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Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) for Quantitative Analysis in Environmental and Life Sciences: A Review of Challenges, Solutions, and Trends

This focal point review provides an overview of recent developments and capabilities of inductively coupled plasma mass spectrometry (ICP-MS) coupled with different separation techniques for applications in the fields of quantitative environmental and bio-analysis. Over the past years numerous technical improvements, which are highlighted in this review, have helped to promote the evolution of ICP-MS to one of the most versatile tools for elemental quantification. In particular, the benefits and possibilities of using state-of-the-art hyphenated ICP-MS approaches for quantitative analysis are demonstrated with a focus on environmental and bio-analytical applications.

Index Headings: **Inductively couple plasma-mass spectrometry; ICP-MS; Hyphenated techniques; Isotope dilution; Quantification; Environment; Proteomics; Chromatography; Trace elements; Heteroelements.**

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INTRODUCTION

The accurate quantification of biologically relevant molecules, such as proteins or even hazardous substances in the environment, becomes more and more an essential prerequisite for monitoring and understanding biological processes or comparatively assessing our environment in terms of, for example, contaminant concentrations or the presence of novel emerging compounds. In particular, the recent developments within the prominent fields of genomics, proteomics, and metallomics have enhanced the understanding of the complex interplay of genes and proteins in cellular processes, which are often reflected by time-dependent changes in absolute protein concentrations or the degree of a site-specific post-translational modification.[1] However, even though different techniques for the quantification of proteins have been suggested during recent years, this still represents a challenging task.[2] In addition, the quantitative analysis of priority hazardous substances in the environment becomes more and more challenging because new legislation often requires more sensitive methods, or even completely new approaches, for the determination at very low concentrations (pg/L levels) of already defined priority compounds or newly emerging contaminants that show up in the environment as substitutes for already banned substances or as a result of changing industrial processes.[3]

Since its introduction in the 1980s, inductively coupled plasma mass spectrometry (ICP-MS) has evolved to become arguably the most versatile, element-specific detection technique.[4,5] In parallel, because of the fast developments in the field of elemental speciation, the utilization concept of ICP-MS has undergone a significant change.

Within this context, trace metals, metalloids, semimetals, and hetero elements play important roles, since nature as well as organic chemists have learned to skillfully combine these elements with hydrogen (H), carbon (C), nitrogen (N), and oxygen (O) to form an unimaginably large number of sometimes beneficial, but also sometimes hazardous, substances.

Although it was initially used for the total quantification of trace metals in liquid samples, ICP-MS has matured into a powerful chromatographic detector and therefore an essential part of state-of-the-art hyphenated detection schemes, allowing the detection of all kinds of compounds via their characteristic (hetero) element content. In particular the development of hyphenated techniques has established the importance of ICP-MS in the field of environmental speciation analysis during recent years,[6,7] which continuously emerged to the field of bio-inorganic speciation.[8–10] Here the field of metallomics, which focuses on the global analysis of the entirety of all metal and metalloid species within a cell or tissue type, represents one of the most dynamic research areas that recently emerged from trace element speciation.[11–13] Within this context, the utilization of ICP-MS has been changed, since for the first time this technique is now used to detect organic compounds,[14] organometallic compounds,[15] or even more complex bio molecules, such as nucleic acids,[16–19] phospholipids,[20] and metal–proteins[9] via their specific and often characteristic element content. As a result the concept of hetero (element) tagged proteomics has been proposed, in which analytical information is generated by the complementary application of elemental and molecule-specific mass spectrometry, as well as the utilization of hetero atoms such as phosphorus, sulfur, and selenium for screening and quantification purposes.[13,21–23]

This progress was realized due to a number of instrumental developments during the last decade, which will be highlighted in the following sections. Their availability finally induced a paradigm shift, since it became possible to analyze elements that are highly susceptible to interference, which finally allows the number of elements that are easily detected by ICP-MS to be extended to covalently bound hetero atoms such as phosphorus, sulfur, selenium, or even halogens, which show a widespread distribution in all classes of chemical substances.[24]

More recently, labeling approaches that utilize an ICP-MS detectable element have gained a great deal of interest, in particular in the life sciences, as such approaches can be used to allow those molecules that naturally contain no detectable element tag to be detectable by ICP-MS.[24–27] Meanwhile appropriate reaction chemistry has been developed that allows the direct covalent labeling of a bio molecule with elements such as Hg[28–30] or I,[31,32] both of which can be detected with high sensitivity. In terms of flexibility, labeling with lanthanides using bi-functional chelating agents, which are covalently bound to the targeted bio molecule, and which form highly stable complexes with the metal ion, represents the current state of the art within this field.[33–35] Lanthanides are ideal elements for such labeling approaches because they can be detected with high sensitivity via ICP-MS due to a negligible background, as well as the absence of interferences.

In consequence, all kinds of molecules that naturally contain covalently bound (hetero) elements, or which have been chemically labeled with an ICP-MS detectable element, could be easily quantified, whenever their final molecule-tag stoichiometry is known.[22,24,36] However, in parallel this underlines again that a successful application of such approaches strongly depends on the complementary application of molecule-specific detection techniques, such as matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization mass spectrometry (ESI-MS), to gain the necessary information related to the tag stoichiometry.[21,24]

The following sections of this focal point review will provide an overview and critically discuss the recent progress made in using ICP-MS hyphenated to different separation techniques for quantitative analysis in environmental and life science, via the utilization of specific element tags. In addition, trends and the current state of the art of suitable analytical techniques will be highlighted. Whenever possible review articles are referenced, which may provide a good starting point to gain more detailed, topic-related information.

ICP-MS FUNDAMENTALS

Ionization: The Potential of a Plasma Ion Source. In contrast to other popular mass spectrometric ionization techniques such as the so-called "soft" electrospray (ESI) or MALDI, elemental MS utilizes a high-temperature plasma discharge as source for mainly singly, positively charged ions.[37] In consequence ICP-MS has matured to a powerful, important technique, allowing the determination of most elements present in the periodic table (See Fig. 1).

Inductively coupled plasmas mostly utilize noble gases, such as argon as plasma gas, in which the efficient vaporization, dissociation or atomization, excitation, and final ionization of the sample constituents to be analyzed takes place. In addition, this high-temperature process leads to a complete fragmentation of every sample molecule, leaving only their detectable, atomic constituents, namely metals, metalloids, or heteroatoms, which could then be used as surrogates to also detect complex molecules, such as proteins, nucleic acids, or even small organic molecules, as illustrated in Fig. 2. Due to the high temperature of the plasma, which may exceed 7000 K, ICP-MS is particularly well suited to handle liquid samples when hyphenated with introduction techniques such as flow-injection analysis (FIA), high-performance liquid chromatography (HP-LC), or capillary electrophoresis (CE).[9] However, gaseous or solid samples can also be analyzed using gas chromatography (GC)[38] or laser ablation (LA)[39] as sample introduction techniques, respectively. In summary ICP-MS provides a number of attractive properties as a detector for quantitative analysis, such as high sensitivity (ng L^{-1} range), wide linear dynamic detection range (up to nine orders of magnitude depending on the instrument and the application), and specificity for the accurate detection and quantification of metals, metalloids, and heteroelements (including non-metals, semi-metals, and halogens). In addition,

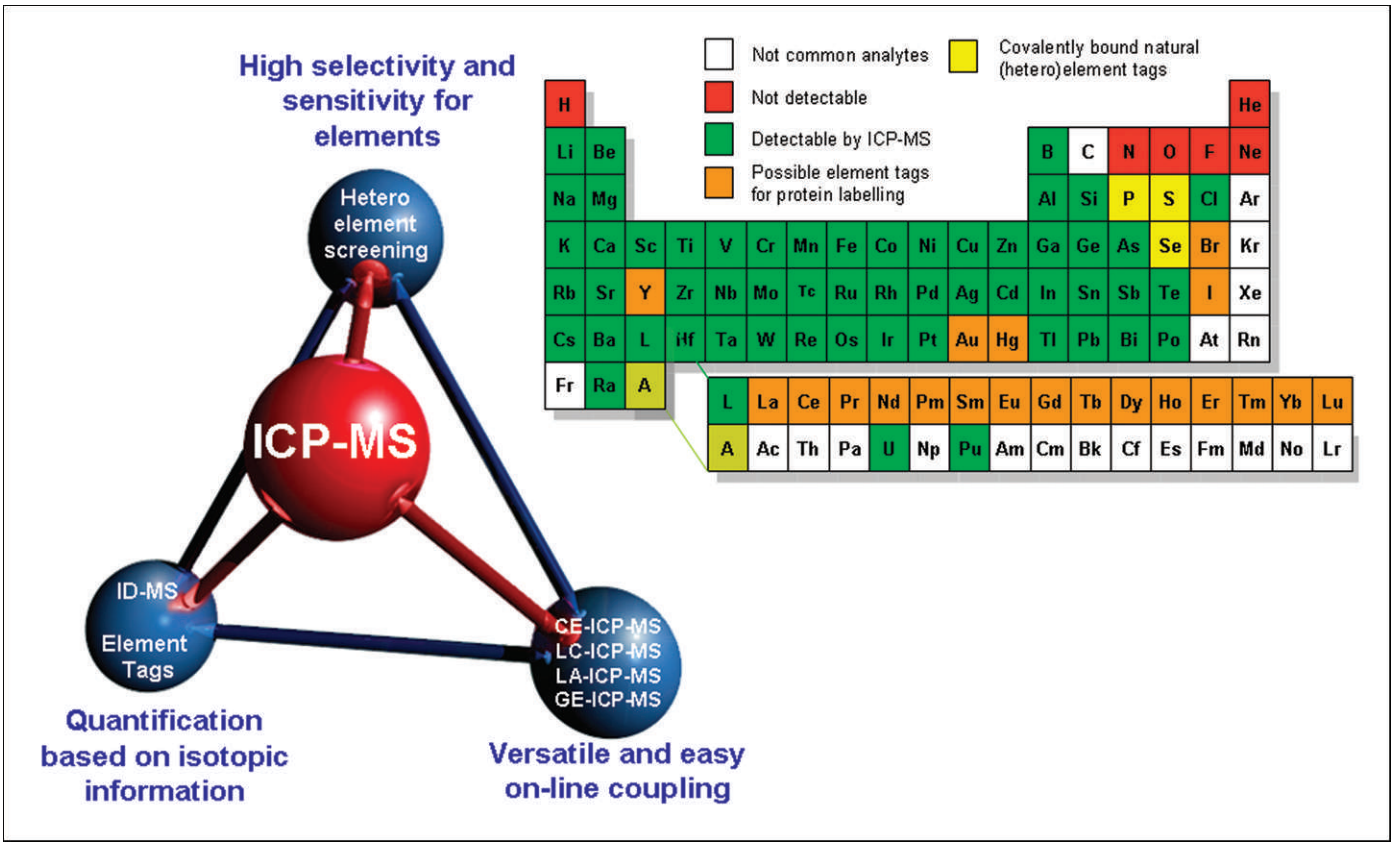

FIG. 1. Illustration of the specific features of ICP-MS as a (hetero)element-specific detector. The instrumental progress in high-resolution ICP-MS, as well as the development of collision/reaction cells, during the last decade has minimized the interference problem that naturally delimits the accurate detection and quantification of many elements. Meanwhile, most elements of the periodic table including metals, metalloids, semimetals, non-metals, or halogens can be accurately determined by ICP-MS, allowing their application as surrogate standards for the quantification of all kinds of molecules whenever the stoichiometry of the used element tag is known.

ICP-MS can be used to obtain precise isotope ratio information for those elements that feature multiple stable isotopes. As a result, isotope dilution analysis has matured to the method of choice when the goal is the most accurate quantitative results.[40] Despite its current versatility, however, ICP-MS is still widely known as only a "metal" detector and it is hoped that this article will change this state of affairs.

Sample Introduction: Current Status and Trends in Hyphenated Techniques. At first sight, and in comparison to other techniques such as ESI or MALDI-MS, respectively, ICP-MS seems to be only interesting as a pure elemental detector, since all molecule-specific information is lost, due to the specific properties of the plasma ion source. However, the necessary molecular specificity can be obtained by the hyphenation of ICP-MS with different state-of-the-art separation techniques, such as HPLC or GC as well as electrophoretically driven techniques such as capillary or one- and two-dimensional gel electrophoresis (1D or 2D GE).

High-Performance Liquid Chromatography–Inductively Coupled Plasma–Mass Spectrometry. The hyphenation of LC to ICP-MS for the separation and (hetero)element specific detection is relatively straightforward and can be easily achieved via the direct connection of the separation column and the nebulizer that is part of the spray chamber. Despite this simplicity, the composition of the liquid matrix often complicates the detection of many elements, due to the enhanced formation of interferences. The introduction of high amounts of salts or organic solvents into an ICP is known to change a number of fundamental parameters, such as plasma temperature, electron density, aerosol generation or analyte transport, as well as the overall ionization processes that take place inside the plasma. Plasma instabilities until its extinction, carbon deposition on the cones and lens system, or signal suppression are well-known further detrimental effects related to the introduction of high amounts of organic solvents into an ICP, which often limits the hyphenation of standard reversed-phase (RP) HPLC setups with ICP-MS detection, in particular when working with solvent flow rates between 0.1 and 1 mL min^{-1}. Membrane desolvation,[41] oxygen addition,[42] spray chamber temperatures below 0 °C,[20] or reduced injector tubing inner diameters[42] are often-used tools to minimize the organic load of the plasma, in particular when utilizing RP-HPLC conditions, which are necessary for many

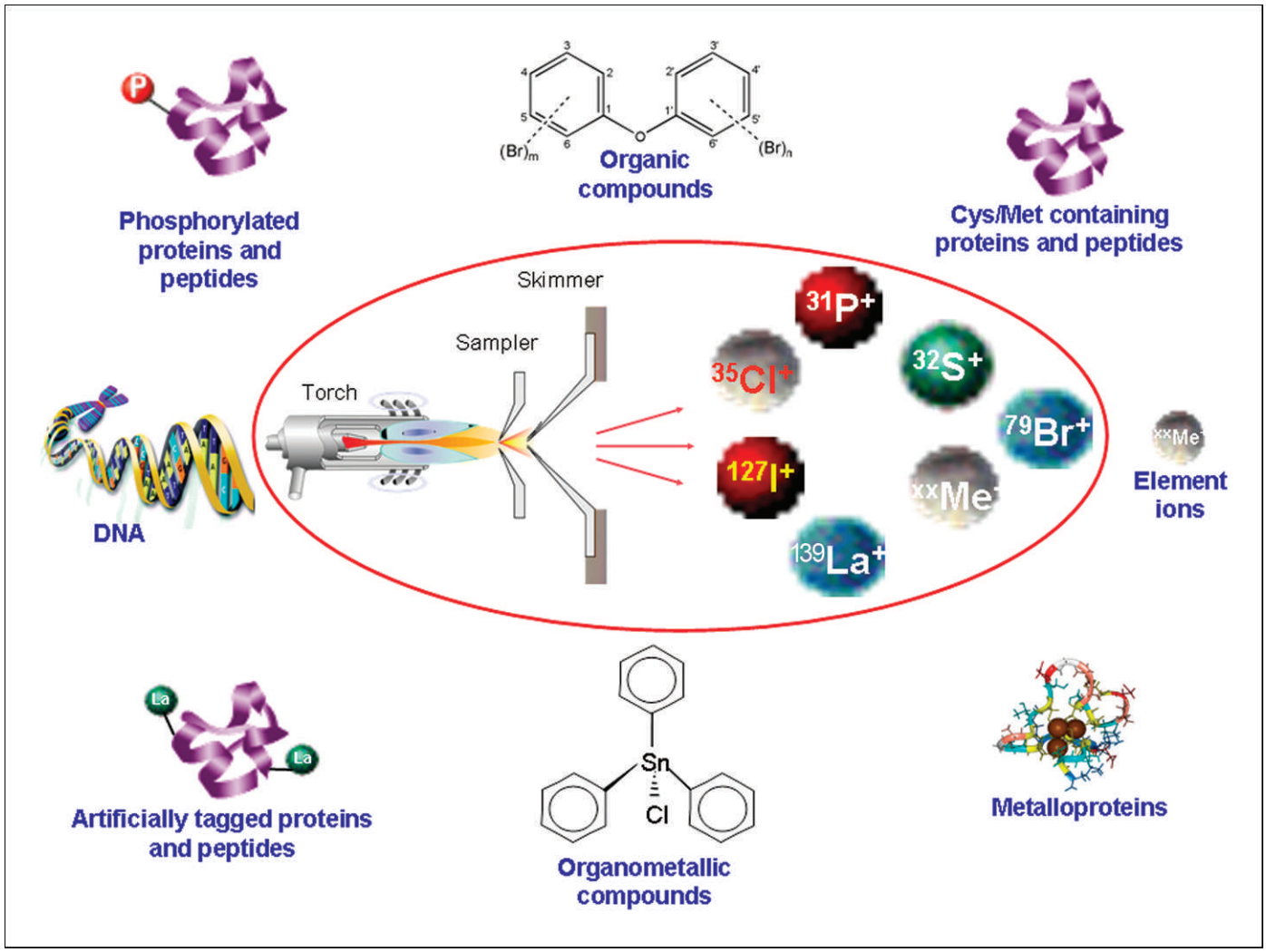

FIG. 2. The high-temperature process inside an ICP leads to complete fragmentation of every sample molecule, leaving only their detectable, atomic constituents, namely metals, metalloids, or heteroatoms that could be used as surrogates to detect complex molecules such as proteins, nucleic acids, or even small organic molecules.

separations in environmental and life science applications.

The recent development of new interface systems that allow the hyphenation of capillary or nano LC to ICP-MS have helped to overcome the problems related to the introduction of reversed-phase gradients into the ICP during (hetero)element specific detection.[43] First attempts have been made by using conventional low-flow micro-concentric nebulizers, combined with a low volume spray chamber[44] or modified direct injection high efficiency nebulizer (DIHEN).[45]

More recently the utilization and modification of a total consumption micro-concentric nebulizer, which was originally developed and optimized for the hyphenation of capillary electrophoresis (CETAC CEI 100, Omaha, Nebraska) as an interface for capillary and nano HPLC to ICP-MS, has been described.[46,47] Due to its zero dead-volume design and the possibility to directly connect standard chromatographic tubing to the nebulizer capillary, this device is well suited for the direct hyphenation of capillary HPLC to ICP-MS.[48]

Recently a modified CEI-100 nebulizer, with a different nebulizer capillary and a further reduced internal dead volume, that allows the application of solvent flow rates between 0.5 and 6 μL min^{-1} has been introduced.[49]

Giusti et al. even introduced a new nebulizer working at flow rates less than 500 nL min^{-1}, allowing the sheathless hyphenation of nano-HPLC to ICP-MS. A nano-electrospray emitter needle has been used as a nebulizer capillary to reduce the internal dead volume and to obtain a suitable back pressure, which is necessary for stable nebulization.[50] In general, these capillary or nano-LC interface systems allowed for the first time the direct introduction of gradients with up to 100% organic solvent into the ICP, without the known negative effects such as carbon buildup or even plasma extinction.

This evolution is particularly important because it facilitates the comple-

mentary application of electrospray-based MS techniques under exactly the same chromatographic conditions, which is mandatory to elucidate the (hetero)element stoichiometry, especially during quantitative studies of biomolecules.

Despite the advantages of such miniaturized separation approaches, it has to be kept in mind that ICP-MS (in contrast to ESI-based MS techniques) represents a mass-flow-dependent detection technique. Therefore, the sample ion signal of an ICP-MS is proportional to the total number of atoms detected per unit of time. As a result, the gain in analyte concentration obtained by using miniaturized separation columns occurs at the expense of a reduced solvent flow that enters the detector. In consequence, the improved peak concentration due to the application of capillary or nano-HPLC does not necessarily result in an enhanced response in the ICP-MS system, as observed for ESI-MS.[51]

Gas Chromatography–Inductively Coupled Plasma–Mass Spectrometry. Gas chromatography as a sample introduction technique offers significant advantages compared to standard liquid sample introduction approaches, since 100% transport efficiency can be realized. In addition, an improved sensitivity can be achieved due to the dry plasma conditions, since nearly no plasma energy is needed for sample desolvation and vaporization, allowing also the efficient analysis of interesting high heteroelements with a high ionization potential such as P, S, Cl, Br, or I. Furthermore, intensities of most plasma- and matrix-based polyatomic interferences are negligibly small, due to the absence of an aqueous liquid solvent, which provides outstanding sensitivity for most elements. In consequence the instrumental settings of GC-ICP-MS often indicate strong differences in comparison to normal wet plasma conditions (e.g., lower plasma power often below 1000 W, highly negative extraction lens voltages, introduction of additional gases into the plasma such as He or N_2[14,52–54]), which have to be considered to obtain high sensitivity. Appropriate interface technology has been extensively developed in academic research, especially within the elemental speciation community; however, in the meantime different companies such as Agilent Technologies and Thermo Fisher have also released robust interface systems. Here two concepts have to be distinguished. While most interface systems benefit from the utilization of the dry plasma conditions, recently a dual-mode interface has been introduced. This sample introduction system facilitates the simultaneous introduction of both liquid and gaseous samples into the ICP-MS. Therefore, the dual sample introduction system allows the connection of the GC transfer line to the torch, while a conventional nebulizer/spray chamber combination is mounted above the GC transfer line and connected to a third leg of the GC-ICP-MS torch. The nebulizer is used for the continuous introduction of a liquid matrix, which is utilized for calibration and to maintain wet plasma conditions. This setup may have the advantage to use unspecific standards for a compound-independent calibration and it allows the continuous introduction of an internal standard; however, these wet plasma conditions also eliminate the advantages of GC as a separation technique.

Maintaining a constant temperature over the whole transfer line in order to prevent analyte condensation, as well as preserving high peak resolution by using capillary GC are the most critical points in the hyphenation of GC and ICP-MS. Meanwhile, all commercially available GC-ICP-MS interfaces allow transfer line temperatures above 300 °C, thereby facilitating the analysis of high-boiling-point compounds, such as multiply brominated flame retardants like polybrominated diphenyl ethers (PBDEs).[55] Under such conditions, even such highly interfered isotopes as ^{32}S can be measured directly[56,57] with high sensitivity, which is impossible under wet plasma conditions.

Capillary Electrophoresis/Gel Electrophoresis–Inductively Coupled Plasma–Mass Spectrometry. In general, capillary electrophoresis hyphenated to ICP-MS provides interesting capabilities as a separation technique for environmental and life science applications due to its high separation efficiency (up to 200 000 theoretical plates), the ability to handle the smallest sample amounts (nL range), and the absence of a packed stationary phase, which is prone to negative interaction with the sample.[58,59] The electrophoretic movement of the analytes, which is overlaid by the electroosmotic flow, allows the separation of positively, neutral, and negatively charged ions and compounds in one run. This makes it in particular interesting for the separation of the smallest sample amounts of labile complexes, such as metalloproteins,[9,60] whose integrity is often affected when using other separation techniques, such as LC.[61] Despite these interesting features the application of CE-ICP-MS as well as related instrumental developments indicates a certain stagnation, so the reader is referred to some good reviews focusing on the hyphenation of CE to ICP-MS.[60,62,63] This stagnation may be caused in particular by the complexity of such CE-ICP-MS systems, since different parameters have to be considered, such as the utilized buffer and make-up solutions, which ideally should be totally decomposed in the plasma without leaving too much residue on the cones and the lens system. In addition, buffer concentrations should be kept as low as possible to avoid any detrimental crystallization effects at the nebulizer tip. Also, a high ionic strength of the buffer should be avoided, which will result in the production of excessive Joule heat. This also helps to reduce the risk of air bubble production inside the interface, which may lead to a breakdown of the electrical current or insufficient signal stability.

The interface itself represents a further critical issue, since it has to maintain an effective electrical contact to the outlet of the CE capillary in order to provide a stable electrical current for reproducible electrophoretic separations. Furthermore, any laminar flow inside the CE capillary due to the suction effect of the used nebulizer should be avoided, by adapting the flow rate of the electroosmotic flow (EOF) (a few nL min^{-1} depending on the CE capillary i.d. and the voltage) to those of the nebulizer (normally ranging from 1 to 1000 μL min^{-1}). Additionally, zero dead volumes are anticipated to reduce peak broadening, which has a detrimental

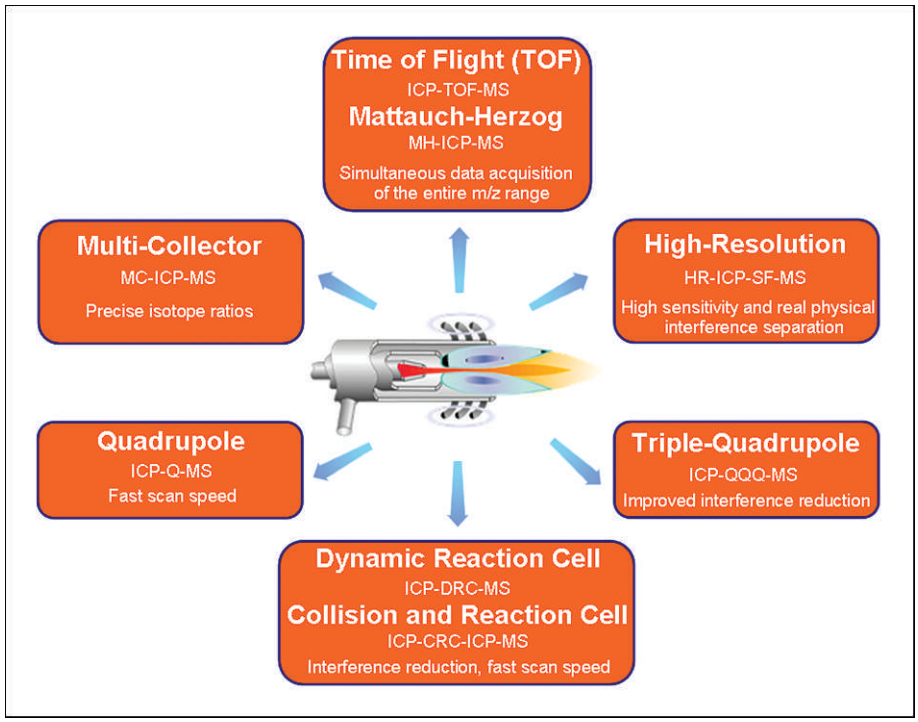

FIG. 3. Illustrative overview of the most recent commercially available ICP-MS platforms.

effect on the high separation efficiency of CE.

Up to now various setups have been suggested to solve the above-mentioned problems in coupling CE and ICP-MS. A conductive, coaxial sheath liquid (e.g., introduced via a cross piece), which is mixed with the CE capillary effluent, is frequently used to provide a stable electrical connection at the CE capillary outlet.[46,47,64–72] Even though a number of interface designs have been suggested during the last decade, to address the mentioned challenges, most recent applications utilize commercially available interface systems.[46,47,73] In summary it must be said that despite the exceptional separation capabilities of CE and the high-level research studies that utilize CE-ICP-MS, more developments are necessary to make CE-ICP-MS a technique suitable for routine analysis.

Recently the application of laser ablation (LA) ICP-MS as interface for the utilization of one- or two-dimensional gel electrophoresis (GE) as a separation technique has gained much interest for either the analysis of naturally[74] or artificially[75] tagged proteins.[76] In particular, 2D GE represents an attractive tool for the separation of complex protein mixtures. The combination of an isoelectric focusing step, which separates the sample constituents according to their isoelectric point, with a second separation dimension according to the molecular weight, allows highly resolved maps of complex proteomes to be generated. Laser ablation is then used to screen the different spots for the presence of specific covalently bound (hetero)elements, e.g., phosphorus, which may indicate the presence of a phosphorylated protein species.[74] Such approaches have also been used for the separation of and screening for metal proteins; however, due to the need to work under native GE conditions, only a poor separation quality can be obtained in comparison to the normally used denaturating conditions.[77] Since the purity of many biochemical reagents in terms of possible element contaminations is often not sufficient, elevated background levels during the ablation process represent a further critical issue. To overcome this limitation, membrane blotting is frequently used to transfer the separated species to a high purity, e.g., nitrocellulose, membrane, whose matrix is rather uncritical during the ablation process.[78,79] Elemental fractionation processes caused by the ablation have to be addressed, since they influence in particular the accuracy of quantitative LA-ICP-MS analysis.[39]

MASS ANALYZERS: STATUS AND RECENT DEVELOPMENTS

Depending on the analytical requirements in terms of mass resolution, sensitivity, isotope ratio precision, or data acquisition speed, several different ICP-MS platforms are commercially available, as summarized in Fig. 3. For many years quadrupole ICP-MS has represented the most frequently used instrumentation for multi-element analysis or as a detector for hyphenated approaches. However, due to the presence of interfering argides, hydrides, carbides, nitrides, and oxides, which are naturally formed in an argon plasma operated under normal lab conditions or due to the matrix constituents of a typical environmental or clinical sample, accurate multi-element determination was always challenging. Table I provides an overview of some of the most frequently used element tags in classical speciation and metallomics analysis, elements that are in focus as covalent tags for absolute protein quantification (P, S, Se), and those showing potential in particular for environmental analysis (P, S, Cl, Br, I) as well as their most prominent interferences. The interference problems have been overcome by the availability of high-resolution double-focusing sector field ICP-MS (HR-ICP-SF-MS) or more recently due to the introduction of collision/reaction cell or the dynamic reaction cell ICP-MS (CC-ICP-MS or DRC-ICP-MS, respectively). By utilizing a medium resolution of $\Delta m/m = 4000$ or by the application of cell gases such as H_2, He, Xe, O_2, or NH_3 most polyatomic interferences can be physically separated from the targeted ions or minimized to an insignificant level utilizing different gas-phase mechanisms.[80] This includes collision-induced dissociation (CID),[81] kinetic energy discrimination (KED),[81,82] charge or proton transfer reactions,[82] or mass shift reactions.[83] Even though HR-

TABLE I. Selected metals, metalloids, and (hetero)elements utilized as tags for ICP-MS based quantification in environmental and life sciences, their isotopes, and prominent polyatomic interferences.

Isotope	Abundance	Accurate mass (Da)	Most prominent polyatomic interferences
^{31}P	100	30.97376	$^{14}N^{16}O^{1}H^{+}$
^{32}S	94.93	31.97207	$^{16}O_2^{+}$
^{34}S	4.29	33.96787	$^{16}O^{18}O^{+}$
^{35}Cl	75.78	34.96885	$^{34}S^{1}H^{+}$
^{37}Cl	24.22	36.96590	$^{36}S^{1}H^{+}$, $^{36}Ar^{1}H^{+}$
^{51}V	99.75	50.94396	$^{35}Cl^{16}O$, $^{37}Cl^{14}N$, $^{40}Ar^{11}B$
^{52}Cr	83.79	51.94051	$^{36}Ar^{16}O$, $^{40}Ar^{12}C$, $^{35}Cl^{16}OH$, $^{37}Cl^{14}NH$
^{54}Fe	5.845	53.93961	$^{40}Ar^{14}N$, $^{38}Ar^{16}O$, $^{37}Cl^{16}OH$, $^{40}Ca^{14}N$
^{55}Mn	100	54.93805	$^{40}Ar^{14}NH$, $^{39}K^{16}O$, $^{23}Na^{32}S$, $^{37}Cl^{18}O$
^{56}Fe	91.75	55.93494	$^{40}Ar^{16}O$, $^{40}Ca^{16}O$
^{59}Co	100	58.93320	$^{36}Ar^{23}Na$, $^{24}Mg^{35}Cl$, $^{42}Ca^{16}OH$, $^{23}Na^{35}ClH$
^{63}Cu	69.17	62.92960	$^{40}Ar^{23}Na$, $^{40}Ca^{23}Na$
^{64}Zn	48.63	63.92915	$^{40}Ar^{24}Mg$, $^{40}Ar^{23}NaH$, $^{32}S^{16}O^{16}O$
^{65}Cu	30.83	64.92779	$^{40}Ar^{25}Mg$, $^{40}Ar^{24}Mg^{1}H$
^{66}Zn	27.90	65.92604	$^{40}Ar^{26}Mg$
^{75}As	100	74.92160	$^{40}Ar^{35}Cl$, $^{40}Ca^{35}Cl$
^{78}Se	23.77	77.91730	$^{40}Ar^{38}Ar$, $^{40}Ar^{37}ClH$, $^{38}Ar^{40}Ca$
^{79}Br	50.69	78.91834	$^{63}Cu^{16}O^{+}$, $^{40}Ar^{39}K^{+}$
^{80}Se	49.61	79.91652	$^{40}Ar^{40}Ar$, $^{40}Ar^{40}Ca$, $^{79}Br^{1}H$
^{81}Br	49.31	80.91629	$^{40}Ar^{40}Ar^{1}H^{+}$,
^{82}Se	8.73	81.91671	$^{40}Ar^{40}Ar^{1}H_2^{+}$, $^{40}Ar^{42}Ca^{+}$
^{111}Cd	12.8	110.90418	$^{79}Br^{32}S$
^{114}Cd	28.73	113.90336	$^{98}Mo^{16}O$
^{120}Sn	32.59	119.90220	
^{127}I	100	126.90448	
^{202}Hg	29.80	201.97063	

ICP-SF-MS operated in the high-resolution mode ($\Delta m/m > 10\,000$) allows most target elements to be resolved from interfering polyatomic ions, the enhanced mass resolution also results in a reduced ion transmission and in consequence a strong loss of sensitivity. Depending on the cell gas used, collision/reaction cells allow the effective suppression of many interfering polyatomic ions, leaving the ion transmission more or less unaffected.

Recently a collision/reaction cell ICP-MS-MS (ICP-QQQ-MS) has been introduced to the market.[84] In comparison to the already available DRC-ICP-MS, which utilizes a radio frequency/direct current (rf/dc) quadrupole reaction cell, which may be pressurized with a reactive gas, in order to promote ion-molecule reactions, and which also features an adjustable DRC bandpass, allowing the suppression of new interferences produced through sequential reactions within the cell,[85–87] this new technique includes two real independent quadrupole mass filters connected via an octopole collision/reaction cell. In contrast to the DRC approach, this device shows comparable operation modes, as known from the frequently used ESI triple quadrupole instrumentation, such as neutral gain scan, product ion scan, or precursor ion scan, which allows an improved reduction in interference as well as better control of the gas-phase reactions or even completely new detection schemes to handle specific interferences.[88]

Also during the last decade multicollector sector field ICP-MS (MC-ICP-MS) and more recently Mattauch-Herzog (MH-ICP-MS)[89–91] instruments have gained much attention, as they allow, for example, the accurate determination of isotopic ratios or the fully simultaneous acquisition of the entire m/z range covered by the elements of the periodic table. These technological developments strongly promote applications such as marine geochemistry, geochronology, cosmochemistry, or provenance studies.

To make the picture complete, ICP-TOF-MS and ICP-IT-MS should also be mentioned as possible analyzers; however, from a commercial point of view they play an insignificant role. Recently the combination of an ICP source with an ultra-high-resolution Orbitrap mass analyzer ($\Delta m/m > 100\,000$) has been demonstrated; however, such instrumentation is not currently commercially available and is still under further development.[92]

QUANTIFICATION STRATEGIES USING ICP-MS

As a mass-flow-dependent detector utilizing an argon plasma for the total decomposition of the sample, as well as the continuous generation of element ions, which finally reflect the composition of the sample, ICP-MS opens some advantages in terms of calibration and quantification. In particular its compound-independent response allows the application of simple element standards to perform a calibration and finally to quantify virtually every compound as long as it contains an ICP-MS detectable element at a known, thermodynamically stable stoichiometry.[93,94]

External Calibration or Standard Addition. Depending on the sample matrix complexity, the simplest way to quantify the targeted element species during ICP-MS analysis is to use available, synthesized standards to generate external calibration curves for each compound. Alternatively the standard addition approach can be applied to consider possible interferences originating from the sample matrix. In many cases such standard addition calibrations can be transferred to an external matrix-match calibration, which could be applied to a larger set of samples without the need to apply the time-consuming standard addition procedure for every sample. This strategy has been extensively applied in the field of environmental speciation analysis of defined organometallic compounds of mainly anthropogenic origin and their degradation products, such as methylmercury, alkyllead, butyl- and phenyltin compounds, or simple arsenic species.[9] However, this concept fails, in particular in the field of quantitative bioanalysis such as proteomics, due to the often high complexity of related samples and therefore the lack of suitable, commercially available standards.

Compound-Independent Calibration and Internal Standards. In contrast to other ionization techniques, such

as electron ionization (EI), ESI, or MALDI, under certain conditions a complete compound-independent ionization can be obtained when using an ICP as ion source. Under such conditions the instrumental sensitivity is proportional to the number of detectable atoms in the molecule investigated, independent of their chemical form. This allows a compound-independent calibration and quantification (CIC) to be performed based on non-sample-specific standards (e.g., inorganic salts[93,95] or small organic molecules[96]) that contain a known or even certified concentration of the target element, thus eliminating the need for specific and often expensive standards, which are still rare for many applications. Such calibration strategies are in particular interesting for the absolute quantification of biomolecules, e.g., in proteomics or metallomics studies.

CIC approaches have been used, e.g., for the quantification of metallothioneins (MT) via their sulfur and cadmium content during CE-ICP-MS experiments using thiourea (as S standard) and cadmium nitrate, respectively,[95] sulfur-labeled yeast,[97] or more recently for the quantification of transferrin isoforms via use of simple, certified iron standards.[98] CIC is in particular interesting for the quantification of monoisotopic element tags such as phosphorus, which cannot be quantified by isotope dilution analysis. Recently the application of an automatic routine for flow-injection analysis (FIA) of a simple inorganic phosphorus standard implemented into a chromatographic separation has been developed, to obtain an instrumental response factor for phosphorus, which finally allows absolute quantification of the separated phosphorylated peptide species whenever their tag stoichiometry is known.[93]

A further possibility to obtain the necessary instrumental response factors is to use internal standards, which are added as a spike to the sample. These standards are separated together with the sample under the same chromatographic conditions and again allow response factors to be computed; these response factors could then be used to perform an absolute quantification of the targeted molecules. The successful application of such strategies has been demonstrated for the quantification of sulfur-containing proteins such as insulin[99] or more recently for the absolute quantification of phosphorylated peptides[96,100] using thiamine (as the internal S standard) or bis(4-nitro-phenyl)phosphate (as the internal P standard), respectively. In general such internal standardization provides a straightforward way to obtain the necessary response factor for CIC; however, it is mandatory to find an internal standard spike showing suitable chromatographic properties (no interference with the target analytes, no co-elution). This could be challenging, in particular when analyzing complex, e.g., biological, samples, which may show a broad elution window during their chromatographic separation. Also, gradient effects during the separation have to be taken into account and need to be compensated, since they will influence the ionization behavior of the targeted element and therefore result in gradient-dependent, changing response factors.[96]

Isotope Dilution Analysis (IDA). The utilization of isotopically labeled surrogate standards, which incorporate varying numbers of stable isotopes, such as ^{2}D, ^{13}C, ^{15}N, or ^{18}O, has a long tradition and reflects the current state of the art, in particular within the field of precise, targeted quantitative environmental analysis, which often includes complex sample extraction and preconcentration schemes. Known amounts of these isotopically labeled standards are added to each sample, ideally directly at the beginning of the sample preparation process, to account for the possible loss of the analytes during the whole analytical process. Since the labeled surrogate standards show the same chemical properties as their native counterparts, both will co-elute during their initial chromatographic separation, while showing a mass spectrum characterized by a specific mass shift of some neighboring peaks whose intensity ratios are utilized for quantification.

Comparable approaches for an absolute quantification have also been developed within the field of bioanalysis, utilizing proteotypic, isotopically labeled peptides [AQUA peptides (absolute quantification peptides),[101] PASTA peptides (phosphorus-based absolutely quantified standard peptides),[102] PolySIS (poly protein stable isotope standard),[103] or QconCAT (Quantification concatamer)[104]] as internal standards for the targeted proteins. While AQUA peptides are quantified by classical amino acid analysis, the recently developed PASTA peptides are quantified by LC-ICP-MS, utilizing their phosphorus tag, which is chemically or enzymatically cleaved before their final utilization. Such quantification approaches are particularly interesting for targeted quantitative studies focusing on the study of a defined set of proteins, characterized by a number of proteotypic peptides. In particular HPLC-ESI-MS-MS methods, utilizing the multiple reaction monitoring (MRM) capabilities of such instrumentation, are frequently used for such targeted approaches.

In contrast the often used PolySIS or QconCAT proteins consist of several isotopically labeled proteotypic peptides, which are synthesized in a concatenated form as a single "non sense" amino acid chain. Afterwards these standard proteins are purified and quantified via amino acid analysis. Such internal standard proteins are than added to the sample after protein extraction. During the enzymatic cleavage reaction a set of labeled, internal standard peptides is generated, allowing absolute quantification of their unlabeled counterparts, which in consequence allows the quantification of the targeted proteins. These approaches allow the production and quantification of multiple standard peptides in a single step; however, such standards can only partly mimic the behavior of the targeted individual intact proteins, which should be reflected by the concatenated standard, in particular during extraction and enzymatic cleavage.[105] Recently it has been demonstrated that fully traceable analytical results can be achieved with such quantification approaches.[106,107]

Heumann and co-workers pioneered the development of comparable IDA approaches for hyphenated ICP-MS setups.[108] In general two different modes, namely non-species-specific IDA as well as species-specific IDA have to be differentiated.[21,40,109]

Non-Species-Specific Isotope Dilution. The non-species-specific isotope

dilution approach represents the most flexible IDA method that can be used for ICP-MS based quantification, in particular for the analysis of biomolecules.

During non-species-specific IDA an isotopically enriched spike solution, which includes the targeted element, is added post column with a constant mass flow to the flow coming from the applied separation technique (e.g., HPLC, CE, or GC). This approach is in particular interesting for the accurate quantification of compounds for which there is a lack of suitable, stable standards, or even unknown compounds. For non-species-specific IDA it is necessary to maintain a complete mixing of the isotopically enriched post-column spike, which contains the targeted elements as well as the sample after its elution from the column; however, this is easily achieved with specific chromatographic hardware such as mixing tees or knot reactors. After separation the isotope ratio between the target element present in the sample with a natural isotopic abundance and the isotopically enriched spike is calculated for each data point of the chromatogram. The isotope dilution equation finally allows the calculation of the mass flow of the targeted element during the whole separation process. The integration of the individual peaks present in the mass flow chromatogram allows the direct calculation of the absolute amount of the compounds reflected by the different peaks.

$$MF_s = c_{sp} \times d_{sp} \times f_{sp} \times \frac{M_s}{M_{sp}} \times \frac{A^b_{sp}}{A^a_s} \times \left(\frac{R_m - R_{sp}}{1 - R_m \times R_s} \right) \quad (1)$$

where MF_s is the mass flow rate (normally ng min^{-1}) of the targeted element, c_{sp} is the concentration of the targeted element in the spike solution, d_{sp} is the density (g mL^{-1}) of the spike solution, f_{sp} is the flow rate (mL min^{-1}) of the spike solution, and M_s and M_{sp} are the atomic weights of the element in the sample and in the spike, respectively. The isotope taken as reference in the sample is referred to as a and the used stable enriched isotope in the spike is referred to as b. R_m is the experimental isotope ratio (a/b) measured in the chromatographic peak of the targeted species after post-column mixing of the sample and the spike, and R_s and R_{sp} are the elemental isotope ratio (b/a) and (a/b) in the sample and the isotopic spike, respectively.

The potential of non-species-specific IDA has been demonstrated in particular for the quantification of proteins that naturally contain elements such as sulfur[59,110] or selenium[111] in a covalently bound form; however, the analysis of stable metal-containing proteins such as transferrin via iron IDA has also been recently demonstrated.[112,113] The non-species-specific IDA approach has been further refined to improve the mass-flow stability, which is a critical parameter influencing the accuracy of the quantification procedure.[114,115]

The main drawback of this IDA approach is that any chemical or physical loss of the analyte during the sample preparation as well as the separation procedure cannot be compensated. In addition, the thermodynamic and kinetic stability of the targeted protein has to be ensured during the entire analytical process. It is also mandatory to know the (hetero)element stoichiometry, to be able to calculate the concentration of the target protein via the measured (hetero) element content.[116]

Species-Specific Isotope Dilution. The above-mentioned limitations can be overcome by the application of the species-specific isotope dilution approach, since it allows any losses during the analytical procedure to be compensated, assuming that the species-specific isotopically labeled spike compound and the targeted analyte reach chemical equilibrium prior to sample extraction. For species-specific IDA it is mandatory that the targeted chemical species are known and that a corresponding isotopically enriched spike material is available or could be synthesized with high yields and simple reactions. Normally such spikes are added to the sample at the beginning of the sample preparation procedure. Such isotopically enriched compounds act as ideal internal standards, since they show the same chemical structure as the target analyte, except that they are enriched with a certain isotope. In consequence, they coelute with the target compound, which also allows nebulization or ionization effects due to the sample matrix, or changes in the mobile-phase composition, to be compensated. Since these spikes show a unique isotopic composition that differs significantly from the natural isotopic abundances of an element, it is not possible that they will be interfered by other sample constituents. The concentration of the targeted species can then be calculated by using the species-specific isotope dilution equation below.

$$c_s = c_{sp} \times \frac{m_{sp}}{m_s} \times \frac{M_s}{M_{sp}} \times \frac{A^b_{sp}}{A^a_s} \times \left(\frac{R_m - R_{sp}}{1 - R_m \times R_s} \right) \quad (2)$$

where c_s and c_{sp} are the concentrations of the targeted element in the sample and spike solution, respectively, m_{sp} and m_s are the masses taken from the sample and the spike in the mixture, respectively, and M_s and M_{sp} are the atomic weights of the element in the sample and in the spike, respectively. Isotope a is taken as reference in the sample and b is the stable enriched isotope in the spike; R_m is the experimental isotope ratio (a/b) measured in the chromatographic peak of the targeted species after post-column mixing of the sample and the spike, and R_s and R_{sp} are the elemental isotope ratios (b/a) and (a/b) in the sample and the isotopic spike, respectively.

Species-specific IDA has been frequently applied for environmental analysis, in particular for the accurate quantification of different organometallic compounds such as tin,[117] lead,[118] or mercury[119] species, due to the availability of suitable, highly enriched isotopes of the specific elements, as well as the more or less simple synthesis of these species. Recently the synthesis of (hetero)element labeled organic compounds, such as polybrominated flame retardants (PBDEs), and their application for the quantitative analysis of water samples via species-specific IDA has also been demonstrated.[120–122]

The application of species-specific IDA for the quantification of selected metal-containing bio molecules represents a more challenging approach. The production of the isotopically enriched

metalloproteins is usually carried out under in vitro conditions, via incubation of the apo protein (metal-free) with a spike solution, which contains the isotopically enriched metal.[123–125] Recently the in vivo synthesis of isotopically enriched metal proteins such as ^{57}Fe-ferritin[126,127] or ^{65}Cu-plastocyanin[128] has also been successfully demonstrated, just to give a few examples. Despite the inherent advantage of such species-specific standards it is important to ensure that during the demetallation process, which is necessary for the in vitro generation of the apo protein, no degradation of the protein occurs. Also, the final storage environment, as well as the chromatographic conditions, has to be considered in order to avoid any isotopic exchange, which will alter the synthesized species-specific protein standard.

TRENDS IN APPLYING ICP-MS FOR QUANTITATIVE ANALYSIS

Due to the ongoing progress in the field of ICP-MS based quantification, it is almost impossible to provide a truly comprehensive overview. The selected examples are intended to indicate the versatility of using ICP-MS as a tool for quantitative analysis, as well as challenges related to the different applications and hyphenation approaches.

Environmental Analysis. Since its introduction ICP-MS has been widely used for environmental-orientated applications, such as trace element determination in a variety of sample matrices. This still represents the most common area for the routine application of ICP-MS. However, refined analytical strategies, new instrumentation, and the development of suitable interface systems to combine chromatographic and electrophoretic separation techniques with ICP-MS detection open a number of new analytical possibilities, which will be highlighted in the following section. In particular, the potential of using (hetero)elements as surrogates for the quantification of all kinds of chemical substances will be discussed.

Analysis of Halogenated Volatile Organic Compounds. The transfer of brominated as well as iodinated or chlorinated volatile organic compounds from different environmental compartments into the atmosphere, where their decomposition due to photochemical reactions takes place, is strongly related to the ongoing reduction of the tropospheric ozone layer. Within this background a two-dimensional on-line detection scheme for brominated, iodinated, and chlorinated volatile organic compounds has been developed using GC hyphenated to an electron capture detector (ECD) and ICP-MS.[129] Under optimized conditions ECD showed lower detection limits for the measured halogens (below 1 pg); however, with only ECD detection it was not possible to distinguish between co-eluting species that contain different halogens. Seawater samples from the North Sea have been analyzed with respect to quantification of the different halogenated volatile organic compounds, released into the water phase or the atmosphere by seaweed or algae.

Pesticides. Since almost all pesticides contain heteroatoms, element-specific detection techniques such as atomic emission detectors (AED) are often utilized for pesticide detection after capillary GC separation.[130–132] Its overall low sensitivity as well as its matrix susceptibility represent the main drawbacks of AED as a (hetero)element specific detector.[133] Here ICP-MS represents a powerful alternative due to its special properties.

One of the first papers regarding the application of capillary GC and collision cell ICP-MS for pesticide analysis was published by Vonderheide et al., in which they demonstrated the element-specific detection of organophosphorus pesticides such as Terbufos, Diazinon, Fonofos, Disulfoton, and Ronnel.[54] Helium has been used as cell gas at a flow rate of 1.25 mL min^{-1} to reduce polyatomic interferences on the mass of phosphorus. In addition, nitrogen has been added to the plasma for sensitivity enhancement, which unfortunately also results in a substantially increased phosphorus background due to the formation of nitrogen-containing interfering polyatomic ions such as $^{14}N^{16}O^{1}H^{+}$ or $^{15}N^{16}O^{+}$. Instrumental limits of detection in the mid ng L^{-1} range have been obtained for the different pesticides measured.

To fully utilize the potential of ICP-MS, in particular its multi-element capability, Pröfrock et al. used GC-CC-ICP-MS for the simultaneous determination of up to 23 phosphorus, sulfur, chlorine, bromine, and iodine containing pesticides.[14] Low plasma power and hot extraction conditions have been used to improve the overall sensitivity of the proposed setup. Even though the background for all measured elements was extremely low due to the dry plasma conditions, helium at a flow rate of 2.5 mL min^{-1} was added to the collision cell to further reduce the background, especially on the sulfur isotope ^{32}S. In contrast to Ref. 54, helium was used as additional plasma gas to further improve the sensitivity without increasing the ^{31}P background. Depending on the element, detection limits in the low ng L^{-1} (P, Br, I) to μg L^{-1} (S, Cl) range have been obtained. Pesticides in fruit and vegetable samples have been quantified using a standard calibration as well as a compound-independent calibration approach. In addition, retention time locking has been applied by locking the retention time of selected compounds to those specified in a pesticide database, allowing the identification of additional compounds without the need for compound-specific standards. Figure 4 shows the simultaneous separation and (hetero)element-specific detection of a multi-compound pesticide mixture (CUS 3217, 0.1% in n-hexane, 1 μL pulsed splitless injection) by GC-ICP-MS. This example clearly indicates the high resolving power of GC and the outstanding multi-element capabilities of collision cell ICP-MS.

Since many pesticides are water soluble and nonvolatile, which requires their error-prone derivatization prior to GC analysis, Sadi et al. used ion-pairing reversed-phase liquid chromatography coupled to ICP-MS to quantify phosphorus-containing herbicides, such as α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), Glufosinate, and Glyphosate.[134] Phosphorus has been detected as ^{31}P using He as cell gas to reduce interference. Detection limits in the low ng L^{-1} range for the different herbicides have been obtained. Unfortunately, only spiked water samples have been analyzed so far to simulate an

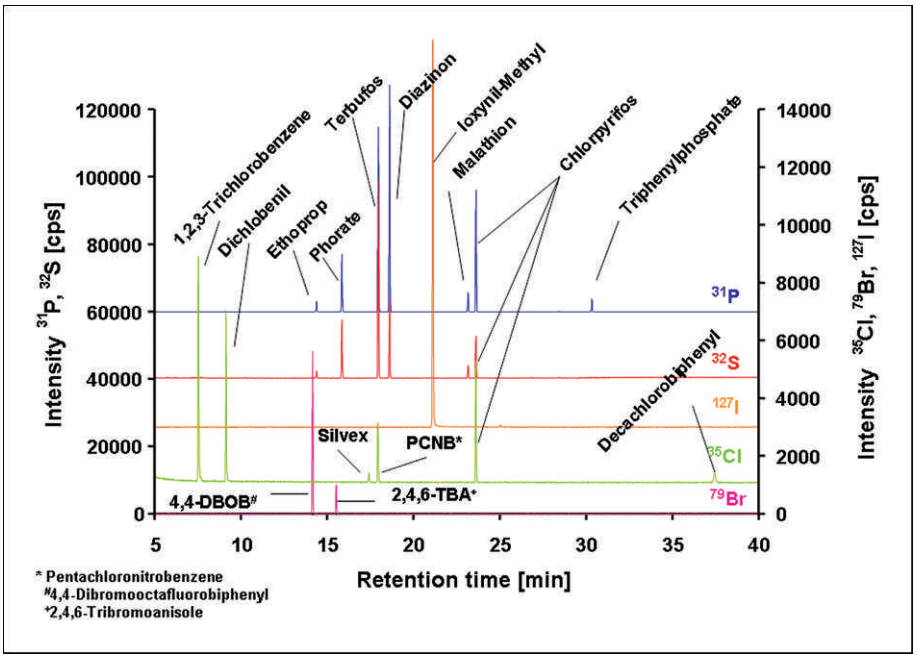

FIG. 4. Separation and (hetero)element-specific detection of a multi-compound pesticide mixture (CUS 3217, 0.1% in n-hexane, 1 µL pulsed splitless injection) by GC-collision cell-ICP-MS. Peak assignment and retention order: (a) 1,2,3-Trichlorobenzene, (b) Dichlobenil, (c) 4,4-Dibromooctafluorobiphenyl, (d) Ethoprop, (e) 2,4,6-Tribromoanisole, (f) Phorate, (g) Silvex, (h) Pentachloronitrobenzene, (i) Terbufos, (j) Diazinon, (k) Ioxynil-Methyl, (l) Malathion, (m) Chlorphyrifos, (n) Triphenylphosphate, and (o) Decachlorobiphenyl. (Taken from Ref. 14, reprinted with permission from Journal of Analytical Atomic Spectrometry 2004, 19, 623-631. Copyright, The Royal Chemical Society 2004.)

environmentally relevant matrix, so the potential of the method for the detection of trace amounts in real samples remains unclear. Also, no comparison has been made of the detection limits of other methods that can utilize an MRM to achieve outstanding sensitivity and selectivity and in parallel that may also allow the direct identification of unknown chromatographic peaks (e.g., HPLC-MS).

Chemical Warfare Agents. Richardson and Caruso published two papers dealing with the analysis of organophosphorus nerve agent degradation products, using either reversed-phase ion pairing HPLC[135] or GC[136] after trimethylsilyl (TMS) and tert-butyldimethylsilyl (TBDMS) derivatization for their separation. Phosphorus-specific detection during HPLC experiments has been carried out using collision cell ICP-MS and helium as the cell gas. In addition, phosphorus has been also detected as $^{31}P^{16}O^{+}$ at *m/z* 47. In contrast, no cell gas was needed for the GC experiments because of the dry plasma conditions obtained by using GC-ICP-MS, which results in an overall low background compared to HPLC-based approaches. The authors indicate a significant improvement of the detection limits, especially when using GC-ICP-MS after sample derivatization. However, only spiked water and soil samples have been analyzed so far to simulate an environmentally relevant matrix, so again the potential of the method for the detection of trace amounts in real samples remains unclear. Also no comparison to other, e.g., LC-MS based, methods in terms of detection limits is given. Recently the same authors described the analysis of organophosphorus nerve agent degradation products spiked into pesticide mixtures using GC-ICP-MS.[137]

Flame Retardants. With the enactment of the 2000 European Community (EC) Water Framework Directive (WFD), the most significant piece of European water legislation for over 20 years is coming into effect. Along with a number of other compounds such as tin species, flame retardants such as PBDEs have now become priority hazardous substances due to their persistent character as well as their already widespread distribution within the environment.

For PBDEs, high-performance liquid chromatography with mass spectrometric detection via an atmospheric pressure chemical ionization source (HPLC-APCI-MS) is the method of choice. However, GC-MS has also been widely used for PBDE analysis.[138,139] The current main challenges for these methods are the required lower detection limits (pg/L range) and the extension of the number of detectable congeners due to the improved chromatographic resolution. Here ICP-MS indicates some advantages since in comparison to GC-MS setups, the interface outlet is operated at atmospheric pressure, which reduces possible band broadening effects due to the vacuum of the mass analyzer. In particular the commercial availability of robust GC-ICP-MS interface systems that allow transfer line temperatures over 300 °C opens this field of analytics for the ICP-MS.

Vonderheide et al. described for the first time the application of GC-ICP-MS for the analysis of different PBDE congeners.[55] A normal quadrupole ICP-MS has been used for bromine specific detection. Again the dry plasma conditions result in an overall low bromine background, allowing detection limits in the medium parts per trillion range. The optimized method has been used for the quantification of selected brominated flame retardants in sewage sludge samples from a local water treatment plant. In a more recent paper the same author used GC-ICP-MS for the analysis of the breakdown of brominated flame retardants by microorganisms in soil samples[140] or to quantify them in different marine reference materials.[141] Recently the synthesis and application of bromine labeled PBDEs as species-specific standards has been demonstrated for the accurate quantification of priority PBDEs in water samples.[120–122]

Shah et al. used solid-phase microextraction (SPME) and GC-ICP-MS to overcome the limitations of other methods, such as liquid-liquid extraction combined with GC-MS, for the analysis of phosphorus-based flame retardants in

human plasma samples, which often requires large sample amounts.[142] Extraction parameters such as salt content, pH, temperature, and extraction time and their effects on the recovery of the different phosphorus compounds have been evaluated to obtain a maximum extraction efficiency. Helium at a flow rate of 1.2 mL min^{-1} has been used as cell gas to reduce the phosphorus background during analysis, allowing detection limits down to 17 ng L^{-1} for selected compounds such as tributyl phosphate.

In a comparable paper Ellis et al. extended the approach published by Shah et al. by combining a microwave-assisted extraction protocol with a previously described SPME procedure for the extraction of organophosphorus triesters and their analysis using GC-ICP-MS.[143] Wastewater samples have been analyzed with the optimized method. Unfortunately an extraction efficiency of only 40% has been achieved, which complicates accurate quantification. In addition, GC-TOF-MS has been used to confirm the presence of the different species.

Petroleum Products. Environmental concerns about the effects resulting from the combustion of sulfur-containing fuels has led to a drastic reduction of the legal limits for sulfur in fuels within the last years.[144] The effective removal of sulfur species from crude oil requires accurate quantitative knowledge about the sulfur species composition of the different oil fractions during the refinery process. This requires robust analytical methods that are able to provide specific information on sulfur-containing compounds present in petroleum products at the ng g^{-1} range. In comparison to other techniques often used for sulfur analysis such as atomic emission or chemiluminescence detection, ICP-MS ideally combines high sensitivity and robustness, which allows the complex hydrocarbon matrix to be handled.

Bouyssiere et al. showed for the first time the application of GC-ICP-MS for petroleum analysis. The on-line coupling of capillary GC with ICP-collision cell-MS was proposed for the speciation of sulfur compounds such as different thiophenes in hydrocarbon matrices. The technique showed an absolute sulfur detection limit of 0.5 pg for a 1 μL sample injected in the splitless mode, which is about two orders of magnitude lower compared to currently used techniques.[145]

Recently, the development of a non-species-specific isotope dilution GC-ICP-MS method for the quantification of sulfur species in petroleum products using a single ^{34}S labeled spike compound has been described. In addition EI-IT-MS has been applied for structural characterization of the sulfur species. Detection limits for sulfur of 9 ng g^{-1} were obtained. Different reference materials such as BCR107 or SRM-2296 have been analyzed with the developed method with respect to their sulfur species composition. Results were in good agreement with the specified values, indicating the high accuracy obtainable by non-species-specific IDMS.[56]

Tao et al. developed a GC-ICP-MS method for sulfur analysis in petroleum liquids. The operating conditions of the ICP-MS and their effects on the background intensities at *m/z* 32 and 34 were investigated to decrease possible contamination and interference. The detection limit was around 0.6 ng S mL^{-1}, corresponding to 0.05 pg S. The present method was successfully applied to petroleum liquids, such as naphtha, gasoline, kerosene, and light oil.[146]

Miscellaneous Environmentally Related Applications. Caruso and coworkers published two papers dealing with the analysis of iodinated phenols in water samples, which are byproducts produced during drinking-water disinfection and whose chemistry and toxicology are not well understood. Either GC or CE hyphenated to ICP-MS, in both cases combined with solid-phase microextraction, has been used for the analysis of the iodinated compounds.[147,148] Carboxen poly(dimethylsiloxane) SPME fibers provide the best extraction rates and recoveries for the iodophenol species analyzed. Detection limits around 0.1 ng L^{-1} have been obtained for the different compounds using GC-ICP-MS, while CE-ICP-MS provides detection limits around 40 ng L^{-1}. In comparison, the GC-based approach indicates a much higher sensitivity in comparison to CE, also allowing the detection of low-abundant, iodine-containing species. Even though the CE approach has been optimized for short separation times, no advantage was gained in comparison to GC, which also allows baseline separation of the different iodophenols within 6 minutes. From the analytical point of view the utilization of CE-ICP-MS for this application is quite interesting. However, bearing in mind the extraordinary detection limits obtained by GC-ICP-MS and its robustness, this approach seems to be more suited for real-life samples.

More recently Shah et al. used SEC, IC, and RP-HPLC hyphenated to ICP-MS to separate and specifically detect iodinated compounds extracted from commercially available seaweeds.[149] Three extraction procedures have been optimized, followed by the application of different chromatographic approaches to separate various molecular-weight fractions of iodinated compounds. Peak identification has been carried out only by retention time matching, using a number of known iodine containing standards. Unfortunately, the authors only provide information on analytical parameters such as detection limits for the applied IC-ICP-MS setup. Here a direct comparison with techniques such as GC and CE-ICP-MS, which have been utilized in some previous papers, could be interesting.[147,148]

Pharmaceuticals. The application of ICP-MS during drug development and quality control as well as to study their behavior in the environment represents a further future field of application that might also benefit from its particular properties with respect to specifically detecting and quantifying (hetero)elements.

Quantitative information on the drug itself and possible metabolites, as well as the number, nature, and concentration of impurities, will provide valuable information to improve drug safety and product quality. In particular chlorine, bromine, and iodine are essential parts of various pharmaceuticals, which facilitates the application of ICP-MS.

Iodine represents an essential part of the thyroid hormones 3,3′,5,5′-tetraiodothyronine (T4) and 3,5′,5-triiodothyronine (T3), which are synthesized by the thyroid gland. Both are essential

parts of the thyroglobulin proteins. In 1993 Takatera and Watanabe used iodine as a (hetero)element tag to detect and quantify the content of iodinated amino acids in an enzymatic digest of bovine thyroglobulin.[150] Absolute detection limits in a range from 35 to 130 pg as iodine have been obtained, which is an order of magnitude lower than those in conventional methods using the stable isotope of iodine.

One of the first papers dealing with the (hetero)element-specific detection of pharmaceuticals was published by Axelsson and co-workers in 2001. They used LC-ICP-MS for the analysis of impurities in different iodine-containing contrast agents, such as Omnipaque™ (Iohexol) or Visipac™ (Iodixanol).[94] Membrane desolvation, as well as oxygen addition, have been utilized to reduce the acetonitrile vapor, which may cause plasma instabilities and carbon buildup. Detection limits of 40 μg L^{-1} were obtained, which corresponds to 0.4 ng of iodine.

Jensen et al. used reversed-phase HPLC hyphenated with ICP-MS to analyze a number of halogen-containing drugs, such as furosemid, diclofenac, or bromazepam.[151] Membrane desolvation has been used for sample introduction to reduce the organic solvent load of the plasma during isocratic and gradient separation of selected compounds. They observed the partial loss of different chlorine, bromine, and iodine containing compounds in the desolvation system, which complicates the quantitative analysis of analytes with unknown properties and for which standards are not available. Absolute detection limits for chlorine, bromine, and iodine of 8 ng, 2 pg, and 2 fg, respectively, have been obtained.

HPLC-ICP-MS was utilized by Kannamkumarth et al. for the determination of levothyroxine, an iodine-containing hormone, and its degradation products in different batches of pharmaceutical tablets.[152] Isocratic separation conditions using 22% (v/v) acetonitrile in a trifluoroacetic acid solution allowed complete resolution of the different iodine species without compromising the quantification, due to gradient-related effects on the elemental response for ^{127}I. Instrumental detection limits below 200 ng L^{-1} have been reported for the different forms of the hormone, also allowing the detection of low abundant degradation products that could not be detected by UV detection.

Recently gadolinium (Gd) chelates used in magnetic resonance imaging (MRI) and their behavior during sewage treatment, as well as their final distribution in the environment, have been analyzed via the coupling of hydrophilic interaction chromatography (HILIC) with ICP-MS to account for the special chemical properties of such compounds.[153,154]

Life Sciences Applications. In general life science related applications that utilize ICP-MS for quantification can be separated into three different main directions: (i) applications that use noncovalently bound metals (e.g., Cu, Zn, Cd, Fe, ...), (ii) applications based on the utilization of covalently bound (hetero)elements such as P, S, Se, and I, and (iii) applications that include chemical labeling with ICP-MS detectable elements (e.g., I, Hg, lanthanides, etc.)

Due to the importance of (hetero)elements such as phosphorus and sulfur for biological systems, in particular life and bio-science orientated applications originated the ongoing development and improvement of ICP-MS as a (hetero) element-specific detection technique. The analysis of phospholipids, DNA/RNA, protein phosphorylation, and sulfur-based absolute protein quantification can be identified as the main research directions that will be emphasized in the following section.

Phospholipids. Despite their importance for biological systems and the possible advantages of using ICP-MS for phospholipid analysis over other approaches in terms of detection sensitivity and method linearity, only a few examples of the application of ICP-MS for phospholipid analysis can be found in the recent literature. Phospholipids are the main building blocks for all pro- and eukaryotic cell membranes. Also various biological processes, such as cellular signaling cascades, are based on phospholipids. However, neither the qualitative nor quantitative analysis of phospholipids is straightforward due to their complexity and special chemical properties, such as hydrophobicity or the ability to form micellar structures in aqueous solutions. In particular, the application of ICP-MS for phospholipid analysis represents a challenge because phospholipids are only soluble in organic solvents and high organic solvent loads are necessary for their chromatographic separation. Membrane desolvation, oxygen addition, low-flow LC systems, or flow splitting have been used to reduce the carbon load of the plasma during ICP-MS-assisted phospholipid analysis.

Axelsson et al. published the first reports on phospholipid analysis, by liquid chromatography hyphenated to a hexapole collision/reaction cell ICP-MS system via an ultrasonic nebulizer and a membrane desolvator, using phosphorus as an element tag.[94] The authors clearly showed the advantages of this generic detection approach over other techniques such as ultraviolet, refractive index, evaporative light scattering detection, or MS in terms of sensitivity and linearity.[94]

More recently Kovacevic et al. described the application of normal-bore HPLC hyphenated to an octopole reaction cell ICP-MS system for the separation and phosphorus-specific detection of different phospholipid standards, as well as lipid extracts from the yeast *Sacharomyces cerivisiae*.[20] Instead of membrane desolvation, solvent splitting, spray chamber chilling down to −5 °C, and the addition of oxygen were utilized to reduce the carbon load of the plasma. To reduce polyatomic interferences on the mass of phosphorus, helium was used as cell gas. Absolute detection limits for phosphorus between 0.21 and 1.2 ng phosphorus were obtained.[20]

Nucleic Acid Analysis. In one of the first papers on the application of natural (hetero)element tags, Siethoff and coworkers used phosphorus for the quantitative determination of in vitro generated adducts of styrene oxide and the four nucleotides 2′-deoxyguanosine-5′-monophosphate (dGMP), 2′-deoxythymidine-5′-monophosphate (dTMP), 2′-deoxycytidine-5′-monophosphate (dCMP), and 2′-deoxyadenosine-5′-monophosphate (dAMP). High-resolution ICP-MS and ESI-MS coupled to normal-bore RP-HPLC has been used

for the identification and quantification of the different adducts. The application of a mathematical correction function to compensate for changes of the instrumental phosphorus response during the HPLC gradient was necessary to obtain accurate quantitative results. In addition, oxygen has been added to the spray chamber to reduce the carbon build-up within the system. Inorganic phosphorus standards were used for the quantification of the different adducts, allowing the detection of one modified nucleotide within 3.5×10^5 un-modified nucleotides, which is comparable to standard radioactive post-labeling techniques.[155]

More recently Edler et al. used a hexapole collision cell ICP-MS combined with a membrane desolvation system and a microbore HPLC setup for the quantification of DNA adducts generated by reaction with either styrene oxide or Melphalan.[16] In comparison to the work of Siethoff et al., no mathematical corrections were needed to compensate for changes in the instrumental response, due to the membrane desolvator. As an example Fig. 5 shows the LC-ICP-MS chromatograms monitored at *m/z* 31 of Melphalan adducts of single nucleotides. Gradient elution and bis(4-nitrophenyl)phosphate (BNPP) as internal standard were used for the separation and quantification of the different modified nucleotides. Even though quadrupole-based instrumentation has been used, comparable analytical figures of merit to those presented by Siethoff et al. have been obtained.[16,155,156]

As an alternative to HPLC, different authors demonstrated the use of capillary electrophoresis (CE) hyphenated to ICP-MS for the separation and phosphorus-specific detection of mono-phosphorylated deoxynucleotides or RNA nucleotides.[17,157] In parallel, HPLC-ESI-MS has been used to identify the separated compounds.[17]

Recently Brüchert and Bettmer developed an interface for the online coupling of 1D slab gel electrophoresis and ICP-MS that allows the separation and quantification of DNA strands.[158] Detection limits of 1 ng DNA absolute have been achieved, which corresponds to 96 pg of phosphorus. In comparison to CE, relatively poor separation efficiency can be obtained with a 1D agarose gel, which represents the main limitation of this setup. To indicate the versatility of the interface, the authors recently applied their approach for phosphorylation analysis of selected model proteins.[159]

SEC-ICP-MS has been used to investigate the interaction of chromium with DNA extracted from metal-contaminated soil samples. Helium was used as cell gas to reduce polyatomic interferences on the masses of phosphorus (^{31}P) and chromium (^{52}Cr). In this case phosphorus has been monitored to trace the DNA-containing fraction during the SEC separation.[160]

Protein Phosphorylation Analysis. As already mentioned, a number of initial studies focusing on the application of ICP-MS as a (hetero) element-specific detector have been conducted in the field of protein phosphorylation analysis.

The reversible phosphorylation of proteins at their serine, threonine, and tyrosine residues is one of the most important post-translational modifications in eukaryotic organisms that regulate cell signaling as well as the enzymatic activity, localization, complex formation, and degradation of proteins.[161,162]

Phosphorylation is regulated by the complex interaction of kinases and phosphatises that catalyze protein phosphorylation and de-phosphorylation, respectively.[163] However, despite the outstanding methodological and instrumental developments, especially within the field of mass spectrometry, the analysis of protein phosphorylation is still not straightforward.

ESI-based tandem MS systems provide various scan modes that can be used for qualitative phosphorylation analysis. These include neutral loss scanning in the positive ion mode after collision-induced dissociation (CID) experiments for H_3PO_4 (−98 Da) or HPO_3 (−80 Da), which is characteristic for the presence of phosphoserine or phosphothreonine containing peptides. The screening for a diagnostic immonium ion at *m/z* 216.043 is characteristic for phosphotyrosine-containing peptides. Precursor ion scanning in the negative ion mode for fragments that generate the loss of PO_3^- (−79 Da) represents a further sensitive mode for the selective detection of phosphorylated peptides.[164–166]

The quantitative determination of phosphorylation, e.g., in selected signaling proteins, will permit a true systems biology approach, which will provide valuable data on regulatory pathways and networks based on phosphorylation.[165] However, the accurate quantification of phosphorylation events especially in signal transduction pathways is still not straightforward and requires synthetic internal phosphopeptide standards. ICP-MS represents a valuable complementary approach for protein phosphorylation analysis with respect to the fast screening of complex samples for phosphopeptides, as well as their absolute quantitative determination.

With a number of papers, Wind and co-workers pioneered the field of ICP-MS assisted protein phosphorylation analysis.[41,44,45,99] In this context they also introduced the successful application of capillary HPLC hyphenated to ICP-MS under the same chromatographic conditions, which is a prerequisite for the real complementary application of ESI and ICP based MS techniques in life sciences related research.

As already stated, the application of nano or capillary HPLC dramatically reduces the problems related to the introduction of the frequently used RP organic gradient solvents, such as acetonitrile or methanol, into the ICP.

Wind et al. used capillary LC hyphenated to either high-resolution ICP-MS and a hexapole collision-reaction cell ICP-MS or ESI-MS-MS for the phosphorus-specific detection of different synthetic phosphopeptides and tryptic digests of β-casein or activated MAP kinase. Membrane desolvation was used to reduce the organic solvent load within the plasma, especially during the hexapole ICP-MS experiments. However, incomplete peptide recovery from the desolvation systems has been observed, especially for late eluting compounds.[41]

A further paper focused on the demonstration of phosphorylation degree determination of different model proteins via simultaneous measurement

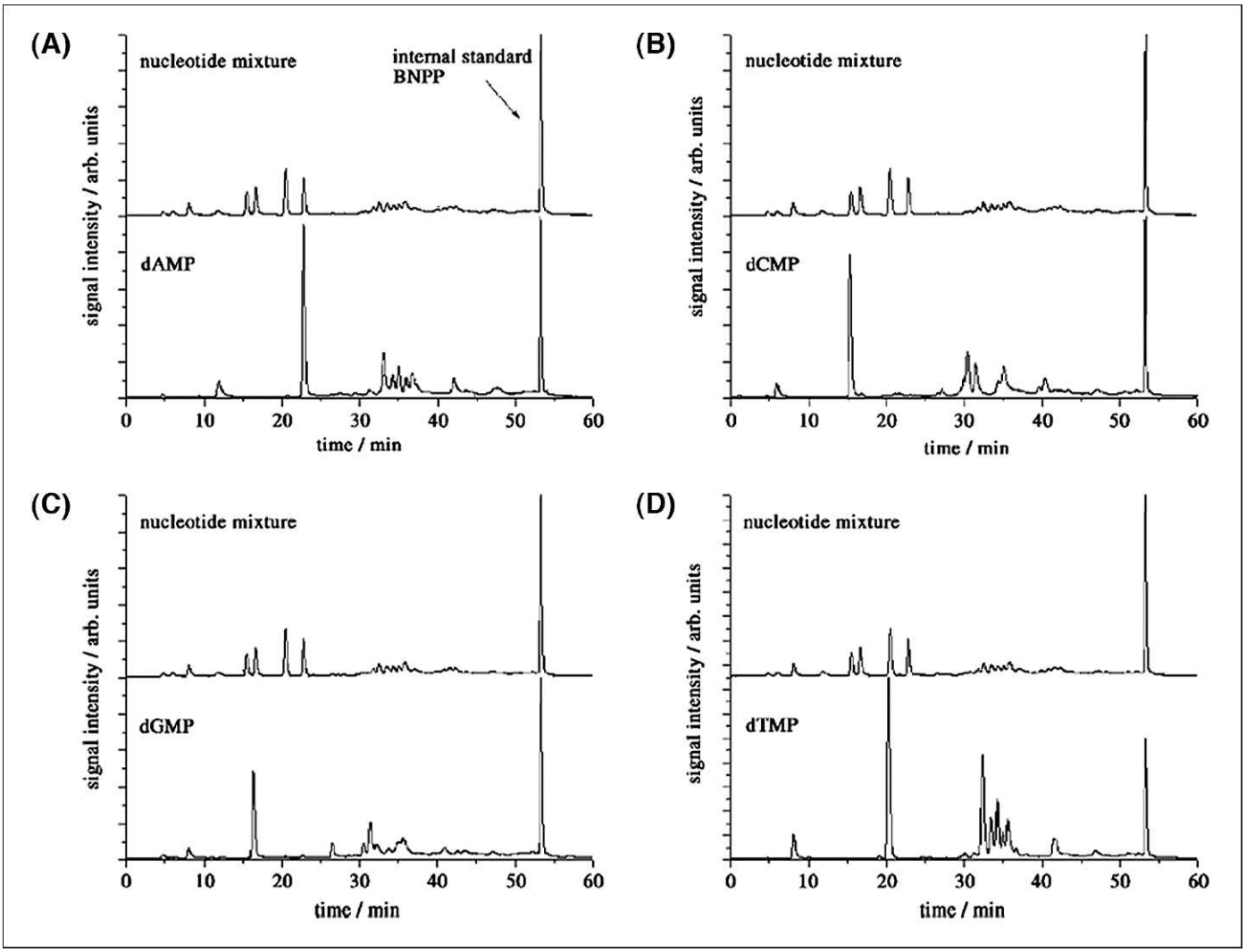

FIG. 5. LC-ICP-MS (^{31}P) chromatograms of Melphalan adducts in the nucleotide mixture (top trace) and of single nucleotides; all with internal standard (last signal). The phosphate adducts (first signal) and the base adducts (smaller following signals) are clearly distinguishable. (A) dAMP, (B) dCMP, (C) dGMP, and (D) dTMP. (Taken from Ref. 16, reprinted with permission from Journal of Mass Spectrometry 2006, 41, 507-516. Copyright 2006 John Wiley & Sons Ltd.)

of the phosphorus and sulfur content of the eluting peptide fractions.[44]

Minimized dead volumes are essential for highly resolved nano or capillary LC separation of phosphopeptides. Within this background, Wind et al. modified a direct injection nebulizer (DIHEN) to minimize the dead volume of the device. In comparison with their previously used spray chamber nebulizers, the DIHEN was slightly less sensitive but showed better chromatographic resolution, superior signal stability, and more robustness with respect to changing gradient conditions.[45]

In a number of further papers the same authors extensively demonstrated the complementary application of elemental and molecule specific MS for the investigation of protein phosphorylation of real samples, such as the so-called polo-like kinases Plx1 and Plx2, human fibrinogen and fetuin subunits, or the phosphorylation state at His-48 of the chemotaxis protein CheA, which influences the stability of this protein.[167–169]

To overcome some of the limitations related to the different described interface systems, Pröfrock and co-workers introduced a new interface system for the successful direct hyphenation of capillary and nano HPLC to a collision cell ICP-MS. This interface allows the direct introduction of organic gradients into the ICP-MS, without the need for using membrane desolvation or oxygen addition.[49] Helium was used as cell gas to minimize polyatomic interferences at the mass of phosphorus, while maintaining a good overall instrumental sensitivity. With this new interface, 100% transport efficiency, good nebulization stability, and minimized dead volumes at capillary as well as nano HPLC flow rates (below 1 μL min^{-1}) have been realized.

The well-known protein β-casein was used as a model to demonstrate the

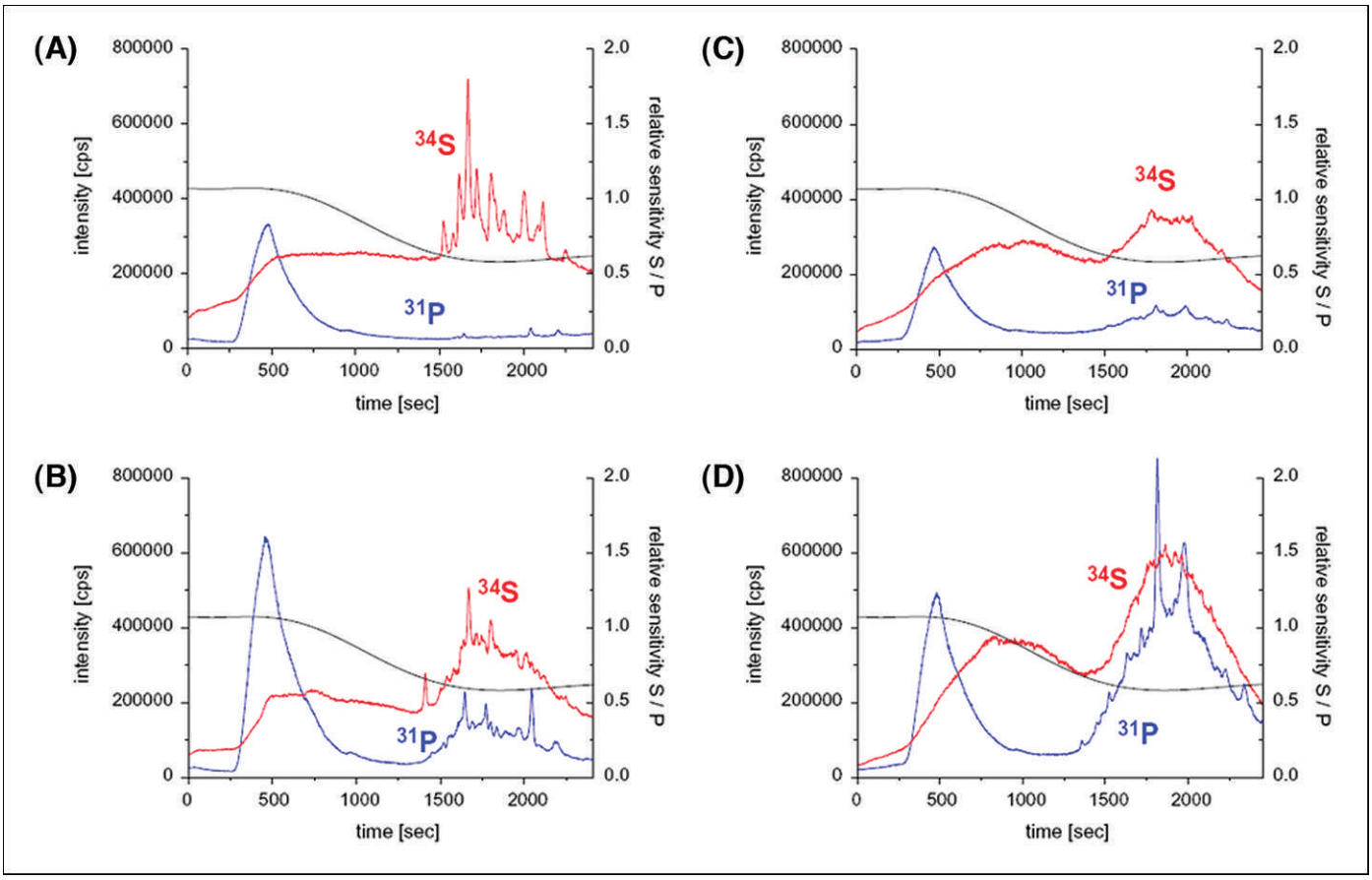

Fig. 6. LC–ICP-MS analysis of digested protein extracts before and after phosphoprotein enrichment by metal oxide affinity chromatography (MOAC). Left row: A. thaliana seeds (A) before and (B) after MOAC enrichment; right row: C. reinhardtii (C) before and (D) after MOAC enrichment. (Taken from Ref. 170, reprinted with permission from Biochemical and Biophysical Research Communications 2007, 355, 89-96. Copyright Elsevier 2007)

potential of the setup for the phosphorylation profiling of tryptic protein digests. In addition capillary LC-ESI-MS-MS was used to further characterize the pre-selected peptides, due to their phosphorus content. Four hundred femtomoles (400 fmol) of the singly phosphorylated peptide with the sequence FQpSEEQQQTEDELQDK derived by the tryptic digestion of β-casein could be detected, while detection limits of 1.95 μg L^{-1} (1.95 pg absolute) for phosphorus were obtained.[49]

More recently Krüger et al. analyzed protein phosphorylation levels of *Arabidopsis thaliana* and the algae *Chlamydomonas reinhardtii* as representatives for multicellular and unicellular green, photosynthetically active organisms with the aim of quantifying differences in the cellular protein phosphorylation level of both species.[170] Therefore, capillary HPLC and high-resolution ICP-MS in the medium resolution mode were employed for the separation and element-specific detection of phosphorylated peptides after enzymatic digestion of the protein extracts. In parallel, the corresponding sulfur traces were also monitored, which allows the conversion of the molar phosphorus-to-sulfur ratio into a stoichiometric protein phosphorylation degree.

In addition, metal oxide affinity chromatography (MOAC) has been used to specifically enrich phosphoproteins from the different plant extracts. Unfortunately the authors did not present additional ESI data with respect to phosphoprotein/peptide identification, even though the ICP-MS based screening of the samples indicated the presence of a number of phosphorylated peptides. As an example Fig. 6 shows the LC–ICP-MS analysis of digested protein extracts, before and after phosphoprotein enrichment by MOAC.[170]

The quantification of unknown phosphorylated peptides requires the correction of the well-known gradient impact on the instrumental phosphorus response, in particular during reversed-phase separations, e.g., by the application of mathematical correction functions.

To overcome the need for a mathematical correction of the phosphorus response, Pereira Navaza et al. introduced a new strategy for the accurate quantification of protein phosphorylation using simple organic compounds as a phosphorus standard.[96] They described the utilization of a constant post-column sheath flow with a constant acetonitrile content to buffer gradient composition changes that influence the ionization

efficiency of phosphorus within the plasma, even at capillary LC or nano-LC flow rates. They achieved a constant elemental response over the used capillary LC gradient (10–50% B), allowing the application of phosphorus-containing compounds, such as bis(4-nitrophenyl) phosphate (BNPP) as internal standards for accurate phosphopeptide quantification.[96]

Recently a new approach for the compensation of gradient-related changes of the instrumental response for phosphorus during reversed-phase gradient separation of phosphorylated peptides has been developed. Instead of a constant sheath flow, a second precise capillary LC pump has been used to generate a countercurrent reversed gradient that is mixed post-column with the RP column outflow before entering the capillary and nano-LC-ICP-MS interface. The experimental design allows the application of gradient separations, while the element-specific detection is carried out under isocratic conditions with a constant organic solvent intake into the plasma during the whole separation. This helps to eliminate any changes in the elemental response during reversed-phase separations, which is a general prerequisite for the application of ICP-MS for absolute quantification of proteins and peptides via their (hetero) element content, especially when no corresponding high-purity standards are available. Highly reproducible separations have been obtained, with retention time and peak area RSDs of 0.05% and 7.6% ($n = 6$), respectively. Detection limits for phosphorus of 6.24 μg L^{-1} (6.24 pg absolute) have been realized. In addition, an automatic routine for flow injection analysis (FIA) at the end of each chromatographic separation has been developed to calibrate each chromatographic separation. This makes absolute quantification of the separated species possible whenever their tag stoichiometry is known. As proof of concept, phosphorylated peptides have been used as model compounds for method development and to demonstrate the applicability of the proposed setup for phosphopeptide quantification (see Fig. 7) on the basis of simple inorganic phosphorus standards.[93,171] Beside the application of capillary LC, laser ablation ICP-MS (LA-ICP-MS) as an interface to combine high-resolution separation techniques such as 1D or 2D gel electrophoresis has also gained much interest, especially for protein phosphorylation analysis.

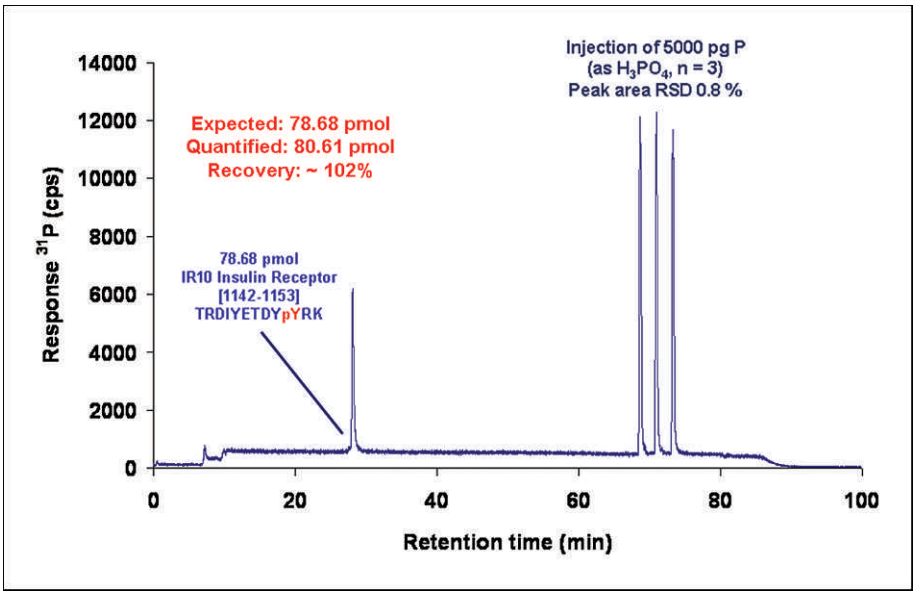

FIG. 7. Quantification of an IR10 Insulin Receptor [1142–1153] standard sample using a simple inorganic phosphorus standard and translation of the phosphorus content into molar peptide amounts, based on the known phosphorus stoichiometry of the peptide. (Taken from Ref. 93, reprinted with permission from Journal of Chromatography A 2009, 1216, 6706–6715. Copyright Elsevier 2009).

The first report with respect to the utilization of LA-ICP-MS for protein phosphorylation analysis was published by Marshall et al., who described the investigation of membrane-blotted phosphorylated proteins by LA-ICP-MS.[172] β-casein was used as model protein and detection limits of 16 pmol were obtained.

Also a direct analysis of gels containing the destained protein spots has been performed. However, due to the high phosphorus background of the gel matrix it was not possible to determine the location of the phosphorylated proteins.[172]

To overcome the contamination problem, Wind et al. improved this approach by adding a washing step after the blotting process into their strategy for the LA-ICP-MS based analysis of gel-separated phosphoproteins.[173] $Ga(NO_3)_3$ was used as complexing agent to remove any non-covalently bound phosphate from the membrane, which is known to result in non-specific interactions with other non-phosphorylated sample constituents, leading to false positive results. Detection limits of 5 pmol phosphorus were obtained. The authors also presented quantitative data indicating the possibility of using this approach for the quantification of gel-separated phosphoprotein species.[173]

A new strategy for the analysis of gel-separated phosphoproteins by combining whole gel elution with flow-injection ICP-MS detection has been published by Elliott and colleagues. Phosphorus was measured as $^{31}P^{16}O^+$ at m/z 47. Results were compared with the phosphorus-specific screening of 1D PAGE gels by LA-ICP-MS.[174] Along with the contamination problem, the relatively small size of the standard ablation cell represents a further limitation of LA-ICP-MS, since gels or blot membranes had to be cut into pieces to facilitate their whole analysis.

To overcome this shortcoming, Feldmann and co-workers developed and optimized a new laser ablation cell for the detection of phosphoproteins blotted on to nitrocellulose membranes.[175] In comparison to other ablation cells, their new device allowed the direct analysis

of whole blot membranes with dimensions up to 8.5 cm height and 10 cm length. Detection limits for the model proteins pepsin and β-casein down to 5 and 3 pmol, respectively, were obtained. The quantitative results calculated for a commercially available protein marker standard on the basis of a previously acquired calibration function were also in good agreement with the concentrations specified by the manufacturer. Overall this approach is mainly limited by the possibility of additional sample loss and the additional time needed for the blotting process.[175]

Based on the ablation cell described by Feldmann and co-workers, Venkatachalam et al. introduced a new calibration approach for the quantitative detection of phosphorylated proteins, blotted onto membranes.[78] For the calibration procedure different standards (β-casein, pepsin, phosphate solution) containing different known amounts of phosphorus were dotted directly onto the blotting membrane. The results indicate that the dotting procedure leads to recoveries of the test proteins of only around 55%. As an alternative, the standards were separated and blotted together with the test sample. A total amount of 126 pmol of phosphorus was determined with the second approach. These results were in good agreement with the known calculated total phosphorus amount of 139 pmol of the test sample. Additionally, a higher dynamic range in comparison to previous work has been obtained. The standard deviation of the whole method (electrophoretic separation, blotting process, laser ablation procedure) was 6%.[78]

In a recent publication Krüger et al. showed for the first time the protein and proteome phosphorylation stoichiometry analysis of the cytoplasmatic proteome of bacterial cells (*Corynebacterium glutamicum*) and eukaryotic cells (*Mus musculus*) by a combined approach based on one-dimensional gel electrophoresis, in-gel digestion, and capillary reversed-phase LC-ICP-MS, as well as an additional strategy that includes the application of 1D gel electrophoresis, protein blotting, and LA-ICP-MS.[176] Consistent quantitative results have been obtained with both approaches; however, higher sensitivities were achieved with the capillary LC based setup. In this study the eukaryotic proteome of *Mus Musculus* was found to be significantly more phosphorylated in comparison to the bacterial proteome (around 0.8 mol of P/mol of protein versus around 0.01 mol of P/mol of protein). This study demonstrated for the first time the direct and rapid determination of a global phosphorylation degree of a complex protein mixture by using different ICP-MS methodologies.[176]

The examples given above clearly show the possibilities that arise because of the complementary application of ICP-MS in protein phosphorylation studies. In addition, ICP-MS can help to replace standard procedures, e.g., the in vivo or in vitro incorporation of radioactive ^{32}P for phosphorylation analysis, which is still restricted to selected laboratories.[177,178] A very valuable review article also dealing with this topic was recently published by Navaza et al.[36]

Absolute Protein and Peptide Quantification. The accurate absolute quantification of proteins and peptides is still a challenge. Knowledge about the dynamic change of protein abundance directly reflects the status of biological systems and therefore can provide valuable information in particular for life science orientated research.

Wind et al. proposed sulfur as a key element for the absolute quantification of proteins and peptides.[99]

Recent calculations on the basis of the human proteome have revealed that 26.6% and 25.5%, respectively, of the resulting tryptic peptides include at least one of the sulfur-containing amino acids cysteine or methionine within their sequence.[2] These peptides represent 96.1% and 98.9%, respectively, of all human proteins. This facilitates the quantitative determination of the majority of proteins on the basis of their natural sulfur content, once the sulfur stoichiometry is clarified by the complementary application of ESI or MALDI based MS approaches.[99]

Insulin has been used as a model protein to demonstrate its absolute quantification, while thiamine has been used as an internal sulfur standard. The authors also showed the complementary application of capillary LC HR-ICP-MS and capillary LC ESI-MS-MS for the quantification and characterization of tryptic protein digests of two functional domains of the bacterial chemotaxis protein cheAH (3-137) and cheA-C (257-513) over-expressed in *E. coli*.[99,179]

Over the last few years Heumann and co-workers have promoted the application of isotope-dilution approaches for accurate quantitative elemental speciation analysis using either non-species-specific or species-specific strategies.[108,180–182] Based on this development, Prange and Schaumlöffel et al. pioneered the field of sulfur isotope-dilution analysis by combining post-column non-species-specific isotope dilution and capillary electrophoresis hyphenated to sector field ICP-MS for the highly resolved separation of different metallothionein (MT) isoforms, which allowed determination of the metal stoichiometry of the different isoforms as well as their absolute quantification.[183,184] A non-species-specific element spike containing ^{34}S, ^{65}Cu, ^{68}Zn, and ^{116}Cd introduced via the make-up flow of the CE interface was used for on-line isotope ratio measurement of $^{32}S/^{34}S$, $^{63}Cu/^{65}Cu$, $^{64}Zn/^{68}Zn$, and $^{114}Cd/^{116}Cd$. Reversed isotope dilution, using a standard solution containing S, Cu, Zn, and Cd with natural isotopic abundances, has been used for mass flow calibration of the different elements. Based on the known MT-to-sulfur stoichiometry, molar ratios between sulfur and the different metals, as well as the absolute amounts of the different isoforms were quantified.[183,185]

This initial study has been further improved by Wang and Prange by using surface-modified CE capillaries to obtain a better separation of the different MT isoforms, which can be found in the commercially available preparations.[186]

Polec-Pawlak et al. demonstrated the application of the CE-ICP-IDSF approach developed by Prange and Schaumlöffel for the analysis of metallothionein complexes in rat liver extracts. In addition, ESI-MS was used as a complementary technique to further identify the separated protein species.[187]

A comparable setup has been used by Van Lierde et al. for the quantification and determination of the stoichiometric

Zn/protein ratio for the *Aeromonas hydrophila* (AE036) metallo-beta-lactamase.[188] However, in this study simple non-species-specific element standards such as albumin (for S) and $ZnCl_2$ have been utilized for quantification. Protein detection limits of 8 ng calculated on the basis of sulfur were obtained.[188]

The introduction of collision and reaction cell technology induced a paradigm shift since it became possible to analyze highly interfered elements such as sulfur with quadrupole-based instrumentation either when using GC, LC, or CE for sample introduction.

Pröfrock et al. demonstrated the first application of a quadrupole-based collision/reaction cell ICP-MS with xenon as cell gas to reduce the interferences at the main isotope ^{32}S.[95] Xenon as cell gas helped to decrease the spectral background by about six orders of magnitude, which is a prerequisite for the determination of sulfur using its main isotope ^{32}S. Instrumental detection limits of 1.3 ng mL^{-1} (^{34}S) and 3.2 ng mL^{-1} (^{32}S) were obtained. The authors also demonstrated the sulfur specific detection and quantification of metallothionein isoforms isolated from non-incubated fish samples after on-line CE-ICP-MS, using non-species-specific element standards.[95]

To overcome the $^{16}O_2^+$ interference problem Bandura and co-workers applied quadrupole ICP-MS with a dynamic reaction cell for the determination of sulfur by using oxygen as cell gas to generate $^{32}S^{16}O^+$ ions. Due to the oxidation of $^{32}S^+$ a mass shift of +16 was generated, allowing the detection of sulfur at *m/z* 48, which suffers less interference.[83]

Yeh et al. used this strategy for the determination of sulfur-containing amino acids by using capillary electrophoresis coupled to dynamic reaction cell ICP-MS.[189]

In general CE-based approaches pioneered the application of sulfur as an element tag for absolute protein quantification; however, this technique is still limited by the small sample amounts used in CE, the relatively high detection limits, and the reduced long-term stability of most interface systems.

In a very recent paper Wang et al. used normal bore HPLC coupled to hexapole collision cell ICP-MS for the quantitative analysis of intact proteins (BSA, SOD, MT-II) via their sulfur content. Interferences have been minimized by using oxygen as cell gas. Post-column isotope dilution analysis has been used to quantify the different model proteins. Detection limits of 8, 31, and 15 pmol have been obtained.[190]

SEC coupled to either ICP-DRCMS (oxygen as cell gas) or ICP-SFMS (medium resolution) has been used by Hann et al. for the determination of metal sulfur ratios in selected metalloproteins, such as myoglobin or Mn superoxide dismutase.[191] Detection limits for sulfur of 4.3 μg L^{-1} were obtained with the ICP-DRCMS approach in comparison to 14 μg L^{-1} obtained with ICP-SFMS instrumentation. Non-species-specific calibrants such as Fe^{3+}, Mn^{2+}, or SO_4^{2-} were tested for quantification; however, in terms of measurement uncertainty, the best results were obtained by using metalloprotein standards. Unfortunately this application may be restricted to pure or purified protein samples due to the limited chromatographic resolution obtainable with SEC.[191]

Both LC-ICP-MS and LC-ESI-TOF-MS have been used by the same author for the investigation of the metal stoichiometry of native and recombinant copper proteins derived from the cyanobacterium *Synechocystis*. Sulfur was measured as $^{32}S^{16}O^+$ using oxygen as the cell gas. An altered sulfur/metal ratio caused by the removal of an N-terminal methionine indicates the heterogeneous expression of two of the recombinant proteins. Mainly influenced by the chromatographic separation method, detection limits for sulfur between 4.6 μg L^{-1} (SEC-ICP-MS) and 16 μg L^{-1} (IC-ICP-MS) have been obtained.[128]

Recently Ellis and colleagues demonstrated the complementary molecule- and element-specific detection of thioarsenicals by using collision cell ICP-MS and xenon to enable a sulfur-specific detection. Spiked, NIST, freeze-dried urine was used as sample matrix. Sulfur detection limits below 0.05 mg kg^{-1} have been obtained.[192]

Newly developed molecule complexes allow the cross-membrane transport of gadolinium complexes, which are widely used as paramagnetic contrast agents for the imaging of intracellular compartments. Krüger et al. used a combined approach based on elemental and molecular mass spectrometry for the characterization of a gadolinium-tagged modular contrast agent for MRI.[193] SEC-ICP-MS has been used to investigate the Gd saturation of the synthetic transporter complex via the measurement of the sulfur-to-gadolinium ratio. The contrast agent was further characterized by static nano-ESI-QTOF-MS.[193]

In 2008 Zinn et al. reported the coupling of capillary LC to high-resolution ICP-MS for the absolute quantification of intact proteins (human apolipoprotein A1, α-fetoprotein) via post-column sulfur isotope dilution analysis.[194] Oxygen has been added to the spray chamber to improve the signal stability, due to oxidation of the carbon originating mainly from the organic solvents used. The sulfur blank derived from the instrument itself, the solvent, and the used gases restricted the sensitivity of the setup; however, absolute detection limits for sulfur of 350 pg have been realized.

Garijo Anobe and colleagues used sulfur as an additional natural tag during the investigation of different iron-containing metalloprotein standards.[195] They introduced a new interface comparable to those described by Brüchert and Bettmer for on-line isotope-dilution GE-ICP-MS. Iron–sulfur ratios were measured to monitor possible iron loss during the sodium dodecyl sulfate–polyacrylamide gel electrophoresis separation.[158,195,196]

In summary it is fair to say that most examples regarding the absolute quantification of a biomolecule via its sulfur content have been carried out with proteins. In consequence, currently only protein samples of low complexity can be quantified with a sulfur-based approach due to the problems related to the chromatographic separation of intact proteins. In particular, column recoveries have to be considered for reliable quantification, since this parameter strongly depends on the chemical properties of the investigated proteins, such as molecular weight, amino acid composition, pI, or hydrophobicity.

To overcome this problem, a protein

can also be quantified on the peptide level. Recently Schaumlöffel et al. used pre-column isotope dilution analysis and nano-HPLC-ICP-MS for the absolute quantification of sulfur-containing peptides.[197] In parallel, nano-HPLC-ESI-QTOF-MS has been utilized to identify and elucidate the sulfur stoichiometry of the separated peptides, which is mandatory for the precise, absolute quantification based on the natural sulfur tag, especially when working with unknown proteins. As introduced by Pröfrock et al., they also utilized xenon as cell gas to overcome the sulfur interference problem. Different sulfur-containing peptides, as well as tryptic digests of human serum albumin (HSA) and the salt-induced protein (SIP 18), isolated from selenium-rich yeast, were used as model compounds for method development and validation. A detection limit for sulfur of 45 μg L^{-1}, which corresponds to 1–2 pmol of the individual peptide species, has been reported. Recovery rates of 103% of the individual peptides and good precision of the quantification with an RSD of 2.1% were obtained.

In summary the selected examples clearly indicate the variety of possibilities that arise due to the quantification of proteins or peptides using their natural sulfur tag. Unfortunately the overall number of examples that can be found in the literature is still quite limited. Currently most applications are focused on the quantification of selected, intact, model proteins.

(Hetero)element-Labeled Compounds. Despite the widespread distribution of ICP-MS detectable (hetero) elements such as phosphorus or sulfur within biomolecules, a multiplicity of molecules may still not contain the mentioned elements. However, selected chemical reactions can be utilized to derivatize selected amino acids with the aim to covalently attach a (hetero)element tag, which makes the molecule detectable and therefore visible for ICP-MS. Different good reviews are meanwhile available, indicating the potential of this approach.[24,27]

Early labeling studies used mercury species for the specific labeling of thiol residues. More recently such approaches have been used for the successful quantification of proteins such as insulin or ovalbumin, respectively.[28,29]

Cartwright et al. described the application of tris(2,4,6-trimethoxyphenyl)-phosphonium propylamine bromide (TMPP) to label compounds containing carboxylic acid residues with phosphorus.[198] HPLC hyphenated to high-resolution sector field ICP-MS operated at medium resolution has been used to detect the labeled compounds via their phosphorus content. Absolute detection limits for phosphorus ranging from 1.4 to 7.8 ng have been obtained. The proposed approach helps to make compounds detectable by ICP-MS; however, the selected element tag is not ideally suited due to the well-known limitations related to the detection of phosphorus using ICP-MS. In addition chemical activation of the carboxylic acids is required, which complicates the overall procedure.

Unfortunately only 10–20% of the carboxylic acids were derivatized even under optimized reaction conditions, which is insufficient especially when attempting a real quantitative analysis of carboxylic-acid-containing compounds such as amino acids in peptides.

More recently Venkatachalam et al. used stable iodine isotopes for the labeling of antibodies, which specifically targets different members of the cytochrome P450 enzyme family. Laser ablation ICP-MS has been used for the detection of membrane-blotted microsomal cytochromes via specific, iodinated antibodies.[199] A similar approach has been recently used for the absolute quantification of iodinated peptides using capillary LC hyphenated to ICP-MS.[32]

During the last few years labeling using lanthanides and bi-functional chelating agents gained much interest and also represents the latest trend in ICP-MS based quantitative analysis.[24,27] Their application for absolute protein quantification,[35] multiplexed immunohistochemical detection of tumor markers,[200] peptide quantification,[201] or multiplexed bioassays[202] indicate the future potential of such labeling approaches.

CONCLUSION

The outstanding instrumental developments during the last decade in the field of elemental mass spectrometry and its application as a (hetero)element-specific detector, as well as the continuous progress in the development of new hyphenated techniques, has resulted in a growing interest in such technologies and their unique analytical properties. As indicated by the broad range of examples ranging from environmental analysis of emerging compounds to proteomics related topics, such as protein phosphorylation studies or absolute protein quantification, ICP-MS has the potential to become a key technology for quantification, especially because of its unique characteristics, such as compound-independent ionization behavior, sensitivity, and robustness, which represent the main strengths of ICP-MS. However, it has to be kept in mind that ICP-MS can be used for quantification only when suitable standards for calibration are available. In the case of a compound-independent calibration, knowledge about the (hetero)element tag stoichiometry is always mandatory. This clarifies that ICP-MS has to be a part of an integral concept that also includes detection techniques such as ESI or MALDI-MS that can provide the essential molecule-specific information in terms of molecular weight, structure, or (hetero)element composition, which are necessary for the successful application of ICP-MS for quantification in both environmental as well as life science research disciplines.

From the above summary of the published literature, it can be seen that the coupling of GC to ICP-MS has great potential for (hetero)element-based applications due to the resulting dry plasma conditions and its robustness. However, the recent improvements in the hyphenation of capillary and nano-LC to ICP-MS have helped to overcome some of the well-known problems related to "normal" LC-ICP-MS setups. As recently reviewed, elemental labeling strategies can help to overcome the limitations of natural (hetero)element tags in terms of occurrence and detectability.[24,27] This technique shows interesting potential, especially for the design of accurate multiplexed quantification schemes as needed for future biomedical applications such as high-throughput biomarker screening and quantification.

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