JAAS

ROYAL SOCIETY OF CHEMISTRY

ASU REVIEW

View Article Online
View Journal | View Issue

Cite this: *J. Anal. At. Spectrom.*, 2026, 41, 819

Received 29th January 2026

DOI: 10.1039/d6ja90007k

rsc.li/jaas

Atomic spectrometry update: review of advances in the analysis of clinical and biological materials, foods and beverages

Marina Patriarca, *[a] James P. C. Coverdale, [b] Alan Cross, [c] Montserrat González-Estecha, [d] Sarah Hill, [e] David Milde [f] and Julian Tyson [g]

[a]*Rome, Italy. E-mail: m.patriarca@outlook.it; Web: https://www.linkedin.com/in/marina-patriarca-361b832b*

[b]*School of Pharmacy, School of Health Sciences, College of Medicine and Health, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*

[c]*Thames Water PLC, Spencer House, Manor Farm Lane, Reading, Berkshire, RG2 0JN, UK*

[d]*Hospital General Universitario Gregorio Marañón, Calle del Doctor Esquerdo no. 46, 28007 Madrid, Spain*

[e]*LGC, The Priestley Building, 10 Priestley Road, Guildford, GU2 7XY, UK*

[f]*Department of Analytical Chemistry, Faculty of Science, Palacky University Olomouc, 17. listopadu 12 771 46, Olomouc, Czech Republic*

[g]*Department of Chemistry, University of Massachusetts Amherst, 710 North Pleasant Street, Amherst, MA 01003, USA*

In this Update period, there were no important developments for AAS, whereas, for OES, options such as alternative excitation sources and miniaturisation were investigated. The multielement capabilities and low detection limits of ICP-MS continued to attract the attention of most users and this was also the technique of choice for developments in pharmaceutical and dietary supplement analysis. Sustained interest was observed for spICP-MS and scICP-MS applications, focussing on sample preparation and introduction, calibration strategies and approaches to interference removal. The ability of LIBS to measure molecular species was exploited to develop an isotope dilution analysis method for the detection of Ca in tap water and milk powder and to tackle bacterial detection in urine. Numerous examples of EDXRF applications were reported including a systematic evaluation of the next generation of portable monochromatic excitation EDXRF systems for the analysis of food, plant and biological CRMs. Both XRF and LA-ICP-MS play a key role to identify element spatial distribution in cancer tissues. Several papers addressed correction and quality control approaches for tissue imaging. Beside instrumental improvements, a substantial number of publications reported digestion, extraction and preconcentration procedures, mostly applied to food. Vapour generation procedures and AFS were employed by several researchers to obtain better detection limits for both elements and their species in various biological matrices. Measurements of nanoparticles, as well as their application for extraction or preconcentration of chemical elements, are now widespread and the relevant developments are dealt with in the analytical technique or application sections, rather than in a separate section. Following the trend seen in previous years, the application of atomic spectrometry to the indirect measurement of complex molecules continued and expanded from research in the clinical area to the detection of pathogens in food. An approach, based on reaction cell chemistry, enabled the determination of volatile organic compounds by ICP-MS. Multielement applications to clinical samples with improved detection limits were reported. A few studies revisited the use of more accessible specimens, such as dried blood spots and samples from the respiratory tract or from biobanks. Element speciation in food, beverages and clinical samples was investigated by several researchers. Beside As, Hg and Se, which appear regularly in this series of Updates, new approaches to the speciation of Cu in serum in relation to Wilson's disease and Alzheimer's disease were reported. Following the trend seen in the last few years, the number of papers focusing on chemometrics to identify origin and authenticity of foods and traditional medicines has significantly increased. Both LIBS and EDXRFS are frequently used for this purpose, but the focus of the work is often the processing of the obtained analytical data. A new section describes applications to "novel food ingredients", particularly plant-based food and alternative protein sources, in which consumers' interest is growing. To support the quality of analytical measurements, the satisfactory analyses of CRMs is highlighted and publications reporting innovative calibration strategies, novel interlaboratory comparisons and new or revisited CRMs are addressed.

1 Introduction

This is the 41st annual review of the application of atomic spectrometry to the chemical analysis of clinical and biological materials, foods and beverages. The Update follows on from last year's [1] and is a critical review of the relevant literature published from the second half of 2024 through the first half of 2025. It continues the series of Atomic Spectrometry Updates (ASU) on this topic and should be read in conjunction with the five other ASUs published during the same period, reporting advances in X-ray fluorescence spectrometry and its special applications,[2] analysis of metals, chemicals and materials,[3] environmental analysis,[4] atomic spectrometry and related techniques,[5] and elemental speciation.[6] Conventions used in this and other ASUs include the use of an italicised word or phrase close to the beginning of each paragraph to help the reader to identify its subject area. Different tenses are used to clarify whether the information presented comes from the authors (past tense) or represents the views of the ASU reviewers (present tense). Abbreviations following a list shared among all ASUs are listed at the end without further explanation in the text. Non ASU abbreviations are defined in the text at their first appearance and also listed at the end, except those that appear only in one paragraph or section.

2 Reviews

Following the fast developments of applications of atomic spectrometry to clinical specimens, drugs and traditional medicines, food and beverages, including indirect measurements of molecules, novel food and authenticity studies, we note a number of reviews addressing general trends or specific aspects relevant to these topics over this Update's period. Some of them are briefly mentioned here, whereas more specific reviews are discussed in greater detail in the context of the relevant sections.

The role of elements and their species in relation to human health is an important area of research demanding improved and reliable analytical methods. Several techniques are available, but ICP-MS attracts most attention because of its high sensitivity and the ability for speciation, single cell and single particle analysis. A group of researchers[7] reviewed applications of this technique for cancer research (105 references). In particular, they noted that beside the role of ICP-MS in the direct measurement of element changes in biological fluids and tissues of cancer patients *vs.* control subjects, *emerging features of the technique, such single cell analysis, enabled insights of biological events occurring at the cellular level*. This capability also provided support to research devoted to the design of metal-based anticancer drugs and the evaluation of their uptake, distribution and mechanism of action inside cells. Furthermore, the authors highlighted the emerging exploitation of ICP-

MS as a detector to identify target biomolecules, through elemental labelling (*e.g.* of antibodies or nucleic acids), which is further discussed in Section 6 of this Update. Given the capability of CE-ICP-MS for the simultaneous detection of elemental species, Matczuk *et al.*[8] investigated the application of this technique in biomedical research, in particular focussing on the detection of metal-based anticancer drugs, metal-based nanomaterials, and metal ions relevant for human health in samples of biological origin (*e.g.* cancer cells, CSF, serum). Therefore, they reviewed the latest literature for the period between 2019 and early 2024 (91 references), specifically addressing examples of element speciation supported by reliable information on method performance as well as any technical issues or limitations. A tutorial approach to the technique was not deemed necessary, but the paper included a scheme showing the workflow of CE-ICP-MS, and presented best approaches for separation strategies and ICP-MS settings, based on real-world examples. Another group of researchers[9] noted that AAS may play *an important role in antibacterial research* and presented a review of 49 references to highlight the versatility of this technique, illustrated by a variety of practical examples. Beside the measurement of metal-containing drugs, AAS had been applied also to the determination of antibiotics of solely organic origin (*e.g.* tetracyclines, cephalosporins, and fluoroquinolones) after complexation with a metal, providing a suitable alternative for molecules that cannot be detected by other conventional techniques, such as UV-VIS or GC. Applications spanned from routine quality control of pharmaceutical preparations to investigations of drug mechanisms of action and pharmaceutical profiles, requiring their measurement in biological fluids and cells. They noted that, in most cases, sample preparation was simple, the analysis was fast and did not require large amounts of organic solvents, thus qualifying as a "green" approach. In a review covering 160 references, Adesina *et al.*[10] addressed *recent developments enhancing the capabilities of ICP-MS and XRF for the determination of elements in human specimens* (*e.g.* hair, nails, blood, bone, and tissues) and stressed the importance of a careful selection of the technique more suitable for the task at hand, considering analytical sensitivity, the complexity of the analysis, time requirements and cost-efficiency. They highlighted that, although the sensitivity of ICP-MS remained unmatched, XRF offered the advantages of less demanding and faster sample preparation and non-destructive analysis and also noted the increasing popularity of portable XRF instruments.

Blaimer and Leopold[11] focussed on *metal-containing NPs, present in biological and biomedical samples, as well as in food and personal care products*. They examined 112 references over a period of five years to provide an overview of the analytical methods proposed for the identification, characterisation, and quantification of metal NPs in these types of samples. The findings were summarised in a table, providing analytical details and the relevant LODs and LOQs for size and concentration of metal NPs. They reported that element specific detection was achieved primarily using ICP-MS, in a variety of combinations with other techniques (AF4) or specific settings (ICP-TOF-MS, spICP-MS, LA-spICP-MS). However, they noted that GFAAS, allowing direct analysis of a variety of samples, often after minimal preparation, offered advantages over more complex techniques, in terms of simpler and faster analysis, and, more importantly, limiting the risk of unwanted changes in metal NP size, aggregation or dissolution during sample preparation. Appropriate strategies based on different atomisation temperatures also enabled discrimination of metal NPs of different sizes. In addition, they mentioned a few successful applications of both ICP-OES and benchtop TXRF.

Plants play an increasingly important role in both human nutrition and as herbal medicines, but concern is also rising for their content of trace elements, that may, in some cases, pose a risk to human health. A comprehensive review of the advancements of methodologies for the determination of trace elements in plants and plant extracts, based on 329 references, was presented by Rawat *et al.*[12] The authors discussed the advantages and drawbacks of analytical techniques (AAS, ICP-OES, ICP-MS, NAA and XRF) and sample preparation methods for various herbal parts (*e.g.* roots, stems, bark, fruits), such as MAD and SPME, providing several examples of their applications. Another group of researchers[13] reviewed determinations of chemical elements by GFAAS in medicinal plants and plant-based remedies (97 references). The authors highlighted the main advantages of GFAAS, as a versatile, simple, sensitive, and comparatively low-cost technique for this task. They reported numerous examples of the analysis of raw materials, *e.g.* herbs, leaves, flowers, roots, rhizomes, barks, seeds, soil, as well as prepared medicines such as tablets, capsules, syrups, and ointments. For each application, details of the type of sample analysed, sample preparation (ashing, digestion, dilution, solid sampling), temperature programme, chemical modifiers used (mostly $Mg(NO_3)_2$, Ni, Pd, $NH_4H_2PO_4$, ascorbic acid), and analytical performance were given.

Over the past years, the quality of food, linked to species *authenticity and origin traceability is gaining increasing interest*. Elemental profiles coupled with advanced data analysis techniques play a key role for classification and discrimination between original products and adulterated ones. New developments in this area are described elsewhere in this Update (Section 10.2.10 and Table 2). It is also worth noting the work of Soni *et al.*[14] who specifically reviewed more than 200 papers reporting analytical techniques and statistical methods for multivariate analysis applied to determine the origin of soybeans, a type of food in increasing demand, which in turn increases the risk for lower quality and adulteration. The authors described applications of FTIR, GC-FID, GC-TOF-MS, HPLC-MS, IRMS, LC-Orbitrap-MS, NIR, NMR, and terahertz spectroscopy, as well as techniques for trace element analysis (EDXRF, ICP-MS, NAA), and discussed their relative benefits and shortcomings.

3 Metrology: inter-laboratory studies, reference materials and calibration strategies

The word "metrology" addresses terms and concepts forming a recognised part of the harmonisation of good practices in the

work of analytical laboratories and in related areas, such as the production of RMs and the organization of interlaboratory comparisons (ILCs) or PTs. In this Update, we report advances in the areas of ILCs, CRMs and two innovative calibration strategies.

Comparing results among laboratories can help identifying errors in laboratory work and *ensure their measurement results are reliable and consistent with other laboratories or international standards.* A recent ILC has focused on the determination of the content of 21 elements (As, Br, Ca, Cl, Co, Cr, Cu, Fe, Hg, K, Mn, Mo, Ni, P, Pb, Rb, S, Se, Sr, Ti, and Zn) in human hair.[15] The authors evaluated results from six laboratories from five countries, applying TXRF (three laboratories), μEDXRF (one laboratory), ICP-OES (one laboratory), and ICP-MS (two laboratories). Despite the small number of participants, the authors implemented statistical analysis of the results separately, for the TXRF measurements and for the results obtained using all other measurement techniques. The laboratories using TXRF were required to analyse the sample according to the procedure proposed by the ILC organisers (25 mg of hair mixed with 100 μL of 100 mg L^{-1} Ga as IS and concentrated HNO_3, left for 24 h at room temperature, then put into a microwave oven at 900 W for 10 s). Quality control for the TXRF measurements was performed using a RM (IAEA-086, Human hair). Results from all methods passing the outlier rejection procedures were used to calculate the consensus mean value (as an arithmetic mean) and the corresponding SD. Moreover, for TXRF, the average interlaboratory RSDs were calculated separately and were found to be between 30 and 40%. The smallest RSDs (<30%) were observed for Br, Ca, Cl, Cr, Pb, Rb, S, Se, Sr and Zn. These elements included both light and heavy elements, with low and high concentrations in hair samples. The Asia Pacific Metrology Program and the Asia Pacific Laboratory Accreditation Cooperation set up an international PT program for Cd in infant formula,[16] using Cd values assigned independently using ID-ICP-MS. The organiser prepared the test material by spiking 40 kg of infant formula with a ^{111}Cd solution. The PT items' homogeneity and stability were tested according to ISO 13528. The assigned value was 0.255 ± 0.016 mg kg^{-1} Cd (expanded uncertainty, $k = 2$) and the average of participants' results was 0.216 mg kg^{-1} with an SD of 0.064 mg kg^{-1}. The performance of participating laboratories was evaluated using a z score, where the standard deviation for performance assessment (σ_{PT}) was set based on the Horwitz equation. Out of 62 laboratories that met the deadline for returning results, 49 achieved acceptable performances ($|z$ score$| \leq 2$) and five provided unsatisfactory results ($|z$ score$| > 3$). For information, laboratory performance was also assessed using the zeta (ζ) score, that includes the measured result's uncertainty. Thirty-two laboratories reported satisfactory results with $|\zeta| \leq 2$. This scoring method could not be applied to 13 laboratories, which did not report measurement uncertainty.

The preparation of a new CRM for Hg in fish (CRM INM-039-1) was recently reported, describing its development and certification.[17] The chosen matrix was striped catfish from the Colombian Amazon. Five specimens, approximately 7 kg each, from Leticia (Colombia), were used for production. After filleting, skin removal, and bone extraction, the fish muscle tissue underwent freeze-drying, degreasing, grinding, homogenization, aliquoting and packaging, and sterilisation. The normalised production yielded 119 bottles containing 15 g of the CRM candidate. The process of characterisation involved raw material selection, sample preparation, homogeneity, and stability testing, as well as establishment of a certified value and associated expanded uncertainty for the total Hg mass fraction (3.94 ± 0.27 mg kg^{-1}, 95% confidence, $k = 1.97$) and an informative value for MeHg (3.79 ± 0.31 mg kg^{-1}). The main sources of uncertainty identified during production were characterisation and long-term stability. Characterisation for total Hg involved two techniques (ICP-MS and CV-AAS), implemented in one laboratory, after MAD with HNO_3 and H_2O_2. For MeHg, only GC-MS was applied after TMAH extraction, and therefore, this value was not certified. The developed CRM exhibited comparable total Hg and MeHg mass fractions to ERM-CE464 (total and methyl mercury in tuna fish) and additionally, in terms of stability, homogeneity, and particle size, it was also comparable to other similar CRMs such as NRCC DOLT (Dogfish liver) 4 and DORM 4 (Fish protein).

Naturally occurring uptake from the environment or inadvertent introduction of toxic elements (such as As, Cd, Hg, and Pb) is a potential source of exposure to consumers of botanical dietary supplement products, the majority of the dietary supplement RMs produced by NIST do not have certified values for these elements. Fong Sam *et al.*[18] set *the determination of As, Cd, Pb, and Hg content in the current inventory of NIST botanical dietary supplement SRMs/RMs* as an objective of their research. Results for As, Cd, Pb and Hg, using MAD with HNO_3 followed by quantification by ICP-QQQ-MS (reporting isotopes ^{75}As, ^{111}Cd, ^{201}Hg and sum of ^{206}Pb, ^{207}Pb and ^{208}Pb for Pb) were provided for 27 dietary supplement SRMs/RMs, and 11 candidate materials, still in preparation. Two test portions from three packets or bottles of each material were analysed, yielding six analytical results for each of 33 SRMs/RMs treated as unknowns for this study, whereas five SRMs with certified values for all four elements were selected for use as control materials. The results of this study demonstrated that the available botanical SRMs/RMs provided a consistent, continuous range of Cd, Hg and Pb mass fractions (10^1–10^4 mg kg^{-1}). One identified mass fraction gap for these SRMs/RMs was for As, where there was two orders of magnitude difference between most of the botanical materials (10^2 mg kg^{-1}) compared to the ginger and kelp materials, with extremely high As mass fractions (10^4 mg kg^{-1}).

In this Update, we report on *innovative calibration strategies* implemented in the field of atomic spectrometry. Zhou *et al.*[19] presented an evaluation of the application of online multiple IS calibration for the determination of I concentrations by ICP-QMS. The proposed approach streamlined the calibration process by requiring only a single standard solution, thereby enhancing sample throughput and minimising liquid waste. Eight IS elements (Ge, In, Lu, Re, Rh, Sc, Tb, and Y) in aqueous solution, along with a single I calibration standard, were introduced into the ICP-MS *via* a Y-connection. Briefly, by plotting ratio of signal intensities ($I_{sample} : I_{IS(i)}$ *vs.* ratio of signal intensities $I_{standard} : I_{IS(i)}$) for all eight elements used as ISs, the

slope of that plot equalled $c_{sample}/c_{standard}$, which may be rearranged and used to determine the concentration of analyte in the sample. The new methodology was validated through the analysis of six CRMs for I in water (09113i, 09114i), urine (09109n, 09110i), and salt (1006 h, 1007 h), respectively, obtained from the National Iodine Deficiency Disorder Laboratory in Beijing, China. For liquid CRMs, the agreement with certified values, ranging from 8.5 to 226 μg of L^{-1} I, was between 96.6 and 102.5%. The measured values for the two salt CRMs, corresponded to 100% and 103.5% of the certified values of 11.5 and 23.7 mg kg^{-1} I, respectively. The achieved LOD (3 s approach) was 0.18 μg L^{-1} and linearity for calibration lines gave r^2 = 0.9997. The developed multiple IS calibration was shown to provide results comparable to those obtained with the Chinese national standard method – based on the catalytic action of I^- on the redox system cerium^{IV}–arsenic^{III} – for water and urine, the titration method for salt as well as an ICP-MS method with external standard calibration and a single IS. Calibration by Proxy, a matrix-matched calibration strategy using multiple IS species to build a calibration curve, was described by Jones *et al.*[20] The technique required only two solutions: solution 1 containing a sample solution and a suite of ISs (Ge, In, Sc, Tm, Y and Yb) at known concentrations, and solution 2 identical to solution 1, plus an aliquot of a standard containing all analytes of interest and an additional aliquot of a solution containing the ISs. The concentration of both analytes and ISs in solution 2 was 1 mg L^{-1} higher than that in solution 1. The calibration curve was prepared by plotting the signal measured for each IS in solution 1 divided by the signal arising due to the aliquot of IS added to solution 2 on the *y*-axis. Therefore, the *y*-axis value measured for a specific IS was identical to the value that would be measured for any other element present at the same concentrations in the two solutions (each IS serves as a proxy for analyte values). The concentrations of IS in solution 1 were plotted on the *x*-axis, and these corresponded to any analytes present in solution 1 at the same concentration. This calibration method was applied to the determination of As, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Mo, Sr, and Zn in five CRMs (NIST 1568a Rice flour, 1566b Oyster tissue, 1577b Bovine liver, 1573a Tomato leaves and 1547 Peach leaves) by ICP-OES after MAD with a mixture of HNO_3–H_2O_2–H_2O. The elements used as IS to construct the calibration curve were Ge, In, Sc, Tm, Y and Yb. Percent recoveries for analytes ranged from 89 to 106%, with RSDs of the order of 1%. The LODs for 18 elements (As, Be, Ca, Cd, Co, Cr, Cu, Mg, Mn, Mo, Ni, Pb, Sb, Se, Tl, U, V and Zn) obtained from 10 determinations of a sample blank varied from 2 μg L^{-1} to 10 μg L^{-1}. A further study by the same authors[21] explored multi-channel dilution analysis. This novel, matrix-matched analytical calibration technique, built upon the concept of standard dilution analysis and combining traditional standard addition and IS calibration methods, allowed to correct for both matrix interferences and fluctuations in signal levels associated with variations in biological samples. The method required the preparation of only two solutions: the first one consisting of 50% sample and 50% blank with one IS (Y), and the second consisting of 50% sample and 50% of a standard solution containing all analytes of interest at known concentrations and a second IS (Sc). Multiple dilutions of the standard were made automatically as the solutions travelled from the autosampler to the ICP-OES instrument, through multiple channels of different tubing diameters and lengths. This setup resulted in a signal "stairstep" as portions of solution are measured at different points in time. A calibration curve was prepared from the plateau regions of the signal stairstep. Ten elements (As, Ba, Cd, Cu, Fe, Mg, Mn, Mo, Sr, Zn) were successfully measured in three CRMs (NIST 1566b, Oyster tissue; NIST 1577b, Bovine liver; NIST 1573a, Tomato leaves) after MAD digestion with HNO_3–H_2O_2–H_2O. The LODs for this calibration technique using ICP-OES were similar to those obtained through traditional ICP-OES measurements, on the order of single-digit μg L^{-1} for most analytes. Multi-channel dilution analysis simplified the analytical process, increasing sample throughput by decreasing the time required for the preparation of the solutions. An unconventional calibration strategy, combining standard addition and IS, was implemented for the determination of Cr, Li, and Ni in blood serum, cough syrup, and a protein supplement by means of HR-CS-AAS.[22] Cobalt and Mn were used as ISs. Only two solutions were required: the first corresponded to a sample fortified with the element of interest and IS, and the second was a mixture of the same sample and the corresponding blank. Both solutions were gradually mixed (and diluted using a syringe pump), emulating an automatic standard addition procedure. Adding IS allowed the method to correct the sensitivity variations produced by possible physical interferences generated during nebulisation. No sample pretreatment, except for sample dilution, was needed. Conditions such as physical fluctuations of absorbance due to EtOH content (0–100% v/v), linearity range, and matrix effect (standard addition studies with real samples) were evaluated. The developed method introduced some improvements, such as automation of the process, substantial reduction of the volume generally used in FAAS, and an effective reduction of interferences. The achieved LODs were 0.71 mg L^{-1}, 0.41 mg L^{-1}, and 0.03 mg L^{-1} for Cr, Li, and Ni, respectively. The results were compared with data obtained with conventional external calibration, unfortunately no matrix CRMs were analysed.

In a proof-of-concept study by Bazo *et al.*,[23] both intra- and extracellular Se content in yeast CRM cells (NRCC CRM SELM-1, Se-enriched yeast cells) were accurately, directly, and simultaneously *determined for the first time from the time-resolved scICP-MS signal with two novel NP based calibration strategies.* Regarding intracellular determination, firstly, a widely used strategy for calibrating scICP-MS signals was applied, consisting of calculating the transport efficiency (TE) with the particle size method and correlating the intensity of the cell events with their corresponding mass. This procedure yielded an average intracellular Se content of 68.9 ± 7.3 fg per cell (1.23 ± 0.13 mg kg^{-1}). Secondly, two alternative NP-based calibration strategies, independent from TE, were evaluated for the quantitative determination of the intracellular Se content: (i) external calibration, which gave a value of 61.7 ± 1.1 fg Se per cell (1.10 ± 0.02 mg kg^{-1}) and (ii) a relative method using NP standards of the same target element, providing a result of 60.7 ± 3.0 fg Se

per cell (1.08 ± 0.05 mg kg^{-1}). In addition, the extracellular Se (dissolved fraction) was determined directly and simultaneously, using the average background from the scICP-MS time-resolved signal, yielding a value of 0.96 ± 0.03 mg g^{-1}. After the determination of the extracellular Se contents, the accuracy of the different calibration strategies was evaluated by comparing the sum of the intracellular and extracellular Se (external calibration: 2.06 ± 0.03 mg g^{-1}; relative method: 2.04 ± 0.05 mg g^{-1}) with the results obtained for total Se by ICP-MS after cell digestion (2.030 ± 0.020 mg g^{-1}), and the certified total Se mass fraction 2.031 ± 0.070 mg g^{-1} of the SELM-1, achieving very good agreement ($102 \pm 2\%$ and $101 \pm 3\%$, for the external calibration and relative method, respectively).

4 Sample collection and preparation

4.1 Collection, storage and preliminary preparation

In the period covered by this Update, there have been fewer publications presenting novel aspects regarding the collection, storage, and preliminary preparation of matrices that have been used for a long time or on a routine basis. The most novel studies focused on other *more accessible matrices that have been the subject of controversy in the past*, such as biobank blood matrices, dried blood spots (DBS) or samples from the respiratory tract. The use of archived blood samples from biobanks for trace element analysis is incredibly valuable for retrospective environmental exposure assessment and epidemiological studies. However, this practice is fraught with both technical and ethical/legal controversies, such as risk of contamination in the collection devices or during long-term storage, as well as long-term stability and preanalytical variability. This variability includes factors like time of processing, the type and concentration of anticoagulants or chemical additives, and freeze–thaw cycles. Further challenges include the difficulty in comparing elements across different matrices (plasma, serum, or whole blood), and controversies arising from the use of stored samples for a purpose other than that for which they were obtained.

In this regard, Guerra *et al.*[24] featured a comprehensive assessment of matrix effects on the measurement of the concentrations of 27 metals (Al, As, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, I, K, Mg, Mn, Mo, Ni, Pb, Rb, Se, Sn, Sr, Ti, Tl, U, V and Zn) by ICP-MS *in human blood serum as well as in plasma samples, collected with different anticoagulant agents* (citrate, EDTA and heparin). The study innovation, offering insights for biobank planning, lies in comparing blood serum with three distinct plasma types in samples collected from 20 healthy volunteers, with all four matrices obtained from the same venipuncture. The procedure involved a rapid "dilute and shoot" approach, also reducing potential contamination. Serum and heparin-plasma samples were diluted 1 + 9 with 0.1% HCl. A higher dilution rate (1 + 19) was necessary for citrate-plasma and EDTA-plasma, due to their elevated sodium content and chelating properties, which suppressed ionization and crystallized in the sampler vials and the injector requiring more rigorous maintenance. To evaluate the influence of anticoagulants, serum was selected as the reference matrix. Heparin-plasma and serum offered the most consistent measurements. Citrate- and EDTA-plasma analysis displayed higher variability, when compared with their corresponding concentration in serum. In the case of trisodium citrate, only As, Mo, Pb and Rb were found to have concentrations not significantly different from serum levels. Dipotassium EDTA showed similar behaviour to citrate, but also prevented the measurement of K in the sample. Additionally, K_2EDTA caused elevated levels of haemolysis compared to other anticoagulants, resulting in higher Fe concentrations. Specifically, Ca, K and Mg and are not recommended to be determined in EDTA and citrate plasma. The higher variability in citrate and EDTA plasma is likely due to contamination from collection tubes, metal complexation with the anticoagulants or haemolysis effects. These findings provided critical evidence for the planning of large-scale epidemiological studies and biobank design, emphasizing the necessity of selecting the appropriate blood matrix and collection tubes to ensure accurate and reliable elemental analysis.

The use of DBS, as a straightforward sample collection method for metals analysis, is experiencing renewed interest, having been used in the past particularly for the screening of PTEs in large-scale epidemiological studies, especially those focusing on children's health. In addition to being a less invasive sampling method, DBS cards are easy to store and transport without refrigeration, which dramatically reduces costs and simplifies logistics. They also present great potential for retrospective analysis of archived newborn screening spots. However, their use also implies challenges and limitations, such as the haematocrit effect on the sample, contamination of the filter paper, ensuring precision and accuracy for the measurement of low, baseline environmental levels of metals compared to traditional venous blood samples, the lack of universal standardization for DBS collection, storage, and extraction protocols across laboratories, as well as ethical controversies around the secondary use of residual newborn DBSs. Two publications reported using this type of sample collection. Cheng *et al.*[25] described an analytical method using the DBS technique coupled with ICP-MS for the measurement of whole blood concentrations of Cu, Fe, Mg, Se and Zn. The procedure involved collecting 20 μL of whole blood onto a DBS card, drying it, cutting out the blood spot, and placing it into a 15 mL centrifuge tube. These elements were extracted with a solution of 0.5% (v/v) HNO_3–1 g L^{-1} cysteine–0.01% (v/v) Triton X-100 by ultrasonic shaking for 40 min and vortex shaking for 1 min, followed by centrifugation (8000 *g*, 5 min). The extracts were filtered through 0.22 μm PTFE syringe filters and were analysed by ICP-MS. The method developed for the measurement of elements by the DBS technique met US FDA validation criteria[26] for linearity, short term and intermediate precision (RSD < 10%), and recovery of spikes added to DBS samples (93.8–99.3%). The linearity ranged up to 2000 ng mL^{-1} for Fe, Mg and Zn. The LODs ranged from 0.002 ng mL^{-1} (Cu) to 0.34 ng mL^{-1} (Mg). The primary limitation is that the DBS technique is restricted to whole blood samples. Another study by Krmpotic *et al.*[27] presented a systematic investigation of the direct analysis of dried spots of human whole blood and serum samples for multielement determination by PIXE. The analytes quantified were: Ca, Fe, K, Mg, Na, P,

S and Zn in whole blood, and Ca, K, Mg, Na, P and S in plasma. The procedure involved spiking 50 μL of sample with V as the IS, to a concentration of 20 mg L^{-1} V, depositing the mixture onto filter paper discs, drying under ambient conditions, and performing direct PIXE measurement *in vacuo*. The method's reliability was assessed analysing RMs (Seronorm® Trace Elements Whole Blood and Trace Elements Serum; IAEA-A-13 Animal blood) under the same conditions. Results showed good agreement when compared to ICP-OES after acid digestion. The LODs in whole blood were within the range 2–5 mg L^{-1} for Ca, K, Mg, P and S and at the level of 1 mg L^{-1} for Cu, Fe and Zn. The highest LOD (25 mg L^{-1}) was obtained for Na. The LOD for Cu was too high for reliable quantification by PIXE. The essential element Cl was not addressed due to a lack of RMs, despite being detectable by PIXE at relevant concentrations. Filter paper impurities contributed significantly to the background, particularly for Ca and Fe, necessitating blank subtraction. Significant differences were observed between PIXE and ICP-OES results, for Fe concentrations in some real whole blood samples, potentially due to Fe loss/precipitation during the acid digestion, used for the ICP-OES comparison, requiring further investigation.

Aiming to provide a practical and efficient alternative to more invasive sampling methods for assessing respiratory metal exposure, Adesina *et al.*[28] described a novel procedure for *quantifying metals and metalloids in the upper airway using nasosorption and benchtop EDXRF*. The combination of non-invasive nasal epithelial lining fluid (NELF) collection on an absorbent fibrous matrix with rapid, non-destructive benchtop EDXRF analysis offered an easier, non-destructive, lower cost and quicker alternative to other techniques, such as ICP-MS, for investigating the levels of several elements, including As, Cu, Fe, Hg, Mn, Pb and Zn. The primary advantage of NELF collection over other biological samples lies in its simplicity, comfort, and feasibility for repeated monitoring, that requires no specialized medical setting or trained staff, unlike invasive methods such as bronchoalveolar lavage. This non-invasiveness makes NELF collection rapid, cost-effective, and highly scalable for large cohort studies, while still providing a valuable and concentrated snapshot of biomarkers—including inflammatory mediators, pathogens, and environmental contaminants—directly from the respiratory mucosal surface. The authors described the procedure that involved preparing spiked NELF collection strips that were directly analysed by the EDXRF system. Initially, blank NELF collection strips were measured before spiking with the elements. Strips were then individually spiked with known amounts of elements to create standard samples. This was used to verify that the novel benchtop EDXRF technique could accurately and precisely quantify those concentrations directly from the strips. No extraction or complex sample preparation was required, as the analysis is non-destructive. The standard unit for the measurement results on NELF collection strips is ng per strip as the most suitable unit for this methodological framework to ensure comparability of the results. The average minimum LOD was 0.1 ng per strip, with accuracy within 5% and precision within 10% RSD across triplicates. The authors acknowledged the limitation of the study, that did not include a comparison against an alternative method, such as ICP-MS, for these specific types of samples. In addition, they noted that the calibration method relied on the assumption of a consistent matrix effect, but potential non-uniformity in real-world NELF samples could present a challenge. This approach established a practical and efficient alternative to more invasive sampling methods and more time-consuming analytical techniques, such as ICP-MS for assessing respiratory metal exposure.

4.2 Digestion, extraction and preconcentration

This Update period has seen substantial numbers of publications relating to digestion, extraction and preconcentration. This is especially true for preconcentration by LLE or SPE in methods for the determination of trace elements in food. These, together with the much smaller number of papers describing the analysis of clinical materials, are summarised in Table 1, which is discussed in more detail later in this section. It is now common for researchers to discuss the "greenness" (the environmental and sustainability characteristics) of their new methods, as calculated by one or more green metric scales. Only publications in which the results of the analysis of a CRM (where available and appropriate) were reported are included in this section of the Update, though spike recoveries are considered acceptable when a CRM is not available. There are still publications that do not provide important details of the sample preparation procedure, such as the final volume obtained after solubilising a solid sample prior to implementation of the next stages of the method. This makes it impossible for readers to ascertain the LOD in the original sample, as almost always LODs are given in mass/volume units for the solution produced by the first step of the analysis. Often there is no mention whether (or how) the moisture content of the sample was accounted for, an important step if results are to be compared with those on the certificate of a CRM, which are almost always given on a dry weight basis. Readers are also interested in the throughput of a method, but the time required for an analysis is rarely readily available; readers have to add up the times given for each of the individual steps, and then work out how many samples are being processed in parallel.

4.2.1 Reviews. Two *reviews feature sample preparation* prominently. Grebneva-Balyuk *et al.* examined[29] the preparation of biological samples in the determination of NPs by spICP-MS (114 references). For fluids, the reviewers discussed dilution, filtration (both micro and ultra), centrifugation, sedimentation, fractionation (HPLC, FFF) and various combinations of these. In the case of tissues, the authors discussed hydrolysis (enzymatic and alkaline) and the role of ultrasound in accelerating such processes. They considered LA to be a kind of sample preparation procedure that yielded spatially resolved information, though they pointed out the potential problem of changes in NP size distribution on interaction with laser radiation above a power density of 1 J cm^{-2}. The authors cited examples of the determination of NPs of Ag, Au, Pd and Pt and oxides of Ce, Fe, Si, Ti and Zn. In reviewing the determination of Be at ultra-trace concentrations by procedures in which a spectroscopic

technique (both atomic and molecular) was used, Nemcek and Hagarová[30] covered (117 references) mainly environmental waters, but did include several examples of the analyses of food and beverages. They pointed out that even for techniques with low LODs, such as ETAAS, preconcentration was needed and they comprehensively covered all types of LLE and SPE, highlighting that with vesicle-mediated DLLME and ETAAS, LODs as low as 0.01 ng L^{-1} could be obtained.

4.2.2 Modified microwave aided digestion. A number of papers have reported on relatively minor variations to the standard procedure of digestion with HNO_3 and H_2O_2 in a sealed vessel in a scientific microwave oven.

To improve the green characteristics of a method for the determination of multiple elements in biological matrices by either ICP-OES or ICP-MS, da Silva *et al.*[31] devised a *miniaturised MAD with only H_2O_2*. They analysed human urine and serum and a plant material (soybean callus). For the liquid samples, 100 μL was taken and digested with 300 μL of concentrated H_2O_2, and for the solid, 50 mg was digested with 500 μL of H_2O_2 in sealed PTFE vessels. The heating programme was 5 min at 400 W, 20 min at 790 W, and 3 min at 320 W. The final volume was specified as 3–5 mL. The method validation included the analysis of two RMs: RECIPE ClinChek® serum control (lyophilised, for trace elements, level II) and ClinChek® urine control (lyophilised, for trace elements, level II). For serum, the relative errors ranged from −28% (Cd, ICP-MS) to +5% (Mg, ICP-OES), whereas for urine they ranged from −18% (Ni, ICP-MS) to +22% (Zn, ICP-MS). For the plant material, they compared the results with those of a MAD involving both HNO_3 and H_2O_2, obtaining results that were not significantly different. They measured the residual carbon content by ICP-OES against citric acid standards to be 0.8%. The LODs ranged from 0.001 (Cd by ICP-MS) to 60 (Ca by ICP-OES) μg L^{-1} and they measured Ca, Cd, Co, Cu, Fe, Mg, Mn, Mo, Ni, Se, and Zn in 20 urine samples, 10 of which were from patients with COVID-19. They found that the concentrations of Ca, Cd, Co, and Mg were significantly higher in the urine of patients with COVID-19.

For the determination of Ca, Cu, Fe, K, Mg, Na and Zn in several processed meats by MIP-OES, Luckow *et al.*[32] developed a *miniaturised MAD procedure for a domestic microwave oven* with only HNO_3 as the reagent. They first mapped the homogeneity of the energy distribution inside the oven, identifying six locations at which digestion vials could be placed. They also calibrated the oven settings in terms of power. For the optimized procedure they placed 40 mg of ground sample into PTFE minivials to which were added 400 μL of concentrated HNO_3. To exert fighter control over the heating rate, a central cup containing 200 mL of H_2O was also placed in the microwave oven, which was then run for 3 min at power level 7 corresponding to 440 W. The digests were diluted to 5 mL and filtered before analysis. The validation included the analysis of two CRMs (NIST SRM 1546, meat homogenate and NRCC TORT-2, lobster hepatopancreas), for which they obtained relative measurement errors ranging from −19% to +18%. The LODs were 0.27, 0.17, 0.33, 0.13, 0.59, 0.86 and 0.25 mg kg^{-1} for Ca, Cu, Fe, K, Mg, Na and Zn, respectively. They applied their method to the analysis of 20 samples of processed meats, including sausage, chicken patè, hamburger, breaded steak, mortadella (Italian cured pork sausage), ham, salami and turkey breast. They found Ca, K, Mg and Na in all samples, Fe and Zn in 19 samples, and Cu in 14 samples.

For the determination of 13 (Ag, As, Ba, Cd, Co, Cr, Cu, Ni, Pd, Pb, Se, Tl and V) elements in 24 foodstuffs by ICP-MS, Pothuraju *et al.*[33] proposed what they called a *microwave-assisted extraction procedure*, but it turns out that their optimized procedure is a MAD with HNO_3 and H_2O_2 in a sealed vessel in a microwave oven. A similar lack of novelty (in the analytical methodology, at least) is found in a paper entitled "improving methods for sample preparation of biological fluids",[34] whose authors described only MADs with a very limited range of acid reagents (HNO_3 and HCl) for the preparation of human blood samples for the determination of As, Cu, Ga, Mg, Ni, and Se by ICP-MS. Similarly for the determination of As, Cd, Hg and Pb in herbal medicines by ETAAS. To *et al.*[35] applied a chemometric optimization to the MAD and found that for a sample mass of 0.809 g, the volume of concentrated HNO_3 was 8.431 mL and that concentrated H_2O_2 was not needed. The digestion parameters of time and temperature were not investigated, nor was the final volume, which was 50 mL. The LODs were 1.0, 0.5, 0.1 and 10 ng mL^{-1} for As, Cd, Hg and Pb, respectively. They measured recoveries (ranging from 90 to 112%) from a blank sample matrix, a commercial herbal medicine that had no detectable concentrations of the elements concerned, which was spiked with three different concentrations of each analyte corresponding to 80, 100 and 120% of the allowable concentration limits. They applied the procedure to the analysis of four herbal medicines, finding Cd and Hg in all of them, and As and Pb in some of them. Much the same approach was adopted by Saber *et al.*[36] for the preparation of tea samples for subsequent determination of As and Pb by ICP-MS. Using the microwave oven manufacturer's recommended heating program and a fixed sample mass of 0.25 g, they investigated the effect of the volume of HNO_3 concluding that 9 mL was best. It is not clear whether H_2O_2 was included; at one point the text indicates that 1 mL was also added. For a reason that was not explained, they diluted the digested solutions to a final volume of 50 mL with a solution of 2% HNO_3 and 0.5% HCl, which could contribute to an $^{40}Ar^{35}Cl^+$ inter reference on $^{75}As^+$. The LODs were not given, but the LOQs were 0.25 and 1.5 μg kg^{-1} for As and Pb, respectively. Recoveries of spikes (50, 100, 400 μg kg^{-1}) ranged from 75 to 89%. They analysed 244 tea samples collected in Egypt from 2023 to 2024 and found As in 95% and Pb in 98% of the samples.

For total element determinations of Hf, Ti and Zr oxides in clinical samples, Gerken *et al.*[37] investigated *dissolution procedures that did not involve HF*. All of the reactions were carried out in sealed quartz vessels that could be pressurized to 120 bar in a microwave oven system. Various combinations of H_2SO_4, H_2O and H_2O_2 were investigated and the results compared with those obtained by digestion with HF. The initial experiments were performed with pure metal oxide nanoparticulate powders that were dispersed in water at a concentration of 1 mg mL^{-1}, but the volumes of these suspensions were not given. For cell spiking experiments, 10 μL of the homogeneous NP slurry were

added to 100 HT1080 cancer cells and for tissue spiking, 20 mg of the CRM EC JRC IRMM BCR®-185R (bovine liver) powder were mixed with 200 μL of the particle dispersion. The method selected consisted of digestion with 1.5 mL 97% H_2SO_4 and 1 mL 30% H_2O_2 (so-called piranha solution) that involves the formation of peroxomonosulfuric acid (Caro's acid) in the pressurized microwave oven at 120 bar and 250 °C for 30 min, after an initial ramping from room temperature to 250 °C over 12 min. They acknowledged that this solution required special precautions, and so H_2SO_4 was first added to the vessels in a cold water bath and then the H_2O_2 was added slowly. As the reagents didn't spontaneously mix, a short vortex agitation was applied when an exothermic reaction was observed, though this was not violent (*i.e.* no foaming, effervescence or deflagration). The recoveries of all metals were between 80 and 100% for both nanoparticulate and microparticulate material. The LODs were given in "ppb" and it was not clear if this referred to solution concentrations (*i.e.* μg L^{-1}) or sample concentration (*i.e.* μg kg^{-1}). The values were 1.9, 0.21, 0.71 ppb for Hf, Ti and Zr, respectively.

4.2.3 Extraction. Despite the ready availability of commercial scientific MAD devices designed to completely mineralize organic matrices, there is still considerable interest in alternative methods in which the analytes are extracted by greener reagents with reduced energy consumption.

Brito *et al.*[38] investigated *extractions with DESs* in the determination of As, Cd, and Pb in plant and food matrices by ICP-MS. They prepared two solvents based on citric acid, malic acid, and xylitol and made a comprehensive chemometric-based optimization study of the conditions for both ultrasound assisted and microwave assisted extractions. They compared the performance of the DES methods with that of conventional MAD with HNO_3 and H_2O_2, obtaining results that were significantly higher than those of the DES methods (the worst case was for Cd, for which the DES result was only 68% of that by the MAD method). However, a comparison of methods on three green metric scales (the Analytical Eco-Scale, GAPI and AGREEprep) showed the DES methods to be superior. Ferreira *et al.*[39] reported the results of a very similar study: the performance of methods in which three DESs based on citric acid plus either xylitol, β-alanine, or malic acid (and water) were used in the determination of the same three elements by ICP-MS. The only sample analysed was a forage grass CRM (*Brachiaria brizantha* cv. Marandu, E1001a) supplied by the Brazilian Agricultural Research Corporation (Embrapa, São Paulo, Brazil). They found that the performance of some of the DESs (in terms of extraction efficiency) was dependent on the method of preparation and that, in a microwave-assisted procedure, none of the solvents could satisfactorily extract all three analytes. For the determination of Se in rice by ICP-MS, Zhang *et al.*[40] investigated the performance of four DESs, selected from 10 different composition of each, for their low viscosities and densities: DES1 (34% guanidine hydrochloride, 21% fructose, 45% water), DES2 (23% guanidine hydrochloride, 32% glycerol, 45% water), DES3 (27.5% citric acid, 27.5% proline, 45% water), and DES4 (30% choline chloride, 25% citric acid, 45% water). Rice samples (0.3 g) were extracted with 9 mL of DES in an ultrasonic bath for 45 min. After dilution to 15 mL, the extract was further cleaned up by centrifugation (10 min) and filtration (0.22 μm). The concentrations determined in a Se-enriched (approximately 60 μg kg^{-1}) rice (and hence extents of extraction) were compared with that obtained by conventional MAD with HNO_3 and H_2O_2, but which was modified by adding a 6-hour heating step (hot plate, 115 °C) to "drive off the acid". The percentage relative extraction efficiencies were 136, 89, 95 and 120%, respectively. They explained the high results as possibly due to partial loss of Se during the conventional strong acid digestion process, a hypothesis that could have been readily tested by the analysis of a CRM. The spike recoveries were about 90% for the MAD, but the spike concentrations were given in μg L^{-1}, which is hard to understand.

Many of these *alternatives-to-conventional-MAD procedures involve UAE*. For the determination of Ca, Mg, Zn, Na, and K in dairy products (milk, yoghurt, cheese) by FAAS, Costa-Santos *et al.*[41] optimized (face centred central composite design) a UAE procedure. Sample (0.5 g) was mixed with 5 mL of 50% (v/v) HNO_3 and allowed to stand overnight before bath ultrasonication for 30 min at either 45 °C (milk) or 70 °C (yoghurt and cheese). The mixture was diluted to 25 mL and filtered. The results were compared with those obtained for a multi-stage, digestion-block procedure with HNO_3 and H_2O_2 for which samples were also predigested with 50% HNO_3 overnight. No significant differences were found except for K in milk and cheese, for which the UAE method provided significantly lower results. Part of the validation procedure involved the analysis of a CRM (NIST SRM 1549, non-fat milk powder), for which the results at 45 °C are clearly significantly lower than those obtained at 70 °C, apart from those for Zn. At 70 °C, the relative measurement errors ranged from −9 to +2%, most of which are probably not significant. They examined the extent to which protein and fat matrix components had been degraded during the procedure with confocal microscopy after tagging of these components with fluorescent dyes and found that the UAE procedure effectively degraded organic matter. In the determination of REEs, together with Sc and Y, in coffee by ICP-MS, Savic *et al.*[42] developed a UAE procedure with HNO_3. Sample (0.25 g) was extracted with 10 mL of 1 mol L^{-1} HNO_3 in an ultrasonic bath (37 kHz, 95 W, 80 °C) for 15 min followed by dilution to 25 mL. The procedure was applied to no fewer than 92 samples comprising ground roasted coffees (37), ground roasted coffees in capsules (12), instant coffees with additives (24), instant coffees (15) and coffee surrogates, and the results compared with those obtained with a conventional MAD procedure with HNO_3 and H_2O_2. The results were shown as log–log plots for which r^2 values of >0.9 were obtained for Ce, La, Nd, Pr, Sc and Y; between 0.7 and 0.9 for Eu, Dy, Gd and Tb; with lower values for Er, Ho, Sm and Tm. The method was also applied to the analysis of a CRM (NIST SRM 1547, Peach leaves). A visual comparison of the results indicates that there were no significant difference between the results and the "values of potential interest to users" (which are given to just one significant figure in Appendix B of the certificate document) for Ce, Eu, Gd, La, Nd, Sc, Sm, Tb and Yb (the only elements for which values are provided by NIST). They concluded that application

of multivariate analysis techniques allowed different types of coffee to be distinguished with clear separation of surrogates from 100% coffee. For the determination of Ca, K, Mg, and P in cocoa honey by MIP-OES, Cavalcante *et al.*[43] optimized a bath UAE procedure with the help of a Doehlert design with multiple responses. The variables investigated included the concentration of HNO_3, the volume of H_2O_2 and the irradiation time, but did not include temperature, ultrasound power, sample volume or final volume. All experiments were carried out at room temperature and presumably at maximum power (170 W, 42 kHz). For a 1.5 mL sample, the optimized conditions were 5.62 mol L^{-1} HNO_3 (4 mL), 1.5 mL of H_2O_2 (30%) and 29.61 min. After extraction, the volume was made up to 20 mL and the mixture centrifuged. The LODs were 2.7, 0.87, 0.51 and 0.01 mg L^{-1}, for Ca, K, Mg, and P, respectively. The results obtained for 11 samples were compared with those obtained by a procedure involving conventional MAD and ICP-OES; although no discussion of the agreement was given, visual inspection of the results indicates that there were no significant differences. In contrast, for the determination of major and trace elements in red wines by ICP-OES, Dlangalala *et al.*[44] included both sample volume and extraction temperature in their optimization. The optimum conditions were sample volume 2 mL, HNO_3 concentration (4 mL) 3 mol L^{-1}, extraction time 30 min and temperature 70 °C. After filtering (0.45 μm), the solution was diluted to 10 mL with H_2O. The method validation included measurement of spike recoveries, for which no detailed results were presented, but they were summarised as being between 85 and 112% for a spike concentration of 10 μg L^{-1}. It is possible that the recoveries were measured for only four elements (Ba, Ca, Na and Mg), the LODs for which were 0.28, 5.50, 0.47, 0.46 μg L^{-1}, respectively. As the concentrations of these elements in the real samples ranged from 29 to 506 mg L^{-1}, spiking at 10 μg L^{-1} would seem to be undetectable. Results were presented for these elements together with those for 13 other elements (Al, As, B, Ba, Cd, Co, Cr, Cu Ni, Pb, Sb, Ti and V) in two samples.

To determine Ba, Ca, Cu, Cr, Mg, Mn, Sr and Zn in handmade chocolates by MIP-OES, Guimaraes *et al.*[45] devised *an extraction procedure involving emulsion breaking*. The optimized procedure involved forming an emulsion by agitating 0.25 g of sample with 5 mL of extractant, consisting of 1.5 mol L^{-1} HNO_3 and 1.5% (m/v) Tween 80, for 5 min in an ultrasonic bath (no details were provided). The emulsion was broken by heating for 4 min in a water bath at 80–90 °C. After separation, the aqueous phase was diluted to 5 mL. They showed that agitation with ultrasound was superior to vortex mixing. Part of the validation involved the analysis of a CRM (NIST SRM 2384, baking chocolate) for the concentrations of Ca, Cu, Fe, Mg, Mn and Zn, and the values obtained were not significantly different from the certificate values. They also showed that the results were not significantly different from those obtained by a method based on dry ashing, dissolution in HNO_3 and measurement by FAAS. Although a MAD system was listed among the apparatus, the experimental section contained no mention of MAD. They applied their method to the analysis of 10 chocolates with different cocoa contents (ranging from 50 to 85%) and found all analytes in all samples. The LODs ranged from 0.01 (Ba) to 0.46 (Fe) mg kg^{-1}.

4.2.4 Speciation. Several studies of the elemental speciation of seafoods have appeared during this Update period. Three of them are concerned with As speciation.

For the determination of As species in marine fish by HPLC-ICP-MS, Wu *et al.*[46] devised a *microwave extraction* procedure for which the optimized conditions were 30% MeOH, 32 min, 85 °C, and a solid–liquid ratio of 1 : 20 (g : mL) for 1 g of minced and homogenized sample. They compared the results obtained with a, presumably non-optimized, UAE procedure, showing that the extent of extraction was higher for all six As species (As^{III}, As^{V}, AB, AC, DMA and MMA). The validation included measurement of recoveries of spikes of the previously mentioned six species, together with carbazone and roxarsone, at 40, 100 and 500.00 μg kg^{-1} from Pacific saury, for which values ranging from 82.1 to 103.2%, were obtained. In addition to Pacific saury, they also analysed yellow croaker, pomfret, ribbonfish, cod and toothfish, finding all species except carbazone and roxarsone. The sum of species expressed as a percentage of the tAs concentration ranged from 85 to 92%, but it was not clear how the total As values were obtained. The slope of the regression equations show clear evidence of compound-dependent responses, and the LODs (expressed as As) ranged from 0.7 (DMA) to 11 (carbazone) μg kg^{-1}.

Despite the obvious importance of sample preparation in speciation analysis, Thang *et al.*[47] provided remarkably few details in their report of a method for *As speciation in seaweed* by HPLC-ICP-MS. There is no mention of any apparatus related to sample preparation and after an optimization, they selected 20 mL of 50% MeOH (v/v) at 50 °C for 10 min, though they also considered 2% HNO_3 and the mobile phase (300 mmol L^{-1} $(NH_4)_2CO_3$, 3% MeOH, pH 10.3). The sample mass and volume of extractant were not included in the optimization; the former may have been 20 mg (dried material) but there is no mention of the volume of extractant or of how the mixture was heated or of how the extract was separated from the residue. The method accuracy was assessed by spike recoveries of As^{III}, As^{V}, AB, DMA and MMA, at 50, 200, and 500 μg kg^{-1}, which ranged from 91 to 107%. The procedure was applied to 15 seaweed samples: only one of which contained As^{V} and DMA; As^{III} was not found in six samples; AB was found in all but one samples, and DMA was found in all. The sum of species expressed as a percentage of the total As concentration ranged from 80 to 108%, but no information was given as to how the total As values were obtained. The slope of the regression equations showed clear evidence of compound-dependent responses, and the LODs (expressed as As) ranged from 2.3 (As^{III}) to 21 (AB) μg kg^{-1}. Four of the samples, including the one with 108% recovery, contained a substantial fraction of an unknown compound that had been quantified with the As^{III} calibration.

For the determination of *As and Cr species in shrimp, fish and bivalve molluscs* by HPLC-ICP-MS, de Sa *et al.*[48] optimized separate extraction and chromatographic procedures for the species of each element. Samples were first prepared by lyophilisation and cryogenic grinding. To extract As species, 100 mg were heated at 90 °C for 40 min with 5 mL of 0.03 mol

L^{-1} HNO_3 in closed vessels in a digester block; Cr species were extracted by 5 mL of 0.045 mol L^{-1} EDTA (pH 10.0) under the same conditions. For As speciation, the extract was 5-fold diluted with mobile phase, and for Cr species a 10-fold dilution was applied. For As the only parameters optimized were time and temperature, but for Cr the optimization included these together with the mass of sample and EDTA concentration. The methods were applied to several CRM: NRCC DORM-4 (Fish protein), NRCC DOLT-5 (Lobster hepatopancreas), NRCC TORT-3 (Lobster), NIST SRM 1566b (Oyster tissue) and a fish tissue RM developed by the Institute for Energy and Nuclear Research (IPEN), São Paulo-SP, Brazil. Visual inspection of the As results suggested that the sums of species are significantly lower than the certificate total values for all of the CRMs, but for most of the 12 real samples, the sums of species are probably not significantly different from the total values (determined following a conventional MAD). For Cr, only Cr^{III} was detected in the extracts, but this only accounted for between 7 and 57% of the total Cr. The LOQs were 52, 83, 5.3, 16, 2.8, 114, and 54 μg kg^{-1} for, As^{III}, As^{V}, AB, DMA, MMA, Cr^{III} and Cr^{VI}, respectively. Recoveries of the various species spiked into three samples (shrimp, fish and bivalve mollusc) were given, but no details were provided; visual inspection suggests that some of the values were significantly less than 100%, though none was lower than 87% even for Cr^{VI}.

For the *determination of Hg species in fish by microplasma OES*, Cai *et al.*[49] devised a "one-pot" pretreatment. Both fresh and freeze-dried fish material were analysed, and iHg and MeHg were extracted from either 100 mg of powdered or 1 g of fresh fish by TMAH, which was added together with $SnCl_2$ to a quartz reaction vessel through whose lid was inserted an activated carbon electrode tip. On sonication for 30 min, iHg was converted to volatile Hg^0 that was trapped on the electrode tip. Then the electrode was replaced and any MeHg, liberated by PVG on irradiation with UV light from a mercury vapour lamp, trapped on this second electrode. Subsequently the Hg was determined by OES following desorption and excitation in a DBD. A CRM (ZKQC8236, aquid powder, supplier not given) was accurately analysed and the procedure applied to five real samples, in all of which both analytes were detected, apart from iHg in the yellow croaker. Spike recoveries were between 95 and 100%. The LODs were 2 and 1.2 μg kg^{-1} for iHg and MeHg, respectively in freeze-dried fish. The pretreatment and analysis of a batch of 12 samples were completed in under 50 min, with overall extraction efficiencies, based on total Hg, of 90% for 100 mg of freeze-dried fish and 86% for 1 g of fresh fish.

In a comprehensive study of the Se speciation in Se-enriched yeast, Angaïts *et al.*[50] applied a six-step *sequential extraction procedure* with quantification and characterisation of the species in each extract by ICP-MS and HPLC-ICP-MS The material studied was the CRM NRCC SELM-1 (Se-enriched yeast, certified for total Se and SeMet). The six steps were as follows: (1) UAE with H_2O (100 mg, 5 mL) for 1 h, followed by centrifugation, separation and repeat of the UAE; (2) suspension of the pellet from step 1 in 2 mL 0.1 mol L^{-1} TRIS–HCl (pH 7.5) buffer and incubation with protease solution (30 mg) at 37 °C for 17 h, followed by centrifugation, separation and repeat of the procedure and finally centrifugation, separation and "washing" as described in step 1; (3) resuspension of the pellet as described in step 2, addition of 50 μL of 0.5 mol L^{-1} iodoacetamide, shaking for 1 h, addition of 150 μL of 0.2 mol L^{-1} DTT and further shaking for 1 h, the extraction was repeated and the remaining pellet washed; (4) combination of (2) and (3) in which the iodoacetamide and DTT were first added together, followed an hour later by more DTT and then an hour later by protease (37 °C for 17 h), finally the pellet was washed; (5) UAE (1 h) with SDS (5 mL, 4%) and after centrifuging and separation, the pellet was (6) hot-plate digested with HNO_3 and H_2O_2. Four different overall procedures were created by varying the order of the steps (though, obviously, the first and last steps were (1) and (6), respectively). Also in each procedure step (5) was next to last. They described step (1) as extraction of the selenometabolome, step (2) as proteolysis, step (3) as carbamidomethylation derivatization (to break S–S bridges and block Cys residues). They concluded that SELM-1 contains 14.8 ± 0.7% Se-metabolome fraction that includes. 1.5% SeMet and 2% SeCys, 64.7 ± 2.7% water-insoluble SeMet, 10.5 ± 1.5% water-insoluble SeCys and 9.7 ± 1.7%, iSe (Se^{IV}, Se^{VI}, and Se^0), thereby accounting for 99.8% of Se.

For the speciation of iAs in waters, Chillè *et al.*[51] selectively *extracted As^{III} into a polymer inclusion membrane* with subsequent quantification by XRF. They prepared 1 cm diameter membranes from cellulose triacetate containing Cyanex 301 (bis(2,4,4-trimethylpentyl) dithiophosphinic acid) by a solvent casting method. The As^{III} was extracted from up to 500 mL of sample at pH 1 by stirring for 24 h. They determined As^{V} after reduction to As^{III} with sodium thiosulfate + KI in a sequential procedure in which the As^{III} was first extracted, and then any As^{V} remaining was reduced, a second membrane added and the 24 h extraction repeated. The LOQ was 2 μg L^{-1}. Their validation included measurement of spike recoveries of both species (at concentrations from 20 to 100 μg L^{-1}) in two samples of ultrapure and mineral water (not containing any As) that ranged from 98 to 116%, and they applied the method to two real samples (spring and ground water) neither of which contained an As^{III} concentration above the LOQ.

There have been several studies of the *determination of NPs in biological matrices*. For the determination of CuO NPs in seafood by spICP-MS, Bartolomé *et al.*[52] devised an enzymatic UAE. Optimum conditions, selected by response surface methodology, were 1 g of sample, 20 mL of enzyme solution (2.9 g L^{-1} lipase, 2.9 g L^{-1} pancreatin in 0.2 mol L^{-1} phosphate buffer), heating at 37 °C (water bath), continuous sonication (probe) at 60 W (62% amplitude) for 8 min. Extracts were diluted 1 + 99 with 1% glycerol and filtered (0.45 μm). They compared the results obtained for native and spiked razor clam samples with those of an alkaline hydrolysis method, involving reaction of 0.25 g (fresh weight) of sample with 20 mL of (20% w/w) TMAH for 24 h at room temperature, for which they found that the mass and number concentrations and mean particle size were much lower. They concluded that the TMAH method converted the NPs to dissolved Cu ions. They applied the enzyme method to nine different specimens representative of a range of seafoods: bivalve molluscs (mussels, cockles, razor clams, clams,

oysters and scallops), edible seaweeds (wakame, nori) and processed seafood (surimi sticks) finding CuO NPs in all of them except the edible seaweeds. On the other hand, in a study of the uptake of Ag NPs by *E. coli*, Gimenez-Ingalaturre *et al.*[53] found that these NPs were stable in the presence of TMAH. Following incubation with particles of different sizes, they centrifuged 100 μL of bacterial suspensions to remove the PBS medium then added 400 μL of 25 mmol L^{-1} $CaCl_2$ and 1.6 mL of TMAH (25% w/w) to the resulting pellet. After digestion for 24 h at room temperature in the dark, the solution was made up to 10 mL with H_2O so that the final concentration of TMAH was 4%. The Ag species were characterised by sp and scICP-MS and hydrodynamic chromatography ICP-MS. Total Ag was determined after digestion of the centrifuged pellet with HNO_3 and H_2O_2 for 24 h at room temperature. They found that 10 nm particles were taken up by the bacterium, whereas 60 nm particles were not, and thus their bactericidal effect was due to ionic silver released into the culture medium.

To extract TiO_2 NPs from human serum prior to characterisation by spICP-MS, Justo-Vega *et al.*[54] *treated samples with a mixture of enzymes* (pancreatin and lipase) followed by centrifugal ultrafiltration. In the preparation of the 0.1% solution of the enzymes (sonication at 35 kHz, 325 W for 5.0 min) not all the pancreatin dissolved and the remaining enzyme, which could cause NP agglomeration, was removed by centrifuging. Four mL of the enzyme solution was mixed with 0.5 mL of serum and the mixture heated at 37 °C for 4 h. For the second step, the hydrolysed serum was centrifuged (3900 rpm, 2364 g, at 20 °C) for 40 min in 30 kDa nominal molecular weight limit ultrafiltration tubes. The retained material was recovered by placing the filter container upside down in a clean microcentrifuge tube and centrifuging for 2 min. The recovered hydrolysed serum (around 50–100 μL) was diluted to 2.0 mL and 1.0 mL with 1% (v/v) glycerol. The LODs were 36 nm and 5.70×10^3 particles mL^{-1}, for size and number concentration, respectively. They applied the procedure to four samples, but did not find any TiO_2 NPs. To evaluate accuracy and demonstrate the need for the enzyme extraction step, they measured the recoveries of spikes of 100 nm TiO_2 NPs at concentrations of 63.5 and 127.0 μg L^{-1}, both with and without the enzymatic hydrolysis. The recoveries increased from 82 ± 8% to 97 ± 3% when the hydrolysis step was included. The same researchers devised a preconcentration procedure that is discussed in the preconcentration section. Espada-Bernabé *et al.*[55] studied the same enzymes (lipase and pancreatin), but used separately at concentrations of 3 mg mL^{-1}, in an investigation of procedures for the extraction of SiO_2 and TiO_2 NPs from confectionary products prior to determination by spICP-MS. They also investigated Tris–HCl (30 mmol L^{-1}, pH 7.4) and H_2O as extractants. For each reagent, the effects of manual shaking, or ultrasonic agitation (both by probe and in a bath) were evaluated. In the case of sugar paste, for which a high background signal due to ionic Ti interfered with the sp measurements, they also applied an ultrafiltration step. They found, by TEM, that neither the extraction agents nor the shaking, ultrasonication, or ultrafiltration affected the size, morphology or aggregation state of either type of particle. However, they found significant differences in the percentages and number-based concentration of particles and in the total amount of Si and Ti extracted, and concluded that extraction by shaking with H_2O was the best method. In particular, they found higher backgrounds when the enzymes were used that resulted in higher particle-size LODs. They applied their method to the analysis of pure and soluble cocoa powder, powdered custard, sugar paste and sugar powder, finding NPs of both elements in all samples. The mass balance studies were supported by measurements of the total element concentrations following MAD of 100 mg of sample with 4.4 mL of a mixture of 3.6 mL concentrated HNO_3, 0.6 mL of concentrated H_2O_2 and 0.2 mL of concentrated HF. Only results for Si were presented: the sums of ionic and particulate concentrations were not significantly different from those for total Si for all samples.

To *determine elements in individual cells* by scICP-MS/MS, the sample preparation must ensure the separation of the intact cells, together with the introduction of these intact cells into the spectrometer. For the determination of Fe and Zn in human umbilical vascular endothelial cells by scICP-MS/MS, Leal *et al.*[56] investigated the effects of washing the cells and of the resuspension medium. They found that a single wash with PBS with centrifugation at 250×*g* for 5 min was satisfactory, and that on resuspension in H_2O, or PBS the cells were not stable. However, when resuspended in 10% paraformaldehyde in PBS followed by centrifugation (1200×*g*, 5 min) and resuspension in H_2O, stable cells were obtained. Analysis of the bulk cells was performed after UAE of 1 mL of the cell suspension with 100 μL of a mixture of concentrated HNO_3 and H_2O_2 (proportions not given) with dilution to a final volume of 5 mL. The LODs were 4 fg per cell and 8 fg per cell for Fe and Zn, respectively. Curiously, the supplier of the paraformaldehyde (or of the 10% solution in PBS) was not given.

A method for the determination of nanoparticulate Hf, Ti and Zr oxides in clinical materials that did not involve HF in the sample preparation was described by Gerken *et al.*[37] Their procedure did not distinguish between nanoparticulate and dissolved metals and so is discussed earlier in this section (*vide supra*).

4.2.5 Comparison of digestions/extractions. Many of the publications describing a new sample preparation procedure described elsewhere in this section contain the results of a comparison of the new procedure with an established one, such as MAD with HNO_3 and H_2O_2, so the selection of papers for this section is somewhat arbitrary.

Melo *et al.*[57] *compared three methods (MAD, UAE and alkaline solubilisation)*, for the possible determination of Ca, Fe, K, Mg, Na, P and Zn in Brazilian artisanal cheeses, by ICP-OES with a reference method in which samples (1 g) were dry ashed and the residue dissolved in 2.5 mL of 12 mol L^{-1} HCl and diluted to 50 mL. The relevant parameters of each procedure were optimized by a central composite design method. All samples were first grated, dried, milled and homogenized. For the MAD procedure the parameters studied were sample mass and volume of concentrated HNO_3 (all digestions involved the same heating program with maximum temperature 200 °C). The optimal values were 1.58 mL of 14 mol L^{-1} HNO_3 (plus enough

H_2O to make 3 mL total volume) and 0.570 g sample. The final volume was 30 mL. For the UAE procedure, the parameters were concentrations of HNO_3 and HCl and time and all experiments featured 0.3 g of sample and sonication at 50 Hz and room temperature. The optimized conditions were 3.90 mol L^{-1} HNO_3, 1.0 mol L^{-1} HCl (total volume 5 mL) and 2.5 min. After separation (centrifugation) 3 mL of this solution was diluted to 10 mL. For the alkaline solubilization, the parameters were concentration of TMAH, time and temperature; all experiments were performed with 0.3 g of sample. The optimum conditions were 21.8% (w/v), 103 min and 50 °C. After extraction, the original 3 mL was diluted to 5 mL and after centrifuging, 3 mL of this solution was diluted to 10 mL. The results of recovery experiments ranged from 80.5 to 126%. The outlying high value of 126% was for Na by the alkaline solubilisation method. None of the methods was able to satisfactorily extract all seven elements, although the MAD was suitable for all except Zn (but the reason for this was not discussed); the UAE method worked for Ca, K, Na and Zn, and the alkaline extraction worked only for K, Na and Zn. For the relevant elements, there were no significant differences (based on a paired *t*-test) between the results obtained by the three methods and those obtained following dry ashing. The LODs varied quite markedly from one procedure to the other, but overall ranged from 0.001 (Zn by UAE) to 0.5 (Na by MAD) μg g^{-1}.

For the determination of As, Cd, Cr, Cu, Fe, Pb, Ni, Sn, V, and Zn in vegetable oils by ICP-OES, Munjanja *et al.*[58] developed a *MAD with only H_2O_2*. The optimized conditions were 0.1 g sample, 7.5 mL of 2.0 mol L^{-1} H_2O_2, 156 °C and 50 min. The digests were diluted to 10 mL. The residual carbon content was between 0.84 to 1.60% (m/m). No details were given as to how this was measured, but as C was listed in the table of wavelengths, presumably C was determined by ICP-OES (against citric acid standards). If the numbers refer to the percentage of the original C left undigested, then 1 g of sunflower oil (approximately 83% carbon) would give rise to approximately 0.013 g of C for 1.6% residual carbon. As the final volume was 10 mL, the concentration of carbon would be 1300 mg L^{-1}, which is rather high. The results of spike recoveries (at 2.5 and 5 μg L^{-1}) were compared with those obtained by a MAD with concentrated HNO_3 "under the same digestion conditions", but no details were given. No significant differences were observed. The LODs ranged from 0.003 (Cd) to 14 (Sn) μg L^{-1} (so it is not clear how the Sn spikes could have been quantified), and the procedure was applied to the analysis of samples of sunflower, peanut and olive oils. All the analytes, except Sn were found in all samples.

As part of the development of a method for the determination of 19 elements (Ag, Al, B, Ba, Cd, Ca, Cr, Co, Cu, Fe, K, Li, Mg, Mn, Ni, P, Pb, Sr and Zn) in 346 cultivars of quinoa by MIP-OES, Cladis *et al.*[59] *compared MAD with dry ashing*. They found that dry ashing gave more complete digestions and more consistent analytical signals, which they ascribed to the larger sample mass and lower acid volume, resulting in decreased dilution compared with that of the MAD procedure. Samples (1 g) were subjected to a single ashing cycle in unlidded crucibles; the temperature was ramped (2 °C min^{-1}) to 275 °C, held for 2 h then ramped (3 °C min^{-1}) to 600 °C for a further 2 h. After cooling, the ash was dissolved in 1 mL concentrated HNO_3 and diluted to 25 mL with H_2O. They considered quinoa to be a low moisture crop with little variation in moisture content across cultivars, and so they did not dry the samples, which had moisture contents ranging from 6.4 to 10.2%. As part of the validation, they analysed a CRM (NIST SRM 1567b, Wheat flour), obtaining relative measurement errors that ranged from −28% (K, 766.491 nm) to +6% (Mn). The LODs ranged from 0.027 (Mg) to 14 (P) mg kg^{-1}, and the concentrations of Ag, Cd, Co, Cr and Ni in the real samples were all < LODs.

Following a comparison of the residual carbon content of the digests of rice by procedures in which *heating was either in a water bath or in a microwave oven*, Puente la Cruz *et al.*[60] concluded, not surprisingly, that MAD was the better option for a procedure in which 41 elements (those whose concentrations in NIST SRM 1568b are certified, Al, As, Ca, Cd, Cu, Fe, Hg, K, Mg, Mn, Mo, P, Rb, S, Se and Zn together with B, Ba, Be, Bi, Ce, Co, Cr, Cs, Ga, La, Li, Na, Nb, Ni, Pb, Sb, Sn, Sr, Te, Ti, Tl, U, V, W, and Zr) were determined by ICP-MS or CV-AFS. They investigated several different reagent combinations and concluded that for 0.5 g of sample, 2 mL HNO_3, 0.5 mL H_2O_2, and 0.5 mL deionized water were the best conditions. The validation included the analysis of a CRM (NIST SRM 1568b, Rice flour), for which relative measurement errors ranging from −7 to +10% were obtained, and measurements of spike recoveries, for the non-certified elements, that ranged from 90 to 120%. The LODs ranged from 0.00003 (U) to 30 (Na) mg kg^{-1}, and the method was applied to several different rice types purchased locally. The residual carbon content (determined by ICP-MS against citric acid standards) was 180 mg L^{-1} and the residual acidity was < 5%.

In the development of a method for the determination of multiple elements (Al, Be, Co, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Sb, Se, V, Zn and REEs) in extra virgin olive oil (EVOO) by ICP-OES and ICP-MS, Inaudi *et al.*[61] improved the precision of results by *incorporating some additional steps prior to MAD*. Samples (25 mL) were agitated for 120 min, then sonicated (bath) for 10 min followed by cold reaction of 0.5 g in the microwave vessels with 4 mL of HNO_3 and 2 mL of H_2O_2 for 240 min. A five-step MAD programme was then applied and, after cooling, the digests were made up to 30 mL. The validation included the analysis of a CRM (NIST SRM 1573a, Tomato leaves), for which the relative errors ranged from −27% (Ni) to +5% (Co). The LODs ranged from 0.001 (several REEs) to 3 (Li) μg L^{-1} and the procedure was applied to the determination of Al, Be, Co, K, Li, Mg, Na, Sb, Se and V by ICP-OES and Cu, Fe, Mn, Ni, Zn and REEs by ICP-MS in 13 real samples. In addition, attempts to determine some other elements, such As, B, Ba, Cd, Cr, Pb, Si and Sr were made, but their concentrations were below LODs. Thirteen non-REE elements were found in all samples. Several of the REE concentrations were below LOD, though every REE was found in several of the samples.

4.2.6 Ashing and microwave induced combustion. Although innovation in sample preparation was not the primary aim of their study, Coelho *et al.*[62] showed that *microwave-induced combustion was suitable for the preparation for fish*

tissues for subsequent determination of the Hg content by VG-AAS. Dried powdered samples (500 mg) were pelletized (4 tons, 2 min) then placed on a filter paper disc moistened with NH_4NO_3 solution (combustion igniter), in a closed quartz vessel containing 6 mL of a 0.5 mol L^{-1} HNO_3 absorption solution that was pressurized with O_2 at 20 bar. The combustion and reflux (to leach any adsorbed analyte) steps took 5 min followed by 20 min cooling. The final solution was made up to 25 mL. The main novelty of the study was the use of a DBD for plasma-mediated VG, and this is discussed further in Section 5.4. The validation included the measurement of spike recovery (104%), the accurate analysis of a CRM (NRCC DOLT-4, Dogfish liver) and comparison of the results with those of an ICP-MS method for which the sample introduction was by conventional CVG. The LOD was 3.2 μg L^{-1}, corresponding to 0.16 μg g^{-1} in a solid sample. The procedure was not applied to any real samples.

To determine Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in coffee by ICP-OES, Gomes *et al.*[63] *first dry ashed the beans or the dried residue obtained after a hot water extraction* (the so-called cupping protocol). Beans (1 g) were ashed at 550 °C for 4 h and the residue dissolved in 1.0 mL concentrated HNO_3 and 40 mL of 1.9 mol L^{-1} HCl, then diluted with H_2O to 100 mL. For the hot water extraction, a 5.5% (m V^{-1}) solution was prepared, and 10 mL was filtered, evaporated at 10 °C and ashed as described for the beans. The ash was dissolved in 0.5 mL concentrated HNO_3 and 20 mL of 1.9 mol L^{-1} HCl and diluted to 50 mL. The LODs ranged from 0.001 (Mn) to 0.3 (Na) mg L^{-1}. All elements were detected in both the beans and the extracts in all 10 samples of specialty coffees and all 8 samples of traditional coffees. The accuracy of the method was not investigated, which may not have been all that relevant as their goal was to show that the coffees could be differentiated, with the help of PCA, on the basis of the elemental concentrations.

4.2.7 Mineralization without microwaves. Prior to the determination of Bi, Pb, Sb, Sn, in a variety of biological samples by HG point discharge OES, Cai *et al.*[64] digested the samples in a home-made device in which samples were *mineralized by the application of a magnetic field assisted DBD*. The device was described in detail in the SI that included a time-lapsed video of the entire operation. To 50 mg sample was added 1 mL of digestion reagents (a mixture of 600 μL H_2O, 400 μL H_2O_2 and between 40 and 120 μL of HNO_3, depending on the sample) and the discharge applied for 15 min. A further 600 μL of reagent mixture was then added and the discharge applied for a further 10 min. After cooling, the digestion vessel became the reaction vessel for HG and 1 mL of 3.5% (m/v) $K_3Fe(CN)_6$ was added. The vessel was sealed with a septum and the point discharge DBD detection unit was placed on top of the vessel. Immediately following initiation of the plasma, 0.5 mL of 3% (m/v) $NaBH_4$ was injected, and the resulting transient signals recorded. The LODs were 8.2, 2.4, 4 and 2 μg L^{-1} for Bi, Pb, Sb and Sn, respectively. The authors reported LODs in the solid that were 20 times higher, presumably based on the dissolution of 50 mg of sample in 1 mL of solution. Part of the validation included the accurate analysis of two CRMs: GBW 10044 (rice) and GBW 10023 (laver) though only Pb could be measured in the rice and only Pb and Sn in the laver. They applied the procedure to real samples of rice, blood, milk, laver and fish. Of the 20 possible results, 13 were below the LODs. They compared the results with those obtained by a conventional MAD digestion followed by ICP-MS, for which only four results were < LOD, finding no significant differences. Spike recoveries of concentrations ranging from 0.1 mg kg^{-1} (Bi in fish) to 100 mg L^{-1} (Pb and Sn in blood) were 95–106%. The researchers point out that compared with direct DBD digestion, the application of the magnetic field shortened the digestion time by 40–69%, so that a batch of six samples was digested in 25–40 min. As the device can be battery powered, it could be applied to the pretreatment and determination of toxic elements in biological samples in the "field".

For the determination of As, Cd, Cu, Fe, and Zn in bivalve molluscs by ICP-OES, Costa *et al.*[65] applied *infrared radiation to help digestion* of the samples with HNO_3. Operating conditions were optimized by a full factorial design in which the residual carbon content (determined by ICP-OES against citric acid standards) was the figure of merit. Samples were first lyophilised and ground, then 200 mg was taken and 14 mL of concentrated HNO_3 added. After heating (three halogen heat lamps, 127 V, maximum power 1200 W) for 25 min, 1 mL of 30% (w/w) H_2O_2 was added and the solution diluted to 20 mL. The validation included the analysis of CRM NRCC TORT-2 (Lobster hepatopancreas), but no spike recovery experiments or comparison with another method were performed. The results for the CRM were not significantly different from the certificate values. The LODs were 2, 90, 5, 270 and 15 μg L^{-1} for As, Cd, Cu, Fe and Zn, respectively, and they applied the method to the analysis of six real samples (thick lucine (3), oyster, clam and mussel). In none of the samples was Cd found, but Fe and Zn were found in all samples; surprisingly, As was not found in the three thick lucine (*Lucina pectinata*) samples; Cu was found in all except one thick lucine sample. They also measured the residual acid concentration (by acid–base titration), but no results were presented other than a comment to the effect that there was considerable loss of acid by volatilization because of the high heating rate. A somewhat similar system was developed by Peixoto *et al.*[66] for the digestion of biological matrices prior to analysis by ICP-OES. They constructed a home-made system to carry out wet digestions in closed vessels mounted in a heating block and irradiated with light from a 500 W halogen lamp, which they described as simple to assemble, eco-friendly and low-cost. They applied a full factorial design and came up with optimized conditions of ramping over 15 min to 200 °C, then maintaining 200 °C for 5 min. The sample mass was 0.25 g, the reagents were 2 mL of concentrated HNO_3 and 1 mL of 30% (w/w) H_2O_2 (though there is some ambiguity over whether H_2O_2 was in fact used), and the final volume was 50 mL. They applied their method to the determination of Ca, Cu, Fe, K, Mg and Zn in two RMs (MR-Agro E3002A, Fish tissue and MR-Agro E3001a, Bovine liver) obtaining results that were not significantly different from the assigned values reported in the RMs documents. They also compared their results with those of a conventional MAD, in sealed vessels with HNO_3 and H_2O_2, in terms of the residual carbon content, which was determined by a dedicated TOC analyser. They found that the residual carbon

at 56 ± 3 mg L^{-1} was about 25% higher for the IR system than those of the digests with the microwave system. No real samples were analysed and no LODs were reported.

Digestions featuring *reactive species produced by reactions catalysed by iron^{II}* have been described. To determine, Al, Cd, Co, Cr, Cu, Mn and Zn in honey by ICP-OES, Reis *et al.*[67] decomposed samples by reaction with activated persulfate. They investigated two possible procedures: the production of reactive sulfate radicals by reaction with either (1) iron in basic solution or (2) iron under ultrasonic irradiation. They applied a simplex centroid design, a two-level full factorial design, together with a Doehlert matrix response surface methodology for the optimization. The sample mass was 0.2 g for each method. For method (1) the sample was shaken for 30 s with a ferrous ammonium sulfate ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$), KOH and ammonium persulfate solution ($(NH_4)_2S_2O_8$) (total volume 10 mL). For method (2) the sample was sonicated with ammonium persulfate, ferrous ammonium sulfate and HCl for 10 min and the diluted to 10 mL with H_2O. Four samples were also analysed following conventional MAD with HNO_3 and H_2O_2 (0.2 g and final volume 20 mL). They found that the UAE method resulted in significant matrix effects, which they interpreted as resulting from less effective decomposition of organic matter compared with that produced by the basic solution method. The LODs for method (1) were 60, 2, 30, 10, 30, 30 and 10 μg kg^{-1} for Al, Cd, Co, Cr, Cu, Mn and Zn respectively. The validation of this procedure included measurement of spike recoveries of additions of 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 50 mg kg^{-1} that ranged from 80 to 120%. The concentrations of Cd and Co were below the LOQs for all samples, but for the other elements there were no significant differences (paired *t*-test) between the results for the persulfate method and those of the MAD for four samples. They evaluated their procedure on both the AGREE and AGREEprep scales, showing significantly better scores that those of five previously described methods for the analysis of honey. To digest a urine matrix prior to the determination of REEs by ICP-MS, Nong *et al.*[68] applied a UV Fenton reaction in which the digestion with dilute H_2O_2 was catalysed by ferrous ions and UV irradiation. As the second stage of the sample pretreatment involved preconcentration by coprecipitation of ferrous hydroxide, further details of the procedure are given in the preconcentration section.

Ferreira *et al.*[69] determined Ba, Ca, Mg, Mn, Ni, P, S, Sr, V, and Zn in tropical fruit pulps by ICP-OES after *digestion with HNO_3 and H_2O_2 in closed vessels in a block digester*. They applied a constrained-mixture design for the optimization with extent of organic matter decomposition as the figure of merit. The optimization was carried out with mango fruit pulp, which has a high fibre content, and whose total organic carbon content was determined by CHN analysis to be 44.9%. Following digestion, the residual carbon content was determined by ICP-OES at 193.027 nm against citric acid standards. For the optimized conditions (described below), the residual carbon was 0.71%. As the sample mass was 0.2 g and the final volume was 10 mL, the residual carbon in solution would have been 64 mg L^{-1}. To 0.2 g of dried or 1.3 g wet sample were added 4.0 mL of 2 mol L^{-1} HNO_3 and 2.0 mL of 30% H_2O_2 and the sealed vessel heated at 180 °C for 2 h. The residual acid concentration, determined by acid–base titration was 0.75 mol L^{-1}; based on simple dilution of the HNO_3 added, the concentration would be 0.8 mol L^{-1}. As they used phenolphthalein as the indicator for the strong acid/strong base titration, their results are biased high. The LODs ranged from 0.01 (Mn) to 7.0 (Ca) mg kg^{-1}. The validation included the analysis of the CRM NCS DC 73351 (Tea leaves) for which they obtained relative measurement errors that ranged from −19 to 3%. They applied the procedure to the analysis of 17 different sample types from four different suppliers, for a total of 41 samples, and presented results for seven of the elements; Ba, Ni and V were not determined. They evaluated the procedure on both the AGREE (score: 0.54) and AGREEprep (score: 0.38) scales, but did not make any comparisons with other methods. A very similar procedure was adopted by de Jesus *et al.*[70] for the determination of Ba, Ca, Cu, K, Mg, Mn, Na, P, Sr, V and Zn in 25 Brazilian paprika samples by ICP-OES. The sample mass was 0.25 g to which was added 2 mL concentrated HNO_3, 2 mL concentrated H_2O_2 and 4 mL H_2O. After digestion, the final volume was made up to 15 mL. The validation included the analysis of three CRMs: Centro de Energia Nuclear e Agricultura-Universidade de Sao Paulo (CENA-USP, Piracicaba, SP, Brazil) CRM-Agro C1005a (sugarcane leaves) and CRM-Agro C1003a (tomato leaves) and NIST SRM 1515 (apple leaves). Although the authors claimed that no significant difference at a 95% confidence level (Student's *t*-test) between the results and the certified values, visual inspection of the reported data indicates significant differences for some of the elements measured in the NIST material (Ca, Cu, K, Mn, P and Sr), the C1003a material (Cu) and the C1005a material, (Mn, P and Zn). The LODs ranged from 0.02 (Sr and V) to 6 (Ca) μg g^{-1} and all elements were detected in the real samples. The residual carbon was 5.8% and the residual acid concentration was 1.79 mol L^{-1}.

To determine Ca, K, Na, Mg, and P in milk by MIP-OES, Soares de Araujo *et al.*[71] showed that sample preparation by *simple dilution with formic acid* was sufficient to obtained accurate results when combined with aerosol dilution sample introduction. To 0.2 g of whole milk (3% fat) was added 0.2 mL of 100% (w/w) formic acid and, after stirring, the volume was made up to 10 mL. This concentration of formic acid did not cause precipitation of proteins. As part of the validation, they analysed a skimmed milk powder CRM (ERM BD150) that required additional dilution: to 0.1 g sample was added 5 mL of H_2O and 1 mL of formic acid. After making up to 10 mL, the solution was diluted a further 10 times before introduction to the spectrometer, which was equipped with a home-made aerosol dilution system that allowed a diluent gas to merge with the aerosol from the spray chamber prior to introduction to the plasma. The dilution gas flow rate was varied between 0.2 and 0.7 L min^{-1} and the nebuliser gas flow rate between 0.3 and 0.8 L min^{-1} such that the sum of the flow rates was 1 L min^{-1}. They claimed that dilution factors from 10 to 200 were evaluated, but it is hard to see how these factors arise, as the highest dilution listed was for diluent flow 0.7 and nebuliser flow 0.3 L min^{-1}, which corresponds to a dilution of factor of 3.3. They found that both auto background correction and an IS (Sc) were

necessary, and that a spectral interference from zinc on P could be overcome by measurement at 214.92 nm. The LODs were 0.006, 0.13, 0.018, 0.1 and 3.4 mg L^{-1} for Ca, K, Na, Mg and P, respectively. For the analysis of the CRM, only the result for Mg was significantly different from the certificate value, and spike recoveries (at 5 and 10 mg L^{-1}) were between 96 and 109%. They applied the method to the analysis of 10 real milk samples.

A somewhat similar approach was adopted by Dlangalala *et al.*,[44] who determined both major (Al, B, Ba, Ca, K, Mg and Na) and trace elements (As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Ti and V) in red wine by ICP-MS after *ultrasound-assisted dilution*. However, as the procedure involved adding (to 2 mL of wine) 3 mol L^{-1} HNO_3 and heating at 70 °C for 30 min, it might be more accurately described as a digestion. The final volume was 10 mL. The validation included measurement of recoveries of 10 μg L^{-1} spikes for four elements: Ba, Ca, Na and Mg, which ranged from 85 to 112%. The LODs for these elements were 0.28, 5.5, 0.47 and 0.76 μg L^{-1}, respectively. The procedure was applied to two real samples, in both of which all of the analytes were found at concentrations ranging from about 1 (Al, Co, Cr, Cu, Sb) to several hundred (K, P) mg L^{-1}.

4.2.8 Solid sample analysis. Just two articles were concerned with aspects of the preparation of solid samples for analysis by XRFS and one article described a procedure in which the samples were first digested and then the analyte transferred *via* SPE to a solid matrix for determination by LIBS.

For the *determination of Pb in aquatic products by LIBS*, Wang *et al.*[72] incorporated a preconcentration by SPE prior to measurement. Following digestion (sample mass not given) with HNO_3 and $HClO_4$, the solution was made up to 25 mL, and 2 mg of UiO-66-NH2@TA (a solid material consisting of tannic acid modified with the metal organic framework consisting of zirconium and 2-aminobenzene-1,4-dicarboxylic acid linkers). After extraction at 37 °C for 30 min, the solid was separated by centrifugation. The authors conducted an extensive comparison of five different LIBS sample preparation procedures involving (a) dropping onto a substrate; (b) drying, adhering to sticky tape and attaching to a silicon substrate; (c) compressing with microcrystalline cellulose; (d) compressing with stearic acid; (e) mixing with a carboxymethyl chitosan solution and drying on a silicon substrate. They also investigated the effects of silicon, zinc or glass slides. The most intense signals were obtained when the extractant was dropped directly onto a silicon wafer. Under these conditions, the LOD was 0.18 μg mL^{-1}. They applied the method to the analysis of two real samples: fresh large yellow croaker and scallop, but did not find Pb in either of them. Spike recoveries (0.5 to 4 μg g^{-1}) ranged from 75 to 99%. They also compared the results with those of an AAS method (no details were given, but it was Chinese national standard GB 5009.12-2023), which was able to detect the Pb in the two samples. Visual inspection of the results indicates that the recoveries for the AAS method were mostly significantly higher than those of the LIBS method.

4.2.9 Preconcentration and matrix separation. The majority of papers are descriptions of preconcentration methods in which a solid sample (typically a food) is solubilised (typically by MAD with HNO_3 and H_2O_2), the analytes are selectively extracted by one or other of the various LLE or SPE procedures and are finally transferred to a relatively small liquid phase that is compatible with the operation of the instrument. This work is discussed later in this section and is summarised in Table 1. The smaller number of procedures not involving LLS or SPE are discussed below together with one procedure in which the analyte was separated from interfering matrix components by SPE.

To determine Cd and Cr in sugar by ETAAS, do Amaral *et al.*[73] devised *a LLE procedure mediated by a semipermeable membrane*. The analytes were extracted as their APDC complexes from 30 mL of a 10% sugar solution into 3 mL of $CHCl_3$, contained within the tubular membrane, followed by back extraction into 2 mL of 4.2 mol L^{-1} HNO_3. The membrane was made from low-density PE tubing with wall thickness 80 μm, but the length was not given (although the diameter was specified as 3.0 cm), and the solution was shaken for 150 min. The accuracy was established by measuring recoveries of spikes of 2.0 and 4.0 μg L^{-1} for Cd, and 10 and 15 μg L^{-1} for Cr that ranged from 90.0 to 102% (for Cd) and from 85.2 to 103% (for Cr). The LODs were 0.4 and 1 μg kg^{-1}, respectively. The procedure was applied to 12 real sugar samples, in which Cd was detected in 11 samples and Cr in six. The authors also applied a similar procedure to the determination of Ag, Cu and Ni in urine.[74] The tubing length was given this time (8 cm), but the analytes were not back extracted from the acceptor solution, instead the 3 mL of $CHCl_3$ was diluted to 5 mL with EtOH. The extraction time was increased to 180 min, but complete extraction was not achieved and it was necessary to take the calibration standards through the extraction procedure. The sample volume was 16 mL, for which the LODs were 0.19, 0.23 and 0.15 μg L^{-1}, respectively. A CRM was not analysed. Recoveries for spikes of 5, 10 and 15 μg L^{-1} ranged from 88 to 106%, from 92 to 105% and from 85 to 103%, for Ag, Cu and Ni, respectively. They applied the method to 5 real samples, in none of which was Ag detected, but Cu and Ni were found in all of them.

To improve the detection capability of spICP-MS in the determination of Ag and TiO_2 NPs in human urine, Justo-Vega *et al.*[75] *compared two preconcentration procedures, surfactant-assisted DLLME and ultracentrifugation*. They optimized each procedure by a single-cycle alternating variable search and concluded that the ultrafiltration procedure gave lower LODs. For this procedure 15 mL of urine was centrifuged at 5000 g in a low-binding regenerated cellulose filter unit (7.6 cm^2, 72 mm length, 29.7 mm diameter). After 25 min, a further 15 mL of sample was added, the combined sample centrifuged again and the retained material washed with 15 mL 1.0% (v/v) glycerol. This material was withdrawn, diluted to 2 mL with 1.0% (w/v) glycerol and vortexed prior to introduction to the mass spectrometer. The number concentration LODs were 2.9×10^2 and 3.1×10^2 particles mL^{-1} for Ag NPs and TiO_2 NPs, respectively. The method validation included measurement of recoveries of NP spikes, from a real urine sample. For Ag, the spikes were 60 nm NP at number concentrations of 1.4 and 2.8×10^4 particles mL^{-1}, and for Ti the spikes were 50 and 100 nm NPs at concentrations of 0.84 and 1.60 μg L^{-1}. The recoveries ranged from 78 to 115%. The NP standards were from nanocomposix

Table 1 Preconcentration by liquid- or solid-phase (micro) extraction[a]

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Ag	Food (nuts, mint, spinach, carrot, scallion, rice potatoes, honey, iced tea, cherry juice, apple juice), water (tap, mineral, drinking)	FAAS	Magnetic SPME on cross-linked polylinoleic acid-polystyrene-polydimethylsiloxane graft copolymer encapsulated with magnetic Fe_3O_4 NPs	Dried ground food samples (1 g) were hot plate digested sequentially with conc. HNO_3 and conc. H_2O_2 and diluted to 50 mL. Liquid samples were filtered, and 35 mL diluted to 50 mL. 300 mg of extractant were added and, after separation, the analyte was dissolved in 0.4 mL of 0.5 mol L^{-1} HCl. Ag was found in all nuts, carrot, scallion and honey	0.4 PF 125	CRM EC JRC IRMM BCR®-032 (phosphate rock) and spike recoveries	223
Al, Ba, Fe, Mn and REEs	Food CRMs (green tea, ginger, rice)	ICP-MS	SPE on a iminodiacetic acid resin column after extraction with a DES sodium hexametaphosphate (SHMP) and D-gluconic acid (GlcA) and elution with HNO_3	The DES was allowed to "dry naturally in a food-grade silicone mold" forming "solidified DES pills", one of which was dissolved in water; solid sample followed by *N*-propylethylenediamine (1 mg, to remove organic acids and pigments) was added. The DES solution was mixed with the sample at a DES volume/sample mass ratio of 51.0 mL g^{-1}. After loading the extract on the SPE column, the analytes were eluted with 10 mL of 1% HNO_3. REEs and Al were not detected in rice, and unsatisfactory results were obtained for As, Cd, Cu, Hg and Pb	0.02–0.08 μg kg^{-1}	CNRM CRMs GBW10052a (green tea), GBW10202 (ginger), and GBW10045a (rice)	224
As, Se	Water (bottled, river, tap), food (nuts)	HG-AAS	Floating drop LLE of complexes with 2-((4-iodophenyl) diazenyl)-8-hydroxyquinoline into supramolecular solvent undecanoic acid and TBAH	Nuts: 20 mg digested with HNO_3 and H_2O_2 at 95 °C for 7 h. The total volume of reagents added was 20 mL, but the final volume, from which 5 mL was taken for preconcentration, was not given. Waters: 5 mL sample taken. 80 (As) or 70 (Se) μL extractant added and mixed by bubbling air. Solidified drop (ice cooling) removed and diluted to 200 μL (diluent not given). Neither analyte found in any sample. Green metrics were calculated on two scales	0.09 (As), 0.07 (Se) PF: 100 (As, Se) EHF: 103 (As, Se)	CNRM CRM GBW08606 (anions in water) and spike recoveries	225

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
iAs	Nut-based beverages (almond, cashew, peanut)	HG-AFS	Floating drop LLE of APDC complex of As^{III} into a hydrophobic natural DES (capric acid and lauric acid)	Samples diluted 5-fold (almond, cashew) or 10-fold (peanut), and 7 mL was taken for preconcentration. 50 μL extractant added and mixed by vortexing. Solidified drop (ice cooling) removed and As^{III} back extracted into 250 μL conc. HCl. Total As was determined after reduction of As^{V}, and hence the As^{V} concentration was determined by difference. As^{III} found in all samples, but As^{V} was not detected in any. A green metric was calculated	0.11 (As^{III}), 0.30 (As^{V}) EHF: 28	NIST SRM 1640a (trace elements in natural water)	226
iAs species and organic As	Rice	ETAAS	LLME of As^{III} complex with DDTP into DES (thymol and decanoic acid)	Sample (0.5 g ground to pass 50-mesh) extracted with 10 mL HNO_3 (0.28 mol L^{-1}) at 90 °C for 2 h. Diluted to 50 mL. To 10.0 mL were added 100 μL of DES extractant and 10 μL of DDTP (to selectively extract As^{III}). Following selective extraction of As^{III}, the DES was solidified by cooling. As^{V} was reduced to As^{III} so by repeating the selective extraction, total iAs was determined. A second 0.5 g sample was subjected to MAD with HNO_3 only to convert all As species to As^{V}, and after reduction to As^{III}, total As was determined. Hence total organic As was calculated as the difference between total As and the sum of As^{III} and As^{V}. All species were found in 16 of the 20 samples. One Iranian white rice contained only organic As, one Iraqi white rice contained only As^{V}, and one Iraqi and one Australian white rice contained no measurable As at all	0.07 μg kg^{-1} ERF 172 EHF 258	NMIJ CRM 7503a (rice flour) and spike recoveries	227
As species	Water (bottled, geothermal, tap), urine	HPLC-ICP-MS	SPME on agarose gel coated silica fibres functionalised with Fe NPs with desorption in 50.0 mol L^{-1} KH_2PO_4	Sample 15 mL (diluted as needed) extracted for 30 min at room temperature. Desorption 15 min at room temperature in 150.0 μL of which 100 μL was injected. In the one real urine sample, only DMA and MMA were detected. In the water samples, only As^{V} was detected, along with As^{III} in the geothermal water. All four species were found in SRM 2669	0.10 (As^{III}) 0.25 (As^{V}) 0.01(DMA) 0.37 (MMA) PF 100	CRM NIST SRM 1643e (trace elements in water) and SRM 2669 (As species in frozen human urine)	176

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] ($\mu g\ L^{-1}$) extent of preconcentration[c]	Validation	Ref.
Bi	Water (tap, spring)	FAAS	SPE on $Ni(OH)_2$ nanoflowers with elution into HNO_3	To 35 mL of sample were added 20 mg of adsorbent; after mixing, and separation, the Bi was dissolved in 250 μL of 6.0 mol L^{-1} HNO_3, which was analysed without further dilution. The analyte was not found in either sample	2.8 EHF 139	CRM NIST SRM 1643f (trace elements in water) and spike recoveries	228
Cd	Dairy products (milk, cheese, and doogh)	FAAS	DSPME on a magnetic ion-imprinted polymer-metal organic framework (Fe_3O_4@ZIF-8@IIP) nanocomposite	Cheese (3.0 g) UAE with 14 mL of hot water; milk and doogh used as received. To 7 mL sample was added 1.5 mL ACN (to precipitate proteins) after separation 10 mg of sorbent added, after mixing and separating, the Cd was dissolved in 125 μL 5% HNO_3, which was injected into the spectrometer. Analyte found in only one doogh sample. The extraction recovery was 98%, which means the PF is 55	0.09 PF 55	CRM NIST SRM 1549 (powder milk) and spike recoveries	229
Cd	Plasma, urine	ETAAS	LLE of the complex with DDTP into IL 1-allyl-3-methylimidazolium bromide	Urine (filtered and diluted 1 + 1 with 0.3 mol L^{-1} HNO_3), then unknown volume taken and diluted to 5 mL. Plasma proteins precipitated with dilute $HClO_4$, but no further details given. To 5 mL of the diluted sample were added 100 μL of 0.02 mol L^{-1} DDTP, 29.2 mg of the IL and, after shaking, 94.74 mg of $NaPF_6$ (to make the IL insoluble). After separation, 22 μL of extractant was diluted to 50 μL with MeOH and 15 μL taken for analysis. Cd was not detected in either sample, but recoveries at realistic concentrations (10–90 ng L^{-1}) were not significantly different from 100%	0.35 ng L^{-1} EHF 95	CRM NIST SRM 955d (human blood, level 1), SRM 2668 (toxic elements in frozen human urine, level 1) and spike recoveries	178
Cd	Food (salami, sausage), water (tap, trout farm)	CS-FAAS	DSPME on magnetic Cu_2O nanocubes with elution into HNO_3	Solid samples (5.0 g) digested on a hot-plate with HNO_3 and H_2O_2 in a two-stage process; after evaporation to dryness, residue dissolved in 10 mL H_2O. To this was added 30 mg extractant; after separation, the Cd was dissolved in 3 mL of 1 mol L^{-1} HNO_3. Total time for preconcentration was only 2.5 min. Cd was found in all samples except the tap water	0.12 PF 14	CRM EC JRC IRMM BCR®-505 (estuarine water), NIST SRM 1577b (bovine liver) and spike recoveries	230

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] ($\mu g\ L^{-1}$) extent of preconcentration[c]	Validation	Ref.
Cd	Sunflower oil	CV-AAS	LLME into HNO_3	Fine droplets of 2% (v/v) HNO_3 were sprayed through 7 mL of oil sample and, after vortexing and centrifugation, 100 μL of the extract was collected. To this was added 100 μL of 1.5 mol L^{-1} HCl and the resulting solution transferred to the CV generation apparatus. Cd was not detected in either of the two samples; recoveries of spikes between 2.5 and 5.3 $\mu g\ g^{-1}$ ranged from 88 to 101%	0.13 $\mu g\ kg^{-1}$ PF not mentioned	Spike recoveries	111
Cd	Vegetables (leek, lettuce), water (mineral, river, sea, tap)	FAAS	DSPME of the ion pair CdI_4^{2-} and alkyl dimethyl benzyl ammonium ion into benzophenone	Dried ground sample (2.0 g) was hot-plate digested with HNO_3 and H_2O_2. After evaporation the residue was dissolved in 50 mL of H_2O. Water samples were filtered and acidified. To 30 mL of sample was added 0.5 mL of 10% (m V^{-1}) KI solution, followed by the ion-pair agent. Then 1 mL of 2% (w/v) benzophenone in EtOH was added. After extraction and separation, the benzophenone sediment was dissolved in 1 mL EtOH, which was introduced into the spectrometer. Cd was detected in all samples, except tap and mineral water	0.4 PF 30 EHF 33.5	NIST SRM 1643d (trace elements in water) and spike recoveries	231
Cd	Food (tea, spinach), water (mineral, river, sea, tap)	FAAS	DSPE of the DTZ complexes on benzoic acid with dissolution in EtOH	The same research group as in the previous entry above devised a second procedure in which samples (2 g of dried ground material) were hot-plate digested with HNO_3 and H_2O_2 and diluted to 50 mL. To 30 mL was added DTZ solution and 40 mg of benzoic acid. A homogeneous solution was obtained at 50 °C; on cooling (ice bath), benzoic acid precipitated as fine particles that were collected (centrifugation) and dissolved in 1 mL EtOH. Cd was detected in all samples except the tap and mineral waters	0.3 PF 30 EHF 32.5	NIST SRM 1643d (trace elements in water) and spike recoveries	232

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] ($\mu g\ L^{-1}$) extent of preconcentration[c]	Validation	Ref.
Cd	Food (tuna, spinach, lentil, anchovy, shrimp), tobacco, water (tap, industry waste)	FAAS	DSPE by MWCNT@$TiSiO_4$ nanocomposite	3 g of food or 1 g of tobacco digested and diluted to 30 mL. 5 mL of the solution or 10 mL water sample taken for preconcentration. After diluting to 40 mL, 5 mg of adsorbent added and after mixing, extraction and separation, the analyte extract? was dissolved in 1 mL of 3 mol L^{-1} HNO_3. The intermediate dilution to 40 mL seems unnecessary. Analyte found in all foods except spinach, but only in one wastewater sample. The concentration of Cd in NCS ZC 73033 (0.19 $\mu g\ kg^{-1}$) is too low to be detected by the procedure as described (250 mg diluted to 40 mL)	0.053 PF: 40	CRM NCS ZC 73033 (scallion), NIST SRM 1570a (spinach leaves), BCR-505 (estuarine water) and spike recoveries	233
Cd, Co	Honey	ICP-OES	Magnetic SPE on Fe_3O_4 NPs coated with (3-aminopropyl)triethoxysilane (APTES) and polyethylene glycol *tert*-octylphenyl ether (TX-114)	5 g sample diluted to 50 mL. Adsorbent added to give a concentration of 2 g L^{-1} Fe_3O_4@APTES@TX-114 (100 mg). After magnetic separation, the analytes were dissolved in 1.5 mol L^{-1} HCl (but, crucially, the volume was not given). Neither analyte was detected in any of the five samples. Recoveries of spikes at 500 and 1000 $\mu g\ L^{-1}$ were quantitative. The details of the procedure are difficult to discern, and there was no discussion of the difference in ERFs	0.889 (Cd), 0.564 (Co) ERF 21 (Cd), 109 (Co)	Spike recoveries	234
Cd, Cu, Zn	Fruit juice (peach, apple), water (tap, well, surface)	FAAS	Precipitation of azathioprine complexes with subsequent DLLME into H_2O	To 20 mL of centrifuged alkaline sample was added 12 mg of azathioprine; after mixing, the complexes were precipitated by the addition of HCl and dissolved in 1.5 mL of ACN. Then 300 μL H_2O were added and the solution was dispersed into 5 mL of ethyl acetate. Following separation, 100 μL of the aqueous phase was injected into the spectrometer. All analytes were found in all water samples, except for Cu in tap water. None was found in peach juice, and Zn was not found in the apple juice	0.35 (Cd), 0.20 (Cu), 0.17 (Zn) ERF 60–64	CRM SPS-WW2 (elements in wastewaters) and spike recoveries	235

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Cd, Pb	Urine	ICP-MS	SPE on 3D-printed monolith modified with thiol-functionalized MOF-808	Sample (10 mL) digested (total of 21 h), evaporated to near dryness and diluted to 8 mL, of which 5 mL was loaded on the MOF-808-SH-3D printed monolith at 200 μL min^{-1}. The analytes were eluted with 250 μL of 0.1 mol L^{-1} HNO_3 containing 2% (m/v) thiourea at 50 μL min^{-1}. The column was regenerated by 1 mL of 1 mol L^{-1} CH_3COONH_4 at 200 μL min^{-1}. Samples were processed by a six-column automated array for which the total time was 95 min. Both analytes found (at normal concentrations) in three samples	3.5 (Cd), 17.6 (Pb) ng L^{-1} EHF 19 (Cd), 20 (Pb)	CRMs: CNRM GBW091032 and GBW09105 (lyophilised human urine–trace metals) and spike recoveries	236
Cd, Co, Cu, Mn, Ni, Pb, Zn	Urine, water (ground, river, sea)	ICP-MS	SPE on a 3D-printed acrylate-based photocurable monolithic foam column incorporating thermally expanded microspheres	Sample (0.5 mL) loaded at 1.0 mL min^{-1}, residual matrix removed by 1.7 mL air at 1.0 mL min^{-1}, and analytes eluted with 3.5 mL of 0.5% (v/v) HNO_3 at 1.0 mL min^{-1} and delivered directly to spectrometer. Enhancement based on peak height response. All analytes detected in all samples except for Cd in groundwater	0.5 to 5.2 ng L^{-1} EHF 13–18	CRMs: NRCC CASS-4 (trace metals in nearshore seawater), SLRS-5 (trace metals in river water), NIST SRM 1643f (trace elements in water) RM Seronorm® trace elements in urine L-2 Spike recoveries	237
Cd	Water (drinking, tap), seafood (salmon, tuna, Indian mackerel, white shrimp, whole squid, blue swimming crab, green mussel, cockle, and jellyfish)	ETAAS	DLLME of 1-(2-pyridylazo) 2-naphthol (PAN) complex into natural DES (menthol and formic acid) with solidified floating drop	Solid samples (0.1 g finely ground) MAD, then diluted to 50 mL. To 10 mL were added 554 μL of 5 mmol L^{-1} PAN, 100 μL of extractant and 500 μL MeOH. After separation (ice bath), the solidified drop was dissolved in 500 μL ethanol and a 20 μL aliquot transferred to the atomiser. The analyte was not found in any of the samples. No details were given of drying and grinding. The results for the LUTS-1 CRM were compared with the wet weight certified value (2.12 mg kg^{-1}) not the dry weight value (14.2 mg kg^{-1})	0.22 ng L^{-1} Enrichment factor (not defined) 112	CRM NRCC LUTS-1 (non defatted lobster hepatopancreas) and Thailand national institute of metrology TRM-F-2005 (elements in drinking water) Spike recoveries	238

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] ($\mu g\ L^{-1}$) extent of preconcentration[c]	Validation	Ref.
Cd, Cu, Pb	Food (leek, spinach, beef bone marrow broth), water (tap)	FAAS	SPE on sodium ferrite NPs ($Na_2Fe_4O_7$ NPs)	Solid samples and broth (mass not given) digested with HNO_3 and H_2O_2 and made up to 200 mL, all of which was passed through a column containing 20 mg adsorbent at 2 mL min^{-1}. Analytes were eluted with 2 mL of 1 mol L^{-1} HCl at 2 mL min^{-1}. All analytes found in all four samples. The NPs were synthesized using a green strategy that involved *Tamarindus indica* fruit extract	0.006 (Cd), 0.008 (Cu), 0.009 (Pb) EHF 100 (Cd, Cu), 99 (Pb)	CRM SPS WW2 (level 2, LOT number 116) and spike recoveries	239
Cd, Cu, Hg, Pb	Food (powdered milk, rice, and wheat groats)	ICP-OES	SPE, adsorbent made by immobilizing a DES (thymol and thionalide) on glass fibres in a microcolumn	Sample (1 g) underwent MAD then were diluted to 50 mL, all of which was passed through the column (containing 120 mg of glass fibres pretreated with 0.5 mL of DES at 1 mL min^{-1}). The analytes were eluted with 2 mL of 5 g L^{-1} thiourea in 0.1 mol L^{-1} HCl at 1 mL min^{-1}. Only Cu found in samples. According to the AGREEprep metric, the methodology achieved an "environmental friendliness index" of 0.7	0.6 (Cd), 4 Cu), 6 (Hg), 6 (Pb) $\mu g\ kg^{-1}$ in solid samples ERF 95 (Cd), 97 (Cu), 98 (Hg), 95 (Pb)	Comparison with an ETAAS reference procedure and spike recoveries	240
Cd	Food (celery, leek, spinach), water (bottled, pond, tap, waste)	FAAS	DLLME in a hydrophobic DES (tetra-*n*-butylammonium bromide and decanoic acid)	Food samples (1 g, dried and ground), MAD and diluted to 40 mL. Soil CRM (0.5 g) digested with aqua regia and $HClO_4$. To 40 mL of digest was added 1 mL of DES (no disperser was needed) and after mixing and separation (total 8 min), the DES phase was diluted to 2 mL with EtOH. Cd was found in all samples, except the tap and bottled water, at concentrations 5 to 9-fold higher than that in the CRM (0.081 $\mu g\ g^{-1}$)	0.7 EHF 18.7	CRMs: SPS-WW2 batch 114 (wastewater) and NCS DC 78302 (Tibet soil)	241
Cd, Co, Cu, Mn, Ni, Pb, Zn	Water (ground, river, sea, waste), urine	ICP-MS	SPE on monolithic 3D-printed column fabricated from Flex 57A resins, comprising tri(propylene glycol) diacrylate, 1,6-hexanediol diacrylate, acrylate oligomers, and diphenyl-(2,4,6-trimethylbenzoyl) phosphineoxide	1.4 mL of sample was loaded onto the column at 1 mL min^{-1} and, after washing, the analytes were eluted with 0.5% HNO_3 at 1 mL min^{-1} with direct introduction to the spectrometer. All analytes found in all samples. The novel feature of the methodology was that valves in the flow manifold were opened and closed by the swelling and shrinking of 3D-printed materials that was controlled by NIR radiation	0.1(Cd)–6.8 (Zn) ng L^{-1} EHF 7.1(Mn)–10.5 (Cd)	CRMs: NRCC CASS-6 (nearshore seawater), NIST SRM 1643f (trace elements in water) NRCC SLRS-5 (trace metals in river water); RMs: Seronorm® (trace elements in urine L-2) and spike recoveries	242

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Cd, Co, Cu, Mn, Ni, Pb, Zn	Water (sea, river, ground), urine	ICP-MS	SPE on monolithic 3D-printed column packing, fabricated from bisphenol a ethoxylate dimethacrylate photocurable resins	The same research group devised a second procedure from the one described above, in which 2.3 mL of sample was loaded onto the column at 1 mL min^{-1} and, after washing, the analytes were eluted with 0.5% HNO_3 at 1 mL min^{-1} with direct introduction to the spectrometer. All analytes found in all samples. The novel feature of the methodology was that valves in the flow manifold were opened and closed by the swelling and shrinking of 3D-printed materials that was controlled by the solution acidity	0.5 (Mn) to 5.9 (Zn) ng L^{-1} EHF 9–9.9	CRMs: NRCC CASS-6 (nearshore seawater), NIST SRM 1643f (trace metals in water) NRCC SLRS-5 (river water) RMs: Seronorm® (trace elements in urine L-2), spike recoveries	243
Cd	Food (dried fruit, peanut), water (canal, river, tap, waste)	FAAS	Magnetic SPE on an Al–Fe_3O_4 nanocomposite	Samples (3.0 g) were digested in stages on a hot plate with HNO_3 and H_2O_2. The final volume was not specified, but 30 mL was taken for the preconcentration stage. To this was added 5 mg of adsorbent and after separation the analyte was eluted in 3.0 mL of 0.1 mol L^{-1} HNO_3. According to the results tables, only waste water, kiwi and orange contained any Cd whereas the abstract indicates that Cd was also found in Granny Smith apples, dried apricots and raisins. The synthesis of the NP involved aluminium foil waste and banana peels	0.068 PF 10	NIST SRM 1573a (tomato leaves), EC JRC IRMM BCR®-505 (estuarine water) and spike recoveries	244
Cd	Food (red chili powder, black pepper powder and turmeric powder), water (tap, waste)	CS-FAAS	DSPME on graphitic carbon nitride modified with a CaFe layer double hydroxide	Solids (0.25 g) subjected to MAD with HNO_3 and H_2O_2 but final volume not given. 25 mL taken for extraction with 15 mg adsorbent with elution with 2 mL 0.5 mol L^{-1} HNO_3. 25 mL of water samples taken. Cd was found in all samples	0.04 PF 12.5 Enrichment factor (not defined) 12.32	CRM NIST SRM 1515 (apple leaves), EC JRC IRMM BCR®-505 (estuarine water) and spike recoveries	245

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Cd, Cu, Ni, Pb	Dietary supplement (Cu), water (bottled, mineral)	FAAS	Magnetic SPE on divinylbenzene/methacrylamide/GO Fe_3O_4	The dietary supplement tablet containing 600 μg Cu was dissolved in a mixture of conc. HNO_3 and HCl (1 + 3, v/v), evaporated to near dryness, then diluted to 25 mL with H_2O. 100 mg of adsorbent was added and after separation, the analytes were eluted with 7 mL 1 mol L HCl. After evaporation the solution was diluted to 5 mL. Only Cu was determined in the dietary supplement. No results were given for the analyses of the water samples, only recovery values. Slightly inferior results were also presented for a second material not containing GO	2.1 (Cd), 11 (Cu), 9.3 (Ni), 2.3 (Pb)	CRM EC JRC IRMM BCR®-146R (stream sediment) and spike recoveries	246
Cd	Food (rice and mate tea), water (mineral tap), tobacco	FAAS with thermospray and tube-in-flame atomisation	Magnetic (Fe_3O_4) DSPME on a silica coated nanocomposite functionalized with 2-aminobenzothiazole	Solid samples (250 mg) were subjected to MAD with HNO_3 and H_2O_2. After evaporation to near dryness samples were diluted to an unknown volume, but possibly 10 mL as this volume was taken for preconcentration. 20 mg of the extractant was added and after separation, the Cd was dissolved in 250 μL of 1.5 mol L^{-1} HNO_3. Cd was not detected in any of the water samples, but found in all three solids. Slightly inferior results were also presented for a second material containing functionalized CNTs	0.02 EHF 19.4	Comparison of results with those obtained by ETAAS method and spike recoveries	247

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Cd, Ni	Food (onion, spinach, and parsley), beverages (mineral water, orange juice, cola), water (waste)	FAAS	DLLME with a ferrofluid composed of a DES (menthol and octylamine) and MNPs modified with 2-methylimidazole	Solid samples (1.0 g) were hot-plate digested with HNO_3 and H_2O_2. It is not clear what the final volume was. For liquid samples it is not clear what volumes were taken: maybe 5 mL for the water. The solutions were extracted with 1.25 mL of ferrofluid and after separation the analytes were dissolved in 2.0 mL of 0.5 mol L^{-1} HNO_3 and filtered. Cd was detected only in one of the three wastewaters (from a fish farm) and the mineral water. Ni was found in all samples except the spinach. Green metrics were calculated on two scales	0.015 (Cd, Ni) PF 5 EHF 4.8 (Cd), 4.7 (Ni)	CRM EC JRC IRMM BCR®-505 (estuarine water), NCS ZC73032 (celery), and NIST SRM 1573a (tomato leaves)	248
Co, Cu, Ni, Pb	Food (cucumber, mint, black pepper), water (dam, river, and wastewater)	FAAS with microsampling	Magnetic SPE on Fe_3O_4 impregnated carboxymethyl-beta-cyclodextrin	Solid samples (0.7 g) were hot plate digested with HNO_3. After evaporation to dryness, the residue was dissolved in 15 mL of water. 20 mg extractant added and after separation the analytes were dissolved in 1 mL 2 mol L^{-1} HNO_3 and 100 μL injected. None of the analytes were found in any of the samples, apart from Co and Ni in the wastewater	0.14 (Co) 0.55 (Cu) 0.5 (Ni) 1.4 (Pb) ng mL^{-1} PF 15 EHF 15	CRM INCT OBTL-5 (oriental basma tobacco leaves) and TMDA 64.3 (environmental water)	249
Co	Water (mineral, tap, waste)	FAAS	LLME of complex with dodecylbenzenesulfonate into 1-dodecanol	Sample volume not specified but after adding KI to give 0.02 mol L^{-1} the volume was 25 mL. To this was added 75 μL of 1-dodecanol containing 25 mmol L^{-1} SDBS after heating and stirring, the solution was cooled to 4 °C and the solidified drop removed and dissolved in 300 μL EtOH. Analyte found only in waste water	2.8 EHF 80	CRM INCT TMDA 61.2 (water) and spike recoveries	250
Cu	Food (rice, cranberry juice), water (tap, lake river, well)	FAAS	Magnetic SPE on β-cyclodextrin/Fe_3O_4 composite with elution by HNO_3	Rice (1 g) was dry ashed (500 °C 5 h), dissolved in HNO_3 and H_2O_2 and diluted to 25 mL. 100 mL of liquid samples were taken. 50 mg of adsorbent was added; after separation, the Cu was dissolved in 2.0 mL of 0.2 mol L^{-1} HNO_3. Analyte was found only in the lake water, which is unexpected given that the typical concentrations of Cu in cranberry juice and rice are well above the LOD	2.2 PF 50	CRM, GBW(E)080039, (trace elements in water) and spike recoveries	251

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] ($\mu g\ L^{-1}$) extent of preconcentration[c]	Validation	Ref.
Cu	Food (olives, dates, coffee), water (tap, drinking, waste), tobacco	CS-FAAS	DSPME on activated graphite@NiO nanoflowers with elution into HNO_3	Solid samples (250 mg) hot-plate digested with HNO_3 and H_2O_2 and diluted to 25 mL. To this (or 40 mL of liquid samples) were added 15 mg of extractant, and after extraction and separation, the Cu was dissolved in 0.5 mL of 2.0 mol L^{-1} HNO_3. Analyte was found only in the coffee and tobacco. In a later study,[252] Cu was found in the dates	4.2 PF 80	CRM SPS-WW2 (wastewater), NCS ZC73032 (celery), IC-INCT-OBTL-5 (oriental basma tobacco leaves) and spike recoveries	253
Cu	Food (dates, coffee, black pepper), water (tap, ground)	CS-FAAS	DSPME on MnO_2 nanowires with elution into HNO_3	Samples (mass not given) were subject to MAD with HNO_3 and H_2O_2, and diluted to 10 mL. To this was added 1 mg of extractant; after extraction and separation, the Cu was dissolved in 0.5 mL of 3 mol L^{-1} HNO_3. Analyte was found in all samples, except the two tap waters	2.9 PF 10	NIST SRM 1573a (tomato leaves), EC JRC IRMM BCR®-505 (river water) and spike recoveries	252
Cu	Fish and seafood, water (drinking, tap, waste, well)	ICP-OES	Magnetic SPME on titanium aluminium carbide (Fe_3O_4–Ti_3AlC_2) nanocomposite with elution into HNO_3	Samples (1 g) were subject to MAD with HNO_3 and H_2O_2, but the final volume was not given. For preconcentration, 30 mL was taken, to which was added 20 mg of adsorbent. After extraction and separation, the Cu was dissolved in 3 mL of 0.1 mol L^{-1} HNO_3. Analyte was found in all samples except the drinking water and one of two wastewaters	0.37 PF 10	CRM NIST SRM 1549 (milk powder) and spike recoveries	254
Cu	Vegetables (onion, bell pepper, arugula)	CS-FAAS	DSPME on carbon nanodots with elution into HNO_3	Sample (0.25 g) were subject to MAD with HNO_3 and H_2O_2, and diluted to 50 mL. To this was added 5 mg of extractant; after extraction and separation, the Cu was dissolved in 1 mL of 2 mol L^{-1} HNO_3. Analyte was found in all samples. The nanodots were synthesized from cows milk	0.4 EHF 47	CRM: NCS ZC 73033 (scallion) and spike recoveries	255
Cu	Food (celery leaves, leeks, chards), water (waste)	CS-FAAS	DSPME on $NiFe_2O_4$ nanoflowers with elution into HNO_3	Samples (0.25 g) were subject to MAD with HNO_3 and H_2O_2, and diluted to 30 mL. To this was added 2 mg of extractant; after extraction and separation, the Cu was dissolved in 1 mL of 1 mol L^{-1} HNO_3. Analyte was found in all samples. At the optimal pH of 8, calcium hydroxide precipitated and was removed by centrifugation	2.1 PF 30	CRM EC JRC IRMM BCR® 505, (estuarine water), NCS ZC73032 (celery), TM-25.3 (fortified water) and spike recoveries (water samples only)	256

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Fe	Beer	FAAS	DLLME of APDC complex into 1,2-dichlorobenzene	To 10 mL of sample were added APDC and 75 μL dichlorobenzene was injected. After separation, the organic phase was mixed with 10 mL of H_2O before introduction to the spectrometer. Analyte was found in all three samples, and there was no significant difference between the results and those obtained by MAD followed by ICP-OES. pH adjustment, salting out and washing were not needed	LOQ 9	Comparison with a MAD ICP-OES method	257
Ga, Ni, Ti, V	Water (tap, sea)	CS-ETAAS	Magnetic DSPME on GO functionalized with methyl thiosalicylate with elution into HNO_3	To 250 mL of sample were added 20 mg of adsorbent; after extraction and separation, the analytes were desorbed into 1 mL of 6% (v/v) HNO_3, 20 μL of which was injected into the furnace together with Nb as modifier. None of the analytes was found in tap water, and Ni and Ga were found in the seawater	0.04 (Ga), 0.66 (Ni), 0.71 (Ti), 0.04 (V)	CRM TMDA 64.3, (fortified lake water) and SPS-SW2 batch 125 (surface water) and spike recoveries	258
Hg, Ni, Zn	Food (including cereals), vegetables, fruit, nuts, honey, salt and sugar, beverages and tap water	ICP-OES	SPE on column of magnetic γ-Fe_2O_3 NPs loaded with mushroom (*Agaricus augustus*) with elution by HCl	Dried sample (1 g) was hot-plate digested with HNO_3 and HCl, then subjected to MAD with HNO_3, HCl and H_2O_2 and finally diluted to 50 mL. This was passed (3 mL min^{-1}) through a column containing 200 mg of γ-Fe_2O_3 MNPs mixed with 200 mg of *A. augustus* powder. The retained analytes were eluted with 5 mL 1.0 mol L^{-1} HCl. Hg was found only in ready-to-use coffee, rice, walnut, spinach and mint; Ni was found in all samples except, tap water, sugar, honey, milk, cheese banana, orange and cherry; Zn was found in all samples. The method did not work for Cd, Co, Cu, Fe, Mn, or Pb. The column could be reused 30 times	0.016 (Hg), 0.011 (Ni) 0.023 (Zn) PF 100 (for 500 mL sample)	CRM NIST SRM 1643e (simulated fresh water), SCP science, Quebec EU-L-2 (waste water), Techlab, France NWTM-15 (fortified water), NCS NCSZC 73350 (poplar leaves), NCSZC 73014 (tea)	259

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Hg species	Wine (red, rosé, white) water (tap, mineral)	CV-AFS	LLME of chlorocomplex into MIL trihexyl(tetradecyl)phosphonium tetrachloroferrate with back extraction into HCl	Red and rosé wines diluted five times. To 5 mL of sample was added concentrated HCl (5 min for formation of chlorocomplex), then 75 μL of MIL added together with 50 mg $NaHCO_3$ liberating CO_2 as effervescing agent. The MIL was collected on a magnetic rod, diluted with 150 μL of $CHCl_3$ and the Hg extracted into 250 μL of concentrated HCl and diluted to 500 μL with H_2O. Total Hg was determined after UV photooxidation of OMC. iHg was found only in the tap water and red wine. OMC were not found in any samples, but recoveries of MeHg and PhHg ranged from 90 to 105%	0.096 (iHg)	CRM NIST SRM 1641e (total Hg in water) and spike recoveries	260
Hg	Edible oils (fish, soybean, sunflower, peanut, corn, canola)	CV-ICP-MS	DLLME into HCl	Sample (5 g) heated to 80 °C (water bath) with 0.5 mL *n*-propanol (disperser), and 0.5 mL 6 mol L^{-1} HCl (extractant). After separation, the aqueous phase was diluted to 10 mL with H_2O. Hg was detected in fish, soybean and corn oils. The MAD method detected Hg in only fish and soybean oils, as the sample dilution factor (×100) was much higher than that of DLLME method (×2)	0.1 ng g^{-1}	CRM Spec-Sol, Brazil OMHG100, (mineral oil) and comparison with a MAD CV-ICP-MS method	109
Mn	Food, including cereals, nuts, pulses, spinach and pumpkin seeds	FAAS	Vortex assisted DSPME of biodegradable polyhydroxy butyrate diethanolamine (PHB-DEA)	0.25 g of sample, MAD with HNO_3 and H_2O_2 and transferred to 50 mL. To 15 mL of digested sample 120 mg of PHB-DEA adsorbent was added, pH adjusted to 6 and shaken. After centrifugation, 0.75 mL of acidic EtOH was added to dissolve the Mn, which was found in all samples	0.15 EHF 182	NIST SRMs 1570a (spinach leaves), 1568b (rice flour), 1515 (apple leaves)	261

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Mn	Water, tea	FAAS	Switchable hydrophilicity solvent-based LLME with triethylamine	To 10 mL of sample in centrifuge tube. 2 mL of acetate buffer (pH 4) was added, next 4 mL of 0.1% (w/v) APDC solution (as a chelating agent) was introduced and vortexed for 30 s. Then 5 mL of switchable polarity solvent was introduced and diluted to 20 mL with H_2O. The tea was deprotonated by adding 2 mL of NaOH and a cloudy solution was readily achieved. Following centrifugation, the upper layer containing the analyte was collected and diluted with 5 mL of 2 mol L^{-1} HNO_3 before analysis	0.413	NIST SRMs 1573a (tomato leaves) and 1515 (apple leaves) spike recoveries	262
Mn	Nuts and roasted chickpeas	SQT-FAAS	MNPs with SPME	Sample (0.2 g) subject to MAD with HNO_3 and diluted to 50 mL to 30 mL of a sample at pH 12 150 mg of MNPs were added. The elution was carried out after magnetic separation with 0.75 mL of 1 mol L^{-1} HNO_3	108	NIST SRM 1515 (apple leaves)	263
Mo	Groundwater, meat, vegetables	ETAAS	On-line flow-batch dispersive LLME	Water samples filtered; solid samples MAD prior to extraction. The reactor of the manifold was loaded with 5 mL of sample, pH adjusted to 1.4. For extraction, the ternary mixture (ethyl xanthane solution, CCl_4 and ACN) was injected into the mixing chamber. In the same step, the formation of the complex and its extraction took place. The organic phase was then separated and 10 μL was taken for ETAAS	0.03 for water, 0.02 μg kg^{-1} for other matrices) EHF 130	NIST SRMs 1640a (water), 1573a (tomato leaves), 1577c (bovine liver)	264
Ni, Pb	Water (tap and mineral), food (including cereals, chicken and vegetables), beverages (black tea and baby milk), tobacco	ICP-OES	SPE with *Sparassis crispa* loaded on hollow mesoporous nano-silica (HMSiNP)	MAD was used to digest solid samples (HNO_3 + HCl + H_2O_2). 100 mg of *S. crispa* loaded HMSiNP was added to 50 mL of the sample (pH 7), then shaken for 2 h. After separation and digestion in concentrated HNO_3, the pellet concentrations of Ni and Pb were measured. The Ni and Pb contents were < LOD for tap water and baby milk	0.019 (Ni), 0.033 (Pb), PF 80 for both metals	NWTM-15, NCS ZC73014 (tea leaves), NCS ZC73350 (poplar leaves)	265

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Pb	Water, medicinal plant *Moringa oleifera* (seeds, leaves, roots)	FAAS	Magnetic DSPE of starch-modified nickel ferrite NP	Plant samples were digested with HNO_3 at 130 °C. Water samples were filtered. 200 mg of the adsorbent was added to 250 mL of sample at pH 6.5. After magnetic separation, the adsorbed analyte was eluted with 1.25 mL of 0.3 mol L^{-1} HNO_3	0.12	NIST SRM 1643e (water) and spike recoveries	266
Pb	Tap water, turmeric	FAAS	Magnetic DSPME with MWCNTs with layered double hydroxide	Solid sample (0.2 g) subject to MAD with HNO_3 and H_2O_2 but final volume not given. To 10 mL of sample (pH 8) were added 5 mg adsorbent; following vortexing for 30 s, the nanotubes were magnetically separated and the Pb desorbed in 2 mL 1.0 mol L^{-1} HNO_3	0.314 PF 11.53	NIST SRM 1515 (apple leaves), EC JRC IRMM BCR®-505 (estuarine water) and spike recoveries	267
Pb	Water (tap and lake), orange juice, edible oil and mushrooms	ETAAS	Magnetic SPE with waste toner-derived magnetic adsorbent	Solid samples were MAD with HNO_3–H_2O_2 prior to extraction (0.1–0.2 g to 250 mL). To 50 mL (pH 7), 10 mg of adsorbent was added. After shaking for 24 h, the supernatant was collected after magnetic separation	0.013 EHF 91	GSB 07-1183-2000 (environmental water), GBW 10016a (tea), GBW(E)100 348–100 362 (rice flour) and spike recoveries	268
Pb	Water (tap, waste), juice powders	FAAS	DSPME with graphitic carbon nitride with Ni and Ag nanocomposites	After MAD of juice powders (0.25 g, with HNO_3–H_2O_2) digests (7 mL) was diluted to 30 mL with water. Extraction involved 5 mg adsorbent, 2 mL buffer (pH 8) and 30 mL of the digested sample. The mixture was vortexed and centrifuged and the Pb desorbed with 2.5 mL of 0.5 mol L^{-1} HNO_3	0.27 PF 12	NIST SRM 1570a (spinach leaves), EC JRC IRMM BCR®-505 (estuarine water) and spike recoveries	269
Sb	Water (bottled, tap, river), food (salmon, tuna, packed chicken, canned meat), beverages (coffee, cappuccino, powdered milk and tea)	HG-AAS	Ultrasound-assisted dispersive solidified floating organic drop microextraction, thiazolyl azo DES	Solid samples (1 g) MAD (HNO_3–H_2O_2) and diluted to 50 mL to 5 mL (pH 5.5) was added 500 μL of 0.5 mol L^{-1} chelating reagent (4,4-dimethyl-2,6-dioxo-*N*-phenylcyclohexan-ecarbothioamide), followed by 100 μL of DES to extract the Sb^{III} complexes. After centrifugation, the DES was solidified (by cooling), separated and dissolved in 200 μL of EtOH. Sb was determined in all samples	0.006 EHF 180	NIST SRM 1643a (trace elements in water) and spike recoveries	270

Table 1 (*Contd.*)

Analyte	Matrix	Technique	Extraction mode/reagents	Procedure/comments	LOD[b] (μg L^{-1}) extent of preconcentration[c]	Validation	Ref.
Se	Food (flour, milk, rice, and vegetables)	HG-AAS	DLLME with MIL	After MAD of solid samples (0.25 g with HNO_3–H_2O_2) were diluted to 50 mL, sulfite reducing agent was added, pH adjusted (5) and 375 μL of 3 per mmol L^{-1} tannic acid (as complexing agent) was added. After complexation, 750 μL of MIL was added repeatedly (5 times). Following magnetic separation, the Se was dissolved in 1500 μL of 1 mol L^{-1} HNO_3 in EtOH, and the MIL magnetically separated before analysis	0.0122, ERF defined but not given	NIST SRM 1573a (tomato leaves), SRM 1568a (rice flour), GBW 10017 (milk powder), GBW 10016 (tea) and spike recoveries	271
Zn	Food (one sample each of cocoa, hazelnut, spinach and strawberry	ICP-OES	DMSPE on NiO nanoflowers	Spinach and strawberry (4 g) and ground cocoa and hazelnut (1 g) were subjected to MAD with HNO_3–H_2O_2 but final volume not given. To 30 mL of digest (pH 7) were added 20 mg of adsorbent. For desorption, 3 mL of 0.5 mol L^{-1} HNO_3 was used, subsequently, the eluent and the nanoadsorbent were separated *via* centrifugation. The upper layer was used for measurement. The Zn concentration in strawberry and spinach was < LOD	0.77 (0.58 μg kg^{-1}), PF 10	NIST SRM 1573a (tomato leaves)	272
ZnO NPs	Infant milk formula, maternal food supplement	ICP-OES	CPE of humic acid complex into Triton X-100	To 0.1 g of powdered infant formula dispersed in 7 mL of H_2O, was added 70 μL of humic acid solution (10 μg mL^{-1}). The mixture was ultrasonicated at 60 °C, the pH was adjusted to 10 and Triton X-100 was then added to reach 5% (w/w). Following further incubation at 60 °C and separation, the Zn concentration in the pellet, supernatant, and total sample was determined. For validation Zn was determined in purchased ZnO NP suspension before and after CPE, recovery was 85.2%. All products analysed showed the presence of ZnO NPs (16 to 40 mg g^{-1} of formula). The percentage of Zn content determined as ZnO NPs varied from 18% to 60%		No CRM available, analysis of commercial ZnO NP	273

[a] No results are given in the column "validation", however, in case of significant differences from the expected values a comment is given in the procedure/comments column. [b] Unless stated otherwise. [c] The preconcentration factor, PF, is based on volume ratio, the enhancement factor, EHF, is based on the ratio of the calibration slopes, the enrichment factor, ERF, is based on concentration ratios.

(Ag) and from US research nanomaterials (TiO_2). The researchers observed a high degree of agglomeration for the TiO_2 NP standards, resulting in mean sizes higher than those given by the manufacturer. The more rapid DLLME procedure, based on taking 10 mL of sample with a final volume of 2 mL, had lower recoveries as well as higher LODs.

For the determination of REEs in urine by ICP-MS, Nong *et al.*[68] first *decomposed the matrix with a photo-Fenton reaction*, involving iron^{II} and H_2O_2, and then preconcentrated the elements in the reaction mixture by co-precipitation with ferrous hydroxide. To 1 mL of urine was added 1 mL of 0.4 mmol L^{-1} iron^{II} oxalate followed by 0.3 mL concentrated H_2O_2 to give a concentration of about 0.3 mol L^{-1} when the final volume was made up to 10 mL. They found the optimum pH to be 4, but did not explain how this parameter was adjusted. Several (exact number not specified) solutions were irradiated with a UV xenon lamp (power not given), mounted 10 cm from the samples, for 2 h. The pH was adjusted to 8 with concentrated NH_4OH solution and the precipitation allowed to proceed for 5 h. After separation by centrifugation, the precipitate was dissolved in 1 mL of 1 mol L^{-1} HNO_3. The validation included the measurement of recoveries of spike at 300 ng L^{-1} to four real samples that ranged from 86 to 102%. The LODs ranged from 0.02 (Tb and Tm) to 4 (Er) ng L^{-1}. Several of the REEs were detected in the real samples.

The measurement of Zn isotopes, which have been used as tracers in studies of biological mechanisms and metabolic processes, is subject to possible artifacts associated with the chemical procedure. To improve the throughput, accuracy and precision of Zn isotope ratio determinations by MC-ICP-MS in biological materials (bone, liver, hair and blood), Retzmann *et al.*[76] devised *a fully automated dual-column SPE procedure* for separating the Zn from interfering matrix components. Samples (100–500 mg) underwent MAD with HNO_3 and H_2O_2 and were then diluted to 50 mL, from which aliquots (volume not given) were evaporated to dryness and redissolved in 1 mL of 2.5 mol L^{-1} HCl. This solution was then loaded onto a DGA resin column that retained the Zn (and some other elements) but allowed barium, calcium, magnesium, nickel, potassium and sodium, to pass through. The retained Zn was washed, by 0.5 mol L^{-1} HCl, into the second column (TK201 resin) that did not retain any iron or gallium. The retained Zn was finally washed off the second column with 4 mL of H_2O. The purified fraction was evaporated to dryness and dissolved in 100 μL of concentrated HNO_3, then evaporated again and dissolved in 2 mL of 2% HNO_3. The validation included the analysis of five CRMs (NIST SRMs 1400 (Bone ash), 1468 (Bone meal), 1577c (Bovine liver); JRC ERM-DB001 (Human hair)) and the RM Seronorm® L-3 (Trace elements whole blood, lyophilised), for which results not significantly different from the assigned values were obtained for all materials. The throughput, of 20 samples per day, was a factor of 2–3-fold better than those of previous methods.

The large number of publications featuring *various forms of LLE and SPE* for preconcentration are summarised in Table 1. Interest in this topic is such that about 95 relevant articles appeared during the present review period, of which about 60 have been discussed in the Table. In an effort to make the summaries more manageable, the descriptions of the procedures omit some details, such as those relating to pH adjustment, cooling, centrifuging and filtering. To allow readers to calculate an LOD in a solid sample, the sample mass and final volume after the first dissolution step are given, if they were available in the paper. Another useful figure of merit is the extent of preconcentration and as far as possible this information is given in the Table (in the same column as the LOD). There is no uniformity in the terminology used by researchers, who gave values for "preconcentration factor", "enhancement factor" or "enrichment factor" often without definition. The following convention has been adopted: the preconcentration factor, PF, is based on the volume ratio, the enhancement factor, EHF, is based on the calibration slopes ratio, and the enrichment factor, ERF, is based on concentration ratio. Many papers still make it difficult for the reader to ascertain the details of the final optimized method, and several papers relegated important information about the chemical analysis to the SI.

5 Progress with analytical techniques

5.1 Mass spectrometry

A sustained interest in the *development of spICP-MS and scICP-MS* was evident during this Update period, focussing on sample preparation and introduction, calibration strategies, and approaches to interference removal. For example, spICP-MS was used to characterise Au NPs spiked into blood and urine (collected from healthy donors).[77] Data were acquired using a PFA nebuliser and quartz cyclonic spray chamber, with a 50 μs dwell time (60 s acquisition) to minimise double-particle events. Stable measurements were achieved for up to 10 days (<17% difference in size and concentration). This approach achieved a size LOD of 13 nm, and a particle number concentration LOD of 147 NP mL^{-1}. Linearity was reported across the particle size range (10–100 nm), with recoveries between 88 and 103%, for NP concentration, and from 100 to 110% for NP size. Relative SDs (short term precision) were below 7% for particle sizes between 20 and 100 nm, though NPs smaller than 10 nm exhibited RSDs of 56%, highlighting a critical limitation of this approach.

To address matrix effects in spICP-MS, Hernández-Postigo *et al.*[78] demonstrated how ID coupled with spICP-MS enabled reliable sizing and counting of Pt NPs in cell culture media and human urine. To monitor both ^{195}Pt and a ^{194}Pt-enriched isotope spike (96.5% isotopic abundance, ISC Science, Spain) by ICP-QQQ-MS, isotopes were analysed sequentially using a 2 ms stabilisation time and a dwell time of 5 ms. In a 20 mmol L^{-1} NaCl matrix, ID-spICP-MS achieved recoveries of 100 ± 1% for 30 nm Pt NPs and 101 ± 2% for 50 nm Pt NPs (an improvement from 42 ± 31% and 36 ± 25%, respectively).

Sample preparation strategies for Ag NPs and TiO_2 NPs spiked into urine were evaluated using either ultrafiltration or a surfactant-assisted (SA) DLLME approach with measurement by ICP-MS.[54] Both methods achieved low size LODs (17–18 nm for Ag NPs, 51–77 nm for TiO_2 NPs) with RSDs < 9% (short term

precision). Analytical recoveries varied by extraction procedure and element (72 to 99% for TiO_2 NPs, 81 to 115% for Ag NPs) but notably Ag NPs were shown to undergo partial dissolution during SADLLME, while ultrafiltration did not affect NP integrity. In another study, TiO_2 NPs spiked into blood and in urine were measured by spICP-MS/MS using NH_3 reaction mode to generate Ti–NH_3 adducts at $m/z = 131$.[79] The use of polyvinylpyrrolidone as dispersant improved particle dispersion, and TE (10.2%) was determined by the particle size method (*i.e.* by comparing the detected mass of a well-characterised reference NP to its known true mass). Dwell times of 100–200 μs were shown to enhance pulse signal uniformity, although longer dwell times reduced S/B. The approach achieved a particle size LOD of 9 nm and a particle number LOD of 2060 NP mL^{-1} with recoveries between 79 and 118%.

In an alternative approach to NP characterisation, Zarei *et al.*[80] developed an AF4-UV-MALS-ICP-MS method for online detection and size characterisation of nanomaterials in spring water, groundwater and bottled drinking water. The instrumentation setup was optimized using Pt and Ag NPs (Nanografi) and TiO_2 and Au NPs (Nanopartz) however there was no apparent validation of the additional 31 elements included in the analysis. The authors also noted challenges distinguishing smaller Mg NPs (1.5 to 10 nm) from ionic Mg^{II}.

A review, covering 114 publications, by Grebneva-Balyuk et al.[29] *provided a useful summary of the application of spICP-MS to biological samples containing NPs*. The authors noted a steady increase in the number of papers since 2012, although commented that many studies focussed on only a limited range of NPs, namely those of Ag, Au, SiO_2 and TiO_2. Emphasis was given to the challenges of sample preparation, especially the quantitative extraction of NPs without altering size or composition. While the discussion of preparation strategies lies beyond the scope of this section, this work highlighted both the rapid growth of interest in spICP-MS and the restricted range of nanomaterials investigated to date.

Applications of scICP-MS also featured strongly, with several studies emphasising calibration strategies and TE. The quantification of intracellular and extracellular Se in yeast cells (SELM-1) was demonstrated using NP-based calibration approaches.[23] A TE-dependent calibration (using Au NPs to calculate TE by the particle size method) was compared to TE-independent calibration with Se NPs (using 150 and 200 nm standard Se NPs). Measurements were acquired using a 20 μL min^{-1} flow rate using a high efficiency single-cell introduction system, achieving an ^{82}Se LOD of 0.26 fg. In another study,[81] the accuracy of Fe quantification in red blood cells by scICP-MS/MS was assessed using different fixation methods and sample introduction systems. Iron was measured on-mass ($m/z = 56$) using hydrogen reaction mode (4 mL min^{-1}). Glutaraldehyde proved a more suitable fixative than PFA, and cell transport efficiencies ranged from 0.5% (Scott-type spray chamber) to 15% (low-volume on-axis chamber). The authors noted that moderate variation in cell density (1.5–6.0 × 10^5 cells mL^{-1}) did not affect results.

A novel metrological strategy was reported to improve discrimination between true single-cell events and non-cellular signals (*i.e.* background noise and transient spikes) non-using scICP-TOF-MS.[82] The approach used Eu and Yb-doped carbon dots which were loaded into HeLa cells. Discrimination of events was based on both a 3.29σ detection threshold and the Eu : Yb ratio in single-cell events. Using a CytoNeb-CytoSpray (Elemental Scientific) high efficiency sample introduction system at a flow rate of 15 μL min^{-1} and 3 ms dwell time, a TE between 40 and 50% was achieved. Limits of detection of 0.106 fg per cell (^{153}Eu) and 0.226 fg per cell (^{172}Yb) were reported. Parallel LA-ICP-MS confirmed high tagging efficiency (99.87%), and a bespoke R algorithm was developed for robust event discrimination.

Several studies highlighted the *continuous improvement of ICP-MS/MS reaction cell strategies* for the determination of analytes prone to severe interferences. Selenium was determined in NIST SRM 1598a (inorganic constituents in animal serum) with improved accuracy using N_2O as the reaction gas compared to oxygen reaction mode.[83] The approach exploited a N-atom transfer reaction to generate SeN^+ thereby eliminating mass spectral interferences including Gd^{2+} from MRI contrast agents in serum. Compared with conventional oxygen reaction mode, sensitivity was enhanced and achieved an LOD of 2.2 ng L^{-1} for the most abundant isotope (^{80}Se). Spike recoveries were between 95 and 106% and short-term precision ranged from 2.5 and 4.2%. Another mass-shift strategy was reported for the rapid determination of *trans*-uranium radionuclides in urine.[84] Calibration was performed using ^{241}Am (NIST 4322C), ^{243}Am (NIST 4332E), ^{237}Np (NIST 4341a), ^{239}Pu (NIST 4330C) and ^{242}Pu (NIST 4334I), although no ^{244}Cm standard was explicitly stated. Using helium-oxygen mode enabled the mass shift of analytes (M → MO or MO_2^+) and suppressed interferences from Bi ($^{209}Bi^{34}S^+$), Hg ($^{199}Hg^{40}Ar^+$), Pb ($^{206}Pb^{35}Cl^+$, $^{207}Pb^{16}O^+$, $^{208}Pb^{31}P^+$), Tl ($^{203}Tl^{36}Ar^+$) and reduced $^{238}U^+$ (*i.e.* no DRC reaction) to < 1 × 10^{-9} and $^{238}U^1H^{16}O_2^+$: $^{238}U^{16}O_2^+$ ratios to < 1 × 10^{-7}. Using 50 mL urine, LODs of 0.27 fg (^{241}Am as $^{241}Am^{16}O^+$, $m/z = 257$), 0.12 fg (^{244}Cm as $^{244}Cm^{16}O^+$, $m/z = 260$), 0.28 fg (^{237}Np as $^{237}Np^{16}O_2^+$, $m/z = 269$), 1.1 fg (^{239}Pu as $^{239}Pu^{16}O_2^+$, $m/z = 271$) and 0.52 fg (^{240}Pu as $^{240}Pu^{16}O_2^+$, $m/z = 272$) were reported. A similar oxygen reaction gas approach was used for the determination of ^{90}Sr in two food CRMs (CNMR GBW10014a, Cabbage and GBW10019, Apple).[85] Isobaric interference from ^{90}Zr was suppressed by exploiting the more favourable exothermic Zr^+ + O reaction (since Sr^+ reacts with oxygen endothermically). Under optimized conditions (40% O_2) an instrumental LOD of 0.09 pg Sr per g was achieved.

Reaction cell chemistry was also used in a unique approach to VOC analysis. In this report, nine VOC spikes of 10 μL (including benzene, toluene, esters, and pyridine) were diluted in helium gas (2 L) and introduced directly into the CRC at a flow rate of 0.1–1 mL over 4 min.[86] Ionisation occurred *via* collisions with Ar^+ ions generated in the ICP-MS plasma, yielding intact molecular ions for detection. For the determination of VOC in coffee, the sample introduction system consisted of a sealed Tedlar bag containing milled beans (7 g) and a flow of helium gas (2 L) that was introduced into the CRC *via* $\frac{1}{4}''$ Tygon tubing. This novel instrumentation setup demonstrated that ICP-MS/

MS can also be used for the soft ionisation of small organics, extending its application beyond inorganic analysis.

Advances in laser ablation ICP-MS have predominantly focussed on improvements to calibration standards for quantitative and isotopic measurements. Thoroë-Boveleth *et al.*[87] developed multielement gelatine films as calibrants for As, Be, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pt, V, Y and Zr (1–10 μg g^{-1}), Al and Zn (10–100 μg g^{-1}), Ca, Fe and Mg (100–1000 μg g^{-1}), K, Na and P (1000–10,000 μg g^{-1}). Samples were analysed using a NWR213 laser (60 μm spot, 20 Hz, 5 ms dwell time, 0.214 s cycle, 70 μm s^{-1} scan rate). The authors varied gelatine content from 0.3 to 20% w/w (optimal range found to be from 2 to 9%) and laser fluences between 0.50 and 1.50 J cm^{-2} (optimal range found to be 1.25–1.50 J cm^{-2}). Higher gelatine content improved correlation coefficients ($r = 0.9988$ and 0.9969 for 15% and 20% gelatine, respectively) but reduced recovery due to film thickness. Similarly, Schannor *et al.*[88] applied LA-MC-ICP-MS to Cu isotope analysis in normal and diseased tissues, using gelatine-based standards. A NWR213 laser (266 nm, 100 Hz, 2000 μm s^{-1}, 10 J cm^{-2}) was coupled to MC-ICP-MS, achieving intermediate precisions of < 0.15‰ and expanded measurement uncertainties between 0.47 and 0.53‰ ($k = 2$) for inorganic CRMs or RMs (NIST SRMs 976 isotopic standard for copper, NIST SRM 3114 copper standard solution, and isotopic RM BAM-i020 Cu in nitric Acid). For biological CRMs, precision RSDs < 0.17‰ and expanded measurement uncertainties ranging from 0.46 and 0.54‰ ($k = 2$) were observed (NIST SRM 1577c Bovine liver and NRCC DOLT-5 Dogfish liver). In contrast, Rubin *et al.*[89] investigated different matrices (cellulose powder, leaf powder, and filter paper) and ISs (^{197}Au, ^{13}C, ^{115}In, ^{103}Rh and ^{205}Tl) for LA-ICP-MS determination of lanthanides in tea. The most effective combination was found to be filter paper with ^{197}Au as IS, which was most optimal when tea powder particle size was < 50 μm and 2 mL of IS solution was added per 500 mg of sample.

Two papers on LA-ICP-MS focussed on single cell applications. Tanaka and Ogra[90] measured seven endogenous trace elements (Cu, Fe, Mg, Mn, P, S and Zn) in differentiated PC12 cells (rat pheochromocytoma) cultured on collagen-coated coverslips. Cells were ablated using a Nd:YAG 213 nm laser (36 μm spot for cells, 90 μm spot for standards, 2 s dwell time, 20 Hz). Elemental masses were calculated from regression curves using FUJIFILM standards, revealing that cellular differentiation significantly altered trace element distribution. In a similar work, Menero-Valdés *et al.*[91] developed a protein imaging workflow using xenobiotic Au-nanocluster antibody probes against CYP1B1 in ARPE-19 cells, which were combined with Ruthenium Red (RR, an indicator of cell volume) and a Rh complex for DNA labelling.

Recent work in isotope analysis has focussed on developments in sample introduction, purification, and stable isotope tracer methods. A microultrasonic single-droplet nebulisation system (MUSDN) coupled to MC-ICP-MS was reported by Dong *et al.*[92] Optimization of the micropore structure of the nebulisation sheet and the addition of a 3D-printed connection ring enabled total nebulisation of microlitre droplets of serum, producing transient signals of several seconds. High-efficiency sampling improved sensitivity (*i.e.* transient signal intensity per analyte mass) by 17-fold for ^{7}Li and 12-fold for ^{63}Cu compared to pneumatic nebulisation, with RSDs of 0.3‰ for δ^{7}Li and 0.08‰ for δ^{65}Cu, although LOD or LOQ were not explicitly provided. The approach was applied to analyse two CRMs (NIST SRM-1400 Bone Ash and SRM-3114 copper standard solution) and three RMs (United States Geological Survey BCR-2 Columbia River Basalt, and Seronorm® Trace Elements in Serum L-1 and L-2). The measured value for δ^{65}Cu in SRM-3114 by MUSDN-MC-ICP-MS (−0.02 ± 0.05‰) was comparable to that achieved by conventional MC-ICP-MS (0.01 ± 0.03‰) and to the published SRM value (−0.06 ± 0.04‰). The method was finally applied to measure δ^{7}Li in serum from patients with Li poisoning, requiring less than 10 ng of serum per analysis. A fully automated dual-column purification procedure for Zn isotope analysis was reported by Retzmann *et al.*[76] using a prepFAST MC system with DGA and TK201 resins. Zn isotope ratios (^{66}Zn : ^{64}Zn) were determined in comparison to isotope CRM JRC IRMM-3702 (zinc in nitric acid) which is certified for ^{66}Zn : ^{64}Zn isotopic composition and was used as δ-anchor for isotopic analysis. Recoveries of 100.1 ± 5.3% (2 s, $n = 22$) were obtained, with stable blanks < 1.5 ng. Isotope ratios were determined in CRMs and RMs including NIST SRM 1400 bone ash (0.67 ± 0.07‰), SRM 1486 bone meal (0.91 ± 0.06‰), SRM 1577c bovine liver (−0.45 ± 0.05‰), ERM-DB001 human hair (−0.35 ± 0.05‰), GBW09101 human hair (−0.32 ± 0.08‰) and RM Seronorm® whole blood L-3 (−0.15 ± 0.05‰), demonstrating method robustness across a range of sample matrices (blood, bone, hair, and liver). Importantly, the isotope analysis showed no on-column fractionation. In a related example, Barad *et al.*[93] used SF TIMS to quantify erythrocyte enrichment following oral administration of ^{57}Fe. The approach, which was applied to nutritional Fe absorption studies, illustrates the continuing role of stable isotope tracers coupled with high precision mass spectrometry.

Advances in internal standardisation and calibration methodology featured studies of IS reaction cell behaviour, and multi-isotope and multi-IS calibration strategies. The performance of common IS elements under mass-shift conditions was systematically evaluated by Hartman and Mawhinney[94] This analysis included ^{209}Bi, ^{69}Ga, ^{74}Ge, ^{165}Ho, ^{115}In, ^{193}Ir, ^{6}Li, ^{187}Re, ^{45}Sc, ^{159}Tb, and ^{89}Y, as ISs under five operating modes (no-gas, hydrogen/helium KED, hydrogen/helium DRC, NH_3 DRC and oxygen DRC) for the determination of As, Fe, S and Zn in biological CRMs (NIST SRM 1573a, Tomato leaves and SRM 1577c, Bovine liver). The authors observed diverse reaction cell behaviours, including signal suppression and product ion formation, with trends correlating to first ionisation energy and mass. Oxygen reaction cell mode reduced signal intensity relative to no-gas mode (except for Bi and Ir) while single-element analysis of Bi revealed a 37% increase in signal due to collisional focussing, with little BiO^{+} formation observed. Using ammonia reaction cell mode, signal suppression was observed for all elements except Bi and Ho. Importantly, the authors noted that an optimal IS for a given element in a particular matrix was not necessarily the optimal IS in a different matrix, emphasising the necessity to consider the behaviour of the IS, analyte and matrix components in the reaction cell. Another

approach to improve calibration robustness was reported by Zhou *et al.*[19] who applied online multi-IS calibration for the determination of I in water, salt and urine. With eight IS (Ge, In, Lu, Re, Rh, Sc, Tb and Y), an LOD of 0.18 μg L^{-1} and an LOQ of 0.59 μg L^{-1} were achieved, offering only modest improvement compared to single-element IS calibration (LOD of 0.20 μg L^{-1} and 0.19 μg L^{-1} for In and Y, respectively). While multielement internal standardisation provided incremental benefits, the magnitude of this improvement may depend strongly on the specific analyte and matrix. The method was applied to measure I in six CRMs (certified *vs.* measured values: 8.5 ± 1.2 μg L^{-1} *vs.* 8.7 ± 0.1 μg L^{-1} – GBW09113L Iodine in Water; 61 ± 6 μg L^{-1} *vs.* 60 ± 1.6 μg L^{-1} – GBW09114L Iodine in Water; 11.5 ± 2 mg kg^{-1} *vs.* 11.5 ± 0.6 mg kg^{-1} – 1006H Iodine in salt; 23.7 ± 2 mg kg^{-1} *vs.* 22.9 ± 0.6 mg kg^{-1} – 1007H Iodine in salt; 116 ± 10 μg L^{-1} *vs.* 119 ± 4 μg L^{-1} – GBW09109N Iodine in lyophilised human urine; 226 ± 15 μg L^{-1} *vs.* 221 ± 4 μg L^{-1} – GBW09110I Iodine in lyophilised human urine). Multi-isotopic strategies for calibration have been explored to improve measurement accuracy across multiple isotopes. Borges de Oliveira *et al.*[95] developed a calibration approach for the determination of Cd, Cr, Hg, Mo, Ni, Pb, Se, Sn and Zn isotopes in blood. Samples were prepared by dilution with Triton X-100 (concentration was not specified by authors) in 25% HNO_3, then combined with either calibration standards (5% sample + 95% standard) or blank (5% sample + 95% blank). Although isobaric interferences led to the exclusion of the ^{54}Cr and ^{58}Ni isotopes, the analysis of RM ClinChek® Whole Blood – Level 3 (RECIPE, Germany) by multi-isotopic calibration (MICal) showed improved agreement of the results with the reference values, compared to external calibration, and was comparable to the standard addition method. For example, the agreement with the RM reference values was 63%, 94% and 97% (Cr) and 53%, 85% and 89% (Mo), for external calibration, the standard addition method and MICal, respectively.

5.2 Atomic absorption and atomic emission spectrometry

In this Update period, there were no important developments for AAS, but we can report on progress in OES implementing *less widespread excitation sources, environmentally friendly sample pretreatment and a miniaturised emission spectrometer*.

Elemental analysis of wine samples by means of MIP-OES is challenging due to both spectral and non-spectral interferences caused by the matrix. Pérez-Vázquez et *al.*[96] applied microwave-sustained inductively coupled atmospheric-pressure plasma (MICAP-OES) for the determination of Ca, Cd, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, and Zn. Wine samples could be analysed directly (after only centrifugation and filtration with 0.45 μm pore size) by means of the MICAP-OES due to the high plasma tolerance to organics and salts. The system provided a robust plasma with an intensity ratio for Mg II (280.270 nm) : Mg I (285.213 nm) higher than 1.8. The sample introduction system consisting of a pneumatic nebuliser and cyclonic spray chamber minimized matrix effects on aerosol generation and transport. Though EtOH and non-volatile organic compounds present in wine gave rise to significant carbon-based spectral interferences, the most sensitive wavelengths for the analytes present in wine were not interfered with, apart for those for Pb. Atomic emission was enhanced up to 50% by wine matrix concomitants (*i.e.*, EtOH and K) whereas ionic emission enhancement was just limited to 10%. It was feasible to simultaneously determine major, minor and trace elements using a single set of experimental conditions. The LODs were mostly within the low μg L^{-1} level, allowing for the determination of the elements regulated by the international code of oenological practices, except for Cd and Pb. For Na it was necessary to dilute the sample if the concentration value was higher than 20 mg kg^{-1}.

The objective of the work presented by Guimaraes[45] was the *multielemental determination using MIP-OES with N_2*, of Ba, Ca, Cu, Cr, Fe, Mg, Mn, Sr, and Zn in chocolate, after extraction induced by emulsion breakage. The most efficient extraction conditions were obtained using 0.25 g of sample, 5 mL of extraction solution consisting of 1.5 mol L^{-1} HNO_3–1.5% m/v Tween 80, submitted to an ultrasonic bath, followed by breaking the emulsion by heating at 90 °C in a water bath. The analytical strategy allowed for the removal of interfering organic components. The LODs obtained, in mg kg^{-1}, were 0.010 (Ba), 0.083 (Ca), 0.35 (Cr), 0.099 (Cu), 0.46 (Fe), 0.016 (Mg), 0.036 (Mn), 0.064 (Sr), and 0.013 (Zn). Validation was performed by analysing a CRM (NIST SRM 2384, baking chocolate) and comparing the proposed procedure with a calcination method. The results obtained for the CRM ranged from 89 to 106% of the certified values, precision expressed as RSD from 7 replicates was between 5.3 and 9.8%. Besides the advantages of shorter process time, flexibility depending on the mass of the sample, and less residue formation, the proposed method did not require concentrated acids and organic solvents and had good LODs, especially when compared to digestion methods.

Progress in miniaturised atomic spectrometers is shown by two devices. A portable magnetic field accelerated nonthermal plasma digestion device using DBD was designed for the rapid and environmentally friendly pretreatment of biological samples, was combined with a miniature injection-detector based on HG point discharge OES.[64] With the assistance of a magnetic field, the DBD plasma digestion of a batch of six samples (50 mg) using a 1.6 mL mixture of H_2O–H_2O_2–HNO_3 could be completed within 25–40 min, achieving digestion efficiencies between 92 and 99%. A $NaBH_4$ solution was used to form hydrides. Under the optimized conditions, the LODs for Bi, Pb, Sb and Sn were within the range of 2.4–8.2 μg L^{-1}, with precisions under repeatability conditions (RSD) < 5% and linearity of calibrations confirmed by r^2 values between 0.9913 and 0.9986. The trueness was verified by measuring CRMs (CNRM GBW10023, laver and GBW10044, rice) with recoveries ranging from 101 to 122%. Metals of interest were successfully determined in real rice, laver, fish, milk, and blood samples. Wang *et al.*[97] introduced a handheld nozzle-electrode point discharge OES device coupled with a self-heating SPME system for on-site determination of Pb in urine. This enabled the integration of sample introduction, detection, and data processing systems with low power consumption (~25 W) and battery-powered operation. Five ml of urine or in a mixture of

HNO_3 and H_2O_2 digested hair sample was mixed with 1 mL of acetate buffer (pH 4.5), and 3.8 mL deionized water in a 40 mL glass vial. The SPME fibres were inserted into the vial headspace and, after sealing, 200 μL of 1% (w/v) solution of tetraethylborate was injected into the sample solution to generate TEL for extraction by the SPME fibre. The analyte could then be directly desorbed by heating the SPME fibre. The customised device was successfully applied to determine Pb in urine and hair, with a LOD of 1.2 μg L^{-1}. The effects of various common ions including Ca^{2+}, Cd^{2+}, Hg^{2+}, K^+, Mg^{2+}, Na^+, Sn^{2+}, and Zn^{2+} on Pb detection were investigated and the device exhibited excellent anti-interference capability. The methodology was validated by analysing two urine matrix CRMs (CNMR GBW09104 and GBW09105) and spiked samples, with recoveries ranging from 92 to 108%. This device could work continuously for 4 h without electricity support.

5.3 Laser induced breakdown spectroscopy

Review articles are discussed in Section 2 of this Update, however, three review papers have specifically covered *the use of LIBS for research applications*. In the first, Zhang *et al.*[98] examined papers from 2017 to 2024 covering biomedical analysis using LIBS. The authors included the benefits of the technique, such as minimal sample preparation, *in situ* rapid measurements and multielement capability, and described examples of its application for biological tissues (specifically cancer and tumour tissues), microorganisms (bacteria, fungi and viruses) and medicinal plants. The papers selected highlighted LIBS as a clinical diagnostic tool, such as for differentiating between cancerous and normal tissues, or for the qualitative and quantitative elemental analysis of tumours. The detection and potential identification of pathogens using LIBS was discussed, although there were limited examples. Finally, analysis of medicinal plants were reviewed which represented the largest area of research applications, particularly for adulteration and origin identification for Chinese traditional medicines. However, in all sample types, calibration, data processing and chemometrics remained the key to obtaining meaningful data, which was also discussed. There was no consensus on the best approach but generally signal pre-processing was required before the use of data models. The authors concluded that LIBS had great potential as an analytical tool (*e.g. in situ* analysis of tissues during surgical operations to confirm complete removal of cancerous tissue), but still had issues to address such as the challenge of highly complex sample types, ensuring accuracy (*e.g.* calibration and RMs), affordability and use in routine settings (*e.g.* in clinical laboratories, QC laboratories). Modlitbová and co-workers[99] also covered biomedical analysis of tissues with a specific focus on implementing LIBS for imaging of microparticles and NPs, noting the benefits such as quick analysis speeds, μm resolution, multielement capability and particle detection. Their review included sample pretreatment, instrumentation advances, data processing and additional advantages of using particles as tags, NPs enhancing signal intensity and microplastic identification. It provided a comprehensive evaluation of LIBS as a tool for imaging biotic tissues. Nanou *et al.*[100] focused their review on food authentication, traceability, and fraud detection for honey, milk and olive oil. The authors also highlighted the benefits of LIBS for fast, non-targeted analysis with minimal sample preparation and potential for real time and *in situ* analysis. As noted above, spectral pre-processing and machine learning models remained critical to obtain optimum models, but, with suitable development, it was possible to determine geographical origin, animal or botanical origins, detect adulteration by lower-quality ingredients or foreign substances, as well as provide supporting QC data. It was noted that well characterised RMs or data obtained from official analytical methods were still required for reliable results, with an emphasis on the need for collaboration with regulatory agencies and expert laboratories to ensure reliable reference samples were obtained and the importance of sharing databases of information. The three food materials reviewed covered a wide range of analytes determined by LIBS. For honey, this included elemental profiling and carbon group identification for botanical and geographical origin classification and adulteration by plant syrups or from cheaper honey sources. For olive oil, the fatty acid content (*via* molecular bands such as CC, CN and CO) enabled geographical and cultivar identification and adulteration from alternative plant oils. For milk, the combination of key elements such as Ca, Fe, K, Mg, Na and Zn, and molecular bands from protein, allowed the detection of adulteration from the addition of whey powders, milk from different animal species, alternative protein sources (egg, soy, wheat) or contaminants (*e.g.* melamine). Interestingly, successful detection of mastitis in cows was demonstrated from the LIBS analysis of Ca, CC, CN and Na in the milk which was compared to the standard test method of somatic cell counts. Likewise to the first review,[98] these authors concluded that there were barriers to mainstream adoption of LIBS, such as the initial cost, validation and lack of standardised protocols from regulatory agencies. However, the conclusion from these reviews was that LIBS is potentially a significantly powerful tool for biomedical research, clinical testing, food safety, authenticity, and fraud prevention.

One advantage of LIBS is the ability to measure molecular species as well as elemental lines which Nakadi *et al.*[101] have implemented *for the novel application of IDA with LIBS*. The emission lines from molecular species experience enhanced differences in the rotational and vibrational levels which differs with the isotopic mass and can be observed when utilising a high resolution spectrometer. In this work, Ca isotopes were detected *via* CaF which was generated by the addition of 1% methyl fluoride in the argon flow through the ablation chamber. The calibration standards and samples used throughout were dried droplets of 1–2 μL. The system operating parameters were optimized to maximise CaF formation and detection. This included adding Sr as an IS to monitor the CaF : Sr ratio and the impact on the spectral background and sensitivity effects. This was tested using a calibration standard of natural Ca abundances (*i.e.* ^{40}Ca at 97.9% and ^{44}Ca at 2.1%) and an isotopically enriched standard of ^{44}Ca at 99.2% and ^{40}Ca at 0.8%. The observed wavelength shift for the CaF vibronic transition at 583.0 nm was ~281 pm which was in reasonably good

agreement with the theoretically calculated value of 292.3 pm for ^{40}Ca and ^{44}Ca. The authors then developed a PLS method with the 'variable influence on projections' protocol which removed wavelengths of less significance in the model. This improved the accuracy of the model but caused a reduction in precision. It was also found that the extremes of the model were more inaccurate. These factors led to the conclusion that the method could not be used for natural isotopic analysis but could be suitable for IDA. This was then assessed using real samples, namely tap water and milk powder, by comparing the Ca concentrations obtained by ID-LIBS to those from FAAS, finding no significant difference at the 95% confidence interval using a *t*-test. The results demonstrated the application of LIBS for Ca detection *via* CaF molecular bands and more importantly, the successful use of IDA in matrix materials without using MS. It will be interesting to see which other elements may also be amenable to this approach.

Two research groups featured in our previous Update[1] have returned with *further investigations and method extensions for LIBS*. Zhang *et al.*[102] described the systematic investigation of the 'coffee ring effect' when drying sample droplets and the benefit of adding NPs for signal enhancement and, interestingly, mitigation of the ring effect. The researchers used a simple silicon wafer substrate and evaluated the impact of adding colloidal Ag NPs (52.7 nm diameter) to human serum before dropping 10 μL onto the substrate to dry before analysis by LIBS. Different patterns were found for the elemental emission lines under investigation, namely C I 247.86 nm, CN 388.34 nm, Ca I 422.67 nm, Ca II 393.36 nm, K I 766.49 nm and Mg II 279.55 nm. It was found that at a serum to Ag NPs ratio of 1 : 6, the coffee ring effect was diminished as the central region accounted for >90% of the total intensity of the whole drop. However, the signal intensity changes were element specific, with the maximum enhancement factors found for the Ca II 393.36 nm and K I 766.49 nm lines at a serum to Ag NP ratio of 1 : 2. The paper again demonstrated the benefit of NP enhanced LIBS for biological samples. Similarly, Blanchette *et al.*[103] extending their method to tackle bacterial detection and identification in urine. Samples of human urine were spiked with *Enterobacter cloacae*, *Escherichia coli* and *Staphylococcus aureus* and were captured and concentrated onto 0.45 μm nitrocellulose filter papers which were then ablated. The researchers employed PCA-ANN to model the spectra collected (from 200 to 590 nm) for the different populations. Overall, the proposed method achieved a classification accuracy of 97.9% when using 80% of the data to build the model, with the remainder used for cross validation testing. In the presence of all three bacteria, the average classification accuracy was 80.6%. This approach demonstrated the potential of LIBS in this area and represented time savings over traditional cell culturing.

Li *et al.*[104] researched various statistical models for *the identification of cancer using non-specific LIBS data*. Serum samples ($n = 58$) from patients with oesophageal, liver or lung cancer, along with healthy controls, were prepared simply by dropping onto an ordered microarray silicon substrate and dried. The raw spectra (180–900 nm) were used with the bagging-voting fusion (BVF) algorithm which combined the predictions from multiple models such as ANN, *k*-nearest neighbours (KNN), quadratic discriminant analysis, random forest (RF) and SVM. The BVF method achieved an accuracy of 92.53% which was significantly improved over the best single model (SVM with an accuracy of 75.86%). The authors noted that the BVF approach required under 3 min processing time from LIBS analysis to detection and identification, highlighting the speed and benefits of using more complex statistical approaches.

As noted above, *LIBS has been used frequently for the adulteration detection and origin discrimination in Chinese traditional medicines*. Chen and co-workers[105] focused on the adulteration of polygonati rhizoma and the assessment of complex data extraction and statistical categorisation tools. Data was simultaneously collected from LIBS and VIS-NIR spectrophotometry from samples of known origin and manually adulterated mixes. The spectra were pretreated before creating the fused model. For LIBS, 18 elemental spectral lines with sufficient signal quality were selected (covering Al, C, Ca, K, Mg, Na, N, O and Si). Peak convolution algorithms were applied using 14 points per peak then submitted to a bidirectional long short-term memory network which identified nonlinear relationships between spectral lines, improving the information extraction. For VIS-NIR spectra, the peaks were much broader with less differentiation so five specific wavebands were selected which represented significant peaks or troughs in the waveform. The first derivative was then calculated to help reduce the data dimensions and mitigate baseline noise. Finally, the two data sets were fused in a deep learning network to obtain classifications. The accuracy of the combined model was 98.75% whereas using the datasets separately resulted in values of 87% for LIBS and 93% for VIS-NIR, and was significantly improved over single simple models such as LDA, SVM and KNN (accuracy ranged between 60.83 and 90.00%). Additional different adulterated samples were tested and achieved classification accuracy of >96%. Overall, the study demonstrated the power of advanced algorithms over simple statistical models which can improve adulteration detection in Chinese medicines.

5.4 Vapour generation procedures and atomic fluorescence spectrometry

Vapour generation procedures and AFS were applied by several researchers to obtain better LODs for both elements and their species in various biological matrices.

Two papers were devoted to *interference studies while using PVG-ICP-MS*. De Oliveira *et al.*[106] carried out determination of I in CRMs of biological origin by platinum-assisted PVG-ICP-MS. The authors assumed that platinum acted as a mediator and signal enhancer in the photochemical process, which is particularly important for I in presence of NO_3^-. Three matrix CRMs of biological origin (IRMM MURST-ISS-A2 Antarctic krill, NIST SRM 1549 non-fat milk powder, and NIST SRM 1566a oyster tissue) with masses 0.25–0.5 g were prepared using MAD with 5 mL HNO_3. Prior to injection in the photochemical reactor, all samples were diluted to contain 0.6 mol L^{-1} HNO_3 and 10% (v/v) CH_3COOH + 0.5 mg L^{-1} platinum and then irradiated using a mercury lamp. The HNO_3 interference on the

PVG was evaluated in the range 0–5% (v/v). Signal suppression due to the photolysis of NO_3^-, generating oxidising species that consume reductive radicals required for the PVG process, was overcome for solutions containing between 0.3 and 0.7 mol L^{-1} HNO_3 upon adding platinum to the solution. This addition also provided a *ca.* 85% signal enhancement for I in comparison with solutions without Pt^{2+}. Matrix-matched external calibration was implemented. Overall, the proposed setup proved efficient for the analysis of digested solid samples, LOD was 0.03 μg per g, which is 5-fold better than that obtained with pneumatic nebulisation. The recoveries for CRMs were 101–103%. The simultaneous determination of Cl, Br and I in several CRMs of biological origin by aerosol-assisted PVG with detection by ICP-QMS was investigated by Bitencourt *et al.*[107] The photoreactor comprised a modified cyclonic spray chamber fitted with a central UV source for irradiation of pneumatically generated sample aerosol. By using an optimized sample medium comprising 1% (v/v) CH_3COOH and 20 mg L^{-1} copper as mediator, the signal intensities for Br, Cl and I were enhanced by 40-, 3- and 30-fold, respectively, compared to those obtained by conventional pneumatic nebulisation. Limits of detection of 6.3 pg mL^{-1}, 4.2 ng mL^{-1} and 1.9 pg mL^{-1} were achieved for Br, Cl and I, respectively, with corresponding estimated overall PVG efficiencies of 99, 10 and 90%. The methodology was evaluated by simultaneous analysis of four different matrix CRMs (NIST SRM 1515 (apple leaves), NIST SRM 1549 (non-fat milk powder), NIST SRM 1632c (coal), and NRCC DORM-5 (fish protein)) digested by microwave-induced combustion with HNO_3. Obtained recoveries ranged from 84% to 115%. Interferences from NO_3^- could be minimised using the UV-assisted spray chamber. Species such as BrO_3^-, ClO_3^- and IO_3^- were also examined, but poor PVG efficiencies (<5%) were encountered. However, addition of 20 mg L^{-1} SO_3^{2-} to the generation medium enhanced response for BrO_3^- and IO_3^-, achieving similar values to those obtained for halides.

Some novel publications described the determination of Cd, Hg and MeHg in food samples and of As and Hg species in blood, combining VG approaches with various analytical techniques (AFS, HR-CS-AAS, ICP-MS).

Two studies implemented CVG to obtain Hg vapours. The development of a new flow batch system for sample introduction to ICP-MS was found to be effective for Hg determination in fish muscle.[108] A FI system with peristaltic mini-pumps was used to propel the solutions and different configurations of flow batch systems (reactor/GLS) were studied. Samples (0.5 g ww) of freshwater fish muscle were decomposed using MAD with a mixture of 3 mL HNO_3–1 mL HCl–2 mL H_2O. Mercury vapours were generated by reaction with $NaBH_4$, argon with a flow rate 1.12 L min^{-1} was used as carrier gas. The developed method had important characteristics, such as small volume of sample (160 μL) and reagent consumption (1000 μL of $NaBH_4$ and 1000 μL of HCl), time per determination of only 40 s, good short term precision (RSD < 5%), good trueness (recovery 110% for NRCC CRM DOLT-4 – dogfish liver) and low cost. The evaluated LOQ of 0.012 μg g^{-1} met the maximum concentration limits for Hg in fish in Brazil and EU legislation. A CVG-ICP-MS system was used by Andriolli *et al.*[109] to determine Hg in edible oils. The described method was based on reversed-phase dispersive LLME followed by CV generation with $NaBH_4$. The operational parameters after optimization were 5 g of edible oil, 0.5 mL of *n*-propanol, and 0.5 mL of 6 mol L^{-1} HCl, heating (10 min at 80 °C), stirring (60 s), and centrifugation (5 min). An LOQ of 0.35 ng g^{-1} and RSD of 7% were obtained. Trueness was tested both by analysis of a CRM OMHG 100 (mineral oil) from SpecSol, Brazil and spike recoveries (at 0.5, 1.0, and 1.5 μg g^{-1}). The results obtained on eight oil samples by the extraction method were compared with those given by a MAD procedure. For five out of eight oil samples, the Hg level was below the LOQ of the MAD method (26 ng g^{-1}). For the remaining three oil samples a very good agreement (98–102%) between results from extraction and MAD methods was reported.

A novel approach based on plasma mediated VG in a tubular DBD, followed by HR-CS-AAS was applied for the determination of Hg in fish tissue.[62] Sample preparation was performed using MIC with 1.4 mol L^{-1} HNO_3. Because plasma mediated VG used only plasma energy to volatilise and atomise the analyte, no chemicals such as $NaBH_4$ or HCl were needed. The operational parameters were optimized as follows: 70 mL per min Ar as the discharge gas, 20 mm electrode distance, primary voltage at 60 V and 50 μL as digested sample volume (in 0.1 mol L^{-1} HNO_3 matrix). The effect of HNO_3 concentration on the plasma mediated VG efficiency was studied. No differences in signal intensity were observed for HNO_3 concentration up to 0.1 mol L^{-1}, while a signal decrease of between 25% and 40% was observed when the acid concentration increased to 1 and 5 mol L^{-1}, respectively. The reported LOD was 0.16 μg g^{-1}. A recovery of 104% was observed for a sample spiked with 2.5 ng of Hg and the result of the analysis of a CRM (NRCC DOLT-4, Dogfish liver) agreed well (97%) with the certified value of 2.58 ± 0.22 mg kg^{-1}.

A methodology combining *MAE, derivatization by phenylation, and preconcentration through liquid phase microextraction, coupled with pyrolysis GC-AFS* was developed by Verdugo *et al.*[110] for the selective quantification of $MeHg^+$ in mouse brain tissue. The derivatisation of $MeHg^+$ was evaluated using two reagents: sodium tetraphenylborate (preconcentration factor 34 ± 3) and phenylboronic acid (preconcentration factor 29 ± 1), while preconcentration was performed using $C_2H_4Cl_2$, CH_2Cl_2, or C_2Cl_4. Several DOE procedures were implemented to find optimal conditions using CRM ERM CE464 (total and methyl mercury in tuna fish) as the sample matrix. The LOD and LOQ values, determined from the calibration curve, were found to be 0.0045 and 0.113 μg L^{-1}, respectively. The result of CRM analysis was in good agreement (104%) with the certified value of 5.50 ± 0.17 mg kg^{-1} MeHg. This approach eliminated the need for mass balance assessments while offering a selective and sensitive quantification of $MeHg^+$. The total Hg in the samples was determined using a direct mercury analyser.

A new analytical protocol for the determination of Cd at trace levels in sunflower oils[111] used fine droplet formation LPME, followed by micro-sampling VG AAS, where Cd volatile species were generated by $NaBH_4$. Sunflower oil sample (30 g) in a centrifuge tube was sprayed with 2% (v/v) HNO_3, vortexed and centrifuged. The aqueous phases from two spraying cycles were

pipetted into a separate tube, acidified with 1.5 moL L^{-1} HCl and then 100 μL of the acidified aqueous phase was pipetted into a micro-sampling cup that was carefully tightened to the GLS. The basic $NaBH_4$ solution was pneumatically transferred onto the mixture of acidified aqueous phase and Cd volatile species were transferred by N_2 flow (140 kPa) to the light path of the AAS. Under the optimum conditions, the LOD was found to be 0.13 μg kg^{-1}, and working dynamic range was between 0.53 and 10.39 μg kg^{-1} with $r^2 = 0.9961$. The percent recovery results for two spiked sunflower oil samples at three levels (from 2.49 to 5.32 μg kg^{-1}) ranged from 87.6% to 101.1%.

Yang *et al.*[112] presented *a novel vacuum UV photochemical reactor* consisting of a quartz tube inserted into a low-pressure mercury lamp to fabricate a high-throughput flow droplet photo digestion device for small amounts of blood samples prior to the determination of As and Hg by HG-AFS. This high-efficiency photo-oxidation reactor significantly improved the transmission of vacuum UV light (185 nm). A mixture of 20 μL blood with 20 μL H_2O_2–10 μL HNO_3 was pumped and passed through the reactor before online As and Hg determination by HG-AFS. The performance of the developed device was confirmed by detecting As and Hg in one blood (Seronorm® whole blood L-2) and two urine CRMs (GDOHNYBZ025-1, GDOHNYBZ025-2) with results in good agreement (94–102%) with the reference values. Also, good recoveries (93–109%) were achieved for blood samples spiked with 2 μg L^{-1} As^{3+} and Hg^{2+}, respectively. Limits of detection of 0.25 and 0.15 μg L^{-1} were obtained for As and Hg, respectively, with precisions under repeatability conditions better than 4% (RSD, $n = 11$). The proposed method was able to determine the following species, added to blood samples, with good spike recoveries of 98–105%: iAs, AB, DMA, MMA, iHg, and MeHg, indicating that the high photo-oxidation efficiency of the proposed photoreactor can efficiently convert these forms to Hg^{2+}, As^{3+}, and/or As^{5+}, respectively.

5.5 X-ray spectrometry

A comprehensive review of recent advances in X-ray spectrometry[2] complements the applications with clinical and biological materials, foods and beverages covered within this Update. Imaging applications of X-ray spectrometry are described separately in Section 7.2.

Review articles are discussed within Section 2 of this Update, but it is worth highlighting relevant reviews describing the *use of XRF spectrometric techniques for research applications in the clinical and food science areas*. In the first paper, Fernández-Ruiz[113] focussed on biomedical applications of TXRF spectrometry, specifically for biomedical, biochemical and pharmacological research. The benefits of the technique were covered such as the high sensitivity, easy sample preparation and low sample quantity requirement. The author reviewed the technique basics and recent developments, including various calibration approaches. Applications were discussed relating to tissue imaging, human fluid analysis, pharmaceutical detection, microbiological samples and NPs. It concluded TXRF spectrometry was a promising and versatile technique. In the second review, Mamtha *et al.*[114] concentrated on EDXRF spectrometry for the analysis of food products. It was noted how the technique has evolved in recent years and is able to handle the analysis of solids, liquids and powders. Applications such as authenticity, food safety and quality control were covered using key papers from the last 15 years. The authors also discussed calibration methods and algorithms, necessary for more complex matrices and in particular for portable devices.

A *systematic evaluation of the next generation of portable monochromatic excitation EDXRF systems* (namely Z-Spec Zmax™ and Emax™) was performed by Johnson-Restrepo *et al.*[115] which included comparisons to a first generation design (HD Mobile®) and standard handheld polychromatic XRF systems (Thermo Fisher Niton™ XL3t and XL5). All instruments implemented the fundamental parameters modelling for calibration *via* proprietary software. For the new Z-Spec analysers, the Zmax™ was specifically optimized to detect As, Cr, Hg, Ni and Pb (although could report data for 16 other elements: Bi, Br, Ca, Co, Cu, Cl, Fe, Gd, Ge, K, Mn, Se, Sr, Ti, Tl and Zn) whereas the Emax™ was optimized for Cd, Mo, Sb and Sn (but could also report values for 13 other elements: Ag, As, Br, Cu, Hg, I, Mn, Pb, Rb, Sr, Y, Zn and Zr). A comparison of LODs showed the Z-Spec systems to be better that the other three under investigation, achieving sub-ppm LODs with no sample preparation and using between 0.15 and 0.5 g of sample. Various food, plant and biological CRMs ($n = 12$) were used to assess the accuracy of the Z-Spec instruments across a broad range of matrices for As, Cd, Cu, Hg, Mn, Ni, Pb and Zn. Overall, the concentrations were within ±10% of the expected values across several orders of magnitude which was impressive. Next, historical food PT samples were tested for Cd, Pb and Tl which included control samples and spiked samples. The agreement with the consensus value from the PT programme was determined, finding acceptable results within ±20%. The precision ($n = 3$) was calculated leading to values from 0.5% to 13.5% RSD. Control samples were correctly identified as non-detects, but when the PT samples were measured on the HD Mobile® device, a positive result of 26 300 μg kg^{-1} Sn was found for the control sample and 62 500 μg kg^{-1} Sn for the spiked material, which was spiked at 2500 μg kg^{-1} Sn, highlighting a significant calibration issue. For the Thermo Fisher devices, except for Sn, all other samples and elements were not detected, even for the spiked samples, due to the poorer LODs. Finally, the Z-Spec systems were used to analyse various medicinal herbs and food samples ($n = 39$) for As, Cd, Cu, Fe, Mn, Ni, Pb, Rb, Se, Sn, Sr and Zn, demonstrating the capability of the second generation of portable monochromatic EDXRF instrumentation. Chen and co-workers[116] also assessed the abilities of an in-house developed monochromatic EDXRF spectrometer for As and Pb detection in grain. The authors described the optimization of the instrument design, calibration equations and interference correction algorithms, as the As Kα line overlaps with the Pb Lα line. The performance was then tested using eight CRMs of corn, rice and wheat, achieving relative deviations from the certified values between 2.6% and 8.0%. The LODs were calculated to be 0.02 mg kg^{-1} and 0.03 mg kg^{-1} for As and Pb respectively, which was below the maximum permitted limits

set by WHO FAO codex alimentarius general standard for contaminants and toxins in food and feed. The within-day and between-day precision was found to be <10%. Finally, real grain samples ($n = 15$) were analysed and the results were compared against those obtained from ICP-MS following MAD, achieving r values > 0.98 for both As and Pb. Overall, it was an impressive demonstration of the developments with portable monochromatic EDXRF devices.

One advantage of TXRF spectrometry is that minimal sample preparation is required. Chua *et al.*[117] described the *validation and application of TXRF spectrometry for the direct analysis of water*. The method was assessed for linearity, precision, LOD and LOQ, reproducibility and robustness using a certified calibration standard and spiked samples for the detection of As, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The results demonstrated sufficient linearity across several orders of magnitude (r^2 > 0.99) with LODs between 0.88 and 2.37 μg L^{-1} and LOQs of 2.92–7.91 μg L^{-1}, which were interestingly inversely proportional to the atomic number. Additionally, short term precision and reproducibility indicated <10% variations. Samples of drinking water and ground water were spiked and recoveries within the range of 81.4–112.8% were obtained. Additionally, the measurement uncertainty was calculated as ~12% for all elements, with the major component due to the sensitivity of the TXRF instrument. Finally, PT results for wastewater achieved z scores < ±0.7, demonstrating excellent performance. Overall, the paper systematically established TXRF spectrometry as a highly capable technique for the determination of key elements relevant to drinking water quality.

The detection of light elements by XRFS can be challenging, more so with portable systems. Xiao *et al.*[118] described the *development of a new second order calibration routine to improve quantification of Ca, K, P and S in food samples with portable XRF instruments*. Spectra were collected from various food CRMs (namely GBW10294 Cadmium contaminated rice, GBW10295 Henan wheat flour, GBW10296 Red bean, GBW10298 Cabbage, GBW10301 Rapeseed, GBW10319 *Panax notoginseng*) which had been mixed with differing amounts of glucose to create calibration materials to train the algorithm (resulting in $n = 31$ samples). The authors generated 36 datasets by varying the current and voltage in increments leading to a three-way data array. This was processed using the alternating penalty trilinear decomposition algorithm for second order calibration. This also enabled deconvolution of interferences from overlapping peaks or the background. The recovery of the various CRMs was calculated for the four elements and ranged between 82% and 119%. The method achieved LOQs of 26 ppm for Ca, 68 ppm for K, 9 ppm for P and 11 ppm for S, which are impressive figures of merit for portable XRF systems with no sample preparation.

This section is rarely complete without papers presenting *new developments for in vivo measurements of bone using XRF*. Strontium was the element of choice for Bickley and co-workers,[119] who developed a new portable system with a silicon drift detector using a ^{109}Cd source in a 180° backscatter geometry and used a 3D-printer to create phantoms for calibration standards. The phantoms were constructed from polylactic acid and designed to taper towards the tip, mimicking a finger. The hollow interiors were filled with Sr-doped calcium hydroxyapatite to represent bone. The researchers assessed the impact of the soft tissue thickness (*i.e.* the polylactic acid substrate) on the signal attenuation, demonstrating that the Compton scatter distribution was sufficient to correct for these effects. The LOD was 22 μg Sr per g Ca for a 30 min measurement time and a radiation dose of 1.1 mSv to the skin, which was in line with other reported studies but this device was portable and minimised dose exposure. The authors also discovered the activity of the X-ray source used was lower than expected, postulating that with higher fluence sources, the system could achieve a minimum LOD of 7 μg Sr per g Ca. Burgos *et al.*[120] developed a new portable system for Pb detection specifically to measure *in vivo* both cortical and trabecular bone. Current portable devices use L-shell fluorescence which limits it to cortical bone whereas systems using K-shell fluorescence can assess trabecular bone but require a radioisotope source or liquid N_2 cooled detectors, limiting the portability. Therefore, the researchers developed a new system using an X-ray tube source and room temperature operable detectors. The paper outlined the technical details and capabilities, whilst checking the radiation dose to the patient and operators. A plaster of Paris phantom, doped with 20 ppm Pb was used during the assessment, along with dosimetry measurements with different shields and distances. The total effective body dose was found to be 1.94 μSv for an adult and 3.28 μSv for a 10 year old child, which was far below recommended levels, and was zero for the operator. The new instrument demonstrated promising capabilities for the on-going *in vivo* monitoring of Pb exposure in the population. In another publication, W was the focus for McHeik and co-workers[121] who presented a theoretical assessment of a K-shell XRF spectrometer for *in vivo* measurement of W in bone. The work was entirely based on *a priori* calculations using Monte Carlo simulations. It was concluded that using a ^{109}Cd X-ray source in a 180° geometry was optimal, potentially able to achieve a LOD of 1 μg W per g Ca. It also expected Compton normalisation to be sufficient for soft tissue effects. Furthermore, the calculated effective dose was estimated as 0.026 nSv. It will be interesting to see if the researchers will be successful in matching the predictions.

6 Elements used for indirect determinations

Following the trend seen in previous years, the application of atomic spectrometry techniques to the indirect measurement of complex molecules continued and expanded from research in the clinical area to food safety issues, including the rapid detection of pathogens responsible for food-borne diseases.

Progress of ICP-MS based immunoassays over the last 10 years were reviewed by Jiang *et al.*,[122] covering 158 publications. The authors highlighted the rapid development of these applications and the key role of designing specific element tags (metal ions and NPs).

Several developments for the *detection of specific proteins inside live cells* were reported, promising new approaches for

clinical research and drug development as well as diagnosis and treatment. The design of molecules for element labelling of specific targets represents the key feature for these applications. Hou *et al.*[123] investigating the expression and activity of Bruton's tyrosine kinase (BTK) which is relevant for B cell signalling, chose the BTK covalent targeting drug, ibrutinib (Ibt), for labelling with Eu. Their Ibt-DOTA-Eu probe did not achieve the scope, due to low cellular uptake. Therefore, they synthesized an azide-modified Ibt probe (Ibt-N_3), able to cross the cell membrane, followed by DBCO-DOTA-Eu labelling through a strain-promoted azide–alkyne cycloaddition reaction. The cells were incubated with the probe in 0.1% DMSO for 1 h, followed by washing with HEPES buffer, cell counting, cell lysis, centrifugation and ultrafiltration. Quantification was achieved by species-nonspecific ID (*via* the $^{151}Eu:{}^{153}Eu$ ratio) HPLC-ICP-QMS. They used a Zorbax 300SB RP C18 column (1.0 × 150 mm length; 3.5 μm particle size) and gradient elution with 0.1% TFA in ultrapure water (mobile phase A) and 0.1% TFA in ACN (mobile phase B). An enriched ^{153}Eu spike solution was mixed with the column effluent, through a four-way connector, together with ultrapure water (0.9 mL min^{-1}) to reduce the effect of ACN on plasma stability and ionization efficiency. Although no details were provided about the analytical performance, the authors claimed the ability to detect BTK at a concentration of 61.28 ng per 10^6 cells *via* the Eu labelled probe. A following paper, by Fang *et al.*,[124] addressed the quantification of the same protein using a newly designed multifunctional antibody-targeted covalent lanthanide tag, consisting of a cell-permeable monoclonal antibody, a covalent Eu tag targeted for BTK and a cathepsin B-cleavable linker. This molecule was intended to specifically recognize the CD19 receptor present on the surface of B-cell lymphoma cells and to be easily internalized into cells, where it would form a covalent bond with BTK. After incubation with cells, the ability of this tag for cell targeting and internalization was investigated through confocal immunofluorescence imaging which demonstrated the selective uptake of the tag by Ramos cells compared to HepG2 cells. Comparison with a competitive binding assay forming stable adducts with cellular BTK confirmed the *in situ* reactivity and selectivity of the proposed tag. The quantification of intracellular Eu-labelled BTK was achieved by species-unspecific ID HPLC-ICP-MS, using a SEC column (waters X bridge protein BEH SEC 200 A 3.5 μm, 7.8 × 300 mm) and 50 mmol L^{-1} NH_4CH_3COO (pH 6.88)–2% ACN as the mobile phase at a flow rate of 0.30 mL min^{-1}. The enriched ^{153}Eu spike solution was mixed with the column effluent through a four-way connector prior to introduction into the ICP-QMS and measurement of the $^{153}Eu:{}^{151}Eu$ ratio. Although no details of the analytical performance of this method were given, the authors stated that the concentration of active BTK measured in Ramos cells (mean ± SD, $n = 3$: 108.7 ± 7.2 ng per 10^6 cells) was consistent with previous literature data, but lower than the results of western blot analysis. Another group of researchers[125] assessed the potential of scICP-QMS coupled with nanoclusters-based immunoprobes for the quantification of low amounts of proteins in individual cells, enhanced with external elemental tags for improved cell event detection. A Rh-DNA intercalator provided better results compared to ruthenium red, as the latter, which binds to the polysaccharides of the cell membrane, could not discriminate between cell events and cellular fragments. The method was then applied to the sequential determination of hypoxia inducible factor-1 alpha (HIF-1 alpha) and vascular endothelial growth factor (VEGF) in individual human Müller glia cells (MIO-M1), using specific antibodies labelled with Au and Ir nanoclusters, respectively. The optimal concentration of both antibodies in MIO-M1 cells in suspension with the two labelled immunoprobes was assessed at five levels. After addition of the Rh-DNA intercalator, cells were diluted to 5 × 10^4 cells mL^{-1} and analysed by scICP-QMS for the sequential measurements of $^{103}Rh^+$, to determine cell events, and of $^{197}Au^+$ and $^{193}Ir^+$ to quantify the proteins detected by the immunoprobes. The ratio of $^{103}Rh^+$ events *vs.* the $^{193}Ir^+$ or $^{197}Au^+$ events was calculated, applying a Poisson threshold ($\varepsilon = 0.5$), selecting reagent concentrations of 0.9 μg mL^{-1} for HIF-1 alpha and 10.5 μg mL^{-1} for VEGF for further experiments. The authors then tested the optimized procedure on control and hypoxic MIO-M1 cells and observed average values of 1.3 ± 0.3 and 0.9 ± 0.1 for the ratios of $^{103}Rh^+:{}^{197}Au^+$ and $^{103}Rh^+:{}^{193}Ir^+$ events respectively, indicating overall acceptable performance, although it was noted that the ratio >1 suggested the need for further improvement of the system's detection threshold.

MicroRNAs (miRNAs) are short single-stranded RNA sequences whose detection and quantification is gaining increasing importance in biomedical research as well as diagnosis and monitoring of diseases, in particular cancers. Four papers proposed *strategies involving elements as tags, with ICP-MS as the detector, for miRNAs quantification*. A group of researchers[126] developed an approach based on a sandwich double–hybridization reaction for the detection of miR-16-5p in cell lysates without amplification reactions. The capture probe was a biotinylated oligonucleotide, conjugated to streptavidin-coated magnetic iron oxide microparticles; the detecting probe was also a biotinylated oligonucleotide, conjugated to 40 nm Au NPs. The authors chose spICP-TOF-MS as the analytical platform, as it enabled detection of both Au and Fe simultaneously, corresponding to the target miRNA and thus enhancing sensitivity. After the preparation of the probes, these were incubated with the sample (100 μL) at 70 °C for 10 min, then the mixture was left to cool for 3 h at room temperature, followed by washing of the sandwich formed by the analyte with TRIS buffer saline, dilution and determination of NP concentration by spICP-TOF-MS. To overcome the complexity of data analysis, a software specifically designed for the processing of large spICP-TOF-MS data (SPCal) was applied. The method achieved an LOD of 160 pmol L^{-1} of miRNA and reproducibility RSD ($n = 4$) at 1500 pmol L^{-1} miRNA of <10%. The authors acknowledged some limitation of the achieved LOD, compared to other published procedures, which was attributed to unspecific adsorption of Au NPs, leading to high blank values. However, they noted that the proposed strategy provided the benefit of absolute quantification of miRNA in biological samples without amplification or transformation reactions of the original sequence. Another group[127] described a platinum group element-bridged DNAzyme dual-walker amplification

system followed by indirect detection of multiple miRNAs *via* the measurement of elements by ICP-MS in the resulting substrate strands. The method, applied to human serum, achieved LODs of 1.8 fmol L^{-1} for miRNA-21, 1.1 fmol L^{-1} for miRNA-199a, and 1.5 fmol L^{-1} for miRNA-499, respectively, as well as a linear range from 5 fmol L^{-1} to 10 pmol L^{-1}. The authors suggested that the high sensitivity and specificity of the proposed strategy held significant potential for further developments and applications. The determination of miRNA-21 in human breast cancer cell lines (SK-BR-3 and MCF-7) and real blood samples from breast cancer patients was achieved by Xu *et al.*[128] applying dual-amplification elemental labelling and spICP-MS. This strategy involved the initial hybridization of two distinct Au NP probes with linker single strand DNA. In the absence of the target, the Au NP probes cross-linked forming large aggregates, that gave high and sparse pulse signals in the spICP-MS. On the contrary, in the presence of miRNA-21, a strand displacement amplification reaction was activated, which products, in turn, triggered the cleavage of the linker single strand DNA, *via* CRISPR/Cas12a's *trans*-cleavage activity. This reaction prevented the cross-link of Au NP probes, resulting in low and dense hopping signals in spICP-MS and enabling the sensitive quantitative detection of miRNA-21. The method achieved linearity in the range 0.5 fmol L^{-1}–100 pmol L^{-1} and an LOQ as low as 0.5 fmol L^{-1}. The authors highlighted the versatility of the strategy, that could, in principle, be adapted to the measurement of other biomarkers, by modifying the specific sequence of the strand displacement amplification template chain that is complementary to the analyte. Jiang *et al.*[129] observed that, although the CRISPR/Cas system is widely used for nucleic acid quantification, it lacked flexibility for multiplex detection. Therefore, the researchers developed a platform for the simultaneous detection of three colorectal cancer-related miRNAs (miR-141, miR-31, and miR-21) by combining spICP-MS with the Thermus thermophilus Argonaute protein (TtAgo), presenting multiple specific cleavage characteristics, and NPs. First, they approached the design of the probes and the identification of optimal TtAgo cleavage substrates; then they assessed a dual-cycle mechanism, involving exponential isothermal amplification (EXPAR) and TtAgo cleavage triggered by reporter DNA-modified NPs, in which the NPs not captured by streptavidin MBs remained in the supernatant after magnetic separation. Target specific nanoprobes (Ag NPs for miR-31, Au NPs for miR-141, Pt NPs for miR-21) allowed the simultaneous detection and quantification of multiple miRNAs. Quantitative detection of a single target miRNA (miR-141) required the incubation of 2 μL of EXPAR reaction mixture with 10 μL TtAgo cleavage system (100 nmol L^{-1} TtAgo, 1× ThermoPol Reaction Buffer, 0.75 mmol L^{-1} $MnCl_2$ and reporter DNA-modified Au NPs) at 80 °C for 15 min, followed by separation on MBs (2.5 μL of the reaction mixture, room temperature, 30 min). The supernatant, containing Au NPs with cleaved reporter DNA, was diluted ~10^4-fold and analysed by spICP-MS. Simultaneous detection of multiple targets (miR-31, miR-141, miR-21) was achieved using additional reporter DNA-modified with Ag NPs and Pt NPs. Selectivity, tested with mismatch sequences of the three target miRNAs, and specificity, assessed under different combinations of the target levels, were satisfactory. Analytical performance indicated linearity from 100 amol L^{-1} to 100 pmol L^{-1}, LODs of 21 (miR-31), 85 (miR-141) and 25 (miR 21) amol L^{-1}, and RSDs within the ranges of 4.4–6.5% (intra-batch) and 6.0–12% (inter-batch) for 100 fmol L^{-1}. The accuracy and reliability of the method with clinical specimens was tested using 1 fmol L^{-1} and 100 fmol L^{-1} concentrations of the three target miRNAs spiked into 100-fold diluted healthy human serum and human embryonic kidney 293 cells (HEK293) total RNA extracts. In human serum, recoveries were within the range of 81.8–110% and in HEK293 total RNA extracts, from 84.3 to 106%. In addition, the results agreed with those obtained on the same samples by Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR). The authors attributed the five-order-of-magnitude improvement in sensitivity to the dual-cycling mechanism and highlighted the benefits of the method, compared with other existing ones, in terms of multiple target capabilities, high specificity, low time consumption and robustness, opening the way to its wider application in clinical practice, although the need for specific instrumentation was also acknowledged as a possible drawback.

Diagnosis of several viral infections often relies on the detection of related antibodies by means of rapid qualitative methods, however, quantitative approaches provide advantages to assess disease severity and progression. Elemental tagging of target molecules coupled with ICP-MS detection was applied *to improve the assessment of viral infections*, devising more sensitive and accurate methods for the measurement of target molecules (*e.g.* virus DNA or specific antibodies). An application of lanthanide tagging for the detection of Hepatitis B Virus (HBV) DNA was reported by Zhao *et al.*[130] The researchers used recombinase polymerase amplification, followed by specific CRISPR/Cas12a reaction and nonspecific cleavage of Tb-single strand DNA modified on MBs. After magnetic separation and dilution to 100 μL with water, the sample was digested with 100 μL of 50% HNO_3 for 2 h at 80 °C, then the digest was diluted with ultrapure water to a final volume of 1 mL and the released Tb^{3+} ions were quantitated by ICP-MS. In comparison with other methods for HBV DNA detection, also based on CRISPR/Cas or CRISPR/Cas12a, the proposed approach could detect a HBV DNA as low as 0.25 fmol L^{-1}, which was 4000-fold lower than assays without amplification. The method specificity was confirmed by the lack of response observed for other virus plasmids and tests on spiked serum samples, showing that it could discriminate at the level of 1 copy μL^{-1} of HBV DNA, thus serving clinical purposes for the early diagnosis of this viral infection. Rubella is a well know virus, responsible for severe health conditions, especially during pregnancy and in newborns. In comparison with existing direct and capture methods using ELISA or chemiluminescent assay for detection, Li *et al.*[131] achieved the quantification of rubella virus IgM in human serum by ICP-MS *via* elemental tagging. To this aim, 50 μL of 0.8 mg mL^{-1} anti-human IgM antibodies, immobilized on MBs, were mixed with 100 μL of serum, incubated at 37 °C for 20 min, followed by addition of 50 μL 5 μg mL^{-1} of Tm^{3+}-

labelled Rubella virus recombinant antigen and incubated at 37 °C for further 10 min. After washing to eliminate excess reagents, 120 μL of 1% HNO_3 were added, and, after gentle shaking for 1 min, the supernatant was analysed by ICP-MS with Re as IS. The method was extensively validated according to CLSI guidelines, showing no cross-reactivity with other antibodies and less than 10% bias in the presence of potential interfering substances (bilirubin, haemoglobin, and triglycerides). It achieved an analytical measurement range between 5 and 500 U mL^{-1}, with good linearity ($r^2 = 0.9944$), and an LOD of 2.84 U mL^{-1}. Overall precision (RSD) and recoveries, assessed at low and high concentrations, were 11.73% and 6.79%, and 95.25 and 102.43%, respectively. The test required 35 min to perform *vs.* 30 min necessary for a commercial electrochemiluminescence immunoassay method. The kappa consistency test for the comparison of the results obtained with two methods on serum samples from 128 subjects yielded a value of 0.86, where values > 0.75 indicate strong agreement (>0.75). The authors concluded that the procedure was suitable to enter routine use in clinical practice.

Tumour markers are involved with the *early and differential diagnosis, prognosis evaluation and progress monitoring of disease states*. Element-labelled immunoassays, coupled with atomic spectrometry, offer a highly sensitive, flexible and specific alternative to established techniques for their quantitative detection, such as ELISA, fluorescence immunoassay, chemiluminescence immunoassay, and electrochemiluminescence immunoassay. Development and analytical and clinical validation of these new approaches are increasingly represented in this Update. The simultaneous detection of alpha-fetoprotein (AFP) and prostate-specific antigen (PSA) in serum, by means of a dual immunoassay based on Eu and Sm labelled antibodies and ICP-MS was reported by Ji *et al.*[132] Magnetic beads coated with specific antibodies for the target proteins were mixed in a 1.5 mL tube, to which 50 μL of serum or standard was added to form the immunomagnetic bead antigen complex. After washing, the Eu^{3+}- and Sm^{3+}- labelled antibodies (for AFP and PSA, respectively) were added to the tube simultaneously, to link to the respective antigens. After washing and magnetic separation, 100 μL of 1% HNO_3 containing Re as the IS, were added to the sandwich complexes and the supernatant solution was analysed by ICP-MS. All reactions were carried out at 37 °C. The method was validated according to CLSI guidelines, showing good linearity ($r^2 > 0.997$), cross-reactivity with other antibodies was < 0.5% and less than 10% bias in the presence of potentially interfering substances (bilirubin, haemoglobin, and triglycerides). The LOD and analytical measurement range for AFP were 0.76 ng mL^{-1} and 1.5–1200 ng mL^{-1}, respectively; whereas values for PSA, were 0.41 ng mL^{-1} and 0.5–250 ng mL^{-1}. Precision, as RSD, was < 4.11% (AFP) and < 4.97% (PSA), and recoveries of AFP and PSA were in the range 95.08–104.18% and 97.65–100.72%, respectively. Analysis of human serum samples by this method and chemiluminescence immunoassay did not reveal significant differences. The authors concluded that the procedure met the CLSI criteria for analytical and clinical validation and offered the basis for the simultaneous detection of more tumour markers and a wider application of mass spectrometry immunoassay in clinical practice. He *et al.*[133] adopted a different approach to facilitate the determination of carcinoembryonic antigen (CEA) at point-of-care, by integrating the well known process of signal amplification based on catalytic Ag deposition on immunogold labels, used for immunoassays, with a specifically designed, 3D-printed, portable capillary liquid electrode GD-microplasma-OES instrument (CLEGD-OES). The procedure involved incubation of 200 μL of 10 μg mL^{-1} CEA primary antibody with coating buffer in a 96-well plate (37 °C for 1 h), followed by discarding and washing, sealing of the uncoated active sites with blocking buffer for 1 h, then addition of 100 μL of diluted serum sample (ratio not given) and 100 μL of 40 μg mL^{-1} CEA colloidal gold-labelled secondary antibody. After 1 h incubation and plate washing, 200 μL of Ag amplification solution was added to each well, to achieve the catalytic deposition of Ag on the surface of the Au NPs within 15 min. After rinsing and drying, the sample was digested with 200 μL 50% (v/v) HNO_3 for 10 min, then diluted to 4 mL with 2% HNO_3. About 0.8 μL of sample was introduced into the plasma *via* a capillary, held for 30 s, whilst Ag was measured by CLEGD-OES at 328.1 nm and CEA concentration calculated. After optimization of the analytical conditions, the device achieved a linear range of 1–500 ng mL^{-1} and an LOD of 0.9 ng mL^{-1} *vs.* that of 0.8 ng mL^{-1} for standard ICP-OES, as well as comparable results for a 100-fold diluted serum sample spiked with CEA at 5 concentration levels (5–120 ng mL^{-1}). Zikmundová *et al.* had previously reported comparable performances of a microtiter plate immunoassay (MTP-ULISA), based on Y-based photon-upconversion NPs (UCNPs) and LIBS detection, with those of established enzyme and fluorescence immunoassays. They presented a follow-up to this work,[134] for the determination of PSA, a biomarker of prostate cancer, using MBs for analyte preconcentration to achieve an LOD of 4.0 pg mL^{-1}, two orders of magnitude lower than that of their equivalent MTP-ULISA (460 pg mL^{-1}). As the amount of MBs used with each sample was constant, it was possible to perform internal standardization of LIBS measurements, based on the intensity ratio Y II (360.07 nm) : Fe I (356.54 nm) in each well, aiming to overcome laser signal fluctuations and bead-bound immunocomplexes loss throughout the washing steps. The procedure involved mixing MBs, conjugated with a monoclonal anti-PSA antibody, with the PSA sample; addition of a biotinylated polyclonal anti-PSA antibody, to form an immunocomplex, that was then labelled with Y, using streptavidin-modified UCNPs. Incubation at room temperature for 1 h was carried out at each step as well as washing steps after each incubation. Magnetic preconcentration reduced the sample volume from 4 mL to 500 μL, of which 100 μL was washed, resuspended in 50 μL and then left to dry at room temperature for double-pulse-LIBS measurements. The authors stated that the PSA concentrations measured with the proposed procedure on clinical human serum samples strongly correlated with those obtained at a hospital setting with an electrochemiluminescence analyser ($r^2 = 0.86$ and 0.99, with and without internal standardization, respectively), but no other detail of analytical performance was provided. Cai *et al.*[135] fabricated an integrated inertial magnetophoresis (the

migration of magnetic particles in a magnetic field) microfluidic chip by combining a contraction–expansion array and active magnetophoresis and coupled online to ICP-MS for rapid separation and reliable detection of circulating tumour cells (CTCs) in blood samples. Their design aimed to overcome the potential interference from white blood cells (WBCs), present in large excess, by a two-step separation process. Before injection into the chip, the WBCs were labelled with antibody modified MBs while the tumour cells were labelled with an Eu-antibody. In the chip, small WBCs were removed in the compression–expansion inertial sorting zone and then any remaining WBCs were captured in the magnetic zone, allowing the Eu-labelled tumour cells to pass directly to the spectrometer. The average recovery for the separation of CTCs from blood samples was 91.6% at a sample flow rate of 200 μL min^{-1}, whereas the WBCs were decreased by about three orders of magnitude. Analytical performance was evaluated as: LOD, 11 CTCs in 1 mL of simulated blood samples, RSD 6.2% (for 100 CTCs, $n = 7$). Cell recovery for blood samples spiked with CTCs at levels between 10 and 200 cells was in the range 92.8–108%, The CTCs in real clinical blood samples were rapidly detected (5 min per 1 mL blood) in all 24 blood samples from patients with different types of cancer. In a following paper,[136] the same group described an alternative approach in which samples were processed in a chip featuring a negative magnetophoresis (the migration of diamagnetic particles in a magnetic medium) sorting zone and a negative magnetophoresis phase-transfer zone. Prior to injection into the cell sorting system, red blood cells were removed and the WBCs and CTCs were labelled by adding the appropriate reagents and waiting for 60 min. The WBCs attached to MBs dispersed in biocompatible ferrofluid were removed by magnetic attractive forces, while CTCs, labelled with Eu, were migrated into the phase-transfer zone by a magnetic repulsive force. In the phase-transfer zone, the CTCs migrated into a buffer under both the magnetic repulsive and inertial lift forces, and were introduced directly into the spectrometer. They achieved CTC enrichment at a flow rate of 100 μL min^{-1} with separation efficiency of 99.6%, while maintaining a cell viability of 99.27%. They achieved a detection rate of 100% when applied to real clinical blood samples from 10 patients with various cancers. Another group of researchers[137] reported the application of magnetic preconcentration and an Au NP probe-based immunoassay coupled with ICP-MS detection for the determination of the aquaporin-1 protein (AQP1) in urine. This protein is thought to play an important role in intracellular and extracellular water homeostasis and may be a biomarker of early-stage kidney and other organ diseases, including cancer. Extraction and preconcentration of the analyte from urine was achieved using MBs modified with epoxy groups (2 mg suspended in 100 μL of phosphate buffer, pH = 7.4), 100 μL urine and 100 μL of 3 mol L^{-1} of $(NH_4)_2SO_4$ in phosphate buffer, incubated at 37 °C for 1 h, under gentle shaking. After repeated washing with PBST (PBS with Tween 20), the MBs-proteins complexes were resuspended in low-fatty-acid bovine serum albumin (1% m/v), gently shaken at 37 °C for 15 min and further washed with PBST. A specific probe, consisting of anti-AQP1 antibody-Au NPs (10 μL), was added and the mixture incubated at 4 °C overnight, toallow the specific immunobinding and form MBs-AQP1-probe complexes. These were separated from the solution, after repeated washing with PBST and addition of 100 μL of 0.1 mol L^{-1} citric acid, heated at 37 °C in a water bath for 5 min, to release the Au NPs, then digested with 100 μL HNO_3 and made up to 2 mL with 2% HNO_3–0.5% HCl (v/v) for ICP-MS analysis. The method achieved a 14 000-fold signal amplification, linearity between 0.3 ng mL^{-1} and 30 ng mL^{-1} ($r^2 = 0.998$) and an LOD of 0.023 ng mL^{-1}, thus was suitable for the discrimination between AQP1 levels in urine of healthy subjects (<1.0 ng mL^{-1}) and renal cell carcinoma patients (137–508 ng mL^{-1}). Reproducibility RSDs ($n = 6$), assessed in urine at three spiked concentrations (0.3 ng mL^{-1}, 3 ng mL^{-1} and 30 ng mL^{-1}, ranged from 7.5% to 15% and recovery of the spiked amounts were between 92 and 110%. The authors noted that this procedure proved more specific, sensitive and accurate compared to other conventional methods, such as western blotting and ELISA, and showed potential for extension to other proteins where specific antibodies are available.

Other applications of elemental-labelling in clinical research or practice were also reported.

Mysková *et al.*[138] investigated the *mechanism of action of candidate drugs for the treatment of obesity and related diseases*, using a lipidated analogue of an anorexigenic prolactin-releasing peptide (palm11-PrRP31), labelled with a lanthanide tag. The lanthanide forms were detected in blood plasma, liver and urine samples in only 6 min, using LC-ICP-MS with an aqueous mobile phase containing 5% (v/v) 1,2-hexanediol–2% formic acid, with LODs ranging from 0.9 to 3.4 ng L^{-1}. The devised procedure proved useful to identify labelled metabolites in biological fluids and tissues thus supporting the understanding of the mechanism of action for the drug.

A new and faster approach to the *mapping of proteins on western blot paper* was presented,[139] based on microextraction sampling and ICP-MS. The proteins were first covalently tagged with Gd, La and Tb, then separated *via* gel electrophoresis, and transferred to western blot paper, which was then analysed directly without any sample preparation. The LODs were 54, 564 and 2.5 fg for Gd, La and Tb, respectively. The procedure proved to be comparable to LA-ICP-MS, for both analysis time (*ca.* 1 min for 2 × 4 mm extraction) and sensitivity and showed potential for further application in the research and development of metal-tagged compounds.

For the *diagnosis of bacteraemia resulting from E. coli or Salmonella,* by means of metalloimmunoassay with ICP-MS detection, Li *et al.*[140] constructed an automated sample pretreatment system. All chemical reactions were carried out under stopped flow conditions on the surfaces of MBs held in place in the serpentine reactor of a microfluidic chip by an external magnet. Following the initial manual loading of the beads coated with reagents to capture the bacterial cells, all fluids were handled by a gas-driven liquid handling system that controlled downstream solenoid valves and the pressure in the sample and reagent bottles. The bacteria capture time was 20 min, the labelling reaction time was 20 min, during which time the immobilized *Salmonella* was labelled with a reagent

loaded with Au NPs and the *E. coli* was labelled with a reagent loaded with Ag NPs, and the desorption time was 15 min. In between each step, there was a 3 min wash with buffer at 5 μL s^{-1}. The desorbed solution was delivered directly to the spectrometer. Recoveries of spikes of various concentrations of colony-forming units (CFU) of each bacteria into three real blood samples ranged from 87 to 106%. The LODs were 200 CFU mL^{-1} for *E. coli* O157 : H7 and 152 CFU mL^{-1} for *Salmonella*. There was no significant difference between these results and those of a blood culture method.

In this Update, we also report t*he application of ICP-MS based immunoassays to food, for the detection of foodborne pathogens*, a major cause of concern for global public health. Li *et al.*[141] achieved the detection of *Salmonella* in food by ICP-MS immunoassay, within one step, using a newly designed "nanorobot", consisting of MB-streptavidin coupled with Au NPs and a double strand DNA (walker DNA-biotin and aptamer). The nanorobots (20 μL) were added to the bacterial suspension, along with buffers and nuclease-free sterile water, to a total volume of 50 μL, and left to react at room temperature for 1 h. The specific recognition of *Salmonella* by the aptamer on the nanorobots, triggered the endonuclease signal amplification and the release of Au NPs. After magnetic separation, supernatant was collected for ^{197}Au detection by ICP-MS. An LOD of 15 CFU mL^{-1}, linear range between 50 and 40 000 CFU mL^{-1} and an RSD of 7.6%, for a bacterial concentration of 2000 CFU mL^{-1}, were reported. The aptamer on the probe did not exhibit specificity towards *E. coli* O157:H7 and *Staphylococcus*. The method was tested on four types of food items susceptible to *Salmonella* contamination (chicken, tomatoes, eggs and milk). Ten g of solid samples and 25 mL of milk were homogenized and filtered, then 200 μL aliquots of each sample type (except eggs, that were 100-fold diluted with sterile water to reduce viscosity) were spiked with 20 μL containing 500, 1,000, and 10 000 CFU mL^{-1} of bacterial cells, achieving satisfactory recoveries ranging from 84.6 to 103%. The authors highlighted that, compared to other procedures, the method allowed the rapid, sensitive and specific detection of *Salmonella* in one-step within 1 h, avoiding contamination risks. Another group of researchers[142] addressed the simultaneous detection of three bacteria – *Salmonella typhimurium* (*S. typhimurium*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), and *Shigella sonnei* (*S. sonnei*) – which are of particular concern. Based on previous work, they chose to employ elemental-labelled nucleic acid probes rather than protein-based ones, that are more prone to cross-react with proteins from similar pathogens, affecting the reliability of the results. The DNA capture probes were immobilized on iron MNPs and corresponding DNA report probes were conjugated to noble metal NPs (Au, Ag and Pt, respectively, for the three target bacteria). After extraction from the bacteria cells, pathogen DNAs were incubated with the probes, sandwich-structure complexes were formed by hybridization, then isolated by magnetic separation and quantified. The researchers applied two detection techniques. Differential pulse voltammetry achieved linear ranges of 10^1–10^{10} copies mL^{-1}, LOQs from 1 to 10 copies mL^{-1} and RSDs <2.9%, for the three target pathogens. The authors highlighted the reliability of the electrochemical detection, attributing the low LOQs to the improved design of the hybridization strategy, and noted the only drawback of the technique was the lack of ability to discriminate between the three elemental labels of the sandwich complexes, confining its application to the detection of single species. However, simultaneous measurement of the three species was achieved by ICP-MS, after digestion of the complexes with aqua regia (1 HNO_3 : 3 HCL, 5 min), volume adjustment to 10 mL and measurement of ^{107}Ag, ^{197}Au and ^{195}Pt. Linear ranges were 10^1–10^{10} copies mL^{-1} (r^2: 0.9919–0.9988), LOQs ranging from 1 to 2 copies mL^{-1}, and RSDs <2.7% for the three bacteria. The method was tested on real food samples (fish, chicken, pork, lobster, egg, potatoes, and Jule® milk) and lake water, spiked with the pathogens. Liquid samples were filtered and diluted 1000-fold with a buffer (10 mmol L^{-1} Tris–HCl, 1 mmol L^{-1} EDTA, pH 8). Solid samples were minced, and 10 g of each was dispersed in 10 mL of PBS, agitated for 30 min, then centrifuged (10 621*g*, 4 °C, 10 min) and diluted 1000-fold with the same buffer. Recoveries, comparable to those reported in previous investigations, ranged from 93.6 to 104.1%, for *S. typhimurium* (pork, eggs and milk), *V. parahaemolyticus* (lobster, lake water and fish) and *S. sonnei* (milk, potato and chicken). The authors concluded that the procedure enabled rapid, simultaneous and highly specific detection, at very low concentration levels, for three pathogens of great concern for food safety. They noted that it offered great potential for the management of outbreak responses, but acknowledged that efforts and cost reduction would be necessary for its widespread use.

Two papers reported the application of separation techniques and ICP-MS based methods to the *determination of P containing molecules present in food and feed samples*. A group of researchers[143] addressed the difficulties of determining phytic acid and inositol phosphates in complex matrices, by developing a procedure based on IC-ICP-OES for the simultaneous determination of 28 inositol phosphate isomers (InsP6 to InsP2) in food and feed (dry grains, pulses, seeds, crushed soy and corn). Samples were ground, then inositol phosphates were extracted from ground samples (1 g) with 10 mL of 0.5 mol L^{-1} HCl, by shaking for 1 h at room, then centrifuged (12 000*g*, 4 °C) and the supernatant passed over a SPE C18 cartridge and filtered. The chromatographic separation of the inositol phosphate isomers in a 100 μL injection volume was achieved at 30 °C, within 33 min, on a CarboPac PA100 column (4 × 250 mm), with gradient elution using H_2O (A) and 0.5 mol L^{-1} HNO_3 (B). The P signal was detected at 177.495 nm and 213.618 nm. The authors reported an LOQ of 208 μg L^{-1} P, corresponding to 740 μg L^{-1} phytic acid, stated to be 4-fold lower than other published IC methods, and reproducibility RSD of < 1% (food) and < 4% (feed). The authors stated that the method simplified the determination of inositol phosphates in complex food and animal feed matrices. Being faster and more sensitive than other existing approaches, it held potential for enhancing the understanding of phytases and the phytic acid degradation processes. Ruiz *et al.*[144] described a novel method for the determination of phospholipids in oily samples (*e.g.* vegetable oils, animal fats, and phospholipid supplements) by HILIC-HR-

ICP-MS. After chromatographic separation of phospholipids on an XBridge BEH HILIC column (4.6 × 100 mm, 3.5 μm), using gradient elution with (A) 10 mmol L^{-1} CH_3COONH_4–0.1% formic acid–5% THF and (B) THF, ^{31}P was detected at a mass resolution of 4000, overcoming the main polyatomic interferences from $^{15}N^{16}O^+$ and $^{14}N^{16}O^1H^+$. Samples were diluted by weight in THF (oils, 5-fold; fats, 25-fold and supplements, 300- or 5000-fold) and 5 μL were injected onto the column. The analysis required 23 min. A comparison with FI-HR-ICP-MS on an asolectin soybean phospholipid extract yielded a 95.8% recovery and an RSD of 0.4% ($n = 3$). With the advantage of application to a wide range of different oily samples after a simple dilution in THF, the method achieved faster and simpler determinations of phospholipids, which are required in both food industry and renewable energy production.

7 Speciation and imaging studies

7.1 Speciation studies

Recent advances in speciation studies and related applications are extensively discussed in a comprehensive Update by Clough *et al.*[6] This section aims to highlight *applications for the analysis of elemental species in clinical and biological materials, foods and beverages*, to complement the topics covered in this Update. The determination of As, Hg and Se species represents the largest part of these applications, however, in the period covered by this Update, new developments were also reported for Cd, Cr, Pb and Zn, and two studies described simultaneous multielement speciation methods. In addition, procedures mainly based on extraction, including those based on nanomaterials, are reported in Section 4.2 and Table 1, the investigation of Cu species in relation to the development of new biomarkers for Wilson's disease and Alzheimer's disease is discussed in Section 8.2.4 and two papers related to Gd species in a contrast agent and the analysis of human milk are covered in the dedicated sections (9 and 10.2.1, respectively).

The largest part of the developments reported during this Update's period were related to food and beverages, with fewer papers addressing the determination of element species in clinical samples.

Whereas the toxic impact of exposure to high As levels is well known, concern is growing for possible adverse health effects, such as cardiovascular disease and diabetes, following long-term exposure to low As concentrations, deriving, for example, from environmental sources, marine food consumption and smoking. A procedure,[145] based on anion exchange HPLC-ICP-MS, was developed and applied *to assess chronic low to moderate As exposure* in 7677 spot urine samples from the US multi-ethnic study of atherosclerosis. Sample preparation consisted of addition of 10 μL H_2O_2 to 100 μL urine, to oxidize As^{III} species to their As^V form, followed by addition of 390 μL of mobile phase and injection (100 μL) onto the column. Species separation was achieved in 10 min on a PRP-X100 column (250 × 4.1 mm, 10 μm particle size), with isocratic elution with 10 mmol L^{-1} $NH_4H_2PO_4$–10 mmol L^{-1} NH_4NO_3–0.5% (v/v) ACN, at pH 9.1, as the mobile phase with a flow rate of 2.0 mL min^{-1}. A total of 11 As species (iAs, AB, DMA, methylarsonic acid, and seven unknown ones, in their As^V form) were detected at LODs ranging from 0.02 to 0.03 μg L^{-1}. The As species were further investigated by means of separation on a AS14A column and gradient elution, followed by analysis by ICP-MS/MS and HR-Orbitrap MS/MS. However, only dimethylarsinoylacetic acid and dimethylarsinoylpropionic acid, potential metabolites of seafood-related arsenicals, could be identified, whereas the others remained unknown. Spike recoveries and 14 human urine RMs, including CRMs (NIST SRM 2668 Level I & II, SRM 2669 Level I & II) were used for method validation, achieving accuracy of 98% with RSDs <6% for the four identified As species in urine. Due to the implementation of the oxidation step, the main As species (iAs, AB, DMA, methylarsonic acid) were detectable in at least 95% of the samples and the minor ones in at least 25% of them. The authors noted the reliability of analytical data as an essential requirement to interpret the findings of epidemiological studies for the investigation of low As exposure and related potential health effects.

Given the different toxicity of Hg species, notably MeHg *vs.* iHg, Hg speciation is a regular feature in this section. *A new approach,*[146] *based on LC coupled with VG-ICP-MS/MS, was explored for the quantification of MeHg and iHg species in whole blood*. Blood samples and matrix-matched working calibration standards (250 μL) mixed with 4.75 mL of 0.1% (v/v) TMAH were placed in an ultrasonic bath for 15 min, then centrifuged (400*g*, 5 min) and filtered. The separation was achieved in 4 min *via* isocratic elution on a C8 Zorbax StableBond (5 μm, 150 mm × 5 mm) column, with a mobile phase consisting of 0.06 mol L^{-1} CH_3COONH_4, 0.05% (v/v) mercaptoethanol, 0.4% (m/v) L-cysteine, 5% (v/v) MeOH, pH ~6.7, at a flow-rate of 1.0 mL min^{-1}. Post-column VG used a low volume gas liquid separator (GLS) with a micro-peristaltic pump, integrated in the ICP-MS design. Mercury species were reacted with 0.01 mol L^{-1} HCl, as the carrier, 0.1% (m/v) NaOH and 0.5% (m/v) $NaBH_4$, as the reductant, both at flow rates of 0.5 mL min^{-1}. The hydrides formed were swept into a specially modified spray chamber by an argon flow (1.1 L min^{-1}). The analysis was carried out in oxygen gas mode, that further improved sensitivity, and achieved an LOD of 0.2 μg L^{-1} for both iHg and MeHg. The assessment of accuracy was carried out using blood CRMs (NIST SRM 955c and 955d, at different concentration levels) and other RMs (*e.g.* PT items) as well as by comparison with LC-ICP-MS/MS. The results of CRM analysis were generally within 30% or the expanded uncertainty of the assigned values, whichever the greater, although most of the reported results were lower than the assigned values, suggesting possible losses of Hg during the process. The authors surmised that the VG step improved the S/N ratio, leading to lower LODs that were further reduced by the use of 40% oxygen as the collision gas. They also noted the benefits of the simplicity of sample preparation and the high sample throughput (~100 samples per 7 h shift), catering for large scale biomonitoring studies.

The capability of ICP-MS for *multielement determinations can potentially provide a significant amount of information from an individual sample*, which can be especially beneficial for the investigations of PTEs in clinical specimens. After an extensive assessment of the optimal analytical conditions, As (As^{III}, As^V,

DMA and MMA) and Hg (Hg^{II}, MeHg and EtHg) species were simultaneously measured in human urine by HPLC-ICP-MS.[147] After dilution of 1 mL urine with 4 mL deionized water, centrifugation (7500 rpm min^{-1}, 5 min) and filtration, 50 μL were loaded onto a Hepu AR C18 column (250 × 4.6 mm, 5 μm). The element species were separated using gradient elution with 0.1% L-cysteine (mobile phase A) and 4 mmol L^{-1} TBAH–5 mmol L^{-1} $NH_4H_2PO_4$ (mobile phase B) and their concentrations measured by ICP-MS in KED mode to reduce remaining matrix interferences. Analytical performance was reported as: linearity within the range of 1–20 μg L^{-1}, ($r > 0.999$; LODs from 0.030 to 0.086 μg L^{-1}), LOQs between 0.10 and 0.29 μg L^{-1} and inter-day RSDs of 0.8–9.2%. The results obtained from NIST SRM 2669 L-2, certified for As species in urine, were in good agreement with the certified values (*t*-test at a 95% confidence level). Because no CRM was available for Hg species, recoveries for all species were evaluated by spiking a urine sample with As and Hg species at concentrations of 2.0, 5.0, and 10.0 μg L^{-1}, resulting in recoveries ranging from 87.0 to 110.3%. The authors noted that the percent ratios of the sum of the species *vs.* total As and total Hg concentrations measured by ICP-MS ranged from 67.5 to 107.1 for As and from 98.8 to 107.7 for Hg, respectively, suggesting the presence of retained unknown As species. However they suggested that the method provided reliable results and was suitable for the analysis of a large number of samples for clinical applications.

Yang *et al.*[148] reported an unusual application of ICP-MS/MS for the *determination of multiple actinides in faeces, as an alternative to radiobioassays*, for the purpose of internal dose assessment in exposed populations. A sample (150 g of synthetic faeces) underwent ashing for 8 h, then the ashes were subjected to borate fusion to release the radionuclides, followed by complete dissolution with 200 mL of 3.5 mol L^{-1} HNO_3, addition of 0.3 g ascorbic acid and flocculation with 5 g of PEG-6000. After centrifugation, coprecipitation of the actinides from the supernatant with CaF_2 and re-dissolution in 8 mol L^{-1} HNO_3 enabled both preconcentration of the analytes and removal of matrix elements. Further purification steps involved sequential column chromatographic separation using AG MP-1 M and DGA resins. After careful optimization of the process ^{241}Am, ^{244}Cm, ^{237}Np, ^{239}Pu, ^{240}Pu and ^{241}Pu were measured with LODs ranging from 0.660 (^{244}Cm) to 4.05 (^{239}Pu) ag g^{-1}, by ICP-MS/MS. Using helium as the collision gas led to a 2.5-fold increase of the sensitivity of the instrumental measurements, to approximately 7 Mcps per ppb. Participation in two ILCs (total 6 PT items), organised by PROCORAD (Association for the PROmotion of Quality COntrol in RADiotoxicological Analysis), France, over a period of two years, yielded satisfactory results (*z* scores < ±2). The reliability and high throughput (18 h for 12 samples) of the proposed method were highlighted as key benefits to expand the number of laboratories involved in screening activities for unexpected actinide exposure.

The *presence and distribution of As species in food* continues to be an interesting area for both research and control activities linked to food safety. Three papers, which have reported advances of measurement techniques, are included in this section. Noting that As speciation in freshwater fish is receiving less attention, due to the challenge represented by accurate detection of As species at trace levels, a group of researchers[149] developed an analytical method fit for this purpose. To prevent possible interconversion of As species, their extraction from 2 g of ground fish fillet was achieved under mild conditions (10 mL of a 1 : 1 MeOH : H_2O mixture, ultrasonication for 1 h in a water bath and centrifugation at 4000 rpm for 40 min), then the same steps were repeated on the solid residue three times. The combined extracts were reduced to approximately 1 mL, by heating at 60 °C then diluted to a final volume of 5 mL with deionized water, centrifuged and filtered. Analysis was performed by HPLC-ICP-MS with an anion-exchange column (PRP-X100, 4.1 × 150 mm, 5 μm), isocratic elution with 35 mmol L^{-1} NH_4HCO_3 (pH 8.25) and helium as the collision gas to eliminate isobaric interferences. Although a higher concentration of NH_4HCO_3 (60 mmol L^{-1}) enabled the complete separation of the five major As species in 9 min, a lower concentration of NH_4HCO_3 led to a longer elution time (~17 min) but revealed the presence of three more As species, that require further investigation. $Arsenic^{III}$, As^V, AB, DMA, MMA were quantified with an impressive LOD of 0.25 μg kg^{-1} ww. The linear range was 0.1–50 μg L^{-1} for the five As species of interest ($r^2 \geq 0.9999$). Accuracy was assessed on three CRMs (NIST SRM 1568b, Rice flour; NRCC DORM-4 and DORM-5, Fish protein) certified for As species. Good agreement was achieved for the fish protein CRMs, but not for the rice flour, due to lower extraction efficiency with this matrix. The method was applied to the determination of As species in 1643 freshwater fish samples, representing 14 common fish species from different locations.[150] The frequency of As species observed in these freshwater fish was AB > DMA>, As^V > MMA and As^{III} was below the LOD in all samples. Beside these species, seven other unknown ones were detected in this large survey of freshwater fish. Monitoring exposure of the general population to potentially toxic substances is part of the actions undertaken to protect consumer health, by establishing and updating maximum allowable levels in the different food commodities. Many data are obtained from food control systems, organised by national authorities to verify compliance with existing legislation. Total diet studies are a more complex tool with the purpose to gain information on the presence and levels of contaminants in specific panels of foods, representing both the type and the consumption level, and involving preparation of foods as consumed. Up to now, they have mostly included adult populations and measurements of the total elemental content, but the Third French Total Diet Study planned to focus on infants and to investigate the elemental species of As, Cr and Hg, due to the differing toxicity. To ensure the reliability and comparability of the results from large batches of different food matrices, over a period of time, it was necessary to develop and validate multi-matrix analytical methods. For As speciation, this was achieved by Ghaffour *et al.*,[151] who applied anion-exchange HPLC-ICP-MS for the determination of As^{III}, As^V, DMA and MMA. To preserve the integrity of the iAs species, avoiding the oxidation of As^{III} to As^V, stronger extraction media (HNO_3, H_2O_2) were excluded and a thiol-selective blocking agent (*N*-ethylmaleimide) was added since sulfur-based proteins, present in some fish, may trigger

the same reaction. After careful optimization of time and temperature, extraction of As species from 0.2 g sample was carried out by either heat-assisted extraction or MAE, under mild conditions: 80 °C, 15 mL or 9 mL of deionised water containing 1.5 mmol L^{-1} (w/v) *N*-ethylmaleimide, for 30 min or 20 min, respectively. The extracts were diluted with ultrapure water to a final volume of 50 mL, 50 μL of which were loaded onto a Dionex IonPac AS7 column (2 × 250 mm), kept at a 30 °C. The four As species were separated within a considerably short time (10 min) using a mobile phase gradient of 0.5 (A) and 5 (B) mmol L^{-1} $(NH_4)_2CO_3$ both with 3% (v/v) MeOH, at pH 9.3, at a flow rate of 0.5 mL min^{-1}. Measurements were carried out at *m*/*z* 75, using an Agilent 7850 ICP-MS. Although this type of instrument features a helium mode CC and half mass correction to remove both polyatomic and doubly charged ions, there was no mention that these tools were applied to eliminate the interference signal overlapping from $^{40}Ar^{35}Cl$. Validation was performed according to the accuracy profile approach,[152] by analysing six different food matrices in duplicate over six separate days within a 4-week period. Analysis of NIST SRM 1568b Rice flour, certified for the four As species, and EC JRC IRMM BCR®-627 Tuna fish tissue, certified for DMA, gave results in good agreement with the certified values. Additionally, spiked samples of molluscs, crustaceans, tuna, cereal products and vegetables were analysed, to build the accuracy profiles. For the four As species, this robust validation approach yielded: LODs of 1.88 μg kg^{-1} (dw); LOQs of 6.25 μg kg^{-1} (dw); intermediate reproducibility RSD ranging from 4.7% to 5.5% and bias < 3%. The authors concluded that the method was fit-for-the-purpose of routine As speciation analysis of the variety of food matrices collected for the Third French Total Diet Study.

The *Cd content and its bioavailability from plant-based food* was the area of concern of Cardini and co-workers,[153] given the need to monitor the exposure to Cd among those consuming this type of products. They explored a qualitative methodology, combining SEC-ICP-MS/MS and SEC-TOF-QMS, to identify Cd-chelating compounds and potential competition from the co-presence of other metals (Ca, Fe, S and Zn) in plant-based food. In this paper, they described the optimization of the process necessary for the analyte extraction from matrices with different protein content in their hydrated state: (a) > 5 g 100 g^{-1} (black-eyed beans and beluga lentils), (b) 2–5 g 100 g^{-1} (tigernut and basmati rice), (c) < 2 g 100 g^{-1}, (sweet potato and beetroot leaves). They explored various parameters such as the content of ACN or MeOH (10, 20 or 30%) in the extraction solvent, solvent volume and extraction times. They observed that extraction efficiency was largely matrix-dependent, ranging from 2.3% (sweet potatoes, basmati rice) to 72.3% (tigernuts), suggesting that Cd may have stronger binding power in certain foods. Organic solvents were found to reduce the recovery of Cd and were therefore excluded. The "best" all-round conditions were then selected as 100 mg of dry sample and 2 mL of 50 mmol L^{-1} CH_3COONH_4, vortexing, ultrasonication for 30–60 min and centrifugation (10 000 *g*, 15 min). A new feature of this study was the extended separation range, achieved by employing two SEC columns – Superdex 30 Increase and Superdex 200 Increase (both 3.2 × 300 mm) – with separation ranges spanning from 600 to 10 kDa and from 10 kDa to 100 Da, respectively, thus enabling the detection of both low and high Mr Cd-binding species. For both columns, the mobile phase was 50 mmol L^{-1} CH_3COONH_4, supplemented with 0.1% formic acid for use in connection with the TOF-QMS, at an optimal flow-rate of 0.075 mL min^{-1}. The IS (Rh, 50 μg L^{-1}) was added as a post-column infusion. The ICP-MS/MS instrument was operated with oxygen as the reaction gas (flow-rate 0.35 mL min^{-1}) for optimal signal enhancement. The average repeatability RSDs for peak areas, evaluated on black-eyed beans, tigernuts, and beetroot leaves was less than 4.9% for ICP-MS/MS and less than 5.9% for TOF-QMS, respectively. The method enabled the identification of various Cd-chelating compounds, including phytic acid, phytochelatins and metallothioneins, as well as different chelating patterns in different food types.

Xu *et al.*[154] proposed a short-column IC-ICP-MS method, using *low-cost cation guard columns as separation columns, to quantify Pb species* (Pb^{2+}, TEL and TML) in seafood, noting its advantages in terms of high sensitivity, shorter analysis time and lower analysis costs. Sample preparation of different species of seaweed, fish and shellfish by MAE showed an extraction efficiency >90%, with no alteration of the Pb species. An aliquot (0.10 g) of dried seafood and 6.0 mL of 12 mmol L^{-1} EDTA were kept at room temperature for 6 h, then subjected to MAE (ramped up to 120 °C over 10 min, then held at 120 °C for 30 min). After cooling to room temperature, the mixture was centrifuged and the supernatant collected. The extraction was repeated on the residue, the two portions were combined, filtered (0.45 mm), and diluted up to 5-fold with water, depending on the concentration of total Pb in the sample, then injected directly onto the column. The species separation was achieved on two consecutive strong cation guard columns (Zorbax 300SCX, 4.6 × 12.5 mm). Literature data suggested a mixture of EDTA and TBAH should be a suitable mobile phase, however, this did not work well for the short columns and the authors suggested this may be due to insufficient time to form the EDTA chelates with the Pb species. They overcame this problem by chelation of the Pb species with 0.3 mmol L^{-1} EDTA, prior to IC separation with 1.0 mmol L^{-1} EDTA–0.25 mmol L^{-1} TBAH, pH 3.0, as the mobile phase, at a flow-rate of 1.0 mL min^{-1}, which achieved the separation of Pb^{2+}, TEL and TML within 10 min. The evaluation of the method LOD yielded values between 0.60 and 0.72 ng g^{-1} dw seafood. The reliability of the method was assessed on six samples of shellfish (*Mytilus edulis* and *Morulauva*), fish (*Ditrema temmincki bleeker* and *Arius sinensis*) and seaweed (*Laminaria japonica* (kelp) and *Ulva lactuca*) spiked with Pb^{2+}, TEL and TML, at 0.275 or 0.550 μg g^{-1}. Recoveries for all species were found to be between 94 and 105%, with RSDs ($n = 5$) <6%. Analysis of a CRM for total Pb (GBW08521, Laver, Puxi-Standard Material Center, Beijing, China) gave a result of 0.790 ± 0.032 μg g^{-1} ($n = 5$) *vs.* the certified value of 0.81 ± 0.03 μg g^{-1} (97% recovery). In addition, the sum of the Pb species in each sample was consistent with the concentration of total Pb, determined by ICP-MS after complete sample digestion with HNO_3. Although the method was tested on a limited number of samples, it shows potential for a wider application.

Considering the advantages of multielement detection for the assessment of their content and speciation in food, a group of researchers[155] focussed on the development of *a comprehensive analytical method for the simultaneous speciation of inorganic and organic As and Se species*. The study was performed using American ginseng (*Panax quinquefolium* L.) root, a traditional Chinese medicine, now increasingly consumed as a functional food, as a model of a complex matrix. Six As species (AB, As^{III}, As^{V}, DMA, MMA and ASA) and five Se species (MeSeCys, Se^{IV}, Se^{VI}, $SeCys_2$ and SeMet) were detected in the same sample within 10 min. First, samples underwent enzymatic hydrolysis: an aliquot of ground dried roots (*ca.* 0.3 g) and 50 mg of proteinase E were diluted to a final volume of 5 mL with deionized water, then ultrasonicated in a water bath (37 °C, 30 min) and centrifuged (8000 rpm, 10 min). After filtration, 20 μL of the supernatant was loaded onto a ZORBAX SB-Aq column (4.6 mm × 250 mm, 5 μm) for ion-pairing RP-HPLC-ICP-MS analysis. The elemental species were separated *via* isocratic elution at a flow-rate of 0.8 mL min^{-1} with 5 mmol L^{-1} sodium 1-hexanesulfonate–20 mmol L^{-1} citric acid–2% (v/v) MeOH, pH 4, as the mobile phase. The ICP-MS was operated in time resolved mode, to monitor the signals at *m*/*z* 75 (As) and 78 (Se), and KED mode, to minimize interferences. Analytical performance was evaluated according to EURACHEM guidelines:[156] linearity in the range 0.1–100 μg kg^{-1} (r^2 between 0.9979 and 0.9999), LODs from 0.058 to 0.60 μg kg^{-1}; LOQs between 0.193 and 1.31 μg kg^{-1} and inter-day precision RSD from 1.07 to 6.67% and recoveries of spiked amounts (5–100 μg kg^{-1}) ranging from 72.13 to 107.2%. The individual data, reported in a figure, indicated the recoveries varied among species with $SeCys_2$ showing the lowest value, DMA and SeMet were around 80%, while AB and ASA recoveries were above 100%. In ten batches of American ginseng analysed by this method, iAs and iSe species and SeMet were present in all samples, $SeCys_2$ in four of them and MMA in only one, whereas all other species were below LODs.

Several trace elements play an important role for human health and an optimal intake with the diet is associated with improved well-being, although a full understanding of their mechanism of action deserves further investigation and excessive intake may be detrimental. The identification of the chemical forms occurring in food, beverages and supplements, as well as their bioavailability would support well-balanced diets, including micronutrient intake. Microalgae are increasingly popular food supplements, that can be easily grown and are a source of numerous substances of nutritional value (*e.g.* amino acids, vitamins and trace elements). The research by Szczęśniak *et al.*[157] focussed on the *qualitative identification of Cr and Zn forms in selected species of microalgae* – diatoms (*Phaeodactylum tricornutum*) and spirulina (*Arthrospira platensis*) – by means of AEC (Cr) or HILIC (Zn) coupled with both ICP-MS/MS and ESI-MS/MS. Different reagents and conditions for the extraction of low Mr Cr and Zn compounds were evaluated using SEC-ICP-MS. Finally, the procedure of choice was enzymatic extraction of 0.05 g of dry microalgae powder vortexed (50 °C for 24 h) with 5 mL of Flavourzyne®. The extracts were centrifuged (15 133*g*, 20 °C, 30 min) and the supernatants filtered prior to analysis. Separation of Cr species (Cr^{III}, Cr^{VI}) was carried out on a Allsep Anion column (150 × 4.6 mm × 7 μm) at 21 °C, with a 14 mmol L^{-1} NH_4NO_3–0.5 mmol L^{-1} EDTA, pH 7.2, mobile phase; the speciation of Zn, was achieved on a ZIC-HILIC column (150 × 2.1 mm × 3.5 μm, 100 A) using gradient elution with 10 mmol L^{-1} CH_3COONH_4 pH 7.3 (A) and ACN (B), as the mobile phases. In both cases, the detection of the elemental species was carried out by ICP-MS/MS, with a hydrogen collision gas flow-rate of 6.0 mL min^{-1}, but, when using HILIC separation, 8% oxygen was added to the carrier gas to avoid carbon deposition on the cones. Analyses were performed in TRA mode. The results confirmed those of several other studies, reported also in our previous Updates, documenting the absence of the toxic species Cr^{VI} in food, due to rapid interconversion to Cr^{III}, whereas Cr^{III} was found in all tested samples. Zinc species were subsequently identified by HILIC-ESI-MS/MS as Zn complexes with nicotianamine, 2′-deoxymugine acid and citrate. Zeng *et al.*[158] reported the simultaneous detection of twelve species of Cd (Cd^{II}), Hg (Hg^{II}, EtHg, MeHg), Pb (Pb^{II}, TEL, TML) and Sn (Sn^{II}, TBT, TET, TMT, TPhT) in shrimp and fish by HPLC-ICP-MS. Their research aimed to achieve an efficient, sensitive, inexpensive and "green" method to support the collection of data for a wider understanding of the chemical species involved in the toxicity of these elements. Giant river prawn (*Macrobrachium rosenbergii*), giant black seabass (*Micropterus salmoides*), crucian carp (*Carassius auratus*), and grass carp (*Ctenopharyngodon idellus*), weighing at least 2 kg each, were purchased locally. Edible parts were isolated and homogenized. Sample aliquots (4.0 g) and 10 mL of 1 mol L^{-1} HCl in 1 : 1 (v/v) MeOH : H_2O were subjected to ultrasonication in water bath (40 °C, 1 h), then centrifuged (3000 rpm, 10 min). The procedure was repeated on the residual sample solid, combining the two supernatants. The extract was neutralised with NH_3, filtered (0.45 μm) and 20 μL analysed. Chromatographic separation was achieved within 24 min on an amphion II column (10 cm × 4.6 mm, 5 μm) with isocratic elution using 4 mmol L^{-1} SDBS–0.1 mmol L^{-1} Cys, pH 2.5, at a flow-rate of 1.5 mL min^{-1}. The IS (100 μg L^{-1} In) was mixed with the eluate at a flow-rate of 0.2 mL min^{-1}, prior to nebulisation. The LOD for Cd^{II} was 0.1 μg kg^{-1}, whereas those for the other metal species were in the ranges 0.08–0.1 (Hg), 0.06–0.1 (Pb) and 0.05–0.7 (Sn) μg kg^{-1}, respectively. The LOQs were < 0.5 μg kg^{-1} for 10 of the metal compounds, except TBT and TPhT. Assessment of precision was limited to repeatability of retention times and peak areas, the latter being < 4.3% RSD ($n = 6$). Two CRMs from the Chinese National Institute of Metrology (GBW10068, Metals and organotins in oyster; GBW10029, Total Hg and MeHg in fish) were analysed, and the results agreed with the certified values within 5%. Due to the lack of CRMs for all analytes, spiking experiments were carried out, using both CRMs (90–111%) and fish/shrimp samples (87–107%). The comparison with four mono-elemental HPLC-ICP-MS methods gave relative differences ranging from −3.3% to 6.2%. The authors noted the advantages of the proposed method, in terms of simultaneous determinations, rapidity, low LODs, low cost and "greenness", as an aqueous mobile phase was used.

7.2 Imaging with MS and X-rays

The *imaging analysis of breast cancer tissues* was the focus of two papers. Escudero-Cernuda *et al.*[159] determined the spatial distribution of essential and non-essential elements in tissues by LA-ICP-TOF-MS. Historical samples were obtained from non-metastatic ($n = 4$) and metastatic ($n = 11$) breast tissues, along with four from healthy patients, which had been formalin fixed and paraffin embedded. These were first assessed by the standard haematoxylin and eosin staining to identify key features in the tissues. Then adjacent microtome slices were deparaffined for analysis by LA-ICP-TOF-MS. The authors acknowledged that the process may impact the elemental distribution in the tissues, however this is the difficulty when using historical sample libraries which are focussed on histology. Gelatin calibration standards were prepared in-house containing Cu, Fe, Sr and Zn, with the concentrations confirmed by bulk analysis by ICP-MS/MS following MAD. After acquisition, *k*-means clustering was applied to mask any irrelevant background or tissue signals, *e.g.* from adipose tissue or holes, to improve the accuracy of the concentration estimation. This was checked against the haematoxylin and eosin staining by visual inspection. The LOQs were calculated as 16 ng g^{-1} for Cu, 58 ng g^{-1} for Fe, 11 ng g^{-1} for Sr and 83 ng g^{-1} for Zn which were impressive for direct solid sampling techniques. It should be noted that the gelatin was pretreated with an ion-exchange resin for cleanup before standard preparation. From the elemental imaging maps, it was possible to clearly identify the tissue features and structures, such as the tumour niche and stroma. Although the sample groups were limited in size, a number of trends were observed. In particular, the elements were found at significantly higher levels in the tumour niche areas compared to the stroma for the non-metastatic and metastatic groups compared to the healthy tissues. For Fe, it was significantly higher in the tumour areas of the metastatic cancer samples compared to the non-metastatic group. Although the work provides some useful insights into the distribution of key essential elements in breast cancer, the authors acknowledged that the limited sample size made it difficult to draw distinct conclusions. Colina-Vegas and co-workers[160] utilised LA-ICP-MS for the quantification of Pt in breast cancer cells to understand the distribution of cancer therapeutic drugs. In this work, the focus was on triple-negative breast cancer cells, specifically MDA-MB-231, which is a particularly aggressive and invasive form which does not contain receptors for oestrogen, progesterone or human epidermal growth factor 2 (HER2). The cells were incubated for 24 h with either carboplatin, cisplatin or a new Pt-phosphine clotrimazole complex (termed PtPCTZ). Prior to analysis, the cells were fixed with paraformaldehyde then dehydrated with EtOH. Calibration was achieved using gelatin droplet standards and interferometric measurements determined the ablation crater volume, allowing the absolute mass of Pt to be calculated. First, the level of Pt uptake was established using scLA-ICP-MS using a 20 μm spot size, finding the average concentration from 100 cells was 16.2 ± 4.6 fg Pt per cell for PtPCTZ, 74.0 ± 19.6 fg Pt per cell for cisplatin and 13.2 ± 4.2 fg Pt per cell for carboplatin. Next, the cellular distribution of Pt was assessed with a laser spot size of 2 μm, leading to clear differentiation between the cell nucleus, cytosol and background. The images showed the highest Pt levels in the cell nuclei which is in line with established knowledge that Pt forms irreversible DNA adducts leading to cytotoxicity and cell death. The new drug, PtPCTZ, demonstrated similar behaviour to cisplatin and carboplatin, showing significant potential in the fight against cancer.

A number of papers addressing *correction approaches and quality control for tissue imaging using LA-ICP-MS* were published. Kronenberg and co-workers[161] described the use of P as an IS to compensate for tissue artifacts such as folding, overlaps and density, which can be a significant factor when examining tumours from murine models, due to their small size. Mouse breast cancer models were used to test this theory, with *in vivo* analysis by MRI following administration of a gadolinium-based contrast agent, namely gadofosveset, along with *ex vivo* histological tissue staining and quantitative LA-ICP-MS imaging. The paper demonstrated the benefits of using P to correct for tissue aberrations for Gd images, as well as for endogenous elements such as Fe and Zn. Furthermore, the impact of immunotherapy could be observed from the presence of necrotic tissue from the T1 weighted MRI images, haemoxylin and eosin staining and the Gd imaging relative to P. Additionally, by calculating the ratio of the area of the necrotic to vital tissue in both MRI and Gd images, similar ratios were found which verified the Gd uptake relative to tissue density, demonstrating the impact of the therapy. Boger *et al.*[162] focused on the detection of intracellular Zn in parietal cells (stomach lining epithelial cells), combined with AFM to assess ablation efficiency between samples and gelatin calibration standards. Firstly, the preparation of the gelatin standards was optimized to minimise bubble formation and provide an even distribution of Zn across the droplet, concluding that 15% w/w gelatin, $ZnCl_2$ in water, droplet volume of 10 μL and 100 °C drying temperature provided sufficient homogeneity, stability and linearity. However, the ablation profile of gelatin drops was different to cells so AFM was used to characterise the ablation craters. It was found that 5 μm square spot size with the average taken from 9 laser shots provided comparable results. With the optimized approach, parietal cells incubated with $ZnCl_2$ were analysed and, in combination with bright field microscopy, it was possible to determine the Zn concentration in viable cells. This was validated by comparison to bulk ICP-MS analysis (following preparation by MAD). The use of AFM was useful to determine the optimum number of laser shots to balance the difference in performance between gelatin standards and real cells, which is not often considered.

Theiner *et al.*[163] described the use of a mixed gas for use in the CRC with LA-ICP-TOF-MS for imaging applications. The work systematically assessed the impact of the mixture 93% He and 7% H_2 compared to standard or no gas mode. Calibration standards with 48 elements present over two orders of magnitude were prepared using gelatin microdroplets (approximately 150–200 μm diameter) with an automated droplet dispenser. Additionally, a serum quality control material (Seronorm® Trace Elements Serum L-1) was also spotted directly on to glass slides to act as a sample matrix. Once the main ICP-TOF-MS

parameters were optimized, the laser spot size was then tested at 1, 2.5, 5 and 10 μm for the elements of interest relevant for serum, namely, Ca, Cu, Fe, Mg, Se and Zn, in both CRC mode and standard mode. The Seronorm spots were then analysed, the concentrations were calculated and compared to the expected values. Generally, the larger spot size performed better than the smaller ones in terms of precision and LOQ in both modes. However, in standard mode, neither ^{78}Se or ^{82}Se produced a linear calibration and the LOQ was insufficient to detect ^{44}Ca at any spot size. The authors focussed on the 10 μm spot size in the main paper (the SI provided all spot size data), with the recovery of the Seronorm® in CRC mode ranged between 89 and 129%. However, in standard mode, the recovery of ^{44}Ca was 45% and 235% for ^{56}Fe (noting that ^{57}Fe was 121%), whereas the recovery for the remaining elements was between 92 and 103%. However, this was to be expected given the presence of major interferences on these isotopes. The researchers went on to test higher mass elements, namely the lanthanides (often used for tagging for imaging with mass cytometry), platinum group elements (metallodrugs) and various other metals (Bi, Pb, Th, Tl and U). It was found that the CRC mode offered up to 2-fold improvement in sensitivity which is beneficial in imaging applications. Overall, the authors demonstrated the suitability of the automated gelatin drop dispenser for producing calibration standards and the use of a serum quality control material to support validation in imaging applications, alongside the use of the gas mix to resolve interferences and improve high mass sensitivity.

A number of papers have utilised *multiple techniques to elucidate or verify imaging data from tissue samples*. Sarretto and co-workers[164] combined LA-ICP-MS for elemental information with atmospheric pressure-MALDI-plasma post ionisation-MS (AP-MALDI-PPI-MS) for lipid identification. The systematic experiments, using mouse brain, demonstrated that the same tissue slice could be used for both instrumental techniques if the AP-MALDI-PPI-MS was performed first, using spray coated 2,5-dihydroxyacetophenone as the matrix. The elemental images were unaffected by the residual matrix and therefore were best when performed second. For data processing, the spectra from both instruments were aligned using FIJI, an open-source platform for biological image analysis based on the popular ImageJ software, and the Multi-Image Landmark Correspondences plugin to link the two datasets pixel to pixel. An in-house written MATLAB script was then used to calculate Pearson's correlation coefficients between the datasets on a pixel basis and were visualised using FIJI. The results showed that elements such as K, Mg, Mn, Na, and P were highly spatially correlated with lipid species such as hexosylceramide (used for glucosyl and galactosyl moieties), ceramides, phosphatidylethanolamines, lysophosphatidylethanolamine and phosphatidylserine. The authors noted the links with myelin-rich white matter and grey matter regions of the brain. The study demonstrated complementary analysis for lipids and elemental information from the same soft tissue slice for the first time. In an interesting combination, Frenzel *et al.*[165] used SXRF and IR spectroscopies for the characterisation of pancreatic cancer tumours. Mouse models were used with induced pancreatic ductal adenocarcinoma and subgroups were also treated with two levels of radiotherapy (2 and 6 Gy), which were all compared to controls. The pancreatic tissue was removed, fresh frozen and 10 μm sections were cryotomed and air dried. First, mid-IR spectra were obtained (3900–900 cm^{-1}) with a specialised setup, with a sample area of 345 μm^2. Then, SXRF was performed on the same tissue section using a beam size of 300 by 150 μm and Cl, K, P and S were quantified using the reference-free calibration approach which was based on fundamental physical parameters using the Sherman equation. Additionally, corrections for any deviations in the sample thickness were performed using the Si Kα fluorescence line (coming from the glass substrate). The FTIR data showed a number of lines consistent with amide bands from proteins, fatty acid bending vibrations and CN/NH bending from proteins. Within the amide bands, it was suggested different frequencies could imply the protein configuration or secondary structures. Differences between healthy, tumours and radiation treated samples could be observed when comparing the ratio of the amide I band with the amide II bands (following peak deconvolution). This also showed the impact of the irradiation treatment as the ratios returned to the same as the control samples but was elevated for the tumours. For the elemental levels, higher concentrations of K and S were seen in pancreatic tumours (46.3 ± 12.4 mg cm^{-3} and 22.9 ± 6.2 mg cm^{-3}, respectively) than in healthy samples (29.6 ± 7.6 mg cm^{-3} and 13.0 ± 3.3 mg cm^{-3}, respectively). Additionally, for the radiation treated samples, some areas with high levels of Cl were co-localised with lower levels of Cl and P, but the authors noted that the biological significance was unknown. However, overall, the study demonstrated the potential benefits of using multiple techniques to aid cancer diagnosis and determine the impact of treatment.

8 Applications: clinical and biological materials

8.1 Multielement applications

Several papers have been published reporting improved LODs for the analysis of multiple elements in whole blood, red blood cells, serum, urine, or exhaled breath. This enables the quantification of clinically relevant concentrations for diagnosis and also for biomonitoring across different biological matrices. For example, the novel development by Huber *et al.*[166] is a *fast-forward dilute-and-shoot multielement method using ICP-MS for the simultaneous analysis of 33 elements* (Ag, Al, As, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cu, Hg, I, Li, Mn, Mo, Ni, Pb, Pd, Pt, Sb, Se, Sn, Sr, Te, Th, Tl, U, V, W, Zn, and Zr) in whole blood, serum, and urine, with a streamlined, single instrumental and reagent setup for all three matrices. The method uses a fast-forward alkaline dilute-and-shoot approach, which includes Au (to stabilize Hg) and Triton X-100 in the diluent for all three matrices. The LODs generally ranged from sub-μg L^{-1} levels, *e.g.*, 0.003 μg L^{-1} (Pt) in whole blood and 0.001 μg L^{-1} (U) in serum. The method's performance was assessed by analysing RMs as internal QC (ClinChek® and Seronorm® whole blood

levels I, II and III, serum levels I and II and urine levels I and II) and participating in External Quality Assessment Schemes (33 elements in whole blood QM-B-Q1911, QM-B-1921, QM-B-Q2001, QM-B-Q2012; 33 elements in serum QM-S-Q1925, QM-S-Q2025, QM-S-Q2116, QM-S-Q2117 and 33 elements in urine QM-U-Q2106, QM-U-Q2114, QM-U-Q2123, QM-U-Q2124). Accuracy was satisfactory across all matrices, with deviations of ≤14% from assigned concentrations for most elements and sample materials, and, in serum, around or far below 10%. In whole blood and serum, minor over- or underestimations were noted for specific elements at certain levels in some controls; however, for clinical applications, a limitation was identified only for Al and Cr at low concentration levels, and the overall accuracy was deemed successful for clinical and biomonitoring purposes. For urine, performance was generally superior with ClinChek® materials—despite a slight underestimation of Al and Hg—compared to Seronorm® samples, where some deviations (*e.g.*, for Co, Hg, Ni and Zn) were observed. Performance in External Quality Assessment Schemes was satisfactory. Intermediate precision (RSDs) was generally below or far below the range 8–15% across all matrices. Short term precision was generally even lower, staying below 8% for most elements in whole blood and serum and below 10% for urine. A limitation was identified for Al and Cr when analysing low concentration levels in serum due to high background contamination from laboratory equipment and reagents, leading to high LODs. For urine analysis, the current data was not creatinine-adjusted, limiting its immediate use for reliable clinical interpretation.

Another study[167] described the *development and validation of an ICP-MS method for quantifying Cu, Mg and Zn in red blood cells*. The procedure involved aliquoting 20 μL of packed red blood cells and diluting them with 750 μL of an alkaline diluent solution (1% NH_4OH–0.1% Triton X-100–0.1% EDTA) with ^{45}Sc and ^{71}Ga added as ISs. The method validation successfully met criteria for accuracy (≤ ±15%), linearity ($r \geq 0.99$), short-term and intermediate precision (RSD ≤15%). Analysis of CRMs was not explicitly stated, but matrix-matched calibrators and quality controls prepared by spiking lysed red blood cells were used. The method was applied to a retrospective analysis of over 25 000 patient samples, stored in a biobank. Although these samples were not individually tested before for these elements, the results could be compared with the reference ranges for Cu, Mg, and Zn in red blood cells, from a reference laboratory to which the authors previously outsourced samples for analysis. However, the study was limited by its retrospective nature and lack of patient medical/nutritional history.

Medvedev *et al.*[168] presented a novel method for multielement analysis of urine samples using ICP-MS and ICP-OES, *incorporating sorption preconcentration with graphene oxide*. A significant development was the ability to perform group preconcentration and multielement analysis for a wide range of 21 analytes (Bi, Cd, Ce, Cu, Dy, Er, Eu, Gd, Ho, In, Lu, Nd, Pb, Pr, Sc, Sm, Tb, Tm, Y, Yb, Zn). The use of GO sorption preconcentration significantly improved the LODs for the analytes by 3 to 60-fold compared to direct ICP-MS and ICP-OES analysis of urine samples. Spike recoveries from 71 to 110% were achieved for most elements, except Cr, Hf, La, Nb, Sn, Ta, Ti, and Zr (6–56%), indicating the method was not universally applicable to the whole panel of elements studied. This methodology achieved high preconcentration factors and significantly improved the LODs of the analytes allowing for more effective biomonitoring and environmental monitoring to control metal content and human exposure.

An innovative method for the sensitive and simultaneous detection of 14 elements (Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Zn) in exhaled breath was described by Li *et al.*[169] The authors used a non-invasive handheld sampler which captured the exhaled breath at an optimized flow rate of 0.2 L min^{-1} through a bottle containing 6.0 mL of a 2% HNO_3 solution for 3 min, while an aspirator pump operating at a flow-rate of 0.1 L min^{-1} pumped the headspace gas out of the bottle and the aerosol metals solution was directly introduced into the ionization source of the ICP-MS. Calibration curves were prepared using standard concentration metal ion aerosols, with linear dynamic responses ranging from 1.0 to 500 ng L^{-1}, for all elements. Both Re and Rh were used as IS. The method demonstrated high analytical performance, achieving LODs for 14 metals between 0.046 and 0.134 ng L^{-1} and good precision (RSDs ranging from 1.18 to 11.93%). Recoveries of additions of the standard aerosols to exhaled breath samples were assessed and ranged between 94.81 and 116.90%. A relatively stable ratio (blood : breath ≈ 3.55 ± 0.23) was found for nine elements (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Zn) suggesting that there is a stable transfer of metals from blood to breath. While the method is novel and highly sensitive, the authors noted that further mechanistic investigation on the underlying changes of metals in breath related to physiological and pathological conditions was needed.

Huang *et al.*[170] developed *a high-throughput method that quantified 40 elements, including essential elements, PTE and REEs, in paired urine and serum samples*. The analysis was performed in 6 min by ICP-MS operated in KED collision mode. The procedure involved a simple direct dilution 1 + 9 of the sample with 1% HNO_3 and subsequent centrifugation to obtain the supernatant for analysis. Bismuth, Ge and Rh were used as IS. The LODs were as low as 2 ng L^{-1} for elements in urine and 20 ng L^{-1} in serum. Recovery rates for spiked (1.0, 5.0 and 40.0 μg L^{-1}) samples were between 80 and 115% for serum and ranged from 82 to 107% for urine, with short term and intermediate precision (RSDs) below 10%. The authors stated that the method was validated according to the US FDA guidelines for biological analysis method,[171] however, analysis of CRMs was not explicitly mentioned. The method was applied to paired urine and serum samples collected from 202 Chinese children. Due to the high number of elements included, the method required relatively higher sample volumes (1 mL urine or 0.35 mL serum) than other reported procedures.

Specimens analysed to investigate leakage of PTEs from metallic implants and biomaterials have featured in a dedicated section of our previous Updates. Due to technological improvements in this clinical area, papers addressing this issue have dramatically decreased. However, *potential metal accumulation in the brain, following metal implants, remains an area of investigation*. Ebner *et al.*[172] examined whether total joint

arthroplasty (TJA) was associated with increased metal accumulation in the brain and histopathologic changes related to Alzheimer's disease. They compared post-mortem brain metal concentrations in TJA patients *vs.* controls and correlated the results with Alzheimer's disease neuropathologic change scores. The analytes measured at ultra-trace levels (pg mL^{-1}) were Al, Co, Cr, Mn, Mo, Ni, Ti, V and Gd as the IS. The matrix was post-mortem frozen brain occipital lobe tissue subdivided into three portions from 177 subjects (88 TJA and 89 controls). The technique used was ICP-QQQ-MS. Sample pretreatment involved drying the tissue (60 to 100 mg per portion) overnight at 90 °C, acid digestion with 1 mL of HNO_3 for ultra trace elemental analysis on a heating block at 80 °C for 30 min. Upon complete digestion of the tissue, 0.5 mL of 30% H_2O_2 was added and placed on the heating block for 30 min. After cooling, 2.5 mL of ultrapure water was added to dilute to a final volume of 4.0 mL. Key findings revealed that TJA subjects had significantly increased brain concentrations of Co and Ti. Increased concentrations of Co, Mn, Mo and Ti were associated with increased odds of both neuritic and diffuse amyloid plaques in all subjects. The authors indicated that the US Clinical Laboratory Improvement Amendments's requirements for method validation were adopted. Although some metals commonly associated with Alzheimer's disease, such as Cu, Fe and Zn, were not assayed—which limits the ability to compare them with the measured cohort—the findings suggested that brain metal concentrations and inter-metal correlations may be altered in the presence of TJA and/or high neuritic plaques.

The development of a *rapid method for measuring actinides in human urine samples for the assessment of internal exposure doses during radiological and nuclear emergencies*, was presented by Wu.[173] The author proposed to measure the concentrations of ^{241}Am, ^{244}Cm, ^{239}Pu and ^{240}Pu, in 100 mL urine samples using ICP-MS/MS after optimizing the sample separation to achieve improved sensitivity and speed for a radiological emergency response. The urine sample (100 mL) was acidified with 5 mL concentrated HNO_3. The sample processing time was significantly shortened by combining a hydrous titanium oxide (HTiO) co-precipitation step with an extraction chromatography column with two tandem small-sized resin columns (TEVA + DGA-N). The sensitivity for the Am and Pu isotopes was improved by using helium and NH_3 as collision/reaction cell gases in MS/MS mode and it was found that pure NH_3 significantly improved the sensitivity for the Pu measurement. The method achieved very low LODs (1.12 fg for ^{239}Pu and 1.46 fg for ^{241}Am in 100 mL urine), meeting the sensitivity requirements for emergency bioassays. The main limitation was that the acid digestion and isotope equilibrium time may need to be prolonged if detoxifying agents (like DTPA) have been administered to the exposed worker, an aspect which was not studied here.

Integration of various types of omics data is currently an important trend in molecular oncology. Alekseeva *et al.*[174] *compared the profiles of trace elements and minerals with gene expression profiles in cancer tissue samples*. The study focused on 85 solid tumour samples, primarily colorectal cancer, and profiled 45 chemical elements and 36 596 known human genes. By integrating two high-throughput technologies—ICP-MS for element concentrations and RNA sequencing for gene and pathway analysis—in the same set of formalin-fixed paraffin embedded tumour tissue samples. This allowed for the identification of the genes that correlated with the concentrations of eight essential elements: Ca, Cu, Fe, K, Mg, Na, P and Zn. The authors stated that their work contributed to establish a baseline for an emerging field: the high-throughput analysis and multiomics integration of trace elements in cancer tissue. A key limit was the small size for the non-colorectal cancer cohort, which included only 18 samples across 16 solid cancer types, making robust conclusions challenging. The study primarily validated the associations by intersecting results from two independent colorectal cancer patient groups, but the overall patient cohort size (85 total) was modest for large-scale omics discoveries. Further studies were explicitly noted as being necessary to explore the functional implications of the novel associations.

8.2 Progresses for individual elements

8.2.1 Arsenic. *A continued interest in As speciation* was evident within this Update period. Oguri *et al.*[175] reported the application of HPLC-ICP-MS for As speciation in toenail clippings. Samples were prepared b8y MAD at 120 °C for 30 min, followed by evaporation to dryness and redissolution in water. Separation was achieved using a C18 column coupled to ICP-MS, optimized using standards for As^{III}, As^{V} (NMIJ 7912-a), AB (NMIJ 7901-a) and DMA^{V} (NMIJ 7913-a). The LODs were 0.040 μg g^{-1} for As^{III}, As^{V}, and AB, and 0.050 μg g^{-1} for MMA and DMA^{V}. Interestingly, despite the use of MAD, the authors report both As^{III} and As^{V} species in sample digests. Spike recoveries (using 10 ng As equivalent per g) ranged from 91 to 118%. Inorganic As was the predominant species (as As^{V}) in toenail samples, while organic As species concentrations were low, and detected in ≤ 3% of samples. Despite the analytical success of the study, the authors concluded that toenail clippings are in fact a poor biomarker for assessing long-term exposure to iAs. Arsenic speciation was also explored in drinking water and urine using SPME with nano-iron-coated fibres.[176] The authors highlighted the importance of controlling pH for efficient extraction and used a PRP-X100 anion-exchange column coupled to ICP-MS for separation of As species. Method validation was performed using CRMs (NIST SRM 2669, As species in frozen human urine; SRM 1643e, Trace elements in water). The analysis of SRM 2669 yielded results for As species in good agreement with the certified values: 99.8% for As^{V}, 93.6% for DMA^{V} and 85.9% for MMA^{V}. The As species present in SRM 1643e are not specified, but As^{V} was found by this study to represent 97.6% of the certified As concentration (60.45 μg L^{-1}, expanded uncertainty 0.72 μg L^{-1}, $k = 2$).

8.2.2 Bromine. *Nano- and micro-XRF imaging* were used to investigate Br distribution in bovine and human ovarian sections.[177] Micro-XRF measurements were carried out at the PUMA beamline of the SOLEIL synchrotron using an effective beam size of 5 μm × 10 μm, with a flux of 1.5×10^{10} photons per s. Samples were raster-scanned in continuous mode with a 10 μm step size at 15 keV. Complementary HR nano-XFM was

then also performed at the ESRF ID16B beamline, where a 17.5 keV beam with a photon flux of 5×10^{11} photons per s was focussed to 70 nm × 60 nm and used for raster scans. Due to a lack of suitable reference standards, these measurements were limited to qualitative investigations only. In these cases, Br was found to be widely distributed, with most prominent enrichment observed in regions surrounding blood vessels.

8.2.3 Cadmium. Advancements in Cd determination in urine and plasma involved the development of an *in situ IL-assisted microextraction procedure coupled with GFAAS*.[178] The method used 1-allyl-3-methylimidazolium bromide as the extraction solvent, with pH and ionic strength optimized to achieve an extraction efficiency of 97.80% by spike recovery. Cadmium was quantified at 228.8 nm, achieving an LOD of 0.35 ng L^{-1} and an LOQ of 1.17 ng L^{-1}, representing a notable improvement compared with previous reported approaches. Recoveries from spiked biological samples ranged from 91.2 to 97.6%, while short-term precisions (RSD) were < 3.7% for urine and < 4.2% for plasma.

8.2.4 Copper. Studies on Cu focussed on the development of *methodologies for serum speciation in relation to Wilson's disease and Alzheimer's disease*. Ott *et al.*[179] compared two approaches for measuring non-ceruloplasmin bound Cu (NCC) in samples from 76 Wilson's disease patients receiving D-penicillamine treatment. The first (NCC-Ex) involved exchangeable Cu determination, where serum was treated with EDTA for 1 h then ultrafiltered using Amicon Ultra-4 filters with a 30 kDa cut-off, followed by ICP-OES analysis of the ultrafiltrate. The second method (NCC-Sp) used HPLC to achieve copper–protein speciation by HPLC, using a MonoQ anion-exchange column. Although no LOD nor LOQ were reported, the measured ranges differed from earlier approaches to quantification (46–213 μg L^{-1} for HPLC speciation and 41–71 μg L^{-1} for ultrafiltration, compared to 50–150 μg L^{-1} by previously reported methodologies). Key findings showed that in patients' serum samples, 39% of the NCC-Sp values and 46% of the NCC-Ex results were below the target range (50–150 μg L^{-1}) while 58% of subjects had 24 h urinary Cu excretion values above the 200–500 μg 24 h^{-1} target. Despite this, no signs of Cu deficiency were found. The authors suggested that NCC targets should be lower than current recommendations and ideally methodology-specific to properly guide Wilson's disease management. Exchangeable Cu was also investigated by Squitti *et al.*[180] who instead used 100 kDa spin cut-off filters, achieving an LOD of 0.0397 μmol L^{-1} (2.5 μg L^{-1}). Validation with Seronorm® Trace Elements Serum L-1 and L-2 showed repeatability RSDs between 2.7 and 4.1% and within-laboratory RSDs ranging from 4.4 to 6.8%. Spiking serum from non Wilson's disease subjects with Cu ranging from 0.5 to 5.0 μmol L^{-1} yielded recoveries of 94.6 and 96.0%, though notably these concentrations are not representative of the serum levels in Wilson's disease' patients, which are usually an order of magnitude lower.

Harrington *et al.*[181] developed a new biomarker: accurate non-ceruloplasmin Cu (ANCC), calculated as total serum Cu minus ceruloplasmin-bound Cu. Using strong anion-exchange chromatography (TSKgel Q-STAT column), with gradient elution using TRIS buffer and CH_3COONH_4, hyphenated to ICP-MS/MS, Cu ($m/z = 63$ as $^{63}Cu^+$) and S ($m/z = 48$ as $^{32}S^{16}O^+$) were measured in oxygen reaction mode. The approach was validated using RMs (LGC8211 Frozen human serum – elements and selenomethionine; ERM-DA250a Creatinine and electrolytes in human serum), achieving a mean Cu recovery of 94.2%. Regression analysis of summed Cu species *vs.* total Cu in patient samples gave a slope of 0.964 ($r = 0.987$). Intra-day precision RSDs at 0.48 μmol L^{-1} and 3.20 μmol L^{-1} Cu were 5.2% and 5.6%, while intermediate precision RSDs at 0.80 μmol L^{-1} and 5.99 μmol L^{-1} Cu were 6.4% and 6.4%. This method achieved an LOD of 0.08 μmol L^{-1} (5.04 μg L^{-1}) and a LOQ of 0.27 μmol L^{-1} (17.01 μg L^{-1}). Its application to 71 patients within a Wilson's Disease Registry study indicated the potential of ANCC as a new biomarker.

Copper speciation by HPLC-ICP-MS was separately developed using a conjoint monolithic column comprised of two 0.1 mL capacity CIMmic α-HAS affinity disks and one 0.1 mL CIMmic diethylaminoethyl disk.[182] An initial gradient elution from 100% MOPS (50 mmol L^{-1}, Buffer A) to 50% NH_4Cl (2 mmol L^{-1}, Buffer B) retained Cu-albumin and separated Cu-ceruloplasmin and Cu-low molecular weight species onto the diethylaminoethyl disk. Subsequent isocratic elution of Cu-albumin used 100% CH_3COOH (0.5 mol L^{-1}, Buffer C) at a flow rate of 1 mL min^{-1} which was online introduced to the ICP-MS. Column recoveries ranged from 86 to 124%. Spike recoveries (10 ng mL^{-1}) were in the range 84–107% and analysis of RMs (Seronorm® Trace Element Serum L-2 and Trace Element Urine L-2) yielded results in good agreement with the reference values (from 96 to 107% and between 83 and 111%, respectively). The method was then applied to measure Cu in serum samples from 21 control subjects and 32 genetically confirmed Wilson's disease patients on stable chelation therapy and/or Zn supplementation.

8.2.5 Iron. Developments in Fe determination have *focussed on both single-cell quantification and stable isotope tracing*. Dejonghe *et al.*[81] applied scICP-MS to measure the Fe content in red blood cells, as discussed in Section 5.1 of this Update. Iron was measured on-mass ($m/z = 56$) using hydrogen in the CRC and levels were consistent over a three-week period in red blood cells from the same donors. Stable isotope approaches were also used to investigate Fe absorption. In another work exploring human Fe uptake, Barad *et al.*[93] used ^{57}Fe (as $^{57}FeSO_4$) mixed with syrup to calculate percentage Fe absorption (based on erythrocyte Fe enrichment) two weeks after dosing. Isotopes were quantified using SF TIMS and normalised to serum ferritin. Interestingly, the authors found serum ferritin-corrected Fe absorption was significantly higher in East Asian participants compared with those of Northern European origin.

8.2.6 Lead. Progress in Pb determination included the development of a *handheld micro-plasma optical emission spectroscopy (μPD-OES) device* coupled with a self-heating SPME function, integrating sample introduction, detection and data processing into a stand-alone battery powered unit.[97] The SPME fibres were inserted into sealed vials and TEL generated by injection of $NaBEt_4$ with stirring, leading to release of Pb for OES at 368.3 nm. The device's LOD was 1.2 μg L^{-1} and

recoveries for spiked urine ranging from 92 to 108% were reported. Analysis of two CRMs for Pb in freeze-dried human urine showed good agreement between measured and certified values for CNRM GBW09104 (106.3 ± 11.5 μg L^{-1} *vs.* 101.0 ± 12.0 μg L^{-1}, $n = 3$) and GBW09105 (304.6 ± 28.2 μg L^{-1} *vs.* 308.0 ± 32.0 μg L^{-1}, $n = 3$). Results of hair analysis were found to be comparable to those obtained by conventional ICP-MS (*e.g.* 1.20 ± 0.13 mg kg^{-1}, by the μPD-OES, *vs.* 1.27 ± 0.16 mg kg^{-1} by ICP-MS). The impact of common matrix elements (Ca, K, Mg, Na, Zn) showed no significant spectral interferences even at concentrations of 100 mg L^{-1} whereas Cd, Hg, and Sn affected Pb response above 2 mg L^{-1}.

8.2.7 Manganese. This year, a retrospective study explored the *association of Mn with non-alcoholic fatty liver disease* in human participants from the 2017–2018 US National Health and Nutrition Examination Survey.[183] Manganese concentrations were determined by ICP-MS in serum samples from 2708 individuals with non-alcoholic fatty liver disease and 1586 control subjects. The Mn levels in patients were found to be significantly elevated compared to the control group (9.33 μg L^{-1} *vs.* 9.06 μg L^{-1}, $p = 0.005$). Mn measurements below 0.990 μg L^{-1} were substituted by the LOD divided by $\sqrt{2}$, though the author's justification for this was not clear. Although the study noted use of DRC technology, no key instrumental parameters (such as gas composition and operating mode), nor formal determination of LOD and LOQ, were specified. Furthermore, the authors did not provide any information about sample collection (trace element tubes, cannulation, contamination control) prior to ICP-MS analysis.

8.2.8 Mercury. A method for the *rapid separation and determination of Hg species in blood* was reported, enabling improved differentiation of MeHg from iHg.[146] Species separation was achieved using a C8 Zorbax StableBond column coupled to ICP-MS/MS, operated in oxygen mode (40% O_2) although no justification for this mode over helium (KED) was provided. The mobile phase consisted of 0.06 mol L^{-1} CH_3COONH_4 with 0.05% mercaptoethanol, 0.4% L-cysteine and 5% MeOH (pH 6.7). Separation was completed in either 4 min for separation of MeHg from iHg or extended to 8 min to further separate EtHg. Incorporation of VG *via* an Elemental Scientific hydrideICP system significantly enhanced S/N ratios and lowered LODs. For LC-ICP-MS/MS alone, LODs were 0.45 μg L^{-1} for iHg, 0.42 μg L^{-1} for MeHg, and 0.84 μg L^{-1} for EtHg, which were reduced to 0.22, 0.23 and 0.33 μg L^{-1} respectively, when VG was included. Method performance was validated using NIST SRM 955c (Toxic metals in caprine blood) and SRM 955d (Toxic elements and metabolites in frozen human blood) and Hg species determined either with or without VG were within ±20% of the certified concentration or within its uncertainty (whichever the greater).

8.2.9 Plutonium. A novel *pseudo-ID strategy was proposed for the determination of Pu isotopes in urin*e without the use of regulated isotopic spikes.[184] The approach used ^{237}Np as a non-isotopic yield tracer to monitor and correct for chemical recovery during sample preparation, with measurements performed by SF-ICP-MS coupled to a high-efficiency sample introduction system to enhance sensitivity and suppress $^{238}UH^+$ interference on ^{239}Pu. The method involved two ICP-MS measurements, before and after standard addition of ^{237}Np, and calculation of Pu concentration was based on the variation in the ^{237}Np : Pu signal intensity ratio. Activity concentrations of ^{239}Pu, ^{240}Pu and ^{237}Np determined using this method were consistent with those obtained by conventional ID using ^{242}Pu, but without the restrictions associated with handling this isotope. Spiked urine samples containing theoretical ^{239}Pu concentrations of 0.028 pg mL^{-1} and 0.014 pg mL^{-1} gave short term precision RSDs of 4.3% and 7.4%, respectively, which were deemed comparable to those obtained by conventional ID (4.2% and 4.5%, respectively). While not performed in this study, the authors emphasised a requirement for future validation using CRMs and application to real urine samples.

8.2.10 Rubidium. *The impact of chronic kidney disease on whole blood Rb concentrations* was investigated in patients either undergoing haemodialysis (60) or pre-haemodialysis (60), and in a healthy control group (60).[185] Samples were prepared by MAD prior to ICP-MS analysis. Measurement accuracy was assessed using three levels of Seronorm® Trace Elements in Whole Blood, although the study did not report analytical performance data (LOD, LOQ, recovery or precision). Rubidium levels were significantly lower in both pre-dialysis patients (2023.51 μg L^{-1}) and those receiving haemodialysis (1188.75 μg L^{-1}) compared to the control cohort (2685.03 μg L^{-1}).

8.2.11 Selenium. Efforts during the period of this Update focussed on *Se speciation and strategies to improve quantitation*. A novel mass-shift approach for the determination of Se in serum using N_2O as a reaction gas to generate SeN^+ by N-atom transfer was discussed in Section 5.1.[83] This methodology achieved an LOD of 2.19 ng L^{-1} with spike recoveries ranging from 94.8 to 106%, using NIST SRM 1598a (Bovine serum), CNMR GBW09131 (Bovine serum) or GBW09141 (Bovine blood) matrices (spiked with 50 to 100 μg L^{-1} Se). Other work focused on total element quantification and parallel speciation. Vinceti *et al.*[186] quantified Se species in CSF samples from patients with amyotrophic lateral sclerosis using a NexSAR LC system fitted with an anion exchange column (AEX AG11 + AS-11, Dionex) coupled to a NexION 300D ICP-MS. Recoveries of single Se species spikes for Se^{IV}, Se^{VI}, SeMet, SeCys, thioredoxin reductase-bound Se, GPx-bound Se, SelP and SeAlb ranged from 89 to 102%, while recoveries from CSF samples were 97 ± 6%. Mass balance between the sum of quantified species and total Se measurements by SF-ICP-MS ranged from 91 to 103%. Rodriguez-Hernandez *et al.*[187] assessed the influence of genetic and non-genetic factors on total serum Se and Se speciation in a male cohort of 1624 car assembly workers in Spain. Se species (including GPx-bound Se, SelP, SeAlb and total selenometabolites) were quantified by HPLC-ICP-MS. Separation was achieved by SEC and affinity chromatography, and quantitation used post-column ID. The authors reported LODs and RSDs of: 0.30 μg L^{-1} and 6% for total serum Se; 0.5 μg L^{-1} and 10.25% for GPx-bound Se; 0.5 μg L^{-1} and 10.35% for SelP; 0.5 μg L^{-1} and 7.56% for SeAlb; and 0.5 μg L^{-1} and 9.98% for selenometabolites, respectively. The study highlighted inverse associations between smoking status and BMI with total serum Se, between

age, smoking status, BMI and meat intake with SeAlb, and between smoking status and selenometabolites.

8.2.12 Strontium. *A non-invasive method to quantify Sr in bone* was reported by Bickley *et al.*[119] A ^{109}Cd excitation source was used, which decays by electron capture to ^{109}Ag, emitting 88 keV γ-rays in approximately 4% of decays. ^{109}Ag emitted K X-rays at 22.2 keV and 24.9 keV, with relative abundances of 84% and 18%, respectively. The dominant excitation source were Ag X-rays, with γ-ray emissions considered negligible from the device. Calibration was performed using 3D-printed polylactic acid phantoms filled with Sr-doped hydroxyapatite, simulating a bone matrix. Under these conditions, a minimum detectable level of 22 μg Sr per g Ca was achieved for a 30 min measurement, with a corresponding skin dose of 1.1 mSv.

8.2.13 Thallium. Heitland *et al.*[188] reported the use of ICP-MS for *segmental hair analysis in a confirmed case of Tl poisoning*. The instrumental LOD was 0.1 ng L^{-1} and LOQ was 0.3 ng L^{-1} which, while greater than the general population Tl hair concentration, were sufficiently low to enable Tl quantitation in the victim's hair when sectioned into 0.3 cm samples (12–16 μg hair). Samples were prepared by washing with water and EtOH, followed by digestion in HNO_3 for 30 min at 90 °C. The method achieved an LOD of 0.008 μg Tl per g hair, with concentrations between 0.6 and 6.5 μg Tl per g hair measured, approximately two orders of magnitude higher than levels observed in the general population. Reference materials Spectrapure Standards SPS-SW1 and SPS-SW2 (Elements in Surface Waters) were used for QC, with deviations from the assigned reference values ranging from 3 to 10% and remaining below the maximum tolerated deviation of 12%. Notably, no hair CRMs were used.

8.2.14 Uranium. A comparative study of U determination in urine examined *the performance of alpha spectrometry vs. ICP-MS*.[189] Both techniques required substantive sample preparation prior to measurement. Alpha spectrometry achieved an LOD of 3.7 mBq L^{-1} per isotope from 1 L of urine, with samples counted for at least 42 h on high-resolution passivated implanted planar silicon detectors, and ^{237}Np identified as a potential interferent. ICP-MS analysis reduced the sample volume to 200 mL and achieved a minimum detectable concentration, which, in this context, is equivalent to a method LOD, of 1 ng L^{-1}. Check standards and QC samples were included, although their origin was not specified. Based on minimal detectable doses, ICP-MS with radiochemical separation was identified as the most sensitive approach for detecting occupational U exposure.

9 Applications: drugs and pharmaceuticals, traditional medicines and supplements

In this Update period, the focus of this topic was ICP-MS applications for the analysis of pharmaceuticals and dietary supplements, including those based on NPs. Single element techniques were not represented because ICP-MS provided simultaneous measurements and better LODs.

In a review paper Klika *et al.*[190] *examined 77 studies, reporting the use of ICP-MS-based assays, both in vitro* and *in vivo*, *in radiopharmaceutical science*. The authors discussed several non-radioactive assays including: the application of DOTA-PSMA-617 (labelled with Eu, Ga or Lu) in cancer research; the separation of metal based DOTA conjugates by HPLC-ICP-MS; the use of hybrid tracers labelled simultaneously with a dye (for fluorescent detection) as well as Ho (for determination by ICP-MS and LA-ICP-MS); and the quantification of $^{99}TcO_4^-$ in urine from patients who had previously undergone scintigraphy with a ^{99m}Tc-MDP radiotracer. The review also addressed the role of ICP-MS measurements in research describing the behaviour of individual cells toward metal-based targeting conjugates and the potential applications for the implementation of metal-based NPs in diagnostics and medical imaging. The authors concluded that ICP-MS emerged as a superior non-radioactive screening method to identify lead candidates for further investigation using radiochemistry. However, ICP-MS may not be applicable in all cases; for example, for fluorine-based probes, due to insufficient F ionisation in Ar based plasmas.

A study focusing on the *speciation of Gd complexes*[191] presented a selective and sensitive HPLC-ICP-MS method for the quantification of Gd multimeric complexes in a tetrameric, macrocyclic Gd-based MRI contrast agent. The separation was achieved on a RP UHPLC column, with phenyl-modified stationary phase as well as a gradient elution (mobile phase: ACN–HNO_3–H_2O), that was connected to ICP-QMS for the monitoring of ^{158}Gd and ^{160}Gd isotopes, and ^{159}Tb as an IS. The quantification was performed *via* an external calibration (0 to 5000 nmol L^{-1}) using gadobutrol as the standard substance. The method allowed for quantitative detection of six different multimeric Gd-containing by-products in a gadoquatrane sample, achieved LOQs of 38 nmol L^{-1} for monomeric, 19 nmol L^{-1} for dimeric, 13 nmol L^{-1} for trimeric, and 10 nmol L^{-1} for tetrameric Gd complexes. Thus, compared to UV detection, which is routinely used in the pharmaceutical industry, HPLC-ICP-MS offered a sensitivity increase of over 15-fold. Additionally, HPLC-ICP-MS allowed for quantification by compound independent calibration by detection of Gd, whereas in HPLC-UV, the species might have different response factors due to varying absorption behaviour.

Three publications addressed the determination of *elemental impurities in pharma products*. In a recent study,[192] a MAD method (500 mg sample, 1 mL HNO_3 and 1 mL of HCl) was developed to allow the interference-free determination of Ir, Os, Pd, Pt, Rh and Ru in five active pharmaceutical ingredients by ICP-QMS. Calibration solutions were prepared in 5% HCl for all metals, except Os for which unsatisfactory recovery (241%) was observed under these conditions. Thus, for Os a stabilising solution of 85 mmol L^{-1} CH_3COOH–10 mmol L^{-1} thiourea–0.6 mmol L^{-1} ascorbic acid was applied for both the digest dilution and the preparation of the calibration solutions. A novel aspect of this paper was the detailed study of carbon interference on the determination of the metals of interest based on the relative signal intensity of standard solutions at 1 μg L^{-1} monitored while increasing carbon concentration up to

2000 mg L^{-1}, in the form of citric acid. It was observed that increasing carbon concentration led to signal increase, with 800 mg L^{-1} being the critical concentration at which signal enhancement was observed for all analytes, except Os. The optimized procedure was validated by comparing its performance with the US Pharmacopoeia specifications.[193] Relevant parameters were working range between 0.5 and 10 μg L^{-1}, r^2 > 0.99, recoveries of spiked samples at three levels between 93 and 110%, RSDs < 13%, under repeatability conditions, and <15%, under intermediate precision conditions. Polyatomic interferences were also addressed by Shen *et al.*[194] They investigated elemental impurities in lanthanum carbonate raw materials and used isotope ratio analysis to overcome polyatomic interferences, confirm specificity and establish an accurate ICP-QMS method using helium collision mode for the determination of 24 elements (listed in ICH Q3D).[195] The isotope ratio $^{78}Se:{}^{82}Se$ was found to be much higher than the theoretical value, indicating that the response intensity at m/z 78 was affected by the presence of other species with the same m/z, such as $LaOH^{2+}$, formed by the large amount of La in the matrix. Therefore, the ^{82}Se isotope was chosen for analysis. The determination of the other 23 elements was not significantly influenced by any polyatomic interference. The validation results fulfilled criteria from the US pharmacopeia.

Nanomedicines, which consist of nanoparticles as active ingredients, are approved for pharmaceutical use. A newly developed AF4-ICP-MS methodology[196] allowed for the assessment of elemental impurities from ions and NPs of different sizes of the same element. It effectively separated Si ions from a mixture of Si ions and silica NPs. Separation of Fe ions from iron oxide NPs and iron hydroxide colloids converted from Fe ions was also successful. The method was applied to Fe determination in Resovist®, a nanomedicine composed of carboxydextran-coated iron oxide NPs. Results showed that 0.022% of Fe was present in ionic form and the active ingredient in Resovist® had a particle size smaller than 30 nm, and some aggregated particles exhibited a hydrodynamic diameter of approximately 50 nm. Particle size was estimated by AF4-ICP-MS and compared with 50 nm carboxydextran-coated iron oxide NPs. Analyses were also performed using spICP-MS and DLS to validate the estimated hydrodynamic diameter of the iron oxide NPs.

A method[197] was described for the *determination and characterisation of Se NPs in dietary supplements* in syrup formulations containing SeO_2 and ascorbic acid by spICP-MS. Adding 3.0% (v/v) IPA to the sample solutions increased the Se NP signal by 3.4-fold. The Se NPs were detected in all five dietary supplements studied, and it was found that from 19.3 to 60.4% of the total Se was in the form of NPs and that their mean diameters were in the range of 50–130 nm. The LODs for NP number concentration and for NP size were 770 particles mL^{-1} and 36 nm, respectively. The overall TE during the study was 7.3 ± 0.2%. For Se NPs with 75 nm diameter a significant increase of the background signal was observed when the concentration of ionic Se was higher than 4 μg L^{-1}, leading to an increase of the LOD for the NP size. In an experiment conducted in solutions of 140 nm Se NPs, spiked with amounts of ionic Se ranging from 0.50 to 20.0 ng mL^{-1}, no significant change was observed in the NP size and NP number concentration LODs.

10 Applications: foods and beverages

Following the trend seen in the last few years, the period covered by this Update has seen a large increase in papers focusing on chemometrics to identify origin and authenticity in a wide range of foods and traditional medicines. Often these papers describe a simple, unvalidated preparation and analysis method, with the focus of the work being involved in the processing of the obtained analytical data. A new feature is the growing number of studies being carried out on new ingredients, particularly on novel protein sources, such as plant-based food or alternative animal proteins such as insects. A new section covers the methods that have been developed in this area.

10.1 Progress for individual elements

10.1.1 Arsenic. *A novel method*[151] *for arsenic speciation* using anion exchange HPLC coupled with ICP-MS, was developed and validated. The procedure enabled the separation and quantification of As^{III}, As^{V}, DMA, and MMA across diverse food matrices. Extraction was performed using microwave-assisted and heat-assisted techniques with ultrapure water containing 1.5 mmol L^{-1} *N*-ethylmaleimide to prevent species interconversion and enhance recovery. Separation was achieved within 10 min using a step gradient of $(NH_4)_2CO_3$ buffers (0.5 and 50 mmol L^{-1}) with 3% MeOH at pH 9.3. Method validation followed the accuracy profile approach[152] which utilises accuracy and precision to generate a tolerance profile for the method. This gave an LOD of 1.88 μg kg^{-1} and an LOQ of 6.25 μg kg^{-1} for all species, with RSDs below 5.5% and bias under 3%. Certified RMs (NIST SRM 1568b, Rice flour and EC JRC IRMM BCR®-627, Forms of arsenic in tuna fish tissue) were also analysed alongside samples and yielded results in good agreement (between 90 and 103%) with the certified values. Overall, the relative expanded uncertainty (U%, $k = 2$) was evaluated to be within the range 2.7–11.8% for the four species studied. The method was successfully applied to samples from the Third French Total Diet Study, confirming its robustness and suitability for routine As speciation in food safety assessments.

10.1.2 Copper. The study by Huang *et al.*[198] focused on *LIBS applied to Tegillarca granosa (a widely consumed shellfish)*. Due to the inherent complexity of LIBS spectra, which contain thousands of wavelengths, a novel strategy was required to effectively screen out non-informative or redundant signals. Due to the minimal preparation required, LIBS was presented as a promising, rapid alternative to other techniques. Samples of *T. granosa* were intentionally contaminated with varying concentrations of $CuSO_4$ for the experiment and reference Cu concentrations were determined using MAD and ICP-MS giving concentrations of Cu in the 103 samples analysed ranging from 5.46 to 162.68 mg kg^{-1}. Before model calibration, LIBS spectra were subjected to preprocessing techniques, including Standard Normal Variate (SNV), moving smooth, and demean

centralization to enhance the S/N ratio and mitigate interferences. An unsupervised kernel minimum regularized covariance determinant (KMRCD) algorithm was proposed and investigated as an effective strategy for selecting informative variables from the complex LIBS spectra. This method was used to identify wavelength points that exhibited behaviour distinct from the majority of uninformative variables. The predictive ability of the model was compared using PLS regression, with the KMRCD-selected variables demonstrating superior performance. The KMRCD algorithm selected 50 variables, resulting in the best model performance with a determination coefficient of prediction (Rp^2) of 0.806 and a RMSEP of 16.496 mg kg^{-1}. This demonstrated that the KMRCD method is an accurate way to eliminate non-informative wavelengths, which is crucial for achieving high quantitative accuracy in LIBS analysis.

The isotopic composition of Cu in 29 human dietary sources was determined using MC-ICP-MS *to assess potential dietary influences on bodily Cu isotope ratios*.[199] Results were given as δ values, *i.e.* the parts per thousand (‰) difference between the $^{65}Cu : {}^{63}Cu$ ratio in the sample compared to that in the NIST SRM 976 (Isotopic Standard for Copper). The $\delta^{65}Cu$ values revealed systematic enrichment of the heavier isotope, ^{65}Cu, in legumes, nuts, and seeds, with $\delta^{65}Cu$ values ranging from 0.44 to 1.00‰, excluding highly processed items like cacao powder and coffee beans. In contrast, fruits and vegetables exhibited lower $\delta^{65}Cu$ values of 0.05 ± 0.21‰, and animal-derived products showed the widest range, from −0.32‰ in chicken egg to 0.89‰ in salmon. The lowest $\delta^{65}Cu$ value was observed in white wheat flour (−0.54‰), while tap water yielded −0.04‰. These findings suggested that individuals following plant-based diets may possess a distinct bodily Cu isotopic signature compared to omnivores, indicating that diet should be controlled for in biomedical studies involving Cu isotope fractionation. In addition, $\delta^{65}Cu$ values in seven matrix RMs were reported for the first time (EC JRC IRMM BCR®-184, bovine muscle; BCR®-189, wholemeal flour; BCR® 191, brown bread; BCR®-668, mussel tissue and BCR® 669, tuna muscle; NRCC DORM-5, fish protein and ANALYTIKA Co. Ltd, Prague, Czechia, Green Algae *Chlorella* sp).

10.1.3 Gadolinium. Černíková *et al.*[200] utilized MAD and ICP-MS to investigate *the presence and amount of anthropogenic Gd, an emerging REE contaminant*, in commonly consumed food products: wheat, spelt, and rye flour, rice, and carrots. Gadolinium from natural sources was assessed using the ratio of REEs in a geological reference standard (post-Archean Australian shale) and using the ratio of Sm and Tb to estimate natural Gd levels to allow Gd_{anth} to be assessed as a mass balance. The method was evaluated analysing CRMs (CNMR GBW 10052 Green Tea and GBW 07603 Bush Twigs and Leaves), which yielded results within 91 and 127% of the certified values, and RSDs < 8%. Researchers analysed a total of 223 samples sourced from various regions in Europe and Asia. A positive Gd_{anth}, which indicates an elevated concentration compared to naturally occurring levels, was confirmed in all tested food types. The median of Gd_{anth} levels present across all sample types ranged from 3.05 to 10.5 μg kg^{-1}. Wheat flour showed the highest median Gd_{anth} concentration (13.2 μg kg^{-1}) and the highest Gd_{anth} values, while rice exhibited the lowest median content of Gd_{anth}. The detection of Gd was attributed largely to the widespread use of Gd-based contrast agents in medical imaging, which are not effectively removed by wastewater treatment plants and subsequently enter the environment and, potentially, the food chain. The authors claimed the findings provided novel evidence of the presence of Gd in food and highlighted the need for continued monitoring of this contaminant in the food chain to address potential risks to human health and food safety. Interestingly a few months later Barrat and Bayon[201] questioned these findings. They argued that the REE data presented for flour and rice were likely erroneous because the resulting normalised REE patterns violated fundamental geochemical principles, such as the Oddo-Harkins effect. The patterns exhibited marked anomalies and unusual "sawtooth" enrichments not observed in natural materials, suggesting analytical problems. They concluded that the inference of anthropogenic Gd accumulation in food was not supported by sufficiently reliable data. The widespread use of normalised REE patterns was advocated as an essential quality control step for future food-related studies.

10.1.4 Iodine. An *under consumption of iodine, an essential element, from the diet may lead to health issues*, particularly relating to thyroid activity. In this study, a comparison of external calibration and ID methods for the ICP-MS determination of I in food was carried out. The team[202] studied 26 different samples of foods with high I content, such as seaweed, seafood and salted products (instant noodles and sausages). Samples underwent alkaline extraction in 1% TMAH prior to analysis. For the ID method, aliquots were spiked with the radioisotope ^{129}I, added either during (D) or post (P) extraction. No significant difference was seen between the D and P spiking methods. The ID-ICP-MS method achieved higher precision (RSD: 3.3% against 4.5%) and a lower LOD (0.01 mg kg^{-1}) than the external calibration approach (0.02 mg kg^{-1}), with both methods yielding strongly correlated results ($r^2 > 0.998$) and no significant differences across food types ($p > 0.970$). As a QC material NIST SRM 1869 (infant formula) was also analysed and returned results in good agreement with the certified value (100% using ID-ICP. MS and 102% with external calibration).

10.1.5 Selenium. *A method integrating biochemical separation and ICP-MS* was developed to quantify and speciate Se in tea samples.[203] Total Se content was determined following MAD with HNO_3 and H_2O_2 using optimized conditions. Selenium species were separated into organic and inorganic forms using AG1 × 4 resin, with Se^{IV} and Se^{VI} further isolated *via* Dowex 1 × 8 resin. Organic Se was fractionated into Se bound to tea polyphenols, tea polysaccharides, and proteins through sequential extraction and purification. Brewing conditions, including temperature, time, water type, and solid–liquid ratio, were shown to significantly influence Se release, with optimal extraction achieved at 100 °C and a 1 : 60 g mL^{-1} ratio. Recovery rates exceeded 95%, and total Se concentrations ranged from 0.126 to 0.132 mg kg^{-1}. The method enabled accurate determination of Se species and assessment of Se bioavailability in tea infusions, supporting the development of Se-enriched functional teas.

Table 2 Applications related to origin and authenticity of food and beverages

Analytes or data output	Matrix	Measurement technique	Statistical analysis	Purpose	Study aim, procedure and comments	Ref.
REEs (13) and $\delta^{13}C$, $\delta^{15}N$	Squid	ICP-MS, IRMS	Classification and regression Trees	Origin	Values for $\delta^{13}C$, $\delta^{15}N$ and Pr enabled successful discrimination of Mediterranean European, Atlantic European, Mediterranean, and Atlantic flying squid, achieving over 90% accuracy, 81% precision, 80% sensitivity, and 93% specificity	274
Spectral	Wines	TXRF	Gaussian and Non-Gaussian curves	Authenticity	Non-adulterated and adulterated wines were selected and their average effective atomic number (Zeff) were estimated. Deconvolution of independent Compton and Rayleigh signals was performed by non-Gaussian and Gaussian curve resolution methods, and the area ratio was evaluated. A calibration curve for Compton/Rayleigh signal ratio *versus* Zeff was established. Wine adulteration was detected in all cases	275
Spectral	Kimchi	LIBS	KNN	Origin	The geographical origin of Kimchi was established with a 92.8% accuracy. The intensities of Mg II (279 nm) and K I (766 nm) were the key markers due to regional variations of these elements in salt used in the preservation process	276
Spectral	Wine	EDXRF	PLS-DA, SIMCA	Origin	111 Red and white wines from Italy, Spain and Croatia were used for this study, with 102 of these from 20 different PDO regions. A PLS-DA model of the spectral patterns identified country, region and PDO with an accuracy of 88%	277
Spectral	Honey	XRF	RF, logical regression	Bee species	Honey samples from 3 different bee species (European honeybee, Philippine giant honeybee and Philippine stingless bee) were analysed using handheld XRF. The RF model could discriminate honeys from the three bee species with an accuracy of 85.2%, whereas the regression models were able to identify honey from stingless bees with 94.1% accuracy and 100% specificity	278

Table 2 (*Contd.*)

Analytes or data output	Matrix	Measurement technique	Statistical analysis	Purpose	Study aim, procedure and comments	Ref.
Spectral	Ginseng	LIBS, NIR	RF, SVM, stochastic gradient descent (SGD), SNV, sequential forward selection (SFS), multiple scattering correction (MSC), least absolute shrinkage and selection operator (LASSO)	Origin	The origin of ginseng was evaluated using LIBS and NIR, to collect data, and multiple statistical tools to model the data. Combining SNV, SFS and SVM provided the best model, achieving accuracy of 99%. Key indicators were Al, Ba, Cl, Cu, K, Mn and O	279
Spectral	Coffee	LIBS	KNN	Species, origin	South Korean roasted coffee beans were analysed by LIBS and data were evaluated by KNN. Emission bands for CN and C–C were identified along with peaks C, Ca, H, K, Li, Mg, Na, O and Rb with Li, Na and Rb being key indicators. The model gave a 98.5% accuracy	211
Spectral	Honey	LIBS	LDA, SVM, RF	Botanical origin	Honey (blossom and honeydew) from 8 different botanical sources were collected from different regions in Greece. Spectral data from the samples was gathered and underwent many permutations using statistical analysis. LDA was found to give prediction accuracies of 96% with Ca, K, Mg and Na being key indicators	280
Spectral	Pistachio	XRF	PCA	Origin	Ca, K and Rb were discriminant markers to establish the origin of pistachio nuts from PDO regions in Sicily	281
Spectral	Soybean	LIBS	Convolutional neural networks (CNN)	Origin	650 samples from 5 Chinese regions were used for this study. 12 key spectral features were identified for the models, which gave a high prediction rate of 99.1% in test samples, demonstrating CNN and LIBS as suitable tools for origin studies in soybeans	282
Spectral	Oregano	EDXRF	PLS-DA, SIMCA	Adulteration	Oregano is a prized herb which is often adulterated with olive leaves. Approximately 50% of 282 samples were deemed suspicious. 90% accuracy in prediction of adulterated samples was achieved, with Cu shown to be a key component to indicate olive leave contamination. SIMCA was able to separate unadulterated samples by geographical origin	283

Table 2 (*Contd.*)

Analytes or data output	Matrix	Measurement technique	Statistical analysis	Purpose	Study aim, procedure and comments	Ref.
Spectral	Olive oil	LIBS, fluorescence	SVM, ANN, PLS regression, RF	Adulteration	EVOOs were adulterated with other oils at ratios between 5 and 95%. LIBS showed better performance for the identification of EVOOs, whereas fluorescence was more efficient in detection of non-EVOOs. Combining data from both techniques, good accuracy was achieved with a 99% prediction	221
Various	Chinese Liquor Baijiu	ICP-MS, ICP-OES	PCA, hierarchical cluster analysis, OPLS-DA	Origin	Samples were diluted with water and analysed by ICP-MS or ICP-OES. The signal intensities were used to fingerprint the wines for use in the models. Al, Ca, K, Mg and Na were identified as the key components and allowed identification of liquors from 3 regions with 100% accuracy	284
Various (7), 10B : 11B and 87Sr : 86Sr	Apples	ICP-MS	ANOVA, PCA, LDA	Origin	Apples from Czechia and Poland were collected over 2 years for analysis. 64 samples were evaluated for a limited range of elements (Ca, Cu, Fe, K, Mg, P and Zn) and B and Sr IRs. Ca, Cu and P levels and the B IR were the biggest indicators of origin, but the models did not achieve a full discrimination	285
Various (8)	Rice	EDXRF	PCA-LDA, ANOVA	Origin	Rice from various regions in China were analysed by EDXRF. 8 elements (Cl, Cu, Fe, K, Mn, P, S and Zn) were key indicators. Classification of 96.6% was achieved	286
Various (10)	Onions	ICP-OES, IRMS	OPLS-DA	Origin	Fifty-eight onion samples from South Korea and China were analysed for element content (Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr and Zn) and $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ IRs. The resulting data underwent OPLS-DA. Only K, Na, $\delta^{34}S$ and Sr were identified as potential markers to classify the onions by country	287
Various (10)	Coffee	EDXRF	PCA, LDA, RF	Origin	Robusta coffee from 4 regions in Philippines were studied. The overall profile of elements was the same in all regions (K > P > Ca > S > Cl > Fe > Cu > Mn > Sr > Zn). Using LDA 79% classification accuracy was obtained, this rose to 84% with RF modelling	288

Table 2 (*Contd.*)

Analytes or data output	Matrix	Measurement technique	Statistical analysis	Purpose	Study aim, procedure and comments	Ref.
Various (20)	Lotus seeds	ICP-MS, UHPLC-TOF-QMS	PCA, PLS-DA, LDA	Origin	Lotus seeds from 7 different geographical origins were analysed for 20 elements and 323 metabolites. 10 minerals and 117 metabolites were identified as potential markers. LDA showed high prediction accuracy	289
Various (22)	Jingbai Pear	ICP-MS, IRMS	ANOVA, Bonferroni test, OPLS-DA, SIMCA	Origin	Highly valued Jingbai pears from 4 regions were studied for IRs (C, H, N, O) and elemental composition. Key markers were Al, Cd, Co, $\delta^{15}N$, $\delta^{18}O$, $\delta^{2}H$, K, Li, Mn, Na and Zn which gave the model an overall accuracy of 92.3%	290
Various (22)	Cockles	TXRF	LDA	Origin/safety	Cockles from 5 regions in Portugal, including one where farming is prohibited due to toxicity resulting from anthropogenic activities were analysed. Discrimination was achieved with 93.2% accuracy for training and 79.5% accuracy for the test datasets, with prohibited site samples showing 100% and 85.7% discrimination, respectively. Cr, Cu, Ni, Pb, and Zn were found to be below the recommended safe levels in all samples. Fe was exceeded in most samples and Mn was high in samples from the prohibited region	291
Various (23)	Wines	ICP-QQQ-MS	PCA, ANOVA	Production	Bioma wine production relies on alternative methods for sterilisation as opposed to the traditional SO2 use. A 90% variance was observed from the first 2 principal components, with As, Mn, Ni, Rb and Sr being key markers. The study showed that production method had a significant impact on elemental composition of wines	292
Various (25)	Rice	ICP-MS, IRMS	Low-level data fusion (LLDF), OPLS-DA	Origin	A total of 170 samples were collected from Thailand, Myanmar, China and Indonesia, including different rice varieties. Out of the elements and IRs ($\delta^{13}C$, $\delta^{2}H$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$) examined, $\delta^{18}O$ was the most powerful indicator of region and variety with OPLS-DA giving accuracies ranging from 90 to 100%	293

Table 2 (*Contd.*)

Analytes or data output	Matrix	Measurement technique	Statistical analysis	Purpose	Study aim, procedure and comments	Ref.
Various (26)	Green tea	ICP-MS, FTIR	PCA	Origin	Four varieties of tea from different regions in Vietnam underwent ICP-MS and FTIR analysis. Two principal components from FTIR and 14 elements accounted for 91.3% of the total variance. Two varieties of tea (Trung Du and hybrid F1) displayed proximate distribution, though still distinguishable. The authors suggested this closeness warranted further investigation	294
Various (27), $\delta^{18}O$ and $\delta^{13}C$	Wine	ICP-MS, IRMS	PCA, PLS-DA, RF, ANN, SVM and R-part	Origin	The aim of this study was to develop a tool to identify the origin of wine, independently of variety or grape type. In order of contribution, Sr, Mg, $\delta^{18}O$, Li, Co, K, Y, Cu, Th, Ce and Pb were the primary indicators of origin. RF was found to give 100% accuracy in prediction of region	295
Various (27) and 86Sr : 88Sr	Cheese (Graviera Naxos)	ICP-MS, MC-ICP-MS	Kernel density estimation (KDE), stable isotope mixing model (SIMM) *via* the Markov chain Monte Carlo (MCMC) algorithm	Origin	Milk, water, salt and rennet as well as the final products were studied to delineate the geochemical signature of Graviera Naxos cheese. A 73.1% contribution of the 86Sr : 88Sr ratio came from the salt used in the production of the cheese. The order of element content was Zn > Fe > Cu > Mn > Mo > Se > Cr, with up to a 25% variation on concentrations through the seasons	296
Various (36)	Asparagus	ICP-MS, LC-MS	RF	Origin, authenticity	Data for 36 elements and 816 metabolites were combined to evaluate the capability of these techniques to establish the origin of asparagus. Using ICP-MS data, a 88.2% accuracy was established, but it was noted that Polish samples often gave false positives when distinguishing German asparagus. Addition of metabolite data into the model improved accuracy to 92.3%	297

Table 2 (*Contd.*)

Analytes or data output	Matrix	Measurement technique	Statistical analysis	Purpose	Study aim, procedure and comments	Ref.
Various (37)	Beans	ICP-MS	Decision tree model	Origin	Gigantes Elefantes from the regions of Kastoria and Prespes are both covered by a PGI. The model displayed an accuracy of 92.86%, sensitivity of 87.50%, and specificity of 96.88%. The elements Ni, followed by Cu, U and Zn were shown to be the features with the highest significance	298
Various (47)	Almonds	ICP-MS (40), ICP-OES (7)	PCA, ANOVA	Origin	Highly valued "Mandorla di Avola" almonds were discriminated from almonds of other geographic provenances. Mn and P were the key indicators accounting for 78% of the total variance. This work was a small study with only 26 samples from 6 categories, but had promising results to support a PDO claim	299
Various (53)	Apple Juice	ICP-MS	SVM, PCA, RF	Origin	Apples from different areas in Germany, Europe and other areas of the world were tested for their elemental composition. Data analysis by SVM gave accuracy of 92.3% for discrimination among regions within Germany. Origin accuracies ranged from 83.2 to 92.3% when fruits from a single country were treated as a single class	300
Various (53)	Apple Juice	ICP-MS	RF, SVM, PCA	Cultivation, adulteration	German apple juices were studied to determine whether organic status could be confirmed by modelling. 16 elements, that had suitable concentrations for evaluation, were used for modelling. RF models achieved 94.2% accuracy in identifying organic juices. The method was able to show adulteration of conventionally produced juice at a level of >20%	301

Table 2 (*Contd.*)

Analytes or data output	Matrix	Measurement technique	Statistical analysis	Purpose	Study aim, procedure and comments	Ref.
Various (53)	Hazelnuts	LA-ICP-MS, ICP-MS	SVM	Origin	Hazelnuts from 5 different countries underwent MAD (500 mg lyophilised sample, 6 mL HNO3, 2 mL H2O2) followed by solution nebulisation ICP-MS. SVM yielded a 92.1% prediction accuracy. To eliminate sample preparation, the method was transferred to LA-ICP-MS which yielded better results allowing for a 100% prediction accuracy	302
Various (60)	Garlic	ICP-MS, ICP-OES	OPLS-DA, classical discriminatory analysis (CDA)	Origin	Garlic samples from China and Korea were analysed in this study. 25 elements (5 ICP-OES and 20 ICP-MS) were shown to have significant differences. The generated heatmap was able to identify the origin with 100% accuracy	303

A mixed-mode RP cation exchange HPLC-ICP-MS method[204] was developed and optimized to separate and quantify Se species in Pleurotus mushrooms enriched with Se^{IV} or Se^{VI}. Methylselenocysteine, Se^{IV}, Se^{VI}, $SeCys_2$ and SeMet were chosen as typical Se species found in mushrooms. Under the optimized conditions, 20 mg of sample underwent enzymatic digestion using UAE with pepsin and protease XIV in Tris–HCl buffer, achieving Se extraction efficiencies between 67 and 102%. Chromatographic separation was completed within 6.5 min under isocratic conditions using a mobile phase of ACN and H_3PO_4 at pH 1.8. The method LOQ was found to be 248 to 337 ng g^{-1} and spiked recovery tests showed an accuracy between 86 and 112%. It was demonstrated that Se^{IV} was more effectively bio-converted into SeMet, supporting Se^{IV} as a preferable supplementation strategy for nutritional enhancement of edible mushrooms, while Se^{VI}-supplemented mushrooms retained higher levels of iSe, raising safety concerns.

A green analytical method was developed by Zhang *et al.*[40] for *determining Se in organically grown rice using deep eutectic solvents coupled with UAE and ICP-MS.* Four DESs were synthesized *via* mixture design using combinations of choline chloride, citric acid, fructose, guanidine hydrochloride, glycerol, proline, and water, and characterised by FTIR and ^{1}H-NMR spectroscopy. Their density and viscosity were optimized to enhance extraction efficiency. With these extraction procedures, Se recovery rates ranging from 85.5% to 106.7% were achieved. The results were validated by comparison with MAD with HNO_3 and H_2O_2. The method demonstrated effective Se extraction while aligning with green chemistry principles, offering a sustainable alternative for trace element analysis in food matrices.

10.2 Single and multielement applications in food and beverages

10.2.1 Human milk and infant formula. Concentrations of As and 17 metals were measured simultaneously, with a validated ICP-MS method, *in samples of human biological fluids, including human milk*, collected from Bac Giang, Vietnam.[205] Sample digestion was performed using MAD on a 500 mg aliquot of sample with 6 mL HNO_3, 2 mL HCl and 2 mL H_2O_2. Method performance, evaluated as LOQ, linearity assessments and spiked recovery studies, confirmed satisfactory linearity ($r >$ 0.9999), LODs, recovery (95–109%), and measurement uncertainty (RSD <1%). Concentration levels varied across matrices, with Ca, Mg, and Zn found in highest abundance. Significant inter-element correlations were observed, suggesting potential shared bioaccumulation or metabolic pathways. The inclusion of human milk provided novel insights into maternal and infant exposure, and the study offered a robust analytical framework for future biomonitoring in regions with limited environmental health data.

Iwai *et al.*[206] addressed *the speciation of Hg in human milk*, by developing a high-throughput HPLC-ICP-MS method, enabling the simultaneous quantification of Hg^{2+} and $MeHg^+$. Human milk samples were pretreated with TMAH with 100 μL of milk being added to 3.9 mL of the TMAH solution. Samples were then sonicated, and ultrafiltered to extract Hg species while minimizing protein and fat interference. Separation was achieved within 300 s using a metal-free C18 column and a mobile phase containing NH_4HCO_3, 2,3-dimercapto-1-propanesulfonic acid, MeOH, and TMAH. The method was validated using the CRM NIST SRM 1953 (Human milk), yielding LODs of 1.9 pg g^{-1} for Hg^{2+} and 14 pg g^{-1} for $MeHg^+$, 99% accuracy and precision of 6.2%. Median concentrations across 80 samples were 0.109 ng g^{-1} for Hg^{2+} and 0.220 ng g^{-1} for $MeHg^+$. The total Hg values obtained *via* HPLC-ICP-MS were strongly correlated with those from ICP-MS ($r^2 = 0.895$). Associations were observed between Hg^{2+} and Se, and between $MeHg^+$ and milk fat, with higher $MeHg^+$ concentrations found in hindmilk.

10.2.2 Dairy products. *A novel method was developed for Ca elemental and isotopic analysis using laser-ablation molecular isotopic spectrometry, based on the formation of CaF molecules in a methyl fluoride-argon gas mixture*. The CaF diatomic molecule was generated in the plasma plume upon ablation of dried liquid samples, and the vibronic transition $X^2\Sigma^+ \rightarrow A^2\Pi$ (0,1) at 583.0 nm was used to resolve isotopic shifts, calculated at 292.3 pm. Quantitative Ca determination was performed in tap water and skimmed milk using internal standardization with Sr and ID, respectively. The PLS technique was applied to improve isotopic data quality. The Ca concentrations found were 47 ± 16 mg L^{-1} in tap water and 1100 ± 140 mg L^{-1} in skimmed milk, showing no significant difference from FAAS reference values. This approach enabled simultaneous acquisition of elemental and isotopic information for Ca, expanding LIBS applications to tracer studies and ID calibration but the authors[101] noted that the method was not yet sufficiently sensitive for natural Ca isotope ratios.

A novel method[207] for *determining total Cl using high-resolution continuum source graphite furnace molecular absorption spectrometry via* the formation of BaCl in an in-furnace reaction was presented. In this approach, 200 μg of Ba as a 5 μL injection of a 40 g L^{-1} solution was added to a 10 μL aliquot of standards or samples, and molecular absorption was measured at 513.894 nm using a zirconium-coated graphite tube with optimized pyrolysis and vaporisation temperatures of 1100 °C and 2300 °C, respectively. The LOD and characteristic mass were found to be 1.1 ng and 0.6 ng of Cl. The method was validated using a wastewater CRM (Spectrapure Standards, SPS-NUTR-WW2, waste water) which gave an accuracy of 95%. The method was then applied to milk samples, yielding Cl concentrations between 556 and 783 mg L^{-1} with repeatability below 10%. The BaCl molecular absorption peak was confirmed to be free from spectral interferences, and calibration using aqueous standards was shown to be accurate, demonstrating the method's suitability for Cl quantification in complex matrices.

Ultrasound-assisted extraction was applied to Brazilian organic dairy products including cheese, milk and yogurt, prior to the determination of essential minerals by FAAS.[41] Sample preparation conditions were optimized through a face-centred experimental design, varying acid volume and ultrasonic bath temperature. Flame AAS was used for the quantification of Ca, K, Mg, Na and Zn, and confocal microscopy was applied to assess organic matter degradation. The UAE procedure

demonstrated comparable accuracy to traditional wet mineralization, with recovery rates ranging from 82 to 109% and precision values below 10%. A CRM (NIST 1549 non-fat milk powder) was used to confirm method reliability, and residual carbon analysis supported effective mineralization. Overall, UAE was shown to be a time-efficient, eco-friendly alternative for mineral analysis in organic dairy matrices.

10.2.3 Cereals. *Microwave induced plasma-AES is a relatively new technique for elemental analysis*, utilising a nitrogen plasma.[208] Samples of cereals and pulses were prepared using MAD (0.25 g sample, 12 mL H_2O, 2 mL HNO_3, 2 mL H_2O_2) and analysed for Al, Ca, Cu, Fe, K, Mg, Mn, P and Zn by MIP-AES. Various CRMs were used to validate the method namely, NRCC DUWF-1 (durum wheat), NRCC BRAN-1 (corn bran), NRCC KINO-1 (quinoa), NIST SRM 1568b (rice), and NIST SRM 3234 (soy) both as spiked and unspiked samples. Good recoveries for both the CRMs (93–120%) and spiked samples (91–115%) were obtained, showing the method to be suitable. A series of spiked recovery studies were carried out on amaranth, barley, fava, lentil, lupin, maize and pea, which showed good recoveries (86–115%) and precisions typically <10%. Indications were that MIP-AES may be a useful and cost-effective tool for analysis of cereals and legumes for some nutritional elements, particularly in regions where access to argon for conventional ICP techniques may be restricted.

An improved method[116] for simultaneously *quantifying As and Pb in grains was developed using a portable monochromatic excitation EDXRF instrument*. Enhanced sensitivity and reduced spectral interference were achieved using a DCC, optimized geometric layout, and algorithmic correction models and a measurement time of 300 s. Detection parameters such as particle size, detection time, moisture content, and tube current were systematically optimized, and matrix effects were addressed using fundamental parameter algorithms. The method was validated against CRMs (GBW(E)100 348 – rice flour, GBW(E)100 377 – brown rice flour, GBW(E)100 380 – corn flour, GBW(E)100 493 – wheat flour) and compared with ICP-MS, showing strong agreement with an r^2 of 0.988, accuracies between 95 and 104% and acceptable precision of <10% RSD. The LODs were determined to be 0.02 mg kg^{-1} (As) and 0.03 mg kg^{-1} (Pb), confirming the method's suitability for routine, non-destructive analysis of toxic elements in grains.

10.2.4 Vegetables, fruit, mushrooms and nuts. Wu *et al.*[155] applied *ion-pairing RP chromatography coupled with ICP-MS to improve the sensitivity* of the simultaneous determination of As and Se species in American ginseng. Enzyme-assisted extraction with proteinase E was optimized to enhance analyte recovery, followed by ultrasonic treatment and filtration. The mobile phase was composed of citric acid, MeOH and sodium 1-hexanesulfonate as an ion-pairing agent at pH 4.0 and an Agilent ZORBAX SB-Aq column was used. Eleven species were identified and quantified (As^{III}, As^{V}, AB, DMA, MMA, ASA, MeSeCys, Se^{IV}, Se^{VI}, $SeCys_2$, and SeMet). The method was validated using spiked ginseng samples which gave recoveries between 72 and 107% and precision from 2.8 to 11.1%. The LODs ranged from 0.058 to 0.6 μg kg^{-1} for As species and from 0.083 to 0.283 μg kg^{-1} for Se species. In American ginseng samples, As^{V} and SeMet were found to be the predominant species. The method was validated according to EURACHEM[156] and EU guidelines, demonstrating high precision, recovery, and stability, and was shown to be effective for safety assessment and quality control of functional foods.

The content of essential and potentially toxic elements in 25 Brazilian paprika (*Capsicum annuum* L.) samples was assessed using *a novel, sustainable analytical method*.[70] The concentrations of 11 elements, namely Ba, Ca, Cu, K, Mg, Mn, Na, P, Sr, V, and Zn, were measured by ICP-OES, following closed-block digestion with 2 mL HNO_3–2 mL H_2O_2–4 mL deionized water. The digestion protocol was optimized to minimize dissolved organic carbon and residual acidity. Method accuracy was validated using 3 CRMs (CRM-Agro C1005a, sugarcane leaves, CRM-Agro C1003a, tomato leaves and NIST SRM 1515 apple leaves), showing agreement between 80 and 105% with the certified values. Statistical techniques (PCA and Hierarchical Cluster Analysis) were applied to explore mineral composition patterns across sweet, spicy, and smoked paprika types, revealing no distinct grouping. Potassium and Na were found to be the most abundant elements, followed by Ca, Mg and P. The method was aligned with Green Analytical Chemistry principles, achieving an AGREE (analytical greenness) score of 0.63, and demonstrated reliability, environmental friendliness, and applicability for multielement food analysis.

Cocoa shell is a natural and deliberate contaminant in cocoa, which affects sensory perception and can cause issues during manufacture of cocoa products. A method for determining cocoa shell content in cocoa products was developed using ICP-MS combined with chemometric modelling.[209] Pure samples were obtained from 15 different South American and African countries and seven different harvests. Samples were roasted and ground under controlled conditions then adulterated with shell up to 10% w/w in 1% increments. The samples then underwent MAD (6 mL HNO_3–2 mL H_2O_2) followed by ICP-MS analysis covering a suite of 54 elements. Twenty elements (Ba, Ca, Cd, Co, Cr, Cu, Eu, Fe, Ga, La, Mn, Mo, Na, Ni, Rb, Sr, Th, Ti, Y, and Zn) were identified as suitable indicators for shell content quantification. A SVR model was constructed, achieving a RMSE of 1.11% and an r^2 of 0.877. Sensory analysis confirmed that shell content above 10% is perceptible, expanding the analytical range to below the current level achievable with sensory analysis. The use of beans from varied origins and harvests demonstrated the robustness and transferability of this analytical strategy. This approach offers a precise, scalable solution for monitoring shell content and mitigating food fraud risks in cocoa-based goods.

A MAD method[210] *for beans, basil, and mint, using very dilute HNO_3 (1 mol L^{-1}) and H_2O_2*, was optimized through factorial design, where digestion efficiency was evaluated by determining the residual carbon content. The selected conditions (0.5 g sample, 8 mL HNO_3, 2.5 mL H_2O_2, and 20 min at 180 °C), enabled effective decomposition of organic matrices while reducing acid consumption by a factor of 10–14 compared to conventional protocols. The method aligned with green chemistry principles by minimizing hazardous waste and improving analyst safety. Validation demonstrated satisfactory linearity (r

> 0.995), low LOQs (0.073–19 mg kg^{-1}), and acceptable precision with typical RSDs <6% for the determination of elements Ca, Cd, Co, Cu, Mg, Mn, Mo, Na, P, Pb, S, V, and Zn by ICP-OES, and K and Na by FAES. Accuracy was confirmed using plant material CRMs, with most analytes showing statistical agreement with certified values. The procedure was applied to real samples, revealing mineral concentrations within regulatory limits for toxic elements and highlighting discrepancies between measured values and those declared on food packaging.

10.2.5 Fish and seafood. A tetra-elemental speciation method[158] was developed for the *simultaneous quantification of Cd, Hg, Pb, and Sn compounds in shrimp and fish* using HPLC-ICP-MS. Twelve chemical species of Cd (Cd^{II}), Hg (Hg^{II}, EtHg, MeHg), Pb (Pb^{II}, TEL and TML) and Sn (Sn^{II}, TBT, TET, TMT and TPhT), respectively, were separated within 24 min using an optimized mobile phase of sodium dodecyl benzene sulfonate and L-cysteine at pH 2.5 and a amphion II column. The LODs ranged from 0.2 to 2.5 μg kg^{-1}, and precision was maintained below 4.3%. In shrimp and fish samples, Cd^{II} was detected only in shrimp (0.043 mg kg^{-1}), while MeHg was found in both matrices (0.8–4.6 μg kg^{-1}); all targeted Pb and Sn compounds were absent. The method was validated through two CRMs (CNMR GBW10068, Oyster Tissue and GBW10029, fish), spike recovery tests (90–109%) and comparison with previous mono-elemental methods, demonstrating high analytical efficiency, low cost, and environmental friendliness.

10.2.6 Drinking water and non-alcoholic beverages. *A classification method for roasted coffee beans was developed using LIBS combined with a novel variable selection approach and the KNN algorithm*.[211] Twelve commercial coffee samples from various geographic origins and cultivars were analysed, and emission lines corresponding to atomic or ionic species, Ca, C, H, K, Li, Mg, Na, O, and Rb, as well as molecular bands of C_2 and CN, were identified. Among these, the emission intensities of Li I (670.8 nm), Na I (589.0 nm), and Rb I (780.0 nm) were selected as key discriminatory variables based on their high inter-to-intraclass variation ratios. A 3-dimensional KNN model using these three variables achieved a classification accuracy of 96.0% at $k = 1$, which was further improved to 98.5% with SD-based scaling at $k = 3$. The results demonstrated that LIBS, leveraging its sensitivity to alkali metals, can be effectively applied for rapid and accurate classification of coffee products with minimal sample preparation.

The multielement analysis of ready-to-drink teas was achieved using a dilute-and-shoot ICP-MS method,[212] enabling direct quantification of 13 elements (Al, As, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Sr, and Zn) without digestion. Tea samples were diluted 20-fold in a HNO_3–HCl–EtOH solution to reduce matrix effects, particularly those for carbon originating from sweeteners and additives, by use of matrix overcompensation calibration, with EtOH serving as the carbon source. A universal calibration curve was constructed and applied across diverse ready-to-drink tea types, regardless of fermentation level or additive content. The method was validated against a procedure based on MAD and standard addition calibration, showing comparable accuracy and recovery rates from spiked samples (typically from 90 to 110% except for Al) in the majority of cases, although some elements, such as Cu and Zn, did show significant differences between the methods. The LOQs for toxic elements were found to be suitable for regulatory monitoring, and the approach offered significant advantages in cost, throughput, and environmental sustainability.

10.2.7 Alcoholic beverages. A comprehensive investigation into the *migration of chemical elements from soil to wine* in thirty monovarietal Bulgarian wines was studied by Mladenova *et al.*[213] Twenty elements were analysed across soil extracts, vine leaves, must, and raw wine. Statistically significant correlations were established to illuminate the complex pathways of elemental uptake and transformation. Insight into bioavailable fractions was provided through the use of CH_3COOH and EDTA soil extracts, while precise quantification was ensured by the application of FAAS, ICP-OES, and ICP-MS. Lead and Zn were identified as reliable indicators of geographical origin, with Zn showing strong correlations in red grape varieties across soil and must. Elemental decreases observed from must to wine were attributed to the impact of vinification processes, including enzymatic activity and clarification treatments. The authors noted that while soil provides the elemental foundation, uptake is regulated by plant-specific mechanisms and environmental conditions. It was suggested that certain elements—particularly Ba, Sr, and Zn—can serve as descriptors for both geographical and botanical origin, although varietal differences influence correlation strength. Overall, valuable methodology for wine authentication and origin tracing was contributed, reinforcing the importance of mineral analysis in quality control and terroir characterisation.

A semi-quantitative method using ICP-MS was applied to determine 41 elements in wine.[214] *A mineral wine profile for origin and variety authentication was developed and tested*. Over 12 000 international wine samples were analysed, using a simple dilution procedure whereby samples were initially diluted 1 + 2 with 1% HNO_3, to stabilise the samples, followed by a further 1 + 4 dilution prior to analysis. Concentrations of elements including Al, As, B, Ba, Ca, Cd, Ce, Cl, Co, Cr, Cs, Cu, Fe, I, K, La, Li, Mg, Mn, Mo, Na, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Ti, Tl, U, V, Y, Zn, and Zr were determined. These mineral wine profiles were used to train machine learning models, with eXtreme Gradient Boosting (XGB) selected for its superior performance. Classification accuracies of 92% for country, 91% for French wine region, and 85% for grape variety were achieved, with corresponding mean area under the receiver operating curve (AUC) scores of 0.964, 0.961, and 0.914. The study demonstrated that elemental composition, particularly REE correlations, can be reliably used for wine authentication, enabling high-specificity origin prediction through artificial intelligence.

10.2.8 Honeys. To investigate the origin of Polish honeys Gręda *et al.*,[215] devised a *simplified sample preparation enabling the reliable quantification of 18 elements by ICP-MS* without wet digestion. The method was optimized and compared to digested samples. The optimal dilution (1% aqueous honey solutions) gave results comparable to the digested samples, with no statistical difference seen between the two sample

preparation methods using a paired *t*-test. This approach allowed 120 honey samples from Lower Silesia, Poland, to be profiled efficiently. Elemental concentrations, particularly of Cu (average content of 315 μg kg^{-1}) and Mn (average content of 3.4 mg kg^{-1}), could be used to discriminate honey by colour, botanical origin, and geographical source. Notably, dark honeys contained higher levels of most elements (except B and Tl), mountain honeys showed elevated Cs (4.8 μg kg^{-1}) and Rb (1.4 mg kg^{-1}), and specific floral types like buckwheat and honeydew were distinguished by high Cu and Mn content. The method achieved LODs up to three times lower than those of microwave digested samples, with LODs as low as 0.001 μg L^{-1} for elements like Cd, Cs and Li and demonstrated stable performance over extended measurement sessions.

To assess the macro element content of cocoa honey, Cavalcante *et al.*[43] optimized an UAE method using Doehlert experimental design with multiple responses. The optimal conditions for the extraction of Ca, K, Mg and P were a sample volume of 1.5 mL added to the extracting solution of 4.0 mL of 5.62 mol L^{-1} HNO_3 and 1.15 mL of H_2O_2, and 29.6 min of sonication. The analysis was carried out using MIP-OES. The procedure was validated by comparison of the results with those obtained for the same samples with MAD and ICP-OES. No significant differences were observed between the extraction and digestion procedures, with good agreement between the two methods when assessed using a *t*-test. Good sample repeatability for the UAE method giving RSDs of < 1% was also established. Eleven cocoa honey samples from Ilhéus, Brazil, were analysed, revealing high concentrations of K (734.4–1001.9 mg L^{-1}), along with substantial levels of Ca, Mg and P. The method was demonstrated to be fast, cost-effective, and environmentally friendly, offering reliable results for nutritional evaluation of cocoa honey.

10.2.9 Alternative protein sources. A sample preparation method based on MIC was developed[216] and validated for the *determination of potentially toxic elements (As, Cd and Pb) in edible insects using ICP-MS.* Sample digestion was optimized by evaluating variables such as sample mass (100–600 mg), HNO_3 concentration (3–14.4 mol L^{-1}), and irradiation time (5–15 min). Optimal conditions were established as 500 mg of sample, 7 mol L^{-1} HNO_3, and 5 min of irradiation. Certified RMs (NRCC BFLY-1, Black Soldier Fly meal; KRIK-1, Cricket flour; and VORM-1, Mealworm powder) were analysed, and results showed no statistical difference from certified values. The achieved LOQs were 0.006 μg g^{-1} (As), 0.003 μg g^{-1} (Cd), and 0.012 μg g^{-1} (Pb). The MIC approach demonstrated comparable accuracy to MAD whilst achieving lower carbon content in the final solutions, thus reducing interference risks in ICP-MS analysis. The method was successfully applied to various insect species, confirming its robustness and suitability for routine monitoring of toxic elements in insect-based foods.

Fourteen trace metals and metalloids (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, and Zn) were *quantified* in *Cornu aspersum* and *Eobania vermiculata land snails* from rural and urban areas of Sicily.[217] Samples underwent a simple MAD (1 g sample, 3 mL HNO_3) with most elements being quantified using ICP-MS, while Hg was measured using a dedicated mercury analyser applying AAS on undigested samples. The ICP-MS method was assessed using a CRM (NRCC DORM-5, Fish protein). Acceptable recoveries (96–105%) were obtained for the CRM, along with r values > 0.999 and acceptable LOQs, thus demonstrating the suitability of the method. The Hg analyser performance was assessed using a PT material (FAPAS, FCCM46-SEA7), as no Hg was found in any samples, this was not progressed any further for this study. All elements except Hg were detected in soft tissues, with concentrations following the order Fe > Al > Zn > Mn > Cu > Ag > Cr > V > Ni > Cd > Pb > Co > Se > As. Significant differences in As and Pb levels were observed between rural and urban sites, though Cd levels remained consistent. Higher concentrations of essential elements such as Co, Cr, Cu, Mn, Ni, and Zn were found in *C. aspersum*, while PCA confirmed that urban environments contributed more to element accumulation. The absence of Hg and low levels of Cd and Pb supported the safety of these snail species for human consumption and their suitability for heliciculture.

The presence, bio-accessibility, and potential health risks of trace elements in plant-based meat analogues sold in Brazil were assessed using ICP-MS, simulated *in vitro* digestion following the INFOGEST 2.0 protocol and MAD.[218] Both the samples and their bio-accessible portions, obtained by means of INFOGEST 2.0 *in vitro* digestion, underwent MAD (0.5 g sample, 4 mL HNO_3, 3 mL H_2O_2), followed by ICP-MS analysis. The method was validated using NIST SRM 1547 (Peach leaves) which returned accuracies ranging from 70 to 112% and precisions of <14% RSD. The elements Al, As, Ba, Cd, Co, Cr, Hg, Mo, Ni, Pb, Sb, and Se were quantified, with measurable levels of Al, Ba, Co, Cr, Mo, Ni, and Se detected in all samples. Bio-accessibility percentages varied depending on product composition. The element content was assessed against European Food Safety Authority (EFSA) guidelines for safe consumption. In five samples, Al exceeded 100% of the Tolerable Weekly Intake, while Co and Ni surpassed 50% of the Health-Based Guidance Value and Tolerable Daily Intake, respectively, in one sample each. The Margin of Exposure for Pb ranged from 1 to 10 in thirteen samples. PCA was applied to group samples based on elemental profiles. These findings highlighted the need for further investigation into the safety of plant-based meat products, particularly regarding child consumption.

10.2.10 Authenticity. *A TOF-SIMS-based screening method was applied to powdered saffron samples.*[219] This approach enabled the discrimination of pure saffron from safflower-adulterated samples at inclusion levels as low as 5%, with safflower-specific triacylglycerol fragments (m/z 381–565) remaining detectable even at 20% (w/w), while turmeric adulteration was not distinguishable due to matrix suppression; PCA captured 98% of variance across 146 peaks, with principal component 1 alone accounting for 93% of group separation.

A deep learning model (LVDLNet) was developed to fuse LIBS and visible-near-infrared spectroscopy (VIS-NIR) for identifying polygonati rhizoma from Protected Geography Indication (PGI) regions, which is often adulterated with polygonati rhizoma from less desirable areas.[105] A range of chemometric tools were applied to the data, including KNN, LDA and SVM, which typically gave prediction accuracies of below 90%. The LVDLNet model gave superior results achieving an accuracy of 98.75%,

macro-precision of 98.78%, macro-recall of 98.75%, and macro-F measure of 98.50, with robustness confirmed across multiple adulteration levels ranging from 0 to 100% w/w.

Kakadu plum, a native Australian fruit growing in popularity, attracted research to help protect producers from fraudulent activity.[220] Elemental fingerprints from 443 samples, collected across 21 regions in northern Australia, were ascertained by use of XRF measuring 30 elements. A RF model was developed achieving an overall classification accuracy of approximately 82%, with Cr, Cu, Mn, and Rb identified as the most important markers for geographic origin authentication. The authors acknowledged that, although their work was useful to protect small-scale farmers, further work would be required to build more robust methods for profiling.

The capabilities of LIBS to detect adulteration of extra virgin olive oils were compared with those of two other techniques. Both LIBS and fluorescence spectroscopy were applied to the same set of samples,[221] including 40 pure EVOOs and 144 binary mixtures adulterated with pomace, corn, soybean, and sunflower oils. Spectral data were analysed using machine learning algorithms such as LDA, SVMs, logistic regression, and gradient boosting, with both techniques proving to be highly efficient in detecting adulteration with a >99% accuracy. Fluorescence spectroscopy was more efficient at predicting the botanical origin of the adulterant oil, typically with a 10% greater accuracy than LIBS. When origin studies were conducted, LIBS was found to have better accuracy with 100% prediction in some cases, whereas fluorescence spectroscopy only achieved 61–80% accuracy. LIBS was found to offer rapid, *in situ* analysis with perfect classification accuracy in distinguishing pure EVOOs from adulterated samples, while fluorescence spectroscopy provided superior performance in identifying the type of adulterant and differentiating samples by geographical origin. The same research group[222] compared the performance of LIBS *vs.* UV-VIS-NIR absorption spectroscopy using the same set of samples (40 pure EVOOs and 144 adulterated binary mixtures). LIBS emission spectra provided elemental information, primarily from atomic lines of C, H, N, and O, and molecular bands of C_2 and CN, while UV-VIS-NIR absorption spectra revealed organic constituents such as carotenoids and chlorophylls. Spectral data were processed using PCA and classified *via* LDA, SVM, and logistic regression. High classification and prediction accuracies (up to 100%) were achieved, with fused data yielding enhanced performance. The LIBS technique was found to be faster and more suitable for real-time EVOO authentication.

A number of other papers, mainly focussed on the application of chemometric methods to investigate the origin, authenticity or provenance of foodstuffs using data generated by multielement spectroscopic methods are briefly described in Table 2.

11 Abbreviations

AAS	atomic absorption spectrometry
AB	arsenobetaine
AC	arsenocholine
ACN	acetonitrile
AEC	anion-exchange chromatography
AES	atomic emission spectrometry
AF4	asymmetric flow-field flow fractionation
AFM	atomic force microscopy
AFS	atomic fluorescence spectrometry
AGREE	analytical GREENnness scale
AGREEprep	analytical GREENnness metric for sample preparation
ANN	artificial neural network
ANOVA	analysis of variance
APDC	ammonium pyrrolidine dithiocarbamate
ASA	*p*-arsanilic acid
ASU	atomic spectrometry update
BMI	body mass index
CE	capillary electrophoresis
CFU	colony-forming unit
CLSI	Clinical & Laboratory Standard Institute
CNN	convolutional neural network
CNRM	National Institute for Metrology, China
CPE	cloud point extraction
cps	counts per second
CRC	collision/reaction cell
CRISPR/Cas	clustered regularly interspaced short palindromic repeats/Cas
CRM	certified reference material
CS	continuum source
CSF	cerebrospinal fluid
CV	cold vapour
CVG	chemical vapour generation
DA	discriminant analysis
DBCO	dibenzocyclooctyne
DBD	dielectric barrier discharge
DBS	dry blood spot
DCC	double curved crystal
DDTP	o,o-diethyldithiophosphate
DES	deep eutectic solvent
DGA	diglycolamide
DLLME	dispersive liquid–liquid microextraction
DLS	dynamic light scattering
DMA	dimethylarsenic
DMSO	dimethysulfoxide
DMTA	4,4-dimethyl-2,6-dioxo-*N*-phenylcyclohexanecarbothioamide
DNA	deoxyribonucleic acid
DOE	design of experiment
DOTA	dodecane tetraacetic acid
DRC	dynamic reaction cell
DSPE	dispersive solid phase extraction
DSPME	dispersive solid phase micro extraction
DTPA	diethylenetriaminepentaacetic acid
DTT	dithiothreitol
DTZ	dithizone
EC	European Commission
EDTA	ethylenediaminetetraacetic acid
EDXRF	energy dispersive XRF
EHF	enhancement factor (based on calibration slope ratios)
ELISA	enzyme-linked immunosorbent assay

ERF	enrichment factor (based on concentration ratios)
ERM	european reference material
ESI	electrospray ionisation
ETAAS	electrothermal atomic absorption spectrometry
EtHg	ethylmercury
EtOH	ethanol
EU	European Union
EVOO	extra virgin olive oil
FAAS	flame atomic absorption spectrometry
FAES	flame atomic emission spectrometry
FAO	food and agriculture organization
FI	flow injection
FID	flame ionization detector
FTIR	Fourier transform infrared
GAPI	green analytical procedure index
GC	gas chromatography
GD	glow discharge
GFAAS	graphite furnace atomic absorption spectrometry
GLS	gas liquid separator
GO	graphene oxide
GPx	glutathione peroxidase
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
HG	hydride generation
HILIC	hydrophilic interaction liquid chromatography
HPLC	high performance liquid chromatography
HR	high resolution
IAEA	International Atomic Energy Agency
iAs	inorganic arsenic
IC	ion chromatography
ICH	International Council on Harmonization (of technical requirements for pharmaceuticals for human use)
ICP	inductively coupled plasma
ID	isotope dilution
IDA	isotope dilution analysis
IgM	immunoglobulin M
iHg	inorganic mercury
IL	ionic liquid
INCT	institute of nuclear chemistry and technology, Warsaw, Poland
IPA	isopropyl alcohol
IR	infrared
IRMM	institute for reference materials and measurements
IRMS	isotope-ratio mass spectrometry
IS	internal standard
iSe	inorganic selenium
JRC	joint research centre
k	coverage factor
KED	kinetic energy discrimination
KMRCD	kernel minimum regularized covariance determinant
KNN	k-nearest neighbour
LA	laser ablation
LC	liquid chromatography
LDA	linear discriminant analysis
LIBS	laser induced breakdown spectroscopy
LLE	liquid liquid extraction
LLME	liquid liquid microextraction
LOD	limit of detection
LOQ	limit of quantification
m/z	mass-to-charge ratio
MAD	microwave-assisted digestion
MAE	microwave-assisted extraction
MALDI	matrix-assisted laser desorption ionisation
MALS	multiangle light scattering
MB	magnetic bead
MC	multicollector
MeHg	methylmercury
MeOH	methanol
MeSeCys	methylselenocysteine
MIC	microwave induced combustion
MICAP	microwave-sustained inductively coupled atmospheric-pressure plasma
MIL	magnetic ionic liquid
MIP	microwave induced plasma
miRNA	micro ribonucleic acid
MMA	monomethylarsenic
MNP	magnetic nanoparticle
Mr	relative molecular mass
MRI	magnetic resonance imaging
MS	mass spectrometry
MS/MS	tandem MS
MWCNT	multiwall carbon nanotube
μEDXRF	micro EDXRF
NAA	neutron activation analysis
NCS	national analysis centre for iron and steel (China)
NIR	near infrared spectrophotometry
NIST	National Institute of Standards and Technology
NMIJ	National Metrology Institute of Japan
NMR	nuclear magnetic resonance
NP	nanoparticle
NRCC	National Research Council of Canada
OES	optical emission spectrometry
OMC	organomercury compounds
OPLS-DA	orthogonal partial least squares discriminant analyses
PBS	phosphate-buffered saline
PCA	principal component analysis
PCR	polymerase chain reaction
PDO	protected designation of origin
PE	polyethylene
PF	preconcentration factor (based on volume ratios)
PFA	perfluoroalkyl
PGI	protected geography indication
PhHg	phenylmercury
PIXE	particle-induced X-ray emission
PLS	partial least squares
ppb	parts per billion (10–9)
ppm	parts per million (10–6)
PT	proficiency testing
PTE	potentially toxic element
PTFE	poly(tetrafluoroethylene)
PLS-DA	partial least squares discriminant analysis
PVG	photochemical vapour generation
QC	quality control
QMS	quadrupole mass spectrometry

QQQ	triple quadrupole
REE	rare earth element
RF	random forest
RM	reference material
RMSE	root mean square error
RMSEP	route mean square error of prediction
RNA	ribonucleic acid
RP	reversed phase
RSD	relative standard deviation
S/B	signal-to-background ratio
S/N	signal to noise ratio
sc	single cell
SD	standard deviation
SDBS	sodium dodecyl benzene sulfonate
SDS	sodium dodecylsulfate
SeAlb	selenoalbumin
SEC	size exclusion chromatography
SeCys	selenocysteine
SeCys2	selenocystine
SelP	selenoprotein
SeMet	selenomethionine
SF	sector field
SIMCA	soft independent modelling of class analogy
SIMS	secondary ion mass spectrometry
SNV	standard normal variate
sp	single particle
SPE	solid phase extraction
SPME	solid phase microextraction
SQT	slotted quartz tube
SRM	standard reference material
SVM	support vector machine
SVR	support vector regression
SXRF	synchrotron XRF microscopy
TBAH	tetrabutyl ammonium hydroxide
TBT	tributyltin
TE	transport efficiency
TEL	tetraethyllead
TEM	transmission electron microscopy
TET	triethyltin
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIMS	thermal ionisation mass spectrometry
TMAH	tetramethylammonium hydroxide
TML	tetramethyllead
TMT	trimethyltin
TOC	total organic carbon
TOF	time-of-flight
TPhT	triphenyltin
TRIS	tris(hydroxymethyl)aminomethane
TXRF	total reflexion XRF
UAE	ultrasound-assisted extraction
UHPLC	ultra high performance liquid chromatography
UN	United Nations
US	United States
US FDA	United States Food and Drug Administration
UV	ultraviolet
UV-VIS	ultraviolet-visible spectrophotometry
UV-VIS-NIR	ultraviolet-visible near infrared spectrophotometry
VG	vapour generation
VIS	visible
vs.	*versus*
WHO	UN World Health Organisation
XFM	X-ray fluorescence microscopy
XRF	X-ray fluorescence
XRFS	X-ray fluorescence spectroscopy

Conflicts of interest

There are no conflicts to declare.

References

1 M. Patriarca, N. Barlow, A. Cross, S. Hill, D. Milde and J. Tyson, *J. Anal. At. Spectrom.*, 2025, **40**(3), 541–664.
2 C. Vanhoof, J. R. Bacon, U. E. A. Fittschen and L. Vincze, *J. Anal. At. Spectrom.*, 2024, **39**(9), 2152–2164.
3 E. Bolea-Fernandez, R. Clough, A. Fisher, B. Gibson and B. Russell, *J. Anal. At. Spectrom.*, 2024, **39**(11), 2617–2693.
4 W. R. L. Cairns, O. T. Butler, O. Cavoura, C. M. Davidson, J. L. Todolí-Torró and M. von der Au, *J. Anal. At. Spectrom.*, 2025, **40**(1), 11–69.
5 E. H. Evans, J. Pisonero, C. M. M. Smith and R. N. Taylor, *J. Anal. At. Spectrom.*, 2025, **40**(5), 1136–1157.
6 R. Clough, C. F. Harrington, S. J. Hill, Y. Madrid and J. F. Tyson, *J. Anal. At. Spectrom.*, 2025, **40**(7), 1615–1644.
7 Z. Zang, X. Liu, M. Wang and Z. Wang, *Crit. Rev. Anal. Chem.*, 2025, **55**(6), 1233–1257.
8 M. Matczuk, L. Ruzik and A. R. Timerbaev, *TrAC, Trends Anal. Chem.*, 2024, **180**, 117967.
9 D. Kruger, J. T. P. Matshwele, M. D. Mukhtar and D. Baecker, *Molecules*, 2024, **29**(13), 3120.
10 K. E. Adesina, C. J. Burgos, T. R. Grier, A. S. M. Sayam and A. J. Specht, *Curr. Environ. Health Rep.*, 2025, **12**(1), 7.
11 D. Blaimer and K. Leopold, *TrAC, Trends Anal. Chem.*, 2024, **181**, 118031.
12 H. Rawat, S. A. Bhat, D. S. Dhanjal, R. Singh, Y. Gandhi, S. K. Mishra, V. Kumar, S. K. Shakya, C. V. Narasimhaji, A. Singh, R. Singh and R. Acharya, *Talanta*, 2024, **10**, 100341.
13 D. Krüger, D. J. Butcher and D. Baecker, *Appl. Spectrosc. Rev.*, 2025, **60**(9–10), 957–977.
14 K. Soni, R. Frew and B. Kebede, *Crit. Rev. Food Sci. Nutr.*, 2024, **64**(19), 6616–6635.
15 D. Banaś, A. Kubala-Kukuś, I. Stabrawa, K. Szary, J. Wudarczyk-Moćko, P. Jagodziński, G. Wesołowski, S. Góźdź, J. Chwiej, P. Wróbel, E. Marguí, J. Jablan, S. Pessanha, H. Stosnach and L. Borgese, *Spectrochim. Acta, Part B*, 2025, **230**, 107236.
16 K. Ma, C. Wei and X. Li, *Microchem. J.*, 2024, **205**, 111332.
17 D. A. Garzon, D. A. Ahumada, C. Paredes and E. Castillo, *Anal. Bioanal. Chem.*, 2025, **417**(12), 2717–2726.
18 J. Fong Sam, A. J. Kuszak, P. J. Gray and S. A. Wise, *J. AOAC Int.*, 2024, **107**(6), 1027–1037.

19 L. H. Zhou, X. C. Lin, L. L. Suo, L. F. Ma, G. X. Hu, X. Xia, M. Li, W. H. Huang and Y. J. Jin, *J. Mass Spectrom.*, 2025, **60**(1), e5109.
20 W. B. Jones, A. J. Crossman and B. T. Jones, *Anal. Chem.*, 2024, **96**(29), 11906–11914.
21 W. B. Jones, R. M. Huff, A. L. Richardson, T. Dessoffy, S. M. Lewis, A. Eddy, A. J. Crossman and B. T. Jones, *J. Anal. At. Spectrom.*, 2024, **39**(9), 2220–2229.
22 D. E. Bustos and R. E. Rivas, *Microchem. J.*, 2025, **212**, 113470.
23 A. Bazo, E. Bolea-Fernandez, A. Rua-Ibarz, M. Aramendia and M. Resano, *Anal. Chem.*, 2025, **97**(26), 13922–13929.
24 G. Guerra, E. Venturelli, A. Polymeropoulos, F. Segrado, D. Morelli, S. Rinaldi, C. Agnoli and S. Sieri, *J. Trace Elem. Med. Biol.*, 2025, **89**, 127667.
25 C. Cheng, Z. Wang, H. Duan, Q. Wu, X. Chen, L. Zhang, D. Wang, M. Yang, Z. Huang, Z. Su, Z. Li, R. He, W. Li, Z. Li and G. Huang, *Talanta*, 2025, **282**, 126963.
26 US Food and Drug Administration, *Bioanalytical Method Validation Guidance for Industry*, 2018.
27 M. Krmpotic, M. Telkhozhayeva, M. N. Tsubery, N. Goldenberg-Cohen and O. Girshevitz, *Talanta*, 2025, **285**, 127394.
28 K. E. Adesina, J. M. Lanzer, H. L. Zetlen, M. E. Rebuli, M. B. Rice and A. J. Specht, *Exposure Health*, 2025, **17**(3), 819–830.
29 O. N. Grebneva-Balyuk, M. S. Kiseleva and I. V. Kubrakova, *J. Anal. Chem.*, 2025, **80**(4), 608–622.
30 L. Nemcek and I. Hagarová, *Toxics*, 2025, **13**(4), 289.
31 A. B. S. da Silva, K. N. da Silva Leal and M. A. Z. Arruda, *Anal. Bioanal. Chem.*, 2025, **417**(7), 1271–1282.
32 A. C. B. Luckow, Y. R. Bloedorn, D. H. Bonemann, S. H. Scherdien, C. G. Gomes, E. R. Lopes, A. S. Ribeiro and A. M. Nunes, *Food Control*, 2025, **169**, 110977.
33 N. Pothuraju, H. K. Pogula, R. Jagdale, U. K. Vadla, R. L. Gajbhiye, V. K. Parihar, R. Velayutham and R. Peraman, *Environ. Monit. Assess.*, 2025, **197**(3), 344.
34 T. K. Nurubeyli, N. S. Jafar and G. N. Mammadova, *Int. J. Mass Spectrom.*, 2025, **507**, 117355.
35 N. D. To, A. Tran Thi Phuong, T. T. Nguyen, H. A. Pham and Q.-K. Truong, *Nat. Prod. Commun.*, 2025, **20**(6), 1934578X251349924.
36 A. Saber, F. Malhat, E.-S. Saber and S. A. Shokr, *Int. J. Environ. Anal. Chem.*, 2025, **105**(19), 7683–7698.
37 L. R. H. Gerken, M. Roesslein, I. K. Herrmann and A. Gogos, *Anal. Methods*, 2025, **17**(26), 5334–5342.
38 T. A. Brito, F. S. Costa, R. C. Oliveira, C. D. B. Amaral, G. Labuto and M. H. Gonzalez, *Food Chem.*, 2025, **464**(Pt 3), 141922.
39 S. S. Ferreira, R. S. Lamarca, L. S. Silva, T. A. L. Burgo, P. C. F. Lima Gomes, C. D. B. Amaral, J. O. Fernandes, S. C. Cunha and M. H. Gonzalez, *ACS Omega*, 2025, **10**(24), 26118–26128.
40 S. Zhang, B. Chen, Y. Liu, H. Sun, H. Zhang, N. Li, Y. Qing, J. Elango, D. Zhao and W. Wu, *Foods*, 2025, **14**(3), 384.
41 A. C. Costa-Santos, E. A. Orlando, A. P. Rebellato, M. C. F. Zambom, G. M. Luz, V. F. Moraes-Neto, E. T. dos Santos Caramês, N. H. Noguera, A. S. do Carmo, M. L. Gigante, M. M. C. Ferreira and J. A. L. Pallone, *Food Anal. Methods*, 2025, **18**(6), 1118–1137.
42 A. Savic, J. Mutic, M. Lucic, J. Veskovic, A. Miletic and A. Onjia, *Foods*, 2025, **14**(2), 275.
43 D. C. Cavalcante, S. O. Pinto, L. S. Ferreira, H. M. Santos, R. M. Jesus, E. G. P. Silva, A. G. Silva and L. N. Santos, *Food Chem.*, 2025, **473**, 143058.
44 C. P. Dlangalala, N. S. Mdluli, M. Sihlahla and N. Mketo, *Food Anal. Methods*, 2025, **18**(6), 1101–1117.
45 G. B. Guimaraes, L. B. Guimaraes, J. C. Romero, S. M. S. Queiroz, D. C. Lima, L. N. Santos, E. G. P. da Silva, R. M. de Jesus, F. S. Dias, F. G. Lepri, A. G. Silva and F. A. C. Amorim, *Food Anal. Methods*, 2024, **17**(11), 1559–1570.
46 X.-q. Wu, G.-Y. Zhu, Z.-w. Chen and M.-h. Ge, *All Life*, 2025, **18**(1), 2520268.
47 N. Q. Thang, H. K. Trung, L. V. Tan, N. T. K. Phuong, N. T. K. Ngan and T. Q. Hieu, *J. Anal. Chem.*, 2025, **80**(5), 877–886.
48 I. P. de Sa, C. A. da Silva and A. R. A. Nogueira, *Braz. J. Anal. Chem.*, 2024, **11**(44), 17–35.
49 J.-Y. Cai, S. Liu, S. Chen, Y.-L. Yu and J.-H. Wang, *Anal. Chem.*, 2023, **95**(26), 9813–9821.
50 A. Angaïts, K. Bierla, J. Szpunar and R. Lobinski, *Anal. Bioanal. Chem.*, 2024, **416**(23), 5111–5120.
51 D. Chillè, E. Marguí, E. Anticó, C. Foti and C. Fontàs, *Anal. Chim. Acta*, 2024, **1324**, 343098.
52 M. Bartolomé, M. J. Villaseñor, D. J. González-Serrano, M. Hadidi and A. Ríos, *Food Chem.*, 2025, **463**(Pt 1), 141101.
53 A. C. Gimenez-Ingalaturre, I. Abad-Álvaro, M. Bakir, P. Chueca, P. Goñi and F. Laborda, *Microchem. J.*, 2024, **205**, 111296.
54 A. Justo-Vega, S. Vazquez-Perez, R. Dominguez-Gonzalez, P. Bermejo-Barrera and A. Moreda-Pineiro, *Talanta*, 2025, **291**, 127766.
55 E. Espada-Bernabé, B. Gómez-Gómez, G. Moreno-Martín and Y. Madrid, *Anal. Chim. Acta*, 2024, **1323**, 343058.
56 K. N. d. S. Leal, A. B. Santos da Silva, A. Z. B. Aragão, C. H. I. Ramos, A. J. Stewart and M. A. Z. Arruda, *Microchem. J.*, 2024, **202**, 110696.
57 M. M. Melo, F. N. Ferreira, A. S. Luna, M. A. P. Langone and J. S. de Gois, *Food Anal. Methods*, 2024, **17**(10), 1402–1410.
58 B. K. Munjanja, O. S. Salami, K. N. Mashale, P. N. Nomngongo and N. Mketo, *Food Anal. Methods*, 2024, **18**(2), 161–171.
59 D. P. Cladis, K. M. Hill Gallant, A. R. Kisch and K. M. Murphy, *J. Food Compos. Anal.*, 2025, **141**, 107270.
60 L. N. Puente De La Cruz, R. Giorgione, F. Marini and M. L. Astolfi, *Food Chem.*, 2024, **461**, 140831.
61 P. Inaudi, I. Certomà, M. Malandrino, L. Favilli, R. Cecire, S. Bertinetti, O. Abollino and A. Giacomino, *J. Food Compos. Anal.*, 2025, **146**, 107846.
62 G. S. Coelho Jr, V. P. Souza, J. Kratzer, J. Dedina and E. M. M. Flores, *Spectrochim. Acta, Part B*, 2024, **221**, 107055.

63 W. P. C. Gomes, G. M. Alves, F. C. O. L. Martins and W. R. Melchert, *Eur. Food Res. Technol.*, 2025, **251**(5), 735–746.
64 J. Y. Cai, H. Shen, J. N. Xu, J. M. Xu, J. H. Wang and Y. L. Yu, *Anal. Chem.*, 2025, **97**(5), 2899–2905.
65 G. C. Costa, A. S. Dos Santos, R. G. O. Araujo, M. G. A. Korn and R. M. M. Santana, *Food Chem.*, 2025, **477**, 143460.
66 F. P. R. Peixoto, D. M. de Abreu, P. G. R. Peixoto, W. O. Matos and G. S. Lopes, *J. Food Compos. Anal.*, 2025, **142**, 107452.
67 M. L. D. Reis, T. d. J. Santos, L. A. d. Albuquerque, C. S. A. Felix, L. S. G. Teixeira and F. d. S. Dias, *Microchem. J.*, 2025, **210**, 112926.
68 L. Nong, M. Li, X. Wang, C. Li and H. Fang, *Talanta*, 2026, **296**, 128455.
69 W. Q. Ferreira, R. F. Jesus, A. S. Jesus, S. A. R. Soares, R. G. O. Araujo and J. B. Pereira Junior, *Food Chem.*, 2025, **488**, 144813.
70 R. C. de Jesus, T. L. de Souza, A. L. O. Latif, E. S. L. B. Souza, A. de Freitas Santos Junior, L. Dos Santos Lobo, J. B. P. Junior, R. G. O. Araujo, L. A. Souza and D. Santos, *Food Chem.*, 2025, **482**, 144152.
71 J. V. Soares de Araujo, E. Arruda Dos Santos, T. S. Santos, R. J. Cassella, C. Duyck and R. R. A. Peixoto, *Talanta*, 2025, **293**, 128096.
72 K. Wang, X. Duan, Y. Tian, H. Lin, L. Cao and J. Sui, *Spectrochim. Acta, Part B*, 2025, **225**, 107120.
73 K. D. do Amaral, G. S. Silva, A. S. Araujo and R. J. Cassella, *Food Chem.*, 2025, **463**(Pt 4), 141527.
74 K. D. do Amaral, P. S. M. Rocha, A. S. Araujo and R. J. Cassella, *J. Pharm. Biomed. Anal.*, 2025, **261**, 116837.
75 A. Justo-Vega, C. Torrente-Seco, L. Janza-Candal, M. Aboal-Somoza, R. Domínguez-González, P. Bermejo-Barrera and A. Moreda-Piñeiro, *Microchem. J.*, 2025, **211**, 113072.
76 A. Retzmann, K. A. Miller, F. A. A. Mohamed and M. E. Wieser, *Anal. Bioanal. Chem.*, 2025, **417**(4), 835–846.
77 M. Cabré, G. Fernández, E. González, J. Abellà and A. Verdaguer, *J. Anal. At. Spectrom.*, 2024, **39**(10), 2508–2513.
78 M. Hernández-Postigo, A. Sánchez-Cachero, M. Jiménez-Moreno and R. C. R. Martín-Doimeadios, *Microchim. Acta*, 2025, **192**(1), 28.
79 Q. Sun, C. Wang, C. Wang, X. Lin and L. Ding, *Microchem. J.*, 2025, **212**, 113283.
80 T. Zarei, M. B. A. Colombo, E. C. Fuchs, H. L. Offerhaus, D. Gebauer and L. L. F. Agostinho, *Water*, 2024, **16**(17), 2419.
81 R. Dejonghe, E. Bolea-Fernandez, A. Lores-Padin, T. Van Acker, A. Rua-Ibarz, O. De Wever and F. Vanhaecke, *Microchem. J.*, 2024, **207**, 112013.
82 G. Redondo-Fernandez, K. Billimoria, S. Cowen, D. Ojeda, D. Bartczak, A. Soldado, J. M. Costa-Fernandez and H. Goenaga-Infante, *J. Anal. At. Spectrom.*, 2025, **40**(5), 1403–1410.
83 H. Yang, L. Fu, J. Huang and L. Chen, *At. Spectrosc.*, 2024, **45**(3), 191–199.
84 C. Chen, J. Sun, K. Hu, B. Chang, X. Du, F. Zhang, C. Peng and S. Xing, *At. Spectrosc.*, 2025, **46**(02), 99–108.
85 N. Zhong, X. Zhao, L. Li, C. Xu, S. Huang, L. Zhao and Y. Zhao, *J. Radioanal. Nucl. Chem.*, 2025, **334**, 131–140.
86 T. Hirata, K. Kobayashi, H. H. Khoo, O. Shikino and H. Asanuma, *Analyst*, 2024, **149**(21), 5174–5183.
87 S. Thoroë-Boveleth, R. Becker, J. Bertram, T. Schettgen, M. Möller, D. Jonigk, T. Kraus and R. Weiskirchen, *Anal. Sci.*, 2024, **41**(3), 237–249.
88 M. Schannor, M. Oelze, H. Traub, Y. B. He, R. Schmidt, L. Heidemann, L. J. Savic, J. Vogl and B. Meermann, *Anal. Chem.*, 2025, **97**(8), 4425–4432.
89 A. C. V. Rubin, V. M. Neves, A. B. Viana, T. B. Riquieri, D. Pozebon and V. L. Dressler, *Quim. Nova*, 2025, **48**(2), 1–7.
90 Y. K. Tanaka and Y. Ogra, *J. Am. Soc. Mass Spectrom.*, 2024, **35**(9), 2082–2089.
91 P. Menero-Valdés, L. Alvarez, H. Gonzalez-Iglesias, B. Fernandez and R. Pereiro, *Anal. Chim. Acta*, 2024, **1317**, 342906.
92 J. H. Dong, Y. Yu, Z. J. Dai, S. Y. Li, L. J. Chen, P. J. Xing, G. Wang, X. Liu, H. T. Zheng and Z. L. Zhu, *Anal. Chem.*, 2024, **96**(50), 19955–19964.
93 A. Barad, Y. Q. Xu, E. Bender, E. K. Pressman, Z. L. Gu and K. O'Brien, *Am. J. Clin. Nutr.*, 2025, **121**(2), 417–426.
94 A. E. Hartman and T. P. Mawhinney, *At. Spectrosc.*, 2024, **45**(3), 181–190.
95 P. Borges de Oliveira, M. Krause, L. Valli Vieira, L. Santos Moreira, A. Virgilio, G. Pedrini Brandão and M. T. Weitzel Dias Carneiro, *Microchem. J.*, 2025, **213**, 113628.
96 J. Pérez-Vázquez, A. García-Juan, R. Serrano, G. Grindlay and L. Gras, *Microchem. J.*, 2025, **213**, 113655.
97 X. Wang, T. Ren, Y. Yang, Y. Lin, Y. Deng and C. Zheng, *At. Spectrosc.*, 2024, **45**(3), 173–180.
98 R. L. Zhang, S. F. Hu, C. F. Ma, T. L. Zhang and H. Li, *TrAC, Trends Anal. Chem.*, 2024, **181**, 118001.
99 P. Modlitbová, E. Zikmundová, P. Pořízka and J. Kaiser, *TrAC, Trends Anal. Chem.*, 2025, **184**, 118110.
100 E. Nanou, N. Pliatsika, D. Stefas, D. Polygenis and S. Couris, *J. Food Compos. Anal.*, 2025, **144**, 107650.
101 F. V. Nakadi, A. Garcia-Garcia, A. Rua-Ibarz and M. Resano, *Talanta*, 2025, **292**, 127920.
102 X. Zhang, X. Li, X. Chen, M. Shi and T. Ren, *Spectrochim. Acta, Part B*, 2025, **227**, 107158.
103 E. J. Blanchette, E. A. Tracey, A. Baughan, G. E. Johnson, H. Malik, C. N. Alionte, I. G. Arthur, M. E. S. Pontoni and S. J. Rehse, *Spectrochim. Acta, Part B*, 2024, **216**, 106944.
104 J. Li, X. Pan, L. Guo and Y. Chen, *Med. Eng. Phys.*, 2024, **132**, 104207.
105 F. Chen, M. Zhang, W. Huang, H. Sattar and L. Guo, *Foods*, 2024, **13**(14), 2306.
106 R. M. de Oliveira, B. S. Soares and D. L. G. Borges, *Anal. Methods*, 2025, **17**(21), 4443–4450.
107 G. R. Bitencourt, P. A. Mello, P. Grinberg and R. E. Sturgeon, *J. Anal. At. Spectrom.*, 2025, **40**(7), 1754–1766.
108 A. B. Viana, C. Pappis, C. K. Pereira and V. L. Dressler, *Talanta*, 2024, **279**, 126600.
109 C. R. Andriolli, A. S. Henn, E. M. M. Flores, E. L. M. Flores and R. S. Picoloto, *Food Anal. Methods*, 2025, **18**(5), 807–814.

110 M. Verdugo, F. Ávila, J. Ruiz, C. Vásquez, N. Roldán, M. G. Lobos and Á. O. Ardiles, *J. Anal. At. Spectrom.*, 2025, **40**(1), 173–185.
111 S. Bodur, B. K. Tutar, S. E. Bodur, O. F. Tutar and S. Bakirdere, *Food Chem.*, 2025, **487**, 144738.
112 X. F. Yang, J. H. Yang, Y. B. Su, Y. R. Deng, X. D. Wen and C. B. Zheng, *Anal. Chem.*, 2025, **97**(4), 1977–1982.
113 R. Fernández-Ruiz, *Int. J. Mol. Sci.*, 2025, **26**(3), 1049.
114 S. I. Mamtha, R. Paranthaman, A. Negi and J. A. Moses, *J. Food Compos. Anal.*, 2025, **140**, 107216.
115 B. Johnson-Restrepo, E. Blain, C. Judd, A. Tysoe and P. J. Parsons, *Radiat. Phys. Chem.*, 2025, **234**, 112749.
116 X. Chen, S. Wang, M. Zhou, J. Wang, W. Song, J. Zhang, Y. Wang, W. Tian and Y. Wu, *Talanta*, 2025, **288**, 127719.
117 L. S. Chua, C. Y. Tee and S. G. Tan, *Spectrochim. Acta, Part B*, 2025, **228**, 107192.
118 J. Xiao, A. L. Xia and T. Geng, *J. Food Compos. Anal.*, 2025, **142**, 107481.
119 L. M. Bickley, E. Da Silva, D. R. Chettle and F. E. McNeill, *J. Anal. At. Spectrom.*, 2025, **40**(4), 1086–1097.
120 C. J. Burgos, D. E. Read, T. R. Grier, M. Khan, M. G. Weisskopf, K. M. Taylor and A. J. Specht, *Health Phys.*, 2025, **128**(4), 273–278.
121 S. McHeik, E. Da Silva and A. Pejović-Milić, *X-Ray Spectrom.*, 2025, **54**(6), 680–692.
122 M. Jiang, C. Zhang, Y. Chen, M. Sun, R. Liu and Y. Lv, *TrAC, Trends Anal. Chem.*, 2025, **191**, 118317.
123 R. X. Hou, Y. X. Gu, Y. Zhao, X. Y. Zhao, L. M. Yang, X. W. Yan and Q. Q. Wang, *At. Spectrosc.*, 2024, **45**(2), 74–82.
124 Z. Fang, X. Zhao, R. Hou, Y. Zhao, L. Wang, L. Yi, L. Yang, X. Yan and Q. Wang, *Anal. Chem.*, 2025, **97**(23), 12125–12132.
125 A. Villa-Vázquez, P. Menero-Valdés, L. Álvarez, H. González-Iglesias, B. Fernández and R. Pereiro, *Microchem. J.*, 2024, **207**, 111745.
126 S. Gonzalez Morales, E. Anon Alvarez, D. Clases, M. Corte-Rodriguez and M. Montes-Bayon, *Mikrochim. Acta*, 2025, **192**(6), 390.
127 T. He, D. Li, Y. Qin, Y. Xiang, Y. Tang, J. Tian and Y. Tang, *Talanta*, 2025, **293**, 128069.
128 Y. Xu, B. B. Chen, M. He, G. L. Yuan and B. Hu, *Anal. Chem.*, 2024, **97**(1), 811–817.
129 M. Jiang, X. Zhao, C. Zhang, R. Liu, J. Hu and Y. Lv, *Anal. Chem.*, 2025, **97**(22), 11907–11916.
130 C. X. Zhao, L. J. Du, J. Hu and X. D. Hou, *Anal. Chem.*, 2024, **96**(37), 15059–15065.
131 H. R. Li, W. C. Jiang, G. W. Sun, L. F. Sheng, R. M. Ma and G. J. Zhang, *Anal. Bioanal. Chem.*, 2025, **417**(15), 3393–3403.
132 X. Ji, J. Wu, J. Zhang, W. Yang and Y. Liu, *J. Clin. Lab. Anal.*, 2025, **39**(2), e25145.
133 J. He, M. Hu, L. Yang, J. C. Yin, Z. X. Zhang, L. Y. Tu, Y. X. Heng, L. Tang, J. He, X. D. Hou and X. Jiang, *Anal. Chem.*, 2024, **96**(49), 19196–19201.
134 E. Zikmundova, D. Sklenarova, E. Koci, T. Zatloukalova, T. Bacova, E. Makhneva, D. Holub, E. Machacova, H. Koprivova, K. Vytiskova, P. Porizka, K. Novotny, P. Skladal, Z. Farka and J. Kaiser, *Mikrochim. Acta*, 2024, **191**(11), 656.
135 J. Cai, B. B. Chen, M. He, G. L. Yuan and B. Hu, *Anal. Chem.*, 2024, **96**(35), 14222–14229.
136 X. Fu, B. Chen, M. He, G. Yuan and B. Hu, *Anal. Chem.*, 2025, **97**(12), 6702–6710.
137 S. Liang, S. Zhao, H. Liu, J. Liu, X. Xie, R. Chen, B. Chen and T. Luan, *Anal. Chim. Acta*, 2024, **1324**, 343101.
138 A. Mysková, T. David, M. Sedinova, I. Demirci, M. Polasek, J. Kunes, L. Maletinska, D. Sykora and A. Kana, *Anal. Chim. Acta*, 2025, **1350**, 343853.
139 J. S. Stanberry, R. Meeks, R. W. Peterson, H. B. Andrews, A. N. Bible, S. Szakas, B. C. Sanders and B. T. Manard, *J. Anal. At. Spectrom.*, 2025, **40**(4), 961–966.
140 X. T. Li, X. Li, G. Y. Wang, C. Wang and L. L. Huang, *At. Spectrosc.*, 2024, **45**(4), 309–315.
141 X. Li, X. Li, K. Hong, D. Liu, B. Li and L. Wang, *Microchem. J.*, 2025, **209**, 112818.
142 Y. Zhou, L. Li, Z. Tang, Y. Zhang, Y. Xu, Z. Zheng, J. Yang, X. Hu, B. Wang, J. Zhang, Q. Jiang and Y. Wang, *Food Chem.*, 2025, **481**, 144076.
143 C. Henninger, T. Stadelmann, D. Heid, K. Ochsenreither and T. Eisele, *Food Chem.*, 2025, **463**(Pt 4), 141437.
144 W. Ruiz, S. Fontagné-Dicharry, S. Verdier, D. C. Dayton, J. Guillemant, R. Moulian, P. Giusti, C. Barrère-Mangote and B. Bouyssiere, *Anal. Chem.*, 2024, **96**(38), 15142–15150.
145 R. A. Glabonjat, K. Schilling, V. N. Slavkovich, C. N. Izuchukwu, O. Balac, S. Dubey, R. Nandakumar, M. Galvez-Fernandez, W. A. Anderson, M. S. Spaur, M. H. Sobel, A. E. Nigra, T. R. Sanchez, M. R. Jones and A. Navas-Acien, *Environ. Res.*, 2024, **262**(Pt 1), 119833.
146 E. J. Pacer, C. D. Palmer and P. J. Parsons, *Anal. Methods*, 2025, **17**(8), 1840–1849.
147 C. Y. Zheng, J. Tang, X. D. Pan, H. T. Shen, Z. Y. Hu, J. S. Zhang, L. X. Wang, P. G. Wu and Y. Tan, *Chemosensors*, 2025, **13**(3), 78.
148 G. Yang, E. Kim, J. Zheng, M. Kowatari and O. Kurihara, *Microchem. J.*, 2025, **209**, 112659.
149 C. Lau, X. F. Lu, K. S. Hoy, T. Davydiuk, J. A. Graydon, M. Reichert and X. C. Le, *J. Environ. Sci.*, 2025, **153**, 302–315.
150 C. Lau, X. F. Lu, X. J. Chen, K. S. Hoy, T. Davydiuk, J. A. Graydon, M. Reichert, A. Leblanc, C. Donadt, G. Jhangri and X. C. Le, *J. Environ. Sci.*, 2025, **153**, 289–301.
151 D. Ghaffour, A. Leufroy and P. Jitaru, *Anal. Bioanal. Chem.*, 2025, **417**(8), 1519–1530.
152 AFNOR, *NF V03-110:2024-07-10 Analysis of Agri-Foodstuffs - Protocol for the Characterization of the Performances of a QuantitativeAnalysis Method for Validation Purposes by Construction of an Accuracy Profile*, 2024.
153 J. Cardini, J. J. Sloth and K. Loeschner, *Anal. Bioanal. Chem.*, 2025, **417**(18), 4147–4161.
154 H. C. Xu, Y. W. Zhu, Y. Peng, C. C. Sun, F. F. Fu and Y. Lin, *J. Anal. At. Spectrom.*, 2025, **40**(5), 1364–1372.
155 J. Wu, Y. Zhang, P. Sheng, C. He, H. Liu, S. Gu, J. Luo and M. Yang, *J. Sci. Food Agric.*, 2025, **105**(9), 4822–4832.
156 *Eurachem Guide: the Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related*

Topics, ed. H. Cantwell, 3rd edn, 2025, available from: http://www.eurachem.org.
157 B. Szczęśniak, I. Sowik, S. Oszwałdowski and L. Ruzik, *Microchem. J.*, 2025, **210**, 113044.
158 P. Zeng, F. Guo, Y. Wang, H. Hu, J. Liu and H. Cheng, *Microchem. J.*, 2025, **208**, 112609.
159 S. Escudero-Cernuda, D. Clases, N. Eiro, L. O. Gonzalez, M. Fraile, F. J. Vizoso, M. L. Fernandez-Sanchez and R. Gonzalez de Vega, *Anal. Bioanal. Chem.*, 2025, **417**(2), 361–371.
160 L. Colina-Vegas, T. Van Acker, W. Villarreal, O. De Wever, A. A. Batista, J. A. Nóbrega and F. Vanhaecke, *Dalton Trans.*, 2024, **53**(47), 18880–18889.
161 K. Kronenberg, E. Hoffmann, L. Hiddessen, B. Maus, M. Gerwing, C. Faber, M. Sperling, M. Wildgruber and U. Karst, *Metallomics*, 2024, **16**(8), mfae034.
162 V. Boger, P. Pirkwieser, N. Orth, M. Koehler and V. Somoza, *Anal. Chim. Acta*, 2025, **1355**, 343999.
163 S. Theiner, E. Foels and G. Koellensperger, *Anal. Chim. Acta*, 2024, **1332**, 343345.
164 T. Sarretto, M. T. Westerhausen, J. C. McKinnon, D. P. Bishop and S. R. Ellis, *Anal. Bioanal. Chem.*, 2025, **417**(4), 705–719.
165 K. Frenzel, Y. Kayser, A. Hornemann, B. Kastner, A. Hoehl, P. Mouratidis, I. Rivens, G. Ter Haar and B. Beckhoff, *PLoS One*, 2024, **19**(9), e0306795.
166 S. Huber, J. Michel, M. Reijnen, M. Averina, B. Bolann, J. O. Odland, S. Hansen and J. Brox, *J. Anal. Methods Chem.*, 2024, **2024**, 9944995.
167 N. Bithi, D. Ricks, B. S. Walker, C. Law and K. L. Johnson-Davis, *J. Mass Spectrom. Adv. Clin. Lab*, 2024, **34**, 21–27.
168 N. S. Medvedev, V. D. Kurbatova, A. O. Frolova, T. Y. Guselnikova, D. Y. Troitskii, V. G. Makotchenko, A. I. Saprykin and I. V. Korolkov, *Int. J. Environ. Anal. Chem.*, 2025, **105**(19), 8309–8325.
169 B. Li, M. He, T. Zhu, T. Yang, L. Hu, X. Zhang, R. Su and H. Chen, *Sci. China:Chem.*, 2025, **68**(7), 3302–3308.
170 Y. Huang, J. Tian, L. Wu, D. Xuan, D. Fang, G. Hu, W. Qu and Y. Zhou, *Talanta*, 2025, **290**, 127765.
171 US FDA. *M10 Bioanalytical Method Validation And Study Sample Analysis*, Guidance for Industry, FDA-2019-D-1469-0016, 2022.
172 B. A. Ebner, S. A. Erdahl, C. S. Lundgreen, M. Vassilaki, W. K. Kremers, D. S. Knopman, R. C. Petersen, D. J. Berry, D. G. Lewallen, P. J. Jannetto, M. E. Murray, R. R. Reichard and H. Maradit Kremers, *Acta Neuropathol.*, 2025, **149**(1), 18.
173 Y. Wu, X. X. Dai, M. Y. Luo, H. M. Guo, N. Yuan, Y. G. Yang, J. J. He and Y. Y. Guo, *J. Anal. At. Spectrom.*, 2025, **40**(2), 513–519.
174 A. O. Alekseeva, M. A. Zolotovskaia, M. I. Sorokin, M. V. Suntsova, G. S. Zakharova, P. A. Pugacheva, A. A. Tinkov, T. V. Korobeinikova, M. I. Sekacheva, E. V. Poddubskaya, A. V. Skalny, N. E. Kushlinskii and A. A. Buzdin, *Biochemistry*, 2024, **89**(12–13), 2274–2286.
175 T. Oguri, N. Shinohara and S. F. Nakayama, *Environ. Health Prev. Med.*, 2024, **29**, 9–10.
176 E. Boyaci, A. Cagir, T. Shahwan and A. E. Eroglu, *Talanta*, 2025, **295**, 128373.
177 A. Gianoncelli, A. Flamigni, S. Schöder, C. Tufoni, V. Bonino, G. Zito, A. Arbo, G. Ricci and L. Pascolo, *X-Ray Spectrom.*, 2025, **54**(5), 488–495.
178 S. Saremi, A. Tamaddon, E. Konoz, A. Ezabadi and H. A. Panahi, *Microchem. J.*, 2025, **212**, 113433.
179 P. Ott, T. Sandahl, A. Ala, D. Cassiman, E. Couchonnal-Bedoya, R. G. Cury, A. Czlonkowska, G. Denk, R. D'Inca, F. de Assis Aquino Gondim, J. Moore, A. Poujois, C. A. Twardowschy, K. H. Weiss, M. Zuin, C. O. F. Kamlin and M. L. Schilsky, *JHEP Rep.*, 2024, **6**(8), 101115.
180 R. Squitti, A. Pal, I. D. Ivanova, M. Marianetti and M. Rongioletti, *Biomolecules*, 2025, **15**(6), 788.
181 C. F. Harrington, G. Carpenter, J. P. C. Coverdale, L. Douglas, C. Mills, K. Willis and M. L. Schilsky, *Clin. Chem. Lab. Med.*, 2024, **63**(2), 320–328.
182 A. Stojsavljevic, K. Kozlica, I. Sarac, U. Lazic, J. Vitkovic, V. Radisic, N. Polovic, M. Svetel and J. Scancar, *J. Pharm. Biomed. Anal.*, 2025, **265**, 117029.
183 Z. He, Y. Zhao and H. Tang, *Front. Nutr.*, 2025, **12**, 1527207.
184 N. Qin, J. Zheng, G. Yang, E. Kim and O. Kurihara, *Talanta*, 2025, **289**, 127768.
185 R. Delipavlova, T. Deneva, D. Davcheva, S. Stoencheva and N. Dimov, *Bratislava Med. J.*, 2025, **126**(7), 1489–1499.
186 M. Vinceti, T. Urbano, T. Filippini, R. Bedin, C. Simonini, G. Soraru, F. Trojsi, B. Michalke and J. Mandrioli, *Biol. Trace Elem. Res.*, 2025, **203**(4), 2355–2364.
187 Z. Rodriguez-Hernandez, A. Paredes-Douton, M. Galvez-Fernandez, M. Grau-Perez, M. Sotos-Prieto, P. Rentero-Garrido, M. Gonzalez-Estecha, M. T. Llorente-Ballesteros, J. L. Gomez-Ariza, B. Callejon-Leblic, P. Fernandez-Navarro, M. Laclaustra, A. Cenarro, F. Civeira, R. A. Glabonjat, D. Monleon, R. Pastor-Barriuso, B. Moreno-Franco, T. Garcia-Barrera and M. Tellez-Plaza, *Free Radicals Biol. Med.*, 2025, **233**, 365–377.
188 P. Heitland, F. Pragst, S. Hartwig and H. D. Koster, *J. Anal. Toxicol.*, 2025, **49**(7), 492–495.
189 B. Rosenberg, A. Johnson, C. G. Potter and C. L. Antonio, *Health Phys.*, 2025, **129**(2), 61–70.
190 K. D. Klika, J. L. Han, M. S. Busse, V. A. Soloshonok, R. Javahershenas, F. Vanhaecke and A. Makarem, *J. Am. Chem. Soc.*, 2024, **146**(45), 30717–30727.
191 S. Weishaupt, W. Holkenjans, S. Balzer, A. Jeremias, M. Sperling, M. Vogel and U. Karst, *J. Chromatogr. A*, 2025, **1746**, 465808.
192 V. H. Cauduro, A. S. Henn, C. A. Bizzi, M. F. Mesko and E. M. M. Flores, *At. Spectrosc.*, 2024, **45**(5), 373–380.
193 US Pharmacopeia, *Elemental Impurities—Procedures*, vol. 233, 2023.
194 M. M. Shen, S. X. Zhou, Y. W. Jing, C. Q. Xiao, S. W. He and J. Yang, *Anal. Methods*, 2024, **16**(47), 8234–8241.
195 *International Conference on Harmonisation, Guideline for Element Impurities Q3D(R2)*, EB/OL, 2024.
196 Y. K. Tanaka, S. Hasegawa and Y. Ogra, *Talanta*, 2025, **293**, 128116.

197 T. N. Akbaba, O. Alp, U. Alshana and N. Ertaş, *Food Anal. Methods*, 2024, **18**(1), 101–112.
198 X. D. Huang, X. J. Chen, G. Z. Huang, Z. H. Xie, W. Shi, S. Ali, L. M. Yuan and X. Chen, *J. Anal. At. Spectrom.*, 2024, **39**(11), 2824–2831.
199 K. V. Sullivan, K. Moser, M. Costas-Rodriguez, E. Bolea-Fernandez and F. Vanhaecke, *Food Chem.*, 2025, **470**, 142673.
200 F. Černíková, A. Krejčová and J. Patočka, *J. Food Compos. Anal.*, 2025, **139**, 107106.
201 J.-A. Barrat and G. Bayon, *J. Food Compos. Anal.*, 2025, **142**, 107506.
202 J. Photi, P. Srisakda, T. Jurkvon, C. Zeder and K. Judprasong, *Food Chem.:X*, 2025, **25**, 102119.
203 K. Hu, L. Zhang, H. Zhou, Y. Liu, S. Liu, B. Chang, X. Gao, J. Yang, K. Shi and X. Hou, *Microchem. J.*, 2025, **212**, 113402.
204 A. P. de Oliveira, J. Naozuka and J. A. Landero-Figueroa, *J. Food Compos. Anal.*, 2025, **142**, 107500.
205 N. Q. Thang, L. Van Tan, N. Van Cuong, N. T. Mai Tho and L. Thanh Tam, *Int. J. Environ. Anal. Chem.*, 2025, **106**(2), 339–351.
206 K. Iwai, M. Iwai-Shimada, N. Tatsuta, Y. Kobayashi, K. Asato, M. Nishimoto, K. Nakai and S. F. Nakayama, *Microchem. J.*, 2025, **211**, 113142.
207 H. Tinas and S. Akman, *Anal. Lett.*, 2025, 1–10.
208 J. L. Braden, E. F. Klarquist and J. A. Kellogg, *Food Chem.:X*, 2024, **24**, 101844.
209 L. Cvancar, M.-S. Müller, M. Oest and M. Fischer, *J. Food Compos. Anal.*, 2025, **146**, 107870.
210 I. J. S. da Silva, M. M. da Silva, R. da Silva, E. J. De Franca, M. J. da Silva and M. T. Kato, *Food Chem.*, 2025, **481**, 143970.
211 Y. Oh, H. Chae, H. Jung, S. Kumar, S. H. Nam and Y. Lee, *Anal. Methods*, 2025, **17**(11), 2437–2445.
212 G. Chen and B. Lai, *J. Food Compos. Anal.*, 2025, **145**, 107768.
213 E. Mladenova, T. Voyslavov, I. Bakardzhiyski and I. Karadjova, *Molecules*, 2025, **30**(3), 475.
214 L. Sarlo, C. Duroux, Y. Clément, P. Lanteri, F. Rossetti, O. David, A. Tillement, P. Gillet, A. Hagège, L. David, M. Dumoulin, R. Marchal, T. Tillement, F. Lux and O. Tillement, *OENO One*, 2024, **58**(4), 1.
215 K. Gręda, A. Leśniewicz, K. Kowalczyk and P. Pohl, *Spectrochim. Acta, Part B*, 2024, **217**, 106978.
216 G. Gohlke, T. C. Pereira, A. P. F. Padilha, C. R. Andriolli, A. S. Henn, R. S. Picoloto, P. A. Mello and E. M. M. Flores, *J. Food Compos. Anal.*, 2025, **144**, 107704.
217 E. M. D. Messina, C. Naccari, C. Alfano, F. G. Galluzzo, G. Cammilleri, L. Pantano, M. D. Buscemi, A. Macaluso, N. Cicero, V. Calabrese and V. Ferrantelli, *J. Food Compos. Anal.*, 2025, **139**, 107159.
218 M. I. A. Fioravanti, R. F. Milani, A. P. Rebellato, M. A. Morgano and A. P. A. Bragotto, *J. Food Compos. Anal.*, 2025, **144**, 107742.
219 E. De Angelis, O. Al-Ayoubi, R. Pilolli, L. Monaci and A. Bejjani, *Foods*, 2024, **13**(13), 2033.
220 C. V. Tadros, D. Mazumder, P. S. Gadd, J. Crawford and P. Saeki, *Food Control*, 2025, **172**, 111158.
221 M. Bekogianni, T. Stamatoukos, E. Nanou and S. Couris, *Foods*, 2025, **14**(6), 1045.
222 E. Nanou, M. Bekogianni, T. Stamatoukos and S. Couris, *Foods*, 2025, **14**(2), 321.
223 S. Yılmaz, B. Hazer and M. Tuzen, *J. Food Compos. Anal.*, 2025, **142**, 107490.
224 Z. Liang, X. Ling, Y. Chen, P. Huo, X. Yao and X. Li, *Anal. Lett.*, 2025, **59**(3), 524–540.
225 N. M. Muslim, F. K. Abbood, N. H. Hammood and E. A. Azooz, *J. Food Compos. Anal.*, 2024, **134**, 106558.
226 M. B. Botella, P. Y. Quintas and R. G. Wuilloud, *J. Food Compos. Anal.*, 2024, **136**, 106748.
227 H. L. Ahmed, S. M. Mohammed-Amin and N. Fattahi, *Int. J. Environ. Anal. Chem.*, 2025, **105**(19), 8497–8510.
228 B. Yildiz, I. Durukan, M. Saylan, B. T. Zaman and S. Bakirdere, *Environ. Monit. Assess.*, 2024, **197**(1), 112.
229 Z. Saeedi, S. M. Sorouraddin, M. A. Farajzadeh and M. R. A. Mogaddam, *Anal. Methods*, 2025, **17**(16), 3331–3343.
230 M. Soylak, T. Zorlu and F. Uzcan, *J. Food Compos. Anal.*, 2025, **143**, 107545.
231 M. R. Jamali, M. Katouk and R. Rahnama, *J. Food Compos. Anal.*, 2025, **145**, 107799.
232 M. R. Jamali, M. Tavakoli, A. Nezhadali and R. Rahnama, *Anal. Lett.*, 2025, **59**(5), 1–15.
233 M. Soylak, A. M. A. Mohammed and H. E. H. Ahmed, *J. Food Compos. Anal.*, 2024, **130**, 106167.
234 C. Caner, M. Y. Ciftci, S. Tabassum, H. Altundag and E. Bulut, *J. Mol. Struct.*, 2025, **1321**, 139836.
235 S. Jabbari, S. M. Sorouraddin and M. A. Farajzadeh, *Int. J. Environ. Anal. Chem.*, 2025, **105**(20), 9159–9174.
236 T. Y. Chen, M. He, B. B. Chen and B. Hu, *Talanta*, 2025, **281**, 126859.
237 Y. H. Cheng and C. K. Su, *Mikrochim. Acta*, 2024, **191**(10), 598.
238 N. Samanpong, S. Wongsaipun, Y. Udnan, A. Sirikulkajorn, P. Masawat and W. C. Chaiyasith, *J. Food Compos. Anal.*, 2024, **136**, 106753.
239 S. Perveen, A. Hol, J. A. Baig, S. T. H. Sherazi, K. Akhtar, S. Hussain and F. Abbasi, *J. Anal. At. Spectrom.*, 2024, **39**(11), 2884–2892.
240 A. Shishov, U. Markova, V. Mulloyarova, P. Tolstoy, N. Shkaeva, D. Kosyakov, N. K. Das and T. Banerjee, *Anal. Chim. Acta*, 2024, **1332**, 343356.
241 G. A. Biltekin, A. Akdogan and Ü. Divrikli, *J. Food Meas. Char.*, 2025, **19**(1), 401–411.
242 C. H. Kuo and C. K. Su, *Anal. Chem.*, 2024, **97**(2), 1281–1290.
243 C. H. Kuo and C. K. Su, *Anal. Chim. Acta*, 2025, **1336**, 343536.
244 H. E. H. Ahmed, M. Konneh and M. Soylak, *Food Chem.*, 2025, **472**, 142922.
245 M. B. Arain, A. Niaz and M. Soylak, *Food Chem.*, 2025, **476**, 143410.
246 A. Bozdağ, E. Şahmetlioğlu, M. Soylak and İ. Narin, *Int. J. Environ. Anal. Chem.*, 2025, **105**(20), 8583–8599.

247 P. C. de Oliveira, L. M. Roveda, L. R. P. Ruis, P. M. dos Santos, C. R. T. Tarley, A. E. de Carvalho, D. Roman and M. Z. Corazza, *Food Anal. Methods*, 2025, **18**(6), 1162–1179.
248 M. Soylak, Q. Salamat and S. Sajjad, *Microchem. J.*, 2025, **211**, 113050.
249 M. Ahmad, A. Badshah, H. Li, M. Asad, M. A. Ibrahim, E. A. Ali, A. A. Shahat, U. Nishan, M. Khan and W. Sun, *J. Sep. Sci.*, 2024, **47**(20), e70004.
250 M. Ünaldı, B. Yıldız and İ. Durukan, *Anal. Lett.*, 2024, **58**(16), 2788–2805.
251 X. Shan, J. Shao, L. Zhang, H. Ye, S. Tan and Y. Le, *Microchem. J.*, 2024, **207**, 111910.
252 M. Soylak, A. M. A. Mohammed and F. Uzcan, *J. Food Compos. Anal.*, 2025, **144**, 107733.
253 M. Soylak, A. M. A. Mohammed and O. Ozalp, *Anal. Lett.*, 2024, **58**(16), 2745–2760.
254 N. Kizil, F. Uzcan, B. B. Beydagi, M. Sahin, B. A. Tokum, M. L. Yola and M. Soylak, *J. Food Compos. Anal.*, 2025, **143**, 107541.
255 F. Uzcan and M. Soylak, *Anal. Lett.*, 2025, **59**(1), 42–56.
256 M. B. Ozen, F. Uzcan and M. Soylak, *Food Chem.*, 2025, **489**, 145018.
257 M. A. Seco, E. T. F. Larruscain, J. R. K. Silveira, R. S. Picoloto and F. A. Duarte, *Braz. J. Anal. Chem.*, 2024, **11**(44), 36–46.
258 L. Vazquez-Palomo, P. Montoro-Leal, J. C. García-Mesa, M. M. L. Guerrero and E. V. Alonso, *Anal. Methods*, 2024, **17**(1), 124–135.
259 M. S. Yalçın, S. Özdemir, E. Kılınç and M. Soylak, *J. Food Compos. Anal.*, 2024, **134**, 106561.
260 A. A. Lemos, C. E. Lujan, M. N. Oviedo, C. Acha and R. G. Wuilloud, *J. Food Compos. Anal.*, 2024, **136**, 106710.
261 N. Altunay, B. Hazer, M. F. Lanjwani and M. Tuzen, *J. Food Compos. Anal.*, 2024, **136**, 106778.
262 Q. Javed, W. A. Khan, N. Shah, M. B. Arain and M. Soylak, *Anal. Lett.*, 2025, **59**(3), 362–372.
263 A. Umaz, K. Umaz, I. Aydin and F. Aydin, *Monatsh. Chem.*, 2025, **156**(4), 381–391.
264 F. Tissot, J. C. Rodriguez and L. Gutierrez, *J. Anal. Chem.*, 2024, **79**(10), 1450–1458.
265 S. Ozdemir, N. Dizge, O. H. Abd-Elkader, G. Plavan and E. Kılınç, *J. Food Compos. Anal.*, 2025, **142**, 107382.
266 H. Abdolmohammad-Zadeh and S. Mousavi, *J. Food Compos. Anal.*, 2024, **136**, 106820.
267 M. B. Arain and M. Soylak, *Food Chem.*, 2025, **463**(Pt 3), 141311.
268 L. Qin, Y. Wang, T. Wan, W. Fan, H. Jiang, B. Zhang, Y. Wu, X. Yu and C. Wang, *Food Chem.*, 2025, **467**, 142324.
269 M. B. Arain, A. Niaz and M. Soylak, *J. Food Compos. Anal.*, 2025, **139**, 107118.
270 E. A. Azooz, S. R. Hussein, I. A. Naguib, M. F. Lanjwani and M. Tuzen, *Food Chem.*, 2025, **485**, 144405.
271 H. U. Haq, B. Eken, B. Çanaklıtaş, R. Dil, B. Yıldırım and N. Altunay, *J. Food Compos. Anal.*, 2025, **144**, 107736.
272 D. Topaloğlu, F. Uzcan, N. Kizil, B. B. Beydagi, D. E. Erbilgin, E. Basaran, M. L. Yola and M. Soylak, *J. Food Compos. Anal.*, 2025, **141**, 107348.
273 G. Salinas-Lucero, K. Juarez-Moreno and R. Vazquez-Duhalt, *J. Food Meas. Char.*, 2024, **18**(11), 9330.
274 M. O. Varra, L. Husakova, P. Iacumin, M. Piroutkova, M. Rossi, J. Patocka, S. Ghidini and E. Zanardi, *Food Chem.*, 2024, **459**, 140303.
275 S. Pérez, R. Vasquez, G. Pascual, J. Araya, J. Neira and C. L. Cespedes-Acuña, *Food Biosci.*, 2024, **61**, 104475.
276 S. Kumar, H. Choi, H. Chae, H. Kim, S. H. Nam, H. Kim, H. Kim, S. H. Han and Y. Lee, *J. Food Compos. Anal.*, 2024, **136**, 106742.
277 M. Ghidotti, S. Papoci, A. Respaldiza, H. Emteborg, F. Ulberth and M. B. de la Calle Guntinas, *Food Chem.*, 2025, **465**(Pt 1), 141989.
278 A. T. Bautista, J. H. D. Aznar, R. A. H. Magtaas, M. M. T. Bauyon, A. J. R. Yu, J. K. G. Balaguer, J. M. Punzalan, J. B. Baroga-Barbecho and C. R. Cervancia, *Food Chem.*, 2025, **474**, 143165.
279 J. Ping, N. Hao, X. Guo, P. Miao, Z. Guan, H. Chen, C. Liu, G. Bai and W. Li, *Food Res. Int.*, 2025, **204**, 115925.
280 G. Kantemiris, P. Kourelias, C. Panagiotopoulou, D. Stefas and S. Couris, *Microchem. J.*, 2025, **212**, 113537.
281 S. Panebianco, G. Barone, M. C. Caggiani, G. Cirvilleri, C. Finocchiaro, G. Lanzafame, P. Mazzoleni, A. Musumarra and M. G. Pellegriti, *Appl. Radiat. Isot.*, 2025, **223**, 111902.
282 L. Wang, T. Galina, L. K. Jing, L. Xu, Y. X. Fu, H. Gao and L. Li, *J. Anal. At. Spectrom.*, 2025, **40**(6), 1526–1535.
283 S. Papoci and M. B. D. Guntiñas, *Curr. Res. Food Sci.*, 2025, **10**, 101099.
284 W. Zeng, Q. Xiong, T. Lin, X. Jiang and X. Hou, *Microchem. J.*, 2024, **207**, 112024.
285 B. Krška, M. Mészáros, T. Bílek, A. Vávra, J. Náměstek and J. Sedlák, *Agronomy*, 2024, **14**(11), 2703.
286 B. Ding, Y. Shi, L. Wang, Z. Liu, S. Wang and H. Zhao, *J. Food Meas. Char.*, 2025, **19**(3), 1886–1897.
287 A. Lee, J. Kwon, S. J. Ahn, J. Lee and H. J. Kim, *Food Addit. Contam., Part A: Chem., Anal., Control, Exposure Risk Assess.*, 2025, **42**(3), 281–292.
288 K. R. S. Gines, E. V. Garcia, R. S. Sagum and A. T. Bautista Vii, *Food Chem.*, 2025, **478**, 143676.
289 H. Su, Z. Li, W. Yu, T. Liu and L. Luo, *Food Chem.*, 2025, **463**(Pt 4), 141486.
290 A. Li, D. Zhao, J. Li, J. Qian, Q. Chen, X. Qian, X. Yang and J. Zhao, *Foods*, 2024, **13**(21), 3417.
291 R. Mamede, I. A. Duarte, S. E. Tanner, V. F. Fonseca and B. Duarte, *J. Food Compos. Anal.*, 2025, **140**, 107291.
292 N. Mercanti, Y. Pieracci, M. Macaluso, A. Zinnai, O. F. X. Donard and V. Vacchina, *Foods*, 2025, **14**(11), 1912.
293 Z. Giannioti, F. I. Brigante, S. Kelly, N. Ogrinc, M. J. Hudobivnik, D. Mazej, A. Tonon, L. Ziller, C. Kukusamude, S. Kongsri, S. Thantar, H. Widyastuti, Y. Yuan and L. Bontempo, *LWT–Food Sci. Technol.*, 2024, **209**, 116752.
294 T. N. Minh, P. Van Thinh, H. L. T. Anh, L. V. Anh, N. H. Khanh, L. Van Nhan, N. Q. Trung and N. T. Dat, *Int. J. Food Sci. Technol.*, 2024, **59**(12), 9234–9244.

295 L. Tian, S. Zheng, H. Zhou, Y. Yang, H. Lu and C. Zhang, *J. Food Qual.*, 2025, **2025**(1), 556138.
296 M. Nikezić, P. Chantzi, J. Irrgeher and T. Zuliani, *Foods*, 2024, **13**(16), 2540.
297 F. Garber, B. Bockmayr, M. Creydt, M. Fischer and S. Seifert, *Anal. Chim. Acta*, 2025, **1357**, 344006.
298 E. C. Mazarakioti, A. Zotos, V. S. Verykios, E. Kokkotos, A. A. Thomatou, A. Kontogeorgos, A. Patakas and A. Ladavos, *Foods*, 2024, **13**(18), 3015.
299 F. Gulino, C. Siragusa, E. Cala, F. Gullo and M. Aceto, *Foods*, 2024, **13**(16), 2634.
300 M. S. Muller, M. Oest, S. Scheffler, A. L. Horns, N. Paasch, R. Bachmann and M. Fischer, *Foods*, 2024, **13**(23), 3783.
301 M.-S. Müller, M. Oest, S. Scheffler, L. Cvancar, A. L. Horns, N. Paasch, R. Bachmann and M. Fischer, *J. Food Compos. Anal.*, 2025, **143**, 107519.
302 M.-S. Müller, C. Springer, E. Middendorf, L. Cvancar, M. Oest and M. Fischer, *J. Food Compos. Anal.*, 2025, **139**, 107168.
303 H. Kim, K. S. Sim, S. H. Hur, J. Song and H. J. Kim, *J. Food Compos. Anal.*, 2024, **136**, 106731.