord ................................................................................................ 38  

7 References ............................................................................................................... 40
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>AES</td>
<td>atomic emission spectrometry</td>
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<tr>
<td>APCI</td>
<td>atmospheric pressure chemical ionization</td>
</tr>
<tr>
<td>API</td>
<td>atmospheric pressure ionization</td>
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<tr>
<td>BNCT</td>
<td>boron neutron capture therapy</td>
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<td>BPA</td>
<td>$p$-boronophenylalanine</td>
</tr>
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<td>BPA-X</td>
<td>a possible metabolite to BPA</td>
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<td>CE</td>
<td>capillary electrophoresis</td>
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<tr>
<td>CFN</td>
<td>cross-flow nebulizer</td>
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<td>DIN</td>
<td>direct injection nebulizer</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
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<tr>
<td>HEN</td>
<td>high-efficiency nebulizer</td>
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<td>IC</td>
<td>ion chromatography</td>
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<td>ICP</td>
<td>inductively coupled plasma</td>
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<tr>
<td>i.d.</td>
<td>internal diameter</td>
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<td>ID</td>
<td>isotope dilution</td>
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<tr>
<td>IS</td>
<td>Ionspray®, pneumatically assisted electrospray</td>
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<tr>
<td>LC</td>
<td>liquid chromatography</td>
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<tr>
<td>MCN</td>
<td>microconcentric nebulizer</td>
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<tr>
<td>MIP</td>
<td>micro-wave induced plasma</td>
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<tr>
<td>MS</td>
<td>mass spectrometry</td>
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<tr>
<td>MS/MS</td>
<td>tandem mass spectrometry</td>
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<tr>
<td>m/z</td>
<td>mass-to-charge ratio</td>
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<tr>
<td>ODS</td>
<td>octadecylsilane</td>
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<tr>
<td>PGC</td>
<td>porous graphitic carbon</td>
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<tr>
<td>Q</td>
<td>quadrupole</td>
</tr>
<tr>
<td>r.f.</td>
<td>radio frequency</td>
</tr>
<tr>
<td>RP</td>
<td>reversed-phase</td>
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<tr>
<td>RSD</td>
<td>relative standard deviation</td>
</tr>
<tr>
<td>SEC</td>
<td>size-exclusion chromatography</td>
</tr>
<tr>
<td>SF</td>
<td>sector field</td>
</tr>
<tr>
<td>TOF</td>
<td>time-of-flight</td>
</tr>
<tr>
<td>USN</td>
<td>ultrasonic nebulizer</td>
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<td>UV</td>
<td>ultraviolet</td>
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</table>
1 Introduction

The technique that is prevalently used in the chemical analysis of mixtures of non-volatile organic substances is liquid chromatography (LC) with ultraviolet (UV) absorption detection. When higher sensitivity and/or analyte mass information is needed LC in conjunction with electrospray ionization mass spectrometry (ESI-MS) is often the choice. However, in certain applications it is not possible to use these methods. The analytes might have low or no absorption in the ultraviolet region or may be difficult to ionize in ESI-MS. Other reasons for applying an alternative detection technique can be if a very accurate trace quantification is needed or simply, if additional information about the analytes is desired. When the substances to be analysed contain an element different from hydrogen, carbon, nitrogen and oxygen, atomic spectrometry may be an alternative detection method. In this thesis such organic substances (called compounds/species/substances containing hetero-atoms) are determined with inductively coupled plasma (ICP) spectrometry. The expression ICP spectrometry is here addressed to both ICP atomic emission spectrometry (AES) and ICP-MS.

This thesis is a contribution to the description and comparison of ICP spectrometry and ESI-MS as detection methods in liquid chromatography. Many features of LC hyphenations also apply to other liquid based separation techniques like capillary electrophoresis (CE). Special emphasis is put on the on-line coupling of liquid separation and ICP spectrometry. In this field, there are three issues that have to be especially considered and that are described in more detail: 1) the presence of organic solvents in the LC eluent (Paper V), 2) low flow rates and 3) quantification without identical standards (Paper III–IV). The parallel hyphenation of LC to ICP spectrometry and ESI tandem MS (MS/MS) is exemplified by selected applications from different fields (Paper I–II).
2 Speciation analysis

The definition of speciation analysis set by *The International Union of Pure and Applied Chemistry* (IUPAC) [1] is “analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample”. A chemical species is a “specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure”. The broad definition of speciation analysis includes almost any of the performed separations, identifications and determinations of compounds involved in analytical chemistry. However, the term speciation analysis is almost solely used by people working with compounds that contain hetero-atoms such as methylated tin species in fish [2], selenium compounds in human urine [3] and iodine containing proteins in breast milk [4]. The scope of a speciation analysis is thus to determine the compositions and concentrations of the various species that contain the hetero-atom, and not only its total concentration.

Analytical problems of speciation character often involve the determination of trace concentrations of analytes in difficult matrices, like biological fluids. Direct determinations by electrochemical techniques, like potentiometry with ion-selective electrodes and voltammetry as well as direct UV-visual molecular spectrophotometry and fluorimetry, have during the last decades drastically lost in importance due to lack of the necessary selectivity and sensitivity required for real-life samples [5]. Combined with a separation of the analyte/s from the matrix prior to the detection, the performance of these techniques is improved, although their selectivity and sensitivity still can be limiting. The element-specific atomic spectrometric techniques have during the same time period gained in importance. This pertains especially to the plasma based techniques, such as ICP spectrometry [6], due to their capability of relatively matrix-independent and sensitive on-line monitoring of effluents from separation systems. Flame atomic absorption spectrometry (FAAS) [7-9] and electrothermal atomic absorption spectrometry (ETAAS) [7-10] are also used in speciation analysis but have, in addition to a high matrix dependence, problems with sensitivity and on-line monitoring, respectively. Finally, the soft ionization techniques, like ESI-MS have become important tools in speciation analysis, especially for identification of unknown compounds. More information about the ICP and soft ionization techniques as such and their relative merits in speciation analysis can be found in Sections 2.1, 2.2, and 2.3, respectively. Some of the
numerous published general reviews on elemental speciation which are relevant to the subject of this thesis can be found in Refs. [8,9,11-16]. Although the separation capabilities and limits of detection have improved substantially with the refinement of old analytical techniques and the development of new techniques during the last decades, speciation analysis at trace concentrations in a complex matrix can still be challenging.

The stability of the chemical species is often an issue of great concern in speciation analysis. Many species in, for example, environmental samples can be altered into new species when their environment is changed such as upon exposure to sunlight, dilution etc. It is thus very important to be aware of that the speciation may change during sampling, storage, sample preparation, and separation. If proper care is not taken the results can be totally erroneous. The stability of chemical species is not considered further in this thesis, but is dealt with in, for example, Refs. [8,17-19].

2.1 Speciation analysis with plasma spectrometry

In the field of speciation analysis, a large number of the researchers use some kind of separation technique connected off-line or on-line to an element specific detector, such as an ICP or microwave induced plasma (MIP) instrument. The MIP-AES [20] and MIP-MS [21] techniques are mainly used together with gas chromatography due to low plasma temperatures and hence low tolerance to liquid solvents. With the invention of the high-power nitrogen MIP techniques, the MIP can, however, compete in sensitivity with low-resolution ICP-MS in the quantification of elements that are prone to spectral interferences from argon polyions. One example is the speciation analysis of arsenic in urine [22].

The inductively coupled plasma is used for atomization, ionization and excitation at atmospheric pressure. In 1974 the first commercially available ICP-AES instruments were introduced and in 1983 the first commercial ICP-MS apparatus entered the market [23]. The ICP techniques were in the beginning used solely to determine the total concentration of a specific element. Speciation analysis and inclusion of a separation step prior to detection was triggered by the discovery that different species of the same element might have different toxicities. For instance, Cr(III) is an essential trace element, while Cr(VI) is toxic [24]. The first published on-line coupling of LC and ICP spectrometry appeared in 1979 [6,25]. The number of publications in this area has steadily increased each year up to 2002 and has exceeded 100 per year since the turn of the century.
2.1.1 The ICP technique

The ICP spectrometer consists of a sample introduction device, a plasma region and a detector. The mainly used plasma gas is argon, since it can efficiently excite and ionize most of the elements in the periodic system simultaneously, which makes multielement analysis possible. A liquid sample is self-aspirated or pumped to a nebulizer where an aerosol is created. In pneumatic nebulization the aerosol is generated through the interaction of a high-speed gas (nebulizer gas) and the liquid at the exit of the nebulizer, seen on the right in Figure 1b. The created aerosol consists of the plasma gas, particles (solid and liquid droplets) and solvent vapour [26]. The nebulizer is often mounted with its tip in a spray chamber, the main task of which is to remove the aerosol droplets that are too large (> 10 µm in diameter) for the plasma [27]. Larger droplets up to 100 µm in diameter do not undergo a sufficiently rapid desolvation, vaporization and atomization in the plasma [27]. They cool the plasma which causes a reduction in precision and an enhancement of interferences. The volume of solvent that enters the plasma must also be restricted to typically 30 µl min\(^{-1}\) or less [28]. Nebulizers working in the ml min\(^{-1}\) range and used in conjunction with LC are often pneumatic, such as the concentric nebulizers [29], which most often are of the Meinhard type (Figure 1a), and the cross-flow nebulizers.

![Figure 1a. Schematic of a Scott double pass spray chamber with a pneumatic concentric (Meinhard) nebulizer (not drawn to scale). b. An enlargement of the nebulizer outlet.](image)
Together with a Scott double pass spray chamber (Figure 1a), generally less than 3% of the sample enters the plasma [26]. Another frequently employed nebulizer in LC-ICP spectrometry is the ultrasonic nebulizer (USN) [30]. This nebulizer creates aerosols by transferring acoustic energy to the liquid. Together with a desolvation system it comprises one of the most efficient conventional nebulizers with an increase in sensitivity of typically a factor of five to ten [26] compared to, for example, the Meinhard nebulizer, since it produces a larger fraction of small droplets. Nebulizers used at lower flow rates are described in Section 3.2.1.

Not only the type of nebulizer, but also the choice of spray chamber will influence the sensitivity. Cyclonic spray chambers (Figure 2) increase the analyte transport efficiency a half time to three times compared to the Scott double pass chamber [31], due to a less efficient filtering action. This results in a somewhat coarser aerosol and also a higher sensitivity [32], at least for aqueous aerosols.

The nebulizer gas transports the aerosol through the spray chamber to the plasma (Figures 1–2). The hot plasma, which has a gas temperature of 5000 – 9000 K [23], is a mixture of electrons, argon ions and argon atoms. It is created and sustained by the argon gas flows and a magnetic field generated by a radio frequency (r.f.) current in the induction coil (Figure 2). To initiate the plasma, a spark from the coil is needed in order to give some electrons enough energy to ionize argon atoms. Electrons released in the collisions participate in the transfer of energy between the coil and the argon gas.

![Figure 2. Schematic of the spray chamber (cyclonic), torch and plasma region in an axial ICP spectrometer (not drawn to scale).](image)

After desolvation of the solvent in the plasma, the chemical compounds in the aerosol are vaporized and finally broken down almost completely into atoms and ions, of which a certain fraction also are excited. The wavelength and the intensity of the light emitted from the plasma on de-excitation are measured in ICP-AES, while the mass-to-charge ratio (m/z) and the intensity of the ions extracted from the plasma are measured in ICP-MS. An ICP-MS
instrument equipped with a quadrupole (Q) provides up to three to four orders of magnitude lower detection limits than the ICP-AES technique [33]. The large difference in sensitivity is mainly due to a high continuum optical background in ICP-AES caused primarily by recombinant radiation processes [33]. A corresponding source of continuum mass to charge ratio ions is not present in ICP-MS [23]. Other differences between AES and MS include the ability of MS to obtain relatively simple spectra [23] and to monitor isotopes.

The ICP spectrometry is a mass-flow sensitive technique, but have been reported to behave like a concentration sensitive detector (independent of flow rate) for a CFN between 0.8 and 2 ml min⁻¹ [6]. This concentration sensitive behaviour has been shown to be the result of a strong decrease in nebulization efficiency at higher flow rates [33]. A high analyte transport efficiency and/or low solvent mass load to the plasma for low-flow nebulizers [34] can result in a reverse mass-flow sensitive behaviour [35] of the plasma. This behaviour is illustrated in Figure 3. Flow injections of an aqueous solution containing some elemental standards to a microconcentric nebulizer coupled to an ICP-AES instrument resulted in an increase in peak area by 60 % as the flow rate decreased from 200 to 50 µl min⁻¹ [36].

The advantages of the plasma techniques are their high sensitivity and selectivity. When, for example, UV detection fails due to low absorbivities of the analytes, ICP spectrometry can be a good choice for compounds that contain hetero-atoms [III]. The ICP technique is very selective, compared to

![Figure 3](image_url). Peak area versus flow rate for flow injections of 20 µl 10 ppm Al, Mg and Mn measured with a microconcentric nebulizer (MCN-100) coupled to an ICP-AES instrument. No optimization of the signal was performed between the changes in the liquid flow rate.
UV detection. In Figure 4 an LC-ICP-AES chromatogram showing the boron signal from a urine sample is compared with the corresponding LC-UV chromatogram. Many compounds in the urine are UV-absorbing at a wavelength of 254 nm, while only the compounds that contain boron are visible in the LC-ICP-AES trace.

![LC-ICP-AES chromatogram of a urine sample monitoring the boron 249.678 nm emission line.](image)

![LC-UV chromatogram at 254 nm. Peak assignment: 1. p-boronophenylalanine (BPA) 2. a possible metabolite to BPA (BPA-X).](image)

**Figure 4a.** LC-ICP-AES chromatogram of a urine sample monitoring the boron 249.678 nm emission line. **b.** LC-UV chromatogram at 254 nm. Peak assignment: 1. p-boronophenylalanine (BPA) 2. a possible metabolite to BPA (BPA-X). Due to a varying day-to-day performance of the column, the retention times are somewhat different [1].

2.1.2. Mass analysers coupled to ICP

The types of mass analysers available in commercial ICP-MS instrumentations are the quadrupole, the time-of-flight (TOF) and the sector-field (SF) (also called double focusing, DF) analyser. Details of their characteristics and operation can be found in Turner et al. [37] and in Refs. [38] (axial TOF), [39] (orthogonal TOF) and [40,41] (SF), respectively. The ICP-QMS is the most common technique in use today, due to its relatively low price and since it often provides sufficiently high speed and sensitivity.

A brief comparison of the features of the three mass analysers is shown in Table 1. In speciation analysis, often one single element or isotope is measured at a time. Hence, when coupled to liquid chromatography, the scanning analysers (Q and SF) generally provides sufficient sampling of the
LC effluent. However, for multiple fast transient peaks in, for example, CE-ICP-MS [42] and different types of flow injection (FI)-ICP-MS systems with [43] or without [44] isotope ratio measurements a faster mass analyser is needed. The TOF mass analyser detects the ions quasi-simultaneously (simultaneous ion sampling but non-simultaneous detection) and can thus sample multiple narrow peaks more accurately.

Table 1. *A general comparison of the Q-, TOF- and SF-ICP-MS techniques.*

<table>
<thead>
<tr>
<th></th>
<th>Q</th>
<th>TOF</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed (per full mass scan)</td>
<td>Min. 0.1 s [45]</td>
<td>50 µs [44]</td>
<td>About 0.5 s [45]</td>
</tr>
<tr>
<td>Resolution</td>
<td>Approx. unit mass (non-sufficient for elements like P, S, Fe)</td>
<td>Approx. unit mass (non-sufficient for elements like P, S, Fe)</td>
<td>About 300, 3000, 10000 (m Åm⁻¹) [37]</td>
</tr>
<tr>
<td>Mass analyser sensitivity</td>
<td>High ppq to low ppt for elements not suffering from spectral interferences [45]</td>
<td>Low ppt (axial TOF) [38] and high ppq to low ppt (orthogonal TOF) [39] for elements not suffering from spectral interferences</td>
<td>Middle to high ppq for most elements [40]</td>
</tr>
<tr>
<td>Purchase price</td>
<td>~1.4 MSEK</td>
<td>~1.4 MSEK</td>
<td>2.5 - 3 MSEK</td>
</tr>
</tbody>
</table>

The low-resolution instruments (Q and TOF) with an approximately unit mass resolution show a lot of spectroscopic interferences especially below a relative mass of 82. Singly and doubly charged ions or polyatomic ions, such as the ArO⁺, ArN⁺ and Ar₂⁺ species from the plasma gas, cannot be resolved from the analyte ions having the same integer m/z. Above a relative mass of 82, spectral interferences from polyatomic ions are essentially negligible. The challenge is that the elements of interest in, for instance, biological applications have relative masses below 82 [46]. Many of these spectral interferences can be eliminated by the SF instrument run at a resolution setting of 3000–4000 [47]. In this way difficult elements such as phosphorus [48-50], sulphur [50,51], calcium [50] and iron [50-52] can be distinguished from spectral interferences. Commercial available are also quadrupole instruments equipped with a collision or reaction cell that can extend the number of determinable isotopes through at least a partial removal of

* Resolution between two adjacent peaks defined as the mean mass 
  m divided by the difference 
  in mass 
  Åm.
polyatomic interferences or by generating less interfered polyions of the analyte isotopes [49,53].

The SF instrument has the highest sensitivity overall (middle to high ppq for most elements) due to a very low noise level and a high ion transmission [41]. At a resolution setting of 300, 10–100 times higher sensitivity compared to Q instruments can be achieved [47]. At a resolution of 3000, a factor of ten is lost in sensitivity, but still the detection limits are lower than for ICP-QMS instruments [41]. The axial ICP-TOFMS instrument used today loses a part of the ions during the modulation of a continuous ion beam to discrete ion packages, which results in detection limits that are about ten times higher for most elements compared to the Q analyser [38]. Similar detection limits as for the Q instruments have been reported for the orthogonal TOF analyser on the market [39]. When choosing instrument also the price may be of importance. The cost of an ICP-QMS or an ICP-TOFMS instrument in 2003 was about one and a half million SEK, while the cost of an ICP-SFMS instrument was nearly twice as high.

2.1.3 LC in speciation analysis with ICP spectrometric detection

The most commonly used LC methods in speciation analysis with ICP spectrometry are ion chromatography (IC) for inorganic ions such as Cr(III) and Cr(VI) [33] and arsenic and selenium species [8,33], and size exclusion chromatography (SEC) for macromolecules [8]. Reversed-phase (RP) LC is used for relatively small non-polar to (medium-)polar compounds such as arsenic and selenium species [8] and boron containing amino acids [I,III]. Ionic, polar species often need an ion-pairing agent added to the mobile phase to be retained in RPLC. RPLC is also used for larger compounds, including peptides and proteins [8]. The non-polar octadecylsilane (ODS) and octylsilane stationary phases are most frequently used in RPLC [9]. The normal-phase LC hyphenation to ICP spectrometry is less employed than the RPLC variant, but has been used in, for instance, phospholipid analysis [54].

The IC and SEC techniques are generally directly compatible with the ICP spectrometer, since buffered aqueous mobile phases are well tolerated by the plasma. High buffer concentrations can, however, erode and clog the cones in ICP-MS instruments [55]. One has also to be aware of possible spectral and non-spectral interferences from the buffer components for certain isotopes, as described in Section 2.3. The reversed- and normal-phase LC mobile phases involve more or less of an organic solvent and special precautions often have to be taken in order to sustain a stable plasma, see the more detailed discussion on organic solvents used especially in RPLC in Section 3.1.

Samples of, for example, biological origin can often be very complex. Multi-dimensional techniques with two or more separation steps off- and/or on-line to the detector can be necessary in order to resolve the components if
interest [14]. A separation based on molecular size is then often followed by a more selective RPLC or IC separation technique.

2.1.3.1 Hyphenation with reversed-phase LC using porous graphitic carbon

A stationary phase in RPLC, which is more hydrophobic than ODS phases for non-polar compounds but also exhibits some special retention mechanisms for polar compounds, is porous graphitic carbon (PGC), now marketed as Hypercarb™. The PGC material was developed by Knox and co-workers [56] and several reviews describe its properties etc. [57,58]. PGC is composed of flat sheets of carbon atoms bound in a hexagonal arrangement. Its unusual retentive behaviour for polar solutes has been denoted the polar retention effect on graphite (PREG) [59] and provides an alternative and attractive complement to IC [60] for anions. The use of the PGC material with ICP spectrometry is scarce, but has been reported for speciation analysis of arsenic [61], selenium [62,63], boron [I,III] and gallium [II].

The conductive property of the PGC material may necessitate special measures to be taken in the coupling to ESI-MS [58]. This is, however, not a problem in conjunction with ICP spectrometry. For relatively non-polar compounds, the increased amount of organic solvent that may be needed in the mobile phase compared with ODS materials, often induce the need for extra precautions in order to retain a stable plasma and a sufficient sensitivity, see Section 3.1.

2.2 Speciation analysis with electrospray ionization mass spectrometry

Soft ionization techniques at atmospheric pressure, like ESI and pneumatically assisted ESI (often called Ionspray®, IS), are suitable for speciation studies. Hereafter, the abbreviation ESI will be used for pure ESI and IS when both nebulization techniques are meant.

In short, the electrospray process is a nebulization of a liquid into charged droplets, which subsequently end up in gas phase ions. The whole process is induced by a large electric field. In IS a gas and/or heat assists in the drying of the droplets. The generated ions in the positive ion mode are normally pseudomolecular [M+H+], or adducts with buffer additives such as sodium and ammonium ions. The electrospray processes are described in Refs. [64-66]. Another atmospheric pressure ionization (API) technique, atmospheric pressure chemical ionization (APCI), is an alternative in speciation analysis. The analytes investigated for speciation analysis purposes are, however, most often highly polar or ionic and therefore ESI is the most useful API
method [66]. A recent review on the use of ESI and APCI in speciation analysis was written by Rosenberg [66].

The ESI can be interfaced to a number of different mass analysers, roughly the same ones as used in ICP instruments. The quadrupole is also here by far most commonly used due to its availability rather than optimal performance. An account of fundamentals of and the instrumentation used in ESI-MS can be found in Ref. [64]. The ESI-MS technique can be used both for molecular and elemental analysis [67]. Corr have used IS-MS/MS in molecular and elemental mode for the quantification of tributyltin and arsenobetaine in two reference materials [68]. However, the great usefulness of API-MS in speciation analysis is the possibility of acquiring structural information preferably using an MS/MS instrument, such as a triple quadrupole, as a complement to the elemental ICP-MS analysis. The use of different operational modes in ESI-MS/(MS) for speciation analysis purposes are summarized in Ref. [67]. One application using a triple quadrupole instrument for structural investigation (Paper I) is presented in Section 2.4.1.

The ESI-MS technique has been used in speciation analysis of non-complex samples without a prior separation step. Stewart et al. determined some sulphur species with direct-infusion ESI-MS [69]. They also quantified sulphate in a waste-water sample by monitoring the hydrogen sulphate and sodium sulphate ions using standard additions of sulphate with iodide as internal standard.

For more complex samples the ESI process may be sensitive towards matrix components, and then a separation step can provide improved data. Sharp et al. [70] have shown that a separation of the analytes from the matrix is highly desirable prior to the ESI-MS determination in speciation analysis, since the ESI-mass spectra can be very complex, even for solutions with few components. In addition, the spectra are very sensitive to alterations in the experimental conditions and new species can be formed in the gas phase.

To improve the detection capability in the MS/MS system, the analyte can be collected from the LC system. The selected fraction is thereafter pre-concentrated and analysed off-line by either ESI-MS/MS [71] or so-called nanospray-MS/MS [62]. In the later case, a few microlitres of the sample are placed in a conducting capillary from which the nanospray is generated at a sufficiently high applied voltage [72,73]. The low sample flow rate of about 20 nl min⁻¹ from the nanospray capillary, makes the sample volume last for measurement during half an hour or longer. Hence, there is a gain in both analyte concentration and the time available for analysis.
2.3 A comparison of ICP-MS and ESI-MS/(MS) as detector principles in liquid separation techniques

“Electrospray and ICP-mass spectrometry: enemies or allies?” is the title of a paper written by Houk [74], where Houk draws the conclusion that the ICP and ESI/IS techniques are complementary and proposes them to be used in parallel and fed from a single LC device. This has not been much realized yet, probably due to logistic reasons. So far, there does not exist any dual ionization instrument with both ICP and ESI/APCI capability on the market. Wilson and co-workers [75,76] have used a hyphenated LC-ICP-MS/ESI-MS/(MS) system in order to identify some metabolites of 4-bromoaniline [75] and to determine the ESI-MS/MS response of some organic platinum species from the simultaneously registered ICP-MS chromatogram [76]. In Paper II, the sensitivity of the LC-ESI-MS system was very low in the scans for the masses of two unknown gallium-desferriisiderophores that had been discovered by LC-ICP-MS. It would hence have been a great help to have had the ICP-MS and ESI-MS/MS instruments monitor the same LC-flow. In this way more exact retention times of the analyte peaks would have been known for the ESI-MS trace.

A subjective comparison of the performances of ICP-MS and ESI-MS techniques and their pros and cons in speciation analysis are listed in Table 2. In the comparison, the ESI device is assumed to be coupled to a single or triple quadrupole mass spectrometer. The techniques are compared in more detail in the text with the schematic comparison in Table 2 as a basis.

Identification. Because of the almost complete destruction of the molecular structure, the identification of a specific species with the LC-ICP technique relies solely on peak matching with an identical standard. As will be further discussed in Section 3.3 the number of reference standards in, for example, the environmental, biological and clinical areas is limited. The possibility of structural elucidation from small amounts of the analyte with ESI-MS/MS makes this technique a good alternative to nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy.

The combination of LC-ICP-MS and (LC-)ESI-MS/MS can provide information on the existence, polarity and even the structure of, for example, a drug metabolite in urine. However, to obtain a more certain identification of the unknown compound, the compound suggested by the MS/MS analysis should be synthesized, if not already available as a standard, and analysed with the same MS/MS settings. Its molecular ion and fragmentation patterns must agree with those of the compound in the sample for a positive identification [77,II].

Sensitivity. When run in the elemental mode ESI-MS is free from many polyatomic interferences that are often present in ICP-MS analysis. On the
Table 2. A subjective and generalized performance comparison of ICP-MS and ESI-MS/(MS) in speciation analysis.

<table>
<thead>
<tr>
<th></th>
<th>ICP-MS</th>
<th>ESI-MS/(MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>Poor</td>
<td>(Reasonably) good</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Good</td>
<td>Moderate/good</td>
</tr>
<tr>
<td>Quantification</td>
<td>Good</td>
<td>Moderate</td>
</tr>
<tr>
<td>Non-volatile additives</td>
<td>Reasonably good</td>
<td>Poor</td>
</tr>
<tr>
<td>Organic solvents</td>
<td>Poor/moderate</td>
<td>Good</td>
</tr>
</tbody>
</table>

On the other hand, the limit of detection is generally two to three times higher than for ICP-MS [67]. The same difference in sensitivity (10–100 ppb and 0.1 ppb, respectively) is often also seen in the molecular mode [78]. The sensitivity in ESI-MS generally decreases with polarity for ions and charged adducts formed in the electrospray process [79]. In ICP-MS the sensitivity is determined mainly by the nebulization and ionization efficiencies of the elements, the isotope abundance, and possible spectral interferences.

The ESI-MS technique has an upper limit in linearity usually at about 10 µM, which might be caused by the droplet surface being either charge or space limiting at this concentration [80]. A linear range of more than four orders of magnitude is rarely seen with continuous infusion of the standard solutions, even for surface-active substances. The detection limits are background limited, but can be improved by making the spectrometer more selective and hence limiting the chemical noise [80]. This is often done by running MS/MS instruments in multiple reaction monitoring (MRM) mode where, for example, the parent ion is selected in the first analyser. Thereafter it is fragmented and one of its daughter ions is monitored in the second mass analyser. In LC-ICP-MS a linear range of five orders of magnitude can sometimes be obtained [76].

**Quantification.** Quantification of a complex sample with external calibration implying identical standards of different concentrations can provide an imprecision and bias close to or within ten percent measured as relative standard deviation (RSD) and percentage of the “true” concentration, respectively, in LC-ESI-MS [81] and LC-ICP-MS [82]. The percentages were based on repetitive measurements on four and eight samples, respectively. At least a certain degree of sample clean-up was performed before the quantification. As a comparison, validated analytical methods for drugs and their metabolites are accepted if they have a bias and
imprecision of 15 % or less, except at the limit of quantification, where the deviation is extended to 20 % [83].

The LC-ESI-MS determination of polar compounds like sugars and other substances containing several hydroxyl, carboxyl or amino functions in complex samples is also more affected by matrix suppression compared to more non-polar compounds [84]. Hence, for polar substances, such as arnesosugars and for medium polar substances in complex samples inclusion of one or several of the following steps in the analytical procedure is advisable: a more extensive sample clean-up [85], a calibration with the standard addition method [86], or a pre- or post-column addition of a stable isotope labelled internal standard [87]. With the rather expensive isotope dilution (ID) technique the imprecision and bias can be within a few percent RSD in LC-ESI-MS [81,88] and LC-ICP-MS [89] analyses. Quantification by means of a structurally similar internal standard in order to improve the precision and accuracy in LC-ESI-MS requires an extensive sample clean-up to eliminate the matrix effects [81,88]. In LC-ICP-MS an internal standard of the same element as the analytes, but not necessarily a structural analogue, can be used for quantification [90]. An increasing number of ICP-users have noted that fairly similar responses can be obtained regardless of the structure of the analyte element containing species [IV and refs. therein]. The use of identical standards can hence be unnecessary when the utmost accuracy is not crucial. Quantification in LC-ICP spectrometry is further discussed in Section 3.3.

Non-volatile additives. Many analytical methods in RPLC, IC, ion-pair RPLC, and SEC with UV detection employ non-volatile mobile phase additives, such as phosphate and carbonate buffers or non-volatile ion-pairing agents. These additives are not very well tolerated in ESI-MS due to suppression effects of the analytes and orifice contamination. It is generally suggested that the more volatile acetate and formate buffers or volatile ion-pairing agents, such as heptafluoro butanoic acid, should replace the non-volatile buffers and additives in the LC-eluent. Post-column addition of an aqueous-organic solution as well as diverting the column effluent away from the ESI interface when no analyte is eluting can be used to decrease the negative effects from non-volatile additives on the ESI interface [91]. With the latter method, the possible suppression effects from the non-volatile additives on the analytes persist, while the addition of a sheath-flow with organic solvent [92] can improve the sensitivity. An MS system equipped with a so-called Z-spray interface allows the use of non-volatile buffers [93,94] with an un-impaired sensitivity [94] from its dual orthogonal construction. Suppression effects as well as source contamination can also be almost eliminated by using a post-column on-line trapping of ion-pairing agents on a strong anion-exchanger [95] or in a self-generating ion-suppressor [96]. Another remedy can be to change the stationary phase to,
for example, PGC that can make non-volatile ion-pair additives unnecessary [97].

The non-volatile additives are much better tolerated by the ICP, but problems with erosion and clogging of the sampler cone can occur from phosphate [55,98], and acetate [98] buffers. The performance of the LC-ICP-MS system can also be improved by replacing sodium with volatile ammonium containing buffers [99]. Moreover, one has to be aware of possible spectral and non-spectral interferences on the analyte masses from the mobile phase additives in LC-ICP-MS. For instance, interferences from phosphorus species from phosphate buffers on the $^{63}$Cu and $^{64}$Zn isotopes can occur in ICP-MS.

**Organic solvents.** Organic solvents have opposing effects on the ICP-MS and ESI-MS performances. While a moderately polar organic additive such as methanol or acetonitrile is almost crucial for maintaining a stable electrospray by reducing the surface tension of the liquid, the organic solvent can easily extinguish the ICP if not special precautions are taken. The use of aqueous-organic aerosols in ICP spectrometry is further discussed in detail in Section 3.1. In RPLC at least one organic solvent is always added to the mobile phase, which makes this technique suitable for ESI-MS. In IC with no organic modifier added to the eluent stability problems of the electrospray can be imposed.

In addition, the liquid flow rate from the LC system has to be considered. In conventional chromatography, 4.6 mm internal diameter (i.d.) columns and flow rates of 0.5 to 2 ml min$^{-1}$ are common. These flow rates are in the same range as the liquid up-take rate in conventional ICP-MS instruments. The electrospray cannot cope with such high rates and the flow has to be split before entering the interface. With an optimized IS interface, flow rates up to 2 ml min$^{-1}$ can, however, be tolerated [35]. The ESI-MS instrument often behaves as a concentration sensitive detector in the low µl min$^{-1}$ range giving an increased sensitivity on decreasing the chromatographic column dimensions and flow rates [100]. The miniaturization of the LC system coupled to an ICP-MS instrument may decrease the sensitivity of the method, although a better tolerance to organic solvents and a higher analyte transport efficiency can result [34]. Low flow rates in LC-ICP-MS are further discussed in Section 3.2.

### 2.4 Selected analytical applications of combined LC-ICP spectrometry and (LC-)ESI-MS/MS

Both LC-ICP spectrometry and (LC-)ESI-MS/MS have a given position in speciation analysis. First, LC-ICP-MS is applied to determine the number of compounds that contain the element of interest and to identify known
analytes by peak matching with standards. Secondly, if sensitive enough [101] (LC-)ESI-MS((/MS) is used to confirm the identity of the known compounds and to collect information on the unknown species [I]. If desired, the compounds of interest can be quantified with LC-ICP spectrometry. The LC-ICP spectrometry can also be a valuable fingerprinting tool for metal-binding compounds in complex matrices prior to structural elucidation by LC-ESI-MS/MS [II]. A review article on combined elemental and molecular MS in speciation analysis has recently been published [102].

2.4.1 Investigation of a possible metabolite of p-boronophenylalanine in urine and plasma samples

In the project described in Paper I, the boron selective LC-ICP spectrometric method facilitated the discovery of an unknown possible metabolite (BPA-X) of p-boronophenylalanine (BPA) in urine and blood plasma samples from patients treated with boron neutron capture therapy (BNCT). Also some boron containing compounds at minor concentrations were found in urine samples collected 24-hours after the start of BPA administration, which could be metabolites or degradation products of BPA. These metabolites were not considered further in this study.

In a trial to produce BPA-X artificially a solution of BPA-fructose was added to urine and heated to 37 °C for eight hours. However, no metabolites were detected with LC-ICP-AES. Isotope measurements by LC-ICP-MS of the boron-10 (enriched) to boron-11 isotope ratios for BPA and its possible metabolite gave similar ratios for the two compounds and provided further evidence that BPA-X probably is a metabolite of BPA. Since the concentrations of BPA and BPA-X were sufficiently high in the urine samples, ICP-AES could be used for quantification of BPA and a determination of the molar BPA-X to BPA ratio in urine at different times after the start of BPA-fructose administration to the patients. The relative concentration of BPA-X increased with time as shown in Figure 5.

Capillary LC-ESI-MS/MS and nanospray-MS/MS (results not published) were used for structural investigation of the metabolite in urine. For the nanospray experiments the urine samples had been fractionated on a 2 mm i.d. PGC-column. The fractions containing BPA and BPA-X, respectively, were evaporated to dryness and diluted to a small volume with a suitable solvent for the nanospray device. Scans of the first quadrupole revealed that
an ion of $m/z = 251.1$ belonged to BPA-X. This fragment might correspond to an acetylated BPA. In a daughter ion scan the species of interest with a special $m/z$ was selected in the first quadrupole, fragmented in the second one and the resulting fragments were scanned in the third quadrupole. The daughter fragments of the ion at $m/z = 251.1$, see the nanospray-MS/MS spectrum in Figure 6, included the molecular ion of BPA (inset in Figure 6) at $m/z = 209.1$ and a fragment of BPA at $m/z = 163.1$, which confirmed that the ion at $m/z = 251.1$ comprises the BPA moiety.

**Figure 5.** Metabolite (BPA-X) to BPA peak area ratios in urine at different times after the start of BPA-fructose administration. Patients A–C and F were exposed to a complete BNCT treatment, while patients I and J received no or a negligible dose of BNCT.

**Figure 6.** Daughter ion scan of $m/z = 251.1$ of BPA-X with nanospray-MS/MS. The ion of $m/z = 209.1$ is the BPA molecular ion (see the inset structure) and $m/z = 163.1$ might be BPA minus a carboxyl group and a hydrogen atom.
In order to detect even higher masses, the first quadrupole was scanned for those ions that fragmented into the ion of $m/z = 251.1$, a so-called parent (or precursor) ion scan. A few masses were found in the parent ion spectra of the BPA-X chromatographic peak from capillary LC-ESI-MS/MS, although the intensities were very low. A proper assignment of the high-mass fragments of the metabolite was therefore not possible. No higher masses than that of $m/z = 251.1$ could be detected in the nanospray experiments, maybe due to aged urine samples. The complete structure of the unknown compound could not be elucidated with any of the techniques.

A PGC column that is not decoupled from the high-voltage applied to the electrospray needle, as was the case in this study, can experience a change in the normal chromatographic behaviour and new species can appear in the spectra due to electrochemical reactions in the system [103]. Since the BPA + 42 mass ion was also present in the off-line spectra from nanospray-MS/MS analysis, this ion is not believed to be an oxidation product of BPA formed on the PGC material during the electrospray process.

### 2.4.2 Siderophore mapping

In Paper II a parallel column-switched LC method for ICP-MS and ESI-MS/MS was set up for some siderophores of the hydroxamate-type. Siderophores are iron-complexing compounds excreted by microbes, fungi, and some plants under iron-deficient conditions to improve the iron-uptake by the organisms. Siderophores are not easily detected in natural samples by LC-ESI-MS. Due to the complexity of these samples, other compounds than the siderophores will give rise to a number of additional peaks in the mass spectra.

The elemental selectivity of the ICP-MS technique makes it suitable for detecting siderophores in complex samples. Unfortunately, the iron sensitivity of the ICP-TOFMS instrument used in this study is very poor, due to a spectral interference from ArO$^+$ on the major iron isotope. However, with an exchange of iron by gallium under reducing conditions, low nanomolar concentrations of the siderophores could be detected by the column-switched LC-ICP-TOFMS system. A field soil solution sample was screened for gallium, showing its siderophore content, see Figure 7. The probable identities of the two unknown siderophores were revealed as Ga-desferrioxamine and Ga-desferricrocin (peak 1 and 2, respectively, in Figure 7) with LC-ESI-MS/MS by comparison with fragmentation patterns of standards.
Figure 7. ICP-MS chromatogram of the siderophore content (peak 1 and 2) in a field soil solution sample treated with Ga(III) under reducing conditions.
3 Aspects of on-line ICP spectrometry in chemical analysis

This chapter describes in more detail how organic solvents and low flow rates affect the plasma and the sensitivity. Various aspects on quantification in on-line LC-ICP spectrometry are also considered.

3.1 Organic solvents

3.1.1 Factors influencing the performance of the plasma
In RPLC methods, the mobile phase contains one or sometimes a mixture of organic solvents. The relative amount of organic solvent can vary from a few percent up to 100 % (v/v) in gradient methods. The solvents most often added to the mobile phase are methanol and acetonitrile [15]. Organic solvents are generally not compatible with the ICP, since the plasma is cooled by the large quantities of solvent vapour [104] and it can become unstable or even extinguish. In addition, carbon deposits can evolve on the torch as well as on the sampler and skimmer cones. The organic solvent also elevates the reflected power, which in the long run may lead to generator break down [105].

The first thing to do when setting up a new method that employs an organic solvent is therefore to verify that the plasma remains stable over prolonged periods of time at the actual mobile phase composition. The ICP has a varying tolerance towards different organic solvents and the maximum tolerable solvent plasma load has been found to be a proper measure when comparing different solvents [106]. According to Molinero et al. [107] the heat of vaporization, density, surface tension, viscosity, heat capacity and heat of atomization of the solvent are the most important parameters in determining the characteristics of the aerosol from a pneumatic nebulizer. Methanol and acetonitrile have a low surface tension and heat of vaporization compared to many other solvents [107] including water. These inherent properties result in small mean diameters of the drops [108], but also in a large solvent mass loading to the plasma. Probably, it is the high energy required to vaporize and atomize methanol molecules in combination
with the high plasma load of methanol that cool and even extinguish the plasma [109].

The vapor pressure can also be useful in the characterization of solvents [106]. Organic solvents that are easily tolerated by the plasma often have a much lower vapor pressures than the more problematic ones, such as methanol and acetonitrile. On the other hand, oxygen containing solvents, such as methanol, is suggested to result in pyrolysis products that are better tolerated by the plasma compared to, for example, an aromatic hydrocarbon like toluene [110]. The addition of a few to ten percent of oxygen gas to the sample aerosol [111, I, II] or to the nebulizer gas before it enters the nebulizer [112] are often used to increase the stability of the plasma and to prevent carbon deposition on the torch and the cones. The oxygen converts the carbon into carbon monoxide and dioxide [113].

With a 100 % organic mobile phase, for instance methanol, at a flow rate of about 1 ml min⁻¹ a special plasma ignition procedure often has to be applied to achieve a plasma discharge at all [114]. A higher r.f. power of typically 500 W compared to aqueous operation may be needed in order to sustain a stable plasma [112]. The increased energy transmitted to the plasma at a higher r.f. power counteracts at least partially the reduction in temperature caused by the high solvent mass loads from the organic solvent [115].

A decrease in the flow rate, see Section 3.2, as well as in the organic content of the mobile phase drastically improves the plasma tolerance [115]. In the study described in Paper III a 35 % aqueous acetonitrile eluent at 150 μl min⁻¹ was used together with an ICP-AES instrument equipped with a low-flow nebulizer. The plasma was ignited after which the LC pump was started. The few seconds that it took for the liquid flow rate to reach 150 μl min⁻¹ were sufficient for the plasma to remain stable. To sustain a steady plasma during the LC-ICP-AES analyses in Papers I and III only a higher r.f. power of 1290 W was needed compared to the normal 1250 W. In the studies reported in Papers I and II, where low-flow nebulizers were used with an ICP-MS instrument, the addition of 2–10 % oxygen gas to the aerosol stream was sufficient to obtain a lasting plasma performance and to prevent carbon deposits on the sampler cone at a liquid flow rate of 150 μl min⁻¹ and 5–48 % acetonitrile in the effluent.

If the LC method requires a relatively high content of organic solvent, a stable plasma can be obtained by adding an aqueous make-up flow post-column, which reduces the content of organic solvent to less than 20 % [116]. Other common approaches, which are used alone or in combination with oxygen addition, to increase the organic or aqueous-organic plasma performance are aerosol cooling [117] and various forms of desolvation. In the cooling and desolvation steps a part to almost all of the solvent is removed. Desolvation can be accomplished by heating and cooling the aerosol one or several times [118]. A membrane that separates the solvent
vapour from the larger aerosol particles of a heated aerosol can be used to increase the desolvation efficiency [119]. In cryogenic desolvation, the temperature of one of the cooling devices is set as low as -80 °C [120], which permits the determination of elements in 100% methanol and acetonitrile at ml min⁻¹ flow rates without the extinction of the plasma[120]. Efficient nebulizers, such as the USN, should not be used without an efficient desolvator even under entirely aqueous conditions [121].

3.1.2 Factors influencing the sensitivity

The effect on the elemental signal when changing from aqueous to purely or partially organic aerosols depends on the nature of the solvent, the element, the nebulizer, the spray chamber type, the sample flow rate and the kind of detector principle used. Apart from the detrimental effects that an unstable plasma or soot depositions on the torch and the cones have on the signal [122], the changes in droplet diameter, plasma temperature and solvent volatility have been proposed to contribute to the difference seen in signal intensity when going from aqueous operation to nebulizing an organic solvent [110].

The effect of the absorption of plasma energy by volatile organic solvents in the aerosol dominates for conventional pneumatic nebulizers at ml min⁻¹ flow rates. The cooling of the plasma at high solvent mass loads results in a decreased excitation and ionization, which can reduce the detection limits of especially the ionic lines in ICP-AES [115] and the analyte isotopes in ICP-MS. Use of a desolvation system after the nebulization of methanol or acetonitrile solutions can result in detection limits close to those for aqueous eluents [120], while the addition of oxygen to the nebulizer gas can enhance the population of analyte oxide species [111], which might impair the sensitivity. The use of cooled spray chambers or other types of condensers can be advantageous in terms of sensitivity also in aqueous operation [118]. Problems with memory effects and analyte losses in desolvation with combined heating and cooling [111,123] or in a membrane separator [119] may occur. The extent of the problems depends on both the element [119,123] and its speciation [124,125]. For low-flow nebulizers, with their relatively small plasma loadings of the organic solvent, the decrease in surface tension and thus median droplet diameter on addition of an organic solvent to the sample or the mobile phase can increase the analyte mass transport and hence the sensitivity [126].

The nebulizer gas flow rate is an important parameter for both the aerosol formation and the properties of the plasma [109]. An increase in nebulizer gas flow rate results in a decreased aerosol droplet mean diameter, which leads to a higher solvent loading of the plasma [127]. The nebulizer gas flow rate that gives maximum net analyte signal can shift in aqueous-organic mixtures compared to aqueous eluents at 150 µl min⁻¹. However, the signal
may at least be partly restored for solutions of less than about 50% of organic solvent by optimizing the nebulizer gas flow rate [36]. The net effect of an increase in the gas flow rate on the signal has been shown to mainly depend on the elemental mass in ICP-MS [128] and on the magnitude of the excitation potentials of the elemental lines in ICP-AES [127].

Often a higher r.f. power is needed to approach the signal intensities obtained in aqueous operation. However, if a thorough optimization of the ICP parameters is done, including all the gas flows and the spray chamber temperature, a lower r.f. power can be optimal for organic solvents [127].

In boron determination with LC-ICP-MS, the sensitivity is severely affected by acetonitrile in the mobile phase, which obstructed a kinetic study and isotope ratio measurements of the minor metabolites found in the investigation presented in Paper I. In Paper V the effect of various solvents on the boron signal was investigated. Aqueous and partly organic solutions containing boric acid, BPA and phenylboric acid (PB) were analysed with an ICP-TOFMS instrument. All boron species were suppressed by acetonitrile compared to aqueous operation (75–95% between about 5 and 30% of acetonitrile) as shown in Figure 8. Methanol and n-propanol also resulted in net signal suppressions, but to a lesser extent. The fact that acetonitrile is less well tolerated by the ICP than methanol has been described by several authors [34,116,129]. The plasma is probably loaded with more acetonitrile than methanol at the same molar flow of solvent to the nebulizer, since both the viscosities [130] and the surface tensions [131] of water-acetonitrile mixtures are lower compared to those of water-methanol mixtures with an

![Figure 8](image-url)

*Figure 8*. Background corrected and normalized boron-11 signal intensity for boric acid, BPA and phenylboric acid (PB) with increasing content of acetonitrile. The solutions were prepared by adding the analytes and acetonitrile to volumetric flasks and diluting with acidified water to a final volume of 100 ml. The nebulizer gas flow rate was optimized for maximum boron signal at each solvent composition. The signals are not corrected for the possible change in analyte transport efficiency with increasing acetonitrile concentration.
organic content of less than 50%. In addition, the enthalpy of atomization is estimated to be about 30% higher per mole for gaseous acetonitrile than for methanol vapour at 298 K (calculations based on data in Ref. [132]). If it is assumed that this relationship in enthalpy of atomization is similar also at the plasma temperature it should be expected that the plasma temperature is more reduced by acetonitrile than by methanol.

An investigation of a possible decrease in plasma temperature and thus a deterioration of its ionization properties on adding acetonitrile to aqueous solutions was performed on the ICP-TOFMS and an ICP-AES instrument with an elemental standard solution of boron (boric acid), cadmium and lithium in different water-acetonitrile mixtures. Lithium was chosen because of its similar mass compared to boron, while cadmium has a higher mass but about the same first ionization potential as boron. The first ionization potentials of the three elements are 8.30 eV (boron), 8.99 eV (cadmium) and 5.39 eV (lithium). The same nebulizer-spray chamber system was used in the analyses with the two ICP instruments. In the ICP-TOFMS measurements, the boron and lithium signals were suppressed almost equally, while the cadmium signal was somewhat less reduced. The intensity of the atomic boron line measured with the ICP-AES instrument was considerably reduced, although less than the boron ion signal in the ICP-MS study. On the contrary, the ICP-AES signals of both the cadmium atomic and ionic lines as well as the intensity of the lithium ionic line were nearly constant or increased with increasing concentration of acetonitrile when no correction for a possible increase in analyte transport efficiency at higher acetonitrile contents was performed. The results of the ICP-AES measurements might indicate that the temperature of the plasma is not lowered to a large extent by the organic solvent. Since cadmium has nearly the same first ionization potential as boron the large reduction in boron signal in the ICP-TOFMS instrument may be caused only to a minor extent by a decrease in ionization efficiency due to a chilled plasma.

However, a space charge effect from carbon species might explain at least a part of the suppression of the boron net signals in ICP-TOFMS. Space charge effects influence the ion extraction and transport through probably the skimmer and in the ion optics region in ICP-MS [133]. They are believed to be caused by a high amount of matrix ions (in this case carbon ions and maybe also other acetonitrile species) in the supersonic beam from the plasma that effects the direction of lighter ions. The existence of a space charge effect is strengthened by the fact that the heavier element cadmium was found to substantially suppress the boron and lithium signals in ICP-TOFMS, while the intensities of the atomic boron and lithium lines were almost unaltered by the addition of cadmium in ICP-AES. The almost equally large signal reductions of the two light mass elements lithium and boron in the ICP-TOFMS analyses further supports the space charge theory.
Another influence from an organic solvent on the analyte signal in ICP-AES is spectral overlap from atomic carbon that potentially could deteriorate the sensitivity of, for example, the arsenic 193.70 nm emission line [115]. Signal enhancements have also been reported in ICP-AES analysis of, for instance, cobalt and iron in acidic solutions containing a few percent of an alcohol [134]. Partially ionized elements in ICP, i.e., elements with high first ionization potentials, such as arsenic and selenium can gain in sensitivity in ICP-MS in the presence of a carbon containing medium such as 3 % methanol in conjugation with an increased r.f. power setting [135]. This is not only due to an increased nebulization efficiency from organic solvents. The signal enhancement is also suggested to result from a transfer of electrons from elements with first ionization potentials a few electronvolts lower than that of carbon (11.26 eV) to carbocations or other carbon containing ions in the plasma [135]. The same charge transfer mechanism is proposed to cause the signal enhancement observed for boron when analysed in the presence of dissolved organic carbon in biological samples [136].

Finally, the type of mass analyser has also been shown to affect the tolerance towards organic solvents and the sensitivity. In a comparative study, an axial ICP-TOFMS instrument was shown to be more tolerant to methanol than an ICP-QMS and an ICP-SFMS instrument [137]. Thus, for medium to high contents of an organic solvent in the mobile phase, the mass analysers may rank in the opposite sensitivity order to that described in Section 2.1.2. However, not the same spray chamber and possibly not even the same nebulizer were used in the three instruments in the comparison. Some caution is hence necessary in the interpretation of these results.

3.2 Low flow rates

Separations with narrowbore, microbore and even nanobore LC operating at flow rates from a few hundred µl min\(^{-1}\) down to a few hundred nl min\(^{-1}\) [35] are becoming more and more common due to lower solvent and stationary phase consumption, less dilution of the analyte, good compatibility with the ESI devices, etc. [138] This trend towards smaller dimensions in LC together with the increasing importance of CE have during the last 20 years stimulated the development of low-flow nebulizers for ICP spectrometry. Since ICP spectrometry is stated to be a mass-flow sensitive technique below 0.8 ml min\(^{-1}\), a decrease in flow rate should not be advantageous, as mentioned earlier. However, the low-flow nebulizers are generally more efficient. The low mass flow is at least to a certain degree compensated for by a higher analyte transport efficiency and/or by an increased excitation or ionization efficiency in the plasma since the plasma is loaded with less solvent. The result can hence be a sensitivity close to or even better at 50 µl min\(^{-1}\) with a low-flow nebulizer than what is obtained with a
conventional nebulizer at a flow rate of 0.5–1 ml min\(^{-1}\) [139]. As already mentioned, lower flow rates can also make the plasma tolerant to higher concentrations of an organic solvent by decreasing the solvent load to the plasma. With a good micronebulizer, the ICP-MS instrument can handle µl min\(^{-1}\) flow rates even in gradient elution with up to at least 50 % of organic solvent without a severe deterioration in sensitivity [140].

3.2.1 Low-flow nebulizers

Low-flow nebulizers have a smaller liquid aperture than nebulizers of conventional size. A number of nebulizers working in the ml min\(^{-1}\) flow rate range have been miniaturized. As a comparison, a Meinhard nebulizer has an aperture of 0.22–0.32 mm i.d. while its low-flow analogue, the high-efficiency nebulizer (HEN) has an opening of 0.10 µm [141]. It is the reduced cross-sectional area of the nebulizer gas [108] and the liquid outlets and the thinner wall [142] of the liquid capillary that make the liquid-gas interaction more efficient, so that an aerosol with smaller droplets is created by the low-flow nebulizers. Table 3 lists some low-flow nebulizers that are or have been commercial available. Almost all of the listed nebulizers can be used in narrowbore LC with flow rates of 20–300 µl min\(^{-1}\) as well as in capillary microbore LC with flow rates in the range of 2–20 µl min\(^{-1}\) [35].

The low-flow nebulizers can be divided into direct injection nebulizers and low-flow nebulizers with a spray chamber. Direct injection nebulizers are mounted inside the torch (Figure 2) with the outlet close to the plasma, which provides a 100 % analyte transport efficiency. A mass median diameter\(^*\) of 18.3 µm in aqueous solution and 9.6 µm in 30 % methanol have been measured for a direct injection nebulizer (DIN) [126]. Since all droplets from the DIN are transported to the plasma a cooling of the plasma from non-vaporized droplets probably occurs. A signal corresponding to a 100 % transport efficiency from the DIN is generally not seen in the continuous aspiration mode [150]. In conjunction with LC, the detection limits can be lower than for conventional nebulizer-spray chamber systems due to the small dead volume of 1.5 µl of the DIN [144], which results in a less broadening of the chromatographic peaks.

Low-flow nebulizers with spray chambers produce droplets that are on average smaller than the drops created by a Meinhard nebulizer. This results in a higher analyte transport efficiency of the low-flow nebulizers, as shown in Table 3, than the 2–8 % reported for a Meinhard nebulizer with a Scott double pass or a cyclonic spray chamber at ml min\(^{-1}\) flow rates [31]. The transport efficiencies reported by Ref. [31] might be positively biased since they are obtained from a method that has been shown to measure the solvent

\(^*\) Half of the total liquid mass is in droplets smaller than the mass median diameter.
Table 3. Analyte transport efficiencies and typical flow rates for some low-flow concentric nebulizers that are or have been commercial available.

<table>
<thead>
<tr>
<th>Low-flow nebulizer</th>
<th>Abbreviation</th>
<th>Typical flow rate/µl min⁻¹</th>
<th>Analyte transport efficiency %</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>without spray chamber</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct injection nebulizer</td>
<td>DIN</td>
<td>10–200</td>
<td>100</td>
<td>[143,144]</td>
</tr>
<tr>
<td>Direct injection high efficiency nebulizer</td>
<td>DIHEN</td>
<td>1–100</td>
<td>100</td>
<td>[145]</td>
</tr>
<tr>
<td>with spray chamber</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High efficiency nebulizer</td>
<td>HEN</td>
<td>10–120</td>
<td>55–8b</td>
<td>[142,146]</td>
</tr>
<tr>
<td>MCN-100</td>
<td></td>
<td>10–120</td>
<td>42–6b</td>
<td>[142]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−1000</td>
<td></td>
<td>[147]</td>
</tr>
<tr>
<td>MicroMist</td>
<td></td>
<td>10–120</td>
<td>43–7b</td>
<td>[142]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−600</td>
<td></td>
<td>[148]</td>
</tr>
<tr>
<td>PFA-ST Microflow ST nebulizer</td>
<td></td>
<td>100–700</td>
<td>“High”</td>
<td>[149]</td>
</tr>
</tbody>
</table>

a. In aqueous media  
b. Ryton double-pass Scott-type spray chamber

transport efficiency rather than the analyte transport efficiency [151]. A comparison of a HEN, a microconcentric nebulizer (MCN-100), a MicroMist (MM) nebulizer, and a conventional Meinhard nebulizer with the same double pass Scott-type spray chamber in an aqueous liquid flow rate range of 10–120 µl min⁻¹, has been made by Todoli et. al. [142] They showed that the HEN has both the highest analyte transport efficiency (Table 3) and solvent transport efficiency of the four nebulizers and also the lowest detection limits. The analyte transport efficiencies of the Meinhard nebulizer varied between 23 and 5% in the same flow rate range. In terms of analytical figures of merit, such as detection limit, the three other nebulizers followed the order MM < MCN < Meinhard nebulizer.

In Papers I, II and IV an MCN-100 nebulizer and in Paper II a low-flow nebulizer from Elemental Scientific called PFA-ST MicroFlow ST were used at flow rates of 150 or 300 µl min⁻¹. These flow rates were found to be a good compromise between the optimal flow rate for the LC columns used on the one hand and the retention times of the analytes and thus the total analysis time and argon consumption on the other hand. An MCN-100 nebulizer was also modified by exchanging the capillary from 140 µm i.d. to a 77 µm i.d. capillary (unpublished results). The signals were stable at a flow
Figure 9. Capillary LC-ICP-TOFMS at a flow rate of 1 µl min⁻¹ with a modified MCN-100 nebulizer mounted in a cyclonic spray chamber. Column: 310 x 0.2 mm ODS, 10 mM ammonium acetate, pH 4.0, 20 % methanol. Peak assignment: 1. Co elemental standard, 2. cyanocobalamin (vitamin B₁₂). About 0.5 ng as cobalt was injected of each species.

rate of 1–20 µl min⁻¹ and capillary LC could be run at 1 µl min⁻¹ both with (see Figure 9) and without a cyclonic spray chamber.

In the coupling of an LC system to an ICP spectrometer, the volume from the injection valve, where the sample is injected onto the chromatographic column, to the plasma should be kept as small as possible in order to minimize bandbroadening, especially when working with flow rates in the middle to low µl min⁻¹ range [152]. The nebulizer-to-plasma distance is often fixed. A long transport of the aerosol to the plasma may result in signal losses but contributes little to the bandbroadening, while the column-to-nebulizer transfer volume has a larger negative impact on the peak width [153]. In addition, the spray chamber volume should be kept as small as possible. Some low-volume spray chambers have been developed. However, two low-volume spray chambers were found to have performances in terms of sensitivity and sample wash-out times at an aqueous liquid flow rate of 10 - 160 µl min⁻¹ that were only somewhat better, and in certain respect worse, than for a conventional cyclonic spray chamber [154]. A cyclonic spray chamber together with the modified MCN-100 described above only contributed slightly to the peak broadening at 1 µl min⁻¹ compared to a “no spray chamber” set-up [36]. Łobinski et al. [155] have reported less bandbroadening and thus higher efficiency for separated cobalamin analogues for an LC-ICP-MS instrument with a DIN interface and a total flow rate of 100 µl min⁻¹ than what was obtained with either UV or ESI-MS detection.
3.3 Quantification

The quantification of analytes to which identical standards of well-defined concentrations are available is normally straightforward. In the external calibration mode the standard is analysed at different concentrations and a calibration curve is drawn showing analyte signal versus analyte concentration. The sample concentration of the analyte is evaluated from the curve or the equation of the curve. For samples subject to matrix effects the method of standard addition \[156\] can improve the accuracy. In conjunction with LC the analytes may be separated from the matrix and the time-consuming method of standard addition may become unnecessary. If the precision or accuracy needs to be improved in speciation analysis by LC-ICP spectrometry one of the approaches mentioned in Section 3.3.3 can be used. To check the accuracy of an analytical method the analysis of a certified reference material, if available, is very useful.

3.3.1 Quantification without identical standards

For many sample types and substances reference materials or standards are lacking. This problem is often encountered in environmental, biological and clinical analyses \[9\] as well as in drug development \[157\].

Sometimes a determination of the relative amount of the different analytes is sufficient. One prerequisite in this type of determination as well as in the other examples in this section is that the response per hetero-atom (the response factor) of the different analytes are equal. A relative quantification approach was applied in Paper III in a kinetic study of the degradation of \(o\)-carboranylalanine into its two diastereomeric analogues (Figure 10). Since only one boron atom differs between the mother compound and its degradation products the assumption of equal response factors should be fairly accurate. Of course, the different numbers of boron atoms in \(o\)-carboranylalanine and the \(nido\)-carboranes had to be corrected for in the calculations.

![Figure 10. Degradation of \(o\)-carboranylalanine into its \(nido\)-analogues.](image-url)
For species that resemble each other closely, such as in impurity and metabolic studies fairly accurate quantification of the concentrations can be performed using the drug as standard also for the impurities and metabolites [157].

Post-column injection of inorganic elemental standards and samples can be useful in the case of quantification in SEC-ICP-MS, since free metal ions often are retained on the size-exclusion column, while metal ions strongly bound to, for example, a protein follow the elution of the protein [158]. The total concentration of the element of interest in the sample can also be measured by external calibration with an inorganic elemental standard using direct infusion or flow injection analysis. The concentration of the unknown species can then be evaluated from the peak area of each species in relation to the total area of the peaks in the chromatogram [159]. These strategies requires that the correct number of atoms of the element of interest can be assigned to each compound. To get a reasonably accurate quantification of the different species, the measurement of the total elemental concentration must not be subjected to any severe matrix or spectral interferences. Furthermore, all the compounds in the sample that contain the element of interest must be retained and eluted from the chromatographic column. Compounds eluting with the front in RPLC and IC might have erroneous peak areas due to base line disturbance from, for example, salts or a modified content of the organic solvent present in the mobile phase.

When gradient elution RPLC is applied one has to be aware of possible changes in the response factor during the run due to the change in the content of organic solvent. In a comparative study, Packert Jensen et al. [125] showed that a direct injection nebulizer gave structure independent response factors only under isocratic conditions. For those compounds that were not lost during the desolvation in an MCN-100 nebulizer equipped with a membrane desolvator, the response factors were dependent on the molecular structure but independent of the methanol concentration.

### 3.3.2 Generic detection

The use of inorganic standards for the quantification of hetero-atoms in biomolecules by ICP spectrometry has been proposed [52], which, of course, presupposes that the detectors are generic, i.e, give the same response factor for different species of the same element or that the relative response factors of the element in the inorganic standards and the biomolecules are known. In a comparison of the response factors of a number of chemical species UV detection and ESI-MS were found not to be generic detection techniques, while ICP-MS showed a much higher degree of genericness [157], even though a USN with a membrane desolvator was used.
However, the opinions diverge on the issue whether the ICP spectrometry is a generic detection technique or not for non-volatile species. Many speciation analysts maintain that this is not the case [160], not even for small and structurally rather similar compounds, such as met in arsenic [55,135,161] and selenium [162] speciation. For quantification, a species-specific measurement or alternatively, the use of an isotope dilution technique is considered necessary. The differing response factors of arsenic species obtained with conventional nebulizers and spray chambers have been explained to be caused by the instrument used or the instrument optimization applied [135] as well as on different volatility of the species [159]. The eluent composition or the stationary phase might also have an influence on the elemental response factors [162].

Other researchers including [90,125,163,164] and Refs. in Paper IV have found that the elemental response factors can be considered approximately equal for different species, even in arsenic [82,129,159] and selenium [165] speciation analysis, sometimes also under gradient elution. A likely provision for this behaviour is that the compounds behave similarly in the nebulization and transport processes. As mentioned earlier, this is not always the case when a desolvation unit is used together with the nebulizer or a gradient eluent from a RPLC separation is monitored. Another prerequisite for the genericness is that the response factor and concentration calculations are based on peak areas and not on peak heights, since the latter is dependent on the chromatographic retention time. If a chromatographic method and a sample introduction device are used that does not discriminate against any of the species to any significant degree, it is probably the matter of accuracy that separates the two views on the genericness of the LC-ICP spectrometry.

In Paper IV the genericness of an ICP-AES and an ICP-TOFMS instrument was investigated using direct aspiration, flow injection and LC coupled to the instruments. A Meinhard or an MCN-100 nebulizer was used without desolvation. It was found that each instrument gave the same response factor for cobalt and sulphur, respectively, in inorganic standards and large biomolecules with, in the worst case, a bias of 10 % in the concentration intervals 1–8 ppm for ICP-AES and 1–8 ppb (pulse counting detection) and 1–40 ppb (analogue detection) for ICP-TOFMS. The imprecision (n = 2–4) was less than 3 % except for the flow injection analyses, for which the imprecision was 3.3 and 8 % at the most for ICP-AES and ICP-TOFMS, respectively.

According to the generic behaviour of the ICP-AES instrument in Paper IV, a sulphur containing 6 kDa peptide (murine epidermal growth factor, mEGF) conjugated to a dextran chain of 9 kDa could be determined with FIA-ICP-AES and sulphate standards by monitoring the sulphur signal [166]. The sensitivity of the ICP-AES detector was, however, insufficient for an extended analysis scheme. An ICP-SFMS [164] in high-resolution mode or an ICP-QMS with a dynamic reaction cell [167] are likely to be more
useful detectors in terms of sensitivity (detection limit at low ppb [164] to subppb (direct aspiration) [167] for sulphur) in the quantification of sulphur containing peptides and proteins.

3.3.3 Accuracy and precision

Apart from a possible uncertainty of the identity in a chromatographic peak in LC-ICP spectrometric analysis, the accuracy and precision in the quantification may be fairly poor, due to time-dependent instabilities in the plasma. The accuracy and precision in the quantification can be improved by using some kind of internal standard. Correction for the long-term instrumental drift or slightly varying sample injection volumes can be made by using an internal standard compound containing the analyte element [48,168] or a different element [169]. Long- and short-term variations in the plasma spectrometer during the chromatographic separations can be corrected for by a post-column continuous addition of an internal elemental standard [8,170]. To find an appropriate internal standard can, however, be a challenge.

Analogous to these “low-budget” variants are the ID methods. Either an identical standard enriched in one of its ICP-MS measurable isotopes is used (species-specific ID) [171] or an enriched isotope of the analyte element is continuously added post-column, so called species-unspecific ID [160]. A variant of the species-specific ID is the use of just one enriched standard for all species containing the analyte element in the sample [172]. This may be considered as pseudo species-specific ID and can be employed for correction of variations in injection volume and instrumental drift [172] or, like the species-unspecific ID, when identical standards are unavailable. Species-unspecific ID can also correct for possible signal changes during the monitoring of the effluent from a RPLC gradient [137]. The ID methods are very accurate since they are based on ratios instead of absolute intensities. Matrix effects and drifts in the signal are thereby effectively reduced. If properly homogenized in the sample prior to analysis, the species-specific spike can also compensate for losses during the sample preparation and analysis. The ID techniques often require an ICP instrument with a high-resolving mass analyser in order to overcome spectral interferences [47]. Furthermore, the ID techniques can only be employed for elements that have two or more stable or long-lived isotopes that are free from spectral interferences. For monoisotopic elements like aluminium, arsenic and cobalt some of the other quantification approaches must be used.
4 Concluding remarks and future aspects

In this thesis, it is shown that the combination of LC-ICP spectrometry and (LC-)ESI-MS/(MS) enable enhanced analytical information for screening, accurate and precise quantification and identification of substances that involve or can bind hetero-atoms in complex samples. Furthermore, it is clearly visualized that a separation step prior to the ICP spectrometric analysis provides additional valuable information about the sample, which would remain undetected using conventional total elemental analysis.

The mobile phase additives used in the various LC techniques impose some, but different, restrictions on the ICP and API techniques, respectively. Organic solvents can thus impair the performance of the ICP spectrometers, while non-volatile buffers and ion-pair reagents can be troublesome in ESI-MS. Measures to solve or to reduce the incompatibility problems between the liquid mobile phase and the detectors exist. However, some further research and development are desirable in, for example, the combination of organic solvents and ICP spectrometry.

ICP-MS has the required power to complement the soft ionization MS techniques as a screening and quantification tool, not only in typical speciation analysis, but also in, for example, protein and gene research. The technique is available today, but needs to be made more visible for the soft ionization mass spectrometrists. The elements of interest in element selective proteomics and genomics is sulphur and phosphorus, which, due to spectral interferences, require the use of a high-resolution ICP-MS or a low-resolution instrument equipped with a reaction or collision cell. Future studies will hopefully establish that it is possible to use inorganic elemental standards or small organic compounds in the quantitative determination of nucleotides and sulphur- or phosphorus containing peptides and proteins at appropriate concentrations with an acceptable accuracy, as has been indicated in this thesis and elsewhere [157,164]. If so, the present struggle for finding good quantification methods in, for example, proteomics could have come appreciably closer to an end. Another potential use of LC coupled to both high- and low-resolution ICP-MS is screening experiments of metal-free biomolecules that can bind metal ions, provided that the complexes are stable during sample preparation and separation.
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6 Summary in Swedish:
Kopplad vätskeseparation och spectrometrisk
detektion av organiska föreningar innehållande
deterner hetero-atomer

Den här avhandling beskriver och jämför vätskekromatografi (LC) kopplad
 till induktivt kopplat plasma atomemissionsspektrometri (ICP-AES), ICP-
 masspektrometri (MS) och jonisering med elektrospray (ESI) kombinerad
 med MS för bestämning av substanser som innehåller eller kan binda andra
 grundämnen än väte, kol, kväve och syre.

6.1 Kort om ICP-spektrometri och ESI-MS
Den hårdasjonisationen i ICP-spektrometrin resulterar i att de i provet
 ingående föreningar bryts sönder till atomer och joner, varav en andel
 också blir exciterade. Detektionen sker antingen med AES där intensiteten
 av emissionen mäts när de utvalda exciterade atomerna och joner
 deexciteras, eller med MS med vilken antalet joner och deras massa
 dividerat med laddning (m/z) detekteras. Tre typer av massanalysatorer finns
 på marknaden: kvadrupoler, flygtuber och magnetiska sektor-instrument. De
två förstnämnda är lågupplösande tekniker (joner som skiljer med en ungefär
 en massenhett kan särskiljas) medan den sistnämnda är högupplösande.
Kvadrupolerna är vanligast förekommande. Alla grundämnen i periodiska
 systemet till och med uran kan analyseras, förutom några lätta grundämnen
 som till exempel väte, kväve och syre. ICP-MS-teknikerna har en överlägen
 känslighet för de flesta grundämnena (ned till pg–fg ml⁻¹) [40,45] och kan ge
 en kvantifiering med stor riktighet och precision över ett stort
 koncentrationsintervall. De lågupplösande instrumenten har problem med
 spektrala interferenser för många isotoper under m/z = 82, vilket medför att
 känsligheten för element som svavel, fosfor och järn inte är så mycket större
 än vad den är för ICP-AES-instrumenten. Vätskekromatografi kopplad till
 ICP-spektrometri används idag framför allt för identifiering och
 haltbestämning av små organiska och organiska föreningar innehållande
 grundämnena som arsenik, selen och tenn, samt för att bestämma vilka
 metallor som binder in till biomakromolekyler som proteiner. Eftersom
molekylstrukturen förstörs vid atomiseringen och joniseringen kan identifieringen av kromatografiska toppar bara göras genom att jämföra retentionstiderna med de från identiska standarder.


![Molekylära jonen av p-boronphenylalanine](image.png)

Figur i. Den molekylära jonen av p-boronphenylalanine.

6.2 Aspekter på kopplingen av LC och ICP-spektrometri respektive ESI-MS

Beroende på vilken typ av vätskekromatografi samt flödehastighet och dimension på kolonn som används kan LC-metoden eller övergången mellan LC-systemet och detektorn behöva modifieras för att vara kompatibel med detektorn.

ICP-teknikerna fungerar generellt sett bra ihop med de traditionella LC-metoderna med höga flöden (ml min\(^{-1}\)) och icke-flyktiga mobilfaskomponenter som fosfatsalter. För vissa isotoper av till exempel
koppar och zink får man dock se upp med spektrala interferenser från fosfatbuffert i ICP-MS.

Mobilfaser som innehåller organiska lösningsmedel kan försämra plasmats stabilitet och metodens känslighet. Det lätta grundämnet bor har funnits få starkt reducerad känslighet i ICP-MS vid tillsats av ett par procent organisk modifierare till mobilfasen [V]. Minskningen i signal orsakas troligtvis till stor del av att den stora mängden kolföreningar i jonstrålen ger borjonerna en delvis felaktigt riktad bana under färden in till massanlysatorn, vilket resulterar i att färre borjoner når detektorn. Känslighetsminskningen visade sig vara större för acetonitril än för metanol och n-propanol. För vissa element kan dock en ökning i känslighet fås med några procent organiskt lösningsmedel i eluenter [134,135]. Om organiska lösningsmedel måste användas i eluenter och om de påverkar plasmat så att det socknar eller analytkänsliheten försämrar kraftigt, kan den negativa effekten på plasmat reduceras genom att till exempel 1) syrgas tillsätts förstoftargasen, 2) eluentflödet sänks, 3) eluenten spåds ut efter kolonnen till att innehålla mindre än 20 % organisk modifierare eller 4) genom att ta bort mer eller mindre av lösningsmedlet efter förstoftningen genom kylning av aerosolen, eller värming följd av kylning i en eller flera cykler.

Så kallade mikro- eller lågflödesförstoftare används ofta vid lägre eluentflöden (1–300 µl min-1). Lågflödesförstoftarna ger upphov till mindre droppar i aerosolen, vilket medför att en större andel av provet (10–100 %) [142,143] när plasmat jämfört med konventionella förstoftare, som till exempel den pneumtiskt koncentriska Meinhard-förstoftaren (1–8 %) [31]. Trots att ICP-spektrometrin egentligen är en massflödeskänslig teknik, behöver en sänkning av flödet, i kombination med byte till en lågflödesförstoftare, inte alltid betyda att känsligheten försämras.

Elektrospray har en principi motsatt tolerans mot eluenttillsatserna jämfört med ICP. En viss del organisk modifierare är oftast nödvändig för att få en stabil spray. Däremot förorenar icke-flykiga mobilfaskomponenter inloppet till massanlysatorn och de kan även orsaka en minskning av analytisignalen. Ett par åtgärder som kan tas till om icke-flyktiga komponenter i mobilfasen inte byts ut mot flykiga buffertsalter eller jonparsreagens är kontinuerlig tillförsel av organiskt lösningsmedlet efter kolonnen [92] eller användning av en stark anjonbytarkolonn som binder de önskade tillsatserna [95].

Ren elektrospray klarar inte flöden på mer än några få µl min -1, vilket medför att flödet från kolonner med innerdiametrar på 1–4.6 mm måste delas av in till ESI-MS-instrumentet. Hela flödet (c:a 1–5 µl min -1) från kapillärkolonner med innerdiametrar omkring 200–300 µm kan däremot ledas till elektrosprayen. I detta flödesområde beter sig LC-ESI-MS som om den vore koncentrationskänslig [100], vilket medför att känsligheten kan förbättras när man använder kapillär-LC, eftersom utspädningen av provet blir mindre ju smalare kolonnen är. Med pneumatiskt assisterad elektrospray, också kallad jonspray, kan eluentflöden på upp till 2 ml min -1 användas.
6.3 Kvantifiering med LC-ICP-MS

ICP-spektrometri har i ett flertal studier visat sig vara en generisk detektionsteknik för ickeflyktiga substanser [IV och ref. däri], vilket betyder att responsfaktorn för ett och samma grundämne är detsamma oavsett molekylstrukturen. Även en oorganisk standard som sulfat kan ge god riktighet vid kvantifieringen av stora biomolekyler som proteiner [IV]. Det är vanligt att standarder saknas inom miljöanalys samt biologisk och klinisk analys. Den generiska egenskapen kan utnyttjas då man vill få en någorlunda bra uppskattning av koncentrationen hos en förening som saknar en väldefinierad standard. I en kinetikstudie av nedbrytningen av o-karboranylalanin till dess \textit{nido}-analoger kunde till exempel en relativ kvantifiering göras mellan start- och slutprodukterna för att få en uppfattning om mekanismen bakom nedbrytningen [III].

6.4 Slutord

Kombinationen av LC-ICP-MS och (LC-)ESI-MS/MS utgör ett kraftfullt verktyg för identifiering och kvantifiering av de flesta substanser som innehåller hetero-atomer. Med LC-ICP-MS kan man också relativt enkelt och snabbt få en överblick av de substanser som innehåller ett visst grundämne (se figur ii), vilket kan underlätta identifieringen med LC-ESI-MS/MS [II].


Det finns även en möjlighet att ICP-MS-teknikerna med deras höga känslighet kan användas för detektion av till exempel låga halter av proteiner som inte innehåller metaller naturligt, men som kan binda metaller, förutsatt att de bildade komplexen är så stabila att de klarar provbearbetning och separation utan att dissocieras.
Figur ii. ICP-MS-kromatogram av siderofor-innehållet (topp 1 och 2) i ett markvattenprov behandlat med Ga(III) i reducerande miljö. Utifrån analys av samma prov med LC-ESI-MS/MS kunde topp 1 bestämmas till galliumdesferioxamin och topp 2 till galliumdesferrikrocin.
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A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to October, 1993, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science”.)