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Atomic Spectrometry Update: review of advances in elemental speciation

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This is the 14th Atomic Spectrometry Update (ASU) to focus on advances in elemental speciation and covers a period of approximately 12 months from December 2020. This ASU deals with all aspects of the analytical atomic spectrometry speciation methods developed for: the determination of oxidation states; organometallic compounds; coordination compounds; metal and heteroatom-containing biomolecules, including metalloproteins, proteins, peptides and amino acids; and the use of metal-tagging to facilitate detection *via* atomic spectrometry. As with all ASUs, the focus of the research reviewed includes those methods that incorporate atomic spectrometry as the measurement technique. However, because speciation analysis is inherently focused on the relationship between the metal(loid) atom and the organic moiety it is bound to, or incorporated within, atomic spectrometry alone cannot be the sole analytical approach of interest. For this reason, molecular detection techniques are also included where they have provided a complementary approach to speciation analysis. This year, the number of publications covered has fallen, quite possibly due to the SARS-CoV-2 pandemic restricting laboratory access, but the number of elements covered remains high at over 30. The most popular elements are still As, Hg and Se, whilst more workers are using some form of separation to reduce the dissolved ion signal when undertaking analysis for NPs. There has also been an increase in the number of papers reporting on field deployable miniaturised devices for elemental speciation, a trend which will surely continue as the associated technologies develop more robust and less power hungry portable excitation sources.

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1 Topical reviews

This latest update adds to that from last year[1] and complements the five other annual Atomic Spectrometry Updates, advances in environmental analysis,[2] advances in the analysis of clinical and biological materials, foods and beverages,[3] advances in atomic spectrometry and related techniques,[4] advances in X-ray

fluorescence spectrometry and its special applications[5] and advances in the analysis of metals, chemicals and materials.[6]

Several *relevant books or book chapters* have appeared in the current review period. An open-access e-book, "Advances in Analytical Techniques and Methodology for Chemical Speciation Study" contains 16 chapters that cover a wide range of areas in which the application of atomic spectrometry techniques are described. An online editorial provides a helpful overview of the e-book contents, and the e-book can be accessed from ref. 7. Although most of the text is devoted to miniature and portable analytical devices with quantification by techniques other than atomic spectrometry, the coupling of miniaturised separation devices with ICP-MS for speciation applications is reviewed in one chapter.[8] A number of texts with an environmental focus have been published. A 17-chapter e-book "Remediation of Heavy Metals" features a substantial introductory chapter[9] devoted to "Analytical Methods for the Determination of Heavy Metals in Water," whose authors stress the importance of speciation analysis, which they described as "a fundamental yet still undervalued aspect of the integral evaluation of the presence and impact of heavy metals in waters." A number of chapters in a book devoted to bioremediation are concerned with the removal of metals, and the importance of speciation is particularly highlighted in the chapter devoted to phytoremediation of mine waste disposal sites.[10] The 15 chapters in "Arsenic Toxicity: Challenges and Solutions" are concerned with aspects of sources of As contamination, its impact on human health, and on prospective bio- and phytoremediation. Applications of genetic engineering and nanotechnology to the problems of As contamination in ground water and river basins are also discussed. Chemical analysis is specifically highlighted in a chapter on seafood.[11] Relevant solid-state speciation topics are discussed in several chapters in "Metallurgical Slags: Environmental Geochemistry and Resource Potential".[12] In the past, slags have been discarded as waste and, through the release of potentially toxic trace elements, represent environmental and human health hazards. However, the resource potential of slags is being increasingly recognised, and this book is aimed at all those "interested and inspired by a circular economy and minimising our environmental footprint on planet Earth." Chemical analysis, including solid-state speciation, is highlighted in Chapter 3. Solid-state speciation is also featured in a text devoted to magma redox geochemistry.[13] Part three of the book (7 chapters) is devoted to "tools and techniques to characterise the redox and its effect on isotope partitioning." The most recent text in the series "Geochemical Tracers in Earth System Science" is entitled "The Iron Speciation Paleoredox Proxy".[14] Details of the contents are difficult to find, but there is some discussion of the analytical chemistry measurements needed to support work on this topic. A book dealing with the analysis of foods has been published: the most recent text in the Food Bioactive Ingredient series, "Analytical Methods in the Determination of Bioactive Compounds and Elements in Food", contains a substantial chapter on elemental speciation[15] and a chapter devoted to the "Two Sides of Selenium: Occurrence and Determination of Selenium Forms in Food and Environmental Samples Using Analytical Methods".[16] Two texts devoted to toxicology have appeared, one of which is devoted to the toxicology of metals. The fifth edition of the two-volume "Handbook on the Toxicology of Metals" contains a well-written introductory chapter in the first volume (General Considerations[17]) and a second that discusses the relevant chemistry of metals, sampling, analytical methods, and speciation.[18] The second volume consists of 38 chapters each devoted to an individual element each of which contains a section entitled "Methods and problems of analysis." The importance of speciation is mentioned several times in a text on the essentials of toxicology[19] but there is no coverage of how information on speciation is obtained.

The *review articles appearing during the current review period are mostly concerned with environmental topics*. Some of these topics are quite broad; following the introduction of environmetallomics, featured in last year's elemental speciation ASU,[1] this year has seen the arrival of "agrometallomics" and the term "clinimetallomics"[20] has also appeared though in an article with a rather narrow focus, namely As speciation by HPLC-ICP-MS in the urine of patients with arsenism (discussed in Section 3.2). The article which coins the term agrometallomics is a review (283 references with titles) of analytical methodologies for the characterisation of the "entirety of metal and metalloid species that can induce healthy or toxic effects on living organisms at agriculturally relevant levels and ranges" in, one assumes, agriculturally relevant materials.[21] These would appear to include "agricultural plants, animals and edible fungi, seed, fertiliser, pesticide, feedstuff, as well as the agricultural environment and ecology, and even functional and pathogenic microorganisms." The reviewers identified the following categories of methods: ultrasensitive and high-throughput analysis, elemental speciation and state analysis, and spatial- and microanalysis, and thus the review is constructed primarily around the analytical techniques and not the sample material or analytes. There is a substantial sub-section devoted to single cell and microparticle analysis within the "spatial and microanalysis" section. The reviewers concluded with some suggestions for areas of further development that included the significant challenge of transformation-free, and high-efficiency sample pre-treatment for elemental speciation. There was almost no discussion of the needs for portable equipment, though a number of determinations by LIBS or pXRFS were described. Viana *et al.*, in a review of extraction techniques for elemental speciation in environmental materials,[22] also considered that maintaining the chemical stability of the species and avoiding equilibrium disruptions during the sample treatment is one of the biggest challenges in chemical speciation, especially in environmental matrices where the concentrations of potential interferents are normally high. They confine their review (146 references with titles) to methods for waters, soils and sediments. Following a fairly standard introduction to sampling and sample preservation, the bulk of the review is concerned with several extraction techniques for aqueous samples. These include SPE, SPME (including the use of magnetic materials), hollow-fiber extraction, CPE, DLLME, and headspace single-drop extraction. The section devoted to the extraction of species from soils and sediments is much

shorter. The review is organised by technique and so following progress for a particular element is difficult. The reviewers concluded that because obtaining "comprehensive information about the species distribution of a given element in a complex environmental sample is virtually impossible," researchers should aim to "meet the needs involved, respecting the available resources, and should inform (the end user about) the limitations inherent to the methods and techniques used."

Two *reviews have focused on separation techniques less commonly used in conjunction with atomic spectrometry*. For the characterisation of engineered elemental metal NPs in the environment, Bai *et al.*[23] examined (103 references with titles) the performance of various FFF procedures coupled with element-specific ICP-MS detection. The reviewers focused on the AF4 and hollow-fibre methods, pointing out that a major advantage of AF4 methods over SEC is the application to a wide range of particle sizes, from 1 nm to 100 μm, which covers the complete size range of engineered metallic NPs. In addition to discussing material published in the recent research literature, the reviewers also provided tutorial introductions to the various techniques, which readers unfamiliar with AF4 separation may find useful. The reviewers concluded that differentiating between NPs and their coexisting derivatives, such as their corresponding ions, free ions, and complexes of the corresponding ions in real biological matrices, still represents significant challenges in the field of environmental nanotoxicology. They also pointed out that laboratory experiments on the transformation or uptake of NPs in detectable concentrations do not represent real scenarios and that further improvements in the performance of AF4-ICP-MS are required, such as increasing the sensitivity by the injection of large volumes (50 mL), which could allow elucidation of the various processes that govern their fate and behaviour during aging or long-term exposure studies. In reviewing the prospects of hydrodynamic chromatography, Brewer[24] cites only 21 references (with titles). Separation occurs within the parabolic laminar flow velocity profile in an open tube or in the interstitial spaces of a packed column, whereby analytes access the various streamlines based on size and, as a result, the larger analytes in the faster moving streamlines elute prior to the smaller ones. A range of detection methods, RI, UV absorption, MALS, quasi-elastic light scattering, differential viscometry and ICP-MS, coupled in various combinations have been described, indicative of the primary area of application to date, namely the characterisation of high molecular mass synthetic polymers. Possible application in the determination of NPs was briefly mentioned.

A review (118 references with titles) on the *applications of ICP-MS in the development of metal-based drugs and diagnostic agents* discussed[25] monitoring drug–biomolecule interactions, studying cellular and tissue distribution, probing the intracellular transformation and profiling active metabolites. The review also emphasised ICP-MS-based methodologies that provide (a) insights into drug activation and targeting chemistries, (b) monitoring of the cellular response to a drug and (c) high-resolution, quantitative tissue imaging. The reviewer concluded that part of the reason that not all of the "streamlined methodologies" (high resolution and triple quadrupole mass spectrometry or isotope dilution) have become widely accepted (as was forecast in a 2014 review of the topic by the same author) is that the majority of researchers are not trained analytical chemists. Consequently, "there is a common lack of basic quality control, which is made worse by the lack of CRMs." The reviewer also concluded that supporting the development of metal-containing compounds for therapeutic and diagnostic practice will continue to be an important application of ICP-MS.

2 CRMs and metrology

The NMIJ have added the phosphate ion to the certified analytes in two existing seawater CRMs, 7602-a and 7603-a.[26] In the original certification exercise, phosphate was determined by continuous flow analysis (CFA), due to the analyte levels being too low for an ICP-MS based approach. Due to the potential for interferences (*e.g.* As and Si) to bias results, the workers decided that a more sensitive ICP-MS based method was required to confirm the CFA data. This was achieved by coupling an AEC system with a sector field ICP-MS instrument equipped with the Jet interface cones and rotary vacuum pump to enhance sensitivity by a factor of 18 compared to that obtainable with the standard instrument set up. The separation of phosphate from the seawater matrix used IonPac AS23 AEC, with a guard column of the same stationary phase, a mobile phase of 4.9 mmol L^{-1} sodium carbonate and 0.5 mmol L^{-1} sodium hydrogen carbonate flowing at 1.5 mL min^{-1}. The column oven temperature was 30 °C and the regenerant was 25 mmol L^{-1} sulfuric acid flowing at 3 mL min^{-1}. A six-port valve was inserted after the AEC column to allow only the eluent or a rinse solution to be directed to the ICP-MS instrument. To achieve maximum stability and sensitivity, the nebuliser gas flow was optimised by using the residual $^{23}Na^{+}$ signal in the eluent. The ICP-MS was operated at a resolution of 4000, with a dwell time of 10 ms and a total run time of 21 minutes. The seawater samples were diluted 10-fold with high purity water, to minimise the impact of the tail of the large Cl^{-} signal on instrument sensitivity, and calibration was by a standard additions approach by spiking with phosphate at three levels. Internal standardisation, to negate the effects of instrumental drift, was undertaken by ratioing the $^{31}P^{+}$ peak area to that of the $^{75}As^{+}$ signal, which eluted approximately six minutes later, and this ratio had an RSD of 1.8% and the phosphate LOD was 0.005 μmol kg^{-1}. The uncertainty of the measurements was estimated by combining the uncertainties of the calibration curve slope, the measurement repeatability, dilution repeatability, the concentration of the calibration solutions and sample homogeneity. Method validation was by use of the MOOS-3 seawater CRM, with the result obtained, 1.56 ± 0.03 μmol kg^{-1}, being in good agreement with the certified value of 1.55 ± 0.14 μmol kg^{-1}. The values of phosphate in the CRMs 7602-a and 7603-a were found to be 1.00 ± 0.02 and 3.00 ± 0.03 μmol kg^{-1}, respectively, which compared favourably with the previously determined values by CFA of 1.07 ± 0.02 and 3.03 ± 0.03 μmol kg^{-1}, respectively. The phosphate content of CRM 7601-a, 0.016

$\pm$ 0.009 μmol kg^{-1} CFA value, was too low to be determined by the described method due to the need to dilute the samples 10-fold. The paper gives a large amount of detail on the method development stages and would be well worth reading by workers interested in using AEC-ICP-MS for seawater phosphate measurements.

With the present interest in *SeMet in nutritional supplements, it is of special importance to have CRM verified in SeMet* in order to ensure the quality of the results. The NRC of Canada has produced two SeMet CRMs: SENS-1, a powdered material of natural isotopic abundance and with purity traceable to the SI, and SEES-1, a concentrated solution of ^{82}Se-enriched SeMet for IDA measurements.[27] The material used for SENS-1 was a commercially available SeMet powder whereas SEES-1 was in-house synthesised from metallic Se through a three-step process described in detail in the paper. The total Se concentration in SENS-1 and SEES-1 was determined by IDA-ICP-MS measurements after MAD of the samples. The SeMet content and impurities were evaluated by RP-HPLC-ICP-MS and quantitative ^{1}H NMR. For HPLC-ICP-MS measurements, a separation system including a Zorbax XBB C18 column and a mobile phase of 10 mmol L^{-1} ammonium formate and MeOH with 1% formic acid at 0.25 mL min^{-1} at 40 °C was employed. The MeOH carbon load from the mobile phase was removed with 20% oxygen in Ar during ICP-MS analysis. The CRM solutions were also analysed by HPLC-orbitrap-MS using the same chromatographic conditions. The three major containing impurities detected and identified were selenohomocystine, 2-amino-4-(methyldiselenyl)butanoic acid and methyl 2-amino-4-(methyldiselenyl)butanoate. With the information obtained, along with the NMR data, the SeMet concentration in SENS-1 was established as 1.0961 $\pm$ 0.007 μg g^{-1}. Once its purity and concentration was determined, SENS-1 was used as the primary standard for reverse isotope dilution-HPLC-ICP-MS to determine the concentration of SeMet in SEES-1, which was found to be 1556 $\pm$ 60 μg g^{-1}.

3 Elemental speciation analysis

3.1 Antimony

The *environmental chemistry of Sb has been comprehensively reviewed* (169 references with titles).[28] The review also included extensive coverage of the relevant analytical methodologies and a discussion of common remediation/removal technologies. The section on speciation analyses is rather short: only 14 papers are very briefly mentioned. However, the reviewers concluded that as many questions about the species of Sb and their effects on organisms and humans are still not fully settled, future research is expected to make great progress as a result of improvements in analytical and characterisation techniques. The article contains several colourful, eye-catching illustrations that summarise topics such as the chronology of Sb research, biogeochemical cycling, water treatment, and soil bioremediation.

Three *reports of Sb speciation, each of which represents one of the three major approaches to making such measurements*: selective SPE or LLE, selective HG and HPLC-ICP-MS. In the first of these, Sb^{III} was selectively preconcentrated by extraction of a chelate complex into a MIL.[29] The researchers evaluated three different complexing agents and three different MIL before choosing DDTP and trihexyl(tetradecyl)phosphonium tetrachloroferrate, respectively. A chemometric optimisation strategy was applied in the section on the operating conditions giving the highest extraction efficiency, which for most parameters investigated was equivalent to the ETAAS signal obtained. Total Sb was determined after reduction of Sb^{V} by KI, whose concentration appeared to be crucial. The authors reported that concentrations above 1% (w/v) KI caused "high spectral interference," but did not explain the basis for this. Concentrations below 1% resulted in incomplete reduction. Further details are given in Table 2. Pentavalent antimonials are now the basis for all leishmaniasis treatments, whereas Sb^{III}, present as residues or produced through tissue reduction, is widely accepted as responsible for side effects, antileishmanial action and drug resistance. For the speciation of Sb in injectable leishmanicidal drugs by selective HG AAS the signal from Sb^{V} was suppressed by reaction with the citric acid.[30] Previously reported problems of decreasing sensitivity (due to suppression of active sites on the interior to the heated quartz tube walls) and memory effects, were eliminated by careful control of the citric acid concentration, which was substantially decreased compared with those typically recommended by other researchers. Values for the various operating parameters were selected on the basis of a multivariate optimisation consisting of a factorial design followed by a central composite design. The LOD for Sb^{III} was 0.05 μg L^{-1}, and for total Sb (determined after reduction with 1% KI) was 0.15 μg L^{-1} for total Sb. The procedure was validated by spike recoveries and applied to the analysis of seven meglumine antimoniate samples; Sb^{III} was detected in all samples at concentrations ranging from 0.1 to 0.4% of the total Sb which ranged from 80 to 100 mg mL^{-1}. The possibility that a thiolated methyl Sb compound (trimethylmonothioantimony, TMMTSb) could occur as a result of microbial action in the environment was investigated in a laboratory-based study.[31] A range of analytical techniques were brought to bear on the characterisation of the products of (a) the synthesis reaction and (b) the products of incubating TMSb with microbiota enriched from hot spring sediments and paddy soil, including HPLC-ICP-MS. The species were separated on a Hamilton PRP-X100 column with 100 mmol L^{-1} NH_4HCO_3 + MeOH (99 + 1). No citation to prior chromatographic work was made, nor was any chromatographic method development reported. Peaks were observed at retention times of 3.1 and 26.3 min that were attributed to TMSb and TMMTSb, respectively. Fractions were collected around these retention times, and the identity of the species confirmed by FT-ICR-MS/MS. The researchers found that *Clostridia* bacteria, which are sulfate-reducing, were involved in the formation of TMMTSb.

3.2 Arsenic

There has been one *review focusing on As speciation* published this year in an article entitled "The Need to Unravel Arsenolipid Transformations in Humans," (88 references with titles).[32] The

review covers the state our knowledge of (a) the identity of the arsenolipids that are ingested from seafood and (b) the associated potential risks, as evidenced by accumulation in breast milk, ability to cross the blood–brain barrier (and accumulate in the brain), with possible development of neurodegenerative disorders. It concluded that emerging evidence indicates that there are risks associated with human exposure to arsenolipids. The author points out that because of their complex chemical structure, research has been constrained by a lack of certified standards and analytical methods. Such methods involve advanced extraction procedures and techniques such HPLC-ICP-MS, ES-QQQ-MS, and HR-MS, which, it was noted, are expensive and available in only a few laboratories across the world. The reviewer concluded that future toxicological and epidemiological studies of representative patterns of arsenolipid exposure in different populations and at environmentally relevant concentrations are needed to generate data for health risk assessments. Thus, an interdisciplinary collaboration between organometallic chemists, analytical chemists, gut microbiologists, toxicologists, and epidemiologists is the way forward to successfully unravel arsenolipid transformations in humans.

The research trends observed in recent year regarding the focus of interest for As speciation studies (*e.g.* food materials, marine matrices, and clinical issues due to both treatment and ingestion) have largely continued over the current review period, although the volume of work published has decreased. A number of groups have reported on the *development or modification of analytical techniques for As speciation*. The use of lasers to study As speciation has increased this year. Quantitative iAs speciation at the ng kg^{-1} level using SPE and femtosecond LA-ICP-MS has been reported by Lee *et al.*[33] The iAs species in water samples were separated by SPE using a SAX membrane filter prior to LA. The pH value of the sample was adjusted to pH 4 with ammonium hydroxide and phosphoric acid to facilitate the complete separation of iAs from the organic As species. The LDR was from 0.5 to 1000 μg kg^{-1}, recovery efficiency was 96 to 106% and the LOD for iAs was 0.028 μg kg^{-1}. The application of μ-TLC for the speciation of iAs in aqueous media by LA-ICP-MS has also been reported.[34] An As^V imprinted polymer was synthesised by copolymerisation of itaconic acid and ethylene glycol di-methacrylate in the presence of As^V in acetonitrile assisted by ultrasound. The method used a TLC plate fabricated with the As^V imprinted polymer nanoparticles. A groove, created on a glass slide *via* HF assisted etching, was filled with a paste composed of the ion imprinted polymer and calcium sulfate. The glass slide was then utilised as a TLC plate for iAs species using an acetonitrile/water mixture at pH 4 as the mobile phase. The separated zones were transferred to the ICP-MS using LA. The surface morphology, functionality, and bulk composition of the As-synthesised copolymer were confirmed utilising XRD, SEM, and FTIR. The effect of the experimental conditions such as pH, mobile phase composition, and ion imprinted polymer/$CaSO_4$ mass ratio on the separation efficiency were also optimised. Once optimised, the LOD, RSD of responses, and LDR of the method were 0.3 μg L^{-1}, 3.8%, and 0.2 to 100 μg L^{-1}, respectively. Two groups have reported on the application of functionalised magnetic nanoparticles for speciation analysis of iAs. In the first study, mercapto- and amino-functionalised magnetic nanoparticles were used as magnetic SPEs to directly extract As^{III} and As^V from a range of water samples.[35] Nanocomposites comprising silica-coated magnetic nanoparticles (SMNPs) modified with 3-mercaptopropyltrimethoxysilane (MPTMS) or 3-aminopropyltriethoxysilane (APTES) were used. Various SPE parameters were optimised including the dose of magnetic adsorbent, pH of sample solution, loading and elution conditions of analytes, adsorption capacity and reusability of SMNPs-MPTMS and SMNPs-APTES for As^{III} and As^V respectively. Good selectivity and no oxidation or reduction prior to the capture of the two As species was reported. With a 25 fold enrichment factor, the LOD values for As^{III} and As^V were 23.5 and 10.5 ng L^{-1}, respectively. A CRM, NRCCRM material GBW08605 environmental water, was analysed, and the results for iAs species were in close agreement with the certified values. The iAs was evaluated in spiked tap, river, lake and rainwater samples. Good recoveries of 89 to 96% and 90 to 102% were achieved for As^{III} and As^V, with RSDs of 3.2 to 8.0% and 2.5 to 7.6%, respectively. In the second study, a magnetic functionalised material based on graphene oxide and magnetic NPs (previously reported by the authors) was used to develop a magnetic SPE method to pre-concentrate inorganic and organic As species *via* a FI system prior to their indirect determination by HPLC-ICP-MS.[36] During a 180 s sample loading period, at a flow rate of 4 mL min^{-1}, As species were retained on the functionalised magnetic nanomaterial and then eluted into a chromatographic vial for subsequent introduction to the HPLC-ICP-MS. The method was evaluated for use with environmental waters and biological samples and under optimised conditions gave LOD values of 3.8 ng L^{-1} for AB, 0.5 ng L^{-1} for cacodylate, 1.1 ng L^{-1} for As^{III} and 0.2 ng L^{-1} for As^V with RSDs of <5%. The results obtained for the NRCC CRMs fortified lake water TMDA 64.3 and seawater CASS-6 were within the certified ranges. A multi-mode sample introduction system (MSIS) designed to simultaneously separate and detect both non-hydride forming and hydride forming elements has been proposed.[37] The unit acts as an interface for two HPLC columns with ICP-OES and is basically a conventional spray chamber with pneumatic nebuliser and a HG unit in one device. The HG process was possible due to inlets located vertically in the centre of the device. The HG reagents were delivered to the chamber, mixed at the top of the inlets and the volatile hydrides formed carried in an Ar gas stream from the MSIS unit to the ICP torch. The upper inlet of the unit was used to introduce $NaBH_4$ solution, and the lower inlet provided HCl solution. Additionally, the MSIS unit functioned as a gas liquid separator with the excess liquid carried from the chamber using a peristaltic pump. The waste liquid flow rate was proportional to the sum of HG reagents flow rates. Simultaneous separation and detection of non-hydride forming and hydride forming elements was possible using two types of HPLC column, a cation-exchange and anion-exchange column, respectively. The method was able to determine 15 elements quantitatively, including three As and two Fe species and LOD values of 2.67 to 28.7 μg L^{-1} and recoveries of 80 to 120% were achieved for As species. The

device was evaluated with a range of different matrices. A LLME method, based on two MILs as extraction solvents for the determination of iAs species in water, soil and sediment samples, for use with ETAAS detection has been reported.[38] First, As^{III} species were separated by chelation with APDC and then extracted by an MIL trihexyl(tetradecyl)phosphonium hexachlorodisprosiate $\{P_{6,6,6,14}\}(3)DyCl_6$. The As^{V} species remaining in the aqueous phase were pre-concentrated by extraction in the MIL trihexyl(tetradecyl)phosphonium tetrachloroferrate $\{P_{6,6,6,14}\}FeCl_4$ in 2 mol L^{-1} HCl. The MIL phase containing As^{V} was separated by a magnetic rod and diluted in chloroform and an aliquot was injected into the ETAAS. Total iAs was determined using the MIL $\{P_{6,6,6,14}\}FeCl_4$ as the extraction solvent at 0.5 mol L^{-1} HCl to achieve the same extraction efficiency for both species. The concentration of As^{III} was calculated by the difference between total iAs and As^{V} concentration. An extraction efficiency of 99% for As^{V} and an enhancement factor of 35 were obtained with 5 mL of sample. The method LOD values were 17 ng L^{-1}, 0.11 ng g^{-1} and 0.13 ng g^{-1} for As^{V} and 20 ng L^{-1}, 0.13 ng g^{-1} and 0.14 ng g^{-1} for As^{III} in water, soil and sediment samples, respectively. The RSDs for six replicate measurements of 3 μg L^{-1} for As^{V} and As^{III} were 3.1% and 3.9%, respectively. The calibration linear range was 0.05 to 13 μg L^{-1}. In a second application of LLME to determine As species, a method based on the use of an APDC bonded IL for chelation with As^{III}, followed by conversion of the As^{III} chelated task-specific IL to a hydrophobic IL using KPF_6 as an anion-exchange reagent has been reported.[39] Any As^{V} present was reduced to As^{III} using a 2 : 1 w/w blend of KI and $Na_2S_2O_3$ and then the total As was measured using ETAAS. Under optimal conditions, a LDR of 0.2 to 15 ng mL^{-1} and 0.2 to 20 ng mL^{-1} were observed for As^{III} and total As, respectively. The RSD ($n = 5$) for the determination of As^{III} (10 ng mL^{-1}) was 3.2% and the LOD was 0.01 ng mL^{-1}.

The *separation of As species using chromatography* is by far the most popular approach for use with atomic spectroscopy, but few publications focus on the analytical utility of the columns used. The determination of 13 As species (AB, AC, As^{III}, As^{V}, DMA, MMA, oxo-arsenosugar-glycerol, oxo-arsenosugar-phosphate, oxo-arsenosugar-sulfonate, and oxo-arsenosugar-sulfate, TMAO, tetramethylarsonium, thio-dimethylarsinic acid) separated using HPLC with a fluorocarbon stationary phase coupled to ICP-MS has been reported by Miyashita *et al.*[40] The study evaluated four different types of fluorocarbon columns with silica gel-based fluorinated phases, including three pentafluorophenyl columns with C_6F_5 phases and one perfluoroalkyl column with a C_6F_{13} branched phase. Following optimisation, a 4.6 mm × 15 cm Discovery HS F5 column was selected, but changed to one with the same packing but smaller size (2.1 mm × 15 cm) for later work. Water (pH 7.0), HNO_3 at pH 3.0, and several kinds of organic acids at typical concentrations (*i.e.* 0.1% FA, 0.5 to 1% EtA, 0.035 to 0.05% TFA, 0.05 to 0.1% PFPA, and 0.05 to 1% HFBA) were evaluated in the mobile phase at various flow rates (0.2 to 0.3 mL min^{-1}), temperatures (35 to 50 °C) and organic modifiers (*i.e.* 1 to 5% MeOH, 1% EtOH, 1% IPA, and 1% ACN). A mobile phase of 0.05% heptafluorobutyric acid–methanol (99 : 1, v/v) proved optimum. Amongst the benefits resulting from this work, the absence of ion-pair reagents (including those containing S and P), enables As–P–S detection, and thus As species including P and/or S, oxo-PO_4, thio-DMA_V, oxo-SO_3, and oxo-SO_4.

A method for the determination of *iAs species in natural waters* using noncovalently aminated silica has been proposed.[41] The polyamines poly(hexamethyleneguanidine), poly(4,9-dioxadodecane-1,12-guanidine), hexadimethrine, and poly(diallyldimethylammonium) were evaluated as silica modifiers for the preconcentration of As^{III} and As^{V} from aqueous solutions. Once eluted from the collection cartridges using 2 mol L^{-1} HNO_3, the As was determined by ICP-MS and ICP-OES. It was found that As^{V} could be quantitatively extracted from solutions at pH 4.5 to 7.0 by the anion exchange mechanism in less than 5 min, whilst As^{III}, neutral at this pH, was not adsorbed. Reaction with 2,3-dimercapto-1-propanesulfonic acid, which resulted in the formation of the negatively charged complex of As^{III}, was used for extraction from solutions with a pH of 3.5 to 6.5. A system of two cartridges filled with poly(diallyldimethylammonium) modified silica and the on-line reaction of As^{III} with 2,3-dimercapto-1-propanesulfonic acid proceeding between the cartridges was used for separate preconcentration and determination of As^{V} and As^{III} at pH 5. The stability, preservation and storage of As^{III}, As^{V}, DMA and MMA in water samples has been studied as part of the development of ISO TS 19620:2018, a method for the determination of As^{III} and As^{V} species in waters by LC-ICP-MS or HG-AFS.[42] Four As species were studied: As^{III}, As^{V}, DMA, and MMA, in three different water types: deionised water, mineral water and natural river water. The effect of sample bottle material, light, storage temperature, and acidification were evaluated. The study found that when samples were acidified and refrigerated, they could reliably be stored for up to 12 weeks without significantly affecting the As concentration and speciation. The sample bottle material and light had no effect on the speciation integrity or stability.

There have been few *geochemical applications of As speciation* reported in the last year. The geochemical characterisation of dust from As-bearing tailings at the abandoned Giant Mine, Canada have been studied utilising microanalytical mineralogical analysis combined with quantitative measurements of phase distribution and bulk concentration.[43] Surface tailing material was sampled from three tailings impoundments on site and sieved to <63 μm as a proxy for dust. Total suspended particulate (TSP) samples were collected continuously over two months to represent the airborne material generated from the tailings. Analysis by ICP-OES and ICP-MS revealed elevated concentrations of As, Sb, Zn, Pb, Cu and Ni in all samples, and comparison of the results for sieved and unsieved samples showed that As was more concentrated in the <63 μm fraction of the tailings. The XANES results for the tailings indicated that the As in the samples is a mixture of As, As^{III} and As^{V}. An SEM-based mineral liberation analysis showed that roaster-generated iron-oxides (*i.e.*, maghemite), calcium-iron As^{V} (*i.e.*, yukonite), and arsenopyrite comprised most of the As-bearing particles in the surface tailings. Of the three solid phases, calcium-iron As^{V} posed the greatest risk to human health since it exhibited the highest relative bioaccessibility. A rare lead As^{V}

phase, which may be bioaccessible, was also detected in minor quantities in the surface tailings. This phase was identified as mimetite using μ-XRD analysis. Very little As trioxide was found in the surface tailings samples, and no As trioxide was found in the total suspended particulate samples. An assessment of As and Pb mobility in surface water, pore water, and sediment from eight samples from the Ribeira do Iguape Valley, SE Brazil has also been reported.[44] The concentration ranges for As and Pb were 0.11 ± 0.02 μg L^{-1} to 0.44 ± 0.02 μg L^{-1} and 0.31 ± 0.01 μg L^{-1} to 2.02 ± 0.01 μg L^{-1}, respectively. One river sediment sample collected revealed As (113 ± 1 mg L^{-1}) and Pb (672 ± 9 mg L^{-1}) at concentrations above the acceptable standards adopted by the local authorities. Using sequential extraction, this sample showed that more than 90% of Pb seemed to be strongly bound to the sediment matrix, while As showed higher mobility of between 60 and 90%, probably due to the action of acid agents. Arsenic speciation in pore water from this sample obtained using HPLC-ICP-MS, revealed the presence of As^{III} as the predominant species. The development of a etched silicon microfluidic device to study metal geochemistry *in situ* using XRF microprobe spectroscopy has been reported.[45] The device was sealed with a 30 μm thin glass window that was sufficiently transparent for XRF spectrometry. Both fluorescence mapping and spot XAS were performed on the device. The desired X-ray energies were generated by a three-pole wiggler, pre-focused using a toroidal mirror and vertical collimating mirror before passing through a (111) Si double-crystal monochromator. The final focusing was performed using a set of Kirkpatrick–Baez focusing mirrors which achieved X-ray spot sizes of 5 to 9 μm × 5 mm. The resultant X-ray flux was 10^{11} photons per s. X-ray fluorescence for both mapping and XANES spectroscopy was measured using a Hitachi Vortex ME4 detector with Quantum Detectors XSpress3 electronics. The use of the device was demonstrated using an Fe (hydr)oxide solid loaded with As and then infused with sulfide, resulting in time-variant Fe precipitation reactions and As sorption. Key results from the study include *in situ* XRF time-series maps of Fe, As and a Br flow tracer, as well as spot XANES at both the Fe K edge and As K edge. Additionally, multiple energy mapping was used to examine the spatial speciation of As over time. The distribution and formation of monothioarsenate, dithioarsenate, and trithioarsenate (the three main forms of thioarsenate in fresh groundwater) have been studied in As rich groundwater from the Datong Basin in N China.[46] A total of 30 groundwater samples were collected to conduct hydrochemistry and As speciation analysis (thioarsenates, As^{III} and As^{V}) by HPLC-ICP-MS. The results showed thioarsenate detected in 40% of the groundwater samples at concentrations ranging from 2.36 to 210 μg L^{-1}; the highest concentration accounting for 68% of total As. The thioarsenate mainly occurred in the discharge area of the Datong basin, where the groundwater was characterised by strongly reducing conditions (Eh up to −175.3 mV) and higher concentrations of As (up to 3450 μg L^{-1}) and sulfide (up to 8480 μg L^{-1}). A strong positive correlation ($r = 0.81$, $p < 0.05$) was observed between thioarsenate and sulfide. The formation mechanisms of monothioarsenate, dithioarsenate, and trithioarsenate were discussed with respect to how a better understanding of the different formation conditions of thioarsenate species can help explain the migration and enrichment of As in sulfide-rich groundwater.

The distribution and chemical speciation of *As in atmospheric particulate material*, including total suspended particles (TSP), PM_{10}, and $PM_{2.5}$, collected from Baoding, China have been investigated.[47] The average total mass concentrations of As in $PM_{2.5}$, PM_{10} and TSP were 54.1, 35.3, 31.5 μg g^{-1}, respectively, showing that As is prone to accumulating on fine particles. In the study, the HPLC-ICP-MS system was optimised for As speciation to include TMAO from the particulate material. An anion exchange column (Hamilton PRPX100) with a mobile phase of 90 mmol L^{-1} NH_4HCO_3 at pH 8.6 was used to separate As^{V}, DMA and MMA, while a cation exchange column (Hamilton PRP-X200) with a mobile phase of 4 mmol L^{-1} pyridine at pH 2.8 was used to separate As^{III} and TMAO. The results showed that As^{V} was the dominate component in all the samples, corresponding to 79.2% ± 9.3% of the total extractable species, whilst As^{III}, TMAO and DMA made up the remaining 21%. The study also demonstrated that As^{III} accounted for about 14.4% ± 11.4% of the total extracted species, with an average concentration of 1.7 ± 1.6 ng m^3. It was noted that TMAO was widely present in the samples (84 out of 97), supporting the author's assumption that TMAO is reasonably ubiquitous in atmospheric particles. Two X-ray based techniques, SR-XRF and XANES, have been used to assess low concentrations of As in ambient total suspended particulates and the $PM_{2.5}$ fraction from samples collected in Cairo, Egypt.[48] Using the tunability of the synchrotron source, excitation photon energies below or above the As K edge (13.6 and 12.5 keV) were employed together with spectral de-convolution procedures to elucidate the occurrence of As in the spectra, and avoid the contribution of spectral interferences in between As–K emission lines and those of Ga-K_β, Bi-L, and L_{12} series of Pb. In the case of total suspended particulates collected from an industrial area, high levels of As were found (2.9 ± 0.7 ng m^{-3}). The As speciation was investigated further using XANES at the As K-edge. By applying linear combination fitting procedures to the XANES data, the authors determined that 82% of the As species existed as As^{III}, whereas only 18% was present as As^{V}. The XANES measurements could not be achieved for the areas with lower levels of As (1.6 ± 0.6 ng m^{-3}).

The *speciation of As in plants* continues to be a popular area of research, although the number of plant species studied has reduced this year. Two groups have reported work on the hyperaccumulator fern *Pteris vittate*. The interactive effects of iAs species and PAHs on their uptake, accumulation and translocation have been studied hydroponically by Liao *et al.*[49] The presence of PAHs hindered As uptake and acropetal translocation by *P. vittata*, decreasing As concentrations by 29.8 to 54.5% in pinnae, regardless of the initial As speciation. The inhibitive effect of PAHs was 1.6 to 8.7 times greater for As^{III} than for As^{V}. Similarly, iAs inhibited the uptake of fluorene and benzo(*a*)pyrene by *P. vittata* roots by 0.4 to 21.7% and by 33.1 to 69.7%, respectively. Interestingly, co-exposure to As and PAHs slightly enhanced the translocation of PAHs by *P. vittata* with their concentrations increased 0.3 to 0.8 times in shoots, except

for the As^{III} + benzo(*a*)pyrene treatment. The antagonistic interaction between As and PAH uptake was suggested to be caused by competitive inhibition or oxidative stress injury. Using SXRF, high concentrations of As were found distributed throughout the microstructures far from main vein of the pinnae when co-exposed with PAHs, the opposite of what was observed when only exposed to As. The PAHs could also significantly inhibit the accumulation and distribution of As in vascular bundles in rachis treated with As^{III}. Two-photon laser scanning confocal microscopy revealed that PAHs were mainly distributed in the vascular cylinder, epidermal cells, vascular bundles, epidermis and vein tissues, and this was independent of As speciation and treatment. Kashiwabara *et al.*[50] used μ-XRF imaging of frozen hydrated sections of a root of *Pteris vittata* to study the mechanism of As uptake. The As distribution was successfully visualised in cross sections of different parts of the root, which showed that the major pathway of As uptake changes from symplastic to apoplastic transport in the direction of root growth, and that As and K have different mobilities around the stele before xylem loading, despite their similar distributions outside the stele in the cross sections.

In recent years, *As in rice* from specific countries or regions has been a popular topic for research and this trend has continued this year. Several reports on rice grown in Brazil have been published such as an analysis and risk assessment of As in rice from different regions.[51] A total of 268 rice samples of different subtypes (white, parboiled and brown) were collected from different regions of Brazil over 51 month period. These were analysed for total As and iAs by HPLC-ICP-MS. The calculated average of total As in all analysed rice subtypes was 0.109 mg kg^{-1}. When evaluated by subtype, the range of As concentrations were: 0.004 to 0.288 mg kg^{-1} for white rice; 0.100 to 0.296 mg kg^{-1} for brown rice; and 0.100 to 0.220 mg kg^{-1} for parboiled rice. Of the analysed samples, less than 10% of the samples had values greater than 0.200 mg kg^{-1} and iAs species represented 58% of the total As measured in the samples. The accuracy and precision of the method were assessed using reference material Rice flower NIST 1568b. A method for As speciation in Brazilian rice using LC-ICP-MS has also been reported.[52] Four As species, As^{III}, As^{V}, DMA^{V}, and MMA^{V}, were separated in less than 8 min using 10 mmol L^{-1} $(NH_4)_2 \cdot HPO_4$ as the mobile phase, at pH 6 and a flow rate of 1.4 mL min^{-1}. The LOD values for As^{III}, DMA^{V}, MMA^{V} and As^{V} in rice were 1.87, 2.89, 0.54 and 3.00 ng g^{-1}, respectively. Method accuracy was assessed through analyte recovery tests and analysis of CRMs (SRM 1568b (rice flour) from NIST, NMIJ 7532-a (As compounds and trace elements in brown rice flour) from the National Measurement Institute of Japan (NMIJ), and ERM-BC211 (rice flour) from the European Reference Materials (ERM)). Once validated, the method was used to determine As speciation in 640 rice samples, comprising polished (white) rice, parboiled rice and brown rice collected from different mesoregions in the Rio Grande do Sul State in Brazil. The average concentrations of total iAs and DMA in brown and parboiled rice were similar but higher than those in white rice. Only one sample (a white rice) gave a total As concentration higher than 300 ng g^{-1}, the maximum total As concentration allowed by current Brazilian legislation. This sample had a total As of 306 ng g^{-1}, although the iAs concentration in the sample was 58.3 ng g^{-1} which was well below the maximum iAs concentration under current European and Chinese legislation (200 ng g^{-1}). For parboiled and brown rice, the maximum iAs concentrations found were 112.5 ng g^{-1} and 115.1 ng g^{-1}, respectively. Vietnamese rice has been studied to compare the As speciation in Sengcu rice from terraced paddies in mountainous terrain with commercial rice from lowland paddies.[53] The experimental conditions were determined by response-surface methodology for optimal extraction of all As species by MAE and then HPLC-ICP-MS-MS for speciation. The method yielded 95% accuracy and <6.1% precision for the CRM material NIST SRM1568b rice flower. The method LOD values for As^{III}, As^{V}, DMA and MMA were 0.1, 0.2, 0.5 and 0.1 μg kg^{-1}, respectively. The predominant species was As^{III} in both Sengcu and commercial lowland rice, which may be due to its abundance in the aquatic environment of paddy fields and thus being readily absorbed by the roots of rice plants. In contrast, differences in irrigation practices, microbial activities of farming fields and soil properties lead to variations in As^{V} and DMA concentrations between Sengcu and commercial rice. Further work is necessary to provide a more comprehensive understanding of the effect of farming practices on As assimilation in rice grains. On-column internal standardisation as an alternative calibration strategy for speciation analysis has been demonstrated using the analysis of iAs in rice.[54] The calibration method of choice for high accuracy speciation analysis is ssIDMS because it can correct for detector sensitivity drift, matrix effects, and analyte loss during sample preparation and analysis. However, in many cases, ssIDMS calibration is either not applicable (*e.g.* for mono-isotopic elements) or not feasible (*e.g.* limited by the cost and availability of like-for-like isotopically enriched species). This study was based on the chromatographic injection of the same species of the analyte as the IS, after the sample injection. The approach can compensate for on-column analyte losses and signal drift and can be applied with any detector capable of recording time-resolved data, provided that enough species resolution can be achieved. A variety of rice materials with iAs fractions ranging from 60 to 300 mg kg^{-1}, including white rice, brown rice and rice intended for baby food were used. Two subsamples from each material were spiked with approximately 50 mg kg^{-1} As^{V} standard just before the extraction. The iAs spike recoveries were within 5% of the spiked concentration were obtained in all cases. The data from the HPLC-ICP-MS were processed using laboratory developed MS Excel™ spreadsheets. The method was reported to be used for the certification of iAs in baby food matrices to support EU Commission Regulation 2015/1006 regarding the maximum levels of iAs in foodstuffs. The effect of applying different concentrations of P (0, 10, 20, 30, and 40 mg kg^{-1}) to soils used to grow rice has been studied.[55] The yield parameters, As species accumulation, and polyphenol levels in the grain of rice grown under As spiked soil (10 mg kg^{-1}) were also investigated. The As species (As^{V}, As^{III}, DMA and MMA) and polyphenols in rice grain samples were determined using LC-ICP-MS and LC-MS/MS, respectively. The P treatments significantly reduced the toxic

effects of As on agronomic parameters such as root weight and length, shoot and spike length, straw, and grain yield. Among the treatments studied, only the treatment of 30 mg kg^{-1} P helped to decrease the elevated levels of As^{III}, As^{V}, and DMA in rice grains due to As application and As-linked toxicity on agronomic parameters and chlorophyll biosynthesis. The levels of *trans*-ferulic acid, chlorogenic acid, caffeic acid, and apigenin-7-glucoside increased in response to accumulation of As in the rice grain. The overall conclusion was that the precise use of P may help to mitigate As linked phytotoxicity and enhance the food safety of rice grain.

Continuing on the theme of *As speciation in rice and rice products*, a SPE procedure has been developed for the speciation of iAs in rice samples using FI-HG AAS.[56] A small amount of tetra-*n*-butylammonium bromide (TBAB) was used as the phase-transfer agent and injected into the sample solution. After shaking, a small volume of perchlorate was added as ion-pairing agent. Due the interaction between these agents, fine particle solids were formed. The As^{III} present was selectively extracted after complexation with APDC. The suspension was centrifuged and the sedimented phase leached with HCl and introduced into the FI-HG-AAS to recover the adsorbed analyte. The variables were optimised by fractional factorial design and response surface methodology. The LOD and LOQ were 0.01 and 0.04 μg g^{-1}, respectively. The procedure showed potential for the determination of As^{III} with a preconcentration factor of 17 and RSD of 5.5%. The method was applied to the CRM IRMM-804 rice flour and no significant difference ($p > 0.05$) to the certified results were observed. The As bioaccessibility in rice grains following use of a modified physiologically-based extraction test (MPBET) has been investigated.[57] Compared to soil, rice has a different composition and higher intake by humans but lower As concentration. Thus, in this study, the As bioaccessibility in rice samples was evaluated using a MPBET at a rice : solution ratio of 1 : 20 (w/v) which is 5 times greater than the original ratio of 1 : 100 (w/v), with other parameters of the assay staying the same. Using the modified extraction test for rice, As bioaccessibility in raw rice samples (44 to 88% in the gastric phase and 47 to 102% in the intestinal phase) was similar to those in cooked rice (42 to 73% and 43 to 99%). The As bioaccessibility in rice was generally higher in the intestinal phase than in the gastric phase, with Fe and Ca concentrations in rice being negatively correlated with As bioaccessibility in the gastric phase ($R^2 = 0.47$ to 0.49). In addition, for cooked rice, a strong positive correlation was observed between bio-accessible As and iAs ($R^2 = 0.63$ to 0.72) suggesting iAs in rice was easier to dissolve than organic As in gastrointestinal digestive fluids. Due to limited variation in As bioaccessibility and As bioavailability among the 11 samples, a weak correlation was observed between them ($R^2 = 0.01$ to 0.03). However, As bioaccessibility values measured by the gastric phase of the MPBET agreed with As bioavailability values based on a mouse bioassay, suggesting MPBET gastric phase had potential to predict the As bioavailability in rice. The author acknowledged that further work with additional samples was needed to ascertain the robustness of the MPBET gastric phase in predicting As bioavailability in rice for routine use. The concentrations of total As and As speciation in Chinese rice wine and the associated potential health risk has been assessed.[58] A total of 79 rice wine samples from various regions in China were analysed by HPLC-ICP-MS to determine total As and As species concentrations. The average concentration of total As found was 14.6 μg L^{-1} and the concentration of As^{III}, As^{V}, DMA, and AB were 2.86 μg L^{-1} (0.970 to 6.08 μg L^{-1}), 7.22 μg L^{-1} (2.24 to 22.9 μg L^{-1}), 3.92 μg L^{-1} (1.58 to 7.82 μg L^{-1}) and 0.620 μg L^{-1} (<LOD to 0.950 μg L^{-1}), respectively. No MMA or AC were detected. The target hazard quotients for chronic noncarcinogenic risks (skin lesions) were below 1, suggesting that the Chinese population did not encounter a significant noncarcinogenic risk. However, the mean values of margin of exposure for lung cancer were below 100 (62.1 to 75.1) for male drinkers, indicating a potential carcinogenic risk. A comparison of the As species of rice wines with the raw ingredients found that the methylation increased DMA during fermentation.

A number of papers have also reported on *As speciation in various matrices associated with rice cultivation*. The dynamics of DMMTA in paddy soils and its subsequent accumulation in rice grains has been studied.[59] The authors first optimised a HPLC-ICP-MS method to quantify methylated thioarsenate species. After optimisation, it was found that the various methylated thioarsenates could be well separated using a RP C18 column, a mobile phase of 20 mmol L^{-1} $NH_4H_2PO_4$ (pH 3.0) and a flow rate of 1.0 mL min^{-1}. The LOD was between 0.4 and 0.8 nmol L^{-1}, depending upon the As species. For quantifying the iAs and methylated oxyarsenic species, AEC using a PRP-X100 column was also used. None of the methylated thioarsenate species could be detected using this PRP-X100 column. External spiking with synthetic methylated thioarsenates to porewater samples (preserved with 10 mmol L^{-1} DTPA) showed that recoveries using the C_{18} column were 88 to 101% for MMMTA, 98 to 103% for DMMTA, and 86 to 96% for DMDTA, all ($n = 6$). The recoveries for the analyses of different inorganic and methylated oxyarsenic species using the PRP-X100 column ranged from 81 to 102%. Finally, the sum of the As species in all samples obtained by HPLC-ICP-MS using the two columns accounted for 92 to 104% of the total As measured by ICP-MS. Using this method together with 10 mmol L^{-1} DTPA to preserve As speciation, methylated thioarsenate species in pore waters of seven As-contaminated soils incubated under flooded conditions and of two paddy fields were investigated. The main methylated thioarsenate species in the pore waters in both incubated soils and paddy fields was found to be DMMTA, with concentrations ranging from 0.2 to 36.2 μg L^{-1} and representing *ca.* 58% of its precursor DMA. The temporal production and dynamics of DMMTA were linked with the DMA concentrations. When soils were drained, DMMTA was converted to DMA. In the two non-flooded paddy fields, DMMTA concentrations in rice grains were 0.4 to 10.1 μg kg^{-1}. The addition of sulfur fertiliser and rice straw increased grain DMMTA by 9% to 28%.

Arsenic speciation in other food stuffs has also been investigated. Magnetic dispersive microsolid phase extraction (MDMSPE) has been coupled with dispersive LLME for the separation and preconcentration of As^{III} and As^{V} in dairy products with ETAAS used for detection.[60] In the first step,

magnetic $ZnFe_2O_4$ nanotubes were employed to adsorb As^V and the adsorbent was separated from the aqueous phase using an external magnetic field. Then As^{III} in the upper aqueous phase from MDMSPE was enriched by dispersive LLME. Samples were prepared with artificial gastric juice to avoid inter-conversion of the As species. The parameters affecting the separation and preconcentration of the determined species were optimised. The LOD values of the method were determined to be 1.3 and 2.0 pg mL^{-1} for As^{III} and As^V respectively, with RSDs of 4.5% and 5.6% for a concentration of 1.0 ng mL^{-1} ($n = 9$). The enrichment factors were 175-fold for As^{III} and 163-fold for As^V and the As recovery from spiked samples ranged from 90.8% to 105%. A milk powder CRM was also analysed by this approach and the measured results were in good agreement with the certified value. Clay products for oral use form a particular group of food supplements in relation to potential As toxicity since all As in these supplements is likely to be in the iAs form. The health risk from consumption of these products has been the subject of a study by Demaegdt *et al.*[61] Clay products for oral use were bought on the Belgian market and analysed for total As, As^{III}, As^V, AB, DMA and MMA and bioaccessible As, in order to perform an exposure assessment and risk characterisation. Total As concentrations differed considerably between the samples and ranged from 0.20 to 6.4 mg kg^{-1}. The bioaccessibility of iAs, determined *via* UBM (an extraction using digestive enzymes) varied between 8% and 51%. The iAs concentration determined *via* HPLC-ICP-MS after extraction with diluted $HNO_3 + H_2O_2$ (as in the CEN method for foodstuffs) was only a poor predictor of the bioaccessible iAs fraction, despite the significant relationship ($R^2 = 0.36$; $p < 0.05$). The risk characterisation did not reveal acute risks related to iAs exposure, although a potential concern regarding chronic iAs intake was identified for the general population in 42% of the analysed food supplements, and for sensitive population groups in 67% of the samples. Overall, the data presented illustrated that consumption of some of these clay products may contribute significantly to dietary iAs intake. Data from the first German total diet study (BfR MEAL Study) regarding the total As and water-soluble As species in a variety of foods (870 pooled samples) has been presented.[62] Total As, iAs, AB, DMA and MMA were determined by HPLC-ICP-MS/MS. The highest levels of total As were found in fish, fish products and seafood (mean: 1.43 mg kg^{-1}; $n = 39$; min–max: 0.01 to 6.15 mg kg^{-1}), with AB confirmed as the predominant As species (1.23 mg kg^{-1}; $n = 39$; min–max: 0.01 to 6.23 mg kg^{-1}). In contrast, iAs was determined as the prevalent As species in terrestrial foods (0.02 mg kg^{-1}; $n = 38$; min–max: 0.00 to 0.11 mg kg^{-1}). The data from the study provided a useful database for refining the exposure and risk assessment of As content in food when considering different As species.

The *speciation of As in mushrooms* continues to attract attention. The group at the University of Graz in Austria have published two reports this year. The first, in conjunction with the University of Ghent, utilised the potential of HPLC-ICP-MS-MS to investigate non-targeted elemental metabolomic analysis to many non-metals of biological importance. This study looked at the non-targeted and parallel speciation analysis of As, P, S and Se, in mushrooms.[63] Contrasting levels of diversity were found in the metabolomic profiles of the four investigated elements among the various species along with sharp discrepancies among related elements (*e.g.* P *vs.* As in certain mushroom species). The study showed that ICP-MS-MS offers a new dimension in non-targeted metabolomic analysis and enabled a unique comparative approach in investigating and tracking the biochemistry of related elements in moderately complex organisms. In the second study, the As speciation in wild-grown samples of the parasitic mushroom *Tolypocladium ophioglossoides* was investigated using HPLC-ICP-MS.[64] The As concentrations were 0.070 to 3.44 mg kg^{-1} dry mass, although interestingly up to 56% of the extracted As was found to be an unknown As species, which was marginally retained under anion and also cation-exchange conditions. After evaluating the chromatography, the compound was isolated and identified as 2-(sulfoxyethyl) trimethylarsonium ion (AC-*O*-sulfate) using HR-MS. The compound was synthesised and further quantified in all investigated samples *via* ion-pair chromatography coupled to ICP-MS. In addition to the high abundance of AC-*O*-sulfate in *T. ophioglossoides*, small amounts of this As species were also detected in one sample of the host mushroom, *Elaphomyces asperulus*. In a sample of another parasitic mushroom, *Ophiocordyceps sinensis*, AC-*O*-sulfate could not be detected, but the main species was another unknown compound that was oxidised to inorganic As^V with hydrogen peroxide. This is the first report of AC-*O*-sulfate to be found in nature. The authors suggested that it is possible that it is present in many other organisms, at least in low concentrations, but has not been detected because of its unusual chromatographic behaviour. The existence of AC-*O*-sulfate raises questions about the biotransformation pathways of As in the environment and the specific behaviour of fungi. The As speciation (As^{III}, As^V, DMA, MMA, AB, and AC), and distribution in wild *Cordyceps sinensis* has been studied.[65] Four techniques were used in this work, ICP-MS and HPLC-ICP-MS to determine the total As and the As speciation, and XANES and μ-XRF imaging to characterise As valence and distribution. The total As range in the wild *C. sinensis* samples was 5.77 to 13.2 μg g^{-1} with an average of 8.85 ± 2.5 μg g^{-1}. The main As species detected in the wild *C. sinensis* samples were As^{III} and As^V, however the iAs only accounted for 4.47 to 11.4% of the extracted As.

The *speciation of As in seafood* is again a popular subject for study this year. Arsenic speciation and bioaccessibility in raw and cooked seafood has been assessed using a physiologically based extraction test combined with the Simulator of Human Intestinal Microbial Ecosystems model.[66] In the study, 34 seafood samples (fishes, shellfishes, and seaweeds) were collected from different markets in China and analysed for total and speciated As before and after boiling. Six As species (As^{III}, As^V, MMA, DMA, AB, and AC) were determined using anion exchange HPLC-ICP-MS. The results showed that the total As contents of seaweeds (raw: 44.1; boiled: 31.1 μg g^{-1} dw) were higher than those of shellfishes (raw: 8.34; boiled: 5.14 μg g^{-1} dw) and fishes (raw: 6.01; boiled: 3.25 μg g^{-1} dw). Boiling significantly decreased the As content by 22.2% for seaweeds, 32.3% for shellfish, and 41.4% in fish ($p < 0.05$). During *in vitro*

digestion, the bioaccessibility of total As and AsB significantly varied between the investigated species of seafood samples in gastric and small intestinal phases ($p < 0.05$). A higher total As bioaccessibility (gastric: 68.6%, small intestinal phases: 81.9%) was obtained in fish compared to shellfish (gastric: 40.9%, small intestinal phases: 52.5%) and seaweeds (gastric: 31%, small intestinal phases: 53.6%). However, there was no significant differences in colonic phase ($p > 0.05$), and with gut microbiota, As^{V} was transformed into MMA and As^{III} in the colonic phase. An unknown As compound was found in the seaweeds. The determination of total As and hydrophilic As species in seafood has been reported by Luvonga *et al.*[67] Five different marine-based food types cutting across the food chain from microalgae, macroalgae, bivalve clam, crustaceans and finfish were studied. Total As was determined using ICP-MS whilst As speciation analysis was performed using HPLC-ICP-MS. The total As contents ranged from 133 ± 11 ng g^{-1} to 26 630 ± 520 ng g^{-1}. The mass fractions of iAs, AB, DMA, and the four commonly occurring arsenosugars were reported. Extractable hydrophilic As species accounted for 10% (aquacultured shrimp) to 95% (kelp) of the total As and DMA was established to be a by-product of the decomposition of As sugars in acid extracts of samples known to contain these species. A universal MAE method has been developed to extract As^{V}, As^{III}, MMA, DMA, AB, and arsenosugars from seafood with an extraction efficiency of 95% without altering their original speciation and IC-ICP-MS was used to determine the As species in various seafood (seaweed, fish, shellfish and shrimp).[68] The method LOD was from 8.0 to 12.0 ng As g^{-1} for dried seafood. The optimised extraction method involved a pre-soaking stage in which 0.1 g of dried seafood sample was soaked with 6.0 mL of 20 $mmol^{-1}$ HNO_3 for 12 h. The previously soaked mixture was then extracted using MAE for 30 min at 120 °C. After the extract was separated and collected, MAE was again repeatedly used on the residue for 30 min with 4.0 mL of 20 $mmol^{-1}$ HNO_3 at 120 °C without previous soaking. The method facilitated the extraction of As^{V}, As^{III}, MMA, DMA, AB, and arsenosugars from seaweed, fish, shellfish and shrimp samples with a recovery of 92 to 104% and a RSD ($n = 5$) < 5%. To confirm whether the As species were altered during the extraction, a mixed standard of 5 ng mL^{-1} As^{V}, As^{III}, MMA, DMA, and AB, was pre-treated with the same MAE procedure and then the results were compared with that of the mixed standard without MAE pre-treatment. No change in speciation was found. The biochemical metal accumulation effects and metalloprotein metal detoxification in environmentally exposed tropical *Perna perna* mussels has been investigated.[69] Samples were collected from four sites in two bays in SE Brazil, one anthropogenically impacted and one previously considered a clean reference site for metal contamination. Gill metallothionein, reduced GSH, carboxylesterase and lipid peroxidation were determined by UV-vis, and metal and metalloid contents were determined by ICP-MS. Metalloprotein metal detoxification routes in heat-stable cellular gill fractions were assessed by SEC-HPLC-ICP-MS. Several associations between metals and oxidative stress endpoints were observed at all four sampling sites using PCA. The As, Cd, Ni and Se contents seemed to be particularly affected by carboxylesterase activity. Gill metallothionein was implicated in playing a dual role in both metal detoxification and radical oxygen species scavenging. Differential SEC-HPLC-ICP-MS metal-binding profiles, and thus detoxification mechanisms, were observed. The *Perna perna* mussels proved to be potential tropical bioindicator, although further work is required.

Further papers published on the *speciation of As in aquatic species* include a study to develop and validate a method for As speciation analysis of a broad range of water-soluble organo-arsenic species in marine matrices.[70] Optimum extraction conditions were identified through a $2^{(7-3)}$ fractional factorial design using blue mussel as the test sample. The effects of sample weight, type and volume of extraction solution, addition of H_2O_2 to the extraction solution, extraction time, temperature, and use of UAE were investigated. The highest As recoveries were obtained using 0.2 g of sample, 5 mL MeOH : H_2O, 50% v/v as the extractant, an extraction temperature of 90 °C for 30 min, and no UAE. Anion- and cation-exchange HPLC-ICP-MS settings were also optimised. The method detected a total of 33 known and unknown As species within a run time of 23 min for CEC and 20 min for AEC. Several marine CRMs were used for validation: BCR 627 (tuna fish tissue), ERM-CE278k (mussel tissue), DORM-4 (fish protein), DOLT-5 (dogfish liver), SQID-1 (cuttlefish), TORT-3 (lobster hepatopancreas), and CRM7405-b (hijiki seaweed). Based on *t*-test results, the obtained total As concentrations were not significantly different from the certified values (95% confidence level). Various chromatographic columns were also tested, and a Metrosep C6 was chosen as optimal for CEC and PRP-X100 for AEC work. The simultaneous speciation of As (As^{III}, As^{V}, DMA, MMA and AB) and Hg (Hg^{II} and MeHg) in fish by DRC HPLC-ICP-MS has been reported.[71] The separation was complete in less than 4.5 min using a ZORBAX SB-Aq C18 column in combination with two mobile phases: 5 mmol L^{-1} 1-octanesulfonate, 5 mmol L^{-1} acetate buffer and 1% (v/v) IPA at pH 4.0 as mobile phase A and 2 mmol L^{-1} L-cysteine in 1% (v/v) IPA (pH 4) as mobile phase B. With O_2 as the reactive gas in the DRC, sensitivity of both As and Hg were improved due to the measurement of the former as $^{75}As^{16}O^{+}$ at m/z 91 and collision damping for both. The LOD values were in the range 0.005 to 0.007 ng As per mL and 0.013 to 0.015 ng Hg per mL. To determine the accuracy, CRM NRCC DORM-3 fish protein was analysed and the sum of the concentrations of individual species agreed with total certified concentrations of As and Hg. The developed method was applied to a variety of fish samples. The As and Hg species in fish were quantitatively extracted into a solution of 1% (v/v) HCl and 0.1% (m/v) Protease XIV in a closed centrifuge tube and kept in a water bath which was microwave heated to 70 °C for 60 min. The spike recovery of individual As and Hg species was between 97 and 103%. The precision between sample replicates was better than 7%. An iron-magnetic nanomaterial functionalised with dimethyl triamine-pentamethylene phosphonic acid was used for non-chromatographic speciation of As in wild shrimp (*Farfantepenaeus brasiliensis*) by ICP-MS.[72] This material was used for the SPE for As species, optimisation using central composite design indicated a pH 4.0, 15 min extraction time, and 20 mg of mass of material for optimal extraction. The iAs extracted using

the nanoparticles gave concentrations of between 20 and 100 μg kg^{-1} in the evaluated samples. Method validation was achieved using CRMs dogfish liver DOLT-5 NRCC and fish protein DORM-4 NRCC. It was possible to reuse the same magnetic nanomaterial for 6 successive cycles, with an LOD of 16.4 ng kg^{-1}. The use of this non-chromatographic method was reported by the authors to improve the LOD and LOQ by about 1000 times when compared with chromatographic methods. The determination of iAs in fish oil (Japanese sardine oil, krill oil, Japanese common squid oil, and anchovy oil) and fish oil capsules has been reported.[73] The workers used LC-ICP-MS with a ODS column with a mobile phase of 10 mmol L^{-1} sodium 1-butanesulfonate, 4 mmol L^{-1} malonic acid, 4 mmol L^{-1} TMAH, and 0.05% methanol at pH 3.0. The iAs was extracted from the fish oil by heating at 80 °C in 1.6% TMAH–EtOH. The LOD (0.005 and 0.004 mg kg^{-1}), LOQ (0.016 and 0.011 mg kg^{-1}) repeatability (4.2 and 3.5%), and trueness (recoveries 94 to 109%) for fish oil and fish oil capsules respectively, were based on spiked samples. Only iAs was detected in the anchovy oil (0.7 μg kg^{-1}), all other samples being below the LOD.

Several groups have published work on *As speciation in clinical studies* during the review period. Hypertension in patients exposed to high As areas in western China has been investigated.[74] The study focused on genetic susceptibility and urinary As metabolism characteristics. A case-control study was conducted involving individuals exposed to high As levels (the As content in the pressurised well water was up to 510 μg L^{-1}, and that in the mechanical well water was 167 μg L^{-1}) in two adjacent high-As areas in W China. A total of 699 samples were collected: 192 case samples (patients with hypertension) and 507 control samples (no hypertension). Blood pressure measurement data obtained from an epidemiological survey were used to determine whether the subjects had hypertension, and a logistic regression model was used to analyse the association between lipid metabolism gene polymorphisms and hypertension susceptibility. Blood and urine samples were collected based on epidemiological methods, single nucleotide polymorphisms (SNPs) were genotyped using a SNPscan™ multiple SNP typing kit, and urinary As concentrations were determined using HG-AFS. The distribution of the urinary As secondary methylation ratio DMA/MMA was found to be different between hypertensive patients and controls indicating differences in urinary As metabolism. Early-life exposure to iAs has been found to be associated with impaired immune function and a decreased lung function in children; however the results have been inconsistent. Tsai *et al.*[75] have evaluated the effect of prenatal and childhood exposure to iAs on allergic diseases in children using a 15 year study period. Children born to women enrolled in the Taiwan Maternal and Infant Cohort Study from December 2000 to November 2001 were recruited and further investigated every 2–3 years until the age of 14. Urinary specimens were collected in the pregnant women during the 3rd trimester and from the children. Diagnoses of allergic diseases were based on physician diagnoses using the International Study of Asthma and Allergies in Childhood questionnaire. Urinary As speciation was performed using HPLC-ICP-MS. Of the 261 children from 358 mother–infant pairs used in this study, those with asthma and allergic rhinitis reported a higher prevalence of maternal allergy (49.47%) than did non-allergic children (29.81%). In the fully adjusted model, levels of maternal urine (iAs + MMA + DMA) greater than the median were found to be significantly associated with an increased risk of asthma. Levels of urinary As (iAs + MMA + DMA) in children higher than the median were associated with an increased risk of allergic rhinitis. Overall, the study found prenatal and childhood exposure to iAs were found to be significantly associated with the occurrence of asthma and allergic rhinitis in children, respectively. Protein-bound As is less readily excreted from the body due to complexation with biological macromolecules. Wang *et al.*[76] have developed a method utilising SEC and IEC combined ICP-MS and multiple reaction mode monitoring to determine bound-As species. After exposure to As_4S_4, DMA^V was identified as bound As in rat livers subsequent proteomics analysis showed that the potential binding partners included hemoglobin, glutathione S-transferases, superoxide dismutase (Cu–Zn) and (Mn), thiosulfate sulfurtransferase, and metallothionein-2.

Every year there are several *clinical studies on the use of* As_2O_3 to treat patients with acute promyelocytic leukemia (APL). A study to evaluate levels and distributions of As species in leukocytes and granulocytes of APL patients was reported this year.[77] Leukocytes were collected from 21 patients treated with As_2O_3 during induction, consolidation, and drug-withdrawal period and iAs, MMA, and DMA were measured by HPLC-ICP-MS. During induction, consolidation, and drug-withdrawal period, the general trend of As species was iAs > MMA > DMA ($P < 0.05$) in leukocytes. The predominant As species was iAs, with median concentration of 10.8 (6.03 to 14.6) ng mL^{-1}. The major methylated metabolite was MMA, with median concentration of 0.94 (0.60 to 2.50) ng mL^{-1}. The arsenicals were also detected in leukocytes during drug-withdrawal (LOQ 0.5 ng mL^{-1}). In granulocytes, iAs was found during the induction period with median concentration of 1.08 ng mL^{-1}, while MMA and DMA were not detected. The results indicated that iAs was the primary As species in leukocytes and granulocytes from APL patients treated with As_2O_3. A method to determine As species in red blood cells collected from 97 APL patients has also been reported.[78] A mixture of H_2O_2 and $HClO_4$ was used to release the haemoglobin bound As and precipitate protein. Four As species, As^{III}, As^V, MMA^V and DMA^V, in plasma and red blood cells were then detected by HPLC-HG-AFS. Free and bound As species in red blood cells were separated by 30 kDa molecular mass cut-off filters and determined to evaluate haemoglobin binding capacity of different As species. The As species in red blood cells followed the trend iAs > MMA > DMA ($p < 0.01$), while the concentration of DMA was significantly higher than iAs and MMA in plasma ($p < 0.01$). The correlation between iAs concentration in plasma and corresponding red blood cells As level was weak and the concentrations of DMA and MMA in plasma were moderately positive correlated with those in red blood cells. Haemoglobin-binding ratios of iAs, MMA and DMA were all over 70%. In a third study, the researchers focused on the As methylation metabolism and its relationship with chronic hepatic toxicity among APL patients who had finished

treatment with As_2O_3.[79] A total of 112 *de novo* APL patients who had completed the As_2O_3 treatment were enrolled in the study. The As species As^{III}, As^{V}, MMA^{V} and DMA^{V} in patients' plasma, urine, hair and nails were determined by HPLC-ICP-MS. Eighteen single nucleotide polymorphisms (SNPs) of the As^{III} methylation transferase (AS3MT) gene, which was known as the main catalyser for As methylation, were tested using PCR. The results showed that total As decreased to normal concentrations 6 months after cessation of treatment with As_2O_3, although the As speciation demonstrated significantly higher portion of As^{III} in patient's urine (40.1% *vs.* 1.94%, $P < 0.001$), hair (29.3% *vs.* 13.3%, $P = 0.002$) and nails (30.2% *vs.* 13.6%, $P = 0.003$) when compared to the healthy controls, thus indicating a decreased capacity for As methylation metabolism after the treatment with As_2O_3. The urine primary methylation index (PMI) was also significantly lower in patients with both chronic liver dysfunction (0.14 *vs.* 0.28, $P = 0.047$) and hepatic steatosis (0.19 *vs.* 0.3, $P = 0.027$), suggesting that insufficient methylation of As might be related to chronic liver disorders. The authors suggested that urine PMI could be a useful monitoring index for chronic adverse effects following As_2O_3 treatment. Table 1 shows other applications of As speciation presented in the literature during the time period covered by this ASU.

3.3 Chromium

This year, publications describing Cr speciation analysis are about equally divided between those featuring HPLC and those featuring a solid- or liquid-phase extraction separation step. Most of the methods featured ICP-MS as the instrumental technique, with smaller numbers using FAAS, ET-AAS or ICP-OES. The previously reported presence of Cr^{VI} in some food samples has, as was noted last year, been called into question; but, nonetheless, in the past year researchers have reported the presence of Cr^{VI} in both rice[86] and cow milk.[87]

Several *procedures based on HPLC-ICP-MS* have been described. A methodology for determining inorganic Cr species in sewage sludge by HPLC-ICP-MS/MS, involved optimisation of the extraction procedure (for which FAAS was used).[88] The researchers investigated the effects of MAE, vortex mixing and UAE, for a variety of times and at various temperatures and finally chose a procedure that involved a "shaking water bath at 100 rpm" at 50 °C for 16 h, in which 0.2 g of sample was extracted with 5 mL of 50 mmol L^{-1} EDTA at pH 9.5. The final volume was 25 mL, and the sample was diluted 10-fold before HPLC injection. The ICP-MS solution LOD and LOQ were 0.060 and 0.20 μg L^{-1}, respectively, corresponding to 3 and 10 μg kg^{-1} in the solid. Species were separated by AEC on an Agilent Technologies G3268 polymethacrylate resin column (30 × 4.6 mm, 10 μm) by isocratic elution at 30 °C with a mobile phase of 5 mmol L^{-1} EDTA, 15 mmol L^{-1} Na_2SO_4 and 5 mmol L^{-1} NaH_2PO_4 at pH 7. It was observed that all Cr^{VI} was spontaneously reduced to Cr^{III} in the sludge sample and the extraction efficiency was improved to 53.3% compared with the 4.09% of the EPA 1311 method. The method was validated by "fortification/recovery assays at 10 and 50 μg L^{-1}" for which results between 70 and 120% were obtained, but no other details were provided. In a study of the determination of Cr species in rice, initial experiments allowed the extraction and LC conditions and the plasma instrument operating parameters to be optimised.[86] The researchers used the mobile phase, to which HF had been added, as the extractant, noting that at concentrations in excess of the 1% (v/v) used, Cr^{VI} was reduced to Cr^{III}. Samples (300 mg) were heated with 5 mL of 0.5 mmol L^{-1} tetrabutyl ammonium phosphate, 2% (v/v) methanol, 2 mmol L^{-1} EDTA and 1% (v/v) HF at 90 °C for 50 min with MAE. After cooling, the digests were centrifuged, diluted (1–12-fold) with mobile phase and filtered. The mobile phase composition was as described for extraction, minus the HF, and with the pH adjusted to 6.9, and the isocratic RP separation occurred in about 5 min on a C18 column at room temperature. The column eluent was introduced in the plasma *via* a USN, whose operating parameters were also optimised. Spectral interferences due ArC^+, $ClOH^+$ and ClO^+ on the Cr isotopes at *m*/*z* 52 and 53 were removed by reaction with ammonia gas in a DRC. The LOD was 0.01 μg L^{-1}, and the method was validated by the analysis of NIST SRM 1573a (tomato leaves), which contains 1990 ± 60 μg kg^{-1} of total Cr, as the current NIST rice flour material (SRM 1568b) is not certified for Cr. The total Cr in the extract of all samples examined was not significantly different from (a) the sum of species or (b) total Cr determined after closed-vessel MAD with nitric acid. Spike recoveries of both species ranged from 95 to 105%. Interestingly, not only was Cr^{VI} found in both the CRM and all three real samples (3 polished rice and 1 rice cereal), but also an unknown Cr species was found in all samples except the CRM. Concentrations (in the rice samples) of the unknown ranged from 11–53 μg kg^{-1} (as Cr), and those for Cr^{III} and Cr^{VI} ranged from 20–210 and 6–135 μg kg^{-1}, respectively. The detection of Cr^{VI} is in direct contradiction of the findings of other researchers, as is discussed below.

In a series of four papers, Jitaru, Sloth and co-workers described the *development of a double-spike SSID HPLC-ICP-MS method* for the determination of Cr species[89] and its application to the analysis of meat and dairy products,[89,90] bread and breakfast cereals[91] and rice.[92] The method consisted of sequential extraction/complexation of the two species: Cr^{III} with EDTA and Cr^{VI} with DPC (though the researchers consider that DPC reduces Cr^{VI} to Cr^{III} before complexation). Each sample (300 mg) was mixed with the $^{50}Cr^{III}$ and $^{53}Cr^{VI}$ spikes in a 50 mL polypropylene tube, and, as the equilibration of analytes and the corresponding spikes in the sample matrix is a critical step, the blended solution was thoroughly mixed by shaking for 10 min followed by standing for at least 15 min. Then, 16 mL of EDTA solution was added so that the final concentration was 2.4 mmol L^{-1} and the mixture was maintained at 70 °C (heating block) for 25 min. After cooling, 200 μL of DPC solution was added to give a final concentration of 0.02 mmol L^{-1} when made up to 20 mL with water, and the mixture was again heated at 70 °C for 25 min, cooled and filtered (0.45 μm polyvinylidene fluoride). The species were separated in under 3 min on a short microbore anion-exchange column (Dionex IonPac AG7 2 mm × 50 mm, 10 μm) by isocratic elution at 30 °C with a mobile phase of 0.01 mol L^{-1} HNO_3 + 2.5% (v/v) MeOH + 0.30 mol L^{-1} EDTA (at pH 2). Speciation measurements were made on

Table 1 Applications of speciation analysis: arsenic

Analyte species	Matrix	Technique	Sample treatment	Separation	LOD	Validation	Reference
As^{III}, As^{V}, MMA^{V}, DMA^{V}, arsenosugars	Seaweeds	HPLC-HG-AFS with post-column UV oxidation	Washed, dried, ground. UAE 120 min, 1% HNO_3	AEC, 29 °C, gradient, (A) 20 mmol L^{-1} $(NH_4)_2CO_3$, pH 9; (B) water; (C) 20 mmol L^{-1} $(NH_4)_2CO_3$ pH 10.3	3–6 ng g^{-1}	BCR-279 recoveries (6–83%)	80
As^{III}, As^{V}, DMA^{V}, MMA^{V}	Urine	HPLC-ICP-MS Total As by HG-AAS	Filtered (0.22 μm) and diluted with dextrose solution	AEC, isocratic elution, 10 mmol L^{-1} $(NH_4)_2CO_3$ + 10 mmol L^{-1} trizma base + 15 mmol L^{-1} $(NH_4)_2SO_4$	0.02 (DMA), 0.2 (As^{V}) μg g^{-1} creatinine	NIST SRM 2669 NIES CRM No. 18. No data given	81
As^{III}, As^{V}, DMA^{V}, MMA^{V}, AB, AC	Urine	HPLC-ICP-MS	Diluted 20-fold with 20 mmol L^{-1} Na_2EDTA containing 0.1% Triton	AEC, 30 °C gradient, (A) 50 mmol L^{-1} $(NH_4)_2CO_3$ pH 9, (B) water	1–2 ng mL^{-1}	None given	20
As^{III}, As^{V}, DMA^{V}, MMA^{V}, AB, AC	Rice	HPLC-ICP-MS	Extraction with H_2O, shaking (2 h)	AEC, gradient, (A) 15 mmol L^{-1} $(NH_4)_2CO_3$, pH 8.7; (B) 50 mmol L^{-1} $(NH_4)_2CO_3$	0.7–1.4 μg kg^{-1}	Spike recovery, 93–109%. No As species detected in samples tested	82
As^{III}, As^{V}, MMA^{V}, DMA^{V}, AB, AC, Se^{VI}, Se^{IV}, SeMet, SeCys	Seafood, onion	HPLC-ICP-MS	Freeze dried, extraction with H_2O, 40 °C, 20 min	AEC, CEC, gradient, 1 and 75 mmol L^{-1} NH_4NO_3, pH 9.0, 1% MeOH, 40 °C Nine columns evaluated	0.2–2 μg L^{-1} (As), 0.4–2 (Se) μg L^{-1}	BCR-627. No data given	83
As^{III}, As^{V}, MMA^{V}, DMA^{V}, AB, Se^{VI}, Se^{IV}, Br^{-}, BrO_3^{-}, I^{-}, IO_3^{-}	Bottled water, fruit juices	HPLC-ICP-MS	Waters direct. Fruit juice diluted with H_2O, shaken, centrifuged, filtered	AEC, gradient, (A) 20 mmol L^{-1} $(NH_4)_2CO_3$; (B) 50 mmol L^{-1} $(NH_4)_2CO_3$ both at pH 10	LOQ 0.17–1.2 μg L^{-1} (water), 0.34–2.4 μg L^{-1} (fruit juice)	Spike recovery, 86–102%	84
iAs, iCr, iSe	Fly ash (from coal from six mines); solid and water extracts	XRD, XRF, XANES, HPLC-ICP-MS	For XAFS, diluted with BN, pelletised. For HPLC, stirred (6 h) with H_2O (solid–liquid weight ratio of 1 + 9), filtered (0.45 μm)	As: RP C18 isocratic, 10 mmol L^{-1} butane-1 to sulfonic acid, 4 mmol L^{-1} malonic acid, 0.05% TMAH in methanol (pH 3.0). Cr: AEC, isocratic, 10 mmol L^{-1} Na_2CO_3 + 10 mmol L^{-1} $NaHCO_3$ Se: AEC, isocratic, 10 mmol L^{-1} ammonium citrate	Not given	Spike recovery, 99–113%	85

a Thermo iCAP Q instrument and total Cr, for which samples were subject to acidic MAD, was determined on an Agilent 7700 instrument with He as the cell gas in each case. The procedure was applied to the analysis of beef steak and baby and semi-skimmed milk. The researchers showed that Cr^{VI} spikes (baby milk 0.25 μg kg^{-1}, beef steak 0.5 μg kg^{-1}, semi-skimmed milk 1.0 μg kg^{-1}) were quantitatively recovered, even though they calculated the interconversion factor (for Cr^{VI} to Cr^{III}) to be 100% for each sample type. They found Cr^{III} in all samples, but the concentration of Cr^{VI} for all samples was below the LOQ, which was 0.049 μg kg^{-1}. The LOQ for Cr^{III} was 0.013 μg kg^{-1}. The researchers then examined the effect of cooking on these three samples[90] and found that Cr^{III} was not oxidised to Cr^{VI}. They also examined a greater range of samples: 10 different items from each of the sample type (semi-skimmed milk, infant formula milk and meat/meat products). No Cr^{VI} was found in any of the samples, whereas the Cr^{III} concentrations ranged between 0.22 and 7.61 μg kg^{-1} in the milk, though considerably higher values were found in meat samples (1.40–79.7 μg kg^{-1}) with the highest concentrations in the processed meats (pork sausage and Spanish chorizo). The researchers stressed that although the Cr^{VI} spiked in the milk and meat samples was entirely reduced during the analysis, the SSID approach allowed for accurate quantification of this species. They also noted that Cr^{VI} spiked into the samples was stable for a week. When the procedure was applied to the analysis of bread and breakfast cereals,[91] they found that most of the Cr was erroneously identified as Cr^{VI}. They suggested that Cr^{III} was not released during the first step of the extraction (EDTA chelation) but was released during the second, DPC-derivatisation step and was thus erroneously identified as Cr^{VI}. They then modified the extraction/derivatisation procedure by increasing the temperature of each stage to 90 °C and the time for the first step to 60 min. They analysed 11 different types of each sample material, in none of which was any Cr^{VI} detected, whereas the Cr^{III} concentrations ranged from 5–176 μg kg^{-1} (bread) and from 24–350 μg kg^{-1} (breakfast cereal). They showed that Cr^{VI} spiked into French bread at concentrations up to 20 μg kg^{-1} was quantitatively recovered as Cr^{III}. They included a critical commentary on several previous reports of the presence of Cr^{VI} in bread, concluding "the previously reported findings of Cr^{VI} are most probably erroneous and caused by analytical artefacts." Finally, they determined the Cr species in rice,[92] for which they used the extraction procedure with the higher temperatures and longer times. The LOD values were 0.004 and 0.014 μg kg^{-1} for Cr^{III} and Cr^{VI}, respectively. For the 10 samples of different origin and colour analysed, the Cr^{VI} concentrations were below the LOD, whereas the Cr^{III} concentrations ranged from 0.59 (whole grain rice) up to 104 μg kg^{-1} (brown rice). Total Cr was also determined by ICP-MS (following MAD with HNO_3) and the results were equal to the Cr^{III} concentrations. To assess the stability of the species in rice, one sample was spiked individually with Cr^{III} and Cr^{VI} at concentrations of 5.0, 10, 15 and 20 μg kg^{-1} and then analysed after 2 h by SSID HPLC-ICP-MS. The results showed a complete reduction of Cr^{VI} to Cr^{III}. As for the previous results for bread, they described previously published results reported by other researchers showing that rice does contain Cr^{VI} is likely to be an analytical artefact. Although the work of Chen and Jiang[86] discussed above was published before the researchers submitted this fourth paper, it is not cited; it is the results disclosed in an earlier paper from this group, mentioned in last year's ASU, that are considered erroneous.

A method based on *electrokinetic sample pre-treatment* has been devised.[93] Although the researchers describe this as a "paper-based analytical device", the separation was, in fact, carried out on a glass fibre filter membrane cut to give a 35 mm × 4 mm rectangle that was wetted with triammonium citrate and placed above two reservoirs 33 mm apart, into which 300 μL of the same sample solution was placed. Platinum wire electrodes 0.5 mm diameter were inserted in each reservoir and a DC potential of 250 V applied for the required time (about 3 min), after which the two 1 mm ends immersed in the anode and cathode reservoirs were discarded, and the remainder divided into equal parts (about 16 mm each). The two sample strips were inserted into 1.5 mL centrifugal tubes, a rinse solution added, and the tubes sonicated for 10 min. The nature of the rinse solution was not explicitly given but might have been water. The volume was not given either. Following extraction, the Cr in each tube was determined by either ETAAS or ICP-OES. The researchers discussed the possible nature of the Cr species present in each reservoir as the pH changed due to the electrolysis of water (the anode reservoir becomes acidic and the cathode reservoir becomes basic). The results showed that no residual Cr^{VI} was detected in the cathode reservoir, which was consistent with the Cr^{VI} recovery of close to 100%. However, about 14% of the Cr^{III} was found in the anode reservoir, in agreement with the lower recovery for Cr^{III} of 86%. The procedure was applied to the analysis of tap and lake waters (pH = 7) and an electroplating wastewater sample (diluted 1000 times) with deionised water, all of which were stored at 4 °C prior to analysis. Neither species was found in the river or lake water, but spikes of each at concentrations of 100, 200 and 300 μg L^{-1} were 88–110% recovered. Both species were found in the electroplating wastewater.

As part of the *validation of the results obtained by a smartphone-enabled field monitoring tool* for Cr^{VI} in water, samples were also analysed by IC-ICP-MS. No details of this aspect of the work were provided other than a Dionex system was used with 1% HNO_3 eluent that was also the matrix for the calibration standards over the range of 0–0.1 mg L^{-1}. None of the real samples taken contained Cr^{VI}, but out of the 20 spiked uncontaminated groundwater samples, a set of 12 samples covering the linear range were analysed by IC-ICP-MS and the results were compared with data recorded from the other two techniques (smartphone and visible absorption spectrophotometry following reaction with DPC). No significant differences were observed between the ICP-MS results and the smartphone results. Full details of the smartphone device, which fits over the camera lenses and flash lamp, were provided. The LOD of smartphone-enabled colorimetric method ranged from 1–10 μg L^{-1}, which were considered significantly better than those of commercially available field test kits.

In the development of a *method to determine Cr^{VI} in organic fertilisers* by alkaline extraction followed by CPE and FAAS, the

researchers used HPLC-ICP-MS to demonstrate that Cr^{VI} was not produced during the extraction step.[94] Species were separated on an anion-exchange column (G3268-80001, Agilent Technologies), with a mobile phase of 25 mmol L^{-1} $(NH_4)_2SO_4$ and 1 mmol L^{-1} NaOH at pH 8.0. Few further details were provided, though readers were referred to methods EPA 3050A, and ISO 17075-2:2017 (IULTCS/IUC 18-2:2017), developed for the determination of Cr^{VI} in leather.

As a possible way of overcoming the species-interconversion problems of conventional soil solution sampling for inorganic contaminants such as Cr^{VI}, Hamilton *et al.* coupled *microdialysis with HPLC-ICP-MS*.[95] They also hoped that the coupling would provide better temporal resolution allowing dynamic interactions to be captured. As the researchers explained, microdialysis uses a probe containing a specific molecular weight cutoff semipermeable membrane. Pumping a perfusate solution into the probe creates a diffusion gradient within the sampled medium causing solutes to diffuse across the membrane and the dialysate exiting the probe, containing the sampled solutes, can then be analysed by a suitable analytical technique. They pointed out that the minimal disruption to the soil, coupled with the ability of the technique to sample soil solution at representative water contents (50% water holding capacity and higher) makes microdialysis an attractive tool for studying small-scale inorganic solute availability. In this case, they determined Cr^{VI} in the dialysate by ICP-MS after separation by AEC (Hamilton PRP X-100 column) with isocratic elution by a mobile phase of 40 mmol L^{-1} NH_4NO_3, 50 mmol L^{-1} TRIS buffer, and 5 mmol L^{-1} NH_4-EDTA, adjusted to pH 7.0 with concentrated HNO_3, probably at room temperature, though this was not specified. No citations to previous chromatographic work were given, so it is not clear to what extent this separation was novel. No chromatographic optimisation was reported. The QQQ mass spectrometer instrument was operated in collision cell mode with He as the collision gas. The LOD was 0.2 μg L^{-1}. The setup was applied to the sampling of native Cr^{VI} in three soils with differing geochemical properties at 15 min intervals, offering improved temporal resolution and a significant reduction in analysis time over offline microdialysis. The researchers concluded that their system could be applied to studies of (a) solute turnover/removal at root- and microbe-relevant scales in near real time, (b) the mechanisms governing rapid soil fixation and (c) speciation changes, for important redox-active micronutrients such as I and Se. Finally, they added As, Hg and Tl to the list of elements that could be studied in the future.

In a study of *Cr speciation in cow milk*, a digestible fraction was identified by adding 10 mL of artificial gastric juice (recipe given) to 2.0 mL of milk followed by heating (water bath) for 3 h at 37 °C and filtering through a 0.45 μm membrane filter.[87] Total Cr was determined in the residue, by MAD with $HNO_3 + H_2O_2$, as well as in the filtrates and the original milk. The Cr species in the digestible fraction were separated *via* pH control by sequential DMSPE with a fibrous nanocomposite material prepared by *in situ* growth of graphitic carbon nitride on the surface of TiO_2 nanofibers ($g\text{-}C_3N_4@TiO_2$), whose preparation and characterisation were described in detail. The pH of a 20 mL sub-sample of the digestible fraction was adjusted to either pH 8.0 for Cr^{III} or 3.0 for Cr^{VI} and 10 mg of extractant added. The mixture was dispersed by ultrasound for 4 min, centrifuged (for 4 min at 4500 rpm) and the target species desorbed by 1.0 mL eluent (0.5 mmol L^{-1}) HNO_3 for Cr^{III} or 0.1 mmol L^{-1} NaOH for Cr^{VI} by vortexing (4 min), followed by centrifugation. The LOD values were milk powder: 110 (Cr^{III}), 260 (Cr^{VI}), cow milk: 5 (Cr^{III}), 13 (Cr^{VI}) ng kg^{-1}. The procedure was applied to one sample and to a milk powder CRM (GBW 10017 from the National Research Center for Geology, Beijing, China), in both of which both species were found, with Cr^{III} being the predominant species. Spike recoveries ranged from 92 to 105% and showed no evidence of interspecies conversions. The authors considered that the artificial gastric juice prevented such conversions. Table 2 shows other applications of Cr speciation presented in the literature during the time period covered by this ASU.

3.4 Gadolinium

There are currently *two distinct areas in Gd speciation that are the focus of analytical development, both relating to Gd-containing Magnetic Resonance Imaging (MRI) contrast agents* these include: the development of methods for the investigation of their environmental fate and degradation; and studies on the biomedical effect and action of new and existing contrast agents. Both areas have been developed around the use of chromatography coupled to ICP-MS.

Two recent studies have developed *methods for the analysis of Gd-based contrast agents (GBCA) in water discharged from municipal water-treatment works*, where this unusual and rare class of chemicals is appearing due to increased medical usage and disposal into waste-water streams. Horstmann *et al.*[101] developed a method for the determination of 4 common GBCA in fresh and oceanic waters of Australia, including effluent, river and seawater samples. The approach was based on HILIC-ICP-MS, which seems to be a popular and effective LC mode for these complexes, providing rapid separation aligned with good quantification. Separation in HILIC is based on a compound's polarity and degree of solvation and requires hydrophilic stationary phases with reversed-phase type eluents. In this study, the column was a HILIC silica column (Accucore 100 × 2.1 mm, 2.6 μm particle size) using a mobile phase containing an ammonium acetate buffer (10 mmol L^{-1}) at pH 5.3, with acetonitrile added as an organic modifier. The separation was carried out at a column temperature of 40 °C to reduce back pressure. To mitigate the effects of the organic modifier and reduce build-up of carbon on the cones, an Ar/O_2 (20% O_2) gas mixture was added pre-torch, with the spray chamber cooled to −5 °C. To improve the sensitivity, ion extraction and transport processes were optimised and the quadrupole mass filter was operated with an increased mass bandpass, lowering the LOD to between 18 and 24 ng L^{-1} for the individual GBCAs. This allowed detection of Gd-DOTA, Gd-BT-DO3A and Gd-DTPA-BMA at concentrations of up to 160 ng L^{-1} in water samples collected from rivers within the proximity of effluents of local wastewater treatment plants. No Gd-DTPA was observed in the

Table 2 Applications of speciation analysis: chromium

Analyte species	Matrix	Technique	Procedure	LOD	Validation	Reference
Cr^{VI}	Water, toy migration solutions	ICP-MS	Samples acidified with HNO_3. SAX of Cr^{III} in 60 mm column. Tolerated up to 50 mg kg^{-1} Cr^{III}	0.03 μg kg^{-1}	Spike recovery, "close to 100%"	96
Cr^{VI}	Waters	FAAS	Sample pH adjusted to pH 5. Magnetic DSPME on multiwalled CNTs functionalised with 3-aminopropyltrimethoxysilane (APTMS) with vortex mixing, magnetic separation, adsorbed Cr^{VI} eluted with 1.0 mL of 2.0 mol L^{-1} HNO_3	3 μg L^{-1}	Spike recovery 91–111%	97
Cr^{III}, Cr^{VI}	Waters	LIBS	SPE Cr^{III} on chelate resin (Lewatit TP207) and Cr^{VI} anion-exchange resin (Lewatit MP68). Sequential extraction on glass columns (100 mm length, 4 mm i.d.) packed with each resin	Cr^{III} 88 μg L^{-1}, Cr^{VI} 270 μg L^{-1}	Spike recovery, Cr^{III} 93–108%, Cr^{VI} 98–100%	98
Cr^{VI}, total Cr	Waters, urine	ETAAS	Cr^{VI}, DES, phenol and chlorine chloride at pH 5.7 with THF as emulsifier, centrifugation, deposited phase diluted with H_2O Total Cr after oxidation with 0.02 mol L^{-1} $KMnO_4$ solution and 0.5 mL of conc. H_2SO_4	0.096 μg L^{-1}	Spike recovery 95–106%	99
Cr^{VI}	Organic fertiliser, sewage sludge	FAAS	CPE complex with DPC in triton X-100 0.5 mol L^{-1} NaOH, 0.28 mol L^{-1} Na_2CO_3 pH ≥ 11.5, $MgCl_2 \cdot 6H_2O$, and 0.5 mol L^{-1} pH 7 phosphate buffer. NaCl DPC, Triton X-100 ethanol	0.6 μg g^{-1}	SRMs 2701 and 695, 94–101% recovery. Spike recovery from fertiliser, 82 to 90%	94
Cr^{VI}	Air, urine	FAAS	DLLME of DPC complex in presence of SDS, acid and salt with solidified floating organic drop (1-undecanol, 2-dodecanol or *n*-hexadecane) and EtOH as disperser	0.02–0.05 μg L^{-1}	Spike recovery 98%	100

environmental water samples tested. The analysis of GBCAs in sea water required the development of a novel automated micro-SPE method for matrix elimination and analyte pre-concentration, enabling the detection of Gd-DOTA and Gd-BT-DO3A. Sample clean-up using micro-SPE improved the peak shape and resolution, resulting in a much better signal-to-noise ratio and improved LOD for the four Gd-containing species investigated. Without this step, the chromatographic separation was severely compromised and it was not possible to distinguish some of the species from each other due to peak broadening. The second study[102] focused on the measurement of GBCAs in river water samples from near to, and 2 km downstream of, a water-treatment works. Once again, HILIC was chosen as the separation mode, except in this case the eluent was developed so that it was 100% aqueous, which meant the precautions required when using an organic modifier and ICP-MS were not required. The column stationary phase was phosphorylcholine bound to silica (150 × 4.6 mm, 3.0 μm particle size) and the mobile phase was ammonium acetate buffer (10 mmol L^{-1}) at pH 5.3, with the column held at 25 °C. The method was developed for the measurement of six GBCAs including Gd-DTPA, Gd-HP-DO3A, Gd-DTPA-BMA, Gd-DOTA, Gd-BT-DO3A and Gd-EOB-DTPA in river water and provided LOD in the range 3.4 to 22 ng L^{-1}. Only Gd-DOTA and Gd-BT-DO3A were found in the samples from the treatment plant effluent, however two unidentified Gd-containing peaks were present, which were thought to result from the breakdown of other industrial Gd-chemicals during treatment.

Progress on Gd-speciation in the biomedical area has recently been focused on investigating the Gd-containing compounds present in tissue and methods for their efficient extraction, but also the fate of a new ultra-small Gd-containing NPs with potential clinical applications. The safe use of GBCAs has become of interest to regulatory agencies such as the Food and Drug Administration (FDA) because there is evidence that their repeated use can lead to an accumulation of Gd in some tissues, particularly the brain and kidney. In an effort to study the processes that lead to a build-up of Gd, it is necessary to carry out speciation analysis of the Gd-containing compounds present in these organs to determine the form of Gd present and whether it is the intact GBCA that was administered, a metabolite, or some other degradation product. The first step in doing this is to develop suitable extraction methods that can efficiently isolate the compounds, without their degradation; this is no mean feat from a practical perspective. Working on brain tissues from rats[103] injected with either gadoterate

meglumine, gadobenate dimeglumine, gadodiamide, or no injection (control group), urea was used as the extractant and compared to the use of water. Total Gd concentrations were determined in the original brain tissue and the resulting soluble and insoluble fractions by ICP-MS and used to calculate the Gd accumulation and extraction efficiency. Separation by SEC with ICP-MS detection was then used to monitor the speciation of Gd in the soluble fractions. The Gd-containing species were isocratically eluted either from a Superdex 75 or a Superdex 200 column (300 × 10 mm) with an ammonium acetate (100 mmol L^{-1}) buffer at pH 7.4 over 45 minutes at a flow rate of 0.7 mL min^{-1}. Because of the limited separation range of the Superdex 75 column (3 to 80 kDa), the Superdex 200 column (10 to 660 kDa) was used to better estimate the molecular weight of the detected Gd species. The identity of the eluted species was investigated by using molecular size and retention time matching with Gd chelates and a ferritin standard. The LOQ was estimated using standard GBCA solutions to be 0.32 pmol mL^{-1} for all 3 GBCAs using a signal-to-noise >5 criterion. To control the background level of Gd, the supernatants obtained from the samples of the control group were analysed systematically at the beginning and at the end of the sequence as the blanks. The average blank chromatogram per run was calculated and subtracted from the chromatograms unless specified otherwise. The combination of sequential water and urea extractions solubilised the brain tissue with an efficiency of 97%, much higher than previous work, while preserving the stability of the initially injected form of GBCA. Clearly more work in this complex area is required, but the approach described shows considerable promise. Labied *et al.*[104] used Taylor dispersion analysis (TDA), which is a technique for rapidly determining the diffusion coefficients of molecules from which the hydrodynamic radii can be determined. In this work they coupled the TDA to ICP-MS and also used CE-ICP-MS, to study the degradation pathway of AGuIX, an ultra-small Gd-containing nanoparticle (<8 nm in diameter) as part of a phase 2 clinical trial. The NPs comprised a polysiloxane substrate as a carrier for Gd, for use as a radiosensitiser in the treatment of various cancers. This approach allowed for the close monitoring of the biodegradation of the particles in a wide range of media, from aqueous buffers to highly complex matrices such as blood serum. The results of TDA-ICP-MS for particle biodegradation were found to be in agreement with those obtained from CE-ICP-MS separation. The CE-ICP-MS method was then used to monitor the Gd-containing biodegradation products and appeared to be a useful complementary tool, as these compounds can be fully separated. Indeed, this hyphenation demonstrated that the AGuIX biodegradation main product is likely to be 2,2,2″-(10-(1-carboxy-4-oxo-4-((3-(trihydroxysilyl)propyl)amino)butyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid-Gd (APTES-DOTAGA-Gd). Moreover, no evidence of free Gd release upon biodegradation was provided by the CE-ICP-MS experiments, indicating that the renal toxicity experienced with earlier Gd-containing contrast agents may be minimised with the use of this type of nanoparticle formulation.

3.5 Germanium

A method for the *speciation analysis of the three main Ge species*, iGe, monomethyl germanium (MMGe) and dimethyl germanium (DMGe), in environmental waters by HG-CryoTrap-ICP-MS has been developed.[105] The analytes were volatilised by HG prior to their pre-concentration/separation in a semi-automated cryogenic trap followed by detection by ICP-MS/MS. Two clean up procedures, involving either HG or $Mg(OH)_2$ co-precipitation, to minimise the iGe reagent blanks were evaluated and after this step one mL of water could be analysed without any pre-treatment. After optimisation of all experimental variables, which are fully described in the paper, LOD values of 0.015, 0.005 and 0.003 ng L^{-1} were obtained for iGe, MMGe and DMGe, respectively. Standard addition experiments did not show any significant matrix effect, and, therefore, external calibration was used for sample analysis. In the Tris–HCl + L-cysteine HG reaction media used, additional experiments did not reveal any significant demethylation of MMGe to iGe in the process of HG-cryotrapping. The method was applied to the analysis of iGe, MMGe and DMGe in eight CRMs: CASS-4, CASS-5 and CASS-6 (nearshore seawater); NASS-5 and NASS-7 (seawater); SLRS-4, SLRS-5 and SLRS-6 (river water) and the values obtained were similar to those reported in the literature by other workers. The paper is an interesting read for those new to the field of Ge speciation in natural waters and more generally as a guide for method development and validation when no CRMs are available.

3.6 Gold

A study which reports on the *coupling of hydrodynamic chromatography (HC) with ICP-MS for the simultaneous determination of dissolved and nanoparticulate species of gold and silver* has been published this year.[106] The optimal mobile phase for the separation of the Au species was found to be 0.45 mmol L^{-1} SDS and 0.05 mmol L^{-1} penicillamine flowing at 1.6 mL min^{-1} with a run time of 11 minutes. This allowed the quantitative recovery of ionic Ag and Au NPs up to 50 nm in diameter and the resolution achieved between ionic Au and 10 nm Au NPs was 0.7 with an LOD of 0.05 μg L^{-1}. The method was applied to the detection of Au NPs and dissolved Au species in a dietary supplement, with the Au NP mass fraction determined HC-ICP-MS showing good agreement with the results obtained by ICP-MS after a sample digestion step involving a 2 : 1 mixture of HCl and HNO_3, being 20.4 ± 0.8 and 21.0 ± 0.8 mg L^{-1}, respectively. However, the ionic Au fraction in this sample, which found to be less than 2.3 μg L^{-1} by an ultrafiltration based method, could not be determined by HC-ICP-MS due to a peak overlap which the authors attributed to the small size of the Au NPs and the low ionic Au concentration. A spike recovery value of 88.7 ± 4.5% was obtained at a spike level of 155 μg L^{-1}. The measured Au NP size, found to be 6.5 ± 0.1 nm, agreed with that obtained by with TEM, 5.1 ± 2.4 nm, and that obtained by cited values from other workers although it differed from the manufacturers value of 3.2 nm. The results obtained for Ag NP containing supplements can be found in Section 3.17.

3.7 Halogens

The growth in the development and use of *lithium ion batteries (LIB), which has arisen from their wide spread use in mobile electronic devices and power generation networks, has lead to the development and use of new analytical methods to investigate the degradation products that are formed during their use*. Due to their structural similarity to chemical warfare agents like sarin, organo(fluoro)phosphates are potential hazardous decomposition products formed from the widely used conducting salt $LiPF_6$, present in the liquid electrolytes used in commercial cells. The use and development of on-line SPE has proven to be essential to achieve the required LOD and LOQ when the low concentration organo(fluoro)phosphate (O(F)P) degradation products are in the presence of a high concentration of the hexafluorophosphate ($LiPF_6$) conducting salt.[107] Using optimised SPE conditions, $LiPF_6$ could be removed quantitatively from the sample by allowing the degradation products to bind to the SPE column, whilst the unretained $LiPF_6$ was flushed to waste. This allowed for an increase in the injection volume by up to a factor of 50, while the dilution factor could be decreased up to a factor of ten. The O(F)P were identified using an isocratic HPLC separation on an C_{18} column at 40 °C, using a water-based eluent containing acetonitrile (17% v/v) with a flow-rate of 0.35 mL min^{-1}, coupled to an ion-trap-TOF-MS. For substance independent quantification with a plasma-based technique, the same isocratic separation method was implemented as described above and a SF-ICP-MS instrument was used to monitor the O(F)P degradation products, which were quantified by the measurement of ^{31}P in medium resolution mode ($R > 4000$) at a plasma power of 1250 W (shielded torch, Pt sample/skimmer cones) and oxygen was added to the nebuliser flow to reduce carbon build up on the interface. As the O(F)P are potentially toxic and, therefore, standards are not available quantification with ES-MS was not possible. Due to the number of compounds present and the lack of resolution between them in the chromatographic system used, the O(F)P degradation products were quantified as two broad "bulk" elutions, using external 6-point calibration with trimethyl phosphate as the calibration standard.

It is encouraging to note that a *method for the measurement of synthetic brominated flame retardants (BFR) and their proposed hydroxylated metabolites in human samples* has been published.[108] Most studies on BFR have focused on their measurement in environmental samples. Once again on-line SPE was used to pre-concentrate the analytes, prior to measurement, in this case using RP HPLC-ICP-MS. The main parameters such as loading rate, elution time and elution solvent were optimised, although chemometric approaches do not seem to have been used to achieve this, instead the authors relied on a one-step-at-a-time design, which is inefficient and does not always locate the global optimal conditions. The final method used an Oasis HLB SPE column and a loading solvent of water at a rate of 3.0 mL min^{-1}. The elution solvent was MeOH–ACN–H_2O (54 : 26 : 20, v/v), at a rate of 1.0 mL min^{-1}. The HPLC column was a Zorbax Eclipse Plus C_{18}. The LOQ for the method were between 0.14 to 0.74 ng L^{-1}, however the recoveries were low, between 61.8 to 77.7%, and the short-term precision (RSD) was between 3.7 to 10.1% ($n = 3$). The method was applied to the analysis of 30 serum samples and a total of 4 kinds of PDBEs and 2 kinds of hydroxy PBDE were detected in the human serum samples, confirming that individuals are being exposed to these persistent chemicals.

The investigation of I-containing species in ground water and other environmental samples has been the focus of a study using IC-ICP-MS. A rapid technique (<15 min duration) for the simultaneous I speciation (iodide/iodate) and isotopic ratio ($^{129}I/^{127}I$) measurements by IC-ICP-MS, used O_2 as the reaction cell gas.[109] This eliminated >99% of ^{129}Xe present in the Ar supply by a charge–exchange reaction and allowed for high precision analysis of ^{129}I. The downside to the use of O_2 was that Mo, which eluted as a peak from the column, reacted with the O_2 to form a molybdate polyatomic at m/z 127 and 129 due to $^{95}Mo^{16}O_2$ and $^{97}Mo^{16}O_2$, respectively. Fortunately, this did not affect the speciation results as the Mo conveniently eluted between the peaks for iodide and iodate, however it did affect the measurement of total I for ground water samples containing Mo at high $Mo/^{129}I$ ratios (>20). The knock-on effect of this interference was that the organo-I species could not be quantified by the difference between the total I and the two measured species. Speciated standard reference materials yielded LOD for ^{127}I of approximately 24 ng L^{-1} for iodate and iodide, and for ^{129}I of approximately 1.8 ng L^{-1} for iodate and 2.6 ng L^{-1} for iodide. The method was used to investigate radioiodine contamination in groundwater samples from six wells at the Hanford Site, in Washington State, USA. Iodate was the primary species for both I isotopes, with small quantities of ^{127}I-iodide detected in most of the samples, but all ^{129}I-iodide results were below the LOD.

There is currently considerable *interest in the determination of per- and polyfluoroalkyl substances* (PFAS) in the environment. Feldmann and co-workers point out that although more than 4700 PFAS may occur, only 40–50 compounds are routinely determined in targeted analysis by ES-MS using isotopically labelled standards.[110] They demonstrated the application of simultaneous HPLC-ICP-MS/MS with HR-ES-MS to evaluate the biodegradation of a model organofluorine compound, 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decanol (8 : 2 FTOH), in sewage sludge. This work used a newly developed module, for the data evaluation software MZmine, for a non-targeted analysis for PFASs and their degradation compounds. Analytes were separated on a C18 column, with gradient elution by mobile phases (a) (H_2O/ACN, 95 + 5, 20 mmol L^{-1} $NH_4CH_3CO_2$, pH 6.6) and (a) (H_2O/ACN, 5 + 95, 20 mmol L^{-1} $NH_4CH_3CO_2$). The eluent flow (0.5 mL min^{-1}) was split 4 : 1 between the ICP and ES instruments, and a 50 mg L^{-1} Ba solution was merged (1 + 1) with flow to the ICP MS instrument so that F could be detected as BaF^+, which enabled untargeted analysis for PFAS *via* the retention time information obtained from the ICP-MS/MS chromatograms. This approach, together with other software-based approaches, such as removal of blank and control sample features, decreased the 5115 features initially detected to 15, thereby greatly increasing the detection efficiency of unknown organofluorine compounds. The researchers point out that F

speciation *via* ICP-MS/MS is currently limited by the achievable LOD values, 0.06 to 2.8 mg L^{-1} were reported, and that improved sensitivity would reduce the risk of ES-MS features being falsely excluded from further analysis due to F signals being undetected by ICP-MS/MS. They also called for the development of negative mode instruments ICP-MS, which were available from one manufacturer in the past. The introduction to the article is a useful tutorial overview of the current situation regarding, and challenges facing, the determination of PFAS.

3.8 Iron

Analytical *methods for Fe speciation continue to be developed* and reported due the important biological role of this element. Iron was one of the elements, the others being Cu, Fe, Mn, Ni and Zn, included in a study of the identification and characterisation of labile metal pools in cells, due to the important role these metals have in signalling, and regulation.[111] The paper explains that metal chelators and buffers that coordinate metals should be excluded from the analytical procedure and that the interaction of metals with size-exclusion columns, which contain carboxylate groups that bind metals must be minimised. This can be achieved by increasing the ionic strength of the mobile phase, which can be problematic for ICP-MS and ES-MS. Therefore, a new strategy to overcome these secondary interactions, that involved saturating the column binding sites with ^{67}Zn prior to the analyses, was developed. Metal species were separated on either a single SEC column or on two such columns linked in series and the mobile phase was either 20 or 50 mmol L^{-1} ammonium acetate at pH 6.5, flowing at either 0.6 or 0.25 mL min^{-1} for the single or double column set ups, respectively. Following DNA hydrolysis, cell lysates were centrifuged (100 000 g, 60 min), transferred to a chilled anaerobic glove box (1–10 mL m^{-3} O_2, 4–8 °C) and filtered (regenerated cellulose 3 kDa). The researchers found 2–5 Fe species with apparent masses between 300 and 5000 Da and that the labile iron pool in the *E. coli* cells accounted for 8% of total cell Fe and 20% of the cytosol Fe. The authors concluded that a major problem in identifying labile metal compounds in aqueous cellular solutions by ES-MS was signal suppression by high concentrations of salts. Intravenous iron–carbohydrate complex preparations (IVIP) are non-interchangeable prodrugs, the pharmacokinetics (PK) of which influence their pharmacodynamics (PD) and, thus, their efficacy and safety. As part of a study to examine the PK/PD relationships of three IVIP a two-pathway model of transient non-transferrin-bound iron (NTBI) generation, following single dose administration, has been identified using a range of analytical techniques of which one was HPLC-ICP-MS to analyse serum samples for total Fe, IVIP-Fe and transferrin-bound Fe (TBI).[112] These were separated using a SEC column with a mobile phase of 50 mmol L^{-1} ammonium acetate at pH 7.4 flowing at 0.75 mL min^{-1} for 45 minutes and the $^{56}Fe^{+}$ signal detected by ICP-MS with ammonia as the cell gas. Patients were given 200 mg Fe as either Fe-carboxymaltose, Fe-sucrose, Fe-iso-maltoside-1000 or a placebo. It was found that the IVIP-dependent increases Fe returned to baseline in 48–150 hours, except for s-Ferritin and TSAT. The NTBI was low with Fe-isomaltoside-1000 (0.13 μmol L^{-1} at 8 h), rapidly increased with Fe-sucrose (0.8 μmol L^{-1} at 2 h, 1.25 μmol L^{-1} at 4 h), and delayed for Fe-carboxymaltose (0.57 μmol L^{-1} at 24 h). The NTBI area under curve (AUC) levels were 7-fold greater for Fe-carboxymaltose and Fe-sucrose than for Fe-isomaltoside-1000. The hepcidin peak time varied, but not AUC or mean concentration. s-Ferritin concentration and AUC were highest for Fe-carboxymaltose and greater than placebo for all IVIP. Two mechanisms for the observed NTBI kinetics were proposed: rapid and delayed NTBI appearance consistent with direct (circulating IVIP-to-plasma) and indirect (IVIP-to-macrophage-to-plasma) Fe release based on IVIP plasma half-life and s-Ferritin dynamics.

Two papers from the same researchers report on *the use of HPLC-MIP-OES and or HPLC-ICP-OES for Fe speciation studies*. In the first of these papers a system which involves both AEC and CEC HPLC systems interfaced to an ICP-OES instrument.[37] The interface consisted of a modified spray chamber with allowed two sample stream inputs. The AEC eluent was mixed with hydride generation reagents before being introduced *via* a vertical inlet, allowing the spray chamber to act as the gas/liquid spray chamber, whilst the CEC eluent was conventionally nebulised. The AEC mobile phase consisted of 2.5 mmol L^{-1} Na_2HPO_4 and 25 mmol L^{-1} $KH_2PO_4 \cdot 2H_2O$, pH 6.0 ± 0.2, whilst the CEC mobile phase was 7.0 mmol L^{-1} pyridine-2,6-dicarboxylic acid, 66 mmol L^{-1} KOH, 5.6 mmol L^{-1} K_2SO_4 and 74 mmol L^{-1} formic acid at pH 4.2 ± 0.2 with the flow rate being 1.0 mL min^{-1} in each case. Two Fe species, Fe^{II} and Fe^{III}, with LOD values of 10.4 and 13.4 μg L^{-1}, were separated by the CEC system in 13 minutes, with a suite of 10 other metals also being analysed for with similar LOD values being obtained. To prevent Fe species interconversion, the CEC column was periodically conditioned with 100 mmol L^{-1} Na_2SO_3 to remove O_2. The AEC system was used to separate As, Ge and Se species. The method was applied to extracts, prepared using the CEC mobile phase and UAE, of holly, pottery, sediments and soils. In the second paper by this group the chromatographic set up was used but interfaced to an MIP-OES instrument and the same types of sample extracts analysed.[113] In this case, the LOD values were approximately 10-fold higher than those obtained with ICP-OES detection due to the lower sensitivity of the MIP-OES instrument. There is a wealth of optimisation details contained within each paper should the reader have further interest.

A *non-chromatographic method for Fe speciation* has also been reported.[114] The sample preparation method is described as an 'in syringe-supramolecular dispersive liquid–liquid microextraction' and Fe quantification was by FAAS by injecting the extracts into a carrier stream. The Fe^{III} in samples was complexed with 4,5-dihydroxy-1,3-benzendisulfonic acid (Tiron), neutralised through the formation of the ion pair with the ionic liquid of 1-hexadecyl-3-methylimidazolium bromide, and extracted into the reverse micelles of decanoic acid in tetrahydrofuran. The total Fe content was determined after oxidation of Fe^{II} to Fe^{III} with H_2O_2 and the Fe^{II} concentration determined by difference. Under optimal conditions, the linear range was 3.5 to 45.0 μg L^{-1} of Fe^{III} and the LOD and LOQ values were 1.04

μg L^{-1} and 3.45 μ L^{-1}, respectively. The method was applied to the analysis of Fe species in various river, tap and well water samples which were found to contain between 6 and 30 μg Fe^{II} per L and between 10 and 34 μg Fe^{II} per L. *In situ* μ-Raman, μ-XANES and μ-XRF have been used to investigate Fe speciation and the radial Fe profile in black coral, with the aim of assessing the use of this material as a proxy for the environmental impact of past mining activities.[115] The preliminary results from the μ-Raman and μ-XANES analysis demonstrated that Fe in black coral was mainly combined with 3,4-dihydroxyphenylalanine (DOPA) as tris-DOPA-Fe complex. Also, the elevated Fe concentration in the Fe profile recorded with synchrotron μ-XRF, with a 2.5 μm resolution, corresponded well with the exploitation history of the adjacent onshore Tiandu Iron Mine (Sanya, China) from 1939 to 1960. Other distinct Fe peaks detected coincided with war activities in 1970s. The authors suggest that these findings indicate that the high-resolution Fe record with low annual growth rate (~17.8 μm per year) of black coral may serve as a proxy of marine environmental record.

3.9 Lead

Only one report of Pb speciation has appeared in this review period, describing the *EXAFS speciation in urban residential soils* impacted by multiple legacy sources.[116] The report is of a very detailed study of the Pb contamination in the city of East Chicago, IN, an industrial centre with a wide range of Pb sources, including a former smelter. Portable XRF measurements of 358 randomly sampled soils across the city revealed a highly heterogeneous distribution of Pb concentrations ranging from 24 to 14 428 mg kg^{-1} (many of which were above the US EPA regulatory limit for soil) across all neighbourhoods, and mostly uncorrelated with distance from the former smelting site, but which were negatively correlated with median household income. Speciation by EXAFS ($n = 44$) at the SSRL beamline 4–1 indicated that the Pb was present primarily as metastable forms, including Fe oxide-bound Pb, Mn oxide-bound Pb, organic complexes of Pb and some Pb-hydroxycarbonate. Significant concentrations of crystalline insoluble species, such as galena and pyromorphite, were not detected. The researchers concluded that the unique chemical forms of potential Pb sources, such as paint, ore or slag had not persisted and had been converted to acid-soluble forms of Pb that have greater bioavailability. The researchers also noted that Pb concentrations were significantly correlated with those of Cr, Cu, Fe, Mn and Zn; however, although Pb appeared to be correlated with As, they thought this was spurious because of interferences from the L_α emission for Pb and the K_α emission for As. They noted that portable XRF instruments are able to correct for this at lower Pb : As ratios, but at the higher ratios encountered in this study, the deconvolution procedure had failed and As concentrations were overestimated.

3.10 Manganese

Two reports of Mn speciation have appeared in this review period. The first described the *sequential DSPME of Mn^{VII} on a magnetic nanomaterial* followed by the DMLLE of Mn^{II} with collection in a solidified floating drop of organic solvent.[117] Briefly, the magnetic DSPME (for Mn^{VII}) with $ZnFe_2O_4$ nanotubes (ZFONTs) involved desorption into NaOH with UAE and the DLLME (for Mn^{II}) of the 1-phenyl-3-methyl-4-benzoyl-5-pyrazone (PMBP) involved complex formation with a solidified floating organic drop (1-undecanol). The procedure was applied to the analysis of a number of beverages, in none of which was any Mn^{VII} detected (the LOD was 0.007 μg L^{-1}). Spike recovery experiments showed no evidence for possible interspecies conversion. The details are given in Table 2. The work emanates from the same research group who reported the presence of Cr^{VI} in cow's milk based on a method also involving DSPME.[87] Manganese was one of the elements included in a study of the identification and characterisation of labile metal pools in cells, which are extremely important in understanding metal ion trafficking, signalling, and regulation; however, the analyses are challenging due to the inherent lability of these complexes. The development of a *SEC-ICP-MS procedure for the study of low-molecular-mass labile metal (Cu, Fe, Mn, Ni and Zn) pools in E. coli* represents significant progress.[111] The researchers explain that metal chelators and buffers that coordinate metals should be excluded and that the interaction of metals with size-exclusion columns, which contain carboxylate groups that bind metals must be minimised. This is typically done by increasing the ionic strength of the mobile phase, but doing so here would have been problematic for ICP-MS (and ES-MS, which was also used in the study). They developed a new strategy to overcome these secondary interactions that involved saturating the column binding sites with ^{67}Zn prior to the analyses. Metal species were separated on either a single SEC column or on two such columns linked in series. The mobile phase was either 20 mmol L^{-1} or 50 mmol L^{-1} ammonium acetate at pH 6.5 at flow rates of 0.6 mL min^{-1} for the single column and 0.25 mL min^{-1} for the double column. Following DNA hydrolysis, the cells lysate was centrifuged at 100 000 g for 60 min, transferred to a chilled anaerobic glove box (MBraun Labmaster 120, 1–10 ppm O_2, 4–8 °C) and filtered (Ultracel regenerated cellulose 3 kDa ultrafiltration disc). The researchers found 2–5 Fe, at least 2 Ni, 2–5 Zn, 2–4 Cu, and at least 2 Mn species with apparent masses between 300 and 5000 Da. They concluded that a major problem in identifying labile metal compounds in aqueous cellular solutions by ES-MS was suppression by the high concentrations of salts. They identified the major low-molecular-mass S- and P-containing species to be reduced and oxidised glutathione, methionine, cysteine, orthophosphate, and common mono- and di-nucleotides such as ATP, ADP, AMP, and NADH, and found that *E. coli* grown in media supplemented with one of these metal salts, the size of the labile metal pools was sensitive to the concentration of nutrient metals.

3.11 Mercury

The *measurement of Hg and its species in natural waters* is challenging due to the very low concentrations usually present. Thus, a pre-concentration step is usually required and a fully validated procedure, to the requirements of ISO 17025 and

Eurachem guidelines, has been reported this year.[118] The Hg species present in seawater were twice extracted with dichloromethane (DCM), back extracted into a 0.01 mmol L^{-1} $Na_2S_2O_3$ and ethylated before quantification by GC-AFS. The entire measurement process was described by mathematical equations, allowing an uncertainty budget to be formulated, and all factors that could influence the results were systematically investigated. The working range of the method was 0.2 to 7 pg, with LOD and LOQ values of 0.05 and 0.17 pg respectively, which equated to 0.0004 ng kg^{-1} and 0.0014 ng kg^{-1}, respectively for a typical analytical run whilst repeatability was 3.3–4.5% and the intermediate precision was 2.9%. As there is no CRM certified for MeHg content available the method was validated by spike recovery experiments at two levels, 0.005 and 0.05 ng kg^{-1} and recoveries were 94 ± 4%. In addition, the MeHg content in BCR 579 coastal seawater CRM, which is certified for total Hg only, was measured and compared with previously published values and these were found to be in statistical agreement. The relative expanded uncertainties for the method ranged from 16 to 25% ($k = 2$). The analytical procedure was then used in an inter laboratory comparison exercise organised under the Geotraces programme and the results obtained from this work were in statistical agreement with the assigned values. The authors conclude that the analytical procedure is fit for purpose for routine monitoring studies on the dissolved MeHg content in the costal and open ocean seawaters. As with previous papers by these authors, the paper could be used as a guide for method development and validation to international standards. An field portable on-line FI-CVAFS system for measuring Hg^0, iHg and tHg in seawaters has been reported on this year.[119] The system comprises a dual-channel purge-and-trap system which combines reduction with $SnCl_2$, two-stage trapping onto Au followed by thermal desorption prior to detection of the released Hg^0. To measure Hg^0, samples were purged with N_2 gas prior to trapping whilst for iHg, the reduction step was used before purging and samples were treated with BrCl for tHg measurements. Measurement precision was stated to be <5% RSD ($n = 30$) and the LOD was ≤0.05 ng L^{-1}. The system was validated by analysis of BCR-579 coastal seawater and the recovery was 101 ± 4% ($n = 10$) and sample throughput of was 10 samples per hour. The system was then deployed on ship for the measurements of surface seawaters of the East China Sea which were in the ranges of 13 to 90 pg L^{-1} for Hg^0, 0.07 to 0.7 ng L^{-1} for iHg and 1.4 to 14.0 ng L^{-1} for tHg. A method for the pre-concentration of Hg species from waters by CPE followed by quantitation with HPLC-HG-AFS has been presented.[120] For the CPE procedure the water borne Hg species were chelated with sodium diethyldithiocarbamate, extracted into Triton X-114 micelles and then back extracted into L-cysteine under UAE. Aliquots of this final solution were then injected onto a C18 column. The paper is mainly concerned with the optimisation of the CPE procedure and the HPLC mobile phase is not explicitly stated but seems to be 0.1% w/v L-cysteine. No other chromatographic details are given but from a chromatogram given the retention time of iHg and MeHg were 3.4 and 4.5 minutes, respectively. After optimisation, the linear range was 0.10–5.0 μg L^{-1} and the LOD values for iHg and MeHg were 0.004 and 0.016 μg L^{-1}, respectively. The final paper covered this section on Hg speciation in waters reports on the use of 3D printing to fabricate a field portable CV and photo-chemical vapour generator (PVG) μ-point discharge (PD)-OES instrument.[121] The system was compact with a total dimension of 230 × 38 × 84 and a complete description on the design and fabrication of the system is given in the paper and ESI. For Hg speciation, iHg was selectively reduced to Hg^0 with 0.001% (m/v) KBH_4 and 5% (v/v) HCl whereas the reduction of both iHg and MeHg was obtained by PVG in presence of formic acid and using a nano-TiO_2 coated quartz coil. Hence, the MeHg content was determined by difference. The LOD value was 0.1 μg L^{-1} for all Hg measurements, and the linear range was 20 to 150 μg L^{-1}. Method validation was achieved through the analysis of three CRMs (DORM-4, DOLT-5 and GBW(E)080395), with Hg species extraction using 10% (v/v) HCl and UAE. The measured concentrations for each CRM were in statistical agreement (*t*-test) with the certified values. Spike recovery studies of different environmental water samples gave recoveries ranging from 93 to 103%. The system was also used for iSe speciation, and this is covered in Section 3.16.

High precision *isotope ratio measurements of Hg species* are used for both high accuracy quantification by IDA and for determining reaction pathways, such as the *in situ* methylation of iHg in sediments. Three different ICP-MS instrument types, with either quadrupole, sector-field or time-of-flight mass analysers, have been evaluated for the obtainable precision of Hg isotope ratio measurements.[122] The target analyte was MeHg, quantification was by species specific isotope dilution analysis (ssIDMS) and GC was used as the separation technique as this provides short transient signals to test the instrumental data acquisition modes. Sediment samples were freeze-dried and sieved to collect the <250 μm fraction before MeHg extraction by a previously reported procedure involving $CuSO_4$, HNO_3, DCM, $Na_2S_2O_3$, and propylation of the extracted Hg species before injection onto the GC column. Two spike solutions, ^{201}Hg-enriched Hg and MeHg were used and mass bias correction was performed using a Tl solution, which was aspirated into a dual entry spray chamber to which the heated transfer line from the GC was also connected. The results showed that the isotope ratio precision obtainable using GC-ICP-MS is similar, within a broad range of peak signal-to-noise ratios, when analysing one isotopic system with instruments equipped with either a Q, SF or ToF mass analyser. The authors suggest though that, due to the simultaneous detection capability of ICP-ToF-MS compared with the scanning required for the Q and SF mass analysers, that this might not be the case if several isotopic systems are analysed within one measurement. This, of course, could have been tested by monitoring more than two Hg isotopes during the analytical run. The procedural LOQ for MeHg was similar for all three instruments and ranged between 0.003 and 0.016 μg kg^{-1}. The GC-ICP-ToF-MS coupling was used to quantify MeHg in sediment extracts from the Finow Canal, Germany. Mass fractions between 0.180 and 41 μg kg^{-1}. Method validation was by the analysis of sediment CRMs ERM-CC020, ERM-CC580, IAEA-456 and the results obtained for each CRM

agreed with the certified values within uncertainty limits. The work also investigated the contributions that various measurement parameters contributed to the combined uncertainty of the determined MeHg mass fractions. At lower MeHg concentrations, the major uncertainty contributors were the measured isotope ratio and the spike mass fractions, with the contribution of the former reducing until at signal to noise ratios of >10 the latter became the major contributor. This was observed for all three instruments used in the study. The paper contains a good amount of detail on the measurements made and is worth reading for the discussions presented. A reduced precision of isotope ratio measurements is usually observed for speciation work due to the need to monitor transient signals rather than continuous aspiration of a sample solution. Therefore, workers in this field often seek to selectively extract individual Hg species or use some form of selective sample introduction system such as CV and one of these approaches has recently been reported on.[123] In this work, Hg species were extracted from various biological CRMs, BCR-414, DOLT-5, DORM-4, ERM-CE-464 and SRM 1946, and fish tissue samples by US EPA method 1630 and then subjected to a multi-step reduction–oxidation–reduction process. Initially, $SnCl_2$ was used to reduce iHg to Hg^0, which was removed from the system by purging with N_2, followed by oxidation of organo-Hg species to iHg with BrCl and finally a second reduction step with $SnCl_2$ but in this case the liberated Hg^0 was trapped in a 40% inverse *aqua regia* solution. For comparison purposes the samples were also extracted using the well-known toluene/H_2SO_4/NaBr/$CuSO_4$/$Na_2S_2O_3$ method. The Hg concentrations and isotope ratios in these solutions were measured using CVAFS and MC-ICP-MS, respectively. After optimisation of the procedure, which is discussed in detail, a precision (2SD) of 0.03 to 0.15‰ and the measured isotopic ratios for organo-Hg extracted from the CRMs were in agreement with those previously published in the literature whilst recoveries from the CRMs used were 101 ± 8%, ($n = 18$).

A number of papers published in the period covered by this ASU have *focused on the extraction of Hg species from different matrices*, with two of these reporting on the development of magnetic SPE. To this end an S containing metal organic polymer (MOP) (Fe_3O_4@BD-TpMA-S-SH) was synthesised as a magnetic solid-phase extraction (MSPE) sorbent for mercury speciation.[124] The paper covers the synthesis and characterisation of the MOP, optimisation of the SPE conditions and then application of the MOP for determining Hg species in lake water and locally purchased fish by HPLC-ICP-MS. The HPLC separation used a C18 column with a mobile phase of 8.0 mmol L^{-1} L-cysteine, 12.5 mmol L^{-1} $(NH_4)_2 \cdot HPO_4$, 0.05% triethylamine, pH 7.0, 8% MeOH flowing at 1.5 mL min^{-1}, which separated EtHg, iHg, MeHg and PhHg in 20 minutes. The MOP is 25 wt% S, providing a high number of adsorption sites for Hg, with capacities of 636, 656, 649 and 366 mg g^{-1} for iHg, MeHg, EtHg and PhHg, respectively. Under optimal conditions, LOD values of 0.43, 0.55, 0.69 and 1.1 ng L^{-1} for iHg, MeHg, EtHg and PhHg, respectively, were obtained, with enrichment factors in the range of 346–383. The method was validated using DORM-2 CRM and the found values for total Hg and MeHg were in statistical agreement (*t*-test) with the certified values and spike recovery experiments in lake water and fish tissue extracts (5 mol L^{-1} HCl, UAE 30 minutes) were also successful. Only iHg was detected in the lake water sample, 2.8 ng L^{-1}, whereas both iHg and MeHg were detected in the fish samples, all at mass fractions of less than 100 ng g^{-1}. The synthesis, characterisation and use of a different MOP, (Fe_3O_4@UiO-66-SH) prepared by coating Fe_3O_4 NPs with S functionalised UiO-66, has also been described.[125] Again, Hg species quantification was by HPLC-ICP-MS, using a C18 column and a mobile phase of 0.1% L-cysteine, 0.5% MeOH and 10 mmol L^{-1} ammonium acetate at pH 7 flowing at 1.0 mL min^{-1} for a six minute run time. After optimisation of the SPE conditions, which is fully described, the MOP allowed LOD values of 1.4 and 2.6 ng L^{-1} for iHg and MeHg, respectively for water samples and the enrichment factors were 45.7 and 47.6 for iHg and MeHg, respectively. A fish tissue CRM, GBW10029, was used for method validation, with Hg species extraction by 5 mol L^{-1} HCl and UAE for 10 minutes, and the determined values were in statistical agreement with the certified values (*t*-test) and spike recoveries from water samples were in the range of 91–95% with an RSD range of 4.3–5.7%. The method was applied to 2 water samples, one environmental and one tap, and no Hg species were detectable, whilst for a Sea Perch sample, only iHg was detected, 8.41 μg g^{-1}. The preparation and characterisation of an ion imprinted polymer (IIP) has been reported on, with the IIP then being applied for the pre-concentration of Hg species from seaweed extracts by HPLC-ICP-MS.[126] The HPLC conditions were a C18 column and a mobile phase of 0.4% mercaptoethanol and 10% methanol at pH 2.0 flowing at 0.3 mL min^{-1} for 8.5 minutes. The IIP was synthesised by a precipitation polymerisation method, using a ternary pre-polymerisation mixture containing the template (MeHg), a non-vinylated monomer (phenobarbital), and a vinylated monomer (methacrylic acid). Mercury species were extracted from seaweed samples 0.1% (v/v) HCl, 0.12% (w/v) L-cysteine, 0.1% (v/v) 2-mercaptoethanol (2-ME) and UAE at 45 kHz for 30 minutes. Under optimised conditions, the LOD values obtained were 0.007 and 0.02 μg kg^{-1} for MeHg and iHg, respectively, with a pre-concentration factor of 50. The proposed methodology was finally applied for the selective preconcentration and determination of MeHg and Hg(II) in a BCR-463 certified reference material, tuna fish, as there is no seaweed CRM available for Hg species, and in several edible seaweeds. The found and certified MeHg mass fractions for BCR-463 are 2.86 ± 0.05 mg kg^{-1} and 3.01 ± 0.06 mg kg^{-1} whilst spike recoveries from seaweed extracts ranged between 89 to 112% for MeHg and 86 to 108% for iHg. The actual iHg and MeHg in the seaweed samples ranged from 0.06 to 0.1 and 0.01 to 0.06 mg kg^{-1}.

Two different approaches have been investigated for the quantification of Hg species in blood and hair.[127] The first used HPLC-HG-ICP-MS to quantify EtHg, iHg, MeHg, and PhHg, with these species being separated on a C18 column with a two-component mobile phase, (A) 0.5 g L^{-1} of L-cysteine hydrochloride monohydrate and 0.5 g L^{-1} L-cysteine at pH 2.3 and (B) MeOH, and a gradient elution over 14 minutes. The use of post-column HG provided a 30 to 40 fold increase in sensitivity

compared to a system with no HG involved and LOD values between 3 and 6 ng L^{-1}. The Hg species were extracted from blood using a combination of mobile phase A, HNO_3, heating at 85 °C for two minutes followed by the addition of more of the mobile phase and UAE at 40 °C for five minutes and finally, filtration. Recoveries of better than 90% (iHg and MeHg) and 80% for EtHg, respectively, were obtained from the Seronorm Trace Elements Whole Blood L-1 and L-2 RM. The second approach was a selective extraction of MeHg from hair samples with 2 mol L^{-1} HCl, with the selectivity being verified by analysis using HPLC-ICP-MS. For extracts of IAEA-086 human hair CRM tHg and MeHg recovery was 100 ± 10%. An automated ethylation-purge and trap-GC-pyrolysis-ICP-MS procedure has been described and used to monitor bacterial Hg methylation under anaerobic conditions.[128] Sample extracts were added to an acetate buffer in a sample vial along with aliquots of a $NaBEt_4$ solution and the ethylated species purged from the sample vials with N_2, supplied *via* an autosampler needle, onto a Tenax trap followed by thermal desorption with a stream of Ar gas to the GC-pyrolysis ICP-MS combination. Sample throughput was eight per hour and the LOD for MeHg was 0.03 ng L^{-1} with a typical RSD of 4%. Method validation was by the analysis of DORM-4, a fish protein CRM, by IDA with a $Me^{201}Hg$ spike, and the found results were in statistical agreement ($p = 0.05$) with the certified value. Spike recovery of MeHg into seawater was poor, 32%, when external calibration was used, which the authors attributed to a Cl^- interference on the ethylation step, but was 106% when IDA was used for quantification, demonstrating the ability of this technique to overcome some types of interference. For the experiments on Hg methylation, involving *Geobacter sulfurreducens* and double-enriched isotope tracing $^{199}Hg^{2+}$ and $Me^{201}Hg$, it was found that the amount of $Me^{201}Hg$ increased with time whilst that of $Me^{201}Hg$ reduced with time. Table 3 shows examples of other applications of Hg speciation presented in the literature during the time period covered by this ASU.

3.12 Nickel

The role of metalloproteins in metal detoxification in environmental exposed *Perna perna* myfilid mussels have been explored.[69] The following metal/metalloids were evaluated: Se, Cd, Cu, As, Zn Pb and Ni. Mussels were collected from two tropical bays in Southeastern Brazil, dissected and the gill samples of 10 individuals pooled and frozen at −20 °C. Gill metallothioneins (MT), reduced glutathione (GSH), carboxylesterase protein (CarbE) and lipid peroxidation were determined by UV-vis measurements. For metallothionein determination, samples were extracted using tris-2 carboxyethylporfine (TCEP) under heating at 70 °C for 10 minutes. After centrifugation, the supernatants containing the MT were analysed by SEC-HPLC-ICP-MS by using a SEC column with Tris–HCl 0.02 mol L^{-1} (pH = 7.0) as the mobile phase flowing at 0.7 mL min^{-1}. The data obtained showed a differential binding profile and thus detoxification mechanisms depending on the metal/metalloid. Three elements, As, Cu and Ni, were found to be mostly bound to LMM binding proteins (<0.3 kDa) and GSH, whereas Cd, Se, Pb and Zn were only bound to LMM metalloproteins. Data analysis by PCA revealed strong association between metal and antioxidant parameters. As, Cd, Ni and Se directly affected CarbE activity and MT seems to play a dual role: metal detoxification and ROS scavenging.

3.13 Phosphorus

Recent work involving the certification of phosphate in three seawater CRMs (National Metrology Institute of Japan (NMIJ) CRM 7601-a, 7602-a, and 7603-a) has used IC coupled to SF-ICP-MS.[26] The use of AE separation allowed for the reduction of the severe matrix effects due to the high concentration of Cl present in seawater, such that phosphate could be completely separated from Cl in the chromatograms for a 10% and 1% dilution of seawater. This separation was achieved using an IonPac AS23 AE analytical column (4 × 250 mm, Dionex) with an IonPac AG23 guard column (4 × 5 mm, Dionex). The column oven temperature used was 30 °C and the flow rate of the eluent, which was a mixture of sodium carbonate (49 mmol L^{-1}) and sodium hydrogen carbonate (0.5 mmol L^{-1}), was 1.5 mL min^{-1}. The eluent from the IC column was introduced into the nebuliser by controlling a four-way, six-port Teflon rotary switching valve. In this approach, the IC eluent, including chloride, was first pumped to waste and the switching valve was then turned to the ICP introduction system just prior to the appearance of the phosphate peak. This helped reduce the impact of the high salt matrix on the stability of the ICP-MS signal. To obtain a higher sensitivity for ^{31}P, which, due to its relatively high 1st ionisation potential has a poor ionisation efficiency (<30%) in the plasma, a jet-sampler cone and an X-skimmer cone were loaded into the interface of the SF-ICP-MS instrument, with a booster pump exhausting the interface. This resulted in a factor of 18 increase in the sensitivity compared to conventional cones. Due to P being a mono-isotopic element, it was not possible to use IDMS, so standard addition was the calibration approach used. By using As as an internal standard (as the oxide anion As^{5+}), time-dependent fluctuation of the ^{31}P sensitivity were corrected and a better calibration curve was obtained. Although P in NMIJ CRM 7601-a could not be determined because its concentration after dilution for the IC separation was <LOD (0.005 μmol kg^{-1}), the analytical results for NMIJ CRM 7602-a and 7603-a were in good agreement with the values obtained *via* continuous flow analysis, considering their expanded uncertainties with the coverage factor of 2. The developed method was validated by analysing an existing certified reference material (MOOS-3). This method enabled the measurement of sub-μmol kg^{-1} levels of phosphate with high accuracy and sensitivity.

3.14 Platinum

The *use of different MS approaches to the characterisation of the binding of Pt-based anti-cancer drugs with proteins in blood* has been reviewed.[140] It covers analytical developments in this area over the last 30 years (since 1990) and includes 89 references in total. It covers methods to investigate protein binding to cisplatin but also its analogues, including carboplatin,

Table 3 Applications of speciation analysis: Hg

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
As^{III}, As^{V}, MMA, DMA, AsB, iHg, MeHg	HPLC-ICP-MS	Fish	1% (v/v) HCl and 0.1% (m/v) protease XIV, MAE, 70 °C, 60 minutes	RP C-18, gradient (a) 5 mmol L^{-1} 1-octanesulfonate, 5 mmol L^{-1} acetate buffer, 1% (v/v) IPA (pH 4.0), (b) 2 mmoL L^{-1} L-cysteine in 1% (v/v) IPA (pH 4)	As species; 0.005–0.007 ng mL^{-1} Hg species 0.013–0.015 ng mL^{-1}	Spike recovery, 97–103%	71
MeHg, tHg	CV-AAS, GC-AFS	Dolphin blubber and skin	tHg: 1 : 1 H_2SO_4 : HNO_3, 60 °C, 1 hour MeHg: EPA method 1630	Not given	tHg: 0.040 μg L^{-1} MeHg: 0.011 ng L^{-1}	BCR463, recovery 96 ± 6%	129
iHg, EtHg, MeHg, PhHg	HPLC-AFS	Waters	SPE, thiolated resin. Elution with 4 mL of 7 mol L^{-1} HCl	RP C-18, gradient, (A) MeOH, (B) 60 mmol L^{-1} $NH_4CH_3CO_2$, 10 mmol L^{-1} L-cysteine	Between 0.05–0.1 μg L^{-1}	Spike recovery, 87–111% Tort-2, 86–90% recovery	130
iHg, MeHg	GC-ID-ICP-MS	Plankton	tHg, HNO_3 Hg species, 50% v/v MeOH, MAE, ethylation	Not given, cited reference	iHg: 047 pmol L^{-1} MeHg; 0.007 pmol L^{-1}	None used	131
iHg, MeHg	LC-CV-AFS LC-ICP-MS	Blood	0.2 mL sample, 0.25 mL 7% (v/v) HCl, 1.5% (w/v) L-cysteine, vortex mixing, UAE	RP-C18, 5% (v/v) MeOH, 0.1% (v/v) 2-ME, 0.018% (v/v) HCl	Not given	Seronorm whole blood level 2. Quebec blood (PC-B-M 1201, 1203 and 1601). All found values within CRM ranges	132
iHg MeHg	LC-ICP-MS	Sediment	10% 2-ME and 7% HCl, vortex mixing, UAE ± 60 °C, 7 minutes	RP-C18, 0.4% (w/v) L-cysteine, 0.06 mol L^{-1} $NH_4CH_3CO_2$, 0.05% (v/v) 2-ME, 5% (v/v) MeOH (pH 5.5)	iHg, 0.090 ng g^{-1} MeHg, 0.076 ng g^{-1}	SRM 2709a. Total Hg (sum of species) 86% recovery	133
MeHg	LC-ICP-MS HR-XANES	Bird, earthworm, fish	Bird: MeHg, 4.5 mol L^{-1} HNO_3, 55 °C, 8 h tHg, above then BrCl XANES: pressed pellets from freeze dried samples	Not given, cited references	tHg 0.003 ng. HPLC-ICP-MS not given	IAEA-436, recovery 98%	134
Hg binding proteins	SEC-ICP-MS	Tuna, salmon	99% RIPA buffer, 1% inhibitor °C, 60 min, centrifuged Protein layer, 0.1 mmol L^{-1} TCEP, 1% cocktail, 5% SDS, and 4.2 mmol L^{-1} HEPES buffer	50 mmol L^{-1} Tris–HCl and 50 mmol L^{-1} NH_4NO_3, pH 7.5 Full method given in cited reference	Not given	Not used	135
iHg, EtHg, MeHg	LC-ICP-MS	Mustard, pak-choi, soil	tHG: 3 : 1 HNO_3 : HCL, MAE iHg, MeHg. Soils. 0.5 mol L^{-1} HNO_3, UAE, 60 min 25 °C Plants.1% thiourea, 2% HCl, 1% KCl, 90 min, 25 °C	RP C-18, 60 mmol L^{-1} $NH_4CH_3CO_2$, 5% MeOH, 0.3% (v/v), 2-ME	tHg, 0.017 μg L^{-1}	GBW07401. tHg 103% recovery Spiking, 76 tom 92% recoveries	136

Table 3 (*Contd.*)

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
MeHg, tHg	GC-CV-AFS	Sediment	tHg, HF/HNO_3/HCl digestion MeHg, H_2SO_4/KBr/$CuSO_4$–CH_2Cl_2 extraction, propylation	Full methods given in cited references	tHg, 0.05 ng	BCR-580, IAEA 433 MeHg recovery 100% for each CRM	137
iHg, MeHg, PhHg	ETAAS	Waters	Hg species extracted using a Zr-based metal–organic framework then selectively eluted	None. Selective elution	0.006 μg L^{-1} for a 10 mL sample volume	Spiking, recoveries ranged from 90 to 100%	138
Various Hg species	XAFS, XANES	Sediment	Pressed pellets	None required	N/A	N/A	139

oxaliplatin, nedaplatin, lobaplatin, and heptaplatin, which have all been used clinically at some point. After intravenous administration of these drugs, a substantial amount of Pt based drugs will bind with proteins in the blood, which facilitates transport, distribution, and metabolism within the body. However, toxicity can also occur from the irreversible binding between biologically active proteins and these Pt-containing drugs. The review charts the development of the MS based methods, from using SEC, CE and then 2-dimensional LC, coupled to ICP-MS for the identification and quantification of Pt-species binding to proteins *via* comparison with standard proteins. The review then details more recent methods based on the combination of multi-dimensional LC with ES-MS/MS. Initial work in this area focused on the high-abundance proteins such as HSA, but more recently attempts have been made, unsuccessfully, to identify low abundance proteins that bind these drugs. This has used proteomic approaches whereby tryptic digestion generates Pt-drug bound peptides, which are characterised using LC-MS/MS and database searches. Often in these studies the identification of the Pt-containing peptide was not based on the search engine data, but after manual identification of the fragments based on the characteristic isotopic pattern present due to Pt. The authors make the reasonable point that methods based on MS have superior selectivity and sensitivity; when combined with multidimensional separation methods and efficient software, they can play an even greater role in identifying the protein targets of metal-based drugs both *in vitro* and *in vivo*. Additionally, this will be helpful to understand the pharmacokinetics and toxicity of metal-based drugs and optimise their structures.

A method for the *measurement of potassium hexachloroplatinate, a low concentration impurity in the pharmaceutical preparation of carboplatin, using HPLC-ICP-MS* has been presented.[141] A 4-way valve switching system was integrated with HPLC-ICP-MS, based on which a portion of the injected sample could be discarded by switching the valve, thus eliminating the influence of the high content of Pt from carboplatin on the quantification of the impurity. In this way, the high concentration of carboplatin would not contaminate the ICP-MS instrument, which could lead to sample carry over, a high background and less than ideal analytical performance. However, the system described in the paper would still lead to high levels of carboplatin entering the HPLC column used, which could lead to other unwanted chromatographic effects (although the results did not imply that this was the case). Carboplatin and potassium hexachloroplatinate standards were used to determine the retention time using a PRP-X100 AE column and isocratic elution with an eluent containing dihydrogen phosphate (50 mmol L^{-1}) at pH 5.5. This is an unusual choice of buffer, as non-volatile compounds such as phosphate salts are usually replaced with reagents containing ammonium salts, as these are more volatile and so lead to less problems with blocking of the sample cone often experienced with prolonged use. The results showed that the linear range of potassium hexachloroplatinate was 1.0 to 100 μg L^{-1}, the LOD ($3 \times S/N$) was 0.1 μg L^{-1}, the spike recovery rate was between 90 to 99%, and the short term precision ($n = 6$) was 2.7 to 3.4% RSD.

The *growing importance of nanoparticles (NP) in Pt-pharmacology, either as Pt-NP or Pt-drug delivery systems, has created a need for analytical platforms that can provide information on their efficacy, toxicity and mode of action*. A fundamental study on the effect of various cell-culture media used in toxicological studies on Pt-NP, has used HPLC coupled to triple quadrupole ICP-MS to characterise 5 and 30 nm sized particles, as well as ionic Pt.[142] The chromatographic and acquisition conditions were optimised to achieve the separation of these nano-sized particles and their corresponding ion in less than 8 min with LOD at the μg L^{-1} level and adequate recoveries. Separation was performed using a RP C_{18} column (Nucleosil 7 μm particle size, 1000 Å pore size, 250 × 4.6 ID mm). The mobile phase consisted of PBS (2 mmol L^{-1}, pH 7.3) with the addition of SDS (10 mmol L^{-1}) at a flow rate of 0.5 mL min^{-1}. The separation mechanism was based on SEC, so retention was related to hydrodynamic volume, where the larger entities elute first. However, without the addition of SDS, there was a strong and irreversible interaction of the Pt-NPs with the stationary phase, but the level of SDS required had to be kept low to reduce deposition on the sample cone. Without the cell culture media, the LODs were 1.3, 2.4, and 0.3 μg L^{-1}, whereas LOQ were 4.5, 7.9, and 0.9 μg L^{-1}, for 5 and 30 nm Pt-NPs and ionic Pt, respectively. However, the chromatographic resolution between the peaks for each Pt-containing entity was poor and it is clear from the chromatograms in the report that the LC approach chosen resulted in significant tailing of the peaks. The fundamental use of Fe-NPs (<10 nm diameter) as a nanocarrier drug delivery system for *cis*-diamminetetrachloroplatinum(IV) (a cisplatin prodrug) has been investigated.[143] These Fe oxide nanostructures show magnetic and biocompatible properties and fulfill the requirements to be classified as ''smart'' multifunctional "nano-platforms" with properties including: prolonged circulation in the blood; effective intracellular incorporation; and responsiveness to local physiological stimuli, such as changes in pH, which may result in an improved and localised drug release mechanism. Improvements in drug delivery may be a way to reduce the cytotoxic effect of these drugs experienced by patients undergoing chemotherapy. The formulation of these NPs and their interaction with cellular DNA were determined using HPLC coupled to triple quadrupole ICP-MS. The chromatographic separation of the particles was conducted using a Nucleosil C_{18} column (7 μm particle size, 250 × 4.6 mm i.d., pore size 1000 Å), and a mobile phase of SDS (10 mmol L^{-1}) in water at 0.5 mL min^{-1}. For the separation of the cisplatin–oligonucleotide complex, a Superdex Peptide 10/300 GL (30 cm × 10 mm i.d.) SEC column with a fractionation range from 100 to 7000 Da and a mobile phase of ammonium acetate (10 mmol L^{-1}) was used at a flow rate of 0.7 mL min^{-1}. The columns were interfaced to the ICP-MS using a 15 cm long polyether ketone (PEEK) tube, which was connected to the polytetrafluoroethylene (PTFE) sample tube of the nebuliser. Furthermore, the cellular uptake of the synthetic nanoconjugate was explored by single cell-ICP-MS which was used in sensitive and resistant ovarian cancer cell models. This revealed intracellular Pt concentrations of 12 fg/cell and 4 fg/cell, respectively, which were 4-fold higher with respect to the control system, which was based on the uptake of cisplatin alone in both models.

3.15 Rhenium

An analytical procedure based on the use of CE-ICP-MS was developed for Re speciation.[144] The first part of the paper was devoted to selection of the appropriate conditions for CE separation (nature and concentration of buffer solution, separation voltage, sheath fluid) and its coupling to the ICP-MS *via* an interface consisted of a sprayer with a direct injection nebuliser. The electrophoresis experiments were carried out in a fused-silica capillary column with 10 mmol L^{-1} K_2CO_3 (pH = 11) as mobile phase along with a separation voltage of 27 kV. Special attention was paid to the composition of the sheath fluid, which was employed to match the low CE flow rate with the uptake rate of ICP-MS. Subsequently, 2% KNO_3 was introduced as the sheath fluid flowing at 1.5 mL min^{-1}. Under the optimised conditions, LOD values of 0.02 μg L^{-1} and 0.01 μg L^{-1} were obtained for total Re and Re^{IV}, respectively, with an RSD better than 30%. The method was employed to determine total Re, Re^{IV} and Re^{VII} in spiked ground water samples and spike recoveries ranged from 96 to 100%. Although Re^{IV} was completely separated and detected by CE-ICP-MS, its accurate quantification was obtained by subtracting the concentration of Re^{VII} from total Re as a standard solution of Re^{IV} was not available.

3.16 Selenium

The *characterisation of Se NPs in environmental and biological samples* is still a topic of interest. A method based on the use of CE-ICP-MS/MS for detecting SeNPs in aqueous solution and human plasma has been reported.[145] In-house prepared polyvinyl alcohol (PVA) coated SeNPs, synthesised by the reduction of SeO_2 with ascorbic acid, and commercially available SeNPs were used as model nanoparticles in this work. The technical challenge of coupling CE to ICP-MS was solved by the design of an interface consisting of an ES connected to the ICP-MS by an in-house fabricated cap-end. A sheath liquid flow set up 1.4 μL min^{-1} was also employed to produce sufficient spray in the sample introduction system. Fused capillaries and 30 mmol L^{-1} tetraborate buffer (pH 9.2) were used for separation. The separation efficiency of the system was evaluated using three LMM Se compounds: the positively charged TMSe, the neutral selenosugar SeGa and the negatively charged SeO_3^{2-} and baseline separation of these three compounds was achieved within 7 minutes by using a voltage of 20 kV and a pressure of 50 mbar. Peak area and migration time precision (RSD) were 1.4–3% and 1.0–2.6%, respectively. Different migration times were obtained when analysing aqueous diluted suspension of PVA-SeNPs (3.7 min) and the commercial SeNPs (5.0 min) demonstrating the influence of the stabilising agent employed in the properties of nanoparticles. Subsequently, the method was applied to determine the chemical fate of SeNPs in plasma but with modifications. In brief, PVA-SeNPs were incubated in 50% plasma and the samples were measured at 5 h, 48 h and 5 days. Due to the high protein content of the plasma, a coated fused silica

capillary column and an applied pressure of 10 mbar was used. After 48 h a degradation product was identified as SeO_3^{2-}. The SeO_3^{2-} and SeNPs were subsequently quantify by applying a correction factor for the ICP-MS signal of PVA-SeNPs and dissolved Se due to the different ICP-MS response obtained for both Se analytes. In an outstanding study, metallomic, metabolomics and metagenomics tools were combined to establish the metabolisms of Se^{IV} and SeNPs in rats through the evaluation of faeces samples.[146] Rats were orally exposed to SeNPs (39 ± 12 nm) and Se^{IV} at two levels of concentration, 3.14 and 6.28 mg kg^{-1}. After 24 hours, rats were euthanised and the brain, kidneys, liver, stomach, small and large intestine, gastrointestinal content, and fresh faecal samples were collected. The impact of Se on intestinal microbiota and metabolites in faecal samples was evaluated by 16 rRNA and HILIC-UHPLC-QTOF-MS analyses and ICP-MS, SR-XRF and XANES measurements used to determine the concentration and transformation of Se in gastrointestinal tract and faeces. The SeNPs showed a less toxic effect on intestinal microorganisms than SeO_3^{2-} and facilitated the growth of beneficial intestinal microbes. The Se^{IV} also seemed to affect metabolites related to the immune system and neurodegenerative processes, while SeNPs induce changes in nucleotide metabolisms and cellular processes. Data from SR-XRF and XANES evidenced the presence of Se in the duodenum wall with Se–C and Se^{VI} as the main species found in the SeNP-treated rats and Se^{IV}-treated rats, respectively. All these findings suggest a low toxicity of SeNPs against gut microbiota. The proposed protocol constitutes a powerful tool and opens new insights into the safety evaluation of nanomaterials. A paper reports on the accumulation and transformation of HgSeNPs (tiemanite) in Se-deficient rats.[147] Rats were orally administered with selenite and/or MeHg or HgSeNPs. After feeding, rats were sacrificed and the following organs were collected: brain, liver, kidney, spleen, testis, faeces, large intestines, and serum. The organs were subsequently homogenised with 50 mmol L^{-1} Tris–HCl (pH 4.0), treated with HNO_3 or HNO_3 : H_2SO_4 and CVAAS and ICP-MS/MS were used for total Se and Hg determination, respectively. Additionally, two serum selenoproteins, GPX3 and SeLP, were analysed for by LC-ICP-MS. For this purpose, a SEC (Shodex GC-520HQ, 300 kDa, 7.5 i.d. × 300 mm) column was employed. The mobile phase was 50 mmol L^{-1} Tris–HCl (pH 7.4) at a flow rate of 0.6 mL min^{-1}. The data revealed that Se^{IV} either administered alone or co-administered with MeHg was mainly employed in the biosynthesis of serum selenoproteins, whereas HgSeNPs appeared to be biologically inert as selenoproteins were not produced. The administration of HgSeNPs did not increase Hg and Se in organs, except in the large intestine. Most HgSeNPs were recovered in faeces, indicating their low bioaccessibility and biological inertness. A separate paper describes the quantification of Se^0 in commercial selenised yeast by HPLC-ICP-MS after its transformation into $SeSO_3^{2-}$.[148] A detailed discussion of about the efficiency of this transformation was presented. The Se transformation was achieved by adding an excess of sodium sulfite at high temperature. About 200 mg of yeast were extracted with 10 mL of sodium sulfite solution in 10 mmol L^{-1} ammonium citrate (pH 7) at 90 °C for 1 hour. The resulting supernatants were subsequently measured for their total Se content (ICP-MS) and Se species (PRPX-100 column coupled to ICP-MS). The efficiency of conversion was determined to be 104 ± 6% and only one Se-containing peak corresponding to selenosulfite was detected. The presence of sulfite did not influence the retention time of other selenocompounds usually present in Se-enriched yeast such as Se^{IV}, SeMet and Se^{VI}, the presence of which did not interfere either with Se^0 detection. The LOD for Se^0 was evaluated as 0.3 ng kg^{-1}. The precision of the method was calculated in terms of reproducibility and repeatability with RSD values below 8%. The proposed method enabled the quantification of Se^0 in different selenised yeast batches representing on average 10–15% of total Se, which was in the range of 2–3 g Se per kg. The presence of Se^0 in these samples was confirmed by sp-ICP-MS with NP sizes between 80–150 nm detected. Unfortunately, the high dissolved Se content prevented the detection of NPs with sizes lower that 60 nm and consequently, quantification of Se^0 by this technique.

Studies of the determination of *Se speciation in food, feed and Se-enriched supplements have been reported this year*. One interesting approach is the combination of magnetic dispersive microsolid phase extraction (MDMSPE), using $ZnFe_2OCu$ nanotubes, with dispersive liquid–liquid microextraction followed by GFAAS measurements.[149] The method was employed to quantify Se^{IV} and Se^{VI} in different types of milk. Liquid samples were heated at 37 °C with artificial gastric juice composed of NaCl, pepsin and HCl at pH 2. The resulting extracts were submitted to MDMSPE where $ZnFe_2OCu$ nanotubes were employed to selectively retained Se^{IV} while Se^{VI} remained in solution. The sorbent containing the retained Se^{VI} was isolated from the aqueous solution by using an external magnetic field and treated with 0.5 mL of 0.25 mol L^{-1} NaOH to desorb Se^{VI}. In the next step, the upper aqueous phase from the MDMSPE was subjected to the preconcentration of Se^{IV} by DLLME by employing 100 μL of APDC as chelating agent, 0.3 μL of chloroform as dispersed agent and 50 μL as extraction solvent. After centrifugation of the mixture, the small droplet of $CHCl_3$ containing Se^{IV} was sedimented and introduced into the GFAAS for the detection of Se^{IV}. The main factors affecting the determination of the analytes were evaluated in detail. This analytical approach offers LOD values of 1.0 and 1.3 pg mL^{-1} and RSDs of 4.6 and 5.15% for Se^{IV} and Se^{VI}, respectively and an enrichment factor of 200 was obtained. The method was successfully applied to cow milk, soy milk, milk tea and yoghurt. Recovery values of between 91 and 107% were obtained for the different samples. The authors compared the developed method with other non-chromatographic processes compiled in the literature and highlighted the advantages of the current method in terms of the low detection limits and simplicity as pre-oxidation and pre-reduction steps are not required. Atasoy *et al.*[150] developed a Au-coated W-coil trap to increase the sensitivity of conventional HGAAS. The method was used to determine iSe in a great variety of food samples including chicken meat, eggs and fish. In brief, Se hydride formed by using 1% (m/v) $NaBH_4$ was collected at 172 °C on the trap which was placed in the middle of the vertical arm of the quartz tube of the HG system. Afterwards, the trapped hydride was released by increasing the

temperature up to 680 °C and introduced to the atomisation system *via* an Ar gas stream and H_2 was employed to protect the trap from oxidation. The W coil was obtained from a commercial lamp and coated with Au using an electrodeposition procedure. Several experimental conditions were optimised (collection and releasing temperatures, flow rates of Ar and H_2, collection time and $NaBH_4$ concentration). The durability of the trap was assessed by SEM measurements and set at 300 times. Accuracy was assessed by analysis of DOLT-5 CRM and there was good agreement between the certified content (83 ± 18 mg Se per kg) and the found value (78.7 ± 0.08 mg Se per kg). The method was then applied to determine total Se and Se^{IV} in eggs and chicken of different origins and fish tissues (skin, meat and liver). The method offers a great potential for those laboratories equipped with only a flame AAS as it provides an LOD as low as 0.021 μg Se per L.

One paper reports the distribution of Se and Hg biomolecules and Se speciation in the protein fractions of fish muscle of highly consumed fish: tuna, swordfish, farmed salmon and wild salmon.[151] Three types of proteins were sequentially extracted and separated based on their different solubility in buffer solutions: (1) sarcoplasmic proteins (0.05 mol L^{-1} phosphate buffer, pH 7.5), (2) myofibrillar proteins (0.05 mol L^{-1} phosphate buffer and 0.45 mol L^{-1} KCl) and (3) alkali soluble proteins (0.1 mol L^{-1} NaOH). Each of the protein extracts were analysed for total Se (ICP-MS) and Hg (direct Hg analyser) and for Se and Hg speciation with HPLC-ICP-MS and HPLC-ES-MS/MS. The Se and Hg concentrations ranged from 0.68 to 2.2 mg kg^{-1} and 0.011 and 1.45 mg kg^{-1}, respectively, with tuna and swordfish as the fish species with the highest content of both elements. Moreover, ANOVA analysis evidenced statistically significant differences between Se and Hg values for farmed and wild salmon, suggesting the effect of the type of feed and growing conditions on Se and Hg content and their distribution through protein fraction. Analysis using SEC-ICP-MS, with a Superdex 200 (100–600 kDa) column, revealed that Hg and Se were mostly associated with proteins of 2–12 kDa and up to 574 kDa, respectively, and that Se and Hg appeared bound to proteins of the same MW. Only MeSeCys was found in all the protein fractions when speciation analysis was performed by HPLC-ICP-MS and HPLC-ES-MS/MS by means of using a RP Kinetic EVO C18 (150 × 3.0 mm, 5 μm) column.

Enzymatic probe sonication followed by HPLC-ICP-MS has been applied for the *simultaneous determination of Se species in animal feed* at natural concentration levels.[152] Different separation mechanisms were tested: Zorbax Eclipse XDB C18 (150 mm × 4.6 mm, 5 μm), Atlantis Silica HILIC (50 mm × 4.6 × 5 μm), Hamilton PRP-X100 (250 mm × 4.1 mm, 10 μm) and Ion Pac AS 7 (50 mm × 4 mm, 5 μm). The best results were attained by employing an Ion Pac AS7 column and a mobile phase composed of increasing concentrations of $(NH_4)_2CO_3$ under gradient elution at a flow rate of 1 mL min^{-1}. Under these conditions six Se species, Se^{IV}, $Se^{V}I$, $SeCys_2$, MeSeCys, SeMet and SeEt, were completely separated within 15 minutes. Critical parameters affecting enzymatic probe sonication were investigated (enzyme type, extraction time, temperature, ultrasonic power and sample/enzyme ratio). The results were compared with hot water extraction (90 °C), enzymatic hydrolysis (10 mg of protease XIV and 10 mg trypsin at 37 °C for 24 h) and sequential extraction (hot water followed by enzymatic hydrolysis of the solid residue). Three kinds of protease were tested: protease XIV, subtilisin A and trypsin. Optimal Se recoveries (>90%) were obtained when using protease XIV. Recovery of Se species was highly dependent on the ultrasonic power applied and 20 W for 60 seconds was selected as this provided quantitative extraction of the different species without affecting their integrity. The LOD and LOQ values for Se species were in the ranges of 0.21–0.56 μg kg^{-1} and 0.69 and 1.87 μg kg^{-1}, respectively. The proposed method was validated using the CRM materials GBW 10010, GBW10011 and GBW 10012 certified for total Se and SELM-1 certified for total Se and SeMet. The speciation analysis of feed samples collected from markets and local farms detected Se^{IV}, SeMet and $SeCys_2$ as the major Se species.

Two papers cover the *simultaneous determination of metal/metalloids, including Se*, in foodstuffs by employing HPLC-ICP-MS. One describes the determination of As and Se in onions and seafood samples.[83] A complete and detailed description on the conditions affecting separation is given after eight chromatographic columns and various mobile phases were critically evaluated. The separation of both inorganic and organic forms of As and Se was completed using two analytical separation modes: AEC and double bed CEC with a mobile phase of NH_4NO_3 at pH 9.0 used as mobile phase under gradient elution flowing at 1.0 mL min^{-1} and 0.1% (v/v) MeOH was added to enhance the signal response of As. The procedure allowed authors to separate As and Se species (As^{III}, As^{V}, DMA, MMA, AsB, Se^{IV}, Se^{VI}, SeMet and $SeCys_2$) within 10 minutes with a suitable resolution. The LOD values ranged from 2.3 μg g^{-1} (for MMA) to 23.07 μg g^{-1} (for $SeCys_2$). The applicability of the method was verified by analysing As and Se species in water extracts of a great variety of onions and seafood samples (squid and shrimps). In onions, As^{III} was found to be the dominant arsenic species, at lower than 0.2 mg As per kg, whereas AsB was the predominant species in seafood samples. Regarding Se, SeMet was the major species in both onions and seafood samples. In the second paper, an anion exchange chromatographic column (Dionex Ion Pac As 14) with $(NH_4)_2CO_3$ (pH 10) as the mobile phase under gradient elution enabled the separation of 11 compounds: AsB, As^{III}, As^{V}, DMA, MMA, Se^{IV}, Se^{VI}, BrO_3^-, Br^-, IO_3^- and I^-, in less than 17 minutes.[84] The developed methodology was applied to analyse these analytes in drinking water and fruit juices. The LOD values for the 11 species ranged from 0.08 to 0.25 μg L^{-1}. Method validation was by spike recovery with recoveries ranging from 86 to 114%, thus demonstrating the suitability of the proposed method for the simultaneous determination of 11 species of nutritional and toxicological interest.

A method based on the use of a Zorbax SB-C18 (4.6 × 20 mm, 5 μm) coupled to ICP-OES was developed for performing Se speciation in Se-enriched kefir.[153] Kefir grains were spiked at three different concentrations of Se^{IV} (20, 30 and 50 mg kg^{-1}). Afterwards, the Se^{IV}-fed kefir grains were kept in dark conditions for 4 days to complete the fermentation process. The Se-enriched kefir grains were subsequently separated from the

liquid phase and subjected to an extraction with TMAH and UAE for 4 hours. No SeMet was detected in the fed kefir grains at the different concentrations of Se^{IV} supplementation, while inorganic or elemental Se was determined to be 1597–3116 mg Se per kg. To confirm the identity of the Se-containing peak detected by RP analysis, the extracts were also analysed by AEC with 2.1 mmol L^{-1} $NaHCO_3$/1.6 mmol L^{-1} $NaCO_3$ (pH 10) as the mobile phase flowing at 1.5 mL min^{-1}. The presence of selenite was identified by retention time matching with available standards. Unlike other fermentative microbes, kefir was not able to metabolise selenite to more bioactive and less toxic Se species such as selenoamino acids.

The *metabolism of Se in plants remains of research interest*. Two papers have appeared, each employing HPLC coupled to UV-HG generation with detection by AAS in one or AFS in the second. The former paper evaluated the conditions affecting the separation and determination of Se species in Se-enriched crops (rice, soybean and sweet potato), by foliar application of Se^{IV} (as sodium selenite), using ion pair-reversed phase (IP-RP-HPLC) and UV-HGAAS.[154] Optimal separation conditions were achieved by using a Water Sunfire C18 (250 × 4.6 mm, 5 μm) column and a mobile phase composed of 25 mmol L^{-1} phosphate buffer solution, 5 mmol L^{-1} tetrabutylammonium bromide (TBAB) and methanol (pH 6.0), flowing at 0.6 mL min^{-1}. The column temperature was set at 35 °C. Under these conditions, six Se species (SeCy, MeSeCys, Se^{IV}, Se^{VI}, SeMet and SeUr) were separated within 15 minutes. The signal of most Se species reached the maximum with 10% (m/v) HCl and 2% (m/v) KBH_4 as the HG reagents. Special attention was paid on the procedure to extract Se species from plant tissues. Eight enzymatic treatments, such as single enzyme (protease K, protease XIV, lipase, pepsin, and trypsin) and mixed enzymes (K + XIV, pepsin + trypsin, trypsin + K + XIV, lipase + XIV), were evaluated. The best extraction efficiency was obtained when using MAE and protease K. This approach offered LOD values for the six Se species between 0.77 and 1.77 μg Se per L. Recovery experiments performed in each of the grain crops provided values ranged from 82 to 97% with RSD values of 10%. The accuracy of the method was verified by analysing two CRMs, SELM-1, certified for SeMet, and a rice flour (GBW10045) certified for total Se, and no significant differences between the measured and certified values were reported. The major Se species found was SeMet, which represented 77–90% of the total Se content. No iSe was found in the Se-enriched crops. In the second paper, AEC (PRP-X100) was employed to separate the Se species, followed by UV-HG-AFS measurements.[155] Four different mobile phases were evaluated, $NH_4H_2PO_4$, NH_4Ac, NH_4NO_3 and $(NH_4)_2 \cdot HPO_4$, and the use of a mobile phase containing 60 mmol L^{-1} $(NH_4)_2 \cdot HPO_4$, at pH 10, enabled separation of Se^{IV}, Se^{VI}, SeCy and SeMet within 10 minutes. The method was applied to determine Se species in roots and leaves of tobacco plants grown in soil pots containing two levels of selenite (2.2 and 22.2 mg kg^{-1}). The Se species were extracted from plant tissues using 1 : 2 MeOH : H_2O and UAE for 15 minutes. Three species, Se^{VI}, SeMet and SeCys were predominant in the tobacco roots and leaves. It is well known that $SeCys_2$ and SeMetO co-elute when using the PRP-X100 column. Unfortunately, this consideration was not taken into account in the current study and the presence of SeCys was addressed without performing further experiments to validate the data obtained. A paper reports on the effect of several plant biostimulants on Se uptake by plants hydroponically grown in presence of Se^{IV}, Se^{VI} and a mixture of both.[156] Biostimulants consist of a mixture of hetero-polyanions such as phosphomolybdate, silicotungstate, borovanadate, titanomolybdate esterified by humic acids. These additives are usually employed in agriculture to enhance nutrition efficiency, abiotic stress tolerance and crop quality. In the current study, the biostimulants were foliarly applied at two growing stages (tillering or heading stage) of wheat crops. Microfocused X-ray spectroscopy was used to determine the chemical form and location of Se in the plant tissues. Regardless of the chemical form of Se and type of bio stimulant applied, μ-XRF measurements revealed organic Se as the main Se species found in wheat grain and Se was preferentially located with other minerals in the outer layer and embryo parts of the grain. The proportion of organic Se was slightly affected by the application stage. However, grains from plants treated at the tillering stage contain higher ratio of C–Se–C and lower ratio of C–Se–Se–S than grain treated at the heading stage where the ratio of C–Se–C and C–Se–Se–C was equal. The authors postulated the benefits of biostimulants in increasing the amounts of grain produced without either diminishing the Se concentration or altering the Se species formed. Biostimulants seem to be a good tool to favour Se supplementation efficiency.

Analysis for *Se species in biological samples* remains of interest to researchers due to the essentiality and health benefits of Se compounds. An interesting study identified liver proteins regulated by Se in rats.[157] Male rats were exposed to different dietary sodium selenite concentrations ranging from deficiency (0, 0.08 mg Se per kg), adequate (0.24 mg Se per kg) to toxic levels (0.8, 2 and 5 mg Se per kg). Upon completion of the treatment period, rats were euthanised and livers were removed and treated with a mixture of Tris buffered saline (TBS) solution (50 mmol L^{-1} Tris–HCl, pH 10, 150 mmol L^{-1} NaCl and EDTA). Extracts containing soluble proteins were then analysed for total Se content (ICP-MS), Se species (SEC-ICP-MS) and protein identification and quantification (LC-MS/MS). Bioinformatics tools were also used to statistically compared results from the different diets. Separation of the liver soluble fractions by SEC was performed using a BioSEC 3-column (Agilent 4.6 × 300 mm, 150–0.5 kDa) with 200 mmol L^{-1} NH_4NO_3 at pH 7.5 as the mobile phase, flowing at 0.4 mL min^{-1}. Except for the control group, different Se-containing peaks were detected, the retention time of which was dependent on the level of Se supplemented. The Se-containing peaks from SEC were collected, treated with iodoacetamide and trypsin digested. The resulting Se containing peptides were analysed by LC-MS/MS. Proteins were identified as GPX-1, Se binding protein 1 (SELENOB1) and Se binding protein 2 (SELENOB2). Label free proteomic analysis was also performed to identify those proteins regulated by dietary Se. Peptides corresponding to 571 proteins were identified by LC-QTOF-MS and a Mascot data base search. The extreme diets presented the highest number of proteins

differentially expressed (0 mg Se per kg: 45 proteins; 5 mg Se per kg: 59 proteins) and 13 proteins commonly affected. Network analysis revealed that overexpressed proteins were linked to the metabolisms of gluthatione, xenobiotic processes (*e.g.* the role of Se in metabolising enzymes expressed in the liver such as the cytochrome P450 family) and amino acid metabolisms. The similarities found constitute an important finding and support the hypothesis that Se supplementation to a Se-deplete population could produce adverse effects. A comparative study between HPLC with a fluorescence detector (FLD), with a post-column Köning reaction, and HPLC-ICP-MS/MS in terms of simultaneous detection and quantification of selenocyanate (SeCN) and thiocyanate (SCN) has appeared this year.[158] The SeCN and SCN were well separated within 20 minutes on a Scherzo SS-C18 column (3 μm, 4.6 i.d. × 250 mm) and by using a mobile phase of 0.1 mol L^{-1} acetate buffer (pH 5.0) and a mixture of 12.5 mmol L^{-1} $NaClO_4$/MeOH. The column eluent was then mixed with the Köning reagent (pyridine, barbituric acid and chloramide T) to develop fluorescence. For HPLC-ICP-MS measurements, the same column was employed but with a mobile phase composed of 0.1 mol L^{-1} $AcNH_4$ at pH 5.0. Under these conditions, SeCN and SCN were separated within 45 minutes. The best analytical performance was obtained by HPLC-FLD, which provided LOD values of 5.90 and 9.97 nmol L^{-1} for SeCN and SCN, respectively, whereas LOD values of 2.09 × 10^3 and 0.5 × 10^3 nmol L^{-1}, respectively, were attained by HPLC-ICP-MS/MS. Thus, HPLC-FLD was used to quantify SCN and SeCN in different mammalian cell lines exposed to selenite. The results revealed that selenite induced the production of SeCN by the replacement of S for Se in SCN. The capability of selenoneine (SeN) (the Se analogue of the sulfur-containing antioxidant ergothioneine) to cross the *in vitro* blood–brain barrier (BBB) model was evaluated by HPLC-ICP-MS/MS and ICP-MS/MS.[159] A SeN standard was isolated from a genetically modified fission yeast, *Schizosacharomyces pombe*. For cytotoxic effects, a well-established *in vitro* BBB model system was applied by utilising primary porcine brain capillary endothelial cells (PBCECS) incubated with Se concentrations ranging from 10–100 μmol L^{-1} of each Se species (SeN, MeSeCys and Se^{IV}) for 48 h. To evaluate Se transfer across the BBB model, PBCECS were grown as monolayer on inserts resulting in two compartments, the apical (blood–brain) and basolateral (brain parenchyma), with Se applied to the apical compartment. Both SeN and MeSeCys exerted no cytotoxic effects on PBCECs in concentrations up to 100 μmol L^{-1}, whereas selenite caused substantial cytotoxic effects. Cell lysate samples were analysed by HPLC-ICP-MS with different separation mechanisms used depending on the Se species supplemented. Media and cell lysate samples treated with SeN were analysed by RP-HPLC-ICP-MS with a Water Atlantis of C18 (4.6 mm × 150 mm) column and 20 mmol L^{-1} ammonium formiate-3% MeOH and with and without the use of 0.1 mmol L^{-1} tris-(2-carboxyethyl) phosphine hydrochloride (TCED) whereas MeSeCys treated samples were measured without TCED. Samples exposed to selenite were determined by AEC. The ICP-MS was operated in H_2 reaction mode and 1% CO_2 in Ar was employed to enhance the Se signal. Incubation with SeN provided eight unknown Se-peaks in addition to SeN. Only the major unknown Se-peak was identified as a pyruvate adduct by HPLC-ES-orbitrap-MS. Pyruvate is usually present in the culture media and hence the SeN-pyruvate adduct was not considered a true metabolite of SeN. The other minor unknown peaks suggest a negligible metabolism of SeN by PBCECs. Time dependent Se transfer experiments revealed the low transfer of SeN. It seems to be that SeN has a low transfer rate to brain and remains almost unaltered when exposed to brain endothelial cells.

Lately, *the use of ICP-MS/MS for metalloid determination has focused the attention of several researchers as it offers important advantages in terms of selectivity and improvement in the LOD*. The quantification of oxytocin (OT) and its diselenide containing analog selenoxitocin (SeOT) in human plasma was achieved by using HPLC-post column isotope analysis-ICP-MS.[160] Separation was performed on a Aeris peptide XB C18 (3.6 μm, 100 mm × 2.1 mm i.d.) column set at 40 °C using a mobile phase of (A) 0.1% TFA in water and (B) 0.1% TFA in AcN under gradient elution. The eluent was mixed with either ^{77}Se or ^{34}S *via* a static mixing piece and introduced directly into the nebuliser or the ICP-MS/MS, operated with O_2 as a reaction gas, to detect $^{32}S^{16}O$, $^{34}S^{16}O$, $^{77}Se^{16}O$ and $^{80}Se^{16}O$. The analytical performance of post-column IDA was compared with external calibration based on matrix-matched standards. The developed procedure enabled the sensitive determination of OT and SeOT with similar LOD values for the two calibration methods. For SeOT, LOD values of 0.018 μmol L^{-1} for external calibration and IDA were obtained, respectively, whereas for OT, 0.51 μmol L^{-1} and 0.35 μmol L^{-1} for external calibration and IDA were found, respectively. Moreover, a degradation study of OT and SeOT in plasma was performed. For SeOT, several degradation products were observed after 24 h of incubation, while for OT, only a minor degradation product within the incubation time of 72 h was detected, suggesting the low stability of SeOT. The potential of ICP-MS/MS to carry out non-targeted metabolomic analysis of As, P, S and Se in a variety of mushroom species has been explored by Jalin *et al.*[63] Non-targeted speciation analysis, as well as the quantification of methionine and SeMet, were performed using a RP Phenomenex Synergy Polar (25 mm × 4.6 mm, 4 mm) column and a mobile phase of 100 mmol L^{-1} ammonium formate (pH 3.5) at a flow-rate of 1.0 mL min^{-1} and a column temperature of 40 °C. Due to the insufficient retention of negatively charged ionic species, a Hamilton PRP-X100 column with a mobile phase of 5 mmol L^{-1} of $AcNH_4$ at pH 6.6 was employed to determine arsenic acid, DMA, MMA, PO_4^{3-} and SO_4^{2-}, and O_2 was used as a reaction gas in the ICP-MS. A total of 13 mushrooms were included in the study. The major S and P species found in aqueous extracts of all the mushrooms were PO_4^{3-} and SO_4^{2-}, whereas organic forms were dominant for As (DMA and AsB) and Se (SeMet). The speciation data revealed a great diversity in the number of chemical species when comparing between pair of elements (P/As and S/Se) within certain mushrooms. The S and P metabolomic profiles were found to be generally more complex than their As and Se metalloid counterparts. The number of chemical species was different across different mushroom species. For instance, SeMet was the major species detected in *M. procea*, whereas in

B. edulis, more than 10 Se-species were detected (LOD 0.06 μg L^{-1} in the mushroom extract or 6 μg g^{-1} dry mass). The use of ICP-MS/MS enabled a comparative evaluation of the metabolisms of elements that share chemical similarity.

The production of *matrix-matched pig brain homogenates containing Fe and Se as calibrants for quantitative imaging of these elements using LA-ICP-MS* has recently been undertaken.[161] In this report, the effect of elemental species calibration (inorganic *versus* metalloprotein spiked) is discussed in detail. In-house matrix-matched tissues were prepared by spiking pig brain with varying volumes of either inorganic salts (Fe^{II} from $FeCl_2$ and Se^{IV} from $SeCl_4$) or proteins (ferritin and a mixture of Se proteins obtained from the water soluble fraction of selenised yeast) to yield Fe and Se concentrations ranging from 50–2000 mg kg^{-1} and 0.8–1.2 mg kg^{-1}, respectively. Spiked tissues were homogenised, frozen and sectioned at −20 °C to a thickness of 30 μm and the total Fe and Se concentrations, measured with ICP-MS, agreed with the theoretical values estimated from gravimetric preparations. A good linearity of the calibration graphs of both $FeCl_2$ and ferritin spike tissues was obtained at laser energy <3 J cm^{-2}. Unlike Fe, Se was highly affected by laser energy; at 1 J cm^{-2}, the regression slope for selenoprotein calibration was about 36% lower than that of inorganic Se calibration. The laser fluence also had an impact on the RSD for both elements. The higher the laser fluence, the larger the within line RSD achieved. Consequently, a careful optimisation of this laser parameter proved to be crucial to get reliable quantitative imaging data for both Fe and Se in tissues. The LOD of Se was improved (0.032 mg kg^{-1} *versus* 0.068 mg kg^{-1}) by mixing 25% (v/v) MeOH with the laser induced aerosol prior introduction into the ICP-MS. The use of methanol enabled the detection of Fe and Se at physiologically relevant concentrations.

Most studies related to Se are focused on its essentiality, however, as Se is also a toxic element at higher concentrations, it is a subject of great importance in environmental samples, especially its *determination in water samples*. Two papers based on the use of portable liquid electrode plasma optical emission spectrometry (LEP-OES) have been launched this year to determine Se^{IV} and Se^{VI} in waters. The authors claimed the advantages of this technique over ICP-MS-based techniques including reduced cost, minimal sample volume and portability, which is of special relevance for performing on-field measurements. However, its poor selectivity makes it necessary to perform a selective sorbent extraction before measurements. In the first paper, eight different SPE systems containing diverse supramolecules, such as modified crown ethers (lariat ethers derivates) immobilised on silica or polymeric supports, were evaluated.[162] The paper contains detailed information on the effect of different parameters (solution pH, sample loading flow rate, elution type and volume and LEP-OES operating parameters). Quantitative elution was achieved by using 1 mol L^{-1} NaOH solution. Total Se was transformed to Se^{IV} *via* heat treatment using different acidic reducing agents and the best results were obtained by heating for 20 minutes at 120 °C with 6 mol L^{-1} HCl. The LOD value was 2.16 μg mL^{-1}. The method was validated by spike recoveries and accurate analysis of the standard reference material of wastewater (SPS-WW1). The measured concentrations were in good agreement with the certified values. In the second paper, a dithiocarbamate-modified cellulose (DMC) was employed as a selective adsorbent of Se^{IV}.[163] The factors affecting adsorption are detailed: pH, contact time, sample volume and interferences. More than 97% of Se^{IV} was absorbed over a wide pH range (1.0–8.0). The retained Se^{IV} was subsequently desorbed with 1.5 mol L^{-1} KOH solution. The enrichment factor was reported to be 833. The LOD was determined as 2.5 μg L^{-1} with an RSD value for Se^{IV} of 4.4%. The accuracy of the proposed methodology was evaluated through the determination of total Se in a CRM water (DWS-2). The method was also applied to determine Se in synthetic fluegas desulfurisation wastewater spiked with Se^{IV} and Se^{VI} at a concentration of 5.0 mmol L^{-1} with recoveries of 96.2 ± 1.8% and 105.8 ± 1.8%, respectively. In recent years, a homogeneous Co/Ni photocatalyst has been introduced to enhance the photovapour generation of several elements including Se. Compared to another commonly used catalyst, (TiO_2 NPs), a metal ion photocatalyst has the advantages of a homogeneous nature, low cost, and good repeatability. However, this methodology has not been reported yet as a post-column derivatisation tool. In this line, Li *et al.*[164] developed a methodology based on HPLC-photochemical vapor generation (PVG)-AFS using homogeneous Cd ions as the photocatalyst. The method was applied to determine Se^{IV} and Se^{VI} in natural waters using two separation systems: an Ion Pac AS19 column (7.5 μm, 4 mm × 250 mm) and 5 mmol L^{-1} carbonate buffer as mobile phase and a PRPX-100 (10 μm, 4.1 mm × 250 mm) and 50 mmol L^{-1} Na_2HPO_3 as the mobile phase. The best results were obtained using the Ion Pac column. After separation, the Se species in the effluent were delivered into the PVG reactor, reduced in presence of HAc and Cd ions under UV irradiation and measured by AFS. Under the optimal conditions of 60 μg mL^{-1} Cd, 3.5 m reaction coil and 40% HAc, the proposed method provides LOD values of 0.16 and 0.21 ng mL^{-1} for Se^{IV} and Se^{VI}, respectively. The applicability of the method was evaluated for the speciation analysis of iSe in environmental samples, including mineral water, river water and water reference materials (GBW(E)080395 and BWB2261-2016). The results obtained, 993 ± 5 and 82.8 ± 2 ng Se per mL of Se^{IV}, were found to be in good agreement with the certified values (1000 ± 90 and 84 ± 4 ng Se per mL). The LOD values obtained were lower than those provided by other methodologies using photocatalysts such as TiO_2 and ZnO_2. A procedure that used of HG-GFAAS measurements to determine total iSe, Se^{IV} and Se^{VI} in waters has also been described.[165] The simple protocol consisted of two stages. The total Se content was measured directly and then Se^{VI} samples were treated with of 3% (m/v) BH_4Na in HCl and the formed SeH_2 swept to the GFAAS with N_2 gas, with the Se^{IV} concentration calculated by difference. The LOD values were determined to be 0.32 μg L^{-1} for Se^{IV} and 0.11 μg L^{-1} for Se^{VI}. Spike recoveries in diluted synthetic irrigation water were 97 and 99% for Se^{VI} and Se^{IV}, respectively. The accuracy of the method was also tested by using two standard reference materials (SRM1604a and SRM1643) certified in Se^{IV}. The obtained values agreed with the reference concentrations. The use of 3D printing to fabricate

a field portable CV and photo-chemical vapour generator (PVG) μ-point discharge (PD)-OES instrument.[121] The system was compact with total dimensions of 230 × 38 × 84 and a complete description of the design and fabrication of the system was given in the paper and ESI. Selenium speciation was evaluated by PVG-μPD-OES in the presence of 50 mmol L^{-1} of nitrate and with and without adding nano-TiO_2NPs. The results evidenced that the determination of total inorganic Se and selective determination of Se^{IV} was accomplished in the presence and absence of TiO_2NPs, respectively. The LOD values were 5.2 and 3.5 μg L^{-1} for Se^{IV} and Se^{VI}, respectively. Validation of the method was achieved through the analysis of three CRMs (DORM-4, DOLT-5 and GBW(E)080395). The measured concentrations were in good agreement with the certified values and spike recovery studies into different environmental water samples gave values ranging from 93 to 103%, demonstrating the accuracy of the developed 3D printed system. The system was also used to determine Hg species and this is covered in Section 3.11. Finally, a paper reports on the identification of selenopolythionates in waters by high resolution mass spectrometry (ES-FT-ICR-MS).[166] Selenopolythionates ($SenSxO_6^{2-}$) are derivatives of polythionates in which Se atoms are inserted into the polysulfide chain. These compounds are produced from $SeSO_3^{2-}$ *via* oxidative additions. The use of ES-FT-ICR-MS allowed the authors to perform the first MS characterisation of these compounds (selenotrithionate, $NaSeS_2O_6$, diselenotetrathionate, $Na_2Se_2S_2O_6$, and triselenopentathionate, $NaSe_3S_2O_6$) in aged solutions of $SeSO_3^{2-}$. Moreover, CID was applied to selenotritionate to distinguish between isomers. The authors emphasised the need of using HR-MS based techniques in speciation analysis to ensure the correct characterisation of unknown complex species as analytical tools usually employed for ionic Se compounds, such as IC-ICP-MS, failed in the determination of selenopolythionates.

3.17 Silver

Five papers report on *Ag speciation* this year, with the first of these reports covering the development and use of a CE-ICP-MS system for the intracellular speciation of dissolved Ag^I and Ag NPs.[167] An in-house fabricated fused silica CE column, with a number of different running buffers evaluated, nebuliser and spiral flow spray chamber were used for the separations and coupling with the ICP-MS. The optimal running buffer was found to be 15 mmol L^{-1} sodium dodecylbenzene sulfonate (SDBS) and 10 mmol L^{-1} TRIS at a pH of 9.0, D-penicillamine was added to the samples under investigation as a complexing agent for the Ag^I ions and the total run time of 10 minutes. Using the developed system, the speciation of dissolved Ag^I and AgNPs in a culture medium (OPTI-MEM) and HepG2 cells was performed, with the NP concentration being estimated from the difference between the total Ag content and the Ag^I content determined by CE-ICP-MS. Spike recoveries, at six different concentrations between 50 and 200 μg L^{-1}, of 94% in opti-MEM and 94 to 107% in HepG2 cell lysate were obtained, with an LOD value of 87 ng L^{-1} and RSD values of <3% for relative peak area and <2% for migration time. The paper gives a wealth of information on the development of the system and a brief discussion of the chemistry of the interactions between the various running buffer combinations, the Ag species and the fused silica CE capillary. The use of SEC-ICP-MS and XAS to investigate the biochemical fate of Ag^I ions in *Staphylococcus aureus*, *Escherichia coli*, and biological media has been reported.[168] The SEC column operated in the size range of 5 to 1200 kDa and a 100 mmol L^{-1} of 2-[4-(2-hydroxyethyl)piperazin-1-yl] ethanesulfonic acid (HEPES) was used as the mobile phase at a flow rate of 0.3 mL min^{-1}. Separately, a 0.1 to 120 kDA SEC was used to separate size fractions of the samples under study, with the fractions being collected after UV detection and analysed by offline ICP-MS for their Ag content. Using the analytical approaches described, it was found that at micromolar concentrations of $AgNO_3$ in lysogeny broth, Ag^I was complexed by thiolate-containing species of MW ~30–50 kD whilst no formation of AgCl was observed. Neither the XANES nor EXAFS spectra of $AgNO_3$ in broth changed appreciably across a 20-fold $AgNO_3$ concentration range. The SEC-ICP-MS data indicated that higher Ag^I concentrations caused the aggregation of lower molecular weight species that bound Ag. The Ag K-edge XANES of *E. coli* and *S. aureus* cell pellets treated with sublethal doses of Ag ions did not differ significantly from that of the Ag-spiked broth, suggesting the same predominantly thiolate local coordination of Ag. Minor contributions from nitrogen-based (or less likely oxygen-based) donor-atom ligands were identified in Ag treated *S. aureus* cells, and in culture broth with higher concentrations of silver present. The SEC-ICP-MS data indicated that Ag in both bacterial lysates was redistributed from the binding targets after uptake from culture medium, and bound by higher molecular weight, most likely proteinaceous, biomolecules.

Two papers report on *Ag speciation in seafood* samples. In the first of these, a method based on asymmetric flow field-flow fractionation (AF4) coupled to UV-vis and ICP-MS detectors was used to detect and quantify Ag NPs in bivalve molluscs.[169] The samples were solubilised using an enzymatic hydrolysis procedure (pancreatin and lipase at 37 °C for 12 h). The AF4 separations used a regenerated cellulose (RC) membrane (10 kDa, 350 mu m spacer) and aqueous 5 mmol L^{-1} Tris–HCl pH = 7.4 as the carrier solution. The separation utilised a focusing step followed by size fraction elution using a cross flow of 3.0 mL min^{-1} for 15 min, followed by a linear cross flow decrease for 7.5 min, and a washing step for 9.4 min with no cross flow. Several bivalve molluscs (clams, oysters and variegated scallops) were analysed for total Ag content (by ICP-MS after acidic MAE) and for Ag NPs by the AF4 method. The detected Ag NPs eluted at the same time as proteins (UV monitoring at 280 and 405 nm), suggesting a certain interaction occurred between Ag NPs with proteins in the enzymatic extracts. The AF4-UV-ICP-MS fractograms suggested different Ag NP size distributions for selected samples. Membrane recoveries, determined by peak area comparison of fractograms with and without application of cross flow, ranged between 49% and 121%. The presence of Ag NPs in the enzymatic extracts was also shown by SEM after an oxidative pre-treatment based on H_2O_2 and MAE. The paper gives plenty of detail on the AF4 optimisation process and the

authors note that attention must be given to the sample pretreatment procedure as matrix components can influence the AF4 separation. A survey has been undertaken for the Ag^{I} and Ag NP content of canned seafood samples, anchovy, clam, mackerel and tuna, available on the Italian market.[170] The analytes of interest were extracted by alkaline hydrolysis, TMAH for 24 hours with initial UAE for 30 minutes at 37 °C followed by dilution with 1% TMAH and analysis by sNP-ICP-MS. The LOD of the method, evaluated using procedural blanks, was 1.5×10^3 particles per mL, which equated to 3.3×10^5 particles per g after the sample mass and TMAH volumes used were accounted for. Spike recoveries, using 40 nm Ag NPs in high purity water at a concentration of 20 5.7×10^7 particles per mL gave a median diameter of 41.8 ± 3.5 nm and a recovery of 94.1%. No statistical difference (*t*-test) was observed with these results when the Ag NPs were spiked into 1% TMAH. Spike recoveries into seafood samples were 88.5 ± 4.2%. The total Ag content in the seafood samples was determined by ICP-MS after an acidic MAE digestion procedure. The most frequent mean Ag NP size in all of the samples was 27 nm with the mean diameter ranging from 31 nm (anchovy) to 36 nm (tuna). No statistical difference was observed between the total Ag content and the dissolved Ag^{I} content, which was presumably assessed from the baseline of the sNP-ICP-MS particleograms, as the Ag NP content was <1% of the total Ag content. Analysis of the packaging materials by SEM did not reveal the presence of Ag NPs, suggesting that these were either formed *in situ* in the biota, as part of a detoxification mechanism, or ingested during normal life activities.

A study which reports on the *coupling of hydrodynamic chromatography (HC) with ICP-MS for the simultaneous determination of dissolved and nanoparticulate species of Au and Ag* has been published this year.[106] The optimal mobile phase for the separation of the Ag species was found to be 0.45 mmol L^{-1} SDS and varying amounts of penicillamine flowing at 1.6 mL min^{-1} with a run time of 11 minutes. This allowed the quantitative recovery of ionic Ag and Au NPs up to 50 nm in diameter and the resolution achieved between ionic Ag and 10 nm Ag NPs was between 0.31 and 0.93 depending on the mobile phase composition, with an LOD of 0.75 μg L^{-1}. The method was applied to the detection of Ag NPs and dissolved Ag species in three dietary supplements, with the Ag NP mass fraction determined by HC-ICP-MS showing good agreement with the results obtained with FAAS after a sample digestion step involving HNO_3 for two samples, whilst for the third sample, the total Ag content could not be determined due to peak overlapping. Spike recovery experiments, for ionic Ag and Ag NPs, showed that different penicillamine mobile phase concentrations were needed to achieve satisfactory results for each species. The measured Ag NP sizes, under optimal mobile phase conditions for this measurement, agreed with that obtained by TEM for two samples whilst no AG NPs were detected by TEM for the third sample. The results obtained for an Au NP containing supplement can be found in Section 3.6.

3.18 Thallium

The low LOD values needed to monitor Tl species in environmental water samples were achieved by preconcentration by *magnetic DSPME with ETAAS detection*;[171] however, the speciation was achieved by a previously published LLE method for Tl^{III}. The Tl^{I} remained in the upper aqueous phase, which was then subjected to the preconcentration step. As both Th^{I} and Tl^{III} were extracted in this step, total Tl was determined in a separate experiment and Tl^{III} obtained by difference. The LLE method involved 1 mol L^{-1} HNO_3, 2 mol L^{-1} NaBr, 0.05 mol L^{-1} CPC then MeOH : $CHCl_3$ (9 : 1 v/v), shaking (5 min) and centrifugation, whilst the preconcentration method utilised 1 mol L^{-1} HNO_3, 2.5 mol L^{-1} KI, 0.01 mol L^{-1} Aliquat 336 magnetic NPs, shaking, magnetic separation and treatment of the collected NPs with 0.1 mol L^{-1} EDTA at pH 9 by vortexing and further magnetic separation. Both CS (276.786 nm, with the integrated absorbance summed over three pixels) and HCL-source instruments were used. The LOD was 0.01 μg L^{-1} and the method was applied to the analysis of 5 CRM waters and several tap, spring, river, sea and bottled water samples. The results, which curiously are in the ESI, showed that Tl^{I} was detected in all samples, and that Tl^{III} was present in all samples except tap water and one bottled water. Recoveries of spikes at 0.2 and 0.5 μg L^{-1} were between 95 and 116%.

3.19 Tin

A method for the determination of *TBT in seawater by IDA GC-ICP-MS* has been developed that only requires 12 mL of sample solution.[172] The researchers employed a triple ID procedure involving two calibration blend solutions with different isotope ratios, in addition to the single ID solution. The calibration blend solutions contained both a standard of a known concentration and an isotopically enriched (spike) standard, while the sample blend solution contained both sample and isotopically enriched standard. The researchers pointed out that one of the advantages of this triple ID method is that the isotope ratio and the concentration of the spike material are not required, since they do not appear in the model equation, and thus, triple ID is the most appropriate method if the isotope ratio or concentration of spike material is not well characterised. The TBT standards, including those made from the ^{117}Sn-enriched TBT (isotopic abundance 92.1%), were prepared by a two-step procedure in which an intermediate stock solution was prepared in 1-propanol, (which, with a bp of 97 °C, is relatively involatile) and subsequent dilutions were made with 0.1% (v/v) HCl in ultra-pure water. To a seawater sample (12 mL) were added 100 μL of the enriched spike solution (2.8 μg L^{-1} TBT) and 500 μL of 0.5 mol L^{-1} pH 5 acetate buffer, and the solution agitated by rotation at 1000 rpm for 20 min. Then, 20 μL of 0.05% (w/w) $NaBEt_4$ solution in THF were added with further agitation (1000 rpm for 5 min) followed by LLE with 500 μL of hexane, (agitated at 2000 rpm for 5 min). Following phase separation (5 min), 200 μL of the organic phase was transferred to a GC vial containing the glass inset. Separation was carried out with an instrument fitted with a programmable temperature vaporisation (PTV) inlet, a capillary column (cross-linked 5% phenylmethyl siloxane, 30 m × 0.25 mm i.d. × 0.25 μm coating) operated in large volume injection mode (100 μL) with a 6-baffle glass liner 2.0 mm × 2.75 mm × 120 mm. The operating

conditions were optimised with a chemometric procedure. The LOD and LOQ, based on the ^{120}Sn signal and a sample volume of 12 mL, were 0.015 and 0.045 ng L^{-1}, respectively. Trueness (and precision) were evaluated by analysing QC solutions at 1.5 and 4.8 ng L^{-1} prepared in artificial seawater (3% m/v NaCl), and the method was applied to the analysis of seawater from the Bay of Izmit, Kocaeli, Turkey, which was sampled three times during a calendar year at 13 locations; TBT was detected in almost all samples, with average concentrations at each station ranging from 1 to 3 ng L^{-1}.

The *stability of SnF_2 in toothpaste has been studied by XAS*.[173] The compound is added as an antimicrobial agent and fluoride carrier to dental enamel, but is known to be quite reactive towards O_2 and the variety of organic and inorganic compounds commonly present in toothpaste. The XAS experiments were conducted at the Sn K-edge (29.2 keV) on the bending magnet beamline of the DuPont, Northwestern, Dow Collaborative Access Team, located at sector 5 of the APS at Argonne National Laboratory (Lemont, IL, USA). The Sn K-edge XAS spectra were calibrated, averaged, normalised, analysed and plotted using a series of in-house notebooks written in Python (references given). The percentage of Sn^{II} and Sn^{IV} in the samples were quantified by linear combination fitting (LCF) analysis of the XANES spectra and their first derivatives. To further characterise Sn^{II} compounds, the average coordination environments were examined by EXAFS. The first coordination shells of the reference compounds selected were characterised by fitting to theoretical scattering signals (also by LCF), to determine chemical identity, coordination number, and interatomic distance of the nearest-neighbour atoms to Sn. Four different toothpastes were examined, and the researchers deduced from the XANES spectra that after opening the tubes, oxidation occurred, which was significant for one of the samples. They suggested that stabilising agents, such as gluconate and phosphate species, have different efficacies in preventing Sn^{II} oxidation as soon as the paste was exposed to O_2 and that phosphate additions provided better stabilisation. They also noted (from the EXAFS results) that there was little or no evidence for the presence of SnF_2 and $SnCl_2$, and they deduced that Sn undergoes ligand exchange reactions to form predominantly oxide species that ultimately end up as amorphous $Sn(OH)_4$.

3.20 Vanadium

All three of the reports of V speciation describe *chromatographic separation with ICP-MS* detection. Anion-exchange HPLC was applied to speciation in *Amanita muscaria* (fly agaric mushroom)[174] and in estuarine waters.[175] *A. muscaria* is unusual in being able to accumulate concentrations of V up to 100 mg kg^{-1} dw, most of which is present as a compound known as amavadin, whose biological function is not known. In amavadin, V exists in the rather unusual non-oxo V^{IV} ion (the vanadyl ion VO^{2+} is more common) and is coordinated by two tetradentate ligands that can coordinate in two distinct orientations, and thus amavadin exists in two isomers. For speciation analysis, an anion-exchange HPLC method was optimised (the effects of citric acid concentration, pH and temperature were investigated) for a Zorbax SAX column (4.6 × 150 mm, 5 μm). The mobile phase was 10 mmol L^{-1} citric acid, 10 mol L^{-1} Na_2EDTA, at pH 5 (adjusted with aqueous NH_3), the elution was isocratic and the column temperature was 13 °C. An IS of Ge (200 mg L^{-1} in 10% nitric acid) was merged post-column *via* a T-piece using tubing whose diameter was 1/4 of that of the sample tubing in which the eluent was flowing at 1 mL min^{-1}. Under these conditions, the amavadin isomers eluted (not quite baseline separated) between 13.5 and 17 min, whereas vanadyl acetate eluted around 6.5 min. Samples (2 g of cap, stipe and gills, and 1 g of bulb and skin) were extracted with 20 mL (10 mL in the case of the bulb and skin) of the mobile phase with ultrasonic agitation (20 min), followed by centrifuging (10 min), and then filtering (0.22 μm nylon). The LOD values were 0.04 and 0.05 μg L^{-1} for vanadyl acetate and amavadin, respectively, and the method was validated by analyses of CRMs NIST SRM 1573a (tomato leaves) and SRM 1640a (trace elements in natural water), for which relative measurement errors of 10 and 5%, respectively, were obtained. The procedure was applied to the analysis of three samples. The extraction efficiency was 74 ± 12%, and amavadin, which was found in all parts of all three samples, accounted for 75–96% of the extracted V. All samples also contained several additional, unknown V compounds, including one eluting as late as 52 min. To determine V^V in estuarine waters of varying salinity, Knezevic *et al.*[175] optimised an anion-exchange HPLC separation with HR ICP-MS detection with quantification by standard additions. They could not reliably quantify V^{IV} with the method, and so this was determined as the difference between the V^V concentration and the total V concentration, which was determined by direct introduction of a sample, diluted 10 times with HNO_3, into the spectrometer with calibration against standards prepared in 10-times diluted CRM CASS-5 (nearshore seawater) with In as IS. The instrument was operated at medium resolution (4000) to avoid the major isobaric interference from $^{35}Cl^{16}O^+$, and V was measured at m/z 50.942. Species were separated on a Metrosep A Supp 5–50/4.0 column (50.0 mm × 4.0 mm), maintained at 22 °C, by isocratic elution with an eluent composed of 40 mmol L^{-1} NH_4HCO_3, 40 mmol L^{-1} $(NH_4)_2SO_4$, 8 mmol L^{-1} EDTA and 3% ACN. The LOD was 102 ng L^{-1} in the seawater and 31 ng L^{-1} in the freshwater, and the method was applied to the analysis of V redox speciation in the vertical salinity gradient of the highly stratified estuary of the Krka River in Croatia. Preservation and storage tests showed that, unlike the situation with the previously suggested addition of EDTA, the speciation was maintained for at least 7 days without any preservatives, provided samples were kept at natural pH and 4 °C. The researchers found that the reduced V species were stable even in oxic conditions, which they attributed to interaction with organic or inorganic ligands present in the water column. The effect of Ag triflate (AgOTf) on the interaction between V and asphaltene nanoaggregates in the atmospheric residues of several crude oils was investigated by GPC with HR ICP-MS.[176] The separation was achieved on three GPC columns (from 1000 to 600 000 Da) connected in series preceded by a Styragel guard column (30 mm × 4.6 mm, 10 000 Da exclusion limit) with a mobile

phase of THF stabilised with 250 mg L^{-1} butylated hydroxytoluene flowing at 1 mL min^{-1}. The eluent was split so that 40 μL min^{-1} was directed to the double focusing SF ICP-MS fitted with a modified CETAC DS-528-30 microflow total consumption nebuliser mounted with a laboratory-made, single-pass, jacketed glass spray chamber thermostated at 60 °C by water circulated from a temperature-controlled water bath. To avoid carbon deposition, O_2 was added at 0.08 L min^{-1}. Samples were diluted 50 times in THF and 20 μL were injected. The effects of different concentrations of AgOTf were studied along with the kinetics of the interaction. In addition to V at *m*/*z* 51, Ag (*m*/*z* 109), Fe (*m*/*z* 56) an S (*m*/*z* 32) were also monitored. The researchers concluded that the addition of Ag triflate caused disaggregation of some V compounds linked to asphaltene nanoaggregates, and that Ag^+ can partially move some porphyrins from the high-molecular-weight region to the low-molecular weight region, from which it was inferred that the interaction between Ag^+ and the porphyrins surroundings led to a decrease in the size of the nanoaggregates in the high-molecular-weight region and an increase in the "free" V porphyrin compounds.

3.21 Zinc

The *research interest in Zn is focused on its determination in biological organisms*. A study investigated the metal concentration profile (Zn, Cu, Cd, Pb, Cr and AS) of contaminated Pacific oysters (*Crassostrea gigas*) collected in a highly contaminated coastal are of China due to the emission of a semiconductor manufacturing plant.[177] Several techniques, including ICP-OES, EDX, EXAFS, FTIR, XPS and XANES were applied in this work. The ICP-OES measurements of oyster soft tissues revealed concentrations of Cu and Zn as high as 1100–1400 mg kg^{-1} and 500–700 mg kg^{-1}, respectively, whereas for other elements tested, the concentrations were lower than the maximum levels allowed by Chinese regulations (As (0.11–0.35 mg kg^{-1}), Cr (0.80–1.60 mg kg^{-1}) and Pb (0.10–0.20 mg kg^{-1})). A seasonal variation of the trace metal concentration was observed, with the highest values detected in October as in this month, the dry season coincides with the increasing production of semiconductors. The XANES/EXAFS results showed that CuO and ZnO were the compounds mainly found in contaminated oysters, while Cu_2S and ZnS were predominant in non-contaminated oysters. The authors postulate that ROS produced by the high Cu/Zn mass fractions force Cu and Zn to bind to O donors. In an outstanding study, the difficulties in determining labile low molecular mass (LMM) transition metal complexes by LC-ICP-MS and LC-ES-MS/MS were addressed and partially solved. These unstable complexes can be altered during sample preparation or during migration through chromatographic columns. Moreover, the presence of salts suppresses ES-MS signals, making the identification of these complexes very difficult. In the current work, *E. coli* was used as a biological model and a procedure to isolate *E. coli* cytosol without using EDTA in the lysis buffer was developed, as the migration of Zn from LMM-Zn to EDTA was observed.[111] However, EDTA removal decreased the effectiveness of cell lysis. To avoid this problem, a strain of *E. coli* which contained holin and endolysin proteins that enable cell lysis without EDTA was used. To minimise adsorption/desorption problems in SEC columns, a saturation of the basic sites of the column with ^{67}Zn was performed before separation. As a result of that, a Superdex peptide 10/3000GL column loaded with $^{67}ZnSO_4$ was employed to separate the LMM compounds. A detailed description of the loading procedure is given in the work. To prevent ES-MS signal suppression by the salt content in the cytosol preparation, two SEC columns linked in tandem were applied when the collected SEC fractions were analysed by ES-MS/MS. After SEC analysis, the *E. coli* cytosolic fraction exhibited 2–5 Fe, 2 Ni. 2–5 Zn, 2–4 Cu and 2 Mn-containing peaks associated to masses between 300–5000 Da. Some LMM-P and LMM-S complexes were identified in the cytosolic fraction by ES-MS/MS. Unfortunately, the authors failed to establish the chemical identity of Fe, Cu, Zn and Mn complexes detected by SEC-ICP-MS, even when using a longer column. The use of a longer column overcome the problems of salt contents but also diluted the samples. It is important to highlight the analytical improvements obtained in this work for determining these challenging analytes. Finally, SEC-ICP-MS was applied to determine HMM and LMM Cu and Zn compounds in dairy cow blood serum.[178] A total of 17 5–6 year old female cows were examined. Blood samples were collected, centrifuged and the separated serum was stored at −70 °C. Prior to analysis, samples were diluted with a solution containing 1% butanol-1, 1% TRITON X-100 and 0.07% HNO_3. Samples were injected onto the LC equipment with a SEC column (Agilent BiOSEC-5, 78 × 300 mm, pore size 300 A) and a mixture of 50 mmol L^{-1} NH_4Ac and 20 mmol L^{-1} TRIS solution with the addition of 50 mmol L^{-1} NH_4Ac and 5% MeOH as mobile phase. The eluate from the column was analysed by ICP-MS. Results revealed four major Cu-containing peaks that the authors assigned, by comparing the retention times with those of the standard, to tetrameric and dimeric macroglobulin, ceruloplasmin, albumin and LMM copper compounds. Four Zn-containing peaks were also detected and attributed to tetrameric and dimeric macroglobulin, albumin and Zn amino acid compounds. The Cu and Zn were mostly bound to HMM ligands, such as albumin, ceruloplasmin and macroglobulin, with a very small fraction associated with LMM ligands. No attempts were carried out to identify by ES-MS/MS the compounds detected by SEC-ICP-MS.

4 Biomolecular speciation analysis

A comprehensive review in an emerging human health related application area includes work covering the interplay between metal(loid)s and the host microbiota.[179] Significantly, it brings together the approaches of metabolomics, metallomics, metatranscriptomics and metaproteomics to investigating this relationship. The review, with 172 references, has a focus on dysbiosis (abdominal cramping, diarrhoea, and constipation) and the imbalance in gut bacteria related to metal(loid)s, in particular, the gut–brain axis. The review goes into significant detail regarding the different work-flows involved in these -omics approaches, in particular hetero-atom tagged

proteomics for the "absolute-quantification" of metal-containing biomolecules by using ICP-MS as a chromatographic detector. A second review, containing 68 references, includes methods for the analysis of metalloproteins.[180] In particular, it proposes "metrometallomics" as a branch of metallomics, which is defined as the measurement activities required for metallic analytes with metrological measurement strategies, including the establishment of reference methods with associated uncertainty evaluation, CRM development, and their application in the life and environmental sciences. The review highlights the current trend of metrometallomic research being towards the development of absolute quantitative strategies for the determination of the metal-transport proteins and metalloenzymes. Focusing on the main protein quantification techniques developed using ICP-MS in recent years, it covers several methods for quantitative protein analysis. These include: direct heteroatom measurement; elemental labelling; direct protein tagging with I, Hg or other complexes; immunological tagging with nanoparticles; and the specific method of metal-coded affinity tags. The advantages of using ID-MS calibration methods for the quantitation of different biomolecules, including different metalloproteins, is also highlighted.

As described in the aforementioned review, *the use of ID-MS calibration approaches for the measurement of macromolecules* is an important advantage offered by inorganic instrumentation in the field of biometric applications, because it can provide results of the highest accuracy and traceability. Unfortunately, this area has not advanced particularly quickly since it was first suggested, mainly due to the lack of suitable isotopically enriched standards, the costs involved in using such standards and, possibly, a lack of knowledge and training in this area. It is therefore encouraging to see two papers recently published using different aspects of ID-MS for the measurement of important clinical biomarkers. The accurate measurement of β-amyloid peptide, a biomarker for Alzheimer's disease, by HPLC-ICP-MS has used ssID-MS for the characterisation of the Aβ40 and Aβ42 peptide standards.[181] Since the majority of proteins contain S *via* the amino acids cysteine and methionine or are phosphorylated, an amino acid analysis based on the S/P content can be carried out using HPLC-ICP-MS. With the advent of triple quadrupole ICP-MS instrumentation, it is also now possible to measure the appropriate isotopes with a suitably low LOD, using O_2 in the reaction cell mode. Importantly, the measurement of S or P allows the direct traceability to SI units, which is often not possible in clinical assays because of the lack of available reference standards. The method is based on the separation of the S-containing amino acids methionine and cysteine after oxidation and hydrolysis of the peptide standards. Using strong AEC, both amino acids could be separated from each other, as well as from their oxidised forms and sulfate. An IonPac-AS22 AE column (2 × 250 mm, 6 μm) and a gradient elution using an eluent containing ammonium acetate buffer at pH 8.0 was used for the separation. The S content was determined *via* ICP-MS/MS using O_2 as reaction gas. The use of ssIDMS was enabled by using a ^{34}S-labeled yeast hydrolysate containing methionine sulfone and cysteic acid with different isotopic compositions. The peptide contents of synthetic β-amyloid standards (β-amyloid$_{1–40}$ and β-amyloid$_{1–42}$), as well as myoglobin and lysozyme with different degrees of purity, were determined. For validation purposes, the SRM NIST 2389a, which contains the amino acids in a similar concentration, was subjected to the developed sample preparation and analysis method. The LODs for methionine and cysteine were 0.04 and 0.05 μmol L^{-1}, respectively. Analysis of the NIST CRM using conventional external calibration gave recoveries of 70 and 66% for cysteine and methionine, whereas using ssIDMS improved the recoveries to 99 and 100%, respectively. The second paper also used ssIDMS, in this case to determine the concentration of serum albumin bound Cu as part of an assay to determine the "exchangeable" Cu fraction, which is a marker used in the monitoring of patients with Wilson disease (WD).[182] The serum proteins were separated using a FPLC MonoQ AE column, with a gradient elution using Tris pH 7.4 at a flow rate of 1.0 mL min^{-1}, which was coupled to a triple quadrupole ICP-MS instrument. A protein quantification approach was used where the concentration of Cu associated with the protein fraction was based on its relative peak area distribution and the total Cu concentration in the sample. The methodology was characterized in terms of selectivity, sensitivity, precision, and robustness. Due to the lack of speciated-Cu reference materials, protein recovery was assessed by comparison with that of ssIDMS. For this, a double spike HPLC-ICP-ssIDMS method for Cu-albumin was developed using an in-house prepared isotopically enriched albumin standard, containing enriched ^{65}Cu. Three human sera (two frozen, LGC8211 and ERM (R)-DA250a, and the lyophilised Seronorm™ Human) were analysed using both the relative and IDMS quantification methods. The validated relative approach, with expanded uncertainties ($k = 2$) of between 5.7 and 10.1% for Cu-albumin concentrations ranging from 112 to 455 μg kg^{-1} Cu, was found to be able to discriminate between healthy and WD populations in terms of Cu-albumin content.

Speciation *methods for the investigation of the interaction of Cu with biomolecules have been reported, in studies of both human and animal health*. The interaction between the β-amyloid peptide and Cu^{2+} is reported to play an important role in Alzheimer's disease.[183] However, the affinity constant between them is still controversial and a wide range of values from 10^7 to 10^{11} mol^{-1} have been reported. Most studies have focused on the short peptide β-amyloid$_{1\text{-}16}$, which was proved to contain the high-affinity site for Cu complexation. With the aim of determining a more accurate and reliable affinity constant value, a method based on CE-ICP-MS was developed and competitive binding experiments conducted in the presence of nitrilotriacetic acid. The effect of a neutral or positively charged capillary surface and the nature of the buffer (Tris or Hepes) were studied. Tris buffer was found to be inappropriate for such measurements, as it enhanced the dissociation of the Cu^{2+}-complexes, which was already occurring in the presence of an electric field in the CE system. Using the Hepes buffer, a value of 10^{10} mol^{-1} was found for the affinity of the small β-amyloid$_{1–16}$ for Cu^{2+}, which is in agreement with the values obtained for other proteins involved in neurodegenerative diseases. A

method for the assessment of the major Cu and Zn containing molecular species in dairy cow blood serum used SEC for their separation with detection using ICP-MS.[178] Speciation of serum Cu and Zn was performed using a Bio SEC-5 column (7.8 × 300 mm, pore size 300 Å) packed with spherical, high purity porous silica with a hydrophilic polymeric coating. This gave a separation profile in the range of 5 to 1250 kDa. The 55 : 45 mixture (pH 7.4) of 50 mmol L^{-1} ammonium acetate and 20 mmol L^{-1} Tris solution with addition of 50 mmol L^{-1} ammonium acetate and 5% (v/v) methanol was used isocratically as the eluent. Analysis of serum ^{63}Cu species revealed four major fractions containing 2.5% (A), 15.6% (B), 75.6% (C), and 11.9% (D) of the total Cu. The four fractions could be assigned to tetrameric and dimeric macroglobulin, ceruloplasmin, albumin, and low molecular mass (LMM) Cu compounds, respectively. A minor fraction (E) containing <1% of total serum Cu levels was thought to be related to low-molecular mass Cu species. Speciation analysis also revealed four Zn fractions containing 6.3% (A), 16.9% (B), 71% (C), and 3% (D) of total Zn, which were attributed to Zn-bound tetrameric and dimeric macroglobulin, albumin, and Zn-amino acid compounds. The results demonstrated the particular features of Zn and Cu transport in dairy cows, which may be used for assessment of dietary status of trace elements. Whilst clearly a useful system, the method of assigning the peaks relies on the use of size *vs.* retention time calibration equations. There is therefore the potential to misassign peaks if the retention behaviour of a particular protein is not related to its size in solution. There should be a general approach to the characterisation of different metalloprotein species whereby at least 2 different chromatographic separations are used, rather than just relying on a single mode as in this work.

An alternative approach to using 2 orthogonal chromatographic systems is the confirmation of metal species by using molecular MS approaches alongside inorganic MS. This approach has been used to *characterise the Cd-containing biomolecules in marine short-necked clam using SEC hyphenated with ICP-MS and ESI-Q-TOF-MS*.[184] The paper exemplifies the practical difficulties in using molecular methods to identify metal-containing species, when there are few authentic molecular standards available and the sample matrix has a strong effect on ionisation of the molecules understudy. To overcome the matrix issues, the authors used an in-house prepared gel filtration column of Sephadex G-75 and enriched the collected fractions by lyophilization to isolate the compounds of interest. These were then analysed by SEC coupled to ICP-MS and then by ES-Q-TOF-MS using a polymer-based gel filtration column (TSK gel G4000PWXL, 7.8 mm i.d. × 30 cm, 10 μm) and an eluent containing 10 mmol L^{-1} ammonium acetate at pH 7.8, at a flow rate of 0.6 mL min^{-1}. The results showed three separate Cd-containing peaks by ICP-MS detection and these were then isolated as 3 fractions from the Sephadex G-75 column. The major Cd-containing peak had a molecular mass of 32 695 Da by ES-Q-TOF-MS. The other Cd peaks were shown after deconvolution of the mass spectra to contain a mixture of five main Cd-binding species, with masses of 10 764 Da, 17 984 Da, 18 156 Da, 19 236 Da and 20 978 Da, in which the species with 19 236 Da was the most abundant species. The final peak was shown to contain some small Cd species with molecular weights less than 1000 Da. In order to verify the reliability of the two hyphenated methods, a commercially available Zn-binding metallothionein from rabbit liver, with a protein purity >70%, was analysed using the same conditions. The results demonstrated that the use of the two developed hyphenated methods using SEC-ICP-MS and SEC-ES-Q-TOF-MS were able to characterise the Cd species in marine shellfish as most likely being metallothioneins or analogues of metallothioneins (*i.e.*, MT-like proteins) which were thought to play significant roles in the detoxification of Cd in cells.

New *methods for the measurement of vitamin B12 in different foodstuffs have used HPLC coupled to either ICP-MS or ICP-OES* depending on the concentration expected in the sample. As highlighted previously, the use of inorganic measurement methods provides the opportunity of greater traceability of the analytical method, compared to those currently routinely used, which can suffer from the unavailability of authentic standards. The term "vitamin B12" designates a family of Co-containing compounds called cobalamins, amongst which methylcobalamin, adenosylcobalamin and hydroxocobalamin are the most commonly found in nature. Due to the human body's inability to synthesize this essential vitamin and because it is not present in all foodstuffs, there is a requirement to develop methods that are applicable to measuring it at the concentrations found in a particular food source. In the area of infant nutrition, the assessment of the vitamin B12 intake in exclusively breastfed babies depends on the reliability of its determination in milk. An accurate and robust method based on HPLC-ICP-MS has been developed to measure the low concentrations that can be encountered when measuring this vitamin in mothers with poor nutrition.[185] Extremely low levels (below 50 pmol L^{-1}, corresponding to 68 ng L^{-1}) can be found in specific populations, such as non-supplemented vegetarians and vegans. Milk samples were first reacted with KCN to convert all the forms of cobalamin present to the most stable cyanocobalamin species. The samples were then heated to 120 °C for 30 min to denature the bound proteins. The derivatised samples were then passed down an immunoaffinity cartridge to isolate the analyte from the matrix. Separation of cyanocobalamin from free Co or other potential Co containing species was carried out with a silica-based C_{18} column (Atlantis T3, 15 mm × 2.1 mm, 3 μm) at 45 °C, under isocratic conditions. The mobile phase was composed of water–methanol 75 : 25 (v/v) containing 5 mmol L^{-1} EDTA disodium salt hydrate and 10 ng mL^{-1} of Ge in order to control any signal drift. Flow rate was 0.3 mL min^{-1} and the injection volume was set at 50 μL. A triple quadrupole ICP-MS instrument was used and in order to maintain plasma stability and avoid any carbon deposit on the cone surface, a low amount of oxygen (5.5%) was added to the argon carrier gas. A plasma torch with narrower diameter was used (1.5 mm i.d. instead of the 2.5 mm i.d.) to maintain plasma stability. The LOQ, defined as the minimum concentration that can be quantitated with acceptable precision, was thus established at 40 pmol L^{-1} (54 ng L^{-1}). Cow's milk and infant formula SRMs with reference vitamin B12 values were used to

determine the method's accuracy and showed recoveries ranging from 103 to 120%. Another paper proposed a novel analytical method for the separation and determination of cobalamin and Co in kefir samples by HPLC-ICP-UV-OES.[186] The LOD values for Co in cobalamin and Co by HPLC-ICP-OES were 0.07 mg kg^{-1} and 0.06 mg kg^{-1}, (as Co) respectively. Recovery results for cobalamin and Co detected by the HPLC-ICP-OES system were calculated in the range of 87–100 and 99–115%, respectively, while recovery results for cobalamin were found to be between 89 and 98% for an HPLC-UV system.

5 Abbreviations

AAS	atomic absorption spectrometry
AB	arsenobetaine
AC	arsenocholine
ADP	adenosine diphosphate
AEC	anion-exchange chromatography
AES	atomic emission spectrometry
AF4	asymmetric flow-field flow fractionation
AFS	atomic fluorescence spectrometry
AMP	adenosine monophosphate
APDC	ammonium pyrrolidine dithiocarbamate
APL	acute promyelocytic leukemia
APTES	3-aminopropyltriethoxysilane
ASU	Atomic Spectrometry Update
BFR	brominated flame retardants
CarbE	carboxylesterase protein
CE	capillary electrophoresis
CEC	cation-exchange chromatography
CEN	European Committee for Standardisation
CFA	continuous flow analysis
CPE	cloud point extraction
CRM	certified reference material
CV	cold vapour
DCM	dichloromethane
DDTP	*o*,*o*-diethyldithiophosphate
DLLME	dispersive liquid–liquid microextraction
DMA	dimethylarsenic (include oxidation state if known)
DMC	dithiocarbamate-modified cellulose
DMDTA	dimethyldithioarsinic acid (include oxidation state if known)
DMMTA	dimethylmonothioarsinic acid
DNA	deoxyribonucleic acid
DTPA	diethylenetriaminepentaacetic acid
EDTA	ethylenediaminetetraacetic acid
EDX	energy-dispersive X-ray
ERM	European reference material
ES	electrospray
ETAAS	electrothermal atomic absorption spectrometry
EtHg	ethylmercury
EtOH	ethanol
EXAFS	extended X-ray absorption fine structure
FAAS	flame atomic absorption spectrometry
FI	flow injection
FT	Fourier transform
FTIR	Fourier transform infrared
GBCA	Gd-based contrast agent
GC	gas chromatography
GF	graphite furnace
GPC	gel permeation chromatography
GSH	glutathione
HAS	human albumin serum
HC	hydrodynamic chromatography
HG	hydride generation
HILIC	hydrophilic interaction liquid chromatography
HMM	high molecular mass
HPLC	high performance liquid chromatography
HR	high resolution
iAs	inorganic arsenic
IC	ion chromatography
ICP	inductively coupled plasma
ICR	ion cyclotron resonance
ID	internal diameter
IDA	isotope dilution analysis
IDMS	isotope dilution mass spectrometry
iHg	inorganic mercury
IP	ion pair
ISO	International Organization for Standardisation
LA	laser ablation
LC	liquid chromatography
LDR	linear dynamic range
LEP	liquid electrode plasma
LIB	lithium ion batteries
LIBS	laser-induced breakdown spectroscopy
LLE	liquid–liquid extraction
LLME	liquid–liquid microextraction
LMM	low molecular mass
LOD	limit of detection
LOQ	limit of quantification
MAD	microwave-assisted digestion
MAE	microwave-assisted extraction
MC	multicollector
MDMSPE	magnetic dispersive micro solid phase extraction
MeHg	methyl mercury
MeOH	methanol
MeSeCys	methylselenocysteine
MIL	magnetic ionic liquid
MIP	microwave induced plasma
MMA	monomethylarsenic
MMMTA	monomethylmonothioarsonic acid
MOP	metal–organic polymer
MPBET	modified physiologically-based extraction test
MPTMS	3-mercaptopro-pyltrimethoxysilane
MRI	magnetic resonance imaging
MS	mass spectrometry
MSIS	multi-mode sample introduction system
MSPE	magnetic solid-phase extraction
MT	metallothionein
MW	molecular weight
NADH	nicotinamide adenine dinucleotide
NIST	National Institute of Standards and Technology
NMIJ	National Measurement Institute of Japan
NMR	nuclear magnetic resonance
NP	nanoparticle
NRCC	National Research Council of Canada

NRCCRM	National Research Centre for Certified Reference Materials (China)
OT	oxytocin
PAH	polyaromatic hydrocarbon
PD	point discharge
PFA	perfluoroalkyl
PhHg	phenylmercury
PMBP	1-phenyl-3-methyl-4-benzoyl-5-pyrazone
PMI	primary methylation index
PM_x	particulate matter (with an aerodynamic diameter of up to x μm)
PVA	polyvinylalcohol
PVG	photochemical vapour generation
pXRFS	portable X-ray fluorescence spectrometry
Q	quadrupole
RI	refractive index
RNA	ribonucleic acid
RP	reversed phase
RSD	relative standard deviation
SAX	strong anion exchange
SCN	thyocianate
SDS	sodium dodecylsulfate
SEC	size exclusion chromatography
SeCN	selenocyanate
$SeCys_2$	selenocystine
SeEt	seleno-D,L-ethionine
SELENOB1	Se binding protein 1
SELENOB2	Se binding protein 2
SEM	scanning electron microscopy
SeMet	selenomethionine
SeUr	selenourea
SF	sector field
SMNP	silica-coated magnetic nanoparticles
sNP	single nanoparticle
SPE	solid phase extraction
SPME	solid phase microextraction
SR	synchrotron radiation
SRM	standard reference material
ssIDMS	species specific isotope dilution mass spectrometry
TBAB	tetra-*n*-butylammonium bromide
TBT	tributyltin
TCED	tris-2-carboxyethyl phosphine hydrochloride
TCEP	tris-2 carboxyethylporfine
TDA	Taylor dispersion analysis
TEM	transmission electron microscopy
THF	tetrahydrofuran
TLC	thin layer chromatography
TMAH	tetramethylammonium hydroxide
TMAO	trimethylarsine oxide
TMMTSb	trimethylmonothioantimony
TMSb	trimethylantimony
ToF	time-of-flight
TRIS	tris(hydroxymethyl)aminomethane
TSP	total suspended particles
UAE	ultrasound-assisted extraction
UBM	unified bioaccessibility method
UV	ultraviolet
UV-vis	ultraviolet-visible spectrophotometry
XANES	X-ray absorption near-edge structure
XAS	X-ray absorption spectroscopy
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction
XRF	X-ray fluorescence

Conflicts of interest

There are no conflicts to declare.

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